Muneeb U. Rehman
Sabhiya Majid *Editors*

Therapeutic Applications of Honey and its Phytochemicals

Volume II

Springer

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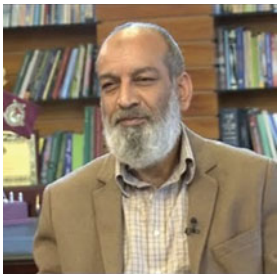
Dedicated to our Parents

Foreword



Honey is one of the nature's marvellous gifts to mankind finding mention in various valued ancient texts. This delicious edible substance produced by honey bees has been consumed by humans since times immemorial to supplement diet and cure various ailments. For its innumerable medicinal properties and health benefits, from ancient times honey has been used in traditional medicines to cure wounds, eye diseases, hiccups, constipation, piles, eczema, ulcers, etc. The various medicinal benefits of honey based on its antioxidant, anti-inflammatory, anti-cancerous, neuroprotective, anti-fibrotic and anti-diabetic properties are attributed to the presence of certain active ingredients in it. Flavonoids and polyphenols, the main bioactive compounds found in honey, are known potent antioxidants. Recent and modern studies have attributed the presence of these bioactive compounds to its therapeutic effects against illnesses related to nervous system, cardiovascular system, diabetes mellitus, gastrointestinal system and even the most dreaded cancers.

The book entitled *Therapeutic Applications of Honey and its Phytochemicals* (Volume II) is an in-depth compilation of recent research on this subject. The editors Dr. Muneeb U. Rehman and Prof. Sabhiya Majid have compiled the book splendidly shedding light on all the valuable research literature currently available on the topic. Volume II is based on prevention and treatment of various diseases by honey and its phytochemicals, providing finest details related to their possible mechanism of action. The main highlights of this volume are book chapters by collaborators from around the globe. Scientists from USA and Saudi Arabia have detailed the molecular mechanistic approach of anti-leukemic bioactive compounds in honey. Another chapter deals with the neuro-protective effect of honey and its mechanistic

basis. A group from Saudi Arabia and UAE has discussed neuro-protection via NAD^+ pathway in various neuro-degenerative diseases. Collaborators from India and Saudi Arabia have discussed the molecular mechanisms underlying prevention and treatment of cancers by honey and its phytochemicals. Another group from India has elaborated upon the role of the phytochemicals from honey as MAP-kinase inhibitors. Besides these, the other major chapters included in this book are focussed on mechanistic basis of prevention and treatment of various diseases including fibrosis, diabetes, metabolic disorders, dermatitis, cardiovascular diseases, arthritis, wound healing and fatty liver disease by honey and its phytochemicals.

Therapeutic Applications of Honey and its Phytochemicals (Volume II) is an exhaustive and finest compilation of its kind. It specifically presents a holistic view of the available literature on honey and its medicinal value. The editors have incredibly provided solid foundation of the subject to meet the requirements of researchers, medical practitioners and entrepreneurs. For medical practitioners including those of alternative medicine, herbal therapists, and dieticians this book will be of immense clinical benefit. For academicians, scientists, teachers and students, this book will be a rich source of information from where they can satiate their quest for knowledge.

University of Kashmir Khurshid Iqbal Andrabi
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Contents

About the Editors

Muneeb U. Rehman PhD is a faculty member at College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. He holds a doctorate in Toxicology (specialization in cancer biology and natural product research) from Jamia Hamdard, New Delhi, India Dr. Rehman has more than 10 years of research and teaching experience in the field of toxicology, biochemistry, cancer biology, natural product research and pharmacogenomics. He is the recipient of several national and international fellowships and awards. He has published 90 research papers in peer-reviewed, international journals, and 25 book chapters. Dr. Rehman is an on the editorial boards and is a reviewer of several high-impact, international scientific journals. He is also a life member of various international societies and organizations. Currently, Dr. Rehman is engaged in studying the molecular mechanisms of cancer prevention by natural products and the role of pharmacogenomics and toxicogenomics in evaluating the effectiveness and safety of drugs.

Sabhiya Majid PhD is a Professor and Chairperson of the Department of Biochemistry, Govt. Medical College (GMC-Srinagar), J&K, India. She is also the Nodal Officer of the Multi-disciplinary Research Unit (ICMR-MRU) funded by Indian Council of Medical Research. Prof. Majid has received numerous grants from various Government of India funding agencies, and has implemented various research projects and infrastructure development schemes, including FIST–DST-GOI (Department of Science & Technology, Fund for Improvement of Science &Technology Infrastructure, Govt. of India. She is a member of several scientific associations and Boards of Undergraduate, Postgraduate and Research Studies. Prof. Majid has 25 years of experience in teaching, research and supervision of diagnostic laboratories at the Govt. Medical College Srinagar, including teaching MBBS, BDS, nursing and medical laboratory technology students. Her current research focuses on identifying non-invasive markers for cancer diagnosis.

Molecular Mechanistic Approach of Important Antileukemic Compounds Present in Honey

1

Insha Amin, Arif Ali, Bilal Ahmad Mir, Rayeesa Ali, Sheikh Bilal Ahmad, Manzoor Ur Rahman Mir, Wajhul Qamar, Azher Arafah, Muneeb U. Rehman, and Tahir Maqbool Mir

Abstract

Homeostasis is a collective name for the self-regulating cellular processes that maintain cell stability and survival. Any variation from normal functioning in any biological process can lead to different diseases or syndromes such as cancer, diabetes, and metabolic syndromes. Cancer results by the uncontrolled division of cells in any organ or tissue and can metastasize to other organs as well. Derailment in the process of cell division is the main cause for caner development. Leukemia—a type of cancer in which the function and production of blood cells gets affected, is one of the leading cancers-related mortalities throughout the world. Scientific research has witnessed a great interest in the pharmacodynamics of naturally occurring food products or other plants or plant products of medicinal

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value in order to make them novel drug agents to target various diseases including cancer. This chapter vividly describes phenolic compounds that can be used as signature drugs to target leukemias. Gene expression and microarray studies have depicted the various signaling pathways regulated by these compounds and, hence, serve in inducing decreased cell growth and malignancy in leukemias. Honey is well-known for its nutritional and medicinal properties since ages and is continuously being explored for its wide pharmacological properties. Studies have attributed significant anti-cancerous action to honey, but very less literature is available on its antileukemic action. In this present chapter, we have summarized the anti-leukemic activities associated with bioactive components present in honey. Honey is a storehouse of biologically essential phenolic compounds such as phenolic acids, tannins, flavonoids, terpenoids, and coumarins. These compounds show tumor reduction or inhibitory action by arresting the cell cycle, up- or downregulating mRNA expression of proteins involved in apoptotic cascades like Bax, caspase-3, Bcl-2, NOXA, MCL-1, rTRAIL, FAS, SCF/c-Kit complex, p-ATM, p-ATR, 14-3-3 proteins sigma, MGMT, and HDACs; deactivating drug efflux ABC transporters; various cyclins and CDKs; and decreasing mitochondrial membrane potential. Till date no study elucidating the effect of raw honey-derived phenolic compounds has been undertaken and, therefore, a wide scope exists for studying the effective chemotherapeutic mechanisms of these compounds.

Keywords

Cancer · Leukemia · Blood cancer · Honey · Phytochemicals · Apoptosis · Chemoprevention · Chrysin · Quercetin · Apigenin · Kaempferol · Galangin · Hesperetin · Coumarin

1.1 Introduction

Several self-regulating biological processes take place in cells to maintain stability with survival conditions (homeostasis). To maintain cell homeostasis, many physiological processes take place inside the cell. Any derailment or fluctuation in the basic mechanisms maintaining cellular metabolism leads to different diseases (Biswal et al. 2017). Cancers occur due to the overgrowth of cells of any organ and can metastasize leading to various malignancies (Cooper 2000). Leukemia is one of the leading cancers affecting blood cell components and resulting in high risks to the life of patients, with a global prevalence of approximately 300,000 new cases every year. The cellular growth in these cancer cells can be kept under control by either apoptosis or autophagy. Hence, the escape of cells from apoptosis or autophagy stimulates proliferation of cells that can continue to grow rapidly leading to cancer, which can eventually metastasize to other organs. Scientific research has witnessed a great interest in the pharmacodynamics of naturally occurring food products or other plants or plant products of medicinal value in order to make them novel drug agents

to target various diseases. In this regard, phenolic compounds have been expressed notable potential. The antioxidant and antiinflammatory properties of phenolics make them potential candidates for therapeutic agents against remarkable diseases including cancers.

Honey is well known for its nutritional and medicinal importance and is continuously being explored for its wide pharmacological properties. Studies have revealed, potential anti-cancerous action to honey, but very less literature is available related to its anti-leukemic action. Honey is considered as a storehouse of biologically essential phenolic compounds such as phenolic acids, tannins, flavonoids, terpenoids, and coumarins. All these compounds have been studied to possess pronounced antitumorigenic action in various cancers including leukemias of different types. These compounds show tumor reduction or inhibitory action by arresting the cell cycle (Fig. 1.1), up- or downregulating mRNA expression of proteins involved in apoptotic cascades like Bax, caspase-3, Bcl-2, NOXA, MCL-1, rTRAIL, FAS, SCF/c-Kit complex, p-ATM, p-ATR, 14-3-3 proteins sigma, MGMT, and HDACs. Moreover, these compounds exhibit their anti-cancer action by deactivating drug efflux ABC transporters, various cyclins and CDKs, and decreasing mitochondrial membrane potential. But, in particular, further research is required to elucidate the antileukemic properties of these compounds after being isolated and characterized from honey. This chapter summarizes the molecular mechanisms associated with the antileukemic action of bioactive compounds present in natural honey.

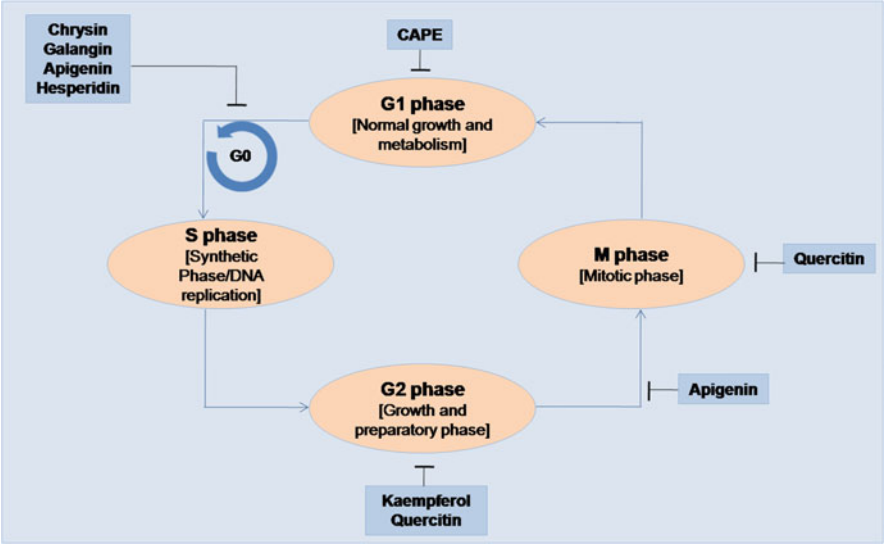

Fig. 1.1 Different chemical components present in honey causing cell cycle arrest at different phases of cell division during leukemia. Caffeic acid phenylethyl ester (CAPE) leads to cell cycle arrest at the G1 phase of cell division. Chrysin, galangin and hesperidin arrest cell cycle at the G1/G0 stages while apigenin does so at the G1/G0 and G2/M stages. Kaempferol induces arrest of the cell cycle at the G2 phase and quercitin at the G2/M phases of the cell cycle during leukemia

1.2 Leukemias

Hematologic cancers include myeloproliferative neoplasms, acute myeloid leukemia (AML), Hodgkin lymphoma, myelodysplastic syndromes, and chronic lymphocytic leukemia (CLL) (Genovese et al. 2014). AML is a major blood cell malignancy causing increase in the proliferation of clonal myeloid cell precursors and finally increased concentration of myeloid cells in the bone marrow (Zhu et al. 2019). It has been studied that most commonly CLL occurs due to an imbalance of lymphocyte apoptotic mechanisms leading to the abnormal proliferation of lymphocytes (Billard 2014).

Cancers occur due to any dysregulation in the normal physiological suicide programs. Programmed cell deaths can be widely of two types, viz., I and II, and are quite essential for the maintenance of steady cellular state (Nikoletopoulou et al. 2013). Hence, execution of these cellular death programs helps in the prevention of tumorigenesis. Apoptosis is an important suicidal program and is regarded as type I programmed cell death whereas cell death due to autophagy is regarded as type II programmed cell death (Towers et al. 2020). Interplay between apoptosis and autophagy is a vital phenomenon for cellular homeostasis (Li et al. 2020). Apoptosis in a cell is characterized by mitochondrial membrane potential loss, cytoplasmic blebbing, DNA fragmentation, and apoptotic body formation (Li et al. 2020). Another mechanism for cell death is autophagy characterized by lysosomal activation and the subsequent activation of degradation or phagocytotic pathways. A large set of proteins are associated with the activation of the apoptotic cascade or signaling in a cell. There may be proapoptotic and antiapoptotic classes of proteins which execute simultaneously to induce apoptosis. Cell death or apoptosis is notably induced by the upregulation of proapoptotic proteins and concomitant downregulation of antiapoptotic proteins. Bcl-2 family of proteins like Bcl-2, Bcl-XL, and Mcl-1 are regarded as antiapoptotic and are present in mitochondria (Bae et al. 2020). On the other hand, proapoptotic molecules like Bax, Bad, Bck, and BH3 domain (Puma, Noxa, Bid, Bim) are upregulated in cancer cells and cause decrease in the survival of cells (Bae et al. 2020; Yan et al. 2020).

Furthermore, any derangement in the cell cycle progression has a pivotal role in cancer development (Pirtoli et al. 2020). Cellular growth involves a series of molecular events in which a parent cell converts into new daughter cells, enabling cells to grow. The cell cycle has four important phases, namely gap 1 or the G1 phase, synthesis or the S phase, gap 2 or the G2 phase, and mitosis or the M phase. At the molecular level, two enzyme complexes, viz., cyclin A–cyclin T and the cyclin-dependent protein kinases (CDK 1–CDK 9) (Abubakar et al. 2012) are involved in the course of cell cycle.

1.3 Anticancer Compounds in Honey

Honey is a miraculous food and is a depot of almost 200 substances such as sugars including fructose and glucose, amino acids, proteins, trace amount of vitamins and enzymes, water, etc. (Wang and Li 2011) Phenolic acids and flavonoids are the two

Table 1.1 Phenolic Compounds Found in Honey

S. no.	Main bioactive compound	Type
1.	Phenolic acids	Caffeic acid
		Ellagic acid
		Ferulic acid
		p-Coumaric acids
2.	Flavonoids	Chrysin
		Apigenin
		Kaempferol
		Galangin
		Quercetin
		Pinocembrin
		Hesperetin
3.	Coumarins	Coumarin

major pharmacopotent classes of compounds present in honey (Stephens et al. 2010) (Table 1.1). Catalase, superoxide dismutase, reduced glutathione, tocopherols, and ascorbic acid are the major compounds present in honey with antioxidative properties. Honey and its many bioactive compounds possess antioxidative, antiinflammatory, chemopreventive, immunoregulatory, antiatherogenic, and wound healing properties (Fig. 1.2).

Phenolic acids are bioactive molecules present in many valuable foods including honey. They possess many essential biological activities like antiinflammatory, anticancerous, antioxidative, and antiatherogenic. Protocatechuic, p-coumaric, caffeic, and vanillic acids are the various constituents of honey derived from hydroxybenzoic acid and have potential antitumorigenic activity (Rocha et al. 2012; Tanaka et al. 2011).

1.4 Honey in Other Cancers

Honey has been used in its raw form to treat a number of cancers and has shown significant activity in combating cancerous growth. Several studies have reported potent anticancerous activities against many cancers like liver (Baig and Attique 2014), cervical (Fauzi et al. 2011), oral, bladder (Swellam et al. 2003), bone, and breast (Fauzi et al. 2011) cancers (Fig. 1.2). Vascular cell adhesion molecules (VCAM-1) and intercellular adhesion molecule or cluster differentiation 54 (ICAM-1 or CD54) are the important endothelial cell–associated adhesion molecules which are downregulated in prostrate (PC-3) and breast (MCF-7) cancer cell lines when exposed to different types of Greek honey (Spilioti et al. 2014). Furthermore, honey is a treasure of many compounds with properties of mitigating oxidative stress in cells. Spilioti and colleagues have reported that anticancer properties of honey were associated with its oxygen radical absorbance capacity (ORAC).

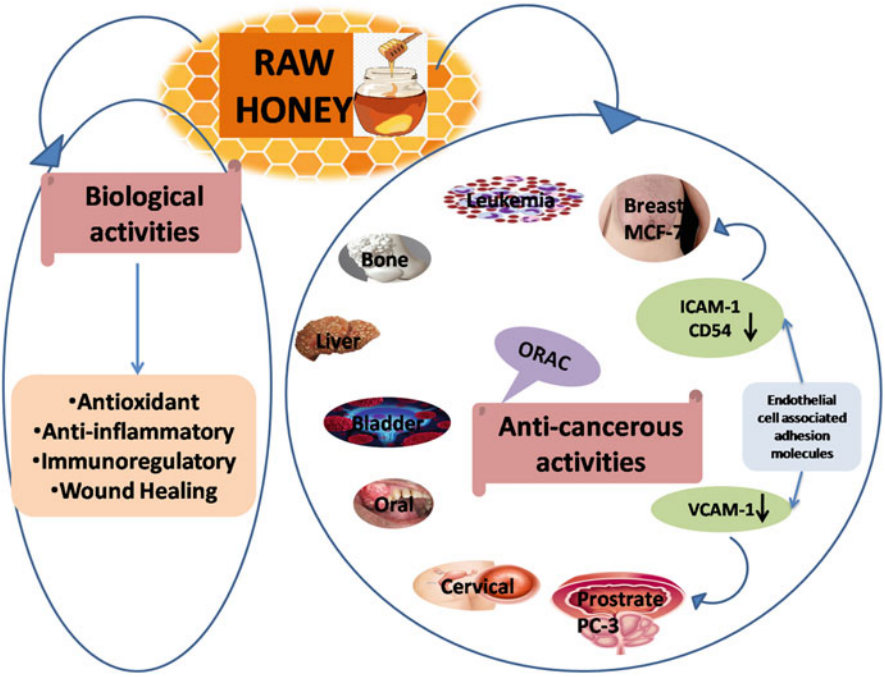

Fig. 1.2 Pharmacological activities of raw honey. Natural honey possesses numerous biological activities such as scavenging of toxic free radicals in the body by its antioxidant activity. Honey is a potent antiinflammatory agent resulting in the downregulation of key inflammatory markers (iNOS, Cox-2). Honey is a natural immunostimulant that protects cells from several pathogens. Honey enhances the wound healing process that may be interrelated with its antioxidant, antiinflammatory, and immunoregulatory activities. In addition, honey has been proved to be very beneficial against various cancers including leukemia, and bone, liver, oral, and prostate cancers

1.5 Honey in Leukemia

Till now very little literature is available reporting honey as an antileukemic substance in either in vitro or in vivo trials. Tualang honey (TH) is a widely studied variety of honey obtained from wild honey bees. Tualang trees present in the rain forests of Malaysia are home to these wild honey bees. It is the source of a number of biologically active compounds. It shows significant antileukemic effects by inducing apoptosis in leukemic cell lines. Microscopically apoptotic changes like membrane blebbing, apoptotic bodies, cell roundness, and fragmentations were seen in the TH-treated cell lines, which clearly depict the apoptosis-inducing ability of TH (Man et al. 2015). However, TH showed more pronounced antileukemic effects in acute leukemia as compared to chronic leukemic models.

An in vivo study revealed that raw honey and one of its phenolic compounds, eugenol, could not produce significant antileukemic activity against the rat leukemia

model. The median survival time (MST) showed nonsignificant increase using all the honey samples in comparison to the positive control (Jaganathan et al. 2014). However, additional research designed to validate the effective molecules in honey possessing antileukemic effects needs to be conducted in future.

1.6 Kaempferol

Bestwick et al. (2007) deduced the antiproliferative action of kaempferol in promyelocytic leukemia cells (HL-60), leading to various changes in cell cycle. A significant increase in the S-phase of cell cycle showed apoptotic changes like increased caspase-3 activity and decreased antiapoptotic Bcl-2 expression. In acute promyelocytic leukemia (APL), kaempferol treatment led to increase in apoptotic gene expression and concomitantly inhibited multidrug resistance. In HL-60 and NB4 leukemia cell models, kaempferol induced apoptosis by Akt and BCL2 downregulation while causing CASP3 and BAX/BCL 2 ratio upregulation (Moradzadeh et al. 2018). Cancer cells show prominent multidrug resistance that makes the anticarcinogens ineffective in these cells. ABC (ATP-binding cassette) transporters are upregulated in these cells causing efflux of the anticancerous drugs from the cancer cell, thus impeding the action of drug in the cancer cell (Chang et al. 2020). Kaempferol lead to a concentration-dependent decrease in the expression of ABCB1and ABCC1, which indicated inhibition of multiple drug resistance in leukemic cell lines (Moradzadeh et al. 2018). This suggests that kaempferol can be used as a potential anticancer drug substitute in cells that show resistance to chemotherapeutic drugs. Kim et al. (2016) found that G2 cell cycle arrest and mitochondrial system apoptosis led to cytotoxic effects due to kaempferol in leukemia. It was proposed that the antitumor activity of kaempferol might be due to hyperactivation of the ATM/ATR-Chk1/Chk2 pathway, which is important for inducing DNA damages in the cell. Furthermore, increase in phosphorylation at Ser-15 of the tumor suppressor p53 gene; upregulation of proapoptotic genes like Bak, PUMA (p53 upregulated modulator of apoptosis), and caspase enzyme (caspase 3, 8, and 9); and significant loss in mitochondrial membrane potential ($\Delta\psi m$) were responsible for antitumorigenic activity in leukemia cells (Fig. 1.3).

Comet tail formation and fragmentation of cellular DNA depicting cell apoptosis occurred in human promyelocytic leukemia cells (HL-60) when treated with kaempferol. Inhibition of the expression of a set of DNA damage repair associated protein like 14-3-3 proteins sigma (14-3-3σ), p-ATM, p-ATR, O6-methylguanine-DNA methyltransferase (MGMT), DNA-dependent protein kinases, p53 and MDC1, have been depicted in kaempferol-treated leukemic cell lines (Wu et al. 2015).

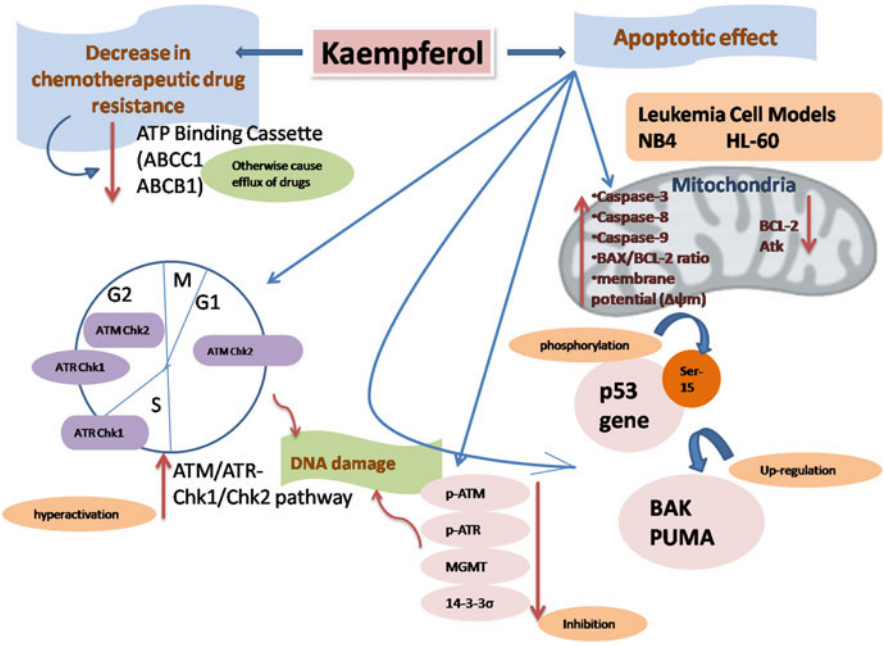

Fig. 1.3 Role of kaempferol in cell signaling pathways in leukemia cells. In leukemia cell models, kaempferol induces apoptosis by downregulating Akt and BCL2 and upregulating caspases and the BAX/BCL 2 ratio. Furthermore, kaempferol enhances the effect of other chemotherapeutic drugs (when given in combination) by inhibiting the ATP binding cassette (such as P-gp efflux pump), thus leading to increasing bioavailability of chemotherapeutic drugs during the treatment of leukemias. Kaempferol halts cell division by arresting cell cycle at the G1 stage resulting in cytotoxicity in leukemia. The antitumor activity of kaempferol is due to hyperactivation of the ATM/ATR-Chk1/Chk2 pathway, which is important for inducing DNA damages in the cell. Furthermore, increase in phosphorylation at Ser-15 of the tumor suppressor p53 gene; upregulation of proapoptotic genes like Bak, PUMA (p53 up-regulated modulator of apoptosis), and caspase enzyme (caspase 3, 8 and 9); and significant loss in mitochondrial membrane potential (Δψm) were responsible for the antitumorigenic activity in leukemia cells

1.7 Quercitin

Chemically, quercetin (3,3′,4′,5,7-penta-hydroxyflavone) is an important bioactive compound found in honey. Quercitin shows proapoptotic synergistic effect with cisplatin, when given in combined treatment in murine leukemia cell lines (L1210) (Čipák et al. 2003). Quercetin resulted in time- and dose-dependent decreases in the proliferation of HL-60 cells by trapping of cells in G(2) and M phases of cell cycle and over expression of apoptotic genes (Ren et al. 2010). Potentiating the effect of antileukemic drugs has been found with quercetin treatment in human leukemic cell lines like U937, HL-60, and THP-1. It has been reported that several anti-cancer drugs exert their anti-cancer action by inducing apoptosis in cancer cells due to loss

of mitochondrial membrane potential and decrease in reduced glutathione (GSH) content (Ramos and Aller 2008). Apoptotic cascade-like activation of Bax, caspase-8, cytochrome c, and Omi/Htra2 with Bcl-XL downregulation might be the driving mechanisms for the antileukemic effect in these cells. Kang and Liang demonstrated inhibition of proliferation of HL-60 cells by quercetin in a dose- and time-dependent manner via inhibition in the activities of TPK (tyrosine protein kinase) in the membrane and PKC (protein kinase C) in the cytosol (Kang and Liang 1997).

Quercitin led to enhanced Bax and caspase-3 expression activity with concomitant downregulated expression of Bcl-2 and NF-κB p65 mRNA levels, leading to cell cycle arrest at S phase in leukemic rat models (Han et al. 2015). Quercitin is a highly advantageous pharmacologically active compound showing prominent antileukemic effects but suffers due to its slow solubility and, therefore, decreased bioavailability. Therefore, to potentiate the pharmacokinetics and pharmacodynamics of quercetin, pharmaceutical nanotechnology comes to the rescue. Recently, quercetin-loaded polymer-lipid hybrid nanoparticles were used to potentiate the antileukemic effect of quercetin (Yin et al. 2019). Epigenetic modification, including posttranslational modifications, and DNA methylation of various proapoptotic genes also led to the antileukemic effects of quercitin. STAT-3-dependent increase in the expression of DNA methyltransferase (DNMT1 and DNMT3a) downregulated class I histone deacetylases (HDACs) and increased demethylation of apoptosis inducers, BCL2L11; DAPK1 genes were also seen in AML models in both in vivo and in vitro studies (Alvarez et al. 2018).

A crosstalk between apoptosis and autophagy has been reported by many studies for the pronounced antileukemic effect of quercetin. Quercetin in combination with green tea was able to cause significant reduction in BCL-2, BCL-XL, and MCL-1 proteins, overexpression of BAX, and caspase-3 stopping tumor growth in HL-60 (Calgarotto et al. 2018). Cells were trapped in the G1 phase of the cell cycle, and the activity of autophagy-inducing proteins was also enhanced by quercitin treatment. Chang et al. (2017) further demonstrated inhibition of CDK2/4, hence halting the cell cycle progression. Activation of proapoptotic signaling like the caspase pathway and poly (ADP ribose) polymerase (PARP)-1 cleavage are regarded as key mechanisms for tumor regression. Activation of the autophagic cascade marked by upregulated expression of LC3-II (light chain 3), downregulation of p62, and formation of acidic vesicular organelles were found to be associated with antileukemic effects in HL-60. Recently, a study on the CML cell line K-562 inferred the antiproliferative effect of quercetin as there was a reduction in the expression of some of the vital prosurvival proteins, especially heat shock proteins (HSP70), Bcl-X(L), and Forkhead box protein M1 (FOXM1), and simultaneous upregulation in proapoptotic genes like caspases (3 and 8) and Bax (Hassanzadeh et al. 2019). This suggests quercetin can be a candidate flavonoid for attenuating the proliferative mechanism by increasing apoptosis and antisurvival mechanism in leukemic cells.

Quercitin results in a dose-dependent reduction in levels of inositol 1,4,5-triphosphate (IP3) and expression of oncogenes, viz., c-myc and ki-ras, leading to a fragmentation of nucleosomal DNA in K562 human leukemia cells (Csokay et al. 1997). G2/M arrest associated with significant decline of cyclin D, cyclin E, and

elongation factors (E2F1and E2F2), and cyclin B overexpression occurred in quercetin-treated HL60 cells (Lee et al. 2006). Caspase-3 activation indicated by proteolytic cleavages of its target, i.e., PLC-γ1, led to DNA lysis and death in these leukemic cells. Spagnuolo et al. (2012) demonstrated that transcriptional activity of cell death-inducing proteins like recombinant tumor necrosis factor-alpha-related apoptosis-inducing ligand (rTRAIL) and CD95/FAS/apoptosis antigen 1 (APO-1) was upregulated in ALL when exposed to quercetin. Also, both mRNA and protein levels of Mcl-1 were decreased depicting the proapoptotic activity of quercetin.

Multiple myeloma (MM), a hematological cancer of plasma cells of bone marrow, incidence has increased recently all over the world. Quercitin led to the activation of a proapoptotic pathway in MM by upregulating p21, caspases (3,9), and poly(ADP-ribose)polymerase expression; c-myc downregulation; and G2/M cell cycle arrest (He et al. 2016). Furthermore, in vivo studies in xenograft models also revealed tumor growth inhibition using quercetin.

1.8 Chrysin

Chrysin or 5,7-dihydroxyflavone is a bioactive flavonoid found in honey and possesses a wide range of pharmacological activities including anticancer effects. Chrysin treatment in leukemic BALB/c mice enhanced the T and B cell populations and increased macrophage-induced phagocytosis and natural killer cell cytotoxicity, ascertaining the probable mechanism for antileukemia (Lin et al. 2012). These immunological effects were seen by increased number of cell surface markers of CD3 which is a T-cell maker, CD19, a B-cell marker, and Mac-3, indicating initiation of phagocytosis in the cells. Methylated chrysin, viz., 5,7-dimethoxyflavone (DMF), could be a potential candidate to treat ALL. DMF produces antileukemic effects by arresting the cell cycle (G0/G1), downregulates phosphorylated retinoblastoma-associated protein 1, and induces apoptotic changes in ALL (Goto et al. 2012). The apoptotic effect of chrysin has been established in ALL cell lines such as U937, MO7e, THP-1, and HL-60. However, Zaric et al. (2015) found that chrysin can induce proapoptotic and decrease antiapoptotic mechanisms in chronic leukemic cell lines like MOLT-4 and JVM-13 and lymphocytes isolated from B-CLL patients. A decrease in cell viability, lowered expression of Bcl-2, and activation of Bax, caspases, and mitochondrial cytochromes led to apoptosis in leukemic cell lines (Fig. 1.4). Stem cell factor (SCF) in combination with c-Kit executes its function in the proliferation and differentiation of hematopoietic stem cells. Chrysin shuts down the SCF/c-Kit-complex mediated pathways of cell differentiation via downregulation of the PI3K pathway and a concomitant upregulation of ERK5, CREB, and STAT3 (Lee et al. 2007). Woo et al. (2004) found an overexpression of caspase 3 and myr-Akt signaling pathways to be associated with apoptosis induction in U937 leukemic cell lines.

Decreased expressional activity of myeloid cell leukemia-1 (MCL-1) is associated with antileukemic properties of chrysin (Polier et al. 2011). MCL-1 belongs to the BCL-2 family and is, thus, a key regulator in the maintenance of

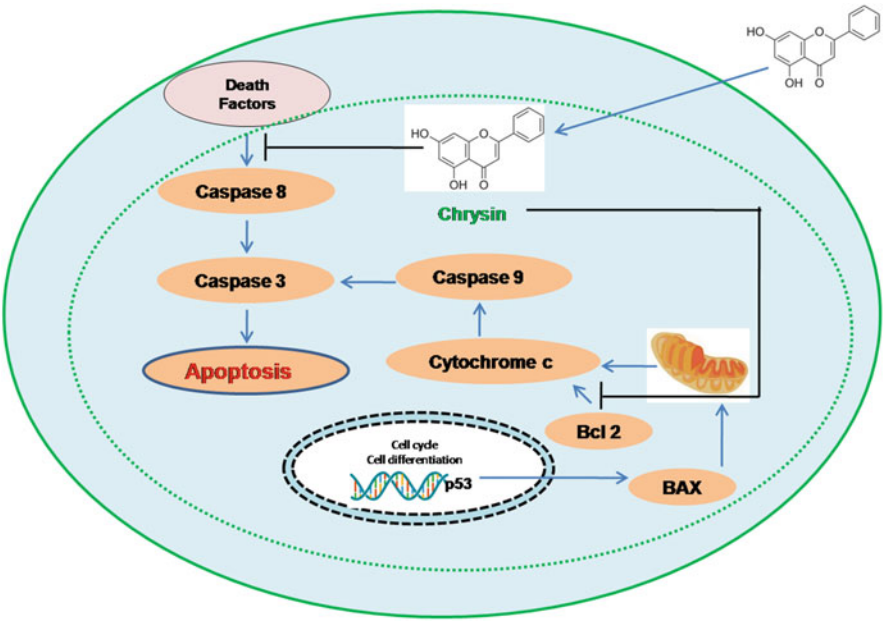

Fig. 1.4 Anticancer activity of chrysin, endorsing apoptosis. Chrysin influences death factors present on cell membranes (such as TNF-related apoptosis inducing ligands—TRAIL), downregulating antiapoptotic protein Bcl 2, and activation of Bax, caspases and mitochondrial cytochromes leading to apoptosis in leukemic cell lines

homeostasis and growth (Xiang et al. 2018). Hence, inhibition of MCL-1 is a target for designing drugs effective for cancer prevention. Polier et al. (2011) found the association of Mcl-1 gene downregulation with the inhibition of cyclin-dependent kinase 9 (CDK9) and Ser2 phosphorylation at the COO^{-} (carboxy) end of RNA polymerase II (RNA pol II). This decreased transcriptional activation of Ser2 finally stops the mRNA synthesis and protein formation. Chrysin-mediated apoptosis occurs due to the induction of mitochondrial membrane collapse, increased reactive oxygen species, caspase 3 activation, and selective inhibition of complex II and complex V (ATPases) in CLL (Salimi et al. 2017). Activation of caspase 3 and 8 pathways in human leukemia cell lines (U937) has also been linked with the chrysin-dependent antileukemic effect (Monasterio et al. 2004).

1.9 Galangin

Galangin's antileukemic effect in human leukemia cell lines (U937) was found to be associated with overactivation of caspases 3 and 8 inducing apoptosis and DNA fragmentation of cancerous cells (Monasterio et al. 2004). Tolomeo et al. (2008) found galangin potentiated imatinib's apoptotic and antiproliferative activity in sensitive and resistant leukemic cell lines. This was linked to an arrest of the cell

cycle at the G0–G1phase with a concomitant decrease in the level of cyclins (cdk1, cdk4, and cycline B), retinoblastoma (pRb) and BCL-2. Galangin (1–100 μM) exerted a dose- and time-responsive antiproliferative effect in HL-60, a human leukemia cell line (Bestwick and Milne 2006). Furthermore, DNA abnormalities like increased hyopodiplody, membrane disruptions, and caspase-3 activation in cancer cells were attributed with the antileukemic efficacy of galangin. Galangin has significantly shown antiproliferative potential in other cell lines like human hepatocellular carcinoma (HepG2) as well, by inhibiting protein kinase C/extracellular signal-regulated kinase (PKC/ERK)-mediated signaling pathway (Chien et al. 2015). It was also found that G2/M or G1 phase of the cell cycle are inhibited in colorectal cell lines (HCT116) on exposure to galangin (Sulaiman 2016).

1.10 Apigenin

Apigenin is a vital dietary flavonoid and has a chemical formula of 4′,5,7-trihydroxyflavone. Budhraja et al. (2012) found that apigenin treatment led to a pronounced apoptotic pathway through Akt, JNK, and caspase hyperactivation with concomitant cytochrome c release from mitochondria in human leukemic cells in dose- and time-responsive ways. In vivo administration resulted in decreased proliferation and subsequent apoptosis of tumors in U937 xenografts. Activation of caspase (9 and 3) and PKCδ, MAPKs, p38, and ERK as a result of their phosphorylation and induction of oxidative stress (ROS) inside the leukemic cells led to apoptosis and cellular death (Vargo et al. 2006). This study signifies the essential and pronounced role of PKCδ for the antileukemic nature of apigenin as was also observed after PKCδ silencing in leukemia cells.

Another mechanism for apigenin's apoptotic activity might be its potent CDK inhibition which, in turn, led to the downregulation of the prosurvival or antiapoptotic gene, Mcl-1 (Polier et al. 2011). Both MCL-1 and PI3K/AKT inhibition is known to cause apoptosis and, hence, significant antileukemic effects in CLL [Shehata et al. 2010]. Along with CDK, apigenin is believed to produce pronounced proteasomal inhibition which is linked in attenuating tumor growth, therefore, producing antileukemic actions in CLL cells (Chen et al. 2005).

NOXA or phorbol-12-myristate-13-acetate-induced protein 1, one of the major proteosome inhibitors, led to proapoptotic action in CLL when exposed to apigenin (Fennell et al. 2008). NOXA is responsible for stimulating various processes like caspase activation, changes in mitochondrial membrane constitution and potential, and proapoptotic cascade leading to apoptosis in cells. The Noxa/Mcl-1 axis serves as an effective target for generating apoptotic signals in CLL as NOXA is linked to proteosomal degeneration of MCL1 (Billard 2014) leading to decrease in survivability in cells.

Ruela-de-Sousa and colleagues have reported apigenin as a potent chemopreventive agent in erythroid and myeloid leukemic cell lines. Apigenin induced a G2/M and G0/G1 phase arrest of the cell cycle in myeloid myeloid (HL60) and erythroid (TF1) cells, respectively, which might be through

downregulation of the JAK/STAT pathway. Antiproliferation through the PI3K/PKB pathway inhibition which is, in turn, due to the mechanistic activation of PTEN and apoptotic caspase pathway activation was observed in myeloid cells only. On the other side, only increased autophagic activity was seen in erythroleukemic cells, indicating a dominant effect of apigenin in leukemia. Apigenin also induced autophagy through mTOR and P70S6K downregulation, in turn, reducing S6 protein phosphorylation. TF1 cells also showed reduction of autophagy-related genes, viz., Atg5, 7, and 12 with apigenin treatment.

1.11 Hesperidin

Hesperidin, a flavanone, is a bioactive component in many citrus fruits and honey that activates proapoptotic mechanisms in tumor cells leading to decrease in cell proliferation. However, very less research has been conducted until now regarding the antileukemic potential of hesperidin. One study by Ghorbani et al. (2012) showed the antileukemic effects of hesperidin in human pre-B cell lines (NALM-6). Further, significant growth inhibition was seen in these cell lines particularly through the overexpression of PPARγ under a hesperidin dosage of 10–50 μM. Apoptosis was induced by upregulation of antisurvival or proapoptotic protein, Bax, and a concomitant reduction in Bcl-2 and XIAP expression under a dose of 10–100 μM. Furthermore, involvement of p53 in tumor suppression was suggested as there was upregulated expression of p53 when NALM-6 cells were pretreated with hesperidin. Another study available suggests that hesperidin causes reduced cell proliferation in human CML cells (K562) by arresting cell cycle at G0/G1 phase and triggering programmed cell death (Adan and Baran 2016). Microarray analysis revealed involvement of downstream signaling pathways like JAK/STAT, KIT receptor, and growth hormone receptor for the antiproliferative tools of hesperidin. Interestingly, mitochondrial membrane depolarization and activation of caspase-3 led to apoptosis and cellular death in CML cells. This suggests hesperidin as an effective target for chemotherapeutic strategy in CML treatment protocols.

1.12 Caffeic Acid Phenylethyl Ester (CAPE)

Chemically CAPE is 2-phenylethyl, 3-(3,4-dihydroxyphenyl) acrylate and is also known as phenethyl caffeate or phenylethyl caffeate (Kumazawa et al. 2010). CAPE shows distinct pharmacological actions due to its varied properties, viz., antiinflammatory, antimicrobial, and cytotoxic (Murtaza et al. 2014). Probably the first study conducted by Chen and coworkers demonstrated decrease in cellular progression in HL-60 with exposure to CAPE in a dose-dependent manner. Almost about 74% growth inhibition was observed with 10 μM of CAPE exposure within 48 h. CAPE treatment was found to induce cytoplasmic blebbing, membrane disruptions, and chromatin condensation in HL-60 cell lines with about 25% and 67% growth reduction following 6 and 72 h treatment, respectively (Chen et al.

2001). Furthermore, apoptotic mechanisms like the activation of caspase-3 and Bax simultaneously with Bcl-2 expression caused inhibition to occur in CAPE-treated HL-60 cells. Mitochondria-mediated apoptosis involved increase in cytosolic cytochrome c, downregulation of Bcl-2 expression, and activation/cleavage of caspase-3 and PARP with overexpression of Bax in U937 cells when treated with CAPE (Jin et al. 2008).

Differentiation is a leading mechanism in cancerous cells that decreases malignancies, generating benign tumors and decreases self-renewal properties in cancer cells (Deng et al. 2018). CAPE enhances the granulocytic differentiation property of ATRA (all-trans retinoic acid) in HL-60 by causing arrest at the G1 phase via inhibition of cdk2-cyclin E complex formation (Kuo et al. 2006). Also, RARα, p21, and C/EBPε proteins showed enhanced activity leading to differentiation in ALL cells.

Recently CAPE exposure in lymphoblastoid cell line, PL104, lead to the activation of apoptotic mechanisms like loss of mitochondrial potential (Δψm), nuclear fragmentation, and G1 stage arrest (Cavaliere et al. 2014). Transcriptional analysis showed that survivin and Bcl-2 expressions were downregulated with subsequent increase in Bax, and caspases 3, 7, and 9.

1.13 Conclusion

Dietary phenolic acids and flavonoids, in general, and, specifically, in honey can be widely exploited as chemotherapeutic agents that can help in alleviating diseases and fight critical health conditions including cancer. Most of these antiproliferative compounds cause decrease in cell growth by putting the cell into an apoptotic environment, halting cell cycle, and activating tumor suppressor pathways. However, the mechanism of action of these compounds is still very ambiguous. This chapter vividly describes phenolic compounds that can be used as signature drugs to target leukemias. Gene expression and microarray studies have depicted the various signaling pathways regulated by these compounds and, hence, serve in inducing decrease cell growth and malignancy in leukemias. Till date no study elucidating the effect of raw honey–derived phenolic compounds has been undertaken and, therefore, a wide scope exists for studying the effective chemotherapeutic mechanisms of these compounds.

References

Abubakar MB, Abdullah WZ, Sulaiman SA, Suen AB (2012) A review of molecular mechanisms of the anti-leukemic effects of phenolic compounds in honey. Int J Mol Sci 13 (11):15054–15073

Adan A, Baran Y (2016) Fisetin and hesperetin induced apoptosis and cell cycle arrest in chronic myeloid leukemia cells accompanied by modulation of cellular signaling. Tumor Biol 37 (5):5781–5795

Alvarez MC, Maso V, Torello CO, Ferro KP, Saad STO (2018) The polyphenol quercetin induces cell death in leukemia by targeting epigenetic regulators of pro-apoptotic genes. Clin Epigenetics 10(1):139

Bae CS, Lee CM, Ahn T (2020) Encapsulation of apoptotic proteins in lipid nanoparticles to induce death of cancer cells. Biotechnol Bioproc Eng 25:264–271

Baig S, Attique H (2014) Cytotoxic activity of honey in hepatoma cells: in vitro evaluation. Pak J Med Dent 3(1):12–16

Bestwick CS, Milne L (2006) Influence of galangin on HL-60 cell proliferation and survival. Cancer Lett 243(1):80–89

Bestwick CS, Milne L, Duthie SJ (2007) Kaempferol induced inhibition of HL-60 cell growth results from a heterogeneous response, dominated by cell cycle alterations. Chemico-Biol Interact 170(2):76–85

Billard C (2014) Apoptosis inducers in chronic lymphocytic leukemia. Oncotarget 5(2):309

Biswal BN, Das SN, Das BK, Rath R (2017) Alteration of cellular metabolism in cancer cells and its therapeutic prospects. J Oral Maxillofac Pathol 21:244–251

Budhraja A, Gao N, Zhang Z, Son YO, Cheng S, Wang X, Shi X (2012) Apigenin induces apoptosis in human leukemia cells and exhibits anti-leukemic activity in vivo. Mol Canc Therapeut 11(1):132–142

Calgarotto AK, Maso V, Junior GCF, Nowill AE, Latuf Filho P, Vassallo J, Saad STO (2018) Antitumor activities of Quercetin and Green Tea in xenografts of human leukemia HL60 cells. Sci Rep 8(1):1–7

Cavaliere V, Papademetrio DL, Lombardo T, Costantino SN, Blanco GA, Álvarez EM (2014) Caffeic acid phenylethyl ester and MG132, two novel nonconventional chemotherapeutic agents, induce apoptosis of human leukemic cells by disrupting mitochondrial function. Target Oncol 9(1):25–42

Chang JL, Chow JM, Chang JH, Wen YC, Lin YW, Yang SF, Chien MH (2017) Quercetin simultaneously induces G0/G1-phase arrest and caspase-mediated crosstalk between apoptosis and autophagy in human leukemia HL-60 cells. Environ Toxicol 32(7):1857–1868

Chang L, Hou Y, Zhu L, Wang Z, Chen G, Shu C, Liu Y (2020) Veliparib overcomes multidrug resistance in liver cancer cells. Biochem Biophys Res Commun 521(3):596–602

Chen D, Daniel KG, Chen MS, Kuhn DJ, Landis-Piwowar KR, Dou QP (2005) Dietary flavonoids as proteasome inhibitors and apoptosis inducers in human leukemia cells. Biochem Pharmacol 69(10):1421–1432

Chen YJ, Shiao MS, Hsu ML, Tsai TH, Wang SY (2001) Effect of caffeic acid phenethyl ester, an antioxidant from propolis, on inducing apoptosis in human leukemic HL-60 cells. J Agri Food Chem 49(11):5615–5619

Chien ST, Shi MD, Lee YC, Te CC, Shih YW (2015) Galangin, a novel dietary flavonoid, attenuates metastatic feature via PKC/ERK signaling pathway in TPA-treated liver cancer HepG2 cells. Cancer Cell Int 15(1):15

Čipák L, Rauko P, Miadoková E, Čipáková I, Novotny L (2003) Effects of flavonoids on cisplatin-induced apoptosis of HL-60 and L1210 leukemia cells. Leukemia Res 27(1):65–72

Cooper GM (2000) The cell: a molecular approach, 2nd edn. Sinauer Associates, Sunderland, MA. The Development and Causes of Cancer. https://www.ncbi.nlm.nih.gov/books/NBK9963/. Accessed 5 May 2020

Csokay B, Prajda N, Weber G, Olah E (1997) Molecular mechanisms in the antiproliferative action of quercetin. Life Sci 60(24):2157–2163

Deng X, Zhang X, Li W, Feng RX, Li L, Yi GR, Lu B (2018) Chronic liver injury induces conversion of biliary epithelial cells into hepatocytes. Cell Stem Cell 23(1):114–122

Fauzi AN, Norazmi MN, Yaacob NS (2011) Tualang honey induces apoptosis and disrupts the mitochondrial membrane potential of human breast and cervical cancer cell lines. Food Chem Toxicol 49(4):871–878

Fennell DA, Chacko A, Mutti L (2008) BCL-2 family regulation by the 20S proteasome inhibitor bortezomib. Oncogene 27(9):1189–1197

Genovese G, Kähler AK, Handsaker RE, Lindberg J, Rose SA, Bakhoum SF, Purcell SM (2014) Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med 371(26):2477–2487

Ghorbani A, Nazari M, Jeddi-Tehrani M, Zand H (2012) The citrus flavonoid hesperidin induces p53 and inhibits NF-κB activation in order to trigger apoptosis in NALM-6 cells: involvement of PPARγ-dependent mechanism. Eur J Nutr 51(1):39–46

Goto H, Yanagimachi M, Goto S, Takeuchi M, Kato H, Yokosuka T, Yokota S (2012) Methylated chrysin reduced cell proliferation, but antagonized cytotoxicity of other anticancer drugs in acute lymphoblastic leukemia. Anti-Cancer Drugs 23(4):417–425

Han Y, Yu H, Wang J, Ren Y, Su X, Shi Y (2015) Quercetin alleviates myocyte toxic and sensitizes anti-leukemic effect of adriamycin. Hematology 20(5):276–283

Hassanzadeh A, Hosseinzadeh E, Rezapour S, Vahedi G, Haghnavaz N, Marofi F (2019) Quercetin promotes cell cycle arrest and apoptosis and attenuates the proliferation of human chronic myeloid leukemia cell line-K562 through interaction with HSPs (70 and 90), MAT2A and FOXM1. Anticancer Agents Med Chem 19(12):1523–1534

He D, Guo X, Zhang E, Zi F, Chen J, Chen Q, Yang Y (2016) Quercetin induces cell apoptosis of myeloma and displays a synergistic effect with dexamethasone in vitro and in vivo xenograft models. Oncotarget 7(29):45489

Jaganathan SK, Mondhe D, Wani ZA, Supriyanto E (2014) Evaluation of selected honey and one of its phenolic constituent eugenol against L1210 lymphoid leukemia. Sci World J 2014:912051

Jin UH, Song KH, Motomura M, Suzuki I, Gu YH, Kang YJ, Kim CH (2008) Caffeic acid phenethyl ester induces mitochondria-mediated apoptosis in human myeloid leukemia U937 cells. Mol Cell Biochem 310(1-2):43–48

Kang TB, Liang NC (1997) Studies on the inhibitory effects of quercetin on the growth of HL-60 leukemia cells. Biochem Pharmacol 54(9):1013–1018

Kim KY, Jang WY, Lee JY, Jun DY, Ko JY, Yun YH, Kim YH (2016) Kaempferol activates G2-checkpoint of the cell cycle resulting in G2-arrest and mitochondria-dependent apoptosis in human acute leukemia Jurkat T cells. J Microbiol Biotechnol 26(2):287–294

Kumazawa S, Ahn MR, Fujimoto T, Kato M (2010) Radical-scavenging activity and phenolic constituents of propolis from different regions of Argentina. Nat Prod Res 24(9):804–812

Kuo HC, Kuo WH, Lee YJ, Wang CJ, Tseng TH (2006) Enhancement of caffeic acid phenethyl ester on all-trans retinoic acid-induced differentiation in human leukemia HL-60 cells. Toxicol App Pharmacol 216(1):80–88

Lee SJ, Yoon JH, Song KS (2007) Chrysin inhibited stem cell factor (SCF)/c-Kit complex-induced cell proliferation in human myeloid leukemia cells. Biochem Pharmacol 74(2):215–225

Lee TJ, Kim OH, Kim YH, Lim JH, Kim S, Park JW, Kwon TK (2006) Quercetin arrests G2/M phase and induces caspase-dependent cell death in U937 cells. Cancer Lett 240(2):234–242

Li B, Zhou P, Xu K, Chen T, Jiao J, Wei H, Xiao J (2020) Metformin induces cell cycle arrest, apoptosis and autophagy through ROS/JNK signaling pathway in human osteosarcoma. Int J Biol Sci 16(1):74

Lin CC, Yu CS, Yang JS, Lu CC, Chiang JH, Lin JP, Chung JG (2012) Chrysin, a natural and biologically active flavonoid, influences a murine leukemia model in vivo through enhancing populations of T-and B-cells, and promoting macrophage phagocytosis and NK cell cytotoxicity. In Vivo 26(4):665–670

Man N, Khuzaimi NM, Hassan R, Ang CY, Abdullah AD, Radzi M, Sulaiman SA (2015) Antileukemic effect of tualang honey on acute and chronic leukemia cell lines. Bio Med Res Int 2015:307094

Monasterio A, Urdaci MC, Pinchuk IV, Lopez-Moratalla N, Martinez-Irujo JJ (2004) Flavonoids induce apoptosis in human leukemia U937 cells through caspase-and caspase-calpain-dependent pathways. Nutr Cancer 50(1):90–100

Moradzadeh M, Tabarraei A, Sadeghnia HR, Ghorbani A, Mohamadkhani A, Erfanian S, Sahebkar A (2018) Kaempferol increases apoptosis in human acute promyelocytic leukemia cells and inhibits multidrug resistance genes. J Cell Biochem 119(2):2288–2297

Murtaza G, Karim S, Akram MR, Khan SA, Azhar S, Mumtaz A, Hassan MH (2014) Caffeic acid phenethyl ester and therapeutic potentials. Bio Med Res Int 2014:145342

Nikoletopoulou V, Markaki M, Palikaras K, Tavernarakis N (2013) Crosstalk between apoptosis, necrosis and autophagy. Biochim Biophys Acta 1833(12):3448–3459

Pirtoli L, Belmonte G, Toscano M, Tini P, Miracco C (2020) Comment on "Everolimus induces G1 cell cycle arrest through autophagy-mediated protein degradation of cyclin D1 in breast cancer cells". Am J Physiol Cell Physiol 18(2):448–449

Polier G, Ding J, Konkimalla BV, Eick D, Ribeiro N, Kohler R, Li-Weber M (2011) Wogonin and related natural flavones are inhibitors of CDK9 that induce apoptosis in cancer cells by transcriptional suppression of Mcl-1. Cell Death Dis 2(7):182–182

Ramos AM, Aller P (2008) Quercetin decreases intracellular GSH content and potentiates the apoptotic action of the antileukemic drug arsenic trioxide in human leukemia cell lines. Biochem Pharmacol 75(10):912–1923

Ren HJ, Hao HJ, Shi YJ, Meng XM, Han YQ (2010) Apoptosis-inducing effect of quercetin and kaempferol on human HL-60 cells and its mechanism. Zhongguo Shi Yan Xue Ye Xue Za Zhi 18(3):629–633

Rocha LD, Monteiro MC, Teodoro AJ (2012) Anticancer properties of hydroxycinnamic acids—a review. Cancer Clin Oncol 1(2):109–121

Salimi A, Roudkenar MH, Seydi E, Sadeghi L, Mohseni A, Pirahmadi N, Pourahmad J (2017) Chrysin as an anti-cancer agent exerts selective toxicity by directly inhibiting mitochondrial complex II and V in CLL B-lymphocytes. Cancer Investig 35(3):174–186

Shehata M, Schnabl S, Demirtas D, Hilgarth M, Hubmann R, Ponath E, Gaiger A (2010) Reconstitution of PTEN activity by CK2 inhibitors and interference with the PI3-K/Akt cascade counteract the antiapoptotic effect of human stromal cells in chronic lymphocytic leukemia. Blood 116(14):2513–2521

Spagnuolo C, Russo M, Bilotto S, Tedesco I, Laratta B, Russo GL (2012) Dietary polyphenols in cancer prevention: the example of the flavonoid quercetin in leukemia. Ann N Y Acad Sci 1259 (1):95–103

Spilioti E, Jaakkola M, Tolonen T, Lipponen M, Virtanen V, Chinou I, Kassi E, Karabournioti S, Moutsatsou P (2014) Phenolic acid composition, antiatherogenic and anticancer potential of honeys derived from various regions in Greece. PLoS One 9(4):e94860

Stephens JM, Schlothauer RC, Morris BD, Yang D, Fearnley L, Greenwood DR, Loomes KM (2010) Phenolic compounds and methylglyoxal in some New Zealand manuka and kanuka honeys. Food Chem 120(1):78–86

Sulaiman GM (2016) Molecular structure and anti-proliferative effect of galangin in HCT-116 cells: in vitro study. Food Sci Biotechnol 25(1):247–252

Swellam T, Miyanaga N, Onozawa M, Hattori K, Kawai K, Shimazui T, Akaza H (2003) Antineoplastic activity of honey in an experimental bladder cancer implantation model: in vivo and in vitro studies. Int J Urol 10(4):213–219

Tanaka T, Tanaka T, Tanaka M (2011) Potential cancer chemopreventive activity of protocatechuic acid. J Exp Clin Med 3(1):27–33

Tolomeo M, Grimaudo S, Di Cristina A, Pipitone RM, Dusonchet L, Meli M, Simoni D (2008) Galangin increases the cytotoxic activity of imatinib mesylate in imatinib-sensitive and imatinib-resistant Bcr-Abl expressing leukemia cells. Cancer Lett 265(2):289–297

Towers CG, Wodetzki D, Thorburn A (2020) Autophagy and cancer: modulation of cell death pathways and cancer cell adaptations. J Cell Biol 219(1):e201909033

Vargo MA, Voss OH, Poustka F, Cardounel AJ, Grotewold E, Doseff AI (2006) Apigenin-induced-apoptosis is mediated by the activation of PKCδ and caspases in leukemia cells. Biochem Pharmacol 72(6):681–692

Wang J, Li QX (2011) Chemical composition, characterization, and differentiation of honey botanical and geographical origins. Adv Food Nutr Res 62:89–137

Woo KJ, Jeong YJ, Park JW, Kwon TK (2004) Chrysin-induced apoptosis is mediated through caspase activation and Akt inactivation in U937 leukemia cells. Biochem Biophys Res Commun 325(4):1215–1222

Wu LY, Lu HF, Chou YC, Shih YL, Bau DT, Chen JC, Chung JG (2015) Kaempferol induces DNA damage and inhibits DNA repair associated protein expressions in human promyelocytic leukemia HL-60 cells. Am J Chin Med 43(02):365–382

Xiang W, Yang CY, Bai L (2018) MCL-1 inhibition in cancer treatment. Onco Targets Ther 11:7301–7314

Yan YY, Shi KY, Teng F, Chen J, Che JX, Dong XW, Zhang B (2020) A novel derivative of valepotriate inhibits the PI3K/AKT pathway and causes Noxa-dependent apoptosis in human pancreatic cancer cells. Acta Pharmacol Sin 41:835–842

Yin J, Hou Y, Song X, Wang P, Li Y (2019) Cholate-modified polymer-lipid hybrid nanoparticles for oral delivery of quercetin to potentiate the antileukemic effect. Int J Nanomedicine 14:4045

Zaric M, Mitrovic M, Nikolic I, Baskic D, Popovic S, Djurdjevic P, Zelen I (2015) Chrysin induces apoptosis in peripheral blood lymphocytes isolated from human chronic lymphocytic leukemia. Anticancer Agents Med Chem 15(2):189–195

Zhu B, Xi X, Liu Q, Cheng Y, Yang H (2019) MiR-9 functions as a tumor suppressor in acute myeloid leukemia by targeting CX chemokine receptor 4. Am J Transl Res 11(6):3384

2 Possible Therapeutic Potential of Flavonoids and Phenolic Acids from Honey in Age-Related Neurodegenerative Diseases Via Targeting NAD^+ Degradation

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Abstract

Neurodegenerative disorder is the major age-related problem with no specific cure. The drugs available in the market relieve the severity of the disease and increase the average life span. There is always room for more novel therapeutic strategies for the prevention of neurodegenerative diseases. Honey has been used since ages for various disease prevention, and recently it has been used to combat these diseases too. The flavonoids and the phenolic acids of honey have preventive effects and have been known to exhibit antioxidant, anti-inflammatory, and anti-apoptotic effects. These phytochemicals can prevent the neurodegeneration via NAD pathway where they are responsible for inhibiting CD38, PARP and

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activating SIRT1 overall by increasing the concentration of NAD in the neurons. With an increase in the amount of NAD, Sirtuins can be activated which protects age-related neurodegenerative diseases. Therefore, honey can be used as "NAD compounds" in clinics but with further mechanistic research.

Keywords

Neurodegenerative disorders · Honey · Neuroprotection · NAD^+ pathway · SIRT1

2.1 Introduction

Neurodegenerative disorders including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), temporal lobe epilepsy, and ischemia/hypoxia are major problems with aging. All these diseases are a cause of increased burden on individuals, families, society, and country as a whole (Rotermund et al. 2018). A lot of studies are conducted to underline the biological and genetic cause of these diseases but still there are only symptomatic treatments for majority of them. Moreover, the chemical-induced treatment has introduced many side effects and drug resistance in patients on long-term usage (Ahmed et al. 2018). Therefore, despite new researches in the development of the prevention of these diseases, still there is room for more novel therapeutic strategies.

Alternative treatment has profound advantages over traditional western medicines as they have less side effects. A lot of studies are going on to introduce the complementary and alternative medicines (CAM) in all forms of diseases. Honey is a product of nature which has pull off the attention of investigators as a complementary and alternative medicine (Othman 2012a, b; Ahmed and Othman 2013).

Honey is employed for its sanative effects since ages. It has around 200 substances (Eteraf-Oskouei and Najafi 2013). It is a trusted source for vitamins and minerals (White 1979). Honey constituents vary as bees feed on different plants. Almost all kinds of honey contain flavonoids and antioxidant enzymes which can work together to improve the condition of any disease (Alvarez-Suarez et al. 2010; Rakha et al. 2008; Turkmen et al. 2006; Johnston et al. 2005; Al-Mamary et al. 2002).

In many recent studies, Honey *per os* has been indicated for anorexia, insomnia, intestinal and stomach ulcers, osteoporosis, and respiratory tract infections. Applied externally, honey can be used to treat many skin disorders including eczema, lip sores, athlete's foot, and different kinds of wounds (like accidents, surgery, bedsores, or burns) (Olaitan et al. 2007). Regrettably, research on neuropharmacological effects of honey is exiguous, still a fact that honey has memory enhancer properties cannot be ignored (Rahman et al. 2014). The isolated flavonoids and polyphenols from natural products possess antioxidant activity as suggested by these studies (Jimenez-Del-Rio et al. 2010; Ortega-Arellano et al. 2011). Still we do not have adequate amount of knowledge regarding the use of

honey in neurodegenerative disorder, we tried to summarize some in this book chapter.

Neurodegenerative disorder is multifactorial and has many pathways working together to exhibit the neurological systems. One of the main pathways which is attributed to neurodegenerative disorder is NAD^+ metabolism (Johnson and Imai 2018). Biogenesis and regulation of NAD^+ have attracted competing interests in recent years. It has been found that aging is accentuated by reduction in NAD^+ throughout various tissues (Johnson and Imai 2018). Many studies have implicated NAD biosynthesis as a promising target for preventing neurodegenerative diseases.

Mitochondria dysfunction is a diagnostic factor of many neurodegenerative diseases and leads to NAD^+ depletion, which is monitored with aging in humans too (Kerr et al. 2017). NAD^+ is a vital coenzyme for many enzymes responsible for various metabolic processes particularly with regard to glycolysis, TCA cycle, and oxidative phosphorylation (Imai and Yoshino 2013; Verdin 2014; Haigis and Sinclair 2010). It is also a crucial substrate for NAD^+-exhausting enzymes like sirtuins (SIRT), poly-ADP-ribose polymerases (PARPs), and cluster of differentiation 38 (CD38) (Johnson and Imai 2018) which are already known to regulate numerous fundamental and cellular processes (Imai and Guarente 2014). In mammals, NAD^+ biosynthesis is done by three processes: salvage pathway from nicotinamide (NIC), de novo pathway from tryptophan, and Preiss-Handler pathway from nicotinic acid. Several enzymes in metabolic pathways use this generated NAD^+. From all these enzymes, three are very important, viz. CD38, PARPs, and SIRTs. Maximum cellular degradation of NAD^+ is attributed to CD38 and PARPs, whereas SIRTs have minor role in NAD^+ degradation (Chini et al. 2017).

NAD^+-dependent pathway activation has multiplied oxidative stress to several folds as a consequence of intonation of mitochondrial function. As major or auxiliary component of pathological processes, dysfunction of mitochondria and cellular oxidative stress simultaneously are responsible for pathogenesis of AD, PD, ALS, and HD (Sasaki et al. 2009; Summers et al. 2014; Yang et al. 2015; Dellinger et al. 2017). Hence, NAD^+ metabolism has come into existence as a new therapeutic focus for aging and neurodegenerative disorders.

There is reduction of NAD^+ with age and the significant cause of this decline is the depletion of nicotinamide phosphoribosyltransferase (NAMPT)-mediated NAD^+ biosynthesis (Yoshino et al. 2011; Stein and Imai 2014). Another cause for this decline is the increased breakdown of NAD^+ by activation of NAD exhausting enzymes like PARP and CD38. Together, the decrease in synthesis and elevation in the consumption of NAD^+ is responsible for a variety of age-affiliated pathologies (Ramsey et al. 2008; Mouchiroud et al. 2013; Stein and Imai 2014).

All these studies summarize that NAD^+ depletion signaling is a key process in aging, still a detailed mechanism of this pathway is not available. It can be hypothesized that increasing the availability of NAD^+ can lead to protection of neurodegenerative disorders. This chapter replenishes the subsisting literature in this area with emphasis on mechanisms dealing with regulation of NAD^+ homeostasis and how this equilibrium is disrupted in aging and neurodegeneration with focus on AD, PD, and ALS will be discussed. It also focuses on the supplementation with

honey to combat the adverse effects of these diseases by the NAD^+ homeostasis leading to prevention of disease.

2.2 Age-Related Neurodegenerative Pathologies (ANP)

Age-related neurodegenerative pathologies (ANP) affect aging individuals is a known stuff now. The rise in the age of people over 65 years was 703 million in 2019 which is likely to be higher than two billion in 2050 (UN 2019). It is estimated that in aging demographics, by 2050, there will be 120% change between 2019 and 2050 and for first-time ever in history, young people in the world will undershoot in number than the aged people (World Health Organization 2018).

With increase in the population age, ANPs like AD and PD have turned to be most prevalent (Reitz et al. 2011; Reeve et al. 2014), and ALS also seems to have followed the similar trend but still has to be proved (Beghi et al. 2006).

Aging can be defined as the physical variations established in adulthood, causing decline in efficiency of functions, decline homeostasis, leading to death. Aging of living beings is a continuous process, but its rate varies from individual to individual. The nervous system is damaged with age accounting to the shrinking of the brain mass with less or nonfunctional neurons. The nervous system network is deeply impaired leading to alterations in the nervous circuits and synapses. The most important areas of hypothalamus lose nerve cells which may lead to various physiological changes. These changes could be impaired metabolism and circadian rhythm leading to mental and emotional vagary in such people. Aging contributes to a decrease of important neurotransmitters like dopamine, tyrosine hydroxylase, serotonin, noradrenaline, and cholinesterase and an elevation in the activity of monoamine oxidase (Hung et al. 2010).

The major risk factor of neurodegenerative pathologies is aging. It leads the patients to various neurological problems with inability of self-repair. Bishop et al. 2010 in order to control aging have proposed various signaling pathways. To name a few are target of rapamycin (TOR) signaling, SIRTs, insulin/IGF-1 signaling, and mitochondrial function. Recent investigations have proved the engagement of these pathways in ANP.

At cellular level, many harmful processes like oxidative stress, dysfunction of mitochondria, DNA damage, and death of cells lead to aging (Sahin and Depinho 2010). It was found in pathological reports that oxidative stress is present in the brain samples of patients with common ANPs including AD, PD, and ALS (Andersen 2004). Smith et al. 2010 suggested that the cortex and medulla of the brain of patients with preclinical Alzheimer and mild cognitive impairment have elevated iron and free radical generation. Numerous reports suggest that antioxidant enzyme activities (catalase, glutathione peroxidase, glutathione reductase, and superoxide dismutase (SOD)) were significantly inhibited in the AD brains (Zemlan et al. 1989; Pappolla et al. 1992). Glutathione, the master antioxidant in the brain, is also reported to reduce in PD brains particularly in substantia nigra region (Pearce et al. 1997; Perry et al. 1982; Perry and Yong 1986). The major cause of

neurodegenerative disorders is elevation of oxidative stress which results due to the deterioration of antioxidant defense and repair mechanism. Longevity and epigenetics have an association between each other. It is found that the SIRT1 gene promotes longevity of mammals (Frye 1999; Blander and Guarente 2004).

2.2.1 Alzheimer's Disease

With the modern lifestyle at ease, the span of life increases due to which dementia is a rising issue in developed countries. The most widespread of dementias is Alzheimer's. Due to variability in humans, Alzheimer's disease has been reported to affect people in a variety of ways, but forgetfulness is considered to be the most common symptom. This could be due to brain cell disruption mainly in new memory forming region, viz. hippocampus and frontal cortex. Alzheimer's disease is a multifactorial disease having more than one cause with age as the major risk factor.

Neurofibrillary tangles and senile plaques are the major pathological characteristics of AD in the cortex and hippocampus. In AD brain, there is accumulation of beta-amyloid protein outside neurons and the accumulation of the tau protein in the cytosol of nerve cells. In this disease, the cell-to-cell communication is lost due to decrease in synapses and neurotransmitters and accumulation of beta-amyloid protein in extracellular matrix and eventually the neurons die. Inside the neuron, there is accumulation of abnormal tau protein forming neurofibrillary tangles blocking the nutrient and essential molecules transport leading to the cell death. Van Ham et al. 2009 suggested that the cellular changes as a result of aging may contribute to the protein misfolding and aggregation mechanisms. Aged brain is more prone to oxidative stress and mitochondria dysfunction. The brain is highly susceptible to oxidative damage due to its high fat content and defective mitochondrial function due to high bioenergetics demands (Bishop et al. 2010). The main target contributing to the aging of brain is disruption of mitochondrial functions of neurons which make neurons more susceptible to age-dependent pathological changes (Bishop et al. 2010). Numerous studies have revealed that antioxidants may play a protective role in the prevention of the pathogenesis of AD (Javed et al. 2012; Khan et al. 2012; Ishrat et al. 2009).

2.2.2 Parkinson's Disease

Second commonest neurodegenerative disease in aged population is PD. It is stemming disorder of the nervous system consisting of both motor and nonmotor systems. It involves formation of Lewy bodies by accumulation of misfolded protein called α-synuclein (van Ham et al. 2009). These Lewy bodies are located in lower brain stem and olfactory regions, cortex, and midbrain (Braak et al. 2003, 2006). In PD, degeneration of dopaminergic neurons is confined to substantia nigra pars compacta that project to the striatum (Samii et al. 2004). PD like AD is multifactorial

with aging, family history, pesticide exposure, and environmental chemicals (e.g., synthetic heroin use) to name a few.

The main signs and symptoms of PD include rest tremor, bradykinesia, rigidity, and stoopy posture which becomes evident when striatal dopamine (80%) and nigral neurons (50%) are diminished (Fearnley and Lees 1991). Other symptoms include dementia, depression and anxiety, speech changes, writing changes, orthostasis, and hyperhidrosis.

Hindle 2010 stated in his study the greatest risk factor for PD is progressive age. The clinical course and pathological progression of PD can be influenced by age-related factors. In one of the longitudinal cohort study by Halliday and McCann 2010, it was found that dementia appeared earlier in PD patients with age >70 years with much shorter course as compared to the younger onset patients. The other finding describes that in older onset patients, more α-synuclein-containing Lewy bodies were found in the whole brain contributing to age-related plaque in the older onset patients (Halliday and McCann 2010). Even the responsiveness to different medications was also affected by the age.

2.2.3 Amyotrophic Lateral Sclerosis (ALS)

ALS is heterogeneous age-related neurodegenerative disease triggered by the continuous degeneration of upper motor neurons and lower motor neurons leading to inexorably weakness. There is muscle atrophy and fasciculation due to the death of lower motor neurons, whereas the death of upper motor neurons causes spasticity and elevated reflex activity. The resulting effect of these neuronal loss leads to the death of the patient within 1–5 years from symptoms onset. Generally the etiology of ALS is unknown. Mostly this disease has sporadic nature (SALS) with unknown nature (Renton et al. 2014), but around 5–10% of all the reported cases are genetic contributing to familial ALS (FALS). There are around 30 different genes which have been linked to the familial form of ALS (Renton et al. 2014), but the main enzyme responsible for FALS is superoxide dismutase 1 (SOD1) (Rosen et al. 1993).

The clinical diagnosis of the disease is still a challenge, hence there are substantial delays in the treatment. A substantial research has to be done to eliminate these disease mimics. At present, the only drugs available are Rilutek (riluzole) and Radicava (edavarone) which are disease-modifying therapies. As a result, ALS is an effective area of research aiming at exploring novel therapeutic strategies.

2.3 Nicotinamide Adenine Dinucleotide (NAD^+): A Key Metabolic Regulator

NAD^+ is a pyridine nucleotide which serves as energy provider to the cells by catabolism of fuel substrates. Sir Arthur Harden and colleagues discovered NAD^+ (Harden and Young 1906) more than a 100 years ago. It is a vital coenzyme that serves two functions: it act as coenzymes for enzymes that are responsible for

reduction–oxidation reactions in many metabolic pathways like glycolysis and oxidative phosphorylation, where electron is carried by this molecule from one reaction to the other, and second function is that it acts as a cofactor for several other enzymes like DNA repair protein poly(adenosine diphosphate–ribose) polymerases (PARP), for deacetylases of the sirtuins, for activity of cyclic ADP-ribose hydrolases CD38 and for normal functioning of sterile alpha and toll/interleukin-1 receptor motif-containing 1 (SARM1) (Essuman et al. 2017).

2.3.1 NAD^+ Concentration in Cells

There is always homeostasis between consumption and biosynthesis of NAD^+ making its concentration stable in cells. There are many enzymes involved in these processes which have different roles in maintaining the homeostasis.

2.3.2 Biosynthesis of NAD^+

Nikiforov et al. 2015 and Yang and Sauve 2016 have extensively studied the NAD^+ synthesis mechanisms. In this chapter, we will focus on the effect of NAD^+ on the conditions related to aging and neurodegeneration.

The synthesis of NAD^+ can be done by five major precursors: tryptophan, nicotinic acid, nicotinamide, nicotinamide mononucleotide (NMN), and nicotinamide riboside (NR). Different pathways are responsible for the NAD^+ synthesis. The three main pathways synthesizing NAD^+ are Preiss-Handler pathway, de novo pathway, and salvage pathways which are described in Fig. 2.1.

In **Preiss-Handler pathway,** dietary nicotinic acid is converted to nicotinic acid mononucleotide (NAMN) in the presence of the enzyme nicotinic acid phosphoribosyltransferase (NAPRT). NAMN transferase (NMNAT) converts NAMN into NAAD. NMNAT have three isoforms: NMNAT1, -2, and -3 which are found in distinct subcellular localizations. NAAD thus formed is then transformed to NAD^+ by NAD^+ synthase (NADS) enzyme.

NAD^+ is formed by essential amino acid L-tryptophan in **de novo *pathway***. This process of conversion is completed in eight steps (Nikiforov et al. 2015). The first reaction constitutes the tryptophan conversion to N-formylkynurenine, which in mammals can be catalyzed by two enzymes (tryptophan-2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO)). This step is considered to be the rate-limiting step of the process. TDO is found in liver and leads to production of NAD^+ in liver while IDO is expressed in extrahepatic tissues including lungs, intestine, and spleen (Yamazaki et al. 1985; Kudo and Boyd 2000). Formamidase (KFase) converts N-formyl kynurenine into kynurenine. Kynurenine is converted into 3-OH kynurenine in a reaction catalyzed by kynurenine 3-hydroxylase (K3H). Kynureninase (Kyase) then forms 3-hydroxyanthranilate, which gets transformed into α-amino-β-carboxymuconate-γ-semialdehyde (ACMS) by 3-hydroxyanthranilate 3,4-dioxygenase (3HAO). This is the branching point of de

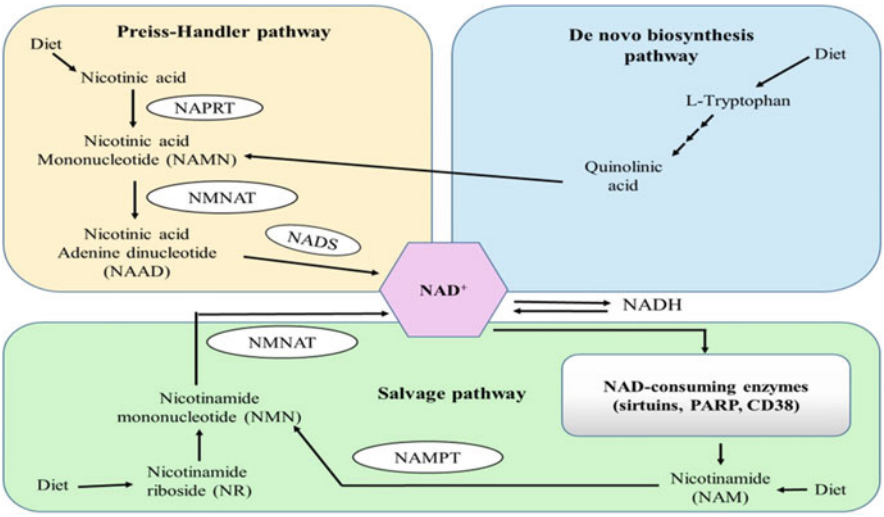

Fig. 2.1 The pathways for maintaining the NAD^+ levels in neurons. The cells maintain the NAD^+ concentration by maintaining the homeostasis between the catabolism and anabolism of NAD^+. The generation of NAD^+ is done by three pathways. First is the Preiss-Handler pathway where dietary nicotinic acid is converted to nicotinic acid mononucleotide (NAMN) in the presence of the enzyme nicotinic acid phosphoribosyltransferase (NAPRT). NAAD is generated from NAMN by NAMN transferase (NMNAT). The final step is carried out in the presence of NAD^+ synthase (NADS) by conversion of NAAD into NAD^+. Second pathway is de novo synthesis where the formation of NAD is done from tryptophan. Tryptophan is converted to quinolinic acid in a number of steps generating NAMN as final product. NAMN generated then converges to Preiss-Handler pathway. The NAD^+ salvage pathway is the third pathway wherein the nicotinamide produced as a by-product of the enzymatic activities of sirtuins, PARPs, and the cADPR synthases CD38 is recycled. Initially, nicotinamide is converted into nicotinamide mononucleotide (NMN) by nicotinamide phosphoribosyltransferase (NAMPT) which is then converted into NAD^+ via different nicotinamide mononucleotide adenylyltransferases (NMNATs)

novo NAD^+ synthesis pathway (Bender 1983; Houtkooper et al. 2010). By cyclization, the ACMS formed will be converted to quinolinic acid (QA), which is then converted to NAMN by quinolinate phosphoribosyltransferase (QPRT). At this point, NAMN fuses with the Preiss–Handler pathway and produced NAD^+. The other fate of ACMS could be removal of its carbon group which either leads to the production of picolinic acid or is directed to total oxidation to CO_2 and H_2O.

In **salvage pathway**, nicotinamide (NAM) is converted to form nicotinamide mononucleotide (NMN) by nicotinamide phosphoribosyltransferase (NAMPT). This step is rate limiting (Revollo et al. 2004). NMN adenylyltransferases (NMNAT) then transforms NMN into NAD^+ (Wang et al. 2006; Bogan and Brenner 2008). Many enzymes consume NAD^+ to generate NIC again along with various products. This NIC can again be used in the salvage pathway.

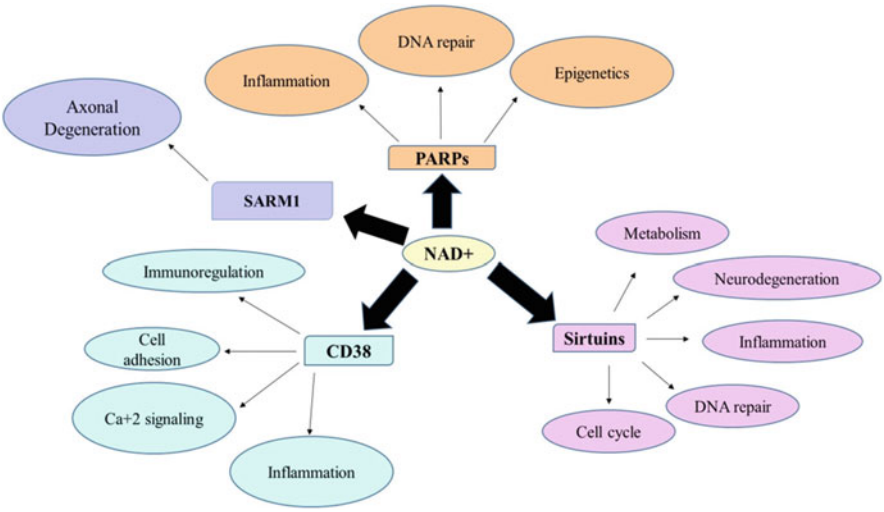

Fig. 2.2 NAD^+-consuming enzymes in neurodegeneration. NAD^+ concentration in the cell has the ability to affect the activities of regulator enzymes: the sirtuins, the poly(ADP-ribose) polymerases (PARPs), cyclic ADP-ribose synthases, and sterile alpha and Toll/interleukin-1 receptor motif-containing 1 (SARM1). These enzymes serve to regulate various significant processes by using NAD^+, thus limiting its concentration in the cell

2.3.3 Catabolism of NAD^+

The consumption of NAD^+ is involved in many proteins and pathways (Osborne et al. 2016; Kupis et al. 2016; Gibson and Kraus 2012; Malavasi et al. 2008) but only briefly described here (Fig. 2.2).

2.3.3.1 Sirtuins

These are family of proteins that regulate the cellular health by maintaining the cellular homeostasis. It can work only in the presence of NAD^+, hence acts as NAD^+-dependent deacetylases or deacylases which is responsible for regulation of many regulating proteins. In mammals, there are seven sirtuin members, SIRT1-7. The localization of these members is in different subcellular regions having different enzymatic functions (Bheda et al. 2016). SIRT1 is located in the nucleus as well as cytosol (Tanno et al. 2007). Cytosol is the main location of SIRT2, but it can also be found in the nucleus (Vaquero et al. 2006). SIRT5 is localized in the mitochondrial compartments (Verdin et al. 2010), while the locations of SIRT6 (Mostoslavsky et al. 2006) and SIRT7 are nucleus and nucleolus, respectively (Ford et al. 2006). All the functions carried out by these proteins require NAD^+ compulsorily.

2.3.3.2 PARPs

PARPs are NAD^+-consuming enzymes which act by breaking it into nicotinamide and ADP-ribose (ADPR). PARP1 and 2 are major enzymes responsible for DNA

repair, consuming major NAD^+ molecules in the nucleus (Morales et al. 2014). Exaggerated PARP-1 activation leads to cellular NAD^+ and ATP pools depletion. PARPs and SIRT1, both have NAD^+ as common substrate and as a result there is always a competition in their activities. When there is an increase in DNA damage, PARP activation decreases the functions of SIRT and vice versa. PARP1 inhibition by genes and drugs increases the total cellular NAD^+ levels (Bai et al. 2011; Pirinen et al. 2014).

2.3.3.3 CD38

Another molecule known to catabolize NAD which is found in mammals is CD38. It acts on NAD^+ hydrolyzing it into ADPR and nicotinamide. The secondary role of CD38 mediating cellular signaling is generation of cyclic ADPR (cADPR), a potent Ca^{2+} inducer (De Flora et al. 2004). CD38 also degrades the precursors of NAD^+, viz. NMN and NR along with consumption of NAD^+ directly, thus decreasing the total cellular NAD^+ content (Grozio et al. 2013; Preugschat et al. 2014).

2.3.3.4 SARM1

Sterile alpha and toll/interleukin-1 receptor motif-containing 1 (SARM1) is a new class of NAD^+ hydrolases (Essuman et al. 2017). SARM1 is responsible for axonal degeneration after injury. NAD^+ depletion is found in axonal injury, and SARM1 function loss delays axonal degeneration. In this enzyme, presence of the toll/interleukin-1 receptor (TIR) domain promotes axonal degeneration by acting as NAD^+ hydrolase enzyme (Essuman et al. 2017).

2.3.4 Neurodegenerative Disease and NAD^+

Neurodegenerative disease is a large title for any damage to nerve cells in the human brain. It comprises most common nervous system diseases like Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Huntington's disease, temporal lobe epilepsy, prion disease, spinocerebellar ataxia, and hypoxia/ischemia. Metabolic dysfunction is one of the dominant causes of neurodegenerative diseases which alter the homeostasis of energy in mitochondria leading to the progression of disease (Procaccini et al. 2016; Karbowski and Neutzner 2012). A handful of studies suggested the relationship between aging and metabolic dysfunction (Harman 1981; Sun et al. 2016; Bratic and Larsson 2013). As discussed, reactive oxygen species (ROS) generation is the main culprit that carry off aging. Metabolic dysfunction and elevation of ROS are observed in various neurodegenerative diseases (Uttara et al. 2009; Pehar et al. 2018). According to some reports, the mutation in mitochondrial proteins causes neurodegenerative diseases (Lin and Beal 2006; Zuo and Motherwell 2013). DNA damage and inflammation are also leading to aging. Glucose and lipid metabolism abnormalities are also linked with these diseases (Clarke et al. 2015; Kennedy et al. 2016; Dunn et al. 2014; Knight et al. 2014). Thus, almost all neurodegenerative diseases have disrupted energy metabolism in neurons which could be a potential target for drugs to treat these diseases (Fig. 2.3).

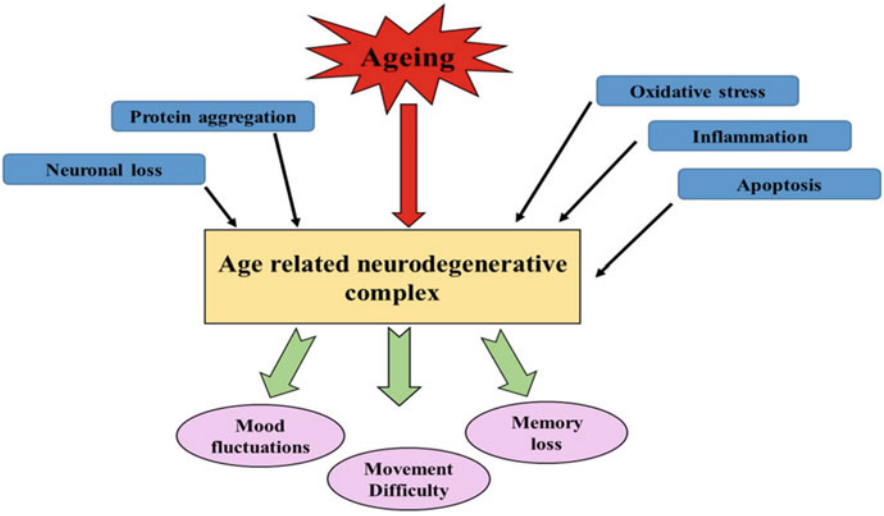

Fig. 2.3 Detailed schematic representation of the causes and effects of aging: Aging is caused by various factors (like neuronal loss, protein aggregation, oxidative stress, inflammation, and apoptosis) leading to the formation of age-related neurodegenerative complex which produces various symptoms like mood fluctuations, movement difficulty, and memory loss

The contributing factor for neurodegenerative diseases may be NAD^+ depletion which is also found with aging (Kerr et al. 2017). This depletion can be due to less production of NAD^+ or overconsumption of it by NAD^+-consuming enzymes (Fig. 2.4).

NAD^+ reportedly wanes over the years in the human brain, and several animal studies imply that boosting it could extend lifespan (Zhu et al. 2015; Zhang et al. 2016). Differentially altered Sirtuin levels were also observed in postmortem brain and spinal cord samples suggesting role of NAD^+ in neurodegeneration (Körner et al. 2013).

2.4 Honey and Its Components

Honey is a naturally occurring product by honeybees which they form from the nectar of flowers. Honey has different varieties which depends mainly on the variety of the plant which honey bees feeds on (Mijanur Rahman et al. 2014). Evidence suggest that it has more than 200 substances which gives it medicinal importance. The main constituents of Honey are enlisted in Table 2.1. Almost all natural honey are enriched in flavonoids and phenolic acids together with antioxidant agents. The list of the common flavonoids and phenolic acids is given in Table 2.2.

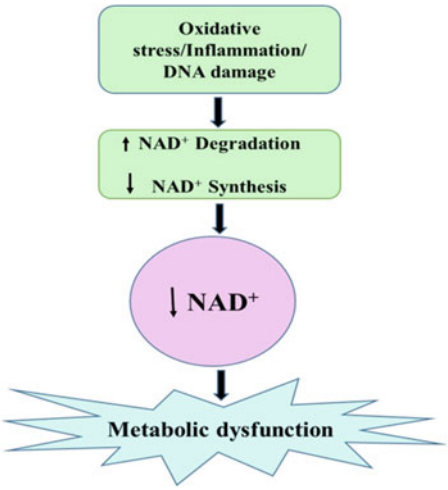

Fig. 2.4 A pathway suggested for tissue NAD^+ decline and damage during ageing. During aging and neurodegenerative disorders, oxidative damage, inflammation, and DNA damage lead to activation of NAD^+-consuming enzymes contributing to scarcity of NAD^+ in tissue. At the same time, these processes decrease the NAD^+ synthesis enzymes again contributing to NAD^+ deficiency causing metabolic dysfunction and ultimately causing disease

Table 2.1 Conventional constituents of honey

Honey (nutritional value/100 g)	
Constituents	Average (g)
Sugars	80–87
Fat	0
Water	15–20
Protein	<1
Vitamins	0.05–0.11
Minerals	0.5–0.9
Ash	0.1–0.4

2.4.1 Medicinal Importance of Honey as Neuroprotective Gel

Honey is extensively utilized as folk medicine in traditional therapies (Molan 2001). The limited use of honey in modern therapy is based on the lack of scientific data (Ali et al. 1991). With recent research, it was found that honey is an enigmatic gel with several health benefits including antioxidant (Jeffrey and Echazarreta 1996; Ahmed and Othman 2013), wound healing and antibacterial activity (Medhi et al. 2008), anti-fungal (Tonks et al. 2003), anti-inflammatory (Al-Waili and Boni 2003), gastroprotective (Ezz El-Arab et al. 2006), cardioprotective (Khalil and Sulaiman 2010), hepatoprotective (Meda et al. 2004; Al-Waili 2003b; Kilicoglu et al. 2008), antidiabetic (Al-Waili 2004; Bansal et al. 2005) and antihypertensive (Bilsel et al. 2002), and many more. There are a lot of reports suggesting the free radical scavenging property of honey (Beretta et al. 2007). It has proved to be an effective

Table 2.2 Common flavonoids and phenolic acids in honey

Flavonoids	
Apigenin	$C_{15}H_{10}O_5$
Chrysin	$C_{15}H_{10}O_4$
Galangin	$C_{15}H_{10}O_5$
Kaempferol	$C_{15}H_{10}O_6$
Luteolin	$C_{15}H_{10}O_6$
Pinobanksin	$C_{15}H_{10}O_5$
Pinocembrin	$C_{15}H_{10}O_5$
Quercetin	$C_{15}H_{10}O_4$
Rutin	$C_{27}H_{30}O_{16}$
Acacetin	$C_{16}H_{12}O_5$
Hesperetin	$C_{16}H_{14}O_6$
Triacetin	$C_9H_{14}O_6$
Myricetin	$C_{15}H_{10}O_8$
Isorhamnetin	$C_{16}H_{12}O_7$
Phenolic acids	
Caffeic acid	$C_9H_8O_4$
Cinnamic acid	$C_9H_8O_2$
Chlorogenic acid	$C_{16}H_{18}O_9$
Ferulic acid	$C_{10}H_{10}O_4$
Gallic acid	$C_7H_6O_5$
p-Coumaric acid	$C_9H_8O_3$
Syringic acid	$C_9H_{10}O_5$
Ellagic acid	$C_{14}H_6O_8$
Vanillic acid	$C_8H_8O_4$
Benzoic acid	$C_7H_6O_2$

antioxidant both in vivo and in vitro (Erejuwa et al. 2012). Antioxidant property of honey may be allocated to the presence of flavonoids and phenolic acids in it (Ahmed et al. 2018). It also comprises many other small compounds like sugars, proteins, amino acids, carotenes, and other components in traces contributing to its antioxidant effect (Nagai et al. 2001; Diplock et al. 1994). Honey increases stimulation and production of monocytes, phagocytes, lymphocytes, and/or macrophages which in turn releases different cytokines and interleukins such as TNF-α, IL-6, and IL-1β and hence will lead to healing process (Tonks et al. 2007). Inflammation is the defensive way of response by tissues against any pathogen or xenobiotics which causes injury in the body. Honey has anti-inflammatory effect which is well documented in cell cultures (Jaganathan and Mandal 2009) as well as in animal models and clinical trials (Subrahmanyam 1998; Al-Waili and Boni 2003). Inflammation starts by activation of nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) pathways leading to the release of various mediators of inflammation like enzymes, cytokines, cyclooxygenase-2 (COX-2), C-reactive protein (CRP), and interleukins. All of these inflammatory markers lead to stepwise processes causing inflammation in the cells (Erejuwa et al. 2010; Erejuwa et al. 2012). The anti-inflammatory mechanisms of honey have been enumerated in

several in vivo studies by decreasing edema, swelling, fluid infiltration, and plasma levels of proinflammatory cytokines. There are studies reporting that the phenolic acids and flavonoid contents of honey are able to ameliorate the proinflammatory enzymes activity, like COX-2, iNOS, and prostaglandins (Murtaza et al. 2014; Candiracci et al. 2012). Honey also demonstrates immunomodulatory activities (Al-Waili 2003a). Apart from all these qualities of honey, it also has remarkable effect on nervous system. Unfortunately, nootropic and neuropharmacological effects of honey have scare report, but nevertheless, it is believed that honey is a memory booster tonic (Mijanur Rahman et al. 2014). In one of the studies, it is reported that honey has nootropic activity as it helps in mental development in newborn babies and preschool children which later developed improved memory, decreased anxiety, and increased IQ levels late in life (Cantarelli et al. 2008). Another experiment suggests that honey consumption decreased the degenerated neurons in CA1 region, mainly responsible for memory (Cai et al. 2011). As enlisted above, honey is a very strong antioxidant, and many neurological diseases like AD, MCI, PD, ALS, and HD have evidence of oxidative stress in them (Mariani et al. 2005), hence honey could be used as an ameliorating agent for these diseases too. Honey has also been used as nutraceutical exhibiting its neuropharmacological effect. Reports indicate that honey possesses antinociceptive, anxiolytic, anticonvulsant, and antidepressant effects (Akanmu et al. 2011). The common symptom of various biochemical insults to functional and structural integrity of neurons like aging, neuroinflammation, and neurotoxicity is oxidative stress, which is due to the presence of high fat contents in brain and more oxygen demand (Schmitt-Schillig et al. 2005). The neuroprotective effect of honey has been found by many experiments as to be mediated by dopaminergic and non-opioid central mechanisms (Oyekunle et al. 2010; Young and Gauthier 1981). Not only neural cells but glial cells have also responded to honey treatment as a study shows honey having neuroprotective effect in the MCAO model of ischemia in rats (Z'arraga-Galindo et al. 2011; Fig. 2.5).

2.5 Flavonoids and Phenolic Acids in Honey Preventing Neurodegeneration by NAD^+ Pathway

In age-related neurodegenerative diseases, NAD^+ plays an imperative role. If the amount of NAD^+ can be maintained in the cell either by decrease in its catabolism or increase in its production, mitochondrial health can be maintained and aging and its associated conditions could be prolonged. The main NAD^+ase in humans are CD38, PARP, and SARM1 as discussed in this chapter. Any compound which is inhibitor of the abovementioned NAD^+ase can lead to an increase in NAD^+ levels. The dire paucity of these inhibitors, however, translates the search for new molecular tools highly desirable in the field of prevention of neurodegenerative diseases. Honey is a natural gel containing a large quantity of flavonoids and phenolic acids which is thought to give all the medicinal properties.

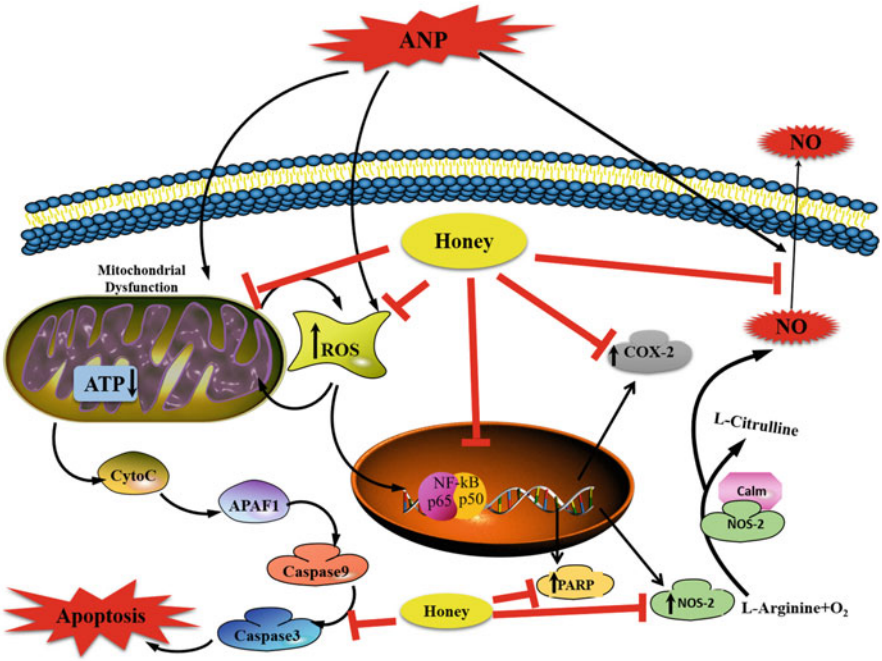

Fig. 2.5 Illustrates the medicinal uses of honey in neuroprotection by acting as anti-oxidant and anti-inflammatory agent leading to protection against age related neurodegenerative diseases. ANPs lead to the increase of oxidative stress, inflammation and subsequently apoptosis. Honey and its components have been shown protection against these effects by decreasing mitochondrial dysfunction and overall oxidative stress. It has been known to decrease COX-2, NF-κB, iNOS and NO production as a result of ANP pathogenesis. By combating these effects Honey can behave as anti-apoptotic agent leading to the protection of neuronal loss during these diseases

Apigenin, a natural flavonoid of honey, is reported to be an inhibitor of NAD^+ase, CD38 (Escande et al. 2013). He recorded that flavonoids including luteolin, quercetin, and apigenin have lowered the major NAD^+ase CD38 at low micromolar range in vitro with a strong impact on SIRT1 activity. These flavonoids have also targeted CD38 in vivo. As CD38 is a major molecule responsible for NAD^+ catabolism hence its decrease availability by honey can lead to the increase of NAD^+ pool in the cells, leading to the decrease in the mitochondrial damage. Camacho-Pereira et al. 2016 also reported that NAD^+ levels were elevated in many tissues by apigenin which decreased the overall proteome acetylation through sirtuin activation. Luteolin, an another CD38 inhibitor, had neuroprotective effects on children suffering from autism (Tsilioni et al. 2015). These studies indicate that CD38 could be a novel therapeutic target for the treatment of neurodegenerative diseases by blocking it with its inhibitor leading to increase in the total NAD^+ levels.

Another target could be PARP, an enzyme responsible for DNA repair and various other cellular processes. The flavonoids quercetin, rutin, and tricetin present in honey are natural inhibitors of PARP1 (Geraets et al. 2007). PARP activity inhibition by flavonoids in honey can ameliorate the cancer, and this could be a

new approach (Pasupuleti et al. 2017). Many scientists including Kauppinen et al. 2011, Martire et al. 2013, and Strosznajder et al. 2012 demonstrated relationship between PARP-1 and neurodegenerative diseases. Recent reports have already reported that inhibition of PARP-1 inflects mitochondrial activity by NAD^+ pathway (Bai et al. 2015; Ying et al. 2005). In various experimental models of neurological injuries both acute and chronic in nature, the use of PARP-1 inhibitors can reveal an enormous potential as a treatment (Bai and Virag 2012; Rom et al. 2015). Acacetin found in honey has also decreased the level of PARP activity in in vitro model of PD (Kim et al. 2017). Wu et al. (2015) studied the cytoprotective effect of apigenin and luteolin and reported that they could restore the cell viability and inhibited the activation of both caspase-3 and PARP-1 in 4-HNE-treated PC12 cells. One side of the coin describes PARP-1 inhibition as an effective approach for neurodegenerative disorders, the other side uses PARP-1 inhibitors as clinical treatment for cancer-inducing cell death as well. Thus, these two opposite paths of PARP 1 underline its complex role in fate of cell subject to types of cell and experimental methodology (Virag and Szabo 2002).

SARM1 could be a promising therapeutic target for several neuropathies including axonopathy (Essuman et al. 2017), but still its preclinical development is ongoing and no drugs are available which could target SARM1.

It is believed that increasing NAD^+ levels can increase sirtuin activity which acts by multiple pathways to stop the neurodegeneration in various diseases. SIRT1 is known as NAD^+ deacetylase which deacetylates proteins contributing to longevity (Satoh et al. 2011). It is also known to regulate autophagy in the brain by activation of AMP-activated protein kinase (AMPK) pathway (Salminen and Kaarniranta 2012). In case of AD, SIRT1 is known to upregulate the α-secretase ADAM10 and decrease NF-κB leading to downregulation of β-secretase β-site AβPP-cleaving enzyme 1 (BACE1) (Gao et al. 2015). This also reduces the Aβ levels, oxidative stress, and neuronal loss in AD pathogenesis (Godoy et al. 2014) with protection of synaptic loss too (Godoy et al. 2014). The leptin-dependent Tau phosphorylation is also known to be inhibited by SIRT1 (Greco et al. 2011). Sirtuins are also known to have influence on the pathways involved in neuroprotection and brain tissue renewal by inducing Notch receptor cleavage (Costa et al. 2005). Notch pathway is thought to be indispensable element of neurogenesis and differentiation of new cell development in case of any pathogenesis. SIRT1 is known to have protective effects in PD too, by restoring several pathways linked to general stress resistance and more specifically to α-synuclein (ASN) metabolism. The role of SIRT is somewhat controversial in case of Huntington's disease. So, leucine, isoleucine, and valine of honey which have been known to enhance the SIRT levels and reduced ROS generation results in an increase in the lifespan of male mice (D'Antona et al. 2010). Quercetin have also activated SIRT1, thus exhibiting potential in multiple sclerosis treatment (Davis et al. 2009). The neuroprotective effects of quercetin by activation of SIRT1 would lead to repression of Bax-dependent apoptosis and multiple proapoptotic transcription factors. Similar finding was reported by Leyton et al. (2015) in which the effects of quercetin on this pathway were studied which inhibited *herpes simplex* virus type 1-induced neurodegeneration by activating

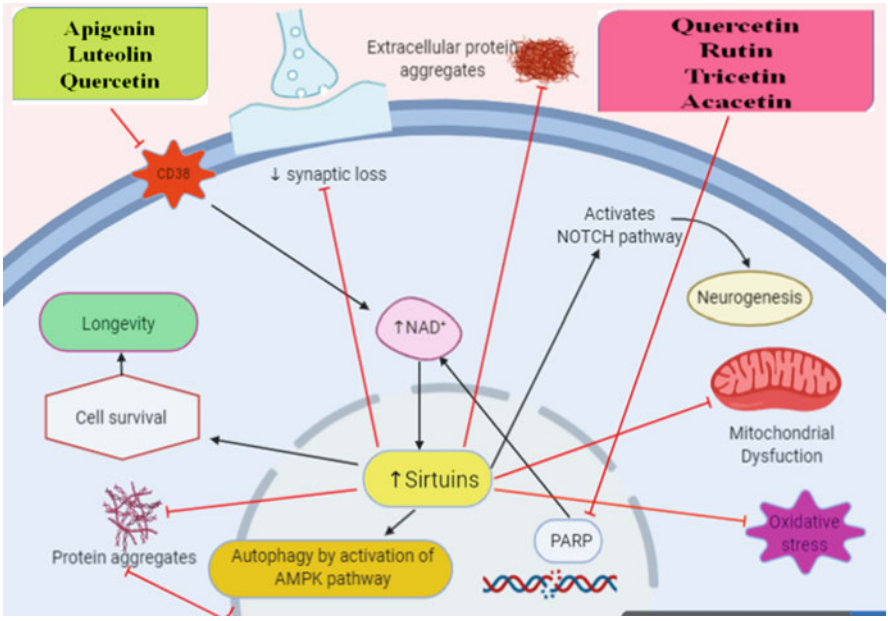

Fig. 2.6 A summarized diagram of the damage in neurons due to age-related neurodegenerative pathology (ANP) and the effect of components of honey in maintaining NAD levels in cells and the prevention by Sirtuin activation: Age-related neurodegenerative disease decreases NAD^+ pool in the cells. NAD^+ decrease leads to mitochondrial dysfunction and overall ATP decrease, oxidative stress elevating the ROS generation, inflammatory markers, and PARP activation. The components of honey (apigenin, luteolin, quercetin, rutin, tricetin, acacetin) has ameliorated these effects by maintaining the overall NAD^+ pools in the cells. The adequate amount of NAD leads to activation of Sirtuins which are specially activated at stress conditions leading to several neuroprotective pathways. Sirtuins are known to decrease mitochondrial dysfunction, oxidative stress, intracellular and extracellular protein aggregation, and loss of synapse. Sirtuins are also known to activate neurogenesis by activating the Notch signaling pathway and leads to cell survival increasing longevity of neurons

SIRT1. Several preclinical data have reported polyphenols as exhibiting potential to activate SIRT1 pathway blocking neural inflammation and alteration of inflammatory cytokines. Chrysin can exert the activation of SIRT1/NRF2 in carrageenan-induced pleurisy and pulmonary injury (Zhiping et al. 2018). All the studies clearly signify that honey and its constituents have potential effect on the NAD^+ catabolic pathway. These reports support that the enzymes responsible to degrade NAD^+ are downregulated leading to increase NAD^+ to the total pool in the cells. With increase in the amount of NAD^+, sirtuins can be activated which protects age-related neurodegenerative diseases (Fig. 2.6).

2.6 Future Prospective

This review describes the preventive effect of honey and its constituents on age-related neurodegenerative disorders via NAD^+ pathway. The flavonoids and phenolic acids have supportive roles related to the disease, but the data available is less and requires further studies. A totally new area of research with extensive clinical potential has come into existence by the discovery of the NAD^+ sirtuin mitochondria axis. Few hypotheses suggest the direct relationship of the NAD^+ sirtuin pathway on mitochondrial stability and metabolic homeostasis in neurodegenerative diseases, still, future research is needed to increase the insight in the clinical use of honey to combat these pathways. Honey can be expected to be used as "NAD^+ compounds" in clinics with further mechanistic research. "NAD^+ compounds" like honey may not be wonderful drugs to cure the disease but could be promising agent to maintain the mitochondrial health and supporting healthier lifestyles.

2.7 Conclusion

The preponderance of age-related neurodegenerative disease like Alzheimer's disease, Parkinson's disease, and ALS is increasing in the population with increased life expectancy. This chapter summarizes that decreased NAD^+ concentrations in the cells can lead to the aging causing various pathogenesis of the age-related disorders. It also suggests that if NAD^+ supplementation is provided, it can ameliorate the aging effects and their associated conditions. Honey has been reported to be a health booster preventing neurological disorders and memory as well. This book chapter has underlined the pathway of this prevention of honey in age-related neurodegenerative disorder. The studies discussed in this book chapter have torched keen interest in maintaining the total NAD^+ concentrations in the cells which could be therapeutic efforts leading to prevention of disease and increasing overall life expectancy. Future meticulous clinical trials in humans are necessary to come to any conclusion related to the hypothesis discussed above and suggesting whether this early promise can be changed to a reality or not.

References

Ahmed S, Othman NH (2013) Honey as a potential natural anticancer agent: a review of its mechanisms. Evid Based Complement Alternat Med 2013:829070–829077

Ahmed S, Sulaiman SA, Baig AA, Ibrahim M, Liaqat S, Fatima S et al (2018) Honey as a potential natural antioxidant medicine: an insight into its molecular mechanisms of action. Oxidative Med Cell Longev 2018:8367846

Akanmu MA, Olowookere TA, Atunwa SA et al (2011) Neuropharmacological effects of Nigerian honey in mice. Afr J Tradit Complement Altern Med 8(3):230–249

Ali AT, Chowdhury MN, Al Humayyd MS (1991) Inhibitory effect of natural honey on Helicobacter pylori. Trop Gastroenterol 12:139–143

Al-Mamary M, Al-Meeri A, Al-Habori M (2002) Antioxidant activities and total phenolics of different types of honey. Nutr Res 22:1041–1047

Alvarez-Suarez JM, Tulipani S, Romandini S, Bertoli E, Battino M (2010) Contribution of honey in nutrition and human health: a review. Mediterr J Nutr Metab 3:15–23

Al-Waili NS (2003a) Intravenous and intrapulmonary administration of honey solution to healthy sheep: effects on blood sugar, renal and liver function tests, bone marrow function, lipid profile and carbon tetrachloride-induced liver injury. J Med Food 6:231–247

Al-Waili NS (2003b) Effects of daily consumption of honey solution on hematological indices and blood levels of minerals and enzymes in normal individuals. J Med Food 6:135–140

Al-Waili NS (2004) Natural honey lowers plasma glucose, c-reactive protein, homocysteine, and blood lipids in healthy, diabetic, and hyperlipidemic subjects: comparison with dextrose and sucrose. J Med Food 7:100–107

Al-Waili NS, Boni NS (2003) Natural honey lowers plasma prostaglandin concentrations in normal individuals. J Med Food 6:129–133

Andersen JK (2004) Oxidative stress in neurodegeneration: cause or consequence? Nat Med 10: S18–S25

Bai P, Virag L (2012) Role of poly(ADP-ribose) polymerases in the regulation of inflammatory processes. FEBS Lett 586:3771–3777

Bai P, Cantó C, Oudart H, Brunyánszki A, Cen Y, Thomas C et al (2011) PARP-1 inhibition increases mitochondrial metabolism through SIRT1 activation. Cell Metab 13:461–468

Bai P, Nagy L, Fodor T, Liaudet L, Pacher P (2015) Poly(ADP-ribose)polymerases as modulators of mitochondrial activity. Trends Endocrinol Metab 26:75–83

Bansal V, Medhi B, Pandhi P (2005) Honey-A remedy rediscovered and its therapeutic utility. Kathmandu Univ Med J 3:305–309

Beghi E, Logroscino G, Chió A, Hardiman O, Mitchell D, Swingler R et al (2006) The epidemiology of ALS and the role of population-based registries. Biochim Biophys Acta 1762:1150–1157

Bender DA (1983) Biochemistry of tryptophan in health and disease. Mol Asp Med 6:101–197

Beretta G, Orioli M, Facino RM (2007) Antioxidant and radical scavenging activity of honey in endothelial cell cultures (EA.hy926). Planta Med 73(11):1182–1189

Bheda P, Jing H, Wolberger C et al (2016) The substrate specificity of Sirtuins. Annu Rev Biochem 85:405–429

Bilsel Y, Bugra D, Yamaner S, Bulut T, Cevikbas U, Turkoglu U (2002) Could honey have a place in colitis therapy? Effects of honey, prednisolone and disulfiram on inflammation, nitric oxide, and free radical formation. Dig Surg 19:306–311

Bishop NA, Lu T, Yankner BA (2010) Neural mechanisms of ageing and cognitive decline. Nature 464:529–535

Blander G, Guarente L (2004) The Sir2 family of protein deacetylases. Annu Rev Biochem 73:417–435

Bogan KL, Brenner C (2008) Nicotinic acid, nicotinamide, and nicotinamide riboside: a molecular evaluation of NAD^+ precursor vitamins in human nutrition. Annu Rev Nutr 28:115–130

Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN et al (2003) Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 24:197–211

Braak H, Bohl JR, Muller CM, Rub U, de Vos RA et al (2006) Stanley Fahn lecture 2005: the staging procedure for the inclusion body pathology associated with sporadic Parkinson's disease reconsidered. Mov Disord 21:2042–2051

Bratic A, Larsson NG (2013) The role of mitochondria in aging. J Clin Invest 123(3):951–957

Cai M, Shin BY, Kim DH et al (2011) Neuroprotective effects of a traditional herbal prescription on transient cerebral global ischemia in gerbils. J Ethnopharmacol 138(3):723–730

Camacho-Pereira J, Tarrago MG, Chini CCS, Nin V, Escande C, Warner GM, Puranik AS, Schoon RA, Reid JM, Galina A et al (2016) CD38 dictates age-related NAD decline and mitochondrial dysfunction through an SIRT3-dependent mechanism. Cell Metab 23:1127–1139

Candiracci M, Piatti E, Dominguez-Barragán M et al (2012) Anti-inflammatory activity of a honey flavonoid extract on lipopolysaccharide-activated N13 microglial cells. J Agric Food Chem 60 (50):12304–12311

Cantarelli MA, Pellerano RG, Marchevsky EJ, Camina JM (2008) Quality of honey from Argentina: study of chemical composition and trace elements. J Argent Chem Soc 96(2):33–41

Chini CCS, Tarragó MG, Chini EN (2017) NAD and the aging process: role in life, death and everything in between. Mol Cell Endocrinol 455:62–74

Clarke JR, Lyra ESNM, Figueiredo CP, Frozza RL, Ledo JH, Beckman D et al (2015) Alzheimer-associated abeta oligomers impact the central nervous system to induce peripheral metabolic deregulation. EMBO Mol Med 7(2):190–210

Costa RM, Drew C, Silva AJ (2005) Notch to remember. Trends Neurosci 28(8):429–435

D'Antona G, Ragni M, Cardile A, Tedesco L, Dossena M, Bruttini F, Caliaro F, Corsetti G, Bottinelli R, Carruba MO, Valerio A, Nisoli E (2010) Branched-chain amino acid supplementation promotes survival and supports cardiac and skeletal muscle mitochondrial biogenesis in middle-aged mice. Cell Metab 12(4):362–372

Davis JM, Murphy EA, Carmichael MD, Davis B (2009) Quercetin increases brain and muscle mitochondrial biogenesis and exercise tolerance. Am J Phys Regul Integr Comp Phys 296(4): R1071–R1077

De Flora A, Zocchi E, Guida L et al (2004) Autocrine and paracrine calcium signaling by the CD38/NAD+/cyclic ADP-ribose system. Ann N Y Acad Sci 1028:176–191

Dellinger RW, Santos SR, Morris M, Evans M, Alminana D, Guarente L, Marcotulli E (2017) Repeat dose NRPT (nicotinamide riboside and pterostilbene) increases NAD(+) levels in humans safely and sustainably: a randomized, double-blind, placebo-controlled study. NPJ Aging Mech Dis 3:17

Diplock AT, Rice-Evans CA, Burdon RH (1994) Is there a significant role for lipid peroxidation in the causation of malignancy and for antioxidants in cancer prevention? Cancer Res 54 (7):1952–1956

Dunn L, Allen GF, Mamais A, Ling H, Li A, Duberley KE et al (2014) Dysregulation of glucose metabolism is an early event in sporadic Parkinson's disease. Neurobiol Aging 35 (5):1111–1115

Erejuwa OO, Sulaiman SA, Wahab MS, Sirajudeen KN, Salleh MS, Gurtu S (2010) Antioxidant protection of Malaysian tualang honey in pancreas of normal and streptozotocin-induced diabetic rats. Ann Endocrinol 71(4):291–296. https://doi.org/10.1016/j.ando.2010.03.003

Erejuwa OO, Sulaiman SA, Ab Wahab MS (2012) Honey: a novel antioxidant. Molecules 17 (4):4400–4423

Eteraf-Oskouei T, Najafi M (2013) Traditional and modern uses of natural honey in human diseases: a review. Iran J Basic Med Sci 16(6):731–742

Escande C, Nin V, Price NL, Capellini V, Gomes AP, Barbosa MT et al (2013) Flavonoid apigenin is an inhibitor of the NAD+ase CD38: implications for cellular NAD+ metabolism, protein acetylation, and treatment of metabolic syndrome. Diabetes 62:1084–1093

Essuman K, Summers DW, Sasaki Y, Mao X, DiAntonio A, Milbrandt J (2017) The SARM1 toll/Interleukin-1 receptor domain possesses intrinsic NAD+ cleavage activity that promotes pathological axonal degeneration. Neuron 93(6):1334–1343

Ezz El-Arab AM, Girgis SM, Hegazy ME, Abd El-Khalek AB (2006) Effect of dietary honey on intestinal microflora and toxicity of mycotoxins in mice. BMC Complement Altern Med 6:1–13

Fearnley JM, Lees AJ (1991) Ageing and Parkinson's disease: substantia nigra regional selectivity. Brain 114(Pt 5):2283–2301

Ford E, Voit R, Liszt G et al (2006) Mammalian Sir2 homolog SIRT7 is an activator of RNA polymerase I transcription. Genes Dev 20(9):1075–1080

Frye RA (1999) Characterization of five human cDNAs with homology to the yeast SIR2 gene: Sir2-like proteins (sirtuins) metabolize NAD and may have protein ADP-ribosyltransferase activity. Biochem Biophys Res Commun 260(1):273–279

Gao R, Wang Y, Pan Q, Huang G, Li N, Mou J, Wang D (2015) Fuzhisan, a Chinese herbal medicine, suppresses beta-secretase gene transcription via upregulation of SIRT1 expression in N2a-APP695 cells. Int J Clin Exp Med 8(5):7231–7240

Geraets L, Moonen HJ, Brauers K, Wouters EF, Bast A, Hageman GJ (2007) Dietary flavones and flavonoles are inhibitors of poly(ADP-ribose)polymerase-1 in pulmonary epithelial cells. J Nutr 137:2190–2195

Gibson BA, Kraus WL (2012) New insights into the molecular and cellular functions of poly (ADP-ribose) and PARPs. Nat Rev Mol Cell Biol 13:411–424

Godoy JA, Zolezzi JM, Braidy N, Inestrosa NC (2014) Role of Sirt1 during the ageing process: relevance to protection of synapses in the brain. Mol Neurobiol 50(3):744–756

Greco SJ, Hamzelou A, Johnston JM, Smith MA, Ashford JW, Tezapsidis N (2011) Leptin boosts cellular metabolism by activating AMPK and the sirtuins to reduce tau phosphorylation and β-amyloid in neurons. Biochem Biophys Res Commun 414(1):170–174

Grozio A, Sociali G, Sturla L et al (2013) CD73 protein as a source of extracellular precursors for sustained NAD+ biosynthesis in FK866-treated tumor cells. J Biol Chem 288(36):25938–25949

Haigis MC, Sinclair DA (2010) Mammalian sirtuins: biological insights and disease relevance. Annu Rev Pathol 5:253–295

Halliday GM, McCann H (2010) The progression of pathology in Parkinson's disease. Ann N Y Acad Sci 1184:188–195

Harden A, Young WJ (1906) The alcoholic ferment of yeast-juice. Proc R Soc B Biol Sci 78:369–375

Harman D (1981) The aging process. Proc Natl Acad Sci U S A 78(11):7124–7128

Hindle JV (2010) Ageing, neurodegeneration and Parkinson's disease. Age Ageing 39:156–161

Houtkooper RH, Canto C, Wanders RJ, Auwerx J (2010) The secret life of NAD+: an old metabolite controlling new metabolic signaling pathways. Endocr Rev 31:194–223

https://www.who.int/gho/publications/world_health_statistics/2015/en/

https://www.who.int/whr/2006/whr06_en.pdf

Hung CW, Chen YC, Hsieh WL, Chiou SH, Kao CL (2010) Ageing and neurodegenerative diseases. Ageing Res Rev 1:S36–S46

Imai S, Guarente L (2014) NAD+ and sirtuins in aging and disease. Trends Cell Biol 24(8):464–471

Imai S, Yoshino J (2013) The importance of NAMPT/NAD/SIRT1 in the systemic regulation of metabolism and ageing. Diabetes Obes Metab 15(3):26–33

Ishrat T, Hoda MN, Khan MB, Yousuf S, Ahmad M, Khan MM et al (2009) Amelioration of cognitive deficits and neurodegeneration by curcumin in rat model of sporadic dementia of Alzheimer's type (SDAT). Eur Neuropsychopharmacol 19:636–647

Jaganathan SK, Mandal M (2009) Honey constituents and their apoptotic effect in colon cancer cells. J ApiProd ApiMed Sci 1(2):29–36

Javed H, Khan MM, Ahmad A, Vaibhav K, Ahmad ME, Khan A et al (2012) Rutin prevents cognitive impairments by ameliorating oxidative stress and neuroinflammation in rat model of sporadic dementia of Alzheimer type. Neuroscience 210:340–352

Jeffrey AE, Echazarreta CM (1996) Medical uses of honey. Rev Biomed 7:43–49

Jimenez-Del-Rio M, Guzman-Martinez C, Velez-Pardo C (2010) The effects of polyphenols on survival and locomotor activity in drosophila melanogaster exposed to iron and paraquat. Neurochem Res 35(2):227–238

Johnson S, Imai SI (2018) NAD^+ biosynthesis, aging, and disease. F1000Res 7:132

Johnston JE, Sepe HA, Miano CL, Brannan RG, Alderton AL (2005) Honey inhibits lipid oxidation in ready-to-eat ground beef patties. Meat Sci 70:627–631

Karbowski M, Neutzner A (2012) Neurodegeneration as a consequence of failed mitochondrial maintenance. Acta Neuropathol 123(2):157–171

Kauppinen TM, Suh SW, Higashi Y, Berman AE, Escartin C, Won SJ et al (2011) Poly (ADP-ribose) polymerase-1 modulates microglial responses to amyloid beta. J Neuroinflammation 8:152

Kennedy MA, Moffat TC, Gable K, Ganesan S, Niewola-Staszkowska K, Johnston A et al (2016) A signaling lipid associated with Alzheimer's disease promotes mitochondrial dysfunction. Sci Rep 6:19332
Kerr JS et al (2017) Mitophagy and Alzheimer's disease: cellular and molecular mechanisms. Trends Neurosci 40:151–166
Khalil MI, Sulaiman SA (2010) The potential role of honey and its polyphenols in preventing heart diseases: a review. Afr J Tradit Complement Altern Med 7:315–321
Khan A, Vaibhav K, Javed H, Khan MM, Tabassum R, Ahmed ME et al (2012) Attenuation of Aβ-induced neurotoxicity by thymoquinone via inhibition of mitochondrial dysfunction and oxidative stress. Mol Cell Biochem 369(1–2):55–65
Kilicoglu B, Gencay C, Kismet K, Serin Kilicoglu S, Erguder I, Erel S et al (2008) The ultrastructural research of liver in experimental obstructive jaundice and effect of honey. Am J Surg 195 (2):249–256
Kim SM, Park YJ, Shin MS, Kim HR, Kim MJ, Lee SH, Yun SP, Kwon SH (2017) Acacetin inhibits neuronal cell death induced by 6-hydroxydopamine in cellular Parkinson's disease model. Bioorg Med Chem Lett 27(23):5207–5212
Knight AL, Yan X, Hamamichi S, Ajjuri RR, Mazzulli JR, Zhang MW et al (2014) The glycolytic enzyme, GPI, is a functionally conserved modifier of dopaminergic neurodegeneration in Parkinson's models. Cell Metab 20(1):145–157
Körner S, Böselt S, Thau N, Rath KJ, Dengler R, Petri S (2013) Differential sirtuin expression patterns in amyotrophic lateral sclerosis (ALS) postmortem tissue: neuroprotective or neurotoxic properties of sirtuins in ALS? Neurodegener Dis 11(3):141–152
Kudo Y, Boyd CA (2000) Human placental indoleamine 2,3-dioxygenase: cellular localization and characterization of an enzyme preventing fetal rejection. Biochim Biophys Acta 1500:119–124
Kupis W, Palyga J, Tomal E, Niewiadomska E (2016) The role of sirtuins in cellular homeostasis. J Physiol Biochem 72:371–380
Leyton L, Hott M, Acuña F, Caroca J, Nuñez M, Martin C, Zambrano A, Concha MI, Otth C (2015) Nutraceutical activators of AMPK/Sirt1 axis inhibit viral production and protect neurons from neurodegenerative events triggered during HSV-1 infection. Virus Res 205:63–72
Lin MT, Beal MF (2006) Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature 443(7113):787–795. https://doi.org/10.1038/nature05292
Malavasi F, Deaglio S, Funaro A et al (2008) Evolution and function of the ADP ribosyl cyclase/CD38 gene family in physiology and pathology. Physiol Rev 88:841–886
Mariani E, Polidori MC, Cherubini A, Mecocci P (2005) Oxidative stress in brain aging, neurodegenerative and vascular diseases: an overview. J Chromatogr B Anal Technol Biomed Life Sci 827(1):65–75
Martire S, Fuso A, Rotili D, Tempera I, Giordano C, De Zottis I et al (2013) PARP-1 modulates amyloid beta peptide-induced neuronal damage. PLoS One 8:e72169
Meda A, Lamien EC, Millogo J, Romito M, Nacoulma OG (2004) Ethnopharmacological communication therapeutic uses of honey and honeybee larvae in Central Burkina Faso. J Ethnopharmacol 95:103–107
Medhi B, Prakash A, Avti PK, Saikia UN, Pandhi P, Khanduja KL (2008) Effect of Manuka honey and sulfasalazine in combination to promote antioxidant defense system in experimentally induced ulcerative colitis model in rats. Indian J Exp Biol 46(8):583–590
Mijanur Rahman M, Gan SH, Khalil MI (2014) Neurological effects of honey: current and future prospects. Evid Based Complement Alternat Med 2014:958721
Molan P (2001) Manuka Honey as a medicine, Global Bioactives Summit, Hamilton
Morales J, Li L, Fattah FJ et al (2014) Review of poly (ADP-ribose) polymerase (PARP) mechanisms of action and rationale for targeting in cancer and other diseases. Crit Rev Eukaryot Gene Expr 24(1):15–28
Mostoslavsky R, Chua KF, Lombard DB et al (2006) Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. Cell 124(2):315–329

Mouchiroud L, Houtkooper RH, Moullan N et al (2013) The NAD+/Sirtuin pathway modulates longevity through activation of mitochondrial UPR and FOXO signaling. Cell 154(2):430–441
Murtaza G, Karim S, Akram MR et al (2014) Caffeic acid phenethyl ester and therapeutic potentials. Biomed Res Int 2014:145342–145349
Nagai T, Sakai M, Inoue R, Inoue H, Suzuki N (2001) Antioxidative activities of some commercially honeys, royal jelly, and propolis. Food Chem 75(2):237–240
Nikiforov A, Kulikova V, Ziegler M (2015) The human NAD metabolome: functions, metabolism and compartmentalization. Crit Rev Biochem Mol Biol 50:284–297
Olaitan PB, Adeleke OE, Ola IO (2007) Honey: a reservoir for microorganisms and an inhibitory agent for microbes. Afr Health Sci 7(3):159–165
Ortega-Arellano HF, Jimenez-del-Rio M, Velez-Pardo C (2011) Life span and locomotor activity modification by glucose and polyphenols in *Drosophila melanogaster* chronically exposed to oxidative stress-stimuli: implications in Parkinson's disease. Neurochem Res 36(6):1073–1086
Osborne B, Bentley NL, Montgomery MK, Turner N (2016) The role of mitochondrial sirtuins in health and disease. Free Radic Biol Med 100:164–174
Othman NH (2012a) Does honey have the characteristics of natural cancer vaccine? J Tradit Complement Med 2(4):276–283
Othman NH (2012b) Honey and cancer: sustainable inverse relationship particularly for developing nations—a review. Evid Based Complement Alternat Med 2012:410406
Oyekunle OA, Akanmu MA, Ogundeji TP (2010) Evaluation of anxiolytic and novelty induced behaviours following bee-honey consumption in rats. J Neurosci Behav Health 2(4):38–43
Pappolla MA, Omar RA, Kim KS, Robakis NK (1992) Immunohistochemical evidence of oxidative [corrected] stress in Alzheimer's disease. Am J Pathol 140(3):621–628
Pasupuleti VR, Sammugam L, Ramesh N, Gan SH (2017) Honey, Propolis, and Royal Jelly: a comprehensive review of their biological actions and health benefits. Oxidative Med Cell Longev 2017:1259510
Pearce RK, Owen A, Daniel S, Jenner P, Marsden CD (1997) Alterations in the distribution of glutathione in the substantia nigra in Parkinson's disease. J Neural Transm (Vienna) 1997104 (6–7):661–677
Pehar M, Harlan BA, Killoy KM, Vargas MR (2018) Nicotinamide adenine dinucleotide metabolism and neurodegeneration. Antioxid Redox Signal 28(18):1652–1668
Perry TL, Yong VW (1986) Idiopathic Parkinson's disease, progressive supranuclear palsy and glutathione metabolism in the substantia nigra of patients. Neurosci Lett 67(3):269–274
Perry TL, Godin DV, Hansen S (1982) Parkinson's disease: a disorder due to nigral glutathione deficiency? Neurosci Lett 33(3):305–310
Pirinen E, Cantó C, Jo YS, Morato L, Zhang H, Menzies KJ et al (2014) Pharmacological inhibition of poly(ADP-ribose) polymerases improves fitness and mitochondrial function in skeletal muscle. Cell Metab 19:1034–1041
Preugschat F, Carter LH, Boros EE et al (2014) A pre-steady state and steady state kinetic analysis of the N-ribosyl hydrolase activity of hCD157. Arch Biochem Biophys 564:156–163
Procaccini C, Santopaolo M, Faicchia D, Colamatteo A, Formisano L, de Candia P et al (2016) Role of metabolism in neurodegenerative disorders. Metabolism 65(9):1376–1390
Rahman MM, Gan SH, Khalil MI (2014) Neurological effects of honey: current and future prospects. Evid Based Complement Alternat Med 2014:958721
Rakha MK, Nabil ZI, Hussein AA (2008) Cardioactive and vasoactive effects of natural wild honey against cardiac malperformance induced by hyperadrenergic activity. J Med Food 11:91–98
Ramsey KM, Mills KF, Satoh A et al (2008) Age-associated loss of Sirt1-mediated enhancement of glucose-stimulated insulin secretion in beta cell-specific Sirt1-overexpressing (BESTO) mice. Aging Cell 7(1):78–88
Reeve A, Simcox E, Turnbull D (2014) Ageing and Parkinson's disease: why is advancing age the biggest risk factor? Ageing Res Rev 14:19–30
Reitz C, Brayne C, Mayeux R (2011) Epidemiology of Alzheimer disease. Nat Rev Neurol 7:137–152

Renton AE, Chiò A, Traynor BJ (2014) State of play in amyotrophic lateral sclerosis genetics. Nat Neurosci 17:17–23

Revollo JR, Grimm AA, Imai S (2004) The NAD biosynthesis pathway mediated by nicotinamide phosphoribosyltransferase regulates Sir2 activity in mammalian cells. J Biol Chem 279 (49):50754–50763

Rom S, Zuluaga-Ramirez V, Dykstra H, Reichenbach NL, Ramirez SH, Persidsky Y (2015) Poly (ADP-ribose) polymerase −1 inhibition in brain endothelium protects the blood–brain barrier under physiologic and neuroinflammatory conditions. J Cereb Blood Flow Metab 35:28–36

Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hentati A, Donaldson D et al (1993) Mutations in cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 362:59–62

Rotermund C, Machetanz G, Fitzgerald JC (2018) The therapeutic potential of metformin in neurodegenerative diseases. Front Endocrinol (Lausanne) 19(9):400

Sahin E, Depinho RA (2010) Linking functional decline of telomeres, mitochondria and stem cells during ageing. Nature 464:520–528

Salminen A, Kaarniranta K (2012) AMP-activated protein kinase (AMPK) controls the aging process via an integrated signaling network. Ageing Res Rev 11(2):230–241

Samii A, Nutt JG, Ransom BR (2004) Parkinson's disease. Lancet 363:1783–1793

Sasaki Y, Vohra BP, Baloh RH, Milbrandt J (2009) Transgenic mice expressing the Nmnat1 protein manifest robust delay in axonal degeneration in vivo. J Neurosci 29(20):6526–6534

Satoh A, Stein L, Imai S (2011) The role of mammalian sirtuins in the regulation of metabolism, aging, and longevity. Handb Exp Pharmacol 206:125–162

Schmitt-Schillig S, Schaffer S, Weber CC, Eckert GP, Muller WE (2005) Flavonoids and the aging brain. J Physiol Pharmacol 56(1):23–36

Smith MA, Zhu X, Tabaton M, Liu G, McKeel DW Jr, Cohen ML et al (2010) Increased iron and free radical generation in preclinical Alzheimer disease and mild cognitive impairment. J Alzheimers Dis 19(1):363–372

Stein LR, Imai S (2014) Specific ablation of Nampt in adult neural stem cells recapitulates their functional defects during aging. EMBO J 33(12):1321–1340

Strosznajder JB, Czapski GA, Adamczyk A, Strosznajder RP (2012) Poly(ADP-ribose) polymerase-1 in amyloid beta toxicity and Alzheimer's disease. Mol Neurobiol 46:78–84

Subrahmanyam M (1998) A prospective randomised clinical and histological study of superficial burn wound healing with honey and silver sulfadiazine. Burns 24(2):157–161

Summers DW, DiAntonio A, Milbrandt J (2014) Mitochondrial dysfunction induces Sarm1-dependent cell death in sensory neurons. J Neurosci 34(28):9338–9350

Sun N, Youle RJ, Finkel T (2016) The mitochondrial basis of aging. Mol Cell 61(5):654–666

Tanno M, Sakamoto J, Miura T et al (2007) Nucleocytoplasmic shuttling of the NAD+-dependent histone deacetylase SIRT1. J Biol Chem 282(9):6823–6832

Tonks AJ, Cooper RA, Jones KP, Blair S, Parton J, Tonks A (2003) Honey stimulates inflammatory cytokine production from monocytes. Cytokine 21:242–247

Tonks AJ, Dudley E, Porter NG et al (2007) A 5.8-kDa component of manuka honey stimulates immune cells via TLR4. J Leukoc Biol 82(5):1147–1155

Tsilioni I, Taliou A, Francis K, Theoharides TC (2015) Children with autism spectrum disorders, who improved with a luteolin-containing dietary formulation, show reduced serum levels of TNF and IL-6. Transl Psychiatry 5(9):e647

Turkmen N, Sari F, Poyrazoglu ES, Velioglu YS (2006) Effects of prolonged heating on antioxidant activity and colour of honey. Food Chem 95:653–657

UN (2019) United Nations. World population ageing 2019. United Nations. Department of Economic and Social Affairs Population Division

Uttara B, Singh AV, Zamboni P, Mahajan RT (2009) Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. Curr Neuropharmacol 7(1):65–74

van Ham TJ, Breitling R, Swertz MA, Nollen EA (2009) Neurodegenerative diseases: lessons from genome-wide screens in small model organisms. EMBO Mol Med 1:360–370

Vaquero A, Scher MB, Lee DH et al (2006) SirT2 is a histone deacetylase with preference for histone H4 Lys 16 during mitosis. Genes Dev 20(10):1256–1261

Verdin E (2014) The many faces of sirtuins: coupling of NAD metabolism, sirtuins and lifespan. Nat Med 20(1):25–27

Verdin E, Hirschey MD, Finley LW et al (2010) Sirtuin regulation of mitochondria: energy production, apoptosis, and signaling. Trends Biochem Sci 35(12):669–675

Virag L, Szabo C (2002) The therapeutic potential of poly(ADP-ribose) polymerase inhibitors. Pharmacol Rev 54:375–429

Wang T, Zhang X, Bheda P et al (2006) Structure of Nampt/PBEF/visfatin, a mammalian NAD+ biosynthetic enzyme. Nat Struct Mol Biol 13(7):661–662

White JW (1979) Composition of honey. In: Crane E (ed) Honey: a comprehensive survey. Heinemann, London, pp 57–192

World Health Organization (2018) Ageing and Health. https://www.who.int/news-room/fact-sheets/detail/ageing-and-health

Wu PS, Yen JH, Kou MC, Wu MJ (2015) Luteolin and Apigenin attenuate 4-Hydroxy-2-Nonenal-mediated cell death through modulation of UPR, Nrf2-ARE and MAPK pathways in PC12 cells. PLoS One 10(6):e0130599

Yamazaki F, Kuroiwa T, Takikawa O, Kido R (1985) Human indolylamine 2,3-dioxygenase. Its tissue distribution, and characterization of the placental enzyme. Biochem J 230:635–638

Yang Y, Sauve AA (2016) NAD^+ metabolism: bioenergetics, signaling and manipulation for therapy. Biochim Biophys Acta 1864(12):1787–1800

Yang J, Wu Z, Renier N, Simon DJ, Uryu K, Park DS et al (2015) Pathological axonal death through a MAPK cascade that triggers a local energy deficit. Cell 160(1–2):161–176

Ying W, Alano CC, Garnier P, Swanson RA (2005) NAD+ as a metabolic link between DNA damage and cell death. J Neurosci Res 79:216–223

Yoshino J, Mills KF, Yoon MJ et al (2011) Nicotinamide mononucleotide, a key NAD+ intermediate, treats the pathophysiology of diet- and age-induced diabetes in mice. Cell Metab 14 (4):528–536

Young SN, Gauthier S (1981) Effect of tryptophan administration on tryptophan, 5-hydroxyindoleacetic acid and indoleacetic acid in human lumbar and cisternal cerebrospinal fluid. J Neurol Neurosurg Psychiatry 44(4):323–328

Z'arraga-Galindo N, Vergara-Arag'on P, Rosales-Mel'endez S et al (2011) Effects of bee products on pentylenetetrazole-induced seizures in the rat. Proc West Pharmacol Soc 54:32–39

Zemlan FP, Thienhaus OJ, Bosmann HB (1989) Superoxide dismutase activity in Alzheimer's disease: possible mechanism for paired helical filament formation. Brain Res 476(1):160–162

Zhang H, Ryu D, Wu Y, Gariani K, Wang X, Luan P, D'Amico D, Ropelle ER, Lutolf MP, Aebersold R, Schoonjans K, Menzies KJ, Auwerx J (2016) NAD^+ repletion improves mitochondrial and stem cell function and enhances life span in mice. Science 352(6292):1436–1443

Zhiping Y, Yifu G, Jun L, Li L, Zhipeng L (2018) Chrysin attenuates carrageenan-induced pleurisy and lung injury via activation of SIRT1/NRF2 pathway in rats. Eur J Pharmacol 836:83–88

Zhu XH, Lu M, Lee BY, Ugurbil K, Chen W (2015) In vivo NAD assay reveals the intracellular NAD contents and redox state in healthy human brain and their age dependences. Proc Natl Acad Sci U S A 112(9):2876–2881

Zuo L, Motherwell MS (2013) The impact of reactive oxygen species and genetic mitochondrial mutations in Parkinson's disease. Gene 532(1):18–23

Neuroprotective Effects of Honey: A Mechanistic View

3

Nawab John Dar

Abstract

Honey has been used as food and medicine by humans since the beginning of civilizations. It has been used to treat a multitude of ailments due to its numerous biological activities like anti-bacterial, anti-inflammatory, anti-mutagenic, anti-tumor, and anti-oxidant. More recent reports have shown that honey possess neuroprotective potential as well. Several pre-clinical reports have demonstrated that honey is able to modulate the neurobehavioral outcomes and improve learning and memory in mice models by regulating the anti-oxidant mechanisms, neurotrophic factors, and cholinergic system. Honey is a rich source of pharmacologically potent natural compounds like polyphenols that have been demonstrated to attenuate microglia-induced inflammation and improve memory deficits in different neurotoxicity models. In this chapter, I will be summarizing the neuroprotective potential of honey in various paradigms of neurological ailments and discuss its mechanism of action with possible therapeutic applications.

Keywords

Honey · Neurodegeneration · Oxidative stress · Neurotoxicity · Nootropic effect · Neuroprotection

Abbreviations

6-OHDA	6-hydroxydopamine
AChE	Acetylcholinesterase activity

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BDNF	Brain-derived neurotrophic factor
CAT	Catalase
GPx	Glutathione peroxidase
GSH	Glutathione
H_2O_2	Hydrogen peroxide
iNOS	inducible nitric oxide species
MCAO	Middle cerebral artery occlusion
MK	Manuka honey
NO	Nitric oxide
OVX	Ovariectomized
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TH	Taulang honey

3.1 Introduction

Honey is a viscous, sweet, and natural food product produced from the nectar of flowers by worker honeybees (*Apis mellifera*; Family: Apidae). It is a rich blend of different bioactive compounds like polyphenols, flavonoids, organic acids, minerals, peptides, enzymes, and vitamins. However, the composition as well as the pharmacological potential of honey from different regions of the world varies. Studies have shown that Malaysian tualang honey, which is produced by the rock bee (*Apis dorsata*) by building hives on tualang trees (*Koompassia excelsa*) found mainly in the northwestern region of peninsular Malaysia, is the richest source of bioactive compounds. It was reported that tualang honey contains more phenolic acids and flavonoids than manuka honey and other local Malaysian honey. In addition, it has demonstrated higher anti-oxidant activity compared to gelam honey, Indian forest honey, and pineapple honey (Kishore et al. 2011). Most of the pharmacological studies have been carried out using tualang honey. Due to its rich nutraceutical composition, it has demonstrated anti-oxidant potential against different ailments including brain-related disorders (Erejuwa et al. 2012, 2011; Alvarez-Suarez et al. 2010). Oxidative stress is one of the common contributors in neurological ailments and, in particular, degenerative disorders like Alzheimer's, Parkinson's, and Huntington's diseases where disturbed equilibrium between pro-oxidants and anti-oxidants with associated disruption of redox circuitry and macromolecular damage results in reactive oxygen species (ROS) and other free radical generation that ultimately leads to brain injury. Oxidative stress continues to remain a key therapeutic target for neurological diseases (Halliwell 1992; Patel 2016). Oral intake of honey has been shown to increase anti-oxidant, vitamin C, and other trace element levels in plasma as well as brain (Al-Waili 2003). Moreover, it has increased the production of neurotrophic factors such as brain-derived neurotrophic factor (BDNF), attenuating oxidative stress and anxiety-like behavior (Fig. 3.1) in stressed ovariectomized rats (Al-Rahbi et al. 2014a). Honey has been shown to attenuate the kainic acid-induced oxidative stress and neurodegeneration in the cortical region of the rat

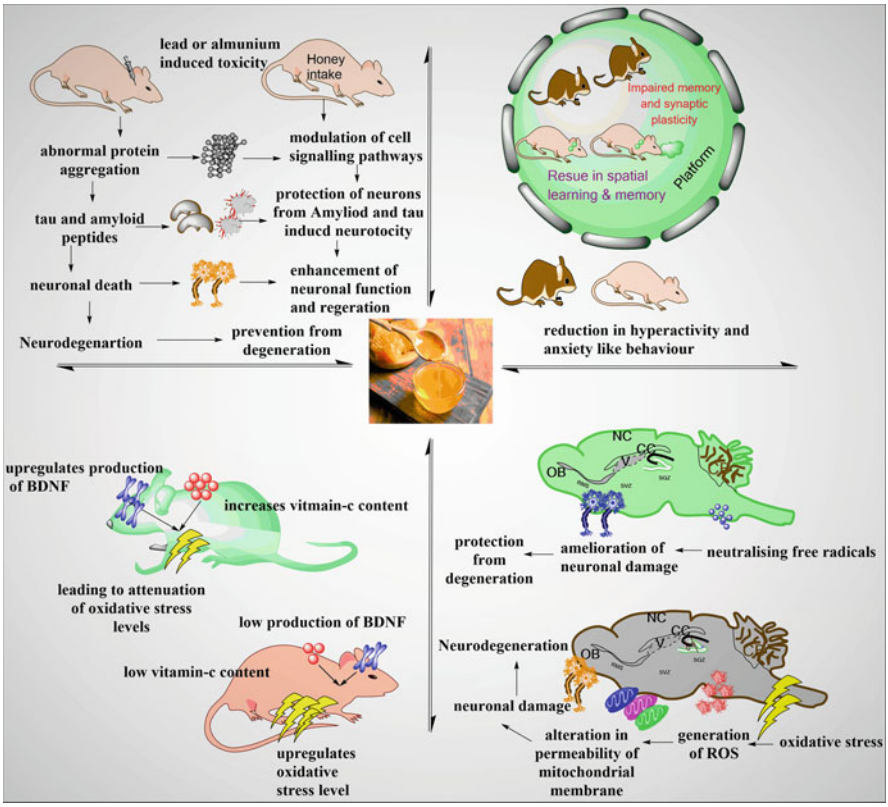

Fig. 3.1 Graphical depiction of neuroprotective effect of honey: Honey and its active constituents have been demonstrated to possess anti-oxidant properties, enhance learning and memory, regulate neurodevelopment and neurobehavioral activities, and rescue neuronal cells from various regions of the brain against different neurotoxic insults via regulating anti-oxidant enzymes, neurotrophic levels, protein aggregation and subsequent inflammation, and other detrimental cascades in neurodegenerative diseases like Alzheimer's disease

brain (Sairazi et al. 2017). It has been found that honey also contains choline and acetylcholine, which are neurotransmitters in nature performing vital brain functions (White Jr 1975). It has demonstrated significant potential at early developmental stages in newborns and pre-school age children, improving their memory, growth, and enhance their performance at later stages of life (Cantarelli et al. 2008; Sairazi et al. 2017). Studies using animal models have shown that early feeding with honey is beneficial and may improve age-associated cognitive decline and memory loss (Chepulis et al. 2009). Honey has been shown to protect neuronal cells and prevent neurobehavioral deficits (Abdulmajeed et al. 2016) as well as other tissues against different toxic insults (Fig. 3.1) like lead acetate-induced hepatorenal toxicity (Elmenoufy 2012) acetaminophen-induced acute renal hepato-nephrotoxicity (Afroz et al. 2014) and bisphenol A-induced ovarian toxicity in pre-pubertal rats

(Zaid et al. 2014). Although, honey has numerous biological activities; here I will be specifically discussing the neuroprotective effects of honey in general from a mechanistic point of view.

3.2 Chemical Composition of Honey

Honey is a mixture of numerous components such as sugars, enzymes, organic acids, vitamins, amino acids, and trace elements. It contains phenolic compounds and flavonoids, and the anti-oxidant activity of honey is mostly attributed to these compounds. The chemical structures of some flavonoids and phenolic compounds

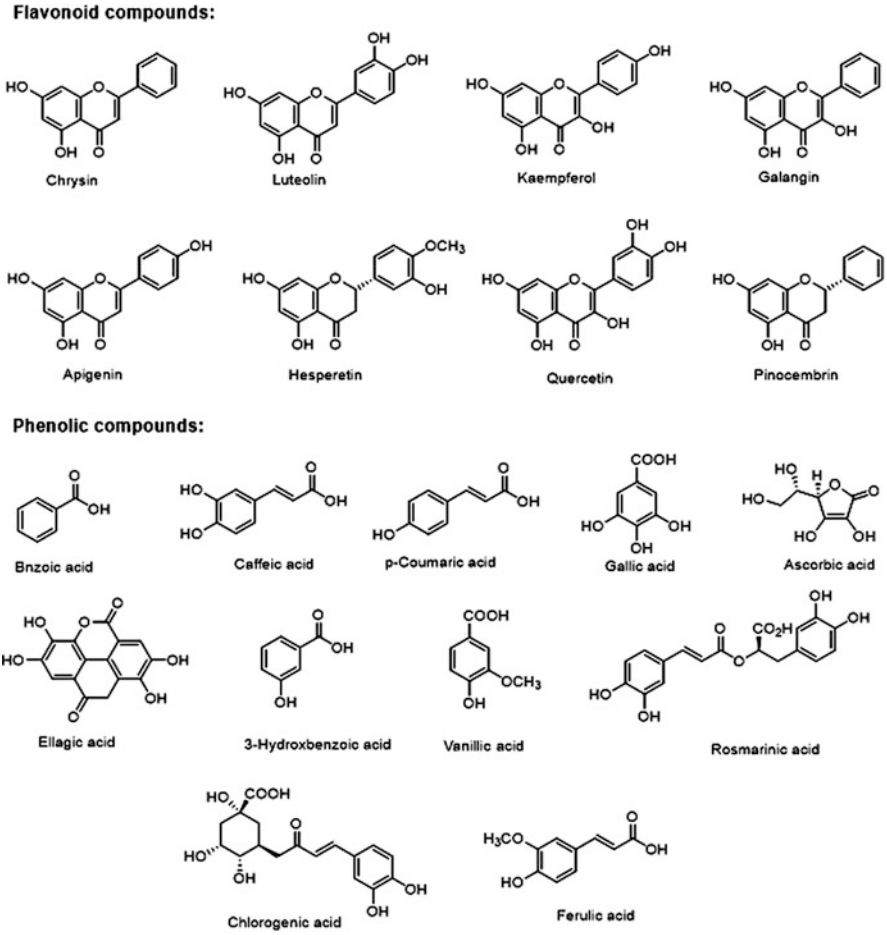

Fig. 3.2 Chemical structures of pharmacologically active components present in honey with neuroprotective activities

with pharmacological potential and neuroprotective activities are shown in (Fig. 3.2) as follows.

3.3 Honey and Oxidative Stress

Honey is a natural anti-oxidant due to the presence of flavonoids, phenolic acids, enzymes (e.g., glucose oxidase, catalase, ascorbic acid, carotenoid-like substances, and organic acids. It is assumed that the primary anti-oxidant potential of honey is due to the presence of phenolic compounds (Kenjerić et al. 2007). These polyphenols have demonstrated their potential by neutralizing free radicals, which is achieved by donating an electron or hydrogen atom (Rice-Evans et al. 1996). The cellular architecture of central nervous system is vulnerable to oxidative stress because of its high oxygen consumption and relative insufficiency of reactive oxygen species (ROS) scavenging, and this oxidative stress has been shown to play an important role in age-associated neurodegenerative diseases such as Alzheimer's disease (Floyd 1991; Zhu et al. 2014). Honey has been shown to attenuate the oxidative stress in different brain regions and improve cognitive deficits after kainic acid– and paraquat-induced toxicity in rats (Sairazi et al. 2017; Tang et al. 2017). It has been further demonstrated to reduce lead-induced oxidative stress and prevent neurobehavioral deficits in Wistar rats. (Abdulmajeed et al. 2016). Manuka honey has been shown to protect HDFa fibroblasts against apoptosis, intracellular ROS production, and lipid peroxidation. It has been further demonstrated to protect mitochondrial functionality, promoted cell proliferation, and activated the AMPK/Nrf2/ARE signaling pathway, as well as the expression of the anti-oxidant enzymes such as SOD and CAT (Alvarez-Suarez et al. 2016). Oxidative stress and inflammation are fundamentally connected in pathophysiological events where redox imbalance occurs due to the disruption of redox homeostasis (Shao et al. 2012). Honey polyphenols have been demonstrated to prevent chronic diseases with associated oxidative stress and inflammation. It has been shown to reduce ROS and stress markers and restore anti-oxidant enzymes. Moreover, honey polyphenols have been shown to ameliorate mitochondrial functions and modulate inflammatory processes. These beneficial properties are mediated in part through their ability to target numerous signaling pathways, such as p38 MAPK, AMPK, PI3K/Akt, NF-κB, and Nrf2 (Battino et al. 2020). Tualang honey treatments in ovariectomized (OVX) rats exposed to social instability stress have shown to improve both short-term and long-term memories and enhanced the neuronal proliferation of hippocampal CA2, CA3, and DG regions compared to untreated stressed OVX rats (Al-Rahbi et al. 2014b). Taulang honey in combination with DHA-rich fish oil has been demonstrated to reduce pro-inflammatory cytokine levels (TNF-a, IL6, and IFN-g) in the brain homogenates of rats under chronic stress conditions compared to untreated groups; however, there was no significant difference when these agents were consumed separately (Asari et al. 2019). Taulang honey treatment in loud-noise-induced stressed rats has shown significant decrease in stress hormone levels and brain oxidation indices. Further, there was an increase in memory, anti-

oxidant enzymes activities, and neuronal density in medial prefrontal cortex (mPFC) and hippocampus in comparison to vehicle-treated stressed rats. These restoration in functionalities were more prominent in younger rats compared to aged ones (Azman et al. 2018).

3.4 Honey and Neurotoxicity

Honey has been shown to protect neuronal cells and ameliorate neuronal deficits in different neurotoxicity models. Excitotoxicity is a neurotoxic process where post-synaptic glutamate receptors are overactivated in various neurological ailments leading to oxidative stress (Dar et al. 2017). Polyphenolic compounds and flavonoids present in honey have demonstrated diverse pharmacological potential. Apigenin (a common flavonoid present in honey) has been shown to protect hippocampal neurons in a concentration-dependent fashion by attenuating reactive oxygen species (ROS) and inhibiting the depleted levels of reduced glutathione (GSH) in kainic acid-induced excitotoxicity (Han et al. 2012). It has been further shown to improve and rescue primary hippocampal neurons by modulating sodium/potassium-ATPase (Na+/K+-ATPase) activities in oxygen-glucose deprivation/reperfusion-induced injury models (Liu et al. 2010). Pre-treatment with honey has been shown to prevent cell death and protect astrocytes against H_2O_2-induced stress compared to the H_2O_2 only group (Ali and Kunugi 2019). Another study using rat models has reported that pre-treatment with taulang honey significantly reduced neuronal degeneration in the piriform cortex but failed to prevent the kainic acid-induced seizures. It has been further shown to attenuate locomotor activity and hyperactivity in kainic acid-induced excitotoxicity (Sairazi et al. 2017). Animals fed with taulang honey for 2 weeks and then exposed to paraquat (dopaminergic neurotoxin) by intraperitoneal injections have shown to ameliorate glutathione peroxidase activity and a number of tyrosine-hydroxylase immune-positive neurons in the midbrain region compared to paraquat-only treated group (Tang et al. 2017). Studies have reported that aluminum chloride ($AlCl_3$), which is a neurotoxic agent when administered alone, has shown to reduce the anti-oxidant levels and increase lipid peroxidation in mice. While as honey syrup administered at 500 mg/kg b.w. for 45 days has resulted in the amelioration of anti-oxidant enzyme levels (SOD, CAT, GSH) and lipid peroxidation levels (Shati et al. 2011), honey has been shown to protect the brain against lead-induced toxicity by enhancing anti-oxidant activities as demonstrated by increasing superoxide dismutase (SOD), glutathione-*S*-transferase (GST), and glutathione peroxidase (GPx) activities. Moreover it has been shown to reduce the lipid peroxidation levels in the brain after lead exposure (Abdulmajeed et al. 2016). Studies have suggested that microglial-associated neuroinflammation could be a therapeutic target in neurodegenerative disorders. Honey extract has been demonstrated to decrease the secretion of pro-inflammatory mediators such as interleukin (IL-1β) and tumor necrotic factor (TNF-α) in N13 microglia after lipopolysaccharide induction. It has also been shown to reduce inducible nitric oxide synthase expressions and reactive oxygen species levels (Candiracci et al. 2012). Chrysin (flavonoid present in honey)

has been shown to attenuate the neuroinflammatory processes by modulating COX-2 and protected rats against formalin-induced neuropathic pain by regulating corticosterone and noradrenaline levels in serum (Farkhondeh et al. 2015; Rauf et al. 2015). It has been further shown to protect rats against 6-hydroxydopamine (6-OHDA)-induced neurotoxicity by modulating anti-oxidant capacity in the striatum, tyrosine hydroxylase, BDNF, dopamine, and glial cell line-derived neurotrophic factor levels. Moreover, it has reduced inflammatory markers like TNF-α, IL-1β, IL-2, IL-6, and NF-KB and increased IL-10 levels (Goes et al. 2018). In addition, chrysin has demonstrated anti-convulsant potential in pentylenetetrazole (PTZ)-induced convulsions in rats (Sharma et al. 2019).

3.5 Honey and Neurodegeneration

Honey is a nootropic agent which helps in the growth and development of the central nervous system predominantly in newborns, improving memory and growth. It has been shown to enhance the overall rational performance at later stages of life (Cantarelli et al. 2008). Studies have demonstrated that taulang honey protected neurons and could improve spatial learning and memory in chronic cerebral hypoperfusion-induced neurodegeneration (Saxena et al. 2016, 2014). Honey has been shown to reduce the number of degenerating neurons in CA1 hippocampal region that are highly susceptible to oxidative stress (Cai et al. 2011). In addition, its consumption could prevent hippocampal morphological impairment in adult male rats and improve hippocampal morphological impairment in ovariectomized rats (Kamarulzaidi et al. 2016; Al-Rahbi et al. 2014b). Due to its anti-oxidant potential and ability to modulate the cholinergic system, honey consumption has been shown to improve cognitive functions in middle-aged, adult, and old-aged rats (Azman et al. 2016). Further, a 5-year pilot study in human subjects with mild cognitive impairment aged 65 and older have shown improvements in cognitive behavior and decline in dementia development after daily consumption of one teaspoon of honey (Al-Himyari 2009). Animal studies have concluded that honey is a functional food that improves spatial working memory and possesses anxiolytic, anti-nociceptive, anti-convulsant, and anti-depressant effects (Akanmu et al. 2011). Recent studies have reported that honey could be a potential source of cholinesterase inhibitors and may play a vital role in inhibiting pathogenesis of degenerative disorders like Alzheimer's disease. Cholinergic neurotransmission is found to be deregulated in Alzheimer's disease due to depletion of acetylcholine via acetylcholinesterase activity (AChE), therefore inhibiting acetylcholinesterase activity and increasing acetylcholine levels in neurons is hypothesized to inhibit the progression of this disease (Baranowska-Wójcik et al. 2020; Tundis et al. 2016). A recent study has evaluated the anti-AChE and anti-BChE activity of 47 Polish honeys and found that the highest potential for AChE inhibition was in the case of buckwheat honey (39.51% inhibition) while multi-floral honey showed the highest capacity for BChE inhibition (39.76%) (Baranowska-Wójcik et al. 2020). This report is consistent with previous studies where Acacia honey consumption for 1 week has been shown to

significantly decrease AChE activity in the cerebrum and cerebellum of male Wistar albino rats (Warad et al. 2014). Luteolin (a flavonoid found in honey) has been demonstrated to protect neurons against microglia-associated inflammation and improve spatial working memory in aged rats. Further, it has ameliorated basal synaptic transmission via modulation of long-term potentiation (LTP) and activated cAMP response element-binding protein (CREB), thereby restoring synaptic function and memory in neurodegenerative disorders (Jang et al. 2010; Xu et al. 2010). Kaempferol, a natural flavonol present in honey, has been shown to prevent 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP)-induced parkinsonism by preventing the loss of TH-positive neurons (Li and Pu 2011). Taulang honey has been shown to attenuate the hypoxia-induced neuronal damage and improved short-term, spatial, and long-term memories compared to normoxic and hypoxic groups (Qaid et al. 2020). Ferulic acid (polyphenol in honey) has demonstrated neuroprotective effects by regulating phospho-PDK1, pAkt, and p-Bad levels in the middle cerebral artery occlusion (MCAO) injury model. It has been further shown to attenuate the escalated levels of caspase-3 as well. Moreover, ferulic acid has reduced the oxidative stress levels and inhibited ICAM-1 mRNA expression, thereby decreasing the number of microglia/macrophages and apoptosis in cerebral ischemia/reperfusion injury in rats (Koh 2012; Cheng et al. 2008). Cholinergic acid from honey has been shown to protect neuronal cells against methyl mercury-induced apoptosis by attenuating reactive oxygen species (ROS), modulating the glutathione peroxidase (GPx) levels and inhibiting the activation of caspase-3. It has also been demonstrated to protect neurons against scopolamine-induced toxicity by ameliorating the learning and memory impairments and impeding acetylcholine esterase activity. It has further decreased malonaldehyde (MDA) levels in frontal cortex and hippocampal regions in the mouse brain (Li et al. 2008; Kwon et al. 2010). Honey has shown a myriad of pharmacological activities against several other ailments and needs to be evaluated thoroughly with a specific set of active constituents to develop a therapeutic strategy targeting specific pathways in disease progression.

3.6 Novel Phenolic and Flavonoid Compounds with Neuroprotective Potential

Among the active constituents present in honey, it has been reported that polyphenols and flavonoid are the most potent anti-oxidants (Khalil et al. 2011; Kishore et al. 2011). These potent molecules have demonstrated significant pharmacological actions against several disease models when given either singly or in combination. Ferulic acid (FA) (a phenolic compound present in honey) has shown to have scavenging potential toward free radicals like superoxide radicals, peroxynitrite, hydroxyl radical, and oxidized low-density lipoprotein. It has been demonstrated to have the anti-oxidant potential due to its ability to form a resonance stabilized phenoxy radical. It has been further shown to attenuate lipid peroxidation levels and counter alkoxyl and peroxyl radicals in synaptosomal and neuronal cell

populations (Trombino et al. 2004; Kanski et al. 2002; Kikuzaki et al. 2002). In vitro studies have shown that ferulic acid pre-treatment protected neurons against hydroxyl- and peroxyl-induced oxidative damage and further attenuated protein carbonyls, 4-hydroxynonenal (HNE) protein adduct, and 3-nitrotyrosine (3-NT) levels in gerbils (Kanski et al. 2002). Moreover, it has attenuated 4-hydroxynonenal (HNE) levels and protected neurons against noise-induced oxidative stress and hearing loss in guinea pigs (Fetoni et al. 2010). Ferulic acid has been shown to ameliorate cognitive impairments and reduce protein carbonyl levels in buthionine sulfoximine (BSO)-treated mice (Griffith 1982). Reports have demonstrated the sodium ferulate was able to protect neurons in cerebral ischemia/reperfusion injury in rats (Wang et al. 2003), and long-term administration of ferulic acid was able to improve learning and memory impairments against Aβ-induced toxicity in mice (Kim et al. 2004). Additionally, ferulic acid has reduced the standard length of Aβ fibrils in transgenic *C. elegans* expressing human Aβ in their body walls (Jagota and Rajadas 2012). Ferulic acid has been shown to modulate the levels of pro- and anti-apoptotic proteins like Bax, tBid, and Bcl2, thereby regulating mitochondrial apoptosis. Moreover, it has been shown to exert its neuroprotective effects via downregulation of the JNK pathway (Kim and Lee 2012). Quercetin (flavonoid present in honey) has shown a myriad of pharmacological activities and is regarded as one of the potent molecules to regulate different biological pathways. Quercetin has been demonstrated to protect neuronal cells SHSY5Y against 6-OHDA-induced toxicity and cell death; however, its effect was found to be time dependent and for longer exposure either no significant protection or detrimental effects were found (Ossola et al. 2008). It has been shown to improve GSH concentration in SHSY5Y cells and decrease oxidative damage to DNA, lipids, and proteins (Lee et al. 2016). Quercetin has been demonstrated to ameliorate the tau protein–associated neuropathology by inhibiting cyclin-dependent kinase 5 (CDK5) activity, which acts as a crucial enzyme in regulating tau protein and blocks the Ca2+-calpain-p25-CDK5 signaling pathway. It has further attenuated tau protein hyperphosphorylation at the Ser396, Ser199, Thr231, and Thr205 sites in okadaic acid-induced tau hyperphosphorylation in HT22 hippocampal cells (Shen et al. 2018). Quercetin has been shown to decrease β-amyloidosis in aged 3xTg-AD mice and has been revealed to increase AMPK activity in the APPswe/PS1dE9 transgenic mouse model of AD promoting Aβ intracellular clearance (Espargaró et al. 2017; Sabogal-Guáqueta et al. 2015). It has been shown to ameliorate the endoplasmic reticulum stress-induced apoptosis and rotenone-induced behavioral impairments (El-Horany et al. 2016). In addition, quercetin has been demonstrated to promote pro-survival signals in dopaminergic cells by activating polycystin 1, transient receptor potential channel interacting (PKD1). Moreover, it induces Akt and CREB phosphorylation and modulates BDNF expression, thereby affording neuroprotective effects. It has an ability to bind with α-syn and the resulting quercetin-α-syn adducts have revealed to attach to the α-syn oligomers or monomers, increasing the surface hydrophilicity, and inhibiting further fibrillation. It has been shown to modulate the impaired respiratory chain function in dopaminergic neurons and afforded protection against dopaminergic neurodegeneration in the MitoPark transgenic mouse model of

Parkinson's disease (Ay et al. 2017; Zhu et al. 2013). Together with resveratrol, it has been shown to attenuate the decrease of dopaminergic neurons induced by a dopaminergic neurotoxin, 1-methyl-4-phenyl pyridinium (MPTP) (Okawara et al. 2007). Apigenin (a phenolic compound in honey) has been demonstrated to reduce the activity of caspase-3, up-regulated Bcl-2, and down-regulated Bax levels. It has been further shown to inhibit p38 MAPK (mitogen-activated protein kinase) signaling during ischemic/reperfusion injury, thereby decreasing myocardial infarction and conferred cardioprotective effects (Hu et al. 2015). It has been shown to recue neuronal cells against oxidative stress and reduce Aβ burden via down-regulating BACE1 and β-CTF levels (Zhao et al. 2013a). Additionally, apigenin has been shown to attenuate the mitochondrial machinery by impeding ROS-induced activation of p38-MAPK and stress-activated protein kinase (SAPK)/c-JNK pathways (Zhao et al. 2013b). Apigenin has been further demonstrated to inhibit β-secretase activity, which is crucial in Alzheimer's disease progression in enzyme as well as cell-based assays (Ebrahimi and Schluesener 2012; Shimmyo et al. 2008). Apigenin in combination with other anti-oxidants has been demonstrated to rescue the tyrosine hydroxylase (TH) and neurotrophic factor levels in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced toxicity (Patil et al. 2014). Apigenin has been revealed to stimulate neurogenesis and synaptogenesis in human pluripotent stem cells by regulating estrogen receptors and correspondingly via modulation of retinoic acid receptors a and b and retinoic X receptor c levels (Souza et al. 2015). Gallic acid (a phenolic compound present in honey) has been demonstrated to inhibit Aβ oligomerization and free radicals attenuating brain amyloid neuropathology, neural damage, and ameliorate cognitive functions (Hajipour et al. 2016). It has been shown to attenuate learning and memory deficits and enhance cognitive functions in aged rats following intra-cerebroventricular (ICV) infusion of Aβ (McDaid et al. 2005). Gallic acid has been demonstrated to have protective effects against NMDA-induced excitotoxicity, thereby protecting neurons against toxicity insults (Korani et al. 2014). Pinocembrin (a flavonoid present in honey) has been shown to rescue neuronal cells against H_2O_2-induced oxidative stress and neurotoxicity. Moreover, it has been demonstrated to protect neuronal cells against Aβ, 6-OHDA, and MPTP-induced toxicity, thereby ameliorating the neuronal functionalities (de Oliveira et al. 2018; Jin et al. 2015; Wang et al. 2016a, b). Pinocembrin has been demonstrated to regulate the Nrf2-HO-1 axis and further inhibit NF-κB, iNOS, COX-2, and prostaglandin-E2 in LPS-stimulated BV-2 microglial cells (Wang et al. 2016b; Zhou et al. 2015). In addition, pinocembrin has been shown to decrease protein nitration, carbonylation, and lipid peroxidation in mitochondrial membranes in methylglyoxal-treated SHSY5Y cells, thereby ameliorating mitochondrial dysfunctions (de Oliveira et al. 2017a). Moreover, it has been demonstrated to reduce pro-apoptotic Bax, and inhibit cytochrome-C release and caspase-3-9 activation in paraquat-treated SHSY5Y cells (de Oliveira et al. 2017b). Pinocembrin has been shown to improve respiratory activity by increasing ADP/O, state 3 respiration state, oxidative phosphorylation rate, and respiration control rate index in global brain ischemia/reperfusion (four-vessel occlusion I/R) rat model. Moreover, it has shown to increase mitochondrial ATP content in SHSY5Y cells (Shi et al. 2011).

Based on promising neuroprotective activities of pinocembrin, it has been approved by China Food and Drug Administration (CFDA) as a new treatment drug for ischemic stroke and is currently in phase II clinical trials (Shen et al. 2019).

3.7 Conclusion

The brain is extremely vulnerable to oxidative damage due to its high oxygen demand, low anti-oxidant system, and remarkable vascularization with polyunsaturated fatty acids. Oxidative stress is the underlying cause for most of the neurological insults and neurodegenerative diseases; therefore strategies directed towards regulating the anti-oxidant systems are always encouraged for improving the brain health. Honey, due to its rich blend of phenolic compounds and flavonoids, has been demonstrated to be a potent stress-relieving agent in numerous experimental models. Due to its ample and pharmacologically useful composition, each component present in the formulation has been shown to protect neurons against several neurotoxic conditions and reduced the toxicity-induced oxidative stress via a multitude of biological pathways, thereby impeding the progression of disease. However, further studies are warranted to evaluate the promising phenolic and flavonoid compounds targeting specific pathways in neurodegeneration.

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References

Abdulmajeed WI, Sulieman HB, Zubayr MO, Imam A, Amin A, Biliaminu SA, Oyewole LA, Owoyele BV (2016) Honey prevents neurobehavioural deficit and oxidative stress induced by lead acetate exposure in male wistar rats-a preliminary study. Metab Brain Dis 31(1):37–44

Afroz R, Tanvir E, Hossain M, Gan SH, Parvez M, Islam A, Khalil M (2014) Protective effect of Sundarban honey against acetaminophen-induced acute hepatonephrotoxicity in rats. Evid-Based Complement Alternative Med 2014:143782

Akanmu MA, Olowookere TA, Atunwa SA, Ibrahim BO, Lamidi OF, Adams PA, Ajimuda BO, Adeyemo LE (2011) Neuropharmacological effects of Nigerian honey in mice. Afr J Tradit Complement Altern Med 8(3):230–249

Al-Himyari FA (2009) The use of honey as a natural preventive therapy of cognitive decline and dementia in the middle east. Alzheimers Dement 5(4):P247

Ali AM, Kunugi H (2019) Bee honey protects astrocytes against oxidative stress: a preliminary in vitro investigation. Neuropsychopharmacol Rep 39(4):312–314

Al-Rahbi B, Zakaria R, Othman Z, Hassan A, Ahmad AH (2014a) Protective effects of Tualang honey against oxidative stress and anxiety-like behaviour in stressed ovariectomized rats. Int Sch Res Notices 2014:521065

Al-Rahbi B, Zakaria R, Othman Z, Hassan A, Ismail ZIM, Muthuraju S (2014b) Tualang honey supplement improves memory performance and hippocampal morphology in stressed ovariectomized rats. Acta Histochem 116(1):79–88

Alvarez-Suarez JM, Giampieri F, Cordero M, Gasparrini M, Forbes-Hernández TY, Mazzoni L, Afrin S, Beltrán-Ayala P, González-Paramás AM, Santos-Buelga C (2016) Activation of AMPK/Nrf2 signalling by Manuka honey protects human dermal fibroblasts against oxidative damage by improving antioxidant response and mitochondrial function promoting wound healing. J Funct Foods 25:38–49

Alvarez-Suarez JM, Tulipani S, Díaz D, Estevez Y, Romandini S, Giampieri F, Damiani E, Astolfi P, Bompadre S, Battino M (2010) Antioxidant and antimicrobial capacity of several monofloral Cuban honeys and their correlation with color, polyphenol content and other chemical compounds. Food Chem Toxicol 48(8–9):2490–2499

Al-Waili NS (2003) Effects of daily consumption of honey solution on hematological indices and blood levels of minerals and enzymes in normal individuals. J Med Food 6(2):135–140

Asari MA, Zulkaflee MH, Sirajudeen K, Yusof NAM, Sairazi NSM (2019) Tualang honey and DHA-rich fish oil reduce the production of pro-inflammatory cytokines in the rat brain following exposure to chronic stress. J Taibah Univ Med Sci 14(4):317–323

Ay M, Luo J, Langley M, Jin H, Anantharam V, Kanthasamy A, Kanthasamy AG (2017) Molecular mechanisms underlying protective effects of quercetin against mitochondrial dysfunction and progressive dopaminergic neurodegeneration in cell culture and MitoPark transgenic mouse models of Parkinson's disease. J Neurochem 141(5):766–782

Azman KF, Zakaria R, Abdul Aziz CB, Othman Z (2016) Tualang honey attenuates noise stress-induced memory deficits in aged rats. Oxid Med Cell Longev 2016:1549158

Azman KF, Zakaria R, Othman Z, Abdul Aziz CB (2018) Neuroprotective effects of Tualang honey against oxidative stress and memory decline in young and aged rats exposed to noise stress. J Taibah Univ Sci 12(3):273–284

Baranowska-Wójcik E, Szwajgier D, Winiarska-Mieczan A (2020) Honey as the potential natural source of cholinesterase inhibitors in Alzheimer's disease. Plant Foods Hum Nutr 75:30–32

Battino M, Giampieri F, Cianciosi D, Ansary J, Chen X, Zhang D, Gil E, Forbes-Hernández T (2020) The roles of strawberry and honey phytochemicals on human health: a possible clue on the molecular mechanisms involved in the prevention of oxidative stress and inflammation. Phytomedicine 11:153170

Cai M, Shin BY, Kim DH, Kim JM, Park SJ, Park CS, Hong ND, Kang DH, Yutaka Y, Ryu JH (2011) Neuroprotective effects of a traditional herbal prescription on transient cerebral global ischemia in gerbils. J Ethnopharmacol 138(3):723–730

Candiracci M, Piatti E, Dominguez-Barragán M, García-Antrás D, Morgado B, Ruano D, Gutiérrez JF, Parrado J, Al C (2012) Anti-inflammatory activity of a honey flavonoid extract on lipopolysaccharide-activated N13 microglial cells. J Agric Food Chem 60(50):12304–12311

Cantarelli MA, Pellerano RG, Marchevsky EJ, Camiña JM (2008) Quality of honey from Argentina: study of chemical composition and trace elements. J Argent Chem Soc 96:1–2

Cheng C-Y, Su S-Y, Tang N-Y, Ho T-Y, Chiang S-Y, Hsieh C-L (2008) Ferulic acid provides neuroprotection against oxidative stress-related apoptosis after cerebral ischemia/reperfusion injury by inhibiting ICAM-1 mRNA expression in rats. Brain Res 1209:136–150

Chepulis LM, Starkey NJ, Waas JR, Molan PC (2009) The effects of long-term honey, sucrose or sugar-free diets on memory and anxiety in rats. Physiol Behav 97(3–4):359–368

Dar NJ, Bhat JA, Satti NK, Sharma PR, Hamid A, Ahmad M (2017) Withanone, an active constituent from Withania somnifera, affords protection against NMDA-induced excitotoxicity in neuron-like cells. Mol Neurobiol 54(7):5061–5073

de Oliveira MR, da Costa FG, Brasil FB, Peres A (2018) Pinocembrin suppresses H_2O_2-induced mitochondrial dysfunction by a mechanism dependent on the Nrf2/HO-1 axis in SH-SY5Y cells. Mol Neurobiol 55(2):989–1003

de Oliveira MR, Peres A, Ferreira GC (2017a) Pinocembrin attenuates mitochondrial dysfunction in human neuroblastoma SH-SY5Y cells exposed to methylglyoxal: role for the Erk1/2–Nrf2 signaling pathway. Neurochem Res 42(4):1057–1072

de Oliveira MR, Peres A, Gama CS, Dal Bosco SM (2017b) Pinocembrin provides mitochondrial protection by the activation of the Erk1/2-Nrf2 signaling pathway in SH-SY5Y neuroblastoma cells exposed to paraquat. Mol Neurobiol 54(8):6018–6031

Ebrahimi A, Schluesener H (2012) Natural polyphenols against neurodegenerative disorders: potentials and pitfalls. Ageing Res Rev 11(2):329–345

El-Horany HE, El-latif RNA, ElBatsh MM, Emam MN (2016) Ameliorative effect of quercetin on neurochemical and behavioral deficits in rotenone rat model of Parkinson's disease: modulating autophagy (quercetin on experimental Parkinson's disease). J Biochem Mol Toxicol 30 (7):360–369

Elmenoufy GA (2012) Bee honey dose-dependently ameliorates lead acetate-mediated hepatorenal toxicity in rats. Life Sci J 9(4):780–788

Erejuwa OO, Sulaiman SA, Ab Wahab MS (2012) Honey: a novel antioxidant. Molecules 17 (4):4400–4423

Erejuwa OO, Sulaiman SA, Wahab MSA, Sirajudeen KN, Salleh MSM, Gurtu S (2011) Differential responses to blood pressure and oxidative stress in streptozotocin-induced diabetic Wistar-Kyoto rats and spontaneously hypertensive rats: effects of antioxidant (honey) treatment. Int J Mol Sci 12(3):1888–1907

Espargaró A, Ginex T, Vadell MM, Busquets MA, Estelrich J, Muñoz-Torrero D, Luque FJ, Sabate R (2017) Combined in vitro cell-based/in silico screening of naturally occurring flavonoids and phenolic compounds as potential anti-Alzheimer drugs. J Nat Prod 80(2):278–289

Farkhondeh T, Samarghandian S, Azimin-Nezhad M, Samini F (2015) Effect of chrysin on nociception in formalin test and serum levels of noradrenalin and corticosterone in rats. Int J Clin Exp Med 8(2):2465

Fetoni AR, Mancuso C, Eramo SLM, Ralli M, Piacentini R, Barone E, Paludetti G, Troiani D (2010) In vivo protective effect of ferulic acid against noise-induced hearing loss in the Guinea-pig. Neuroscience 169(4):1575–1588

Floyd RA (1991) Oxidative damage to behavior during aging. Science 254(5038):1597–1598

Goes AT, Jesse CR, Antunes MS, Ladd FVL, Ladd AAL, Luchese C, Paroul N, Boeira SP (2018) Protective role of chrysin on 6-hydroxydopamine-induced neurodegeneration a mouse model of Parkinson's disease: involvement of neuroinflammation and neurotrophins. Chem Biol Interact 279:111–120

Griffith O (1982) Mechanism of action, metabolism, and toxicity of buthionine sulfoximine and its higher homologs, potent inhibitors of glutathione synthesis. J Biol Chem 257(22):13704–13712

Hajipour S, Sarkaki A, Farbood Y, Eidi A, Mortazavi P, Valizadeh Z (2016) Effect of gallic acid on dementia type of Alzheimer disease in rats: electrophysiological and histological studies. Basic Clin Neurosci 7(2):97

Halliwell B (1992) Reactive oxygen species and the central nervous system. J Neurochem 59 (5):1609–1623

Han J-Y, Ahn S-Y, Kim C-S, Yoo S-K, Kim S-K, Kim H-C, Hong JT, Oh K-W (2012) Protection of apigenin against kainate-induced excitotoxicity by anti-oxidative effects. Biol Pharm Bull 35 (9):1440–1446

Hu J, Li Z, Xu L-t, A-j S, X-y F, Zhang L, L-l J, Lu A-d, Dong Y-f, Z-p J (2015) Protective effect of apigenin on ischemia/reperfusion injury of the isolated rat heart. Cardiovasc Toxicol 15 (3):241–249

Jagota S, Rajadas J (2012) Effect of phenolic compounds against Aβ aggregation and Aβ-induced toxicity in transgenic C. elegans. Neurochem Res 37(1):40–48

Jang S, Dilger RN, Johnson RW (2010) Luteolin inhibits microglia and alters hippocampal-dependent spatial working memory in aged mice. J Nutr 140(10):1892–1898

Jin X, Liu Q, Jia L, Li M, Wang X (2015) Pinocembrin attenuates 6-OHDA-induced neuronal cell death through Nrf2/ARE pathway in SH-SY5Y cells. Cell Mol Neurobiol 35(3):323–333

Kamarulzaidi MA, Yusoff M, Mohamed AM, Adli D (2016) Tualang honey consumption enhanced hippocampal pyramidal count and spatial memory performance of adult male rats. Sains Malaysiana 45(2):215–220

Kanski J, Aksenova M, Stoyanova A, Butterfield DA (2002) Ferulic acid antioxidant protection against hydroxyl and peroxyl radical oxidation in synaptosomal and neuronal cell culture systems in vitro: structure-activity studies. J Nutr Biochem 13(5):273–281

Kenjerić D, Mandić ML, Primorac L, Bubalo D, Perl A (2007) Flavonoid profile of Robinia honeys produced in Croatia. Food Chem 102(3):683–690

Khalil M, Alam N, Moniruzzaman M, Sulaiman S, Gan S (2011) Phenolic acid composition and antioxidant properties of Malaysian honeys. J Food Sci 76(6):C921–C928

Kikuzaki H, Hisamoto M, Hirose K, Akiyama K, Taniguchi H (2002) Antioxidant properties of ferulic acid and its related compounds. J Agric Food Chem 50(7):2161–2168

Kim H-S, Cho J-Y, Kim D-H, Yan J-J, Lee H-K, Suh H-W, Song D-K (2004) Inhibitory effects of long-term administration of ferulic acid on microglial activation induced by intracerebroventricular injection of β-amyloid peptide (1—42) in mice. Biol Pharm Bull 27 (1):120–121

Kim H-Y, Lee S-M (2012) Ferulic acid attenuates ischemia/reperfusion-induced hepatocyte apoptosis via inhibition of JNK activation. Eur J Pharm Sci 45(5):708–715

Kishore RK, Halim AS, Syazana MN, Sirajudeen K (2011) Tualang honey has higher phenolic content and greater radical scavenging activity compared with other honey sources. Nutr Res 31 (4):322–325

Koh P-O (2012) Ferulic acid prevents the cerebral ischemic injury-induced decrease of Akt and Bad phosphorylation. Neurosci Lett 507(2):156–160

Korani MS, Farbood Y, Sarkaki A, Moghaddam HF, Mansouri MT (2014) Protective effects of gallic acid against chronic cerebral hypoperfusion-induced cognitive deficit and brain oxidative damage in rats. Eur J Pharmacol 733:62–67

Kwon S-H, Lee H-K, Kim J-A, Hong S-I, Kim H-C, Jo T-H, Park Y-I, Lee C-K, Kim Y-B, Lee S-Y (2010) Neuroprotective effects of chlorogenic acid on scopolamine-induced amnesia via anti-acetylcholinesterase and anti-oxidative activities in mice. Eur J Pharmacol 649(1–3):210–217

Lee M, McGeer EG, McGeer PL (2016) Quercetin, not caffeine, is a major neuroprotective component in coffee. Neurobiol Aging 46:113–123

Li S, Pu X-P (2011) Neuroprotective effect of kaempferol against a 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine-induced mouse model of Parkinson's disease. Biol Pharm Bull 34 (8):1291–1296

Li Y, Shi W, Li Y, Zhou Y, Hu X, Song C, Ma H, Wang C, Li Y (2008) Neuroprotective effects of chlorogenic acid against apoptosis of PC12 cells induced by methylmercury. Environ Toxicol Pharmacol 26(1):13–21

Liu S-Q, Su F, Fang L-M, Xia Q, Zhang X (2010) Protective effect of apigenin on neurons against oxygen-glucose deprivation/reperfusion induced injury. FASEB J 24:604–615

McDaid D, Kim E-M, Reid R, Leslie J, Cleary J, O'Hare E (2005) Parenteral antioxidant treatment preserves temporal discrimination following intrahippocampal aggregated Aβ (1–42) injections. Behav Pharmacol 16(4):237–242

Okawara M, Katsuki H, Kurimoto E, Shibata H, Kume T, Akaike A (2007) Resveratrol protects dopaminergic neurons in midbrain slice culture from multiple insults. Biochem Pharmacol 73 (4):550–560

Ossola B, Kääriäinen TM, Raasmaja A, Männistö PT (2008) Time-dependent protective and harmful effects of quercetin on 6-OHDA-induced toxicity in neuronal SH-SY5Y cells. Toxicology 250(1):1–8

Patel M (2016) Targeting oxidative stress in central nervous system disorders. Trends Pharmacol Sci 37(9):768–778

Patil SP, Jain PD, Sancheti JS, Ghumatkar PJ, Tambe R, Sathaye S (2014) Neuroprotective and neurotrophic effects of Apigenin and Luteolin in MPTP induced parkinsonism in mice. Neuropharmacology 86:192–202

Qaid EY, Zakaria R, Yusof NAM, Sulaiman SF, Nazlahshaniza S, Othman Z, Ahmad AH, Aziz CBA, Sangu M (2020) Tualang honey ameliorates hypoxia-induced memory deficits by

reducing neuronal damage in hippocampus of adult male Sprague-Dawley rats. Turk J Pharm Sci 17(3)

Rauf A, Khan R, Raza M, Khan H, Pervez S, De Feo V, Maione F, Mascolo N (2015) Suppression of inflammatory response by chrysin, a flavone isolated from Potentilla evestita Th. Wolf. In silico predictive study on its mechanistic effect. Fitoterapia 103:129–135

Rice-Evans CA, Miller NJ, Paganga G (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med 20(7):933–956

Sabogal-Guáqueta AM, Munoz-Manco JI, Ramírez-Pineda JR, Lamprea-Rodriguez M, Osorio E, Cardona-Gómez GP (2015) The flavonoid quercetin ameliorates Alzheimer's disease pathology and protects cognitive and emotional function in aged triple transgenic Alzheimer's disease model mice. Neuropharmacology 93:134–145

Sairazi NSM, Sirajudeen K, Asari MA, Mummedy S, Muzaimi M, Sulaiman SA (2017) Effect of tualang honey against KA-induced oxidative stress and neurodegeneration in the cortex of rats. BMC Complement Altern Med 17(1):31

Saxena A, Phyu H, Al-Ani I, Oothuman P (2016) Improved spatial learning and memory performance following Tualang honey treatment during cerebral hypoperfusion-induced neurodegeneration. J Transl Sci 2(5):264–271

Saxena AK, Phyu HP, Al-Ani IM, Talib NA (2014) Potential protective effect of honey against chronic cerebral hypoperfusion-induced neurodegeneration in rats. J Anat Soc India 63 (2):151–155

Shao D, Oka S-i, Brady CD, Haendeler J, Eaton P, Sadoshima J (2012) Redox modification of cell signaling in the cardiovascular system. J Mol Cell Cardiol 52(3):550–558

Sharma P, Kumari A, Gulati A, Krishnamurthy S, Hemalatha S (2019) Chrysin isolated from Pyrus pashia fruit ameliorates convulsions in experimental animals. Nutr Neurosci 22(8):569–577

Shati A, Elsaid F, Hafez E (2011) Biochemical and molecular aspects of aluminium chloride-induced neurotoxicity in mice and the protective role of Crocus sativus L. extraction and honey syrup. Neuroscience 175:66–74

Shen X, Liu Y, Luo X, Yang Z (2019) Advances in biosynthesis, pharmacology, and pharmacokinetics of Pinocembrin, a promising natural small-molecule drug. Molecules 24(12):2323

Shen XY, Luo T, Li S, Ting OY, He F, Xu J, Wang HQ (2018) Quercetin inhibits okadaic acid-induced tau protein hyperphosphorylation through the Ca2+-calpain-p25-CDK5 pathway in HT22 cells. Int J Mol Med 41(2):1138–1146

Shi L, Qiang G, Gao M, Zhang H, Chen B, Yu X, Xuan Z, Wang Q, Du G (2011) Effect of pinocembrin on brain mitochondrial respiratory function. Yao Xue Xue Bao 46(6):642–649

Shimmyo Y, Kihara T, Akaike A, Niidome T, Sugimoto H (2008) Flavonols and flavones as BACE-1 inhibitors: structure–activity relationship in cell-free, cell-based and in silico studies reveal novel pharmacophore features. Biochim Biophys Acta 1780(5):819–825

Souza CS, Paulsen BS, Devalle S, Lima Costa S, Borges HL, Rehen SK (2015) Commitment of human pluripotent stem cells to a neural lineage is induced by the pro-estrogenic flavonoid apigenin. Adv Regen Biol 2(1):29244

Tang SP, Kuttulebbai Nainamohamed Salam S, Jaafar H, Gan SH, Muzaimi M, Sulaiman SA (2017) Tualang honey protects the rat midbrain and lung against repeated paraquat exposure. Oxid Med Cell Longev 2017:4605782

Trombino S, Serini S, Di Nicuolo F, Celleno L, Andò S, Picci N, Calviello G, Palozza P (2004) Antioxidant effect of ferulic acid in isolated membranes and intact cells: synergistic interactions with α-tocopherol, β-carotene, and ascorbic acid. J Agric Food Chem 52(8):2411–2420

Tundis R, Bonesi M, Menichini F, Loizzo MR (2016) Recent knowledge on medicinal plants as source of cholinesterase inhibitors for the treatment of dementia. Mini Rev Med Chem 16 (8):605–618

Wang H, Wang Y, Zhao L, Cui Q, Wang Y, Du G (2016a) Pinocembrin attenuates MPP+-induced neurotoxicity by the induction of heme oxygenase-1 through ERK1/2 pathway. Neurosci Lett 612:104–109

Wang Q, Xiong L, Chen S (2003) Effect of sodium ferulate on activation of extracellular signal regulated kinase after cerebral ischemia/reperfusion injury in rats. Zhongguo Zhong Xi Yi Jie He Za Zhi 23(12):918–921

Wang Y, Miao Y, Mir AZ, Cheng L, Wang L, Zhao L, Cui Q, Zhao W, Wang H (2016b) Inhibition of beta-amyloid-induced neurotoxicity by pinocembrin through Nrf2/HO-1 pathway in SH-SY5Y cells. J Neurol Sci 368:223–230

Warad VB, Shastri R, Habbu P, Katti P, Jagannath AB, Kulkarni VH, Chakraborty M (2014) Preparation and screening of Swarnaprashana for nootropic activity. Int J Nutr Pharmacol Neurol Dis 4(3):170

White J Jr (1975) In: Crane E (ed) Composition of honey—a comprehensive survey. Heinemann, London

Xu B, Li X-X, He G-R, Hu J-J, Mu X, Tian S, Du G-H (2010) Luteolin promotes long-term potentiation and improves cognitive functions in chronic cerebral hypoperfused rats. Eur J Pharmacol 627(1–3):99–105

Zaid SSM, Othman S, Kassim NM (2014) Potential protective effect of Tualang honey on BPA-induced ovarian toxicity in prepubertal rat. BMC Complement Altern Med 14(1):509

Zhao L, Wang J-L, Liu R, Li X-X, Li J-F, Zhang L (2013a) Neuroprotective, anti-amyloidogenic and neurotrophic effects of apigenin in an Alzheimer's disease mouse model. Molecules 18 (8):9949–9965

Zhao L, Wang J-l, Wang Y-r, X-z F (2013b) Apigenin attenuates copper-mediated β-amyloid neurotoxicity through antioxidation, mitochondrion protection and MAPK signal inactivation in an AD cell model. Brain Res 1492:33–45

Zhou L-t, Wang K-j, Li L, Li H, Geng M (2015) Pinocembrin inhibits lipopolysaccharide-induced inflammatory mediators production in BV2 microglial cells through suppression of PI3K/Akt/NF-κB pathway. Eur J Pharmacol 761:211–216

Zhu J, Mu X, Zeng J, Xu C, Liu J, Zhang M, Li C, Chen J, Li T, Wang Y (2014) Ginsenoside Rg1 prevents cognitive impairment and hippocampus senescence in a rat model of D-galactose-induced aging. PLoS One 9(6):e101291

Zhu M, Han S, Fink AL (2013) Oxidized quercetin inhibits α-synuclein fibrillization. Biochim Biophys Acta 1830(4):2872–2881

Molecular Mechanisms of Phytochemicals from Honey in Prevention and Treatment of Cancer

4

Rabia Farooq, Aamir Hanif, Andleeb Khan, Azher Arafah, Muneeb U. Rehman, and Sabhiya Majid

Abstract

Honey—an important natural compound and a renowned native food product—is exploited since immemorial times for its medical properties. Its medicinal properties have fascinated the humans so much as it has earned a dominating spot in conventional medicine. Currently more research is going on in exploring the natural compounds present in the honey for promoting human health. Honey contains more than 200 bioactive components besides glucose, fructose, minerals, vitamins, amino acids and enzymes (Da Silva et al., Food Chem 196:309–323, 2016). The composition of honey depends on the type of plants on which honeybee (*Apis mellifera*) nourishes. Different kinds of phytochemicals present in the honey with high phenolic content, polyphenols and flavonoids mainly contribute to its high medicinal properties including its antioxidant and anticancer action (Iurlina et al., Food Chem 115:1141–1149, 2009; Pyrzynska and Biesaga, TrAC Trends Anal Chem 28:893–902, 2009; Yao et al., Food Chem

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81:159–168, 2003). The polyphenolic compounds present in the honey have been found to possesses anti-proliferative effects against various types of cancers (Jaganathan and Mahitosh, J Biomed Biotechnol 2009:830616, 2009). These studies summarized the significant role of the bioactive species present in honey for different diseases. In this review, we critically examine the anticancer properties and molecular mechanisms of different bioactive components present in honey.

Keywords

Propolis · Carcinogensis · Angiogenic · Anti-inflammatory

4.1 Introduction

Honey—an important natural compound and a renowned native food product—is exploited since immemorial times for its medical properties. Its medicinal properties have fascinated the humans so much as it has earned a dominating spot in conventional medicine. Currently more research is going on in exploring the natural compounds present in the honey for promoting human health. Honey contains more than 200 bioactive components besides glucose, fructose, minerals, vitamins, amino acids and enzymes (Da Silva et al. 2016). The composition of honey depends on the type of plants on which honeybee (*Apis mellifera*) nourishes. Different kinds of phytochemicals present in the honey with high phenolic content, polyphenols and flavonoids mainly contribute to its high medicinal properties including its antioxidant and anticancer action (Iurlina et al. 2009; Pyrzynska and Biesaga 2009; Yao et al. 2003). The polyphenolic compounds present in the honey have been found to possesses anti-proliferative effects against various types of cancers (Jaganathan and Mahitosh 2009). These studies summarized the significant role of the bioactive species present in honey for different diseases. In this review, we critically examine the anticancer properties and molecular mechanisms of different bioactive components present in honey.

4.2 Honey and Its Composition

Honey is an absolute food containing more fructose than glucose and other disaccharides like maltose ~ (glucose + glucose), sucrose ~ (glucose + fructose), isomaltose, nigerose, turanose and maltulose. Nigerose is a rare sugar with potential immune-boosting and probiotic effects. Turanose is as an analogue of sucrose. So, all these carbohydrates (76%) are responsible for sweet taste of honey as well for its high caloric value (300 kcal/100 g). Besides these carbohydrate moieties, honey contains minerals and vitamins in trace amounts like tin, calcium, chromium, lithium, nickel, phosphorus, sodium and zinc. The pH of honey is around 3.9 and is so acidic, which was once considered to be due to formic acid present in bee venom, but later studies showed the type of acid is like that in apple or lemon fruits and its specific gravity is around 1.4.

The secondary metabolites including various phytochemicals present in the honey include phenolic acids (e.g., *p*-coumaric acid, ellagic acid, vanillic acid, syringic acid, gallic acid) and flavonoids (apigenin, pinocembrin, Luteolin, chrysin, galangin, Pinobanksin, hesperetin, kaempferol, luteolin, myricetin and quercetin) (Nicolson et al. 2007). Hydroxybenzoic and hydroxy cinnamic acids are phenolic compounds which possess antioxidant properties along with other phenolic groups (Balasundram et al. 2006). The antioxidant and free radical scavenging properties of non-flavonoid compounds and flavonoids in honey make it a key molecule in the treatment and prevention of various types of cancers (Jaganathan et al. 2015).

4.3 Bioavailability and Biotransformation of Phytochemicals from Honey

Polyphenolic compounds and phenolic compounds are the result of secondary metabolism of plants, which possess inevitable role in various functions in plants. These compounds possess antiapoptotic and antioxidant properties, so the bioavailability of these compounds is important to understand. Many aspects play role in the metabolism of these dietary compounds present in the honey like the type of flower on which nectar is taken, structure, environmental factors, combination with other compounds and gut microflora (D'Archivio et al. 2010). Regarding the metabolism of honey, one study demonstrated that when about 1.5 g/kg of buckwheat honey is taken, there occurs significant increase in phenolics in blood which remains high up to 6 h (Schramm et al. 2003). But limited studies are done on the bioavailability of secondary metabolites of honey (D'Archivio et al. 2010).

As honey is digested, the intestinal enzymes and intestinal bacteria cause β-hydrolysis of flavonoids present in the honey. As the absorption of phenolic compounds present in the honey in the upper GIT is very less (Clifford 2004), the enterocyte specific enzyme lactase phlorizin hydrolase (LPH) present in the enterocytes causes hydrolysis which leads to the formation of aglycon, which is more easily absorbed by epithelial cells. Hydrolysis of flavonoid by other enzyme cytosolic β-glucosidase (CBG) leads to formation of polar glucosides, which are also easily transported inside the epithelial cells for further hydrolysis (Spencer et al. 1999; Gee et al. 2000). After absorbed by the intestinal epithelial cells, second phase of metabolism starts causing formation of sulphates and uridine in the form of transferases, the catechol-*O*-methyltransferases (COMTs). There occurs third phase of metabolism where various proteins are required (Del Rio et al. 2010). The various phenolic compounds act as prebiotics, which are highly important for microflora of bowels (Jonsson and Backhed 2017).

4.4 Anticancer Mechanism of Action of Honey

Anticancer effect of honey includes the phytochemicals present in the honey like various phenolic compounds and flavonoids. Various pathways are exploited in various cancer cell lines, which has been reported by various studies (Fauzi et al.

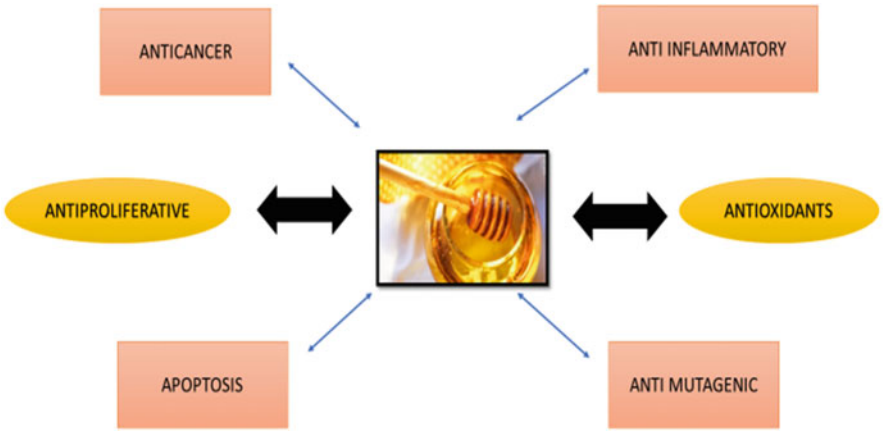

Fig. 4.1 Various anticancer mechanisms of honey compounds. Honey shows various anticancer mechanisms by acting as anti-proliferative, apoptotic, antimutagenic, antioxidant and anti-inflammatory agent

2011). The probable anticancer mechanisms involved in honey supplementation is briefly described in Fig. 4.1.

1. Honey possesses apoptotic effects and is known to upregulate caspase 3 and Bax protein, which have antiapoptotic action.
2. Honey is known to upregulate tumour suppressor gene p53 (Jaganathan and Mandal 2009).
3. Royal jelly protein present in honey shows effect on pro-inflammatory cytokines TNF-α, IL-6, IL-1, and thus showed antitumour action.
4. Components in honey are shown to have antioxidant and free radical scavengers which otherwise may lead to many inflammatory diseases and even cancer (Van Acker et al. 1996).
5. Honey is shown to possess anti-proliferative activity as well by its effect on cell cycle (Tomasin and Cintra Gomes-Marcondes 2011).
6. Honey acts as an anti-inflammatory agent which causes prevention of carcinogenesis via various pathways.
7. Honey acts as antimutagenic compound as well.

4.5 Anticancer Effect of Honey Via Modulation of Signalling Pathway

The main reason for death nowadays in the world is cancer, as this dreadful disease not only causes death of an individual but also all the money he or his family have earned. The treatment of this disease is very high. The available anticancer drugs in the market are costly, non-specific, besides possessing high side effects. So, the need

of hour is to switch on to the plant-based compounds which are economical and with minimum or no side effects at all. Various dietary agents are observed to have apoptotic, anti-proliferative and anti-inflammatory effects: the pathways of which are found to be deregulated in carcinogenesis. Honey: full of antioxidants, readily available, economical can be exploited for anticancer treatment. The anticancer effect of honey by modulation of certain cell signalling and metabolic pathways is discussed as follows.

4.5.1 Anticancer Activity of Honey by Interfering with Oxidative Stress (Antioxidant)

Formation of reactive oxygen species (ROS), hydroxyl ions (OH^-), superoxide radicals and other free radicals is considered as a potent threat for the development of not only cancer but other chronic diseases like coronary diseases, inflammatory disorders, neurological degeneration, ageing as well. So, in order to counteract these free radicals, antioxidants are needed. And honey is one such compound which shows high antioxidant properties (Antony et al. 2000). Phenolics and flavonoids in this naturally occurring compounds have such strong antioxidant properties as observed by various studies that these compounds like chrysin and pinocembrin are actively studied for the same (Viuda-Martos et al. 2008; Sun et al. 2015).

Consumption of honey is known to activate the levels of glutathione peroxidase, Vitamin C and beta-carotene which are antioxidants (Al-Waili 2003). Quercetin at high levels leads to decrease in ROS and increase in the production of H_2O_2. A dihydroflavonoid pinobanksin-3-acetate is considered as the strongest antioxidant in honey (Boisard et al. 2014). Several studies put forth that when these polyphenols are tested in vitro separately for their antioxidant properties, they showed less activity than when present in honey so one can conclude in honey these work synergistically (Gheldof and Engeseth 2002).

Not only flavonoids and phenolics in honey have anti-scavenging properties but it also contains vitamins (C and E), enzymes (catalase, peroxidase and glucose oxidase), carotenoids and melanoidins which also leads to its antioxidant properties. Honey melanoidins are high-molecular-weight proteins which are mostly present in abundance in heat-treated honey and possess high antioxidant effect (Katrina and Danielle 2011). These flavonoids in honey act as substrates for free radicals like *OH and nitric oxides to limit free radicals in the body or called as free radical sequestration method and thus helps in the prevention of various diseases including cancer. The other mechanisms may include hydrogen donation and metal ion chelation.

Anticancer signalling pathway for Caffeic acid (CA) and Caffeic acid Phenyl esters (CAPE): A phenolic compound present in honey propolis possesses anticarcinogenic properties via modulating antioxidant and anti-inflammatory pathways. CAPE stops the formation of carcinogenic compounds in the body like nitrosamines and nitroamides (Damasceno et al. 2017). One of the studies of antioxidant effect of CAPE is on hepatocellular carcinoma (HCC) where it acts as both primary as well as secondary oxidant (Gu et al. 2016; Won et al. 2010). CAPE

due to its chemical structure helps in stopping chain reaction by donating its hydrogen or electrons to free radicals in the body. It also forms complexes with other metal ions like copper and iron, so preventing formation of peroxides and other free radicals which otherwise would lead to attack on cell membranes, amino acids and DNA (Angelo and Jorge 2007). Increase in ROS production causes damage to mitochondrial membrane and proteins via lipid peroxidation, which leads to the change in inner membrane permeability causing damage to mitochondrial DNA as well as genetic/epigenetic changes in DNA which are known to be the causes of various cancers (Dhanasekaran et al. 2016). Intracellular ROS are capable of inducing damage and, in severe cases, cell death through mitochondrial alterations leading to the release of cytochrome c (Berman and Hastings 1999; Halestrap et al. 2000), through activation of the JNK pathway (Tournier et al. 2000) or by activation of nuclear factor-κB (NF-κB) transcription factors.

4.5.2 Anticancer Effects of Honey on Cell Proliferation

Deregulation of any proteins which are involved in cell cycle pathway leads to tumorigenesis. Cell cycle pathway is a highly regulated and controlled mechanism. Cell cycle phases include G_0/G_1/S and M. The transition from G_1 to S phase is highly important as when the cells pass to S phase, it has to undergo proliferation, differentiation and programmed cell death. This pathway is controlled by various cyclin-dependent kinases (cdks) (Diehl 2002). Ki-67—a protein present in the nucleus is present in G_1/S/M phases but absent in G_0 phase—is a new biomarker to mark cell proliferation (Scholzen and Gerdes 2000).

Any agent which have the tendency to block cell proliferation can be used as anticancer agents. Phenolics and flavonoids which includes chrysin, quercetin and kaempferol present in the honey show effects on cell cycle by causing cell cycle arrest at G_0 or G_1 phase (Waheed et al. 2019). Ki-67 is shown to have less expression when honey is added to cancer cell lines, so it means cell is not in active phase which grows unchecked in cancer cell (Tomasin and Cintra Gomes-Marcondes 2011).

The various cell signalling mechanisms are known to be involved in the antiproliferation action of honey like tyrosine cyclooxygenase, STAT 3 inhibition, Wnt inhibition pathways (Lirdprapamongkol et al. 2013). Caffeic acid and other phenyl esters in honey are shown to have profound effect on various regulatory enzymes of cell division via various pathways like protein tyrosine kinase cyclooxygenase and ornithine decarboxylase pathways (Rao et al. 1993). Artepillin C in the similar way has been shown to cause antiproliferation of prostate cancer cells by reducing TNF-related, apoptosis-inducing ligand (TRAIL) resistance and impeding NF-κB (Zhang et al. 2013). Flavonoid chrysin in honey has also exerted antiproliferative effect in various cancer cell lines like human Hep-3B, TCC, A549, HeLa and colorectal cancer cells (Patel 2016). Apart from chrysin and galangin, other flavones and phenolics present in the honey like caffeic acid phenethyl ester (CAPE), benzyl ferulate, benzyl isoferulate, pinostrobin, 5-phenylpenta-2,4-dienoic acid and tectochrysin showed anti-proliferative effects on various cancers like gastric, esophagus and colon (Catchpole et al. 2015). CAPE causes upregulation

of ROR2 pathway and causes cell cycle arrest at G_1 or M phase of cell cycle (Motawi et al. 2016).

Catchpole et al. studied the anti-proliferative effects of chrysin, galangin and CAPE besides other smaller compounds like benzyl ferulate, benzyl isoferulate, pinostrobin, 5-phenylpenta-2,4-dienoic acid and tectochrysin on colon cancer cell line DLD-1 and gastrointestinal cell lines (Owen et al. 2015). Benzyl caffeate, a propolis, also showed anti-proliferative activity against murine colon cancer cell line 26-L5 (Usia et al. 2002). CA and CAPE showed anti-proliferative effects on tyrosine protein kinase (TPK), lipoxygenase and cyclooxygenase pathways.

Quercetin, a flavanone, is known to decrease the cell proliferation rate in glioma cells by either causing cell division stoppage at G_2 cell phase or by leading to its apoptosis. Quercetin and Ellagic acid present in the honey via Wnt-β-catenin pathway reduce cell proliferation and thus act as anti-tumorigenic compounds, as shown in DU145 prostate cancer cell line (Giuseppe et al. 2019). Wnt signalling pathway acts on cyclin D1 protein (cell cycle protein) important for cell division. Ellagic acid and quercetin inhibits the formation of cyclin D1 and thus blocks cell proliferation process. Quercetin prevents many proliferative pathways like protein kinase c, tyrosine kinases and PI-3 kinases by inhibiting many enzymes in many cancers like prostate, colon, gastric and leukaemia (Ranelletti et al. 1992; Yoshida et al. 1992). Figure 4.2 shows how cyclins at different phases of cell cycle are

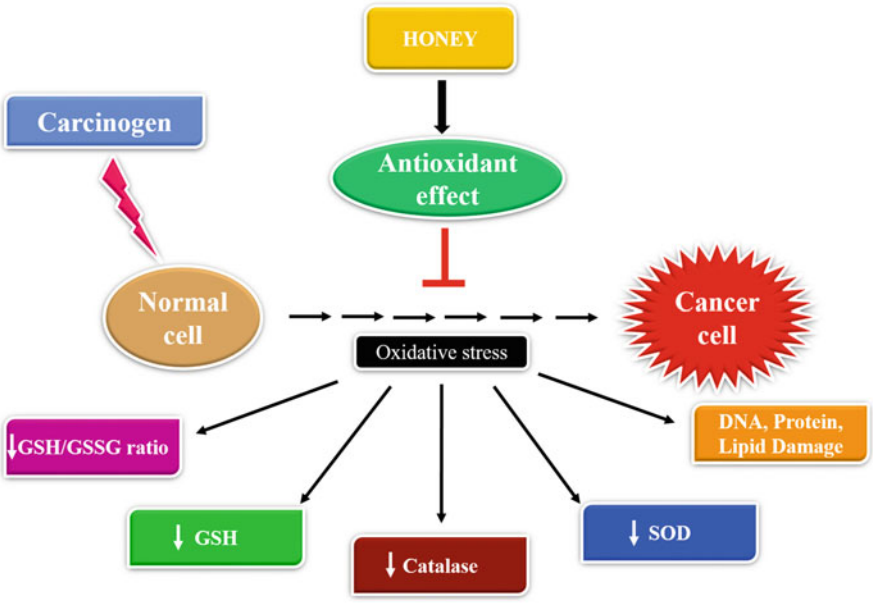

Fig. 4.2 Figure representing oxidative stress and antioxidant effect of honey. The induction of carcinogen (UV rays, chemical carcinogens, etc.) on the normal cell converts it to cancerous cell by different intracellular pathways. Oxidative stress is one of the hallmarks of cancerous cells leading to increased ROS generation and depletion of cellular antioxidants like GSH and dependent enzymes, catalase and SOD. There is damage to DNA, protein and lipids in the cells due to the deleterious effect of carcinogen. It is reported that honey and its components have powerful anti-oxidative effects leading to decrease in total ROS generation in the cells

inhibited by Quercetin. Like at G_1 phase, Quercetin inhibits cyclin D an p21, which is a cyclin-dependant kinase inhibitor, which deactivates cyclin K2-E complex formation at G_1 to S phase transition. P21 also inhibits CDK2-cyclin A and CDK1-cyclin B complex formations which are needed for orderly progression through S phase and G_2/M (Rather and Bhagat 2019).

The attachment of β-catenin and the cell adhesion molecule E-cadherin also regulates migration part of cell cycle process. This type of regulation is observed among colon cancers, gastric cancers, prostate cancers, etc. (Bullions and Levine 1998; Refolo et al. 2015; Park et al. 2005). *p*-Coumaric acid also is observed to possess anti-proliferative activity in colon cancer cell lines, by affecting cell cycle phases (de Lana et al. 2018).

4.5.3 Anticancer Activity of Honey by Modulating Inflammatory Pathways

Inflammation is actually body's defence against outside stimuli, but chronic inflammation leads to various diseases like diabetes, heart diseases and even can be the cause of cancer. Various cytokines and macrophages are released in the body in response to inflammation. Even the generation of free radicals in the body could lead to tissue inflammation through various pathways which causes increase in pro-inflammatory cytokines like cyclooxygenase-2 (COX-2), lipoxygenase-2, IL-6, IL-12 and C-reactive proteins (CRP). Honey contains compounds which have profound effect on various inflammatory pathways like mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF-κB) (Al-Waili 2003). Pro-inflammatory cytokines like TNF-α and others activate NF-κB pathway which in turn upregulates (COX-2), lipoxygenase-2, IL-6, nitric oxide, etc., which leads to tumour progression (Sugarman et al. 1985). The pathway showing how honey compound quercitin inhibits the inflammatory pathways at various checkpoints is shown in Fig. 4.3.

Gangrein and Chrysin decrease inflammation by blocking an inflammatory protein COX-2, lipoxygenase-2 (Rossi et al. 2002; Ko et al. 2003). CAPE acts as an anti-inflammatory compound in honey by preventing the release of arachidonic acid from the cell (Mirzoeva and Calder 1996). It has been observed that CAPE decreases the effect of radiations on skin cells due to its anti-inflammatory properties (Staniforth et al. 2006). In rat models having breast cancer, honey is shown to reduce TNF-α and other pro-inflammatory levels. Chrysin inhibited proliferation of HTH7 and KAT18 cells of anaplastic thyroid carcinoma (Tram Anh et al. 2011). CAPE is a potent and a specific inhibitor of NF-κB activation, and this may provide the molecular basis for its multiple immunomodulatory and anti-inflammatory activities of CAPE (Natarajan et al. 1996). Inflammatory agents like TNF, ceramide, hydrogen peroxide and phorbol esters activate NF-κB, but CAPE blocks its activity

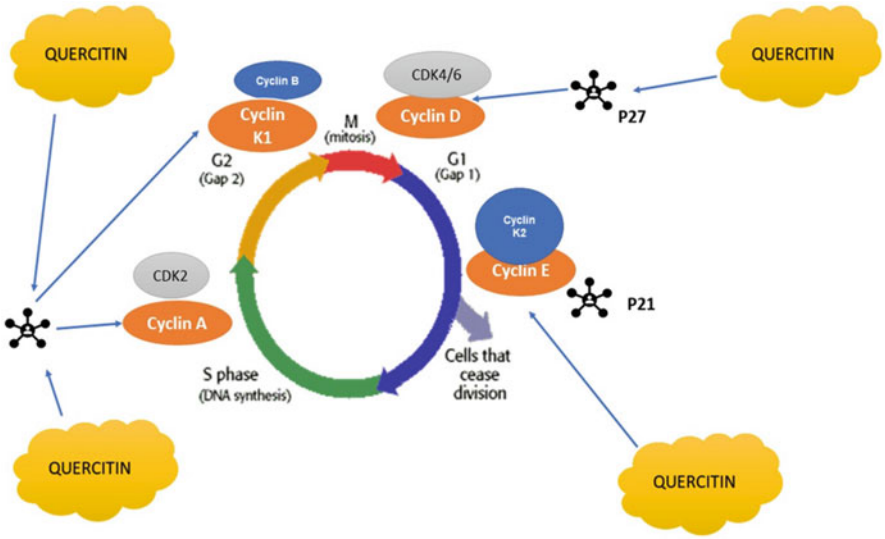

Fig. 4.3 Representative diagram shows inhibition by Quercetin by acting on different proteins in cell cycle. In cell cycle, various cyclins are responsible for cell division. Quercetin inhibits these cyclins at different phases. At G_1 phase, Quercetin inhibits cyclin D and p21 which in turn by various factors deactivates cyclin K2-E complex formation at transition phase of G_1 and S stopping cell division. p21 also inhibits cyclin A-CDK2 complex and cyclin K1-cyclin B complex in S phase and G_2 phase/M phase leading to arrest of cell division

by acting on its sulphydryl groups. CAPE also prevents the translocation of p65 to the nucleus, which is a subunit of NF-κB, which further stops the formation of NF-κB as demonstrated in Fig. 4.4. The blocking and inactivation of NF-κB by CAPE provides the molecular basis for its immunomodulatory and anti-inflammatory activities. NF-κB signalling besides causing formation of COX-2 and lipoxygenase also leads to the transition of epithelial cells to mesenchymal cells, which further causes the slackening of ECF via metalloproteinases leading to metastasis (Hoesel and Schmid 2013).

Chrysin also inhibits NF-κB at various check points like blocks formation of COX-2, iNOS, TNF and cytokines and hence shows anti-inflammatory effect (Rauf et al. 2015; Xiao et al. 2014).

Various research studies have observed that other secondary metabolites from honey like daidzein, apigenin, luteolin, kaempferol and quercetin possess inflammatory activity. They have the potential to decrease the production of TNF-α and IL-1β in N13 microglia cell line (Candiracci et al. 2012). Honey is known to have protective effect against neuro-degenerative diseases especially Alzheimer's/Parkinson's, where inflammation is known to be the cause. Other proteins in the honey such as apalbumin-1, Major Royal Jelling Proteins (MRJP-1) an MRJP-3, have been shown to decrease pro-inflammatory adipocytokines by either blocking

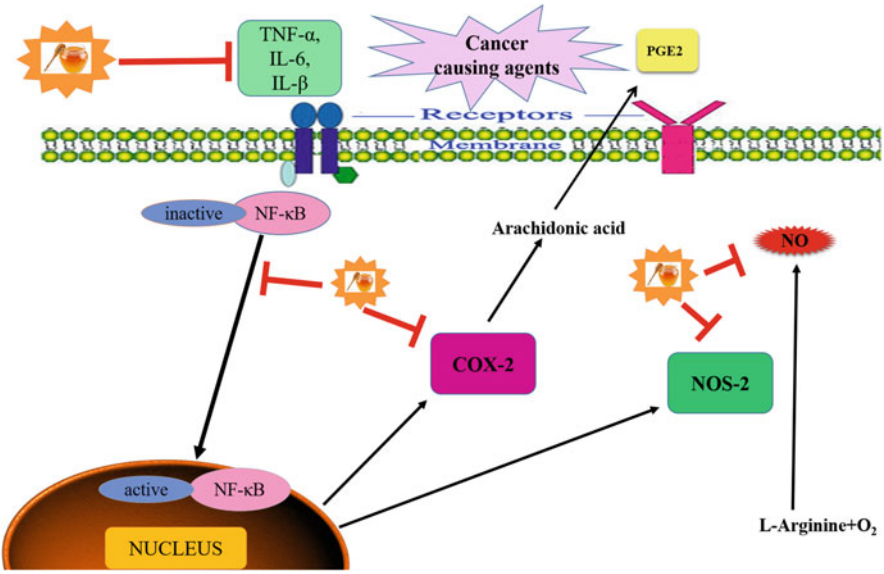

Fig. 4.4 Inflammatory pathway is inhibited by honey (products) at various checkpoints. The inflammation in cancerous cells is a combined effect of multiple factors acting together. The carcinogens increase pro-inflammatory cytokines (TNF-α, IL-6, IL-12, IL-1β) in the cells which activated NF-κB pathway which in turn upregulates COX-2 and NOS-2 pathways leading to inflammation in the cells. Honey and its components have been reported to decrease these activations of inflammatory markers leading to anti-inflammatory effect of honey

mannose receptors of phagocytic cells or activating antigen-stimulated T-cells (Okamoto et al. 2003; Molan and Rhodes 2015).

4.5.4 Anticancer Effect of Honey Via Regulating Angiogenesis

Angiogenesis plays an important role in tumour formation. It is the formation of new blood vessels to provide more oxygen to tissues. But in some mechanisms in the body, this process is important like wound healing. During angiogenesis, the inflammatory cytokine like vascular endothelial growth factor (VEGF) level and prostaglandin E2 (PGE2) acts as an important marker of angiogenesis. Researchers observed the angiogenetic effects of honey but at less concentration, so we can conclude that at low concentration the honey helps in angiogenesis, but at high concentration it is shown to decrease the levels of VEGF and PGE2 (Eteraf-Oskouei et al. 2014). However, some type of honey is so potent that even as low as 0.2 g/kg concentrations the cancer cells showed decrease in growth and VEGF and hence minimized vasculature around tumour. A study was conducted to observe the effect of honey products on bladder cancer in rats, and 300 mg/kg of propolis was found to inhibit bladder cancer angiogenesis in these animals (Dornelas et al. 2012). CAPE

also leads to decrease in colon cancer progression by downregulation of VEGF gene expression and PGE-2 synthesis (Michaluart et al. 1999). Genes like GLI3, CRABP-II, calmodulin, galectin-7, conexin-26, cytokeratin I and E-cadherin, which are expressed during angiogenesis, are found to be regulated by honey products.

Phenolics as Antiangiogenic compound: CA in honey as discussed works in number of ways as anticancer agent. CAPE via c-Jun N-terminal kinases (JNK-1 pathway) leads to decrease in HIF-1α (hypoxia-inducible factor 1) by dephosphorylation of JNK-1 protein causing less VEGF production and thus decrease in vascularisation (Gu et al. 2016). Alike chrysin, epigallocatechin-3-gallate (EGCG) also acts as angiogenic compound and works via the same pathway causing dephosphorylation of gp130/JNK-1/STAT-3 leading to reduction of VEGF and IL-6 (Chiu-Mei et al. 2010; Choi et al. 2008). This type of anti-angiogenesis is observed in breast, liver, prostate cancers and renal cell carcinoma (Jung et al. 2007). Another flavonoid silibinin is shown to have antiangiogenic effect in lung cancer via STAT-3 pathway causing upregulation of antiangiogenic compounds like Ang-2 and Tie-2 (Tyagi et al. 2009). Figure 4.5 shows how Chrysin downregulated VCAM-1 protein expression and chrysin blocked adhesion of monocytes in cerebral vascular endothelial cells. NF-κB, p38 mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase, which are all activated by lipopolysaccharide, were significantly inhibited by chrysin.

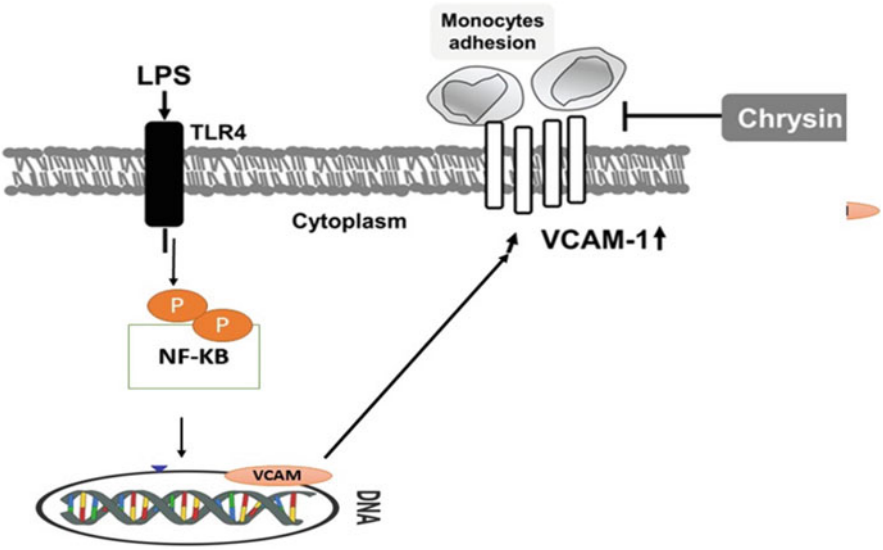

Fig. 4.5 Representative diagram showed the inhibition of Chrysin in downregulation of VCAM. Chrysin blocked monocytes adhesion in cerebral vascular endothelial cells. NF-κB, p38 mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase, which are all activated by lipopolysaccharide, were significantly inhibited by chrysin

4.5.5 Anticancer Effect of Honey on Immunity

Honey is considered as 'super-charged immunity booster' by University of Michigan. Honey is a powerhouse of antioxidants, effective for exclusion of free radicals from the body. Persons with low immune status in the body are more prone to develop not only cancer but also a number of diseases. Honey products provoke production of antibodies against both thymus-independent as well as thymus-dependent antigens. Daily consumption of honey is known to increase production of prostaglandins among AIDS patients (Al-Waili et al. 2006). Among humans, it has known to increase monocyte production and even causes neutrophil migration, eventually lessening tumour growth (Miki et al. 2010). Flavonoids and phenolics in natural honey increase various pro- and anti-inflammatory cytokines like TNF-α, interleukin (IL)-1β and IL-6 release which activate the immune response to fight against infection. Honey has been shown to induce the number of B-cells, T-cells, natural killer cells, neutrophils and monocytes, all of which boosts immunity in the body (Hussein et al. 2012).

Honey is metabolized into short chain fatty acids in the body, which are shown to have immunomodulatory effects. Not only phenolics and flavones, even oligosaccharides present in the honey show prebiotic effects in the gut and increases the gut microbe population like bifidobacterial and lactobacilli. Nigeria honey is shown to have high immunomodulating effects (Chepulis 2007). Given to its positive effects on immune system, the body has less chance of getting diseases, tumour cells at first are recognized and destroyed by the cytokines and so less chance of getting cancer. CAPE in propolis is effective in treating asthma and allergic reactions by reducing TNF-α, intercellular adhesion molecule (ICAM)-1, IL-6, IL-8, leukotrienes and prostaglandin E2 (PGE2) via inhibiting NF-κB pathway (Wang et al. 2010).

4.5.6 Anticancer Activity of Honey on Telomerase

Telomeres are part of chromosomes containing short tandemly repeats (STRs) and important constituents in a chromosome which have associated proteins to preserve chromosome integrity and stability. Telomerase, a reverse transcriptase enzyme, is known to maintain telomere length. During ageing, the length of telomere shortens causing tissue dysfunction. During carcinogenesis, there occurs upregulation of these telomerase, so more cell proliferation. So, by blocking or decreasing telomerase expression can be used as anticancer mechanism. Honey is known to have this effect on telomerase activity. Flavones in honey like acacetin and chrysin were known to mitigate telomere position effect (TPE genes): a heterochromatic region which causes transcriptional silencing of telomerase activity, and hence leads to decrease in tumour formation (Amina et al. 2013). An another study conducted on ethanol extracts of propolis (EEP) showed decreased expression of telomerase by about 93% by silencing telomerase reverse transcriptase gene in T-cell acute lymphoblastic leukaemia cell line (Gunduz et al. 2005).

4.5.7 Anticancer Activity of Honey by Causing Apoptosis

During carcinogenesis, antiapoptotic activity of cells increases due to deregulation of apoptotic pathways. Inadequate apoptosis or programmed cell death results in carcinogenesis, but it has been observed that honey showed apoptotic effect. In various cancer cell lines like in T24, RT4, 253J and MBT-2 honey is known to induce apoptosis (Jaganathan and Mandal 2009; Tareq et al. 2003). Through various pathways like via caspase or p53 pathways, the various metabolites of honey cause apoptosis. Caspases are known to possess proapoptotic effect and works via death signalling pathway or mitochondrial pathway. In one way, galangin and chrysin in honey leads to the regulation of caspase 3 and then cell apoptosis (Hsu et al. 2003). Chrysin also leads to decrease in Akt and p-38 in various cancer cell lines (Woo et al. 2004). Apigenin-constituent of honey causes downregulation of Bcl 2- and Akt, an antiapoptotic protein, and increases expression of Bax (proapoptotic protein) (Tomasin and Cintra Gomes-Marcondes 2011). p53 is activated by reactive oxygen species (ROS) generated by caffeic acid, p-coumaric acid and eugenol (phenolic compounds of honey), which in turn activates apoptotic proteins (Jaganathan 2012; Jaganathan et al. 2013). One mechanism involves decrease in mitochondrial membrane potential which is caused by caffeic acid present in honey and is observed in among breast and colon cancer cases. So, honey or secondary metabolites present in it works in both ways to have apoptotic effect on cells. Honey is therefore used as apoptotic inducer which makes it a best chemotherapeutic agent (Figs. 4.6 and 4.7).

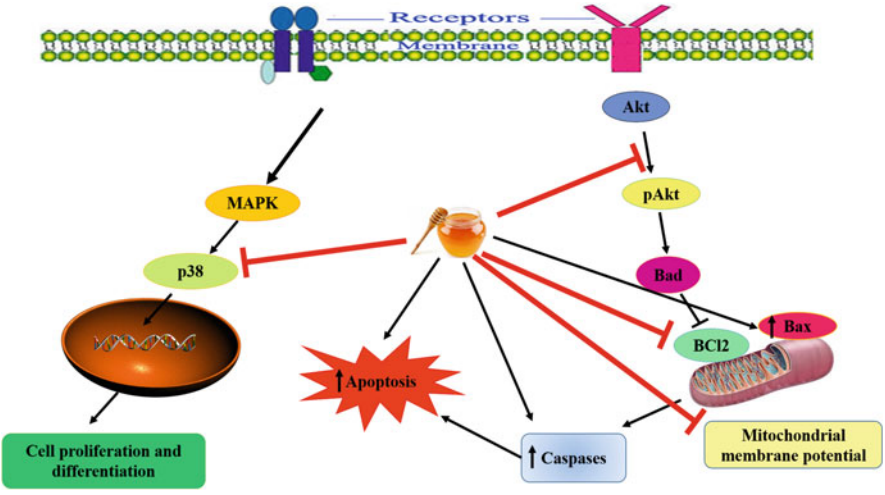

Fig. 4.6 Apoptotic effect of honey. In cancerous cells, there is antiapoptotic activity of cells by activation of MAPK activating p38 which leads to cell proliferation and differentiation. Along with this, another pathway activating Akt is working in cancerous cells where BCl2 is increased and Bax is decreased leading to Caspases inactivation and inhibiting apoptosis. In this figure, honey and its components have been shown to activate different cascades leading to apoptosis of the cells

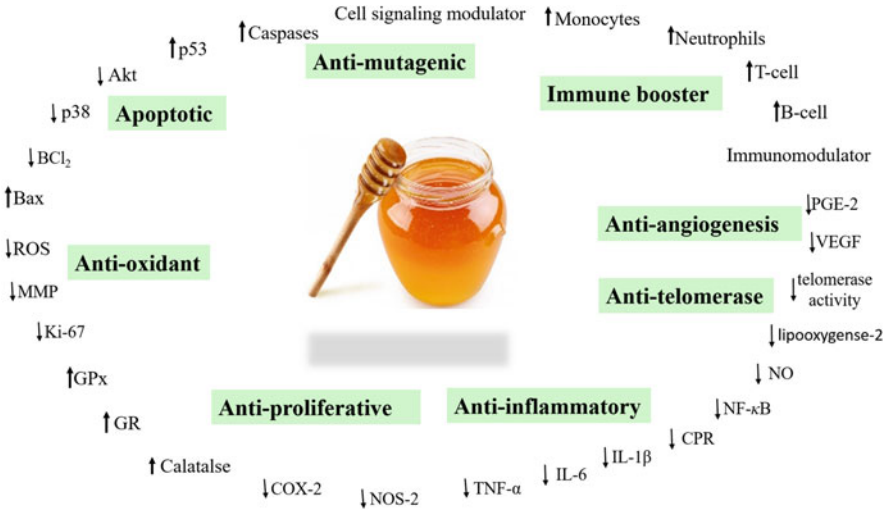

Fig. 4.7 Overall anticancerous mechanisms of honey and its components. This figure is summarized effects of honey and its components on the cancerous cell. Honey acts as antimutagenic, apoptotic, antioxidant, anti-proliferative, anti-inflammatory, anti-angiogenesis, and immune booster in cancerous cell inhibiting factors required for cancer and activation of anticancerous factors. ↑ means activation and ↓ means suppression

Sirtuin family protein (SIRT-1), a histone deacetylase, is found to be highly expressed in various cancers like colon, acute myeloid leukaemia, prostate (Jang et al. 2008; Tseng et al. 2009). Ellagic acid in honey causes inhibition of SIRT-1 protein and in turn activates apoptotic genes like Bax, p53 and retinoblastoma (Rb) protein and thus supresses cancer growth (Giuseppe et al. 2019). Vanillic acid also leads to apoptosis by regulating procaspase-3, procaspase-9, Bax and p53 proteins as shown in small cell lung carcinoma and colon cancers (Kumar et al. 2013).

4.5.8 Antimutagenic Effect of Honey on Different Cancers

Mutation or mutagenicity is a process which causes damage/lesions to the DNA that result in cell death or some undesirable changes which are permanent and leads to carcinogenesis. The compounds in honey are shown to have antimutagenic effects and hence makes it as an anticancer potential contender. Very few studies are conducted to check the antimutagenic effect of honey on cells. In one such study, *E. coli* after exposing them with UV radiation (as UV radiation causes DNA damage) and in order to check the mutation, Selectable--In-Vivo Expression Technology (SIVET) assay frequency was found to be high. Supplementing the cells with honey, SIVET levels which measure damage were observed to be low, so confirming its antimutagenic effect (Saxena et al. 2012). In fact, bee pollen is also known to have

mutagenic effect and so anticarcinogenic as well (Denisow and Denisow-Pietrzyk 2016). Trp-p-1 is a mutagen present in numerous foods and is considered as a carcinogen. But honey also showed a significant decrease of Trp-p-1 with increase in its concentration (Wang et al. 2002). The exact mechanism is still a mystery.

4.6 Effect of Honey on Different Carcinomas

4.6.1 Effect of Honey on Leukaemia

Leukaemia is a common malignancy of white blood cells, which is further categorized into chronic myeloid leukaemia (CML) and acute myeloid leukaemia (AML). The current treatment besides chemotherapy is imatinib (a tyrosine kinase inhibitor) but with time patients develop resistance to this drug. So new, cost-effective drug is need of the hour, and honey is such a natural compound which have tremendous effect on cancer cells.

Various reports suggested that honey compounds like galangin and kaempferol act as anti-proliferative agents on promyelocytic leukaemia cells line (HL60) (Bestwick et al. 2007). Another flavonoid quercetin acts on both CML and AML cell lines (K562 and MV4-11) but via apoptosis (Akan and Garip 2011).

4.6.2 Effect of Honey on Breast Cancer

Breast cancer is the second largest causing death among women. The cells, i.e. cancer stem cells (CSC), from which this deadly disease is known to cause are resistant to chemotherapy. So, to find a novel therapeutic drug is important to decrease the mortality associated with breast cancer (Rathore and Wang 2013). Different types of honey like Manuka honey (UMF 10+), Tualang honey and Thyme honey were shown to inhibit abnormal cell division at a concentration of 0.6% (*w*/*v*) in multiple cell lines (human breast cancer MCF-7 and MDA-MB-231). Quercetin showed anti-proliferative action on MCF-7 cell lines. CAPE downregulates breast cancer cells by inhibiting the phosphorylation of epidermal growth factor receptor (EGFR) (Wu et al. 2011). EGFR in carcinogenesis binds with EGR (receptors) and via mitogen-activated protein kinase (MAPK), protein kinase B (Akt) and c-Jun N-terminal kinase (JNK) pathways, resulting in abnormal DNA synthesis and proliferation (Wagner and Nebreda 2009). Honey compounds in breast cancer cells work by increase in apoptotic cells and decrease in VEGF levels, so decrease in angiogenesis (Kadir et al. 2013). In one of the study supplementation of breast cancer patients with honey overall showed a good effect on WBC and platelet counts (Zaida et al. 2018).

4.6.3 Effect of Honey on Gastrointestinal Carcinomas

Gastrointestinal cancers include cancers of stomach, oesophagus, gallbladder, pancreas, liver, intestine, colon, and rectum, which are the most common human cancers in both men and women worldwide. Gastric cancer is the second leading cause of death in the world. Due to the failure of conventional therapies, there has been a shift to dietary and lifestyle modifications since several years (Oh et al. 2010). Helicobacter pylori (*H. pylori*) is considered as a risk factor for developing gastric cancer and other associated diseases. Ellagic acid, gallic acid, and other phenolic constituents in honey are known to act as anti-inflammatory and antioxidant agents to decrease GIT-related cancers. These compounds decrease the inflammation of the tumour cells by causing deactivation of NF-κB pathway which in turn leads to decrease in pro-inflammatory markers (Abdel-Latif et al. 2005; Hussein et al. 2012). As inflammation is reduced so does the cancer cells. Even gastric ulcers, gastritis is healed by honey, as it showed sucralfate effects on epithelial cells of stomach lining. Chrysin works by decreasing matrix metalloproteinases (MMP-9) expression in gastric cancer (Xia et al. 2015). In colon carcinoma, honey along with ginger acts on various cancer-related pathways like Ras/ERK and P13K/AKT (Tahir et al. 2015). Ellagic acid downregulates insulin-like growth factor 2 (IGF-II) signalling pathway to inhibit colon carcinogenesis (Narayanan and Re 2001). Quercetin shows anti-proliferative effect on colon cancer cell line Caco-2 (Salucci et al. 2002), and via NF-κB and MAPK pathways it helps to reduce pancreatic cancer as well (Batumalaie et al. 2013).

4.6.4 Effect of Honey on Oral Cancer

Infections in mouth by a fungi *Candida albicans* is one of the causes of oral cancer (Hooper et al. 2009). Honey is known to have effect on fungal infections and other aerobic pathogens. One of the side effects of chemotherapy is oral mucositis. In one study the use of honey among chemotherapeutic patients shows significant effect on oral mucositis among paediatric cancer patients (Soad et al. 2017). Honey is economical and easily available and can be used to treat inflammation associated with oral mucosa, ulcers, etc.

4.6.5 Effect of Honey on Prostate Cancer

One of the common cancers among males is prostate cancer, and many deaths are associated with it annually. It was reported that honey shows anti-metastatic effects on prostate cancer cell lines. The phenolic compounds like caffeic acid reduce the cell proliferation and invasion in prostate cancer cell lines around 50% (Lansky et al. 2005). Not only phenolic compounds but sugars present in honey shows inhibition in cell invasion process: which is one of the important characteristics of metastasis (Ho et al. 2010). Gallic acid causes inhibition of MMPs via NF-κB pathway: which

leads to breakdown of extracellular matrix (ECM) of the cell. The honey compounds are known to reduce both enzymatic action of MMPs as well as translocation of NF-κB. Quercetin is also shown to have same effect on prostate cancer cell lines (Vijayababu et al. 2006).

4.7 Clinical Trials

Trials on mice models have indicated potential anticancer and antitumour properties of honey (Maria et al. 2013). A 50% solution of honey in saline injected in the mice showed about 33% inhibition of tumour growth. Further studies suggest that direct injection of honey could have potent antitumour properties. The high sugar content in honey is suggested to shrink the size of tumour through osmolarity effect. However, further studies are needed to evaluate the effectiveness of this approach.

Other studies (Al-Waili 2004) on the immunomodulatory effects of honey on humans have indicated that the consumption of 1.2 g/kg bodyweight leads to 50% increase in blood monocytes count and a marginal rise in lymphocytes and eosinophils. These encouraging studies suggest that further studies are required to firmly establish the utility in clinical trials.

4.8 Conclusion

This chapter summarizes the mechanisms of phenolics and flavonoids of honey in preventing the process of carcinogenesis by multiple mechanisms like acting as apoptotic agent, anti-proliferative agent, antiangiogenetic agent and antioxidant agent. The compounds in the honey work cumulatively to combat cancers. From wound healing to boosting immunity, honey compounds work synergistically adopting different mechanisms. However, limited data is available on its effect on humans, but in animal models its anticancer effect is well cited. Therefore, it could be developed as a natural cancer 'vaccine' or anticancer drug in future. So it is highly advisable to have daily consumption of honey in our diets.

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References

Abdel-Latif MM, Windle HJ, Homasany BS, Sabra K, Kelleher D (2005) Caffeic acid phenethyl ester modulates Helicobacter pylori-induced nuclear factor-kappa B and activator protein-1 expression in gastric epithelial cells. Br J Pharmacol 146:1139–1147

Akan Z, Garip A (2011) Protective role of quercetin: antioxidants may protect cancer cells from apoptosis and enhance cell durability. Web Med Central 2(1):1

Al-Waili NS (2003) Effects of daily consumption of honey solution on hematological indices and blood levels of minerals and enzymes in normal individuals. J Med Food 6(2):135–140
Al-Waili NS (2004) Effects of daily consumption of honey solution on Hematological indices and blood levels of minerals and enzymes in normal individuals. J Med Food 6(2):135–140
Al-Waili NS, Al-Waili TN, Al-Waili AN, Saloom KS (2006) Influence of natural honey on biochemical and hematological variables in AIDS: a case study. Sci World J 6:1985–1989
Amina B, Caroline B, Robert N, Adelaïd SL, Natacha B, Alexandre O, Arva F, Yiming L, Laurence L, Eric G, Frédérique M, Jing Y (2013) Acacetin and chrysin, two polyphenolic compounds, alleviate telomeric position effect in human cells. Mol Ther Nucl Acids 2(8):e116
Angelo PM, Jorge N (2007) Compostos fenólicos em alimentos—uma breve revisão. Rev Inst Adolfo Lutz 66:1–9
Antony SM, Han IY, Rieck JR, Dawson PL (2000) Antioxidative effect of maillard reaction products formed from honey at different reaction times. J Agric Food Chem 48(9):3985–3989
Balasundram N, Sundram K, Samman S (2006) Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. Food Chem 99:191–203
Batumalaie K, Zaman SS, Mohd YK, Shah II, Devi SS, Qvist R (2013) Effect of gelam honey on the oxidative stress-induced signaling pathways in pancreatic hamster cells. Int J Endocrinol 2013:367312
Berman SB, Hastings TG (1999) Dopamine oxidation alters mitochondrial respiration and induces permeability transition in brain mitochondria, implications for Parkinson's disease. J Neurochem 73(3):1127–1137
Bestwick CS, Milne L, Duthie SJ (2007) Kaempferol induced inhibition of HL-60 cell growth results from a heterogeneous response, dominated by cell cycle alterations. Chem Biol Interact 170(2):76–85
Bullions LC, Levine AJ (1998) The role of beta-catenin in cell adhesion, signal transduction, and cancer. Curr Opin Oncol 10(1):81–87
Boisard S, Le Ray AM, Gatto J, Aumond MC, Blanchard P, Derbre S et al (2014) Chemical composition, antioxidant and anti-AGEs activities of a French poplar type propolis. J Agric Food Chem 62:1344–1351
Candiracci M, Piatti E, Dominguez-Barragan M, Garcia-Antras D, Morgado B, Ruano D et al (2012) Anti-inflammatory activity of a honey flavonoid extract on lipopolysaccharideactivated N13 microglial cells. J Agric Food Chem 60:12304–12311
Catchpole O, Mitchell K, Bloor S, Davis P, Suddes A (2015) Antiproliferative activity of New Zealand propolis and phenolic compounds vs human colorectal adenocarcinoma cells. Fitoterapia 106:167–174
Chepulis LM (2007) The effect of honey compared to sucrose, mixed sugars, and a sugar-free diet on weight gain in young rats. J Food Sci 72:S224–S229
Chiu-Mei L, Shyu K-G, Wang B-W, Chang H, Chen Y-H, Chiu J-H (2010) Chrysin suppresses IL-6-induced angiogenesis via down-regulation of JAK1/STAT3 and VEGF: an in vitro and in ovo approach. J Agric Food Chem 58(11):7082–7087
Choi JS, Choi YJ, Shin SY, Li J, Kang SW, Bae JY, Kim DS, Ji GE, Kang JS, Kang YH (2008) Dietary flavonoids differentially reduce oxidized LDL-induced apoptosis in human endothelial cells: role of MAPK- and JAK/STAT-signaling. J Nutr 138:983–990
Clifford MN (2004) Diet-derived phenols in plasma and tissues and their implications for health. Planta Med 70:1103–1114
D'Archivio M, Filesi C, Varì R, Scazzocchio B, Masella R (2010) Bioavailability of the polyphenols: status and controversies. Int J Mol Sci 11(4):1321–1342
Da Silva PM, Gauche C, Gonzaga LV, Costa ACO, Fett R (2016) Honey chemical composition, stability and authenticity. Food Chem 196:309–323
Damasceno SS, Dantas BB, Ribeiro-Filho J, Antônio M, Araújo D, Galberto M, da Costa J (2017) Chemical properties of caffeic and ferulic acids in biological system: implications in cancer therapy. A review. Curr Pharm Des 23:3015–3023

de Lana SR, Nathállia AJ, da Nathália CPS, da Joelma F, Mariana M, Anderson JT (2018) Pharmacokinetic, antiproliferative and apoptotic effects of phenolic acids in human colon adenocarcinoma cells using in vitro and in silico approaches. Molecules 23(10):2569

Del Rio D, Borges G, Crozier A (2010) Berry flavonoids and phenolics: bioavailability and evidence of protective effects. Br J Nutr 104(Suppl 3):S67–S90

Denisow B, Denisow-Pietrzyk M (2016) Biological and therapeutic properties of bee pollen: a review. J Sci Food Agric 96:4303–4309

Dhanasekaran R, Bandoh S, Roberts LR (2016) Molecular pathogenesis of hepatocellular carcinoma and impact of therapeutic advances. F1000Res 5:879

Diehl JA (2002) Cycling to cancer with cyclin D1. Cancer Biol Ther 1(3):226–231

Dornelas CA, Fechine-Jamacaru FV, Albuquerque IL, Magalhães HIF, Dias TA, Faria MHG, Alves MK, Rabenhorst SH, de Almeida PR, Lemos TL et al (2012) Angiogenesis inhibition by green propolis and the angiogenic effect of L-lysine on bladder cancer in rats. Acta Cir Bras 27:529–536

Eteraf-Oskouei T, Najafi M, Gharehbagheri A (2014) Natural honey: a new and potent anti-angiogenic agent in the air-pouch model of inflammation. Drug Res (Stuttg) 64:530–536

Fauzi AN, Norazmi MN, Yaacob NS (2011) Tualang honey induces apoptosis and disrupts the mitochondrial membrane potential of human breast and cervical cancer cell lines. Food Chem Toxicol 49(4):871–878

Gee JM, DuPont MS, Day AJ, Plumb GW, Williamson G, Johnson IT (2000) Intestinal transport of quercetin glycosides in rats involves both deglycosylation and interaction with the hexose transport pathway. J Nutr 130:2765–2771

Gheldof N, Engeseth NJ (2002) Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples. J Agric Food Chem 50:3050–3055

Giuseppe C, Giuseppe S, Mariarita S, Valeria S, Luca VG (2019) Antiproliferative effects of ellagic acid on DU145 cells. Open Biochem J 13:23–31

Gu W, Yang Y, Zhang C, Zhang Y, Chen L, Shen J et al (2016) Caffeic acid attenuates the angiogenic function of hepatocellular carcinoma cells via reduction in JNK-1-mediated HIF-1α stabilization in hypoxia. RSC Adv 6:82774–82782

Gunduz C, Biray C, Kosova B et al (2005) Evaluation of Manisa propolis effect on leukemia cell line by telomerase activity. Leuk Res 29:1343–1346

Halestrap AP, Doran E, Gillespie JP, O'Toolee A (2000) Mitochondria and cell death. Biochem Soc Trans 28(2):170–177

Ho HH, Chang CS, Ho WC, Liao SY, Wu CH, Wang CJ (2010) Anti-metastasis effects of gallic acid on gastric cancer cells involves inhibition of NF-κB activity and downregulation of PI3K/AKT/small GTPase signals. Food Chem Toxicol 48:2508–2516

Hoesel B, Schmid JA (2013) The complexity of NF-κB signaling in inflammation and cancer. Mol Cancer 12:86

Hooper SJ, Wilson MJ, Crean SJ (2009) Exploring the link between microorganisms and oral cancer: a systematic review of the literature. Head Neck 31:1228–1239

Hsu YL, Kuo PL, Lin CC (2003) Acacetin inhibits the proliferation of Hep G2 by blocking cell cycle progression and inducing apoptosis. Biochem Pharmacol 67:823–829

Hussein SZ, Mohd Yusoff K, Makpol S, Mohd Yusof YA (2012) Gelam honey inhibits the production of proinflammatory, mediators NO, PGE (2), TNF-a, and IL-6 in carrageenan-induced acute paw edema in rats. Evid Based Complement Alternat Med 2012:109636

Iurlina MO, Saiz AI, Fritz R, Manrique GD (2009) Major flavonoids of Argentinean honeys. Optimisation of the extraction method and analysis of their content in relationship to the geographical source of honeys. Food Chem 115:1141–1149

Jaganathan SK (2012) Growth inhibition by caffeic acid, one of the phenolic constituents of honey, in HCT 15 colon cancer cells. Sci World J 2012:372345

Jaganathan SK, Mahitosh M (2009) Antiproliferative effects of honey and of its polyphenols: a review. J Biomed Biotechnol 2009:830616

Jaganathan SK, Mandal M (2009) Honey constituents and their apoptotic effect in colon cancer cells. J ApiProd ApiMed Sci 1(2):29–36
Jaganathan SK, Supriyanto E, Mandal M (2013) Events associated with apoptotic effect of p-Coumaric acid in HCT-15 colon cancer cells. World J Gastroenterol 19(43):7726–7734
Jaganathan SK, Balaji A, Vellayappan MV, Asokan MK, Subramanian AP, John AA, Supriyanto E, Razak SI, Marvibaigi MA (2015) Review on antiproliferative and apoptotic activities of natural honey. Anti Cancer Agents Med Chem 15:48–56
Jang KY, Hwang SH, Kwon KS, Kim KR, Choi HN, Lee NR, Kwak JY, Park BH, Park HS, Chung MJ, Kang MJ, Lee DG, Kim HS, Shim H, Moon WS (2008) SIRT1 expression is associated with poor prognosis of diffuse large B-cell lymphoma. Am J Surg Pathol 32(10):1523–1531
Jonsson AL, Backhed F (2017) Role of gut microbiota in atherosclerosis. Nat Rev Cardiol 14:79–87
Jung JE, Kim HS, Lee CS, Park D, Kim Y, Lee M, Lee JW, Park J, Kim M, Ye SK, Chung MH (2007) Caffeic acid and its synthetic derivative CADPE suppress tumor angiogenesis by blocking STAT3-mediated VEGF expression in human renal carcinoma cells. Carcinogenesis 28:1780–1787
Kadir EA, Sulaiman SA, Yahya NK, Othman NH (2013) Inhibitory effects of Tualang honey on experimental breast cancer in rats: a preliminary study. Asian Pac J Cancer Prev 14:2249–2254
Katrina B, Danielle M (2011) The recognition of high molecular weight melanoidins as the main components responsible for radical-scavenging capacity of unheated and heat-treated Canadian honeys. Food Chem 125:570–575
Ko HH, Tsao LT, Yu KL, Liu CT, Wang JP, Lin CN (2003) Structure–activity relationship studies on chalcone derivatives: the potent inhibition of chemical mediators release. Bioorg Med Chem 11:105–111
Kumar PPBS, Ammani K, Azhar M, Jaya G (2013) Vanillic acid induces oxidative stress and apoptosis in nonsmall lung cancer cell line. Int J Recent Sci Res 24:1077–1083
Lansky EP, Jiang W, Mo H et al (2005) Possible synergistic prostate cancer suppression by anatomically discrete pomegranate fractions. Investig New Drugs 23(1):11–20
Lirdprapamongkol K, Sakurai H, Abdelhamed S, Yokoyama S, Athikomkulchai S, Viriyaroj A et al (2013) Chrysin overcomes TRAIL resistance of cancer cells through Mcl-1 downregulation by inhibiting STAT3 phosphorylation. Int J Oncol 43:329–337
Maria JFC, Rkia EK, Fawaz T, Ghada B, Junu A, Hakam ET, Al-Ramadi BK (2013) Intravenous administration of Manuka honey inhibits tumor growth and improves host survival when used in combination with chemotherapy in a melanoma mouse model. PLoS One 8(2):e55993
Michaluart P, Masferrer JL, Carothers AM, Subbaramaiah K, Zweifel BS, Koboldt C, Mestre JR, Grunberger D, Sacks PG, Tanabe T (1999) inhibitory effects of caffeic acid phenethyl ester on the activity and expression of cyclooxygenase-2 in human oral epithelial cells and in a rat model of inflammation. Cancer Res 59(10):2347–2352
Miki F, Kengo K, Yuriko H, Mayuko M, Takahiro I, Emenike CE, Masaharu S, Pinkerton KE, Minoru T (2010) Jungle honey enhances immune function and antitumor activity. Evid Based Complement Alternat Med 2011:908743
Mirzoeva OK, Calder PC (1996) The effect of propolis and its components on eicosanoid production during the inflammatory response. Prostaglandins Leukot Fatty Acids 55:441–449
Molan P, Rhodes T (2015) Honey: a biologic wound dressing. Wounds 27:141–151
Motawi TK, Abdelazim SA, Darwish HA, Elbaz EM, Shouman SA (2016) Modulation of tamoxifen cytotoxicity by caffeic acid phenethyl ester in MCF-7 breast cancer cells. Oxidative Med Cell Longev 2016:3017108
Narayanan BA, Re GG (2001) IGF-II down regulation associated cell cycle arrest in colon cancer cells exposed to phenolic antioxidant ellagic acid. Anticancer Res 21:359–364
Natarajan K, Singh S, Burke TR Jr, Grunberger D, Aggarwal BB (1996) Caffeic acid phenethyl ester is a potent and specific inhibitor of activation of nuclear transcription factor NF-κB. Proc Natl Acad Sci U S A 93(17):9090–9095
Nicolson SW, Nepi M, Pacini E (2007) Nectaries and nectar, vol 4. Springer, New York

Oh B, Butow P, Mullan B, Beale P, Pavlakis N, Rosenthal D, Clarke S (2010) The use and perceived benefits resulting from the use of complementary and alternative medicine by cancer patients in Australia. Asia Pac J Clin Oncol 6:342–349

Okamoto I, Taniguchi Y, Kunikata T, Kohno K, Iwaki K, Ikeda M et al (2003) Major royal jelly protein 3 modulates immune responses in vitro and in vivo. Life Sci 73:2029–2045

Owen C, Kevin M, Stephen B, Paul D, Amanda S (2015) Antiproliferative activity of New Zealand propolis and phenolic compounds vs human colorectal adenocarcinoma cells. Fitoterapia 106:167–174

Park CH, Chang JY, Hahm ER, Park S, Kim HK, Yang CH (2005) Quercetin, a potent inhibitor against beta-catenin/Tcf signaling in SW480 colon cancer cells. Biochem Biophys Res Commun 328(1):227–234

Patel S (2016) Emerging adjuvant therapy for cancer: propolis and its constituents. J Diet Suppl 13:245–268

Pyrzynska K, Biesaga M (2009) Analysis of phenolic acids and flavonoids in honey. TrAC Trends Anal Chem 28:893–902

Ranelletti FO, Ricci R, Larocca LM et al (1992) Growth-inhibitory effect of quercetin and presence of type-II estrogen-binding sites in human colon-cancer cell lines and primary colorectal tumors. Int J Cancer 50:486–492

Rao CV, Desai D, Simi B, Kulkarni N, Amin S, Reddy BS (1993) Inhibitory effect of caffeic acid esters on azoxymethane-induced biochemical changes and aberrant crypt foci formation in rat colon. Cancer Res 53:4182–4188

Rather R, Bhagat M (2019) Quercetin as an innovative therapeutic tool for cancer chemoprevention: molecular mechanisms and implications in human health. Cancer Med. https://doi.org/10.1002/cam4.1411

Rathore K, Wang HC (2013) Mesenchymal and stem-like cell properties targeted in suppression of chronically-induced breast cell carcinogenesis. Cancer Lett 333:113–123

Rauf A, Khan R, Raza M et al (2015) Suppression of inflammatory response by chrysin, a flavone isolated from *Potentilla evestita* Th. Wolf in silico predictive study on its mechanistic effect. Fitoterapia 103:129–135

Refolo MG, D'Alessandro R, Malerba N, Laezza C, Bifulco M, Messa C, Caruso MG, Notarnicola M, Tutino V (2015) Anti proliferative and pro apoptotic effects of flavonoid quercetin are mediated by CB1 receptor in human colon cancer cell lines. J Cell Physiol 230 (12):2973–2980

Rossi A, Ligresti A, Longo R, Russo A, Borrelli F, Sautebin L (2002) The inhibitory effect of propolis and caffeic acid phenethyl ester on cyclooxygenase activity in J774 macrophages. Phytomedicine 9(6):530–535

Salucci M, Stivala LA, Maiani G, Bugianesi R, Vannini V (2002) Flavonoids uptake and their effect on cell cycle of human colon adenocarcinoma cells ($CaCO_2$) Br. J Cancer 86:1645–1651

Saxena S, Gautam S, Maru G, Kawle D, Sharma A (2012) Suppression of error prone pathway is responsible for antimutagenic activity of honey. Food Chem Toxicol 50(3–4):625–633

Scholzen T, Gerdes J (2000) The Ki-67 protein: from the known and the unknown. J Cell Physiol 182(3):311–322

Schramm D, Karim M, Schrader H, Holt R, Cardetti M, Keen C (2003) Honey with high levels of antioxidants can provide protection in healthy human subjects. J Agric Food Chem 51:1732–1735

Soad KAJ, Mohammad SAM, Abear H, Iman E, Rajaa AR, Saad MAM, Saad A, Mohammad AM, Steve H (2017) Effects of honey on oral mucositis among pediatric cancer patients undergoing chemo/radiotherapy treatment at King Abdulaziz University Hospital in Jeddah, Kingdom of Saudi Arabia. Evid Based Complement Alternat Med 2017:5861024

Spencer JP, Chowrimootoo G, Choudhury R, Debnam ES, Srai SK, Rice-Evans C (1999) The small intestine can both absorb and glucuronidate luminal flavonoids. FEBS Lett 458:224–230

Staniforth V, Chiu L-T, Yang N (2006) Caffeic acid suppresses UVB radiation-induced expression of interleukin-10 and activation of mitogen-activated protein kinases in mouse. Carcinogenesis 27(9):1803–1811

Sugarman BJ, Aggarwal BB, Hass PE (1985) Recombinant human tumor necrosis factor-α: effects on proliferation of normal and transformed cells in vitro. Science 230(4728):943–945

Sun C, Wu Z, Wang Z, Zhang H (2015) Effect of ethanol/water solvents on phenolic profiles and antioxidant properties of beijing propolis extracts. Evid Based Complement Alternat Med 2015:595393

Tahir AA, Sani NFA, Murad NA, Makpol S, Ngah WZW, Yusof YAM (2015) Combined ginger extract & Gelam honey modulate Ras/ERK and PI3K/AKT pathway genes in colon cancer HT29 cells. Nutr J 14:1–10

Tournier C, Hes P, Yang DD, Xu J, Turner TK, Nimnual A, Bar-Sagi D, Jones SN, Flavella RA, Davis RJ (2000) Requirement of JNK for stress-induced activation of the cytochrome cmediated death pathway. Science 288(5467):870–874

Tomasin R, Cintra Gomes-Marcondes MC (2011) Oral administration of *Aloe era* and honey reduces walker tumour growth by decreasing cell proliferation and increasing apoptosis in tumour tissue. Phytother Res 25(4):619–623

Tram Anh P, Xiao-Min Y, Muthusamy K, Herbert C (2011) Antiproliferative effect of chrysin on anaplastic thyroid cancer. J Surg Res 170(1):84–88

Tseng RC, Lee CC, Hsu HS, Tzao C, Wang YC (2009) Distinct HIC1-SIRT1-p53 loop deregulation in lung squamous carcinoma and adenocarcinoma patients. Neoplasia 11(8):763–770

Tyagi AS, Ramasamy RP, Raina K, Redente EF, Dwyer-Nield LD, Radcliffe RA, Malkinson AM, Agarwal R (2009) Growth inhibition and regression of lung tumors by silibinin: modulation of angiogenesis by macrophage-associated cytokines and nuclear factor-κB and signal transducers and activators of transcription 3. Cancer Prev Res 2:74–83

Usia T, Banskota AH, Tezuka Y, Midorikawa K, Matsushige K, Kadota S (2002) Constituents of Chinese propolis and their antiproliferative activities. J Nat Prod 65:673–676

Van Acker SABE, Van den Berg B, Tromp MNJL et al (1996) Structural aspects of antioxidant activity of flavonoids. Free Radic Biol Med 20(3):331–342

Vijayababu MR, Arunkumar A, Kanagaraj P, Venkataraman P, Krishnamoorthy G, Arunakaran J (2006) Quercetin downregulates matrix metalloproteinases 2 and 9 proteins expression in prostate cancer cells (PC-3). Mol Cell Biochemistry 287:109–116

Viuda-Martos M, Ruiz-Navajas Y, Fernandez-Lopez J, Perez-Alvarez JA (2008) Functional properties of honey, propolis, and royal jelly. J Food Sci 73:R117–R124

Wagner EF, Nebreda ÁR (2009) Signal integration by JNK and p38 MAPK pathways in cancer development. Nat Rev Cancer 9:537–549

Waheed M, Hussain MB, Javed A, Mushtaq Z, Hassan S et al (2019) Honey and cancer: a mechanistic review. Clin Nutr 38(6):P2499–P2503

Wang XH, Andrae L, Engeseth NJ (2002) Antimutagenic effect of various honeys and sugars against Trp-p-1. J Agric Food Chem 50(23):6923–6928

Wang LC, Chu KH, Liang YC, Lin YL, Chiang BL (2010) Caffeic acid phenethyl ester inhibits nuclear factor-κB and protein kinase B signalling pathways and induces caspase-3 expression in primary human CD4+ T cells. Clin Exp Immunol 160(2):223–232

Won C, Lee CS, Lee JK, Kim TJ, Lee KH, Yang YM et al (2010) CADPE suppresses cyclin D1 expression in hepatocellular carcinoma by blocking IL-6-induced STAT3 activation. Anticancer Res 30:481–488

Woo KJ, Jeong YJ, Park JW, Kwona TK (2004) Chrysin-induced apoptosis is mediated though caspase activation and Akt inactivation in U937 leukemia cells. Biochem Biophys Res Commun 325:1215–1222

Wu J, Omene C, Karkoszka J, Bosland M, Eckard J, Klein CB, Frenkel K (2011) Caffeic acid phenethyl ester (CAPE), derived from a honeybee product propolis, exhibits a diversity of anti-tumor effects in pre-clinical models of human breast cancer. Cancer Lett 308:43–53

Xia Y, Lian S, Khoi PN, Yoon HJ, Joo YE, Chay KO, Kim KK, Do Jung Y (2015) Chrysin inhibits tumor promoter-induced MMP-9 expression by blocking AP-1 via suppression of ERK and JNK pathways in gastric cancer cells. PLoS One 10:e0124007

Xiao J, Zhai H, Yao Y et al (2014) Chrysin attenuates experimental autoimmune neuritis by suppressing immuno-inflammatory responses. Neuroscience 262:156–164

Yao L, Datta N, Tomás-Barberán FA, Ferreres F, Martos I, Singanusong R (2003) Flavonoids phenolic acids and abscisic acid in Australian and New Zealand leptospermum honeys. Food Chem 81:159–168

Yoshida M, Yamamoto M, Nikaido T (1992) Quercetin arrests human leukemic T-cells in late G1 phase of the cell cycle. Cancer Res 52:6676–6681

Zaida Z, Zairos F, Zainal A, Siew HG, Wan ZW, Abdul HM, Mahaneem M (2018) Effects of honey supplementation on safety profiles among postmenopausal breast cancer patients. J Taibah Univ Med Sci 13(6):535–540

Zhang W, Lan Y, Huang Q, Hua Z (2013) Galangin induces B16F10 melanoma cell apoptosis via mitochondrial pathway and sustained activation of p38 MAPK. Cytotechnology 65:447–455

An Assay on Mechanisms of the Anti-Fibrotic Effects of Honey

5

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Abstract

Fibrosis is a consequence of diverse continual compensation of various connective tissue in the body. That results in the formation of extracellular matrix that is why it forms a sandwiched between matrix and deterioration. It is well resolute and articulated as scarring and as of particular cell lines fibrosis is developed, such conditions are known as a fibroma. Immune cells produce several factors like cytokines, chemokines, etc. that serves as the best factors which affects the inflammation. A variety of synthetic and herbal drugs are accessible in the market that are specially designed for the treatment of fibrosis. Throughout the remedies, honey is one of the most common and easily available ingredient of our kitchen that contains numerous enzymes, vitamins, organic acids, flavonoids, phenolic acids, amino acids, and volatile components. These phytochemicals are responsible for different pharmacological activities including anti-fibrotic effect. In this chapter, we focused on mechanisms of the anti-fibrotic effects of honey and we conclude that how honey inhibits the inflammatory cytokines, chemokines, and other factors viz. PDGF, IL-4, TGF-β, and interleukin-13. These biomarkers are incorporated with proliferation and causes activation of myofibroblast cells. Balance for the extracellular matrix degradation and its synthesis helps determine ECM homeostasis. At last, these immune cells, endothelial or epithelial cells lead

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to apoptosis, proliferation, and repair at the damage site, resulting in the healing of the wound and showed a strong anti-fibrotic effect.

Keywords

Honey · Fibrosis · Anti-fibrotic effect · Extracellular matrix · Chemokines

5.1 Introduction

Nowadays numerous herbal preparations are used by the physicians for the treatment of different diseases due to various side effects of allopathic preparations. The research is going on in search of natural drugs having less or minimal side effects. In this journey, we can't forget the name of honey as it is used for the preparation of various ancient Egyptian and Greeks along with numerous Unani, Ayurvedic, Naturopathic, and Traditional Chinese preparations (Eteraf-Oskouei and Najafi 2013; Miguel et al. 2017). Honey is naturally sweet and viscous food product prepared by honey bees with the help of nectar obtained for flowers.

It is determined by color and odor. Light color honeys have high value of marketed price as compare to darker one. Sweetness of honey is due to presence of monosaccharides, glucose, and fructose. Rest of constituents are various enzymes, vitamins, organic acids, flavonoids, phenolic acids, amino acids, and volatile components (Alqarni et al. 2014).

Fibrosis can be defined as the development, overgrowth, hardening, and/or scarring of additional connective tissue (fibrous) in any part of the human body especially organ or tissue which has a role in a reactive process (Birbrair et al. 2014). When we focus on fibrosis we found that there is a deposition of large amount of components, especially at the matrix portion behind it (Glick et al. 2010; Wynn 2011). It is well determined and expressed as scarring and from specified cell lines fibrosis is developed, such conditions are known as fibroma (Birbrair et al. 2014). When chronic inflammation is developed in the body then, at last, it is referred to fibrosis due to various stimuli, especially immune reactions, allergic reactions, persistent infections, radiations, and various tissue damages. Recent facts show that there are several novel pathogenic mechanism that plays a critical role in the progression and reverse establishment of fibrosis (Wynn 2011). There are different types of fibrosis depending on the organ they are present. Some of them are enlisted in Table 5.1.

5.1.1 Diagnosis

It can be diagnosed with the help of computerized tomography images of fibrosis, gross pathology of fibrosis, histopathology of fibrosis, X-rays of fibrosis, etc. (Sica et al. 2019).

Table 5.1 Major organs affected by fibrosis, types of fibrosis, and possible contributing factors

Organ	Type of fibrosis	Possible contributing factors	References
Liver	Cirrhosis Bridging fibrosis	Viral hepatitis, hepatocellular carcinoma, schistosomiasis, and alcoholism	Dwivedi and Jena (2018), Li et al. (2017)
Lung	Cystic fibrosis, replacement fibrosis, focal fibrosis, diffuse parenchymal lung disease, idiopathic pulmonary fibrosis, radiation-induced lung injury	Sarcoidosis, silicosis, drug reactions, rheumatoid arthritis, systemic sclerosis, and idiopathic pulmonary fibrosis	Rockey et al. (2015) Borthwick et al. (2013)
Kidney	Renal fibrosis Cystic fibrosis Nephrogenic systemic fibrosis	Chronic kidney diseases, diabetes and hypertension	Rockey et al. (2015), Li et al. (2017)
Heart	Atrial fibrosis Endomyocardial fibrosis Old myocardial infarction	Cardiac fibrosis, hypertrophic cardiomyopathy, heart attack, hypertension, atherosclerosis and arrhythmia	Eddy (2000) Li et al. (2017)
Eye	Subretinal fibrosis, Epiretinal fibrosis	Macular degeneration, retinal and vitreal retinopathy	Li et al. (2017)
Skin	Keloid (skin fibrosis) Nephrogenic systemic fibrosis	Hypertrophic scars, systemic sclerosis, scleroderma, multiple cancers, and pulmonary arterial hypertension	Li et al. (2017)
Pancreas	Pancreatic fibrosis	Autoimmune and hereditary causes	Wynn (2011)
Intestine	Crohn's disease	Inflammatory bowel disease and pathogenic organisms	Wynn (2011)
Brain	Glial scar	Alzheimer's disease and AIDS	Li et al. (2017)
Bone marrow	Myelofibrosis	Chronic myelogenous leukemia, Myelodysplastic syndrome and aging	Li et al. (2017)
Others	Arthrofibrosis (knee, shoulder, other joints) Dupuytren's contracture (hands, fingers) Mediastinal fibrosis (soft tissue of the mediastinum) Peyronie's disease (penis)	Surgical complications, scar tissues, chemotherapeutic drug-induced fibrosis, radiation-induced fibrosis, and mechanical injuries	Parish and Rosenow (2002), Li et al. (2017)

5.1.2 Physiology

In case of fibrosis, myofibroblast is the cellular mediator. These fibroblasts serve as initial collagen developing cells and upon maturation, they produce mesenchymal, endothelial, and epithelial cells. Cellular mediators were activated with the help of various mechanisms, especially paracrine signals developed from various macrophages and lymphocytes. Myofibroblast secretes autocrine factors and pathogen species produce pathogen-associated molecular patterns (PAMPS). These

PAMPS then interacts with recognition receptors, chemokines, cytokines, monocytes, chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein-1 β (MIP-1 β).

Cytokines that are present in human body are transforming growth factor-beta 1 (TGF-β1) and interleukins (IL). Growth factors are platelet-derived growth factors (PDGF) and angiogenic factors are vascular endothelial growth factor (VEGF), caspase, acute-phase proteins, and peroxisome proliferator-activated receptors (PPARs). Rennin angiotensin aldosterone II acts as a manager of fibrosis and targeted for drugs which were used for the treatment of fibrosis.

5.1.3 Prevalence and Incidence

There are several studies reported for the evaluation of incidence and effectiveness of fibrosis. According to different agencies, the most afferent data range from 4.6 to 7.4 cases/100,000 and 13 to 20 cases/100,000 for females and males, respectively (Flume et al. 2009). On the basis of earlier data, it is understandable that in Europe, fibrosis affect near about 7500 people. There is an unknown concept for the incidence and prevalence of the fibrosis, that is why geographical and racial factors influenced it most (Kim et al. 2015). Level of prevalence is increased nowdays due to strong optimization of latest diagnostic methods and lifestyle increment.

5.1.4 Prognosis and Etiological Causes

The prognosis of fibrosis is underprivileged and the survival rate is only 2–3 years after its diagnosis (Kim et al. 2015). Delay in progression leads to chronic respiratory failure. In rest of cases, there are periods of comparable stability with particular worsening in complications and sometime death also. In small number of patients, the duration of disease is very short with fast progression. Generally average rate of survival is 2–5 years as determined in symptoms (Raghu et al. 2011).

The etiological causes of fibrosis are not well determined due to diagnostic complications. As this disease occurs due to various predetermined factors like genetic, environment, immunological, and others; that plays an important role in the etiology of fibrosis. Genetic mutation is one of the most effective alterations that maintain the length of telomeres. Fibrosis is commonly due to the presence of various surfactants like mucin 5B promoter region and protein C gene. It is determined that there is no well-established genetic test to carry out predisposition for fibrosis (Wu et al. 2018). Apart from genetic, environmental factors also a predominant risk factor for the development of fibrosis. Examples like exposure of silicon, steel, brass, lead, farming, smoking, and wooden houses (Cystic Fibrosis Trust 2014). Some studies also determine that gastroesophageal reflux, autoimmune diseases, and immunosuppressive therapy are major and effective risk factors for progression and predisposition of fibrosis (Ng and Moore 2016; Michael et al. 2015; Abdalla et al. 2009). The other factors like radiology and histology are also well-

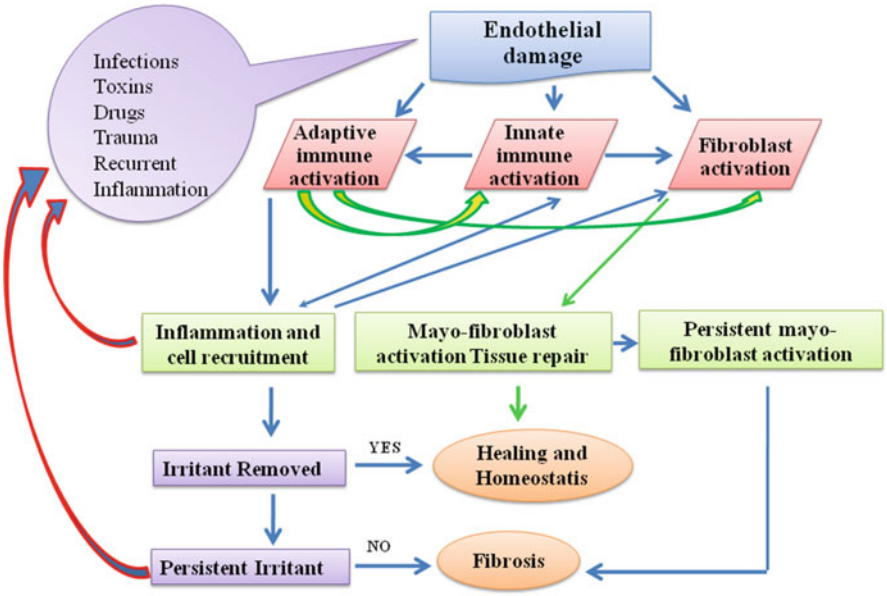

Fig. 5.1 Diagrammatic representation of how honey acts in the pathogenesis of fibrosis

established manifestation of fibrosis along with the factors associated with connective tissue create fibrosis (Al-Momani et al. 2016).

5.1.5 Pathogenesis

Fibrosis is the pathological result of normal wound healing process. Generally, when injuries generated at that time endothelial and epithelial cells are destroyed and release pro-inflammatory cytokines with the help of cascade coagulation and immune cell replacement (Darby and Hewitson 2007). The most important of them are macrophages and neutrophils. Tissue damage and dead cells are removed by the determined immune cells due to acute inflammation (Klingberg et al. 2013). At the same time, these immune cells produce several factors like cytokines, chemokines, which serve as best factors that affected inflammation. The other factors are like growth factors viz. PDGF, IL-4, TGF-β, and interleukin-13 which are incorporate a limited amount of proliferation and activation of myofibroblast cells (Wynn and Vannella 2016). Myofibroblasts were derived from various multiple cells like epithelial cells, bone marrow cells, and epithelial endothelial mesenchymal transitional cells. These cells are properly related to various blood vessels except resident fibroblast. Precursor cells such as pancreatic stellate cells (PSC) and hepatic stellate cells (HSC) may also develop myofibroblastic phenotype in the liver and pancreas.

Therefore, myofibroblast may achieve the balance for the extracellular matrix (ECM) degradation and its synthesis is helpful in determining ECM homeostasis. At last, these immune cells, endothelial, or epithelial cells lead to apoptosis, proliferation, and repair at the damage site, resulting in healing of wound (Darby et al. 2014; Ghosh et al. 2013) (Fig. 5.1).

5.2 Factors Affecting the Progression of Fibrosis

5.2.1 Extracellular Factors

Most of the extracellular factors associated with fibrosis are ligands that bind with receptors, like growth factors and cytokines. In endocrine signaling pathways, autocrine and paracrine are the factors which target distant cells and adjacent cells (Gabbiani 2003). They instead bind to specific cell membrane receptors which activate intracellular signaling, culminating in cellular level pro-fibrotic responses. Various extracellular factors, particularly enzymes which are effective for their property such as metalloproteinases matrix (MMPs), can diminish ECM to prevent excessive level of accumulation (Vettori 2012).

5.2.2 Growth Factors

These are the large protein molecules that trigger the proliferation and growth of various cells. They are released by different cells to produce cellular responses as well as fibroblast proliferation (Kendall and Feghali-Bostwick 2014; Marvig et al. 2015). Transforming growth factor beta (TGF-β) is the chief determinant which plays an important role in fibrogenesis by regulating cell growth through small mothers against decapentaplegic (Smad) (Scales and Huffnagle 2013). Along with that, it also causes the overexpression of α-hallmark muscle actin (α-SMA) myofibroblast. However, TGF-β signaling responds to collagen I and III gene transcription contributing to ECM accumulation (Luchetti et al. 2014). Platelet-derived growth factor (PDGF) can induce the hematopoietic stem cell derived proliferation and collagen expression. Hepatocyte growth factors (HGF) are also responsible for fibrosis which helps reduce overexpression (Fan et al. 2018). In case of inflammatory type of wound healing process, excessive level of cytokines especially chemokines are released by the immune cells which include neutrophil, T cells, and macrophages. When we compare growth factors to that of cytokines, it acts as a transmitter for the cell signaling and results in increased immunological response of cell and ultimately leads to inflammation in cells. Chemokines having the property to migrate into liver and there they produce fibrosis (Gressner and Gressner 2008). Interleukin 6 is also reported as agent that moves various tissue conservation via signal transducer and transcription activator 3 that type of signaling pathway in some peritoneal type of fibrosis to a chronic inflammatory state (Luchetti et al. 2014).

5.2.3 Broad Range of Proteins/Peptides

Wide range of protein and peptides are other extracellular variables that differentiate myofibroblast with growth factor. For example, during embryonic growth and responds to defect through Snail and Twist's inhibition of epithelial-cadherin signal. Hh signaling pathway epigenetic regulation refers to biliary level of fibrosis along with that it is also associated with liver fibrosis. The up determinant type of signaling was well reported in an ample supply of β catenin in either fibrotic kidney. The pathways control epithelial-to-mesenchymal transition (EMT) induced genes like Twist, LEF 1, which aggravates disorder (Van Mourik 2017).

5.2.4 Matrix Metalloproteinases (MMPs)

Extracellular endopeptidases (laminins, fibronectin, proteoglycans, and collagens) are the factors that degrade the ECM metalloproteinase tissue inhibitors (TIMPs) can also function as MMPs inhibitors. To maintain the homeostasis, MMPs and TIMPs modulate the system either by development of fibrosis or cell injuries. It can be in balance position by the activation of various latent immune cells and some myofibroblast (Barr et al. 2018).

5.2.5 Intracellular Factors

Intracellular factors also modulate and relocate the expression of some extracellular factors, such as cytokines and growth factors. Certain inflammatory pathways and metabolize them from cells to enhance immune responses. In addition, epigenetic factors function as a new way of manipulating gene expression correlated with fibrosis (Xu et al. 2016).

5.2.6 Nuclear Receptors

These types of receptors are located in the cytoplasm and nucleus. They receive various signals from intercellular ligands to bind with specific receptor inside the DNA and regulate genetic expression (Rieger et al. 2015). Some examples are like proliferator-activated receptor γ (PPAR-γ), peroxisome which may completely and directly manage and regulate type I collagen gene expression and can block TGF β signaling pathways. Other receptors are also found in nucleus like farnesoid-X receptor (FXR) which can exhibit an fibrotic protecting effect via the reduction of various proliferation type of cholangiocytes and subsequent reduction in TGF β (Manley and Ding 2015). The strong activation of FXR which can also decrease and diminish a series of strong type of profibrotic factors especially including α-SMA, collagens, TIMP-1, and MMP-2 (Ali et al. 2015).

5.2.7 Multiple Kinases

There are different intracellular kinase which serves as a reservoir in the inflammatory process like TNF-α, epidermal growth factors, and TGF-β. They initiate the mitogen-activated protein kinase pathway (MAPK). Among all of them, TNF-α activates another important deterministic mammalian target of rapamycin (mTOR). This mTOR receptor causes activation of transcription factor β-1 and decreases the accumulation of collagen and fibrosis (Afroz et al. 2016a, b).

5.2.8 Galectin-3/Lysyl Oxidase Homolog 2 (LOXL2)/Oxygen Species (ROS)

There are such factors influencing either extra/intracellular mechanisms of fibrosis, particularly species with galectin-3, reactive oxygen species (ROS), and homologous lysyl oxidase. Tissue damages, cell damage, and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activities will produce ROS. The ligand binding oxygen species targets the paptides and triggers TGF-β signals, whereas inteacellular oxidative stress in human body targets p53 cells. That causes apoptosis and activation of mitochondrial caspase inside the cells. So far as galectin-3 is involved, extracellular type of galectin-3 which can induce apoptosis in T type of cells performs as well-defined dual activity in and outermost side of cells (Tzou et al. 2014). The LOXL2 variable is usually considered as extracellular enzymes, which then in reaction to excessive pressure promotes the production of collagen and crosslink with collagen fibers. Moreover, intracellular type of LOXL2 has been well reported to induced EMT in the development of carcinoma. On such basis, the targets involved in these mechanisms will have to be reexamined and their possible functions determined in fibrosis type of treatment across tissues and organs (Table 5.2; Aydin and Akcali 2018).

5.3 A Honey Boon for a Human Being

Honey is a historic item whose background can be directly attributed 8000 years in Europe but still has popular being used as a popular food product (Alvarez-Suarez et al. 2014). Unlike the other traditional food items, honey also has its cultural importance and symbolic significance in certain communities, which has led its effectiveness as therapeutic agent. Overall, honey is a result arising from any insects that store nectar in hives, but perhaps the item produced by honeybees, the subfamily Apis, is usually referred to as honey (Kwakman et al. 2010). Honey in the severe form is generated via a process of network rehashing and evaporation ability to concentrate in the beehive. Honey continues to develop within beehives as the main bee food in wax honeycombs. Honey's deep taste derives from the very high sugar content of fructose and glucose (Jull et al. 2015). The organic mechanism is derived from indicating that honey contains many biochemical and biochemical compounds.

Table 5.2 List of targeting factors for prognosis of fibrosis

Group	Target or mechanism type	Target or mechanism	Organs	References
Targeting intracellular factors				
Enzymes	mTOR	mTOR complex 1/2	Liver, kidney, Lung, heart, Skin, gut	Li et al. (2017)
Nuclear receptors	PPAR	PPAR-γ	Liver, kidney, Lung, heart, Pancreas, skin	Gao and Bataller (2011)
Other proteins	Intracellular TGF-β signaling	SMAD2/3	Liver, kidney, Lung, heart	Verrecchia and Mauviel (2007)
Epigenetics	miRNA	miR-21		Li et al. (2017)
Targeting extracellular factors				
Growth factors	Extracellular TGF-β signaling TGF-β	TGF-β	Liver, kidney, Lung, heat, Pancreas, skin	Biernacka et al. (2011)
Cytokine	Interleukin	IL-13	Liver, kidney, Lung, heart, pancreas, Skin, gut	Borthwick et al. (2013)
MMP/TIMP	MMP/TIMP	MMP-2/ MMP-9/ TIMP-1	Liver, kidney, Lung, skin, Heart, pancreas	Gieseck et al. (2018)
Other proteins and peptides	Endothelin	ET-1 receptor	Liver, kidney, Lung, heart, Skin, gut	Fan et al. (2012)

Honey from its natural state is commonly used as fresh produce. Honey can also be used in many baking procedures and goods where natural tartness is supplied. Honey's physiological structure is that its base is water having high osmolality. That is why pathogens do not develop in honey, their biological source means that they might exhibit bacteria and microorganism spores along with highly poisonous types such as *Clostridium botulinum*, some of the most toxic substances known to mankind (Rybak-Chmielewska 2003). The many substances and growth factors used in honey have led in their use for medical purposes (Fig. 5.2). Various variety of honey with their specification is given in Table 5.3.

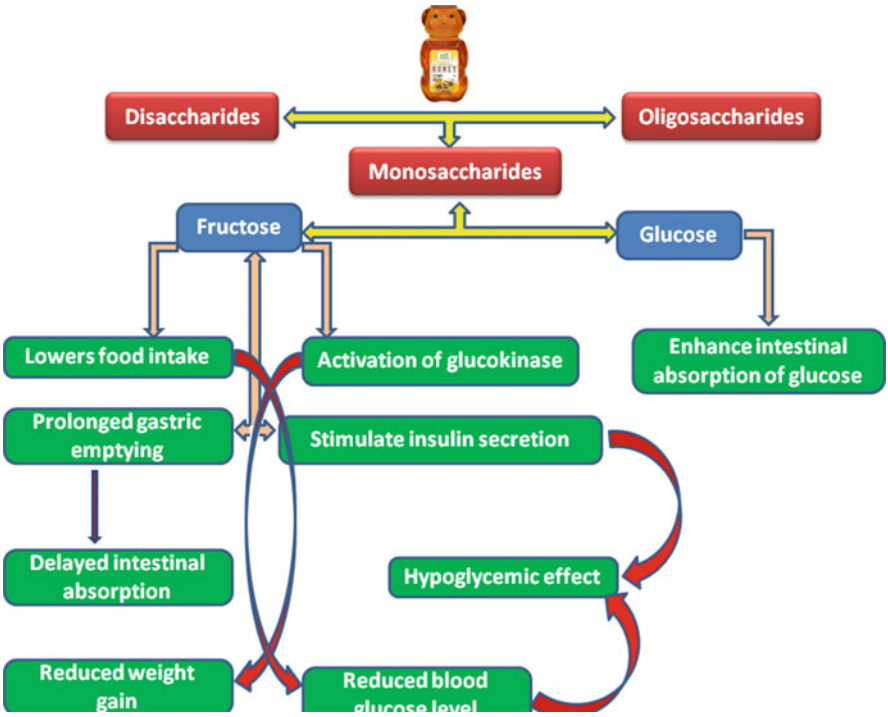

Fig. 5.2 Different uses and health benefits of honey for human beings

5.4 Adulteration of Honey

As the need for any food item is increased as well as its supply is less in that case suppliers opt for adulteration process. The production and cost of honey depend upon weather and harvesting condition. It is subjected to two types of adulteration:

1. Indirect.
2. Direct.

5.4.1 Indirect

Indirectly honey is adulterated by proving factory sugar to the honeybees at natural breeding stage. Such indirect adulteration is very difficult to detect. For discrimination between pure and impure, several chromatographic techniques are used. These chromatographic techniques are useful only when there is an adulteration between 10 and 40% (Siddiqui et al. 2017).

Table 5.3 Different types of honey with their specifications

Type of honey	Specification	Reference
Acacia honey	Most popular honey Identification: light and clear Taste: floral Fructose concentration is high to retain in the liquid state Popular in diabetics	(Muhammad et al. 2016)
Alfalfa honey	Majority production in Canada and the United States Light in color Made from purple–blue blossoms Taste and aroma: mild floral Used for backing purpose	(Akbari et al. 2020)
Aster honey	Light color Honey Extracted from Midsouth region and the United States The thick and smooth consistency Crystallize faster than other honey Used as a natural sweetener	(Akbari et al. 2020)
Avocado honey	Extracted from California avocado blossoms Dark-colored honey Buttery flavor	Almasaudi et al. (2017)
Basswood honey	Found throughout North America Most popular for biting taste White color and exceptional malleability quality Produced from cream-colored basswood blossoms	Sporns et al. (2007)
Beechwood honey	Known as Honeydew Found in New Zealand Produced by aphids on the bark of beechwood tree Used as a syrup for pancakes Regular consumption increase body immunity, better digestive system, high nutritional value	Popek (2002)
Blueberry honey	Pleasant flavor Produced in New England Extracted from white flowers of blueberry bush Light amber-colored	Akbari et al. 2020
Bluegum honey	Eucalyptus honey species Grows in South Australia and Tasmania Dense texture and amber color	Lu et al. (2014)
Buckwheat honey	Strongest and darkest honey Produced in Minnesota, Ohio Rich source of iron and other essential nutrients Antioxidant property	Popek (2002)
Clover honey	Most widely available and popular honey Produced across Canada and New Zealand Classic one because of pleasant and floral sweet taste	Almasaudi et al. (2017)
Dandelion honey	Relatively strong honey Widely produced in New Zealand Bears dandelion aroma considered a medicinal herb in China, Tibet, India due to its healing nature	Cooper et al. (1999)

(continued)

Table 5.3 (continued)

Type of honey	Specification	Reference
Eucalyptus honey	Produced in Australia and California Strong medicinal honey variety Used for cold and headaches The herbal flavor and a slight aftertaste of menthol	(Akbari et al. 2020)
Fireweed honey	Obtained from a tall herb grown in open woods of north-west United States Light color Sweet and complex at the same time The smooth, delicate and buttery taste	Sherlock et al. (2010)
Heather honey	Strongest and most pungent flavors Amber in color and a rich source of protein Almost bitter	(Akbari et al. 2020)
Iron bank honey	Eucalyptus floral variety with bold taste Extracted throughout the year in eastern Australia	Sommeijer (1999)
Jarrah honey	Dark- amber eucalyptus variety Caramel aftertaste An effective remedy for wounds, burns, skin allergies	Lu et al. (2014)
Leatherwood honey	Obtained from leatherwood blossom Available in Southwest region Tasmania, Australia Unique taste and strong flavor Known as Tasmanian honey	Shukrimi et al. (2008)
Linden honey	Light yellow color The very delicate and fresh woody scent Medically rich verities Sedative properties help immensely in cases of anxiety and insomnia Used for treatment of cold, cough, and bronchitis	Gupta and Sharma (2009)
Macadamia honey	Sourced in Australia Floral nectar of Macadamia Nut tree Deep in color with a complex aroma and has a subtle nutty flavor	Kasiotis et al. (2014)
Manuka honey	Produced in New Zealand coastal areas Collected from the flower of Tea Tree bush Rich antibacterial, effectively treating stomach ulcers sore throat, indigestion, and acne and pimples	Avila et al. (2018)
Orange blossom honey	Combination of citrus sources Light in color and mild flavor with a fruity scent Obtained from Spain and Mexico	Bobiş et al. (2018)
Pinetree honey	Mainly comes from Greece Slightly bitter taste with a strong aroma Rich minerals and proteins	Abdulrhman et al. (2013)
Sourwood honey	Light color, caramel kind of taste Sweet and nutty	Kadirvelu and Gurtu (2013)
Sage honey	Heavy bodied Sage honey Produced in California Packed with the property of granulate Delightful taste	Bahrami et al. (2009)

(continued)

Table 5.3 (continued)

Type of honey	Specification	Reference
Tupelo honey	Most premium honey Southern gold tupelo honey Produced in southeastern US swamps Granulating like quality	Umesh et al. (2008)

5.4.2 Direct

Inclusion of other substances in honey is termed as clear adulteration. This type of adulteration is detected by the means of characterization of physical and chemical properties. S86 honey is the example of that type of characterization which requires extensive sample preparation and complex analytical equipment along with it is a time-consuming process. This type of adulteration can be characterized by the help of modern analytical techniques such as nuclear magnetic resonance and calorimetric analysis (Siddiqui et al. 2017; Padovan et al. 2003).

5.5 The Pharmacological Effect of Honey

5.5.1 Antioxidant Activity

A lot of diseases are due to the generation of reactive oxygen species, which are generated during the metabolism. The antioxidants are the agent that intercepts free radicals prior to they get damage. Darkness of honey is directly proportional to its antioxidant activity; hence, we can say that darker the honey, richer the source of antioxidants. Honey is an ample source of phenolic compounds and flavonoids, which act as a reservoir for antioxidant activity. These compounds act as an antioxidant due to its donating ability of hydrogen ion and electrons, which result in the generation of free radical as a consequence due to oxidative stress (Afroz et al. 2016a, b).

5.5.2 Antimicrobial Property

The dynamic cause of antimicrobial property of honey is due to the presence of glucose oxidase enzyme and the physical properties of honey. Rest factors are increase osmotic pressure; down pH; protein in less amount; increase in reducing sugar, carbon, and nitrogen ratio; viscosity; and other phytochemical constituents present in the honey (Abd Jalil et al. 2017; Allen et al. 1991).

5.5.3 Antibacterial Property

The antibacterial effect of honey is due to its property to release the peroxide ion commencing due to glucose oxidation to form gluconolactone and gluconic acid in the existence of oxidase enzyme. This activity was called peroxide activity and constitutes, at variable extent, the mode of action of some honey (Henriques et al. 2006).

5.5.4 Antiviral Property

The antiviral property of honey owes due to the presence of flavonoids (chrysin, acacetin, and apigenin). Several researches suggest that honey can inhibit the human immunodeficiency virus (HIV) either due to the activation via inhibiting the transcription of virus. Chrysin a constituent of honey shows activity against herpes simplex virus (type 1). One study suggests that the chrysin and apigenin have significant antiviral activity against HIV-1 in acutely infected H9 lymphocytes. Apigenin also exhibited antiviral activity against the influenza virus (H3N2) in vitro (Liu et al. 2008).

5.5.5 Anticancer

Honey shows its anticancer property by the potent inhibition on the progression of malignant cells. Its inhibitory action on malignant cell proliferation, tumor, and cancer is due to arrest in cell cycle; commencement of mitochondrial pathway; increase in the permeability of mitochondrial cell wall, apoptosis, and reduction in the development of cancer cells (Erejuwa et al. 2014).

5.5.6 Anti-Inflammatory/Immunomodulatory Activities

Honey is a protective agent, due to its chemical composition especially the phenolic content (Al-Waili and Boni 2003), it shows anti-inflammatory property which is proven by research from in vivo (Fernandez-Cabezudo et al. 2013), in vitro models (Bilsel et al. 2002) and also from the clinical trials (Leong et al. 2012). The presence of flavonoids and phenolic compounds leads to the suppression of cyclooxygenase-2 (COX-2) by altering its pro-inflammatory property (Viuda-Martos et al. 2008). Honey regulates the protein as well as other agents like ornithine, tyrosine, and nitric oxide. In the tissue culture, honey shows increase in natural killer cells and other hemocytoblast (Yaghoobi et al. 2013).

5.5.7 Wound Healing

Experimental research illustrated the use of honey upon wound healing due to the presence of several bioactive products having several activities such as germicidal, protease inhibitor activity, immune modulators, and antiaging. Honey releases the cytokines which work in the tissue repairing process. It also causes the activation of immune system which ultimately prevents the human body from infections (Yaghoobi et al. 2013).

5.5.8 Antidiabetic

Some research suggests the use of honey in diabetes due to its antioxidant property. Apart from its antioxidant property, it also causes a decline in the production of reactive oxygen species, which plays an important key role in the management of diabetes. Data from a research suggest that in a trial with patients of both types of diabetes, the honey-treated group shows a drastic decline in the glycemic index as compared with glucose. The honey-treated group in diabetic patient shows a significant decline in plasma glucose level as compared with dextran-treated group (Rao et al. 2016).

5.5.9 Neuroprotective

Honey shows protective effect on nerves due to the presence of polyphenols. Act by quenching the ROS, which is an important cause of neurotoxicity and deposition of proteins. Polyphenolic contents of honey contradict the oxidative stress, apoptotic effect, and neuroinflammation due to ischemia injury. These polyphenols also play an important role in the memory enhancement (Azman et al. 2018).

5.5.10 Cardioprotective

Honey shows its cardioprotective effect due to the presence of certain constituents viz. flavonoids, phenolic compounds, and vitamin C. The mechanism of action behind this protective role is vasodilation, decrease in coagulation of blood, and by decreasing the level of low-density lipoprotein (LDL) level (Afroz et al. 2016a, b). A number of constituents like caffeic acid, quercetin, phenethyl ester, kaempferol, galangin, and acacetin in honey are responsible for its pharmacological effect as a cardioprotective agent (Rao et al. 2016).

5.5.11 Anti-asthmatic

As listed in the above statement that honey works as a potent anti-inflammatory agent due to the presence of phenolic agents and flavonoids. These agents are also responsible for its preventive effect in asthmatic patients. Some studies on animals show that honey treats unrelieved bronchitis and bronchial asthma. Honey when inhaled at a specific dose cures goblet cell hyperplasia due to the secretion of mucus (Srivastav et al. 2011).

5.6 Pharmacokinetics of Honey

The physical and chemical characteristics of honey specimens were calculated (water content, color, and pH). The second component of honey is water (20%) by weighing and honey's pH is no more than 5. Honey varieties were seen as no diverse in terms of pH and fluid content. The preliminary color of examined honey varies according to several variables such as flower form and its duration (from the period of fermentation to date of the study). Honey the Siam weed flower is noticed to have had the highest color. Although longan and dual-floral honey have the same color spectrum, with the exception of pH 7, which would be induced by the fermentation date discrepancy (Zaworra et al. 2019). Maillard reaction rate and product character are calculated by the chemical nature, along with percentage of water, pH, temperature, and its composition. The essential kinetic variables in reaction of Maillard are temperature and water content. During this analysis, under specific condition, the temperature effect was examined. Therefore, honey water content was steady (Karl-Fischer titrations). For quality control, European Union used hydroxyl methyl furfural (HMF) as a chemical indicator. HMF composition is only affected by temperature. Kinetic studies have tracked the consequences of sugar and amino acid on the time, by the means of bright and dark environments with temperatures from 25 to 85 °C. The kinetic variables were extracted from not only product formation (HMF), as well as from declines in molecules (reduction of sugars and amino acids). From the temperatures test, it is mentioned in literature that carbohydrates and amino acids decreased by 55 and 85% simultaneously by changing the temperature from 25 to 85°. On the other hand, there has also been a substantial increase in HMF content. The reactions show first-order kinetics with amino acids and for HMF it shows zero-order kinetics which determines honey kinetics (Zhang et al. 2018a, b).

5.7 Possible Mechanisms of Honey and Its Components as Anti-fibrotic Agent

The literature review on medicinal properties of honey includes its application as a potent anti-fibrotic agent. There are different types of honey that have their own health benefits; among all, the *manuka honey* is well known for its medicinal

properties. It protects against apoptosis, intracellular ROS production, oxidative damage, mitochondrial dysregulation, and cell differentiation by activating the signaling pathway of adenosine monophosphate-activated protein kinase (AMPK) or nuclear factor erythroid 2 related factor 2 (Nrf2) or antioxidant response elements (ARE). Apart from that it also modifies different antioxidant biomarkers viz. superoxide dismutase (SOD), catalase, and glutathione (GSH) (Alvarez-Suarez et al. 2016). A research on manuka honey suggests that it can inhibit the epidural fibrosis subsequent to laminectomy. Treatment with propolis shows a significant decline ($p < 0.05$) in the rate of fibrosis (epidural). It is well reported for its wound healing property and now the result of this study shows that manuka honey can reduce the development of post-laminectomy epidural fibrosis in rats by stimulating TNF α production in macrophages through the toll-like receptor (Gunaldi et al. 2014). One more work on manuka honey shows that in a concentration ($\leq$10% w/v) along with antibiotics (colistin and tobramycin) it restrains cystic fibrosis development along with the respiratory tract infection (Jenkins et al. 2015). Tonks et al. 2003 suggested that manuka honey stimulates the production of TNF-α in macrophages via toll-like receptor (Tonks et al. 2001). Hence we can say that manuka honey can be used as a possible alternative to treat resistance related to antibiotics in case of cystic fibrosis.

The medicinal qualities are not restricted with manuka only but the other varieties of honey also have tremendous pharmacological effects. As per Afrin et al. (2019), it was reported that *strawberry tree honey* ROS generation by suppressing Nrf2-ARE and nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB) signaling pathways and it also induce damage of cellular macromolecules and decreased antioxidant enzyme activities. *Kanuka honeys* work as an anti-inflammatory agent by affecting the toll-like receptors (TLR1/TLR2) signaling pathway. This effect is possibly due to the presence of phenolic contents in honey (Tomblin et al. 2014). Some reports suggest that honey works as an anti-fibrotic agent by modulating the inflammatory responses along with that it causes proliferation of fibroblasts and epithelial cells. Another study on *buckwheat honey* proposed that different activities are due to the presence of phenolic constituents in it which are most effective in reducing ROS levels (van den Berg et al. 2008).

A study was performed to examine the consequences of honey on both the early detection of the composition of postlaminectomy chronic inflammation in the adult Sprague–Dawley rat model. The results of this study show that honey can act as an anti-inflammatory agent by reducing edema and an amount of exudates by downregulating the inflammatory process. Such a downregulation of excessive inflammatory biomarkers works as a possible target for the prevention of peridural fibrosis and an impact on wound healing without leakage in the brain fluid (Farrokhi et al. 2011) (Fig. 5.3).

Apart from honey as a whole, the individual photochemical also have their own pharmacological properties. A large number of constituents have been reported in honey-like vitamins, minerals, flavonoids, phenolic acid, and many more. The promising anti-fibrotic effects of honey are reported in human as well as in different models of fibrosis possibly due to the presence large amount of flavonoids and phenolic acids. *Apigenin*, a constituent from honey, shows antiproliferative and anti-

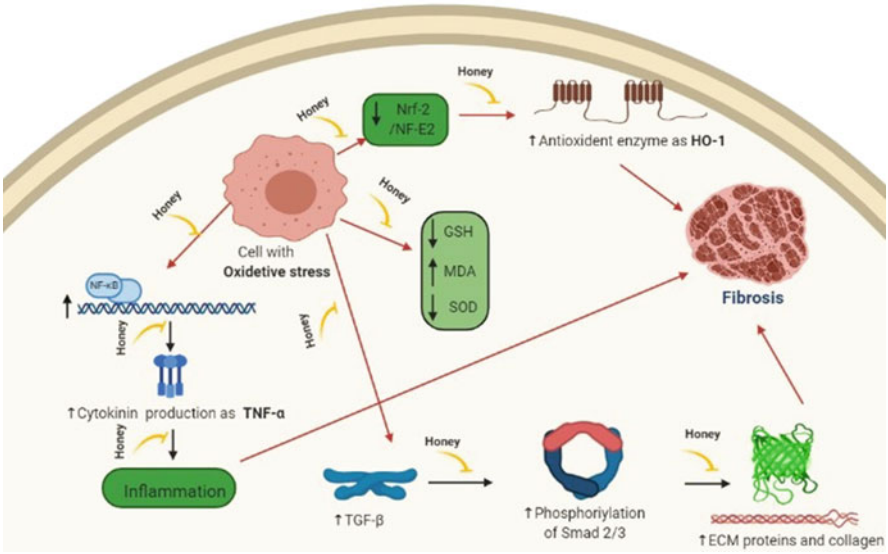

Fig. 5.3 Modulation of various pathways involved in the anti-fibrotic mechanism of honey and its components

inflammatory properties. It suppresses the proliferation and induces apoptosis in pancreatic stellate cells, which reduces the PSCs mediated fibrosis in chronic pancreatitis (Chen et al. 2014). Mrazek et al. (2015) suggest that it reduces the level of parathyroid hormone-related protein (PTHrP) and subsequently elevates the level of protein matrix collagen and fibronectin in PSC. It also causes proliferation of nuclear antigen in cells, TGF-β, and IL-6. It also protects lung fibrosis induced by bleomycin by inhibiting the elevated level of MPO, TGF-β, TNF-α, and collagen (Chen and Chen 2016). Apigenin at a dose of 20 mg/kg attenuates the oxidative stress and fibrosis by decreased transforming growth factor-β1, fibronectin, and type IV collagen. It causes a significant prevention of MAPK activation that ultimately inhibits the inflammatory biomarkers (Malik et al. 2017). It shows its anti-fibrosis action via suppression of MAPK-NF-κB-TNF-α and TGF-β1-MAPK-fibronectin pathways (Qiao et al. 2020). *Hesperidin* is naturally occurring flavanone glycosides that attenuate the liver fibrosis by downregulation of α-SMA and collagen both in vivo and in vitro. Derivative of hesperidine also inhibits fibrosis by inhibiting the activation and proliferation of PGDF via targeting Wnt/β-catenin signaling pathway (Lin et al. 2015). It also exerts protective effect on both lung and liver fibrosis by reducing the obstruction induced due to injury as well as by deposition of ECM and fibronectin in a mouse model. Furthermore, it significantly suppressed EMT, as evidenced by decreased expression of α-smooth muscle actin and E-cadherin. It also prevents the progression of bile duct ligation induced liver fibrosis via inhibiting level of TNF-α, IL-6, collagen, fibrosis indicators (laminin, hyaluronic acid, and

hydroxyproline via TGF-β1/Smad pathway (Kong et al. 2018). It also inhibits the cardiac fibrosis in post diabetic rat model by blocking NF-κB signaling pathway (Yin et al. 2017). *Rutin* is a polyphenolic flavonoid that ameliorates renal fibrosis in nephrectomized animal by its anti-oxidation property along with inhibiting TGFβ1-smad signaling (Han et al. 2015). It also protects hepatic fibrosis induced by bile duct ligation through the reduction in α SMA and Smad. It causes downregulation of NF-κB and TGF-β/Smad signaling, probably via interference of extracellular signal-regulated kinase activation and/or enhancement of nuclear factor erythroid 2 (NF-E2) related factors 2 (Nrf2), hemeoxygenase-1 (HO-1), and AMP-activated protein kinase activity (Pan et al. 2014). One study suggests that it inhibits the ECM by decreasing the level of collagen and fibronectin. It is possible because of the anti-inflammatory property of honey that works by inhibiting the TGF-β1/Smad3 signaling pathway (Wang et al. 2016). *Quercetin* suppressed the TNF-α, IL-1β, and IL-6, thiobarbituric acid reactive substances (TBARS), and reduced GSH level. It may also attenuate the pulmonary fibrosis via anti-inflammation and anti-oxidative properties (Baowen et al. 2010). The anti-inflammatory and anti-fibrotic mechanism is regulated by NF-κB and MAPK signaling pathways (Wang et al. 2017). In a dose-dependent manner, it inhibits fibrotic markers and downregulates the overexpression of TGF-β. Along with that, it causes stimulation of collagen, fibronectin, IL-1β, and MMPs (Yoon et al. 2012). The major role as anti-fibrotic is played by the possible reduction in α-SMA-matrix-and Smad 2/3 activity. Quercetin also attenuated bile duct ligation induced oxidative stress, leukocyte accumulation, nuclear factor (NF)-κB activation, and pro-inflammatory cytokine production (Lin et al. 2014). *Isorhamnetin* is a constituent from honey that protects against hepatic fibrosis by inhibiting TGF-β1 in HSC by blocking the ROS generation through activation of Nrf2 (Yang et al. 2016). It also inhibits collagen and expression of α-SMA in pulmonary fibrosis induced by bleomycin (Zheng et al. 2019). Data of a study show that it reduces collagen production in HSC cells, through inhibiting extracellular-signal-regulated kinase (ERK) signaling pathway (Lee et al. 2008). Another study suggests that it protects liver fibrosis by inhibiting the HSC, ECM, and autophagy via the downregulation of TGF-β1-mediated Smad3 and MAPK signaling pathways (Liu et al. 2019). *Myricetin* inhibits the activation of HSC in an animal model of liver fibrosis induced by carbon tetrachloride. It acts by suppressing α SMA and collagen deposition by inhibiting the phosphorylation of Smad2 and MAPK (Geng et al. 2017). It also reduces the secretion of inflammatory cytokines by activating Nrf2/HO-1 pathway by strengthening the anti-oxidative stress (Zhang et al. 2018a, b). Another research shows that it also inhibits the macrophage and hence protecting the hepatic fibrosis in mice model (Yao et al. 2020). *Pinocembrin* is a flavonoid that improves the oxidative stress by increasing the level of glutathione level and superoxide dismutase along with reduction in malondialdehyde. It also stimulates the nuclear factor erythroid -2 (NF-E2) and its related factor 2 (Nrf2). It also induces hemeoxygenase-1 (HO-1) enzyme, TNF-α, and different biomarkers (α-SMA and collagen) of fibrosis. It also inhibits the TGF-α/Smad signaling pathway by downregulation of TGF-α and p-Smad2/3 (Stine et al. 2018). *Galangin* shows its anti-fibrotic mechanism by attributing the

antioxidant action and inhibition of HSCs proliferation and collagen gene expression. Wang et al. (2013) reported that it causes downregulation of the overexpression of α-smooth muscle actin (α-SMA) and transforming growth factor 1 (TGF-β1) by removing the free radicals and inhibiting hepatic stellate cells activation and proliferation. Its mechanism may be related to the upregulation of MMP1, downregulation of TIMP1, and promotion of collagen degradation (Wang et al. 2013). *Luteolin* causes significant inhibition of PDGF and TGF β1, whereas it stimulates the Smad pathway (Jie et al. 2014). Another study suggests that luteolin at a dose of 10 mg/kg suppresses the neutrophil infiltration as well as TNF-α and IL-6 elevation. It also inhibited TGF-β1-induced α-SMA, type I collagen, and vimentin expression in primary cultured mouse lung fibroblasts (Chen et al. 2010). *Kaempferol* suppresses skin fibrosis by reducing the amount of ROS, number of myofibroblasts, T-cells, macrophages, inflammatory/pro-fibrotic cytokines including IL-6, TGF-β, and TNFα (Sekiguchi et al. 2019). Kaempferol significantly inhibits collagen synthesis and causes the activation of HSCs by downregulating the Smad2/3 phosphorylation in a dose-dependent manner (Xu et al. 2019). *Chrysin* modulates the ECM and tissue inhibitors of metalloproteinase 1 (TIMP) or modulation of MMP rebalance and decrease collagen deposition in a dose-dependent manner (Balta et al. 2018). It also causes the downregulation of phosphatidylinositol-3-kinase (PI3K)/Akt/Nrf2 and ERK/Nrf2 pathway (Gao et al. 2013). *Acacetin* is a flavonoid compound that shows significant decline in fibrotic markers by targeting the MAPK and PI3K/Akt signaling pathways (Chang et al. 2017). It alleviates myocardial fibrosis via TGF-β1/Smad3 signaling pathway (He et al. 2020).

Other flavonoid components from honey such as pinobanksin and 12-acetoxyviscidone show reduced cardiac fibrosis by downregulating the expression of fibrosis factors (collagen, MMPs, TGF-β1, and p-Smad2/3) which coincided with the upregulation and expression of silent information regulator 1 (SIRT1) in the hearts of rats with myocardial infarction (Wang et al. 2018).

Apart from the flavonoids, some phenolic acids are also present in honey that also plays an important role in the mechanism of anti-fibrosis. *Gallic acid* is the phenolic acid which is present in high concentration in honey and shows its anti-fibrotic action by attenuating the irregular deposition of collagen. It shows a potent inhibitory action on fibrosis when it is coadministered with advanced glycated end products possibly due to its mechanism of upregulation of profibrotic genes and ECM (Umadevi et al. 2014). It attenuates bleomycin-induced fibrosis due to the presence of high amount of antioxidants present in it. It also results in an increased level of collagen, malondialdehyde (MDA), and different cytokines (TNF-α and IL) as well as ameliorates total thiol content, glutathione peroxidase (Nikbakht et al. 2015). One of the recorded data shows that it has progression on hepatic fibrosis through reduction in the proliferation of HSC (El-Lakkany et al. 2019). Gallic acid prevents cardiac fibrosis via regulating c-Jun N-terminal kinase (JNK2) signaling and Smad3 binding activity (Ryu et al. 2016). *Ellagic acid* at the dose of 60 mg/kg body weight can use a significant decrease in the expression of fibrotic markers in alcohol-induced toxicity either by creating a balance between MMP/TIMP or by blocking the activation of PSC (Devipriya et al. 2007). It shows its anti-fibrotic

mechanism by inhibiting the level of IL and TNF-α induced activation in MAPK (Masamune et al. 2005). It also prohibits the overproduction of ROS in PSC along with a response in modification of TGF-β1 and PDGF (Suzuki et al. 2009). It also inhibits the degenerative changes in hepatocytes by increasing their survival in prolonged culture (Buniatian 2003). *Caffeic acid* shows its anti-fibrotic effect by modifying the level of TGF-β1, TNF-α, and PGE2 level. It also prevents pulmonary fibrosis by balancing pro-fibrotic or anti-fibrotic contents (Larki-Harchegani et al. 2013). It also suppresses the formation of myofibroblast and collagen in a human dermal model case (Mia and Bank 2016). It is suggested by the researcher that phenethyl ester of caffeic acid prevents the induction of fibrosis induced by bleomycin due to its potent action as free radical scavenger along with high contents of antioxidants present in that (Ozyurt et al. 2004). Zaeemzadeh et al. (2011) suggest that at a dose of 5 μmol/kg, caffeic acid shows potent anti-fibrotic effect. It attenuates fibrosis via inhibition of TGF-β1/Smad3 pathway and AKT/mTOR signaling pathways in HSC (Yang et al. 2017; Prasetyo et al. 2019). *Tannic acid* is a natural polyphenol from honey which acts as a potent anti-fibrotic agent for the treatment of pulmonary fibrosis induced by bleomycin in fibroblast culture. It inhibits TGF-β, which is induced by the overexpression of collagen and smooth muscle α-actin as well as force generation by primary cultured human lung fibroblasts (Reed et al. 2019).

After a lot of discussion on the anti-fibrotic action of honey and its components along with their possible mechanism, we came on the conclusion that honey and its components can work as a potent anti-fibrotic agent and can be used for the future prospective.

5.8 Future Prospective

No pharmacotherapy so far has received specific suggestions for the treatment of fibrosis, as is clear from the 2015 guidelines. The major side effect of synthetic drugs taken by fibrosis patients is these drugs produce severe comorbid conditions which can't cure. The environment for the management of fibrosis is clearly on fake grass and the stability of the disease (i.e., worsening of the disease and development of the fibrosis). From the above descriptive study, it is clear that honey has numerous health benefits viz. antioxidants, anxiolytics, anticonvulsant, antinociceptive, and antidepressant activity. Due to its properties, it improves the fibrosis's antioxidant status. Several studies of honey supplementation indicate that the polyphenol compounds present in honey show its anti-fibrotic effect. Polyphenol components of honey quench natural reactive oxygen species causing toxicity to nerves and aging along with protein deposition. The knowledge includes assessing the effects of fresh honey and its constituents in various fibrosis disorders, as well as researching the biochemical effects of honey on mitochondrial dysfunction, cell death, and necrobiosis. In addition, studying the actual cell signaling cascades related with synaptic plasticity may provide honey for more precise therapeutic interventions. It is also necessary to determine the honey outcome on plasticity under regular and

disease circumstances. More consideration should be given to the neural circuits and receptors involved in the fibro pharmacological effects of honey.

References

Abd Jalil MA, Kasmuri AR, Hadi H (2017) Stingless bee honey, the natural wound healer: a review. Skin Pharmacol Physiol 30:66–75

Abdalla AF, Zalata KR, Ismail AF, Shiha G, Attiya M, Abo-Alyazeed A (2009) Regression of fibrosis in paediatric autoimmune hepatitis: morphometric assessment of fibrosis versus semi-quantitative methods. Fibrogenesis Tissue Repair 2:2

Abdulrhman M, El Hefnawy M, Ali R, Hamid IA, Abou El-Goud A, Refai D (2013) Effects of honey, sucrose and glucose on blood glucose and C-peptide in patients with type 1 diabetes mellitus. Complement Ther Clin Pract 19:15–19

Afrin S, Forbes-Hernández TY, Cianciosi D, Pistollato F, Zhang J, Pacetti M, Battino M (2019) Strawberry tree honey as a new potential functional food. Part 2: strawberry tree honey increases ROS generation by suppressing Nrf2-ARE and NF-κB signaling pathways and decreases metabolic phenotypes and metastatic activity in colon cancer cells. J Funct Food 57:477–487

Afroz R, Tanvir EM, Karim N, Hossain MS, Alam N, Gan SH, Khalil MI (2016b) Sundarban honey confers protection against isoproterenol-induced myocardial infarction in Wistar rats. Biomed Res Int 2016:643764, 10 pages

Afroz R, Tanvir EM, Zheng W, Little PJ (2016a) Molecular pharmacology of honey. Clin Exp Pharmacol 6:3

Akbari E, Baigbabaei A, Shahidi M (2020) Determination of the floral origin of honey based on its phenolic profile and physicochemical properties coupled with chemometrics. Int J Food Prop 23 (1):506–519

Ali AH, Carey EJ, Lindor KD (2015) Recent advances in the development of farnesoid X receptor agonists. Ann Transl Med 1:5

Allen KL, Molan PC, Reid GM (1991) A survey of the antibacterial activity of some New Zealand honeys. J Pharm Pharmacol 43:817–822

Almasaudi SB, Al-Nahari AA, El Sayed M, Barbour E, Al Muhayawi SM, Al-Jaouni S, Azhar E, Qari M, Qari YA, Harakeh S (2017) Antimicrobial effect of different types of honey on Staphylococcus aureus. Saudi J Biol Sci 24:1255–1261

Al-Momani H, Perry A, Stewart CJ, Jones R, Krishnan A, Robertson AG, Bourke S, Doe S, Cummings SP, Anderson A, Forrest T, Griffin SM, Brodlie M, Pearson J, Ward C (2016) Microbiological profiles of sputum and gastric juice aspirates in cystic fibrosis patients. Sci Rep 1:26985

Alqarni AS, Owayss AA, Mahmoud AA, Hannan MA (2014) Mineral content and physical properties of local and imported honeys in Saudi Arabia. J Saudi Chem Soc 18:618–625

Alvarez-Suarez J, Gasparrini M, Forbes-Hernández T, Mazzoni L, Giampieri F (2014) The composition and biological activity of honey: a focus on Manuka honey. Foods 3:420–432

Alvarez-Suarez JM, Giampieri F, Cordero M, Gasparrini M, Forbes-Hernández TY, Mazzoni L, Afrin N, Beltrán-Ayala P, González-Paramás AM, Santos-Buelga C, Varela-Lopez A, Battino M et al (2016) Activation of AMPK/Nrf2 signalling by Manuka honey protects human dermal fibroblasts against oxidative damage by improving antioxidant response and mitochondrial function promoting wound healing. J Funct Food 25:38–49

Al-Waili NS, Boni NS (2003) Natural honey lowers plasma prostaglandin concentrations in normal individuals. J Med Food 6:129–133

Avila S, Beux MR, Ribani RH, Zambiazi RC (2018) Stingless bee honey: quality parameters, bioactive compounds, health-promotion properties and modification detection strategies. Trends Food Sci Technol 81:37–50

Aydin MM, Akcali KC (2018) Liver fibrosis. Turkish J Gastroenterol 29:14–21

Azman KF, Zakaria R, Othman Z, Abdul Aziz CB (2018) Neuroprotective effects of Tualang honey against oxidative stress and memory decline in young and aged rats exposed to noise stress. J Taibah Univ Sci 8:1380–1389

Bahrami M, Ataie-Jafari A, Hosseini S, Foruzanfar MH, Rahmani M, Pajouhi M (2009) Effects of natural honey consumption in diabetic patients: an 8-week randomized clinical trial. Int J Food Sci Nutr 60:618–626

Balta C, Ciceu A, Herman H, Rosu M, Boldura OM, Hermenean A (2018) Dose-dependent antifibrotic effect of chrysin on regression of liver fibrosis: the role in extracellular matrix remodeling. Dose-Response 16:155932581878983

Baowen Q, Yulin Z, Xin W, Wenjing X, Hao Z, Zhizhi C et al (2010) A further investigation concerning correlation between anti-fibrotic effect of liposomal quercetin and inflammatory cytokines in pulmonary fibrosis. Eur J Pharmacol 642:134–139

Barr J, Yadav L, Schuen J, Zea-Hernandez J (2018) Cystic fibrosis. Chron Dis Disabil Pediatr Lung 12:273–284

Biernacka A, Dobaczewski M, Frangogiannis NG (2011) TGF-β signaling in fibrosis. Growth Factors 29:196–202

Bilsel Y, Bugra D, Yamaner S, Bulut T, Cevikbas U, Turkoglu U (2002) Could honey have a place in colitis therapy? Effects of honey, prednisolone, and disulfiram on inflammation, nitric oxide, and free radical formation. Dig Surg 19:306–311

Birbrair A, Zhang T, Files DC, Mannava S, Smith T, Wang ZM, Messi ML, Mintz A, Delbono O (2014) Type-1 pericytes accumulate after tissue injury and produce collagen in an organ-dependent manner. Stem Cell Res Ther 5:122

Bobiş O, Dezmirean DS, Moise AR (2018) Honey and diabetes: the importance of natural simple sugars in diet for preventing and treating different type of diabetes. Oxidative Med Cell Longev 1:1–12

Borthwick LA, Wynn TA, Fisher AJ (2013) Cytokine mediated tissue fibrosis. Biochim Biophys Acta Mol basis Dis 1832:1049–1060

Buniatian GH (2003) Stages of activation of hepatic stellate cells: effects of ellagic acid, an inhibiter of liver fibrosis, on their differentiation in culture. Cell Prolif 36:307–319

Chang W, Wu QQ, Xiao Y, Jiang XH, Yuan Y, Zeng XF, Tang QZ (2017) Acacetin protects against cardiac remodeling after myocardial infarction by mediating MAPK and PI3K/Akt signal pathway. J Pharmacol Sci 135:156–163

Chen C-Y, Peng W-H, Wu L-C, Wu C-C, Hsu S-L (2010) Luteolin ameliorates experimental lung fibrosis both in vivo and in vitro: implications for therapy of lung fibrosis. J Agric Food Chem 58:11653–11661

Chen H, Mrazek AA, Wang X, Ding C, Ding Y, Porro LJ et al (2014) Design, synthesis, and characterization of novel apigenin analogues that suppress pancreatic stellate cell proliferation in vitro and associated pancreatic fibrosis in vivo. Bioorg Med Chem Lett 22:3393–3404

Chen L, Chen L (2016) Apigenin protects against bleomycin-induced lung fibrosis in rats. Exp Ther Med 11:230–234

Cooper RA, Molan PC, Harding KG (1999) Antibacterial activity of honey against strains of Staphylococcus aureus from infected wounds. J R Soc Med 92:283–285

Cystic Fibrosis Trust (2014) UK cystic fibrosis registry annual data report 2013. Registry

Darby IA, Hewitson TD (2007) Fibroblast differentiation in wound healing and fibrosis. Int Rev Cytol 257:143–179

Darby IA, Laverdet B, Bonté F, Desmoulière A (2014) Fibroblasts and myofibroblasts in wound healing. Clin Cosmet Investig Dermatol 7:301–311

Devipriya N, Sudheer AR, Srinivasan M, Menon VP (2007) Effect of ellagic acid, a plant polyphenol, on fibrotic markers (MMPs and TIMPs) during alcohol-induced hepatotoxicity. Toxicol Mech Method 17:349–356

Dwivedi DK, Jena GB (2018) Glibenclamide protects against thioacetamide-induced hepatic damage in Wistar rat: investigation on NLRP3, MMP-2, and stellate cell activation. N-S Arch Pharmacol 391:1257–1274

Eddy AA (2000) Molecular basis of renal fibrosis. Pediatr Nephrol 15:290–301
El-Lakkany NM, El-Maadawy WH, Seif el-Din SH, Saleh S, Safar MM, Ezzat SM, Hammam OA (2019) Antifibrotic effects of gallic acid on hepatic stellate cells: in vitro and in vivo mechanistic study. J Tradit Complement Med 9:45–53
Erejuwa OO, Sulaiman SA, Ab Wahab MS (2014) Effects of honey and its mechanisms of action on the development and progression of cancer. Molecules 19:2497–2522
Eteraf-Oskouei T, Najafi M (2013) Traditional and modern uses of natural honey in human diseases: a review. Iran J Basic Med Sci 16:731–742
Fan D, Takawale A, Lee J, Kassiri Z (2012) Cardiac fibroblasts, fibrosis and extracellular matrix remodeling in heart disease. Fibrogenesis Tissue Repair 5:15
Fan W, He N, Lin CS, Wei Z, Hah N, Waizenegger W, He MX, Liddle C, Yu RT, Atkins AR, Downes M, Evans RM (2018) ERRγ promotes angiogenesis, mitochondrial biogenesis, and oxidative remodeling in PGC1α/β-deficient muscle. Cell Rep 22:2521–2529
Farrokhi MR, Vasei M, Fareghbal S, Bakhtazad A (2011) Effect of honey on peridural fibrosis formation after laminectomy in rats: a novel experimental study. Evid Based Complement Alternat Med 2011:504967
Fernandez-Cabezudo MJ, El-Kharrag R, Torab F, Bashir G, George JA, El-Taji H, al-Ramadi BK (2013) Intravenous administration of Manuka honey inhibits tumor growth and improves host survival when used in combination with chemotherapy in a melanoma mouse model. PLoS One 8:1–15
Flume PA, Mogayzel PJ, Robinson KA, Goss CH, Rosenblatt RL, Kuhn RJ, Marshall BC, Bujan J, Downs A, Finder J, Goss C, Gutierrez H, Hazle L, Lester M, Quittell L, Sabadosa K, Vender RL, White TB, Willey-Courand DB, Saldanha I, Oyegunle M, Shankar MB, Mckoy N, Sengupta S, Odelola OA, Waybright S (2009) Cystic fibrosis pulmonary guidelines: treatment of pulmonary exacerbations. J Respir Crit Care Med 180:802–808
Gabbiani G (2003) The myofibroblast in wound healing and fibrocontractive diseases. J Pathol 200:500–503
Gao B, Bataller R (2011) Alcoholic liver disease: pathogenesis and new therapeutic targets. Gastroenterology 141:1572–1585
Gao AM, Ke ZP, Shi F, Sun GC, Chen H (2013) Chrysin enhances sensitivity of BEL-7402/ADM cells to doxorubicin by suppressing PI3K/Akt/Nrf2 and ERK/Nrf2 pathway. Chem Biol Interact 206:100–108
Geng Y, Sun Q, Li W, Lu ZM, Xu HY, Shi JS, Xu ZH (2017) The common dietary flavonoid myricetin attenuates liver fibrosis in carbon tetrachloride treated mice. Mol Nutr Food Res 61:1600392
Ghosh AK, Quaggin SE, Vaughan DE (2013) Molecular basis of organ fibrosis: potential therapeutic approaches. Exp Biol Med 227:301–314
Gieseck RL III, Wilson MS, Wynn TA (2018) Type 2 immunity in tissue repair and fibrosis. Nat Rev Immunol 18:62
Glick D, Barth S, Macleod KF (2010) Autophagy: cellular and molecular mechanisms. J Pathol 221:3–12
Gressner OA, Gressner AM (2008) Connective tissue growth factor: a fibrogenic master switch in fibrotic liver diseases. Liver Int:28, 1065–1079
Gunaldi O, Erdogan S, Guclu DG, Tugcu B, Ofluoglu E, Baydin S (2014) "Honey" can prevent epidural fibrosis development after laminectomy: an experimental study. Turk Neurosurg 24:849–854
Gupta JK, Sharma R (2009) Production technology and quality characteristics of mead and fruit-honey wines: a review. Nat Prod Radiance 8:345–355
Han Y, Lu JS, Xu Y, Zhang L, Hong BF (2015) Rutin ameliorates renal fibrosis and proteinuria in 5/6-nephrectomized rats by anti-oxidation and inhibiting activation of TGFβ1-smad signaling. Int J Clin Exp Pathol 8:4725
He R, Zhang J, Luo D (2020) Acacetin alleviates myocardial fibrosis via TGF-β1/Smad3 signaling pathway. J Mol Cell Cardiol 140:42–43

Henriques A, Jackson S, Cooper R, Burton N (2006) Free radical production and quenching in honeys with wound healing potential. J Antimicrob Chemother 58:773–777
Jenkins R, Wootton M, Howe R, Cooper R (2015) A demonstration of the susceptibility of clinical isolates obtained from cystic fibrosis patients to manuka honey. Arch Microbiol 197:597–601
Jull AB, Cullum N, Dumville JC, Westby MJ, Deshpande S, Walker N (2015) Honey as a topical treatment for wounds. Cochrane Database Syst Rev 6:CD005083
Kadirvelu A, Gurtu S (2013) Potential benefits of honey in type 2 diabetes mellitus: a review. Int J Collab Res Intern Med Public Health 5:199
Kasiotis KM, Anagnostopoulos C, Anastasiadou P, Machera K (2014) Pesticide residues in honeybees, honey and bee pollen by LC–MS/MS screening: reported death incidents in honeybees. Sci Total Environ 485:633–642
Kendall RT, Feghali-Bostwick CA (2014) Fibroblasts in fibrosis: novel roles and mediators. Front Pharmacol 27:123
Kim HJ, Perlman D, Tomic R (2015) Natural history of idiopathic pulmonary fibrosis. Respir Med 109:661–670
Klingberg F, Hinz B, White ES (2013) The myofibroblast matrix: implications for tissue repair and fibrosis. J Pathol 229:298–309
Kong R, Wang N, Luo H, Lu J (2018) Hesperetin mitigates bile duct ligation-induced liver fibrosis by inhibiting extracellular matrix and cell apoptosis via the TGF-β1/Smad pathway. Curr Mol Med 18:15–24
Kwakman PHS, Te Velde AA, De Boer L, Speijer D, Vandenbroucke-Grauls CMJE, Zaat SAJ (2010) How honey kills bacteria. FASEB J 24:2576–2582
Larki-Harchegani A, Hemmati AA, Arzi A, Ghafurian-Boroojerdnia M, Shabib S, Zadkarami MR, Esmaeilzadeh S (2013) Evaluation of the effects of caffeic acid phenethyl ester on prostaglandin E2 and two key cytokines involved in bleomycin-induced pulmonary fibrosis. Iran J Basic Med Sci 16:850
Lee MK, Yang HK, Ha NR, Sung SH, Kim YC (2008) Isorhamnetin from Oenanthe javanica attenuates fibrosis in rat hepatic stellate cells via inhibition of ERK signaling pathway. Nat Prod 14:81–85
Leong AG, Herst PM, Harper JL (2012) Indigenous New Zealand honeys exhibit multiple anti-inflammatory activities. Innate Immun 18:459–466
Li X, Zhu L, Wang B et al (2017) Drugs and targets in fibrosis. Front Pharmacol 8:855
Lin SY, Wang YY, Chen WY, Chuang YH, Pan PH, Chen CJ (2014) Beneficial effect of quercetin on cholestatic liver injury. J Nutr Biochem 25:1183–1195
Lin X, Kong LN, Huang C, Ma TT, Meng XM, He Y et al (2015) Hesperetin derivative-7 inhibits PDGF-BB-induced hepatic stellate cell activation and proliferation by targeting Wnt/β-catenin pathway. Int Immunopharmacol 25:311–320
Liu AL, Liu B, Qin HL, Lee SMY, Wang YT, Du GH (2008) Anti-influenza virus activities of flavonoids from the medicinal plant Elsholtzia rugulosa. Planta Med 74:847–851
Liu N, Feng J, Lu X, Yao Z, Liu Q, Lv Y, Zhou Y (2019) Isorhamnetin inhibits liver fibrosis by reducing autophagy and inhibiting extracellular matrix formation via the TGF-β1/Smad3 and TGF-β1/p38 MAPK pathways. Mediat Inflamm 31:6175091
Lu J, Turnbull L, Burke CM, Liu M, Carter DA, Schlothauer RC, Whitchurch CB, Harry EJ (2014) Manuka-type honeys can eradicate biofilms produced by Staphylococcus aureus strains with different biofilm-forming abilities. PeerJ 2:e326
Luchetti F, Canonico B, Bartolini D, Arcangeletti M, Ciffolilli S, Murdolo G, Piroddi M, Papa S, Reiter RJ, Galli F (2014) Melatonin regulates mesenchymal stem cell differentiation: a review. J Pineal Res 56:382–397

Malik S, Suchal K, Khan SI, Bhatia J, Kishore K, Dinda AK, Arya DS (2017) Apigenin ameliorates streptozotocin-induced diabetic nephropathy in rats via MAPK-NF-κB-TNF-α and TGF-β1-MAPK-fibronectin pathways. Am J Physiol Renal 313:F414–F422

Manley S, Ding W (2015) Role of farnesoid X receptor and bile acids in alcoholic liver disease. Acta Pharm Sin B 5:158–167

Marvig RL, Sommer LM, Molin S, Johansen HK (2015) Convergent evolution and adaptation of Pseudomonas aeruginosa within patients with cystic fibrosis. Nat Genet 47:57–64

Masamune A, Satoh M, Kikuta K, Suzuki N, Satoh K, Shimosegawa T (2005) Ellagic acid blocks activation of pancreatic stellate cells. Biochem Pharmacol 70:869–878

Mia MM, Bank RA (2016) The pro-fibrotic properties of transforming growth factor on human fibroblasts are counteracted by caffeic acid by inhibiting myofibroblast formation and collagen synthesis. Cell Tissue Res 363:775–789

Michael D, Valentine VG, Nydia C, Gibson KF, Raval JS, Melissa S, Jianmin X, Yingze Z, Steven RD (2015) Autoantibody-targeted treatments for acute exacerbations of idiopathic pulmonary fibrosis. PLoS One 10:e0133684

Miguel MG, Antunes MD, Faleiro ML (2017) Honey as a complementary medicine. Int Med Insights 12:1178633717702869

Mrazek AA, Porro LJ, Bhatia V, Falzon M, Spratt H, Zhou J et al (2015) Apigenin inhibits pancreatic stellate cell activity in pancreatitis. J Surg Res 196:8–16

Muhammad A, Odunola OA, Ibrahim MA, Sallau AB, Erukainure OL, Aimola IA, Malami I (2016) Potential biological activity of acacia honey. Front Biosci 8:351–357

Ng SM, Moore HS (2016) Drug therapies for reducing gastric acidity in people with cystic fibrosis. Cochrane Database Syst Rev 22:CD003424

Nikbakht J, Hemmati AA, Arzi A, Mansouri MT, Rezaie A, Ghafourian M (2015) Protective effect of gallic acid against bleomycin-induced pulmonary fibrosis in rats. Pharmacol Rep 67:1061–1067

Ozyurt H, Sogut S, Yıldırım Z, Kart L, Iraz M, Armutçu F et al (2004) Inhibitory effect of caffeic acid phenethyl ester on bleomycine-induced lung fibrosis in rats. Clin Chim Acta 339:65–75

Padovan GJ, De Jong D, Rodrigues LP, Marchini JS (2003) Detection of adulteration of commercial honey samples by the 13C/12C isotopic ratio. Food Chem 82:633–636

Pan PH, Lin SY, Wang YY, Chen WY, Chuang YH, Wu CC, Chen CJ (2014) Protective effects of rutin on liver injury induced by biliary obstruction in rats. Free Radic Biol Med 73:106–116

Parish JM, Rosenow Iii EC (2002) Mediastinal granuloma and mediastinal fibrosis. Semin Respir Crit Care 23:135–144

Popek S (2002) A procedure to identify a honey type. Food Chem 79:401–406

Prasetyo DH, Sarsono S, Nurwati I, Putranto PA, Martini M, Prasetyo NA (2019) Hepatoprotective and antifibrotic effects of Indonesian Propolis. Majalah Kedokteran Bandung 51:134–140

Qiao M, Yang J, Zhu Y, Zhao Y, Hu J (2020) Transcriptomics and proteomics analysis of system-level mechanisms in the liver of apigenin-treated fibrotic rats. Life Sci 248:117475

Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, Colby TV, Cordier JF, Flaherty KR, Lasky JA, Lynch DA (2011) An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med 183:788–824

Rao PV, Krishnan KT, Salleh N, Gan SH (2016) Biological and therapeutic effects of honey produced by honey bees and stingless bees: a comparative review. Brazilian J Pharmacogn 129:24–30

Reed EB, Ard S, La J, Park CY, Culligan L, Fredberg JJ, Dulin NO (2019) Anti-fibrotic effects of tannic acid through regulation of a sustained TGF-beta receptor signaling. Respir Res 20:168

Rieger S, Zhao H, Martin P, Abe K, Lisse TS (2015) The role of nuclear hormone receptors in cutaneous wound repair. Cell Biochem Funct 33:1–13

Rockey DC, Bell PD, Hill JA (2015) Fibrosis—a common pathway to organ injury and failure. N Engl J Med 2:373

Rybak-Chmielewska H (2003) Honey. In: Chemical and functional properties of food saccharides. CRC Press, Boca Raton, FL

Ryu Y, Jin L, Kee HJ, Piao ZH, Cho JY, Kim GR, Jeong MH et al (2016) Gallic acid prevents isoproterenol-induced cardiac hypertrophy and fibrosis through regulation of JNK2 signaling and Smad3 binding activity. Sci Rep 6:34790

Scales BS, Huffnagle GB (2013) The microbiome in wound repair and tissue fibrosis. J Pathol 229:323–331

Sekiguchi A, Motegi SI, Fujiwara C, Yamazaki S, Inoue Y, Uchiyama A, Ishikawa O (2019) Inhibitory effect of kaempferol on skin fibrosis in systemic sclerosis by the suppression of oxidative stress. J Dermatol Sci 96:8–17

Sherlock O, Dolan A, Athman R, Power A, Gethin G, Cowman S, Humphreys H (2010) Comparison of the antimicrobial activity of Ulmo honey from Chile and Manuka honey against methicillin-resistant Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. BMC Complement Altern Med 10:1–5

Shukrimi A, Sulaiman AR, Halim AY, Azril A (2008) A comparative study between honey and povidone iodine as dressing solution for Wagner type II diabetic foot ulcers. Med J Malaysia 63:44–46

Sica A, Casale B, Spada A, Di Dato MT, Sagnelli C, Calogero A, Troiani T (2019) Differential diagnosis: retroperitoneal fibrosis and oncological diseases. Open Med 15:22–26

Siddiqui AJ, Musharraf SG, Choudhary MI, Rahman A-U (2017) Application of analytical methods in authentication and adulteration of honey. Food Chem 130:162–164

Sommeijer MJ (1999) Beekeeping with stingless bees: a new type of hive. Bee World 80:70–79

Sporns O, Honey CJ, Kötter R (2007) Identification and classification of hubs in brain networks. PLoS One 2:e1049

Srivastav S, Singh P, Mishra G, Jha KK, Khosa RL (2011) Achyranthes aspera—an important medicinal plant: a review. J Nat Prod Plant Resour 1:1–14

Stine JG, Wang J, Cornella SL, Behm BW, Henry Z, Shah NL et al (2018) Treatment of Type-1 hepatorenal syndrome with pentoxifylline: a randomized placebo controlled clinical trial. Ann Hepatol 17:300–306

Suzuki N, Masamune A, Kikuta K, Watanabe T, Satoh K, Shimosegawa T (2009) Ellagic acid inhibits pancreatic fibrosis in male Wistar Bonn/Kobori rats. Dig Dis Sci 54:802–810

Tomblin V, Ferguson L, Han DY, Murray P, Schlothauer R (2014) Potential pathway of anti-inflammatory effect by New Zealand honeys. Int J Gen Med 7:149–158

Tonks A, Cooper RA, Price AJ, Molan PC, Jones KP (2001) Stimulation of TNF-α release in monocytes by honey. Cytokine 14:240–242

Tonks AJ, Cooper RA, Jones KP, Blair S, Parton J, Tonks A (2003) Honey stimulates inflammatory cytokine production from monocytes. Cytokine 21:242–247

Tzou M, Gazeley DJ, Mason PJ (2014) Retroperitoneal fibrosis. Vasc Med 116:194–197

Umadevi S, Gopi V, Elangovan V (2014) Regulatory mechanism of gallic acid against advanced glycation end products induced cardiac remodeling in experimental rats. Chem Biol Interact 208:28–36

Umesh Hebbar H, Rastogi NK, Subramanian R (2008) Properties of dried and intermediate moisture honey products: a review. Int J Food Prop 11(4):804–819

van den Berg AJ, van den Worm E, van Ufford HC, Halkes SB, Hoekstra MJ, Beukelman CJ (2008) An in vitro examination of the antioxidant and anti-inflammatory properties of buckwheat honey. J Wound Care 17:172–178

Van Mourik IDM (2017) Liver disease in cystic fibrosis. Paediatr Child Heal 2:340–349

Verrecchia F, Mauviel A (2007) Transforming growth factor-β and fibrosis. World J Gastroenterol 13:3056

Vettori S (2012) Role of MicroRNAs in fibrosis. Open Rheumatol J 6:130–139

Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, Pérez-Álvarez JA (2008) Functional properties of honey, propolis, and royal jelly. J. Food Sci 73:117–124

Wang B, Liu D, Zhu QH, Li M, Chen H, Guo Y, Zhao M et al (2016) Rutin ameliorates kidney interstitial fibrosis in rats with obstructive nephropathy. Int Immunopharmacol 35:77–84
Wang Q, Sui X, Sui DJ, Yang P (2018) Flavonoid extract from Propolis inhibits cardiac fibrosis triggered by myocardial infarction through upregulation of SIRT1. Evidence Based Complement Alternat Med 2018: 4957573
Wang R, Zhang H, Wang Y, Song F, Yuan Y (2017) Inhibitory effects of quercetin on the progression of liver fibrosis through the regulation of NF-κB/IκBα, p38 MAPK, and Bcl-2/Bax signaling. Int Immunopharmacol 47:126–133
Wang X, Gong G, Yang W, Li Y, Jiang M, Li L (2013) Antifibrotic activity of galangin, a novel function evaluated in animal liver fibrosis model. Environ Toxicol Pharmacol 36:288–295
Wu CW, Chang HC, Chen TH, Huang HT, Liu CJ, Wang HC (2018) Idiopathic pulmonary fibrosis. J Intern Med Taiwan 106:1566–1574
Wynn TA (2011) Integrating mechanisms of pulmonary fibrosis. J Exp Med 208:1339–1350
Wynn TA, Vannella KM (2016) Macrophages in tissue repair, regeneration, and fibrosis. Immunity 44:450–462
Xu F, Liu C, Zhou D, Zhang L (2016) TGF-β/SMAD pathway and its regulation in hepatic fibrosis. J Histochem Cytochem 64:157–167
Xu T, Huang S, Huang Q, Ming Z, Wang M, Li R, Zhao Y (2019) Kaempferol attenuates liver fibrosis by inhibiting activin receptor-like kinase 5. J Cell Mol Med 23:6403–6410
Yaghoobi R, Kazerouni A, Kazerouni O (2013) Evidence for clinical use of honey in wound healing as an anti-bacterial, anti-inflammatory anti-oxidant and anti-viral agent: a review. Jundishapur J Nat Pharm Prod 8:100–104
Yang JH, Kim SC, Kim KM, Jang CH, Cho SS, Kim SJ, Ki SH (2016) Isorhamnetin attenuates liver fibrosis by inhibiting TGF-β/Smad signaling and relieving oxidative stress. Eur J Pharmacol 783:92–102
Yang N, Dang S, Shi J, Wu F, Li M, Zhang Xand Zhai S (2017) Caffeic acid phenethyl ester attenuates liver fibrosis via inhibition of TGF-β1/Smad3 pathway and induction of autophagy pathway. Biochem Biophys Res Commun 486:22–28
Yao Q, Li S, Li X, Wang F, Tu C (2020) Myricetin modulates macrophage polarization and mitigates liver inflammation and fibrosis in a murine model of nonalcoholic steatohepatitis. Front Med 7:71
Yin Y, Xu Y, Ma H, Tian X (2017) Hesperetin ameliorates cardiac inflammation and cardiac fibrosis in streptozotocin-induced diabetic rats by inhibiting NF-kB signaling pathway. Biomed Res 28:223–229
Yoon JS, Chae MK, Jang SY, Lee SY, Lee EJ (2012) Antifibrotic effects of quercetin in primary orbital fibroblasts and orbital fat tissue cultures of Graves' orbitopathy. Invest Ophth Vis Sci 53:5921–5929
Zaeemzadeh N, Hemmati A, Arzi A, Jalali M, Rashidi I (2011) Protective effect of caffeic acid phenethyl ester (CAPE) on amiodarone-induced pulmonary fibrosisin rat. Iran J Pharm 10:321
Zaworra M, Koehler H, Schneider J, Lagojda A, Nauen R (2019) Pharmacokinetics of three neonicotinoid insecticides upon contact exposure in the Western honey bee, Apis mellifera. Chem Res Toxicol 32:35–37
Zhang MJ, Su H, Yan JY, Li N, Song ZY, Wang HJ, Qu MH (2018b) Chemopreventive effect of Myricetin, a natural occurring compound, on colonic chronic inflammation and inflammation-driven tumorigenesis in mice. Biomed Pharmacother 97:1131–1137
Zhang Y, Wang M, Yang J, Li X (2018a) The effects of the honey-roasting process on the pharmacokinetics of the six active compounds of Licorice. Evid Based Complement Alternat Med 2018:5731276, 9
Zheng Q, Tong M, Ou B, Liu C, Hu C, Yang Y (2019) Isorhamnetin protects against bleomycin-induced pulmonary fibrosis by inhibiting endoplasmic reticulum stress and epithelial-mesenchymal transition. Int J Mol Med 43:117–126

A Mechanistic Perspective on Chemopreventive and Therapeutic Potential of Phytochemicals in Honey

6

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Abstract

Honey is naturally obtained from honeybees. Honey is well-known for its therapeutic and nutritional values since ancient times. It has been used for treating infectious diseases for ages. It contains various chemical constituents such as carbohydrates, amino acids, proteins, phenols, and flavonoids. Phenolic and flavonoid contents of honey are very well-known for various biological and clinical applications. Honey is used for therapeutic purposes like wound healing, skin disinfection, ulcers, asthma, cancer, eye diseases, fungal infections, etc. It is very sensitive to heat and light which may diminish its therapeutic benefits. Its physicochemical properties like acidity, high level of glucose, peroxide content, etc. are responsible for its pharmacological actions. Honey shows antioxidant and immune-boosting ability by preventing lipid peroxidation and inhibiting the generation of oxidative stress. In this book chapter, we have described all the major studies conducted for supporting the health benefits of honey such as wound healing, anti-inflammatory, antioxidant, anticancer, antibacterial, antidiabetic, neurological disorders, nephroprotective, cardioprotective, and gastroprotective agent.

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Keywords

Honey · Polyphenols · Anticancer · Antibiotic · Wound healing · Immune booster

6.1 Introduction

Honey bees are marvel alchemists that are useful for the humans and their health-related issues. They have been used in traditional medicine since ancient times. Bee keeping is not new to the human civilization. A man probably knew how to collect honey from honey bees about 9000 years ago. A rock painting found near Spain, Valencia, dating back to 7000 BC shows a man collecting honey from a large hive. Ancient literatures including the Talmud and the Bible scrolls from the Orient, Greece, and Rome all laud honey as a source of youthfulness and health. The oldest medicine book of Egypt papyri recorded that honey was used for the treatment of wounds in 1553–1550 BC. In the Holy Quran, a Chapter is titled by the name "The Bee"; it quotes honey's benefits, and medicinal uses for the treatment of several diseases. The ancient Indian medicine literatures of Ayurveda and Vedas have already reported the therapeutic and nutritional value of honey about 4000 years ago (Zaidi and Sharma 2019). Honey and milk diet was believed to prolong the life (Sci et al. 2015).

Honey is a golden viscous natural substance produced by honeybees. It is the nectar that exudates from flowers, plants, and trees, which are processed by honey bees (Jaganathan et al. 2011). A worker bee forages 55,000 miles to collect nectar from around two million flowers for accruing one pound of honey. The honey produced by the bees is stored in honeycombs inside the beehive. To avoid fermentation of the honey, the worker bee uses its wings to fan the honeycomb to evaporate water from the nectar (Xiang et al. 2006). Beekeeping (apiculture) is a practice known for the domestication of bees, commonly in man-made hives. Honey is collected from these hives. There are two main types of honey, forest, and apiary honey, honey produced by honeybees, Apis dorsata and wild nest of Apis cerana *indica* which were collected by crude method of squeezing the honeycomb is regarded as forest honey, are turbid and contaminated with wax, pollen, broad and plant residues, whereas honey produced in apiaries by Apis mellifera and Apis cerana gathered by modern extraction is known as apiary honey, are pure and transparent (Afroz et al. 2016). Honey possesses a very high nutritional and therapeutic value well documented from ancient times in the ancient literature (Yoon et al. 2013). This valuable bee product has traditionally been consumed directly and used in various medical practices. Honey is produced all over the globe; the annual production of honey all over the world is around 1.20 million tons. Turkey, China, Argentina, Ukraine, Mexico, and the United States are the main producers. Honey consumption is more in the developed nations as compared to the developing nations (Calixto-Campos et al. 2015) (Figs. 6.1 and 6.2).

Honey is mainly composed of sugar. It is an important source of energy due to its high sugar content. As 100 g of honey provides approximately 300 kcal, the regular

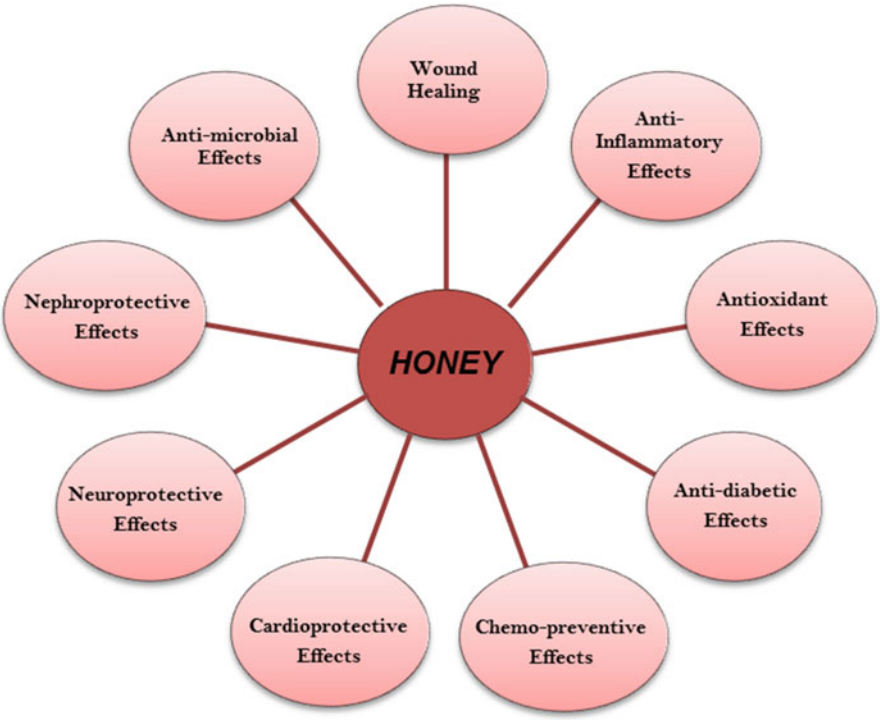


Fig. 6.1 Schematic representation of the different therapeutic effects of honey

intake of 20 g honey covers about 3% daily requirement of energy (Baltrušaityte et al. 2007). Honey contains monosaccharides, fructose, and glucose. It contains roughly the same sweetness as that of sugar, due to which many people use honey instead of sugar. It has been used as a dietary supplement and is now being used in innumerable food and beverages as a sweetening and flavouring agent (Jaganathan 2012). Honey contains several phytochemicals that vary depending on the species and the origin of bees. The difference in the composition of honey is due to its floral sources, harvesting seasons, and the climatic and geographical origin (Yoon et al. 2013). The colour of the honey bee varies according to the floral sources and minerals. Honey's flavour depends upon the colour; darker honey contains stronger flavour content (Xiang et al. 2006). Honey contains almost similar types of phenolic acid contents globally; each constituent has different nutritional and medicinal properties, and due to its phytochemical content it can be used for different applications such as nutritional, medicinal, food additive, etc. (Zhu et al. 2018).

Honey has been ascribed with medicinal properties by ancient people. In the contemporary world, the use of natural products in health care is becoming popular; honey is being used in various disorders and is considered a natural cure (Liu et al. 2014). Information regarding the use of honey for the treatment of many diseases has been mentioned in beekeeping journals, magazines, and natural leaflets. Honey is

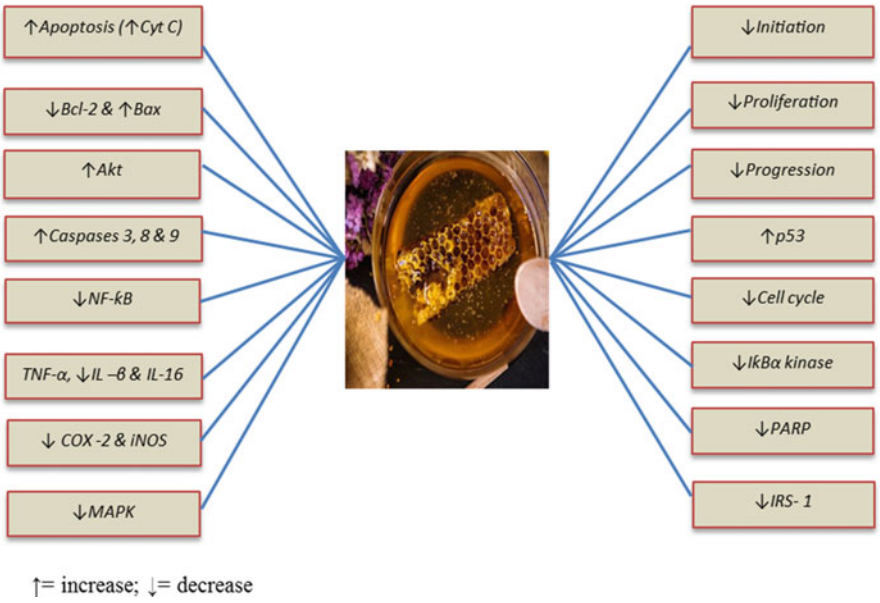


Fig. 6.2 Regulatory effects of honey on different hallmarks and biomarkers/molecules of cancers

Table 6.1 Chemical composition of honey

S. No.	Compounds	Amount (%)
1.	Water	17–18
2.	pH	3–5
3.	Total sugar	73–82.4
4.	Fructose	31–42
5.	Glucose	25–31
6.	Other minor compounds	0.50–1

imported and exported globally in present times due to its medicinal and nutritional benefits. An alternative medicine branch, called apitherapy has been developed contributing treatment based on honey and bee products for many diseases (Jaganathan 2012). Recent advances in research emphasized that honey has potential biological activities with favourable health-promoting properties. Various polyphenols present in honey have been proven to curb the development of several diseases. Various studies have been conducted for the detection of its chemical and biological properties such as anti-inflammatory, anti-oxidant, anti-diabetic, anti-tumour, anti-bacterial, and wound-healing effects (Baltrušaityte et al. 2007) (Table 6.1).

6.2 Phytochemicals in Honey

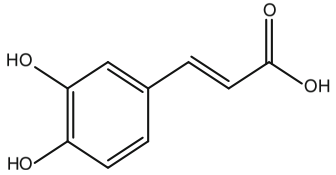
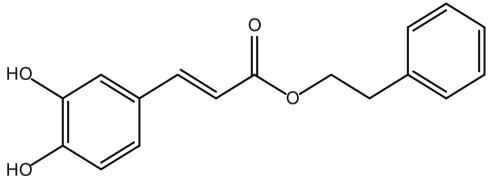
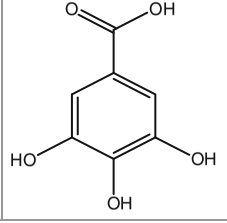
The composition of honey varies extensively depending upon the geographical origin, botanical origin, seasonal variation, environmental factors, floral sources, and processing (Calixto-Campos et al. 2015). Honey is a supersaturated solution containing at least 181 substances, which include water, sugar, proteins, enzymes, minerals, vitamins, and various phytochemicals. It is mainly composed of sugars such as fructose and glucose as a major constituent and water; to a lesser extent honey includes protein, amino acids, minerals, vitamins and a little amount of other components (Jaganathan 2012; Baltrušaityte et al. 2007; Mani and Natesan 2018; Cho et al. 2004; Weng et al. 2005; Zhu et al. 2018; Geraets et al. 2007; Hsu et al. 2004) (Table 6.2).

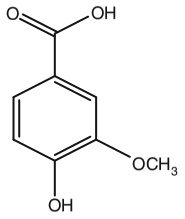
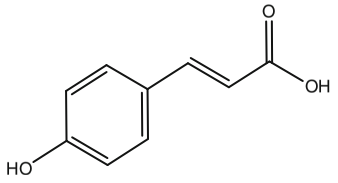
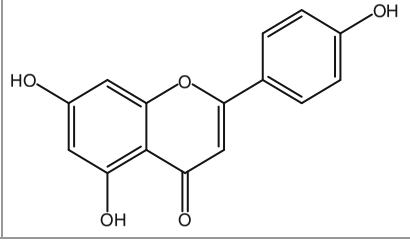
Depending on the origin of honey the phytochemicals found in honey are characterized as phenolic compounds. Phenolic compounds are an important group of compounds found in plants as secondary metabolites. Some of these polyphenols in plants are incorporated into honey by forager's honeybee. The phytochemicals present in honey are mainly phenolic acids, flavonoids, and their derivatives, which are present in smaller quantities. The phenolic constituents of honey have different biological activities and are the major contributors to health benefits (Weng et al. 2005; Xiang et al. 2006; Mani and Natesan 2018). The major types of phenolic compounds are flavonoid and phenolic acid. A total of 56–500 mg/kg of polyphenols are present in various honey and the flavonoid content of honey is between 60 and 460 μg/100 g (Lodi et al. 2009). Flavonoids are classified into flavones, flavonols, flavanones, flavanols, flavanonols, isoflavones, anthocyanins, and anthocyanidins based on the degree of oxidation of the C-ring. Flavones (apigenin, luteolin, and chrysin) and flavonols (quercetin, myricetin, and kaempferol) are profusely found in honey (Baltrušaityte et al. 2007). The polyphenol content indicates a specific biomarker for the origin of honey. The most common phenolic compounds present in honey are listed below.

6.2.1 Wound Healing Effect

In early times, honey was used for the treatment of wounds and fostered the process of healing wounds. The use of honey as a treatment for wounds was first mentioned and found in *Edwin Smith Papyrus*. During the First World War, the Russian and Germans were found to use honey in wound treatment which remained popular until the arrival of antibiotics in 1940 (Vandamme et al. 2013). Honey has been used as a remedy for the treatment of ulcers, burns, and wound healing. The use of honey as a drug and an ointment has continued till date. Due to high osmolarity, honey creates a moist environment around the wound, which provides a protective barrier and prevents bacterial aggregation (Miguel et al. 2010). Honey shows remarkable effects in wound healing as it is proved to be very effective in the treatment of wounds. The role of Malaysian Tualang honey on colon anastomosis healing in Wistar rats was

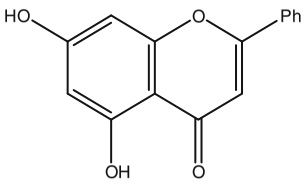
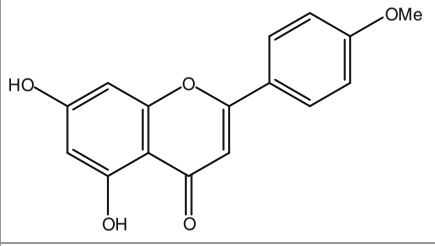
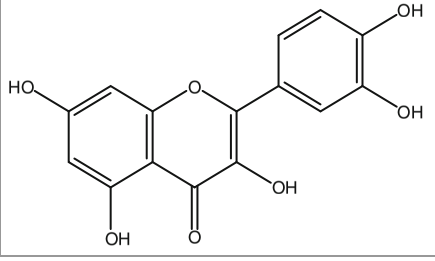
Table 6.2 Some of the widely reported phenolic acid and flavonoid contents identified in honey from various sources listed in the table

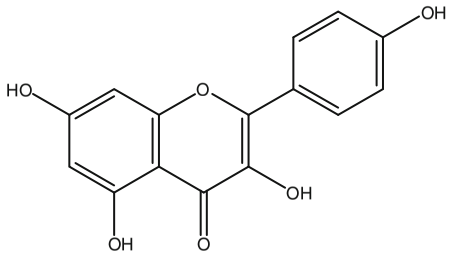
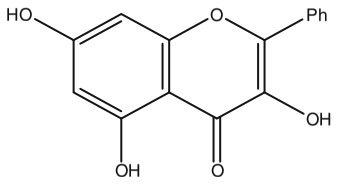
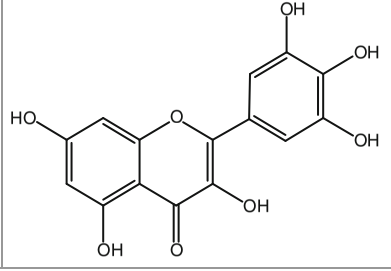
S. No.	Polyphenols	Structure	Biological significance	References
1.	Caffeic acid		Anti-cancer, anti-microbial, cardioprotective, antioxidant	Jaganathan (2012)
2.	Caffeic acid phenyl ester		Anti-inflammatory, immunomodulatory, anti-carcinogenic, cardioprotective	Xiang et al. (2006)
3.	Gallic acid		Anti-inflammatory, anti-mutagenic, anti-cancer, antioxidant, cardioprotective	Afroz et al. (2016), Yoon et al. (2013)

4.	Vallinic acid		Antioxidant, anti-microbial	Calixto-Campos et al. (2015)
5.	*p*-coumaric acid		Anti-cancer, antioxidant, anti-inflammatory	Baltrušaityte et al. (2007), Jaganathan et al. (2013), Zhu et al. (2018)
6.	Apigenin		Antioxidant, anti-tumour	Baltrušaityte et al. (2007)

(continued)

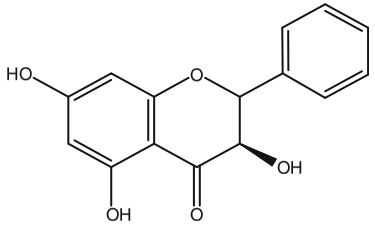
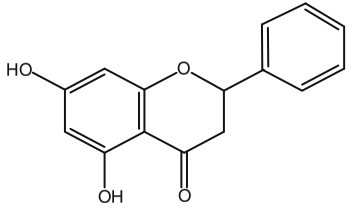
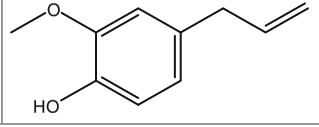
Table 6.2 (continued)

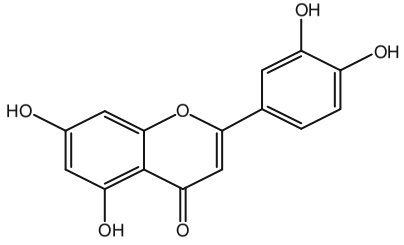
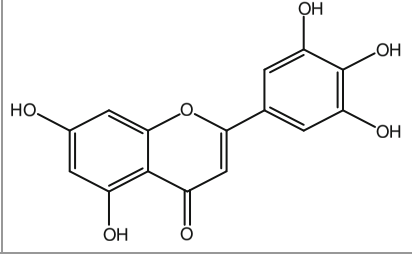
S. No.	Polyphenols	Structure	Biological significance	References
7.	Chrysin		Anti-inflammatory, neuroprotective, immunomodulatory, anti-diabetic, anti-oxidant, anti-microbial, anti-cancer	Liu et al. (2014), Mani and Natesan (2018),Cho et al. (2004), Weng et al. (2005)
8.	Acacetin		Cardioprotective, anti-proliferative	Hsu et al. (2004)
9.	Quercetin		Anti-cancer, antioxidant, anti-proliferative, cardioprotective	Lodi et al. (2009), Nicholson et al. (2010)

10.	Kaempferol		Anti-cancer, cardioprotective, anti-oxidant, anti-inflammatory	Hoon et al. (2012)
11.	Galangin		Anti-proliferative	Bestwick and Milne (2006)
12.	Myricetin		Anti-diabetic, anti-cancer, anti-inflammatory, anti-oxidant	Semwal et al. (2016)

(continued)

Table 6.2 (continued)

S. No.	Polyphenols	Structure	Biological significance	References
13.	Pinobanksin		Anti-oxidant, anti-cancer	Lan et al. (2016)
14.	Pinocembrin		Anti-cancer, anti-oxidant, neuroprotective	Lan et al. (2016)
15.	Eugenol		Anti-cancer	Jaganathan et al. (2011)

16.	Luteolin		Anti-oxidant, anti-inflammatory, anti-cancer, cardioprotective	Si et al. (2014)
17.	Tricetin		Anti-cancer, anti-inflammatory	Geraets et al. (2007), Mei et al. (2009)

examined; oral administration of Tualang honey elevates wound healing by reducing inflammatory cells and enhancing the fibroblast.

Similarly, the potential of honey to facilitate speedy healing of wounds formed at the time of surgery with an electroscope and cold scalpel was evaluated but the cold scalpel group treated with honey was found to have reduced hyperemia as compared to silver sulphadiazine and the control group. Silver sulphadiazine was the drug of choice to treat the wound. Fibroelastic and fibroplasia were increased and continuous in the electroscope group treated with honey and silver sulphadiazine than that of the control group. The honey treated group showed an improved level of epithelialization as compared to silver sulphadiazine (Eyarefe et al. 2017). In another study, the variation in the efficacy of honey and sugar in wound dressing was investigated. The median percentage of healing after 14 days of treatment was 57% and 31% in honey and sugar treated group, respectively. It was found that the healing rate was higher in the patients treated with honey. The release of hydrogen peroxide by honey as compared to sugar facilitates the reduction of microbial contamination in culture from 55% to 23% in the honey treated group and from 56% to 39% in the sugar treated group, as observed in 1 week (Mphande et al. 2007). It was found that honey helps in controlling microbial contamination and promoting epithelisation, and appears to be better in aiding quick healing and minimizing pain, but the exact mechanism involved in wound healing is yet to be explored in detail. Honey is also reported as an anti-bacterial agent in many studies. Honey helps in wound healing through immune-boosting capability which is an additional benefit. (Calixto-Campos et al. 2015).

6.2.2 Anti-Microbial Effect

Tetragonisca angustula (*T. angustula*) is a variety of honey, which is found widely in Brazil and Mexico. It has been reported for its anti-microbial action against various bacterial strains such as *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, and *E. coli*. It has been reported for its anti-microbial activity against Gram-positive, Gram-negative, and coagulase-negative methicillin-sensitive bacteria. It is equally effective on yeasts such as *Saccharomyces cerevisiae*, *Aspergillus niger*, and *Candida albicans*. Furthermore, honey types such as those obtained from *Trigona carbonaria* and *Trigona laeviceps* (Thailand), a stingless bee and others like Tualang honey (Malaysia), Manuka honey, and honey bee honey have also been reported for their anti-bacterial activity against bacteria, yeast, and fungus (Miorin et al. 2003; Rao et al. 2016; Sgariglia et al. 2010). In a study, it is reported that honey is effective against various drug-resistant strains of bacteria, for example, methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *enterococci,* and haemolytic *streptococci*. It is very effective against several human pathogens, including *Salmonella typhimurium*, *Escherichia coli* (E. coli), *Enterobacter aerogenes*, and *S. aureus* (Mandal and Mandal 2011). Recently, honey has been reported by many scientists for its anti-bacterial activity. They find promising results for the broad-spectrum anti-bacterial

activity in the natural unheated form against various pathogens (Mandal and Mandal 2011). Phenolic acids and Flavonoids are responsible for honey's anti-microbial activity. It contains flavonoids such as pinocembrin, pinobanksin, and chrysin (Zaidi and Sharma 2019).

Honey consists of low acidity (low pH), high osmolarity, low hydrogen peroxide (H_2O_2) content, and non-peroxide content, which are the major contributors for honey's anti-bacterial activity. Most of the honey produces H_2O_2, when it is diluted due to glucose oxidase enzyme activation, which further oxidizes glucose to gluconic acid and H_2O_2. Apart from the H_2O_2 content, endogenous enzymes like glucose oxidase and several other non-peroxide factors i.e., the presence of phyto-chemical components such as methylglyoxal (MGO), are responsible for its anti-bacterial action (Weston 2000; Mavric et al. 2008). The Disc diffusion method is used for the qualitative detection of the anti-bacterial activity of honey, and Minimum inhibitory concentration (MIC), Zone diameter of inhibition (ZDI), and time-kill studies are used for the quantitative detection of anti-bacterial activity in the in vitro system. Honey is reported for the treatment of skin disorders such as wound healing, burns, skin inflammation, etc. Along with that, it possesses anti-microbial activity, which is an additional feature of honey that aids in quick recovery (Mandal and Mandal 2011; McLoone et al. 2016). Honey is never reported bacterial resistance till now due to that it is a very promising anti-bacterial agent for future research. Anti-microbial resistance is growing day by day, which indicates the emerging trends and scope for new anti-bacterial agents for burn treatment and for the challenges that arise due to burning wound pathogens (Church et al. 2006). In future, Honey may also provide major breakthrough for the treatment of bacteria-resistant effectively.

6.2.3 Anti-Diabetic Effect

Diabetes is allied with a deterioration of glycemic control and progressive metabolic derangements. Many studies have been conducted on the experimentally induced diabetic model that showed the protective effect of honey by controlling the glycemic rate and lipid metabolism, and it also decreases oxidative stress which contributes to organ damage. The exact mechanism is not clear but several studies have been reported on the positive effect of honey in the diabetic control group (Kadirvelu and Gurtu 2013). In a study, it was reported that the amount of fructose in honey may be responsible for the hypoglycemic activity, as honey is mainly composed of fructose along with other chemical constituents (Erejuwa et al. 2012a). In the Diabetic Mellitus model due to chronic hyperglycemia, there is an increase in oxidative stress, as evidenced by an increased level of malondialdehyde along with superoxide dismutase and glutathione peroxide but the catalase activity was down-regulated. Administration of honey ameliorates oxidative stress, reduces the elevated malondialdehyde levels, and it also restores oxidative enzymes like catalase and the superoxide dismutase activity of the pancreas. The honey-treated diabetic group showed a reduced blood glucose level (8.8 mmol/L) compared to the

diabetic control group (17.6 mmol/L), producing hypoglycemic effect in streptozotocin-induced diabetic rats but no such effect was seen in the non-diabetic pancreas. In another study, the addition of honey in the diet as a supplement resulted in a reduction of the glucose level in the serum of diabetic alloxanine-induced rats. The hypoglycemic effect of honey may be due to insulin sensitization and anti-oxidant activity (Fasanmade and Alabi 2008). Besides, the synergistic effect of honey with hypoglycemic agents was evaluated in which honey as an adjuvant to glibenclamide and metformin results in an elevated insulin level and decreases hyperglycemia and fructosamine. However, glibenclamide or metformin alone reduces hyperglycemia (13.9 ± 3.4 or 13.2 ± 2.9 mmol/L, respectively) but the combining effects with honey are much higher (88 ± 2.9 or 9.9 ± 3.3 mmol/L) (Erejuwa et al. 2011). Also, a clinical study on a diabetic patient was conducted in which the consumption of honey for 8 weeks resulted in the reduction of body weight, total cholesterol, LDL, and triglyceride but an increase in HDL providing the beneficial effects of honey on the diabetic patient (Bahrami et al. 2009). It has been suggested that honey has a low glycemic index as compared to glucose, and the consumption of honey for 2 h significantly lowers the serum glucose level, and it also decreases the insulin level (Soylu et al. 2015). Similarly, in Type 1 diabetes mellitus patients, honey lowers the glycemic index as compared to the sucrose group. On the other hand, it increases the C-peptide level in the same group as compared to the glucose or sucrose group. The study shows that honey might have the ability to stimulate the insulin released from the diseased β-cells (Abdulrhman et al. 2013).

6.2.4 Anti-Inflammatory Effect

Honey from stingless bees and various other sources are renowned for its anti-inflammatory effect, which is obtained from distinct countries e.g., Egypt, Malaysia, Russia, and Germany (Nweze et al. 2016). Stingless bee honey is very beneficial for human health from ancient times as it contains various phenolic compounds, which are derivatives of cinnamic acid and para-hydroxybenzoic acid. Flavonoid content of honey is naringenin, hesperetin, chrysin, pinocembrin, galangin, quercetin, and kaempferol (Truchado et al. 2008). Inflammation is the most important part of tissue injury or infection. When tissue injury or infection is prolonged, it can prevent the repair of the damaged tissue or organ. Inflammation generates free radicals. These free radicals generate leucocytes, which are stimulated as a part of the inflammatory process. Furthermore, inflammation stimulates various cellular processes, which produce growth factors and control the process of angiogenesis and proliferation of fibroblasts and epithelial cells. Proteins, lipids, and nucleic acids are an important part of normal cell functioning, which can be damaged by the inflammation of cells due to injury or infection (Yuksel 2011). Chronic inflammation causes diseases like diabetes mellitus, cancer, cardiovascular diseases, and atherosclerosis. Polyphenol content of natural honey helps in preventing neuroinflammation via oxidative stress inhibition, and additionally, it impacts neurodegenerative diseases, neuronal

apoptosis, necrosis, and synaptic plasticity (Rahman et al. 2014). Honey is very effective in the prevention of these chronic inflammatory pathological conditions. The primary function of the phenolic group in honey is anti-inflammatory activity. These two phenolic group and flavonoid group compounds are reported for suppressing pro-inflammatory activities of inducible action of nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). When the bee honey is administered in diluted form to humans, it decreases prostaglandins, which include prostaglandin E2 (PGE2), thromboxane B2, and prostaglandins F2à (PGF2á) (Viuda-Martos et al. 2008). Honey administration in Inflammatory Bowel Diseases (IBDs) shows more significant results. Corticosteroids and NSAIDs show more side effects as compared to honey's anti-inflammatory effects. It is reported that honey shows protective effects on hypertensive rats due to its anti-inflammatory and anti-oxidant effects, which mainly control blood pressure by reducing lipid peroxidation such as MDA concentration and upregulating the Nrf2 level (Nooh and Nour-Eldien 2016). On the other hand, scientific research of honey's activity in in-vitro and in-vivo investigations on the anti-inflammatory effect is still very limited; it requires further research to explore the pharmacological effects of honey and its constituents (Carlisle et al. 2013).

6.2.5 Anti-Oxidant Effect

It is reported in many studies that honey is a good antioxidant, both in in vitro and in vivo systems. Honey obtained from various geographic regions like Turkish red pine honey, Saudi Arabian honey, and Peruvian honey are known for its antiradical activity. In an in vitro system, all these regional honey shows anti-oxidant activity, as assayed using oxygen radical absorbance capacity (ORAC) assay, 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay, and ferric reducing anti-oxidant power (FRAP) assay. These are the markers for anti-oxidant activity which gets elevated by a natural or synthetic agent. In in vivo systems, it showed an anti-oxidant effect on the tissues such as liver, GIT, kidneys, pancreas, eyes, plasma, red blood cells, and reproductive organs. It showed its protective effect by ameliorating the oxidative stress inside tissues (Erejuwa et al. 2012b).

Honey is well documented for its importance in protecting the cell defense system against oxidative damage. Free radicals and other oxidizing agents such as ROS play a key role in toxin's mode of action (Nagai et al. 2001). There are a lot of endogenous antioxidants like superoxide dismutase (SOD), catalase, alpha-lipoic acid (ALA), coenzyme Q10 (CoQ10), and glutathione peroxidase (GPX)) present in the human body apart from endogenous antioxidants and exogenous antioxidants are taken from the diet source. Honey's anti-oxidant activity is measured as the anti-oxidant capacity (AOC), which represents that these are bioactive molecules (Cianciosi et al. 2018).

The anti-oxidant potential of honey is the ability to reduce oxidative damage by scavenging the free radical which is generated due to an imbalance in the homeostasis of the anti-oxidant and the pro-oxidant in the human body. Honey contains many

chemical constituents which have been reported earlier for their free radical scavenging properties (Gheldof and Engeseth 2002). Honey exerts its anti-oxidant activity by forming a complex with metal ions e.g., Cu and Fe and free radicals. Honey contains phenolic and flavonoid groups which significantly help in neutralizing the free radicals by complex formation (Yuksel 2011). These phenolic groups contain phenolic acid content including derivatives of hydroxybenzoic acid and hydroxycinnamic acid and flavonoids like naringenin, hesperetin, pinocembrin, galangin, chrysin, quercetin, and kaempferol (Truchado et al. 2008). Besides these, carbohydrates, proteins, amino acids, carotenes, organic acids, and other minor constituents are responsible for its anti-oxidant effect (Gheldof and Engeseth 2002). Honey contains several Vitamins such as thiamine, pyridoxine, riboflavin, p-aminobenzoic acid, folic acid, pantothenic acid, and vitamins A, C, and E which are very potent for their anti-oxidant effects. These Vitamins have been reported in many studies previously for their anti-oxidant activities (Calixto-Campos et al. 2015). In a study, it was reported that honey supplementation in Wistar rats reduced the major oxidative stress marker MDA in the plasma by 35% (approx.) after 7 days when it is administered (5 g/kg body weight once daily) (Cianciosi et al. 2018). The anti-oxidant effect also contributes to its anti-inflammatory nature as it removes the free radicals which involve inflammation as well (Henriques et al. 2006).

6.2.6 Immunoprotective Effect

In addition to honey being a good anti-bacterial agent, it also helps in protecting from infection by activating the immune system to fight with intruders. Honey stimulates the proliferation of B and T lymphocytes in cell culture. It is reported in multiple studies that B60 lymphocytes and T lymphocytes help in increasing the number of culture cells and trigger the immune cells as well as increase their number (Aznan et al. 2016). It is reported that NO plays a key role in immune system defence against the infection (Al-Waili et al. 2011). NO inhibits the replication of viruses and kills the intracellular pathogens (Baldik et al. 2002). Honey has been reported to show increased production of NO excretory products like urine, saliva, and biological fluids which help in killing the pathogens. It triggers the release of immune cells TNF-α, IL-1, and IL-6. These are cell messengers that can activate the immune system against infection (Al-Waili et al. 2011; Al-Waili and Boni 2004).

IFN-γ also plays a major role in the body's both innate and adaptive immune defence systems. It works as a pro-inflammatory mediator. It is involved in many immunological disorders along with the other cytokines. Stingless bee honey is reported for its anti-inflammatory effect by elevating the concentration of the IFN-γ even better than the standard drug (Rožman and Švajger 2018). In a study, it was reported that bee honey contains salicylic acid, p-coumaric acid, aromadendrin, and taxifolin which are the major contributors for the protective effect of bee honey in acetaminophenon-induced hepatic inflammation by regulating the pro-inflammatory (Cha et al. 2018). Daily intake of honey 80 g/day up to 3 weeks increased the prostaglandin levels in an AIDS patient compared to a normal

individual. Many studies have indicated that daily intake of honey helps in boosting the immune system (Al-Waili et al. 2006).

6.2.7 Respiratory Effect

Cough is a major issue for doctors as it leads to further respiratory tract diseases such as asthma. Children are very susceptible to cough as their immune system is not strong enough to fight allergens (McLoone et al. 2016). Honey has been used for cough treatment traditionally. It is reported in many studies that honey is very beneficial for respiratory tract diseases, especially asthma. In a study, it was reported that honey elevates the structural changes that occur due to allergen-induced chronic asthma. When it was administered through the inhalation route it improved the lower respiratory tract inflammation in ovalbumin-induced chronic asthma in the rabbit model. It showed preventive as well as improved asthma conditions (Cianciosi et al. 2018).

It has been reported that honey improved the clinical effects when compared to various treatment groups. It reduced the cough frequency and improved the symptoms, which could have led to diseases like asthma. All the reviewed and reported literatures indicated that honey is very healthy and effective in the treatment of respiratory tract infections (Meo et al. 2017).

6.2.8 Chemopreventive Effect

6.2.8.1 Cardioprotective Effect

"Prevention is better than cure". As defined, chemoprevention is the treatment by supplementing or administrating agents to prevent or suppress the development of the disease.

In the era of cardiovascular diseases, the drugs used in controlling such diseases have a certain constraint. Therefore, there is a strong tendency toward natural products. It has been reported that honey minimizes the cardiovascular risk factors in healthy people and patients with heightened risks factor. Numerous studies have revealed an association between the consumption of honey and the reduced risk of cardiovascular ailments. Honey enriched in flavonoids and vitamin C has been revealed to have a cardioprotective effect by different mechanisms: reducing the activity of blood platelets, preventing the oxidation of low-density lipoprotein, improving coronary vasodilation, and by reducing the cholesterol levels in hyperlipidemic patients (Cianciosi et al. 2018). The inhibitory effects of honey on the aggregation of platelets and blood coagulation were evaluated and it was shown that honey inhibits coagulation by interfering with intrinsic, extrinsic, and common coagulation cascades indicating anti-coagulating activity and also prolongs the formation of thrombus, the formation of thrombus on atherosclerotic plaque leads to cardiovascular accidents. Thus, honey can be considered an excellent counteracting agent (Khan et al. 2011).

Honey is very effective against reducing the cholesterol level. It decreases the cholesterol level due to its phenolic content. It is reported in many studies that continuous intake of phenolic compounds is directly linked with heart disease, and it helps in reducing the risk in these pathological conditions as well. There are strong associations between low high-density lipoprotein cholesterol levels and its connection with cardiovascular diseases (Drexel 2006). The reduction in cardiovascular risk factors such as C-reactive proteins, cholesterol, triglyceride, and low-density lipoprotein and an increase in high-density lipoprotein were observed when 75 g of honey was ingested for 15 days as compared to the same amount of artificial honey (fructose and glucose) (Al-waili 2004). Besides, ingestion of 10% honey for a long time results in the elevation of HDL levels, which results in the reduction of cardiovascular disease; therefore, the ability of honey to improve HDL is valuable (Fasanmade and Alabi 2008). Similarly, the consumption of honey for a longer period reduces the risk of cardiovascular factors in a normal individual and especially in individuals with elevated risk factors. In a study, 55 obese patients were fed with 70 g of natural honey against sucrose as control for a duration of 30 days. It reduced the body weight (1.3%) and consistent the reduction of total cholesterol (3%) and body fat (1.1%) were measured and triglyceride (11%), LDL-C (5.8%), C-reactive protein (3.2%) and increase in HDL-C (3.3%) in normal individuals whereas in patients, it reduced total cholesterol by (3.3%), triglyceride (19%) and C-reactive (3.3%) (Yaghoobi et al. 2008). Meanwhile, the wild type honey treatment causes enhancement of the endothelial-derived relaxing factor nitric oxide, which has a vasodilation effect which arised due to influence of epinepherine. (Rakha et al. 2008).

6.2.9 Anti-Cancer Effect

Cancer is a large group of diseases categorized as hyperproliferation of mutated cells. Cancer chemoprevention is the preventive measure for the treatment of populations susceptible to develop cancer or at risk by supplementing or administrating agents to prevent or suppress the development of the disease by acting on different stages of cancer such as initiation, promotion, and progression (Muhammad et al. 2016). The available literature shows that honey is endowed with anti-cancer activity in terms of prevention, progression, and treatment. Various polyphenols present in honey showed anti-tumour effects against numerous types of cancers. Honey has gained importance in this area, as it is rich in polyphenols. Some of the polyphenols present in honey, namely caffeic acid, caffeic acid phenyl esters, galangin, quercetin, chrysin, kaempferol, acacetin, pinocembrin, pinobanksin, and apigenin were considered as favourable pharmacological agents in the treatment of cancer (Jaganathan and Mandal 2009). Honey shows its action at different stages of carcinogenesis such as initiation, proliferation, and progression. The anti-tumoural effect of honey is characterized by different mechanisms due to the initiation of apoptosis, modulation of oxidative stress, cell cycle arrest, induction

of mitochondrial outer membrane permeabilization, amelioration of inflammation, and inhibition of angiogenesis (Cianciosi et al. 2018).

Apoptosis is the favoured process by which honey induces cell death in tumour condition. The anti-tumour effects of Tualang honey and Manuka honey were evaluated against breast cancer. The administration of honey 1.0 g/kg for 120 days showed a reduction in the tumour size and weight. Honey-treated rats showed a less aggressive tumour. Honey exerts anti-cancer effect by activation of intrinsic apoptotic pathways by increasing the expression of pro-apoptotic protein such as Caspase 9, p53, APAF-1, INF-γ, and INFGR1. It also works through the down-regulation of anti-apoptotic proteins such as Bcl-xL, TNF-α, and COX2 as compared to the untreated groups (Ahmed et al. 2017). Polyphenolic compounds in honey having anti-proliferative effects have been reported. The eugenol content of honey can inhibit the growth of Ehrlich ascites carcinoma by mitochondrial and reactive oxygen species-mediated apoptotic mechanisms. Eugenol at 100 mg/kg dose inhibits the growth of solid tumour by 24.35% and Ehrlich ascites by 28.88%. Gelam honey has been evaluated for its anti-cancer effect as it is proved to be with chemopreventive potential against colorectal cancer and several other cancer types. In-vitro studies on a combination of gelam honey and ginger show the chemotherapeutic activity against HT29 colon cell lines. The treatment displayed a significant suppressive effect by inducing early apoptosis by increasing the caspase 9 and IκB gene regulation and decreasing the KRAS, ERK, ATK, BCL-xL, and NFκB (Tahir et al. 2015). Oxidative stress promotes carcinogenesis by generating free-radical, which further leads to cancer development. Carcinoma development takes place due to oxidative stress and apoptosis. The anti-proliferative activity of honey against human hepatoma cells (HepG2) reduces survival in a dose-dependent manner. NO is an oxidant molecule that causes hepatic injury through reactive oxygen species (ROS) generation and inhibiting the apoptosis process. Treatment of HepG2 cell with 20% honey for 72 h showed an increase in caspase-3 activity and the anti-oxidant profile. Few studies were reported that showed anti-oxidant activity efficiently tackles free radical generation and oxidative damage which also might play a role in impeding the initiation and promotion of cancer (Hassan et al. 2012). It has also been postulated that Kelulut honey has chemopreventive property against azomethane-induced colon cancer. Azomethane-induced aberrant crypt foci formation, which is the biomarker for colon cancer, indicates the progression of the same. When Kelulut honey is administered orally (1183 mg/kg body weight) it reduces the total number of aberrant crypt foci, aberrant crypt, and crypt multiplicity. Caffeic acid ester content is responsible for the anti-cancer property of Kelulut honey. Another study also demonstrated that the presence of abscisic acid in honey evidences its anti-mutagenic role against ethyl methanesulfonate in *E. coli* MG1655. The maximum anti-mutagenicity observed was 78% through rifampicin resistance assay. Further to re-confirm, the human lymphoblast cell line (TK6) was used, and the mutagenicity was reduced by 55% at ABA 50 μg/mL and 75% at 200 μg/mL, indicating a dose-dependent anti-mutagenic potential (Yazan et al. 2016).

6.2.10 Gastroprotective Effect

Gastric ulcer is a very common disease affecting humans globally. It is characterized by mucosal damage with immune cell infiltration. Various factors contribute to the advancement of ulcers such as smoking, *H. Pylori* infection, extensive use of alcohol, and physiological stress. Many drugs have been used in the treatment of the disease but prolonged use of drugs is associated with side effects. Herbal drugs are attracting popularity due to their fewer side effects as compared to synthetic drugs. The natural honey extract known for its anti-oxidant properties has been widely evaluated for its gastroprotective and anti-microbial effects. A study concluded that honey exerts antiulcer activity by the preservation of gastric mucosal glutathione, decreases lipid peroxidation, and restores endogenous antioxidants (Almasaudi et al. 2016). Similarly, the anti-bacterial activity of different types of honey against *Heliobacter pylori,* responsible for gastro-duodenal ulcers, has been evaluated in-vitro; *H. pylori* isolated from patients with the gastric disease was assessed by measuring MBC and MIC for its susceptibility to various types of honey, and the honey was found to possess an anti-bacterial effect with evident therapeutic potential (Ndip et al. 2007). In another study, the gastro-protective effect of honey against indomethacin-induced gastric lesion was evaluated; pre-treatment of an animal with a dose of (2 mg/kg) Alimento Supervis (AS), Alimento Mieleucalipto (AM), and honey prevented the gastric lesions by reducing the ulcer index, microvascular permeability, and myeloperoxidase as compared to indomethacin treated alone. The reduction in ulcer index is due to the radical scavenging activity of honey and by modulating leucocyte function; reduced neutrophil infiltration was also observed in pre-treated animals because of the anti-inflammatory properties of honey. Honey helps in mucosal healing by its anti-oxidant, anti-inflammatory, and cytoprotective mechanism (Nasuti et al. 2006).

6.2.11 Effect on Gut Microbiota

"Let food be thy medicine and medicine be thy food"—the age-old quote by Hippocrates which completely justifies honey's role in the present time. It possesses prebiotic, probiotic, and synbiotic activities which are proved from the literature. Probiotics are very effective in many pathological conditions such as diarrhoea prevention, constipation, improving bile salt conjugation; they have anti-bacterial effect, anti-inflammatory, and anti-oxidative effects besides being used in allergy, cancer, AIDS, respiratory tract infection, urinary tract infection, aging, diabetes, osteoporosis, etc. (Pandey et al. 2015).

Gut microbiota is largely unexplored, and it holds out many healthy metabolic activities that impact many key elements of the human physiology and it adds nutrients and energy through anaerobic fermentation. Prebiotics, probiotics, and synbiotics play a very significant role in the human gut which is involved in many pathological conditions. The use of prebiotics and probiotics has increased for research purposes over the past few years. Some of the major bacterial strains

found in our stomach, small intestine, and large intestine are *Lactobacillus*, *Enterococcus*, *Bifidobacterium*, *Bacteroides*, *Clostridium*, *Eubacterium*, *Staphylococcus*, *Coliform*, etc. (Mohan et al. 2017). Probiotic cells provide growth factors like oligosaccharides. In the present time, synbiotics are frequently used in the form of supplements. These are available as food ingredients and dietary supplements. Prebiotics and probiotics combination give the synergistic effect. The use of prebiotics and probiotics is intended to stimulate the microflora in the stomach. A prebiotic activates the microflora in the large intestine. Thus, the combination of prebiotics and probiotics synergistically provides overall protection to the stomach (Pandey et al. 2015).

Honey contains a supersaturated solution of sugars, primarily glucose and fructose, and apart from these, it has numerous other minor components. It contains disaccharides, such as maltose, sucrose, and several higher oligosaccharides which contain 3–10 monosaccharide units. It is also a good prebiotic as it contains oligosaccharides that help in the promotion of bacterial strains like *Lactobacilli* and *Bifidobacteria*; additionally, it also works as an anti-microbial agent which can show a synergistic effect against probiotics for protecting our body from several pathogens (Sanz et al. 2005). Honey promotes probiotic growth when it is incubated with honey under optimal conditions along with that it also shows inhibitory effects against pathogens and intestinal microbes. Many study reports have confirmed its ability to work as a prebiotic by modifying the gut microflora composition in the human intestine. (Mohan et al. 2017).

6.2.12 Neuroprotective Effect

The central nervous system is very prone to oxidative stress as compared to other vital organs due to its higher amount of oxygen consumption and comparative inadequacy in the presence of endogenous anti-oxidant and reactive oxygen species (ROS) scavenging enzymes. The brain's protective mechanism against oxidative stress is not adequate to prevent increasing oxidative damage. Alzheimer's disease is an old-age-related pathological condition in which neurons degenerate, and oxidative stress and apoptosis play a key role in the development of such neurodegenerative disease. However, anti-oxidant intake in the form of a nutraceutical may protect the brain from oxidative damage. Stress exposed rats produce oxidative stress in the brain which results in reactive oxygen species (ROS) generation. ROS further ultimately damages the nerve membrane resulting in neuronal loss and leads to neurodegenerative diseases. Tualang honey administration in the stressed rats showed improved memory in the brain's oxidative stress condition by increasing the anti-oxidant enzymes and by reducing the oxidative markers and decreasing the hormonal stress level. Thus, honey protects neuronal cell damage (Azman et al. 2018). Similarly, the potential effects of honey flavonoid extracts against neurodegenerative diseases involving neuroinflammation have been studied. The honey extract has a potent inhibitory effect against microglial activation aiding neuroinflammation, by attenuating the production of pro-inflammatory mediators

such as IL-1β and TNF-α. Moreover, the expression of iNOS and the production of ROS were also inhibited. Thus, combining the effect of the anti-oxidant and anti-inflammatory activities of honey in the neurodegenerative process can produce an additive neuroprotective effect (Candiracci et al. 2012). Besides, hyperglycemia and increased oxidative stress impede the neurological function in streptozotocin-induced diabetic rats due to the generation of ROS; the use of honey and insulin as an anti-oxidant significantly inhibits apoptosis of neuronal cells by neutralizing the free radicals and preventing the neurodegeneration in the hippocampus of the diabetic rats (Jafari Anarkooli et al. 2014).

6.2.13 Nephroprotective Effect

Honey is a source of natural antioxidant which contains a large number of phenolic acids and flavonoids. Antioxidants are found in two forms, enzymatic and non-enzymatic. Superoxide dismutase (SOD), catalase, glutathione reductase (GR), glutathione peroxidase (GPx), thioredoxin reductase (TR), and peroxiredoxin-2 (PRDX2) are the enzymatic antioxidants, while tocopherol, glutathione (GSH), and ascorbic acid are non-enzymatic antioxidants. Honey contains polyphenols like chrysin, pinobanksin, pinocembrin, and luteolin, which are reported for the reduction in oxidative stress previously. All the phytochemicals present in honey exert a combined anti-oxidant effect, which helps to scavenge free radicals and thus help in inhibiting renal injury. In a study, it was concluded that honey exerts a kidney protective effect against lead-induced oxidative stress, which increases the MDA level and decreases the anti-oxidant enzyme glutathione peroxidase activity and serum nitric oxide. The administration of natural honey ameliorates the increased level of urea and serum creatinine and decreases the MDA level. Honey modulates adverse damage in kidney cells induced by lead toxicity, by restoring the glutathione activity through its anti-oxidant mechanism (Halawa et al. 2009). In another study, the protective effect of honey against nephrotoxicity induced by amikacin—an antibiotic—was evaluated; the use of the drug induces free radical production, which results in elevation of serum creatinine and urea, MDA and a decrease in the serum glutathione level where honey acts as free-radical scavengers, reduced the nephrotoxic effect of the drug by decreasing the serum creatinine and urea, MDA and elevation of glutathione levels. Honey significantly improves the renal function by interfering with oxidative stress (Ali and Ismail 2012). The role of honey in carbon tetrachloride-induced hepatonephrotoxicity was also investigated and honey's potential role in significantly decreasing MDA levels and elevating the total anti-oxidant capacity was confirmed (El Denshary et al. 2012). It was observed that the nephroprotective mechanism conferred by the anti-oxidant activities of the phenolic and flavonoid content. Nephroprotective activity take place through these mechanisms such as free-radical scavenging, hydrogen donation, singlet oxygen quenching, metal ion chelation, and acting as a substrate for radicals such as superoxide.

6.3 Conclusion

In this chapter, we try to include all the major benefits of using honey as a therapeutic agent. Since ancient times, honey has been used as a traditional herbal drug. At present, it seems that its therapeutic and nutritional value has been re-discovered. It contains many compounds that make it very effective for nutritional and therapeutic purposes. Some of the therapeutic benefits of honey are mentioned below:

- It is used for the treatment of wounds. Additionally, it possesses remarkable antibacterial, antioxidant, anti-inflammatory, and immune-boosting properties. All these properties help in quick healing and boosting the body's defence system.
- The free radical scavenging ability of honey helps in preventing oxidation of lipids, and reduction of oxidative stress generation which prevents many diseases. It is a very effective anti-oxidant agent due to its phenolic content.
- Honey has proven very effective in the treatment of diabetes mellitus as it controls the glycemic rate and lipid metabolism which helps in maintaining the blood sugar level.
- It has positive effects on the disease of the respiratory system, erectile dysfunction, gastroprotective, urinary diseases, skin grafts, and other skin-related diseases such as ulcers and psoriasis.
- It shows anti-cancer or anti-tumour activity due to its anti-apoptotic, anti-proliferative, anti-tumour, anti-mutagenic, and estrogenic modulatory activities.
- It is very popular these days in the form of nutraceutical for reducing weight, anti-aging, immune booster, etc.
- It is very effective in conditions like asthma, allergic rhinitis, enhanced fertility, atherosclerosis, and parasitic infection.
- It has been reported for its anti-ulcer activities due to its chemical constituents such as phenolic and flavonoid content which prevents the production of inflammatory mediators released in the ulcer.
- The antiviral activity of honey has been reported in a few studies where it prevents the multiplication of the virus and shows virucidal effects in several viruses such as Herpes simplex type 1, Herpes simplex type 2, vesicular stomatitis and adenovirus type 2, etc.

In the future, it can be very effective for other activities too as best of it yet to be rediscovered. like ancient times.

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References

Abdulrhman M, El M, Ali R, Abdel I, El-goud AA, Refai D (2013) Complementary therapies in clinical practice effects of honey, sucrose and glucose on blood glucose and C-peptide in patients with type 1 diabetes mellitus. Complement Ther Clin Pract 19:15–19. https://doi.org/10.1016/j.ctcp.2012.08.002

Afroz R, Tanvir EM, Zheng W, Little PJ (2016) Molecular pharmacology of honey. Clin Exp Pharmacol 6:3. https://doi.org/10.4172/2161-1459.1000212

Ahmed S, Sulaiman SA, Othman NH (2017) Oral administration of Tualang and Manuka honeys modulates breast cancer progression in Sprague-Dawley rats model. Evid Based Complement Alternat Med 2017:5904361

Ali ARA, Ismail SH (2012) The protective effect of honey against Amikacin-induced nephrotoxicity in rats. Iraqi J Pharm Sci 21:85–93

Almasaudi SB, El-Shitany NA, Abbas AT, Abdel-Dayem UA, Ali SS, Al Jaouni SK, Harakeh S (2016) Antioxidant, anti-inflammatory, and antiulcer potential of manuka honey against gastric ulcer in rats. Oxidative Med Cell Longev 2016:1–10. https://doi.org/10.1155/2016/3643824

Al-waili NS (2004) Natural honey lowers plasma glucose, C-reactive protein, homocysteine, and blood lipids in healthy, diabetic, and hyperlipidemic subjects: comparison with dextrose and sucrose. J Med Food 7:100–107

Al-Waili NS, Al-Waili TN, Al-Waili AN, Saloom KS (2006) Influence of natural honey on biochemical and hematological variables in AIDS: a case study. Sci World J 6:1985–1989. https://doi.org/10.1100/tsw.2006.331

Al-Waili NS, Boni NS (2004) Honey increased saliva, plasma, and urine content of total nitrite concentrations in normal individuals. J Med Food 7:377–380. https://doi.org/10.1089/jmf.2004.7.377

Al-Waili NS, Salom K, Al-Ghamdi AA (2011) Honey for wound healing, ulcers, and burns; data supporting its use in clinical practice. Sci World J 11:766–787. https://doi.org/10.1100/tsw.2011.78

Azman KF, Zakaria R, Othman Z (2018) Neuroprotective effects of Tualang honey against oxidative stress and memory decline in young and aged rats exposed to noise stress. J Taibah Univ Sci 12:273–284. https://doi.org/10.1080/16583655.2018.1465275

Aznan MI, Khan OH, Unar AO, Tuan Sharif SE, Khan AH, Syed SH, Zakaria AD (2016) Effect of Tualang honey on the anastomotic wound healing in large bowel anastomosis in rats—a randomized controlled trial. BMC Complement Altern Med 16:1–7. https://doi.org/10.1186/s12906-016-1003-6

Bahrami M, Ataie-jafari A, Hosseini S (2009) Effects of natural honey consumption in diabetic patients: an 8-week randomized clinical trial. Int J Food Sci Nutr 60:618–626. https://doi.org/10.3109/09637480801990389

Baldik Y, Talu U, Altinel L, Bilge H, Demiryont M, Aykac-Toker G (2002) Bone healing regulated by nitric oxide: an experimental study in rats. Clin Orthop Relat Res 404:343–352. https://doi.org/10.1097/00003086-200211000-00051

Baltrušaityte V, Venskutonis PR, Čeksteryte V (2007) Radical scavenging activity of different floral origin honey and beebread phenolic extracts. Food Chem 101:502–514. https://doi.org/10.1016/j.foodchem.2006.02.007

Bestwick CS, Milne L (2006) Influence of galangin on HL-60 cell proliferation and survival. Cancer Lett 243:80–89. https://doi.org/10.1016/j.canlet.2005.11.025

Calixto-Campos C, Carvalho TT, Hohmann MSN, Pinho-Ribeiro FA, Fattori V, Manchope MF, Zarpelon AC, Baracat MM, Georgetti SR, Casagrande R, Verri WA (2015) Vanillic acid inhibits inflammatory pain by inhibiting neutrophil recruitment, oxidative stress, cytokine production, and NFκB activation in mice. J Nat Prod 78:1799–1808. https://doi.org/10.1021/acs.jnatprod.5b00246

Candiracci M, Piatti E, Garc D, Ruano D, Gutie JF, Parrado J (2012) Anti-inflammatory activity of a honey flavonoid extract on lipopolysaccharide-activated N13 microglial cells. J Agric Food Chem 60(50):12304–12311

Carlisle J, Chan D, Golub M, Henkel S, Painter P, Wu KL (2013) Toxicological Profile for. Oxidative Med Cell Longev 2013:24. https://doi.org/10.1155/2013/286524

Cha H, Lee S, Lee JH, Park JW (2018) Protective effects of p-coumaric acid against acetaminophen-induced hepatotoxicity in mice. Food Chem Toxicol 121:131–139. https://doi.org/10.1016/j.fct.2018.08.060

Cho H, Yun CW, Park WK, Kong JY, Kim KS, Park Y, Lee S, Kim BK (2004) Modulation of the activity of pro-inflammatory enzymes, COX-2 and iNOS, by chrysin derivatives. Pharmacol Res 49:37–43. https://doi.org/10.1016/S1043-6618(03)00248-2

Church D, Elsayed S, Reid O, Lindsay R, Church D, Elsayed S, Reid O, Winston B, Lindsay R (2006) Burn wound infections. Clin Microbiol Rev 19:403–434. https://doi.org/10.1128/CMR.19.2.403

Cianciosi D, Forbes-Hernández TY, Afrin S, Gasparrini M, Reboredo-Rodriguez P, Manna PP, Zhang J, Lamas LB, Flórez SM, Toyos PA, Quiles JL, Giampieri F, Battino M (2018) Phenolic compounds in honey and their associated health benefits: a review. Molecules 23:1–20. https://doi.org/10.3390/molecules23092322

Drexel H (2006) Reducing risk by raising HDL-cholesterol: the evidence. Eur Heart J Suppl 8:23–29. https://doi.org/10.1093/eurheartj/sul037

El Denshary ES, Al-Gahazali MA, Mannaa FA, Salem HA, Hassan NS, Abdel-Wahhab MA (2012) Dietary honey and ginseng protect against carbon tetrachloride-induced hepatonephrotoxicity in rats. Exp Toxicol Pathol 64:753–760. https://doi.org/10.1016/j.etp.2011.01.012

Erejuwa OO, Sulaiman SA, Suhaimi M, Wahab A (2011) Glibenclamide or metformin combined with honey improves glycemic control in streptozotocin-induced diabetic rats. Int J Biol Sci 7:244–252

Erejuwa OO, Sulaiman SA, Wahab MSA (2012a) Fructose might contribute to the hypoglycemic effect of honey. Molecules 17:1900–1915. https://doi.org/10.3390/molecules17021900

Erejuwa OO, Sulaiman SA, Wahab MSA (2012b) Honey: a novel antioxidant. Molecules 17:4400–4423. https://doi.org/10.3390/molecules17044400

Eyarefe DO, Kuforiji DI, Jarikre TA, Emikpe BO (2017) Enhanced electroscalpel incisional wound healing potential of honey in wistar rats. Int J Vet Sci Med 5:128–134. https://doi.org/10.1016/j.ijvsm.2017.10.002

Fasanmade AA, Alabi OT (2008) Differential effect of honey on selected variables in alloxan-induced and fructose-induced diabetic rats. Afr J Biomed Res 11:191–196

Fratellone PM (2015) Apitherapy products for medicinal use. J Nutr Food Sci 5:6–7. https://doi.org/10.4172/2155-9600.1000423

Geraets L, Moonen HJJ, Brauers K, Wouters EFM, Bast A (2007) Dietary flavones and flavonoles are inhibitors of poly (ADP-ribose) polymerase-1 in pulmonary epithelial cells. J Nutr 137:2190–2195

Gheldof N, Engeseth NJ (2002) Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples. J Agric Food Chem 50:3050–3055. https://doi.org/10.1021/jf0114637

Halawa HM, El-nefiawy NE, Makhlouf NA, Mady AA (2009) Evaluation of honey protective effect on Lead induced oxidative stress in rats. JASMR 4:197–209

Hassan MI, Mabrouk GM, Shehata HH, Aboelhussein MM (2012) Antineoplastic effects of bee honey and Nigella sativa on hepatocellular carcinoma cells. Integr Cancer Ther 11:354–363. https://doi.org/10.1177/1534735410387422

Henriques A, Jackson S, Cooper R, Burton N (2006) Free radical production and quenching in honeys with wound healing potential. J Antimicrob Chemother 58:773–777. https://doi.org/10.1093/jac/dkl336

Hoon T, Ku KS, Bae J (2012) Anti-inflammatory effects of kaempferol-3-O-sophoroside in human endothelial cells. Inflamm Res 61:217–224. https://doi.org/10.1007/s00011-011-0403-9

Hsu Y, Kuo P, Lin C (2004) Acacetin inhibits the proliferation of Hep G2 by blocking cell cycle progression and inducing apoptosis. Biochem Pharmacol 67:823–829. https://doi.org/10.1016/j.bcp.2003.09.042

Jafari Anarkooli I, Barzegar Ganji H, Pourheidar M (2014) The protective effects of insulin and natural honey against hippocampal cell death in streptozotocin-induced diabetic rats. J Diabetes Res 2014:491571. https://doi.org/10.1155/2014/491571

Jaganathan SK (2012) Growth inhibition by caffeic acid, one of the phenolic constituents of honey, in HCT 15 colon cancer cells. Sci World J 2012:372345. https://doi.org/10.1100/2012/372345

Jaganathan SK, Mandal M (2009) Antiproliferative effects of honey and of its polyphenols: a review chemistry of honey. Biomed Res Int 2009:830616. https://doi.org/10.1155/2009/830616

Jaganathan SK, Mazumdar A, Mondhe D, Mandal M (2011) Apoptotic effect of eugenol in human colon cancer cell lines. Cell Biol Int 35:607–615. https://doi.org/10.1042/cbi20100118

Jaganathan SK, Supriyanto E, Mandal M (2013) Events associated with apoptotic effect of p-Coumaric acid in HCT-15 colon cancer cells. World J Gastroenterol 19:7726–7734. https://doi.org/10.3748/wjg.v19.i43.7726

Kadirvelu A, Gurtu S (2013) Potential benefits of honey in type 2 diabetes mellitus: a review. Int Jour Coll Res Int Med Pub Health 5:199–216

Khan RA, Azim K, Mesaik AM (2011) Effect of natural honey on human platelets and blood coagulation proteins. Pak J Pharm Sci 24:389–397

Lan X, Wang W, Li Q, Wang J (2016) The natural flavonoid pinocembrin: molecular targets and potential therapeutic applications. Mol Neurobiol 53:1794–1801. https://doi.org/10.1007/s12035-015-9125-2

Liu Y, Song X, He J, Zheng X, Wu H (2014) Synthetic derivatives of chrysin and their biological activities. Med Chem Res 23:555–563. https://doi.org/10.1007/s00044-013-0711-4

Lodi F, Jimenez R, Moreno L, Kroon PA, Needs PW, Hughes DA, Santos-buelga C, Gonzalez-paramas A, Cogolludo A, Lopez-sepulveda R, Duarte J, Perez-vizcaino F (2009) Glucuronidated and sulfated metabolites of the flavonoid quercetin prevent endothelial dysfunction but lack direct vasorelaxant effects in rat aorta. Atherosclerosis 204:34–39. https://doi.org/10.1016/j.atherosclerosis.2008.08.007

Mandal MD, Mandal S (2011) Honey: its medicinal property and antibacterial activity. Asian Pac J Trop Biomed 1:154–160. https://doi.org/10.1016/S2221-1691(11)60016-6

Mani R, Natesan V (2018) Chrysin: sources, beneficial pharmacological activities, and molecular mechanism of action. Phytochemistry 145:187–196. https://doi.org/10.1016/j.phytochem.2017.09.016

Mavric E, Wittmann S, Barth G, Henle T (2008) Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka (Leptospermum scoparium) honeys from New Zealand. Mol Nutr Food Res 52:483–489. https://doi.org/10.1002/mnfr.200700282

McLoone P, Oluwadun A, Warnock M, Fyfe L (2016) Honey: a therapeutic agent for disorders of the skin. Cent Asian J Glob Health 5:241. https://doi.org/10.5195/cajgh.2016.241

Mei C, Medical F, Road C, City Y, Municipal K, Hospital U, Medical K (2009) Tricetin, a dietary flavonoid, inhibits proliferation of human breast adenocarcinoma MCF-7 Cells by blocking cell cycle progression and inducing apoptosis. J Agric Food Chem 57:8688–8695. https://doi.org/10.1021/jf901053x

Meo SA, Al-Asiri SA, Mahesar AL, Ansari MJ (2017) Role of honey in modern medicine. Saudi J Biol Sci 24:975–978. https://doi.org/10.1016/j.sjbs.2016.12.010

Miguel J, Tulipani AS, Romandini S, Bertoli E, Battino M (2010) Contribution of honey in nutrition and human health: a review. Mediterr J Nutr Metab 3:15–23. https://doi.org/10.1007/s12349-009-0051-6

Miorin PL, Levy NC, Custodio AR, Bretz WA, Marcucci MC (2003) Antibacterial activity of honey and propolis from Apis mellifera and Tetragonisca angustula against Staphylococcus aureus. J Appl Microbiol 95:913–920. https://doi.org/10.1046/j.1365-2672.2003.02050.x

Mohan A, Quek S-Y, Gutierrez-Maddox N, Gao Y, Shu Q (2017) Effect of honey in improving the gut microbial balance. Food Qual Saf 1:107–115. https://doi.org/10.1093/fqs/fyx015

Mphande AN, Killowe C, Phalira S, Jones HW, Harrison WJ (2007) Effects of honey and sugar dressings on wound healing. J Wound Care 16:317–319. https://doi.org/10.12968/jowc.2007.16.7.27053

Muhammad A, Odunola OA, Ibrahim MA, Sallau AB, Erukainure OL, Aimola IA, Malami I (2016) Potential biological activity of acacia honey. Front Biosci 8:351–357

Nagai T, Sakai M, Inoue R, Inoue H, Suzuki N (2001) Antioxidative activities of some commercially honeys, royal jelly, and propolis. Food Chem 75:237–240. https://doi.org/10.1016/S0308-8146(01)00193-5

Nasuti C, Gabbianelli R, Falcioni G, Cantalamessa F (2006) Antioxidative and gastroprotective activities of anti-inflammatory formulations derived from chestnut honey in rats. Nutr Res 26:130–137. https://doi.org/10.1016/j.nutres.2006.02.007

Ndip RN, Takang AEM, Echakachi CM, Malongue A, Akoachere J-FTK, Ndip LM, Luma HN (2007) In-vitro antimicrobial activity of selected honeys on clinical isolates of Helicobacter pylori. Afr Health Sci 7:228–232

Nicholson SK, Tucker GA, Brameld JM (2010) Physiological concentrations of dietary polyphenols regulate vascular endothelial cell expression of genes important in cardiovascular health. Br J Nutr 2:1398–1403. https://doi.org/10.1017/S0007114509993485

Nooh HZ, Nour-Eldien NM (2016) The dual anti-inflammatory and antioxidant activities of natural honey promote cell proliferation and neural regeneration in a rat model of colitis. Acta Histochem 118:588–595. https://doi.org/10.1016/j.acthis.2016.06.006

Nweze JA, Okafor JI, Nweze EI, Nweze JE (2016) Comparison of antimicrobial potential of honey samples from Apis mellifera and two stingless bees from Nsukka, Nigeria. J Pharmacogn Nat Prod 2:4. https://doi.org/10.4172/2472-0992.1000124

Pandey KR, Naik SR, Vakil BV (2015) Probiotics, prebiotics and synbiotics—a review. J Food Sci Technol 52:7577–7587. https://doi.org/10.1007/s13197-015-1921-1

Rahman MM, Gan SH, Khalil MI (2014) Neurological effects of honey: current and future prospects. Evid Based Complement Altern Med 2014:958721

Rakha MK, Nabil ZI, Hussein AA (2008) Cardioactive and vasoactive effects of natural wild honey against cardiac malperformance induced by hyperadrenergic activity. J Med Food 11:91–98. https://doi.org/10.1089/jmf.2006.172

Rao PV, Krishnan KT, Salleh N, Gan SH (2016) Biological and therapeutic effects of honey produced by honey bees and stingless bees: a comparative review. Brazilian J Pharmacogn 26:657–664. https://doi.org/10.1016/j.bjp.2016.01.012

Rožman P, Švajger U (2018) The tolerogenic role of IFN-γ. Cytokine Growth Factor Rev 41:40–53. https://doi.org/10.1016/j.cytogfr.2018.04.001

Sanz ML, Polemis N, Morales V, Corzo N, Drakoularakou A, Gibson GR, Rastall RA (2005) In vitro investigation into the potential prebiotic activity of honey oligosaccharides. J Agric Food Chem 53:2914–2921. https://doi.org/10.1021/jf0500684

Semwal DK, Semwal RB, Combrinck S, Viljoen A (2016) Myricetin: a dietary molecule with diverse biological activities. Nutrients 8:90. https://doi.org/10.3390/nu8020090

Sgariglia MA, Vattuone MA, Sampietro Vattuone MM, Soberón JR, Sampietro DA (2010) Properties of honey from Tetragonisca angustula fiebrigi and Plebeia wittmanni of Argentina. Apidologie 41:667–675. https://doi.org/10.1051/apido/2010028

Si H, Wyeth RP, Liu D (2014) The flavonoid luteolin induces nitric oxide production and arterial relaxation. Eur J Nutr 53:269–275. https://doi.org/10.1007/s00394-013-0525-7

Soylu M, Atayoğlu T, İnanç N, Silici S (2015) Ndice glucé mico de mieles turcas multiflorales y efecto de su consumo en el metabolismo de la glucose. J Apic Res 54:155–162. https://doi.org/10.1080/00218839.2015.1131454

Tahir AA, Fathiah N, Sani A, Murad NA, Makpol S, Zurinah W, Ngah W, Anum Y, Yusof M (2015) Combined ginger extract & Gelam honey modulate Ras/ERK and PI3K/AKT pathway genes in colon cancer HT29 cells. Nutr J 14:31. https://doi.org/10.1186/s12937-015-0015-2

Truchado P, Ferreres F, Bortolotti L, Sabatini AG, Tomás-Barberán FA (2008) Nectar flavonol rhamnosides are floral markers of acacia (Robinia pseudacacia) honey. J Agric Food Chem 56:8815–8824. https://doi.org/10.1021/jf801625t

Vandamme L, Heyneman A, Hoeksema H, Verbelen J, Monstrey S (2013) Honey in modern wound care: a systematic review. Burns 39:1514–1525. https://doi.org/10.1016/j.burns.2013.06.014

Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, Pérez-Álvarez JA (2008) Functional properties of honey, propolis, and royal jelly. J Food Sci 73:R117–R124. https://doi.org/10.1111/j.1750-3841.2008.00966.x

Weng MS, Ho YS, Lin JK (2005) Chrysin induces G1 phase cell cycle arrest in C6 glioma cells through inducing p21Waf1/Cip1 expression: involvement of p38 mitogen-activated protein kinase. Biochem Pharmacol 69:1815–1827. https://doi.org/10.1016/j.bcp.2005.03.011

Weston RJ (2000) The contribution of catalase and other natural products to the antibacterial activity of honey: a review. Food Chem 71:235–239. https://doi.org/10.1016/S0308-8146(00)00162-X

Xiang D, Wang D, He Y, Xie J, Zhong Z, Li Z, Xie J (2006) Caffeic acid phenethyl ester induces growth arrest and apoptosis of colon cancer cells via the β-catenin/T-cell factor signaling. Anti-Cancer Drugs 17:753–762. https://doi.org/10.1097/01.cad.0000224441.01082.bb

Yaghoobi N, Al-waili N, Parizadeh SMR (2008) Natural honey and cardiovascular risk factors; effects on blood glucose, cholesterol, triacylglycerole, CRP, and body weight compared with sucrose. Scientific World J 8:463–469. https://doi.org/10.1100/tsw.2008.64

Yazan LS, Muhamad Zali MFS, Ali RM, Zainal NA, Esa N, Sapuan S, Ong YS, Tor YS, Gopalsamy B, Voon FL, Syed Alwi SS (2016) Chemopreventive properties and toxicity of kelulut honey in Sprague Dawley rats induced with azoxymethane. Biomed Res Int 2016:4036926. https://doi.org/10.1155/2016/4036926

Yoon CH, Chung SJ, Lee SW, Park YB, Lee SK, Park MC (2013) Gallic acid, a natural polyphenolic acid, induces apoptosis and inhibits proinflammatory gene expressions in rheumatoid arthritis fibroblast-like synoviocytes. Jt Bone Spine 80:274–279. https://doi.org/10.1016/j.jbspin.2012.08.010

Yuksel P (2011) Comparison of the Versa Trek and BACTEC MGIT 960 systems for the contamination rate, time of detection and recovery of mycobacteria from clinical specimens. Afr J Microbiol Res 5:844–852. https://doi.org/10.5897/ajmr10.008

Zaidi MZ, Sharma J (2019) Honey and its beneficial therapeutic effects: a review. J Pharmacogn Phytochem 8:1056–1061

Zhu H, Liang QH, Xiong XG, Wang Y, Zhang ZH, Sun MJ, Lu X, Wu D (2018) Anti-inflammatory effects of p-coumaric acid, a natural compound of Oldenlandia diffusa, on arthritis model rats. Evid Based Complement Altern Med 2018:5198594. https://doi.org/10.1155/2018/5198594

Phytochemicals from Honey as MAP-Kinase Inhibitors: Current Therapeutic Standing and Future Prospects

7

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Abstract

Honey has been and is being used for medical, pharmaceutical, and domestic needs. Besides, it is used as a conventional medicine and has various pharmacological properties. A variety of polyphenolic compounds are stated in honey and among them important polyphenols are Caffeic acid (CA), Quercetin (QU), Chrysin (CR), Kaempferol (KF), Apigenin (AP), Galangin (GA), Acacetin (AC), Caffeic acid phenyl ester (CAPE), Pinocembrin (PC), and Pinobanksin (PB) that have evolved as potential pharmacokinetic agents in the cure of cancer. Caffeic acid, a naturally occurring phenolic compound commonly found in honey, is being comprehensively studied for its therapeutic use and is being

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described as a cancer-causing agent in preliminary studies, but the same compound in combination with other antioxidants has been revealed to repress colon tumors in rats. CAPE was similarly proposed to have anticarcinogenic, antimitogenic, immunomodulatory, and anti-inflammatory potential. In a related progressive study, influence of CA against UVB (280–320 nm) irradiation-induced IL10 appearance and stimulation of MAPKs (Mitogen-Activated Protein Kinases) in skin of mouse was observed. The findings strongly propose that chrysin exercises growth inhibitory properties either by prompting p38 MAPK leading to buildup of p21Waf1/Cip-1 protein or by arbitrating the repression of proteosome action. It is also a well-established fact that chrysin prompts cell death in association with stimulation of caspase-3 and Akt signal corridor, which plays a vital role in chrysin-incited cell death in U937 cells. Galangin and its antiproliferative outcome on HL-60 cells was expressed in a manner that is dependent on dose, and it also prompted DNA breakage without any loss of integrity of cell membrane. Similarly, quercetin was also shown in an in vitro study to impede HL-60 cell propagation in association with repression of cytosolic PKC (Protein Kinase C) and TPK (tyrosine protein kinase) membrane bound. Acacetin, another important flavonoid, was revealed to impede the propagation of A549 cells, prompt apoptosis, and block cell cycle promotion at G1 cell cycle phase and also heightened the appearance of p53 protein and Fas ligands. Besides was also depicted to impede HepG2 cell propagation and incite cell death by boosting p53 protein and Fas ligands as in case of A-549 cells. Kaempferol-mediated cell death in H-460 cells was complemented by substantial DNA coiling/condensation and amassing ATP content. Besides, it altered the levels of Caspase-3 and AIF (Apoptosis-Inducing Factor). Pinocembrin has been shown

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to induce loss of MMP (mitochondrial membrane potential) with further release of cytochrome c and processing of caspase 3 and 9 in colon HCT116 cancer cells. Apigenin has been shown to exert antiproliferative influence against colon, breast, neuroblastoma, cervical, and liver cancer cell lines. The chapter has clearly put forth certain honey-based compounds that have been tested in laboratory setups and have been revealed to be hopeful pharmacological agent for hindering cancer propagation.

Keywords

Honey · MAP-kinase · Inhibitor · Anticancer

7.1 Introduction

Associated with the genus Apis, honeybees are insects known for making and storing of honey and for various useful ingredients that are supposed to be actually useful to human beings. Two tamed kinds of honeybees are presently known: one the western *Apis mellifera* which is confined to Asia, Africa, and Europe and the other one is eastern *A. cerana* that is dispersed in southern and southeastern parts of Asia. Widely appreciated honeybee product is the honey and is derived by the processing of nectar collected from different flowers and thereafter stockpiled in specialized honeycomb cells. The honey is generally promoted for its beneficial activities and has been promoted as folk tonic since times. Moreover, it is presented as a therapeutic agent in clinical setup (Molan 1999). Additional by-products produced by honeybees include bee venom, propolis, beeswax, royal jelly, and bee pollen. These by-products have been used by humans since early ages for various dietary and remedial measures. Many important biochemical and beneficial characteristics have been perceived in these products besides finding their use as pharmaceutical, nutraceutical, and cosmetic agents (Burlando and Cornara 2013; Viuda-Martos et al. 2008). Many of these honeybee products are being introduced in clinical situations; however, their pharmacological and therapeutic calibration is so challenging because of their high biochemical variability, grounded on honeybee diversities and sources. Many classes of compounds and different molecules have been sequestered from honey and some have been pharmacologically characterized, strongly signifying their reputation for drug finding from natural origin. There is a comprehensive bioactive compound screening in the honey and honeybee products and of their beneficial properties. Thorough studies on bioactivities and healing properties have been examined on the chief ingredients of honeybee products.

7.2 Honey and Its Composition

Honey is made by specialized bees which amass the sap and nectar from several flowering sources and stockpile it as honey that aids as nourishment for bees through cold season. Honey bees rove almost 55,000 miles to collect sap fluid from nearly two million blossoms for amassing single pound of honey. In the beehives, one finds three forms of bees the worker bees, drone, and queen. Solitary worker bees accumulate and regurgitate sap and nectar many times to moderately digest it, before storage in the honeycomb. Throughout gathering of nectar, pollen also is included in honey in many ways. As the honeybee stays several florae in search of nectar, several of the pollen of the florae fall into the sap that is poised by the bee and finds their entry into stomach which is later on vomited along with nectar. Also, some pollens become attached to several body parts of the bee-like antenna, hairs, and legs of visiting bees that get intertwined in the beehive and thereby make its entrance into honey. Air is an additional route of entrance for aerial pollen making their way into honey and gets transported through wind streams. Honey-producing bees by the help of their wings fan comb made by the honeybees to vaporize maximum amount of water from nectar/sap thus evading the unnecessary fermentation of honey which is collected. The shade of honey made differs as per the source of flowers and content of minerals present in the source and frequently varies from watery white to dark amber. Acidic medium present in the stomach and the activities of the enzymes invertase, amylase, and diastase produce highly saturated aqueous solution which is composed of 80% sugars, mostly dextrose and levulose that constitute 31.28% and 38.19%, respectively, remaining are the maltose 7.3% and sucrose 1.3%. Nitrogen present in honey is mostly present in peptide and amino acid form. Minor constituents present in honey makeup for 2.1% of whole honey and include protein (0.266%), amino acids (0.1%), nitrogen (0.043%), small quantity of minerals (0.17%), acids (0.57%), and minute quantities of pigments, sugar alcohols, aroma substances, colloids, phenolics, and vitamins (National Honey Board 2007; Todd and Vansell 1942). Most plentiful amino acid found in honey is proline besides there are other amino acids like phenylalanine, isoleucine, tyrosine, glutamic acid, alanine, leucine, etc. Little amount, usually 0.1–1.5%, of protein in the honey of *A. mellifera* and 0.1–3.0% in the honey of *A. cerana* is also found. Plentiful peptides found in honey are royal jelly protein (MRJP) and defensin-1 isoforms. Acid phosphatase, glucose oxidase, diastase (amylase), catalase, and glucosidase are the chief enzymes found in honey (Di Girolamo et al. 2012; Kubota et al. 2004; Chua et al. 2015). As well claimed is that every honey variety has different peptide design based on ubiquitous ingredients and diverse groups of minor elements. The normal pH of honey is 3.9 which is chiefly because of the occurrence of organic acids, mostly citric acid and gluconic acid. Owing to the presence of ascorbic acid and pollen grains in honey, minute quantity of vitamins are found (mostly vitamin B complex) too in honey. Honey is mostly made up of water and sugars accounting for approximately 17.2% and 79.6%, respectively. Honey, reflecting the mineral level of soils where blossoming plants grow, minerals found in it range between 0.04 and 0.2%. Potassium is main element present in honey, accounting for one-third of whole mineral

content in it. Various other plant-based complexes existing in honey at very little concentrations and in different amounts are based on the floral species of plants stayed at by bees and the milieu of the area where nectar is composed by them. Honey also contains aromatic compounds that are the most diversified part, as affirmed by the discovery of over 500 different volatile complexes in it. The most abundant phytochemicals present in honey are phenolic compounds typically stretching from 50 to 500 mg per kg (Ramanauskiene et al. 2012; da Silva et al. 2016). Flavor is based on the color of honey, more the darker, the robust the flavor and quality of honey. There are greater than 300 exceptional diversities of honey that have been reported in the United States alone based upon the floral sources there.

Honey has been and is being used in domestic, medical, and pharmaceutical needs. Besides, it is used as conventional medicine and has various pharmacological properties. Honey is being used for wound curing and anti-bacteria from early times and is recognized to have various pharmacological activities like anticancer, immune response, and cardioprotective activity. Also, it has been reported as a significant resource for making anti-inflammatory preparations. Recently some important properties including antioxidant property of honey have been brought to the attention. It is the established datum that antioxidants have numerous defensive properties against different ailments including heart-related diseases, neurological deterioration, cancer, aging, and inflammatory disorders. The same has led to the exploration of foods rich in antioxidants. As a chemopreventive measure, various foods rich in phytocompounds which aid as antioxidants are being used. For the same reason, honey takes importance of being rich in phenolic complexes besides antioxidants, e.g., amino acids, proteins, and ascorbic acid. Central polyphenolic compounds present in honey namely, caffeic acid (CA), Kaempferol (KP), Galangin (GA), Quercetin (QU), Chrysin (CR), Apigenin (AP), Pinocembrin (PC), caffeic acid phenyl esters (CAPE), Acacetin (AC), Pinobanksin (PB), etc., have developed as encouraging pharmaceutical and therapeutic compounds in the treatment of various diseases including cancer (Jaganathan and Mandal 2009a, b). As prevention is superior to cure and the same holds good for cancer also. Chemoprevention is well defined as administration of chemical compounds that avert initiation, thwart, or slow down the expansion of cancer (Sporn and Newton 1979) or impede or inverse carcinogenesis at an earlier stage (Kelloff 1999). It utilizes appropriate pharmacological agents (Kelloff and Boone 1994; Kelloff et al. 2004) or dietary components, taken in various forms like micronutrients, macronutrients, or nonnutritive phytocompounds (Ferguson et al. 2004). Phenolics found in honey are important sponsors of the antioxidant capability of honey. The composition of phenolic compounds varies significantly due to floral origin and accordingly honey is anticipated to have a wide array of antioxidant potential (Petretto et al. 2015). The pursuit for foods that are natural and rich in antioxidants has increased because intake of antioxidants has been associated with several defensive properties against diverse ailments like heart diseases, cancer, neurological worsening, aging, and inflammatory disorders (Wollgast and Anklam 2000; Madhavi et al. 1996). Although being used for long, the antioxidant features of honey came to attention recently (FAO 1996). Honey has some minor constituents that are supposed to have

properties like antioxidants (Vit et al. 1997). Among them worth mentioning are flavonoids and phenolics (Cherchi et al. 1994), amino acids, few enzymes (catalase, glucose oxidase), organic acids (Cherchi et al. 1994), ascorbic acid (White and Crane 1975), carotene-related substances (Tan et al. 1989), and proteins (White 1978). Peptides with few amino acid residues frequently found in honey have also been stated to possess strong antioxidant potential. Honey has a number of photoproducts including polyphenolic compounds that act as antioxidants and these compounds are at length explored by scientists for health encouraging prospectus. Propolis another bee product also contains the maximum quantity of phenolic compounds and it has been intensely researched for antioxidant property and radical scavenging feature (Viuda-Martos et al. 2008). Most of these complexes including pinocembrin, chrysin, and pinobanksin possess robust antioxidant and antiradical properties (Sun et al. 2015). Phenolic compounds and polyphenols present in the honey differ according to the climatic and geographical conditions and few of them have been testified as explicit markers for botanical derivation of honey. Composition as well as content of phenolics has substantial differences in dissimilar unifloral honeys (Amiot et al. 1989).

Present treatments utilizing chemotherapeutic drugs pose threats of resistance and additional side effects. This reason justifies the pursuit for alternate therapies and the natural products are perceived as a useful substitute. Honey as a natural product and folk medication has engrossed the consideration by investigators as a harmonizing and alternate medicine. Its use in presently practiced medicine as a prospective therapy is mostly underutilized and underestimated (Sarfaraz et al. 2018). Terpenes, 2-hydroxy-3-phenylpropionic acid, syringic acid (3, 5-dimethoxy-4-hydroxybenzoic acid), benzyl alcohol, methyl 3, 3, 4, 5-trimethoxybenzoic acid, 5-dimethoxy-4-hydroxybenzoate (methyl syringate), 1, 4-dihydroxybenzene and 2-hydroxybenzoic acid are some of the phytochemicals attributed for antimicrobial action of the honey (World Wide Wounds 2002). Phytochemicals and polyphenols existing in honey are also reported to have antiproliferative action.

7.3 Phytochemicals from Honey and Their Antimitotic Potential

Cancer cells mainly have two physiognomies uncontrolled cell growth and poor apoptotic turnover and the drugs normally used for treatment are proapoptotic. Many researchers have elucidated the value of honey in cancer. In one of such investigation by Jaganathan et al., the apoptotic potential of honey was elucidated and the study exhibited that honey induces cell demise in colonic cancer cells of human origin by arresting cellular growth in sub G1 phase. Honey abundant in phenolic compounds and tryptophan content potently repressed cell progression of colonic cancer cells. The study demonstrated that honey mediated apoptosis by cleavage of PARP and by activating Caspase-3. After conducting DNA fragmentation assay, it was found that HT 29 cells presented distinctive ladder design that proved apoptosis (Jaganathan and Mandal 2009a, b). Orsolic et al. in another study described that hydrophilic-

derived products of propolis and related phenolics have antiproliferative consequence before and after the injection of cancer cells. It was also demonstrated by them that the constituents of honey might exert antiproliferative outcome when given prior to tumor cell injection (Orsolic et al. 2004). In other connected in vitro investigation proposed by Tarek et al. honey has been recognized to have actual agent for inhibiting the development of cancer cells of bladder (253J, MBT-2, RT4, and T24). Honey has been found to be highly active when directed orally or intralesionally in MBT 2 bladder cancer cell lines. There existed noteworthy difference among the last tumor size ($P < 0.05$) in the intralesion (IL) honey-treated groups matched to IL saline and variance between final tumor size and heaviness in IL saline and control set was not noteworthy (Tarek et al. 2003). Gribel and Pashinskii conducted research and specified that honey showed modest and noteworthy antiproliferative properties in dissimilar strains of mouse and rat tumor models. Besides the study also depicted that the antitumor properties of various chemo preparations such as cyclophosphamide and 5-fluorouracil were augmented by the honey products (Gribel and Pashinsky 1990). Polyphenols present in honey have been studied alongside numerous illnesses in human and animal models displaying wide-ranging beneficial properties such as antiatherogenic, anti-inflammatory, anticarcinogenic, antithrombotic, immune moderating, and as antioxidants (Cook and Samman 1996; Catapano 1997; Salah et al. 1995; Loku et al. 1995; Vinson et al. 1998; Serafini et al. 1996). Honey as such might be proposed as a natural anticancer preparation as it increases curing of long-lasting ulcers and abrasions, increases immune status, and reduces chronic inflammation. With availability of advanced extraction procedures for several polyphenols that have been accredited with antitumor ability of honey, the researchers focused on polyphenolic fractions taken out from honey in spite of raw honey. Polyphenols or phenolic complexes are found abundantly distributed in plants and plants contain around 8000 known structures (Bravo 1998). These compounds are as well formed by plants as ancillary metabolites and some of these make their way into honey with the help of honeybees. These compounds mostly have been divided into ten types centered on their organization; naphthoquinones, simple phenols, xanthones, phenolic acids, isocoumarins and coumarins, anthraquinones, flavonoids, lignins, and stilbenes. There are 5000 and more flavonoids and their structures have been thoroughly described which constitute the most important polyphenolic class. These flavonoid compounds being the natural antioxidants exhibit widespread biological properties including anti-inflammatory, antibacterial, antithrombotic, antiallergic, vasodilatory, and many others (Cook and Samman 1996). Various polyphenolic compounds present in honey are used as markers for different types of honey, for example, quercetin for sunflower honey (Tomás-Barberán et al. 2001), kaempferol and flavanol for rosemary honey (Ferreres et al. 1998). The hydroxycinnamate compounds like p-coumaric acid, ferulic acid, and caffeic acid have been seen in chestnut honey (Cherchi et al. 1994) while as flavonoid compounds of propolis like pinobanksin, pinocembrin, and chrysin are present in the European honey (Tomás-Barberán et al. 2001). Numerous different compounds present in honey have been researched and reviewed for their anticancerous activity which

includes Quercetin (QU), Pinobanksin (PB), Caffeic acid (CA), Apigenin (AP), Chrysin (CR), Acacetin (AC), Caffeic acid phenyl ester (CAPEs), Kaempferol (KF), Galangin (GA), and Pinocembrin (PC).

7.4 Role of Different Polyphenolic Compounds of Honey in Cancer

7.4.1 Caffeic Acid, Its Esters, and Their Role in Animal Models and Cancer

Caffeic acid which is a natural phenolic complex in honey has been comprehensively studied in lieu of its therapeutic use. Study led by Hirose et al. demonstrated carcinogenic potential of little amounts of antioxidants like 4-methoxyphenol (4-MP), caffeic acid, butylated hydroxyanisole (BHA), catechol, and sesamol taken through diet. For a 2-year period experiment, these antioxidants were extensively scrutinized for their major effects alone or mixtures in different cancer cell lines. Different carcinogenicity experiments were commenced in clusters of 30–31 male F344 rats. These groups of rats were inoculated with 0.4% 4-MP, 0.4% caffeic acid, 0.4% BHA, 0.4% sesamol, and 0.16% catechol either unaccompanied or in different combinations for 104 weeks before being sacrificed. The findings depicted that the typical body masses of rats consuming basic diet were upper than the rats given antioxidants only and were lowest in the different combinational groups. The findings also depicted that the relative kidney or liver masses were higher than earlier in catechol, sesamol, BHA, and different combinational groups. The findings of the study led to the findings in which incidences and frequencies of forestomach histopathological lesions were amplified by exposure to antioxidants excluding the BHA case. The multiplicities or frequencies of forestomach nodular or papillary hyperplasia were noticeably amplified in sets inoculated with caffeic acid, 4-methoxyphenol, and combination of antioxidants, compared with group given the basal diet. Experimental work conducted on multiorgan cancer models depicted an upsurge in the incidence of forestomach papillomas in every large dose group with no similar effect found in different combinational groups. In lower dose case, the frequency of forestomach papillomas was considerably increased in the combination groups. However, the consequence of additional organs mainly colon cancers, was meaningfully reduced only in the high-dose combinational groups. Hence it can be stated from the study that at small dosage, phenolic complexes may display synergistic or additive effect on cancer (Hirose et al. 1998). From such studies, caffeic acid is listed as probable carcinogenic compound. Rao et al. accomplished a comprehensive and exclusive study by creating various caffeic acid esters including phenylethyl caffeate (PEC), methyl caffeate (MC), and phenylethyl dimethylcaffeate (PEDMC) and elucidated their role against DMAB, 3, 2-dimethyl-4-aminobiphenyl, (a mammary gland and colon carcinogen) prompted carcinogenesis in *S. typhimurium (Salmonella)* strains TA 100 and TA 98. Both strains managed to survive (survival more than 98%) to concentration of 150 μM MC, 2500 μM CA,

70 μM PEC, and 80 μM PEDMC/plate. Besides 40–80 μM of PEDMC, 150 μM MC, 40–60 μM PEC meaningfully repressed the DMAB-induced carcinogenesis in these two strains. The findings of the mentioned trials positioned MC level above 225 μM and PEC as well as PEDMC more than 60 μM as toxic. CA displayed substantial harmfulness at more than 2500 μM concentration. Cytotoxicity effect of PEC, CA, PEDMC, and MC was also gauged in cancer cell line colon of the colon (HT-29). Their inhibitory effect on growth was analyzed after exposing cells to these for 48 h. In comparison to the ester analogs of CA, CA has been noticed to be minimally effective in impeding propagation of HT 29 cells. To substantiate the findings further, the inhibitory properties, production of nucleic acid, and protein formation after rearing HT 29 cells with mentioned substances for 48 h were examined. It was witnessed that RNA, protein, and DNA formation was clogged at a concentration of 40 μM of PEC, 60 μM of PEDMC, and 175 μM of MC. Moreover, the activity of the enzyme ornithine decarboxylase (ODC) was repressed at levels of 150 μM MC, 20 μM PEDMC, and 40 μM PEC. Besides the activity of TPK (tyrosine protein kinase), enzymes were repressed at concentrations of 30 μM of PEC, 20 μM of PEDMC, and 100 μM of MC (Rao et al. 1993). The repressive properties of PEC (phenylethyl caffeate) and MC (methyl caffeate) on AOM (azoxymethane) induced enzyme TPK (tyrosine protein kinase), ODC (ornithine decarboxylase), and metabolism of arachidonic acid in colonic mucosa and liver of male F 344 rats was reported in the follow-up studies. Further inhibitory effects of PEC, PEMC (phenylethyl-3-methylcaffeate), caffeic acid, MC, and PEDMC (phenylethyl dimethyl caffeate) on in vitro arachidonic acid metabolomics in colonic mucosa and liver were depicted by them. Finally, the effects of PEDMC, PEMC, and PEC on AOM-induced ACF (atypical crypt foci) development in the colon of F 344 rats were examined. Clusters of F 334 rats were given diets possessing 600 ppm of PEC or MC for biochemical studies and 500 ppm of PEMC, PEDMC, or PEC for ACF studies for five consecutive weeks. Subsequently, 2 weeks after, subcutaneous/hypodermal dose of AOM was injected once a week and was done for two successive weeks, for whole experimental animals excluding vehicle-treated sets. Studies of biochemical in nature were accomplished after sacrificing the animal 5 days after. F 334 rats thereafter were sacrificed 9 weeks later for investigating ACF in colon in ACF study. The liver and colonic mucosal tissue of the rats was investigated for the TPK enzyme activity, ornithine decarboxylase activity, cyclooxygenase, and lipoxygenase metabolic products. Diet of PEC meaningfully repressed AOM induced TPK and ODC activities in colon and liver. It was also witnessed that diet of PEC expressively blocked the AOM induced 12(*S*) hydroxyeicosatetraenoic acid (HETE) and lipoxygenase metabolites 8(*S*). Experimental rats that were given MC diet displayed a reasonable repressive influence on ODC and 5 (*S*), 8 (*S*), 12 (*S*), and 15(*S*) HETEs and substantial influence on colon tyrosine protein kinase action. Although PEC and MC diets exhibited no noteworthy repressive influence on metabolism of cyclooxygenase, ACF was expressively repressed in rats that were given PEDMC (81%), PEMC (82%), or PEC (55%). Research also depicted that PEMC, PEDMC, and PEC existing in honey subdued AOM-induced early neoplastic lesions in colon and also inhibited ODC, lipoxygenase, and TPK activity that were related to the cancer of

colon (Rao et al. 1992). Huang et al. in another research presented the strong suppressive outcome of CAPE application on TPA (12-0-tetradecanoylphorbol-13-acetate) prompted lump formation and creation of HMdU (5-hydroxymethyl-2-deoxyuridine) in the mouse skin DNA. The suppressive consequence of CAPE on TPA-mediated cancer development by the CAPE application in CD I mice hitherto treated with DMBA (7,12-dimethylbenz(a) anthracene) was established. CAPE in concentrations 1, 10, 100, or 3000 nmol together with 5 nmol of TPA was applied two times in a week and the same continued for 20 consecutive weeks. At these levels, it repressed magnitude of skin papillomas by 24, 30, 45, and 70% and mass of tumor in each mouse was reduced by 42, 66, 53, and 74%, correspondingly. Besides the application of 5 nmol of TPA two times a week for consecutive 20 weeks to mice created 12.6 HMdU residues/104 of normal bases on an average in the skin DNA. CAPE application in increasing concentration of 1, 10, 100, or 3000 nmol with 5 nmol of TPA two times a week for 20 consecutive weeks to DMBA-treated mice reduced HMdU levels in DNA of epidermis by 40–93%. At the concentrations of 1.25 μM, 2.5 μM, 5 μM, 10 μM, or 20 μM, CAPE repressed the amalgamation of thymidine-[3H] into the DNA of HeLa cells by 32, 44, 66, 79, and 95% correspondingly. Likewise assimilation of uridine-[3H] in the RNA was repressed by 39, 43, 58, 64, and 75%, whereas assimilation of leucine-[3H] in the protein was subdued by 29, 30 37, 32, or 47%, correspondingly. These findings suggested that CAPE is a strong repressor of DNA production but rather is not potent in preventing RNA production and is minimally effective in preventing protein synthesis (Huang et al. 1996). The molecular mode by which CAPE acts was revealed by Natrajan et al. where they observed the result of CAPE on NF-κB transcription factor in comprehensive way. U-937 cells were preincubated in presence of CAPE in different amounts for 2 h prior to exposing them to TNF (0.1 nM) for 15 min. The treatment repressed the TNF-reliant triggering of NF-κB in dose-reliant way with highest outcome occurring at 25 μg/mL. Study depicted that CAPE also inhibited NF-κB stimulation prompted by the ester of phorbol, okadaic acid, PMA (phorbol-12-myristate 13-acetate), hydrogen peroxide, and ceramide by averting the translocation and movement of p 65 part of NF κB to nucleus without distressing the TNF-induced IκBα degradation. The study has not depicted any repressive consequence on the TFIID, oct-1, and AP-1transcription factors. Additionally, several CAPE structural analogs were also examined to precisely study the importance of CAPE in impeding NF-κB. It has been shown that a rotationally constrained, bicyclic, 5, 6-dihydroxy structure exhibited authority, whereas 6,7-dihydroxy alternative was minimally dynamic in impeding the NF-κB. By these results they established that CAPE is an effective and a precise repressor of NF-κB stimulation and this property might offer molecular base for its manifold anti-inflammatory and immunomodulatory actions (Natarajan et al. 1996). In other study conducted by Lee et al., cellular toxicity potency of CAPE and its molecular activity in glioma cells (C6) were investigated. The results of study specified that after 24 h of inoculation with CAPE (50 μM), C6 glioma cells went through internucleosomal DNA breakage. FACS study of glioma cells treated with CAPE, presented increased buildup of hypodiploid nuclei (24% at 36 h) in a manner dependent on time. Additional results

indicated that after 3 h of treatment, CAPE prompted release of cytochome-c from mitochondria into cytosol which resulted in the stimulation of caspase-3 (CPP32). Moreover, within 12 h after CAPE inoculation, breakdown of PARP (substrate of CPP32) initiated. After 0.5 h of CAPE treatment, the phosphorylation of p53 at serine residues took place and the p53 protein level was augmented after 3 h of treatment. After 36 h of treatment, the expression of Bak and Bax also enhanced resulting in the reduced concentration of Bcl2 (B cell lymphoma/leukemia-2) protein. Likewise, the study stated that CAPE treatment activated ERK (extracellular signal-regulated kinase) and p38 MAPK (p38 mitogen-activated protein kinase) in C6 glioma cells. Further, it was depicted that level of p53, phosphoserine 15 of Bax, inactivate form of CPP32 and p53 were repressed by earlier treatment with SB203580, a specific p38 MAPK inhibitor. Therefore it was concluded that p38 MAPK arbitrated p53 reliant cell death in C6 glioma cells (Lee et al. 2003). Chung et al. indicated that both matrix metalloproteinases 9 (MMP9) and MMP2 were selectively repressed by CA and CAPE. CAPE repressed intensely with IC50 of 2–5 Mm while CA required 10–20 μM. Nonetheless either of these two Cathepsin K and MMP-1,3,7 were not totally repressed. There was an inhibitory effect of CA and CAPE on propagation of HEPG2 cells and it was dependent on dose. In case of HepG2 cell line, CA of 200 μg/mL concentration, decreased cell viability to 61% when matched to control group and CAPE treatment (at low concentration of 20 μg/mL) decreased the cell viability to 72% when matched to control. When exposed to PMA, CA and CAPE repressed the MMP9 expression by hindering the NF-κB action in HEPG2 cells. When administered subcutaneous or orally it was found that CAPE (5 mg/kg) and CA (20 mg/kg) suppressed liver metastasis as well as the development of HepG2 tumor xenografts in bare mice. Finally, the study concluded that CA and its derived product CAPE: (1) repressed the action of MMP9 enzyme that plays a significant part in cancer incursion and its metastasis, (2) obstructed the invasiveness by the suppression of MMP9 transcription by impeding NF-κB role in PMA activated HepG2 cell line, and (3) inhibited the proliferation of xenografts of HepG2 cells in naked mice. Consequently, the above-mentioned two drugs were described as robust contenders for treating cancer and its progression/metastasis through two mechanisms, i.e., inhibition of gene expression and inhibition of metastasis specific enzyme activity (Chung et al. 2004). Hwang et al. in a recently conducted study examined the consequence of CAPE on cancer incursion and metastasis in fibrosarcoma HT 1080 cells by defining how the matrix metalloproteinases (MMPs) are regulated. The cells of HT 1080 were incubated with growing CAPE amount and transcripts of MMP2 and MMP9 in the form of mRNA were observed by RT-PCR and both MMP2 and 9 protein levels were seen to be significantly reduced in a dose-reliant manner. Constitutively produced MMP2 and nine proteins in HT-1080 cells slowly decreased after incubating with CAPE and the same were confirmed by gelatin zymography. Activation studies of proMMP2 were accomplished by using organo-mercuric complex, APMA (4-aminophenylmercuric acetate) to further verify the downregulation of MMP2, and the studies revealed MMP-2 downregulation mediated by CAPE. There was also significant reduction in the levels of mRNA of MT1 MMPs (membrane type matrix

metalloproteinases) and TIMPs (tissue inhibitor of matrix metalloproteinases). Cell movement, cell invasion, and colony establishment of tumors were also inhibited by CAPE, thus turning out to be a vital antimetastatic agent, by deterring the invasive and metastatic properties of cancerous cells (Hwang et al. 2006). Few researchers also explored the potential UVC (280–100 nm) defensive properties of CA (caffeic acid) in human fibroblast (diploid in nature) and A431 epidermal tumor cells. The UVC-protecting outcome of CA at two dissimilar amounts (55.5 μM and 166.5 μM) was undoubtedly revealed together in transformed and normal cells. When cells were grown-up in DMEM medium in the presence of CA, a marked alteration in the propagation of ordinary and transformed cells when exposed to UVC irradiation was witnessed. The defensive effect of CA was found to be discrete in transformed compared to ordinary cells (Neradil et al. 2003). In a connected chronological study led by Vanisree et al. defensive influence of caffeic acid against UVB (280–320 nm) irradiation prompted IL 10 appearance and MAPKs (mitogen-activated protein kinases) activation in the skin of mouse was observed. With the help of in vivo transgenic IL10 promoter luciferase reporter gene-based assay, it was observed that CA inhibited the IL-10 promoter transcription. CA significantly repressed IL10 mRNA production and protein synthesis in skin cells of mouse. Besides the upstream regulators like ERK, p38 MAPK, and down the line transcription factors like AP1, NF–κB, and c-Jun N terminal protein have also been shown to be repressed by CA in skin cells of mouse. From the research, it has been concluded that CA might be used as an instrument against harmful UVB irradiation (Staniforth et al. 2006).

7.4.2 Chrysin and Role of Chrysin By-Products in Cancer

Chrysin also called as 5,7-dihydroxyflavone is a major and a natural organically active flavonoid mined out of honey-based products and plants. It enjoys powerful antioxidant, anti-inflammatory characteristics, and endorses cell death by disturbing cell cycle. Its antitumor mechanisms include the upregulation of Caspase 3 and 9 genes expression and downregulation of Bcl 2, the stimulation of the release of TNF alpha (tumor necrosis factor alpha), the stimulation of p38 MAPK gene expression, and upsurge of p-eIF2α, p-PERK, and ATF 4 levels. In addition to this, chrysin impedes MRP 2 (multidrug resistance-associated protein 2), the BCRP (breast cancer-resistance protein), and the Pgp 170 (phosphoglycoprotein 170) in Caco 2 colon cancer cells (Rouamba et al. 2019). In latest study proposed by Weng et al., the chrysin molecular mechanism of action in C6 glioma cells was studied. Chrysin subdued the cell propagation after 24, 48, and 72 h in an anti-proliferation assay that was accomplished on C6 glioma cells. Ninety percentage of cell proliferation was repressed after 72 h of gestation in 50 μM of chrysin. Flow cytometry testified that via 30 μM and 50 μM treatments, chrysin, after 24 h, augmented the percentage of cells in G1 phase from 69 to 79% and 83% and reduced percentage of cells in S phase from 11.4 to 6.1% and 2.8%, correspondingly. After 30 and 50 μM treatments, the percentage of cells in G2 or M phase altered from 17.9 to 12.2% and

9.2%. The Rb (retinoblastoma) protein phosphorylation levels in C6 glioma cells reduced after giving 30 μM of chrysin. Furthermore, it was also established that p21Waf1/Cip1 a cyclin-dependent kinase inhibitor amount is augmented meaningfully devoid of the change in p53 protein amount in chrysin-treated cells. The researchers using p38 specific inhibitor that ensued in pulling down of p21Waf1/Cip1 level to depict significance of p38 in chrysin arbitrated p21Waf1/Cip1 stimulation. Furthermore, the research depicted that chrysin also repressed proteosome activity and cyclin-dependent kinase 2 and 4 enzymatic activity. The findings firmly advocated that chrysin exercises its special growth repressive effects either by triggering p38MAPK leading to the buildup of p21 Waf1/Cip1 protein level or by arbitrating the repression of proteasome action (Weng et al. 2005). Woo et al. in a related study stated chrysin-arbitrated apoptosis in U937 cancer cells. After 12 h, chrysin-treated cells displayed distinctive internucleosomal breakdown of DNA and the same phenomenon was confirmed by DNA fragmentation assay. FACS analysis of chrysin-treated cells exhibited noticeable upsurge of subG1 cells at the end of 12 h. After chrysin treatment, decreased proenzyme amount of caspase 3 indicated the significance of triggered caspase-3 in programmed cell death. Stimulation of phospholipase Cγ (PLCγ), a downstream effector of caspase3 in cells treated with chrysin cancer further established the importance of caspase 3 in U937 cells. There was decrease in the amount of XIAP (an associate of apoptosis inhibitor proteins) and cytochrome c induction in a manner dependent on dose in chrysin-treated cells and the findings were confirmed by western blotting. Akt signaling has been shown to have substantial importance in apoptosis mediated by chrysin in U937 cells, whereas MAPK does not possess any importance in signaling passageway as revealed by western blotting. Specific PI3K inhibitor, LY294002, has been revealed to hinder Akt phosphorylation in U937 cells and henceforth meaningfully boosted apoptosis. In U937 cells overexpression of a constitutively dynamic Akt (myrAkt) subdued the stimulation of apoptosis, stimulation of caspase 3, and PLCγ1 cleavage induced by chrysin (Woo et al. 2004). Further 13 derived products of chrysin were synthesized by Zheng et al. and tried for anticancerous property against human colorectal adenocarcinoma (HT29 cells) and gastric adenocarcinoma cells (SGC7901). The derived products were designed by methylation, halogenation, alkylation, acetylation, trifluoromethylation, and nitration. As revealed by MTT assay, 5, 7-dimethoxy 8-iodochrysin and 8-bromo 5-hydroxy 7-methoxychrysin possess strongest actions against HT 29 and SGC 7901 cancer cells respectively. Also, derivative 5,7 dihydroxy 8-nitrochrysin was shown to possess robust performances against the HT29 and SGC7901 cells (Woo et al. 2004). To increase the pharmacokinetic properties of chrysin, Zhang et al. conducted a trial for manufacturing tetraethyl bisphosphoric ester of chrysin (CP:C23H28O10P2) and diethyl chrysin 7-yl phosphate (CPE:C19H19O7P) through a simplified Atherton–Todd reaction. Upon mass spectroscopic study, CPE made multiplexes in association to lysozyme and henceforth esters of chrysin phosphate enhanced the communication with other proteins in comparison to original basic chrysin. HeLa cells were combined with 10 μM each of CR, CP, and CPE for 24, 48, and 72 h. There was marked time-dependent decline in cell viability. The consequence of CR, CPE, and

CP treatments to cultured HeLa cells was seen by employing PCNA immunohistochemistry, TUNEL techniques, and methyl green pyronin staining. The results favored the supposition that CR, CP, and CPE might impede propagation and encourage cell death in the subsequent order of effectiveness CP > CPE > CR. Therefore CPE and CP were suggested as new probable candidates for cervical cancer (Zhang et al. 2004).

7.4.3 Role of Galangin in Leukemia

The studies have shown that flavonoids and phenolic acids such as galangin, quercetin, and chrysin are capable to subdue the action of pro-inflammatory enzymes, e.g., prostaglandins, iNOs (inducible nitric oxide synthase), and COX2 (cyclooxygenase 2) (Ahmed et al. 2018). The antiproliferative outcome of galangin on leukemia (HL60) cells was described by Charles et al. and trypan blue exclusion method that was employed that showed the notable reduction in cell sustainability after treatment with 100 μM for 24 h 1–10 μM Galangin exercised antiproliferative importance which was obvious after 48 h of treatment. Early and late cell death was observed by employing gannexin-V-FITC and PI staining by using 100 μM galangin and the outcomes interrelated with outcomes of trypan blue method described previously. A trademark of cell death, active caspase3, was noticed after 24 and 72 h of incubation in galangin of 50 and 10 μM concentration, respectively. Examination of cell cycle showed upsurge in sub-G1 phase cells inoculated with galangin (>10 μM) and the same was demonstrated in DNA fragmentation assay, where they were able to observe characteristic ladder pattern after 24 h of 100 μM galangin introduction. Predominantly forward scatter and side scatter variations were witnessed after 24 and 72 h incubation in 100 μM galangin. Galangin inoculated cells exhibited enhanced side scatter changes revealing increased internal complexity and reduced forward scatter changes indicated decreased relative size. Substantial phagocyte like differentiation was unnoticed and rhodamine median fluorescence strength noted as gauge of ROS levels presented no indication for intracellular oxidative distress as a crucial player of cytotoxicity (Bestwick and Milne 2006).

7.4.4 Role of Quercetin in Cancer

As an anticancer compound, the importance of quercetin ranging between 10 and 80 μM in HL60 cells was investigated by Kang and Liang by experimentation indicating dose-dependent inhibition of HL60 cell propagation. It was revealed that cells inoculated with 10 μM of quercetin exhibited inhibition on the HL60 cellular growth and was 17.1, 27.3, 40.1, and 52.7% after 24, 48, 72, and 96 h of treatment, respectively. Analysis of cellular cycle revealed that 20, 40, and 60 μM quercetin amplified percentage of cells in G2 or M-phase from 7.6 to 12.4%, 19.1 and 23.5% respectively and lessened the percentage of cells in G0 or G1 phase from 46.2 to 40.2%, 32.1% and 34.5% correspondingly, without any noteworthy change

in the cellular percentage in S phase after 24 h treatment. Significant inhibitory consequence was displayed by Quercetin on the events of cytosolic protein kinase C (PKC) and membrane-bound TPK of HL60 cells in vitro, with IC50 values of 30.9 μM and 20.1 μM, correspondingly, without disturbing membrane-bound PKC or cytosolic TPK enzymatic activity. At concentration of 80 μM, quercetin also suppressed the whole action of phosphoinositides like PIP (phosphatidylinositol 4 phosphate), PI (phosphatidylinositol), and PIP2 (Phosphatidylinositol 4,5 bisphosphate). Therefore it led to the conclusion that the inhibition of quercetin on progression of HL60 cells might be linked to its inhibition on PKC or TPK in vitro or on the generation of phosphoinositides (Kang and Liang 1997). The quercetin effect in K562 leukemic cells was studied by Csokay et al. with the findings that at the 5.5 μM, it triggered apoptosis and programs of differentiation as well. After exposing leukemia cells to the drug for 1 h resulted in their apoptosis and differentiation of K562 cells after exposing for 12 h. The effects were accredited to the premature downregulation of Ki-ras and c-myc oncogenes and speedy decrease of inositol 1, 4, 5-triphosphate concentrations (Csokay et al. 1997). Similarly, the consequence of quercetin in A549 cells was illustrated by Robaszkiewicz et al. and it was found that it wielded prooxidant and antioxidant and properties as well based on the concentrations implied. At less concentration (1–20 μM), the cell proliferation was promoted by it, however, at high level (50–200 μM), it led to the cytotoxicity reliant on concentration. More number of living cells was produced by limiting the cell number in the necrotic and apoptotic phases at low concentration (10 μM) of quercetin. However, when the concentration exceeded 50 μM, it lessoned number of living cells by accumulating fractions of apoptotic and necrotic cells. The formation of reactive oxygen species (ROS) in cells generating peroxides in medium was lessened by Quercetin. Also, incubation at less levels of quercetin resulted in minor upsurge in TAC (total antioxidant capacity) of cellular extract but more levels of quercetin resulted in progressive decline in the TAC of cellular extract and total thiol level of cells trailed a similar TAC pattern. Henceforth, the study advocated that effects of quercetin at cellular level are composite and comprise both antioxidant effects and initiation of oxidative stress due to the development of ROS in medium outside the cells (Robaszkiewicz et al. 2007). Quercetin might act contrarily on cancerous and normal neuronal cells and the same was substantiated by another study performed by Braganhol et al. Quercetin reduced cell sustainability in glioma cell cultures by leading to necrosis and apoptosis of cells besides arresting glioma cells in the G2 cell cycle checkpoint also decreasing the mitotic index. Moreover, quercetin gave protection from ischemic-induced damage to hippocampal organotypic cultures. The findings revealed that quercetin encouraged inhibition of growth and led to death of cells in U138MG human glioma cell line but still gave protection to the normal cell cultures (Braganhol et al. 2006). Indap et al. observed the antiproliferative outcome of quercetin in vitro and in vivo both and signposted that it might wield antiproliferative effect against MCF7 cell line in time and dose-reliant mode with IC50 value of 10 μg/mL besides it was also seen to halt MCF7 cellular progression in G2/M phase. Furthermore, quercetin repressed the growth potential of tumor by 58% and more in mice grafted by mammary tumor and

prolonged survival potential of mice bearing sarcoma by 2.3-fold. Besides, it improved mitomycin C's inhibitory effect in mammary carcinoma and these effects were facilitated to some extent by poor vascularization and hypoxic regions of tumors (Indap et al. 2006). Choi and colleagues recently studied the antiproliferative consequence of quercetin on MDA MB 435 (breast cancer cell line) and with the help of MTT assay, it was shown that quercetin revealed inhibitory influence on MDA MB 435 cell growth in a dose- and time-dependent mode. Supplementary analysis of cell cycle in quercetin inoculated cells displayed noteworthy upsurge of cells at subG1 phase of cell cycle and quercetin inoculation augmented Bax expression but reduced the Bcl2 concentration. Quercetin treatment was found to augment PARP and cleaved caspase3 expression (Choi et al. 2008).

7.4.5 Role of Acacetin in Lung and Liver Cancer

The antiproliferative outcome of acacetin in hepatic cancer cell line of human beings (HepG2) was investigated Hsu et al. At 20 μg/mL concentration, the extreme inhibitory consequence (roughly 72%) was detected 48 h after and the IC50 value stood to be 10.44 μg/mL for HepG2 cells. Cytometric results with the help of flow cytometer results specified an upsurge in G1 cellular phase from 31.1 to 61.6 and 76.5% at a level of 10 and 20 μg/mL, correspondingly. After treatment by acacetin and after 48 h, sum of cells suffering apoptosis amplified to nearly fourfold at 10 μg/mL and eightfold at 20 μg/mL and the same was confirmed by DNA fragmentation assay. It has also been revealed that acacetin augmented the stimulation of p53 and it is down the line target, p21/WAF1 as analyzed by enzyme-linked immunosorbent assay (ELISA). In dose-dependent manner, mFasL, sFasL, and FasL amplified as was specified by Fas ligand assay. Bax, a proapoptotic protein content, increased by acacetin treatment at 24 and 48 h. The effect of acacetin in human non-small cell lung cancer (NSCLC), A549 cells, was explained in a continuity study, where antiproliferative consequence was found to be substantial in dose-reliant way and IC50 value was seen to be 9.46 μM. Upon cellular cycle examination, A549 cell line inoculated with 5 and 10 μM of acacetin showed an upsurge in the phase G1 from 34.7 to 42.6% and 61.2%, correspondingly. The quantity of cells suffering apoptosis augmented from 3.2-fold to 8.1-fold at the end of 48 h at 5 μM and 10 μM of acacetin correspondingly and the same findings were confirmed by DNA fragmentation assay. Parallel to the finding in HepG2 cells, as examined by ELISA, acacetin amplified the stimulation of p53 and downstream p21/WAF1target. mFasL, FasL, and sFasL amplified in a dose-reliant manner as indicated by Fas ligand assay. Finally, it was established that p53, Fas–FasL apoptotic mechanism may contribute in antiproliferative action of acacetin in A549 and HepG2 cells (Hsu et al. 2004a, b).

7.4.6 Role of Kaempferol in Lung Cancer and Leukemia

The importance of Kaempferol prompted cell death in human NSCL cells (H460) was discovered by Leung et al. As confirmed by Trypan blue exclusion assay, the changing amounts of kaempferol decreased cell sustainability in a dose-reliant fashion with an IC50 of 50 μM. The death of cells is attributed to apoptosis as there is no discharge of LDH enzymes in cells inoculated with kaempferol and the findings were confirmed by lactate dehydrogenase (LDH) assay. Signal by CM-H2DCFDA, an oxidant sensitive fluorescent probe, not presented any change after kaempferol inoculation, therefore, ROS generation is not the reason for the cytotoxicity witnessed. The membrane potential of mitochondrial noted using fluorescent probe DiOC6 (3,3-dihexyl-oxacarbocyanine) dye, which is voltage based and mitochondrion specific, specified no alteration after giving kaempferol treatments at variable concentrations for 16 h. Apoptosis-inducing factor (AIF), induced by Kaempferol (50 μM), prompted DNA breakdown and condensation in H460 cells. Exposure to kaempferol for 8 h, levels of procaspase 3 were shown to be declining. Inoculation with 50 μM kaempferol for 24 h, amplified the protein content of Cu/Zn SOD and Mn-SOD (Leung et al. 2007). Kaempferol-induced antiproliferation was reported by Bestwick et al. in the promyelocytic leukemia cells (HL60) where exposure to kaempferol for more than 72 h, it inhibited HL60 cells in dose-reliant manner. Cells inoculated with Kaempferol (10 μM) reduced the cell progression and this effect was confirmed by FACS analysis. Compared to decrease in the G1 phase, the amount of cells increased in S-phase after 5 h of treatment. With advancement in the time period from 48 to 96 h, kaempferol in the concentration of 100 μM brought preliminary buildup in S and then G2/M phase. After exposure to phosphatidyl serine devoid of membrane injury as specified by annexin V-FITC binding, indication of prenecrotic period of cell death was spotted only for slight percentage of cells inoculated with $\geq$20 μM kaempferol following 24 or 72 h treatments. There was reduction in mitochondrial potential which was followed by amplified manifestation of caspase 3 activity and the same phenomenon was witnessed after the cells were treated with kaempferol for 24 and 72 h. Multiparametric flow cytometric analysis discovered dissimilar cell subpopulations with reduced size, which is typical of necrosis and apoptosis, possessing amplified caspase 3 activity followed by diminished antiapoptotic Bcl 2 appearance and variations in membrane integrity and asymmetry. The left out population of cells depicted no change or modest rise in Bcl2 appearance with no membrane changes but elevated active caspase-3. Therefore kaempferol prompted growth repression on HL-60 leukemia cells is because of mixed response mostly ruled by alternation in cell cycle though certain level of cytotoxicity is induced by both apoptotic and nonapoptotic processes (Bestwick et al. 2007).

7.4.7 Pinocembrin, Pinobanksin, and Apigenin with Emphasis on Their Role in Cancer

Cell toxicity mediated by pinocembrin against many cancer cell lines including normal fibroblasts of lung in comparison to non-cell toxicity to umbilical cord endothelial cells of human beings has been thoroughly explained by Kumar et al. Pinocembrin prompted loss of MMP (mitochondrial membrane potential) with consequent discharge of cytochrome c and processing of caspase 3/9 in human colonic cancer cell line HCT 116. Bax protein found in cytosol and its translocation to mitochondria seems to be the initial trigger for mitochondrial apoptosis (Kumar et al. 2007). It has been also seen that Pinobanksin employs antioxidant potential by pulling down Fe (II) prompted lipid peroxidation and by hindering MMPT (mitochondria membrane permeability transmission) (Santos et al. 1998). Apigenin is commonly described for antitumor activities in several cell lines and fits in the flavonoid class of compounds. It has been shown to exert antiproliferative effects against cervical, breast, colon, liver cancer, and neuroblastoma cell lines. The consequence of apigenin on cellular progression and cycle in colonic cancer cell lines including Caco-2, SW-480, and HT-29 was studied by Wang et al. Protein content and cell count of the apigenin inoculated cells exhibited decrease in comparison to normal group. The growing cell lines having IC50 values 40, 50, and 70 μM for the SW-480, HT-29, and Caco-2 cells correspondingly were repressed by Apigenin. Flow cytometric examination of apigenin (80 μM) inoculated cells depicted G2/M seizure of 64, 42, and 26 in SW-480, HT-29, and Caco-2 cells correspondingly. The repression of the activity of p34-cdc2 kinase and cyclin B1 in apigenin inoculated cells was also conveyed (Wang et al. 2000). The nature of apigenin as an antiproliferative agent for breast cancer cell line was confirmed by Way et al. and it was stated that apigenin is more effective in inhibiting the HER2/neu over and above expressing cells (MDA-MB-453) compared to basal level HER2/neu expressing MCF-7 cells. Apigenin of 40 μM led to 48% inhibition in MDA-MB-435, whereas in MCF-7 cell line, it produced only 31% growth inhibition. In apigenin prompted apoptosis, role of HER2/HER3 PI3K/Akt pathway was studied and it was shown that it directly repressed PI3Kinase activity first and subsequently inhibited the Akt kinase enzymatic activity. The suppression of HER2 or neu auto-induced phosphorylation and transphosphorylation resulting from diminishing HER2 or neu protein in vivo was demonstrated (Way et al. 2004). In related study conducted by Zheng et al., the cell death prompted by apigenin in human HeLa cervical cancer cell line was elucidated. The findings established that apigenin might lead to the decline in the cell viability with an IC50 of 35.89 μM and induce apoptosis and the same fact was established by DNA fragmentation assay and flow cytometry of apigenin-inoculated cells. Also amplified appearance of p21/WAF1 and p53 was also observed. The apoptosis induction was confirmed by Fas/APO-1 and caspase-3 upsurge and Bcl2 decrease in the apigenin-inoculated HeLa cells (Zheng et al. 2005). Also, apigenin might prompt apoptosis in neuroblastoma cell lines (NUB 7 and LAN 5) as testified by Torkin et al. and suppress the cellular viability in dose-dependent mode among

above-mentioned cell lines with an EC50 = 35 μmol/L in NUB-7 and EC50 = 22 μmol/L in LAN-5 after 24 h. Additionally, it was seen to prevent colony-producing capability and NUB-7 xenograft tumor development in diabetic nonobese mouse models. It was revealed that apigenin prompted apoptosis was facilitated via p53 as it heightened the appearance of p53 and its induced gene products like Bax and p21-WAF1/CIP-1 (Torkin et al. 2005). Another recent study by Chiang et al. proposed antiproliferative consequence of apigenin in Hep-3B, PLC/PRF/5, and HepG2 cells and the study depicted that apigenin might impede the cell progression of the liver cancer cell lines but not normal BNLCL-2 (murine liver cells). IC50 was witnessed to be 2.16 μg/mL for Hep3B, 22.73 μg/mL for PLC/PRF/5, and 8.02 μg/mL for HepG2. Additionally, apoptosis in the HepG-2 cells was shown by flow cytometry and DNA ladder analysis. Cells treated with apigenin were blocked at G2/M point of cellular cycle and increased buildup of p-53 and p-21/WAF-1 in treated cells was observed (Chiang et al. 2006).

7.5 Conclusion

Honey has been and is being used for medical, pharmaceutical, and domestic needs. Besides, it is used as conventional medicine and has various pharmacological properties. Appropriate pharmacological or dietary agents are used for chemoprevention (Kelloff and Boone 1994; Kelloff et al. 2004) and are taken in various forms like macronutrients, micronutrients, or nonnutritive phytoproducts. Variety of polyphenolic compounds are stated in honey and among them important polyphenols are Caffeic acid (CA), Quercetin (QU), Chrysin (CR), Kaempferol (KF), Apigenin (AP), Galangin (GA), Acacetin (AC), Caffeic acid phenyl ester (CAPE), Pinocembrin (PC), and Pinobanksin (PB) and have evolved as potential pharmacokinetic agents in the cure of cancer. Caffeic acid a naturally occurring phenolic compound commonly found in honey is being comprehensively studied for its therapeutic use and is being described as a cancer-causing agent in preliminary studies, but the same compound in combination with other antioxidants has been revealed to repress colon tumors in rats. It was also shown that its and its derivatives upon oral administration reduced metastasis to liver, facilitated by the suppression of activities of both NF-κB and MMP 9 enzymes. CAPE was similarly proposed to have anticarcinogenic, antimitogenic, immunomodulatory, and anti-inflammatory potential. CAPE's anticancer and antiinflammatory properties were revealed to safeguard cells of skin when these cells were exposed to ultraviolet and UVB irradiation. In a related progressive study conducted by Vanisree et al. defensive influence of CA against UVB (280–320 nm) irradiation-induced IL10 appearance and stimulation of MAPKs (mitogen-activated protein kinases) in skin of mouse was observed. These findings strongly propose that chrysin exercises growth inhibitory properties either by prompting p38MAPK leading to buildup of p21Waf1/Cip-1 protein or by arbitrating the repression of proteosome action. It is also well-established fact that chrysin prompts cell death in association with stimulation of caspase-3 and Akt signal corridor, which plays vital role in chrysin-incited cell death in U937 cells.

Galangin and its antiproliferative outcome on HL-60 cells were expressed in a manner that is dependent on dose and it also prompted DNA breakage without any loss of integrity of cell membrane. Similarly, Quercetin was also shown in an in vitro study to impede HL-60 cell propagation in association with repression of cytosolic PKC (protein kinase C) and TPK (tyrosine protein kinase) membrane bound. Also reported was the fact that quercetin in little amount encouraged propagation of A-549 cells, whereas at increased amount it repressed cell propagation and existence/survival. Besides, quercetin also exerted antiproliferative outcome on breast cancer and glioma cells. Acacetin, other important flavanoid, was revealed to impede the propagation of A549 cells, prompt apoptosis, and block cell cycle promotion at G1 cell cycle phase and also heightened the appearance of p53 protein and Fas ligands. Besides was also depicted to impede HepG2 cell propagation and incite cell death by boosting p53 protein and Fas ligands as in case of A-549 cells. Kaempferol-mediated cell death in H-460 cells was complemented by substantial DNA coiling/condensation and amassing ATP content. Besides it altered the levels of Caspase-3 and AIF (apoptosis-inducing factor). Recently it was also reported that inhibitory effect of kaempferol on growth HL60 leukemia cells is owed to heterogeneous response customarily subjugated by cell cycle change though certain degree toxicity of cells results from apoptotic and nonapoptotic processes. Pinocembrin has been shown to induce loss of MMP (mitochondrial membrane potential) with further release of cytochrome c and processing of caspase 3 and 9 in colon HCT116 cancer cells. Apigenin has been shown to exert antiproliferative influence against colon, breast, neuroblastoma, cervical, and liver cancer cell lines. The chapter has clearly put forth certain honey-based compounds that have been tested in laboratory setups and have been revealed to be hopeful pharmacological agent for hindering cancer propagation. After creating further in-depth and comprehensive evidence of honey-based compounds from both in vitro and in vivo studies, further clinical trials and studies are needed to supplement and certify the potential role and importance of honey-based compounds in medical and pharmaceutical industries.

References

Ahmed S, Sulaiman SA, Baig AA, Ibrahim M, Liaqat S, Fatima S, Jabeen S, Shamim N, Othman NH (2018) Honey as a potential natural antioxidant medicine: an insight into its molecular mechanisms of action. Oxid Med Cell Longev 2018:8367846, 19 pages. https://doi.org/10.1155/2018/8367846

Amiot MJ, Aubert S, Gonnet M, Tacchini M (1989) Les composés phénoliques des miels: étude préliminairesur l'identification et la quantification par familles. Apidologie 20:115–125

Bestwick CS, Milne L (2006) Influence of galangin on HL-60 cell proliferation and survival. Cancer Lett 243(1):80–89

Bestwick CS, Milne L, Duthie SJ (2007) Kaempferol induced inhibition of HL-60 cell growth results from a heterogeneous response, dominated by cell cycle alterations. Chem Biol Interact 170(2):76–85

Braganhol E, Zamin LL, Canedo D et al (2006) Antiproliferative effect of quercetin in the human U138MG glioma cell line. Anti-Cancer Drugs 17(6):663–671

Bravo L (1998) Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr Rev 56(11):317–333

Burlando B, Cornara L (2013) Honey in dermatology and skin care: a review. J Cosmet Dermatol 12:306–313. https://doi.org/10.1111/jocd.12058

Catapano AL (1997) Antioxidant effect of flavonoids. Angiology 48(1):39–44

Cherchi A, Spanedda L, Tuberoso C, Cabras P (1994) Solidphase extraction and HPLC determination of organic acid in honey. J Chromatogr 669:59–64

Chiang LC, Ng LT, Lin IC, Kuo PL, Lin CC (2006) Antiproliferative effect of apigenin and its apoptotic induction in human Hep G2 cells. Cancer Lett 237(2):207–214

Choi EJ, Bae SM, Ahn WS (2008) Antiproliferative effects of quercetin through cell cycle arrest and apoptosis in human breast cancer MDA-MB-453 cells. Arch Pharm Res 31(10):1281–1285

Chua LS, Lee JY, Chan GF (2015) Characterization of the proteins in honey. Anal Lett 48:697–709. https://doi.org/10.1080/00032719.2014.952374

Chung TW, Moon SK, Chang YC et al (2004) Novel and therapeutic effect of caffeic acid and caffeic acid phenyl ester on hepatocarcinoma cells: complete regression of hepatoma growth and metastasis by dual mechanism. FASEB J 18(14):1670–1681

Cook NC, Samman S (1996) Flavonoids—chemistry, metabolism, cardioprotective effects, and dietary sources. J Nutr Biochem 7(2):66–76

Csokay B, Prajda N, Weber G, Olah E (1997) Molecular mechanisms in the antiproliferative action of quercetin. Life Sci 60(24):2157–2163

da Silva PM, Gauche C, Gonzaga LV, Costa AC, Fett R (2016) Honey: chemical composition, stability and authenticity. Food Chem 196:309–323. https://doi.org/10.1016/j.foodchem.2015.09.051

Di Girolamo F, D'Amato A, Righetti PG (2012) Assessment of the floral origin of honey via proteomic tools. J Proteome 75:3688–3693. https://doi.org/10.1016/j.jprot.2012.04.029

Ferguson LR, Philpott M, Karunasinghe N (2004) Dietary cancer and prevention using antimutagens. Toxicology 198(1–3):147–159

Ferreres F, Juan T, Perez-Arquillue C, Herrera-Marteache A, Garcia-Viguera C, Tomás-Barberán FA (1998) Evaluation of pollen as a source of kaempferol in rosemary honey. J Sci Food Agric 77(4):506–510

Food and Agriculture Organization (FAO) (1996) Agricultural services bulletin. Food and Agriculture Organization, Rome

Gribel NV, Pashinsky VG (1990) Antitumor properties of honey. Voprosy Onkologii 36 (6):704–709

Hirose M, Takesada Y, Tanaka H, Tamano S, Kato T, Shirai T (1998) Carcinogenicity of antioxidants BHA, caffeic acid, sesamol, 4-methoxyphenol and catechol at low doses, either alone or in combination, and modulation of their effects in a rat medium-term multi-organ carcinogenesis model. Carcinogenesis 19(1):207–212

Hsu YL, Kuo PL, Lin CC (2004a) Acacetin inhibits the proliferation of Hep G2 by blocking cell cycle progression and inducing apoptosis. Biochem Pharmacol 67(5):823–829

Hsu YL, Kuo PL, Liu CF, Lin CC (2004b) Acacetininduced cell cycle arrest and apoptosis in human non-small cell lung cancer A549 cells. Cancer Lett 212(1):53–60

Huang MT, Ma W, Yen P et al (1996) Inhibitory effects of caffeic acid phenethyl ester (CAPE) on 12-O-tetradecanoylphorbol- 13-acetate-induced tumor promotion in mouse skin and the synthesis of DNA, RNA and protein in HeLa cells. Carcinogenesis 17(4):761–765

Hwang HJ, Park HJ, Chung HJ et al (2006) Inhibitory effects of caffeic acid phenethyl ester on cancer cell metastasis mediated by the down-regulation of matrix metalloproteinase expression in human HT1080 fibrosarcoma cells. J Nutr Biochem 17(5):356–362

Indap MA, Radhika S, Motiwale L, Rao KVK (2006) Quercetin: antitumor activity and pharmacological manipulations for increased therapeutic gains. Indian J Pharm Sci 68(4):465–469

Jaganathan SK, Mandal M (2009a) Antiproliferative effects of honey and of its polyphenols: a review. J Biomed Biotechnol 2009:830616. 13 pages. https://doi.org/10.1155/2009/830616

Jaganathan SK, Mandal M (2009b) Honey constituents and its apoptotic effect in colon cancer cells. J Apiprod Apimed Sci 1:29–36
Kang T, Liang M (1997) Studies on the inhibitory effects of quercetin on the growth of HL460 leukemia cells. Biochem Pharmacol 54:1013–1018
Kelloff GJ (1999) Perspectives on cancer chemoprevention research and drug development. Adv Cancer Res 78:320–334
Kelloff GJ, Boone CW (1994) Cancer chemopreventive agents: drug development status and future prospects. J Cell Biochem 20:1–303
Kelloff GJ, Hawk ET, Sigman CC (2004) Cancer chemoprevention: promising cancer Chemopreventive agents, vol 1. Humana Press, Totowa, NJ
Kubota M, Tsuji M, Nishimoto M, Wongchawalit J, Okuyama M, Mori H et al (2004) Localization of alpha-glucosidases I, II, and III in organs of European honeybees, Apis mellifera L., and the origin of alpha-glucosidase in honey. Biosci Biotechnol Biochem 68:2346–2352. https://doi.org/10.1271/bbb.68.2346
Kumar MAS, Nair M, Hema PS, Mohan J, Santhoshkumar TR (2007) Pinocembrin triggers Bax-dependent mitochondrial apoptosis in colon cancer cells. Mol Carcinog 46(3):231–241
Lee YJ, Kuo HC, Chu CY, Wang CJ, Lin WC, Tseng TH (2003) Involvement of tumor suppressor protein p53 and p38 MAPK in caffeic acid phenethyl ester-induced apoptosis of C6 glioma cells. Biochem Pharmacol 66(12):2281–2289
Leung HWC, Lin CJ, Hour MJ, Yang WH, Wang MY, Lee HZ (2007) Kaempferol induces apoptosis in human lung non-small carcinoma cells accompanied by an induction of antioxidant enzymes. Food Chem Toxicol 45(10):2005–2013
Loku K, Tsushida T, Takei Y, Nakatani N, Terao J (1995) Antioxidant activity of quercitin monoglucosides in solution and phospholipid bilayers. Biochim Biophys Acta 1234:99–104
Madhavi DV, Despande SS, Salunkhe DK, Madhavi DL, Deshpande SS, Salunkhe DK (1996) Food antioxidants. Marcel Dekker, New York, NY
Molan PC (1999) Why honey is effective as a medicine. Its use in modern medicine. Bee World 80:80–92
Natarajan K, Singh S, Burke TR Jr, Grunberger D, Aggarwal BB (1996) Caffeic acid phenethyl ester is a potent and specific inhibitor of activation of nuclear transcription factor NF-κB. Proc Natl Acad Sci U S A 93(17):9090–9095
National Honey Board (2007) Honey and Bees. http://www.honey.com/consumers/kids/beefacts.asp
Neradil J, Veselska R, Slanina J (2003) UVC-protective effect of caffeic acid on normal and transformed human skin cells in vitro. Folia Biol 49(5):197–202
Orsolic N, Knezevi A, Sver L, Terzi S, Hackenberger BK, Basi I (2004) Influence of honey bee products on transplantable tumours. J Vet Comp Oncol 1(4):216–226
Petretto GL, Cossu M, Alamanni MC (2015) Phenolic content, antioxidant and physico-chemical properties of Sardinian monofloral honeys. Int J Food Sci Technol 50:482–491. https://doi.org/10.1111/ijfs.12652
Ramanauskiene K, Stelmakiene A, Briedis V, Ivanauskas L, Jakstas V (2012) The quantitative analysis of biologically active compounds in Lithuanian honey. Food Chem 132:1544–1548. https://doi.org/10.1016/j.foodchem.2011.12.007
Rao CV, Desai D, Kaul B (1992) Effect of caffeic acid esterson carcinogen-induced mutagenecity and human colon adenocarcinoma cell growth. Chem Biol Interact 84:277–290
Rao CV, Desai D, Simi B, Kulkarni N, Amin S, Reddy BS (1993) Inhibitory effect of caffeic acid esters on azoxymethane-induced biochemical changes and aberrant crypt foci formation in rat colon. Cancer Res 53(18):4182–4188
Robaszkiewicz A, Balcerczyk A, Bartosz G (2007) Antioxidative and prooxidative effects of quercetin on A549 cells. Cell Biol Int 31(10):1245–1250
Rouamba A, Compaoré M, Kiendrebeogo M (2019) Molecular targets of honey bee's products in cancer prevention and treatment. J Herbmed Pharmacol 8(4):261–268. https://doi.org/10.15171/jhp.38

Salah N, Miller NJ, Paganga G, Tijburg L, Bolwell GP, Rice-Evans C (1995) Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. Arch Biochem Biophys 322(2):339–346

Santos AC, Uyemura SA, Lopes JLC, Bazon JN, Mingatto FE, Curti C (1998) Effect of naturally occurring flavonoids on lipid peroxidation and membrane permeability transition in mitochondria. Free Radic Biol Med 24(9):1455–1461

Sarfaraz A, Siti AS, Atif B, Muhammad IJ, Sana L, Saira F, Sadia J, Nighat S, Nor HO (2018) Honey as a potential natural antioxidant medicine: an insight into its molecular mechanisms of action. Oxidative Med Cell Longev 56:1–19

Serafini M, Ghiselli A, Ferro-Luzzi A (1996) In vivo antioxidant effect of green and black tea in man. Eur J Clin Nutr 50(1):28–32

Sporn MB, Newton DL (1979) Chemoprevention of cancer with retinoids. Fed Proc 38 (11):2528–2534

Staniforth V, Chiu LT, Yang NS (2006) Caffeic acid suppresses UVB radiation-induced expression of interleukin-10 and activation of mitogen-activated protein kinases in mouse. Carcinogenesis 27(9):1803–1811

Sun C, Wu Z, Wang Z, Zhang H (2015) Effect of ethanol/water solvents on phenolic profiles and antioxidant properties of Beijing propolis extracts. Evid Based Complement Alternat Med 2015:595393. https://doi.org/10.1155/2015/595393

Tan ST, Wilkins AL, Holland PT, McGhie TK (1989) Geographical discrimination of honeys though the employment of sugar patterns and common chemical quality parameters. J Agric Food Chem 37:1217–1221

Tarek S, Naoto M, Mizuki O et al (2003) Antineoplastic activity of honey in an experimental bladder cancer implantation model: in vivo and in vitro studies. Int J Urol 10:213–219

Todd FE, Vansell GH (1942) Pollen grains in nectar and honey. J Econom Entomol 35:728–731

Tomás-Barberán FA, Martos I, Ferreres F, Radovic BS, Anklam E (2001) HPLC flavonoid profiles as markers for the botanical origin of European unifloral honeys. J Sci Food Agric 81 (5):485–496

Torkin R, Lavoie JF, Kaplan DR, Yeger H (2005) Induction of caspase-dependent, p53-mediated apoptosis by apigenin in human neuroblastoma. Mol Cancer Ther 4(1):1–11

Vinson JA, Hao Y, Su X, Zubik L (1998) Phenol antioxidant quantity and quality in foods: vegetables. J Agric Food Chem 46(9):3630–3634

Vit P, Soler C, Tomás-Barberán FA (1997) Profiles of phenolic compounds of Apis mellifera and Melipona spp. honeys from Venezuela. Z Lebensm Unters Forsch A 204(1):43–47

Viuda-Martos M, Ruiz-Navajas Y, Fernandez-Lopez J, Perez-Alvarez JA (2008) Functional properties of honey, propolis, and royal jelly. J Food Sci 73:R117–R124. https://doi.org/10.1111/j.1750-3841.2008.00966.x

Wang W, Heideman L, Chung CS, Pelling JC, Koehler KJ, Birt DF (2000) Cell-cycle arrest at G2/M and growth inhibition by apigenin in human colon carcinoma cell lines. Mol Carcinog 28 (2):102–110

Way TD, Kao MC, Lin JK (2004) Apigenin induces apoptosis though proteasomal degradation of HER2/neu in HER2/neu-overexpressing breast cancer cells via the phosphatidylinositol-3-kinase/Akt-dependent pathway. J Biol Chem 279:4479–4489

Weng MS, Ho YS, Lin JK (2005) Chrysin induces G1 phase cell cycle arrest in C6 glioma cells through inducing p21Waf1/Cip1 expression: involvement of p38 mitogenactivated protein kinase. Biochem Pharmacol 69(12):1815–1827

White JW (1978) The protein content of honey. J Apic Res 17:234–238

White JW, Crane E (1975) Honey a comprehensive survey. Heinemann, London, UK

Wollgast J, Anklam E (2000) Review on polyphenols in Theobroma cacao: changes in composition during the manufacture of chocolate and methodology for identification and quantification. Food Res Int 33(6):423–447

Woo KJ, Jeong YJ, Park JW, Kwon TK (2004) Chrysin induced apoptosis is mediated through caspase activation and Akt inactivation in U937 leukemia cells. Biochem Biophys Res Commun 325(4):1215–1222

World Wide Wounds (2002) Honey as a topical antibacterial agent for treatment of infected wounds. http://www.worldwidewounds.com/2001/november/Molan/honey-as-topical-agent.html

Zhang T, Chen X, Qu L, Wu J, Cui R, Zhao Y (2004) Chrysin and its phosphate ester inhibit cell proliferation and induce apoptosis in Hela cells. Bioorganic Med Chem 12(23):6097–6105

Zheng PW, Chiang LC, Lin CC (2005) Apigenin induced apoptosis through p53-dependent pathway in human cervical carcinoma cells. Life Sci 76(12):1367–1379

Clinico-Pharmacological Perspective of Honey and Propolis

8

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Abstract

Honey and propolis are the honey bee-derived products with a long history of consumption by humans for health purposes. Both of these substances consist of a wide spectrum of vital compounds especially phenolics and flavonoids that are capable of exerting beneficial clinical effects on health. The antimicrobial, anti-metastatic, anticancer, anti-inflammatory, antioxidant, and anti-proliferative activities of honey ingredients and antiviral, antifungal, antimicrobial, anesthetic, anti-inflammatory, antioxidant, anti-protozoal, anticancer, antihepatotoxic, anti-hypertensive, and cytotoxic properties of propolis make these substances potential candidates for therapeutics. Honey has been found effective in the treatment of diabetes mellitus, cardiovascular disorders, respiratory ailments, neurological abnormalities, gastrointestinal defects, skin ulcerations, ophthalmic defects, wounds, peptic ulcers, and different types of carcinomas. Likewise, propolis also exhibits potential role in the management of chronic kidney disease, neurological disorders, tumors, ulcers, chronic periodontitis, atherosclerosis, gastrointestinal defects, and wounds. Evidences suggest honey and propolis as potential phyto-derived clinico-pharmacological agents for effective treatment of different diseases.

Keywords

Honey · Clinical · Propolis · Ingredients · Disorders · Pharmacological · Properties

8.1 Introduction

Honey is a natural syrupy viscous organic liquid produced by *Apis mellifera* (Mamo 2018; Olas 2020). Perhaps, honey is the first naturally occurring sweetener ever found and nowadays utilized for medicinal purposes as well as nutritious supplement in dietetics (Olas 2020). The quality and chemical constitution of honey are determined by floral nectar source, processing mode, seasons, and climatic conditions (Khan et al. 2017; Cianciosi et al. 2018; Mamo 2018). Honey is mainly composed of carbohydrates including glucose, fructose, and monosaccharides (Meo et al. 2017). In addition to carbohydrates, honey consists of little quantities of several attractive compounds including phenolics, which possess strong useful effects (Isla et al. 2011). Other important components present in pure honey include flavonoids, alkaloids, polyphenols, cardiac glycosides, reducing and volatile compounds, glycosides, and anthraquinone (Islam et al. 2012). Honey is the lone natural substance which is derived by honey bees and possesses therapeutic, nutritional, industrial, and cosmetic values (Bansal et al. 2005). Traditionally, honey was utilized for the treatment of a wide range of ailments and conditions that include ophthalmic disorders, asthma, hiccups, hepatitis, worm infestation, throat infection, wounds, constipation, fatigue, ulcers, thirst, piles, tuberculosis, eczema, and dizziness (Bergman et al. 1983; Irving et al. 1987; Samarghandian et al. 2017). Propolis is

a sticky material collected by honey bees from plant exudates and buds and transformed with enzymatic action for the purpose of plugging holes in beehive (Viuda-Martos et al. 2008; Anjum et al. 2018). Actually, propolis being antiseptic agent is intended to prevent hive from bacterial and fungal infections as well as prevent intruders (Anjum et al. 2018). The main components that propolis contain include essential oils, wax, and resin (Viuda-Martos et al. 2008). Likewise honey the ingredients present in propolis also show variation depending on botanic source, environmental and climatic conditions (Viuda-Martos et al. 2008; Isla et al. 2011). Honey depicts therapeutic activity due to vitamins, phenolics, amino acids, and antioxidants. Particularly, the antioxidant capability of honey is unraveled by the constituents such as propolis, wax, and pollens because they are the main phenolics sources (Meda et al. 2005; Bertoncelj et al. 2007). Honey contains polyphenols and flavonoids as the two major biologically active compounds that have antioxidant property. Recently about 30 kinds of polyphenols have been reported in honey (Carlos et al. 2011; Nurul et al. 2013). Several in vitro human and animal cell culture studies confirmed the preventive role of polyphenols in various diseases including diabetes, pulmonary, neurodegenerative, liver, and cardiovascular diseases as well as in cancers (Hossen et al. 2017). The honey ingredients have been observed to wield anticancer, antimicrobial, anti-proliferative, anti-metastatic, antioxidant, and anti-inflammatory effects (Samarghandian et al. 2017). Manuka honey shows capability of therapeutics in fungal diseases, diabetes, skin ulcerations, ophthalmic defects, wounds, gastrointestinal disorders, and peptic ulcers (Bansal et al. 2005; Molan 2006). Propolis also exhibits antioxidant and immune-defensive properties owing to biologically active phytocompounds. Many phytocompounds discovered in propolis include esters, fatty acids, alcohols, phenolic acids, diterpenes, lignans, flavonoids, sesquiterpenes, vitamins, minerals, aromatic aldehydes, and amino acids (Batista et al. 2012). Propolis though meant for supporting beehive's sterility and well-being may also present critical health benefits for humans due to protective activity of the biologically active components present in it (Araujo et al. 2012; Cuevas et al. 2013). Propolis together with its components being produced naturally and attributed with fine pharmaceutical as well as pharmacological properties have broad therapeutic applications including healing of atherosclerosis, wounds, neurodegenerative disorders, and skin aliments. There is growing interest in propolis owing to its health benefits that enable it to act as strong protective and remedial agent. Preclinical studies also positively support propolis for anti-inflammatory and antioxidant activities that causes a decline in an array of chronic disorders including hypertension, diabetes, heart ailments, and neurodegenerative disorders like Alzheimer's disease (Braakhuis 2019). Honey exhibits antiviral, antibacterial, and antifungal actions on account of sugars, hydrogen peroxide, pH, and many phytocompounds essentially phenolics (Isla et al. 2011), while propolis shows significantly higher antioxidant and antimicrobial activities due to lager amount of phenolics compared to honey (Meda et al. 2005; Socha et al. 2015). Honey and propolis have been believed to act as natural therapeutic products from the time of ancient civilizations owing to numerous recognized useful activities (Gómez-Caravaca et al. 2006).

8.2 Clinico-Pharmacological Perspective of Honey

Honey is the valued natural product utilized by human race from ancient time. Honey has nutritional as well as medicinal value. Honey contains different components that exert antimicrobial, anti-metastatic, anticancer, anti-inflammatory, antioxidant, and anti-proliferative effects. The contemporary scientific data has reported the protecting effects and value of honey in the management of diabetes mellitus and diseases of cardiovascular, respiratory, nervous and digestive systems, and even cancer since honey contains various kinds of antioxidants. Adequate evidences are present which suggest the application of honey in treating different diseases (Fig. 8.1). Thus, honey may be used as a natural agent for different therapeutic purposes (Samarghandian et al. 2017). Different clinico-pharmacological activities of honey in various health-related clinical conditions have been described (Table 8.1).

8.2.1 Honey in Gastrointestinal Disorders

Honey has been utilized for the treatment and protection against various bacterial and retroviral infections of gastrointestinal tract, for instance, duodenitis, stomach ulcers, and gastritis (Topham 2002; Alnaqdy et al. 2005). The bacterial attachment with the mucosal cells of gastrointestinal epithelium is believed as the initiation of bacterial infectivity development. Several mechanisms (Fig. 8.2) have been shown by means of which honey prevents the bacterial attachment: (a) nonspecific mechanical inhibition wherein honey forms a coating on bacteria, (b) modification of bacterial hydrophobicity/electrostatic charge which is considered as an essential

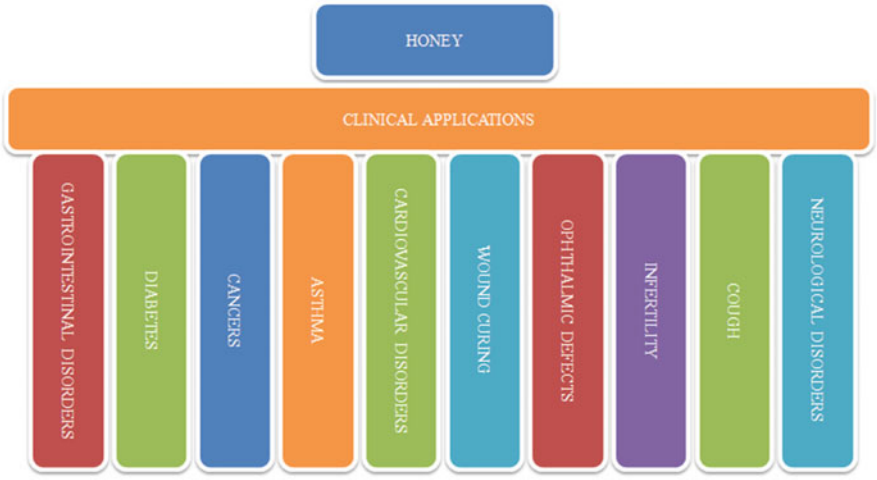

Fig. 8.1 Clinical application of honey

Table 8.1 Clinico-pharmacological activities of honey in different clinical conditions

Clinical condition	Pharmacological activity	References
Gastrointestinal disorder	Honey has been utilized for the treatment of duodenitis, stomach ulcers, and gastritis	Topham (2002), Alnaqdy et al. (2005)
	Natural honey possesses healing properties for the treatment of antral ulcerations and might be utilized similar to sucralfate for the peptic ulcer management	Ali (1995)
	Honey can be used for the treatment of gastro-enteritis and diarrhea at 5% concentration	Bansal et al. (2005), Obi et al. (1994)
	Honey effectively inhibits *Helicobacter pylori* and prevents peptic ulceration and gastritis	Ajibola et al. (2012)
Neurological disorders	Honey and its constituents have been reported to be effective in preventing various cognitive disorders including dementia	Al-Himyari (2009)
	Polyphenols of honey prevent memory-related disorders and stimulate the production of memory at molecular level	Oyefuga et al. (2012)
Diabetes	Honey has been strongly supported with evidence for positive outcome in DM management	Erejuwa et al. (2014)
	Honey has been efficiently confirmed to possess hypoglycemic potential	Ahmed et al. (2018)
Cancer	Honey possess deriding and angiogenic action stimulating potential in different cancer types	Fauzi et al. (2011), Othman (2012), Kustiawan et al. (2014), Hawley et al. (2014)
	Manuka honey has also been found to induce anticancer actions	Fernandez-Cabezudo et al. (2013)
	The essential components of honey including phenolic acids (gallic and caffeic acids) and flavonoids (quercetin, kaempferol, and catechin) show anticancer activities, the most important ingredients of honey with known anticancer activity	Waheed et al. (2019)
Asthma	Honey has the potential for reducing symptoms related to asthma or to act as a deterrent agent for preventing the asthma induction	Bâcvarov (1970)
Cardiovascular diseases	Honey reduces low-density lipoproteins, cholesterol, and triacylglycerol in overweight individuals subsequent to oral administration of 70 g of honey for 30 days	Yaghoobi et al. (2008)

(continued)

Table 8.1 (continued)

Clinical condition	Pharmacological activity	References
	Tualang honey ameliorates the disturbance in cardiac enzyme markers such as aspartate transaminase, lactate dehydrogenase, and creatine kinase-MB	Khalil et al. (2015)
	Honey has a potential to exert protection against cardiovascular disorders especially due to its phenolic constituents	Olas (2020)
Wound healing	Honey has been found effective for treating numerous wound types wherein other remedial techniques fail	Ligouri and Peters (2010)
	Honey reduces the wound infection risk	Wilkinson et al. (2011), Moore and Young (2011)
	Honey dressings enhance the curing of surgical wounds	Goharshenasan et al. (2016)
	Honey vitalizes angiogenesis, promotes fibroblast growth, stimulates the production of fresh epithelial cells, checks the cross-contagion among tissues as well as prevents the bacterial bio-film formation in order to boost the healing and repair of wounds	Halstead et al. (2017)
	Honey dressings successfully enhance the healing of diabetic foot ulcers	Miller (2019)
	Honey effectively cures a wide range of wounds that include non-healing injuries and burns	Martinotti et al. (2019a, b)
	Medical-grade honey securely and effectively treats various abdominal wounds that include burns and dehisced/infected wounds	Smaropoulos and Cremers (2020)
	Honey effectively cures oral ulcers	Hunter et al. (2020)
Ophthalmology	Conjunctivitis, blepharitis, corneal injury, keratitis, and thermal and chemical eye burns has been treated with honey	Meda et al. (2004), Shenoy et al. (2009)
	The symptoms of infective conjunctivitis such as swelling, duration of bacterial elimination, redness, and discharge of pus reduce with the use of honey	Al-Waili (2004a, b), Bansal et al. (2005)
	Honey could cure ophthalmic conditions including bullous keratopathy, dry eye disease, and postoperative corneal edema	Majtanova et al. (2016)

(continued)

Table 8.1 (continued)

Clinical condition	Pharmacological activity	References
	Ophthalmic drops produced from honey have been found to enhance the healing of corneal ulcers induced by foreign bodies	Nejabat et al. (2020)
Cough	Honey possesses an outstanding defense profile and positive effects on the prevention of cough	Paul et al. (2007)
	Honey has been found as effective as dextromethorphan in treating acute cough symptoms among children	Bennett and Daly (2019)
	Honey may lessen the duration of cough better as compared to salbutamol and placebo	Oduwole et al. (2018)
	Honey treats pediatric cough caused by the infection of upper respiratory tract	Tharakan et al. (2019)
Infertility	The application of royal jelly and bee honey to vagina around the occasion of copulation in couples with natural conceiving problem enhances fertility	Abdelhafiz and Muhamad (2008)
	Honey supplement to cryoprotectant solution has been reported to enhance the overall quality of sperms	Fakhrildin and Alsaadi (2014)
	Honey improves fertility, serum concentration of testosterone, and sperm count	Meo et al. (2017)

factor for bacterial interaction with host cells (Edebo et al. 1980; Sakai 1987; Alnaqdy et al. 2005), and (c) neutralizing bacteria due to antibacterial agents present in honey (Alnaqdy et al. 2005).

Helicobacter pylori have been found responsive to honey with antibacterial property in medium level because of hydrogen peroxide at the concentration of 20% (Bansal et al. 2005; Obi et al. 1994). Natural honey possesses healing properties for the treatment of antral ulcerations and might be utilized similar to sucralfate for the peptic ulcer management (Ali 1995). Moreover, honey can be used for the treatment of gastroenteritis and diarrhea at 5% concentration (Bansal et al. 2005; Obi et al. 1994). Pure honey shows bactericidal property against several enteropathogenic microbes that include shigella and salmonella species as well as *E. coli* (Badawy et al. 2004; Adebolu 2005). Honey prevents the adherence of bacteria salmonella to mucosal cells of epithelium (Alnaqdy et al. 2005). Bee honey has been reported to possess a significant antibacterial property and therapeutic utility against infections caused by *Salmonella typhimurium* and *E. coli* 0157:H7 (Badawy et al. 2004). Following the recommendation of World Health Organization (2002),

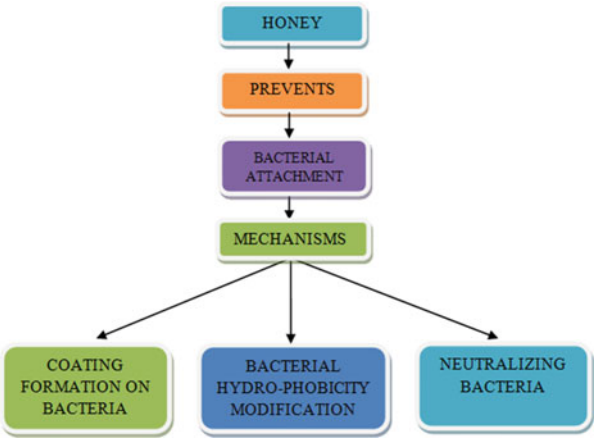

Fig. 8.2 Mechanisms of action of honey for inhibiting bacterial attachment with mucosal cells of gastrointestinal epithelium

Abdulrhman et al. 2010 found that addition of honey to oral rehydration solution (ORS) reduces the rate of bacterial as well as nonbacterial diarrhea. Honey may be utilized for promoting the intestinal absorption of water and sodium due to its elevated sugar level. In addition, honey helps in repairing the damages caused to intestinal mucosa, stimulating the growth of novel tissues, and serves as an anti-inflammatory mediator (Bansal et al. 2005).

8.2.2 Honey in Neurological Disorders

Neural diseases including Parkinson's disease, multiple sclerosis, Huntington's disease, stroke, and Alzheimer's disease are responsible for cognitive damage in elder individuals. These disorders are the outcome of nerve cell dysfunction or death resulting from increase in oxidative stress caused by depleted protein dysfunction, antioxidants, prions, mitochondrial dysfunction, genetic changes, neuroinflammation, and glutamatergic excitotoxicity (Jellinger 2001; Barzilai and Melamed 2003; Spires and Hannan 2005). Oxidative stress acts as a main factor for neuroinflammation that causes apoptosis and fatality of neurons. Honey has been shown to exert useful effects on learning processes and memory. Honey and its constituents have been reported to be effective in preventing various cognitive disorders including dementia (Al-Himyari 2009). Honey is one among various nutraceuticals with potential antioxidant activity which may be used as a novel therapy for neuro-protection (Mijanur et al. 2014). Honey produces anti-depressant, anti-nociceptive, anti-convulsant, and anxiolytic effects as well as improves oxidative content in central nervous system. Numerous honey-related studies have attributed neuroprotective and nootropic property to polyphenols of honey (Akanmu et al. 2011). Moreover, the oxidative stress inhibiting property of honey could be responsible to a certain extent for neuroprotective role against in vivo focal cerebral ischemia and in vivo cell death (Shimazawa et al. 2005). Honey polyphenol

components reduce biological reactive oxidative species (ROS) that causes aging and neurotoxicity as well as morbid accumulation of mis-folded proteins such as amyloid β (Schmitt-Schillig et al. 2005). Polyphenolic components of honey counteract oxidative stress via neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and 5-S-cysteinyl-dopamine, and excitotoxins such as kainic and quinolinic acids (Schmitt-Schillig et al. 2005). Moreover, polyphenolic components of honey counteract direct challenges of apoptosis via methyl mercury, retinoid, and amyloid β (Li et al. 2008). Crude honey as well as polyphenols of honey decrease the neuroinflammation that occurs as a result of immunogenic neurotoxins or damage to ischemia (Akanmu et al. 2009). Most importantly, polyphenols of honey reduce the effect of neuroinflammation of hippocampus. Polyphenols of honey prevent memory-related disorders and stimulate the production of memory at molecular level (Oyefuga et al. 2012).

8.2.3 Honey in Diabetes

Diabetes mellitus is one of the severe problems that results from the defective insulin action or secretion. The insulin deficiency causes disorder in the metabolism of proteins, carbohydrates and fats as well as hyperglycemia (Dimitriadis et al. 2011; Association AD 2014). Other complications of diabetes include retinopathy (Bearse et al. 2004), cardiovascular problems (Saely et al. 2004), and chronic kidney infection (Gupta et al. 2013; Huh et al. 2017). Honey has been strongly supported with evidence for positive outcome in DM management (Erejuwa et al. 2014). Antioxidants are insufficiently produced in patients with type 2 diabetes due to imbalance in oxidative stress and biochemical markers (Yadav et al. 2015, 2017). Thus, the outside antioxidant supplementation to body is vital in order to counter the damage due to oxidation. In different studies, honey has been efficiently confirmed to possess antihypertensive, hepato-protective, anti-inflammatory, antibacterial, antioxidant, and hypoglycemic potential (Erejuwa et al. 2012; Ahmed et al. 2018). Honey antioxidants act as scavenger of free radicals essential for the inflammatory cascade and additionally the creation of reactive oxygen species requiring copper and iron ions as catalysts is prevented as these ions are incarcerated by polyphenolic compounds of honey including flavonoids (Makawi et al. 2009). Honey has been shown to possess hypoglycemic potential in a study on human beings. The glycemic indices of Iranian clover and thyme honeys on healthy individuals under the age of 30 years with mean body mass index (BMI) of 24.3 ± 2.6 kg/m^2 were respectively found as 64.9 and 65.9 which are significantly lower as compared to the glycemic index of glucose (214.4 ± 53 mmol.min/l) (Shishehbor et al. 2013). Australian and Malaysian honeys show a slight difference in their glycemic indices (158 ± 16 and 174 ± 19 mmol×min/l, respectively), nevertheless, considerably lesser than the glycemic index of glucose (Robert and Ismail 2009). Positive effects of honey have been observed in the control of glycemia, hormones for managing appetite and glucose, food ingestion, weight of body, carbohydrate oxidation, and energy expenditure (Erejuwa et al. 2012). Lower glycemic index has been reported in honey

compared to sucrose or glucose in a study on type 1 diabetic patients and healthy individuals (Samanta et al. 1985; Al-Waili 2004a, b). Honey, sucrose, and glucose exhibited the same values in case of type 2 diabetic patients. Honey in comparison to dextrose exhibits critically low increase in the level of plasma glucose in diabetic patients (Eddy et al. 2008), with a decrease in serum levels of homocysteine, C-reactive protein, and triglycerides in hyperlipidemic and healthy individuals (Al-Waili 2004a, b). Honey though has sweet taste, but it has the potential for inducing hypoglycemia because it boosts the secretion of insulin that decreases the level of glucose in blood and thus its use seems positive over sucrose and/or saccharine (Al-Waili and Saloom 1999; Al-Waili 2003, 2004a, b). Regular honey intake causes decline in plasma levels of triglycerides, homocysteine, and prostaglandins, whereas it also boosts antioxidants, level of serum iron and lipid, as well as blood indices in hyperlipidemic and healthy individuals (Al-Waili 2003). Honey as compared to dextrose decreases the plasma level of glucose in healthy individuals. Honey contains antioxidants, fructose, and minerals; fructose boosts the liver uptake of glucose and storage of glycogen while reduces levels of insulin and glycemia. It has been reported that with the increase in intake of fructose from 3 to 20% of calories, the overall serum LDL-C (Low-density lipoprotein cholesterol) and cholesterol rises by 11% and 9%, respectively (Swanson et al. 1992). Therefore, utilizing honey as a dietary component would efficiently evade hyper-insulinemia as well as add to the total dietary fulfillment of diabetic patients (Watford 2002).

8.2.4 Honey in Cancer

Cancer is a global dreadful disease. The effectiveness of honey on different cancer types have been observed in several studies which showed the deriding and angiogenic action stimulating potential of honey (Fauzi et al. 2011; Othman 2012; Hawley et al. 2014; Kustiawan et al. 2014). The studies that investigate the possible role of honey in preventing the growth and progression of cancer/tumor have been increasing in number (Erejuwa et al. 2014). The remarkable anti-angiogenic effects of honey against stability, viability, and even metastasis of cancerous cells have been reported (Fauzi et al. 2011). Malaysian honey has been reported to show good action against different cancers such as cervical cancer (Fauzi et al. 2011), bladder and oral cancers (Swellam et al. 2003), liver cancer (Baig and Attique 2014), and breast and bone cancers (Fauzi et al. 2011). Tualang honey has been reported to be effective in neutralizing tamoxifen-induced harmful effects on noncancerous cells via DNA repair mechanism in Michigan Cancer Foundation 10A (MCF-10A) cells in comparison to MCF-7 cells (Yaacob and Ismail 2014). Manuka honey has also been found to induce anticancer actions (Fernandez-Cabezudo et al. 2013). Manuka honey studied for its effect on improving post-radiation esophagitis symptoms showed its protective effect on lung cancer (Berk et al. 2014). Manuka honey showed remarkable protective effects in squamous cell carcinoma which includes decline in smell from oral cavity wounds and inflammation (Drain and Fleming 2015). Honey is capable of exerting anticancer effects via different mechanisms

(Erejuwa et al. 2014). Studies have shown that honey exerts its anticancer effects by interfering with multiple cell signaling pathways such as apoptotic, anti-inflammatory, anti-mutagenic, and anti-proliferative pathways. Honey brings about modification in immune reactions (Erejuwa et al. 2014). Honey prevents cellular proliferation, induces apoptosis, modifies the progression of cell cycle, and depolarizes mitochondrial membrane in various cancer types including skin cell carcinoma (melanoma) (Pichichero et al. 2010), cervical cell carcinoma, adeno-carcinoma epithelial cells (Yaacob et al. 2013), cancerous cells of endometrium (Tsiapara et al. 2009; Samarghandian et al. 2014a, b), liver cell carcinoma, prostate cell carcinoma, colorectal cell carcinoma (Davoodi et al. 2010; Samarghandian et al. 2011a, b), renal cancerous cells (Samarghandian et al. 2011a, b), bladder cell carcinoma, human non-small cell lung carcinoma (Aliyu et al. 2013), bone cell carcinoma (osteo-sarcoma), leukemia, and oral squamous cell carcinoma (Ghashm et al. 2010).

8.2.5 Honey in Asthma

In traditional medicine, honey has been commonly utilized for the treatment of fever, inflammation, and cough (Bâcvarov 1970). Honey has been shown to possess the potential for reducing symptoms related to asthma or to act as a deterrent agent for preventing the asthma induction. In animal model, oral consumption of honey has been found effective in treating bronchial asthma and chronic bronchitis (Bâcvarov 1970). Moreover, honey as a therapeutic agent effectively prevents inflammation of air passage induced by ovalbumin via the reduction of asthma-linked histopathological alterations in the air passage as well as inhibits the asthma induction (Kamaruzaman et al. 2014). In addition, inhaling honey efficiently removes mucus-secreting goblet cell hyperplasia.

8.2.6 Honey in Cardiovascular Disorders

Cardiovascular diseases (CVDs) constitute a set of blood vessel and heart disorders that include angina, stroke, coronary and congenital diseases of heart that are the most important cause of deaths globally (World Health Organization 2007). Antioxidants of honey including flavonoids, mono-phenolics, polyphenolics, and vitamin C may reduce the risk of cardiovascular failure. Honey flavonoids induce antithrombotic, vaso-relaxant, antioxidant, and anti-ischemic effects in coronary heart diseases and reduce the risk of these disorders via three different mechanisms: (a) improve coronary vasodilatation, (b) reduce the clot-forming ability of blood platelets, and (c) inhibit the oxidation of low-density lipoproteins. Different honey types contain several predominant components that include a broad antioxidant spectrum, phenethyl ester, galangin, caffeic acid, acacetin, quercetin, and kaempferol. Polyphenols of honey possess capable pharmacological utility in decreasing CVDs. However, for further validation of the clinical utility of these

compounds, in vivo and in vitro researches and clinical trials need to be commenced (Khalil and Sulaiman 2010). The utility of honey in decreasing the levels of cholesterol among hyperlipidemic individuals is evident as the significant reduction in lipid levels has been found in case of the constant administration of honey (75 g) suspended in water (250 mL) for 15 days (Al-Waili 2004a, b). Honey-induced effects studied on body weight, fasting blood glucose, high-density and low-density lipoprotein cholesterol, triacylglycerol, C-reactive protein, and total cholesterol demonstrated the reduction of low-density lipoproteins, cholesterol, and triacylglycerol in overweight individuals subsequent to oral administration of 70 g of honey for 30 days (Yaghoobi et al. 2008). Additionally, the intake of honey (10%) for prolonged duration increases the levels of high-density lipoprotein cholesterol, signifying that constant intake of honey improves lipid profile and controls glycemia that indirectly or directly reduce rate of CVD (Chepulis and Starkey 2008). Tualang honey has been reported to ameliorate the disturbance in cardiac enzyme markers such as aspartate transaminase, lactate dehydrogenase, and creatine kinase-MB (Khalil et al. 2015). Honey may contain metabolites of nitric oxide, and elevated nitric oxide level in honey could exert a protective role in CVDs (Bogdanov et al. 2008). Moreover, honey reduces venous blood pressure that may decrease the cardiac preload and as a result may reduce the congestion in venous system (Rakha et al. 2008). Experimental evidences have suggested that dietetic polyphenolic compounds reduce the aggregation of platelets and as a result prevent CVDs. Honey has a rich content of polyphenols such as rutin, luteolin, quercetin, catechin, and apigenin which have been shown to inhibit the in vitro aggregation of platelets via attaching to thromboxane A2 receptor (Guerrero et al. 2005).

8.2.7 Honey in Wound Curing

Honey shows the remedial action mainly because of its antibacterial property, keeping wound moist, and possess high viscosity which assists preventing infection by providing a defensive barrier (Manisha and Shyamapada 2011; Hananeh et al. 2015). The effect of honey on the process of healing has been recognized (Nasir et al. 2010) with many beneficial outcomes on wounds (Jull et al. 2013) as well as believed to boost up circulation and healing. In research studies, great attention has been paid to honey in relation to healing of wounds (Gethin and Cowman 2009) mainly burn wounds (Jull et al. 2013). Honey has been reported effective for treating numerous wound types wherein other remedial techniques fail (Ligouri and Peters 2010). Honey reduces the wound infection risk (Wilkinson et al. 2011; Moore and Young 2011). Additionally, honey boosts skin graft adherence, and exerts anti-inflammatory and antibacterial effects with greater remedial degree. Utilization of honey considerably reduces the rate of infection after few days as well as decreases ache and stay in hospital. Furthermore, honey exerts long-lasting adhesive qualities for the fixation of skin grafts with least contraction (Maghsoudi and Moradi 2015) as well as honey dressings enhance the curing of surgical lesions (Goharshenasan et al. 2016). Honey obtained from Australian stingless honey bee has been reported to

possess extensive antimicrobial property as well as would cure wounds (Boorn et al. 2010). Honey vitalizes angiogenesis, promotes fibroblast growth, stimulates the production of fresh epithelial cells, checks the cross-contagion among tissues as well as prevents the bacterial bio-film formation in order to boost the healing and repair of wounds (Halstead et al. 2017). Honey produces acidic, hypertensive, and moist milieu owing to higher osmotic pressure that drags the protease-rich lymph to wound for the removal of infected or dead tissue or any alien substance that would possibly infect the wound again and impede the process of healing (Molan 2006). The utility of medical-grade honey shows potential to improve the therapeutics of wounds of different severity and origins (Smaropoulos and Cremers 2020).

8.2.8 Honey in Ophthalmic Defects

Globally honey has been utilized in the management of numerous ophthalmological problems including conjunctivitis, blepharitis, corneal injury, keratitis, and thermal and chemical eye burns (Meda et al. 2004; Shenoy et al. 2009). A study reported that using honey as an ointment in patients who suffer from nonresponsive eye diseases showed an improvement in 85% patients, and among the other 15% patients the progression of disease was inhibited. The use of honey reduces swelling, duration of bacterial elimination, redness, and discharge of pus in case of infective conjunctivitis (Obaseiki-Ebor and Afonya 1984; Al-Waili 2004a, b; Bansal et al. 2005).

8.2.9 Honey in Cough

Cough being a key worry for each individual is presented as the most common complaint to nearly every general physician. Cough commonly affects children and has been linked with multiple etiopathological factors. The different factors that determine the etiology of cough include age, environmental, epidemiological, weather, and geographical conditions. However, the cough etiopathology among children differs from adults (Chang and Widdicombe 2007; Chang 2010). Honey possesses an outstanding defense profile and positive effects on the prevention of cough (Paul et al. 2007). Cohen et al. determined the single nocturnal dosage effect of three honey types (eucalyptus, labiatae, and citrus honeys) on cough compared to placebo (silan date extract) and found a remarkable better improvement in each of the three honey groups as compared to the placebo group. Parents reported honey as a better product for symptomatic relief of nocturnal coughs and sleep trouble linked with infections of upper respiratory tract during childhood and as a result preferred honey over placebo (Cohen et al. 2012).

8.2.10 Honey in Infertility

Honey was offered by Egyptians for fertility. Furthermore, several civilizations conventionally used honey for enhancing male vitality. Several factors for infertility along with possible remedies have been described. In a number of observations, honey bee pollens are believed to enhance the general fecundity and fertility as well as the quality of egg due to rich amount of calcium, vitamins, amino acids, iron, other minerals, and immune-boosting properties. Honey intake has been recommended to males with impotence problem and females with infertility problems such as erratic ovulation. Intake of honey-added warm milk is thought to boost up the sperm count significantly in men with sub-fertility or infertility problem. Honey contain rich amount of vitamin B which serves as a vital factor for testosterone production. Honey consumption has been observed to show positive correlation with the concentration of testosterone. High nitric oxide (vasodilatation factor) content in honey has been believed to be capable of producing and improving erection in individuals with impotence or dysfunctional erection. A quantity of 100 g of honey has been depicted adequate for increasing the blood level of nitric oxide by up to 50% (Fakhrildin and Alsaadi 2014). Honey as per the teachings of alternative and complementary medicine enhances the quality of sperms in males and strengthen the uterus and ovaries in females. Recently the honey supplement to cryoprotectant solution has been reported to enhance the overall quality of sperms (Fakhrildin and Alsaadi 2014). In another study, the couples with natural conceiving problem has been observed to depict enhanced fertility on application of royal jelly and bee honey to vagina around the occasion of copulation (Abdelhafiz and Muhamad 2008).

8.3 Clinico-Pharmacological Perspective of Propolis

The biologic properties of propolis rely on its chemical constitution, geographical zones, plant sources, and different seasons. At least 300 compounds like phenolics, amino acids, aromatic acids, and essential oils have been found in propolis (Anjum et al. 2018). The use of propolis has a significant influence on the health of humans and is utilized for several clinical purposes (Fig. 8.3). Currently it is utilized as an antiviral, antifungal, antimicrobial, anesthetic, anti-inflammatory, antioxidant (Boukraâ and Sulaiman 2009; Omar et al. 2017), anti-protozoal, anticancer (Fokt et al. 2010; Abdulrhman et al. 2012; Kuropatnicki et al. 2013; Sforcin 2016), anticarcinogenic and antihepatotoxic and antihypertensive agent besides having cytotoxic property (Toreti et al. 2013). Propolis and its constituents exhibit a range of clinico-pharmacological activities (Table 8.2).

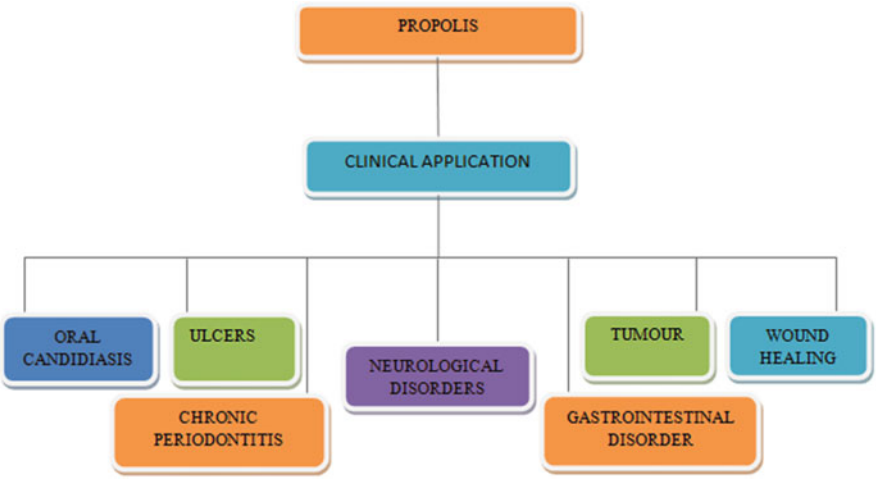

Fig. 8.3 Clinical applications of propolis

8.3.1 Propolis in Tumor

Propolis obtained from stingless bees has been reported to exhibit anticancer effects (Choudhari et al. 2013). Various propolis components have antitumor properties (Komericki and Kränke 2009; Veiga et al. 2017). Several propolis constituents that include caffeic acid phenethyl (CAPE) (Castaldo and Capasso 2002) and artepillin C have been tested and were confirmed to have antitumoral effects (Chan et al. 2013). These propolis ingredients take part in arresting cell cycle, matrix metallo-proteinase inhibition, effect of anti-angiogenesis and additionally prevent the transmission of disease from one part of body to a different part (Sforcin 2016). Propolis is capable of inhibiting tumor cell-DNA synthesis, possesses the tendency to induce tumor cell aging (apoptosis), as well as has the ability to bring white blood cells into action to produce certain agents that can control the role of B, T, and natural killer cells (Salomao et al. 2011; Wagh 2013). Many other constituents like nemorosone, chrysin, cardanol, and galangin play a part in preventing the rapid tumor cell divisions (Sforcin 2016). The tumor-suppressing protein molecules present in CAPE induce apoptosis in C6-glioma cells (De Castro 2001; Watanabe et al. 2011; Sforcin 2016). Furthermore, caffeic acid esters, phenolics, and diterpenoids are capable of destroying cancer cells. The anticancer property of propolis is attributed to the collective action of its polyphenolic components (Sforcin 2007). Reduced glutathione production in cancer cells as a result of radiations is compensated by propolis via synthesizing it in hematopoietic tissues (Chandna et al. 2014). Turkish propolis exerts antitumor action via promoting programmed cell death and also prevents uridine, leucine, and thymidine from being incorporated into cancerous cells, and thus inhibiting synthesis of DNA (Watanabe et al. 2011). Propolis has been shown to exhibit potential for treating breast cancer in humans

Table 8.2 Clinico-pharmacological activities of propolis and its constituents

Clinical condition	Propolis/component	Property	Pharmacological activity	References
Cancer	Turkish propolis	Antitumor/anticancer	It promotes programed cell death	Watanabe et al. (2011)
	Propolis		Stingless bees propolis exhibits anticancer effects	Choudhari et al. (2013)
	Propolis		Possesses the treatment potential for breast cancer in humans via apoptotic activity	Xuan et al. (2014)
	Galangin, nemorosone, chrysin, and cardanol		Inhibits the rapid tumor cell divisions	Sforcin (2016)
	Brazilian red propolis		This propolis subsequent to fractionation is capable of producing cytotoxic effect on cancerous cells	dos Santos et al. (2019)
	Cuban propolis/nemorosone		Inhibits the clonogenic capability of colorectal cancer cells via cell cycle arrest in G0/G1 stage and apoptosis induction	Frión-Herrera et al. (2020)
	Propolis (p-coumaric acid) + green tea (epigallocatechin-3-gallate)		These compounds significantly decrease the viability of four triple-negative breast cancer cell lines (MDA-MB-231, MDA-MB-436, BT-20, and BT-549)	Assumpção et al. (2020)
Ulcers	Propolis (hydro-alcoholic extract)	Anti-ulcers	Brazilian green propolis may be used as a therapeutic agent for gastric ulcer treatment as it increases the production of mucin, mucosal cell proliferation, and re-establishes the oxidative balance	Costa et al. (2019)
	Propolis		Topical application of 5% propolis ointment decreases area of ulceration and enhances the process of healing in 4 weeks	Afkhamizadeh et al. (2018)

	Propolis		Weekly application of topical propolis could boost closure of wounds in human diabetic foot ulcers	Henshaw et al. (2014)
Neurological disorders	Caffeic acid phenethyl ester	Anti-oxidative	Obstructs the neurotoxicity induced by 6-hydroxydopamine	Noelker et al. (2005)
	Caffeic acid phenethyl ester	Anti-oxidative	Prevents cerebellar granule neurons from neurotoxicity induced by glutamate via inhibiting activation of caspase-3 and p38 phosphorylation	Wei et al. (2008)
	Chrysin	Anti-inflammatory	Blocks the activation of microglial c-Jun N-terminal kinase and nuclear transcription factor kappa B	Ha et al. (2010)
	Kaempferol	Ant-oxidative and anti-apoptotic	Autophagic protection to primary nerve and SH-SY5Y cells against acute toxicity induced by rotenone	Filomeni et al. (2011)
Chronic periodontitis	Topical propolis		Topical propolis could be clinically utilized for treating chronic periodontitis	Nakao et al. (2020)
Oral candidiasis	Green propolis		Effective for treating oral candidiasis	Santos (2005)
Wounds	Propolis	Anti-inflammatory, anti-acid, anti-histaminergic, and anti-H pylori	Treat gastric ulcers	Paulino et al. (2015)
	Propolis		Possess clinical therapeutic applicability for wounds	Martinotti et al. (2019a, b)
	Propolis	Anti-oxidative	Reduces wound oxidative stress via improving antioxidant-linked gene expression (ligase-catalytic GCLC, ligase-modifier GCLM, and heme oxygenase-1)	Cao et al. (2017)
Chronic kidney disorder (CKD)	Barazalian propolis	Antiproteinuric	Significantly reduces proteinuria in non-diabetic as well as diabetic CKD patients	Silveira et al. (2019)

owing to its antitumor action via apoptotic induction of breast cancerous cells with no or low toxic effects on normal cells because of its selective toxic action on cancer cells. It is thought that propolis could act as major therapeutic agent for breast cancer (Xuan et al. 2014). Ethanolic extract of Algerian propolis has been shown to significantly induce apoptosis and inhibit melanoma cancer cells due to the presence of galangin (Benguedouar et al. 2015). In addition, Turkish propolis has been reported to exert a selective cytotoxic action on human lung cancer cells via apoptosis, caspase activity, decreasing mitochondrial membrane potential, and inducing stress in endoplasmic reticulum, which shows the capability of propolis for minimizing the proliferation of cancerous cells (Demir et al. 2016).

8.3.2 Propolis in Ulcers

Honey and propolis have been shown to be effective for treating chronic skin ulcerations (Tossoun et al. 1997). Bulgarian propolis exhibits significant antiseptic activity against *Helicobacter pylori* as well as capable of inhibiting growth in *Campylobacter jejuni* and *C. coli* (Kimoto et al. 1998). Boyanova et al. (2003) in an in vitro study also reported that Bulgarian propolis inhibits the growth of *Helicobacter pylori*. Propolis has been demonstrated as a substitute therapeutic agent for the treatment of wounds that promotes the closure of wounds mainly during diabetic foot ulcers in humans (Henshaw et al. 2014).

8.3.3 Propolis in Neurological Disorders

Neurological disorders display defects in the nervous system (nerves, brain, and spinal cord) culminating in signs such as muscular weakening, paralysis, cognitive and sensory impairment, memory failure, epilepsy, discomfort, impaired states of consciousness, and uncertainty. Patients with neurological conditions present various signs based on the portion of the neural system affected. Neural disorders are characterized by increased potency of reactive oxygen species (ROS) production, enhanced formation of pro-inflammatory lipid mediators (platelet-activating factor and eicosanoids), increased secretion of pro-inflammatory cytokine (IL1β, TNFα) as well as ion homeostatic changes, impaired ATP production, and cellular redox alterations throughout the brain (Farooqui and Farooqui 2009; Farooqui 2010). Additionally, mitochondrial impairment is another neurochemical alteration which may lead to neuronal degeneration in neurological diseases. In in vitro and in vivo tests, propolis along with its flavonoid components has been documented for exerting protective action on neurons via their immunomodulatory, anti-inflammatory, and antioxidant effects. Choi et al. examined the possible molecular pathway underpinning the defensive effect mediated by CAPE against reperfusion/ischemia and concluded that CAPE acts on hypoxia-inducible (HIF) pathway as a powerful inhibitor of HIF prolyl hydroxylase (Choi et al. 2010). The neuroprotective activity of CAPE is further supported by its potential to suppress the excitotoxicity

induced by glutamate in cerebellar granule neurons (CGNs) via inhibiting p38 phosphorylation and activation of caspase-3 (Wei et al. 2008). Propolis and its flavonoid ingredients may act as potent therapeutic mediators for the protection of brain in neural disorders (Chen et al. 2008). Microglia-mediated neuroinflammation has been associated with neurodegenerative disorders and thus inhibiting the activity of microglia may enhance the survival of nerve cells. In case of microglia stimulation by lipo-polysaccharides, chrysin has been found responsible for inhibiting the discharge of pro-inflammatory cytokines (TNFα and IL1β) and nitric oxide (NO) to a large extent (Ha et al. 2010). In addition to inhibiting the inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expression, chrysin inhibits the NF-kB and c-Jun N-terminal kinase signaling activation, the processes intimately linked with neuroinflammation induction and maintenance (Ha et al. 2010). While the toll-like receptor (TLR) system defends the host from infections, prolonged stimulation of such receptor molecules is harmful to host cells because of excessive pro-inflammatory cytokine production. Dysregulated TLRs-mediated immunologic reactions may contribute to neuronal cell damage causing neural defects, however, modulating the TRLs signaling pathway via minute molecules including flavonoids might present therapeutic promise against such disorders. Propolis has been observed capable of exerting in vivo immunomodulatory effect via upregulating the expression of TLRs and the synthesis of pro-inflammatory cytokines (Orsatti et al. 2010). Chronic stimulation of microglia, the native brain immune cells, causes and retains an inflammatory reaction, eventually contributing to the death of neuronal cell in neurodegenerative disorders owing to brain sensitivity to neurotoxins that include ROS, protein supplements, pro-inflammatory cytokines, and proteinases (Rezai-Zadeh et al. 2008). Cytokine receptor CD40 signaling vitally participates in the microglia-associated immune reactions in the brain. Luteolin and apigenin demonstrate neuroprotective and anti-inflammatory activities via modulating the activation of microglia by inhibiting the CD40 expression mediated by STAT1 (Rezai-Zadeh et al. 2008). Collective data shows that some flavonoid compounds of propolis could have a possible medicinal function in the diagnosis or prevention of certain neurological disorders in humans (Farooqui and Farooqui 2012).

8.3.4 Propolis in Chronic Periodontitis

Research on patients with periodontal conditions found that using green propolis associated with treatment of chronic periodontitis reduced pus and gingivitis (do Amaral et al. 2006). Another research study found that both in clinical as well as microbial parameters, subgingival irrigation with propolis extract as an adjuvant to the periodontal cure was more successful compared to root preparation and scaling (Coutino and Sanikop 2009).

8.3.5 Propolis in Gastrointestinal Disorders

Infection due to parasites is typically triggered by touch with the contaminated surface. Symptoms of parasitic gastrointestinal tract (GI) infection include stomach discomfort, bloating, and nausea and diarrhea. Ethanolic extract of propolis has been evaluated for in vitro effect on the growth and adherence of Giardia duodenalis trophozoites (Freitas et al. 2006), and it was found that propolis inhibits trophozoite growth and adherence as well as promotes their detachment. Moreover, the clinical efficiency of propolis against giardiasis has been reported via a clinical trial where giardiasis-infected children and adults after treatment with propolis exhibited a recovery rate of 52–60%, while those treated with traditional drugs showed a cure rate of 40%. A different experimental investigation has shown that propolis possesses anti-acid, anti-histaminergic, anti-H pylori, and anti-inflammatory properties that can be used for the treatment of gastric ulceration (Paulino et al. 2015).

8.3.6 Propolis in Wound Curing

Propolis components also have healing abilities in terms of wound healing and injury regeneration (Kuropatnicki et al. 2013) because of its immunomodulatory, antimicrobial, and anti-inflammatory properties (Martinotti and Ranzato 2015; Sforcin 2016). Propolis has also been found capable of reducing free radical amount in inflammatory and improves the formation of collagen along with its components (Kuropatnicki et al. 2013; Martinotti and Ranzato 2015). Additionally, propolis accelerates numerous enzymatic reactions, circulation of blood, cellular metabolism, and even the collagen fiber production owing to the ingredients present in it which include bio-flavonoids, vitamin C, B complex, arginine, provitamin A, and several minerals (Parolia et al. 2010).

8.4 Conclusion

Honey and propolis are attributed with a broad array of medicinal properties owing to their valuable components. These honey bee derivatives have been utilized as natural therapeutic products for the prevention and treatment of large number of diseases since the time of earliest civilization. They are well-known for antimicrobial, anti-metastatic, anticancer, anti-inflammatory, antioxidant, and anti-proliferative properties that may make them potential future clinic-pharmacological agents with further extensive preclinical, clinical, and randomized trail studies. They have already yielded promising therapeutic outcome in different health disorders including cardiovascular disorders, neurological abnormalities, gastrointestinal defects, wounds, ulcers, and carcinomas. Therefore, we recommend for further well-structured studies to find out vital pharmacological mechanisms of these

honey bee derivatives as well as determine the effective dosages for obtaining promising health benefits.

References

Abdelhafiz AT, Muhamad JA (2008) Midcycle pericoital intravaginal bee honey and royal jelly for male factor infertility. Int J Gynaecol Obstet 101:146–149

Abdulrhman MA, Mekaway MA, Awadalla MM (2010) Bee products added to the oral rehydration solution in treatment of gastroenteritis in infants and children. J Med Food 13:605–609

Abdulrhman M, Samir Elbarbary N, Ahmed Amin D, Saeid Ebrahim R (2012) Honey and a mixture of honey, beeswax, and olive oil–propolis extract in treatment of chemotherapy-induced oral mucositis: a randomized controlled pilot study. Pediatr Hematol Oncol 29:285–292

Adebolu TT (2005) Effect of natural honey on local isolates of diarrhea causing bacteria in Southwestern Nigeria. Afr J Biotechnol 4:1172–1174

Afkhamizadeh M, Aboutorabi R, Ravari H, Fathi Najafi M, Ataei Azimi S, Javadian Langaroodi A, Yaghoubi MA, Sahebkar A (2018) Topical propolis improves wound healing in patients with diabetic foot ulcer: a randomized controlled trial. Nat Prod Res 32:2096–2099

Ahmed S, Sulaiman SA, Baig AA, Ibrahim M, Liaqat S, Fatima S, Jabeen S, Shamim N, Othman NH (2018) Honey as a potential natural antioxidant medicine: an insight into its molecular mechanisms of action. Oxidative Med Cell Longev 2018:8367846

Ajibola A, Chamunorwa JP, Erlwanger KH (2012) Nutraceutical values of natural honey and its contribution to human health and wealth. Nut Metabol 9:61

Akanmu MA, Echeverry C, Rivera F, Dajas F (2009) Antioxidant and Neuroprotective effects of Nigerian honey. Proceedings of the Neuroscience Meeting Planner; Washington, DC, USA

Akanmu MA, Olowookere TA, Atunwa SA, Ibrahim BO, Lamidi OF, Adams PA, Ajimuda BO, Adeyemo LE (2011) Neuropharmacological effects of Nigerian honey in mice. Afr J Tradit Complement Altern Med 8:230–249

Al-Himyari FA (2009) The use of honey as a natural preventive therapy of cognitive decline and dementia in the middle east. Alzheimer Dement 5:P247

Ali AM (1995) Natural honey accelerates healing of indomethacin-induced antral ulcers in rats. Saudi Med J 16:161–166

Aliyu M, Odunola OA, Farooq AD, Rasheed H, Mesaik AM, Choudhary MI, Channa IS, Khan SA, Erukainure OL (2013) Molecular mechanism of antiproliferation potential of Acacia honey on NCI-H460 cell line. Nutr Cancer 65:296–304

Alnaqdy A, Al-Jabri A, Al Mahrooqi Z, Nzeako B, Nsanze H (2005) Inhibition effect of honey on the adherence of Salmonella to intestinal epithelial cells in vitro. Int J Food Microbiol 103:347–351

Al-Waili NS (2003) Effects of daily consumption of honey solution on hematological indices and blood levels of minerals and enzymes in normal individuals. J Med Food 6:135–140

Al-Waili NS (2004a) Investigating the antimicrobial activity of natural honey and its effects on the pathogenic bacterial infections of surgical wounds and conjunctiva. J Med Food 7:210–222

Al-Waili NS (2004b) Natural honey lowers plasma glucose, C-reactive protein, homo-cysteine, and blood lipids in healthy, diabetic, and hyperlipidemic subjects: comparison with dextrose and sucrose. J Med Food 7:100–107

Al-Waili N, Saloom K (1999) Glycemic responses to glucose and honey in patients with diabetes mellitus. FASEB J 13:A272

Anjum SI, Ullah A, Khan KA, Attaullah M, Khan H, Ali H, Bashir MA, Tahir M, Ansari MJ, Ghramh HA, Adgaba N (2018) Composition and functional properties of propolis (bee glue): a review. Saudi J Biol Sci 26:1695–1703

Araujo MA, Libério SA, Guerra RN, Ribeiro MN, Nascimento FR (2012) Mechanisms of action underlying the anti-inflammatory and immune modulatory effects of propolis: a brief review. Rev Bras 22:208–219
Association AD (2014) Diagnosis and classification of diabetes mellitus. Diabetes Care 37:S81–S90
Assumpção JH, Takeda AA, Sforcin JM, Rainho CA (2020) Effects of Propolis and phenolic acids on triple-negative breast cancer cell lines: potential involvement of epigenetic mechanisms. Molecules 25:1289
Bâcvarov VI (1970) Treatment of chronic bronchitis and bronchial asthma with honey. Ther Ggw 109:260–268
Badawy OF, Shafii SS, Tharwat EE, Kamal AM (2004) Antibacterial activity of bee honey and its therapeutic usefulness against Escherichia coli O157: H7 and Salmonella typhimurium infection. Rev Sci Tech 23:1011–1122
Baig S, Attique H (2014) Cytotoxic activity of honey in hepatoma cells: in vitro evaluation. Pak J Med Dent 3:12
Bansal V, Medhi B, Pandhi P (2005) Honey—a remedy rediscovered and its therapeutic utility. Kathmandu Univ Med J 3:305–309
Barzilai A, Melamed E (2003) Molecular mechanisms of selective dopaminergic neuronal death in Parkinson's disease. Trends Mol Med 9:126–132
Batista LL, Campesatto EA, Assis ML, Barbosa AP, Grillo LA, Dornelas CB (2012) Comparative study of topical green and red propolis in the repair of wounds induced in rats. Rev Col Bras Cir 39:515–520
Bearse MA, Han Y, Schneck ME, Barez S, Jacobsen C, Adams AJ (2004) Local multifocal oscillatory potential abnormalities in diabetes and early diabetic retinopathy. Invest Ophthalmol Vis Sci 45:3259–3265
Benguedouar L, Lahouel M, Gangloff S, Durlach A, Grange F, Bernard P, Antonicelli F (2015) Algerian ethanolic extract of propolis and galangin decreased melanoma tumour progression in C57BL6 mice. In: Annales de Dermatologie et de Vénéréologie. Elsevier, Amsterdam, p S294
Bennett E, Daly S (2019) Is honey better than dextromethorphan at decreasing acute cough in children? Evid Based Pract 21:1
Bergman A, Yanai J, Weiss J, Bell D, David MP (1983) Acceleration of wound healing by topical application of honey. An animal model. Am J Surg 145:374–376
Berk L, Deshmukh S, Fogh S, Roof K, Yacoub S, Gergel T, Stephans K, Rimner A, DeNittis A, Pablo J (2014) Randomized phase 2 trial of best supportive care: Manuka honey liquid and Manuka honey lozenges for prevention of radiation esophagitis during chemotherapy and radiation therapy for lung cancer. Int J Radiat Oncol Boil Phys 90:S5
Bertoncelj J, Doberšek U, Jamnik M, Golob T (2007) Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. Food Chem 105:822–828
Bogdanov S, Jurendic T, Sieber R, Gallmann P (2008) Honey for nutrition and health: a review. J Am Coll Nutr 27:677–689
Boorn KL, Khor YY, Sweetman E, Tan F, Heard TA, Hammer KA (2010) Antimicrobial activity of honey from the stingless bee Trigona carbonaria determined by agar diffusion, agar dilution, broth microdilution and time-kill methodology. J Appl Microbiol 108:1534–1543
Boukraâ L, Sulaiman SA (2009) Rediscovering the antibiotics of the hive. Recent Pat Anti-Infect Drug Dis 4:206–213
Boyanova L, Derejian S, Koumanova R, Katsarov N, Gergova G, Mitov I, Nikolov R, Krastev Z (2003) Inhibition of Helicobacter pylori growth in vitro by Bulgarian propolis: preliminary report. J Med Microbiol 52:417–419
Braakhuis A (2019) Evidence on the health benefits of supplemental Propolis. Nutrients 11:2705
Cao XP, Chen YF, Zhang JL, You MM, Wang K, Hu FL (2017) Mechanisms underlying the wound healing potential of propolis based on its in vitro antioxidant activity. Phytomedicine 34:76–84
Carlos AU, David H, Carmen G (2011) Role of honey polyphenols in health. J ApiProd ApiMed Sci 3:141–159

Castaldo S, Capasso F (2002) Propolis, an old remedy used in modern medicine. Fitoterapia 73:S1–S6

Chan GC, Cheung KW, Sze DM (2013) The immunomodulatory and anticancer properties of propolis. Clin Rev Allergy Immunol 44:262–273

Chandna P, Adlakha VK, Das S, Singh S (2014) Complementary and Alternative Medicine (CAM): a review of propolis in dentistry. Technology 4:6

Chang AB (2010) Pediatric cough: children are not miniature adults. Lung 188(Suppl. 1):S33–S40

Chang AB, Widdicombe JG (2007) Cough throughout life: children, adults and the senile. Pulm Pharmacol Ther 20:371–382

Chen J, Long Y, Han M, Wang T, Chen Q, Wang R (2008) Water-soluble derivative of propolis mitigates scopolamine-induced learning and memory impairment in mice. Pharmacol Biochem Behav 90:441–446

Chepulis L, Starkey N (2008) The long-term effects of feeding honey compared with sucrose and a sugar-free diet on weight gain, lipid profiles, and DEXA measurements in rats. J Food Sci 73: H1–H7

Choi D, Han J, Lee Y, Choi J, Han S, Hong S, Jeon H, Kim YM, Jung Y (2010) Caffeic acid phenethyl ester is a potent inhibitor of HIF prolyl hydroxylase: structural analysis and pharmacological implication. J Nutr Biochem 21:809–817

Choudhari MK, Haghniaz R, Rajwade JM, Paknikar KM (2013) Anticancer activity of Indian stingless bee propolis: an in vitro study. Evid Based Complement Alternat Med 2013:928280

Cianciosi D, Forbes-Hernández TY, Afrin S, Gasparrini M, Reboredo-Rodriguez P, Manna PP, Zhang J, Bravo Lamas L, Martínez Flórez S, Agudo Toyos P, Quiles JL (2018) Phenolic compounds in honey and their associated health benefits: a review. Molecules 23:2322

Cohen HA, Rozen J, Kristal H, Laks Y, Berkovitch M, Uziel Y (2012) Effect of honey on nocturnal cough and sleep quality: a double-blind, randomized, placebo-controlled study. Pediatrics 130:465–471

Costa P, Somensi LB, Mariano LN, Boeing T, Longo B, Perfoll E, de Souza P, Gushiken LF, Pellizzon CH, Rodrigues DM, Bastos JK (2019) Role of the antioxidant properties in the gastroprotective and gastric healing activity promoted by Brazilian green propolis and the healing efficacy of Artepillin C. Inflammopharmacology 19:1–7

Coutino AO, Sanikop S (2009) Propolis (Honeybee) extract as a subgingival irrigant in periodontal treatment. KSDJ 28:31–35

Cuevas A, Saavedra N, Salazar LA, Abdalla DS (2013) Modulation of immune function by polyphenols: possible contribution of epigenetic factors. Nutrients 5:2314–2332

Davoodi S, Samarghandian S, Tavakkol Afshari J (2010) Modulation of programmed cell death by honey bee in human prostate adenocarcinoma. J Med Plant Res 4:2151–2156

De Castro S (2001) Propolis: biological and pharmacological activities. Therapeutic uses of this bee-product. Annu Rev Biomed Sci 3:49–83

Demir S, Aliyazicioglu Y, Turan I, Misir S, Mentese A, Yaman SO, Akbulut K, Kilinc K, Deger O (2016) Antiproliferative and proapoptotic activity of Turkish propolis on human lung cancer cell line. Nutri Can 68:165–172

Dimitriadis G, Mitrou P, Lambadiari V, Maratou E, Raptis SA (2011) Insulin effects in muscle and adipose tissue. Diabetes Res Clin Pract 1:S52–S59

do Amaral RC, Gomes RT, Rocha WM, Lemos S, Abreu R, Santos VR (2006) Periodontitis treatment with Brazilian green propolis gel. Pharmacol Ther 3:336–341

dos Santos DA, Munari FM, da Silva Frozza CO, Moura S, Barcellos T, Henriques JA, Roesch-Ely M (2019) Brazilian red propolis extracts: study of chemical composition by ESI-MS/MS (ESI+) and cytotoxic profiles against colon cancer cell lines. Biotechnol Res Innov 3:120–130

Drain J, Fleming MO (2015) Palliative management of malodorous squamous cell carcinoma of the oral cavity with Manuka honey. J Wound Ostomy Cont Nurs 42:190–192

Eddy JJ, Gideonsen MD, Mack GP (2008) Practical considerations of using topical honey for neuropathic diabetic foot ulcers: a review. WMJ 107:187–190

Edebo L, Kohlstrom E, Magnusson K, Stendahl O (1980) The hydrophobic effect and charge effects in the adhesion of enterobacteria to animal cell surfaces and the influences of antibodies of different immunoglobulin classes. In: Curtis A, Pitts H (eds) Cell adhesion and motility. Cambridge University Press, Cambridge, pp 65–101

Erejuwa OO, Sulaiman SA, Ab Wahab MS (2012) Honey: a novel antioxidant. Molecules 17:4400–4423

Erejuwa OO, Sulaiman SA, Wahab MS (2014) Effects of honey and its mechanisms of action on the development and progression of cancer. Molecules 19:2497–2522

Fakhrildin MB, Alsaadi RA (2014) Honey supplementation to semen-freezing medium improves human sperm parameters post-thawing. J Family Reprod Health 8:27–31

Farooqui AA (2010) Neurochemical aspects of neurotraumatic and neurodegenerative diseases. Springer, New York

Farooqui T, Farooqui AA (2009) Aging: an important factor for the pathogenesis of neurodegenerative diseases. Mech Ageing Dev 130:203–215

Farooqui T, Farooqui AA (2012) Beneficial effects of propolis on human health and neurological diseases. Front Biosci (Elite Ed) 4:779–793

Fauzi AN, Norazmi MN, Yaacob NS (2011) Tualang honey induces apoptosis and disrupts the mitochondrial membrane potential of human breast and cervical cancer cell lines. Food Chem Toxicol 49:871–878

Fernandez-Cabezudo MJ, El-Kharrag R, Torab F, Bashir G, George JA, El-Taji H, Al-Ramadi BK (2013) Intravenous administration of manuka honey inhibits tumor growth and improves host survival when used in combination with chemotherapy in a melanoma mouse model. PLoS One 8:e55993

Filomeni G, Graziani I, De Zio D, Dini L, Centonze D, Rotilio G, Ciriolo MR (2011) Neuroprotection of kaempferol by autophagy in models of rotenone-mediated acute toxicity: possible implications for Parkinson's disease. Neurobiol Aging 33:767–785

Fokt H, Pereira A, Ferreira A, Cunha A, Aguiar C (2010) How do bees prevent hive infections? The antimicrobial properties of propolis. Curr Res Technol Educ Top Appl Microbiol Microbial Biotechnol 1:481–493

Freitas SF, Shinohara L, Sforcin JM, Guimarães S (2006) In vitro effects of propolis on Giardia duodenalis trophozoites. Phytomedicine 13:170–175

Frión-Herrera Y, Gabbia D, Scaffidi M, Zagni L, Cuesta-Rubio O, De Martin S, Carrara M (2020) The Cuban Propolis component nemorosone inhibits proliferation and metastatic properties of human colorectal cancer cells. Int J Mol Sci 21:1827

Gethin G, Cowman S (2009) Manuka honey vs. hydrogel—a prospective, open label, multicentre, randomised controlled trial to compare desloughing efficacy and healing. J Clin Nurs 18:466–474

Ghashm AA, Othman NH, Khattak MN, Ismail NM, Saini R (2010) Antiproliferative effect of Tualang honey on oral squamous cell carcinoma and osteosarcoma cell lines. BMC Complement Altern Med 10:49

Goharshenasan P, Amini S, Atria A, Abtahi H, Khorasani G (2016) Application of honey on surgical wounds: a randomized clinical trial. Forsch Komplementmed 23:12–15

Gómez-Caravaca AM, Gómez-Romero M, Arráez-Román D, Segura-Carretero A, Fernández-Gutierrez A (2006) Advances in the analysis of phenolic compounds in products derived from bees. J Pharm Biomed Anal 41:1220–1234

Guerrero J, Lozano M, Castillo J, Benavente GO, Vicente V, Rivera J (2005) Flavonoids inhibit platelet function through binding to the thromboxane A2 receptor. J Thromb Haemost 3:369–376

Gupta S, Gambhir JK, Kalra O, Gautam A, Shukla K, Mehndiratta M, Agarwal S, Shukla R (2013) Association of biomarkers of inflammation and oxidative stress with the risk of chronic kidney disease in Type 2 diabetes mellitus in North Indian population. J Diabetes Complicat 27:548–552

Ha SK, Moon E, Kim SY (2010) Chrysin suppresses LPS-stimulated proinflammatory responses by blocking NF-κB and JNK activations in microglia cells. Neurosci Lett 485:143–147
Halstead F, Webber M, Oppenheim B (2017) Use of an engineered honey to eradicate preformed biofilms of important wound pathogens: an in vitro study. J Wound Care 26:442–450
Hananeh WM, Ismail ZB, Alshehabat MA, Abeeleh MA, Ali JF (2015) Effects of Sidr honey on second-intention healing of contaminated full-thickness skin wounds in healthy dogs. Bull Vet Inst Pulawy 59:433–439
Hawley P, Hovan A, McGahan CE, Saunders D (2014) A randomized placebo-controlled trial of manuka honey for radiation-induced oral mucositis. Support. Care Cancer 22:751–761
Henshaw FR, Bolton T, Nube V, Hood A, Veldhoen D, Pfrunder L, McKew GL, Macleod C, McLennan SV, Twigg SM (2014) Topical application of the bee hive protectant propolis is well tolerated and improves human diabetic foot ulcer healing in a prospective feasibility study. J Diabetes Complicat 28:850–857
Hossen MS, Ali MY, Jahurul MH, Abdel-Daim MM, Gan SH, Khalil MI (2017) Beneficial roles of honey polyphenols against some human degenerative diseases: a review. Pharm Rep 69:1194–1205
Huh JH, Yadav D, Kim JS, Son JW, Choi E, Kim SH, Shin C, Sung KC, Kim JY (2017) An association of metabolic syndrome and chronic kidney disease from a 10-year prospective cohort study. Metab Clin Exp 67:54–61
Hunter M, Kellett J, D'Cunha NM, Toohey K, McKune A, Naumovski N (2020) The effect of honey as a treatment for oral ulcerative lesions: a systematic review. Explorat Res Hypot Med 5:27–37
Irving TB, Ahmad K, Ahsan MM (1987) Chapter 5: The story of creation. In: The Qur'an-basic teachings. Pitman Press, Bath
Isla MI, Craig A, Ordoñez R, Zampini C, Sayago J, Bedascarrasabure E, Maldonado L (2011) Physico chemical and bioactive properties of honeys from Northwestern Argentina. LWT–Food Sci Technol 44:1922–1930
Islam A, Khalil I, Islam N, Moniruzzaman M, Mottalib A, Sulaiman SA, Gan SH (2012) Physico-chemical and antioxidant properties of Bangladeshi honeys stored for more than one year. BMC Complement Altern Med 12:177
Jellinger K (2001) Cell death mechanisms in neurodegeneration. J Cell Mol Med 5:1–17
Jull A, Walker N, Deshpande S (2013) Honey as a topical treatment for wounds. Cochrane Database Syst Rev 2013:CD005083
Kamaruzaman NA, Sulaiman SA, Kaur G, Yahaya B (2014) Inhalation of honey reduces airway inflammation and histopathological changes in a rabbit model of ovalbumin-induced chronic asthma. BMC Complement Altern Med 14:176
Khalil MI, Sulaiman SA (2010) The potential role of honey and its polyphenols in preventing heart diseases: a review. Afr J Tradit Compl Altern Med 7:315–321
Khalil MI, Tanvir E, Afroz R, Sulaiman SA, Gan SH (2015) Cardioprotective effects of Tualang honey: amelioration of cholesterol and cardiac enzymes levels. Bio Med Res Int 2015:286051
Khan RU, Naz S, Abudabos AM (2017) Towards a better understanding of the therapeutic applications and corresponding mechanisms of action of honey. Environ Sci Pollut Res 24:27755–27766
Kimoto T, Arai S, Kohguchi M, Aga M, Nomura Y, Micallef MJ, Kurimoto M, Mito K (1998) Apoptosis and suppression of tumor growth by artepillin C extracted from Brazilian propolis. Cancer Detect Prev 22:506–515
Komericki P, Kränke B (2009) Maculopapular exanthem from propolis: case report and review of systemic cutaneous and non-cutaneous reactions. Contact Dermatitis 61:353–355
Kuropatnicki AK, Szliszka E, Krol W (2013) Historical aspects of propolis research in modern times. Evid Based Complement Alternat Med 2013:964149
Kustiawan PM, Puthong S, Arung ET, Chanchao C (2014) In vitro cytotoxicity of Indonesian stingless bee products against human cancer cell lines. Asian Pac J Trop Biomed 4:549–556

Li Y, Shi W, Li Y, Zhou Y, Hu X, Song C, Ma H, Wang C, Li Y (2008) Neuroprotective effects of chlorogenic acid against apoptosis of PC12 cells induced by methylmercury. Environ Toxicol Pharmacol 26:13–21
Ligouri P, Peters KA (2010) New leptospermum honey alginate dressing has a positive impact on a wide variety of wound types. J Wound Ostomy Continence Nurs 36:S46
Maghsoudi H, Moradi S (2015) Honey: a skin graft fixator convenient for both patient and surgeon. Indian J Surg 77:863–867
Majtanova N, Cernak M, Majtan J (2016) Honey: a natural remedy for eye diseases. Compl Med Res 23:364–369
Makawi SZ, Gadkariem EA, Ayoub SM (2009) Determination of antioxidant flavonoids in Sudanese honey samples by solid phase extraction and high performance liquid chromatography. J Chemother 6:S429–SS37
Mamo T (2018) Review on medicinal and nutritional importance of honey. Adv Dent Res 9:1–9
Manisha DM, Shyamapada M (2011) Honey: its medicinal property and antibacterial activity. Asian Pac J Trop Biomed 1:154–160
Martinotti S, Ranzato E (2015) Propolis: a new frontier for wound healing? Burns Trauma 3:9
Martinotti S, Bucekova M, Majtan J, Ranzato E (2019a) Honey: an effective regenerative medicine product in wound management. Cur Med Chem 26:230–240
Martinotti S, Pellavio G, Laforenza U, Ranzato E (2019b) Propolis induces AQP3 expression: a possible way of action in wound healing. Molecules 24:1544
Meda A, Lamien EC, Millogo J, Romito M, Nacoulma OG (2004) Ethnopharmacological communication therapeutic uses of honey and honeybee larvae in Central Burkina Faso. J Ethnopharmacol 95:103–107
Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG (2005) Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem 91:571–577
Meo SA, Al-Asiri SA, Mahesar AL, Ansari MJ (2017) Role of honey in modern medicine. Saudi J Biol Sci 24:975–978
Mijanur RM, Gan SH, Khalil MI (2014) Neurological effects of honey: Current and future prospects. Evid Based Complement Alternat Med 2014:958721
Miller EJ (2019) Effectiveness of honey for healing chronic wounds compared to standard treatments. Lynch J Med Sci 1:8
Molan PC (2006) The evidence supporting the use of honey as a wound dressing. Int J Low Extrem Wounds 5:40–54
Moore C, Young J (2011) Effectiveness of silver in wound care treatment. Phys Ther Rev 16:201–219
Nakao R, Senpuku H, Ohnishi M, Takai H, Ogata Y (2020) Effect of topical administration of propolis in chronic periodontitis. Odontologia 28:1–1
Nasir NA, Halim AS, Singh KK, Dorai AA, Haneef MN (2010) Antibacterial properties of Tualang honey and its effect in burn wound management: a comparative study. BMC Complement Altern Med 10:31
Nejabat M, Soltanzadeh K, Yasemi M, Daneshamouz S, Akbarizadeh AR, Heydari M (2020) Efficacy of honey based ophthalmic formulation in patients with corneal ulcer—a randomized clinical trial. Curr Drug Dis Technol. https://doi.org/10.2174/1570163817666200214113055
Noelker C, Bacher M, Gocke P, Wei X, Klockgether T, Du Y, Dodel R (2005) The flavanoide caffeic acid phenethyl ester blocks 6-hydroxydopamine-induced neurotoxicity. Neurosci Lett 383:39–43
Nurul SM, Gan SH, Halim AS, Shah NS, Sukari HA (2013) Analysis of volatile compounds of Malaysian Tualang (Koompassia excelsa) honey using gas chromatography mass spectrometry. Afri J Trad Compl Alter Med 10:180–188
Obaseiki-Ebor EE, Afonya TCA (1984) In vitro evaluation of the anticandidiasis activity of honey distillate (HY1) compared with that of some antimycotic agents. J Pharm Pharmacol 36:283–284

Obi CL, Ugoji EO, Edun SA, Lawal SF, Anyiwo CE (1994) The antibacterial effect of honey on diarrhoea causing bacterial agents isolated in Lagos, Nigeria. Afr J Med Med Sci 23:257–260
Oduwole O, Udoh EE, Oyo Ita A, Meremikwu MM (2018) Honey for acute cough in children. Cochrane Database Syst Rev. https://doi.org/10.1002/14651858.CD007094.pub5
Olas B (2020) Honey and its phenolic compounds as an effective natural medicine for cardiovascular diseases in humans? Nutrients 12:283
Omar R, Igoli JO, Zhang T, Gray AI, Ebiloma GU, Clements CJ, Fearnley J, Ebel RE, Paget T, De Koning HP, Watson DG (2017) The chemical characterization of Nigerian propolis samples and their activity against Trypanosoma brucei. Sci Rep 7:1
Orsatti CL, Missima F, Pagliarone AC, Bachiega TF, Búfalo MC, Araújo JP, Sforcin JM (2010) Propolis immunomodulatory action in vivo on Toll-like receptors 2 and 4 expression and on pro-inflammatory cytokines production in mice. Phytother Res 24:1141–1146
Othman NH (2012) Honey and cancer: sustainable inverse relationship particularly for developing nations—a review. Evid Based Complement Alternat Med 2012:410406
Oyefuga OH, Ajani EO, Salau BA, Agboola FO, Adebawo O (2012) Honey consumption and its anti-ageing potency in white Wister albino rats. Sch J Biol Sci 1:15–19
Parolia A, Thomas MS, Kundabala M, Mohan M (2010) Propolis and its potential uses in oral health. Int J Med Sci 2:210–215
Paul IM, Beiler J, McMonagle A, Shaffer ML, Duda L, Berlin CM (2007) Effect of honey, dextromethorphan, and no treatment on nocturnal cough and sleep quality for coughing children and their parents. Arch Pediatr Adolesc Med 161:1140–1146
Paulino N, Coutinho LA, Coutinho JR, Vilela GC, da Silva Leandro VP, Paulino AS (2015) Antiulcerogenic effect of Brazilian propolis formulation in mice. Pharmacol Pharm 6:580
Pichichero E, Cicconi R, Mattei M, Muzi MG, Canini A (2010) Acacia honey and chrysin reduce proliferation of melanoma cells through alterations in cell cycle progression. Int J Oncol 37:973–981
Rakha MK, Nabil ZI, Hussein AA (2008) Cardioactive and vasoactive effects of natural wild honey against cardiac malperformance induced by hyperadrenergic activity. J Med Food 11:91–98
Rezai-Zadeh K, Ehrhart J, Bai Y, Sanberg PR, Bickford P, Tan J, Shytle RD (2008) Apigenin and luteolin modulate microglial activation via inhibition of STAT1-induced CD40 expression. J Neuroinflammation 5:41
Robert SD, Ismail AA (2009) Two varieties of honey that are available in Malaysia gave intermediate glycemic index values when tested among healthy individuals. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 153:145–147
Saely C, Aczel S, Marte T, Langer P, Drexel H (2004) Cardiovascular complications in Type 2 diabetes mellitus depend on the coronary angiographic state rather than on the diabetic state. Diabetologia 47:145–146
Sakai D (1987) Adhesion of Aeromonas salmonicida strains associated with net electrostatic changes of host tissue cells. Infect Immun 55:704–710
Salomao K, de Souza EM, Henriques-Pons A, Barbosa HS, de Castro SL (2011) Brazilian green propolis: effects in vitro and in vivo on Trypanosoma cruzi. Evid Based Complement Alternat Med 2011:185918
Samanta A, Burden AC, Jones GR (1985) Plasma glucose responses to glucose, sucrose, and honey in patients with diabetes mellitus: an analysis of glycaemic and peak incremental indices. Diabet Med 2:371–373
Samarghandian S, Afshari JT, Davoodi S (2011a) Chrysin reduces proliferation and induces apoptosis in the human prostate cancer cell line pc-3. Clinics (Sao Paulo) 66:1073–1079
Samarghandian S, Afshari JT, Davoodi S (2011b) Honey induces apoptosis in renal cell carcinoma. Pharmacogn Mag 7:46–52
Samarghandian S, Nezhad MA, Mohammadi G (2014a) Role of caspases, Bax and Bcl-2 in chrysin-induced apoptosis in the A549 human lung adenocarcinoma epithelial cells. Anti Cancer Agents Med Chem 14:901–909

Samarghandian S, Samini F, Taghavi MR (2014b) Antiproliferative and cytotoxic properties of honey in human prostate cancer cell line (PC-3): possible mechanism of cell growth inhibition and apoptosis induction. Afr J Pharm Pharmacol 8:9–15
Samarghandian S, Farkhondeh T, Samini F (2017) Honey and health: a review of recent clinical research. Pharm Res 9:121
Santos VR (2005) Oral candidiasis treatment with Brazilian ethanol propolis extract. Phytother Res 19:652–654
Schmitt-Schillig S, Schaffer S, Weber CC, Eckert GP, Müller WE (2005) Flavonoids and the aging brain. J Physiol Pharmacol 56:23–36
Sforcin J (2007) Propolis and the immune system: a review. J Ethnopharmacol 113:1–14
Sforcin JM (2016) Biological properties and therapeutic applications of propolis. Phytother Res 30:894–905
Shenoy R, Bialasiewicz A, Khandekar R, Al Barwani B, Al Belushi H (2009) Traditional medicine in Oman: its role in ophthalmology. Middle East Afr J Ophthalmol 16:92–96
Shimazawa M, Chikamatsu S, Morimoto N, Mishima S, Nagai H, Hara H (2005) Neuroprotection by Brazilian green propolis against in vitro and in vivo ischemic neuronal damage. eCAM 2:201–207
Shishehbor F, Tehrani M, Jalali M, Latifi M (2013) Comparison of glycemic indices of two varieties of Iranian honey with different fructose to glucose ratios. IJEM 14:479–483
Silveira MA, Teles F, Berretta AA, Sanches TR, Rodrigues CE, Seguro AC, Andrade L (2019) Effects of Brazilian green propolis on proteinuria and renal function in patients with chronic kidney disease: a randomized, double-blind, placebo-controlled trial. BMC Nephrol 20:140
Smaropoulos E, Cremers NA (2020) Medical grade honey for the treatment of paediatric abdominal wounds: a case series. J Wound Care 29:94–99
Socha R, Galkowska D, Bugaj M, Juszczak L (2015) Phenolic composition and antioxidant activity of propolis from various regions of Poland. Nat Prod Res 29:416–422
Spires TL, Hannan AJ (2005) Nature, nurture and neurology: gene–environment interactions in neurodegenerative disease. The FEBS J 272:2347–2361
Swanson JE, Laine D, Thomas W, Bantle JP (1992) Metabolic effects of dietary fructose in healthy subjects. Am J Clin Nutr 55:851–856
Swellam T, Miyanaga N, Onozawa M, Hattori K, Kawai K, Shimazui T, Akaza H (2003) Antineoplastic activity of honey in an experimental blad-der cancer implantation model: in vivo and in vitro studies. Int J Urol 10:213–219
Tharakan T, Bent J, Tavaluc R (2019) Honey as a treatment in otorhinolaryngology: a review by subspecialty. Ann Otol Rhinol Laryngol 128:193–207
Topham J (2002) Why do some cavity wounds treated with honey or sugar paste heal without scarring? J Wound Care 11:53–55
Toreti VC, Sato HH, Pastore GM, Park YK (2013) Recent progress of propolis for its biological and chemical compositions and its botanical origin. Evid Based Complement Alternat Med 2013:697390
Tossoun ZA, Rashed A, Hegazi AG (1997) Honey and propolis as management of chronic skin ulcers. International Symposium on Apitherapy, pp 8–9
Tsiapara AV, Jaakkola M, Chinou I, Graikou K, Tolonen T, Virtanen V, Moutsatsou P (2009) Bioactivity of Greek honey extracts on breast cancer (MCF-7), prostate cancer (PC-3) and solvent extracts of honeys produced in South Africa. Afr J Agric Res 116:702–708
Veiga R, De Mendonça S, Mendes P, Paulino N, Mimica M, Lagareiro NA, Lira I, López BC, Negrão V, Marcucci M (2017) Artepillin C and phenolic compounds responsible for antimicrobial and antioxidant activity of green propolis and Baccharis dracunculifolia DC. J Appl Microbiol 122:911–920
Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, Pérez-Álvarez JA (2008) Functional properties of honey, propolis, and royal jelly. J Food Sci 73:R117–R124
Wagh VD (2013) Propolis: a wonder bees product and its pharmacological potentials. Adv Pharmacol Sci 2013:308249

Waheed M, Hussain MB, Javed A, Mushtaq Z, Hassan S, Shariati MA, Khan MU, Majeed M, Nigam M, Mishra AP, Heydari M (2019) Honey and cancer: a mechanistic review. Clin Nutr 38:2499–2503

Watanabe MA, Amarante MK, Conti BJ, Sforcin JM (2011) Cytotoxic constituents of propolis inducing anticancer effects: a review. J Pharm Pharmacol 63:1378–1386

Watford M (2002) Small amounts of dietary fructose dramatically increase hepatic glucose uptake through a novel mechanism of glucokinase activation. Nutr Rev 60:253

Wei X, Ma Z, Fontanilla CV, Zhao L, Xu ZC, Taggliabraci V, Johnstone BH, Dodel RC, Farlow MR, Du Y (2008) Caffeic acid phenethyl ester prevents cerebellar granule neurons (CGNs) against glutamate-induced neurotoxicity. Neuroscience 155:1098–1105

Wilkinson LJ, White RJ, Chipman JK (2011) Silver and nanoparticles of silver in wound dressings: a review of efficacy and safety. J Wound Care 20:543–549

World Health Organization (2002) Reduced Osmolarity Oral rehydration salts (Ors) formulation. World Health Organization, Geneva

World Health Organization (2007) Prevention of cardiovascular disease. World Health Organization, Geneva

Xuan H, Li Z, Yan H, Sang Q, Wang K, He Q, Wang Y, Hu F (2014) Antitumor activity of Chinese propolis in human breast cancer MCF-7 and MDA-MB-231 cells. Evid Based Complement Alternat Med 2014:280120

Yaacob NS, Ismail NF (2014) Comparison of cytotoxicity and genotoxicity of 4-hydroxytamoxifen in combination with Tualang honey in MCF-7 and MCF-10A cells. BMC Complement Altern Med 14:106

Yaacob NS, Nengsih A, Norazmi MN (2013) Tualang honey promotes apoptotic cell death induced by tamoxifen in breast cancer cell lines. Evid Based Complement Alternat Med 2013:989841

Yadav D, Mishra M, Joseph AZ, Subramani SK, Mahajan S, Singh N et al (2015) Status of antioxidant and lipid peroxidation in type 2 diabetic human subjects diagnosed with and without metabolic syndrome by using NCEP-ATPIII, IDF and WHO criteria. Obes Res Clin Pract 9:158–167

Yadav D, Mishra M, Rana S, Subramani SK, Prasad G (2017) Study of biochemical and oxidative stress markers in the first-degree relatives of persons with type 2 diabetes stratified by glucose tolerance test. Prog Nutr 19:183–190

Yaghoobi N, Al-Waili N, Ghayour-Mobarhan M, Parizadeh S, Abasalti Z, Yaghoobi Z, Yaghoobi F, Esmaeili H, Kazemi-Bajestani S, Aghasizadeh R (2008) Natural honey and cardiovascular risk factors; effects on blood glucose, cholesterol, triacylglycerole, CRP, and body weight compared with sucrose. Sci. World J 8:463–469

Scope of Honey in Diabetes and Metabolic Disorders

9

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Abstract

Metabolic disorders occur when unusual chemical reactions take place in the body amend usual metabolic pathways. Diabetes mellitus a metabolic disorder is generally characterized by high glucose level in blood over longer period of time. In type 1 diabetes, pancreas fails to produce adequate insulin and the same effect is due to the loss of beta cells of pancreas. Type 2 diabetes begins with resistance to insulin and accordingly gives no response to insulin. Gestational diabetes mellitus is similar to type 2 diabetes in various aspects and is having combination of inadequate insulin and sensitivity to it. For many years, honey is being used as a substitute for sugar and for providing medicinal benefits. In animal as well as human studies, convincing evidence specifies that honey displays antidiabetic as well as hypoglycemic effects. Additionally, honey consumption improved other disorders related to metabolism and to diabetes such as reduced levels of HbA1c (glycosylated hemoglobin) and hepatic transaminases and increased HDL cholesterol. The same was in addition to lowering hyperglycemia and oxidative

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stress. Besides depicting hypoglycemic effect, research has indicated that honey improves lipid anomalies in rats and humans suffering from diabetes. The beneficial effects of honey could also be limiting other disorders of metabolism and lessening damaging effects on various organs of the body that ultimately result in diabetic complications. Although there are few studies in the literature which are contrary to the above-depicted discussions regarding the beneficial effects of honey and its use in diabetic disorder. Also the clinical trials or studies on humans (both diabetic and healthy) are rather very sparse. It is anticipated that this book chapter will encourage fundamental investigation intended at explicating the mode of actions by which oligosaccharides present in honey improves antidiabetic/hypoglycemic effects.

Keywords

Metabolic disorders · Diabetes mellitus · Honey · Hypoglycemic

9.1 Introduction

9.1.1 Disorders of Metabolism

Metabolic disorders occur when unusual chemical reactions taking place in the body amend usual metabolic pathways. The metabolic processes take course in our body for getting and making energy from the food we eat and to sustain ourselves. Food that we normally consume is composed of carbohydrates, proteins and lipids. Besides there are also present some other constituents like nucleic acids, minerals, vitamins etc. Digestive juices secreted in our gastrointestinal tract break down the food consumed into simple sugars and acidic compounds which is our body's fuel. Upon complete digestion our body can use this food immediately or can store it in the cells and tissues like liver and muscles in the form of glycogen or fat depending upon the status of the body. Any disorder that occurs in these metabolic reactions may result in the disruption of these processes. When these disorders take place, we may have large quantities of some materials or small quantities of other ones that are needed for staying healthy without any disease. These metabolic disorders have been categorized into different groups. Some metabolic disorders may affect the metabolism of carbohydrates, amino acids, proteins, nucleic acids or lipids. Another group of metabolic disorders involving mitochondrial diseases affects those parts of cells that are involved in the production of the energy needed by the body. The metabolic disorders develop when some organs or cells, such as of liver or pancreas, are affected by some diseases and do not function in a normal way e.g., diabetes. The disorders may also be present hereditarily because of defined inherited gene anomalies which are mostly autosomal recessive (Graef et al. 2008). Metabolic disorders usually are associated with many symptoms like weight loss, lethargy, seizures and jaundice. These symptoms vary with the type of metabolic disorder. The symptoms have been grouped into four categories like acute, late-onset acute,

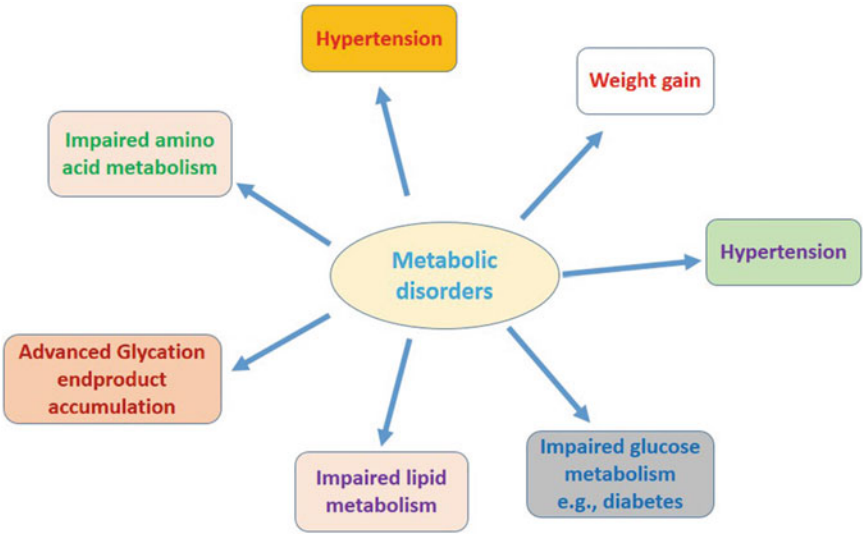

Fig. 9.1 Various disorders associated with metabolism

progressive general and permanent symptoms (Fernandes et al. 2013). In inherited metabolic disorders, because of defective genes, there occurs deficiency of some important enzymes involved in the metabolic processes. These disorders of multiple subtypes are commonly known as inborn errors of metabolism. Some metabolic disorders might also result because of the malfunction of liver or pancreas (Medline Plus 2018). The major groups of metabolic diseases are DNA repair-deficiency disorders, hyperlactatemia, iron metabolism disorders, acid-base imbalance, porphyrias, metabolic brain diseases, lipid metabolism disorders, glucose metabolism disorders, disorders of calcium metabolism, phosphorus metabolism disorders, water-electrolyte imbalance, metabolic syndrome X, malabsorption syndromes, wasting syndrome, mitochondrial diseases, inborn error of metabolism, metabolic skin diseases and proteostasis deficiencies. These disorders are diagnosed by specific screening tools and may be present from birth. If not diagnosed at an early stage, they get missed and get diagnosed at a later stage with the onset of symptoms. The tests available for their diagnoses include specific DNA and blood tests. The microflora residing in the gastrointestinal tract also plays an important role in metabolism with a symbiotic relationship with the host individual. These organisms generally consume undigested food and produce some important by-products for the host organism. Any pathophysiological abnormality in the gut microflora might play a role in metabolic-related obesity (Hur and Lee 2015). These disorders can be screened with the help of routine blood tests, genetic tests, skin tests etc. in newborns and if detected early these can be managed by the management of nutrition. Physicians or dietitians should have the knowledge of the disorder and the genotype of the individual so that they can accordingly plan the treatment that will be most

effective for the individual (Acosta 2010). Figure 9.1 depicts various disorders that are associated with metabolism.

9.1.2 Diabetes Disorder

As per a report in 2017, there were 425 million people who had developed diabetes throughout the world and the occurrence of diabetes has been found to be 8.8% among adults (IDF 2017). Overall, 90% of diabetic cases belong to Type 2 category. Rates have been found to be roughly equal in both genders as indicated by the data. However, males have been found to be more prone to the disease in many populations round the globe (Vos et al. 2012). As per a report of the World Health Organization (WHO), diabetes led to the death of 1.5 million people in 2012, thus making it one of the major causes of death in the world (WHO 2013a, b). As per a report of the International Diabetes Federation (IDF) published in 2017, diabetes caused 4.0 million deaths worldwide and the data were calculated using modelling to evaluate the number of deaths directly or indirectly linked to diabetes. It is also forecasted that globally the number of diabetics might escalate by 48% between 2017 and 2045 (IDF 2017).

Diabetes mellitus or simply diabetes is a metabolic disorder which is generally characterized by a high glucose level in the blood over a longer period of time (WHO 2014a, b). The symptoms of this metabolic disorder mostly include increased thirst, increased hunger and frequent urination. If the disorder is not treated, it can cause more complications which include hyperosmolar hyperglycaemic state, diabetic ketoacidosis or death. Besides, in diabetic patients many long-term complications might also happen over a prolonged period of time including chronic kidney disease, cognitive impairment, stroke, damage to the eyes, foot ulcers, cardiovascular disease and damage to the nerves (Saedi et al. 2016). The disorder mostly manifests itself because of the main reason of the pancreas not producing and secreting enough insulin or the cells of the individual are insensitive towards the insulin secreted by the pancreatic cells (Shoback and Gardner 2011). Diabetes disorder has been categorized into three main subtypes.

Type 1 diabetes: In this subtype of diabetes, the pancreas fails to produce and secrete adequate insulin and the same is due to the loss of beta cells of the pancreas. It was previously also known as insulin-dependent diabetes mellitus (IDDM) or juvenile diabetes. The disease is mainly caused by the loss of beta cells of islets of Langerhans of the pancreas by autoimmunity. The cause of this abnormal autoimmunity is still unknown.

Type 2 diabetes: This disorder begins with resistance to insulin, a condition in which the cells become insensitive to insulin and accordingly give no response to insulin. If the disease continues the insulin deficiency may also develop. It was previously also known as non-insulin-dependent diabetes mellitus (IDDM) or adult-onset diabetes. The disorder mostly arises because of excessive body weight and lack of exercise.

Gestational diabetes: This is the third form of diabetes and is found exclusively in pregnant women who have high blood sugar levels without an earlier history of diabetes (WHO 2013a, b).

Diabetes can be prevented by modifying life style e.g., maintaining normal body weight, avoiding use of tobacco, regular physical exercise, a healthy diet and control of blood pressure. There should be proper eye care and foot care among the people with the disease. Type 1 diabetes is being managed by injecting insulin (WHO 2013a, b) while type 2 is treated by medication with or without the supplementation of insulin. Oral medications and insulin often lead to low blood sugar levels (Rippe and Irwin 2010). Gestational diabetes, which is commonly found in pregnant women usually, resolves of its own after the baby is delivered (Cash 2014).

The typical symptoms shown by a person with untreated diabetes include polyuria (frequent urination), polyphagia (more hunger), polydipsia (more thirst) and weight loss. In type 1 diabetes, the symptoms usually develop rapidly over weeks or months while in in type 2 diabetes the symptoms develop gradually or may be absent till the disease reaches the advanced stage. Diabetes patients also show symptoms of tiredness and weight loss (WHO 2019) besides others which can mark the onset of diabetes although these are not definitive to diabetes. Additionally, the diabetic patients may suffer from fatigue, headache, blurred vision, itchy skin and slow healing of cuts and wounds. Persistent high blood glucose level might lead to the absorption of glucose in the eye lens leading to the changes in it and hampering the normal vision of the person. The condition may also lead to diabetic retinopathy. Besides in diabetics there might occur a condition known as diabetic dermadromes which is characterized by skin rashes (Rockefeller 2015). Persons suffering from type 1 diabetes may also have episodes of diabetic ketoacidosis (DKA), which is a metabolic disorder characterized by vomiting, nausea, abdominal pain and in severe cases a reduced consciousness level and smell of acetone on the breath (Kitabchi et al. 2009).

The different forms of diabetes ultimately escalate the chances of long-term complications. Among these the primary complications which occur because of the damage of the small blood vessels include damage to the nerves, kidneys and eyes. Eye damage, commonly known as diabetic retinopathy, occurs because of the damage to the blood capillaries and vessels supplying to the eye, and this damage can culminate in vision loss and ultimate blindness (WHO 2014a, b). Besides, this disease increases the chances of developing cataracts, glaucoma and other eye-related problems. For the same reason patients with diabetes are recommended to visit an ophthalmologist at least once a year (Medline Plus 2018). Similarly, in diabetics any damage to the kidneys, which is known by diabetic nephropathy, results in urine protein loss, tissue scarring and eventually chronic kidney disease (CKD). These patients require frequent dialysis or permanent kidney transplantation. Also the diabetic patients might suffer from damage to neurons, and the condition is known as diabetic neuropathy which is the most common complication found in diabetic patients (WHO 2014a, b). This condition leads to tingling, pain, altered pain sensation, numbness and can further lead to skin damage.

Table 9.1 Comparative analysis of type 1 and type 2 diabetes (Source: Melmed et al. 2011)

	Diabetes	
Characteristic	Type 1	Type 2
Inception	Sudden	Gradual
Age at inception	Children mostly	Adults mostly
Size of body	Normal or thin	Obese often
Ketoacidosis	Common	Rare
Autoantibodies	Present (usually)	Absent
Insulin produced by body	Absent or low	Normal, increased or decreased
Concordance in identical twins	50%	90%
Incidence	~10%	~90%

Once diabetes was thought of a singular form but today it is a more variable disorder, and patients sometimes have more than one combination of forms (Tuomi et al. 2014). Broadly this disorder is categorized into four variable forms: type 1, type 2, gestational and other specific types (Shoback and Gardner 2011).

9.2 Type 1 Diabetes

In this type of diabetes, the insulin-producing beta cells of the pancreas are lost, which ultimately leads to the insulin deficiency. Type 1 diabetes is further classified as idiopathic or immune-mediated. Mostly this disorder is immune-mediated, where a T-cell-mediated autoimmunity attacks beta cells and destroys them, thus leading to insulin deficiency (Rother 2007). This type of diabetes constitutes about 10% of diabetes mellitus cases in Europe and North America. Mostly the people suffering from this order seem to be healthy and of normal weight when the onset occurs. These patients in the early stages of the disease show normal sensitivity and responsiveness. Due to the frequent occurrence in the children, the disorder was called as juvenile diabetes; however, the majority of the patients living with this disease are adults now (Chiang et al. 2014). The disease can occur at any stage of life. Type 1 diabetes shows inheritance partly linked with multiple genes including HLA genotypes as has been shown by the studies influencing the risk of diabetes.

9.3 Type 2 Diabetes

This type of disorder is characterized mostly by resistance to insulin but there may also be a reduced amount of insulin produced (Shoback and Gardner 2011). The insensitivity towards insulin seems to be because of the defective insulin receptor; however, the exact reasons are not known. The diabetic disorders with unknown reasons have been placed under a separate class. This disorder occurs commonly (WHO 2013a, b). In this type, people show evidence of prediabetic features (impaired blood glucose tolerance or impaired fasting blood glucose) before

developing type 2 diabetes (American Diabetes Association 2017). The progression of this disorder from prediabetes to type 2 diabetes can be reversed or reduced by some lifestyle medications that are known to improve sensitivity towards insulin or decrease the production of glucose by the liver (Carris et al. 2019). There are number of risk factors known that increase the chances of the disease of type 2 diabetes, e.g., stress, obesity (having body mass index (BMI) of more than 30), poor diet, lack of physical activity and urbanization (Melmed et al. 2011). The comparative analysis of type 1 and type 2 diabetes is summarized in Table 9.1.

9.4 Gestational Diabetes

Gestational diabetes mellitus (GDM) is similar to type 2 diabetes in various aspects and has a combination of inadequate insulin and sensitivity to it. It is commonly found in pregnant ladies with an incidence of about 2–10% and usually improves or disappears after delivery of the child. However, once the pregnancy is over, approximately 5–10% of women having GDM suffer from diabetes mellitus, most commonly type 2. This type of diabetes is fully curable; however, it requires careful medical intervention throughout the period of pregnancy. Its management includes dietary modifications, continuous blood glucose examination and in certain cases, insulin intervention is needed (NDIC 2011). If GDM remains untreated it has deleterious effects on the health of the foetus and mother. Risks to which the babies become prone in case of GDM include skeletal muscle defects, macrosomia (increased birth weight) and central nervous system anomalies and congenital heart.

9.5 Other Types

In this category there are a collection of diabetic disorders with few dozens of individual causes. MODY (maturity-onset diabetes of the young) is one of the disorders involving a rare inherited form of diabetes which is autosomal dominant and is because of several single-gene mutations leading to defects in insulin production (National Institute of Diabetes and Digestive and Kidney Diseases 2017). MODY is considerably less prevalent than the above-mentioned three types, accounting for 1–2% of all cases of diabetes. Occurring due to a faulty gene, MODY varies in severity and age at presentation as per the specific gene fault. Hence, 13 different subtypes of MODY are known. The individuals suffering from MODY are able to control the diabetes without insulin intervention (Thanabalasingham and Owen 2011). One form of diabetes arises because the body's receptors do not respond to insulin (although insulin levels are adequate, that is what separates it from type 2 diabetes); this form of diabetes is very rare. Mutations of genes (whether autosomal or mitochondrial) might also lead to faults in beta cells. In some cases abnormal insulin action may also be genetically determined. Damage to the pancreas by any disease has been found to lead to diabetes, e.g., cystic fibrosis and chronic pancreatitis. Besides diseases that are linked with too much

Table 9.2 Diagnostic criteria for diabetes set by WHO (World Health Organization 2006)

Situation	Glucose after 2 h	Glucose at fasting	HbA1c level	
Unit	mmol/L (mg/dL)	mmol/L (mg/dL)	mmol/mol	DCCT %
Normal	<7.8 (<140)	<6.1 (<110)	<42	<6.0
Impaired fasting glycaemia	<7.8 (<140)	≥6.1(≥110) and <7.0(<126)	42–46	6.0–6.4
Impaired glucose tolerance	≥7.8 (≥140)	<7.0 (<126)	42–46	6.0–6.4
Diabetes mellitus	≥11.1 (≥200)	≥7.0 (≥126)	≥48	≥6.5

insulin secretion by antagonistic hormones may cause diabetes, and the same gets resolved once the excess hormone is removed.

The principal hormone that critically regulates the metabolism of glucose is insulin, and this hormone is involved in the regulation of glucose uptake from blood into the majority of cells of the body mostly liver, muscle (except smooth muscle) and adipose tissue. Consequently, the insensitivity of the cell receptors towards it plays a central role in various forms of diabetes (American Diabetes Association 2014). This hormone is used by almost two-thirds of the body's cells to absorb and utilize glucose as fuel from the blood; besides this hormone is also utilized for the conversion of glucose to other related forms required by the cells or for short-term storage. The stored form of glucose is then mobilized by the body during the times of fasting and is utilized for various purposes.

9.6 Characterization of Diabetes

Normally patients suffering from diabetes mellitus have persistent or frequent high levels of glucose in the blood. The disease is identified by following the below-mentioned criteria set by the WHO 1999. The diabetes diagnostic criteria adopted from the criteria set by WHO are also shown in Table 9.2.

- Plasma glucose level in fasting state ≥126 mg/dL (7.0 mmol/L)
- Blood glucose level ≥ 200 mg/dL (11.1 mmol/L) 2 h after the person is given 75 g oral glucose as is given in the oral glucose tolerance test (OGTT)
- Symptoms related to high blood sugar level and blood glucose ≥200 mg/dL (11.1 mmol/L)
- HbA1C (i.e., glycated haemoglobin) ≥ 48 mmol/mol (≥6.5 DCCT %) (Diabetes Care 2009).

For type 1 diabetes there is no recognized protective measure. However, type 2 diabetes that accounts for 85–90% of cases world over can often be delayed or prevented by engaging oneself in physical activity, sustaining normal body weight and consuming balanced diet (WHO 2013a, b). The effective lifestyle modifications

known to be helpful in preventing diabetes include consuming a diet rich in fibre and whole grains and consuming polyunsaturated fats found in nuts, fish and vegetables. Commonly used medications to treat diabetes mostly act by pulling down blood glucose levels through various mechanisms. Consensus is on the fact that when people suffering from diabetes maintain glucose control in the normal range, they do not experience or experience fewer problems like eye-related issues and kidney problems (MacIsaac et al. 2018). Insulin intervention is the only treatment available for type 1 diabetes and is given in the form of regular and NPH insulin or synthetic analogues of insulin. At a later stage, insulin can prove to be helpful in type 2 diabetes also. Some oral medications like metformin are available for type 2 diabetes, while other medications are available in the form of injectables, e.g., GLP-1 agonists. Metformin is being suggested as the drug of choice for type 2 diabetes as it has been shown to decrease mortality due to diabetes. The same drug works by reducing the production of glucose by liver. Besides metformin many other groups of drugs given orally may also reduce blood glucose level in type 2 diabetes. These drugs may upsurge insulin release, e.g., sulfonylureas, reduce absorption of sugar in the intestines, e.g., acarbose, increase the sensitivity of the body towards insulin, e.g., thiazolidinedione and increase elimination of glucose from the body through urine, e.g., SGLT2 inhibitors (Krentz and Bailey 2005).

Currently, the medical industry is shifting more and more on the health-related benefits of products of natural origin, herbs of medicinal importance and also honey for their usage in various ailments. With traditional medicines, including apicultural products like honey, together and in combination with standard medical treatments available, patients with different forms of diabetes can maintain normal insulin levels in the blood and also their overall health status.

9.7 Honey and Its Importance in Diabetes and Metabolic Disorders

Honey is produced by insects known as honeybees. The honeybees are associated with the genus *Apis*, and are known for making and storing of honey and for various ingredients that are actually useful to human beings. A widely appreciated honeybee product is the honey and is derived from the processing of nectar collected from different flowers and thereafter stockpiled in specialized honeycomb cells. Honey is generally promoted for its beneficial activities and has been promoted as a folk tonic since ages. Moreover, it is presented as a therapeutic agent in a clinical set-up (Molan 1999a, b). Honey has been proven very valuable because of its well-established role as an anti-cardiovascular, anti-microbial, anti-diabetic and anti-cancer agent (Alvarez-Suarez et al. 2016).

For many years, there was a myth that honey cannot be added in a diabetic patient's diet as it contains a high content of carbohydrates. As an amalgamated carbohydrate of biological origin, honey is frequently used as a natural sweetening agent and as a traditional therapeutic agent. There are different varieties of honey, and their grades differ in their glycaemic response and some varieties have a low

GI. The difference in glycaemic response has been suggested because of the different floral sources honeybees visit while collecting nectar and the fructose-to-glucose ratio (Bogdanov et al. 2008). With the help of modern or alternative medicine, in the past decade, thorough research had been accomplished so as to overcome the problems as observed in diabetes. For many years, honey is being used as a substitute for sugar and for providing medicinal benefits. In animal as well as in human studies, convincing evidence specifies that honey displays antidiabetic as well as hypoglycaemic effects (Al-Waili 2004a, b; Ahmad et al. 2008; Erejuwa et al. 2010, 2011a, b, c). However, proper mechanisms of anti-diabetic and hypoglycaemic effects are still in infancy and are rather unknown. Honey predominantly is composed of monosaccharides glucose and fructose which are easily absorbed in the small intestine of the GIT (Bogdanov et al. 2008). Honey is made up of more than 200 components with glucose, fructose and water as its main substances. Further, honey also contains many oligosaccharides and polysaccharides that are not digested easily and are not absorbed in the small intestine, but in the large intestine these components get digested by the intestinal microflora residing there (Bogdanov et al. 2008; Astwood et al. 1998; Sanz et al. 2005; Megherbi et al. 2009). Similarly, oligosaccharides and polysaccharides found mostly in plants such as chicory, garlic and onion are highly resistant to gastric juice and cannot be hydrolysed by the digestive enzymes present in the GIT of humans. However these are rich sources of nutrients for microflora present in the large intestine (Blaut 2002; Delzenne 2003; Gibson et al. 2004). Oligosaccharides are commonly viewed as prebiotics, and prebiotics are defined as those ingredients which are not digestible but beneficially affect the host by encouraging the growth or activity of one or limited number of bacteria residing in the colon selectively, thus improving the health of the host. As per the recently updated definition, a prebiotic consumed through diet is a selectively fermented ingredient of the food consumed that marks specific changes in the activity and composition of the microbiota living in the gastrointestinal tract, thus giving benefits to the host's health (Gibson and Roberfroid 1995; Gibson et al. 2010). Among these prebiotics, galacto-oligosaccharides, lactulose and fructo-oligosaccharides are commonly investigated (Gibson et al. 2004). Isomalto-oligosaccharides and xylo-oligosaccharides are similarly other oligosaccharides that have been assessed for their prebiotic effects (Gibson and Roberfroid 1995; van Loo et al. 1999a, b). Certain food substances including meals high in fruits, vegetables and low in fat and other oligosaccharides have been stated to reduce the occurrence of chronic ailments such as diabetes mellitus, hypertension, metabolic syndrome and insulin resistance (Alvarez-Sala Walther et al. 1996; Dall'Agnol and von Poser 2000; Feeney 2004; Heber 2004; Englyst and Englyst 2005; Cani et al. 2007). This chapter brings the latest results that reveal the valuable effects of oligosaccharides in improving diabetes mellitus, insulin resistance and obesity. Because of the similarities of these convincing findings with those of honey, several studies have been undertaken to authenticate the hypothesised findings that oligosaccharides existing in honey might contribute to the beneficial effects related to health and anti-diabetic effects. It is also expected that this will ignite a renewed interest in exploration of these beneficial effects of honey

and will help to encompass the frontiers of this exciting field and supplement the existing literature. Hence, this chapter accredited diverse scientific studies that have been conducted on honey in this regard, validating the beneficial effects of honey in its use in a complex disease like diabetes mellitus in clinical/preclinical studies, and animal and human trials.

9.8 Effect of Fructose and the Hypoglycaemic Effect of Honey

The content of fructose in honey varies from 21 to 43% and the fructose/glucose ratio from 0.4 to 1.6 or even higher. Fructose being the sweetest naturally occurring monosaccharide has a glycaemic index of 19 in comparison to glucose having a glycaemic index of 100 or sucrose which is refined sugar with glycaemic index of 60 (Bahrami et al. 2009; Deibert et al. 2010; Bantle 2009). Many studies have been conducted that have revealed the hypoglycaemic and anti-diabetic effect of the various constituents of honey, but the mechanisms of action of these remain still unclear. It has been suggested that selective mineral ions (copper, vanadium, selenium and zinc), phenolic acids, flavonoids and fructose might have a significant role in the process of benefits to the individuals (Erejuwa et al. 2010; Bahrami et al. 2009; Al-Waili 2003a, b). There is strong indication that fructose tends to reduce blood sugar levels in animal models with diabetes (Kwon et al. 2008; Erejuwa et al. 2012a, b). The mechanisms that seem to be involved in hypoglycaemic and anti-diabetic effects may include reduced degree of intestinal absorption of sugars, prolonged emptying time in stomach and less food consumption (Kellet et al. 2008; Moran and McHugh 1981; Gregory et al. 1989; Thibault 1994; Meirelles et al. 2011). Glucokinase present in hepatocytes has been shown to be activated by fructose and that has a significant part in the glucose uptake and storage in the form of glycogen by the hepatocytes. Glucose present in honey has been found to enhance the absorption of fructose in the intestine and has been noticed to promote its hepatic actions by its improved delivery to the liver cells (Fujisawa et al. 1991; Ushijima et al. 1995). The pancreas is an important organ as far as diabetes is concerned because it secretes two major hormones, insulin and glucagon, that regulate the level of glucose in the blood, and honey has been proposed to protect this important organ against damage and oxidative stress as it is rich in antioxidants. Thus this is an indirect potential mechanism by which honey exerts its antidiabetic effect (Erejuwa et al. 2010). In rat models, fructose administrated in the form of sucrose or alone improved homeostasis of glucose and response to insulin compared to the rats receiving glucose (Prieto et al. 2004). Other related studies demonstrated that in normal or type 2 model diabetic rats, fructose supplementation led to lower levels of blood insulin and blood glucose compared to the rats in which other sugars were administered (Kwon et al. 2008).

Various models of animals have been used for experimental studies depicting the possible glucose-lowering effect of honey, and the frequently used experimental method for prompting type 1 and type 2 diabetes in these models is alloxan and streptozotocin in proper dosages (Srinivasan et al. 2005; Lenzen 2008; Akhtar and

Khan 1989; Fasanmade and Alabi 2008). A study conducted over a period of 6 weeks on non-diabetic healthy rats fed with a diet containing honey depicted promising results. The weight was decreased significantly, but no substantial decrease in glycosylated haemoglobin (HbA1c) or intake of food was perceived (Chepulis 2007). Honey feeding for a longer period of time (i.e., 52 weeks) in Sprague-Dawley rats brought a noteworthy reduction of glycated haemoglobin levels but the same augmented HDL cholesterol levels (Chepulis and Starkey 2008). In rats fed with diet containing sucrose but not sugar, the HDL cholesterol levels were reduced without showing other differences as far as other lipids were concerned. Similarly the rats fed with sugar-free diet containing honey depicted weight gain compared to only sucrose-fed rats. In a study healthy rats were fed with 65/100 g combined fructose and glucose or a diet with honey for 2 weeks and in these rats it was observed that the fructose level in blood, vitamin E in serum and vitamin E/triglycerides in serum increased while blood glucose level remained unaffected and content of triglycerides reduced (Busseroles et al. 2002). In another study conducted by Nemoseck et al., healthy rats were given a diet containing 20% honey for 33 consecutive days. The study depicted significant reduction of leptin content, triglycerides, food/energy intake, epididymal fat weight and body weight but there was not any significant decrease in the glucose level, C-reactive proteins, total cholesterol and adiponectin. This trial depicted that feeding must be used for longer periods so as to get substantial results (Nemoseck et al. 2011a, b). There were no substantial differences in body weight or fasting blood sugar level in rats fed with honey and as honey was proved to have a glucose-lowering effect in healthy animals, the similar helpful effect was witnessed in prompted diabetic models (Erejuwa et al. 2010). There is an important observation with regard to honey and diabetes that in induced diabetic models, honey augments the effect mediated by anti-hyperglycaemic and anti-diabetic drugs (Erejuwa et al. 2010, 2011a, b, c). Alloxan-induced diabetic rabbits were used in one of the experiments, and to these rabbits, three kinds of sweeteners were given along with the diet. Unadulterated honey of *Apis dorsata* and *Apis florea* and adulterated honey were fed to these rabbits and a dose-dependent rise in the blood sugar level was registered in these rabbits (Akhtar and Khan 1989). In other studies, diabetes was induced in rats with the help of alloxan. The diabetic rats were fed with honey and the healthy rats were fed with fructose, and it was observed that the glucose level in blood decreased significantly in diabetic rats and insignificantly in fructose-fed rats (Fasanmade and Alabi 2008). Similar results were again seen in diabetic rats fed with honey which reduced hyperglycaemia (Erejuwa et al. 2009, 2010, 2011a, b, c). Similar to the previous findings of oligosaccharides on insulin level, diabetic rats when given a treatment with honey augmented blood insulin level, and the mass of beta-cell improved (Erejuwa et al. 2011a, b, c). When the healthy and induced type 2 rats were supplemented with honey, there was less body weight gain and reduced food intake (Nemoseck et al. 2011a, b; Bahrami et al. 2009). Alike to the possible effect of oligosaccharides as hepatoprotective agents, honey supplementation also showed hepatoprotective function in STZ-induced diabetic rats (Erejuwa et al. 2011a, b, c). Honey administration was also found to ameliorate aflatoxin-induced

histopathological changes in mice (Ezz El-Arab et al. 2006) besides intravenous honey injection also prompted a defensive effect against carbon tetrachloride (CCl4) induced hepatic damage (Al-Waili 2003a, b, c).

9.9 Effect of Honey and Other Sugars on Diabetes: Human Clinical Trials

Carbohydrates, proteins, lipids, water, minerals, vitamins, bioactive compounds and amino acids are required by humans for all biological activities and these compounds are obtained from the diet. The diet of humans must have all types of ingredients required for the metabolic alterations and for life support. Consumption of each of these ingredients and nutrients is the key factor for maintaining a healthy life in general. As a starting point different kinds of diseases have distortions in the metabolic pathways because of the absence or superfluous amount of one or more nutrients. Diabetes mellitus, as already discussed, manifests in the form of high blood glucose level in blood due to insufficient or no insulin production in the body. Studies on experimental animals proposed the helpful properties of honey as a diet supplement, and the studies also depicted control of diabetes mellitus and additional complications in animal models. However, the studies or trials on human beings both healthy and diabetic are rather sparse. Still there are some published studies that depict the beneficial properties of honey in both diabetic and healthy subjects (Yaghoobi et al. 2008a, b; Bahrami et al. 2009; Ahmad et al. 2008; Abdulrahman et al. 2011; Al-Waili 2003a, b, 2004a, b; Agarwal et al. 2007b). The antioxidant properties of honey are very significant as oxidative stress is associated and chiefly responsible for diabetes disorder (Gheldorf et al. 2003). In a study conducted on healthy, diabetics or patients with hyper-triglycerides in blood depicted promising results when their diet was supplemented with honey compared to the diet with only sucrose and dextrose (Al-Waili 2004a, b). Consequently, the profile of lipids was improved, elevated and normal C reactive protein was reduced. Besides, triacylglycerol and homocysteine value were also reduced in hyper-triglyceridemic patients. In diabetic patients, honey produced significantly lower rise of blood glucose level (BGL) compared with dextrose. Honey boosted insulin in comparison to sucrose, and after consumption at different times, it decreased blood homocysteine, CRP and lipids in normal subjects. The final conclusion was that honey led to lower elevation of PGL in diabetic patients compared to sucrose and dextrose.

Sugar being a polished and refined product is obtained from various natural sources and is processed through technological processes which result in the form of a pure substance called sucrose, which is highly used in various food industries and in modern life. Comparatively honey being also a natural product with sweet taste has a multifarious composition compared to sucrose and is supposed to have a lower energetic value and glycaemic index. The exact chemical composition of refined sugars can be easily stated compared to honey where many aspects have to be considered while stating its composition. Geographical and botanical origins are very helpful in determining the specific properties and composition of various types

of honey. Although the exact mode of action by which honey exerts its helpful effects on the level of blood glucose is not so clear, however from various comparative studies some assumptions depicting the significance of fructose present in honey have been drawn. Fructose in honey has been found to stimulate an important enzyme, glucokinase, in liver cells, which plays a significant part in the glucose uptake and its storage as glycogen by the hepatocytes of liver, thus supporting for its hypoglycaemic effect (Van Schaftingen and Vandercammen 1989).

In a study conducted at Erciyes University, Kayseri, Turkey, 20 healthy volunteers were taken. 50 g of pure glucose dissolved in 250 mL water was given to each of them besides honey in an amount corresponding to 50 g of glucose (calculated according to the physicochemical analysis of honey) was also given to the them. After consumption, on the next morning blood samples (capillary blood samples) were collected from the finger of the volunteers and again after every 15 min and after second consumption of sugars on the next day, until 120 min. Serum insulin and glucose level reduced after 2 h of intake of honey, and C peptide level slightly augmented 2 h after intake of honey. This trial validated that how various honey types of honey with diverse GI values influenced in a different manner on various parameters usually calculated and taken for analysing diabetes control (Soylu et al. 2015). In another related study conducted at Isfahan University of Medical Science, Iran, 60 healthy and normal subjects whose age ranged between 18 and 30 years were enrolled. The subjects received 80 g sucrose dissolved in 250 mL water and 80 g of honey once a day for consecutive 6 weeks. FBS (fasting blood sugar), SBP (systolic blood pressure) and DBP (diastolic blood pressure) were analysed from each participating subject at the start and at the end of the trial. Insignificant change was recorded in SBP and DBP in both groups at the start and at the end of the trial, but FBS enumerated a substantial reduction in the group fed with honey in comparison to the group receiving sucrose at the end of the study (Rasad et al. 2014). As already mentioned earlier, different studies depict that honey intake decreases the weight of the body and blood glucose level in diabetic patients and healthy subjects in comparison to sugar intake. A study conducted on type 2 diabetic patients who consumed natural honey showed that body weight was reduced and so were blood glucose and blood lipids. The study included patients suffering from type 2 diabetes, having fasting blood glucose of 110–220 mg/dL, and were on the same oral hypoglycaemic drug but not on insulin treatment. The investigational group ($n = 25$) received natural honey for 8 consecutive weeks while as the control group ($n = 23$) did not receive natural honey or any other sweetener. The fasting blood sugar levels and weight of the body was measured after every 2 weeks, and continuous decline was registered (Bahrami et al. 2009). Scientific studies also demonstrated that oligosaccharides and fructose in the honey contributed to the hypoglycaemic effect shown by honey. Additionally honey consumption improved other disorders related to metabolism and diabetes such as reduced levels of HbA1c (glycosylated haemoglobin) and hepatic transaminases and increased HDL cholesterol. The same was in addition to lowering hyperglycaemia and oxidative stress (Erejuwa et al. 2012a, b; Bahrami et al. 2009). Further, as stated for oligosaccharides (Yamashita et al. 1984; Cani et al. 2006a, b), honey supplementation showed similar

results and reduced glycaemic response in the postprandial state in type 1 diabetic patients and healthy volunteers (Samanta et al. 1985). Similarly, decline in blood glucose level was testified in subjects with impairment in glucose tolerance (Agrawal et al. 2007a, b). Likewise, as predicted for oligosaccharides (Luo et al. 1996, 2000; Alles et al. 1999; Causey et al. 2000), some studies did not show any substantial effect of honey on the blood glucose levels or on the insulin levels in patients suffering from diabetes (Bornet et al. 1985; Katsilambros et al. 1988; Nemoseck et al. 2011a, b). Bahrami and his co-workers depicted that honey-supplemented diet in patients suffering from type 2 diabetes did not produce any substantial effect on fasting blood sugar levels. However, it increased levels of HbA1c (glycosylated haemoglobin) (Bahrami et al. 2009). Supporting to data depicting the effect of oligosaccharides on lipid metabolism (Fiordaliso et al. 1995; Daubioul et al. 2000), honey supplemented diet substantially decreased LDL cholesterol, TG and TC, while it augmented HDL cholesterol in patients suffering from type 2 diabetes or in healthy persons (Bahrami et al. 2009; Yaghoobi et al. 2008a, b). In obese or overweight individuals, honey-supplemented diet slightly reduced or did not increase the weight of the body (Yaghoobi et al. 2008a, b). Similar results depicting loss of weight or decreased intake of food was reported as a consequence of oligofructose-supplemented diet in obese, overweight or healthy individuals (Delmee et al. 2006; Cani et al. 2006a, b; Parnell and Reimer 2009).

9.10 Honey and Its Role in Diabetic Wound Healing

In addition to the beneficial health effects of consuming or ingesting natural honey in diabetes and other metabolic disorders, honey could also find important beneficial effects in managing wounds of diabetic patients (Alam et al. 2014). These wounds found in diabetic patients are not like classic wounds; they heal very slowly or sometimes these wounds do not heal at all, leading to problems where conventional medicines do not have any effect. Honey has been used as a medicine for the healing of wounds since time immemorial while its use in diabetic wound management is recent. Patients suffering from diabetes sometimes suffer from many other complications like vascular problems, arterial disease, foot complications and ulcerations (Singh et al. 2005; Lavery et al. 2007). Although diabetic wounds are to some extent similar to wounds of normal patients, the healing ability in diabetic wounds is problematic and slow besides the medical expenditures are very high. Honey has been found to be a possible choice to be used in diabetic wounds because of it being natural, its large availability and its inexpensive nature. Honey, when diluted with water and applied at the wound site, forms hypochlorite anions and hydroxyl radicals. Also at the site of the wound the antioxidants found in honey mostly act by two dissimilar mechanisms: first, these fight against microbes and reduce the infection in the wound and second, these antioxidants decrease ROS (reactive oxygen species) generation and inflammation of the wound, thus helping in the healing process (Cooper et al. 1999; Estevinho et al. 2008; Mathews and

Binnington 2002). Also the antibacterial activity of honey is attributed to its osmotic effect, acidic pH, nitric oxide and hydrogen peroxide. The presence of nitric oxide and the production of NO by honey in various body fluids increase the rate of healing (Al-Waili and Saleeb 2003). Scar formation, inflammation control, debridement and wound odour are very important as far as diabetic wound management is concerned (Alam et al. 2014). The slow healing of diabetic wounds is attributed to peripheral neuropathy and peripheral arterial diseases that are associated with diabetes where the blood vessels tend to shrink and, ultimately, decrease the circulation of blood to the respective areas. The nerves become damaged and more vulnerable to injury as these do not receive enough nutrients via blood. The tissue growth stimulus is induced when honey is used because of the chemical composition, the presence of vitamins, assimilable sugars, phenolics and amino acids, which increased the supply of nutrients and oxygen in the diabetic wound area (Molan 1999a, b; Molan 2002). Application of honey decreased the intensity of ulcer pain and reduced the ulcer size along with deodorization at the wound site. Besides it reduced the healing time of wounds and without any side effects. A recent study has brought forth a new indication demonstrating the beneficial properties of Manuka honey in wound management, and the outcomes reported by the researchers in this study depicted that honey improved the responsiveness against oxidative damage and it also stimulated proliferation of cells. This could better help to understand how Manuka honey developed its effect on the healing of wounds (Alvarez-Suarez et al. 2016). Although there are some guidelines for proper honey applications in wound management, it is stated that natural unheated honey should be usually used in treatments and it should be stored in amber-colored glass bottles and in cool places. Different standardised medical grade honey have been formulated with antibacterial activity which find their use in wound management and treatment, e.g., Woundcare 18+ (Comvita: Te Puke, New Zealand), Medihoney (Capilano: Richmonds, Queensland, Australia) and Apiban (Apimed: Cambridge, New Zealand) (Molan 2002). If not available, any dark coloured honey with extraordinary antimicrobial activity may be used.

Based on the findings we hypothesize that saccharides, mostly oligo, present in honey might be contributing to the anti-glycaemic/anti-diabetic effects and may also be involved in reducing other metabolic disorders. Considering the availability of few studies and dearth of large-scale data, especially large controlled randomized clinical trials, we strongly have confidence in that future research on honey should not only be limited to exploring the therapeutic and beneficial potential alone but should also include trials and studies designed at revealing the composite mechanisms of action mediated by honey. It is anticipated that this chapter will encourage fundamental investigation intended at explicating the mode of actions by which oligosaccharides present in honey improves anti-diabetic/hypoglycaemic effects. This will definitely help to further encompass the boundaries of facts with regard to the benefits of honey.

9.11 Summary

Metabolic disorders occur when unusual chemical reactions taking place in the body amend usual metabolic pathways. Diabetes mellitus or simply diabetes is a metabolic disorder which is generally characterized by a high glucose level in blood over a longer period of time. In type 1 diabetes, the pancreas fails to produce and secrete adequate insulin and this is due to the loss of beta cells of the pancreas. Type 2 diabetes begins with resistance to insulin, a condition in which the cells become insensitive to insulin and accordingly give no response to insulin. Gestational diabetes mellitus is similar to type 2 diabetes in various aspects and is having a combination of inadequate insulin and sensitivity to it. It is commonly found in pregnant ladies. For many years, honey is being used as a substitute for sugar and for providing medicinal benefits. In animal as well as in human studies, convincing evidence specifies that honey displays anti-diabetic as well as hypoglycaemic effects. Additionally honey consumption improved other disorders related to metabolism and diabetes such as reduced levels of HbA1c (glycosylated haemoglobin) and hepatic transaminases and increased HDL cholesterol. The same was in addition to lowering hyperglycaemia and oxidative stress. Besides depicting a hypoglycaemic effect, research has indicated that honey improves lipid anomalies in rats and and diabetes in humans. The beneficial effects of honey could also be limiting other disorders of metabolism and lessening the damaging effects on various organs of the body that ultimately result in diabetic complications. There are few studies in the literature which are contrary to the above-depicted discussions regarding the beneficial effects of honey and its use in diabetic disorder. Also the clinical trials or studies on humans both diabetic and healthy are rather very sparse. It is anticipated that this chapter will encourage fundamental investigation intended at explicating the mode of actions by which oligosaccharides present in honey improves anti-diabetic/hypoglycaemic effects.

References

Abdulrahman M, El-Hefnawy M, Hussein R, El-Goud AA (2011) The glycemic and peak incremental indices of honey, sucrose and glucose in patients with type 1 diabetes mellitus: effects on C-peptide level—a pilot study. Acta Diabetol 48(2):89–94

Acosta P (2010) Nutrition management of patients with inherited metabolic disorders. Jones and Bartlett, Sudbury MA, p 2. ISBN 9781449633127

Agarwal OP, Pachauri A, Yadav H, Urmila J, Goswamy HM, Chapperwal A, Bisen PS, Prasad GB (2007b) Subjects with impaired glucose tolerance exhibit a high degree of tolerance to honey. J Med Food 10:473–478

Agrawal OP, Pachauri A, Yadav H et al (2007a) Subjects with impaired glucose tolerance exhibit a high degree of tolerance to honey. J Med Food 10(3):473–478

Ahmad A, Azim MK, Mesaik MA, Khan RA (2008) Natural honey modulates physiological glycemic response compared to simulated honey and D-glucose. J Food Sci 73(7):H165–H167

Akhtar MS, Khan MS (1989) Glycemic responses to three different types of honeys given to normal and alloxandiabetic rabbits. J Pak Med Assoc 39(4):107–113

Alam F, Islam A, Gan SH, Khalil I (2014) Honey: a potential therapeutic agent for managing diabetic wounds. Evid Based Complement Alternat Med 2014:169130, 16 pages

Alles MS, de Roos NM, Bakx JC, van de Lisdonk E, Zock PL, Hautvast GA (1999) Consumption of fructooligosaccharides does not favorably affect blood glucose and serum lipid concentrations in patients with type 2 diabetes. Am J Clin Nutr 69:64–69. Molecules 17:263

Alvarez-Sala Walther LA, Millan Nunez-Cortes J, de Oya OM (1996) The Mediterranean diet in Spain. Legend or reality? (II). Other elements in the Mediterranean diet: vegetables and fruits, fish. Evolution of the diet and cardiovascular diseases in Spain in the last decades. Rev Clin Esp 196:636–646

Alvarez-Suarez JM, Giampieri F, Cordero M et al (2016) Activation of AMPK/Nrf2 signalling by Manuka honey protects human dermal fibroblasts against oxidative damage by improving antioxidant response and mitochondrial function promoting wound healing. J Funct Foods 25:38–49

Al-Waili NS (2004b) Natural honey lowers plasma glucose, C-reactive protein, homocysteine, and blood lipids in healthy, diabetic, and hyperlipidemic subjects: comparison with dextrose and sucrose. J Med Food 7:100–107

Al-Waili N (2003a) Intrapulmonary administration of natural honey solution, hyperosmolar dextrose or hypo-osmolar distilled water to normal individuals and to patients with type 2 diabetes mellitus or hypertension: their effects on blood glucose level, plasma insulin and C-peptide, blood pressure and peaked expiratory flow rate. Eur J Med Res 8(7):295–303

Al-Waili NS (2003b) Identification of nitric oxide metabolites in various honeys: effects of intravenous honey on plasma and urinary nitric oxide metabolites concentration. J Med Food 6(4):359–364

Al-Waili NS (2003c) Intravenous and intrapulmonary administration of honey solution to healthy sheep: effects on blood sugar, renal and liver function tests, bone marrow function, lipid profile, and carbon tetrachloride-induced liver injury. J Med Food 6:231–247

Al-Waili NS (2004a) Investigating the antimicrobial activity of natural honey and its effects on the pathogenic bacterial infections of surgical wounds and conjunctiva. J Med Food 7(2):210–222

Al-Waili NS, Saleeb N (2003) Honey increased nitric oxide and product in saliva of healthy volunteers in FASEB Conference, San Diego, pp 11–15

American Diabetes Association (2014) Insulin basics. Archived from the original on 14 February 2014. Accessed 24 Apr 2014

American Diabetes Association (2017) Classification and diagnosis of diabetes. Diabetes Care 40 (Suppl 1):S11–S24. https://doi.org/10.2337/dc17-S005

Astwood K, Lee B, Manley-Harris M (1998) Oligosaccharides in New Zealand honeydew honey. J Agric Food Chem 46:4958–4962

Bahrami M, Ataie-Jafari A, Hosseini S, Foruzanfar MH, Rahmani M, Pajouhi M (2009) Effects of natural honey consumption in diabetic patients: an 8-week randomized clinical trial. Int J Food Sci Nutr 60(7):618–626

Bantle JP (2009) Dietary fructose and metabolic syndrome and diabetes. J Nutr 139(6):1263S–1268S

Blaut M (2002) Relationship of prebiotics and food to intestinal microflora. Eur J Nutr 41:111–116

Bogdanov S, Jurendic T, Sieber R, Gallmann P (2008) Honey for nutrition and health: a review. J Am Coll Nutr 27:677–689

Bornet F, Haardt MJ, Costagliola D, Blayo A, Slama G (1985) Sucrose or honey at breakfast have no additional acute hyperglycaemic effect over an isoglucidic amount of bread in type 2 diabetic patients. Diabetologia 28:213–217

Busseroles J, Gueux E, Rock E (2002) Substituting honey for refined carbohydrates protects rats from hypertriglyceridemic and pro-oxidative effects of fructose. J Nutr 132:3379–3382

Cani PD, Joly E, Horsmans Y, Delzenne NM (2006b) Oligofructose promotes satiety in healthy human: a pilot study. Eur J Clin Nutr 60:567–572

Cani PD, Knauf C, Iglesias MA, Drucker DJ, Delzenne NM, Burcelin R (2006a) Improvement of glucose tolerance and hepatic insulin sensitivity by oligofructose requires a functional glucagon-like peptide 1 receptor. Diabetes 55:1484–1490

Cani PD, Neyrinck AM, Fava F, Knauf C, Burcelin RG, Tuohy KM, Gibson GR, Delzenne NM (2007) Selective increases of bifidobacteria in gut microflora improve high-fat-diet induced diabetes in mice through a mechanism associated with endotoxaemia. Diabetologia 50:2374–2383

Carris NW, Magness RR, Labovitz AJ (2019) Prevention of diabetes mellitus in patients with prediabetes. Am J Cardiol 123(3):507–512. https://doi.org/10.1016/j.amjcard.2018.10.032. PMC6350898

Cash J (2014) Family practice guidelines, 3rd edn. Springer, New York, p 396. ISBN 978-0-8261-6875-7. Archived from the original on 31 October 2015

Causey JL, Feirtag JM, Gallagher DD, Tungland BC, Slavin JL (2000) Effects of dietary inulin on serum lipids, blood glucose, and the gastrointestinal environment in hypercholesterolemic men. Nutr Res 20:191–201

Chepulis L, Starkey N (2008) The long-term effects of feeding honey compared with sucrose and a sugar-free diet on weight gain, lipid profiles and DEXA measurements in rats. J Food Sci 73(1): H1–H7

Chepulis LM (2007) The effect of honey compared to sucrose, mixed sugars and a sugar free diet on weight gain in young rats. J Food Sci 72(3):S224–S229

Chiang JL, Kirkman MS, Laffel LM, Peters AL (2014) Type 1 diabetes through the life span: a position statement of the American Diabetes Association. Diabetes Care 37(7):2034–2054. https://doi.org/10.2337/dc14-1140. PMC 5865481

Cooper RA, Molan PC, Harding KG (1999) Antibacterial activity of honey against strains of Staphylococcus aureus from infected wounds. J R Soc Med 92(6):283–285

Dall'Agnol R, von Poser GL (2000) The use of complex polysaccharides in the management of metabolic diseases: the case of Solanum lycocarpum fruits. J Ethnopharmacol 71:337–341

Daubioul CA, Taper HS, De Wispelaere LD, Delzenne NM (2000) Dietary oligofructose lessens hepatic steatosis, but does not prevent hypertriglyceridemia in obese zucker rats. J Nutr 130:1314–1319

Deibert P, KOnig D, Kloock B, Groenefeld M, Berg A (2010) Glycaemic and insulinaemic properties of some German honey varieties. Eur J Clin Nutr 64(7):762–764

Delmee E, Cani PD, Gual G, Knauf C, Burcelin R, Maton N, Delzenne NM (2006) Relation between colonic proglucagon expression and metabolic response to oligofructose in high fat diet-fed mice. Life Sci 79:1007–1013

Delzenne NM (2003) Oligosaccharides: state of the art. Proc Nutr Soc 62:177–182

Diabetes Care (2009) Diabetes Care. 33:S3. doi:https://doi.org/10.2337/dc10-S003. PMC 2797388. Archived from the original on 13 January 2010. Accessed 29 Jan 2010

Englyst KN, Englyst HN (2005) Carbohydrate bioavailability. Br J Nutr 94:1–11

Erejuwa OO, Gurtu S, Sulaiman SA, Ab Wahab MS, Sirajudeen KN, Salleh MS (2010) Hypoglycemic and antioxidant effects of honey supplementation in streptozotocin-induced diabetic rats. Int J Vitam Nutr Res 80(1):74–82

Erejuwa OO, Sulaiman SA, Wahab MS (2012a) Fructose might contribute to the hypoglycemic effect of honey. Molecules 17(12):1900–1915

Erejuwa OO, Sulaiman SA, Wahab MS (2012b) Hepatoprotective effect of Tualang honey supplementation in streptozotocin-induced diabetic rats. Int J Appl Res Nat Prod 4:37–41

Erejuwa OO, Sulaiman SA, Wahab MS, Salam SKN, Salleh S, Gurtu S (2011a) Comparison of antioxidant effects of honey, glibenclamide, metformin and their combinations in the kidneys of streptozotocin-induced diabetic rats. Int J Mol Sci 12(12):829–843

Erejuwa OO, Sulaiman SA, Wahab MS, Sirajudeen KN, Salleh MS, Gurtu S (2011b) Hepatoprotective effect of Tualang honey supplementation in streptozotocin-induced diabetic rats. Int J Appl Res Nat Prod 4:37–41

Erejuwa OO, Sulaiman SA, Wahab MS, Sirajudeen KNS, Salleh MS, Gurtu S (2011c) Glibenclamide or metformin combined with honey improves glycemic control in streptozotocin-induced diabetic rats. Int J Biol Sci 7:244–252

Erejuwa OO, Sulaiman SA, Wahab MS, Sirajudeen KNS, Salzihan MS (2009) Effects of Malaysian Tualang honey supplementation on glycemia, free radical scavenging enzymes and markers of oxidative stress in kidneys of normal and streptozotocin-induced diabetic rats. Int J Cardiol 137: S45

Estevinho L, Pereira AP, Moreira L, Dias LG, Pereira E (2008) Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. Food Chem Toxicol 46 (12):3774–3779

Ezz El-Arab AM, Girgis SM, Hegazy EM, Abd El-Khalek AB (2006) Effect of dietary honey on intestinal microflora and toxicity of mycotoxins in mice. BMC Complement Altern Med 6:6. https://doi.org/10.1186/1472-6882-6-6

Fasanmade AA, Alabi OT (2008) Differential effects of honey on selected variables in alloxan-induced and fructose-induced diabetic rats. Afr J Biomed Res 11(20):191–196

Feeney MJ (2004) Fruits and the prevention of lifestyle-related diseases. Clin Exp Pharmacol Physiol 31:S11–S13

Fernandes J, Saudubray J, Berghe G (2013) Inborn metabolic diseases: diagnosis and treatment. Springer Science & Business Media, Berlin Heidelberg, p 4. ISBN 9783662031476

Fiordaliso M, Kok N, Desager JP, Goethals F, Deboyser D, Roberfroid M, Delzenne N (1995) Dietary oligofructose lowers triglycerides, phospholipids and cholesterol in serum and very low density lipoproteins of rats. Lipids 30:163–167

Fujisawa T, Riby J, Kretchmer N (1991) Intestinal absorption of fructose in the rat. Gastroenterology 101(2):360–367

Gheldorf N, Wang XH, Engeseth NJ (2003) Buckwheat honey increases serum antioxidant capacity in humans. J Agric Food Chem 51(5):1500–1505

Gibson GR, Probert HM, Loo JV, Rastall RA, Roberfroid MB (2004) Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutr Res Rev 17:259–275

Gibson GR & Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 125:1401–1412

Gibson GR, Scott KP, Rastall RA, Tuohy KM, Hotchkiss A, Dubert-Ferrandon A, Gareau M, Murphy EF, Saulnier D, Loh G et al (2010) Dietary prebiotics: current status and new definition. J Food Sci Technol 7:1–19

Graef JW, Wolfsdorf JI, Greenes DS (2008) Manual of pediatric therapeutics. Lippincott Williams & Wilkins, New Delhi. ISBN 9780781771665. Metabolic Disorders: Medline Plus. www.nlm.nih.gov

Gregory PC, McFadyen M, Rayner DV (1989) Relation between gastric emptying and short-term regulation of food intake in the pig. Physiol Behav 45(4):677–683

Heber D (2004) Vegetables, fruits and phytoestrogens in the prevention of diseases. J Postgrad Med 50:145–149

Hur KY & Lee MS (2015) Gut microbiota and metabolic disorders. Diabet Metab J 39(3):198–203. https://doi.org/10.4093/dmj.2015.39.3.198. ISSN 2233-6079. PMC 4483604

International Diabetes Federation (2017) IDF diabetes Atlas, 8th edn. International Diabetes Federation, Brussels

Katsilambros NL, Philippides P, Touliatou A, Georgakopoulos K, Kofotzouli L, Frangaki D, Siskoudis P, Marangos M, Sfikakis P (1988) Metabolic effects of honey (alone or combined with other foods) in type II diabetics. Acta Diabetol Lat 25:197–203

Kellet GL, Brot-Laroche E, Mace OJ (2008) Sugar absorption in the intestine: the role of GLUT2. Ann Rev Nutr 28(1):35–54

Kitabchi AE, Umpierrez GE, Miles JM, Fisher JN (2009) Hyperglycemic crises in adult patients with diabetes. Diabetes Care 32(7):1335–1343. https://doi.org/10.2337/dc09-9032. PMC 2699725. Archived from the original on 2016-06-25

Krentz AJ, Bailey CJ (2005) Oral antidiabetic agents: current role in type 2 diabetes mellitus. Drugs 65(3):385–411. https://doi.org/10.2165/00003495-200565030-00005
Kwon S, Kim YJ, Kim MK (2008) Effect of fructose or sucrose feeding with different levels on oral glucose tolerance test in normal and type 2 diabetic rats. Nutr Res Pract 2(4):252–258
Lavery LA, Higgins KR, Lanctot DR (2007) Preventing diabetic foot ulcer recurrence in high-risk patients: use of temperature monitoring as a self-assessment tool. Diabetes Care 30(1):14–20
Lenzen S (2008) The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia 51 (2):216–226
Luo J, Rizkalla SW, Alamowitch C, Boussairi A, Blayo A, Barry JL, Laffitte A, Guyon F, Bornet FR, Slama G (1996) Chronic consumption of short-chain fructooligosaccharides by healthy subjects decreased basal hepatic glucose production but had no effect on insulin-stimulated glucose metabolism. Am J Clin Nutr 63:939–945
Luo J, van Yperselle M, Rizkalla SW, Rossi F, Bornet FR, Slama G (2000) Chronic consumption of short-chain fructooligosaccharides does not affect basal hepatic glucose production or insulin resistance in type 2 diabetics. J Nutr 130:1572–1577
MacIsaac RJ, Jerums G, Ekinci EI (2018) Glycemic control as primary prevention for diabetic kidney disease. Adv Chronic Kidney Dis 25(2):141–148. https://doi.org/10.1053/j.ackd.2017.11.003
Mathews KA, Binnington AG (2002) Wound management using honey. Compend Contin Educ Pract Vet 24(1):53–59
Medline Plus (2018) Diabetes—eye care: Medline Plus Medical Encyclopedia. medlineplus.gov. Accessed 27 Mar 2018
Megherbi M, Herbreteau B, Faure R, Salvador A (2009) Polysaccharides as a marker for detection of corn sugar syrup addition in honey. J Agric Food Chem 57:2105–2111
Meirelles CJ, Oliveira LA, Jordao AA, Navarro AM (2011) Metabolic effects of the ingestion of different fructose sources in rats. Exp Clin Endocrinol Diabetes 119(4):218–220
Melmed S, Polonsky K, Larsen PR, Kronenberg H (2011) Williams textbook of endocrinology, 12th edn. Elsevier/Saunders, Philadelphia, pp 1371–1435. ISBN 978-1-4377-0324-5
Molan PC (1999a) The role of honey in the management of wounds. J Wound Care 8(8):415–418
Molan PC (1999b) Why honey is effective as a medicine. Its use in modern medicine. Bee World 80:80–92
Molan PC (2002) Re-introducing honey in the management of wounds and ulcers-theory and practice. Ostomy Wound Manage 48(11):28–40
Moran TH, McHugh PR (1981) Distinction among three sugars in their effects on gastric emptying and satiety. Am J Physiol Regul Integr Comp Physiol 241(1):R25–R30
National Diabetes Clearinghouse (NDIC) (2011) National Diabetes Statistics 2011. U.S. Department of Health and Human Services. Archived from the original on 17 April 2014. Accessed 22 Apr 2014
Nemoseck TM, Carmody EG, Furchner-Evanson A (2011a) Honey promotes lower weight gain, adiposity, and triglycerides than sucrose in rats. Nutr Res 31(1):55–60
Nemoseck TM, Carmody EG, Furchner-Evanson A, Gleason M, Li A, Potter H, Rezende LM, Lane KJ, Kern M (2011b) Honey promotes lower weight gain, adiposity, and triglycerides than sucrose in rats. Nutr Res 31:55–60
Parnell JA& Reimer RA (2009) Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults. Am J Clin Nutr 89:1751–1759
Prieto PG, Cancelas J, Villanueva-Peñacarrillo ML, Valverde I (2004) Plasma D-glucose, D-fructose and insulin responses after oral administration of D-glucose, D-fructose and sucrose to normal rats. J Am Coll Nutr 23(5):414–419
Rasad H, Dashtabi A, Khansari M et al (2014) The effect of honey consumption compared with sucrose on blood pressure and fasting blood glucose in healthy young subjects. Global J Med Res Stud 1(4):117–121
Rippe RS, Irwin JM (eds) (2010) Manual of intensive care medicine, 5th edn. Wolters Kluwer Health/Lippincott Williams & Wilkins, Philadelphia, p 549. ISBN 978-0-7817-9992-8

Rockefeller JD (2015) Diabetes: symptoms, causes, treatment and prevention. CreateSpace Publishing, Charleston, SC. ISBN 978-1-5146-0305-5
Rother KI (2007) Diabetes treatment—bridging the divide. N Engl J Med 356(15):1499–1501. https://doi.org/10.1056/NEJMp078030. PMC 4152979
Saedi E, Gheini MR, Faiz F, Arami MA (2016) Diabetes mellitus and cognitive impairments. World J Diabetes 7(17):412–422. https://doi.org/10.4239/wjd.v7.i17.412. PMC 5027005
Samanta A, Burden AC, Jones GR (1985) Plasma glucose responses to glucose, sucrose, and honey in patients with diabetes mellitus: an analysis of glycaemic and peak incremental indices. Diabet Med 2:371–373
Sanz ML, Polemis N, Morales V, Corzo N, Drakoularakou A, Gibson GR, Rastall RA (2005) In vitro investigation into the potential prebiotic activity of honey oligosaccharides. J Agric Food Chem 53:2914–2921
Shoback DG, Gardner D (eds) (2011) Chapter 17. Greenspan's basic & clinical endocrinology, 9th edn. McGraw-Hill Medical, New York. ISBN 978-0-07-162243-1
Singh N, Armstrong DG, Lipsky BA (2005) Preventing foot ulcers in patients with diabetes. JAMA 293(2):217–228
Soylu M, Atayoğlu T, Incaç N, Silici S (2015) Glycemic index values of multifloral Turkish honeys and effect of their consumption on glucose metabolism. J Apic Res 54(3):155–162
Srinivasan K, Viswanad B, Asrrat L, Kaul CL, Ramaro P (2005) Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. Pharmacol Res 52(4):313–320
Thanabalasingham G, Owen KR (2011) Diagnosis and management of maturity onset diabetes of the young (MODY). BMJ 343:d6044. https://doi.org/10.1136/bmj.d6044
Thibault L (1994) Dietary carbohydrates: effects on self-selection, plasma glucose and insulin and brain indoleaminergic systems in rat. Appetite 23(3):275–286
Tuomi T, Santoro N, Caprio S, Cai M, Weng J, Groop L (2014) The many faces of diabetes: a disease with increasing heterogeneity. Lancet 383(9922):1084–1094. https://doi.org/10.1016/S0140-6736(13)62219-9
Ushijima K, Riby JE, Fujisawa T, Kretchmer N (1995) Absorption of fructose by isolated small intestine of rats is via a specific saturable carrier in the absence of glucose and by the disaccharide-related transport system in the presence of glucose. J Nutr 125(8):2156–2164
Van Loo J, Cummings J, Delzenne N, Englyst H, Franck A, Hopkins M, Kok N, Macfarlane G, Newton D, Quigley M et al (1999a) Functional food properties of non-digestible oligosaccharides: a consensus report from the ENDO project (DGXII AIRII-CT94-1095). Br J Nutr 81:121–132
Van Loo J, Franck A, Roberfroid M (1999b) Functional food properties of non-digestible oligosaccharides. Br J Nutr 82:329
Van Schaftingen E, Vandercammen A (1989) Stimulation of glucose phosphorylation by fructose in isolated rat hepatocytes. FEBS J 179(1):173–177
Vos T, Flaxman AD, Naghavi M, Lozano R, Michaud C, Ezzati M et al (2012) Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380(9859):2163–2196. https://doi.org/10.1016/S0140-6736(12)61729-2. PMC 6350784
WHO (1999). Definition, diagnosis and classification of diabetes mellitus and its complications. Archived (PDF) from the original on 8 Mar 2003
WHO (2006) Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation (PDF). WHO, Geneva, p 21. ISBN 978-92-4-159493-6
WHO (2013a) Diabetes Fact sheet N°312″. Archived from the original on 26 August 2013
WHO (2013b) The top 10 causes of death Fact sheet N°310″. Archived from the original on 30 May 2017
WHO (2014a) Diabetes programme. Archived from the original on 26 April 2014. Accessed 22 Apr 2014
WHO (2014b) About diabetes. Archived from the original on 31 March 2014

WHO (2019) Diabetes mellitus. Accessed 23 Mar 2019

Yaghoobi N, Al-Waili N, Ghayour-Mobarhan M, Parizadeh SM, Abasalti Z, Yaghoobi Z, Yaghoobi F, Esmaeili H, Kazemi-Bajestani SM, Aghasizadeh R et al (2008b) Natural honey and cardiovascular risk factors; effects on blood glucose, cholesterol, triacylglycerole, CRP and body weight compared with sucrose. Sci World J 8:463–469

Yaghoobi N, Al-Waili N, Ghayour-Mobarhan M et al (2008a) Natural honey and cardiovascular risk factors; effects on blood glucose, cholesterol, triacylglycerole, CRP and body weight compared with sucrose. Sci World J 8:463–469

Yamashita K, Itakura M, Kawai K (1984) Effects of fructo-oligosaccharides on blood glucose and serum lipids in diabetics subjects. Nutr Res 4:961–966

Honey and Its Molecular Pharmacology: An Essay

10

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Abstract

Honey is a sugary, viscous fluid being used nearly 5500 years ago, since prehistoric times. In Sumerian tablet, the first inscribed evidence of honey was found in 2100–2000 B.C. Most olden civilizations like Greeks, Chinese, Egyptians, Romans, Mayans, and Babylonians, used honey mutually aimed at nutrition as well as for medicinal purposes. It exhibits numerous health- benefits which include anti-oxidant, anti-inflammatory, anti-bacterial, anti-diabetic, and protective effects in respiration, gastrointestinal system, cardiovascular, and nervous system. Based on origin, or its way of harvest and processing, honey can be categorized as blossom honey, honeydew or forest honey, monofloral, multifloral honey, raw honey, granulated honey, strained honey, ultra-filtered

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honey, ultrasonicated honey, chunk honey, comb honey, dried honey, whipped or creamed honey. The methods of extraction, processing, packaging, and preservation of honey alter the physical appearance of honey. Nevertheless, some elementary properties allied with honey contribute to it, regardless of the protocols used in the formation like content of H_2O, matter configuration, and retention of water. Other physical structures and features of honey include taste, odor, color, heat, and crystallization. Depending on honey's source, oldness, and storage/packing conditions, liquid honey may be either clear or no color, yellow, amber to dark amber, or black in color. Honey consists of pollen grains, water, waxes, vitamins, sugars, essential minerals, amino acids, proteins, enzymes, pigments, and pollen grains, and numerous phytochemicals with other 180 types of diverse complexes. Chemically, it consists of enzymes, organic acids, and phenolic acids with gluconic acid being the most abundant organic acid. Phenolic acids include non-flavonoids and flavonoids like isoflavones, flavones, anthocyanidins, flavanones, flavonols, chalcones, and enzymes include glucose oxidase, saccharase, catalase, and diastase and others, respectively. The effect of different constituents of honey obtained have been found to inhibit inflammation, oxidative stress, proliferation, metastasis, angiogenesis, and induce apoptosis. Also, honey has been found to regulate diabetes, cardiovascular and neuropharmacological diseases. However, more mechanism- based research needs to be done to promote the consumption of this healthy food in the general population, to promote a healthy lifestyle, and to regulate normal processes of life.

Keywords

Honey · Polyphenols · Cancer · Cardiovascular pharmacology · Neuropharmacology

10.1 Introduction

Honey is a sugary, viscous fluid as defined through the European Union as naturally formed by honeybees (*Apis mellifera*) from the sap of flowers, defecations of plant-sucking insects or plants parts collected by bees, or from emissions of any plant parts which bees gather, alter in joining per explicit constituents of themselves, deposits, desiccate, stock, then place in honeycombs to grow and ripen. Humans have been using honey nearly 5500 years ago, since prehistoric times. In Sumerian tablet, first inscribed evidence of honey was found in 2100–2000 B.C. Most olden civilizations, like Greeks, Chinese, Egyptians, Romans, Mayans, and Babylonians, used honey mutually aimed at nutrition as well as for medicinal purposes (Samarghandian et al. 2017). It has nutritive, cosmetologic, healing, and industrial properties and is the only naturally derived from insects. It serves as a balanced diet and correspondingly beneficial for both men and women in any population. It is not spoiled by high temperature, can be stored in a dry place unopened at room temperature, so does not need refrigeration. At present time, data on the honey usage for curing of various

natural ailments are present in magazines, research papers, and awareness books. Literature reports demonstrate that honey exhibits numerous health benefits which include antioxidant, anti-inflammatory, antibacterial, antidiabetic, and protective effects in respiration, gastrointestinal system, cardiovascular, and nervous system (Carlos et al. 2011; Ediriweera and Premarathna 2012).

10.2 Classification of Honey

Based on origin, or its way of harvest and processing, honey can be categorized. Depending upon its derivation, it is classified into blossom, honeydew, monofloral, and multifloral honeys. *Blossom honey* comes from the juice of flowers mainly.

Honeydew or forest honey is obtained by bees after collecting honeydew from plant juices.

Monofloral honey is principally obtained from a solo plant source having total pollen content of more than 45% from the identical plants and called as per plant-like citrus, manuka, and acacia honey are obtained (Nyuk and Kandhasamy 2020).

Multifloral honey is identified as polyfloral honey having different plant origins where not any is major like meadow blossom honey and forest honey (Alvarez-Suarez et al. 2010). Based on packaging, mode of production, or presentation, honey is categorized separately and characterized differently like raw, ultra-filtered, granulated, chunk, pasteurized, dried, strained, ultrasonicated, comb and whipped (Zielinski et al. 2014).

Raw honey may be obtained by negligible processing with no heat treatment, attained by extraction, settling, or straining of beehive contents (Decaix 1976). Modern beehives can be visualized in Fig. 10.1a.

10.2.1 Granulated Honey

When the glucose content of honey has naturally crystallized/formed granules in a mixture like monohydrate or granulated honey is produced. This granulated or crystallized honey is moved in a vessel kept at 49 °C in warm water to convert it to a liquid state. However, honey is pasteurized by heating to 161 °F (71.7 °C) to destroy fungal cells and melt tiny crystals in the honey, hence delaying the commencement of discernible crystal formation (Bogdanov et al. 2008).

10.2.2 Strained Honey

Strained honey undergoes straining process to get rid of dirt, waxes, exudates from plants, and propolis and not eliminating minerals, pollen, or enzymes from the mixture of honey obtained.

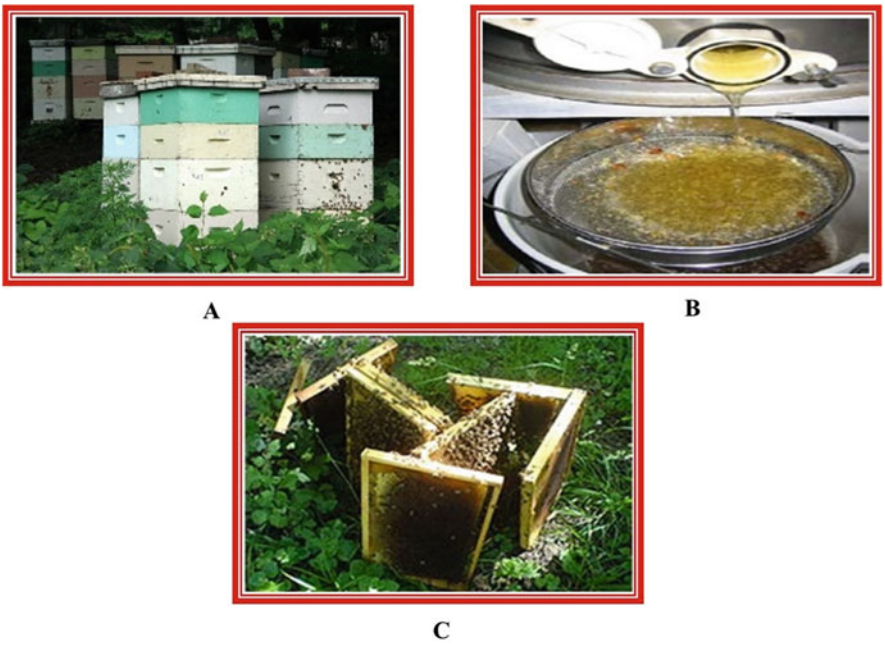

Fig. 10.1 (**a**) Modern beehives, (**b**) filtration of raw honey, and (**c**) typical wood frames

Ultra-filtered honey is achieved by applying 65–77 °C, as it effortlessly melts and passes via fine filter under high pressure in order to get rid of superfluous solids and pollen grains, as shown in Fig. 10.1b (Bogdanov et al. 2008).

10.2.3 Ultrasonicated honey

Ultrasonicated honey attained via non-heating procedure by supposedly kills fungal growth and inhibits crystallization.

Chunk honey filled in large-mouthed bottles having pieces of comb honey engrossed in removed molten honey.

Comb honey is in wax comb of honeybees', conventionally obtained through honey supers before packaging in which the comb is cut in chunks via means of typical wooden frames. Figure 10.1c represents wooden frames (Ajibola 2016).

Dried honey is devoid of moistness to produce entirely compact, nonsticky granules done by aeration and anticaking agents.

Whipped or creamed honey consists of a huge amount of small crystals to produce honey with an even and easily spreadable uniformity.

According to European Union Council Directive which states there is also baker's honey meant for industrial purpose or as a constituent in food industry (Ajibola 2016).

10.3 Physical Properties

The methods of extraction, processing, packaging, and preservation of honey alter the physical appearance of honey (González-Paramas et al. 2000). Nevertheless, some elementary properties allied with honey contribute to it, regardless of the protocols used in the formation-like content of H_2O, matter configuration, and retention of water. It consists of water (16%) and suspended elements (80%) (Ajibola 2016). Viscosity of honey depends on several honey ingredients mainly its water content which is defined as hygroscopicity. Surface tension is dependent on colloidal substances in the honey which is directly proportional to honey's botanical origin serves as additional property influencing the appearance physically. Other physical structures and features of honey include taste, odor, color, heat, and crystallization (White and Doner 1980). Honey is used as a sweetener because of fructose glucose naturally, which contributes to sweetness and has a sweet smell too. Depending on honey's source, oldness, and storage/packing conditions liquid honey may be either clear or no color, yellow, amber to dark amber, or black in color. Nonetheless, pollen or dirt can interfere with transparency or clarity (Olaitan et al. 2007). Honey is also available in bright yellow (sunflower), reddish undertones (chestnut), grayish (eucalyptus), and greenish (honeydew) colors as per its sources. Likewise, external features of honey-like color, crystallization, taste, and fragrance are affected by heat. As a matter of fact, heat converts honey into darker color. Honey crystallization occurs by the development of monohydrate glucose crystals of varying quantity, form, measurement, and excellence with configuration, conservation, and packing procedures of honey (González-Paramas et al. 2000; Decaix 1976). Though regardless of storage conditions over time honey crystallizes.

10.3.1 Crystallization of Honey

Crystallization of honey affects texture and color but would not change the taste, superiority, or nutritive status. Crystallization turns honey lighter in color due to the formation of glucose crystals. The quantity of water also affects rate of crystallization; the lesser water content, the more content of glucose fastens the process (Olaitan et al. 2007). Storage conditions, explicit blend of sugars, and trace components in the honey also influence the rate of crystallization. Tupelo and acacia type honeys extraordinarily crystallize slowly, whereas goldenrod crystallizes in the comb itself often. Heat influences physical characteristics of honey as crystallization, which has been mentioned above. Consequently, at a lower temperature 10 and 21 °C, honey can be crystallized, while as fluidly honey is obtained by heating honey to a higher temperature like 49 °C indirectly to cause the dissolution of the crystals.

Honey is food sweetener, used industrially with no previous processing, and its manufacture is increasing at a global level (Ajibola 2016; Crane 1975).

10.4 Honey: Its Composition and Physicochemical Properties

Honey consists of pollen grains, water, waxes, vitamins, sugars, essential minerals, amino acids, proteins, enzymes, pigments, and pollen grains (PGs) (White and Doner 1980), numerous phytochemicals with other 180 types of diverse complexes. Honey contains a mixture of glucose and fructose in a strenuous aqueous solution but also has various amino and organic acids, at least 22 other intricate carbohydrates, proteins, inhibine containing antibiotics, enzymes, phenol antioxidants, aroma compounds, vitamins, minerals, pigments, etc. (White and Doner 1980). Honey's pH ranges from 3.2 to 4.5, hence is acidic. Sugars which are found in honey are generally monosaccharides like fructose and glucose constituting 75%. Other carbohydrates constituting 10–15% are disaccharides which constitute maltose, maltulose, sucrose, turanose, isomaltose, and trisaccharides (Da Silva et al. 2016). Typically, most predominant sugar in the honey is fructose like in acacia plants, but there are exceptions like dandelion and rape plants which have higher proportion of glucose than fructose (Persano and Piro 2004). Therefore, carbohydrates can help in the recognition of plant-based and/or location-based sources of floral honeys, as well as hydration capacity and other characteristics (Eteraf-Oskouei and Najafi 2013). Apart from percentages of glucose and fructose, its proportion also helps to classify floral honeys and percentages of some trivial oligosaccharides and blended concentrations (Kaškonienė and Venskutonis 2010). Proteins also form part of honey including enzymes like invertase, α-glucosidase, catalase, diastase, sucrase, amylase, and glucose oxidase. Amino acids are present with proline being the maximally present along with other 20 or more amino acids (Da Silva et al. 2016). Table 10.1 consists of the physicochemical composition collected globally around 1000 honey samples. It also contains differing quantity of essential minerals depending upon plant source, location, geographical factors, and processing techniques. Calcium, phosphorus, copper, magnesium, potassium, sodium, zinc, iron, manganese, and selenium are the most abundantly found (Miguel et al. 2017).

Honey consists of small amounts of vitamins which include ascorbic acid (C), riboflavin (B2), niacin (B3), pantothenic acid (B5), and pyridoxine (B6) (Alvarez-Suarez et al. 2013). B complex vitamins are derived mostly from pollen, along with vitamin C which can influence commercial and industrial processes like filtration and oxidation reactions carried out by glucose oxidase (Ciulu et al. 2011). Table 10.2 details about the presence of vitamins/100 g of honey.

Based on plant source and physical location, honey contains minerals and elements of different percentages (Vincēviča-Gaile 2010). Minerals come into existence naturally by decomposition of plant and animal remains via geological processes which occur as inanimate compact elements in the environment (Belitz et al. 2009; Nickel 1995), which happen to be indispensable in controlling metabolism in the living organisms (Gopalan et al. 1989). They are grouped into three categories based on requirements of body as major, trace, and ultra-trace elements. Minerals that are needed in amounts of more than 50 mg/day are major minerals, whereas ones which are required $<$50 mg/day are trace elements and others even less

Table 10.1 Represents the physicochemical composition collected globally around thousand honey samples

Elements	Mean value
Moisture (%)	17.90
pH	3.96
Fructose (%)	39.44
Sucrose (%)	3.19
Glucose (%)	28.15
Reducing sugar (%)	68.31
Minerals (%)	0.36
Total protein (%)	1.13
Lipid amount (mg/g)	215.00
HMFb (mg/kg)	15.49
Vit-C (mg/g)	13.19
Lactone (meq/kg)	8.57
Proline amount (mg/kg)	873.00
Diastase activity (DN)	14.27
Electrical conductance (ms/cm)	0.64
ABS450c (mAU; 50 w/v)	834.00
Acidity (meq/kg)	35.32
Total solids soluble (Brix)	128.00
Refractive index	1.49
Water-insoluble portion (%, w/w)	16.00

Table 10.2 Describes the presence of vitamins/100 g of honey

Vitamins present	Value/100 g
B2	0.038 mg
B3	0.21 mg
B5	0.068 mg
B6	0.024 mg
B9	2 μg
Vit C	0.5 mg
Mixed groups	–

than 1 μg/g and frequently found at <50 ng/g in diet are called as ultra-trace elements (Nielsen 1984; Belitz et al. 2009). Those initiating from natural source in particular micro- or trace minerals are useful for good strength. Instead, having five times specific gravity of H_2O develop toxicity if the source is inorganic or metallic constitute heavy metals (Ajibola et al. 2012). Heavy metals are lethal or noxious at less proportions since they get accrued in the living system (Zugravu et al. 2009). Based on plant source and physical location, honey contains minerals and elements of different percentages (Vincēviča-Gaile 2010).

The volatile compounds include esters, ketones, monoterpenes, sesquiterpenes, benzene derivatives, C13-norisoprenoid and superior alcohols, fatty acids, aldehydes to a lower content (Da Silva et al. 2016). Plant origins and physical location for obtaining honeys can be determined by the presence of some volatile compounds in it like sinensal isomers, etc., which are recognized only in citrus (Alissandrakis et al.

Table 10.3 Depicts about macro, micro, and ultra-trace elements present in honey

Element/units (mg/kg)	Mean
Na	96.48
Cl	302.63
P	84.10
Mg	74.31
Ca	84.36
S	35.27
K	742.43
Trace elements/heavy elements (mg/kg)	Mean
Fe	30.34
Ti	43.40
Si	23.52
Zn	9.33
Al	5.12
Mn	1.42
Ni	1.24
As	0.05
Si	23.52
B	5.36
Mo	0.23
Sr	1.63
V	0.03
Se	0.01
Cd (μg/kg)	89.69
Pb (μg/kg)	424.57
Co (μg/kg)	171.78
Hg (μg/kg)	5.09
Cr (μg/kg)	152.84
Ag (μg/kg)	299.61
Be (μg/kg)	9.93

2007 and Castro-Vázquez et al. 2007) and compound nonanol, etc. are found in acacia honey only (Petretto et al. 2016). Honey from different sources like eucalyptus and chestnut, etc. from varied areas were collected to study the volatile profile and the results were variable, even in the same region. Nevertheless, physicochemical characteristics, major components analysis, clustering analysis, and others are some of the methods to find out plant source and physical location from which honey was obtained by analyzing volatile components of honey samples and it is this method which gives 80% efficacy to classify honey as reported by Oroian et al. (2015) (Table 10.3).

10.5 Chemical Composition

10.5.1 Enzymes and Organic Acids

Organic acids constitute about 0.57% with gluconic acid being the most abundant. Glucose oxidase, saccharase, catalase, and diastase are the enzymes present in honey which are significant in honey formation as well as an efficient food (Cianciosi et al. 2018). Glucose gets converted to δ-gluconolactone by glucose oxidase, which upon hydrolysis converts to gluconic acid, the primary acid, and H_2O_2 having antimicrobial potential. Sucrose is converted to fructose and glucose by invertase. Amylase, an enzyme which acts on long starch chains, makes dextrin and maltose, and hence contributes to honey's quality. Catalase is another enzyme which produces O_2 and H_2O from H_2O_2. Acidity of honey, stability, and its particular taste are due to organic acids present in it (Molan 1999, 2001). Other organic acids found in honey are malic, lactic, formic, fumaric, malonic, aspartic acid, methylmalonic, succinic, citric, oxalic, butyric, acetic, formic, fumaric, glutaric, malonic, formic, acetoglutaric, gluconic, glutamic, galacturonic, butyric, shikimic, isocitric, lactic, malic, quinic, etc. (white and Doner 1980; Mato et al. 2006).

10.5.2 Phenolic Compounds

Polyphenols constitute diverse category of organic substances classified into flavonoids and non-flavonoids (Fig. 10.2). Phenolic acids are non-flavonoids and flavonoids include isoflavones, flavones, anthocyanidins, flavanones, flavonols, and chalcones. They are a by-product of secondary metabolites of plants, which are categorized by numerous phenolic groups present with diverse structural intricacies. The secondary metabolism products have significant ecological functions, yet they are involved in adaptation, respiratory, transporter, and differentiating processes unlike primary metabolites like chlorophyll, amino acids, and simple carbohydrates (Kennedy and Wightman 2011). The phenolic composition may help in classifying and authenticating process like in unifloral varieties, depending mainly on its plant source. The most common phenolic compounds and flavonoids in honey are shown in Fig. 10.3.

These compounds scavenge free radicals by making stable and least harmful molecules, hence having antioxidative activity. The stability of phenolic compounds depends on amount of their hydroxyl groups and they stabilize free radicals by giving away H from OH group (Rice-Evans and Miller 1996).

Flavonoids are generally water-soluble chemicals, bearing low molecular weight and are shaped by combination of benzene rings with alternate straight three carbon chain of atoms (C6–C3–C6); rearrangement repeatedly itself leads to the formation of three rings having 15 carbon atoms called A, B, and C. Largely, these complexes are frequently connected with sugars like glucose with galactose, arabinose, xylose, glucorhamnose, and rhamnose; having two phenolic groups (OH) at least. Aglycones are the compounds when flavonoids are not connected with sugars.

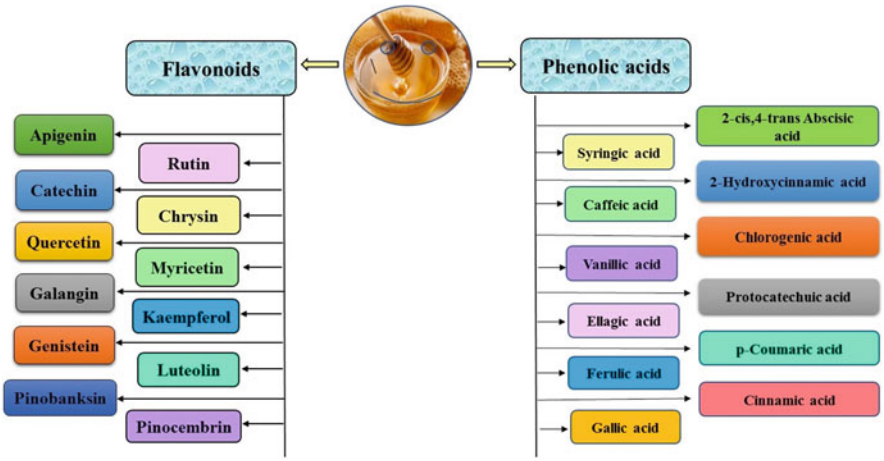

Fig. 10.2 Depicts classification of phytochemicals present in honey

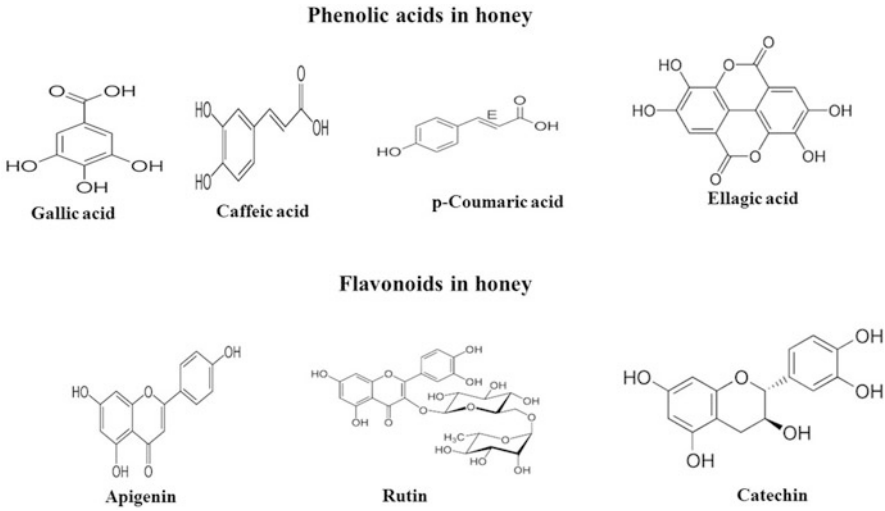

Fig. 10.3 Depicts structure of some compounds present in honey

Therefore, flavonoids are categorized depending on oxidizing of C ring in which we already described above as found in flavanones, flavonols, anthocyanidins, flavanols, flavones, isoflavones, anthocyanins and flavones, flavanols, and flavonols being most copious (Moniruzzaman et al. 2014).

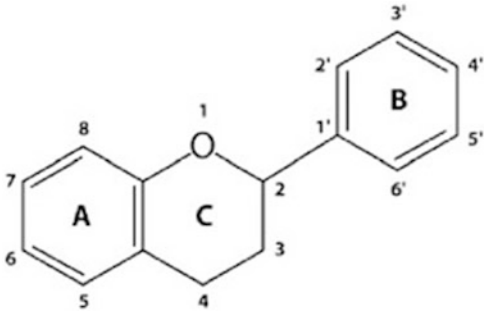

Fig. 10.4 Basic structure of flavonoids

10.5.3 Basic Structure of Flavonoid

Phenolic acids basically comprise of one organic carboxylic acid and a phenolic ring which can further be grouped as per their structure: C6–C3 like ferulic acid and caffeic acid, C6–C2 which include acetophenones and phenylacetic acids and C6–C1 structures which include vanillic and gallic acids. Phenolic compounds or other secondary metabolite structures are typically attached with the structural machineries of the plant-like cellulose, organic compounds like glucose, sugars, or flavonoids (Padayachee et al. 2012; Cianciosi et al. 2018) (Fig. 10.4).

Polyphenols and flavonoids form the most crucial bioactive molecules present in honey. Reports suggest that there may be nearly 30 diverse polyphenols present (Padayachee et al. 2012; D'Archivio et al. 2010) which differ from 50 to 850 mg/kg and have varied flavonoid composition between 36 and 150 mg/kg (Walle 2004), which again depends upon the geographic, climatic, environmental settings as well as floral source. Galangin, quercetin, luteolin, and kaempferol are the kinds of bioactive compounds present in it usually, while hesperetin and naringenin are other constituents found only in explicit types (Spencer et al. 1999; Peternelj and Coombes 2011). However, ellagic acid, quercetin, luteolin, catechin, naringenin, gallic acid, kaempferol, syringic acid, coumaric acid, caffeic acid, benzoic acid, chlorogenic acid, cinnamic acid, myricetin, ferulic acids, apigenin, chrysin, catechin, hesperetin, isorhamnetin, and galangin are generally the most frequently described phenolic acid components and flavonoids present in it (Carlos et al. 2011; Khalil et al. 2011; Erejuwa et al. 2012a, b; Mijanur et al. 2014).

Floral type, physical location, environment around, various bee species around, and then the post collection processing and storage conditions define the elemental, phytochemical constituents, taste, and color of honey (Puscas et al. 2013).

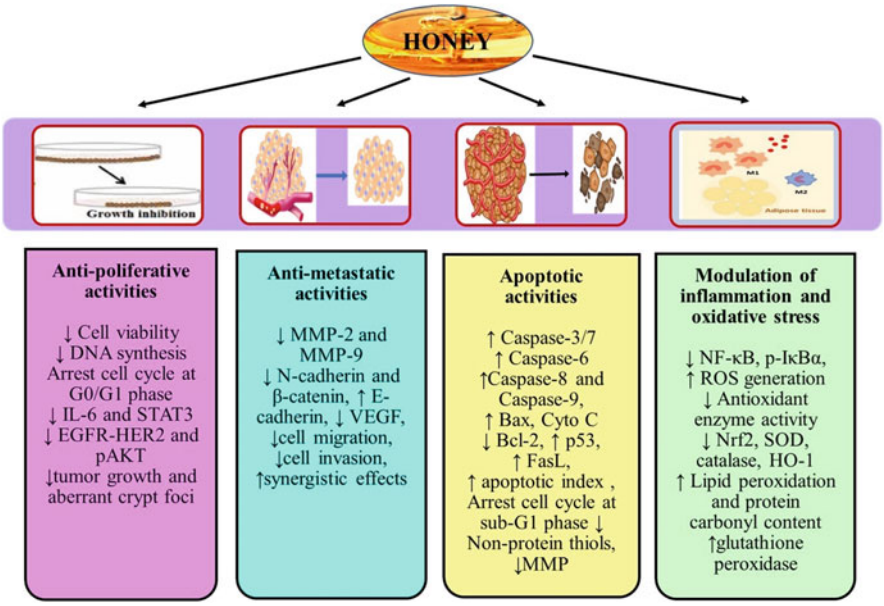

Fig. 10.5 Depicts the possible mechanisms of anticancer effects of honey

10.6 Cancer-Related Mechanistic Studies on Honey

Cancer is a complicated phenomenon commencing with variation at genetic level in healthy cells resulting in uncontrolled propagation, failure to apoptose, leading to development of tumors if the repair pathway does not work (Allan and Travis 2005). Several measures conspire to form cancer-like instigation of oncogenes, suppression of tumor suppressor genes, uncontrolled cell signaling pathways, growth factors and hormones, and other interrelated complex processes (Hanahan and Weinberg 2011). The heterogeneous nature of cellular genotypes and phenotypes and several pathways activate the pathological process of development of cancer. Conventional therapies do not work much because they target one pathway and the cancer cell takes another. The effect of different constituents of honey-like flavonoid or phenolic extracts or fractionated or whole honey extracts from various regions with different dosages on diverse cancer types (Swellam et al. 2003a, b; Khoo et al. 2010; Salucci et al. 2002; Nair et al. 2004; Elattar and Virji 2000) by targeting anti-proliferation, apoptosis initiation process; inhibition of inflammation, oxidative stress, anti-metastasis, and antiangiogenesis (Fig. 10.5) in accordance with type, dose of honey studied in various in vitro and in vivo models. However, it is not limited only to these mechanisms.

Polish honeys with the highest phenolic content decreased MMP-2 and MMP-9 expression, which are the markers of metastasis, in the glioblastoma cell line

Table 10.4 Different kinds of Honey having inhibitory effects on cancer cell lines (anti-proliferation, anti-oxidative, anti-inflammatory, proapoptotic, antimetastatic, and antiangiogenesis pathways)

Honeys	Effects on cancer	In vitro	Dose/ intervention and duration	References
Polish honey	Decrease in cell viability, DNA synthesis, MMP-2 and MMP-9	U87MG glioblastoma cell line	0.5–7.5% polish honey (24–72 h)	Moskwa et al. (2014)
Manuka honey	Decrease NF-κB, p-IκBα	HCT116 and LoVo colorectal cancer cell lines	0–20 and 0–60 mg/mL (48 h)	Afrin et al. (2018a)
	Decreases cell viability, STAT3 phosphorylation, and IL-6 production with decrease in Bcl-2, cell migration, and cell invasion. Caspase-3/ 7, -6, -8, -9, Bax, and Cyto C were elevated	MDA-MB-231 and MCF-7 breast cancer cell lines	0.25–2% (w/v) (24–72 h)	Aryappalli et al. (2017)
	Decrease cell viability and tumor growth in vivo. Increase in apoptosis and coaction with paclitaxel	CT26 colon carcinoma cell line, MCF-7 breast cancer cell line, and B16F1 skin melanoma cell line	0·6–5% for (24, 48, and 72 h)	Fernandez-Cabezudo et al. (2013)
	Decrease in NF-κB, p-IκBα, MMP-2, MMP-9, and cell migration with N-cadherin and β-catenin decrease. Elevation in E-cadherin and synergistic effects	HCT116 and LoVo colorectal cancer cell lines	10–20 and 30–40 mg/mL (48 h) and 5–15 and 20–30 mg/mL (48 h)	Afrin et al. (2018a, b)
Strawberry tree honey	Elevation in p53, caspase-3, -8, -9, c-PARP1, Bax, Cyto C, FasL, and E-cadherin. Decrease in Bcl, cell migration, MMP-2 and MMP-9, N-cadherin, and β-catenin	HCT116 and LoVo colorectal cancer cell lines	3–12 mg/mL (48 h) 10–40 mg/mL (48 h)	Afrin et al. (2019a, b)
	Increased ROS generation, lipid peroxidation, and protein carbonyl content with decrease	HCT116 and LoVo colorectal cancer cell lines	3–12 mg/mL (48 h) 10–40 mg/mL (48 h)	Afrin et al. (2017, 2019b)

(continued)

Table 10.4 (continued)

Honeys	Effects on cancer	In vitro	Dose/ intervention and duration	References
	in antioxidant enzyme activity, Nrf2, HO-1			
Indian honey	Cell cycle arrest, decrease in Bcl-2, nonprotein thiols and MMP with elevation in ROS, p53, caspase-3, PARP cleavage, Bax	HCT15 and HT29 colon cancer cell lines	1–20% (12–48 h)	Jaganathan and Mandal (2010)
Acacia honey	Decrease in cell viability, Bcl-2, p53 arrest cell cycle at G0/G1 phase	NCI-H460 non-small lung cancer cell line	0.5–8% (48 h)	Aliyu et al. (2013)
	Decrease cell viability and arrest cell cycle at G0/G1 phase	A375 and B16-F1 melanoma cell line	0.01–0.2 g/mL (24–72 h)	Pichichero et al. (2010)
	Arrest cell cycle at G0/G1 phase with decrease TNF-α, IL-1β	PC-3 prostate cancer cell line	2–10% (v/v) (48 h)	Aliyu et al. (2012)
	Decrease cell viability with apoptotic cell death	MCF-7 breast cancer cell line	3.12–100% (v/v) (24–72 h)	Salleh et al. (2017)

U87MG (Song et al. 2017). Moreover, the authors suggest a correlation between the quantity of lead and cadmium and found honey having large amount of cadmium to have more potency to inhibit metastasis. It diminished cell viability by decreasing the synthesis of DNA in U87MG glioblastoma cells (Moskwa et al. 2014) (Table 10.4).

Acacia honey (AC) suppresses the development of MCF-7, human breast adenocarcinoma cells, and triggered apoptosis (5.5% v/v) via arrest of the cell cycle at the G0/G1 phase which was revealed through TUNEL assay and live cell view imaging depending upon dose and time (Hegazi and Abd El-Hady 2007) in NCI-H460 (non-small lung) (Aliyu et al. 2013), PC-3 (prostate) (Aliyu et al. 2013) cancer cells. In PC-3 cells, it also upregulated production of cytokines like TNF-α, IL-1β thereby inducing the release of Ca ion from the endoplasmic reticulum (Aliyu et al. 2012, 2013). AC resulted in downregulation of proliferation as well as arresting cell cycle at G0/G1 phase in numerous cell lines (Aliyu et al. 2012; Pichichero et al. 2010) (Table 10.4).

Manuka honey (MH) was found to have antiproliferative effect in time and dose-dependent manner on different cancer cell lines including CT-26, HCT-116, and LoVo (colon), MDA-MB-231 and MCF-7 (breast), and B16-F1 (melanoma) (Aryappalli et al. 2017; Fernandez-Cabezudo et al. 2013; Afrin et al. 2018c),

which were linked with S and G2/M phases cell cycle arrest by modifications in cell cycle–mediated genes. Besides, MH was reported to upregulate phosphorylated p38 mitogen-activated protein kinase (p-p38MAPK) and phosphorylated extracellular signal-regulated kinase 1/2 (p-Erk1/2) pathways and suppress oncogenic signaling pathways like epidermal growth factor receptor (EGFR), human epidermal growth factor receptor (HER2), phosphorylated protein kinase B (p-Akt), and IL-6/signal transducer and activator of transcription (IL-6/STAT3), which results in regulated signaling (Afrin et al. 2018c; Aryappalli et al. 2017). MH and strawberry tree honey (STH) also upregulated molecules associated with intrinsic and extrinsic apoptotic pathways like caspase-8, caspase-9, Bcl-2-associated X protein (Bax), fatty acid synthetase (Fas) ligand (FasL), and cytochrome C (Cyto C) at mRNA level (Afrin et al. 2018c, 2019a). MH was reported to increase caspase-3/7 and caspase-9 enzyme, c-PARP, DNA fragmentation, and inhibited Bcl-2 in mitochondria-dependent apoptotic pathway in a murine melanoma B16-F1 cells (Fernandez-Cabezudo et al. 2013). It also diminished migratory and invasive pathway molecules like MMP-2, MMP-9, N-cadherin, and E-cadherin HCT-116 and LoVo human colon (Afrin et al. 2019b, a), and MDA-MB-231 and MCF-7 breast cancer (Aryappalli et al. 2017) along with inflammatory mediators like NF-κB, p-IκBα resulting in antimetastatic, anti-invasion, and anti-inflammatory effect in HCT-116 and LoVo colon cancer cells (Pichichero et al. 2010) (Table 10.4).

Strawberry tree honey (STH) decreases cell viability in HCT-116 (colon adenocarcinoma cells) and metastatic LoVo cells in a dose- and time-dependent manner (Pichichero et al. 2010) and augments reactive oxygen species production (ROS) (Afrin et al. 2017, 2018a). Apart from increasing oxidative stress allied with apoptosis by diminishing antioxidant armories like glutathione peroxidase, glutathione reductase, superoxide dismutase (SOD), and catalase; in congruent with inhibiting nuclear-related factor 2 (Nrf2), SOD, catalase, and heme oxygenase 1 (HO-1) pathway leading to antioxidant response, upregulating the injury of cellular biomolecules (lipid, protein, and DNA); and mitochondrial respiration and glycolysis disruption which may be attributed to larger amount of polyphenols present in it (Yaacob et al. 2013; Jaganathan et al. 2010a, b; Morales and Haza 2013; Afrin et al. 2018a) (Table 10.4).

Jaganathan and Mandal (2010) showed that the treatment of Indian honey in HCT-15, HT-29 colon, and MCF-7 breast cancer cells induced apoptosis by freezing cell cycle at sub-G1 phase. It also downregulated intracellular nonprotein thiols in congruence with MMP by oxidative stress, improved p53, caspase-3, c-PARP, and Bax, along with downregulation of expression of Bcl-2 protein (Jaganathan and Mandal 2010; Jaganathan et al. 2010a) (Table 10.4).

Jungle honey boosted immunity in mice against tumor inoculated with Lewis lung carcinoma/2 cells. There was a correlation with an enlarged neutrophil-mediated chemotactic response and ROS generation with declined in tumor rate in honey-treated mice (Fukuda et al. 2011) (Table 10.5).

Indian Honey significantly reduced tumor progression (Jaganathan et al. 2010b) in a murine Ehrlich ascites carcinoma model, which may be attributed to its phenolic

Table 10.5 Inhibitory mechanistic effects of honey on in vivo cancer models

Honeys	Effects/mechanism of action on cancer	In vivo	Treatment regimen	References
Jungle honey	Chemotaxis ↑ ROS production	Lewis lung carcinoma	1 mg/day intraperitoneally for 7 days before tumor inoculation	Fukuda et al. (2011)
Indian honey	↓ Tumor growth	Ehrlich ascites carcinoma	25% (v/v) intraperitoneally for 12 days	Jaganathan et al. (2010b)
Bee honey	↓ Lung nodule formation	Spontaneous mammary carcinoma Anaplastic colon adenocarcinoma	2 g/kg (oral), daily for 10 days 1 g/kg (oral), daily for 10 days	Orsolić et al. (2003)
	↓ PCNA ↓ p53 levels	Diethylnitrosamine-induced liver carcinogenesis	2 g/day (oral) for 6 months	El-kott et al. (2012)
	↓ Tumor volume (93)	MBT-2 bladder cancer	6–12% (intralesional), twice weekly; 3 weeks 50% in drinking water, alternate days; 3 weeks	Swellam et al. (2003)
Manuka	Increases Apaf-1, Caspase-9, IFN-γ, IFNGR1, and p53. Decreasing TNF-α, COX-2, and Bcl-xL 1	Female Sprague–Dawley (SD) breast cancer rats	1-methyl-1-nitrosourea (80) mg/kg. 1.0 g/kg body weight/day of TH and MH, respectively, for 120 days	Porcza et al. (2016)
Tualang	↓ Tumor cell growth, ↓ cell proliferation, ↑ Apaf-1, and caspase-9	(DMBA-induced breast cancer	0.2, 1.0, or 2.0 g/kg bodyweight/day of TH, respectively, for 150 days	Fernandez-Cabezudo et al. (2013)

ingredient, whereas it did not show any effect in leukemia cancer (Jaganathan et al. 2014) (Table 10.5).

Bee honey was given orally to rats for 6 months as 2 g honey/day starting 1 week after diethylnitrosamine (DEN) administration had a beneficial result against inflammation, decreased proliferation, and induced apoptosis (El-kott et al. 2012). Because of carbohydrates and sugar intake to rats by administering honey at such dose for a long period may have confounded the findings as they did not take appropriate controlled sugar to rats. Ehrlich ascites carcinoma growth was inhibited by treatment of 25% (v/v) bee honey intraperitoneally 1 day after tumor inoculation significantly inhibited in mice and this tumor inhibitory efficacy was ascribed to the phenolic content and its antioxidant status (Jaganathan et al. 2010b). Lastly, bee honey was explored for its anti-tumor efficacy in MBT-2 bladder cancer model in C3H/He

mice. Tumor volume was diminished by injecting 6 and 12% solutions of honey inside the lesions of tumor. Furthermore, tumor growth was blocked by treating mice with 50% solution of bee honey orally (Swellam et al. 2003a, b). Although the mechanism of action is unknown. Macrophage phagocytic activity and T-Cell activation are generally attributed to Bee honey and may be responsible to activate immune system (Table 10.5).

Manuka honey was administered a week earlier the initiation of breast cancer with N-methyl-N-nitrosourea to rats at a dose of 1 g/kg body weight blocked tumorigenesis (Samarghandian et al. 2011). It upregulated Apaf-1, Caspase-9, IFN-γ, IFNGR1, and p53 leading to induction of apoptosis and diminished TNF-α, COX-2, and Bcl-xL decreasing inflammation. Manuka honey induced strong proapoptotic activity dose and time dependent upon intravenous administration in mice having murine melanoma tumor cells (B16F1) implanted leading to shrinkage of final tumor volume (Fernandez-Cabezudo et al. 2013).

Tualang honey was administered to rats orally after 7,12-dimethyl benzene anthracene (DMBA) for 150 days deferred tumor progress, multiplication, weights, and volumes compared with control animals, thereby preventing 7,12-dimethylbenzeneanthracene (DMBA)-induced mammary tumors in Sprague–Dawley rats. Tualang honey induced proapoptotic proteins like Caspase 9 and p53, reduction of vascular endothelial growth factor (VEGF), marker of angiogenesis, and modulated inflammatory mediators like TNF-α and COX-2 (Kadir et al. 2013) (Table 10.5).

10.7 Cancer-Related Clinical Studies on Honey

Honey improves the quality of life of cancer patients and has been used in alternative medicine. Nevertheless, not much testing/clinical trials in cancer patients have been done to study the potency of honey. Different types of honey manuka and thyme administered orally have been found to alleviate mucositis as an adverse effect of radiation therapy for head and neck cancer patients, which is one of the rare areas which provides evidence (Rao et al. 2017; Cho et al. 2015; Hawley et al. 2014; Charalambous et al. 2018). Notably, but reports did not mention about its effect on cancer promotion. Honey efficiently ameliorated the sternness of mucositis induced by chemotherapy in meta-analysis of randomized trial (Xu et al. 2016). On the other hand, manuka honey was tested against esophagitis induced by radiation in a randomized clinical trial deciphered that it was not better than the standard supportive care (Fogh et al. 2017). Cancer patients who were given 5 mL processed honey and royal jelly honey for 4 weeks, twice daily had reduced fatigue related to cancer in double-blind randomized trial of 52 patients (Mofid et al. 2014). Administration of honey and ardeh (sesame paste) in another 107 patients getting chemotherapy for acute myeloid leukemia amended gastrointestinal problems, neutropenia and declines fever in another double-blind, randomized, placebo-controlled study (Attia et al. 2008). In another study, 40 children of ages 2.5–10 years having acute lymphoblastic leukemia upon treatment with raw clover honey were found to have

reduction in febrile neutropenia and increase in Hb levels in randomized crossover clinical trial (Mabrouk et al. 2002). Generally, honey has been found to have beneficial effect in decreasing adverse side effects associated with chemotherapy/radiotherapy which includes lethargy, mucositis, neutrophil loss, and gastrointestinal toxicity. Additionally, patients of hormone receptor-positive breast cancer when given adjuvant endocrine therapy with anastrozole, aromatase inhibitor along with tualang honey (42% compared with 10% reduction) showed inhibition of breast cancer growth, cancer remission, more efficiently than anastrozole alone treatment in a randomized controlled trial (Hizan et al. 2018). Therefore, well-controlled trials must be designed due to the above mentioned interesting and positive results to directly gauge the potency of honey as an adjuvant treatment in different types of cancer.

10.8 Honey and Its Antidiabetic Properties

Diabetes mellitus is a multifaceted metabolic disease in which either body does not produce sufficient insulin or has insulin resistance wherein enough insulin is produced, but insulin receptors do not respond (Matteucci and Giampietro 2000). It involves differences in lipoprotein and carbohydrate metabolism shooting up glucose in body along with ketoacidosis which may be lethal (Brownlee 1995; Elgawish et al. 1996). Antidiabetic activity of honey has been observed in animal models to clinical trials (Al-Waili 2003; Erejuwa et al. 2010); therefore, it is a potent antidiabetic agent. Studies show that 0.2, 1.2, and 2.4 g/kg/day of honey exerted hypoglycemia in streptozotocin-induced diabetic rats by improving oxidative stress (Erejuwa et al. 2010). Another study shows reduction in glucose level by inhalation of honey as 60% (W/V) in type-2 diabetes mellitus (Al-Waili 2003), which in both studies is associated with the presence of fructose in honey (Erejuwa et al. 2012a, b). Fructose regulates insulin-response system which results in appropriate and normal blood glucose level. Glucose level is decreased by the delay of digestion and absorption by palatinose, an oligosaccharide sucrose as depicted by another hypothesis (Kashimura and Nagai 2007). Fructose ingestion depresses food intake besides delaying absorption, also associated with deferred gastric emptying. Slow fructose absorption in the intestine may lengthen the period of contact and interface between fructose and intestinal receptors resulting in satiety and in more macronutrients to be passed into the large intestine leading to limited intestinal absorption. Additionally, fructose is shown to decrease food intake which may help in the reduction of weight gain as per evidence (Erejuwa et al. 2012a, b). Hydrolysis of carbohydrates preceding to their absorption gives rise to monosaccharides like glucose, fructose, and galactose (Wright et al. 2003). Fructose is taken up by protein and energy-mediated diffusion via GLUT5 and GLUT2 receptors (Schürmann 2008). Glucose and fructose have been shown to increase GLUT2 mRNA expression. But fructose solely increases GLUT5 mRNA expression resulting in its fast absorption (Stelmańska 2008; Henry et al. 1991; Douard and Ferraris 2008). Research shows that mice induced with diabetes fed with fructose leads to hypoglycemic effect (Kwon et al.

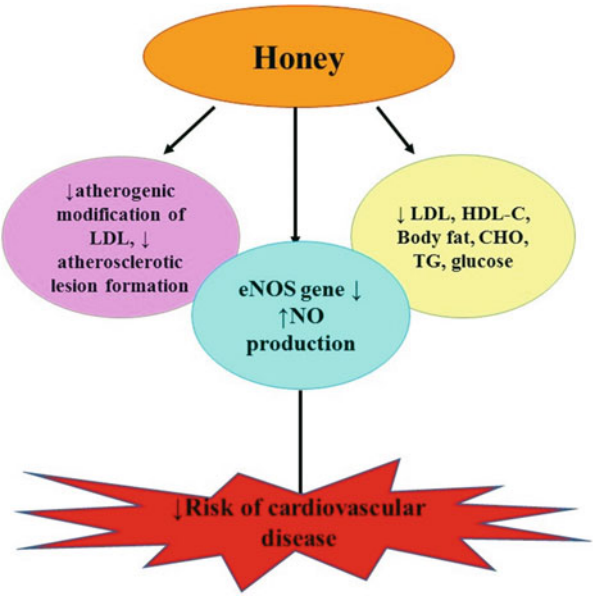

Fig. 10.6 Depicts the possible mechanisms of cardiovascular effects of honey

2008) and also regulates glucose level in the liver by stimulating the phosphorylation enzymes like glucokinase-activating hepatic glucose phosphorylation (Van Schaftingen and Davies 1991). The suppression of these enzymes results in suppression of glycogenolysis. Therefore, fructose regulates whole metabolism of glycogen and glucose depicting its essential vital role to govern hyperglycemia (Youn et al. 1987; Regan et al. 1980). Honey's hypoglycemic effect may be via mediating the insulin signaling pathway is another proposed mechanism (Erejuwa et al. 2010; Batumalaie et al. 2013) having PI3K/Akt as a key component (Carnero et al. 2008) by modulating cell cycle progression, cell survival, and cellular growth. The effect of honey extracts in pancreatic cells under hyperglycemic condition on Akt-activated insulin signaling pathway was recently investigated. Insulin resistance was associated with augmented levels of NF-κB, MAPK, insulin receptor substrate 1 (IRS-1), serine phosphorylation, and decreased Akt expression and insulin contents. Prophylactic treatment with honey and quercetin extract improves expression of Akt and decreased IRS-1 serine phosphorylation, NF-κB, and MAPK (Erejuwa et al. 2012a, b; Vincent et al. 2013; Carnero et al. 2008; Batumalaie et al. 2013) oxidative stress and hyperglycemia. In addition, it was found to alleviate triglycerides, hepatic transaminases, glycosylated hemoglobin (HbA1c), and enhanced HDL cholesterol (Erejuwa 2014). Figure 10.6 is showing the possible mechanisms of antidiabetic effects of honey. Further studies are necessary to explore the exact mechanisms involved in antidiabetic activity of honey.

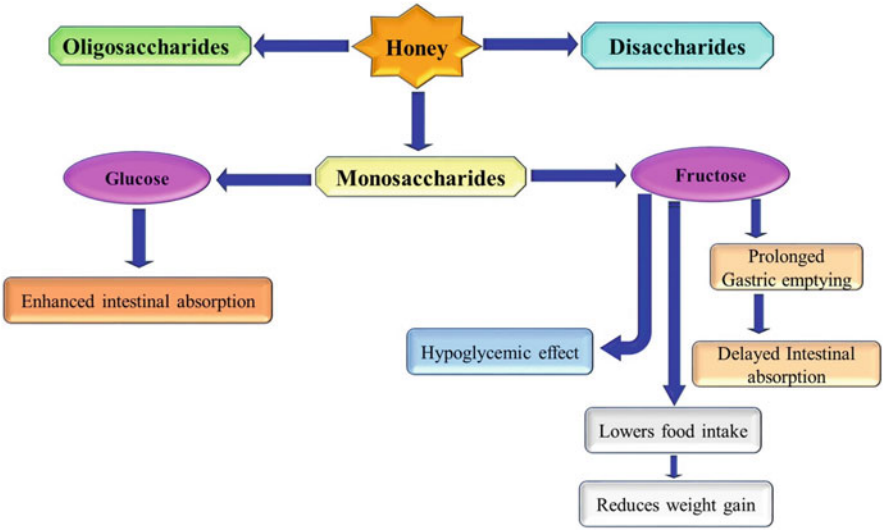

Fig. 10.7 Shows the possible mechanisms of antidiabetic effects of honey

10.9 Cardiovascular Effects of Honey

Some cardiovascular risk factors like blood glucose, cholesterol, CRP (C-reactive proteins), and body weight may be regulated by honey (Yaghoobi et al. 2008) constituents like glucose, fructose, and trace elements including copper and zinc. It was shown that 70 g of honey for 30 days given to cardiac patients and healthy human subjects had decreased LDL (low-density lipoprotein), high-density lipoprotein cholesterol (HDL-C), triacylglycerole, body fat, glucose, and cholesterol levels. Also, reduced CRP level activates nitric oxide production and therefore results in oxidative stress (Yaghoobi et al. 2008). Nitric oxide controls blood pressure, vascular tone, suppresses platelet accumulation, leukocyte accrual, and preclusion of cell proliferation in smooth muscles thereby showing cardioprotection (Naseem 2005). NO is a vital molecule for vasodilation in blood vessels and is initiated by factors which include acetylcholine, shear stress, and cytokines via eNOS synthesis. NO phosphorylates various proteins which cause relaxation of smooth muscles playing a critical role in renal regulation of extracellular fluid homeostasis controlling blood pressure and flow (Naseem 2005; Yoon et al. 2000). Honey modulates cardiovascular risks through some flavonoids by alleviating oxidative stress and augmenting NO bioavailability. Likewise, NO production by rutin increases eNOS gene expression and its activity. Another molecule of honey, Naringin, impedes intercellular adhesion molecule-1 (ICAM-1) expression induced by hypercholesterolemia on endothelial cells. Other major honey flavonoids like catechin and quercetin inhibit aortic atherosclerotic lesions development and atherogenic alteration of LDL (Afroz et al.

2016a). Prophylactic treatment with honey against isoproterenol-induced myocardial infarction in Wistar rats replenished antioxidant armory-like superoxide dismutase, glutathione peroxidase, and glutathione reductase and cardiotoxicity serum toxicity enzymes like creatine kinase-MB, lactate dehydrogenase, aspartate transaminase, and alanine transaminase (Afroz et al. 2016b), thereby providing defense from deleterious effects by free lethal radicals (Khalil et al. 2015). Honey-ameliorated cardiac troponin I (cTnI), triglycerides (TG), total cholesterol (TC), and lipid peroxidation (LPO) and increased antioxidant armory in rat myocardial infraction model (Khalil et al. 2015). Some of the probable mechanisms of cardiovascular effects of honey have been illustrated in Fig. 10.7.

To summarize the cardioprotective mechanism of flavonoids is either by lessening blood platelets activity, preclusion of oxidation of LDLs, and enhancing coronary vasodilatation (Khalil and Sulaiman 2010). Ahmed et al. demonstrated reduction of blood platelets activity in vitro on platelet accumulation and clotting (Ahmed et al. 2011). As honey inhibits all three coagulation cascades and decreases fibrinogen levels. Hence, considered exceptional for thwarting atherosclerotic plaques process resulting in cardiac disorders. However, lipid peroxidation has been found to play an essential role in pathology of atherosclerotic plaques (Ahmed et al. 2011). Therefore, phenolic compounds in honey have prophylactic and mitigating role by counteracting lipid peroxidation (Makedou et al. 2012; Cianciosi et al. 2018).

10.10 Neuropharmacological Effects of Honey

Research demonstrates honey as a mysterious gel having protective effects against gastrointestinal tract, liver, heart, reproductive system as well as anticancer, antidiabetic, antioxidant, antihypertensive, antimicrobial, anti-inflammation, immune mediating, wound healing, cardioprotective, and antineoplastic activities (Manyi-Loh et al. 2011; Cantarelli et al. 2008a, b; Amy and Carlos 1996; Mandal and Jaganathan 2009). However, research on the nootropic and pharmacological effects of honey on brain is rare. Nonetheless, ethno traditional and ancient belief is that honey is a memory-boosting food like Brahma rasayan, an Ayurvedic preparation believed to improve the life span and enhance remembrance, intelligence, attentiveness, and physical strength (Mishra 2011). Honey is found to build and develop complete central nervous system, predominantly among newborns and preschool age children leading to the enhancement of retention and growth, lessening nervousness, and improving intellectual performance later in life (Cantarelli et al. 2008a, b). Besides, human brain also undergoes maturation and restructuring of some edifices like hippocampus and cerebral cortex. Neurogenesis happens after postnatal development occurring principally during childhood, but can encompass puberty through adulthood as per reports (Oyefuga et al. 2012). There was a striking evidence seen in postmenopausal women receiving honey-enhanced instant memory but not after meddling in immediate memory comparatively (Othman et al. 2011). Other report shows normal diet supplemented with honey fed to 2-month-old rats

were examined for their brain function over 1-year period. It was shown honey-fed rats had better spatial memory which was assessed by object recognition tasks and were less anxious during all stages and significantly greater during 9 and 12 months compared to control rats (i.e., 9 and 12) (Chepulis et al. 2009). There was concomitant growth of superoxide dismutase (SOD) and glutathione reductase activity reduction of lipid peroxidation in brain tissue of rats supplemented for short- and long term with honey at a dose of 250 mg/kg body weight as reported previously deciphering role of honey as an anti-oxidative agent (Oyefuga et al. 2012). Additionally, hippocampal CA1 region is susceptible to oxidative stress by diminishing the amount of degenerated neuronal cells, which is prevented due to honey consumption (Cai et al. 2011). Neurodegenerative diseases like Alzheimer's disease (AD), mild cognitive impairment, Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD) have been accompanied by oxidative stress (Oyekunle et al. 2010). Oyekunle et al. conducted study for the first time that honey has neuropharmacological effect and is used as a nutraceutical in which rats were fed with diverse concentrations of honey (10, 20, and 40%) at a dose of 0.5 mL/100 g was found to significantly increase exploratory activities in a hole board test and locomotion, rearing, and grooming activities in an open-field test dose-dependently as compared to control group rats. The above-mentioned results demonstrate that honey consumption alleviates anxiety and causes excitation on the central nervous system even at largely nonsedative dose (Oyekunle et al. 2010). Another study reports the assessment of neurological activities of honey by measuring three-dimensional working memory either by Y-maze test or hypnosis induced by pentobarbital and its evaluation or using hole board and elevated plus maze tests to check anti-anxiolytic activities, or picrotoxin induced seizure model to calculate its convulsant potential or using hot plate and tail-flick tests to check nociceptive effect and forced swimming test is done to evaluate antidepressant effect. It was found out that honey has anti-anxiolytic, antinociceptive, anticonvulsant, and antidepressant potential and is used as a functional food (Akanmu et al. 2011). Neuropharmacological effects of honey provide insights to highlight the neurological features that are modulated by honey treatment. Excitatory neural systems like cholinergic and dopaminergic neurons are responsible for exploratory behaviors, while inhibitory neurons like γ-aminobutyric acid (GABA) are involved in anxious behavior (Ballenger 1999; Lamprea et al. 2003; Patel et al. 2012). Dopaminergic and nonopioid receptor binding involves voltage-gated sodium channel inhibition, instigation of the noradrenergic inhibitory system and/or serotonergic systems, and the GABAergic systems are some neuropharmacological mechanisms which are mediated by honey as experimental evidence supports (Oyekunle et al. 2010; Akanmu et al. 2011; Ballenger 1999; Lamprea et al. 2003; Patel et al. 2012; Young and Gauthier 1981). Besides neural effects, honey also shows neuroprotective effect in the cerebral focal ischemia model in rats through glial cells (Zárraga-Galindo et al. 2011). Likewise, ischemia-induced neuroinflammation was attenuated by honey via triggering microglia, and neuroinflammation is responsible for the growth of neurodegenerative diseases and neuronal injury linked with stroke (Frank-Cannon et al. 2009; Carson et al. 2006). Remarkably, cognitive losses

induced by ischemia due to neuroinflammation induced by microglia and/or astrocyte were downregulated by honey therapy (Akanmu et al. 2009).

10.11 Conclusion

Honey is an organic compound having proteins, enzymes, carbohydrates, amino acids, essential minerals, vitamins, and phytochemicals like phenolic acids and flavonoids that hold exciting pharmacological activities at in vitro and in vivo. Depending on geographical location, floral type, physical location, environment around, various bee species around, and then post collection processing and storage conditions define the elemental, phytochemical constituents, taste, and color of honey. So different types of honey are effective to inhibit malignant cell transformation via mitigating numerous signaling pathways, which include induction of apoptosis, anti-inflammatory pathway, redox signaling pathway, metastasis, and many more. It also diminishes the plasma level of fructosamine, glycosylated hemoglobin, and glucose in diabetes mellitus patients and attenuates numerous risk factors of cardiovascular disease, and benefits the nervous system as well. On the other hand, it is important to consider that it may contain some toxic compounds that should be avoided mainly in childhood. A deeper understanding of the factors and the mechanisms of honey effect will be of crucial importance to promote the consumption of this healthy food in the general population, to promote a healthy lifestyle, and to prevent the most common pathologies.

References

Afrin S, Forbes-Hernández TY, Cianciosi D et al (2019b) Strawberry tree honey as a new potential functional food. Part 2: strawberry tree honey increases ROS generation by suppressing Nrf2-ARE and NF-κB signaling pathways and decreases metabolic phenotypes and metastatic activity in colon cancer cells. J Funct Foods 57:477–487

Afrin S, Forbes-Hernandez TY, Gasparrini M et al (2017) Strawberry-tree honey induces growth inhibition of human colon cancer cells and increases ROS generation: a comparison with manuka honey. Int J Mol Sci 18:E613

Afrin S, Giampieri F, Cianciosi D et al (2019a) Strawberry tree honey as a new potential functional food. Part 1: strawberry tree honey reduces colon cancer cell proliferation and colony formation ability, inhibits cell cycle and promotes apoptosis by regulating EGFR and MAPKs signaling pathways. J Funct Foods 57:439–452

Afrin S, Giampieri F, Forbes-Hernandez TY et al (2018b) Manuka honey synergistically enhances the chemopreventive effect of 5-fluorouracil on human colon cancer cells by inducing oxidative stress and apoptosis, altering metabolic phenotypes and suppressing metastasis ability. Free Radic Biol Med 126:41–54

Afrin S, Giampieri F, Gasparrini M et al (2018a) The inhibitory effect of manuka honey on human colon cancer HCT-116 and LoVo cell growth. Part 2: induction of oxidative stress, alteration of mitochondrial respiration and glycolysis, and suppression of metastatic ability. Food Funct 9:2158–2170

Afrin S, Giampieri F, Gasparrini M et al (2018c) The inhibitory effect of manuka honey on human colon cancer HCT-116 and LoVo cell growth. Part 1: the suppression of cell proliferation, promotion of apoptosis and arrest of the cell cycle. Food Funct 9:2145–2157

Afroz R, Tanvir E, Little P (2016a) Honey-derived flavonoids: natural products for the prevention of atherosclerosis and cardiovascular diseases. Clin Exp Pharmacol 6:3

Afroz R, Tanvir EM, Karim M et al (2016b) Sundarban honey confers protection against isoproterenol-induced myocardial infarction in Wistar rats. BioMed Res Int 2016:6437641, 10 pages

Ahmed A, Khan RA, Azim MK, Saeed SA, Mesaik MA, Ahmed S, Imran I (2011) Effect of natural honey on human platelets and blood coagulation proteins. Pak J Pharm Sci 24:389–397

Ajibola A (2016) Physico-chemical and physiological values of honey and its importance as a functional food. Int J Food Nutr Sci 2:180–188

Ajibola A, Chamunorwa JP, Erlwanger KH (2012) Nutraceutical values of natural honey and its contribution to human health and wealth. Nutr Metab 9:1–12

Akanmu MA, Echeverry C, Rivera F, Dajas F (2009) Antioxidant and neuroprotective effects of Nigerian honey. Proceedings of the Nueroscience Meeting Planner, Washington, DC, USA

Akanmu MA, Olowookere TA, Atunwa SA et al (2011) Neuropharmacological effects of Nigerian honey in mice. Afr J Tradit Complement Altern Med 8:230–249

Alissandrakis E, Tarantilis PA, Harizanis PC, Polissiou M (2007) Aroma investigation of unifloral Greek citrus honey using solid-phase microextraction coupled to gas chromatographic-mass spectrometric analysis. Food Chem 100:396–404

Aliyu M, Odunola OA, Farooq AD et al (2012) Acacia honey modulates cell cycle progression, pro-inflammatory cytokines and calcium ions secretion in PC-3 cell line. J Cancer Sci Ther 4:401–407

Aliyu M, Odunola OA, Farooq AD et al (2013) Molecular mechanism of antiproliferation potential of acacia honey on NCI-H460 cell line. Nutr Cancer 65:296–304

Allan JM, Travis LB (2005) Mechanisms of therapy-related carcinogenesis. Nat Rev Cancer 5:943–955

Alvarez-Suarez JM, Giampieri F, Battino M (2013) Honey as a source of dietary antioxidants: structures, bioavailability and evidence of protective effects against human chronic diseases. Curr Med Chem 20:621–638

Alvarez-Suarez JM, Tulipani S, Diaz D, Estevez Y, Romandini S, Giampieri F et al (2010) Antioxidant and antimicrobial capacity of several monofloral Cuban honeys and their correlation with color, polyphenol content and other chemical compounds. Food Chem Toxicol 48:2490–2499

Al-Waili N (2003) Intrapulmonary administration of natural honey solution, hyperosmolar dextrose or hypoosmolar distill water to normal individuals and to patients with type-2 diabetes mellitus or hypertension: their effects on blood glucose level, plasma insulin and C-peptide, blood pressure and peaked expiratory flow rate. Eur J Med Res 8:295–303

Amy EJ, Carlos ME (1996) Medical uses of honey. Revista Biomédica 7:43–49

Aryappalli P, Al-Qubaisi SS, Attoub S et al (2017) The IL-6/ STAT3 signaling pathway is an early target of manuka honey-induced suppression of human breast cancer cells. Front Oncol 7:167

Attia WY, Gabry MS, El-Shaikh KA et al (2008) The anti-tumor effect of bee honey in Ehrlich ascite tumor model of mice is coincided with stimulation of the immune cells. Egypt J Immunol 15:169–183

Ballenger JC (1999) Current treatments of the anxiety disorders in adults. Biol Psychiatry 46:1579–1594

Batumalaie K, Zaman SS, Yusof MK, Shah II, Sekaran SD, Qvist R (2013) Effect of gelam honey on the oxidative stress-induced signaling pathways in pancreatic hamster cells. Int J Endocrinol 2013:367312, 10 pages

Belitz H, Grosch W, Schieberle P (2009) Food chemistry. 4th revised and extended edn. Berlin: Springer, 1070 pages

Bogdanov S, Jurendic T, Sieber R, Gallmann P (2008) Honey for nutrition and health: a review. Am J Col Nutr 27:677–689
Brownlee M (1995) Advanced protein glycosylation in diabetes and aging. Ann Rev Med 46:223–234
Cai M, Shin BY, Kim DH et al (2011) Neuroprotective effects of a traditional herbal prescription on transient cerebral global ischemia in gerbils. J Ethnopharmacol 138:723–730
Cantarelli MA, Pellerano RG, Marchevsky EJ, Camiña JM (2008a) Quality of honey from Argentina: study of chemical composition and trace elements. J Argent Chem Soc 96:33–41
Cantarelli MA, Pellerano RG, Marchevsky EJ, Camiña JM (2008b) Quality of honey from Argentina: study of chemical composition and trace elements. J Argent Chem Soc 96:33–41
Carlos AU, David H, Carmen G (2011) Role of honey polyphenols in health. J ApiProd ApiMed Sci 3:141–159
Carnero A, Blanco-Aparici C, Renner O, Link W, Leal J (2008) The PTEN/PI3K/AKT signalling pathway in cancer, therapeutic implications. Curr Cancer Drug Targets 8:187–198
Carson MJ, Thrash JC, Walter B (2006) The cellular response in neuroinflammation: the role of leukocytes, microglia and astrocytes in neuronal death and survival. Clin Neurosci Res 6:237–245
Castro-Vázquez L, Díaz-Maroto MC, Pérez-Coello MS (2007) Aroma composition and new chemical markers of Spanish citrus honeys. Food Chem 103:601–606
Charalambous M, Raftopoulos V, Paikousis L et al (2018) The effect of the use of thyme honey in minimizing radiation-induced oral mucositis in head and neck cancer patients: a randomized controlled trial. Eur J Oncol Nurs 34:89–97
Chepulis LM, Starkey NJ, Waas JR, Molan PC (2009) The effects of long-term honey, sucrose or sugar-free diets on memory and anxiety in rats. Physiol Behav 97:359–368
Cho HK, Jeong YM, Lee HS et al (2015) Effects of honey on oral mucositis in patients with head and neck cancer: a metaanalysis. Laryngoscope 125:2085–2092
Cianciosi D, Forbes-Hernández TY, Afrin S, Gasparrini M, Reboredo-Rodriguez P, Manna P, Zhang J, Bravo Lamas L, Martínez Flórez S, Agudo Toyos P, Quiles JL, Giampieri F, Battino M (2018) Phenolic compounds in honey and their associated health benefits: a review. Molecules 23:2322
Ciulu M, Solinas S, Floris I, Panzanelli A, Pilo MI, Piu PC, Spano N, Sanna G (2011) RP-HPLC determination of water-soluble vitamins in honey. Talanta 83:924–929
Crane E (1975) Honey, a comprehensive survey. William Heinemann, London, pp 439–488
D'Archivio M, Filesi C, Varì R, Scazzocchio B, Masella R (2010) Bioavailability of the polyphenols: status and controversies. Int J Mol Sci 11:1321–1342
Da Silva PM, Gauche C, Gonzaga LV, Costa ACO, Fett R (2016) Honey: chemical composition, stability and authenticity. Food Chem 196:309–323
Decaix C (1976) Comparative study of sucrose and honey. Chir Dent Fr 46:59–60
Douard V, Ferraris RP (2008) Regulation of the fructose transporter GLUT5 in health and disease. Am J Physiol Endocrinol Metab 295:E227–E237
Ediriweera ER, Premarathna NY (2012) Medicinal and cosmetic uses of Bee's Honey—a review. Ayu 33:178–182
Elattar TM, Virji AS (2000) The inhibitory effect of curcumin, genistein, quercetin and cisplatin on the growth of oral cancer cells in vitro. Anticancer Res 20:1733–1738
Elgawish A, Glomb M, Friedlander M, Monnier VM (1996) Involvement of hydrogen peroxide in collagen cross-linking by high glucose in vitro and in vivo. J Biol Chem 271:12964–12971
El-kott AF, Kandeel AA, El-Aziz SFA et al (2012) Anti-tumor effects of bee honey on PCNA and P53 expression in the rat hepatocarcinogenesis. Int J Cancer Res 8:130–139
Erejuwa OO (2014) Effect of honey in diabetes mellitus: matters arising. J Diabetes Metab Disord 13:23
Erejuwa OO, Gurtu S, Sulaiman SA, Ab Wahab MS, Sirajudeen KN, Salleh MS (2010) Hypoglycemic and antioxidant effects of honey supplementation in streptozotocininduced diabetic rats. Int J Vitam Nutr Res 80:74–82

Erejuwa OO, Sulaiman SA, Ab Wahab MS (2012a) Honey: a novel antioxidant. Molecules 17:4400–4423
Erejuwa OO, Sulaiman SA, Wahab MS (2012b) Fructose might contribute to the hypoglycemic effect of honey. Molecules 17:1900–1915
Eteraf-Oskouei T, Najafi M (2013) Traditional and modern uses of natural honey in human diseases: a review. Iran J Basic Med Sci 16:731–742
Fernandez-Cabezudo MJ, El-Kharrag R, Torab F et al (2013) Intravenous administration of manuka honey inhibits tumor growth and improves host survival when used in combination with chemotherapy in a melanoma mouse model. PLoS One 8:e55993
Fogh SE, Deshmukh S, Berk LB et al (2017) A randomized phase 2 trial of prophylactic manuka honey for the reduction of chemoradiation therapy-induced esophagitis during the treatment of lung cancer: results of NRG Oncology RTOG 1012. Int J Radiat Oncol Biol Phys 97:786–796
Frank-Cannon TC, Alto LT, McAlpine FE, Tansey MG (2009) Does neuroinflammation fan the flame in neurodegenerative diseases? Mol Neurodegener 4:1–47
Fukuda M, Kobayashi K, Hirono Y et al (2011) Jungle honey enhances immune function and antitumor activity. Evid Based Complement Alternat Med 2011:908743
González-Paramas AM, Gómez-Barex JA, García-Villanova RJ et al (2000) Geographical discrimination of honeys by using mineral composition and common chemical quality parameters. J Sci Food Agric 80:157–165
Gopalan C, Rama Sastri B, Balasubramanian S (1989) Nutritive value of Indian foods. National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, 156 pages
Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144:646–674
Hawley P, Hovan A, McGahan CE et al (2014) A randomized placebo-controlled trial of manuka honey for radiation induced oral mucositis. Support Care Cancer 22:751–761
Hegazi AG, Abd El-Hady FK (2007) Influence of honey on the suppression of human low-density lipoprotein (LDL) peroxidation (in vitro). Evid Based Complement Alternat Med 6:113–121
Henry RR, Crapo PA, Thorburn AW (1991) Current issues in fructose metabolism. Annu Rev Nutr 11:21–39
Hizan NS, Hassan NHM, Haron J et al (2018) Tualang honey adjunct with anastrozole improve parenchyma enhancement of breast tissue in breast cancer patients: a randomized controlled trial. Integr Med Res 7:322–327
Jaganathan SK, Mandal M (2010) Involvement of non-protein thiols, mitochondrial dysfunction, reactive oxygen species and p53 in honey-induced apoptosis. Investig New Drugs 28:624–633
Jaganathan SK, Mandal SM, Jana SK et al (2010a) Studies on the phenolic profiling, anti-oxidant and cytotoxic activity of Indian honey: in vitro evaluation. Nat Prod Res 24:1295–1306
Jaganathan SK, Mondhe D, Wani ZA et al (2010b) Effect of honey and eugenol on Ehrlich ascites and solid carcinoma. Biomed Res Int 2010:989163
Jaganathan SK, Mondhe D, Wani ZA et al (2014) Evaluation of selected honey and one of its phenolic constituent eugenol against L1210 lymphoid leukemia. Sci World J 2014:912051
Kadir EA, Sulaiman SA, Yahya NK et al (2013) Inhibitory effects of tualang honey on experimental breast cancer in rats: a preliminary study. Asian Pac J Cancer Prev 14:2249–2254
Kashimura J, Nagai Y (2007) Inhibitory effect of palatinose on glucose absorption in everted rat gut. J Nutr Sci Vitaminol 53:87–89
Kaškonienė V, Venskutonis PR (2010) Floral markers in honey of various botanical and geographic origins: a review. Compr Rev Food Sci Food Saf 9:620–634
Kennedy DO, Wightman EL (2011) Herbal extracts and phytochemicals: plant secondary metabolites and the enhancement of human brain function. Adv Nutr 2:32–50
Khalil MI, Alam N, Moniruzzaman M, Sulaiman SA, Gan SH (2011) Phenolic acid composition and antioxidant properties of Malaysian honeys. J Food Sci 76:C921–C928
Khalil MI, Sulaiman SA (2010) The potential role of honey and its polyphenols in preventing heart diseases: a review. Afr J Tradit Complement Altern Med 7:315–321

Khalil MI, Tanvir EM, Afroz R, Sulaiman SR, Gan SH (2015) Cardioprotective effects of tualang honey: amelioration of cholesterol and cardiac enzymes levels. BioMed Res Int 2015:286051, 8 pages
Khoo BY, Chua SL, Balaram P (2010) Apoptotic effects of chrysin in human cancer cell lines. Int J Mol Sci 11:2188–2199
Kwon S, Kim YJ, Kim MK (2008) Effect of fructose or sucrose feeding with different levels on oral glucose tolerance test in normal and type 2 diabetic rats. Nutr Res Pract 2:252–258
Lamprea MR, Cardenas FP, Silveira R, Walsh TJ, Morato S (2003) Effects of septal cholinergic lesion on rat exploratory behavior in an open-field. Braz J Med Biol Res 36:233–238
Mabrouk GM, Moselhy SS, Zohny SF et al (2002) Inhibition of methylnitrosourea (MNU) induced oxidative stress and carcinogenesis by orally administered bee honey and Nigella grains in Sprague Dawley rats. J Exp Clin Cancer Res 21:341–346
Makedou K, Iliadis S, Kara E, Gogou M, Feslikidis TH, Papageorgiou G (2012) Honey and its protective role against oxidation of human low density lipoproteins and total serum lipoproteins. Hippokratia 16:287
Mandal M, Jaganathan SK (2009) Antiproliferative effects of honey and of its polyphenols: a review. J Biomed Biotechnol 2009:830616, 13 pages
Manyi-Loh CE, Clarke AM, Ndip RN (2011) An overview of honey: therapeutic properties and contribution in nutrition and human health. Afr J Microbiol Res 5:844–852
Mato I, Huidobro JF, Simal-Lozano J, Sancho MT (2006) Rapid determination of nonaromatic organic acids in honey by capillary zone electrophoresis with direct ultraviolet detection. J Agric Food Chem 54:1541–1550
Matteucci E, Giampietro O (2000) Oxidative stress in families of type 1 diabetic patients. Diabetes Care 23:1182–1186
Miguel MG, Antunes MD, Faleiro ML (2017) Honey as a complementary medicine. Int Med Insights 12:1178633717702869
Mijanur RM, Gan SH, Khalil MI (2014) Neurological effects of honey: current and future prospects Evidence-based complementary and alternative medicine. eCAM 2014:958721
Mishra RN (2011) Rasayan—the ayurvedic perspective. Res J Pharm, Biol Chem Sci 2:269–282
Mofid B, Rezaeizadeh H, Termos A et al (2014) Effect of processed honey and royal jelly on cancer-related fatigue: a double-blind randomized clinical trial. Electron Physician 8:2475–2482
Molan PC (1999) Why honey is effective as a medicine. 1. Its use in modern medicine. Bee World 80:80–92
Molan PC (2001) Why honey is effective as a medicine. 2. The scientific explanation of its effects. Bee World 82:22–40
Moniruzzaman M, Yung AC, Rao PV, Hawlader MN, Azlan SA, Sulaiman SA, Gan SH (2014) Identification of phenolic acids and flavonoids in monofloral honey from Bangladesh by high performance liquid chromatography: determination of antioxidant capacity. BioMed Res Int 2014:737490
Morales P, Haza AI (2013) Antiproliferative and apoptotic effects of Spanish honeys. Pharmacogn Mag 9:231–237
Moskwa J, Borawska MH, Markiewicz-Zukowska R et al (2014) Polish natural bee honeys are anti-proliferative and anti-metastatic agents in human glioblastoma multiforme U87MG cell line. PLoS One 9:e90533
Nair HK, Rao KVK, Aalinkeel R, Mahajan S, Chawda R, Schwartz SA (2004) Inhibition of prostate cancer cell colony formation by the flavonoid quercetin correlates with modulation of specific regulatory genes. Clin Diagn Lab Immunol 11:63–69
Naseem KM (2005) The role of nitric oxide in cardiovascular diseases. Mol Asp Med 26:33–65
Nickel EH (1995) The definition of a mineral. Mineral J 17:346–349
Nielsen FH (1984) Ultratrace elements in nutrition. Annu Rev Nutr 4:21–41
Nyuk LC, Kandhasamy S (2020) A review on analytical methods for honey classification, identification and authentication. Intech Open, London

Olaitan PB, Adeleke EO, Ola OI (2007) Honey: a reservoir for microorganisms and an inhibitory agent for microbes. Afr Health Sci 7:159–165
Oroian M, Amariri S, Rosu A, Gutt G (2015) Classification of unifloral honey using multivariate analysis. J Essent Oil Res 27:533–544
Orsolić N, Knezević A, Sver L et al (2003) Influence of honey bee products on transplantable murine tumours. Vet Comp Oncol 1:216–226
Othman Z, Shafin N, Zakaria R, Hussain NHN, Mohammad WMZW (2011) Improvement in immediate memory after 16 weeks of tualang honey (Agro Mas) supplement in healthy postmenopausal women. Menopause 18:1219–1224
Oyefuga OH, Ajani EO, Salau BA, Agboola F, Adebawo OO (2012) Honey consumption and its anti-ageing potency in white Wister albino rats. Scholar J Biol Sci 1:15–19
Oyekunle OA, Akanmu MA, Ogundeji TP (2010) Evaluation of anxiolytic and novelty induced behaviours following bee-honey consumption in rats. J Neurosci Behav Health 2:38–43
Padayachee A, Netzel G, Netzel M, Day L, Zabaras D, Mikkelsen D, Gidley MJ (2012) Binding of polyphenols to plant cell wall analogues—part 2: phenolic acids. Food Chem 135:2287–2292
Patel JC, Rossignol E, Rice ME, Machold RP (2012) Opposing regulation of dopaminergic activity and exploratory motor behavior by forebrain and brainstem cholinergic circuits. Nat Commun 3:1172
Persano OL, Piro R (2004) Main European unifloral honeys: descriptive sheets. Apidologie 35: S38–S81
Peternelj TT, Coombes JS (2011) Antioxidant supplementation during exercise training: beneficial or detrimental? Sports Med 41:1043–1069
Petretto GL, Tuberoso CI, Vlahopoulou G et al (2016) Volatiles, color characteristics and other physico-chemical parameters of commercial Moroccan honeys. Nat Prod Res 30:286–292
Pichichero E, Cicconi R, Mattei M et al (2010) Acacia honey and chrysin reduce proliferation of melanoma cells through alterations in cell cycle progression. Int J Oncol 37:973–981
Porcza LM, Simms C, Chopra M (2016) Honey and cancer: current status and future directions. Diseases 4:E30
Puscas A, Hosu A, Cimpoiu C (2013) Application of a newly developed and validated high-performance thin-layer chromatographic method to control honey adulteration. J Chromatogr A 1272:132–135
Rao S, Hegde SK, Rao P et al (2017) Honey mitigates radiation-induced oral mucositis in head and neck cancer patients without affecting the tumor response. Foods 6:E77
Regan JJ, Doorneweerd DD, Gilboe DP, Nuttall FQ (1980) Influence of fructose on the glycogen synthase and phosphorylase systems in rat liver. Metabolism 29:965–969
Rice-Evans CA, Miller NJ (1996) Antioxidant activities of flavonoids as bioactive components of food. Biochem Soc Trans 24:790–795
Salleh MAM, Eshak Z, Ismail WIW (2017) Acacia honey induces apoptosis in human breast adenocarcinoma cell lines (MCF-7). J Teknol 79:9–16
Salucci M, Stivala LA, Maiani G, Bugianesi R, Vannini V (2002) Flavonoids uptake and their effect on cell cycle of human colon adenocarcinoma cells (CaCO2) Br. J Cancer 86:1645–1165
Samarghandian S, Afshari JT, Davoodi S (2011) Chrysin reduces proliferation and induces apoptosis in the human prostate cancer cell line pc-3. Clinics 66:1073–1079
Samarghandian S, Farkhondeh T, Samini F (2017) Honey and health: a review of recent clinical research. Pharm Res 9:121–127
Schürmann A (2008) Insight into the odd hexose transporters GLUT3, GLUT5, and GLUT7. Am J Physiol Endocrinol Metab 295:E225–E226
Song S, Su Z, Xu H et al (2017) Luteolin selectively kills STAT3 highly activated gastric cancer cells through enhancing the binding of STAT3 to SHP-1. Cell Death Dis 8:e2612
Spencer JP, Chowrimootoo G, Choudhury R, Debnam ES, Srai SK, Rice-Evans C (1999) The small intestine can both absorb and glucuronidate luminal flavonoids. FEBS Lett 458:224–230
Stelmańska E (2008) The important role of GLUT2 in intestinal sugar transport and absorption. Postepy Biochem 55:385–387

Swellam T, Miyanaga N, Onozawa M, Hattori K, Kawai K, Shimazui T, Akaza H (2003b) Antineoplastic activity of honey in an experimental bladder cancer implantation model: in vivo and in vitro studies. Int J Urol 10:213–219

Swellam T, Miyanaga N, Onozawa M et al (2003a) Antineoplastic activity of honey in an experimental bladder cancer implantation model: in vivo and in vitro studies. Int J Urol 10:213–219

Van Schaftingen E, Davies DR (1991) Fructose administration stimulates glucose phosphorylation in the livers of anesthetized rats. FASEB J 5:326–330

Vincent EE, Elder DJE, Curwen J, Kilgour E, Hers I, Tavaré JM (2013) Targeting non-small cell lung cancer cells by dual inhibition of the insulin receptor and the insulin-like growth factor-1 receptor. PLoS One 8:e66963

Vincēviča-Gaile Z (2010) Macro- and trace elements in honey. LLU-Raksti 25:54–66

Walle T (2004) Absorption and metabolism of flavonoids. Free Radic Biol Med 36:829–837

White JW, Doner LW (1980) Honey composition and properties. Beekeep US Agric 335:82–91

Wright EM, Martın MNG, Turk E (2003) Intestinal absorption in health and disease—sugars. Best Pract Res Clin Gastroenterol 17:943–956

Xu JL, Xia R, Sun ZH et al (2016) Effects of honey use on the management of radio/chemotherapy-induced mucositis: a meta-analysis of randomized controlled trials. Int J Oral Maxillofac Surg 45:1618–1625

Yaacob NS, Nengsih A, Norazmi M (2013) Tualang honey promotes apoptotic cell death induced by tamoxifen in breast cancer cell lines. Evid Based Complement Alternat Med 2013:989841

Yaghoobi N, Al-Waili N, Ghayour-Mobarhan M et al (2008) Natural honey and cardiovascular risk factors, effects on blood glucose, cholesterol, triacylglycerole, CRP, and body weight compared with sucrose. Sci World J 8:463–469

Yoon Y, Song J, Hong SH, Kim JQ (2000) Plasma nitric oxide concentrations and nitric oxide synthase gene polymorphisms in coronary artery disease. Clin Chem 46:1626–1630

Youn JH, Kaslow H, Bergman R (1987) Fructose effect to suppress hepatic glycogen degradation. J Biol Chem 262:11470–11477

Young SN, Gauthier S (1981) Effect of tryptophan administration on tryptophan, 5-hydroxyindoleacetic acid and indoleacetic acid in human lumbar and cisternal cerebrospinal fluid. J Neurol Neurosurg Psychiatr 44:323–328

Zárraga-Galindo N, Vergara-Aragón P, Rosales-Meléndez S et al (2011) Effects of bee products on pentylenetetrazole-induced seizures in the rat. Proc Western Pharmacol Soc 54:32–39

Zielinski L, Deja S, Jasicka-Misiak I, Kafarski P (2014) Chemometrics as a tool of origin determination of polish monofloral and multifloral honeys. J Agric Food Chem 62:2973–2981

Zugravu CA, Parvu M, Patrascu D, Stoian A (2009) Correlations between lead and cadmium pollution of honey and environmental heavy metal presence in two Romanian counties. Bull UASVM Agric 66:230–233

Therapeutic and Prophylactic Effects of Honey on Dermatitis and Related Disorders

11

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Keywords

Honey · Dermatitis · Atopic Dermatitis · Eczema · Manuka Honey · Skin

11.1 Introduction

Honey is a stuff formed when honey bees collect nectar and sweet deposits from plants and further alter and store it in the honeycomb. It is an ideal mixture without allowing other substance (water and other sweeteners) to add on.

Nectar is a floral deposit and sweets are non-floral deposits from plants and upon mixing, modification and storage in the honeycombs by honeybees (*Apis* and *Meliponini*) form an unusual item of very beneficial value called honey (Namias 2003; Al-jabri 2005). Environmental and climatic settings along with botanical source of nectar play a vital part in defining the composition and quality of honey. Honey, based on its quality, can affect the health and nutritional status of people.

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Honey has, antioxidant, anti-inflammatory, and antimicrobial properties, hence beneficial actions have been attributed to it. Interestingly, honey is getting attention for being a complementary and/or an alternate basis of treatment in modern medicine. It has the potential not to select for further resistant strains and is effective against antibiotic-sensitive and antibiotic-resistant strains of microorganisms (White 1992).

11.2 Typical Composition

The composition of honey is highly inconsistent. Table 11.1 contains the various ingredients in natural honey.

11.3 Types of Honey

Comb honey: Honey existing in its unique comb.

Extracted honey: It is generally offered in numerous forms: (1) liquid (2) crystallized or preserved (3) partially crystallized; defined as per the *US Department of Agriculture Standards for Grades.* This is generally referred as "Honey."

11.4 Sources of Honey

Source of honey defines its taste, fragrance, color, and ingredients, e.g.:

Floral: Specifies the nectar-producing flowers for the manufacture of honey.

Non-floral: Specifies extrafloral parts and honeydew (White 1992).

Geographic origin: Determines the area of manufacture and is in agreement with the *Code of Federal Regulations (CFR)*, mixtures containing honey of imported source must be labeled to specify their origin(s) (White 1992).

Table 11.1 Ingredients of honey

Ingredients	Average ± SD	Range
Fructose/glucose ratio	1.23 ± 0.126	0.76–1.86
Fructose (%)	38.38 ± 1.77	30.91–44.26
Glucose (%)	30.31 ± 3.04	22.89–40.75
Minerals (Ash) (%)	0.169 ± 0.15	0.020–1.028
Moisture (%)	17.2 ± 1.46	13.4–22.9
Reducing sugars (%)	76.75 ± 2.76	61.39–83.72
Sucrose (%)	1.31 ± 0.87	0.25–7.57
pH	3.91 ± 0.2	3.42–6.10
Total acidity (meq/kg)	29.12 ± 10.33	8.68–59.49
True protein (mg/100 g)	168.6 ± 70.9	57.7–567

11.5 Forms of Honey

Blended honey: A regular combination of equal to or more than two honeys contradictory in flavor, plant source, geographic origin, shade, or thickness.

Crystallized honey: Also called granulated honey. Its glucose portion has naturally crystallized as monohydrate.

Filtered honey: formed when extraneous solids and pollen grains are filtered from honey.

Organic honey: Created, treated, and parceled in agreement with *State and Federal regulations on honey and organic products* and certified by a *State Department of Agriculture* or an *independent organic farming certification organization.*

Raw honey: As occurring in the hive or as we get without adding heat after mining.

Commercially raw honey: Acquired by least treatment.

Strained honey: Honey containing pollens but without particulate material (bits of wax, propolis, etc.).

Whipped honey: Honey produced by precise crystallization to a flat smooth texture. Also called churned honey, whipped honey, creamed honey, or candied honey (White 1992; Molan 1992).

11.6 Honey Products

Although not meeting the compositional criteria for honey, these are the products containing entire or portion of honey;

Artificial honey: is a combination of dyed and flavored sweeteners to look like honey. This creation does not meet the criteria of honey or honey products.

Deionized honey: Formed on removal of selected ions by extra treatment.

Deproteinized honey: Formed on removal of protein by specific treatment.

Dried honey: On dehydration using drying aids and processing adjuncts, dried honey gets formed. It improves product stability.

Honey extract: It is formed by getting rid of particular constituents. The type of extract is defined by the nature of the constituent.

Honey spread: A diversity of eatable, very glutinous honey products. Spices, fruits, nuts, or aromas are sometimes blended with honey spread.

Ultrafiltered sweetener: When most proteins, enzymes, and polypeptides are removed from honey which do not pass through a submicron membrane give rise to ultrafiltered sweetner.

Honey dew: Secretions of insects who suck sap such as coccids or aphids and possibly secretions of fungi found on the faces of flora (White 1992; Molan 1992).

11.7 Therapeutic and Medicinal Properties of Honey

Natural therapeutic products are in use for times in the handling of numerous illnesses. Even though many have succeeded by orthodox pharmacological methods, presently there is renaissance in using honey and honey products by the common community. This unconventional division of medicine is named as *Apitherapy* (Ghosh and Playford 2003).

Honey has been treated as remedy by numerous humanities over extensive time. However, due to lack of technical report, it has inadequate use in medicine. Nowadays, honey is becoming standard as a trustworthy and operational healing agent. Its usefulness has been attributed to its antioxidant, anti-inflammatory, and antimicrobial properties as well as improving immunity (Krell 1996) (Table 11.2).

11.8 Skin Disorders

There are numerous skin disorders affecting *Homo sapiens*. The most usual disorders could have similar indications to some extent, thus it is imperative to know dissimilarities among them.

Table 11.2 Therapeutic assets of honey and their valuable effects

Properties	Attributions	Activities	References
Antimicrobial (antibacterial, antiviral, antifungal, antiparasitic)	High acidity, osmolarity, hydrogen peroxide, and nonperoxide components	Inhibits or kills	Bansal et al. (2005) Fahey and Stephenson (2002) Irish et al. (2006) Manyi-Loh et al. (2010) Ndip et al. (2007)
Anti-inflammatory	Leucocytes	Reduces inflammation and minimize scarring of wounds	Dunford et al. (2000)
Antioxidant	Phenolic acids Flavonoids	Prevents free radical formation Hunt-free radicals	Gheldof et al. (2002) Baltrušaityt et al. (2007)
Immunological	Leucocytes macrophages	Cytokine formation Substrate for glycolysis	Tonks et al. (2007)

11.8.1 Permanent Conditions

They last for an extended time. Roughly may jump in early age and last till maturity. In some subjects, the ailment will not be existing all the time but will come up few times.

1. Seborrheic dermatitis
   Well-known as *cradle cap* in babies. Slimy and crusty skin patches get formed on the skin, maximally on the scalp. It diminishes on its own and is usually harmless.
   In adults, it occurs at any place and is prone to appear and vanish for rest of lifetime. The exaggerated crust may be greasy, inflamed, and reddish. A yellow film may seem to be formed on surface of the skin as well. Relief from symptoms is brought by specific treatment (Fig. 11.1a) (Deleo 2004).
2. Moles
   Skin cells bunch up with surrounding tissue giving rise to common growths on the surface known as moles. Moles are often present and may develop over a period of time also. Moles are asymptomatic, but should be regularly checked for size, abnormality, and color change (Fig. 11.1b).
3. Rosacea
   It is generally linked with blush. Nevertheless, there are subcategories that give rise to other indications also:
   (a) *Erythematotelangiectatic rosacea* causes typical flushing and redness, prominence of blood vessels.
   (b) *Ocular rosacea* can lead to enflamed eyelids, irritated sore eyes, and indications similar to a style.
   (c) *Papulopustular rosacea* leads to swelling, inflammation, and breakouts looking like acne.
   (d) *Phymatous rosacea* leads to thickening and bumpy texture of skin.
   Rosacea has no treatment, but indications are to be treated to retain the disease (Fig. 11.1c) (Weiss et al. 2017).
4. Lupus
   Lupus is a complex sickness varying considerably. The illness affects the immune system, leading to pain and inflammation. Although it can upset any body part, signs on the skin comprise of sunburn-like rashes on the cheeks and nose, red spots on the surface, or rashes without itching or pain. Other symptoms include stiff or painful joints, fatigue, fever, and headache. Treatment includes various drugs intended to help diminish the injury caused by lupus (Fig. 11.1d) (Khan and Ahmed 2016).
5. Psoriasis
   It is an autoimmune condition. Indications classically comprise blotches of unusual skin. Exaggerated area is usually scaly, sore, and itchy which differ in severity and size.
   Mainly five forms exist:
   (a) *Plaque psoriasis* results in dense and inflamed patchy skin.
   (b) *Pustular psoriasis* gives rise to pustules enclosed by inflamed skin.

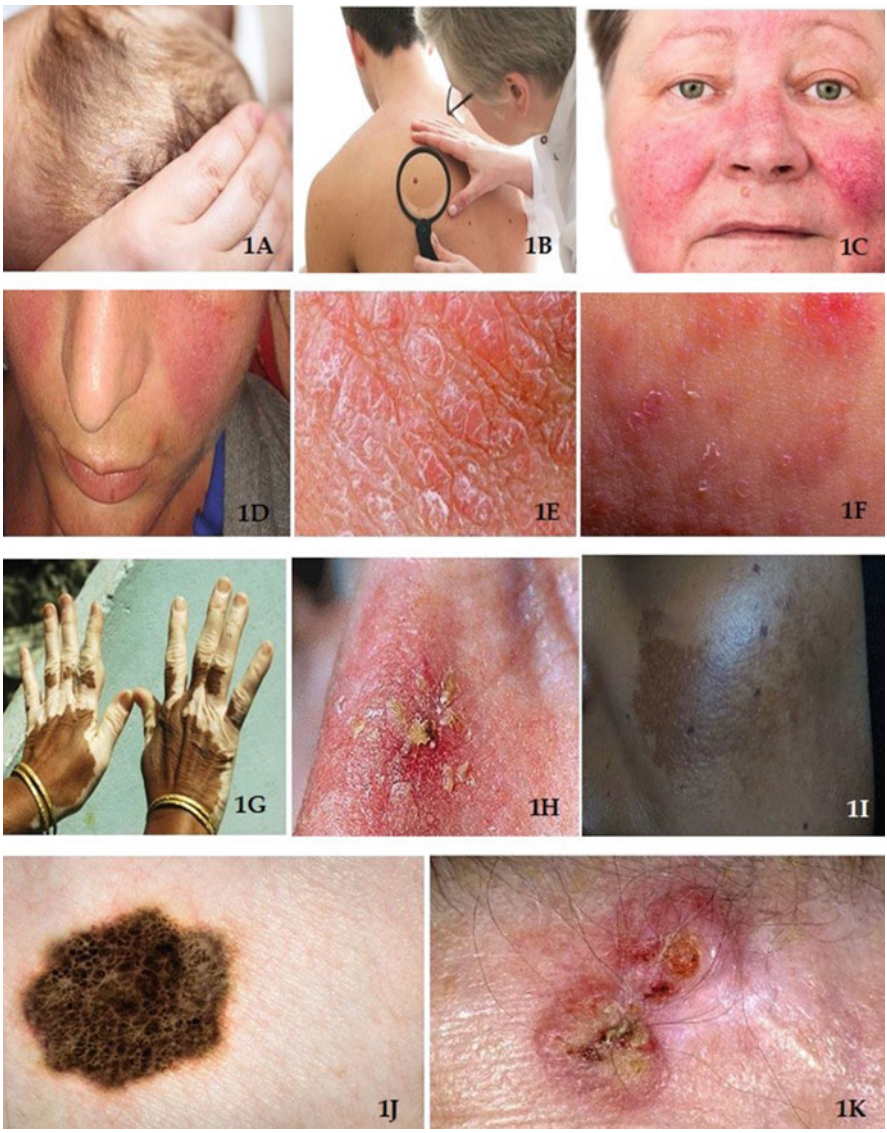

Fig. 11.1 Permanent skin disorders. (**a**) Seborrheic dermatitis, (**b**) Moles, (**c**) Rosacea, (**d**) Lupus, (**e**) Psoriasis, (**f**) Eczema, (**g**) Vitiligo, (**h**) Actinic keratosis, (**i**) Melasma, (**j**) Melanoma, and (**k**) squamous cell carcinoma

(c) *Erythodermic psoriasis* gives rise to severe burn-like patches of skin covering bulky parts of body.
(d) *Inverse psoriasis* gives rise to glossy inflamed rash in the skin folding.
(e) *Guttate psoriasis* gives rise to tiny red plugs on the torso, face, limbs, and scalp (Fig. 11.1e) (Menter et al. 2019).

6. Eczema
   It gives rise to rashes on wrists, ankles, legs, face, behind the elbows, neck, and on the scalp. The rashes may become thickened or bumpy and are very itchy. Generally, found in young age group. In adults, dry skin that is permanently itchy is formed which may cover the further area.

   There are limited diverse forms, triggering their own signs. It either disappears of its own or treated with medicines and ointments. There is no known cure for eczema (Fig. 11.1f) (Oakley et al. 2016).
7. Vitiligo
   Loss of skin color is referred as Vitiligo. Sunlight exposed parts of skin become patchy. People frequently lose their hair color early as well. Symptoms are restricted to one zone in some people while it spreads slowly to different parts in other individuals. Cure is not available. Although not right for everyone, there are rare surgical and medical cure possibilities (Fig. 11.1g) (Nguyen et al. 2016).
8. Actinic keratosis
   Also well known as *Solar keratosis*. It is a coarse, crusty patch on skin developed due to sun exposure for years. Typically, it is less than 2 cm. Usually found on scalp, face, neck, hands, and arms. Typically pink in color but can have a gray, tan, or brown base (Fig. 11.1h) (Ratushny et al. 2012).
9. Melasma
   It causes the appearance of dark patches, usually on the face. It is more frequent in people with darker complexion, pregnant women, and individuals having intense exposure to sunlight (chloasma). Staining of the skin is the only symptom. It may become permanent or go of its own within a year (Fig. 11.1i11.1) (Rajaratnam et al. 2010)
10. Melanoma
    Abnormal moles anywhere on the body that has multiple colors, asymmetrical shape, and irregularly shaped edges may lead to this grave and endangering skin cancer. It is more frequent in fair-skinned people (Fig. 11.1j) (Skin cancer 2019).
11. Squamous cell carcinoma
    This cancer occurs in parts unprotected to UV radiation, as ears, face, and posterior of hands. There is a raised bump which progresses from scaly, reddish patch of skin, and continues to grow. There is a presence of bleeding growth which doesn't heal or heals and then reappears (Fig. 11.1k) (Muranushi et al. 2015).

11.8.2 Temporary Conditions

1. Acne
   It is an extensive short-term skin disorder that may be treated with medicine or ointments.

   It has many forms:

   (a) Presence of pimples with pus at their apex known as *Pustules.*

(b) Presence of raised red bumps called as *Papules* caused due to infection in hair follicles.
(c) Presence of painful *Nodules* lying beneath the skin.
(d) Presence of large painful *Cysts* which are pus-filled lying under the skin (Fitz-Gibbon et al. 2013).

2. Hives (Urticaria)
They are itchy bumps that are upraised from the skin. It is elicited due to illnesses, stress, or even tight clothes or allergic reaction in the body. Antihistamines and preventive practices are used to treat the condition (Yao et al. 2015).
3. Warts
Human papillomavirus (HPV) is responsible for the cracks on the skin. Warts can appear on any part of the body and are contagious. Although they can appear anywhere but typically grow on the feet, hands, and joints. Usually perish on their own, but can be treated with medicated ointments or liquid nitrogen (King-Fan Loo and Yuk-Ming 2010).
4. Fungal nail infection
An ailment in which fungus lives near, under, and around the nails, usually in the feet. The fungal buildup causes the nail's edges to dissolve away, creating white-yellowish scaling and flaking on the exterior of the nails. An antifungal cream or other fungal medicine is usually used for treatment.
5. Cold sore
Typically found near the mouth. It is inflamed, fluid-filled sore. The blister is delicate or painful. Before the sore is visible there is itching or burning sensations on the site. They are produced by *herpes simplex virus*. The last until 2 weeks or may return. Cure comprises of medicines and creams (Usatine and Tinitigan 2010).
6. Candidiasis
Caused by the overgrowth of *Candida albicans* fungus causing exasperated areas of skin. Typically present in the armpits, groin, knees, and under folds of skin. Could be prevented with proper hygiene, evading antibiotics overuse, and home remedies (Hu et al. 2013).
7. Athlete's foot
A rash produced by a fungus that quickly grows in damp environments, such as athletic shoes. Indications comprise of inflamed, dry, and itchy skin. Presence of white, damp, and scaly skin between the toes or under the foot. Medicated ointments and good foot hygiene are the treatment options.

11.8.3 Internal Conditions

1. Carbuncle
When hair follicles get infected with *Staphylococcus aureus* bacteria, a sore, cross lump below the skin gets formed known as Carbuncle which quickly gets filled with pus and becomes puffy. Other indications include fever, itching, and

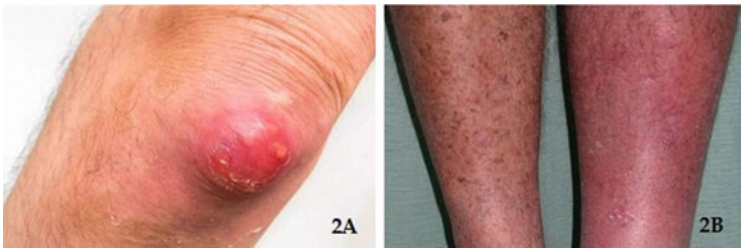

Fig. 11.2 Skin disorders due to internal conditions. (**a**) Carbuncle and (**b**) Cellulitis

tiredness. Treatment options are antibiotics, drainage, and antibacterial washes to which they respond well (Dhar 2019).

2. Cellulitis
   Infection in the inner coat of skin, bacterial in origin, which advances rapidly and could blowout speedily all over. Zone of skin may feel hot, painful, tender, become red, and swollen. It can occur all over the body but mostly in the legs. Mostly treated with antibiotics. This condition is considered as a medical emergency and severe infections may be life-threatening (Baiu and Melendez 2018) (Fig. 11.2).

11.8.4 Age-Related Conditions

11.8.4.1 In Children

1. Hemangiomas

They are bulge out large growths initially arising from small red scratches or bumps. Mostly found on the head, neck, or face of infants. While hemangiomas are mostly found on the skin, they can also be found on various organs especially on the liver. Although some need elimination but usually they disappear by the age of 10.

2. Measles

It is a viral disease, airborne, and highly infectious, with the indication of brown or red rash that spreads to other parts. It affects children and pregnant women. Other symptoms include small reddish spots inside the mouth, runny eyes and nose, fever, and cough. Measles disappears after 7–10 days, but indications should be treated (David et al. 2019).

3. Impetigo

It is the most common and contagious skin infections in young children. It results in blisters and itchy sores around the face and mouth which later on erupts and leaving

a scab which desiccates leaving a mark. The entire development typically takes 3 weeks without action which can be reduced to 1 week with medicines.

4. Dermatomyositis

It is an uncommon inflammatory disease of skin. Children aged between 5 and 15 and adults of 40–60 years of age contract the disease. Typical signs comprise of a purple rash on the elbows, nails, chest, face as well as muscle weakness, and swelling. It can be managed with medicines with no cure available.

11.8.4.2 In Adults

Shingles (Herpes Zoster)

It is caused secondary to chickenpox. It shocks with a pain, followed by a widespread pink, scorching rash within 2 days. Pain can be managed by antiviral treatment within the first 48 h. Vaccines are available against the disease (Ming-Chieh et al. 2017).

Seborrheic Keratoses

Usually as people age, harmless bumps of skin appear with no medical significance. They are black or brown uneven blotches stuck to the skin.

Age Spots

Also called as liver spots. They are level skin spots with extra coloring than adjacent zone due to sun exposure for years. These spots are asymptomatic but disliked by many. Many surgical and medical treatment options are available.

11.9 Benefits of Honey on Skin

It is an unbelievable factor that does miracles for skin. This delicious golden liquid is nature's answer to skin complications. Using honey for skin is at all times a worthy idea. Regular application of honey on the skin results beyond fancy. It has the following benefits on skin.

1. **Moisturizes and hydrates the skin**
   It intensely moisturizes skin because of the presence of factors which assist its entry into the skin thereby preparing and relaxing it and that is the reason why it is a constituent for a wide variety of beauty artifacts. Honey keeps skin moisturized so is a "humectant."
2. **Pore cleanser, anti-acne, and anti-pimples**
   It has antibacterial, antiseptic, and antioxidant possessions, so it assists in removing blackheads by eliminating dust from apertures. It then tightens and hydrates skin apertures for unblemished complexion. It not only removes extra oil from the skin but also clears clogged pores, which will otherwise lead to acne and pimples.

3. **Gentle exfoliator**
   Honey gently removes the dead skin cells and exfoliates skin which in turn creates a brighter complexion.
4. **Lightens scars**
   It reduces swelling and aids in skin healing. Moreover, damaged skin is repaired by its antioxidant property.
5. **Beneficial in sunburn**
   It recognized to be the top therapies for handling blisters and sunburns. It stimulates healing by lessening swelling and delivers nourishment to injured tissues.
6. **Antiaging property**
   Antioxidants limit folds and fine lines present on the face. It increases the elasticity of skin making it look young and radiant (Molan 2001a, b).

11.10 Honey: A Candid Antimicrobial or Microbicide for Skin Disorders

Honey has been documented for its skin-healing properties in old-style medicine. Persians recognized it to be operative in handling of inflammation, eczema, and wounds. The Greeks and Egyptians applied it topically to manage skin burns and wounds (Eteraf-Oskouei and Najafi 2013; Sepehr 2010). Microbes are linked with a series of dermatological ailments. *S. aureus, Pseudomonas aeruginosa* source infections in the wound. Presence of *Escherichia coli* and *S. aureus* is common in atopic dermatitis (Collier 2004; Ong 2014). Atopic dermatitis, psoriasis, Pityriasis versicolor, and Seborrheic dermatitis have the presence of *Malassezia yeasts* (Gaitanis et al. 2012). Conventional treatments like corticosteroids and UV radiation therapy cause skin thinning and skin cancer (Gasparro 2000).

In late 1800s, scientists stated antimicrobial properties of honey but with the advent of antibiotics in 1900s its role has faded away (Molan 2001a, b). Honey has again been researched again for medicinal use as the antibiotic-resistant microbial strains have emerged as a new global health concern (Molan 2001a, b; Fry and Barie 2011). In clinical setting, Manuka honey created by *Apis mellifera* in the form of impregnated dressings, ointments, and gamma-irradiated honey in gels is used topically for treatment of wound infections (Irish et al. 2011). In the Netherlands, wound care remedy is formed in form of Revamil honey and used in clinical practice (Kwakman et al. 2011). So it has a therapeutic potential of being used as antimicrobial agent for infective skin diseases.

A lot of in vitro research is going on antimicrobial action of honeys throughout the globe. A stingless bee, *Apis mellipodae*, in South Gondar, honey from Ethiopia is used in old-style medicine for the treatment of diseases along with skin disorders (Andualem 2013). Andualem et al. confirmed that *E. coli* and *S. aureus* were inhibited with minimal inhibitory concentration (MIC) of 12.5% and 6.25% honey individually by using agar well diffusion (Andualem 2013). In a study by Pimentel et al. using agar well diffusion assays, they showed that honey collected from

Melipona compressipes manaosensis during rains inhibited *E. coli* growth only in concentrated form, while honey collected during the dry season repressed the growth of *E. coli, S. aureus*, *Proteus vulgaris,* and *Klebsiella species* at diluted concentrations clearly indicating the effect of seasons on the antimicrobial action of honey. Seasons influence the health of the bee colonies hence affecting the antimicrobial action of product (Pimentel et al. 2013).

Scientists also compared *agar well diffusion* with *broth dilution assay* for accessing the antimicrobial properties of honey and concluded that the broth dilution assay gives better movement to antimicrobial factors in liquid broth compared to agar so a more sensitive method. By using HPLC, a flavonoid and antibacterial namely Rutin, was isolated from honey (Pimentel et al. 2013). Kuncic et al. found antibacterial action of Slovenian and pasture honeys obtained from Amazon, Brazil. They further reported the MIC of 2.5% against *S. aureus* and 50% against *Candida parapsilosis* and *Candida tropicalis. C. albicans* was not repressed Slovenian honeys (Kuncic et al. 2012). In other studies, growth of *C. albicans* was found to be inhibited by Jujube honey from Albaha, Saudi Arabia. Jujube honey is made by bees nourishing on *Ziziphus jujube* (Ansari et al. 2013; Al-Waili 2005a, b). These studies elucidate the prospectus of honey for treating *C. albicans* related skin disorders such as cutaneous candidiasis. The growth of methicillin-resistant *S. aureus (MRSA), S. aureus, Streptococcus pyogenes, P. aeruginosa,* and *E. coli* was found to be inhibited by Tualang honey, from *Apis dorsata*, Malaysia, in a broth dilution assay (Tan et al. 2009).

Scottish honey, called Portobello honey formed by honey bees in Portobello, Edinburgh, Scotland was tested for its antimicrobial action against *S. aureus, P. aeruginosa,* and *E. coli* using agar disc diffusion and a broth dilution assay in 2013. The results of agar method were inconclusive. The broth assay confirmed the activity of honey at MIC of 50% concluding that Portobello honey is a superior antimicrobial agent (Schneider et al. 2013a, b).

The antimicrobial activity of various honeys from different geographical locations was verified against *MRSA, S. aureus, E. coli, P. aeruginosa,* and *Acinetobacter baumannii* by Carnwath et al. using agar disc diffusion. All of them established antimicrobial activity, with Scottish heather honey being most effective, which suppressed growth of all microbes, with MICs going from $<2\%$ to 6% (Carnwath et al. 2014). Curiously, in vitro experiments establish that Scottish heather honey can backtrack antibiotic resistance, signifying that it might add to therapeutic effects when given in amalgamation with antibiotics (Jenkins and Cooper 2012). Honey might suppress *mecR1* gene product formation, a transducer in MRSA (Jenkins and Cooper 2012). Muller et al. stated that Manuka honey functioned along with rifampicin to inhibit the growth of *MRSA* and *S. aureus* growth (Muller et al. 2013). These experiments explain that honey has a capable antimicrobial action against skin pertinent microorganisms. Undeniably, there is comparable antimicrobial activity of sulfonamide family of antibiotics and honey from Iran (Tajik and Jalali 2009). The bacteria *S. aureus* is responsible for causing wound infections, furuncles, styes, and impetigo which is evidently repressed by honeys of different flower-patterned origins (Tajik and Jalali 2009). Honey may also be used in

the treatment of other skin disorders such as acne. Study of the antimicrobial activity of different types of honeys against other skin disease causing microorganisms ought to be stimulated.

11.11 Honey: Effective Against Microbial Pathogenicity of Skin Relevant Microbes

The capability of pathogenic microorganisms to root illnesses is partially due to making of pathogenicity elements, for instance, *S. aureus* makes enterotoxins, epidermolytic toxins, catalase, and hemolysins (a, b, g, and d). Alphatoxin (a-hemolysin) injures tissue throughout wound infections by generating apertures in host cell crusts and by prompting production of cytokines followed by apoptosis. Amazingly, recent in vitro readings have revealed that honey might decrease bacterial pathogenicity more than being bactericidal. In MRSA, Jenkins et al. described that Manuka honey reduced the expression of a-toxin, genes related to cell division, virulence genes, and quorum sensing genes (Jenkins et al. 2014).

In vitro studies by Lee et al. stated that Korean acacia, Korean polyfloral, and American clover honeys considerably repressed *E. coli* O157: HA biofilm creation at a concentration of 0.5%. Moreover, Korean acacia honey condensed the curli genes (*csgBAC*), quorum sensing genes (AI-2 importer, indole biosynthesis), and virulence genes (*LEE genes*) expression in the microbial strain at low concentrations (Lee et al. 2011a, b). In *P. aeruginosa, Manuka honey* reduced siderophore formation (Kronda et al. 2013). Studies showed cell shape alterations and cell lysis of *P. aeruginosa* following gestation with Manuka honey (Henriques et al. 2011). A honey flavonoid extract was found to disrupt integrity of membrane linked to virulence in *C. albicans* (Canonico et al. 2014). Manuka honey and Slovakian honeys significantly repressed *Proteus mirabilis* and *Enterobacter cloacae* biofilm formation (Majtan et al. 2014). These in vitro studies have made us understand the functions of honey to modify bacterial pathogenicity.

11.12 Honey: A Medicine for Dermatitis

Honey is a nourishing substance that is conventionally known for its medicinal assets. For thousands of years, it has been used in this perspective in varied populations and is still extensively popular. Lately, in vivo and in vitro trials have documented its antimicrobial attributes (Kwakman et al. 2008; Maddocks et al. 2013; Carter et al. 2016) and have been an established wound healer (Lee et al. 2011a, b). In specific, Manuka honey that is chiefly derivative of *Leptospermum scoparium* was revealed to intercept cell partition of *S. aureus* (Jenkins et al. 2011). Additionally, it constrains leukocyte infiltration and cyclooxygenase 2 production apart from inducing nitric oxide synthase expression (Leong et al. 2012) as well as inflammation facilitated through (TLR)1/TLR2 pathway (Tomblin et al. 2014).

Atopic dermatitis (AD) also called as *Eczema*, is a popular continuing atopic inflammatory skin illness described by recurrent episodes of maculopapular rash and intense pruritus (Geha et al. 2011). It is a common long-lasting atopic swelling of outer layer of skin. Its prevalence in children and adults is 10–20% and 1–3%, respectively, and typically the first indicator of a series of allergic ailments as allergic rhinitis and asthma in a marvel known as "Atopic March" (Schneider et al. 2013a, b). Irritating skin, inflammation, blisters, redness, etc. are among the symptoms. Variants of dermatitis are diaper dermatitis, seborrheic dermatitis, etc. During pathogenic process, eosinophils, lymphocytes, mast cells, and macrophages crosstalk with keratinocytes as well as provocations from the external surroundings (Muñiz 2008; Mortz et al. 2015). In 70–90% of patients, skin gets colonized by *S. aureus* compared to only 5% of normal population resulting in secondary infections of dermatitis lesions (Thomsen 2014). Additionally, the making of extremely inflammatory elements such as exotoxins (α, β, γ, and δ cytolysins) and enterotoxins (SEA to SEE) worsen ongoing inflammation because they act as superantigens.

The administration of AD is perplexing in subjects where existing medications don't solve complications (Eichenfield et al. 2014). Some subjects prefer natural preparations and claim to improve when they used honey topically. Still, there is no strong signal in the literature to back these statements mechanistically or clinically. The topical use of honey displayed large progress in the management of disease (Burlando and Cornara 2013; Muñiz 2008). Manuka honey modulates the skin inflammation in AD due to its immunoregulatory and antistaphylococcal properties. As per the experiments by Alangari et al., Manuka honey is helpful in handling the diseases, predominantly, AD. They also detected that IL4-induced CCL26 was considerably decreased in a dose-dependent way by honey (Alangari et al. 2017). CCL26 is a main role player in the harshness of AD (Kagami et al. 2003; Owczarek et al. 2010) along with other illnesses where eosinophils are mainly responsible as eosinophilic esophagitis (Blanchard et al. 2006) and asthma (Larose et al. 2015). It is extra powerful than eotaxin1 (CCL11) and eotaxin2 (CCL24) in appealing eosinophils (Provost et al. 2013). Chemokine receptor (CCR)3 is a collective anti-ligand to all chemokines (Ponath et al. 1996), its manifestation is increased in AD abrasions (Yawalkar et al. 1999), and its obstruction by antibodies prevents enrollment of eosinophils (Shen et al. 2006). CCR3 is also present on basophils (Uguccioni et al. 1997), mast cells (Ochi et al. 1999), and activated Th2 cells (Sallusto et al. 1997). Hence, decrease in IL4-induced CCL26 release by keratinocytes by virtue of honey might clarify the phenomenon. Manuka honey may show its properties on CCL26 at protein levels and needs more learning. Manuka honey contains some flavonoids which are structurally similar to thiazolidinediones, which are PPARg agonists and dose dependently reduce IL4-induced eotaxin release, nevertheless mechanism is unknown (Chan et al. 2013; Zhu et al. 2011). IL4 triggers IL8 release from epithelial cells (Strz et al. 1999; Mullings et al. 2001). This result is arbitrated through extracellular signal-regulated kinase (ERK) pathway, p38 mitogen-activated protein kinase (MAPK) (Ip et al. 2006). The large range of Manuka honey components seems to target

certain pathways, which are evident from the fact that honey did not control IL4-triggered IL8 release at mRNA or protein levels (Mullings et al. 2001).

Dermis and epidermis of AD patients contain an increased number of mast cells which in turn release histamine causing edema, local redness, and itching (Otsuka and Kabashima 2015; Simons and Simons 2011), disturbing skin barrier reliability (Wollenberg et al. 2014). The dose-dependent curb on histamine discharge by Manuka Honey may clarify its medical properties. Yet, its tool of action is controversial. Recently, in vitro experiments have shown that virulence genes in MRSA were repressed by Manuka honey, having extreme effect on *SEC3*, gene coding for Exocyst complex component 1 (Jenkins et al. 2014).

One more study has revealed the efficacy of olive oil, beeswax, and honey (in a ratio of 1:1:1 v/v), during the 7-day trial, for dermatitis, psoriasis, and skin fungal infections wherein they have observed that the mixture eradicated 50% of culture-positive patients infected with *C. albicans* probably owing to anti-inflammatory properties of the ingredients (Al-Waili 2003a, b, 2005a, b). As per another study, honey has bactericidal effect against *S. aureus* derived from canine dermatitis patients (Miorin et al. 2003). Natural raw honey is thought to quash the production and migration of inflammatory cells and in addition, it brings the enrichment in fibroblast/epithelial cell proliferation and production of pro-inflammatory cytokines thereby helping in the healing process through a twin influence on the inflammatory corridor (Mohammed et al. 2018). Honey from diverse floral varieties holds water content between 6 and 14% thus delivers the wanted moisture to the sore skin without producing maceration (Burlando and Cornara 2013; Mohammed et al. 2018).

11.13 Honey, Olive Oil, and Beeswax: Its Topical Application in Diaper Dermatitis

In infants, diaper dermatitis is the most usual skin disease (Hurwitz 1981), present in 25–65% of children (Jodan et al. 1986), and is initiated by wearing a diaper, urine, and feces in a joint venture (Atherton 2001). There is presence of *Candida spp.* in diseased group when related to healthy controls; however, colonization by *S. aureus* does not diverge among the two groups (Ferrazzini et al. 2003). The safety of topical antifungal agents is not proven. So eosin, zinc paste, and corticosteroids are being used topically for the treatment (Hoppe 1997).

Olive oil, beeswax, and honey when applied topically affect formation of cytokines as they contain antioxidants, flavonoids, antibacterial/antifungal compounds (Tuck and Hayball 2002). Several studies have confirmed the effectiveness of this mixture for the treatment of psoriasis, dermatitis, and skin fungal infections (Al-Waili 2003a, b, 2004). In a study conducted by Al-Waili et al., the outcome of consuming this mix to treat the illness was explored. Fungal culture was done on glucose agar. The topical adjunct was made by scrupulously mingling natural honey, olive oil, and beeswax (1:1:1 v/v). In a 7-day trial, the management

was operative in treatment and removed *C. albicans* from 50% of culture-positive subjects (Al-Waili 2005a, b).

Anti-inflammatory properties may be responsible for the relief of symptoms. Osmolality, acidity, and hydrogen peroxide production could be the chief reasons for antimicrobial activity of honey (Molan 1999). Honey reduces prostaglandins and upsurges nitric oxide in biotic fluids as per previous studies (Al-Waili 2003a, b). Olive oil has been revealed to upsurge nitric oxide and lessen arachidonic acid by rat macrophages (Visioli et al. 1998). Nitric oxide released by sweat glands has antimicrobial action (Al-Waili 2003a, b, Akh and Zefirov 1999). Consequently, topical application could increase antioxidant and anti-inflammatory activity, decrease prostaglandin production, rise of nitric oxide levels, and inhibition of microbial sprout (Tuck and Hayball 2002; Noa and Mas 1998).

11.14 Honey and Seborrheic Dermatitis

Honey has antibacterial, antifungal, and antioxidants actions and a high nutrient value. Seborrheic dermatitis is a long-lasting recurring inflammatory ailment resulting from metabolic changes in the cutaneous microflora including yeast, hyperactivity of sebaceous glands, and changed host immune task. In vitro studies have postulated that honey condensed the healing time via a twin influence on the inflammatory pathway. Firstly, honey defeats the manufacture and movement of inflammatory cells to place of damage; lastly, it increases propagation of fibroblasts and epithelial cells and making of proinflammatory cytokines (Tonks et al. 2001; Visavadia et al. 2008; Tomblin et al. 2014). In vitro studies revealed the effect of ingredients of honey on the making of inflammatory cytokines wherein they have established that Manuka honey improved manufacture of inflammatory cytokines, TNF-α, IL-1β/IL-6 via a TLR4-dependent mechanism (Riches 1996; Tonks et al. 2007).

Some other trials explored the possible usage of crude honey in the management of seborrheic dermatitis and dandruff when applied topically and concluded that crude honey could distinctly treat the disease and accompanying hair loss (Al-Waili 2001).

11.15 Honey: A Wound Healer

Wound is an injury that characteristically includes cut or breach of a membrane-like skin. The underline tissue gets damaged causing obliteration of tissue, disturbance of blood vessels, and spillage of blood constituents and hypoxia (Porth 2006). It results in ache, uneasiness, and can lead to dangerous ailment or even death (Cooper et al. 2002). Wound healing is intercepted by endogenous and exogenous influences. Bacterial attack and development favor its infection (Porth 2006). Honey is one of unsurprising preparations useful in the management of wounds. It helps in the regeneration of tissue growth with little or no scars formation hence enhances

wound healing (Al-Waili et al. 2011; Bogdanov 2016; Lund-Nielsen et al. 2011; Misirlioglu et al. 2003). Honey is safe for external use in dressings, etc., with no allergic reactions with fast eradication of wound stink, decrease in quantity of exudates, and purification from microbes (Lund-Nielsen et al. 2011; Misirlioglu et al. 2003; Efem et al. 1992). As per Efem et al., unprocessed honey inhibited most of the fungi and bacteria except *P. aeruginosa* and *Clostridium oedematiens.* Skin defects are usually covered by split-thickness skin grafting which can lead to electrolyte imbalances, infection, delay in healing, and scar formation during the process of healing. The practice of using honey-infused gauzes is safe and effective leading to less pain and faster epithelization time due to conservation of moisture which is indispensable for timely healing (Misirlioglu et al. 2003; Efem et al. 1992). Therapeutic honey dressings have gained extensive recognition due to the influence of honey for curing different types of wounds. *Medihoney® Dressing* with 95% dynamic Manuka honey and 5% calcium alginate, manufactured by Derma Sciences received FDA approval for use in treating wounds like diabetic foot ulcers, venous or arterial leg ulcers, partial or full-thickness pressure ulcers/sores, first and second partial-thickness burns, and traumatic and surgical wounds (Group Health Cooperative 2010). A study by Subrahmanyam et al. showed that honey assists in healing of burns faster with less problems when equated to orthodox silver sulfadiazine gauze dressing (Subrahmanyam 1991). The accelerative influence of honey in ulcer, wound, and skin burn healing course is linked to its intricate chemical composition, hygroscopicity, hypertonicity, and lower pH. Ali-Wali et al. described the antimicrobial activity, nutritional trait, acidity, osmotic influence, antioxidant, and immune-modulating possession, when taken orally, as the chief causes for wound healing signifying that tissue growth factor may be implicated, rather than growth stimulus as a result of wound acidification or enhanced tissue nourishment (Al-Waili et al. 2011).

11.16 Honey: Remedy in Skin Cancer

Management of malignant wounds due to various cancers is efficiently brought about by honey (Simon et al. 2006). Lund-Nielsen et al. proved that use of honey-coated bandages reduced wound size and enhanced wound hygiene in subjects with progressive malignant wounds (Lund-Nielsen et al. 2011).

Fernandez-Cabezudo et al. proved the antiproliferative and antiapoptotic activity of Manuka honey in three cell lines (Fernandez-Cabezudo et al. 2013). In addition, Pichichero et al. identified that acacia honey inducing cell cycle arrest in various cells (Pichichero et al. 2010). In vivo experiments in mice models have proved the tumor-constraining properties of intravenously administered Manuka honey apart from increasing overall animal survival and decreasing chemotherapy prompted toxicity with no changes in hematological and biochemical markers. In another study, UVB exposed keratinocytes treated with Tualang honey had decreased COX-2 and NF-κB activation and showed a drop in DNA damage. Hence, honey may protect the skin against the immunomodulatory and photo oncogenic effects of

sun exposure (Ahmad et al. 2012). Flavonoid and phenolic complexes in honey have been found to obstruct proliferation of melanoma cells through cell cycle seizure and apoptosis (Pichichero et al. 2010; Pichichero et al. 2011). Downregulation of tumor suppressor protein (p53) and antiapoptotic protein (Bcl-2) is brought about by honey in various neoplasms (Placzek et al. 2010).

Inflammation is a hallmark of cancer, so anti-inflammatory effects of honey define its anticarcinogenic activity (Jiang and Shapiro 2014). The anticarcinogenic traits of honey are encouraging but more investigation is essential for an elaborate understanding of the possible effectiveness of honey in the management or prevention of skin cancer.

11.17 Honey: Be Aware of Risks

Top physicians of the world are of the opinion that there might be a possible allergic reaction to honey, predominantly if someone is already allergic to bees or pollen. There could be allergic reactions to proteins secreted by bees and from proteins derived from plant pollen (Simon et al. 2009). So it is imperative to do a patch test before using new skin-care products and look for any redness or allergic reaction in the area of application. Not all skin products contain Manuka honey. Original products are certified by *New Zealand's Unique Manuka Factor Honey Association* and have Manuka factor trademark.

It is worth to take into consideration that according to *The American Academy of Pediatrics* honey is not recommended for infants below 1 year of age. Fresh, unrefined honey increases the occurrence of botulism in infants where the honey is contaminated with spores of *Clostridium botulinum*. After consumption, *C. botulinum* dormant bacterium can sprout, develop, and yield toxin in lower bowel of infants less than 1 year, as the intestinal flora is not advanced (Brown 2000).

11.18 Conclusion

Honey is a very intricate substance with possible therapeutic effect in dealing with various skin disorders. Upcoming explorations must intent to probe whether alike properties could be replicated with other types of honey. Manufacturing extra applied form of honey for topical skin use should accelerate scientific and medical inquiries. Additionally, these discoveries ought to built-up the gateway to the possible character of honey in the management of additional atopic disorders like allergic rhinitis or asthma. In this era of personalized medicine, it will not be wrong to say that honey doesn't do wonders on every individual. So people should not completely depend on honey for the treatment of skin disorders as there are allied allopathic ways to treat various disorders. Double-blinded randomized clinical trials are needed to find the most effective period, regularity, and kind of honey and more

severe technical experiments are needed to authorize its advantages a therapeutic invention of high value in dermatology.

References

Ahmad I, Jimenez H, Yaacob NS, Yusuf N (2012) Tualang honey protects keratinocytes from ultraviolet radiation-induced inflammation and DNA damage. Photochem Photobiol 88:1198–1204

Akh U, Zefirov L (1999) The physiological role of nitric oxide. Usp Fiziol Nauk 30:54–72

Alangari AA, Morris K, Lwaleed BA, Lau L, Jones K, Cooper R et al (2017) Honey is potentially effective in the treatment of atopic dermatitis: clinical and mechanistic studies. Immun Inflam Dis 5:190–199

Al-jabri AA (2005) Honey, milk and antibiotics. Afr J Biotechnol 4:1580–1587

Al-Waili N (2003a) Identification of nitric oxide in sweat and tear. FASEB J 17:342

Al-Waili N (2004) Safety and efficacy of topical application of honey, olive oil and beeswax mixture in patients with tinea versicolor, tinea cruris, tinea corporis and tinea faciei, Pilot study. Complement Ther Med 12:45–47

Al-Waili N, Salom K, Al-Ghamdi AA (2011) Honey for wound healing, ulcers, and burns; data supporting its use in clinical practice. Sci World J 11:766–787

Al-Waili NS (2001) Therapeutic and prophylactic effects of crude honey on chronic seborrheic dermatitis and dandruff. Eur J Med Res 06:306–308

Al-Waili NS (2003b) Topical application of natural honey, beeswax and olive oil mixture for atopic dermatitis or psoriasis: partially controlled, single-blinded study. Complement Ther Med 11:226–234

Al-Waili NS (2005a) Clinical and mycological benefits of topical application of honey, olive oil and beeswax in diaper dermatitis. Clin Microbiol Infect 11:160–163

Al-Waili NS (2005b) Mixture of honey, beeswax and olive oil inhibits growth of Staphylococcus aureus and Candida albicans. Arch Med Res 36:10e3

Andualem B (2013) Combined anti-bacterial activity of stingless bee (Apis mellipodae) honey and garlic (Allium sativum) extracts against standard and clinical pathogenic bacteria. Asian Pac J Trop Biomed 03:725e31

Ansari MJ, Al-Ghamdi A, Usmani S, Al-Waili NS, Sharma D, Nuru A et al (2013) Effect of jujube honey on Candida albicans growth and biofilm formation. Arch Med Res 44:352e60

Atherton J (2001) The aetiology and management of irritant diaper dermatitis. J Eur Acad Dermatol Venerol 15:1–4

Baiu L, Melendez E (2018) Skin abscess. JAMA 319:1405–1410

Baltrušaityt V, Venskutonis PR, Ceksteryte V (2007) Radical scavenging activity of different floral origin honey and bee bread phenolic extracts. Food Chem 101:502–514

Bansal V, Medhi B, Pandhi P (2005) Honey—a remedy rediscovered and its therapeutic utility. Kathmandu Univ Med J 3:305–309

Blanchard C, Wang N, Stringer KF, Mishra A, Fulkerson PC, Abonia JP, Jameson SC, Kirby C, Konikoff MR, Collins MH et al (2006) Eotaxin-3 and a uniquely conserved gene-expression profile in eosinophilic esophagitis. J Clin Invest 116:536–547

Bogdanov S (2016) Chapter 9. Honey in medicine. In: Book of honey, pp 1–27 (Bee Product Science, www.bee-hexagon.net)

Brown KL (2000) Control of bacterial spores. Br Med Bull 56:158–171

Burlando B, Cornara L (2013) Honey in dermatology and skin care: a review. J Cosmet Dermatol 12:306–313

Canonico B, Candiracci M, Citterio B, Curci R, Squarzoni S, Mazzoni A et al (2014) Honey flavonoids inhibit Candida albicans morphogenesis by affecting DNA behaviour and mitochondrial function. Future Microbiol 9:445–456

Carnwath R, Graham EM, Reynolds K, Pollock PJ (2014) The antimicrobial activity of honey against common equine wound bacterial isolates. Vet J 199:110–114
Carter DA, Blair SE, Cokcetin NN, Bouzo D, Brooks P, Schothauer R, Harry EJ (2016) Therapeutic Manuka honey: no longer so alternative. Front Microbiol 07:1–11
Chan CW, Deadman BJ, Manley-Harris M, Wilkins AL, Alber DG, Harry E (2013) Analysis of the flavonoid component of bioactive New Zealand manuka (Leptospermum scoparium) honey and the isolation, characterization and synthesis of an unusual pyrrole. Food Chem 141:1772–1781
Collier M (2004) Recognition and management of wound infections. World Wide Wounds. http://www.worldwidewounds.com/2004/january/Collier/Managementof-Wound-infections.html
Cooper RA, Molan PC, Harding KG (2002) The sensitivity to honey of Gram-positive cocci of clinical significance isolated from wounds. J Appl Microbiol 93:857–863
David AG, Janet KK, Mark RG (2019) Childhood MMR vaccination and the incidence rate of measles infection: a ten year longitudinal cohort study of American children born in the 1990s. BMC Pediatr 19:1–10
Deleo VA (2004) Photocontact dermatitis. Dermatol Ther 17:279–288. https://doi.org/10.1111/j.1396-0296.2004.04026.x
Dhar DA (2019) Folliculitis and skin abscesses. merckmanuals.com/home/skin-disorders/bacterial-skin-infections/folliculitis-and-skin-abscesses
Dunford C, Cooper RA, White RJ, Molan PC (2000) The use of honey in wound management. Nurs Stand 15:63–68
Efem SE, Udoh KT, Iwara CI (1992) The antimicrobial spectrum of honey and its clinical significance. Infection 20:227–229
Eichenfield LF, Tom WL, Berger TG, Krol A, Paller AS, Schwarzenberger K, Bergman JN, Chamlin SL, Cohen DE, Cooper KD (2014) Guidelines of care for the management of atopic dermatitis: section 2. Management and treatment of atopic dermatitis with topical therapies. J Am Acad Dermatol 71:116–132
Eteraf-Oskouei T, Najafi M (2013) Traditional and modern uses of natural honey in human diseases: a review. Iran J Basic Med Sci 16:731–742
Fahey JW, Stephenson KK (2002) Pinostrobin from honey and Thai ginger (Boesenbergia pandurata): potent flavonoid inducers of mammalian phase 2 chemo protective and antioxidant enzymes. J Agric Food Chem 50:7472–7476
Fernandez-Cabezudo MJ, El-Kharrag R, Torab F et al (2013) Intravenous administration of manuka honey inhibits tumor growth and improves host survival when used in combination with chemotherapy in a melanoma mouse model. PLoS One 8:e55993
Ferrazzini G, Kaiser R, Hirsig Chen K, Wehrli M (2003) Microbiological aspects of diaper dermatitis. Dermatology 206:136–141
Fitz-Gibbon F, Tomida S, Chiu BH, Nguyen L, Du C, Liu M, Li H (2013) Propionibacterium acnes strain populations in the human skin microbiome associated with acne. J Investig Dermatol 133:2152–2160
Fry DE, Barie PS (2011) The changing face of Staphylococcus aureus; a continuing surgical challenge. Surg Infect 12:191–203
Gaitanis G, Magiatis P, Hantschke M, Bassukas ID, Velegraki A (2012) The Malassezia genus in skin and systemic disease. Clin Microbiol Rev 25:106–141
Gasparro FP (2000) The role of PUVA in the treatment of psoriasis. Photobiology issues related to skin cancer incidence. Am J Clin Dermatol 01:337–348
Geha R, Niebuhr M, Heratizadeh A, Wichmann K, Satzger I, Werfel T (2011) Intrinsic alterations of proinflammatory mediators in unstimulated and TLR-2 stimulated keratinocytes from atopic dermatitis patients. Exp Dermatol 20:468–472
Gheldof N, Wang XH, Engeseth NJ (2002) Identification and quantification of antioxidant components of honeys from various floral sources. J Agric Food Chem 50:5870–5877
Ghosh S, Playford RJ (2003) Bioactive natural compounds for the treatment of gastrointestinal disorders. Clin Sci 104:547–556

Group Health Cooperative (2010) Clinical review criteria: medihoney dressing for wound management (medihoney primary dressing with active manuka honey, derma sciences medihoney dressing with active manuka honey)
Henriques AF, Jenkins RE, Burton NF, Cooper RA (2011) The effect of manuka honey on the structure of Pseudomonas aeruginosa. Eur J Clin Microbiol Infect Dis 30:167–171
Hoppe E (1997) Treatment of orophyaryngeal candidiasis and candidal diaper dermatitis in neonates and infants: review and reappraisal. Pediatr Infect Dis J 16:885–894
Hu H, Merenstein DJ, Wang C, Hamilton PR, Blackmon M, Chen H et al (2013) Impact of eating probiotic yogurt on colonization by Candida species of the oral and vaginal mucosa in HIV-infected and HIV-uninfected women. Mycopathologia 176:175–181
Hurwitz S (1981) Clinical pediatric dermatology, 1st edn. Saunders, Philadelphia, pp 27–30
Ip WK, Wong CK, Lam CWK (2006) Interleukin (IL)-4 and IL-13 up-regulate monocyte chemoattractant protein-1 expression in human bronchial epithelial cells: involvement of p38 mitogen-activated protein kinase, extracellular signal-regulated kinase 1/2 and Janus kinase-2 but not c-Jun NH2-terminal kinase 1/2 signalling pathways. Clin Exp Immunol 145:162–172
Irish J, Blair S, Carter DA (2011) The antibacterial activity of honey derived from Australian flora. PLoS One 6:e18229
Irish J, Carter DA, Shokohi T, Blair SE (2006) Honey has an antifungal effect against Candida species. Med Mycol 44:289–291
Jenkins R, Burton N, Cooper R (2011) Manuka honey inhibits cell division in methicillin-resistant Staphylococcus aureus. J Antimicrob Chemother 66:2536–2542
Jenkins R, Burton N, Cooper R (2014) Proteomic and genomic analysis of methicillin-resistant Staphylococcus aureus (MRSA) exposed to manuka honey in vitro demonstrated down-regulation of virulence markers. J Antimicrob Chemother 69:603–615
Jenkins RE, Cooper R (2012) Synergy between oxacillin and Manuka honey sensitizes methicillin-resistant Staphylococcus aureus to oxacillin. J Antimicrob Chemother 67:1405–1407
Jiang X, Shapiro DJ (2014) The immune system and inflammation in breast cancer. Mol Cell Endocrinol 382:673–682
Jodan W, Lawson K, Berg R, Franxman J, Marrer A (1986) Diaper dermatitis: frequency and severity among a general infant population. Pediatr Dermatol 3:198–207
Kagami S, Kakinuma T, Saeki H, Tsunemi Y, Fujita H, Nakamura K, Takekoshi T, Kishimoto M, Mitsui H, Torii H (2003) Significant elevation of serum levels of eotaxin-3/ CCL26, but not of eotaxin-2/CCL24, in patients with atopic dermatitis: serum eotaxin-3/CCL26 levels reflect the disease activity of atopic dermatitis. Clin Exp Immunol 134:309–313
Khan D, Ahmed SA (2016) The immune system is a natural target for estrogen action: opposing effects of estrogen in two prototypical autoimmune diseases. Front Immunol. https://doi.org/10.3389/fimmu.2015.00635
King-Fan Loo S, Yuk-Ming TW (2010) Warts (nongenital). Am Fam Physician 81:1008–1009
Krell R (1996) Value added products from beekeeping. Rome: FAO Agricultural Services Bulletin, 124, Food and Agriculture Organization of the United Nations, Rome, Italy
Kronda JM, Cooper RA, Maddocks SE (2013) Manuka honey inhibits siderophore production in Pseudomonas aeruginosa. J Appl Microbiol 115:86–90
Kuncic MK, Jaklic D, Lapanje A, Gunde-Cimerman N (2012) Antibacterial and antimycotic activities of Slovenian honeys. Br J Biomed Sci 69:154–158
Kwakman PH, teVelde AA, deBoer L, Vandenbroucke-Grauls CM, Zaat SA (2011) Two major medicinal honeys have different mechanisms of bactericidal activity. PLoS One 6:e17709
Kwakman PHS, Vanden Akker JPC, GuSclu A, Aslami H, Binnekade JM, de Boe LR, Boszhard L, Paulus F, Middelhoek P, teVelde AA et al (2008) Medical-grade honey kills antibiotic-resistant bacteria in vitro and eradicates skin colonization. Clin Infect Dis 46:1677–1682
Larose MC, Chakir J, Archambault AS, Joubert P, Provost V, Laviolette M, Flamand N (2015) Correlation between CCL26 production by human bronchial epithelial cells and airway eosinophils: involvement in patients with severe eosinophilic asthma. J Allergy Clin Immunol 136:904–913

Lee DS, Sinno S, Khachemoune A (2011a) Honey and wound healing: an overview. Am J Clin Dermatol 12(3):181–190
Lee JH, Park JH, Kim JA, Neupane GP, Cho MH, Lee CS et al (2011b) Low concentrations of honey reduce biofilm formation, quorum sensing, and virulence in Escherichia coli 0157:H7. Biofouling 27:1095–1104
Leong AG, Herst PM, Harper JL (2012) Indigenous New Zealand honeys exhibit multiple anti-inflammatory activities. Innate Immun 18:459–466
Lund-Nielsen B, Adamsen L, Kolmos HJ, Rørth M, Tolver A et al (2011) The effect of honey-coated bandages compared with silver-coated bandages on treatment of malignant wounds-a randomized study. Wound Repair Regen 19:664–674
Maddocks SE, Jenkins RE, Rowlands RS, Purdy KJ, Cooper RA (2013) Manuka honey inhibits adhesion and invasion of medically important wound bacteria in vitro. Future Microbiol 8:1523–1536
Majtan J, Bohova J, Horniackova M, Klaudiny J, Majtan V (2014) Antibiofilm effects of honey against wound pathogens Proteus mirabilis and Enterobacter cloacae. Phytother Res 28:69–75
Manyi-Loh CE, Clarke AM, Munzhelele T, Green E, Mkwetshana NF, Ndip RN (2010) Selected South African honeys and their extracts possess in vitro anti-Helicobacter pylori activity. Arch Med Res 41:324–331
Menter A et al (2019) Joint AAD-NPF guidelines of care for the management and treatment of psoriasis with biologics. J Am Acad Dermatol 80:1029–1072. https://doi.org/10.1016/j.jaad.2018.11.057
Ming-Chieh T, Wan-Ling C, Jau-Jiuan S, Chung-Chien H, Ben-Chang S, Li-Ting K, Herng-Ching L (2017) Increased risk of dementia following herpes zoster ophthalmicus. PLoS One 12(11): e0188490. https://doi.org/10.1371/journal.pone.0188490
Miorin PL, Levy NC, Custodio AR, Bretz WA, Marcucci MC (2003) Antibacterial activity of honey and propolis from Apis mellifera and Tetragonisca angustula against Staphylococcus aureus. J Appl Microbiol 95:913–920
Misirlioglu A, Eroglu S, Karacaoglan N, Akan M, Akoz T et al (2003) Use of honey as an adjunct in the healing of split-thickness skin graft donor site. Dermatol Surg 29:168–172
Mohammed HA, Al-Lenjawi B, Mendoza D (2018) Seborrheic dermatitis treatment with natural honey. Wounds Int 9:36–38
Molan PC (1992) The antibacterial activity of honey. Bee World 73:59–77
Molan PC (1999) The role of honey in the management of wounds. J Wound Care 8:415–418
Molan PC (2001a) Potential of honey in the treatment of wounds and burns. Am J Clin Dermatol 2:13–19
Molan PC (2001b) Why honey is effective as a medicine 2. The scientific explanation of its effects. Bee World 82:22–40
Mortz CG, Andersen KE, Dellgren C, Barington T, Bindslev-Jensen C (2015) Atopic dermatitis from adolescence to adulthood in the TOACS cohort: prevalence, persistence and comorbidities. Allergy 70:836–845
Muller P, Alber DG, Turnbull L, Schlothauer RC, Carter DA, Whitchurch CB et al (2013) Synergism between Medihoney and rifampicin against methicillin-resistant Staphylococcus aureus (MRSA). PLoS One 8:e57679
Mullings RE, Wilson SJ, Puddicombe SM, Lordan JL, Bucchieri F, Djukanovic R, Howarth P, Harper S, Holgate ST, Davies DE (2001) Signal transducer and activator of transcription 6 (STAT-6) expression and function in asthmatic bronchial epithelium. J Allergy Clin Immun 108:832–838
Muñiz AE (2008) Dermatitis. In: Pediatric emergency medicine. WB Saunders, Elsevier, Philadelphia, pp 859–870
Muranushi C, Olsen CM, Pandeya N, Green AC (2015) Aspirin and nonsteroidal anti-inflammatory drugs can prevent cutaneous squamous cell carcinoma: a systematic review and meta-analysis. J Invest Dermatol 135:975–983
Namias N (2003) Honey in the management of infections. Surg Infect 4:219–226

Ndip R, Malange TAE, Echakachi CM, Malongue A, Akoachere J-FTK, Ndip LM, Luma HN (2007) In vitro antimicrobial activity of selected honeys on clinical isolates of Helicobacter pylori. Afr Health Sci 7:228–231

Nguyen CM, Beroukhim K, Danesh MJ, Babikian AK, Leon A (2016) The psychosocial impact of acne, vitiligo, and psoriasis: a review. Clin Cos Invest Dermat 9:383–392

Noa M, Mas R (1998) Effect of D-002 on the pre-ulcerative phase of carrageenan-induced colonic ulceration in the Guinea pig. J Pharm Pharmacol 50:549–553

Oakley A et al (2016) Allergic contact dermatitis. dermnetnz.org/topics/allergic-contact-dermatitis/

Ochi H, Hirani WM, Yuan Q, Friend DS, Austen KF, Boyce JA (1999) T helper cell type 2 cytokine-mediated comitogenic responses and CCR3 expression during differentiation of human mast cells in vitro. J Exp Med 190:267–280

Ong PY (2014) Recurrent MRSA skin infections in atopic dermatitis. J Allergy Clin Immunol Pract 2:396–399

Otsuka A, Kabashima K (2015) Mast cells and basophils in cutaneous immune responses. Allergy 70:131–140

Owczarek W, Paplinska M, Targowski T, Jahnz-Rozyk K, Paluchowska E, Kucharczyk A, Kasztalewicz B (2010) Analysis of eotaxin 1/CCL11, eotaxin 2/CCL24 and eotaxin 3/CCL26 expression in lesional and non-lesional skin of patients with atopic dermatitis. Cytokine 50:181–185

Pichichero E, Cicconi R, Mattei M, Canini A (2011) Chrysin-induced apoptosis is mediated through p38 and Bax activation in B16-F1 and A375 melanoma cells. Int J Oncol 38:473–483

Pichichero E, Cicconi R, Mattei M, Muzi MG, Canini A (2010) Acacia honey and chrysin reduce proliferation of melanoma cells through alterations in cell cycle progression. Int J Oncol 37:973–981

Pimentel RB, da Costa CA, Alburquerque PM, Junior SD (2013) Antimicrobial activity and rutin identification of honey produced by the stingless bee Melipona compressipes manaosensis and commercial honey. BMC Complement Altern Med 13:151

Placzek WJ, Wei J, Kitada S, Zhai D, Reed JC, Pellecchia M (2010) A survey of the anti-apoptotic Bcl-2 subfamily expression in cancer types provides a platform to predict the efficacy of Bcl-2 antagonists in cancer therapy. Cell Death Dis 1:e40

Ponath PD, Qin S, Post TW, Wang J, Wu L, Gerard NP, Newman W, Gerard C, Mackay CR (1996) Molecular cloning and characterization of a human eotaxin receptor expressed selectively on eosinophils. J Exp Med 183:2437–2448

Porth CM (2006) Essentials of pathophysiology: concepts of altered health states. Lippincott Williams & Wilkins, Philadelphia

Provost V, Larose MC, Langlois A, Rola-Pleszczynski M, Flamand N, Laviolette M (2013) CCL26/eotaxin-3 is more effective to induce the migration of eosinophils of asthmatics than CCL11/eotaxin-1 and CCL24/eotaxin-2. J Leukoc Biol 94:213–222

Rajaratnam R et al (2010) Interventions for melasma. Cochrane Database Syst Rev (7):CD003583. https://doi.org/10.1002/14651858.CD003583.pub2

Ratushny V et al (2012) From keratinocyte to cancer: the pathogenesis and modeling of cutaneous squamous cell carcinoma. J Clin Invest 122(2):464–472. https://doi.org/10.1172/JCI57415

Riches DW (1996) Macrophage involvement in wound repair, remodeling and fibrosis. In: Clarke R (ed) The molecular and cellular biology of wound repair. Plenum Press, New York, NY, pp 95–141

Sallusto F, Mackay CR, Lanzavecchia A (1997) Selective expression of the eotaxin receptor CCR3 by human T helper 2 cells. Sci Am Assoc Advanc Sci 277:2005–2007

Schneider L, Tilles S, Lio P, Boguniewicz M, Beck L, LeBovidge J, Novak N, Bernstein D, Blessing-Moore J, Khan D et al (2013a) Atopic dermatitis: a practice parameter update 2012. J Allergy Clin Immunol 131:295–299

Schneider M, Coyle S, Warnock M, Gow I, Fyfe L (2013b) Anti-microbial activity and composition of manuka and Portobello honey. Phytother Res 27:1162–1168

Sepehr S (2010) The most important medicinal uses of honey, and its side effects in the book of the Canon by Avicenna, and in the modern medical literature: a comparative study. J Api Prod Api Med Sci 2:43
Shen HH, Xu F, Zhang GS, Wang SB, Xu WH (2006) CCR3 monoclonal antibody inhibits airway eosinophilic inflammation and mucus overproduction in a mouse model of asthma. Acta Pharmacol Sin 27:1594–1599
Simon A, Sofka K, Wiszniewsky G, Blaser G, Bode U et al (2006) Wound care with antibacterial honey (Medihoney) in pediatric hematology oncology. Support Care Cancer 14:91–97
Simon A, Traynor K, Santos K, Blaser G, Bode U, Molan P (2009) Medical honey for wound care-still the latest resort? Evidence Based Complement Alternat Med 6:165–173
Simons FER, Simons KJ (2011) Histamine and H1- antihistamines: celebrating a century of progress. J Allergy Clin Immun 128:1139–1150
Skin Cancer (2019) https://www.cdc.gov/cancer/skin/index.htm
Strz I, Mio T, Adachi Y, Robbins RA, Romberger DJ, Rennard SI (1999) IL-4 and IL-13 stimulate human bronchial epithelial cells to release IL-8. Inflammation 23:545–555
Subrahmanyam M (1991) Topical application of honey in treatment of burns. Br J Surg 78:497–498
Tajik H, Jalali FSS (2009) In vitro evaluation of antimicrobial efficacy of natural honey in comparison with sulfonamide derivatives. J Anim Vet Adv 8:23–25
Tan HT, Rahman RA, Gan SH, Halim AS, Hassan SA, Sulaiman SA et al (2009) The antibacterial properties of Malaysian tualang honey against wound and enteric microorganisms in comparison to manuka honey. BMC Complement Altern Med 9:34
Thomsen SF (2014) Atopic dermatitis: natural history, diagnosis, and treatment. ISRN Allergy 2014:1–7
Tomblin V, Ferguson LR, Han DY et al (2014) Potential pathway of anti-inflammatory effect by New Zealand honeys. Int J Gen Med 7:149–158
Tonks A, Cooper RA, Price AJ et al (2001) Stimulation of TNF-alpha release in monocytes by honey. Cytokine 14:240–242
Tonks AJ, Dudley E, Porter NG, Parton J, Brazier J, Smith EL, Tonks A (2007) A 5.8 kDa component of manuka honey stimulates immune cells via TLR4. J Leukoc Biol 82:1147–1155
Tuck K, Hayball P (2002) Major phenolic compounds in olive oil. J Nutr Biochem 13:636–644
Uguccioni M, Mackay CR, Ochensberger B, Loetscher P, Rhis S, LaRosa GJ, Rao P, Ponath PD, Baggiolini M, Dahinden CA (1997) High expression of the chemokine receptor CCR3 in human blood basophils. Role in activation by eotaxin, MCP-4, and other chemokines. J Clin Invest 100:1137–1143
Usatine R, Tinitigan R (2010) Nongenital herpes simplex virus. Am Fam Physician 82:1075–1082
Visavadia BG, Honeysett J, Danford MH (2008) Manuka honey dressing: an effective treatment for chronic wound infections. Br J Oral Maxillofac Surg 46:55–56
Visioli F, Bellosta S, Galli C (1998) The bitter principle of olives enhances nitric oxide production by mouse macrophages. Life Sci 62:541–546
Weiss E et al (2017) Diet and rosacea: the role of dietary change in the management of rosacea. Dermatol Pract Concept 7(4):31–37. https://doi.org/10.5826/dpc.0704a08
White JW (1992) Quality evaluation of honey: role of HMF and diastase assays. Am Bee J 132:737–743
Wollenberg A, Seba A, Antal AS (2014) Immunological and molecular targets of atopic dermatitis treatment. Br J Dermatol 170:7–11
Yao Q, Ye Y, Liu X, Qin Z, Liu Z (2015) The effectiveness and safety of acupuncture for patients with chronic urticarial: a systematic review. Biomed Res Int 2016:1–7
Yawalkar N, Uguccioni J, Scharer J, Braunwalder J, Karlen S, Dewald B, Braathen LR, Baggiolini M (1999) Enhanced expression of eotaxin and CCR3 in atopic dermatitis. J Invest Dermatol 113:43–48
Zhu M, Flynt L, Ghosh S, Mellema M, Banerjee A, Williams E, Panettieri RA, Shore SA (2011) Antiinflammatory effects of thiazolidinediones in human airway smooth muscle cells. Am J Respir Cell Mol Biol 45:111–119

Honey Intake and Risk of CVDs: A Mechanistic Disclosure

12

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Abstract

Honey is a naturally occurring sweet substance with gummy consistency produced by honey bees. It is also formed by nectar-producing plants and trees. Honey is well known for its usefulness which is mainly attributed to its flavonoid components. Cardiovascular diseases are the single largest cause of early mortality, especially in developed countries but are also emerging in developing countries at an alarming rate. In most of the cases, atherosclerosis has remained as its main underlying pathological cause. The basic pathology behind atherosclerosis is due to a chronic inflammatory process in the endothelium of arteries. Oxidation of the neo-intimal lipids that releases oxidized molecular species

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eventually leads to vascular atherosclerotic changes. Formation of reactive oxygen species (ROS) in excess amounts due to oxidation of DNA, proteins, carbohydrates, lipids and other bio molecules adds on to these vascular changes. Antioxidant defence mechanisms of our body fail to balance this surplus ROS production, hence producing oxidative stress. Oxidative stress is associated with various metabolic disorders like cardiovascular diseases, systemic hypertension, diabetes mellitus, etc. Honey plays a protective role in a number of metabolic disorders as well as in cardiovascular health mainly due to its rich flavonoid and antioxidant content. Honey is believed to decrease the risk of developing elevated blood pressure. Intake of honey has the capability to decrease systolic blood pressure in healthy subjects. Honey also balances the lipid profile by increasing high-density lipoprotein (HDL) levels and reducing the levels of triglycerides and very low-density lipoproteins (VLDL). In this chapter, we have discussed how honey intake can help in prevention of cardiovascular disorders that has significant mortality and morbidity. A balance between the antioxidant intake and free radical injuries can help our body's defence mechanism to overcome different forms of oxidative stress. Honey may prove as a useful substance that can help in the improvement of biochemical imbalances in the human body.

Keywords

Honey · Flavanoids · Oxidative stress · Atherosclerosis

12.1 Introduction

Honey is a naturally occurring sweet substance with gummy consistency produced by honey bees. It is secreted by all types of honey bees but is formed particularly by the species Apis mellifera (Cortes et al. 2011). It is also produced from nectar or otherwise from exudates of nectar-producing plants and trees (Alvarez-Suarez et al. 2010). Honey is well known for its usefulness attributed mainly to its flavonoid components. Honey samples of various types contain a good quantity of flavonoids such as catechin, kaempferol, rutin, chrysin, acacetin, luteolin, quercetin, etc. (Khalil and Sulaiman 2010). Flavonoids are the compounds with strong biological properties that are beneficial for avoidance of many chronic diseases like atherosclerosis and other forms of cardiovascular diseases (Gross 2004). Most of these flavonoids have therapeutic benefits due to their beneficial properties and may be used as pharmacological agents (Khalil and Sulaiman 2010). According to the recent research, it was established that catechin and quercetin are the two major flavonoids present in honey (Afroz et al. 2016). Aortic atherosclerotic lesion formation is inhibited by the consumption of these flavonoids. These also had an inhibitory outcome on the development of various atherogenic variations of LDLs (Hayek et al. 1997). In addition to the preventive properties against atherosclerosis, honey flavonoids also tend to have anti inflammatory, antioxidant, antiproliferative and even antiplatelet actions. These are believed to lower both cholesterol and blood

pressure levels (Gross 2004). Nitric oxide is present in the vascular endothelium and is produced by nitric oxide synthase. It has good vasodilator properties and prevents atherosclerosis. According to the oxidation hypothesis the main mechanism for atherosclerosis is the oxidative modification of LDLs producing immunogenic stimulus which further results in migration of monocytes to various vessel walls. This is followed by phagocytic uptake of oxidized LDL molecules by macrophages (Nigro et al. 2006). Widespread oxidation of LDLs leads to their accumulation and formation of aggregates (Hoff and O'Neil 1991; Maor et al. 1997; Hayek et al. 1997). In a vascular atherosclerotic injury, both of these modified forms of LDLs have been seen.

Oxidative stress plays a vital role in the pathogenesis of cardiovascular diseases including the initial process of atherosclerosis. Progression of endothelial dysfunction is hindered by nitric oxide. Its deficiency results in the development of atherosclerosis and CVDs. Certain flavonoids like rutin that are present in honey of stingless bees promotes NO production. The mechanism for the production of nitric oxide by rutin is via induction of eNOS gene expression, eNOS protein synthesis and eNOS activity. Increased gene and protein expression of basic fibroblast growth factor (bFGF) is also made possible by giving rutin as treatment (Hayek et al. 1997).

12.2 Brief Idea About Composition of Honey (Al-Waili 2005)

Honey is a nutritive compound as it is composed of numerous contents (Fig. 12.1).

- Carbohydrates: 85% of the solids in honey are mainly contributed by dextrose and fructose. Many disaccharides and trisaccharides are present in honey.
  - Examples of disaccharides: Maltose, maltulose, isomaltose, turanose, kojibiose, B-trehalose, etc.
  - Examples of trisaccharides: Maltotriose, melezitose, panose, isomaltotriose, centose, isopanose, etc.
- Non-aromatic organic acids: Butyric, citric, succinic, fumaric, malic, maleic, oxalic acids, etc. (Anklam 1998).
- Trace elements and minerals: Honey has a good mineral component.
- Examples: Sodium, potassium, calcium, copper, iron, aluminum, sulfur, fluoride, iodide, lead, cobalt, arsenic, lithium, barium, etc. are present in honey (Bogdanov et al. 2008).
- Vitamins: different vitamins like vitamin A, vitamin E, and vitamin C, niacin, thiamine, riboflavin and pyridoxine are found in honey.
- Amino acids: Various essential and non-essential amino acids are present in honey. Some of the examples are proline, glutamic acid, alanine, phenylalanine, tyrosine, etc.
- Enzymes: Honey has good quality of enzymes also. Examples: Glucose oxidase, amylase, acid phosphatase, invertase, catalase, etc. (Baroni et al. 2002).

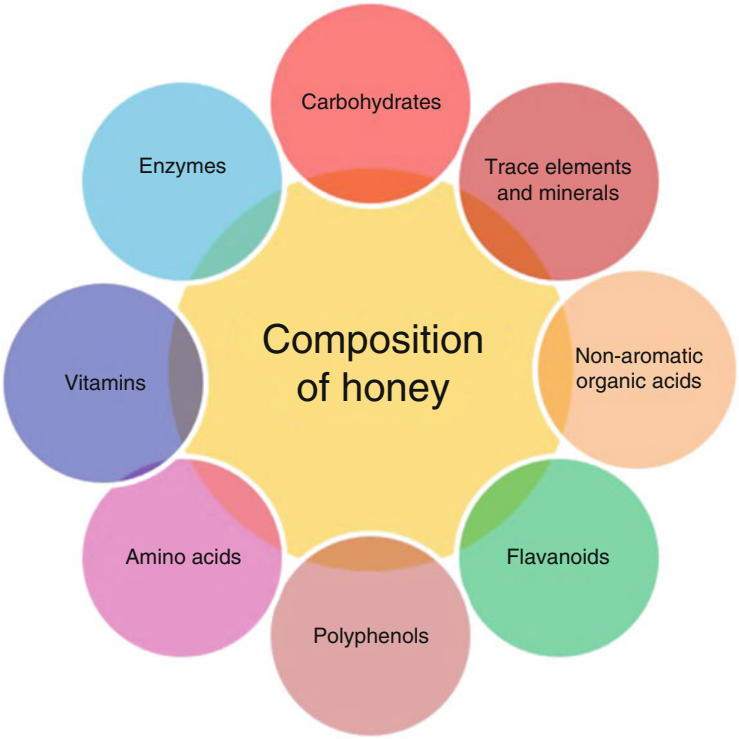

Fig. 12.1 An illustration showing some important phyto-constituents of honey

- Polyphenols: Various polyphenols and their derivatives are present in honey. Examples: quercetin, luteolin, kaempferol, chrysin, etc. (Bertoncelj et al. 2007; Aljadi and Kamaruddin 2004; Islam et al. 2012).
- Nitric oxide: Nitric oxide and its end products are also there in honey.

12.3 Role of Flavanoids in CVDs

The major sources of antioxidants in the diet consist of fruits, leafy vegetables, dark chocolate and green tea. These dietary components have proven protective effects against CVDs (Steinberg et al. 2003). The potential of lowering CVD risks has been revealed by such foods rich in antioxidants and flavonoids. Cocoa products are believed to have greater antioxidant power than most of the teas and red wines. It is imperative to explore the possible beneficial properties of chocolate on CVDs (Lee et al. 2003). Honey has a protective role on a wide range of diseases. The benefits of honey consumption on cardiovascular health are remarkable due to its flavonoid content. Biological and pharmacological activities of honey flavonoids in the prevention and treatment of atherosclerosis and CVDs have been considered in many

studies. In this study, the action of honey on CVDs will be disclosed with a mechanistic approach.

12.4 Cardiovascular Diseases

Cardiovascular diseases (CVDs) are described as a group of disorders of the blood vessels and heart. CVDs are the leading cause of mortality and morbidity worldwide (Gordon et al. 1989; Musselman et al. 1998). In developed countries CVD is the single largest cause of early mortality. In most cases atherosclerosis has remained as its main underlying pathological cause (Little et al. 2011). Untreated CVD can further develop into myocardial infarction and ischemic heart disease. Myocardial infarction being another fatal end point of cardiac diseases is manifested as angina pectoris and other clinical signs and symptoms. CVDs if left untreated can lead to financial and social burden for an individual and eventually to the whole society (Roger et al. 2012). Chronic inflammatory progression in the vascular wall of artery takes place due to oxidation of the neo-intimal lipids that releases oxidized molecular species, eventually leading to vascular atherosclerotic changes. Low density lipoproteins are transported into vascular endothelium and trigger the development of atherosclerosis (Grassi et al. 2009). Lipids present in tunica intima are trapped by proteoglycans that are modified and have hyper elongated glycosaminoglycan chains (Dadlani et al. 2008). Most significant risk factors for cardiac diseases are smoking followed by elevated blood cholesterol, obesity and diabetes mellitus. These all are related to inappropriate eating habits (Kivimäki et al. 2006). Among these, modifiable by dietary changes are elevated blood pressure and high fasting blood glucose (Bostom and Lathrop 1997).

As per various reports it has been established that intake of flavonoids is helpful in the prevention of cardiovascular diseases. This is mainly accomplished by declining oxidative stress and increasing the rising bioavailability of nitric oxide causing vasodilation. Different types of foods that are rich in flavonoids have been identified. Certain polyphenolic compounds like flavonoids work at molecular levels by modulating gene expression linked with human body metabolism, enzymes for the metabolism of drugs and xenobiotics, stress resistance and other detoxification reactions (Grassi et al. 2008). The integrated effect of different flavonoids is protective and overcomes the deleterious effects of cardiovascular risk factors. Therefore, the overall effect is delayed in the onset of atherosclerosis (Grassi et al. 2010).

12.4.1 Prevalence

Cardiovascular disease (CVD) is a group of disorders and a leading cause of death worldwide and in the United States of America (American Heart Association 2004). In India, the CVD death rate is 272 per 100,000 and is higher than the global average of 235 per 100,000 (Prabhakaran et al. 2016). Cardiovascular diseases caused over 16.7 million deaths globally in the year 2002. CVD disease burden is proposed to

rise to 143,000,000 disability-adjusted life-years by the year 2020 (Mackay and Mensah 2004).

12.4.2 Types of CVDs

Cardiovascular diseases are also known as vascular diseases and are of the following types:

1. Coronary artery disease/ischemic heart disease
2. Hypertensive heart diseases
3. Congestive cardiac failure
4. Arrhythmia
5. Peripheral artery disease
6. Cerebrovascular disease-stroke
7. Valvular heart diseases
8. Congenital heart disease
9. Rheumatic heart disease
10. Cardiomyopathy
11. Renal artery stenosis
12. Aortic aneurysm
13. Pulmonary heart disease
14. Inflammatory heart diseases: endocarditis, myocarditis, inflammatory cardiomyopathy and eosinophillic myocarditis (Mendis et al. 2011; Naghavi et al. 2015).

12.4.3 Signs and Symptoms

1. Angina pectoris or chest pain
2. Dyspnoea or breathlessness
3. Pedal oedema (swelling in feet)
4. Puffy face
5. Irregular pulse

12.4.4 Aetiology and Contributing Factors

1. Smoking
2. Systemic hypertension
3. Diabetes mellitus
4. Dyslipidemias progressing into atherosclerosis
5. Excessive alcohol consumption
6. Unhealthy lifestyle
7. Excessive weight gain and obesity
8. Sedentary lifestyle
9. Intake of junk food (Yusuf et al. 2004; Eckel 1997)

12.4.5 Diagnostic Tests

- Biochemistry: KFT, LFT, lipid profile, blood sugar fasting and post parandial, troponin-T, troponin-I, HbA1C in case of diabetic patients (Saenger 2012).
- Haematological and microbiological: CBC, CRP, hsCRP.
- Radiographic: ECG, echocardiography, Holter, etc. (Curry et al. 2018).

12.4.6 Treatment Options

- Diet and lifestyle modification: According to research, lifestyle modifications such as regular exercise and healthy nutrition may prevent cardiovascular diseases (Hu and Willett 2002). American Heart Association, American Diabetes Association, and the U.S. Preventive Services Task Force (Eyre et al. 2004) have established the significance of a healthy diet for the avoidance of CVDs.
- Medical treatment: Commonly, anticoagulants such as aspirin, streptokinase and tissue plasminogen activators are given in medical treatment. Intake of antihypertensives like atenolol comes under conservative management. Further steps include management of myocardial infarction and stroke.

12.5 Molecular Mechanism of Cardiovascular Injury

Oxidative stress and free radicals: A group of molecules that include molecular oxygen and its derived forms in all aerobic cells are called as reactive oxygen species (Herbst et al. 1999). Oxidation of the genetic material i.e. DNA and also other bio-molecules like proteins, carbohydrates and lipids directs excessive formation of reactive oxygen species. This excess production is not balanced by the antioxidant defence mechanisms of our body and is referred to as oxidative stress (Gimbrone 1995). According to research oxidative stress is related to various cardiac diseases, hypertension, diabetes and heart failure (Schachinger et al. 2000). It is also supposed to contribute to the in development of atherosclerosis, dyslipidemias, and hypercholesterolemia. Oxidative stress is believed to alter the normal endothelium functioning (Fig. 12.2) and affect the vascular tone also. This has been referred to a conventional risk that predisposes to atherosclerosis. Nitric oxide, a potent vasodilator, is inactivated by superoxide and other reactive oxygen species. This occurs commonly in systemic hypertension, dyslipidemias, diabetes mellitus and cigarette smoking. Many enzymatic systems are capable of producing reactive oxygen species however; extensive studies have been done on mechanisms like xanthine oxidase, uncoupled endothelial nitric oxide synthase and NADH/NADPH oxidase in vascular cells (Fig. 12.3). Therefore, research on the function of various enzymatic sources of ROS is becoming clearer. This possibly will lead to the improvement of therapies in order to prevent the formation of ROS and ultimate correction of endothelial dysfunction (Cai and Harrison 2000).

Fig. 12.2 An illustration showing some common precursors for oxidative stress and resultant endothelial dysfunction

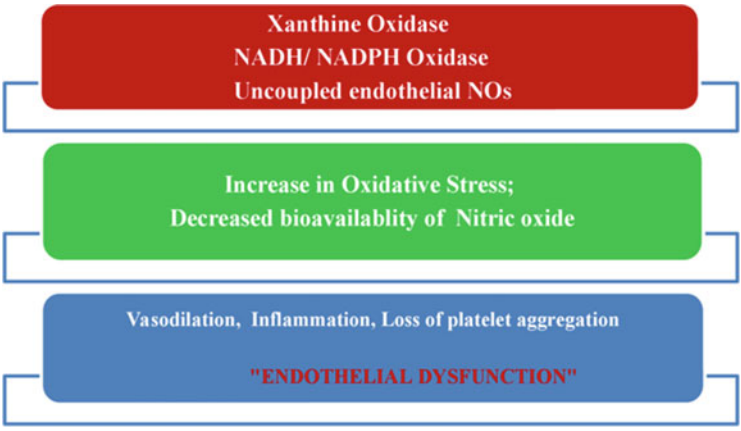

Fig. 12.3 Diagrammatic representation of molecular mechanism of oxidative stress induced endothelial dysfunction

12.6 Protective Action of Honey in CVD

Diet provides fuel to our body. A balanced diet provides nutrition to our body and is beneficial; however unhealthy diet is unfavourable to health. Studies have revealed that drinking beverages and sugar-sweetened drinks is related to the rise in blood pressure levels (Nguyen et al. 2009). Interestingly, honey which is a composite form of sugars is known to have a number of therapeutic and healthy advantages (Alvarez-Suarez et al. 2010). Although consumption of honey on a regular basis can decrease blood pressure, more research work is mandatory to assess the long-term effects of

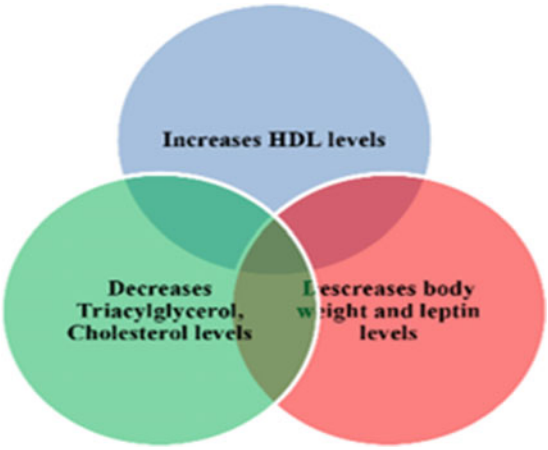

Fig. 12.4 A graphical representation of effect of honey intake on lipid profile

honey on healthy male subjects. Honey has many useful properties such as low pH, flavonoid content, hydrogen peroxide, phenols and sugar concentration that make it a good antimicrobial and antibacterial agent (Molan 1992). The risk of developing hypertension is related to gender because males have a higher risk than their female counterparts (Reckelhoff 2001). According to this study it is understood that honey has the ability to decrease blood pressure (both systolic and diastolic) and also the heart rate in healthy male subjects. Kaempferol is a flavanoid present in honey that shows effects by gene modulation and protein expression of inflammatory molecules (Zeng et al. 2015). Another honey flavonoid chrysin has numerous pharmacological actions and has a protective role in atherosclerosis. Chrysin inhibits the formation of foam cells some of which stimulate cholesterol flow (Kong et al. 2013).

Hypertension is linked to diet. Unhealthy diet being one of the modifiable risk factors linked to high BP. High salty diets have been documented as a cause for high blood pressure (Sacks et al. 2001). Increased cholesterol levels in the blood lead to atherosclerosis. Therefore, foods rich in saturated fats and trans fats are injurious to the heart (Assmann and Schulte 1992). Diets rich in carbohydrate content are harmful for health and lead to diseases of the cardiovascular system (Meena et al. 2007). Honey, though a composite sugar mixture, is described as cardio protective (Maureen 2004) as it improves the lipid profile (Fig. 12.4). According to a study, honey consumption in hypertensive patients decreased both systolic and diastolic blood pressure (Al-Waili 2003). According to a report, honey decreased the blood pressures levels that were raised in rats fed on a carbohydrate diet to induce obesity (Romero-Silva et al. 2011). This was supported by another study that concluded decreased systolic blood pressure in hypertensive rats by honey intake (Erejuwa et al. 2012). The effects that honey intake exert on hypertensive and diabetic patients are summed up in Fig. 12.5.

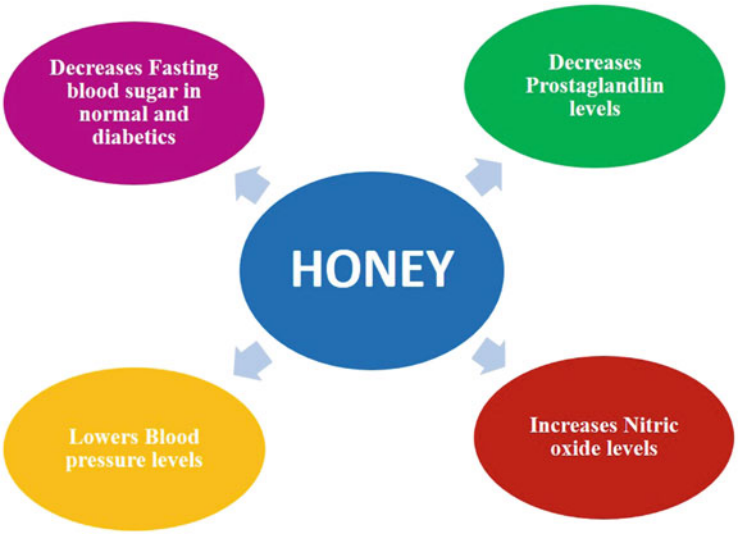

Fig. 12.5 Protective effect of honey on blood sugar and blood pressure

12.7 Other Applications of Honey

1. Prevents gastric ulcers (Ali et al. 1991)
2. Honey is useful as a therapy for diarrhoea (Jeddar et al. 1985)
3. Wound healing (Dumronglert 1983)
4. Used as skin disinfectant (French et al. 2005)
5. Immune inducer (Al-Waili and Haq 2004)
6. Anti-diabetic agent (Akhtar and Khan 1989)
7. Antibacterial agent (Jeddar et al. 1985)
8. Antioxidant (Raloff 1998) and
9. Antimutagenic and antitumour activity (Orsolic 2004).

12.8 Conclusion

Natural honey has many biological functions. The potential of lowering CVD risks is commonly found in foods like honey, green tea, fruits, etc. due to their antioxidant and flavonoid content. A number of flavonoids such as catechin, quercetin etc. that are present in honey are beneficial for CVDs with proven protective effects against metabolic disorders. Honey ingestion increases the levels of vitamin C, beta carotene, glutathione reductase, copper, zinc, NO and its end products. It is also believed to decrease the levels of different prostaglandins like PG E2 and PG F2, thromboxane B2 levels, CRP and homocysteine. Honey consumption improves lipid profiles

and transforms C-peptide levels and insulin secretion. Consumption of honey on a regular basis increases HDL levels and decreases triglycerides and VLDL levels. Honey has dominant antioxidant capacity and its intake prevents oxidative stress, thereby helps in promotion of good health. The flavanoid and polyphenolic content of honey helps to prevent complications in patients with diabetes mellitus, hypertension and cardiac diseases.

Therefore, honey plays a beneficial role in various cardiovascular diseases.

References

Afroz R, Tanvir EM, Paul S, Bhoumik NC, Gan SH, Khalil MI (2016) DNA damage inhibition properties of sundarban honey and its phenolic composition. J Food Biochem 40:436–445

Akhtar MS, Khan MS (1989) Glycaemic responses to three different honeys given to normal and alloxan-diabetic rabbits. J Pak Med Assoc 39:107–113

Ali AT, Chowdhury MN, Al-Humayyd MS (1991) Inhibitory effect of natural honey on Helicobacter pylori. Trop Gastroenterol 12:139–143

Aljadi AM, Kamaruddin MY (2004) Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. Food Chem 85:513–518

Alvarez-Suarez JM, Tulipani S, Romandini S, Bertoli E, Battino M (2010) Contribution of honey in nutrition and human health: a review. Mediter J Nutr Metabol 3:15–23

Al-Waili NS (2003) Intrapulmonary administration of natural honey solution, hyperosmolar dextrose or hypoosmolar distill water to normal individuals and to patients with type-2 diabetes mellitus or hypertension: their effects on blood glucose level, plasma insulin and C-peptide, blood pressure and peaked expiratory flow rate. Eur J Med Res 8:295–303

Al-Waili NS (2005) Mixture of honey, beeswax and olive oil inhibits growth of Staphylococcus aureus and Candida albicans. Arch Med Res 36:10–13

Al-Waili NS, Haq A (2004) Effect of honey on antibody production against thymus-dependent and thymus independent antigens in primary and secondary immune responses. J Med Food 7:491–494

Anklam E (1998) A review of the analytical methods to determine the geographical and botanical origin of honey. Food Chem 63:549–562

Assmann G, Schulte H (1992) Relation of high-density lipoprotein cholesterol and triglycerides to incidence of atherosclerotic coronary artery disease (the PROCAM experience). Am J Cardiol 70:733–737

American Heart Association (2004) Heart Disease and Stroke Statistics: 2004 Update. American Heart Association, Dallas, TX, p 2003

Baroni V, Chiabrando A, Costa C, Wunderlin A (2002) Assessment of the floral origin of honey by SDS–PAGE immunoblot techniques. J Agric Food Chem 50:1362–1367

Bertoncelj J, Dobersˇek U, Jamnik M, Golob T (2007) Evaluation of phenolic content, antioxidant activity, and colour of Slovenian honey. Food Chem 105:822–828

Bogdanov S, Jurendic T, Sieber R, Gallmann P (2008) Honey for nutrition and health: a review. J Am Coll Nutr 27:677–689

Bostom AG, Lathrop L (1997) Hyperhomocysteinemia in end-stage renal disease: prevalence, etiology, and potential relationship to arteriosclerotic outcomes. Kidney Int 52:10–20

Cai H, Harrison DG (2000) Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ Res 87:840–844

Cortes ME, Vigil P, Montenegro G (2011) The medicinal value of honey: a review on its benefits to human health, with a special focus on its effects on glycemic regulation. Cien Inv Agr 38:303–317

Curry SJ, Krist AH, Owens DK, Barry MJ, Caughey AB, Davidson KW et al (2018) Screening for cardiovascular disease risk with electrocardiography: US preventive services task force recommendation statement. JAMA 319(22):2308–2314

Dadlani H, Ballinger ML, Osman N, Getachew R, Little PJ (2008) Smad and p38 MAP kinase-mediated signaling of proteoglycan synthesis in vascular smooth muscle. J Biol Chem 283:7844–7852

Dumronglert EA (1983) Follow-up study of chronic wound healing. Dressing with pure natural honey. J Nat Res Counc Thail 15:39–66

Eckel RH (1997) Obesity and heart disease: a statement for healthcare professionals from the Nutrition Committee, American Heart Association. Circulation 96:3248–3250

Erejuwa OO, Sulaiman SA, Abwahab MS, Kuttulebbai NS, Salzihan S, Gurtu S (2012) Honey supplementation in spontaneously hypertensive rats elicits antihypertensive effect via amelioration of renal oxidative stress. Oxid Med Cell Longev 2012:1–14

Eyre H, Kahn R, Robertson RM, ACS/ADA/AHA Collaborative Writing Committee, ACS/ADA/AHA Collaborative Writing Committee Members, Clark NG, Doyle C, Hong Y, Gansler T, Glynn T, Smith RA (2004) Preventing cancer, cardiovascular disease, and diabetes: a common agenda for the American Cancer Society, the American Diabetes Association, and the American Heart Association. Circulation 109:3244–3255

French VM, Cooper RA, Molan PC (2005) The antibacterial activity of honey against coagulase-negative staphylococci. J Antimicrob Chemother 56:228–231

Gimbrone MA (1995) Vascular endothelium: an integrator of pathophysiologic stimuli in atherosclerosis. Am J Cardiol 75:67B–70B

Grassi D, Aggio A, Onori L, Croce G, Tiberti S, Ferri C et al (2008) Tea, flavonoids, and nitric oxide-mediated vascular reactivity. J Nutrit 138:1554S–1560S

Grassi D, Desideri G, Tiberti S, Ferri C et al (2009) Oxidative stress, endothelial dysfunction and prevention of cardiovascular diseases. Agro Food Ind Hi Tech 20:8–11

Grassi D, Desideri G, Ferri C (2010) Flavonoids: antioxidants against atherosclerosis. Nutrients 2:889–902

Gross M (2004) Flavonoids and cardiovascular disease. Pharm Biotechnol 42:21–35

Gordon DJ, Probstfield JL, Garrison RJ, Neaton JD, Castelli WP, Knoke JD et al (1989) High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. Circulation 79:8–15

Hayek T et al (1997) Reduced progression of atherosclerosis in apolipoprotein E-deficient mice following consumption of red wine, or its polyphenols quercetin or catechin, is associated with reduced susceptibility of LDL to oxidation and aggregation. Arterioscl Thromb Vascul Biol 17:2744–2752

Herbst U, Toborek M, Kaiser S, Mattson MP, Hennig B (1999) 4-Hydroxynonenal induces dysfunction and apoptosis of cultured endothelial cells. J Cell Physiol 181:295–303

Hoff HF, O'Neil J (1991) Lesion-derived low density lipoprotein and oxidized low density lipoprotein share a lability for aggregation, leading to enhanced macrophage degradation. Arterioscl Thromb Vascul Biol 11:1209–1222

Hu FB, Willett WC (2002) Optimal diets for prevention of coronary heart disease. JAMA 288:2569–2578

Islam A, Islam N, Moniruzzaman M (2012) Physicochemical and antioxidant properties of Bangladeshi honeys stored for more than one year. BMC Complement Altern Med 12:177

Jeddar A, Kharsany A, Ramsaroop UG, Bhamjee A, Haffejee IE, Moosa A (1985) The antibacterial action of honey: an in vitro study. S Afr Med J 67:257–258

Khalil M, Sulaiman S (2010) The potential role of honey and its polyphenols in preventing heart disease: a review. Afr J Tradit Complement Altern Med 7:315–321

Kivimäki M, Virtanen M, Elovainio M, Kouvonen A, Väänänen A, Vahtera J (2006) Work stress in the etiology of coronary heart disease—a meta-analysis. Scand J Work Environ Health 32:431–442

Kong L, Luo C, Li X, Zhou Y, He H (2013) The anti-inflammatory effect kaempferol on early atherosclerosis in high cholesterol fed rabbits. Lipids Health Dis 12:115

Lee KW, Kim YJ, Lee HJ, Lee CY (2003) Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. J Agric Food Chem 51:7292–7295
Little PJ, Chait A, Bobik A (2011) Cellular and cytokine-based inflammatory processes as novel therapeutic targets for the prevention and treatment of atherosclerosis. Pharmacol Ther 131:255–268
Mackay J, Mensah G (2004) The atlas of heart disease and stroke. The World Health Organization. http://www.who.int/cardiovasculardiseases/resources/atlas/en/
Maor I, Hayek T, Coleman R, Aviram M (1997) Plasma LDL oxidation leads to its aggregation in the atherosclerotic apolipoprotein E-deficient mice. Arterioscl Thromb Vascul Biol 17:2995–3005
Maureen W (2004) Eating honey can lower the risk of heart disease. J Med Food 1:100–107
Meena S, Beverley A, Abhimanyu G (2007) Effect of high-carbohydrate or high-cis-monounsaturated fat diets on blood pressure: a meta-analysis of intervention trials. Am J Clin Nutr 85:1251–1256
Mendis S, Puska P, Norrving B (2011). Global Atlas on Cardiovascular Disease Prevention and Control (PDF). World Health Organization in collaboration with the World Heart Federation and the World Stroke Organization. pp 3–18. ISBN 978-92-4-156437-3. Archived (PDF) from the original on 2014-08-17
Molan PC (1992) The antibacterial activity of honey. The nature of antibacterial activity. Bee World 73:5–28
Musselman DL, Evans DL, Nemeroff CB (1998) The relationship of depression to cardiovascular disease: epidemiology, biology, and treatment. Arch Gen Psych 55:580–592
Naghavi M, Wang H, Lozano R, Davis A, Liang X, Zhou M (2015) GBD 2013 Mortality and causes of Death collaborators. "Global, regional and national age sex specific all cause specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the global Burden of Disease Study 2013". Lancet 385:117-171
Nguyen S, Choi HK, Lustig RH, Hsu CY (2009) Sugar-sweetened beverages, serum uric acid, and blood pressure in adolescents. J Pediatr 154:807–813
Nigro J, Osman N, Dart AM, Little PJ (2006) Insulin resistance and atherosclerosis. Endoc Rev 27:242–259
Orsolic N (2004) Basic I. Honey as a cancer-preventive agent. Period Biol 106:397–401
Prabhakaran D, Jeemon P, Roy A (2016) Cardiovascular diseases in India: current epidemiology and future directions. Circulation 133:1605–1620
Raloff J (1998) Honey a source of antioxidants. J Apic Res 37:221–225
Reckelhoff JF (2001) Gender differences in the regulation of blood pressure. Hypertension 37:1199–1208
Roger VL et al (2012) Heart disease and stroke statistics--2012 update: a report from the American Heart Association. Circulation 125:e2
Romero-Silva S et al (2011) Effects of Honey against the accumulation of adipose tissue and the increased blood pressure on carbohydrate-induced obesity in rat. Lett Drug Des Discov 8:69–75
Sacks FM, Svetkey LP, Vollmer WM, Appel LJ, Bray GA, Harsha D et al (2001) Effects on blood pressure of reduced dietary sodium and the dietary approaches to stop hypertension (DASH) diet. N Engl J Med 344:3–10
Saenger AK (2012) Universal lipid screening in children and adolescents: a baby step toward primordial prevention? Clin Chem 58:1179–1181
Schachinger V, Britten MB, Zeiher AM (2000) Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. Circulation 101:1899–1906
Steinberg FM, Bearden MM, Keen CL (2003) Cocoa and chocolate flavonoids: implications for cardiovascular health. J Am Diet Assoc 103:215–223
Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F et al (2004) Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the interheart study): case-control study. Lancet 364:937–952
Zeng P, Liu B, Wang Q, Fan Q, Diao JX, Tang J et al (2015) Apigenin attenuates atherogenesis through inducing macrophage apoptosis via inhibition of AKT Ser473 phosphorylation and downregulation of plasminogen activator inhibitor-2. Oxidative Med Cell Longev:2015

Role of Phytochemicals from Honey in Prevention and Treatment of Arthritis and Related Disorders

13

Rabia Farooq, Sabhiya Majid, and Aamir Hanif

Abstract

Honey is a by-product of flower nectar formed by the enzymatic action within the digestive tract of honey bee (Genera Apis). Several compounds in honey are known to possess potent therapeutic properties against several diseases including inflammation, bacterial infections, cardiovascular problems, etc. This chapter specifically summarizes the therapeutic properties of honey for the treatment of rheumatoid arthritis, osteoporosis, and other related diseases. The compounds responsible for these properties and their mechanism of action is also discussed.

Keywords

Rheumatoid arthritis · Osteoporosis · Honey · Flavonoids

13.1 Introduction

Honey: A natural compound, a by-product of flower nectar formed by the enzymatic action in the digestive tract of honey bee (Genera Apis). This natural compound is used as food as well as medicine since ages from the stone age to ancient Egyptians, Chinese, Greeks Assyrians, and Romans (Al-Jabri 2005). Honey was used as

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antibacterial agent and for wound healing (Al-Waili and Haq 2004; Emsen 2007). Honey exhibits bactericidal activity against *Salmonella, Shigella*, *Escherichia coli, Helicobacter pylori,* and around 60 species of bacteria, fungi, and viruses (Jeffrey and Echazarreta 1996; Chowdhury 1999). Honey possesses anti-inflammatory, antioxidant properties, and acts as immunity booster as well (Medhi et al. 2008; Tonks et al. 2003). But it has limited use in modern medicine as more extensive research is not carried yet (Ali et al. 1991).

13.2 Constituents of Honey

Honey has been exploited for various medicinal properties since eternities, ranging from its bactericidal activities to anticancer activity. It is being used against liver diseases to cardiovascular problems. The various properties exhibited by honey are due to wide range of phytochemicals present in it which includes phenolics, non-phenolics, amino acids, enzymes, vitamins, and many other compounds (Johnston et al. 2005; Alvarez-Suarez et al. 2010). Flavonoids in honey include apigenin, pinocembrin, kaempferol, quercetin, galangin, chrysin, and hesperidin and phenolic acids (such as ellagic, caffeic, p-coumaric, and ferulic acids) (Turkmen et al. 2006; Rakha et al. 2008). Water is the second most component present in honey while more than 95% account for easily digestible sugars which mainly contain fructose and glucose (Moundoi et al. 2001). Other sugars like maltose, isomaltose, turanose, nigerose, panose, etc. are present in lower quantities. Among minerals, potassium is the major metal, shadowed by calcium, sodium, magnesium, phosphorus, etc. Among vitamins, Vitamin C is around 0.5 mg followed by other B complex vitamins. In nutshell, honey is a complete food with a lot of carbohydrates, proteins, minerals, vitamins, and even water. All these compounds especially phenolics and flavonoids in honey are responsible for its anticancer, anti-inflammatory, antioxidant, antibacterial, antifungal, and other medicinal properties.

13.3 Role of Phytochemicals of Honey in Diseases

The main cause or risk factor for diseases in the body is oxidative stress, which affects the biomolecules of the body and can lead to the development of diseases like diabetes, metabolic-related disorders, and even cancer (Davies et al. 1982). Oxidative stress in the body is caused when the free radical generation exceeds antioxidant mechanism. Honey is known to contain phytochemicals having various antioxidant properties, which can reduce free radicals in the body and thus decreases oxidative stress and eventually various pathological conditions.

The other main cause of developing diseases is inflammation. Inflammation is caused by any stimuli in the body be it pathogen, tissue injury, which leads to the production of many effector mediators, modulated by cells of both innate and adaptive immunity. Inflammation causes activation of various pathways like nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB), nitric oxide (NO),

hypoxia-induced factor (HIF-), Akt/protein kinase B, extracelluar signal-regulated kinases (ERK-2)/mitogen-activated protein kinases (MAPK), etc. These pathways lead to activation of various pro-inflammatory cytokines like interleukins (IL), leukotrienes, tumor necrosis factor (TNF-α), eicosanoids, and prostaglandins. These mediators are produced by inflammatory cells, like polymorphonuclear leukocytes (neutrophils, eosinophils, and basophils), endothelial cells, mast cells, macrophages, monocytes, and lymphocytes (Czermak et al. 1998; Ley et al. 2007). So, the development of innovative strategies to target inflammatory mediators can lead to prevention and treatment of chronic diseases.

These phytochemical compounds are often the products of secondary plant metabolism and possess complex structures. These secondary metabolites do not help in the processes of assimilation, respiration, transport, and differentiation of the plants. All these compounds, their concentration present in the honey differs from the biological origin of honey, i.e., from its floral origin. In this chapter, we focus on the role of honey in arthritis and related disorders.

13.3.1 Action of Honey Compounds on Arthritis

Arthritis is a disease of joints, in which joints are inflamed, red, painful, and weak. There are three types of arthritis and common one is rheumatoid arthritis (RA). Around 160 million in India alone suffer from this disease. Free radical generation and production of inflammatory cytokines like TNF-α, IL-6, IL-12, etc. are known to be the main cause of the disease. Besides, there is accumulation of prostaglandin E2 (PGE2) and matrix metalloproteinases (MMP)-1 in arthritis condition.

Caffeic acid phenethyl ester (CAPE) is a polyphenol ($C_{17}H_{16}O_4$) present in honey propolis reduces bone resorption, by decreasing inflammatory cytokines via different mechanisms which are illustrated as under:

1. NF-κB pathway induces the transcription of a wide range of cytokines, chemokines, enzymes, and antiapoptotic factors. It also leads to activation of lipoxygenase pathway. As NF-κB pathway is shown to be activated via two pathways: Noncanonical and canonical. Canonical pathway is activated by various substances like hydrogen peroxide, ceramide, phorbol ester, other free radicals, pattern-recognition receptors (PRRs), TNF receptor (TNFR) superfamily members, as well as T-cell receptor (TCR) and B-cell receptor (Natarajan et al. 1996; Zhang et al. 2015). These cause activation of inactive dephosphorylated IκBα–NF–κb complex by IκB kinase (IKK) complex (Bonizzi and Karin 2004; Oeckinghaus and Ghosh 2009; Sun et al. 2013; Sun and Liu 2011; Vallabhapurapu and Karin 2009) as illustrated in Fig. 13.1. The other member of the pathways is: NF-κB1 (also named p50), NF-κB2 (also named p52), RelA (also named p65), RelB and c-Rel which help in the transcription of target genes by binding either as homo or heterodimers to a specific DNA element (Sun et al. 2013).

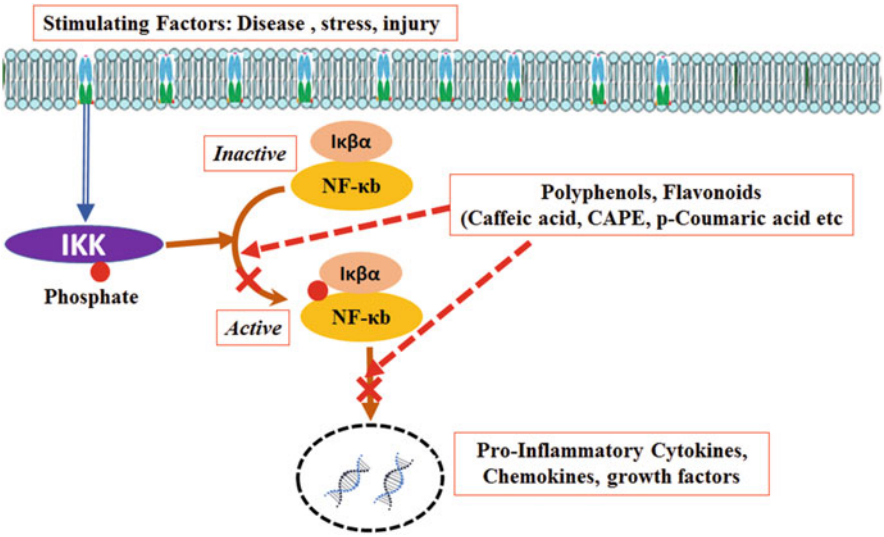

Fig. 13.1 Representative figure shows how polyphenols/flavonoids (caffeic acid, CAPE, pCA) block NF-κB pathways and thus lead to decrease in the transcription of various chemokines, cytokines like NO, TNFα, and IL-6

2. Noncanonical pathway is activated by stimuli which include stimuli, including ligands of a subset of TNFR superfamily members such as LTβR, BAFFR, CD40, and receptor activator of NFκB ligand (RANK) (Sun 2011, 2012).

CAPE inactivates NF-κB pathway by various pathways (Jeong et al. 2002). It blocks production of free radicals which acts as stimuli for NF-κB pathway (Ilhan et al. 2004; Natarajan et al. 1996). It blocks the interaction of NF-κB proteins with the DNA (Song et al. 2002).

CAPE causes dephosphorylation of IκBα–NF–κB complex (Cho et al. 2014; Moon et al. 2009). It also causes the suppression of lipoxygenase pathway (COX-2) of arachidonic acid metabolism in which arachidonic acid forms prostaglandins during process of inflammation. It also causes direct inhibition of the catalytic activity of iNOS (nitrogen synthase) and NO production in macrophages (Surh et al. 2001; Song et al. 2002; Marquez et al. 2003).

Besides the transcription of inflammatory factors, like TNF-α, IFN-γ, IL-2, IL-6, iNOS, and IL-8, was found to be significantly decreased by CAPE treatment in the pyloric mucosa of *H. pylori*-infected Mongolian gerbils (Toyoda et al. 2009; Naito and Yoshikawa 2002). Decreased malondialdehyde (MDA) levels and Xanthine oxidase: an indicator of free radical generation is observed after CAPE supplementation. CAPE inhibits leukotriene formation, which is the cause of inflammation in RA patients (Boudreau et al. 2012).

Chrysin (5,7-Dihydroxyflavone) works in the same way as CAPE to reduce inflammation in the body. Chrysin tablets are recognized to reduce rheumatoid

factors and other inflammatory markers in the body (Vishnu and Krishnan 2018). Chrysin enhances the expression of antioxidant enzymes like SOD, catalase, and glutathione peroxidase in various organs of the body (Pushpavalli et al. 2010; Ciftci et al. 2012; Khan et al. 2012). Chrysin inhibits the excessive production of nitric oxide, IL-6, IL-10, interferon-inducible protein-10 (IP-10), macrophage colony-stimulating factor (M-CSF), monocyte chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP), TNF-α, which are the key players in RA and other diseases (Lee and Park 2015). Vanillin possesses anti-inflammatory properties and prevents arthritis or other like conditions by inhibiting NO synthesis and MMP-9 (Jung et al. 2010).

Chlorogenic acid: A phenolic compound present in honey exerts shown to exert anti-arthritic activity (Kang et al. 2015). During inflammation in RA, in addition to TNF-α, IL-6, IL-1, B cell-activating factor (BAFF) is involved in cartilage destruction and further progresses inflammation (Leah 2011). Overproduction of TNF-α, IL-6 promotes the production of BAFF (Lee et al. 2011; Uzzan et al. 2016). BAFF was known to be produced by immune cells (B-cells) and required for B-cell proliferation, but these are also produced by nonimmune cells like prostate epithelium and fibroblast-like synoviocytes (FLSs) (Ohata et al. 2005; Qin et al. 2018; Jia et al. 2016). These FSLs cause synovial hyperplasia, facilitating the destruction of cartilage in RA (Bottini and Firestein et al. 2013; Duarte 2015; Higgs 2010).

BAFF levels are directly co-related to the level of inflammation among RA cases (Leah 2011; Wei et al. 2015a, b). BAFF also regulates expression of vascular epithelial growth factor (VEGF) in synoviocytes in RA angiogenesis (Reyes et al. 2008; Lee et al. 2013). So, BAFF may serve as a novel molecular therapeutic molecule for the treatment of RA (Moura et al. 2013; Wei et al. 2015a, b). Chlorogenic acid is known to significantly inhibit phosphorylation of IκBα, so blocks the formation of DNA-binding activity of NF-κB to the BAFF promoter region which eventually stops transcription of BAFF proteins. In vivo studies illustrated the decrease in TNF-α and BAFF levels among RA patients (Xiaohong et al. 2019).

Chlorogenic acid is having analgesic property as well apart from anti-inflammatory one. It reduces SOD, peroxynitrite anion, and malondialdehyde levels in mice and showed significant increase in reduced glutathione in paw tissues. So chlorogenic acid can suppress BAFF levels and so it acts as anti-arthritic drug in future and to control oxidative stress (El-Medany et al. 2013). Lixia et al. (2015) observed that both chlorogenic acid (CGA) and luteolin (Lut) act synergistically in the treatment for rheumatoid arthritis (RA) via both NF-κB and JAK/STAT-signaling pathways. This combination showed a promising effect on RA rather than used each singly (Lixia et al. 2015).

Caffeic acid, a phenolic acid present in honey, shows antioxidant and anti-inflammatory properties. It tends to decrease the expression of pro-inflammatory cytokines and even decrease PGE-2 and MMP-1 expression at both RNA and protein levels via NF-κB and mitogen-activated protein kinase (MAPK) pathways (Wang et al. 2017). Caffeic acid and ellagic acid when used for the treatment of arthritis in rats showed that these compounds lower MMP-9, which is the cause of

tissue remodeling and chitinase-3-like protein-1 (CHI3L1), which causes joint destruction. Caffeic and ellagic acid also reduce IL-6, TNF-α, lipid peroxides, etc. (Fikry et al. 2019). Caffeic acid liberates Ca^{2+} ions from osteoclasts, so possess inhibitory effect on osteoclastic function (Shankar et al. 1995; Zaidi et al. 1999). It acts as the most potent inhibitor of osteoclastogenesis in a study conducted on adjuvant induce rats, caffeic acid treatment showed 54% reduction in inflammation and that too without any side effects (Tang et al. 2006).

NFATc1 is regarded as a key protein for the onset of osteoclastogenesis (Takayanagi et al. 2002). Caffeic acid deactivates the formation of mononuclear preosteoclasts by blocking translation of NFATc1 factor so halts osteoclast differentiation during early stages (Tang et al. 2006). Cathepsin K, a protease differentiation marker of osteoclastic bone resorption (Tezuka et al. 1994). Tang et al. also confirmed that caffeic acid blocks transcription of Cathespisn K and so suppresses osteoclast bone resorption (Tang et al. 2006). Caffeic acid also induces apoptosis in several types of cells (Orban et al. 2000; Zaidi et al. 2003). Not only via NF-κB pathway but caffeic acid also suppresses toll-like receptor (TLR-4) inflammatory pathway. In this pathway, caffeic acid blocks IRAK-1 and IRAK-4, which are important proteins in inflammatory signaling pathways, so sequentially blocks AP-1 activation through TAK-1, JNK, MKK4, etc. inhibitions and thus leads to decrease in the production of NO, TNFα, and PGE2 as shown in Fig. 13.2 (Yang et al. 2013).

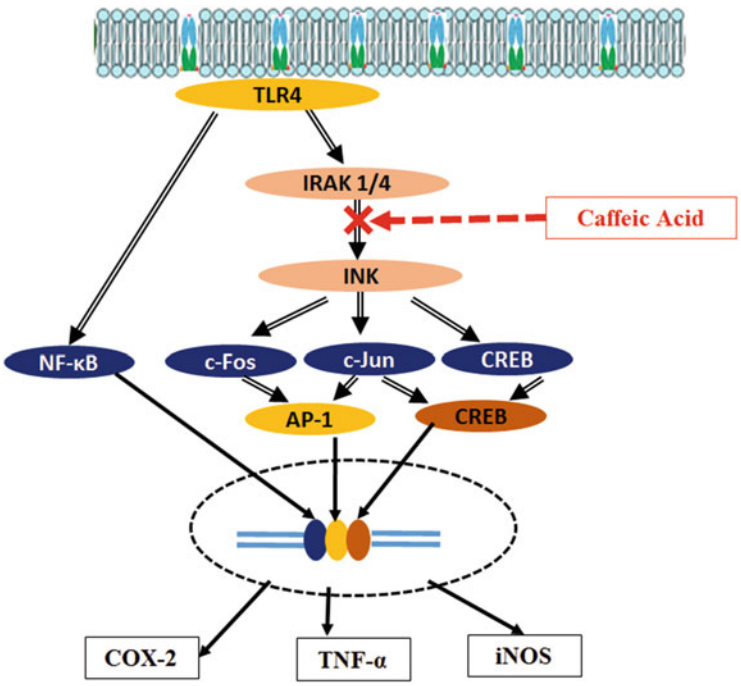

Fig. 13.2 Representative figure shows how caffeic acid blocks IRAK-1/4 via toll-like receptor (TLR-4) inflammatory pathway so blocks AP-1 activation through INK, c-Fos, c-Jun inhibitions, and thus leads to decrease in the production of NO, TNFα, and prostaglandins

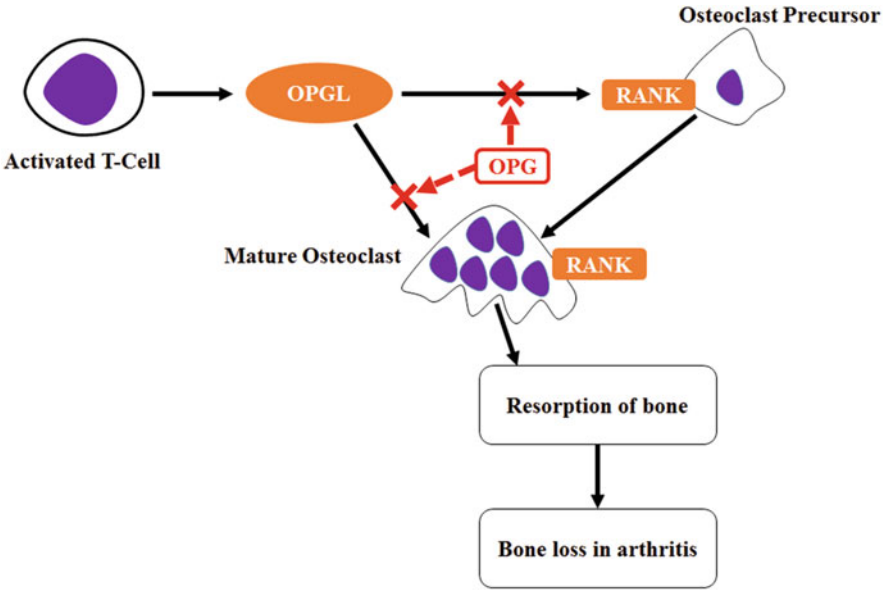

Fig. 13.3 Representative figure shows how activated T cells express RANKL that promote osteoclast formation. OPG blocks RANKL, which further leads to bone resorption

p-Coumaric acid (p-CA) is a hydroxycinnamic acid found in honey. It is known to prevent cell-mediated immune responses in rats (Pragasam et al. 2013) and also decreases TNF-α, IL-6, and immune complexes expression which is inflammatory mediators during oxidative stress and RA. RA-induced mice when supplemented with p-CA shows decrease in symptoms like skin shrinking, alleviation of swelling, clearing of the joint space, and improvement of the range of motion (Hao et al. 2018). In one more study conducted by Samuel et al. 2013 on arthritic rats observed a decrease in TNF-α and circulating immune complexes, cell-mediated immune response, and macrophage phagocytic index showing its both anti-inflammatory as well as immune suppressive property (Samuel et al. 2013). Actually, these pro-inflammatory mediators like TNF-α, IL-1b, IL-17 stimulate various inflammatory signaling and promote osteoclastogenesis. In this process, osteoclast releases enzymes like phosphatases and tartrate-resistant acid phosphatase (TRAP). Osteoclastogenesis progression is expedited by RANKL more than other factors (Wei et al. 2015a, b). However, upregulation of RANKL promotes sequential activation of pathways like MAP kinases, NF-κB, nuclear factor of activated T-cells cytoplasmic 1 (NFATc1), c-Fos (Kim and Kim 2014). This RANKL blocks receptor of osteoprotegerin (OPG), which further leads to bone resorption (Simonet et al. 1997; Hughes et al. 2000), shown in Fig. 13.3. pCA is known to represses activation of NF-κB and MAP kinases through the significant attenuation of NF-jB, NF-κB, ERK1/2, JNK, and p-JNK expression in the joints of arthritic rats. pCA decreases the expression of RANKL-OPG protein and other osteoclastogenic factors

which eventually act as anti-arthritis and anti-osteoporotic compound. pCA also decreases TNF-α and MCP-1, which reduces the severity of synovial hyperplasia in arthritic rats (Neog et al. 2017).

Gallic acid (3,4,5-trihydroxybenzoic acid): $C_6H_2(OH)_3COOH$ is a polyphenol present in abundance in honey. Like other polyphenols, it also possesses anti-inflammatory effects besides anticancer, antioxidant activities. Due to its anti-inflammatory activities, it has an effect on FS from RA patients. Gallic acid suppresses transcription and protein expression of IL-1β, IL-6, CCL-2, CCL-7, COX-2, and even MMP-9. Decrease in these factors acts as protective factor against RA (Yoon et al. 2013). In one study, gallic acid was shown to reduce caspase-3, iNOS levels, COX-2 expressions, which lead to a decrease in NO levels and eicosanoids, which are on the rise in inflammation (Olajide et al. 2014).

Kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one) found in honey propolis, having antioxidant, anti-inflammatory, and moreover anti-osteoclastogenic effects (Wattel et al. 2003; Pang et al. 2006; Yamaguchi et al. 2007). Kaempferol takes free radicals and superoxides and acts as a catalyst for antioxidant enzymes like catalase and glutathione reductase. It blocks the cell division, transcription as well as translation of IL-1β-stimulated FS MMP-1, MMP-3, COX-2, nitric oxide synthase, and PGE2 involved in articular inflammation and destruction in RA disease (Yoon et al. 2013). It also blocks NF-κB, P38 pathways by causing dephosphorylation and thus stops the production of various chemokines and cytokines. Kaempferol supplementation decreases enzyme alkaline phosphatase (ALP), which is a bone damage marker (Trivedi et al. 2009). Kaempferol application also exhibits anti-RA effects to cases having knee arthroplasty. As MMPs are important markers of FS cell migration and invasion among RA patients. These proteases are induced by TNFα from RA FLSs (Cai et al. 2016). Kaempferol via MAPK pathway decreases MMP expression and TNFα levels, which are known to increase cell invasion and cartilage destruction among RA patients (Zeng et al. 2017; Dongmei et al. 2018). Infact, kaempferol exploits its antiproliferative effect via MAPK pathway to reduce RA FLSs (Yoon et al. 2013). It also suppresses IL-1β-induced proliferation of RA FLSs and the production of COX-2 and PGE2 (Kim et al. 2008). Lee et al. conducted a study and observed Kaempferol inhibits NFATc1 and c-Fos transcription factors via blocking phosphorylation of JNK-MAPK, RANKL, ERK1/2, and p38 pathways, which prevent osteoclast formation and differentiation, so suggesting it as a therapeutic agent against RA (Lee et al. 2014).

Hesperidin is a flavanone present in honey possessing unlimited properties which include anti-inflammatory as well. A study was conducted on rats induced with arthritis and Hesperidin treatment was given to them and they observed a decrease in inflammation, RF levels, TNFα, NO, and increase in antioxidant enzyme SOD, GSH, respectively (Khowailed and Sakr 2017). Hesperidin acts via various molecular mechanisms, which include erythroid 2-related factor/extracellular signal-regulated kinase, No/cGFP, and phosphoinositide 3-kinase/mitogen-activated protein kinase (Roohbakhsh et al. 2014). Hesperidin reduces transcription and translation of IL-1β and IL-17A in IL-1β-stimulated human OA chondrocytes.

IL-17 expression is mainly associated with the pain in RA. So decrease in its levels helps to reduce the symptoms associated with this disease to live a better life (Zhaozong et al. 2018). Umar et al. in the year 2013 demonstrated the changes associated with hesperidin treatment to arthritic rats. A reduction in swelling of joints, NO levels and articular elastase (which are a marker for inflammation and are directly related to activation and accumulation of leucocytes) has been observed. Besides, Hesperidin replenished GSH and SOD levels and maintained the integrity of cellular membranes in the injured cartilage.

Apigenin: A flavanone (4′, 5, 7-trihydroxyflavone), having anti-inflammatory, anti-toxicant, anticancer, and antiatherogenic properties (Catarino et al. 2015) and acts as a potent therapeutic agent to overcome diseases such as rheumatoid arthritis, Parkinson's disease, Alzheimer's disease, and various type of cancers. Apigenin due to its slow metabolism in liver makes it as a strong therapeutic agent. Apigenin exerts proapoptotic effects in macrophages to decrease oxidized low-density lipoproteins (LDLs). Apigenin causes dephosphorylation of AKT protein and sequentially causing downregulation of plasminogen activator inhibitor-2 (PAI-2). In the inflammatory pathways, apigenin is known to block iNOS, NO, and COX 2 expression among INF-γ activated C6 astrocytes and LPS-induced RAW 264.7 cell lines (Soliman and Mazzio 1998). Apigenin also declines the expression of IL-1β, IL-6, INF-γ, IL-4, IL-5, TNF-α, MCP-1α (monocyte chemotoactic protein), MIP-1α (monocyte inflammatory protein), etc. via various signal-transducing pathways like PKC, ERK, and MAPK, NF-κB. Like CAPE, chrysin, apigenin also blocks translocation of NF-κB to nucleus so suppresses I-κBα phosphorylation apoptosis in CD4 T-cells, as well as in leukemic Jurkat T-cell lines (Xu et al. 2008). A study conducted on arthritic-induced mouse model, apigenin induces ROS production and activation of NF-κB, AP-1, p53, HIF-1α, PPAR-γ, β-catenin/Wnt, STAT-3, Sp-1, and Nrf2 proteins, which are key regulators in cancer and inflammatory pathways. These molecules lead to transcription and translation of more than 500 cytokines, chemokines involved as regulators in cell cycle regulation and anti-inflammation (Ribeiro et al. 2015). ROS production causes apoptosis through ERK1/2 pathway in fibroblast-like synoviocytes (Shin et al. 2009). Apigenin impedes the processes of osteoblastogenesis, osteoclastogenesis, and exhibited immune modulating in ovariectomized mice (Goto et al. 2015). Immunomodulating effect is caused by stimulating TNF-α in arthritic mice.

Apigenin facilitated activity of antioxidant enzymes like SOD, GSH, catalase, NOS, and glutathione reductase (GR). It protects cells against ROS so reduces lipid peroxidation and protein damage. Apigenin inhibits expression of intracellular adhesion molecules (ICAM), thereby providing protection against inflammation in human aortic endothelial cells. In RA disease, apigenin prevents autoantigen-presenting cells (APCs) necessary for the activation and expansion of overexpressed Th17 cells by causing downregulation of COX-2 and c-FLIP (Kang et al. 2009). In a study conducted by Lee et al. on RAW 264.7 macrophage cells, apigenin is shown to suppress collagenase activity, which occurs in RA disease. It also inhibits transcription of vascular cellular adhesion molecule-1 (VCAM-1)-, intracellular adhesion molecule-1 (ICAM-1)-, and E-selectin. So, acts as anti-inflammatory molecule by blocking COX-2 and monocyte adhesion (Lee et al. 2007).

Quercetin (3,3′,4′,5,7-pentahydroxy flavone: $C_{15}H_{10}O_7$): A flavonoid in honey with anti-inflammatory, antioxidant, and immunomodulator properties which make it a potential agent for RA. Quercetin was associated with decreased levels of TNF-α, IL-1β, IL-17, and MCP-1 and gives protection to joints against inflammation among RA patients (Haleagrahara et al. 2017). It has a property to reduce blood pressure, antiplatelet aggregation, anti-allergy (Sylwia et al. 2012). It reduces the production of macrophage inflammatory mediators through the regulation of NF-κB and moreover it has very less side effects (Ferry et al. 1996; García-Román et al. 2008). Quercetin inhibits MMP-2 activity (Tan et al. 2003), so preventing the degradation of basement membrane and cartilage damage. A study conducted by Tan et al. (2003) shows that Quercetin decreases VEGF levels affect angiogenesis, thereby affecting synovial pannus formation in RA disease. Quercetin depletes reduced GSH, TNFα, IL-1β, COX-2 transcription through various pathways like NF-κB, Nuclear factor erythroid 2-related factor (Nrf2)/home oxygenase (HO-1) pathway making Quercetin a potential candidate for arthritis treatment (Guazellia et al. 2018). Quercetin administration is known to reduce sodium urate crystals which form in gouty arthritis (Ruiz-Miyazawa et al. 2017). It also down-regulates the frequencies of Th17 (T helper) cells and related cytokines while increases the levels of Treg (Regulatory T-cells) cells and related cytokines, the ratio of which (Th17/Treg) is thought to change during RA progression. Quercetin also inhibits the production of proteins like NLRP3, Caspase-1, and IL-1β of synovial tissue in arthritic rats (Yang et al. 2018). Heme oxygenase (HO-1) plays a crucial role in inflammatory response HO-1 induction and inhibits collagen-induced arthritis by downregulation of MMPs in synoviocytes (Muż et al. 2008; Chi et al. 2012). Quercetin induces expression of HO-1 protein so can act as a therapeutic target for RA. Quercetin decreases osteoclast differentiation by inhibiting 1L-17-induced RANKL formation, so prevents bone desorption (Hae-Rim et al. 2019). Javadi et al. conducted a human randomized trial on Quercetin among women with RA and he observed a significant reduction in plasma TNFα levels in the quercetin group when compared to normals. Besides a decrease in tenderness of joints and pain as well (Javadi et al. 2017).

13.3.2 Action of Honey on Osteoporosis

Osteoporosis is decreased in bone mass associated with changes in bone structures, increased fragility, and increased risk of fractures. Osteoporosis is a growing problem in the world, more than 9.9 million Americans suffer from this disease and in country with high population like India, the number has been increased to 36 million as per 2013 data (Wright et al. 2014; Dhanwal et al. 2010; Mithal et al. 2014; Mithal and Lau 2009). It is more prevalent among women than men. Osteoporosis is caused mainly due to inflammation and oxidative stress. Honey is an important food with all the required phytochemicals to be used against oxidative stress and possess anti-inflammatory processes. Phenolics in honey act as antioxidants and work by different mechanisms like by providing electrons to free radicals, metal ion chelation,

accepting hydroxyl ions (Eteraf-Oskouei and Najafi 2013). Even flavonoids in honey are known to create balance between antioxidants and free radicals in the body (Ahmed et al. 2018). Honey also contains antioxidant enzymes like superoxide dismutase (SOD), glutathione reductase, catalase, which gives synergistic effect with other antioxidants (Erejuwa et al. 2012).

Honey contains quercetin and kaempferol, which are known to exert a strong inhibitory effect via receptor activator of NFκB ligand (RANKL) and activator protein 1 (AP-1) activation, a transcription factor highly related to osteoclastic differentiation on osteoclastic bone resorption and apoptosis (Wattel et al. 2003, 2004). Quercetin (3,3′,4′,5,7-pentahydroxyflavone) decreases osteoclastogenesis significantly even at low concentrations. Quercetin prevents bone loss by either inhibiting tumor necrosis factor-alpha (TNF-α) and other cytokines or acts as accelerator of apoptosis mediated by cytokines in osteoblastic cell. TNF-α is pro-inflammatory cytokines believed to be the cause of bone resorption so plays a key role in osteoporosis (Glantschnig et al. 2003). Quercetin helps in TNF-α induced apoptosis in MC3T3-E1 cell line (Son et al. 2006). Quercetin also leads to bone resorption by inhibition of differentiation of osteoclast progenitor cells into preosteoclasts (Woo et al. 2004).

CAPE increases neo-osteogenesis in rat models and hence prevents osteoporosis (Kazancioglu et al. 2015; Duan et al. 2014). It is shown to reduce osteoclasts and improves architecture of tibial metaphyses among ovariectomized mice (Duan et al. 2014). It is known to increase bone healing as well (Alper et al. 2019). CAPE inhibits p70S6K via Akt-driven signaling pathways and RANKL-induced osteoclastogenesis through the interference with NF-κB signaling (Ha et al. 2009). CAPE reduces caspase-3 activity and increases antioxidant activity.

Vanillin: A phenolic aldehyde (4-hydroxy-3-methoxybenzaldehyde) with formula ($C_8H_8O_3$) having antioxidant, antitumor, antimutagenic, anti-inflammatory properties (Imanishi et al. 1990; Ho et al. 2009). Phenolic compound present in honey decreases the number of mature osteoclasts and prevents bone resorption by causing apoptosis RANKL-induced osteoclastogenesis and activate the mitochondrial-dependent apoptosis. Vanillin increases both the transcription and translation of cytochrome c, Bax (apoptotic protein) and Apaf-1, while inhibits the expression of Bcl-2 (Dhanalakshmi et al. 2016). Vanillin promotes mature osteoclast early apoptosis so it acts as a novel anti-osteoporosis agent, by causing decrease in Bcl-2/BAX ratio, which triggers release of cytochrome c from mitochondrial to cytosol. A compound Apoptosome formed by Apaf-1 and cytochrome c leads to the caspase cascade reaction, which eventually leads to apoptosis of mature osteoclasts (Yueqi et al. 2018). Chrysin and naringenin also are known to prevent bone loss and even alleviates hepatic and renal damage caused by retinoic acid in rats (Nada et al. 2014).

Pinocembrin (5,7-dihydroxyflavanone): A flavonoid abundant in propolis is shown to have anti-inflammatory, antioxidant, and neuroprotective effects. It protects the blood–brain barrier from antioxidants, modulates mitochondrial function, and regulates apoptosis. It also inhibits the expressions of proinflammatory cytokines, like TNF-α, IL-1β, intercellular adhesion molecule-1, VCAM-1,

inducible NO-S, and aquaporin-4 (Gao et al. 2010). As previously discussed, the role of pinocembrin in maintaining ECM it reduces translation of MMPs (MMP-1,3,13) in human chondrocyte cell lines. Pinocembrin blocks TNF-α induced NF-κB pathway by dephosphorylation and blocks p65 translocation to nucleus and thus stops production of iNOS, COX-2, PGE_2 production. So, it shows the protective effect of Pinocembrin in osteoporosis in which MMPs are highly released (Zhang et al. 2015). Besides, NF-κB Pinocembrin also showed anti-inflammatory effect by deactivation of JNK and p38MAPK, which also lead to MMP inhibition (Rasul et al. 2013; Soromou et al. 2012). Pinocembrin increases GSH, gluthathione peroxidase levels, decreases mitochondrial dysfunction, and Bcl-2-associated X protein (Bax) (de Oliveira et al. 2017).

13.4 Conclusion

This chapter summarizes the mechanisms of honey compounds, i.e., polyphenols and flavonoids present in the honey in treating rheumatoid arthritis, osteoporois, and other related diseases. The multiple mechanisms include antioxidant activity, immune-modulatory action, and anti-inflammatory pathways, which lead to decrease in the inflammatory cytokines, chemokines mainly involved in the pathogenesis of arthritis. The antiosteoclastic properties of honey compounds are mainly activation of caspases, inhibition of transcriptional factors activity, and release of cytochrome c, apoptotic effects, inhibition of various pathways, which lead to transcription and translation of pro-inflammatory molecules. Limited human trials have been conducted to investigate the anti-arthritic action of polyphenols a flavanone of honey, which shows positive results and without adverse side effects. However, in animal models, its role action is well documented. Therefore, the compounds of honey could be developed as an advanced and future anti-arthritic drugs. The honey compounds show synergistic effects so taking honey on regular basis is highly recommended.

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References

Ahmed S, Sulaiman SA, Baig AA et al (2018) Honey as a potential natural antioxidant medicine: an insight into its molecular mechanisms of action. Oxidative Med Cell Longev 2018:8367846

Ali AT, Chowdhury MN, Humayyd MS (1991) Inhibitory effect of natural honey on Helicobacter pylori. Trop Gastroenterol 12(3):139–143

Al-Jabri AA (2005) Honey, milk and antibiotics. Afr J Biotechnol 4:1580–1587

Alper K, Zildaga TA, Mevlut A, Ufuk T, Erman S, Mukades D, Elif D (2019) Therapeutic effects of caffeic acid phenethyl ester on alveolar bone loss in rats with endotoxin-induced periodontitis. J Dent Sci 114(4):339–345

Alvarez-Suarez JM, Tulipani S, Romandini S, Bertoli E, Battino M (2010) Contribution of honey in nutrition and human health: a review. Mediterr J Nutr Metab 3:15–23

Al-Waili NS, Haq A (2004) Effect of honey on antibody production against thymus-dependent and thymus-independent antigens in primary and secondary immune responses. J Med Food 7:491–494

Bonizzi G, Karin M (2004) The two NF-kappaB activation pathways and their role in innate and adaptive immunity. Trends Immunol 25(6):280–288

Bottini N, Firestein GS (2013) Duality of fibroblast-like synoviocytes in RA: passive responders and imprinted aggressors. Nat Rev Rheumatol 9(1):24–33

Boudreau LH, Maillet J, LeBlanc LM, Jean-Francois J, Touaibia M, Flamand N et al (2012) Caffeic acid phenethyl ester and its amide analogue are potent inhibitors of leukotriene biosynthesis in human polymorphonuclear leukocytes. PLoS One 7:e31833

Cai LY, Kong XL, Xie Q et al (2016) The effects of NF-kappaB signalling on the TNFalpha induced ratios of MMPs to TIMPs in chondrocytes. Sichuan Da Xue Xue Bao Yi Xue Ban 47 (2016):642–648

Catarino DM, Alves-Silva J, Pereira RO, Cardoso MS (2015) Antioxidant capacities of flavones and benefits in oxidative-stress related diseases. Curr Top Med Chem 15:105–119

Chi PL, Chen YW, Hsiao LD et al (2012) Heme oxygenase 1 attenuates interleukin-1βinduced cytosolic phospholipase A2 expression via a decrease in NADPH oxidase/ reactive oxygen species/activator protein 1 activation in rheumatoid arthritis synovial fibroblasts. Arthritis Rheum 64(7):2114–2125

Cho MS, Park WS, Jung W-K, Qian Z-j, Lee D-S, Choi J-S, Lee D-Y, Park S-G, Seo S-K, Kim H-J, Won JY, Yu BC, Choi I-W (2014) Caffeic acid phenethyl ester promotes anti-inflammatory effects by inhibiting MAPK and NF-κB signaling in activated HMC-1 human mast cells. Pharm Biol 52(7):926–932

Chowdhury M (1999) Honey: is it worth rubbing it in? J R Soc Med 92:663–664

Ciftci O, Ozdemir I, Aydin M, Beytur A (2012) Beneficial effects of chrysin on the reproductive system of adult male rats. Andrologia 44(3):181–186

Czermak BJ, Friedl HP, Ward PA (1998) Complement, cytokines, and adhesion molecule expression in inflammatory reactions. Proc Assoc Am Physicians 110(4):306–312

Davies KJ, Quintanilha AT, Brooks GA, Packer L (1982) Free radicals and tissue damage produced by exercise. Biochem Biophys Res Commun 107:1198–1205

de Oliveira MR, Peres A, Gama CS, Bosco SMD (2017) Pinocembrin provides mitochondrial protection by the activation of the Erk1/2-Nrf2 signaling pathway in SH-SY5Y neuroblastoma cells exposed to paraquat. Mol Neurobiol 54(8):6018–6031

Dhanalakshmi C, Janakiraman U, Manivasagam T, Thenmozhi AJ, Essa MM, Kalandar A, Khan MA, Guillemin GJ (2016) Vanillin attenuated behavioural impairments, neurochemical deficits, oxidative stress and apoptosis against rotenone induced rat model of Parkinson's disease. Neurochem Res 41:1899–1910

Dhanwal DK, Cooper C, Dennison EM (2010) Geographic variation in osteoporotic hip fracture incidence: the growing importance of Asian influences in coming decades. J Osteoporos 757102:5

Dongmei P, Nan L, Yanyan L, Qiang X, Qingping L, Yanting Y, Zhenquan W, Yubao J, Minying L, Tianfeng G, Xudong C, Xiaobao L, Qiang W, Mingling L, Xujie L, Mingying Z, Xiaoshan Z, Changsong L (2018) Kaempferol inhibits the migration and invasion of rheumatoid arthritis fibroblast-like synoviocytes by blocking activation of the MAPK pathway. Int Immunopharmacol 55:174–182

Duarte JH (2015) Rheumatoid arthritis: inflammation feeds inflammation—HDAC5 downregulation leads to activation of fibroblast-like synoviocytes in RA. Nat Rev Rheumatol 11(2):64

El-Medany AM, Bassiouni YA, Khattab MM, Mahesar AA (2013) PP257—chlorogenic acid as potential anti-inflammatory analgesic agent. Clin Therapeut 35(8):e98

Emsen IM (2007) A different and safe method of split thickness skin graft fixation: medical honey application. Burns 33:782–787

Erejuwa OO, Sulaiman SA, Ab Wahab MS (2012) Honey: a novel antioxidant. Molecules 17 (4):4400–4423

Eteraf-Oskouei T, Najafi M (2013) Traditional and modern uses of natural honey in human diseases: a review. Iran J Basic Med Sci 16(6):731–742

Ferry DR, Smith A, Malkhandi J, Fyfe DW, deTakats PG, Anderson D et al (1996) Phase I clinical trial of the flavonoid quercetin: pharmacokinetics and evidence for in vivo tyrosine kinase inhibition. Clin Cancer Res 2:659–668

Fikry EM, Gad AM, Eid AH, Arab HH (2019) Caffeic acid and ellagic acid ameliorate adjuvant-induced arthritis in rats via targeting inflammatory signals, chitinase-3-like protein-1 and angiogenesis. Biomed Pharmacother 110:878–886

Gao M, Zhu SY, Tan CB, Xu B, Zhang WC (2010) Pinocembrin protects the neurovascular unit by reducing inflammation and extracellular proteolysis in MCAO rats. J Asian Nat Prod Res 12:407–418

García-Román R, Salazar-González D, Rosas S, Arellanes-Robledo J, Beltrán-Ramírez O, Fattel-Fazenda S et al (2008) The differential NF-kB modulation by Sadenosyl-L-methionine. N-acetylcysteine and quercetin on the promotion stage of chemical hepatocarcinogenesis. Free Radic Res 42:331–343

Glantschnig H, Fisher JE, Wesolowski G, Rodan GA, Reszka AA (2003) M-CSF, TNFalpha and RANK ligand promote osteoclast survival by signaling through mTOR/S6 kinase. Cell Death Differ 10:1165–1177

Goto T, Hagiwara K, Shirai N, Yoshida K, Hagiwara H (2015) Apigenin inhibits osteoblastogenesis and osteoclastogenesis and prevents bone loss in ovariectomized mice. Cytotechnology 67:357–365

Guazellia CFS, Staurengo-Ferraria L, Zarpelona AC, Pinho-Ribeiro FA, Ruiz-Miyazawa KW, Vicentini FTMC, Vignoli JA, Camilios-Neto D, Georgetti SR, Baracat MM, Casagrande R, VerriJr WA (2018) Quercetin attenuates zymosan-induced arthritis in mice. Biomed Pharmacother 102:175–184

Ha J, Choi HS, Lee Y, Lee ZH, Kim HH (2009) Caffeic acid phenethyl ester inhibits osteoclastogenesis by suppressing NF kappa B and downregulating NFATc1 and c-Fos. Int Immunopharmacol 9:774–780

Hae-Rim K, Bo-Mi K, Ji-Yeon W, Kyung-Ann L, Hyun MK, Young SK, Sang-Heon L, Kyoung-Woon K (2019) Quercetin, a plant polyphenol, has potential for the prevention of bone destruction in rheumatoid arthritis. J Med Food 22(2):152–161

Haleagrahara N, SocorroMiranda-Hernandeza S, Md Alim A, Hayes L, Bird G, Ketheesan N (2017) Therapeutic effect of quercetin in collagen-induced arthritis. Biomed Pharmacother 90:38–46

Hao Z, Qing-hua L, Xin-gui X, Yang W, Zhi-hui Z, Mei-juan S, Xun L, Dan W (2018) Anti-inflammatory effects of p-coumaric acid, a natural compound of Oldenlandia diffusa, on arthritis model rats. Evidence Based Complement Alternat Med 2018:5198594

Higgs R (2010) Rheumatoid arthritis: synergistic effects of growth factors drive an RA phenotype in fibroblast-like synoviocytes. Nat Rev Rheumatol 6(7):383

Ho K, Yazan LS, Ismail N, Ismail M (2009) Apoptosis and cell cycle arrest of human colorectal cancer cell line HT-29 induced by vanillin. Cancer Epidemiol 33:155–160

Hughes AE, Ralston SH, Marken J, Bell C, MacPherson H et al (2000) Mutations in TNFRSF11A, affecting the signal peptide of RANK, cause familial expansile osteolysis. Nat Genet 24:45–48

Ilhan A, Akyol O, Gurel A, Armutcu F, Iraz M, Oztas E (2004) Protective effects of caffeic acid phenethyl ester against experimental allergic encephalomyelitis-induced oxidative stress in rats. Free Radic Biol Med 37(3):386–394

Imanishi H, Sasaki YF, Matsumoto K et al (1990) Suppression of 6-TG-resistant mutations in V79 cells and recessive spot formations in mice by vanillin. Mutat Res Lett 243:151–158

Javadi F, Ahmadzadeh A, Eghtesadi S, Aryaeian N, Zabihiyeganeh M, Rahimi Foroushani A, Jazayeri S (2017) The effect of quercetin on inflammatory factors and clinical symptoms in women with rheumatoid arthritis: a double-blind, randomized controlled trial. J Am Coll Nutr 36(1):9–15

Jeffrey AE, Echazarreta CM (1996) Medical uses of honey. Rev Biomed 7:43–49

Jeong HJ, Koo HN, Na HJ, Kim MS, Hong SH, Eom JW et al (2002) Inhibition of TNF-α and IL-6 production by aucubin through blockade of NF-κB activation in RBL-2H3 mast cells. Cytokine 18:252–259

Jia X, Wei F, Sun X et al (2016) CP-25 attenuates the inflammatory response of fibroblast-like synoviocytes co-cultured with BAFF-activated CD4+ T cells. J Ethnopharmacol 189:194–201

Johnston JE, Sepe HA, Miano CL, Brannan RG, Alderton AL (2005) Honey inhibits lipid oxidation in ready-to-eat ground beef patties. Meat Sci 70:627–631

Jung H-J, Song YS, Kim K, Lim C-J, Park E-H (2010) Assessment of the anti-angiogenic, anti-inflammatory and antinociceptive properties of ethyl vanillin. Arch Pharm Res 33(2):309–316

Kang HK, Ecklund D, Michael L, Syamal KD (2009) Apigenin, a non-mutagenic dietary flavonoid, suppresses lupus by inhibiting autoantigen presentation for expansion of autoreactive Th1 and Th17 cells. Arthritis Res Ther 11:R59

Kang TY, Yang HR, Zhang J et al (2015) Corrigendum to "The studies of chlorogenic acid antitumor mechanism by gene chip detection: the immune pathway gene expression". J Anal Method Chem 2015:1

Kazancioglu HO, Aksakalli S, Ezirganli S, Birlik M, Esrefoglu M, Acar AH (2015) Effect of caffeic acid phenethyl ester on bone formation in the expanded inter-premaxillary suture. Drug Des Devel Ther 9:6483–6488

Khan R, Khan AQ, Qamar W et al (2012) Chrysin protects against cisplatin-induced colon. Toxicity via amelioration of oxidative stress and apoptosis: probable role of p38MAPK and p53. Toxicol Appl Pharmacol 258(3):315–329

Khowailed AA, Sakr HI (2017) Hesperidin and experimentally induced arthritis in male rats. J Med Sci Clin Res 5(10):29567–29585

Kim J, Kim N (2014) Regulation of NFATc1 in osteoclast differentiation. J Bone Metab 21:233–241

Kim SK, Kim HJ, Choi SE, Park KH, Choi HK, Lee MW (2008) Anti-oxidative and inhibitory activities on nitric oxide (NO) and prostaglandin E2 (COX-2) production of flavonoids from seeds of Prunus tomentosa Thunberg. Arch Pharm Res 31:424–428

Leah E (2011) Crosstalk in RA synovia—TLR3–BAFF axis sustains B-cell activation. Nat Rev Rheumatol 7(10):559

Lee GH, Lee J, Lee JW, Choi WS, Moon EY (2013) B cell activating factor-dependent expression of vascular endothelial growth factor in MH7A human synoviocytes stimulated with tumor necrosis factor-α. Int Immunopharmacol 17(1):142–147

Lee JH, Zhou HY, Cho SY, Kim YS, Lee YS, Jeong CS (2007) Anti-inflammatory mechanisms of apigenin: inhibition of cyclooxygenase-2 expression, adhesion of monocytes to human umbilical vein endothelial cells, and expression of cellular adhesion molecules. Arch Pharm Res 30 (10):1318–1327

Lee JY, Park W (2015) Anti-inflammatory effect of chrysin on RAW 264.7 mouse macrophages induced with polyinosinic-polycytidylic acid. Biotechnol Bioprocess Eng 20:1026–1034

Lee SM, Kim EJ, Suk K, Lee WH (2011) BAFF and APRIL induce inflammatory activation of THP-1 cells through interaction with their conventional receptors and activation of MAPK and NF-κB. Inflamm Res 60(9):807–815

Lee W, Lee E, Sung M et al (2014) Kaempferol inhibits IL-1β-stimulated, RANKL-mediated osteoclastogenesis via downregulation of MAPKs, c-Fos, and NFATc1. Inflammation 37:1221–1230

Ley K, Laudanna C, Cybulsky MI, Nourshargh S (2007) Getting to the site of inflammation: the leukocyte adhesion cascade updated. Nat Rev Immunol 7(9):678–689

Lixia L, Yujun L, Jingwei Z, Yi W, Jiagang D, Bin D, Limin C (2015) Chlorogenic acid and luteolin synergistically inhibit the proliferation of interleukin-1β-induced fibroblast-like synoviocytes through regulating the activation of NF-κB and JAK/STAT-signalling pathways. Immunopharmacol Immunotoxicol 37(6):499–507

Marquez N, Sancho R, Macho A, Calzado MA, Fiebich BL, Muñoz E (2003) Caffeic acid phenethyl ester inhibits T-cell activation by targeting both nuclear factor of activated T-cells and NF-B transcription factors. J Pharmacol Exp Ther 308(3):1–9

Medhi B, Puri A, Upadhyay S, Kaman L (2008) Topical application of honey in the treatment of wound healing: a meta analysis. JK Sci 10:166–169

Mithal A, Lau E (2009) The Asian Audit Epidemiology, costs and burden of osteoporosis in Asia 2009. International Osteoporosis Foundation, Nyon

Mithal A, Bansal B, Kyer C, Ebeling P (2014) The Asia-pacific regional audit-epidemiology, costs, and burden of osteoporosis in India 2013: a report of international osteoporosis foundation. Indian J Endocrinol Metab 18(4):449

Moon MK, Lee YJ, Kim JS, Kang DG, Lee HS (2009) Effect of caffeic acid on tumor necrosis factor-alpha-induced vascular inflammation in human umbilical vein endothelial cells. Biol Pharm Bull 32:1371–1377

Moundoi MA, Padila-Zakour OI, Worobo RW (2001) Antimicrobial activity of honey against food pathogens and food spoilage microorganisms. NYSAES 1:61–71

Moura RA, Canhão H, Polido-Pereira J et al (2013) BAFF and TACI gene expression are increased in patients with untreated very early rheumatoid arthritis. J Rheumatol 40(8):1293–1302

Muż B, Kontny E, Marcinkiewicz J et al (2008) Heme oxygenase-1 participates in the anti-inflammatory activity of taurine chloramine. Amino Acids 35:397–402

Nada O, Eleonora G, Domagoj Đ, Duje L, Kristijan S, Edi R, Zelko J, Maja VL, Tatjana O (2014) Role of flavonoids on oxidative stress and mineral contents in the retinoic acid-induced bone loss model of rat. Eur J Nutr 53:1217–1227

Naito Y, Yoshikawa T (2002) Molecular and cellular mechanisms involved in Helicobacter pylori-induced inflammation and oxidative stress. Free Radic Biol Med 33:323–336

Natarajan K, Singh S, Burke TR, Grunberger D, Aggarwal BB (1996) Caffeic acid phenethyl ester is a potent and specific inhibitor of activation of nuclear transcription factor NFkappa B. Proc Natl Acad Sci 93(17):9090–9095

Neog MN, Pragasam SJ, Krishnan M, Rasool M (2017) p-Coumaric acid, a dietary polyphenol ameliorates inflammation and curtails cartilage and bone erosion in the rheumatoid arthritis rat model. Biofactors 43(5):698–717

Oeckinghaus A, Ghosh S (2009) The NF-kappaB family of transcription factors and its regulation. Cold Spring Harb Perspect Biol 1:a000034

Ohata J, Zvaifler NJ, Nishio M et al (2005) Fibroblast-like synoviocytes of mesenchymal origin express functional B cell-activating factor of the TNF family in response to proinflammatory cytokines. J Immunol 174(2):864–870

Olajide OA, Kumar A, Velagapudi R, Okorji UP, Fiebich BL (2014) Punicalagin inhibits neuroinflammation in LPS-activated rat primary microglia. Mol Nutr Food Res 58 (9):1843–1851

Orban Z, Mitsiades N, Burke TR Jr, Tsokos M, Chrousos GP (2000) Caffeic acid phenethyl ester induces leukocyte apoptosis, modulates nuclear factor-kappa B and suppresses acute inflammation. Neuroimmunomodulation 7:99–105

Pang JL, Ricupero DA, Huang SA, Fatma N, Singh DP, Romero JR et al (2006) Differential activity of kaempferol and quercetin in attenuating tumor necrosis factor receptor family signalling in bone cells. Biochem Pharmacol 71:818–826

Pragasam SJ, Venkatesan V, Rasool M (2013) Immunomodulatory and anti-inflammatory effect of p-coumaric acid, a common dietary polyphenol on experimental inflammation in rats. Inflammation 36(1):169–176

Pushpavalli G, Kalaiarasi P, Veeramani C, Pugalendi KV (2010) Effect of chrysin on hepatoprotective and antioxidant status in d-galactosamine-induced hepatitis in rats. Eur J Pharmacol 631:36–41

Qin H, Wei G, Sakamaki I et al (2018) Novel BAFF-receptor antibody to natively folded recombinant protein eliminates drug-resistant human B-cell malignancies in vivo. Clin Cancer Res 24 (5):1114–1123

Rakha MK, Nabil ZI, Hussein AA (2008) Cardioactive and vasoactive effects of natural wild honey against cardiac malperformance induced by hyperadrenergic activity. J Med Food 11:91–98

Rasul A, Millimouno FM, Ali Eltayb W, Ali M, Li J, Li X (2013) Pinocembrin: a novel natural compound with versatile pharmacological and biological activities. Biomed Res Int 379850:1–9

Reyes LI, Leon F, Gonzalez P et al (2008) Dexamethasone inhibits BAFF expression in fibroblast-like synoviocytes from patients with rheumatoid arthritis. Cytokine 42(2):170–178

Ribeiro D, Freitas M, Lima JL, Fernandes E (2015) Proinflammatory pathways: the modulation by flavonoids. Med Res Rev 35:877–936

Roohbakhsh A, Parhiz H, Soltani F, Rezaee R, Iranshahi M (2014) Neuropharmacological properties and pharmacokinetics of the citrus flavonoids' hesperidin and hesperidin-a mini-review. Life Sci 113:1–6

Ruiz-Miyazawa KW, Staurengo-Ferrari L, Mizokami SS et al (2017) Quercetin inhibits gout arthritis in mice: induction of an opioid-dependent regulation of inflammasome. Inflammopharmacology. https://doi.org/10.1007/s10787-017-0356-x

Samuel JP, Vijayalakshmi V, Mahaboob KR (2013) Immunomodulatory and anti-inflammatory effect of p-coumaric acid, a common dietary polyphenol on experimental inflammation in rats. Inflammation 36:169–176

Shankar VS, Pazianas M, Huang CL, Simon B, Adebanjo OA, Zaidi M (1995) Caffeine modulates Ca2+ receptor activation in isolated rat osteoclasts and induces intracellular Ca2+ release. Am J Physiol-Ren Physiol 268(3):F447–F454

Shin GC, Kim C, Lee JM, Cho WS, Lee SG, Jeong M, Cho J, Lee K (2009) Apigenin-induced apoptosis is mediated by reactive oxygen species and activation of ERK1/2 in rheumatoid fibroblast-like synoviocytes. Chem Biol Interact 182:29–36

Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS et al (1997) Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 89:309–319

Soliman KF, Mazzio EA (1998) In vitro attenuation of nitric oxide production in C6 astrocyte cell culture by various dietary compounds. Exp Biol Med 218:390–397

Son YO, Kook SH, Choi KC et al (2006) Quercetin, a bioflavonoid, accelerates TNF-α-induced growth inhibition and apoptosis in MC3T3-E1 osteoblastic cells. Eur J Pharmacol 529:24–32

Song YS, Park EH, Hur GM, Ryu YS, Lee YS, Lee JY, Kim YM, Jin C (2002) Caffeic acid phenethyl ester inhibits nitric oxide synthase gene expression and enzyme activity. Cancer Lett 175(1):53–61

Soromou LW, Chu X, Jiang L et al (2012) In vitro and in vivo protection provided by pinocembrin against lipopolysaccharide-induced inflammatory responses. Int Immunopharmacol 14 (1):66–74

Sun SC (2011) Non-canonical NF-kappa B signalling pathway. Cell Res 21:71–85

Sun SC (2012) The noncanonical NF-kappa B pathway. Immunol Rev 246:125–140

Sun SC, Chang JH, Jin J (2013) Regulation of nuclear factor-kappaB in autoimmunity. Trends Immunol 34:282–289

Sun SC, Liu ZG (2011) A special issue on NF-kappaB signaling and function. Cell Res 21:1–2

Surh YJ, Chun KS, Cha HH, Han SS, Keum YS, Park KK, Lee SS (2001) Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NK-kB activation. Mutat Res 480-481:243–268

Sylwia B, Magdalena C, Teresa W et al (2012) In vitro effect of quercetin on human gastric carcinoma: targeting cancer cells death and MDR. Food Chem Toxicol 50(9):3375–3383

Takayanagi H, Kim S, Koga T, Nishina H, Isshiki M, Yoshida H, Saiura A, Isobe M, Yokochi T, Inoue J, Wagner EF, Mak TW, Kodama T, Taniguchi T (2002) Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts. Dev Cell 3:889–901

Tan WF, Lin LP, Li MH, Zhang YX, Tong YG, Xiao D, Ding J (2003) Quercetin, a dietary-derived flavonoid, possesses antiangiogenic potential. Eur J Pharmacol 459(2–3):255–262

Tang QY, Kukita T, Kukita YUA, Nagata K, Sandra F, Watanabe T, Toh K, Okuma Y, Kawasaki S, Rasubala L, Teramachi J, Miyamoto I, Wu Z, Iijima T (2006) Regulation of osteoclastogenesis by Simon extracts composed of caffeic acid and related compounds: successful suppression of bone destruction accompanied with adjuvant-induced arthritis in rats. Histochem Cell Biol 125:215–225

Tezuka K, Tezuka Y, Maejima A, Sato T, Nemoto K, Kamioka H, Hakeda Y, Kumegawa M (1994) Molecular cloning of a possible cysteine proteinase predominantly express in osteoclasts. J Biol Chem 269:1106–1109

Tonks AJ, Cooper RA, Jones KP, Blair S, Parton J, Tonks A (2003) Honey stimulates inflammatory cytokine production from monocytes. Cytokine 21:242–247

Toyoda T, Tsukamoto T, Takasu S, Shi L, Hirano N, Ban H, Kumagai T, Tatematsu M (2009) Anti-inflammatory effects of caffeic acid phenethyl ester (CAPE), a nuclear factor-kappaB inhibitor, on Helicobacter pylori-induced gastritis in Mongolian gerbils. Int J Cancer 125:1786–1795

Trivedi R, Kumar A, Gupta V, Kumar S, Nagar GK, Romero JR, Dwivedi AK, Chattopadhyay N (2009) Effects of Egb 761 on bone mineral density, bone microstructure, and osteoblast function: possible roles of quercetin and kaempferol. Mol Cell Endocrinol 302:86–91

Turkmen N, Sari F, Poyrazoglu ES, Velioglu YS (2006) Effects of prolonged heating on antioxidant activity and colour of honey. Food Chem 95:653–657

Umar S, Kumar A, Sajad M, Zargan J, Ansari MM, Ahmad S, Katiyar CK, Haider AK (2013) Hesperidin inhibits collagen-induced arthritis possibly through suppression of free radical load and reduction in neutrophil activation and infiltration. Rheumatol Int 33:657–663

Uzzan M, Colombel JF, Cerutti A, Treton X, Mehandru S (2016) B cell-activating factor (BAFF)-targeted B cell therapies in inflammatory bowel diseases. Dig Dis Sci 61(12):3407–3424

Vallabhapurapu S, Karin M (2009) Regulation and function of NF-kappaB transcription factors in the immune system. Annu Rev Immunol 27:693–733

Vishnu R, Krishnan R (2018) Preclinical evaluation of anti-rheumatoid effect of chrysin in Freund's complete adjuvant induced arthritis in Wistar albino rats. Asian J Pharm Pharmacol 4(1):74–78

Wang W, Sun W, Jin L (2017) Caffeic acid alleviates inflammatory response in rheumatoid arthritis fibroblast-like synoviocytes by inhibiting phosphorylation of IκB kinase α/β and IκBα. Int Immunopharmacol 48:61–66

Duan W, Wang Q, Li F, Xiang C, Zhou L, Xu J, Feng H, Wei X (2014) Anti-catabolic effect of caffeic acid phenethyl ester, an active component of honeybee propolis on bone loss in ovariectomized mice: a micro-computed tomography study and histological analysis. Chin Med J 127(22):3932–3936

Wattel A, Kamel S, Mentaverri R, Lorget F, Prouillet C, Petit JP et al (2003) Potent inhibitory effect of naturally occurring flavonoids quercetin and kaempferol on in vitro osteoclastic bone resorption. Biochem Pharmacol 65:35–42

Wattel A, Kamel S, Prouillet C et al (2004) Flavonoid quercetin decreases osteoclastic differentiation induced by RANKL via a mechanism involving NFκB and AP-1. J Cell Biochem 92 (2):285–295

Wei F, Chang Y, Wei W (2015a) The role of BAFF in the progression of rheumatoid arthritis. Cytokine 76(2):537–544

Wei Y, Sun X, Hua M, Tan W, Wang F et al (2015b) Inhibitory effect of a novel antirheumatic drug T-614 on the IL-6 induced RANKL/OPG, IL-17, and MMP-3 expression in synovial fibroblasts from rheumatoid arthritis patients. Biomed Res Int 8:1–8

Woo JT, Nakagawa H, Notoya M, Yonezawa T, Udagawa N, Lee IS, Ohnishi M, Hagiwara H, Nagai K (2004) Quercetin suppresses bone resorption by inhibiting the differentiation and activation of osteoclasts. Biol Pharm Bull 27:504–509

Wright NC, Looker AC, Saag KG et al (2014) The recent prevalence of osteoporosis and low bone mass in the United States based on bone mineral density at the femoral neck or lumbar spine. J Bone Mineral Res 29(11):2520–2526

Xiaohong F, Xilin L, Han L, Dan Z, Zhizhen X, Fengtian H, Gang H (2019) Chlorogenic acid inhibits BAFF expression in collagen-induced arthritis and human synoviocyte MH7A cells by modulating the activation of the NF-κB signaling pathway. J Immunol Res 2019:8042097

Xu L, Zhang L, Bertucci AM, Pope RM, Datta SK (2008) Apigenin, a dietary flavonoid, sensitizes human T cells for activation-induced cell death by inhibiting PKB/Akt and NF-κB activation pathway. Immunol Lett 121:74–83

Yamaguchi M, Hamamoto R, Uchiyama S, Ishiyama K (2007) Effects of flavonoid on calcium content in femoral tissue culture and parathyroid hormone-stimulated osteoclastogenesis in bone marrow culture in vitro. Mol Cell Biochem 303:83–88

Yang WS, Jeong D, Young-Su Y, Park JG, Hyohyun S, Sang HM, Sungyoul H, Youl JC (2013) IRAK1/4-targeted anti-inflammatory action of caffeic acid. Mediat Inflamm 518183:1–12

Yang Y, Zhang X, Xu M, Xin W, Feipeng Z, Chengzhi Z (2018) Quercetin attenuates collagen-induced arthritis by restoration of Th17/Treg balance and activation of Heme Oxygenase 1-mediated anti-inflammatory effect. Int Immunopharmacol 54:153–162

Yoon H Y, .Lee E G, Lee H, Cho I J, Choi Y J, Sung M S, Yoo H G, Yoo W H (2013) Kaempferol inhibits IL 1β induced proliferation of rheumatoid arthritis synovial fibroblasts and the production of COX 2, PGE2 and MMPs. Int J Mol Med 32: 971–977

Yueqi C, Ce D, Jin Y, Ruohui T, Tao Y, Lan Z, Wei L, Mengmeng L, Xiaolong Y, Jianmei L, Fei K, Yufeng Z, Shiwu D (2018) Inhibitory effect of vanillin on RANKL-induced osteoclast formation and function through activating mitochondrial-dependent apoptosis signaling pathway. Life Sci 208:305–318

Zaidi M, Blair HC, Moonga BS, Abe E, Huang CL (2003) Osteoclastogenesis, bone resorption, and osteoclast-based therapeutics. J Bone Miner Res 18:599–609

Zaidi M, Moonga BS, Adebanjo OA (1999) Novel mechanism of calcium handling by osteoclast: a review-hypothesis. Proc Assoc Am Physicians 111:319–327

Zeng S, Wang K, Huang M et al (2017) Halofuginone inhibits TNF-alpha-induced the migration and proliferation of fibroblast-like synoviocytes from rheumatoid arthritis patients. Int Immunopharmacol 43:187–194

Zhang D, Huang B, Xiong C, Zhou Y (2015) Pinocembrin inhibits matrix metalloproteinase expression in chondrocytes. IUBMB 67(1):36–41

Honey in Anticancer Drug Toxicity

14

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Abstract

Cancer is a major health issue and the second leading cause of mortality in the world. The most common treatment of cancer is chemotherapy, but adverse effects and chronic sequelae developing from anticancer chemotherapy hamper its clinical use despite its efficiency and adds to the discomfort of cancer patients. The side effects, however, are different for different people depending upon health, dosage and the type of cancer. The most common types of toxicities induced through chemotherapeutic drugs are oral mucositis, gastrointestinal toxicity, hepatotoxicity, nephrotoxicity, cardiotoxicity, and neurotoxicity. Natural products are known for a number of properties, one being prevention and treatment of drug toxicities in cancer. Natural honey is a combination of phenolic acids, flavonoids, antioxidants, etc. and acts as a chemoprotectant and adjuvant during cancer treatment. This book chapter focuses in detail on the efficiency of honey from a potential therapeutic perspective and explains the possible mechanisms of the actions in increasing the tolerance and treatment of chemotherapy-induced side effects in clinical settings. In addition, particular attention is drawn to the role of chrysin, which is a honey flavonoid used in protection against drug therapy-induced toxicities.

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Honey · Drug toxicity · Anticancer · Chemoprotective

14.1 Introduction

Globally, the most common cause of death is cancer (Bray et al. 2018). Chemotherapy and radiotherapy are the most effective and extensive approaches for cancer management. However, chemotherapy and radiotherapy-induced feared side effects including oral mucositis, gastrointestinal toxicity, nephrotoxicity, hepatotoxicity, cardiotoxicity, neurotoxicity and myelosuppression are not only the major concerns for patients but for clinicians as well as they may hinder the clinical usage despite boosting the survival (Shapiro 2016). Even natural chemotherapeutic agents with epirubicin, taxol and irinotecan also result in various problems including bone marrow depression. Half of the cancer patients get mucositis on chemotherapy and more than 50% when chemotherapy is combined with radiotherapy. These harmful side effects along with emotional and psychological stress often worsen the quality of life in cancer patients, and may lead to treatment termination (Ahmed and Othman 2013). Effective management approaches are necessary to increase tolerance for chemotherapy-induced side effects that would lessen the severity. There is increasing evidence that many natural products tend to decrease the side effects resulting from such treatments and may be useful when used together to inhibit overproliferation in tumours by cell cycle arrest and prevent metastatic invasion and stimulation of programmed cell death via activating pathways that increase the expression of tumor necrosis factor alpha (TNF-α) and as well as cause inhibition of lipoprotein oxidation. Natural products may be effective against toxic agents in in vitro and animal experiments (Sanders et al. 2016). Honey has been regarded as one of the natural products that mediates these beneficial effects along with its components. Honey has been used since medieval times as a nutritional product and mainly for its remedial and therapeutic significance in traditional medicine (Braakhuis et al. 2019). It has been shown to have anticancer, anti-inflammatory, antibacterial, antiviral, antidiabetic, antimutagenic and antioxidant properties (Erejuwa et al. 2010; Kishore et al. 2011; Viuda Martos et al. 2008). Honey also has the same antitumor activities as some of the chemotherapeutic drugs. Many types of honey are available worldwide but its chemical and physical composition varies from one place to another depending upon the flora source, season, environment, and geographical origin as well as honeybee species. Honey is a natural liquid consisting of at least 181 ingredients including proteins, minerals, vitamins and natural organic acids (Al-Waili et al. 2012). Honey also contains phenolic acids like caffeic, ferulic, ellagic and coumaric acids; flavonoids such as apigenin, chrysin, galangin, hesperetin, kaempferol, pinocembrin and quercetin; antioxidants, such as tocopherols, ascorbic acid, superoxide dismutase, catalase and glutathione and glycosides, quinone, alkloids, cardiac glycosides, and volatile substances (Grecka et al. 2018). All these constituents have their unique properties and together they

increase the dietetic and homeopathic value of natural honey (Vit et al. 2015). Honey has high content of bioactive plant compounds and various constituents of honey have antioxidant properties as well. Flavonoids such as chrysin in honey have a strong antioxidant property and are present in large content (Islam et al. 2017). According to the recent scientific literature, honey and chrysin may be effective against a wide range of diseases from wound healing to cancer (Lavaf et al. 2017; Song et al. 2019; Yuan et al. 2019) and both play a role in preventing natural and chemical toxicity in different tissues (Van Meeuwen et al. 2007). Several studies have suggested that honey and its components decrease neurotoxicity, lung toxicity, cardiovascular toxicity, hepatotoxicity, nephrotoxicity, reproductive toxicity, genotoxicity and immunotoxicity by modulating oxidative stress and apoptosis in various organs. Drug toxicity is one of the main problems concerned with chemotherapy and causes various illnesses (Nurgali et al. 2018). Honey is suitable and effective for the treatment of oral mucositis and skin reactions, stomatitis, periodontal gum disease, malignant ulcers, external surgical wounds, infections in paediatric oncology patients and chemotherapy-induced palmar-plantar erythrodysesthesia or hand-foot syndrome. It is a dermatologic reaction on the skin of hands and feet caused due to cytotoxic chemotherapy drugs such as fluorouracil or capecitabine (Janusch et al. 2006). Therefore, more clinical studies should be done to confirm the efficacy and safety of honey and chrysin for treating chemotherapy based intoxication. Thus, this book chapter aims to review the protective effects of honey against chemotherapy-induced drug toxicity.

14.2 Chemotherapy-Induced Damages and Honey

The use of either fresh, old or raw natural bee honey has been shown to be effective in the management of various problems caused after chemotherapy such as mucositis, infections such as throat infection, vomiting, diarrhea, constipation and many more (Maiti et al. 2012). Oral mucositis is one of the most significant oral cavity complications in patients undergoing chemotherapy or radiotherapy or a combination of both that triggers ulceration of oral mucosa, painful oral lesions, erythema and systemic infections. This can result in prolonged hospitalization and decreased chances of cure. Most of the cancer patients receiving intensive chemotherapy suffer from this dose limiting complication that significantly affects the patients with malignancies in the head and neck (Simon et al. 2009). Clinical evidence demonstrates that honey can treat the cytotoxic effects of mucositis in children, hence used as a therapeutic measure in paediatric cancer patients (Friend et al. 2018). In a pilot study on patients with acute lymphoblastic leukemia and a condition of oral mucositis, honey alone and a combination of honey, olive oil, propolis and beeswax (HOPE) showed that topical treatment of honey or HOPE resulted in faster healing as compared to the control group, hence both having a role in chemotherapy-induced mucositis (Abdulrhman et al. 2012). A study compared the role of honey + coffee in the treatment of mucositis and found that as compared to betamethasone-treated (steroid) and honey-treated groups, the honey + coffee group

showed significant reduction in the severity of lesions in the hypopharynx mucosal membrane. This may be due to the fact that caffeine in coffee has anti-inflammatory and antioxidant effects on a number of tissues and together with honey it synergistically increases mucosal tissue healing (Raeessi et al. 2014). A study on children suffering from chemotherapy-induced mucositis showed confirmed positive effects and the simple, cost-effective modality role of honey in the management of oral mucositis. As compared to the control group that received antiseptic gel treatment, the experimental group received additional treatment with honey. The latter group showed reduced severity and rapid management of mucositis (Singh et al. 2019). Combinational therapy with the use of honey and tulsi (*Ocimum tenuiflorum*) ice chips together with chemotherapy was found to be superior to chemotherapy alone due to the fact that this treatment before the dose of methotrexate in children showed decreased signs of mucositis (Mishra and Nayak 2017). A study showed the chemopreventive role of Yashtimadhu (*Glycyrrhiza glabra*) together with honey in head and neck malignancies after receiving therapy and that the intensity of mucositis and skin reactions was reduced when this combination was used either orally or locally (Das et al. 2011). A similarly combination of glycerine plus honey in oropharangeal cancer patients showed delayed occurrence of mucositis compared to the control group receiving the standard treatment (Bansal et al. 2017). In patients with oral cancer, which is more common in India, topical application of honey was found to have a contending role in preventing the side effects of chemoradiation-induced mucositis like dysphagia, irritation and dryness as well as helping in speedy recovery (Howlader et al. 2019). Propolis is a resinous mixture found in honey and has anti-ulcer, anti-microbial, healing and anti-inflammatory properties. In a clinical trial, Hypozalix spray and propolis mouthwash efficiently decreased the occurrence of and healed oral mucositis in patients with head and neck malignancies or leukemia (Eslami et al. 2016). In a similar meta-analysis study and propolis mouthwash was found to be an effective and safe treatment (Kuo et al. 2018). Propolis extract was given to patients with breast cancer receiving adjuvant therapy with doxorubicin and cyclophosphamide and was found to prevent the occurrence of mucositis (Piredda et al. 2017). Oral mucositis is caused by pathogens like bacteria, and honey is known for its antibacterial properties against a wide range of bacteria. Its bioactive compounds and components like hydrogen peroxide and the bee-derived antibacterial peptide defensin-1 are majorly known to be responsible for its antibacterial activity. However, due to variation in the concentrations of these compounds, different types of honey show different levels of inhibition (Bucekova et al. 2019). The antibacterial role of honey against *Candida* and aerobic pathogenic bacterial infections showed reduction in the onset of mucositis in pediatric cancer patients when applied topically, hence can be used as a prophylaxis (Al Jaouni et al. 2017). Standard or high-dose cytarabine (Ara-C) has been shown to develop chemotherapy-induced gastrointestinal complications like abdominal pain and diarrhea and together with fever and melena may cause a life threatening condition in acute myeloid leukemia (AML) patients undergoing chemotherapy. There is high incidence of ileothyphlitis and other gastrointestinal infections (28.5%) in such patients as well (Micozzi et al. 1996). In such patients with gastrointestinal

complications, the effect of the combination of honey and ardeh favoured recovery from side effects vs. control. Also, it reduced fever, neutropenia nausea and vomiting in patients with acute myeloid leukemia significantly (Ebrahimi et al. 2016). All therapeutic routines produce varying side effects, including haematological toxicity. The most toxic side effect of the majority of chemotherapeutic agents is myelosuppression (bone marrow suppression) and classically is the dose-limiting factor. It can vary in its severity from mild to severe. A severe type of myelosuppression, called myeloablation, can be lethal. With myelosuppression there can be a decline of some or all of the body's bone marrow producing cells like the red blood cells, white blood cells and platelets. The administration of different chemotherapeutic agents and even those of natural origin, such as epirubicin, irinotecan and taxol causes toxicity in the precursor cells of bone marrow, hence causing early bone marrow depression which is characterized by rapid cell division (Oršolić et al. 2005; Oršolić and Bašić 2008). Febrile neutropenia is a condition that results from systemic chemotherapy and causes opportunistic infections in cancer patients subsequent to chemotherapy. Colony-stimulating factors (CSFs) are known to treat patients with neutropenia by acting as an adjuvant. Administration of Life-Mel honey instead of CSFs in patients treated with chemotherapy decreased the risk of pancytopenia. On administration of honey along with the same chemotherapy schedule there was no need of CSFs in 40% of the patients with no side effects. This treatment proves to be inexpensive, easy and costs only 8% of the CSF course in chemotherapy (Zidan et al. 2006). After chemotherapy the cause of death can be either the infectivity related to drug-induced leucopoenia or from hemorrhage related to thrombocytopenia (Ozer et al. 2000). Similarly, in children with acute lymphoblastic leukemia intake of raw honey of about 2.5 g/kg given twice a week for 12 weeks was considered safe against the adverse effects of chemotherapy-induced febrile neutropenia (Abdulrhman et al. 2016). Honey in combination with chemotherapeutics (5-FU or adriamicin) may prevent chemotherapeutic-induced toxicity on leukocyte populations in the peripheral blood (Orsolic and Basic 2004). *Cis*-diamminedichloroplatinum is a potent drug used in chemotherapy against broad-spectrum tumors but has nephrotoxic side effects. A study was carried out in Wistar rats where bee honey, royal jelly and cisplatin were given to different groups to check the nephroprotective effects of natural products, honey and royal jelly on renal injury. Cisplatin treatment was given beforehand and unlike this group the honey- and royal jelly-treated group showed normal serum biomarkers for renal injury and low expression of transforming growth factor β1, α-smooth muscle actin and bromodeoxyuridine (Brdu) (Ibrahim et al. 2016). Similarly, the nephroprotective effects of honey and royal jelly were seen in cancer patients receiving cisplatin in chemotherapy, which showed promising results (Osama et al. 2017). In a rat model, the role of Manuka and Talh honeys was assessed on cisplatin-induced nephrotoxicity and hepatotoxicity and both showed protective effects by limiting the inflammatory and apoptotic pathway (Neamatallah et al. 2018).

14.3 Honey and Its Components: Chemoprevention and Adjunct to Anticancer Drugs

The chemopreventive role of honey and its components to combat the negative side effects of cancer treatments has been shown to improve the quality of life for patients with variety of cancers. Similarly, the role of honey as an adjuvant with drugs during cancer therapies is shown to prevent progression to malignancy, reduce the required dosage of conventional drugs, lessen the severity of adverse effects and help in the management of cancer therapy overall (Table 14.1). 5-fluorouracil (5-FU) is one of the frequently used chemotherapy drugs against a wide range of cancers including colorectal cancer, and it exerts it anticancer property through inhibition of thymidylate synthase. A study was performed to see the impact of a combined treatment of ginger extract and honey with 5-FU on inhibition of cell growth and cell death, i.e. the chemotherapeutic effect of 5-FU. It was shown that in comparison to the treatment of cells with ginger extract and Gelam honey alone, the co-treatment of Gelam honey and ginger extract with 5-FU shows a higher rate of anti-proliferative effects on cancer cells. It was three times higher than that with 5-FU treatment alone due to a synergism between the ginger extract and the Gelam honey in a dose-dependent manner (Hakim et al. 2014). To unravel the signaling pathways involved during such combination treatments in anticancer mechanism have not been extensively studied. However, in one study on colorectal cancer (HT29) cell line, the cells were treated with ginger extract and Gelam honey in independent and combined therapy. It showed a synergism between the two compounds for curbing the proliferation and activation of apoptotic pathways through increased expression of caspase 9 and IκB genes and decreased expression of KRAS, extracellular-signal-regulated kinase, protein kinase B (Akt), B-cell lymphoma-extra large (Bcl-xL) and NF-κB (p65) genes (Tahir et al. 2015). Another similar study showed the effect of synergism between ginger extract and Gelam honey on colon cancer (HCT116) cells. The effect was seen on various pathways like mTOR, Wnt/β-catenin and apoptosis signaling pathways. The combined therapy down-regulated the expression of mTOR, Raptor, Akt, Rictor, Gsk3β, β-catenin, Tcf4 and cyclin D1 genes, while cytochrome C and caspase 3 genes were up-regulated (Wee et al. 2015). Similarly, the effect of Manuka honey on enhancing the anticancer property of 5-FU on human colon cancer HCT-116 cells and LoVo cells showed a synergistic role in diminishing cell growth through suppression of the epidermal growth factor receptor (EGFR), HER2, p-Akt and p-mTOR expression and transcription factor (NF-κB and Nrf2); increasing cell death by modulation of pro-apoptotic markers like p53, Bax, Cyto c, FasL caspase-3, -8, -9, cleave-PARP and anti-apoptotic Bcl-2 marker; decreasing antioxidant enzyme activity (superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase) and suppressing the expression of metalloproteinases-2, 9 and increasing N-cadherin and E-cadherin in metathesis. Manuka honey acts as an adjuvant in the treatment of colon cancer (Afrin et al. 2018). The phytochemical content and the antioxidant activity of Strawberry tree honey and Manuka honey on human colon adenocarcinoma (HCT-116) and metastatic (LoVo) cell lines were compared and both the Strawberry tree honey and Manuka honey showed an

Table 14.1 Chemo protective effects of honey and its components against toxic agents and honey as an adjuvant to anticancer drugs

Honey	Toxic agents	Function	Cancer type	References
Ginger extract and Gelam honey	5-fluorouracil	Inhibition of thymidylate synthase	Colorectal Cancer	Hakim et al. (2014)
Manuka honey	5-fluorouracil	Suppression of EGFR, HER2, p-Akt and p-mTOR, NF-κB, Nrf2, SOD, CAT, GPx, glutathione reductase, MMP-2, MMP-9 and Bcl-2, activation of p53, Bax, Cyto c, FasL caspase-3, -8, -9 and cleave-PARP, and N-cadherin and E-cadherin	Colorectal Cancer	Afrin et al. (2018)
Manuka honey	Paclitaxel	The rate of apoptosis in tumor cells was alleviated	Melanoma mice	Fernandez-Cabezudo et al. (2013)
Tualang honey Manuka honey	*N*-methyl-*N*-nitrosourea	Manuka honey more effective in inhibiting tumor growth than Tualang honey	Breast cancer	Othman et al. (2016)
Tualang honey	7,12-dimethylbenz (α)anthracene	Tumour size, histological grade, VEGF levels and angiogenesis were decreased	Breast carcinogenesis	Kadir et al. (2013)
Tualang honey	Tamoxifen	Inducing the apoptosis on ER responsive MCF-7 and ER non-responsive MDA-MB-231 cells	Breast cancer	Yaacob et al. (2013)
Tualang honey	4-hydroxytamoxifen	Tualang honey was cytotoxic to MCF-7 and not to normal MCF-10A cells	Breast cancer	Yaacob and Ismail (2014)

(continued)

Table 14.1 (continued)

Honey	Toxic agents	Function	Cancer type	References
Sunflower honey	Tamoxifen and aromatase inhibitors	Reduced the menopausal complaints	Breast cancer	Münstedt et al. (2015)
Honey	Cisplatin, cyclophosphamide and 5-fluorouracil	Decreased the activity of sera ALT, AST and ALP and increased activities of CAT, SOD, GPx, GST and GSH	Liver hepatocarcinogenesis	Mohamed et al. (2019)
Honey	Diethlynitrosamine	Less lesions and less damaged hepatocytes, edema and malignancy	Hepatocellular carcinoma	El-Kott et al. (2012)
Kelulut honey	Azoxymethane	Crypt foci, multiplicity and total aberrant crypts were reduced	Colon cancer	Saiful Yazan et al. (2016)
Iranian propolis	*N*-methyl-*N*-nitro-*N*-nitrosoguanidine	Smaller gastric lesions and a decline in structural abnormality, epithelial stratification and nuclear dispolarity	Gastric cancer	Dinparast-Djadid et al. (2015)
Propolis, caffeic acid, wild flower honey, royal jelly and bee venom	Methylcholanthrene	Honey has antimetastatic effects and less significant tumour nodules in the lungs	Transplantable mammary carcinoma and methylcholanthrene-induced fibrosarcoma	Oršolić et al. (2005)

increased rate of cell death, cytotoxicity in both cancer cell lines as compared to non-cancer cells. In addition, there was an increase in the generation of reactive oxygen species (ROX) by Strawberry tree honey and Manuka honey in HCT-116 and LoVo cells as well. These results suggest a potential chemopreventive agent against colon cancer, and honey can act as an adjuvant in the functioning of drugs already used in cancer treatment (Afrin et al. 2017; Badolato et al. 2017). Manuka honey administration with the antineoplastic drug paclitaxel alleviates the chemotherapy-induced cytotoxicity in the melanoma model of mice (C57BL/6). The study was carried out where mice were administered with Manuka, paclitaxel

or a combination of both. After treatment, the rate of apoptosis in tumor cells was found to be the highest in mice treated with a combination of Manuka and paclitaxel (Fernandez-Cabezudo et al. 2013). In the breast cancer-induced rat model, Tualang and Manuka honey were administered after induction of breast carcinogenesis with *N*-methyl-*N*-nitrosourea. Manuka honey was shown to be more effective in inhibiting the tumor growth than Tualang honey (Othman et al. 2016). But a study on Tualang honey showed that it could be used as an adjuvant in chemotherapy in breast carcinogenesis. On administration of Tualang honey, the size of breast tumor induced by the carcinogen 7,12-dimethylbenz(α)anthracene (DMBA) in rats was reduced as compared to controls (not treated with honey). Many other factors were also reduced like histological grade, VEGF levels and angiogenesis. However, the apoptotic index was high in the honey treated cancer group but in general the average AI was the same for both the TH group and non-TH-treated group (Kadir et al. 2013). Tamoxifen is a drug used as an adjuvant for chemotherapy in women with high susceptibility for breast cancer. Its various toxic side effects were reduced in combination with Tualang honey on healthy cells, non-tumour breast cancer cell line (MCF-10A). Similarly, on estrogen receptor (ER) responsive (MCF-7) and ER-non-responsive human breast cancer (MDA-MB-231) cell lines, Tualang honey increased the effectiveness of the drug tamoxifen by inducing apoptosis on both MCF-7 and MDA-MB-231 cells compared to independent treatments (Yaacob et al. 2013). Similarly, a study showed the effect of Tualang honey on cancerous MCF-7 cells in comparison to normal MCF 10A cells independently and in combination with 4-hydroxytamoxifen, an active metabolite of tamoxifen. 4-Hydroxytamoxifen was cytotoxic to both cell lines, while honey was cytotoxic only to MCF-7 and not to MCF-10A. Tualang honey increased the 4-hydroxytamoxifen-induced DNA damage on MCF-7 cells but decreased the toxic effect of 4-hydroxytamoxifen by increasing the expression of DNA repair proteins Ku70 and Ku80 in MCF-10A cells (Yaacob and Ismail 2014). In patients with breast cancer, Sunflower honey was given along with tamoxifen and aromatase inhibitors that reduced the menopausal complaints in patients with breast cancer (Münstedt et al. 2015). Liver hepatocarcinogenesis is the second cause of cancer-related deaths. The combinational therapy of honey and chemotherapeutic drugs and honey alone has been very effective in chemoprevention against diethylnitrosamine and carbon tetrachloride-induced hepatocellular carcinoma in rats. The chemotherapy drugs used in this study are cisplatin, cyclophosphamide and 5-fluorouracil. These drugs show decreased activity of sera alanine transaminase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) but the induction was more potent in case of treatment with honey plus drugs. Similarly, the co-administration showed a significantly higher increase in the activities of antioxidant enzymes of catalase, superoxide dismutase, glutathione peroxidase, glutathione-*s*-transferase and glutathione as compared to the control (Mohamed et al. 2019). In a study, hepatocellular carcinoma was induced in Sprague Dawley rats through diethlynitrosamine administration, which is considered a liver carcinogen. The groups treated with honey and diethlynitrosamine alone and in combination showed a different liver histology. The liver hepatocytes of the groups treated with diethlynitrosamine + honey and honey alone were normal,

while those treated with diethlynitrosamine had lesions and damaged hepatocytes, edema and malignancy. Also, P-53 tumor protein and proliferating cell nuclear antigen (PCNA) positive nuclei were observed more significantly in immunohistochemistry liver sections for the diethlynitrosamine group than the diethlynitrosamine + honey group and no expression was observed in controls (untreated and honey groups). This indicates honey as a therapeutic agent in hepatocellular carcinoma (El-Kott et al. 2012). Cisplatin is one of the most common chemotherapeutic drugs used for tumors but is known for nephrotoxicity. This study showed that honey protects the kidneys from nephrotoxicity of cisplatin by improving the kidney function, reduces cisplatin-induced tubular epithelial cell death, reduces inflammation in the kidneys and decreases proinflammatory cytokine and chemokine activation such as NF-kB. For this the animals were first treated with orally administered crude honey 500 mg/kg per day for a week followed by cisplatin (Hamad et al. 2015). Another study showed the therapeutic role of *Schisandra chinensis* bee pollen extract (SCBPE) on liver and kidney injury induced by cisplatin (Huang et al. 2017). Kelulut honey produced by the stingless bees from Trigona species is known for its chemopreventive properties. In a study colon cancer was induced in Sprague Dawley rats with azoxymethane administered through the intraperitoneal route. Honey was administered twice orally for 8 weeks followed by azoxymethane that was given once a week for 2 weeks to the treated group, while others were azoxymethane alone, honey alone and untreated control groups. The blood profile and the kidney and liver function for all the groups were similar. However, the aberrant crypt foci, multiplicity and total aberrant crypts were reduced on treatment with kelulut honey, hence overall it is chemopreventive and non-toxic (Saiful Yazan et al. 2016). Propolis is found in honey and has a number of properties including anticancer. Iranian propolis was shown to have a protective effect on *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG)-induced gastric cancer. Propolis was administered before the induction in animals followed by assessment for metastatic tumors and expression of apoptotic genes. As compared to control, the MNNG + propolis-treated group showed smaller gastric lesions by 33%, the number of tumors was reduced by 38% and there was a decline in structural abnormality, epithelial stratification and nuclear dispolarity. Similarly, immunohistochemistry showed increased expression of pro-apoptotic Bax protein and decreased expression of anti-apoptotic Beta-catenin and Bcl-2 in the MNNG + propolis group as compared to the group treated with MNNG only (Dinparast-Djadid et al. 2015). The anti-metastatic and anti-tumour properties of propolis, caffeic acid, wild flower honey, royal jelly and bee venom were examined in the transplantable mammary carcinoma (MCa) model and methylcholanthrene-induced fibrosarcoma mouse models. Honey administration before and after tumor inoculation showed anti metastatic effects and less significant tumour nodules in the lungs. Similarly, propolis and caffeic acid showed significant protective effects before and after tumour inoculation. Royal Jelly showed no protective effects before and after tumour inoculation but showed a significant role in inhibiting the tumour when inoculated together with tumour cells (Oršolić et al. 2005). Cyclophosphamide is a cytotoxic alkylating agent used in treating a number of problems like cancer, autoimmune diseases, etc. and its two

active products are phosphoramide mustard and acrolein, both being highly toxic. A study was carried out to examine the effects of honeybee products such as propolis, royal jelly and pollen grains on a cyclophosphamide-induced tumor model and was found to ameliorate the side effects of the anticancer drug cyclophosphamide (Fahmy et al. 2015). In mice and human bladder cancer cell lines, bee honey reduced the cell number with an increase in the concentration of honey, increased the apoptotic index as compared to controls and decreased cell proliferation than in the untreated cells. Also, oral and intralesional injection of honey showed a decrease in the volume of subcutaneously implanted abdomen tumours in mice treated with honey than in the untreated controls (Swellam et al. 2003).

In future, studies should be commenced to validate the role of varieties of honey and its components, which will have remarkable clinical implications in cancer treatment.

14.4 Role of Chrysin in Drug Toxicity

Chrysin is a flavonoid that occurs naturally in bee propolis and honey and is one of the phytochemicals known to have a wide range of health benefits (Siddiqui et al. 2018; Cheung et al. 2019). It provides protective effects from toxicities caused by various chemotherapeutic drugs in various tissues including the liver, kidneys, heart and lungs (Fig. 14.1) and has pharmacological activities like inhibition of nitric oxide synthase, NF-κB, histone deacetylase and DNA topoisomerases, and anti-inflammatory role by preventing the release of pro-inflammatory cytokines, anticancer role by inducing apoptosis, activation of TNF related apoptosis-inducing ligand (TRAIL), inhibition of TNF-α and IL-1β and antidiabetogenic, antimetastatic and antihypertensive roles (Samarghandian et al. 2017, 2019). Chrysin has protective roles against various chemotherapeutic drugs that induce hepatotoxicity such as methotrexate and cisplatin. Methotrexate is a chemotherapeutic drug used in the treatment of a wide range of tumours and autoimmune diseases but its hepatotoxic effect on the liver limits its clinical use. It exerts its side effect by generating oxidative stress through reactive oxygen species (ROS). Both normal and cancerous cells are subjected to ROS-induced apoptosis. The antitoxic role of chrysin was demonstrated in a study on male wistar rats. Chrysin was given at different dosages and showed anti-hepatotoxic and anti-apoptotic effects through its free radical scavenging property. The chrysin pretreatment group showed normal liver function, the hepatocytes were normal and less distorted. The toxicity markers in the serum like lactate dehydrogenase, alanine transaminase and aspartate-aminotransferase and protein expression of apoptotic markers like p53, Bax protein and caspases 3 were reduced in the chrysin-treated groups and elevated in the methotrexate-treated group. Chrysin also modulates the enzymatic activity of superoxide dismutase and catalase, which are the main antioxidants (Ali et al. 2014). Cisplatin exerts its cis-induced hepatotoxic role through inflammation and oxidative stress. Oral treatment of chrysin in wistar rats pretreated with cisplatin prevented lipid peroxidation, xanthine oxidase activity and glutathione depletion. Chrysin prevented hepatic tissue damage,

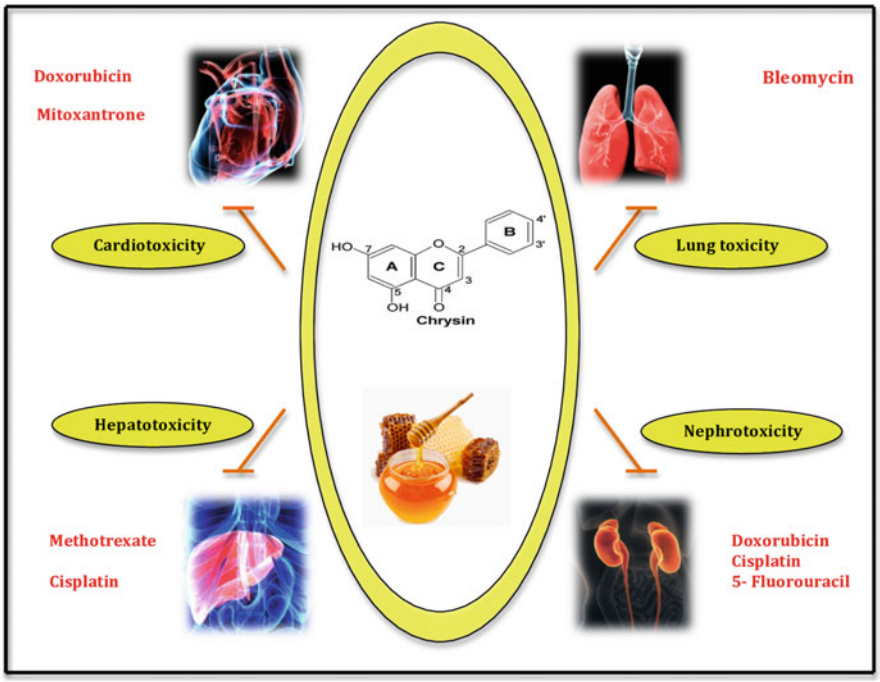

Fig. 14.1 The protective effect of chrysin against chemotherapeutic drug-induced toxicities of the heart, lungs, liver and the kidneys

enhanced expression of cyclooxygenase-2, iNOS and NF-κB, and TNF-α levels (Rehman et al. 2014). Chrysin also has a protective role in cisplatin-induced jejunal toxicity by decreasing the levels of antioxidant enzymes such as catalase, glutathione reductase, glucose-6-phosphate and dehydrogenase and phase II detoxifying enzymes (glutathione-*S*-transferase and quinone reductase) and glutathione, increasing xanthine oxidase activity and attenuating the expression of p53 and apoptotic tissue damage induced by cisplatin (Khan et al. 2012). Chrysin also provides protection against 1,2-dimethyl hydrazine carcinogenesis-induced rat model by reducing preneoplastic colorectal lesion numbers, increasing antioxidant activities and decreasing proliferation and stress (Sequetto et al. 2013). Chrysin plays a protective role against chemotherapy agent-induced cardiotoxicity such as doxorubicin and mitoxantrone. Mitoxantrone treatment causes irreversible congestive heart failure and cardiomyopathy that reduces the left ventricular ejection fraction. It increases the levels of cardiac injury indicator, creatine kinase (CK-MB) and pro-apoptotic markers Bax and caspase-3, while decreases the Bcl-2 expression. Chrysin treatment prevented apoptosis though a decrease in the Bax/Bcl-2 ratio, restoration of desmin disarray (Anghel et al. 2015). Doxorubicin induces cardiovascular toxicity through generation of inflammation, apoptosis and oxidative stress (Minotti et al. 2004). Chrysin was shown to prevent such side effects through

decreasing the pro-apoptotic activity of Bax and cytochrome c and caspase-3, reduced expression of COX-2, NF-κB, iNOS, and the levels of TNF-α and NO and increasing the activity of Bcl-2, antioxidant enzymes (catalase and superoxide dismutase) and lipid peroxidation (Mantawy et al. 2014). Doxorubicin also induces renal toxicity via decreased antioxidant activities. Chrysin prevents nephrotoxicity by managing the levels of aspartate aminotransferase, alanine transaminase and lactic acid dehydrogenase and reversing the histopathological damages (Rashid et al. 2013). Cisplatin is also known for inducing nephrotoxicity through oxidative damage by increasing the antioxidant enzyme (catalase, glutathione peroxidase, glutathione reductase and glutathione S transferases) expression and diminishing markers like serotonin and blood urea nitrogen (Sultana et al. 2012). Cardiovascular toxicity, liver toxicity and renal toxicity by 5-fluorouracil, an antineoplastic drug, limits its clinical use. It causes side effects like apoptosis and oxidative stress in the kidneys through activation of pro-apoptotic proteins and decreases the expression of anti-apoptotic proteins. Administration of chrysin after induction of rats with 5-FU showed a marked decrease in toxicity markers, regulated apoptosis in the kidneys and restored renal histoarchitecture, tubular architecture, etc. (Rashid et al. 2014). In respiratory toxicity, chrysin protects against bleomycin in rats by reversing alveolar congestion and connective tissue infiltration and reducing activities of catalase, superoxide dismutase and glutathione and lung inflammation and fibrosis (Kilic et al. 2014). Since most of these studies are based on animal models, more studies and human clinical trials are required to establish the role of chrysin in human toxicities.

14.5 Conclusion

This book chapter summarizes the role of different types of honey in preventing side effects caused by drugs used in chemotherapy. Conventional honey is shown to have chemo protective effects against chemotherapy-induced mucositis, myelosuppression, nephrotoxicity and neutropenia. Since honey is a combination of various bioactive compounds like phenolic acids, flavonoids and antioxidants, in future many studies are required to identify the role of each component in honey responsible for the prevention of chemotherapy-induced damages. Chrysin is one of the most extensively studied flavonoid that has multiple roles in various diseases and is known for its role in preventing toxicities in the liver, lungs, heart and kidneys induced by chemotherapeutic drugs given during cancer treatment. Since a majority of the studies are based on animal models, human clinical studies are necessary to establish its role. More clinical trials are needed to prove the authenticity of honey treatment either alone or as an adjuvant to understand the role of honey in cancer. Also due to allergic reactions associated with bee products found in honey in different concentrations like bee pollen, propolis and royal jelly, more research is needed to find the appropriate intake dosage for developing them in a potent apitherapeutic agent that can provide health benefits.

References

Abdulrhman M, Samir Elbarbary N, Ahmed Amin D, Saeid Ebrahim R (2012) Honey and a mixture of honey, beeswax, and olive oil–propolis extract in treatment of chemotherapy-induced oral mucositis: a randomized controlled pilot study. Pediatr Hematol Oncol J 29:285–292

Abdulrhman MA, Hamed AA, Mohamed SA, Hassanen NA (2016) Effect of honey on febrile neutropenia in children with acute lymphoblastic leukemia: a randomized crossover open-labeled study. Complement Ther Med 25:98–103

Afrin S, Forbes-Hernandez TY, Gasparrini M, Bompadre S, Quiles JL, Sanna G, Spano N et al (2017) Strawberry-tree honey induces growth inhibition of human colon cancer cells and increases ROS generation: a comparison with Manuka honey. Int J Mol Sci 18:613

Afrin S, Giampieri F, Forbes-Hernández TY, Gasparrini M, Amici A, Cianciosi D et al (2018) Manuka honey synergistically enhances the chemopreventive effect of 5-fluorouracil on human colon cancer cells by inducing oxidative stress and apoptosis, altering metabolic phenotypes and suppressing metastasis ability. Free Radic Biol Med 126:41–54

Ahmed S, Othman NH (2013) Honey as a potential natural anticancer agent: a review of its mechanisms. Evid Based Complement Alternat Med 2013:829070

Al Jaouni SK, Al Muhayawi MS, Hussein A, Elfiki I, Al-Raddadi R, Al Muhayawi SM et al (2017) Effects of honey on oral mucositis among pediatric cancer patients undergoing chemo/radio-therapy treatment at King Abdulaziz University Hospital in Jeddah, Kingdom of Saudi Arabia. Evid Based Complement Alternat Med 2017:5861024

Ali N, Rashid S, Nafees S, Hasan SK, Sultana S (2014) Beneficial effects of Chrysin against methotrexate-induced hepatotoxicity via attenuation of oxidative stress and apoptosis. Mol Cell Biochem 385:215–223

Al-Waili N, Salom K, Al-Ghamdi A, Ansari MJ (2012) Antibiotic, pesticide, and microbial contaminants of honey: human health hazards. Sci World J 2012:930849

Anghel N, Cotoraci C, Ivan A, Suciu M, Herman H, Balta C et al (2015) Chrysin attenuates cardiomyocyte apoptosis and loss of intermediate filaments in a mouse model of mitoxantrone cardiotoxicity. Histol Histopathol 30:1465–1475

Badolato M, Carullo G, Cione E, Aiello F, Caroleo MC (2017) From the hive: honey, a novel weapon against cancer. Eur J Med Chem 142:290–299

Bansal A, Ghoshal S, Kumar B, Bahl A (2017) Exploring the role of "Glycerine plus Honey" in delaying chemoradiation induced oral mucositis in head and neck cancers. Int J Cancer Ther Oncol 5:513

Braakhuis (2019) An evidence on the health benefits of supplemental propolis. Nutrients 11:2705

Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68:394–424

Bucekova M, Jardekova L, Juricova V, Bugarova V, Di Marco G, Gismondi A et al (2019) Antibacterial activity of different blossom honeys: new findings. Molecules 24:1573

Cheung Y, Meenu M, Yu X, Xu B (2019) Phenolic acids and flavonoids profiles of commercial honey from different floral sources and geographic sources. Int J Food Prop 22:290–308

Das D, Agarwal SK, Chandola HM (2011) Protective effect of Yashtimadhu (Glycyrrhiza glabra) against side effects of radiation/chemotherapy in head and neck malignancies. Ayu 32:196

Dinparast-Djadid N, Frankland AC, Saleh Azizian MD (2015) Chemoprotection of MNNG initiated gastric cancer in rats using Iranian propolis. Arch Iran Med 1:18

Ebrahimi M, Allahyari A, Ebrahimi M, Hesam H, Hosseini G, Karimi M et al (2016) Effects of dietary honey and Ardeh combination on chemotherapy-induced gastrointestinal and infectious complications in patients with acute myeloid leukemia: a double-blind randomized clinical trial. Iran J Pharm Res 15:661

El-Kott AF, Kandeel AA, El-Aziz SF, Ribea HM (2012) Anti-tumor effects of bee honey on PCNA and P53 expression in the rat hepatocarcinogenesis. Int J Cancer Res 8:130–139

Erejuwa OO, Sulaiman SA, Wahab MS, Sirajudeen KN, Salleh MM, Gurtu S (2010) Antioxidant protection of Malaysian tualang honey in pancreas of normal and streptozotocin-induced diabetic rats. Ann Endocrinol (Paris) 71:291–296

Eslami H, Pouralibaba F, Falsafi P, Bohluli S, Najati B, Negahdari R et al (2016) Efficacy of Hypozalix spray and propolis mouthwash for prevention of chemotherapy-induced oral mucositis in leukemic patients: a double-blind randomized clinical trial. J Dent Res Dent Clin Dent Prospects 10:226

Fahmy MA, Hassan NH, El-Fiky SA, Elalfy HG (2015) A mixture of honeybee products ameliorates the genotoxic side effects of cyclophosphamide. Asian Pac J Trop Dis 5:638–644

Fernandez-Cabezudo MJ, El-Kharrag R, Torab F, Bashir G, George JA, El-Taji H et al (2013) Intravenous administration of manuka honey inhibits tumor growth and improves host survival when used in combination with chemotherapy in a melanoma mouse model. PLoS One 8(2): e55993

Friend A, Rubagumya F, Cartledge P (2018) Global Health Journal Club: is honey effective as a treatment for chemotherapy-induced mucositis in paediatric oncology patients? J Trop Pediatr 64:162–168

Grecka K, Kuś PM, Worobo RW, Szweda P (2018) Study of the anti-staphylococcal potential of honeys produced in Northern Poland. Molecules 23:260

Hakim L, Alias E, Makpol S, Ngah WZ, Morad NA, Yusof YA (2014) Gelam honey and ginger potentiate the anti cancer effect of 5-FU against HCT 116 colorectal cancer cells. Asian Pac J Cancer Prev 15:4651–4657

Hamad R, Jayakumar C, Ranganathan P, Mohamed R, El Hamamy MM, Dessouki AA et al (2015) Honey feeding protects kidney against cisplatin nephrotoxicity through suppression of inflammation. Clin Exp Pharmacol Physiol 42:843–848

Howlader D, Singh V, Mohammad S, Gupta S, Pal US, Pal M (2019) Effect of topical application of pure honey in chemo-radiation-induced mucositis and its clinical benefits in improving quality of life in patients of oral squamous cell carcinoma. J Maxillofac Oral Surg 18:73–79

Huang H, Shen Z, Geng Q, Wu Z, Shi P, Miao X (2017) Protective effect of Schisandra chinensis bee pollen extract on liver and kidney injury induced by cisplatin in rats. Biomed Pharmacother 95:1765–1776

Ibrahim A, Eldaim MA, Abdel-Daim MM (2016) Nephroprotective effect of bee honey and royal jelly against subchronic cisplatin toxicity in rats. Cytotechnology 68:1039–1048

Islam MR, Pervin T, Hossain H, Saha B, Hossain SJ (2017) Physicochemical and antioxidant properties of honeys from the Sundarbans mangrove forest of Bangladesh. Prev Nutr Food Sci 22:335

Janusch M, Fischer M, Marsch WC, Holzhausen HJ, Kegel T, Helmbold P (2006) The hand-foot syndrome—a frequent secondary manifestation in antineoplastic chemotherapy. Eur J Dermatol 16:494–499

Kadir EA, Sulaiman SA, Yahya NK, Othman NH (2013) Inhibitory effects of tualang honey on experimental breast cancer in rats: a preliminary study. Asian Pac J Cancer 14:2249–2254

Khan R, Khan AQ, Qamar W, Lateef A, Ali F, Rehman MU et al (2012) Chrysin abrogates cisplatin-induced oxidative stress, p53 expression, goblet cell disintegration and apoptotic responses in the jejunum of Wistar rats. Br J Nutr 108:1574–1585

Kilic T, Ciftci O, Cetin A, Kahraman H (2014) Preventive effect of chrysin on bleomycin-induced lung fibrosis in rats. Inflammation 37:2116–2124

Kishore RK, Halim AS, Syazana MN, Sirajudeen KN (2011) Tualang honey has higher phenolic content and greater radical scavenging activity compared with other honey sources. Nutr Res 31:322–325

Kuo CC, Wang RH, Wang HH, Li CH (2018) Meta-analysis of randomized controlled trials of the efficacy of propolis mouthwash in cancer therapy-induced oral mucositis. Support Care Cancer 26:4001–4009

Lavaf M, Simbar M, Mojab F, Majd HA, Samimi M (2017) Comparison of honey and phenytoin (PHT) cream effects on intensity of pain and episiotomy wound healing in nulliparous women. J Compl Integr Med 15:1

Maiti PK, Ray A, Mitra TN, Jana U, Bhattacharya J, Ganguly S (2012) The effect of honey on mucositis induced by chemoradiation in head and neck cancer. J Indian Med Assoc 110:453–456

Mantawy EM, El-Bakly WM, Esmat A, Badr AM, El-Demerdash E (2014) Chrysin alleviates acute doxorubicin cardiotoxicity in rats via suppression of oxidative stress, inflammation and apoptosis. Eur J Pharmacol 728:107–118

Micozzi A, Cartoni C, Monaco M, Martino P, Zittoun R, Mandelli F (1996) High incidence of infectious gastrointestinal complications observed in patients with acute myeloid leukemia receiving intensive chemotherapy for first induction of remission. Support Care Cancer 4:294–297

Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L (2004) Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacol Rev 56:185–229

Mishra L, Nayak G (2017) Effect of flavored (honey and tulsi) ice chips on reduction of oral mucositis among children receiving chemotherapy. Int J Pharm Sci Rev Res 43:25–28

Mohamed NZ, Aly HF, Moneim El-Mezayen HA, El-Salamony HE (2019) Effect of co-administration of Bee honey and some chemotherapeutic drugs on dissemination of hepatocellular carcinoma in rats. Toxicol Rep 6:875–888

Münstedt K, Voss B, Kullmer U, Schneider U, Hübner J (2015) Bee pollen and honey for the alleviation of hot flushes and other menopausal symptoms in breast cancer patients. Mol Clin Oncol 3:869–874

Neamatallah T, El-Shitany NA, Abbas AT, Ali SS, Eid BG (2018) Honey protects against cisplatin-induced hepatic and renal toxicity through inhibition of NF-κB-mediated COX-2 expression and the oxidative stress dependent BAX/Bcl-2/caspase-3 apoptotic pathway. Food Funct 9:3743–3754

Nurgali K, Jagoe RT, Abalo R (2018) Adverse effects of cancer chemotherapy: anything new to improve tolerance and reduce sequelae? Front Pharmacol 9:245

Orsolic N, Basic I (2004) Antimetastatic effect of honey. Mellifera 7(4):38–43

Oršolić N, Bašić I (2008) Polyphenols from propolis and plants in control of allergy and inflammation. In scientific evidence of the use of propolis in ehtnomedicine. Transworld Res Netw 2008:1–39

Oršolić N, Terzić S, Šver L, Bašić I (2005) Honey bee products in prevention and/or therapy of murine transplantable tumours. J Sci Food Agric 85:363–370

Osama H, Abdullah A, Gamal B, Emad D, Sayed D, Hussein E et al (2017) Effect of honey and royal jelly against cisplatin-induced nephrotoxicity in patients with cancer. J Am Coll Nutr 36:342–346

Othman NH, Ahmed S, Sulaiman SA (2016) Inhibitory effects of Malaysian tualang honey and Australian/New Zealand Manuka honey in modulating experimental breast cancers induced by N-methyl-N-nitrosourea (mnu): a comparative study. Pathology 48:S148

Ozer H, Armitage JO, Bennett CL, Crawford J, Demetri GD, Pizzo PA et al (2000) American society of clinical oncology growth factors expert panel. 2000 update of recommendations for the use of hematopoietic colony-stimulating factors: evidence-based, clinical practice guidelines. J Clin Oncol 18:3558–3585

Piredda M, Facchinetti G, Biagioli V, Giannarelli D, Armento G, Tonini G et al (2017) Propolis in the prevention of oral mucositis in breast cancer patients receiving adjuvant chemotherapy: a pilot randomised controlled trial. Eur J Cancer Care 26:6

Raeessi MA, Raeessi N, Panahi Y, Gharaie H, Davoudi SM, Saadat A et al (2014) "Coffee plus Honey" versus "topical steroid" in the treatment of chemotherapy-induced Oral mucositis: a randomised controlled trial. BMC Complement Altern Med 14:293

Rashid S, Ali N, Nafees S, Ahmad ST, Arjumand W, Hasan SK, Sultana S (2013) Alleviation of doxorubicin-induced nephrotoxicity and hepatotoxicity by chrysin in Wistar rats. Toxicol Mech Methods 23:337–345

Rashid S, Ali N, Nafees S, Hasan SK, Sultana S (2014) Mitigation of 5-fluorouracil induced renal toxicity by chrysin via targeting oxidative stress and apoptosis in Wistar rats. Food Chem Toxicol 66:185–193

Rehman MU, Ali N, Rashid S, Jain T, Nafees S, Tahir M, Khan AQ et al (2014) Alleviation of hepatic injury by chrysin in cisplatin administered rats: probable role of oxidative and inflammatory markers. Pharmacol Rep 66:1050–1059

Saiful Yazan L, Zali M, Shyfiq MF, Mohd Ali R, Zainal NA, Esa N et al (2016) Chemopreventive properties and toxicity of Kelulut honey in Sprague Dawley rat s induced with Azoxymethane. Biomed Res Int 2016:4036926

Samarghandian S, Farkhondeh T, Azimi-Nezhad M (2017) Protective effects of chrysin against drugs and toxic agents. Dose-Response 15:1559325817711782

Samarghandian S, Azimi-Nezhad M, Pourbagher SA, Farkhondeh T (2019) Antidotal or protective effects of honey and one of its major polyphenols, chrysin, against natural and chemical toxicities. Acta Biomed 90:533

Sanders K, Moran Z, Shi Z, Paul R, Greenlee H (2016) Natural products for cancer prevention: clinical update 2016. Semin Oncol Nurs 32:215–240

Sequetto PL, Oliveira TT, Soares ÍA, Maldonado IR, Mello VJ, Pizziolo VR et al (2013) The flavonoid chrysin attenuates colorectal pathological remodeling reducing the number and severity of pre-neoplastic lesions in rats exposed to the carcinogen 1, 2-dimethylhydrazine. Cell Tissue Res 352:327–339

Shapiro CL (2016) Highlights of recent findings on quality-of-life management for patients with cancer and their survivors. JAMA Oncol 2:1401–1402

Siddiqui A, Badruddeen, Akhtar J, Uddin MS S, Khan MI, Khalid M, Ahmad M (2018) A naturally occurring flavone (Chrysin): chemistry, occurrence, pharmacokinetic, toxicity, molecular targets and medicinal properties. JBAPN 8:208–227

Simon A, Traynor K, Santos K, Blaser G, Bode U, Molan P (2009) Medical honey for wound care—still the 'latest resort'? Evid Based Complement Alternat Med 6(1):65–73

Singh R, Sharma S, Kaur S, Medhi B, Trehan A, Bijarania SK (2019) Effectiveness of topical application of honey on oral mucosa of children for the management of oral mucositis associated with chemotherapy. Indian J Pediatr 86:224–228

Song HY, Kim HM, Mushtaq S, Kim WS, Kim YJ, Lim ST, Byun EB (2019) Gamma-irradiated chrysin improves anticancer activity in HT-29 colon cancer cells through mitochondria-related pathway. J Med Food 22:713–721

Sultana S, Verma K, Khan R (2012) Nephroprotective efficacy of chrysin against cisplatin induced toxicity via attenuation of oxidative stress. J Pharm Pharmacol 64:872–881

Swellam T, Miyanaga N, Onozawa M, Hattori K, Kawai K, Shimazui T, Akaza H (2003) Antineoplastic activity of honey in an experimental bladder cancer implantation model: in vivo and in vitro studies. Int J Urol 10:213–219

Tahir AA, Sani NF, Murad NA, Makpol S, Ngah WZ, Yusof YA (2015) Combined ginger extract and Gelam honey modulate Ras/ERK and PI3K/AKT pathway genes in colon cancer HT29 cells. Nutr J 14:31

Van Meeuwen JA, Korthagen N, De Jong PC, Piersma AH, Van den Berg M (2007) (Anti) estrogenic effects of phytochemicals on human primary mammary fibroblasts, MCF-7 cells and their co-culture. Toxicol Appl Pharmacol 22:372–383

Vit P, Vargas O, Valle FM (2015) Meliponini biodiversity and medicinal uses of pot-honey from El Oro province in Ecuador. Emir J Food Agric 13:502–506

Viuda Martos M, Ruiz Navajas Y, Fernández López J, Pérez Álvarez JA (2008) Functional properties of honey, propolis, and royal jelly. J Food Sci 73:R117–R124

Wee LH, Morad NA, Aan GJ, Makpol S, Ngah WZ, Yusof YA (2015) Mechanism of chemoprevention against colon cancer cells using combined Gelam honey and ginger extract via mTOR and Wnt/β-catenin pathways. Asian Pac J Cancer 16:6549–6556

Yaacob NS, Ismail NF (2014) Comparison of cytotoxicity and genotoxicity of 4-hydroxytamoxifen in combination with Tualang honey in MCF-7 and MCF-10A cells. BMC Complement Altern Med 14:106

Yaacob NS, Nengsih A, Norazmi M (2013) Tualang honey promotes apoptotic cell death induced by tamoxifen in breast cancer cell lines. Evid Based Complement Alternat Med 2013:989841

Yuan Q, Wen M, Xu C, Chen A, Qiu YB, Cao JG et al (2019) 8-bromo-7-methoxychrysin targets NF-κB and FoxM1 to inhibit lung cancer stem cells induced by pro-inflammatory factors. J Cancer 10:5244–5255

Zidan J, Shetver L, Gershuny A, Abzah A, Tamam S, Stein M et al (2006) Prevention of chemotherapy-induced neutropenia by special honey intake. Med Oncol 23:549–552

A Crosstalk Between Antiinflammatory and Wound-Healing Properties of Honey

15

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Keywords

Inflammation · Honey · Wound healing · Tissue regenration

15.1 Introduction

The human race has been using honey for more than 4000 years. It has been described to have medicinal properties in Islamic, Hindu, and other literatures. The Islamic holy writing, Quran, mentions it as an agent to cure human illness (Khan et al. 2014). Wound healing is a multistep process orchestrated by the recruitment of many cells, cytokines, growth factors and other downstream signaling molecules. Wound management is a matter of high concern in today's modern medicine era. Proper wound healing has a predominant effect on the quality of life. Large resources are utilized around the world for wound management, and, thus, it is a challenge for health care professionals. Skin medicine is a growing field of research and researchers all over the globe do find a great interest in naturopathy. Among various natural medicinal ways to treat wounds, apitherapy, using honey, is of real use. Apitherapy is a type of alternative therapy to treat wounds, infections, and other

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diseases using bee-derived products (Sun et al. 2019). Honey has been used for the treatment of wound and many other ailments since antiquity. Honey-driven wound repair has been studied in a number of experiments. Different types of honey have different pharmacological properties. As far as meta-analysis regarding this chapter that was done, we considered Manuka honey that has been extensively studied and has been found to be highly efficient in wound healing and skin regeneration. Various studies have explained the crosslink between the antiinflammatory, antioxidative, and wound healing actions of honey. Reepithelialization, fibrous tissue generation, neovascularization, release of certain growth factors, and antiinflammatory and anti-infective responses might be responsible in eliciting the wound-healing effects of honey. This might be attributed to a wide range of bioactive compounds present in honey making it suitable candidate for clinicians and researchers to use it as a healing agent. Honey has been used to make different hydrogels with agents like chitosan, pectin, etc., as dressing pads for treating a wide variety of wounds. So far many experimental trials conducted point to honey as a potential agent for wound healing, but further high-end research is required to exactly determine the mechanism of action at the genomic, transcriptomic, and proteomic levels. This chapter gives a vivid insight into the applicability of honey in dermal medicine for wound healing, skin renewal, and regeneration.

15.2 Wounds

A wound is any disruption or a discontinuity in skin which if left unattended can be a potential source of infection for the body. Wounds occurring due to any accident or skin burning can progress to ulcers which are complicated and difficult to manage. Diabetic ulcers occur in patients with chronic diabetes having decreased peripheral blood supply due to severe sensory neuropathy. Diabetic foot in diabetic patients is a major cause of food amputations leading to disability and morbidity. Wound healing poses a high economic burden to all nations and is an area of interest in regenerative medicine (Abou Zekry et al. 2020).

15.2.1 Process of Wound Healing

Wound healing is a complex cascade of cellular and biochemical reactions occurring in tissue soon after any disruption or injury that occurs. It begins with the inflammatory reaction followed by a proliferatory phase and tissue remodeling (Ghuman et al. 2019). Wound healing is a continual mechanism involving a number inflammatory markers and transcription factors. Injury induces inflammatory reactions involving many growth factors, formed blood elements, cytokines, and extracellular matrix.

At the site of the injury, tissue-regenerating signals are released which bring in the neutrophils and monocytes from the blood to the wound area; this marks the beginning of the first phase or the inflammatory phase of the wound-healing process. In the tissue, monocytes become macrophages which induce the release of various

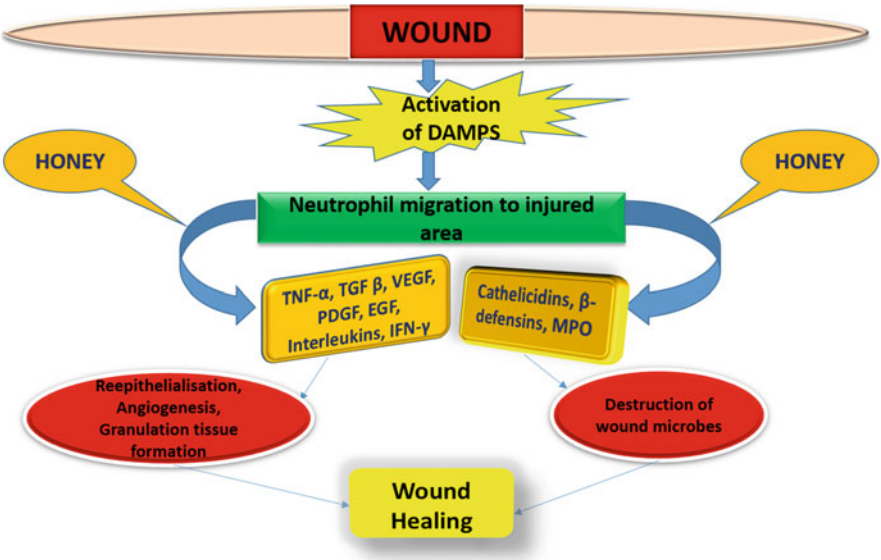

Fig. 15.1 Wound or injury induces neutrophil migration from blood to the injured area leading to the activation of inflammatory mediators which cause wound healing

growth factors like vascular endothelial and platelet-derived growth factors (VEGF and PDGF) (Komi et al. 2019). VEGF induces angiogenesis, a process of generation of new vessels, and also promotes deposition of collagen. On the other hand, PDGF has a role in generating a hemostatic plug by attracting platelets to the injury area. PDGF also has a role in increasing the DNA content and glycosaminoglycans at the wound area. The combined effect of VEGF and PDGF leads to the formation of granulation tissue, which is pivotal for the wound-healing mechanism (Qing 2017).

This inflammatory reaction is followed by the initiation of the proliferatory phase which is marked by epithelialization and angiogenesis in the region of the wound (Dwivedi et al. 2017). The growth of epithelial cells along with the recruitment of fibroblasts to the injured area causes the formation of an extracellular matrix and prominent granulation tissue (Fig. 15.1).

After reepithelialization, neoangiogenesis, and extracellular matrix formation the reconstruction of new tissue in place of wound begins. This is characterized as tissue remodeling stage. In the wound area, fibrolysis of the previously formed fibrin clot occurs, and a new matrix is laid by the fibroblast to support the cells aiding the wound-healing process (Rodrigues et al. 2019). This newly formed extracellular matrix has an abundance of type III collagen fibers which has more crosslinks and is well arranged to give more tensile strength to the affected area.

15.2.2 Treatment Adopted and Drawbacks

There are different classes of wounds depending upon the level of tissue damage inflicted in the injured area. Classically, wounds are treated by the administration of antiinflammatory corticosteroids, antibiotics, antiseptics, and some analgesic agents (Sjöqvist et al. 2019). Photobiomodulation, i.e., using various wavelengths of light including laser and LED, for the treatment of wounds is another noninvasive approach for wound care (Mosca et al. 2019).

15.2.3 Natural Ingredients in Wound Healing

In Ayurvedic medicine, many plant extracts have been known to possess potential wound-healing properties. Data show that almost 164 medicinal plants have been indicated to aid in wound healing (Gupta and Jain 2011). Recently, the leaf extract of *Boerhavia diffusa* was found to enhance wound healing in both in vivo and in vitro models (Juneja et al. 2020). The keratinocyte viability of the human skin cell line, HaCaT, was significantly increased using this extract. *Terminalia catappa* has been reported to cause increased wound healing in rats by increasing hydroxyl proline and DNA content in the wound area (Nugroho et al. 2019). Water extract of roots of *Rheum emodi* cause wound healing by decreasing inflammatory markers, viz., IL-2, IL-6, and TNF-α in the blood and increasing the accumulation of glucosamine, hydroxyproline, and DNA content in the wound tissue (Ahmad et al. 2017). Many other natural plants and their pharmacologically active biocompounds are being studied as potential wound-healing agents.

15.2.4 Honey as a Traditional Medicine

Almost 320 different varieties of honey have been isolated which originate from the floral parts of different plants, each variety possessing different properties (Meo et al. 2017). Honey is a sweet, nontoxic, viscous, and nonallergic natural product synthesized by bees. Honey is a complex of many carbohydrates mainly fructose (about 38%), glucose (about 32%), and also some amount of maltose and sucrose (Bagde et al. 2013). It is a depot of many proteins, B-complex vitamins, vitamin C, and minerals like calcium, potassium, sodium, etc. Honey is a rich source of a varied number of pharmacologically essential bioactive compounds like flavonoids such as pinobanksin, pinocembrin, and chrysin, and certain other compounds in minor concentration such as luteolin, quercetin, 8-methoxykaempferol, isorhamnetin, kaempferol, and galangin (Gill et al. 2019).. The constituents of phenolic acids and volatile norisoprenoids are 4-hydroxybenzoic acid, dehydrovomifoliol, benzoic acid yields, kojic acid, 2-methoxyphenyllactic acid, and methyl syringate (MSYR) (Alvarez-Suarez et al. 2014).

15.2.5 Honey and Wound Healing

Honey has been known as a wound healing agent since ancient periods. It is used in our homes as a healing and soothing base for various skin inflammatory conditions and burns. Wound areas are inflammatory regions of the body and can be seats for oxidative reactions. There exists a predominant crosstalk between oxidative stress and inflammation, which both in turn affect the healing of wounds (Ruiz et al. 2013).

Peroxidation leads to generation of superoxides and peroxynitriles, which trigger the inflammatory cycle inside the cell (Lugrin et al. 2014). Imbalances in the prooxidation and antioxidation mechanisms perpetuate the generation of reactive oxygen and reactive nitrogen species (ROS/RNS) which act as trigger for inflammatory cascade in the body. Inflammation induces the emigration of inflammatory cells like neutrophils and macrophages to the affected area. Signaling molecules like cytokines, inflammatory markers, and other transcription factors regulate the inflammation. Patients suffering from chronic rhinitis and sinusitis when given a nasal spray based on Manuka (*Leptospermum*) or thyme honey showed significant relief from epistaxis, and it was effective in downregulating the expression of IL-6, IL-13, IL-8, MCP-1, and macrophage inflammatory proteins (MIP)—1β in the nasal tissue (Manji et al. 2018; Hashemian et al. 2015). Also, decreased expression of inflammatory markers like IL-8,TNF-α, IL-1β, p-JNK, and IκB kinase β occurs when different types of honey (Manuka and Gelam) were used against *H. pylori*–induced gastric cancer cells (Keenan et al. 2012). In colon cancer, HT-29 cells treated with Gelam honey in combination with ginger exerted antiinflammation by modulating RAS/ERK and PI3K/AKT downstream signaling pathways (Tahir et al. 2015). Antiinflammatory activity of Gelam honey was observed in LPS-treated endottoxemic shock in rats wherein a significant reduction was seen in inflammatory molecules like p65 and high mobility protein group B1 (HMGB1), MPO activity, and neutrophil migration (Kassim et al. 2012). Another honey type called as stingless bee honey was able to decrease NF-κB and MAPK activity and, at the same time, upregulated the expression of transcription factor Nrf2, which is marker for oxidative stress (Ranneh et al. 2019).

The wound-healing property of honey might be due to its multiple inherent properties like maintaining a barrier so as to stop infection entry to wound, antiinflammatory, antibacterial action, and increasing circulation and tissue growth (Hananeh et al. 2015; Wilkinson et al. 2011). Many studies done so far deduce a strong interrelation between the antiinflammatory property of honey and its wound-healing and tissue regeneration effects. Honey decreased the size of the wound and the infiltration of leukocytes and neutrophils in intraoral and second-degree burn ulceration induced experimentally in rats (Bucekova et al. 2017). Downregulated expression of inflammatory mediators (IL-12, TNF-α, IFN-γ) and tumor growth factor (TGF)-β with reduction in neutrophils and leukocyte levels was seen in Lewis rats having corneal abrasion and keratitis (Uwaydat et al. 2011). Cecal abrasions in rats were greatly healed after honey administration as there was a significant reduction in inflammatory parameters and formation of postoperative adhesions in the abdomen (Giusto et al. 2017a, b). Honey dressing used for thermal burns proved

Table 15.1 Various honey types and the signaling proteins involved in wound healing

S. no.	Type of honey	Antiinflammatory pathway	References
1.	Manuka	Downregulation of IL-6, IL-13, IL-8, MCP-1, and MIP-1β expression	Manji et al. (2018), Hashemian et al. (2015)
2.	Manuka and Gelam	Downregulation of IL-8,TNF-α, IL-1β, p-JNK, and IκB kinase β expression	Keenan et al. (2012)
3.	Gelam	RAS/ERK and PI3K/AKT, decrease p65, high mobility protein group B1 (HMGB1), MPO levels	Tahir et al. (2015) Kassim et al. (2012)
4.	Stingless bee honey	Decrease NF-κB and MAPK activity, upregulation of Nrf2	Ranneh et al. (2019)
5.	Manuka	Upregulation of TNF-α, IL-10, VEGF, and TGF-β	Sell et al. (2012)
6.	Acacia, buckwheat, and Manuka	Upregulated expression of MMP-9, CDK2, integrin-β3, cdc25C, and p42/44 MAPK	Ranzato et al. (2012)
7.	Buckwheat honey	Upregulated expression of syndecan, focal adhesion kinase (FAK), and rasGAP SH3 binding protein 1	Ranzato et al. (2012)
8.	Acacia and buckwheat Honey	Upregulated expression of EMT markers like HPRT-1, cadherin-2, WNT family, VIM, and STEAP-1	Ranzato et al. (2012)

to have better wound-repairing activity, lesser infection, faster reepithelialization and decreased inflammatory reaction as compared to mafenide acetate dressings (Maghsoudi et al. 2011). A meta-analysis study of honey inferred that antiinflammatory, antibacterial, and antioxidative properties aid in wound healing. Honey is known to decrease prostaglandins' level which has a major role in inflammation, microbial killing, and the healing process.

Laceration wounds are a serious category of wounds, and medical grade honey has been successfully used in alleviating the infections and decreasing dehiscence in horse (Mandel et al. 2020). Dehiscence of wounds is a common complication after wound repair, occurring due to extra tension due to limb movements (Wilmink et al. 2002). Hence, the intralesional application of honey could help in enhancing wound healing by decreasing the infection rate and degree of dehiscence. Honey from *Teucrium polium*, a medicinal plant, promoted reepithelialization, angiogenesis, and fibrous tissue growth, yielding increased wound healing and tensile strength as observed by histopathology and tensiometry in incision wounds in rats (Alizadeh et al. 2011). A topical application of honey and peppermint (*Mentha pulegium*) combination accelerated the healing of rat cutaneous ulcers (Takzaree et al. 2020). This signifies the synergistic effect of honey (Tables 15.1 and 15.2).

Among the various constituents of honey, hydrogen peroxide (H_2O_2) synthesized in honey by the enzyme glucose oxidase acts as a potent antibacterial agent and can fight against many antibiotic-resistant strains of bacteria (Bucekova et al. 2017; Johnson et al. 2005). On the other hand, it is a known fact that Ca^{2+} has a promising

Table 15.2 List of various honey-based hydrogels

S. no.	Honey-hydrogels	References
1.	PVA/honey hybrid nanofibrous	Kanimozhi et al. (2020)
2.	Honey plus egg white/poly(vinyl alcohol)/clay	Rafati et al. (2020)
3.	Honey, pomegranate peel extract, and bee venom	Abou Zekry et al. (2020))
4.	Honey-chitosan nanofibers	Sarhan and Azzazy (2015) Movaffagh et al. (2019)
5.	Honey-coated nanocellulose	
6.	Pectin-honey hydrogel (PHH)	Giusto et al. (2017a, b)
7.	Hyaluronic acid (HA), acemannan gel (AG), and Manuka honey (MH)	Iacopetti et al. (2020)
8.	Acacia honey with sodium alginate	Iftikhar et al. (2010)

role in wound healing and tissue management (Ranzato et al. 2009). Inhibition of wound healing and closure occurs when the Ca-chelating compound, BAPTA, is added to a culture of immortalized skin keratinocytes (HaCaT), exhibiting a prominent role of Ca ions in tissue regeneration (Ranzato et al. 2008). Investigations have revealed that efflux of H_2O_2 through AQPs causes subsequent release of Ca^{2+}, and Ca^{2+} further helps in the initiation of wound healing and tissue (Martinotti et al. 2019). The intracellular entry of Ca^{2+} is facilitated by melastatin transient receptor potential 2 (TRPM2) and Orai1 channels.

Among the different types of honey, dark brown honey has a maximal concentration of flavonoids and phenolic compounds, which have potent ROS-scavenging potential (Afonso et al. 2020). Afterward, using a scratch wound-healing assay of normal human dermal fibroblasts (NHDF), it was shown that cell migration and, hence, wound healing was potentiated after 36 h of dark brown honey treatment (Afonso et al. 2020). Propolis and honey showed a synergistic wound healing effect as observed in both macroscopic and microscopic changes (Takzaree et al. 2016). Human skin fibroblast showed an increased wound healing after different honey exposures as revealed in scratch wound and chemotaxis assays (Ranzato et al. 2013). Significant interleukin-4 (IL-4), IL-6, and IL-8 upregulation was observed. Honey has many immunomodulatory compounds which help in the activation of different cells responsible for accelerating immune responses during injury and their healings. Immunomodulators like arabinogalactans help in monocytes activation, MRJP1 for macrophages, and a 261 MW component for neutrophil activation (Majtan 2014). Full thickness burns need excision as they do not heal spontaneously, and closure of the resulting wound is a problematic issue. Experimentally infected full thickness burn wounds showed decrease in the wound size and reduction in bacterial growth in *Pseudomonas aeruginosa*–inoculated wounds when treated with Tualang honey (Khoo et al. 2010).

Cutaneous wounds created in rats were healed significantly on topical application of Thyme honey, increasing fibroblasts, which marks its role in tissue proliferation (Takzaree et al. 2017). Recently, abdominal wounds in pediatric patients showed early granulation tissue formation, epithelialization, debridement of necrosed tissue, and no infection when medical grade honey was locally applied at the wound site (Smaropoulos and Cremers 2020). This signifies the broad applicability of honey as a first line of treatment for wounds and also with a merit of causing minimal scars after healing (Smaropoulos and Cremers 2020).

An in vitro study using sterile Manuka honey in culture media revealed the healing properties of honey. In vitro wound healing assay using a lyophilized preparation rich in growth factors (PRGF) supplemented with honey revealed enhanced rates of migration of human fibroblasts (hDF), macrophages, and endothelial cells (hPMEC) (Sell et al. 2012). This might be due to the increased downstream synthesis of cytokines like TNF-α, IL-10, and VEGF, and many physiologically activated growth factors such as TGF-β. This study is of wider importance as it demonstrates how honey can play a role in enhancing the wound-healing action of plasma-derived compounds.

Various other types of honey have been successfully used in treating different wounds. Ulmo honey (*Eucryphia cordifolia*) finds its clinical application in wound healing only when supplemented with vitamin C or ascorbic acid (Schencke et al. 2015). Increased circulation, fibroblastosis, reepithelialization, and renewal of tissue were seen 14 days postburn induction in guinea pigs (Schencke et al. 2015). Ulmo honey has been found to show its significant antibacterial action against methicillin-resistant *Staphylococcus aureus* (MRSA), the main causative agent for wound and skin infections (Acevedo et al. 2017). Therefore, this property makes it a potential candidate for wound-healing actions. Also, venous ulcer healing was accelerated in adult patients using Ulmo honey and oral vitamin C combination (Schencke et al. 2015). Ulmo honey's rapid healing action is attributed to its antiinfection and antiinflammatory properties, proper debridement of the old tissue, and also easy application. The mechanism behind the wound-healing property of honey might be the creation of a high acid milieu and hydrogen peroxide synthesis.

Three different varieties of honey, viz., chestnut (*Castanea sativa*) honey, blossom (multifloral) honey, and rhododendron (*Rhododendron luteum*) honey produced significant healing and wound contraction in full-thickness wounds induced in rabbits (Nisbet et al. 2010). All these types of honey caused maximum epithelialization, collagen and hydroxyproline production, angiogenesis, and fibroplasia as compared to wounds left untreated almost equally.

15.2.6 Effect on Proteins Involved in Tissue Rearrangement and Regeneration

Flavonoids from honey have been successfully able to induce expression of matrix metalloproteinases (MMP-9) in human keratinocytes (Majtan et al. 2013). MMP-9 is a group of matrix endopeptidases which have a prominent role in tissue regeneration

by increasing cell-cell adhesion and remodeling of the basement membrane (Li et al. 2020). Furthermore, honey has strong adhesive characteristics, providing adherence to skin grafts. Honey used in graft fixation allows minimum contracture of grafts, good fixation, and maximum healing growth (Maghsoudi and Moradi 2015). Upregulated expression of MMP-9 occurred with all honey types, viz., acacia, buckwheat, and Manuka in the human keratinocyte cell line (HaCaT) (Ranzato et al. 2012). These cells when scratch wounded recovered and showed reepithelialization with honey treatment.

Syndecan-4, a transmembrane proteoglycan, enhances formation of focal adhesions in the injury site. The role of syndecan-4 has been studied in wound healing through caveolin- and RhoG-regulated integrin endocytosis (Bass et al. 2011). Buckwheat honey showed a significant upregulated expression of syndecan in keratinocyte cell lines (HaCaT) (Ranzato et al. 2012). In the same study, gene expression of focal adhesion kinase (FAK) and rasGAP SH3 binding protein 1 was shown to increase (Ranzato et al. 2012; Meng et al. 2004).

Ranzato et al. (2012) also proposed the activation of cyclin-dependent kinase (CDK2) in a skin keratinocyte model. CDK2 has a predominant role in cell proliferation and tissue regeneration as it is involved in the transition from the G1 to the S phase of the cell cycle. In addition to CDK2, some other peptides that have roles in cytoskeletal rearrangements like vasodilator-stimulated phosphoprotein, integrin-β3, cdc25C, and p42/44 mitogen-activated proteins kinase were shown to increase when treated with honey having different floral origins.

15.3 Epithelial Mesenchymal Transition (EMT)

EMT is a phenomenon in which epithelial cells gain the properties of mesenchymal stem cells which can further dedifferentiate into any other cell type (Lamouille). Its role was first determined in embryogenesis during neural tube formation. EMT is required by cancer cells to metastasize and also in wound healing (Kong). During the wound-healing process the epithelial cells around the wound borders undergo EMT, and, therefore, the mesenchymal cells can undergo migration which is pivotal for regeneration. Snail2 is responsible for the EMT, and its overexpression accelerates wound healing (Hu et al. 2019).

Very few studies are available to validate the effect of honey on EMT. Honey samples from acacia and buckwheat led to upregulated expression of some EMT marker genes, viz., hypoxanthine phosphoribosyltransferase-1 (HPRT-1), cadherin-2, Wingless/Integrated (WNT) family, vimentin (VIM), and six-transmembrane epithelial antigen of the prostate-1 (STEAP-1) at the same time downregulating some other EMT markers, viz., keratin 14 and 19 in HaCaT cells (Ranzato et al. 2012). This suggests how honey helps in epithelial transition and, thus, causes reepithelialization and dedifferentiation in the keratinocytes. Also, a crosstalk between MMP-3 and EMT is also predominant with regard to healing and tissue regeneration. MMP-3 causes induction of EMT via activation of cadherin, Snail, Rac1b activation, VIM, and a-smooth muscle actin in mammary epithelial cells of

the mouse model (Radisky et al. 2005). A meta-analysis postulated honey's vital role in promoting EMT and, hence, aiding a great extent in tissue remodeling and rearrangement (Nordin et al. 2017).

15.4 Honey: A Healer in Diabetic Foot

Diabetes is a growing challenge throughout the world, and India is considered as the diabetic capital of world with 8.7% diabetic people between age 20 and 70 years (Maahs et al. 2010). Recently, a randomized study proposed the healing action of honey when used in dressings for diabetic foot ulcers. The honey dressings showed more prominent reduction in wound size as compared to dressings using povidone iodine (Koujalagi et al. 2020). This proves that honey could be a remarkable agent for ulcer healing and tissue remodeling in diabetic patients having foot ulcers. Manuka honey is a pharmacologically valuable type of honey obtained from *Leptospermum scoparium* (Manuka tree) and, thus, has gained great attention within the scientific community. Recently, the study revealed that topical application of Manuka honey on the excision wound of diabetic rats helped in significant wound closure due to early epithelialization with well-formed keratinized squamous epithelium with normal collagen tissue around hair follicles on histological examination (Gill et al. 2019). Manuka honey is a rich source of methyl syringate (MSYR) to which its myeloperoxidase activity inhibition is owed, leading to the antibacterial action of honey in wounds. Myeloperoxidase has bactericidal action but also causes tissue damage in the area of inflammation due to MPO-derived oxidants (Aratani 2018). Hence, specific MPO inhibition is required for proper tissue regeneration. Myeloperoxidases are known to be active in infected wounds in comparison to noninfected wounds; hence, its inhibition is vital for wound healing (Hasmann et al. 2013). Another compound widely held responsible for the pronounced antibacterial activity of Manuka honey is methylglyoxal (MG) (Mavric et al. 2008). MH, widely marketed as Medihoney, has been found to act as a potent antibacterial agent against *Campylobacter* species (Lin et al. 2009) and many other resistant bacterial strains (George and Cutting 2007). Diabetic ulcers treated with honey showed significant healing by enhancing granulation tissue formation, preventing leg amputations in diabetic patients, a grave complication in chronic diabetes (Eddy and Gideonsen 2005). Mountain honey dressing used for wounds created in mice models was able to induce healing and decrease incision sizes significantly (Reddy and Al Habsi 2020). Beri honey–impregnated dressing applied for healing diabetic foot ulcers led to significant reduction of wound healing duration (Imran et al. 2015). So far, many investigations revealed that honey dressings can be successfully used, but clinicians are reluctant to use them in clinical trials.

15.4.1 Drawback of MG

MG, glyoxal, and 3-deoxyglucosone are dicarbonyl compounds formed by the oxidation or peroxidation of lipids and glucose are reactive species. These dicarbonyl compounds have been found in many foods (Nagao et al. 1986) and also in honey (Adams et al. 2009). The presence of MG in honey at one hand provides the antibacterial properties and is useful in healing as well. MG attacks lysine, arginine (Arg), and cysteine residues of collagens to form complex and irreversible molecules: advanced glycation endproducts (AGEs) (Sassi-Gaha et al. 2010). There are crosslinks in collagen due to increased molecular size which disrupt the normal matrix of the tissue (Sassi-Gaha et al. 2010). Also, AGEs decrease keratinocyte migration (Song et al. 2008). Collagen is important in wound healing and tissue remodeling, and any disruption in collagen structure or physiology can directly diminish the wound-healing process.

In addition to the collagen abnormality, MG has significant effects on derailing peripheral blood circulation which decreases the migration of active cells to the ulcer site (Price and Knight 2009). Blocking AGE receptors could restore impaired wound healing as already reported in diabetic mice (Goova et al. 2001). MG in MH can also significantly decrease wound healing in diabetic patients as MH has the highest concentration of MG as compared to other types of honey (Majtan 2011). Hence, MG can regulate pathogenesis in diabetic ulceration and needs to be considered in future studies for honey's wound healing action.

15.5 Honey Hydrogels for Wound Dressing

Nanotechnology has gained tremendous attention in the scientific world of today. It is a growing field of research, and wound healing using biocompatible nanofibrous materials is in vogue. Nanofibrous dressings are known to have better efficiency of exudate absorption, more surface area, and high porosity as compared to conventional dressings (Miguel et al. 2018). Biocompatible hydrogels possess some unique hydrophilicity and biocompatibility and, therefore, are ideal for wound dressing. High-end techniques like electrospinning, UV and FTIR spectroscopy, and XRD have been used to synthesize PVA/honey hybrid nanofibrous scaffolds that are used as bandaids or dressing pads (Kanimozhi et al. 2020). A hydrogel dressing made from honey plus egg white/poly(vinyl alcohol)/clay accelerated wound healing and also reduced the rate of infection in the wounds (Rafati et al. 2020). Nanofibers generated using honey, pomegranate peel extract, and bee venom have proved to be highly efficacious in wound healing (Abou Zekry et al. 2020). These honey-fabricated nanofibers increased wound closure percentage, enhanced epithelial bridging, and decreased cellular infiltration in the wound area. Manuka honey dressing pads, called as Medihoney®, have been found to be quite helpful in absorbing wound exudates and accelerated wound healing (Fan and Roos 2019). Furthermore, honey-chitosan nanofibers demonstrated antibacterial activity against *S. aureus* and *E. coli*, proposing the potential use of honey nanofibers in protecting

wound infections (Sarhan and Azzazy 2015). Honey-coated nanocellulose was found to be an efficient and rapid delivery medium for noninfectious wound healing (Abu et al. 2020). Pectin is efficiently being used for drug delivery and as a scaffold for cells. Also, it finds application as a skin medicine for wound healing and skin regenerations along with honey. Pectin-honey hydrogel (PHH) served as an effective dressing agent as compared to honey alone (Giusto et al. 2017a, b). Wound contraction was more pronounced, and a histopathological study revealed development of hair follicles and epithelialization, indicating efficient wound healing. Better wound healing due to pectin may be due to direct and continuous contact of the hydrogel with the wound compared to honey only. Another Gelam honey-based hydrogel dressing formulation stimulated wound healing by attenuating wound inflammation, increasing rate of wound contracture, and reepithelialization at a greater efficiency as compared to silver sulphadiazine (SSD) burn healing ointment (Yusof et al. 2007). Application of hyaluronic acid (HA), acemannan gel (AG), and Manuka honey (MH)–based dressing base promoted wound healing of skin wounds by promoting cell proliferation and neovascularization (Iacopetti et al. 2020). Immunohistochemistry showed a positive reaction for CD3, CD20, KI67, vWF, and VEGF, indicating the healing efficacy of MH-based hydrogels. Chitosan, a high-molecular-weight compound, is also used in combination with honey to make hydrogel to potentiate wound healing in rat models with full-thickness wounds. Chitosan/honey hydrogel (1:3) serves as an optimal formulation for effective wound dressing (Movaffagh et al. 2019).

Another topical hydrogel prepared using acacia honey with sodium alginate was able to produce significant wound healing in different wound models (Iftikhar et al. 2010). An early epithelialization and increased wound contracture percentage, breaking strength, and hydroxylproline content were seen in these models. Many honey dressings and gels have been used but still results need proper validation (Jull et al. 2008) so as to bring the products into use at the bed side. Increased wound closure time, synthesis of granulation tissue, and enhanced Ki-67 and HO-1 expression were seen in diabetic wounds on application of hydrogel incorporated with chestnut honey as compared to water hydrogels.

15.6 Conclusion

This chapter discusses vividly the wound-healing property of honey. Honey has been in use for healing since ages. Almost every household utilizes honey keeping in mind its beneficial effects. Traditional medicinal practice utilizes honey for inflammatory diseases, wound healing, as a laxative, etc. It maintains irritable and constipated bowels by acting as a lubricant and increasing peristaltic motions. Inflammation is associated with injuries, and, hence, antiinflammatory drugs are vigorously used for wound healing. Natural medicine derived from various sources has been put into use as antiinflammatory and wound-healing agents. Many experiments have shown that honey can be used as an excellent wound healer due to its antiinflammatory and antiinfective properties. Downregulating the expression

of inflammatory compounds like IL-12, TNF-α, IFN-γ, and TGF-β in injured tissues proves the potency of honey as a wound healer. Being a storehouse of many antioxidative compounds like flavonoids and other phenols, honey has potency in decreasing ROS generated in an injured area. Honey accelerates wound contractions and closure, increases hydroxyproline content, and potentiates epithelialization in a wound site. Studies have shown that the production of H_2O_2 in honey leads to entry of Ca^{2+} ions through AQPs in the wound area, which help in wound healing and subsequent generation of new tissue. Though many published records have mentioned honey's mechanism of action for wound healing robust trials need to be done in this regard. Furthermore, many studies have shown the presence of MG in honey which although an antibacterial agent can decelerate wound healing. Therefore, such compounds need further specification and a detailed study. Gene expression profiles must be formulated which can validate the wound-healing action of honey. Different honey types obtained from varied floral tress need to be studied thoroughly as there exist many differences in the pharmacological properties. Sophisticated techniques like gelatine zymography, reverse transcription PCR, and Western blot should be performed in order to know how honey aids in tissue management and wound healing. This can further help to intricate and formulate honey hydrogels and dressing pads for different wounds. Though wide literature regarding wound healing and tissue management properties of honey can be found the subjacent molecular approaches for its mechanism of action are largely lacking. Various omics approach like genomics, transcriptomics, proteomics, phenomics, etc., should be adopted at the earliest to study the wound healing and tissue regeneration properties of honey so that it can be used a potential wound healer.

References

Abou Zekry SS, Abdellatif A, Azzazy HM (2020) Fabrication of pomegranate/honey nanofibers for use as antibacterial wound dressings. Wound Medicine 28:100181

Abu TM, Zahan KA, Rajaie MA, Leong CR, Ab Rashid S, Hamin NSMN, et al. (2020) Nanocellulose as drug delivery system for honey as antimicrobial wound dressing. Mater Today Proc 31(1):14–17

Acevedo F, Torres P, Oomah BD, de Alencar SM, Massarioli AP, Martín-Venegas R et al (2017) Volatile and non-volatile/semi-volatile compounds and in vitro bioactive properties of Chilean Ulmo (Eucryphia cordifolia Cav.) honey. Food Res Int 94:20–28

Adams CJ, Manley-Harris M, Molan PC (2009) The origin of methylglyoxal in New Zealand manuka (Leptospermum scoparium) honey. Carbohydr Res 344(8):1050–1053

Afonso M, Gonçalves J, Luís Â, Gallardo E, Duarte AP (2020) Evaluation of the in vitro wound-healing activity and phytochemical characterization of Propolis and honey. Appl Sci 10(5):1845

Ahmad SB, Rehman MU, Ahmad SP, Mudasir S, Mir MR (2017) Rheum emodi L. (Rhubarb) promotes wound healing by decreasing inflammatory markers and enhancing accumulation of biomolecules. Ann Phytomed 6(2):114–118

Alizadeh AM, Sohanaki H, Khaniki M, Mohaghgheghi MA, Ghmami G, Mosavi M (2011) The effect of Teucrium Polium Hney on the wound healing and tensile strength in rat. Iran J Basic Med Sci 14(6):499

Alvarez-Suarez JM, Gasparrini M, Forbes-Hernández TY, Mazzoni L, Giampieri F (2014) The composition and biological activity of honey: a focus on Manuka honey. Foods 3(3):420–432

Aratani Y (2018) Myeloperoxidase: its role for host defense, inflammation, and neutrophil function. Arch Biochem Biophys 640:47–52

Bagde AB, Sawant RS, Bingare SD, Sawai RV, Nikumbh MB (2013) Therapeutic and nutritional values of honey (madhu). Int Res J Pharm 4(3):19–22

Bass MD, Williamson RC, Nunan RD, Humphries JD, Byron A, Morgan MR et al (2011) A syndecan-4 hair trigger initiates wound healing through caveolin-and RhoG-regulated integrin endocytosis. Dev Cell 21(4):681–693

Bucekova M, Juricova V, Monton E, Martinotti S, Ranzato E, Majtan J, Microwave Chamani G, Zarei MR, Mehrabani M, Mehdavinezhad A, Vahabian M, Ahmadi-Motamayel F (2017) Evaluation of honey as a topical therapy for intraoral wound healing in rats. Wounds 29:80–86

Dwivedi D, Dwivedi M, Malviya S, Singh V (2017) Evaluation of wound healing, anti-microbial and antioxidant potential of *Pongamia pinnata* in wistar rats. J Tradit Complement Med 7 (1):79–85

Eddy JJ, Gideonsen MD (2005) Topical honey for diabetic foot ulcers. J Fam Pract 54(6):533–535

Fan F, Roos YH (2019) Physicochemical properties, structural transformation, and relaxation time in strength analysis for honey powder models. Food Res Int 122:137–148

George NM, Cutting KF (2007) Antibacterial honey (Medihoney™): in-vitro activity against clinical isolates of MRSA, VRE, and other multiresistant gram-negative organisms including Pseudomonas aeruginosa. Wounds 19(9):231

Ghuman S, Ncube B, Finnie JF, McGaw LJ, Njoya EM, Coopoosamy RM, Van Staden J (2019) Antioxidant, anti-inflammatory and wound healing properties of medicinal plant extracts used to treat wounds and dermatological disorders. S Afr J Bot 126:232–240

Gill R, Poojar B, Bairy LK, Praveen KS (2019) Comparative evaluation of wound healing potential of manuka and acacia honey in diabetic and nondiabetic rats. J Pharm Bioallied Sci 11(2):116

Giusto G, Vercelli C, Comino F, Caramello V, Tursi M, Gandini M (2017a) A new, easy-to-make pectin-honey hydrogel enhances wound healing in rats. BMC Complement Altern Med 17 (1):1–7

Giusto G, Vercelli C, Iussich S, Audisio A, Morello E, Odore R, Gandini M (2017b) A pectin-honey hydrogel prevents postoperative intraperitoneal adhesions in a rat model. BMC Vet Res 13:55

Goova MT, Li J, Kislinger T, Qu W, Lu Y, Bucciarelli LG, Nowygrod S, Wolf BM, Caliste X, Yan SF, Stern DM (2001) Blockade of receptor for advanced glycation end-products restores effective wound healing in diabetic mice. Am J Pathol 159(2):513–525

Gupta N, Jain UK (2011) An update-prominent wound healing property of indigenous medicines. Res J Pharm Technol 4(2):203–213

Hananeh WM, Ismail ZB, Alshehabat MA, Abeeleh MA, Ali JF (2015) Effects of Sidr honey on second-intention healing of contaminated full-thickness skin wounds in healthy dogs. Bull Vet Inst Pulawy 59:433–439

Hashemian F, Baghbanian N, Majd Z, Rouini MR, Jahanshahi J, Hashemian F (2015) The effect of thyme honey nasal spray on chronic rhinosinusitis: a double-blind randomized controlled clinical trial. Eur Arch Otorhinolaryngol 272:1429–1435

Hasmann A, Wehrschütz-Sigl E, Marold A, Wiesbauer H, Schoeftner R, Geweßler U, Kandelbauer A, Schiffer D, Schneider KP, Binder B, Schintler M (2013) Analysis of myeloperoxidase activity in wound fluids as a marker of infection. Ann Clin Biochem 50 (3):245–254

Hu Y, Zheng Y, Dai M, Wu J, Yu B, Zhang H, Yu X (2019) Snail 2 induced E-cadherin suppression and metastasis in lung carcinoma facilitated by G9a and HDACs. Cell Adhes Migr 13 (1):284–291

Iacopetti I, Perazzi A, Martinello T, Gemignani F, Patruno M (2020) Hyaluronic acid, Manuka honey and Acemannan gel: wound-specific applications for skin lesions. Res Vet Sci 129:82

Iftikhar F, Arshad M, Rasheed F, Amraiz D, Anwar P, Gulfraz M (2010) Effects of acacia honey on wound healing in various rat models. Phytother Res 24(4):583–586

Imran M, Hussain MB, Baig M (2015) A randomized, controlled clinical trial of honey-impregnated dressing for treating diabetic foot ulcer. J Coll Physicians Surg Pak 25(10):721–725

Johnson DW, van Eps C, Mudge DW, Wiggins KJ, Armstrong K, Hawley CM et al (2005) Randomized, controlled trial of topical exit-site application of honey (Medihoney) versus mupirocin for the prevention of catheter associated infections in hemodialysis patients. J Am Soc Nephrol 16(5):1456–1462

Jull AB, Rodgers A, Walker N (2008) Honey as a topical treatment for wounds. Cochrane Database Syst Rev 4:CD005083

Juneja K, Mishra R, Chauhan S, Gupta S, Roy P, Sircar D (2020) Metabolite profiling and wound-healing activity of Boerhavia diffusa leaf extracts using in vitro and in vivo models. J Tradit Complement Med 10(1):52–59

Kanimozhi S, Kathiresan G, Kathalingam A, Kim HS, Doss MNR (2020) Organic nanocomposite band-aid for chronic wound healing: a novel honey-based nanofibrous scaffold. Appl Nanosci 10:1639–1652

Kassim M, Yusoff KM, Ong G, Sekaran S, Yusof MY, Mansor M (2012) Gelam honey inhibits lipopolysaccharide-induced endotoxemia in rats through the induction of heme oxygenase-1 and the inhibition of cytokines, nitric oxide, and high-mobility group protein B1. Fitoterapia 83:1054–1059

Keenan JI, Salm N, Wallace AJ, Hampton MB (2012) Using food to reduce H. pylori-associated inflammation. Phytother Res 26:1620–1625

Khan IU, Dubey W, Gupta V (2014) Medicinal properties of honey: a review. Int J Pure Appl Biosci 2(5):149–156

Khoo YT, Halim AS, Singh KKB, Mohamad NA (2010) Wound contraction effects and antibacterial properties of Tualang honey on full-thickness burn wounds in rats in comparison to hydrofibre. BMC Complement Altern Med 10(1):48

Komi DEA, Khomtchouk K, Santa Maria PL (2019) A review of the contribution of mast cells in wound healing: involved molecular and cellular mechanisms. Clin Rev Allergy Immunol 58 (3):298–312

Koujalagi RS, Uppin VM, Shah S, Sharma D (2020) One year randomized controlled trial to compare the effectiveness of honey dressing versus povidone iodine dressing for diabetic foot ulcer at Dr. Prabhakar Kore Hospital and MRC, Belagavi. Int Surg J 7(2):506–513

Li R, Meng Q, Huang J, Wang S, Sun J (2020) MMP-14 regulates innate immune responses to *Eriocheir sinensis* via tissue degradation. Fish Shellfish Immunol 99:301–309

Lin SM, Molan PC, Cursons RT (2009) The in vitro susceptibility of *Campylobacter* spp. to the antibacterial effect of manuka honey. Eur J Clin Microbiol Infect Dis 28(4):339

Lugrin J, Rosenblatt-Velin N, Parapanov R, Liaudet L (2014) The role of oxidative stress during inflammatory processes. J Biol Chem 395:203–230

Maahs DM, West NA, Lawrence JM, Mayer-Davis EJ (2010) Epidemiology of type 1 diabetes. Endocrinol Metab Clin N Am 39(3):481–497

Maghsoudi H, Moradi S (2015) Honey: a skin graft fixator convenient for both patient and surgeon. Indian J Surg 77(Suppl. 3):863–867

Maghsoudi H, Salehi F, Khosrowshahi MK, Baghaei M, Nasirzadeh M, Shams R (2011) Comparison between topical honey and mafenide acetate in treatment of burn wounds. Annal Burn Fire Disast 24(3):132

Majtan J (2011) Methylglyoxal—a potential risk factor of manuka honey in healing of diabetic ulcers. Evid-Based Complement Alternat Med 2011:295494

Majtan J (2014) Honey: an immunomodulator in wound healing. Wound Repair Regen 22 (2):187–192

Majtan J, Bohova J, Garcia-Villalba R, Tomas-Barberan FA, Madakova Z, Majtan T, Majtan V, Klaudiny J (2013) Fir honeydew honey flavonoids inhibit TNF-α-induced MMP-9 expression in human keratinocytes: a new action of honey in wound healing. Arch Dermatol Res 305:619–627

Mandel HH, Sutton GA, Abu E, Kelmer G (2020) Intralesional application of medical grade honey improves healing of surgically treated lacerations in horses. Equine Vet J 52(1):41–45

Manji J, Thamboo A, Sunkaraneni V, Singh A, Tebbutt S, Garnis C (2018) The association of Leptospermum honey with cytokine expression in the sinonasal epithelium of chronic rhinosinusitis patients. World J Otorhinolaryngol Head Neck Surg 5:19–25

Martinotti S, Laforenza U, Patrone M, Moccia F, Ranzato E (2019) Honey-mediated wound healing: H_2O_2 entry through AQP3 determines extracellular Ca2+ influx. Int J Mol Sci 20:764

Mavric E, Wittmann S, Barth G, Henle T (2008) Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka (Leptospermum scoparium) honeys from New Zealand. Mol Nutr Food Res 52(4):483–489

Meng X, Krishnan J, She Y, Ens W, Standing K, Wilkins JA (2004) Association of rasGAPSH3 binding protein 1, G3BP1, and ras-Gap120 with integrin containing complexes induced by an adhesion. J Proteome Res 3(3):506–516

Meo SA, Al-Asiri SA, Mahesar AL, Ansari MJ (2017) Role of honey in modern medicine. Saudi J Biol Sci 24(5):975–978

Miguel SP, Figueira DR, Simões D, Ribeiro MP, Coutinho P, Ferreira P, Correia IJ (2018) Electrospun polymeric nanofibres as wound dressings: a review. Colloids Surf B: Biointerfaces 169:60–71

Mosca RC, Ong AA, Albasha O, Bass K, Arany P (2019) Photobiomodulation therapy for wound care: a potent, noninvasive, photoceutical approach. Adv Skin Wound Care 32(4):157–167

Movaffagh J, Fazly-Bazzaz BS, Yazdi AT, Sajadi-Tabassi A, Azizzadeh M, Najafi E et al (2019) Wound healing and antimicrobial effects of chitosan-hydrogel/honey compounds in a rat full-thickness wound model. Wounds 31(9):228–235

Nagao M, Wakabayashi K, Fujita Y, Tahira T, Ochiai M, Sugimura T (1986) Mutagenic compounds in soy sauce, Chinese cabbage, coffee and herbal teas. Prog Clin Biol Res 206:55–62

Nisbet HO, Nisbet C, Yarim M, Guler A, Ozak A (2010) Effects of three types of honey on cutaneous wound healing. Wounds 22(11):275–283

Nordin A, Sainik NQAV, Zulfarina MS, Naina-Mohamed I, Saim A, Idrus RBH (2017) Honey and epithelial to mesenchymal transition in wound healing: an evidence-based review. Wound Med 18:8–20

Nugroho RA, Utami D, Aryani R, Nur FM, Sari YP, Manurung H (2019) In vivo wound healing activity of ethanolic extract of Terminalia catappa L. leaves in mice (Mus musculus). J Phys Conf Ser 1277(1):012031

Price CL, Knight SC (2009) Methylglyoxal: possible link between hyperglycaemia and immune suppression? Trend Endocrinol Metabol 20(7):312–317

Qing C (2017) The molecular biology in wound healing & non-healing wound. Chin J Traumatol 20 (4):189–193

Radisky DC, Levy DD, Littlepage LE, Liu H, Nelson CM, Fata JE, Werb Z (2005) Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability. Nature 436 (7047):123–127

Rafati Z, Sirousazar M, Hassan ZM, Kheiri F (2020) Honey-loaded egg White/poly (vinyl alcohol)/clay bionanocomposite hydrogel wound dressings: in vitro and in vivo evaluations. J Polym Environ 28(1):32–46

Ranneh Y, Akim AM, Hamid HA, Khazaai H, Fadel A, Mahmoud AM (2019) Stingless bee honey protects against lipopolysaccharide induced-chronic subclinical systemic inflammation and oxidative stress by modulating Nrf2, NF-κb and p 38 MAPK. Nutr Metab (Lond) 16:15

Ranzato E, Patrone M, Mazzucco L, Burlando B (2008) Platelet lysate stimulates wound repair of HaCaT keratinocytes. Br J Dermatol 159(3):537–545

Ranzato E, Martinotti S, Balbo V, Boccafoschi F, Patrone M, Mazzucco L, Burlando B (2009) Platelet lysate-driven in vitro wound healing. Wound Repair Regen 17(4):A78

Ranzato E, Martinotti S, Burlando B (2012) Epithelial mesenchymal transition traits in honey-driven keratinocyte wound healing: comparison among different honeys. Wound Repair Regen 20:778–785

Ranzato E, Martinotti S, Burlando B (2013) Honey exposure stimulates wound repair of human dermal fibroblasts. Burn Trauma 1(1):32–38

Reddy SH, Al Habsi FS (2020) A comparative study on the role of Omani honey with various food supplements on diabetes and wound healing. J King Saud Univ Sci Res J Pharm Technol 4 (2011):203–213

Rodrigues M, Kosaric N, Bonham CA, Gurtner GC (2019) Wound healing: a cellular perspective. Physiol Rev 99(1):665–706

Ruiz S, Pergola PE, Zager RA, Vaziri ND (2013) Targeting the transcription factor Nrf2 to ameliorate oxidative stress and inflammation in chronic kidney disease. Kidney Int 83:1029–1041

Sarhan WA, Azzazy HM (2015) High concentration honey chitosan electrospun nanofibers: biocompatibility and antibacterial effects. Carbohydr Polym 122:135–143

Sassi-Gaha S, Loughlin DT, Kappler F, Schwartz ML, Su B, Tobia AM, Artlett CM (2010) Two dicarbonyl compounds, 3-deoxyglucosone and methylglyoxal, differentially modulate dermal fibroblasts. Matrix Biol 29(2):127–134

Schencke C, Vasconcellos A, Salvo J, Veuthey C, del Sol M (2015) Healing effect of Ulmo honey (Eucryphia cordifolia) supplemented with ascorbic acid as treatment in burns. Int J Morphol 33 (1):137–143

Sell SA, Wolfe PS, Spence AJ, Rodriguez IA, McCool JM, Petrella RL et al (2012) A preliminary study on the potential of manuka honey and platelet-rich plasma in wound healing. Int J Biomater 2012:313781

Sjöqvist S, Ishikawa T, Shimura D, Kasai Y, Imafuku A, Bou-Ghannam S, Kanai N (2019) Exosomes derived from clinical-grade oral mucosal epithelial cell sheets promote wound healing. J Extracell Ves 8(1):1565264

Smaropoulos E, Cremers NA (2020) Medical grade honey for the treatment of paediatric abdominal wounds: a case series. J Wound Care 29(2):94–99

Song ZQ, Wang RX, Yu DM, Wang PH, Lu SL, Tian M, Xie T, Huang F, Yang GZ (2008) Impact of advanced glycosylation end products-modified human serum albumin on migration of epidermal keratinocytes: an in vitro experiment. Zhonghua Yi Xue Za Zhi 88(38):2690–2694

Sun Y, Shen Z, Jia Z, Miao X (2019) Safety evaluation of an apitherapy formulation, Bao-Yuan-Ling: acute and sub-acute oral toxicity in Wistar rats. Curr Top Nutraceut Res 17(1):85–93

Tahir AA, Sani NF, Murad NA, Makpol S, Ngah WZ, Yusof YA (2015) Combined ginger extract & Gelam honey modulate RAS/ERK and PI3K/AKT pathway genes in colon cancer HT29 cells. Nutr J 14:31

Takzaree N, Hadjiakhondi A, Hassanzadeh G, Rouini MR, Manayi A (2016) Synergistic effect of honey and propolis on cutaneous wound healing in rats. Acta Med Iran 54:233–239

Takzaree N, Hassanzadeh G, Rouini MR, Manayi A, Hadjiakhondi A, Zolbin MM (2017) Evaluation of the effects of local application of thyme honey in open cutaneous wound healing. Iran J Public Health 46(4):545

Takzaree A, Goodarzi M, Shahbabae S (2020) Investigating histopathologic the synergistic effect of topical use of Mentha pulegium essential oil and honey in healing of cutaneous ulcers. J Med Plant 4(72):274–287

Uwaydat S, Jha P, Tytarenko R, Brown H, Wiggins M, Bora P, Bora NS (2011) The use of topical honey in the treatment of corneal abrasions and endotoxin-induced keratitis in an animal model. Curr Eye Res 36:787–796

Wilkinson LJ, White RJ, Chipman JK (2011) Silver and nanoparticles of silver in wound dressings: a review of efficacy and safety. J Wound Care 20:543–549

Wilmink JM, Herten JV, Weeren PV, Barneveld A (2002) Retrospective study of primary intention healing and sequestrum formation in horses compared to ponies under clinical circumstances. Equine Vet J 34(3):270–273

Yusof N, Hafiza AA, Zohdi RM, Bakar MZA (2007) Development of honey hydrogel dressing for enhanced wound healing. Radiat Phys Chem 76(11–12):1767–1770

Phytochemicals from Honey: Novel Weapon for the Prevention and Treatment of Cancers

16

Nusrath Yasmeen and Aga Syed Sameer

Abstract

Honey is one of the naturally available food which is well renowned for its medicinal and therapeutic properties because of its various phytochemical constituents of phenolic and flavonoid origin. These phytochemicals have been shown to contribute to its various medicinal properties like antimicrobial, antiviral, anti-inflammatory, and antioxidant activities. Numerous polyphenols present in honey are demonstrated to possess antiproliferative activities against various cancer types. This chapter focuses on the most recent reports regarding the pivotal part played by various phytochemicals present in honey during various phases of carcinogenesis, both in vivo and in vitro. Numerous epidemiological and experimental studies have demonstrated that honey is highly effective as a potential therapeutic agent that can be used as an alternative medicine for various biodiverse ailments. Although there is a huge limitation of translating the active phytochemicals in honey as drugs and to carry them from bench to bedside to be used in clinical practice, still it is well demonstrated that they can be utilized to boost the immune system of patients receiving chemotherapy.

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Keywords

Honey · Honeybee · Anticancer · Phytochemicals · Chemotherapy · Alternative medicine

Abbreviations

CAR-T	Chimeric antigen receptor T cells
CASPASES	Cysteine aspartate specific proteases
CDKs	Cyclin dependent kinases
COX	Cyclooxygenases
DISC	Death inducing signaling complex
ECM	Extracellular matrix
ER	Estrogen receptors
GBD	Global burden of disease
IARC	International Agency for Research on Cancer
IFN	Interferon
IL	Interleukin
MAPKs	Mitogen activated protein kinases
MOMP	Mitochondrial outer membrane permeabilization
NAC	*N* acetyl L cysteine
NO	Nitric oxide
O_2^-	Superoxide
OH^-	Hydroxyl
PGE	Prostaglandin E
PI3K	Phosphatidylinositol-4,5-bisphosphate 3-kinase
PR	Progesterone receptors
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TNF	Tumor necrosis factor
TP53	Tumor protein 53
VEGF	Vascular endothelial growth factor

16.1 Introduction

The malady cancer has derived its name from the Greek word "*karkinos*" (Καρκινός), or "crab" initially used by Hippocrates, the father of medicine (Skuse 2015). However, cancer is best considered as an umbrella term, and it encompasses nearly 100 different types of disease entities with marked differences in genetic, transcriptional, histological, and clinical parameters (Kapinova et al. 2018). It is regarded as one of the most dreadful diseases of the twenty-first century leading the mortality rates at the second position next to cardiovascular diseases (de Oliveira

Júnior et al. 2018). The data pertaining to the worldwide statistics of 2018 reflects that 9.6 million people succumbed to cancer and also reported that new cases of cancer increased to 18.1 million (IARC Report 2018). According to Global Burden of Disease (GBD), by 2040, the number of new cancer cases detected would reach upto 27.5 million with 16.3 million cancer deaths. This projection on cancer burden is attributed to the increasing number of the geriatric population (Bray et al. 2018).

Cancers are defined by the National Cancer Institute as a multitude of diseases wherein abnormal cells proliferate and metastasize (spread to the nearby tissue) ("What Is Cancer?" Skuse 2015). It is a complex neoplastic disease manifested by risk factors of which some are nonmodifiable (genetic factors) and some modifiable factors such as environmental factors, e.g., smoking, physical inactivity, obesity, diabetes, and infections amongst others (Aggarwal et al. 2009). Carcinogenesis (cancer development processes) mechanisms are driven by numerous genetic and epigenetic factors working in concomitance with each other. Carcinogenesis mechanisms involve three sequential steps namely: initiation, promotion, and progression. Initiation as the name suggests is the first step of carcinogenesis, wherein mutated DNA are formed due to genetic damage that is irreversible. The second step—promotion, which results due to aberrant proliferation of mutated cells outside their normal niches causing tumor formation called tumorigenesis. The last step—progression involves invasion of the tumor cells into the extracellular matrix (ECM), nonneoplastic cells and surrounding tissue reinforcing cancer metastasis (Sever and Brugge 2015). Finally, progress toward malignant tumors by altering the cell physiology through changes in tempo-spatial arrangements (Yarla et al. 2016). These steps occur because the cell escapes homeostatic control mechanisms that normally regulate cellular growth, proliferation, adhesion, invasion, survival, apoptosis, and finally death.

The plethora of cancer treatment modalities include conventional cancer chemotherapy, radiotherapy, surgical treatment, CAR-T cell therapy, stem cell therapy, and molecular targeted therapy. (Shaked 2016). Unfortunately, these conventional treatment strategies, during the last few decades, have not provided any substantial improvement due to the financial cost, availability issues of cytotoxic agents, detrimental effects to the normal cell physiology, unprecedented systemic side effects, and the development of resistance against therapies (Kydd et al. 2017). Together with this, the increase in cancer incidence and mortality rates presents significant obstacles, which mandates a closer look toward the search of novel, more tolerable treatments in order to overcome this burgeoning healthcare problem (Zeligs et al. 2016). These challenges in the field of oncology arouse the inquisitive behavior of researchers who dabble with numerous natural compounds and dietary products that may have promising effects toward cancer treatment.

Substantial evidence gathered from the research of the past few decades for safer and more effective treatments diverted the focus toward the usage of numerous plant-based and dietary compounds that have demonstrated medicinal properties. These natural products serve as tremendous resources with the proven potential of promising drugs which can be utilized for preventing numerous diseases, including cancer (Rajamanickam and Agarwal 2008). However, the practical use of plants,

plant components, herbs, and spices has been in existence since ancient times. Hippocrates nearly 2500 years ago aptly quoted "*Let food be thy medicine and medicine be thy food*" (Mehta et al. 2010). Epidemiological data has shown strong evidence of the association between cancer and diet. More recently, phytochemicals have been regarded as the most promising chemopreventive treatment options for the management of tumorigenesis. "*Chemoprevention*", the term was first proposed by Sporn at the end of the 1970s (Amin et al. 2009), refers to the usage of natural, biological, or synthetic mediators to inhibit or reverse the primary phase of carcinogenesis, to prevent premalignant cells from invading other cells and to reverse the occurrence of carcinogenesis (Ranjan et al. 2019). As a consequence, a large number of health professionals are getting persuaded to use complementary and alternative medicine (CAM) for treating cancers (Yates et al. 2005). People indulging in CAM account up to 29–91% irrespective of several anticancer treatment modalities available. The reasons attributed to this shift are multifaceted, e.g., promote emotional well-being, improve immunity, usage of single CAM modality, and reduce adverse effects of conventional treatment.

16.2 Chemical and Structural Elucidation of Phytochemicals in Honey

Since time immemorial, honey was a part of diet, and well utilized due to its health promoting and healing properties across civilizations. Honey is gathered by honey bees (especially *Apis mellifera*) from flowers as nectar. *Apis mellifera* belongs to the family Apidae (Habib et al. 2014), which produces a variety of products out of nectar on which it feeds, like honey, royal jelly, pollen, and propolis that are stored in the beehive (Badolato et al. 2017). Its composition varies with variation in plant types. Natural honey is a sweet flavored syrup comprising nearly 200 distinct chemical constituents. Owing to its healing effects and unique taste, honey is extensively consumed and hence occupies an esteemed position in traditional medicine (Küçük et al. 2007). The main constituent of honey is sugar accounting for 79.6%, of which levulose is 38.19% and dextrose is 31.28%, and the rest is sucrose 1.3%, maltose 7.3%, and small amounts of fructo-oligosaccharides (Eteraf-Oskouei and Najafi 2013). Water accounts for nearly 17.2%. The other constituents of honey are proteins, vitamins (especially, niacin, riboflavin and pantothenic acid), several amino acids (mainly proline), enzymes (peroxidases, catalases, amylases, oxidases, etc.), and several minerals (like calcium, zinc, copper, potassium, and so on). Apart from these, honey also has several biologically active constituents like polyphenols which are of two main types: flavonoids and phenolic acids (Ajibola 2015). Honey's health benefits are primarily due to the presence of several phytochemical antioxidants, of which polyphenolic constituents like flavonoids, phenolic acids, and carotenoid derivatives are noteworthy (Alvarez-Suarez et al. 2013; Porcza et al. 2016). The composition of the numerous phytochemicals present in honey alongside its flavor, fragrance, and color varies markedly with the geographical regions, climate, types of flowers, and species of honey bee involved in its production.

Table 16.1 Chemical constituents of honey

Category	Compound
Flavonols	Quercetin, Kaempferol, Galangin, Fisetin, Myricetin
Flavanones	Pinocembrin, Naringin, Naringenin, Hesperidin Pinobanksin
Flavones	Apigenin, Acacetin, Chrysin, Luteolin Genkwanin, Wogonin, Tricetin
Phenolic acids	Caffeic acid, Chlorogenic acid, Cinnamic acid, *p*-Coumaric acid, Vanillic acid, Ferulic acid, *p*-Hydroxybenzoic acid, Gallic acid, Syringic acid, Rosmarinic acid and derivatives, 2-*Cis*, 4-*Trans* Abscisic acid, 2-Hydroxycinnamic acid, Ellagic acid, Protocatechuic acid, Sinapic acid

Additionally, physical conditions like weather, processing methods, handling and manipulation, packaging, and storage time also affect the composition of honey to a great extent. Also, depending upon the source/origin different types of honey exist, which are expected to have different biological activities (Missio et al. 2016). Table 16.1 depicts the classification of polyphenolic constituents of honey. The molecular structures of the polyphenolic constituents—flavonoids and phenolic acids that can be obtained from honey—are portrayed in Fig. 16.1.

16.3 Therapeutic Properties of Honey

It is apparent from the review of the literature that honey is a best sweetener, with enormous health benefits, and hence being used all over the world from the early epochs till date. Various constituents of honey have been reported to possess a wide array of therapeutic properties, of which the main include antibacterial, anti-inflammatory (Tsang et al. 2015), antioxidant (Beretta et al. 2005), antimicrobial, antiulcer (Almasaudi et al. 2016) antimutagenic, and antitumor activities (Bogdanov et al. 2008). It is also used for treatment of cough and rhinosinusitis (Lee et al. 2017), urinary diseases (Bouacha 2018), gastrointestinal diseases, eczema, psoriasis and dandruff (Samarghandian et al. 2017). Honey is also used to treat cataracts, burns and for wound healing (Al-waili 2003). Research data suggests that natural honey can be used to decrease the plasma levels of glucose, lipids and homocysteine along with c-reactive protein in healthy, diabetic and hyperlipidemic subjects (Al-waili 2004). The phenolic contents of honey also exhibit antileukemic effects in the cell lines of acute and chronic leukemia origin (Nik Man et al. 2015). Figure 16.2 summarizes various medicinal and therapeutic effects mediated by honey.

16.4 Honey: A Potential Cancer Chemopreventive Agent

Phytochemical and pharmacological evidence revealed the plethora of health benefits that are associated with honey and supported the ethnopharmacological use of honey in complementary medicine to cure numerous diseases, including cancer. There is a tremendous increase in research interest in honey's chemopreventive effects and its associated possible mechanisms. Evidently, the

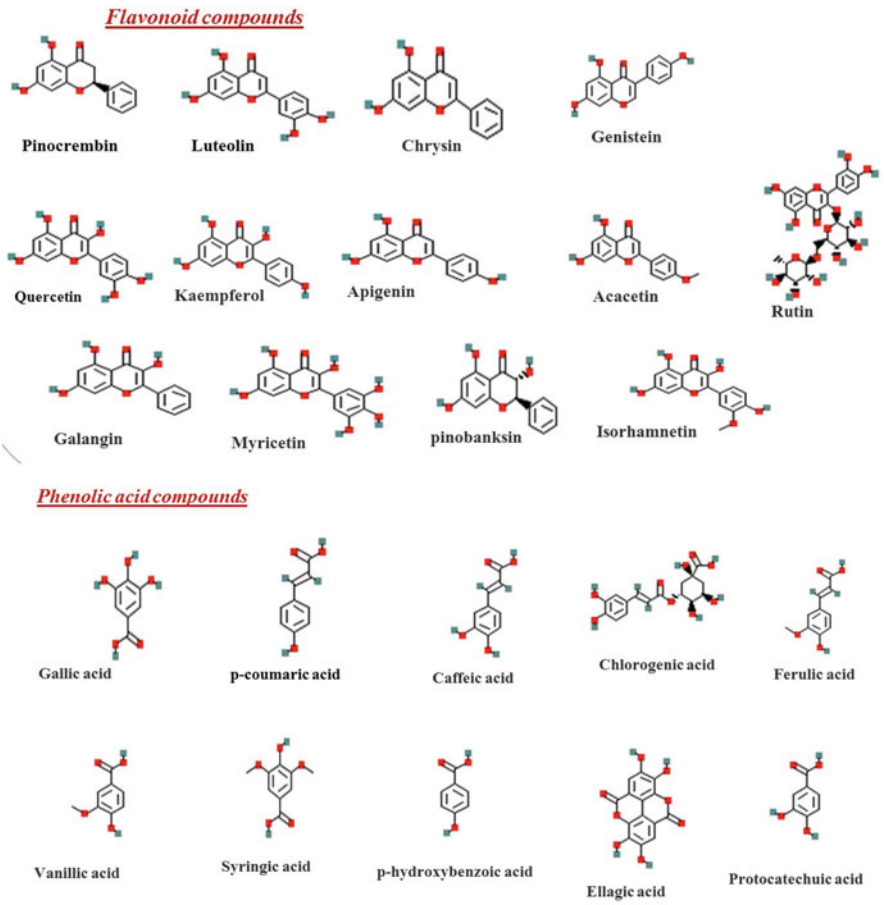

Fig. 16.1 Chemical constituents of honey and their molecular structures

chemopreventive effects of honey are mostly attributed to its polyphenol constituents (like phenolic acids and flavonoids) (Fresco et al. 2006). Chemoprevention could be achieved by blocking one of the three phases of carcinogenesis pathways, i.e., initiation, promotion, and progression (Abubakar et al. 2012). Anticancer, antiproliferative and antimetastatic effects of honey could be achieved through any one of the following probable mechanisms:

- Preventing the formation or activation of a mutagen
- Inhibiting uncontrolled cellular growth
- Arresting the cell cycle
- Inhibiting cellular proliferation
- Activation of mitochondrial apoptotic pathways
- Permeabilization of the outer mitochondrial membrane

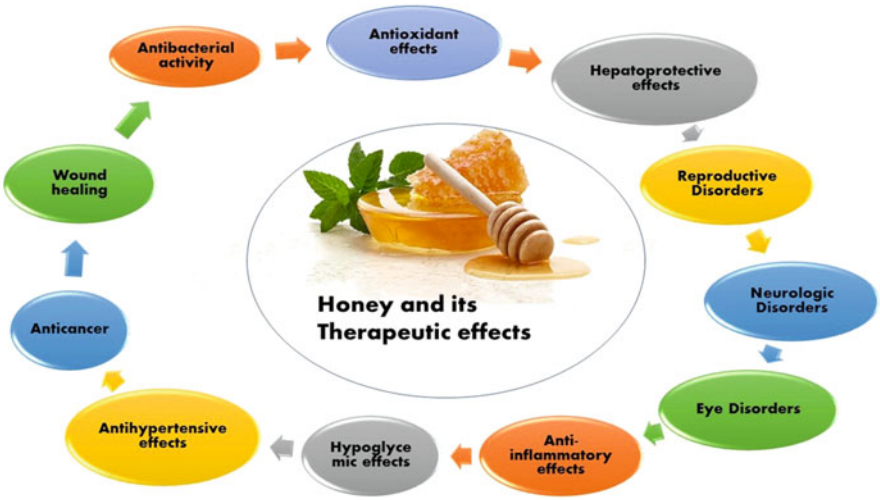

Fig. 16.2 Honey and its various therapeutic effects

- Inducing apoptosis and differentiation
- Oxidative stress modulation and quenching
- Curbing inflammation
- Modulating insulin signaling
- Inhibiting angiogenesis

16.4.1 Effects of Honey on Cell Cycle Arrest and Antiproliferative Activity

Cell cycle is regarded as one of the highly regulated and coordinated processes which ensures cellular growth and division by controlling proper duplication of the genetic material (DNA replication). Several regulatory, molecular and biochemical signaling cascades of the cell cycle prompt cellular division. Cell cycle comprises five sequential phases—G_o (gap_o—the quiscent phase), G_1 (gap_1), S (DNA synthesis), G_2 (gap_2) and M (the mitotic phase) (Alimbetov et al. 2018). This entire process is under the direction of a cascade of protein kinases and several checkpoints. The checkpoints are significant regulatory nodes of the cell cycle (Chao et al. 2017). In cellular cycle, two pivotal checkpoints exist: one at the G_1/S phase and the other at the G_2/M phase (Dominguez-brauer et al. 2015). Generally, it has been observed that cancer cells have defective checkpoint mechanisms, which leads to uninhibited cell proliferation. Numerous studies using different honey types and cell lines were performed, which document the anticancer effect of honey by induction of cell arrest. In one of such studies, Fauzi et al. (Fauzi and Yaacob 2016) proved that honey causes growth arrest of breast cancer cells MCF-7 (ER α-positive) and MDA-MB-231 (ERα-negative) at the G_2/M and S phase of the cell cycle,

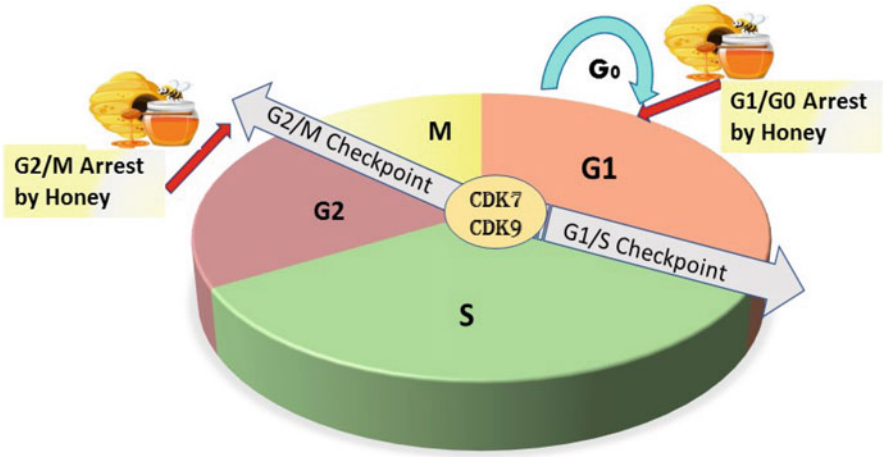

Fig. 16.3 Varied effects of honey on the cell cycle

respectively. This arresting of growth might result from the induction of DNA damage by honey. Figure 16.3 provides a pictorial description of the effects of honey on the cell cycle.

Aliyu et al. 2012 reported that the anticancer activity of honey on the PC-3 cell line is attributed to the modulation of the G_o/G_1 phase of cell cycle, expression of IL-1β and TNF-α, secretion of calcium ions, and in vitro desensitization of prostate-specific antigens. In a similar study, Afrin et al. 2018 reported that in HCT-116 cells and in LoVo cells there was induction of cell cycle arrest in the S phase and in the G_2/M phase through the modulation of cell cycle regulator genes (CDK2, CDK4, cyclin D1, cyclin and Ep21, p27 and Rb) respectively. Experimental data shows that several phenolic compounds and flavonoids (chrysin, quercetin, and kaempferol) in honey are able to cause cell cycle arrest at the G_o/G_1, G_1 and G_2/M phases as seen in human colon, glioma, cervical, esophageal, renal, hepatoma melanoma and adeno-carcinoma cell lines (Priyadarsini et al. 2010; Pinheiro et al. 2019; Pratheeshkumar et al. 2016; Zhang et al. 2008). Figure 16.4 summarizes the effects of honey as an antiproliferative, antioxidant and anticancer agent.

The hallmarks of cancer cells are high proliferative activity and aberrant genotype in comparison with the normal cells. The absence or dysfunction of one or more regulatory proteins of the cell cycle, like cyclin dependent kinase (CDK) forming complexes with cyclins, permanently turns on "molecular switch," leading to carcinogenesis or tumor development (Diehl 2002). The nuclear protein Ki67 is absent in the quiescent G_o phase of the cell cycle but its expression during other phases (G_1, G_2, S, and M) is strongly associated with cellular growth and proliferation (Li et al. 2015). Research done by Tomasin and Gomes-Marcondes (2011) proved that aloe vera on co-administration with honey causes modulation of tumor growth by decreasing the overexpressed Ki67nuclear protein in turn leading to reduction in the tumor weight and cellular proliferation (Tomasin and Gomes-Marcondes 2011).

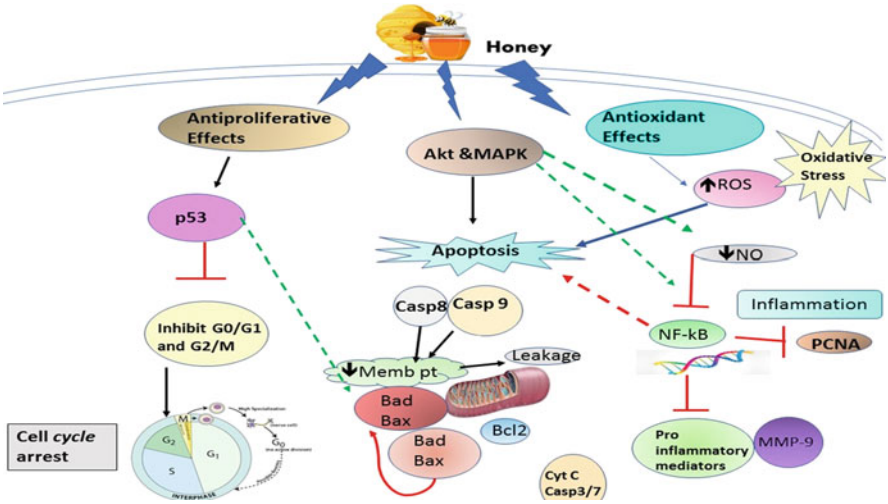

Fig. 16.4 Molecular mechanisms/targets mediating the antiproliferative, antioxidant, and anticancer effects of honey

Honey was reported to exhibit antiproliferative effects on murine (B16-F1) and human (A375) melanoma cell lines in a time- and dose-dependent manner which was proved by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay and the trypan blue test. This effect was supposed to be due to the presence of a chemical constituent in honey called chrysin (Pichichero et al. 2010). Analysis of the cell cycle demonstrated that arrest in the sub-G_1 phase of the cell cycle is responsible for the apoptotic action of honey and its constituents (Jaganathan 2009).

16.4.2 Effects of Honey on Apoptosis

The two most prominent features of cancer are uncontrolled cellular proliferation and decreased apoptosis. Accordingly, apoptosis is defined as the programmed cell death—a process that efficiently eliminates dysfunctional cells and regulates cell growth. Evading/escaping apoptosis is recognized as the hallmark for the development of cancer, and inhibition of apoptotic processes can lead to decreased treatment sensitivity/increased drug resistance (Hanahan and Weinberg 2011). Hence, apoptosis is regarded as a double-edged sword. Apoptosis is hallmarkedly represented by numerous morphological and biochemical changes within the cell, which include cell shrinkage, blebbing of membranes, condensation of the genetic material and collapse of the nucleus, and finally fragmentation of the cell (Lowe and Lin 2000).

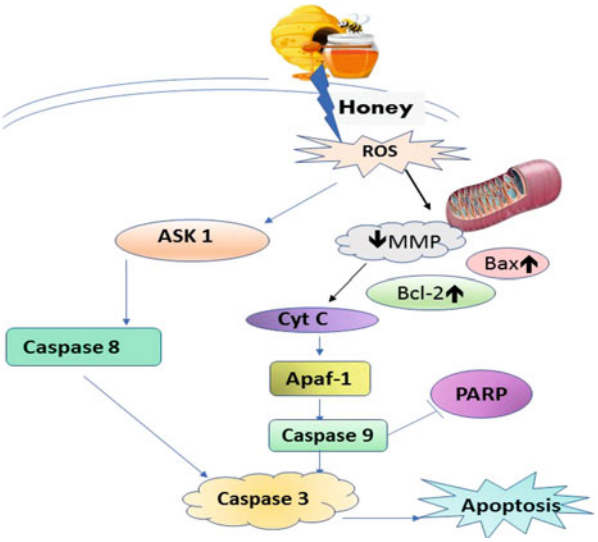

Fig. 16.5 The effect of honey on the apoptotic pathway and the antioxidant pathway. The effect of honey on the apoptotic pathway. Honey exerts apoptotic effect through up-regulation and modulation of proapoptotic proteins (p53, Bax, caspase 3, and caspase 9) and down-regulation of antiapoptotic proteins (Bcl-2). *Bcl-2* B cell lymphoma 2 protein, *Cyt. C* cytochrome C, *Apaf-1* apoptotic protease activating factor 1, *TNF* tumor necrosis factor, *TRAIL* TNF related apoptosis-inducing ligand, and *TRADD* TNFR associated death domain protein

Anticancer agents usually induce apoptosis. Three cardinal features of apoptosis include hydrolysis of the protein constituents of the cell, fragmentation of the nuclear DNA, and recognition of the dying cellular remains by phagocytic cells. Of which, protein cleavage occurs primarily due to activation specific enzymes called caspases. Caspases (cysteine aspartate-specific proteases) belong to the cysteine protease family, which plays a prominent role during apoptosis; more specifically in the phases of initiation and execution. Activation of the inactively synthesized caspases is through specific initiation mechanisms powered by three pathways (Power et al. 2002). Apoptosis proceeds either through the receptor-mediated pathway extrinsic pathway (caspase 8 or death receptor) or through the intrinsic pathway (caspase 9 or mitochondrial) centered on mitochondrial outer membrane permeabilization (Ghobrial et al. 2005). Finally, both the pathways converge to a central mechanism referred to as the endoplasmic reticulum pathway which serves as the executioner of the apoptosis process (O'Brien and Kirby 2008). Figure 16.5 summarizes the effects of honey on apoptotic and antioxidant pathways.

Apoptosis may be triggered by signals from the inside the cell or by death activators present attached to surface receptors, like TNF-alpha, lymphotoxin, and Fas Ligand (FasL) constituting death inducing signaling complex (DISC) which serves to initiate the activity of caspases, leading to the "extrinsic" pathway for apoptosis. Other mechanisms of induction are the intrinsic pathways, which are dependent upon mitochondrial outer membrane permeabilization (MOMP) and

concomitant release of intermembrane space proteins called cytochrome-c (cyt-c) into the cytosol. This released cyt-c then assembles a multiprotein caspase activating complex called "apoptosome" that promotes caspase-independent (nonapoptotic) cell death (Hassan et al. 2014). Tumor cells might suppress apoptosis through distinct molecular mechanisms. Tumor cells exhibit resistance to apoptosis due to imbalance in the antiapoptotic and proapoptotic proteins such as Bcl-2 and BAX, respectively. The presence of both Bcl-2 and BAX during apoptosis is controlled by p53 (Wong 2011). The p53 protein (or TP53) also referred to as the "guardian of genome" within the cell is the first tumor suppressor gene involved in induction of apoptosis and cell cycle regulation. The occurrence of many different kinds of human cancers is linked to downregulation or inactivation of p53.

Several investigations were carried out to prove the effect of honey on apoptosis and its use as an anticancer agent. The anticancer effects of honey are attributed to its effective ability of inducing apoptosis via increased expression of numerous pro-apoptotic proteins such as Bax, caspase 3, caspase 9, BAX, and p53 coupled with the decreased expression of Bcl-2 (antiapoptotic) protein in addition to the prevention of the proliferation of cancerous cells. In human renal adenocarcinoma cell lines (ACHN), where apoptosis serves as the pivotal mechanism for the induced cell death, honey has been demonstrated to increase its effectiveness several fold (Samarghandian et al. 2011). Research has demonstrated that honey induces apoptosis by many mechanisms like induction of DNA damage, increased expression of p53, and finally activation of numerous caspases, which brings about the death of HepG2 cell lines (Cheng et al. 2019). Swellam et al. showed that honey could be used to induce apoptosis in three (T24, 253 J and RT4) human bladder cancer cell lines and one (MBT-2) murine bladder cancer cell line. They showed that 1–25% honey significantly decreased the proliferation of T24 and MBT-2 cell lines and 6–25% honey decreased the proliferation of RT4 and 253 J cell lines. Further, in vivo studies showed that intralesional injection with 6% and 12% honey, as well as oral ingestion of honey significantly suppressed tumor growth (Swellam et al. 2003). Research shows that honey exhibits apoptosis via the death receptor pathway (Fauzi and Yaacob 2016). Furthermore, overexpression of p53, activation of intrinsic (caspase-9) and extrinsic (caspase-8) pathways along with caspase 3 and cleaved-PARP treated two genetically well recognized human colon adenocarcinoma cell lines, HCT-116 and LoVo, which confirms the apoptotic action of honey (Afrin et al. 2018).

Honey is known to induce apoptosis in different cancer cell lines and types through induction of MOMP causing reduction in mitochondrial membrane potential. Cancers caused due to defective DNA damage repair can be treated by the flavonoids in honey which is effective in inhibiting the activity of PARP. Honey has the potential to cause the upregulation of Bax, downregulation of Bcl-2, activation of caspases 3 and 9 and induction of p53, thereby inhibiting cancer (Pasupuleti et al. 2017). Honey exhibited significant anticancer activity against human breast and cervical cancer cell lines. This was possible through a decrease in mitochondrial membrane potential ($\Delta\Psi m$), activation of caspase-3/7 and -9 in all honey treated cancer cells lines signifying the involvement of mitochondrial apoptotic pathway

(Fauzi et al. 2011). Honey helped in modulating the anticancer activities of tamoxifen via depolarization of the mitochondrial membrane and induction of caspase-dependent apoptosis in ERα-dependent MCF-7 and ERα-independent MDA-MB-231 breast cancer cell lines (Yaacob et al. 2013). Quercetin a constituent of cancer may induce caspase-dependent apoptosis in HepG2 cell lines by direct activation of the mitochondrial pathway and inhibition of survival signaling. Apoptotic activity of honey can be attributed to flavonoids such as Quercetin causing MOMP (Chien et al. 2009).

16.4.3 Effects of Honey on Inflammation

Chronic inflammation has proven harmful and is found to be associated with a wide variety of cancers (Wang and Karin 2015). Chronic inflammation is linked to several endogenous mediators like chemokines, pro-inflammatory cytokines, inflammatory enzymes, and adhesion molecules (Aggarwal et al. 2006). Cancer-related inflammation is generally of two types: tumor-extrinsic inflammation and cancer-intrinsic or cancer-elicited inflammation. Several factors that can trigger tumor-extrinsic inflammation are autoimmune diseases, smoking and obesity; bacterial and viral infections (e.g., bladder cancer by schistosomes) (Zaghloul 2012); oral infections by human papilloma virus (HPV) (Shaw and Robinson 2011); and tobacco, alcohol, and asbestos exposure, which are responsible for stimulating malignant progression and thereby increasing the cancer risk. Similarly, cancer-initiating mutations by the recruitment and activation of inflammatory cells lead to malignant progression and thus cancer-elicited inflammation (Allavena et al. 2008). These two interrelated inflammatory processes can result in immunosuppression. The expansion of inflammatory-induced cancers is restricted within the locale of the neoplastic cells or tissues. Chronic inflammations due to inflammatory bowel diseases (IBDs) and ulcerative colitis are significant contributors to the risk of induction, development and advancement of colorectal cancer (CRC) (Aggarwal et al. 2009). In various types of cancers, numerous key inflammatory molecules have been identified that have been shown to induce and sustain tumorigenesis, at the intersection of the intrinsic and extrinsic pathways. Chronic inflammation and cascades of tumorigenesis have been demonstrated to be elicited by a series of cross-talk amid multiple signaling pathways that include the nuclear factor kappa-B (NF-κB) pathway, the stress-responsive mitogen-activated protein kinase (MAPK), and STAT3 signaling pathways (Aggarwal et al. 2009).

Key chemical or inflammatory mediators/pathways that promote proliferation and malignant cell survival and angiogenesis and metastasis include cytokines such as NFκB, tumor necrosis factor (TNF-α), and interleukins (IL-1, IL-6, IL-11, IL-17, IL-21, IL-22, and IL-23). Also several other mediators such as chemokines CCL2 and CXCL8, matrix metalloproteinase 9 (MMP9), cyclo-oxygenase 2 (COX-2), interferon (INF-γ), inducible nitric oxide synthase (iNOS), vascular endothelial growth factor (VEGF), and PGE 2 are identified. Furthermore, cancer development can be ablated by TGFβ and IL-10 (Del Prete et al. 2011; Nikolaou et al.

2013). Inflammatory cells involved in the progression of carcinogenesis secrete TNF, which because of its pro or antitumorigenic effects acts as a double-edged sword (Aggarwal 2003). In human gastrointestinal cancers, IL-11 is the dominant inducing factor, which exhibits a significant association with STAT3 activation in comparison to IL-6 in promoting tumorigenesis (Singh et al. 2019; Coussens and Werb 2002).

The recent in vivo studies conducted showed that honey has anti-inflammatory properties. Honey is helpful in reducing the promotion as well as the progression of cancers by decreasing MAPK and NF-κB expression in neoplastic cell lines. The induction of apoptosis in cancer cells and consequent halting of the release of various inflammatory molecules like COX-2, iNOS, IL-1β, IL-6, and TNF-α have been shown to be because of flavonoids in honey. In HT-29 cells, treatment with different varieties of honey down-regulated the expression of NF-κB and up-regulated that of IκBα, and the action was comparable to common anti-inflammatory drugs (Tahir et al. 2015; Wen et al. 2012). Several research studies proved that Manuka, Pature, Nigerian Jungle, and Royal Jelly honey varieties increased IL-1β, IL-6, and TNF-α expression (Ahmed and Othman 2013; Tonks et al. 2001). Gelam honey also exhibits anti-inflammatory effects by significantly decreasing the production of pro-inflammatory cytokines such as NO, PGE2, TNF-α, and IL-6 in the plasma, which tallies with the reduction in the expression of the corresponding genes and proteins in carrageenan-induced acute paw edema in rat. The possible mechanism for this anti-inflammatory effect might be the inhibition of COX-2 and iNOS mediators of inflammation (Hussein et al. 2012). These anti-inflammatory mechanisms of honey make it a promiscuous therapeutic agent that can be used to modulate disease.

16.4.4 Effects of Honey on Modulation of Oxidative Stress

Oxidative stress is referred to as the "*imbalance between oxidants and antioxidants.*" It is due to excessive production of oxidants or free radicals like superoxide anions (O_2•) and hydroxyl (•OH), peroxyl (ROO•), and alkoxyl radicals (RO•) and failure to remove oxidants by protective mechanisms called antioxidants, which ultimately leads to potential damage to the cell. The agents that quench reactive oxygen species (ROS) and reactive nitrogen species (RNS) are called antioxidants (Orrenius et al. 2007). Balance between oxidation and reduction states is pivotal for normal cell growth and proliferation. The relationship between ROS and tumor progression or carcinogenesis is well documented in the literature. ROS may lead to carcinogenesis due to persistent DNA damage and mutations in p53 (Saeidnia and Abdollahi 2013). Various types of cancers might occur due to exhaustive cell proliferation because of the decreased levels of ROS, in contrast higher levels of ROS would result in oxidative damage. The participation of ROS and hence their production especially of hydrogen peroxide (H_2O_2) in promoting tumor initiation and progression is supported by both in vivo and in vitro studies (Lee and Lee 2006). The "hypocritical" character of ROS is substantiated by evidence that ROS not only induces and maintains the oncogenic phenotype of cancer cells, but can also induce apoptosis and

cellular senescence, hence acts as antitumorigenic species (Valko et al. 2006). Honey is a potent antioxidant associated with good radical scavenging properties (Ahmed et al. 2018). Among the various constituents of honey, vitamin C and E, phenolic compounds, flavonoids, peroxides, catalase, glucose oxidase enzymes and carotenoids have been found to possess a high level of antioxidant activity (Lewoyehu and Amare 2019). Although the precise mechanism by which honey exerts its antioxidant activity is unclear, many proposed ones include flavonoid substrate action for hydroxyl free radical sequestration, chelation of metallic ion, donation of hydrogen, and superoxide radical action. Manuka honey was reported to be cytotoxic to MCF-7 breast cancer cells in vitro correlating that the effects are due to the presence of the total phenol content and antioxidant power (Portokalakis et al. 2016). Antitumor activity of Jungle honey fragments was proved to be due to chemotactic induction for neutrophils and reactive oxygen species (ROS) (Fukuda et al. 2011).

Modulation of oxidative stress is one of the main reasons for the anticancer effect of honey. It has been reported that treatment of colon tumor cells (HCT-15 and HT-29) with honey leads to the depletion of nonprotein thiol, thereby decreasing their free radical scavenging capacities. Furthermore, honey also leads to the increased production of ROS which gives it an effective antiproliferative activity. In addition, treatment with honey leads to cell death because of the fragmentation of DNA. The activity is inhibited by treating the cells with antioxidant, *N*-acetyl-L-cysteine (NAC) (Jaganathan and Mandal 2010).

16.4.5 Effects of Honey on the Insulin Signaling Pathway

The binding of insulin to the insulin receptor on the cellular surface is responsible for its diverse actions. The insulin mediated signaling pathway is responsible for the mitogenic, pro-migratory and the metabolic effects of insulin (Vella et al. 2018). Deregulation of the insulin signaling pathway directly leads to insulin resistance, which further causes hyperinsulinemia. Hyperinsulinemia and other obesity-related factors have been strongly linked to both clinically and epidemiologically as the major risk factors responsible for the initiation and progression of a variety of malignancies (Poloz and Stambolic 2015). Studies also implicated that cancer development and progression may be due to an aberrant insulin level and insulin-mediated signaling pathways, including cancers of breast, colon, esophagus, endometrium, kidney, liver, and pancreas (Ray et al. 2014). Recent investigations revealed the concept that the insulin receptor is involved in carcinogenesis. The PI3K/Akt signaling pathway is another key component of insulin signaling responsible for the metabolic actions of insulin along with insulin receptor-mediated cell proliferation and survival. However, insulin exhibits mitogenic effects through the activation of the MAPKs cascade. Moreover the two cascades—PI3K/Akt and MAPKs—are interrelated and do converge to the common mTOR/p70S6K pathway, which is responsible mainly for regulating cell growth, survival, and metabolism (Cheng et al. 2010). Another important component responsible for various effects in

IR signaling is c-Abl tyrosine kinase. Insulin has been demonstrated to stimulate c-Abl phosphorylation and FAK dephosphorylation in normal cells. However, in tumor cells with dysfunctional c-Abl, insulin induces FAK phosphorylation and cell proliferation, survival, and migration (Belfiore and Malaguarnera 2011). Lori et al., proved that the treatment of HepG2 cells with honey extracts increases the expression of insulin receptors, and stimulates glucose uptake (Lori et al. 2019). Pretreatment of subjects with quercetin and Gelam honey extract improved insulin resistance and insulin action by increasing the expression of Akt, while reducing the expression of IRS-1 serine phosphorylation (ser307), MAPK, and NF-κB. The anticancer properties of honey are due to its modulatory action on the insulin signaling cascade (Batumalaie et al. 2013).

16.4.6 Effects of Honey on Angiogenesis

Angiogenesis is one of the complex physiological processes involving the interdependent interactions of numerous kinds of cells and their mediators, which in turn results in the development of new blood vessels from preexisting vessels (Oskouei et al. 2014). In the repair processes, apoptosis occupies an important aspect especially during the restoration of blood vessels. For proper functioning and viable growth of the reparative cells, the restoration process plays an essential role in providing ample oxygen and nutrients to the damaged tissue. Additionally, cancer development and progression to advanced stages, angiogenesis, has been found to play an essential part in driving the processes (Toshiro et al. 2014). In numerous morbid diseases, neovascularization has been shown to be the sentinel characteristic of the affected tissue. (Al-Husein et al. 2012). Neovascularization is one of the highly regulated processes which involves a number of complex phases like sprouting, splitting, and remodeling, resulting in a change of the existing blood vessels to meet the demands of the disease burden, and hence when angiogenesis becomes unregulated and uncontrolled, it almost always is shown to promote tumorigenesis. In contrast, inadequate angiogenesis is reported to be involved in coronary artery disease pathogenesis (Polverini 2002).

The process of tumorigenesis involves disturbance in the balance between pro-angiogenic and antiangiogenic factors, for the conversion and development of normal cells to the tumor masses and then further advance them to metastasis. Thus a loss of tight control of the angiogenesis called as “angiogenic switching” is pivotal for the carcinogenesis process to be established and then further driven to metastatic end by secondary growth factors and cytokines (Naik et al. 2015). Several proangiogenic molecules such as fibroblast growth factors (FGFs), vascular endothelial cell growth factors (VEGFs), and angiopoietins and their counterparts, i.e., inhibitors of angiogenesis such as endostatin, platelet factor-4, vasostatin, and angiostatin exist to drive the process (Pandya et al. 2006). Angiogenesis process in tumor development has been demonstrated to be highly effective target for preventing and treating cancers effectively. Basic mechanism for the antiangiogenic effects of honey is unclear; however it can be credited to the existence of various

phytochemicals and Vitamin E in natural honey, both of which contribute to decrease VEGF concentration. Eteraf-Oskouei T et al. have reported a potent antiangiogenic effect of honey in his in vivo models, when they were treated with honey for chronic inflammation. Furthermore, honey has been demonstrated to inhibit COX and VEGF in pouch fluid leading cessation of production of PGE 2. This effect is in turn reflected in the reduction of angiogenesis (Oskouei et al. 2014).

16.5 Conclusion

Honey, a natural food supplement, has several phytochemicals with medicinal and health benefits. Several epidemiological and experimental studies have proven the efficacy of honey as a potential therapeutic agent that can be used as an alternative medicine for various biodiverse ailments. Investigational data from several studies consolidated that honey can be effectively used as an antibacterial, antifungal, antiviral, anti-inflammatory and antidiabetic alternative drug. It also exhibits strong wound healing and immunomodulatory action, retains estrogenic regulatory action, and anticancer, antimutagenic, and abundant other vital effects. Evidence-based research suggests that honey exhibits all the abovementioned potential therapeutic effects via modulation of numerous signal transduction pathways and multiple molecular targets.

With the development of varied resistances to drugs coupled with the numerous side effects of conventional therapies, researchers have shifted their focus to chemopreventive agents. Honey in this regard is considered to be a good anticancer and chemopreventive agent. Though there is lack of exploratory data revealing the full mechanism of honey as an anticancer agent, the existing data suggests that it exerts antiproliferative, anticancer, antitumor, and antimutagenic effects all of which are facilitated by various mechanisms, some of which include activation of the mitochondrial pathway; cell cycle arrest; permeabilization of the mitochondrial outer membrane; caspase activation in apoptosis; inflection of oxidative stress; enhancement of inflammation; intonation of insulin signaling; angiogenesis inhibition in cancer cells; stimulation of IL-1β, TNF-α, IFN-γ, IFNGR1 and p53; inhibition of IL-1, IL-10, COX-2, and LOXs in inflammation; and modulation of numerous other miscellaneous targets. However, there are many more open apertures regarding the therapeutic benefits and the associated mechanisms of honey that need to be explored in depth. Further clinical and experimental research is warranted to corroborate the pharmaceutical benefits of using honey either alone or as an adjuvant treatment. Moreover, there is an urgent need to disseminate the acquired knowledge about honey to the medical community to facilitate its use as a chemopreventive agent.

References

Abubakar MB, Abdullah WZ, Sulaiman SA, Suen AB (2012) A review of molecular mechanisms of the anti-leukemic effects of phenolic compounds in honey. Int J Mol Sci 13 (11):15054–15073. https://doi.org/10.3390/ijms131115054

Afrin S, Giampieri F, Gasparrini M, Forbes-Hernández TY, Cianciosi D, Reboredo-Rodriguez P, Amici A, Quiles JL, Battino M (2018) The inhibitory effect of Manuka honey on human colon cancer HCT-116 and LoVo cell growth. Part 1: the suppression of cell proliferation, promotion of apoptosis and arrest of the cell cycle. Food Funct 9(4):2145–2157. https://doi.org/10.1039/c8fo00164b

Aggarwal BB (2003) Signalling pathways of the TNF superfamily. Nat Rev Immunol 3:745–756. https://doi.org/10.1038/nri1184

Aggarwal BB, Shishodia S, Sandur SK, Pandey MK, Sethi G (2006) Inflammation and cancer: how hot is the link? Biochem Pharmacol 72:1605–1621. https://doi.org/10.1016/j.bcp.2006.06.029

Aggarwal BB, Vijayalekshmi RV, Sung B (2009) Targeting inflammatory pathways for prevention and therapy of cancer: short-term friend, long-term foe. Clin Cancer Res 15(2):425–430. https://doi.org/10.1158/1078-0432.CCR-08-0149

Ahmed S, Othman NH (2013) Honey as a potential natural anticancer agent: a review of its mechanisms. Evid Based Complement Alternat Med 2013:1–7. https://doi.org/10.1155/2013/829070

Ahmed S, Sulaiman SA, Baig AA, Ibrahim M, Liaqat S, Fatima S, Jabeen S, Shamim N, Othman NH (2018) Honey as a potential natural antioxidant medicine: an insight into its molecular mechanisms of action. Oxidative Med Cell Longev 2018:1. https://doi.org/10.1155/2018/8367846

Ajibola A (2015) Money and health. Malaysian J Med Sci 22(5):7–22. https://doi.org/10.1038/217797a0

Al-Husein B, Abdalla M, Trepte M, DeRemer DL, Somanath PR (2012) Anti-angiogenic therapy for cancer: an update. Pharmacotherapy 32(12):1095–1111. https://doi.org/10.1002/phar.1147.Anti-angiogenic

Alimbetov D, Askarova S et al (2018) Pharmacological targeting of cell cycle, apoptotic and cell adhesion signaling pathways implicated in chemoresistance of cancer cells. Int J Mol Sci 19 (6):1690. https://doi.org/10.3390/ijms19061690

Aliyu M, Odunola OA, Farooq AD, Mesaik AM, Choudhary MI, Fatima B, Qureshi TA (2012) Cancer science & therapy acacia honey modulates cell cycle progression, pro-inflammatory cytokines and calcium ions secretion in PC-3 cell line. J Cancer Sci Ther 4(12):401–407. https://doi.org/10.4172/1948-5956

Allavena P, Garlanda C, Borrello MG, Sica A, Mantovani A (2008) Pathways connecting inflammation and cancer. Curr Opin Genet Dev 18:3–10. https://doi.org/10.1016/j.gde.2008.01.003

Almasaudi SB, El-shitany NA, Abbas AT, Abdel-dayem UA, Ali SS, Jaouni S, Al K, Harakeh S (2016) Antioxidant, anti-inflammatory, and antiulcer potential of manuka honey against gastric ulcer in rats. Oxidat Med Cellular Longevity 2016:3643824. https://doi.org/10.1155/2016/3643824

Alvarez-Suarez J, Giampieri F, Battino M (2013) Honey as a source of dietary antioxidants: structures, bioavailability and evidence of protective effects against human chronic diseases. Curr Med Chem 20(5):621–638. https://doi.org/10.2174/092986713804999358

Al-waili NS (2003) Topical application of natural honey, beeswax and olive oil mixture for atopic dermatitis or psoriasis: partially study. Complement Ther Med 11(4):226–234. https://doi.org/10.1016/S0965-2299(03)00120-1

Al-waili NS (2004) Natural honey lowers plasma glucose, C-reactive protein, homocysteine, and blood lipids in healthy, diabetic, and hyperlipidemic subjects: comparison with dextrose and sucrose. J Med Food 7(1):100–107

Amin ARMR, Kucuk O, Khuri FR, Shin DM (2009) Perspectives for cancer prevention with natural compounds. J Clin Oncol 27(16):2712–2725. https://doi.org/10.1200/JCO.2008.20.6235

Badolato M, Carullo G, Cione E, Aiello F, Caroleo MC (2017) From the hive: honey, a novel weapon against cancer. Eur J Med Chem 142:290–299. https://doi.org/10.1016/j.ejmech.2017.07.064

Batumalaie K, Safi SZ, Yusof KM, Ismail IS, Sekaran SD, Qvist R (2013) Effect of gelam honey on the oxidative stress-induced signaling pathways in pancreatic hamster cells. Int J Endocrinol 2013:367312

Belfiore A, Malaguarnera R (2011) Insulin receptor and cancer. Endocr Relat Cancer 18(4):R125–R147. https://doi.org/10.1530/ERC-11-0074

Beretta G, Granata P, Ferrero M, Orioli M, Maffei R (2005) Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. Anal Chim Acta 533:185–191. https://doi.org/10.1016/j.aca.2004.11.010

Bogdanov S, Jurendic T, Sieber R, Gallmann P (2008) Honey for nutrition and health: a review. J Am Coll Nutr 27(6):677–689. https://doi.org/10.1080/07315724.2008.10719745

Bouacha M (2018) Honey bee as alternative medicine to treat eleven multidrug-resistant bacteria causing urinary tract infection during pregnancy. Sci Pharm 86:14. https://doi.org/10.3390/scipharm86020014

Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68(6):394–424. https://doi.org/10.3322/caac.21492

Chao HX, Poovey CE, Privette AA, Grant GD, Chao HY, Cook JG, Purvis JE, Chao HX, Poovey CE, Privette AA, Grant GD, Chao HY, Cook JG (2017) Orchestration of DNA damage checkpoint dynamics across the human cell cycle article orchestration of DNA damage checkpoint dynamics across the human cell cycle. Cell Syst 5(5):445–459.e5. https://doi.org/10.1016/j.cels.2017.09.015

Cheng Z, Tseng Y, White MF (2010) Insulin signaling meets mitochondria in metabolism. Trends Endocrinol Metab 21(10):589–598. https://doi.org/10.1016/j.tem.2010.06.005

Cheng N, Zhao H, Chen S, He Q, Cao W (2019) Jujube honey induces apoptosis in human hepatocellular carcinoma HepG2 cell via DNA damage, p53 expression, and caspase activation. J Food Biochem 43(11):1–13. https://doi.org/10.1111/jfbc.12998

Chien SY, Wu YC, Chung JG, Yang JS, Lu HF, Tsou MF, Wood W, Kuo SJ, Chen DR (2009) Quercetin-induced apoptosis acts through mitochondrial- and caspase-3-dependent pathways in human breast cancer MDA-MB-231 cells. Hum Exp Toxicol 28(8):493–503. https://doi.org/10.1177/0960327109107002

Coussens LM, Werb Z (2002) Inflammation and cancer. Nature 19:860–867. https://doi.org/10.1038/nature01322.Inflammation

de Oliveira Júnior RG, Christiane Adrielly AF, da Silva Almeida JRG, Grougnet R, Thiéry V, Picot L (2018) Sensitization of tumor cells to chemotherapy by natural products: a systematic review of preclinical data and molecular mechanisms. Fitoterapia 129(January):383–400. https://doi.org/10.1016/j.fitote.2018.02.025

Del Prete A, Allavena P, Santoro G, Fumarulo R, Corsi MM, Mantovani A (2011) Molecular pathways in cancer-related inflammation. Biochem Med 21(3):264–275

Diehl JA (2002) Cycling to cancer with cyclin D1. Cancer Biol Ther 1(3):226–231. https://doi.org/10.4161/cbt.72

Dominguez-brauer C, Thu KL, Mason JM, Blaser H, Bray MR, Mak TW (2015) Review targeting mitosis in cancer: emerging strategies. Mol Cell 60(4):524–536. https://doi.org/10.1016/j.molcel.2015.11.006

Eteraf-Oskouei T, Najafi M (2013) Traditional and modern uses of natural honey in human diseases: a review. Iran J Basic Med Sci 16(6):731–742. https://doi.org/10.22038/ijbms.2013.988

Fauzi AN, Yaacob NS (2016) Cell cycle and apoptosis pathway modulation by Tualang honey in ER-dependent and -independent breast cancer cell lines. J Apic Res 55(5):366–374. https://doi.org/10.1080/00218839.2016.1249149

Fauzi AN, Norazmi MN, Yaacob NS (2011) Tualang honey induces apoptosis and disrupts the mitochondrial membrane potential of human breast and cervical cancer cell lines. Food Chem Toxicol 49(4):871–878. https://doi.org/10.1016/j.fct.2010.12.010

Fresco P, Borges F, Diniz C, Marques MPM (2006) New insights on the anticancer properties of dietary polyphenols. Med Res Rev 26(6):747–766. https://doi.org/10.1002/med.20060

Fukuda M, Kobayashi K, Hirono Y, Miyagawa M, Ishida T, Ejiogu EC, Sawai M, Pinkerton KE, Takeuchi M (2011) Jungle honey enhances immune function and antitumor activity. Evid Based Complement Alternat Med 2011:908743. https://doi.org/10.1093/ecam/nen086

Ghobrial IM, Witzig TE, Adjei AA (2005) Targeting apoptosis pathways in cancer therapy. CA Cancer J Clin 55(3):178–194. https://doi.org/10.3322/canjclin.55.3.178

Habib HM, Al Meqbali FT, Kamal H, Souka UD, Ibrahim WH (2014) Bioactive components, antioxidant and DNA damage inhibitory activities of honeys from arid regions. Food Chem 153:28–34. https://doi.org/10.1016/j.foodchem.2013.12.044

Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144(5):646–674. https://doi.org/10.1016/j.cell.2011.02.013

Hassan M, Watari H, Abualmaaty A, Ohba Y, Sakuragi N (2014) Apoptosis and molecular targeting therapy in cancer. Biomed Res Int 2014:150845. https://doi.org/10.1155/2014/150845

Hussein SZ, Yusoff KM, Makpol S, Anum Y, Yusof M (2012) Gelam honey inhibits the production of proinflammatory, mediators NO, PGE 2, TNF-α, and IL-6 in carrageenan-induced acute paw edema in rats. Evid Based Complement Alternat Med 2012:1–14. https://doi.org/10.1155/2012/109636

Jaganathan SK (2009) Honey constituents and their apoptotic effect in colon cancer cells. J ApiProduct ApiMed Sci 1(2):29–36. https://doi.org/10.3896/ibra.4.01.2.02

Jaganathan SK, Mandal M (2010) Involvement of non-protein thiols, mitochondrial dysfunction, reactive oxygen species and p53 in honey-induced apoptosis. Investig New Drugs 28 (5):624–633. https://doi.org/10.1007/s10637-009-9302-0

Kapinova A, Kubatka P, Golubnitschaja O, Kello M, Zubor P, Solar P, Pec M (2018) Dietary phytochemicals in breast cancer research: anticancer effects and potential utility for effective chemoprevention. Environ Health Prev Med 23(1):1–18. https://doi.org/10.1186/s12199-018-0724-1

Küçük M, Kolayli S, Karaoğlu Ş, Ulusoy E, Baltaci C, Candan F (2007) Biological activities and chemical composition of three honeys of different types from Anatolia. Food Chem 100 (2):526–534. https://doi.org/10.1016/j.foodchem.2005.10.010

Kydd J, Jadia R, Velpurisiva P, Gad A, Paliwal S, Rai P (2017) Targeting strategies for the combination treatment of cancer using drug delivery systems. Pharmaceutics 9(4):46. https://doi.org/10.3390/pharmaceutics9040046

Lee KW, Lee HJ (2006) Biphasic effects of dietary antioxidants on oxidative stress-mediated carcinogenesis. Mech Ageing Dev 127:424–431. https://doi.org/10.1016/j.mad.2006.01.021

Lee V, Im H, Pl P, Manuka DGE (2017) Manuka honey sinus irrigation for the treatment of chronic rhinosinusitis: a randomized controlled trial. Int Forum Allergy Rhinol 7(4):365–372. https://doi.org/10.1002/alr.21898

Lewoyehu M, Amare M (2019) Comparative evaluation of analytical methods for determining the antioxidant activities of honey: a review. Cogent Food Agricult 5(1):1685059. https://doi.org/10.1080/23311932.2019.1685059

Li LTAO, Jiang G, Chen Q, Zheng JUNN (2015) Ki67 is a promising molecular target in the diagnosis of cancer (review). Mol Med Rep 11:1566–1572. https://doi.org/10.3892/mmr.2014.2914

Lori G, Cecchi L, Mulinacci N, Melani F, Caselli A, Cirri P, Pazzagli L, Luti S (2019) Honey extracts inhibit PTP1B, upregulate insulin receptor expression, and enhance glucose uptake in human HepG2 cells. Biomed Pharmacother 113:108752. https://doi.org/10.1016/j.biopha.2019.108752

Lowe WS, Lin WA (2000) Apoptosis in cancer. Carcinogenesis 21(3):485–495

Mehta RG, Murillo G, Naithani R, Peng X (2010) Cancer chemoprevention by natural products: how far have we come? Pharm Res 27(6):950–961. https://doi.org/10.1007/s11095-010-0085-y

Missio P, Gauche C, Gonzaga LV, Carolina A, Costa O (2016) Honey: chemical composition, stability and authenticity. Food Chem 196:309–323. https://doi.org/10.1016/j.foodchem.2015.09.051

Naik AA, Juwekar OD, Ansulkar PV, Deshpande S, Chowdhary A (2015) Evaluation of the anti-angiogenic and antioxidant activity of Indian honeys in comparison with manuka honey from New Zealand. Int J Pharm Sci Res 6(4):1813–1818. https://doi.org/10.13040/IJPSR.0975-8232.6(4).1813-18

Nik Man NMK, Hassan R, Ang CY, Abdullah AD, Mohd Radzi MAR, Sulaiman SA (2015) Antileukemic effect of Tualang honey on acute and chronic leukemia cell lines. Biomed Res Int 2015:307094. https://doi.org/10.1155/2015/307094

Nikolaou K, Sarris M, Talianidis I (2013) Molecular pathways: the complex roles of inflammation pathways in the development and treatment of liver cancer. Clin Cancer Res 19(11):2810–2817. https://doi.org/10.1158/1078-0432.CCR-12-1961

O'Brien MA, Kirby R (2008) Apoptosis: a review of pro-apoptotic and anti-apoptotic pathways and dysregulation in disease. J Vet Emerg Crit Care 18(6):572–585. https://doi.org/10.1111/j.1476-4431.2008.00363.x

Orrenius S, Gogvadze V, Zhivotovsky B (2007) Mitochondrial oxidative stress: implications for cell death. Annu Rev Pharmacol Toxicol 47:143–183. https://doi.org/10.1146/annurev.pharmtox.47.120505.105122

Oskouei TE, Najafi M, Ginal GA (2014) Natural honey: a new and potent anti-angiogenic agent in the air-pouch model of inflammation. Drug Res 64:530–536

Pandya NM, Dhalla NS, Santani DD (2006) Angiogenesis—a new target for future therapy. Vasc Pharmacol 44:265–274. https://doi.org/10.1016/j.vph.2006.01.005

Pasupuleti VR, Sammugam L, Ramesh N, Gan SH (2017) Honey, propolis, and royal jelly: a comprehensive review of their biological actions and health benefits. Oxidative Med Cell Longev 2017:1. https://doi.org/10.1155/2017/1259510

Pichichero E, Cicconi R, Mattei M, Muzi MG, Canini A (2010) Acacia honey and chrysin reduce proliferation of melanoma cells through alterations in cell cycle progression. Int J Oncol 37(4):973–981. https://doi.org/10.3892/ijo_00000748

Pinheiro L, Florack A, Faustino C (2019) Honey inhibitory effect on cancer. Nov Tech Nutri Food Sci 4(1):315–316. https://doi.org/10.31031/NTNF.2019.04.000580

Poloz Y, Stambolic V (2015) Obesity and cancer, a case for insulin signaling. Cell Death Dis 6:1–11. https://doi.org/10.1038/cddis.2015.381

Polverini PJ (2002) Angiogenesis in health and disease: therapeutic opportunities. J Dent Educ 66(8):962–975

Porcza L, Simms C, Chopra M (2016) Honey and cancer: current status and future directions. Diseases 4(4):30. https://doi.org/10.3390/diseases4040030

Portokalakis I, Yusof HIM, Ghanotakis DF, Nigam PS (2016) Manuka honey-induced cytotoxicity against MCF7 breast cancer cells is correlated to total phenol content and antioxidant power. J Adv Biol Biotechnol 8(2):1–10. https://doi.org/10.9734/JABB/2016/27899

Power C, Fanning N, Redmond HP (2002) Cellular apoptosis and organ injury in sepsis: a review. Shock 18(3):197–211. https://doi.org/10.1097/00024382-200209000-00001

Pratheeshkumar P, Sreekala C, Zhang Z, Budhraja A, Ding S, Son Y, Wang X, Hitron A, Hyun-jung K, Wang L, Lee J (2016) HHS Public Access 12(10):1159–1184

Priyadarsini RV, Murugan RS, Maitreyi S, Ramalingam K, Karunagaran D, Nagini S (2010) The flavonoid quercetin induces cell cycle arrest and mitochondria-mediated apoptosis in human cervical cancer (HeLa) cells through p53 induction and NF-κB inhibition. Eur J Pharmacol 649(1–3):84–91. https://doi.org/10.1016/j.ejphar.2010.09.020

Rajamanickam S, Agarwal R (2008) Natural products and colon cancer: current status and future prospects. Drug Dev Res 69(7):460–471. https://doi.org/10.1002/ddr.20276

Ranjan A, Ramachandran S, Gupta N, Kaushik I, Wright S, Srivastava S, Das H, Srivastava S, Prasad S, Srivastava SK (2019) Role of phytochemicals in cancer prevention. Int J Mol Sci 20 (20):1–17. https://doi.org/10.3390/ijms20204981

Ray A, Alalem M, Ray BK (2014) Insulin signaling network in cancer. Indian J Biochem Biophys 51(6):493–498. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/25823221

Saeidnia S, Abdollahi M (2013) Antioxidants: friends or foe in prevention or treatment of cancer: the debate of the century. Toxicol Appl Pharmacol 271(1):49–63. https://doi.org/10.1016/j.taap.2013.05.004

Samarghandian S, Afshari JT, Davoodi S (2011) Honey induces apoptosis in renal cell carcinoma. Pharmacogn Mag 7(25):46–52. https://doi.org/10.4103/0973-1296.75901

Samarghandian S, Farkhondeh T, Samini F (2017) Honey and health: a review of recent clinical research. Pharm Res 9(2):121–127. https://doi.org/10.4103/0974-8490.204647

Sever R, Brugge JS (2015) Signal transduction in cancer. Cold Spring Harb Perspect Med 5(4): a006098. https://doi.org/10.1101/cshperspect.a006098

Shaked Y (2016) Balancing efficacy of and host immune responses to cancer therapy: the yin and yang effects. Nat Rev Clin Oncol 13(10):611–626. https://doi.org/10.1038/nrclinonc.2016.57

Shaw R, Robinson M (2011) The increasing clinical relevance of human papillomavirus type 16 (HPV-16) infection in oropharyngeal cancer. Br J Oral Maxillofac Surg 49(6):423–429. https://doi.org/10.1016/j.bjoms.2010.06.023

Singh N, Baby D, Rajguru JP, Patil PB, Thakkannavar SS, Pujari VB (2019) Inflammation and cancer. Ann Afr Med 18(3):121–126. https://doi.org/10.4103/aam.aam_56_18

Skuse A (2015) What was cancer? Definition, diagnosis and cause. Palgrave Macmillan, London, UK, pp 20–39

Swellam T, Miyanaga N, Onozawa M, Hattori K, Kawai K, Shimazui T, Akaza H (2003) Antineoplastic activity of honey in an experimental bladder cancer implantation model: in vivo and in vitro studies. Int J Urol 10(4):213–219. https://doi.org/10.1046/j.0919-8172.2003.00602.x

Tahir AA, Fathiah N, Sani A, Murad NA, Makpol S, Zurinah W, Ngah W, Anum Y, Yusof M (2015) Combined ginger extract & Gelam honey modulate Ras/ERK and PI3K/AKT pathway genes in colon cancer HT29 cells. Nutr J 14:1–10. https://doi.org/10.1186/s12937-015-0015-2

The International Agency for Research on Cancer (IARC) Report (2018) Latest global cancer data: cancer burden rises to 18.1 million new cases and 9.6 million cancer deaths in 2018. In: International Agency for Research on Cancer, September, 13–15. WHO, Geneva, http://gco.iarc.fr/

Tomasin R, Gomes-Marcondes MCC (2011) Oral administration of Aloe vera and honey reduces Walker tumour growth by decreasing cell proliferation and increasing apoptosis in tumour tissue. Phytother Res 25(4):619–623

Tonks A, Cooper RA, Price AJ, Molan PC, Jones KP (2001) Stimulation of TNF-release in monocytes by honey. Cytokine 14(4):240–242. https://doi.org/10.1006/cyto.2001.0868

Toshiro O, Kazuhiro K, Tomomi Kobayashi MS, Kaji K (2014) Propolis suppresses tumor angiogenesis by inducing apoptosis in tube-forming endothelial cells. Biosci Biotechnol Biochem 72(9):2436–2440. https://doi.org/10.1271/bbb.80169

Tsang K, Kwong EW, Woo KY, To TS, Chung JW, Wong TK (2015) The anti-inflammatory and antibacterial action of nanocrystalline silver and Manuka honey on the molecular alternation of diabetic foot ulcer: a comprehensive literature review. Evid Based Complement Alternat Med 2015:218283. https://doi.org/10.1155/2015/218283

Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 160:1–40. https://doi.org/10.1016/j.cbi.2005.12.009

Vella V, Milluzzo A, Scalisi NM, Vigneri P, Sciacca L (2018) Insulin receptor isoforms in cancer. Int J Mol Sci 19:1–20. https://doi.org/10.3390/ijms19113615

Wang K, Karin M (2015) Tumor-elicited inflammation and colorectal cancer. Adv Cancer Res 128:173–196. https://doi.org/10.1016/bs.acr.2015.04.014

Wen PC, Hussein SZ, Abdullah S, Karim A, Makpol S, Anum Y, Yusof M (2012) Gelam and Nenas honeys inhibit proliferation of HT 29 colon cancer cells by inducing DNA damage and apoptosis while suppressing inflammation. Asian Pac J Cancer Prev 13:1605–1610

Wong RSY (2011) Apoptosis in cancer: from pathogenesis to treatment. J Exp Clin Cancer Res 30 (1):87. https://doi.org/10.1186/1756-9966-30-87

Yaacob NS, Nengsih A, Norazmi MN (2013) Tualang honey promotes apoptotic cell death induced by tamoxifen in breast cancer cell lines. Evid Based Complement Alternat Med 2013:989841. https://doi.org/10.1155/2013/989841

Yarla NS, Bishayee A, Sethi G, Reddanna P, Kalle AM, Dhananjaya BL, Dowluru KSVGK, Chintala R, Duddukuri GR (2016) Targeting arachidonic acid pathway by natural products for cancer prevention and therapy. Semin Cancer Biol 40–41:48–81. https://doi.org/10.1016/j.semcancer.2016.02.001

Yates JS, Mustian KM, Morrow GR, Gillies LJ, Padmanaban D, Atkins JN, Issell B, Kirshner JJ, Colman LK (2005) Prevalence of complementary and alternative medicine use in cancer patients during treatment. Support Care Cancer 13(10):806–811. https://doi.org/10.1007/s00520-004-0770-7

Zaghloul MS (2012) Bladder cancer and schistosomiasis. J Egypt Natl Canc Inst 24(4):151–159. https://doi.org/10.1016/j.jnci.2012.08.002

Zeligs KP, Neuman MK, Annunziata CM (2016) Molecular pathways: the balance between cancer and the immune system challenges the therapeutic specificity of targeting nuclear factor-kB signaling for cancer treatment. Clin Cancer Res 22(17):4302–4308. https://doi.org/10.1158/1078-0432.CCR-15-1374

Zhang Q, Zhao X, Wang Z (2008) Flavones and flavonols exert cytotoxic effects on a human oesophageal adenocarcinoma cell line (OE33) by causing G2/M arrest and inducing apoptosis. Food Chem Toxicol 46:2042–2053. https://doi.org/10.1016/j.fct.2008.01.049

Beneficial Effects of Honey Flavonoids in Nonalcoholic Fatty Liver Disease: An Update

17

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Keywords

Flavonoids · NAFLD · Enzyme inhibitors · Carcinogenic · Fibrosis

17.1 Introduction

Honey is a natural substance which is used as a source of energy and has a lot of therapeutic effects. More than 200 substances are present in the honey that formed from the nectar of flowers by honeybees especially from the species *Apis mellifera* (Family: Apidae). Formation of honey is a stepwise process, in the whole process honey bees play a key role in transforming flower nectar into honey by the process of regurgitation, evaporation, and enzymatic alteration of saccharides present in nectar. The solute portion is mainly fructose (38–55%) and glucose (about 31%) monosaccharide's solvated by the solvent is higher as compared to normal, so the solution is called supersaturated solution and sweetness is because of these solutes.

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Honey had been used by prehistoric populations, including the Greeks, Chinese, Egyptians, Romans, Mayans, and Babylonians, as a nutritional source and for its therapeutic effects. The main substance is fructose and glucose and also contains fructooligosaccharides (Chow et al. 2002), and apart from these it also contains many amino acids, vitamins, minerals, and enzymes (White 1979). This natural substance is very popular, insect-derived with several nutritional, cosmetic, therapeutic, and industrial values. As food, it is a balanced diet for both genders of all ages, not required to refrigerate, not at all ruin, stored at room temperature in a dry place and must be unopened (Samarghandian et al. 2017; Bansal et al. 2005). The standard physical properties which maintain the natural identity of honey are water activity of 0.56–0.62 and a pH value of 3.9 (Hassapidou et al. 2006; Babacan and Rand 2007). The high content of fructose makes it a natural sweetener, used from very ancient period of time (Babacan and Rand 2007), and is used as a sweating agent in beverage industries (Pataca et al. 2007). Globally, scientific advertisements given, in general magazines, journals, and natural products' leaflets explain the therapeutic values as well its nutritional importance, convincing the human being to use it regularly (Inglett 1976). Composition variation of honey totally depends upon plants from where bees are feed. Apart from carbohydrates, the other natural substances like flavonoids, secondary metabolites of plants, frequently bound to sugars (glycosides) also available as aglycones. The structures of flavonoids have two aromatic rings and one heterocyclic ring. Based on the heterocyclic ring, flavonoids are divided into subclasses flavones, isoflavones, flavanols, flavanones, anthocyanidins, proanthocyanidin, and chalcones. Substances like phenolic acids, ascorbic acid, tocopherols, catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), Millard reaction products and peptides are present in honey that work against unwanted oxidation in the body, these have a synergistic antioxidant effect. All compounds of honey work against unwanted oxidation in the body, and have a synergistic antioxidant effect (Alvarez-Suarez et al. 2010; Johnston et al. 2005; Turkmen et al. 2006; Rakha et al. 2008; Al-Mamary et al. 2002). Flavonoids are a group of nutraceuticals, and about 5000 different flavonoid compounds are available in the seven classes.

All over the world, particularly in western countries, nonalcoholic fatty liver disease (NAFLD) is a common disease with ubiquity ranging from 6% to 35%, with a median of 20% (Vernon et al. 2011). Basically, this disease indicates a metabolic syndrome and includes a spectrum of diseases such as steatosis, nonalcoholic steatohepatitis, liver fibrosis, cirrhosis, and hepatocellular carcinoma. Patients of fatty liver have about 10–20% inflammation and fibrosis, possibly because a failure of antilipotoxic protection; nevertheless, inflammation may precede steatosis. The risk factor of NAFLD is obesity, hyperglycemia, insulin resistance, and hypertriglyceridemia. The most effective cause of morbidity and mortality is the development of the symptoms of diabetes and metabolic syndrome (Tilg et al. 2010; Farrell et al. 2006).

NAFLD is developed from multiple factors, the pathophysiological model does not follow a strict sequence (Fig. 17.1). In this model, metabolic disorders, oxidative stress, and inflammation (local and systemic) are major causes involved in the

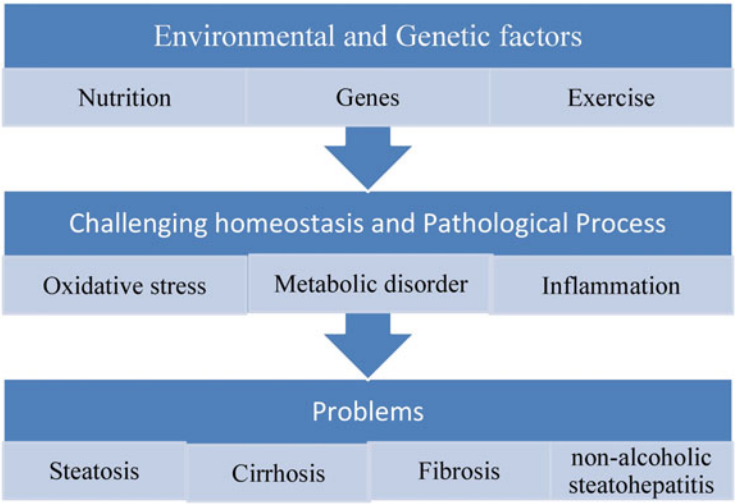

Fig. 17.1 Pathophysiological model of NAFLD

progression of NAFLD also, involved in insulin resistance and genetic susceptibility with histological characteristics of alcoholic liver (Ullah et al. 2019). This disease has been identified in four stages: (a) hepatic fat deposition (hepatic steatosis), (b) hepatic fat deposition with inflammation, (c) fibrosis, and (d) cirrhosis. The first and second stages are also known as nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH), respectively. In NASH condition over hepatic fat deposition and inflammation occur. Tenacious inflammation (hepatitis) liver cause's scar tissue formation in the liver is called fibrosis. The acute form of NAFLD is cirrhosis, which is the fourth stage. Fibrosis leads to the cirrhosis where maximum liver cells' structure and function are compromised. Finally the condition is failure of liver. In fibrosis condition, hepatocytes function properly, but in cirrhosis they fail to function. Generally, in adult population the NAFLD percentage is 5–20%, but this increase above 40% have patients of obesity and type 2 diabetic (Ullah et al. 2019; Clark 2006; Lazo et al. 2008). Because of the several factors accumulation of lipids in the liver. As the lipolysis increase, fatty acid uptake enhanced from the adipocytes or if the diets have high fat containing such phenomenon takes place. In liver deposition of triglyceride takes place if de novo lipogenesis increases, oxidation of fatty acid lessens and secretion of very-low-density lipoproteins occurs (VLDL). Accumulation of triglyceride droplets inside the hepatocytes may be protective, otherwise their conversion to nontriglyceride lipotoxic metabolites will be the cause of liver injury. The enzyme involved is in different steps of hepatic de novo lipogenesis is fatty acid synthase (FAS), and its functions are controlled by a complex network of nuclear receptors (Koo 2013; Neuschwander-Tetri 2010).

For treatment of the NAFLD examining the important pathways which materialize the necessary in the pathogenesis of the disease. It directed the patients to reduce

the body mass index and improve insulin resistance by adopting physical exercise, dietary modification, bariatric surgery, and pharmacological treatments. Many times, NAFLD pathogenesis of conventional "two-hit" hypothesis is updated. In general, maximum patients have accumulation of lipid or steatosis which is because of obesity and insulin resistance. Development of steatohepatitis and fibrosis occurs because of several factors such as FFAs, inflammatory cytokines and adipokines, oxidative stress, and mitochondrial dysfunction in a complex interplay with a genetic predisposition. Plan for the treatment of NASH concentrates on enhancing the components of the metabolic syndrome like obesity and insulin resistance, and still no liver-specific agents are available. But the fine tune of any kind of mechanism is tangled in NASH pathogenesis which gives useful targets to prevent the development of fibrosis and its associated complications. The understandings of pathogenesis of NASH are helpful for the development of novel therapeutic strategies (Dowman et al. 2010). If the treatment of NASH not be done on time, it further grows to liver fibrosis, cirrhosis, and ultimately it is converted to hepatocellular carcinoma. At present, no any proper therapeutic approaches for the treatment of NAFLD and NASH has been found, but honey flavonoids are recommended as home remedy for the treatment to cure the disease. The other strategy in controlling and monitoring of NSFLD relies on diet, physical activity, and lifestyle modifications and metabolic disorder associated with NAFLD including hyperglycemia, insulin resistance, and hyperlipidemia. A regular use of honey flavonoids studied in patients of NAFLD found beneficial effects. Apart from the honey, foods that have flavonoids have several advantages for health. Basic role of flavonoids against the pathological condition are to their antioxidant activity. Sometimes these compounds directly interfere to block the signaling pathways which develop the disease process. All different subgroups of flavonoids have hepatoprotective effects (Akhlaghi 2016). Not only antioxidant but also other pharmacological effects such as anti-inflammatory and metabolic effects have been revealed, and this compound is considered as a bioactive compound. The different effects (Fig. 17.2) and subclasses (Table 17.1) are illustrated.

17.2 Pathophysiology of Nonalcoholic Fatty Liver Disease (NAFLD)

Nonalcoholic fatty liver disease (NAFLD) is a heterological disease, which as its name implies is deposition of fat in liver, which can be categorized into nonalcoholic fatty liver and nonalcoholic steatohepatitis (NASH), histologically. Presence of at least 5% hepatic steatosis without evidence of hepatocellular injury in the form of hepatocyte blooming is considered to be nonalcoholic fatty liver. While a least of 5% hepatic steatosis with inflammation and hepatocyte injury with or without fibrosis is considered to NASH (Ekstedt et al. 2015; Angulo et al. 2015). NASH clinical research network has given a spectrum of several histological patterns of NAFLD (Yeh and Brunt 2014) (Fig. 17.3) Out of all the patients diagnosed with NAFLD,

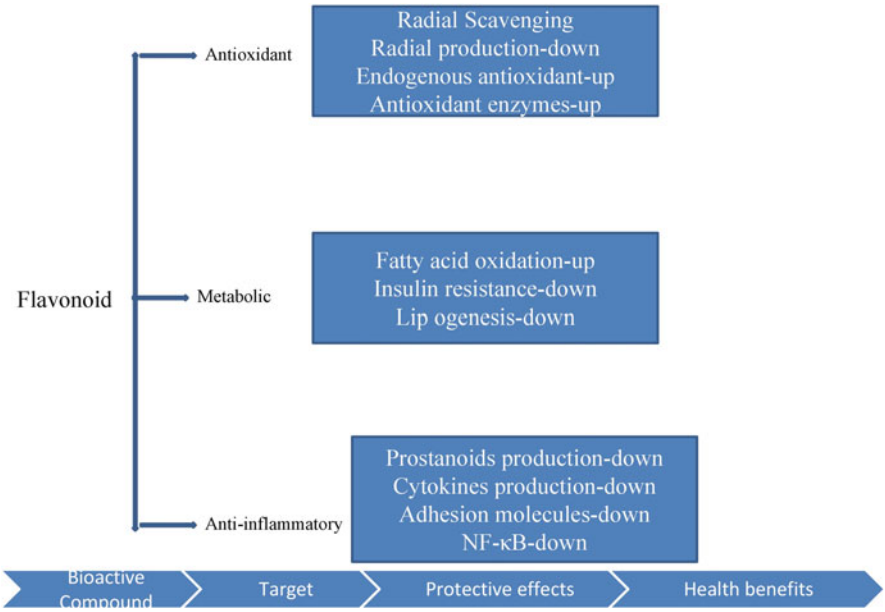

Fig. 17.2 Therapeutic approach of flavonoid

only 5–10% patients will progress to NASH which makes it easier to transform into fibrosis, cirrhosis, and consequently hepatocellular cancer (Buzzetti et al. 2016).

Hepatic steatosis or fatty change is a major pathological marker for NAFLD. It is basically accumulation of fat droplets in the hepatocytes (Cairns and Peters 1983). Fat mainly includes free fatty acids and triglycerides which makes it a hallmark feature for NAFLD (Townsend and Newsome. 2016). There are various factors that can contribute to accumulation of fats in the hepatocytes which can broadly be categorized into genetics and unhealthy lifestyles. An unhealthy lifestyle mainly includes high calories intake mainly of glucose, fructose, and saturated fat, and this can further be accompanied by sedentary lifestyles and aging (Fig. 17.4) (Stefan et al. 2019). Earlier two-hit model of NAFLD has been proposed for the progression of the disease in which first hit included insulin resistance and hyperinsulinemia which altered hepatic pathways for uptake, synthesis, degradation of fat (FFA) in the liver and leads to fat accumulation in it (Buzzetti et al. 2016). The second hit is associated with fibro genesis which is a consequence of activation of several inflammatory events (Peverill et al. 2014). But the two-hit model has lost its importance for it being too simplistic to describe the intricacy of the NAFLD as NAFLD includes a multitude of factors in its development. Currently, a "multiple hit" theory describes the progression of NAFLD. These multiple factors mainly include unhealthy and sedentary lifestyles, diet, genetic, epigenetic, environmental factors, insulin resistance, and type-2 diabetes mellitus (Ayonrinde et al. 2015; Holterman et al. 2013). Complex involvement of hepatic resident cells and activated immune cells which includes Kupffer cells, T cells, and hepatic stellate cell (HSC)

Table 17.1 Subclass of flavonoid

S. no	Class	Substance	Natural source	Pharmacological role
1.	Flavones	Luteolin, apigenin, and tangeretin	Fruits and medicinal plants	Glucosides
2.	Flavonols	Kaempferol, quercetin, myricetin and fisetin	Vegetables and fruits	Proanthocyanins
3.	Flavanones	Hesperitin, naringenin and eriodictyol	Citrus fruits	Free-radical scavenging properties
4.	Isoflavonoids	Genistein and daidzein	Soybeans and other leguminous plants	Inducing hormonal and metabolic changes, effects on various disease pathways
5.	Anthocyanins	Cyanidin, delphinidin, malvidin, pelargonidin, and peonidin	Cranberries, black currants, red grapes, merlot grapes, raspberries, strawberries, blueberries, bilberries, and blackberries	Fight against disease
6.	Chalcones	Phloridzin, arbutin, phloretin, and chalconaringenin	Tomatoes, pears, strawberries, bearberries and certain wheat products	Fight against disease

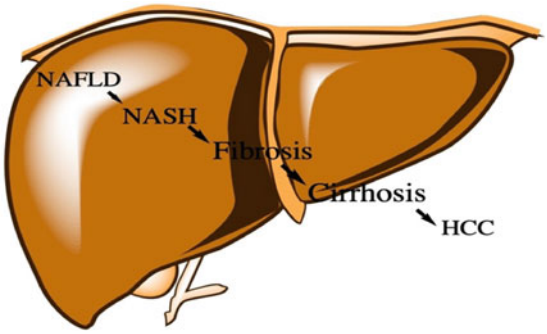

Fig. 17.3 Progression and spectrum of nonalcoholic fatty liver disease (NAFLD), *NASH* nonalcoholic steatohepatitis, *HCC* hepatocellular cancer

with inflammatory factors leads to progression and pathogenesis of NAFLD (Chen et al. 2016; De Vito et al. 2012) (Fig. 17.5) Markers for hepatic fibrosis and cirrhosis are chronic activation of hepatic stellate cells and apoptosis. While hepatic progenitor cells (HPCs) are associated with NASH and fibrosis (Nobili et al. 2012). Some evidences suggest that Kupffer cells mediate NAFLD pathogenies including immune tolerance and lipid homeostasis, differently (Dattaroy et al. 2016; Mouralidarane et al. 2013). A study reported that Kupffer cell triggers TGs

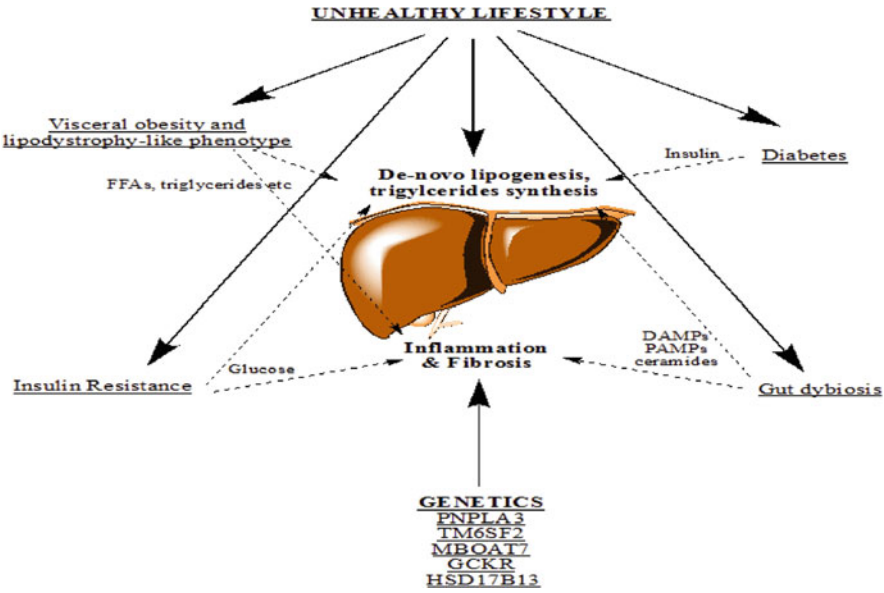

Fig. 17.4 Causes of NAFLD

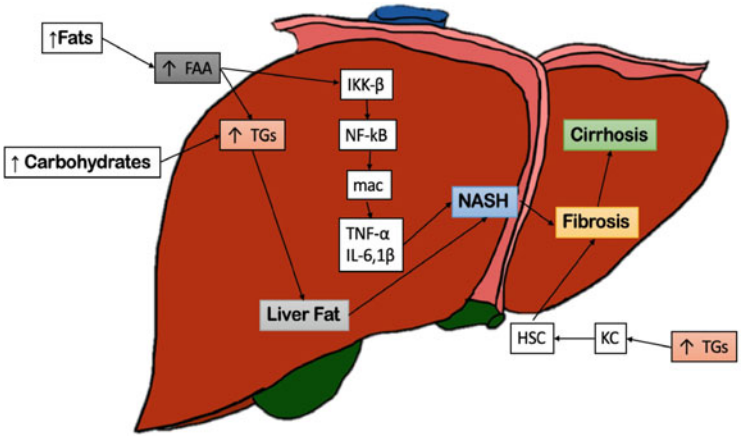

Fig. 17.5 Pathogenesis of nonalcoholic fatty liver disease. Abbreviations *FFAs* free fatty acids, *IKK β* inhibitor of κB kinase-β, *NF-κB* nuclear factor kappa B, *Mac* macrophages, *TNF-α* tumor necrosis factor-α, *IL-6* interleukin 6, *IL-1 β* interleukin 1 β, *TGs* triglycerides, *NASH* nonalcoholic steatohepatitis, *HSC* hepatic stellate cells, *KC* Kupffer cells

accumulation and hepatic steatosis by interleukin-1 beta (IL-1β)-mediated suppression of peroxisome proliferator-activated reactor-α (PPAR-α) actions (Stienstra et al. 2010). Another study reported the altered functionality of peripheral T cells subpopulations in NASH (FerreyraSolari et al. 2012) Pathophysiology of NAFLD is still not certain and requires further investigation.

17.3 Therapeutic Effects of Flavonoids

Honey exerts a wide range of biological activity including anti-inflammatory, immune-strengthening, antitumor, and antioxidant effects (Cheng et al. 2007). Honey exerts neuroprotective effect via its antioxidant potential. Tualang honey has known to exert neuroprotective effect which was attributed to its high flavonoids content and some enzymes such as glucose oxidase, catalase and peroxidase, and non-enzymatic antioxidants such as ascorbic acid, α-tocopherol, and carotenoids (Azman et al. 2018).

Polyphenol play a central role in exerting the protective effect of honey in the nervous system. Polyphenol is able to counteract neurotoxicity induced by the ROS, which are neurotoxic, the deposition of misfolded proteins, such as beta amyloid hence imparting neuroprotection (Cianciosi et al. 2018).

Various microorganisms such as *H. influenza*, *P. aeruginosa*, *S. aureus*, *K. pneumonia*, and *S. pyogenes* are known to cause respiratory infections such as influenza, pneumonia, nosocomial infection, pneumonia in debilitated individual, and pharyngitis (Reham et al. 2016) Buckwheat honey which is higher in phenolic content is known to relief nocturnal cough and quality of sleep in children and parents, and the effect was found to be superior than dextromethorphan (Paul et al. 2007).

Honey is a rich source of natural antioxidants such as flavonoids, polyphenols (like quercetin, caffeic acid, acacetin, phenethyl ester (CAPE), galangin, and kaempferol), and monophenolics which are reported to exert cardioprotective effects (Khalil et al. 2010; Samarghandian et al. 2017). Some flavonoids such as rutin present in honey are known to enhance bioavailability of nitric oxide, a potent vasodilatation by increasing eNOS gene expression whose activity promotes the production of NO. Similarly, catechin and quercetin present in honey have negative effect on aortic atherosclerotic lesion development. Oxidative stress is the key cause of various diseases and disorders such as cancer, mutagenesis, aging, atherosclerosis, and many other degenerative diseases (Ahmed et al. 2008). Antioxidants seize the free radicals before any damage caused. Honey is known to be a rich source of antioxidant. Darker the honey, more is its antioxidant value. Antioxidant property of honey which is attributed to the presence of flavonoids, polyphenolics, Vitamin C, and monophenolics may be associated with the reduced cardiac failure. Flavonoids are known to exert antioxidant, antithrombotic, anti-ischemic, and vasorelaxant effects which exert protective effect against coronary heart disease. Flavonoids present in honey reduce the chances of coronary heart disorders via improving coronary vasodilatation, inhibiting oxidation of low-density lipoprotein, and reducing the ability of platelet to form blood clot. The flavonoids which are rich in honey are caffeic acid, quercetin, phenethyl ester, kaempferol, galangin, and acacetin (Samarghandian et al. 2017).

Antioxidant property of honey is attributed to its chemical constituents such as flavonoids and phenolic acids, carotenes, organic acids, sugars, amino acids, protein, Maillard reaction products. It is rich in several phenolics (viz., ferulic acids, p-coumarin, caffeic acids, ellagic acids), flavonoids (viz., quercetin, kaempferol,

apigenin, pinocembrin, hesperetin, chrysin, and galangin), vitamins C and E, superoxide dismutase, and catalase. These antioxidants coordinate positively with each other to exert antioxidant potential (Vallianou et al. 2014). An antioxidant property of honey is related to its brightness. Darker honey has more antioxidant value. Phenolic compounds present in honey are responsible for its antioxidant property as phenolic level is correlated with its higher radical absorbance activity (Samarghandian et al. 2017).

Phenolic compounds such as pinocembrin and syringic acid present in honey exert anti-microbial effects (Cianciosi et al. 2010). The anti-fungal effect of honey was attributed to production of H_2O_2 and presence of volatile and phenolic compounds (Anand et al. 2019).

Honey with greater phenolic content exerts more antitumor effect (Erejuwa et al. 2014). The polyphenols and phenolic compounds present in honey are known to exert anti-leukemic potential against various leukemic cell lines (Ahmed et al. 2008). Honey is known to arrest cell cycle in the sub-G1 phase in bladder cancer cell lines, and this capacity of honey is attributed to presence of several flavonoids and phenolic compounds. Some of the flavonoids such as chrysin, quercetin, and kaempferol present in honey arrest the cell cycle in G0/G1, G1, and G2/M in various cancer cell lines (Anand et al. 2019). The phenolic component of honey also exerts anticancer effect by inducing apoptosis through mitochondrial membrane depolarization and modulating the expression of pro- and anti-apoptotic protein. It also increases the activation of caspase 3 and cleavage of poly (ADP-ribose) polymerase (PARP) in human colon cancer cell lines (Samarghandian et al. 2017). High levels of flavonoids present in honey exert anti-secretory mechanisms which contribute to its gastroprotective effect (Erejuwa et al. 2014). Honey also acts as a potent anti-inflammatory agent. Chrysin, galangin, and quercetin present in honey suppresses the activity of enzymes such as inducible nitric oxide synthase (iNOs) and cyclooxygenase-2 (COX-2) which are associated with the production of inflammatory cytokines. Manuka honey has been known to activate IL-10, IL-1, IL-6 (anti-inflammatory cytokine), TNF-α, and IL-1β (pro-inflammatory cytokines) through toll-like receptors (TLR) and growth factors PDGF and TGF-β.

17.4 Advantages of Honey Flavonoids

Honey is a natural product which is formed by honey bees from nectar of flower. Honey has been consumed by many civilization from ancient times and till now for its nutritional and medicinal benefits (Adebolu 2005; Ashrafi et al. 2005). There are evidences that indicate the therapeutic effects of honey as antioxidant (Ahmed et al. 2013), anti-inflammatory (Khalil et al. 2012), antibacterial (Attia et al. 2008), antidiabetic (Estevinho et al. 2008), respiratory, gastrointestinal (Abdulrhman et al. 2008) cardiovascular, and nervous (Ghosh and Playford 2003) system protective effects. Besides rich in carbohydrates, honey is also a source for flavonoids, polyphenols, reducing compounds, alkaloids, glycosides, cardiac glycosides, anthraquinone, and volatile compounds (White 1962). There are several flavonoids found

in honey which include quercetin, kaempferol, myricetin, chrysin, galangin, and luteolin (Zand et al. 2000). Phenolic and flavonoid compounds have been shown to have anti-inflammatory and immunomodulatory effects in animal models, cell cultures (Fernandez-Cabezudo et al. 2013; Candiracci et al. 2012; Bilsel et al. 2002), and clinical trials (Leong et al. 2012). Honey flavonoids have shown to regulate/suppress the proteins like cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), ornithine decarboxylase, and tyrosine kinase. These are proteins in the process of inflammation (Viuda-Martos et al. 2008). Flavonoids and phenolic compounds also produce antioxidant effects and inhibit low-density lipoproteins from oxidizing (Kamaruzaman et al. 2014). Different types of honey have also shown inhibition of pro-inflammatory factors such as TNF-α, IL-6, and IL-1 β production (Hussein et al. 2012). Honey has shown immunomodulatory effects by short chain fatty acid (SCFA) fermentation agents, which is produced by slow absorption of honey (Samarghandian et al. 2017). Sufficient data exists which recommends the use of honey in a diseased condition, but further clinical examination can strengthen the therapeutic use of honey.

17.5 Limitations of Honey Flavonoids

Flavonoids interfere with a lot of molecular pathways that are involved in the progression of NAFLD, therefore it has shown beneficial effects. But these evidences are majorly based on pre-clinical studies where the disease model is artificially induced. This might differ from real NAFLD in humans. Another issue arises as flavonoid doses for animal studies were very high, extrapolation of these higher doses to human may cause ethical issues. Flavonoid–drug interaction is yet to be explored and studied (Srinivas et al. 2015; Akhlaghi 2016).

17.6 Conclusion

NAFLD pathophysiological model has multiple pathways, line up with steatosis and inflammation, and ultimately fibrosis, cirrhosis, liver failure, and hepatocellular carcinoma. Different flavonoids act on the pathways in different steps to control the disease. Flavonoids are bioactive compounds and are naturally present in plants. These bioactive compounds have been found to control lipid metabolism, insulin resistance, inflammation, and oxidative stress, the most important pathological processes in the etiology of NAFLD.

References

Abdulrhman M, El-Hefnawy M, Ali R, El-Goud AA (2008) Honey and type 1 diabetes mellitus. In: Liu CP (ed) Type-1 diabetes—complications, pathogenesis, and alternative treatments. Open InTech, Croatia

Adebolu TT (2005) Effect of natural honey on local isolates of diarrhea causing bacteria in Southwestern Nigeria. Afr J Biotechnol 4:1172–1174

Akhlaghi M (2016) Non-alcoholic fatty liver disease: beneficial effects of flavonoids. Phytother Res 30(10):1559–1571

Al-Mamary M, Al-Meeri A, Al-Habori M (2002) Antioxidant activities and total phenolics of different types of honey. Nutr Res 22:1041–1047

Alvarez-Suarez JM, Tulipani S, Romandini S, Bertoli E, Battino M (2010) Contribution of honey in nutrition and human health: a review. Mediterr J Nutr Metab 3:15–23

Anand S, Deighton M, Livanos G et al (2019) Agastache honey has superior antifungal activity in comparison with important commercial honeys. Sci Rep 9:181–197

Angulo P, Kleiner DE, Dam-Larsen S et al (2015) Liver fibrosis, but no other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty liver disease. Gastroenterology 149:389–397

Ashrafi S, Mastronikolas S, Wu CD (2005) Use of honey in treatment of aphthous ulcers IADR/AADR/CADR 83rd general session. Baltimore, MD, USA, pp 9–12

Attia WY, Gabry MS, El-Shaikh KA, Othman GA (2008) The anti-tumor effect of bee honey in Ehrlich ascitetumor model of mice is coincided with stimulation of the immune cells. J Egypt Public Health Assoc 15:169–183

Ayonrinde OT, Olynyk JK, Marsh JA, Beilin LJ, Mori TA, Oddy WH et al (2015) Childhood adiposity trajectories and risk of nonalcoholic fatty liver disease in adolescents. J Gastroenterol Hepatol 30:163–171

Azman KF, Zakaria R, Othman Z, Aziz CBA (2018) Neuroprotective effects of Tualang honey against oxidative stress and memory decline in young and aged rats exposed to noise stress. J Taibah Univ Sci 12(3):273–284

Babacan S, Rand AG (2007) Characterization of honey amylase. J Food Sci 72:C050–C055

Bansal V, Medhi B, Pandhi P (2005) Honey—a remedy rediscovered and its therapeutic utility. Kathmandu Univ Med J (KUMJ) 3:305–309

Bilsel Y, Bugra D, Yamaner S, Bulut T, Cevikbas U, Turkoglu U (2002) Could honey have a place in colitis therapy? Effects of honey, prednisolone, and disulfiram on inflammation, nitric oxide, and free radical formation. Dig Surg 19:306–311

Buzzetti E, Pinzani M, Tsochatzis EA (2016) The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). Metabolism 65(8):1038–1048

Cairns SR, Peters TJ (1983) Biochemical analysis of hepatic lipid in alcoholic and diabetic and control subjects. Clin Sci (Lond)() 65:645–652

Candiracci M, Piatti E, Dominguez-Barragán M, García-Antrás D, Morgado B, Ruano D et al (2012) Anti-inflammatory activity of a honey flavonoid extract on lipopolysaccharide-activated N13 microglial cells. J Agric Food Chem 60:12304–12311

Chen WEI, Wang X, Huang LI, Liu BO (2016) Hepcidin in non-alcoholic fatty liver disease regulated by the TLR4/NF-κBsignaling pathway. Exp Ther Med 11:73–76

Cianciosi D, Forbes-Hernández TY, Afrin S et al (2018) Phenolic compounds in honey and their associated health benefits: a review. Molecules 23(9):2322

Clark JM (2006) The epidemiology of nonalcoholic fatty liver disease in adults. J Clin Gastroenterol 40:S5–S10

Dattaroy D, Seth RK, Das S, Alhasson F, Chandrashekaran V, Michelotti G et al (2016) Sparstolonin B attenuates early liver inflammation in experimental NASH by modulating TLR4 trafficking in lipid rafts via NADPH oxidase activation. Am J Physiol Gastrointest Liver Physiol 310:G510–GG25

De Vito R, Alisi A, Masotti A, Ceccarelli S, Panera N, Citti A et al (2012) Markers of activated inflammatory cells correlate with severity of liver damage in children with nonalcoholic fatty liver disease. Int J Mol Med 30:49–56

Dowman JK, Tomlinson JW, Newsome PN (2010) Pathogenesis of non-alcoholic fatty liver disease. QJM 103(2):71–83

Ekstedt M, Hagström H, Nasr P et al (2015) Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. Hepatology 61:1547–1554

Erejuwa OO, Sulaiman SA, Wahab MS (2014) Effects of honey and its mechanisms of action on the development and progression of cancer. Molecules 19(2):2497–2522

Estevinho L, Pereira AP, Moreira L, Dias LG, Pereira E (2008) Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. Food Chem Toxicol 46:3774–3779

Fernandez-Cabezudo MJ, El-Kharrag R, Torab F, Bashir G, George JA, El-Taji H et al (2013) Intravenous administration of manuka honey inhibits tumor growth and improves host survival when used in combination with chemotherapy in a melanoma mouse model. PLoS One 8: e55993

Ghosh S, Playford RJ (2003) Bioactive natural compounds for the treatment of gastrointestinal disorders. Clin Sci (Lond) 104:547–556

Hassapidou M, Fotiadou E, Maglara E, Papadopoulou SK (2006) Energy intake, diet composition, energy expenditure, and body fatness of adolescents in Northern Greece. Obesity (Silver Spring) 14:855–862

Holterman A-XL, Guzman G, Fantuzzi G, Wang H, Aigner K, Browne A et al (2013) Nonalcoholic fatty liver disease in severely obese adolescent and adult patients. Obesity 21:591–597

Hussein SZ, Mohd Yusoff K, Makpol S, Mohd Yusof YA (2012) Gelam honey inhibits the production of proinflammatory, mediators NO, PGE (2), TNF-a, and IL-6 in carrageenan-induced acute paw edema in rats. Evid Based Complement Alternat Med 2012:109636

Inglett GE (1976) A history of sweeteners—natural and synthetic. J Toxicol Environ Health 2:207–214

Johnston JE, Sepe HA, Miano CL, Brannan RG, Alderton AL (2005) Honey inhibits lipid oxidation in ready-to-eat ground beef patties. Meat Sci 70:627–631

Kamaruzaman NA, Sulaiman SA, Kaur G, Yahaya B (2014) Inhalation of honey reduces airway inflammation and histopathological changes in a rabbit model of ovalbumin-induced chronic asthma. BMC Complement Altern Med 14:176

Khalil I, Moniruzzaman M, Boukraâ L, Benhanifia M, Islam A, Islam N et al (2012) Physicochemical and antioxidant properties of Algerian honey. Molecules 17:11199–11215

Koo SH (2013) Nonalcoholic fatty liver disease: molecular mechanisms for the hepatic steatosis. Clin Mol Hepatol 19:210–215

Leong AG, Herst PM, Harper JL (2012) Indigenous New Zealand honeys exhibit multiple anti-inflammatory activities. Innate Immun 18:459–466

Mouralidarane A, Soeda J, Visconti-Pugmire C, Samuelsson A-M, Pombo J, Maragkoudaki X et al (2013) Maternal obesity programs offspring nonalcoholic fatty liver disease by innate immune dysfunction in mice. Hepatology 58:128–138

Neuschwander-Tetri BA (2010) Nontriglyceride hepatic lipotoxicity: the new paradigm for the pathogenesis of NASH. Curr Gastroenterol Rep 12:49–56

Nobili V, Carpino G, Alisi A, Franchitto A, Alpini G, De Vito R et al (2012) Hepatic progenitor cells activation, fibrosis, and adipokines production in pediatricnonalcoholic fatty liver disease. Hepatology 56:2142–2153

Pataca LC, Borges Neto W, Marcucci MC, Poppi RJ (2007) Determination of apparent reducing sugars, moisture and acidity in honey by attenuated total reflectance Fourier transform infrared spectrometry. Talanta 71:1926–1931

Paul IM, Beiler J, McMonagle A, Shaffer ML, Duda L, Berlin CM Jr (2007) Effect of honey, dextromethorphan, and no treatment on nocturnal cough and sleep quality for coughing children and their parents. Arch Pediatr Adolesc Med 161(12):1140–1146

Peverill W, Powell LW, Skoien R (2014) Evolving concepts in the pathogenesis of NASH: beyond steatosis and inflammation. Int J Mol Sci 15:8591–8638

Rakha MK, Nabil ZI, Hussein AA (2008) Cardioactive and vasoactive effects of natural wild honey against cardiac malperformance induced by hyperadrenergic activity. J Med Food 11:91–98

Samarghandian S, Farkhondeh T, Samini F (2017) Honey and health: a review of recent clinical research. Pharm Res 9(2):121

Stefan N, Häring HU, Cusi K (2019) Non-alcoholic fatty liver disease: causes, diagnosis, cardiometabolic consequences, and treatment strategies. Lancet Diabetes Endocrinol 7 (4):313–324

Stienstra R, Saudale F, Duval C, Keshtkar S, Groener JEM, van Rooijen N et al (2010) Kupffer cells promote hepatic steatosis via Interleukin-1 beta-dependent suppression of peroxisome proliferator-activated receptor alpha activity. Hepatology 51:511–522

Townsend SA, Newsome PN (2016) Non-alcoholic fatty liver disease in 2016. Br Med Bull 119:143–156

Turkmen N, Sari F, Poyrazoglu ES, Velioglu YS (2006) Effects of prolonged heating on antioxidant activity and colour of honey. Food Chem 95:653–657

Ullah R, Rauf N, Nabi G, Ullah H, Shen Y, Zhou YD, Fu J (2019) Role of nutrition in the pathogenesis and prevention of non-alcoholic fatty liver disease: recent updates. Int J Biol Sci 15 (2):265

Vallianou NG, Gounari P, Skourtis A, Panagos J, Kazazis C (2014) Honey and its anti-inflammatory, anti-bacterial and anti-oxidant properties. Gen Med (Los Angel) 2:2

Vernon G, Baranova A, Younossi ZM (2011) Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Aliment Pharmacol Ther 34:274–285

Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, Pérez-Alvarez JA (2008) Functional properties of honey, propolis, and royal jelly. J Food Sci 73:R117–R124

White JW (1962) Composition of American honeys. Agricultural Research Service, USDA, Washington, DC

White JW (1979) Composition of honey. In: Crane E (ed) Honey: a comprehensive survey. Heinemann, London, pp 157–192

Yeh MM, Brunt EM (2014) Pathological features of fatty liver disease. Gastroenterology 147:754–764

Zand RS, Jenkins DJ, Diamandis EP (2000) Steroid hormone activity of flavonoids and related compounds. Breast Cancer Res Treat 62:35–49

Honey Products and Their Potential in Wound Healing

18

Omar Sarheed and Manar Samir Debe

Abstract

Honey dressings attract attention as a therapeutic alternative for wound care due to its antibacterial, antioxidant, and immunomodulatory action. To obtain better medicinal properties, the formulation of honey requires the characterization of physicochemical and mechanical properties. This chapter aims to provide an in-depth account of the value of characterizing the honey dressing. Physical tests, such as swelling capacity, water vapor transmission rate, and thermal studies, are described. Therapeutic testings of honey wound dressings and their clinical applications have been covered. Recent developments in the formulation of honey dressings are also discussed in this chapter.

Keywords

Honey · Physico-chemical characterization · Formulation · Wound dressings

18.1 Introduction

In ancient cultures, natural products were the only available sources for treating most clinical conditions. Honey, for example, was used as a medicinal component for its antibacterial, antioxidant, and immunomodulatory effects which can be utilized in wound healing. However, the discovery of many synthetic drugs surpassed the use of the traditional "folk medicine." The use of antibiotics became the most common practice in the clinical field for treating all sorts of infections. This overuse of antibiotics led to the emergence of antibiotic-resistant bacteria, an issue that caused

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a comeback for the use of natural products in clinical medicine nowadays (Minden-Birkenmaier and Bowlin 2018).

Honey is the natural product of honeybees, *Apis mellifera*, which collect nectar from floral plants. It is a sweet syrup supersaturated with sugars in addition to the presence of some minerals, polyphenols, vitamins, carotenoids, amino acids, proteins, and enzymes, which differ depending on the floral source (Miguel et al. 2017). Honey is made up of nearly 200 constituents with sugar accounts for 95–99% of the total content. Glucose and fructose are the main carbohydrates present in honey making it readily absorbed in the GIT, along with other disaccharides and oligosaccharides like maltose, sucrose, isomaltose, . . . etc. Besides sugar, water is an important part of honey as well. The presence of organic acids like gluconic acid contributes to the acidity and the taste of honey. Vitamins like B and C are also present whose composition in honey differs based on the type of the honeybee. Minerals are present in low concentrations, 0.1–1%, where potassium is the main mineral. Calcium, magnesium, sodium, sulfur, and phosphorus are also present (Eteraf-Oskouei and Najafi 2013). Out of the different amino acids found in honey, proline is the most important one. Multiple phenolic acids and flavonoids are present that are dependent on the origin of the honey and, hence, used as chemical markers for origin identification (Miguel et al. 2017).

The effectiveness of honey in treating wounds is due to these various components. It is found to have a broad range of antibacterial effect. The enzyme glucose oxidase in honey breaks glucose into gluconic acid and hydrogen peroxidase; the former lowers the pH of honey reducing protease activity, increasing tissue oxygenation and stimulating macrophage and fibroblast activity, while the later kills the bacteria sterilizing the wound. Invertase is another enzyme found in honey that hydrolyzes sucrose to glucose and fructose increasing the osmotic potential. Flavonoids remove the free radicals preventing tissue damage. Furthermore, honey being a viscous fluid provides coverage over the wound forming a barrier against the surrounding bacteria while keeping the wound hydrated (Minden-Birkenmaier and Bowlin 2018).

As the use of honey in treating wounds and infections has been recognized by our ancestors, scientists have performed numerous studies and extensive research to elaborate on this effect and the mechanisms behind it. Based on this, researchers have found multiple applications for honey as a therapeutic wound dressing and formulated the dressing using multiple techniques. This chapter will discuss the use and efficacy of honey as a wound dressing in several clinical conditions including its characteristic properties which make it possible. In addition, the chapter will discuss the different dosage forms of honey available that enable its use as a wound dressing and their performance in the light of healing rate and pain alleviation.

18.2 Types of Wounds

Skin is made up of multiple layers which cover the entire body that consists of muscles, bones, ligaments, and internal organs. By covering the body, the skin protects it from the external environment including microbes, heat, light, and injury. However, if the structure or the function of the skin is compromised it leaves the body susceptible to infection. This disruption in the skin due to an injury known as wound that can sometimes be simple and heals rapidly but can also be as serious as being life threatening (Ibrahim et al. 2018; Tang et al. 2019).

Injuries are very common in the worldwide population; most of the wounds can heal within a limited period of time by a series of well-organized processes. Different components work together to repair the skin tissue including platelets, keratinocytes, immune cells, microvascular cells, and fibroblasts. Usually, the healing process occurs without complications or significant intervention; such injuries are known as acute wounds.

However, when patients who get injured suffer from other diseases, this can impair the healing process and prolong it dramatically. This known as chronic wounds. With the extension of the period of wound healing and closure, wounds become vulnerable to excessive inflammatory phase, persistent infections, formation of drug-resistant microbial biofilms, and the inability of epidermal cells to respond to reparative stimuli (Demidova-Rice et al. 2012; Sarheed et al. 2016).

Thus, treatment is required to stimulate wound healing. Ideally, the treatment is required to provide, first, removal of the dead and necrotic tissue from the wound site to allow for new tissue cells to form, a process known as debridement. Then, microorganisms at the wound site need to be eradicated as their proliferation at the wound causes infection and inflammation. Finally, the epithelial cell needs to be stimulated to migrate to the wound and repair it, a process known as epithelialization. The application of dressing over the wound which is medicated with agents that promote these cellular and molecular processes is a very common practice (Demidova-Rice et al. 2012). A detailed description of wounds types in which honey is used for is mentioned later.

18.3 Wound Dressing

As a response to the injury of the skin, the wound-healing process commences, which is a complex multistep process to regain the normal structure of the skin. For a wound to heal first, an inflammatory response is initiated which is characterized by blood coagulation and attraction of immune cells to the injury site. The cells secrete cytokines, protecting against developing an infection. Endothelial cells also travel to the injury site secreting growth factors. Then, in the proliferative stage, an epithelial layer covers the wound to fill the spaces in addition to collagen deposition which contributes to reduction in wound size. Finally, the remodeling starts to restore the original integrity and structure of the skin.

The normal healing process can take up to 10 days, but some alterations and disturbances can occur to the process prolonging the healing, and leading to chronic infections. Thus, some substances can be applied onto the wound to facilitate the process of healing (Ibrahim et al. 2018). Applying a wound dressing is a common way to promote healing. It can consist of simple substances like cotton and gauze which only provide the advantage of forming a protective layer over the wound. A wound dressing may also contain a material with bioactive properties such as antimicrobial and antioxidant effects (Tang et al. 2019).

Ideally, a dressing should provide sufficient hydration to the wound, absorb the discharge formed in the wound site, be placed and removed easily avoiding any discomfort, and be porous enough to allow gaseous exchange. Preferably, the dressing should contain an agent with antimicrobial properties while being nontoxic and biocompatible (Wang et al. 2012). Many synthetic and natural candidates are considered as viable options to be incorporated in wound dressings. Honey is a common example because of its antibacterial, antiinflammatory, and antioxidant properties along with its ability to maintain wound closure and increasing the rate of reepithelialization (Tang et al. 2019).

18.4 Physical Characterization of Wound Dressings

To ensure patient adherence to the dressing applied onto the wound, certain desirable properties should be provided. To be proven as a good candidate for use in wound care by clinical professionals, the dressing should act as an enhancer of wound healing. This can be accomplished by offering enhancement in cell migration like growth cells, epidermal cells, leukocytes, etc. The dressing should also provide a moist environment and gaseous exchange to support angiogenesis while not permitting the entry of bacteria or any foreign matter. From the consumer's perspective, the dressing should also be easy to apply and is not painful upon removal. It is also preferred to be cost-effective in which the frequency of the application is reduced or the dressing is less expensive while still being sufficiently useful in wound treatment.

There are various standardized tests available that should be carried out before the formulated product is put on the market in order to assess the performance of the dressing and ensure safe and effective product for the intended use. Such tests include the following:

18.4.1 Swelling Tests

An important characteristic the dressing is the absorption of exudate from the wound while maintaining moist conditions and avoiding leakage. Upon absorption, the dressing normally swells. The swelling behavior and the degree of swelling are measured during the development of the dressing to evaluate the extent of fluid absorption. Swelling depends on the composition of the dressing, temperature, pH, time, types of fluid, ...etc. The presence of polymeric and cross-linked molecules

increases the degree of swelling. The swelling is measured by placing the dressing in solutions of varying pH because the wound pH changes during healing and moves toward the acidic region. The amount of fluid retained is measured and expressed as swelling degree (Roy et al. 2010; Agrawal and Purwar 2018). The incorporation of honey in wound dressings increases the swelling ratio due to its high sugar content which increases the osmolarity of the formulation (Mohd Zohdi et al. 2012).

18.4.2 Fluid Handling Capacity

This is mainly performed for hydrocolloid, alginate, and polyurethane foam dressings and it measures the amount of fluid retained in the dressing as well as the amount lost as moisture vapor. This enables to quantify the absorbency of the dressing and the moisture vapor loss. The presence of a gel-like structure on the dressing improves the fluid handling properties of the dressing. Dressings with increased fluid handling capability is advantageous for chronic wounds healing, as they minimize the incidence of skin maceration due to the immobilization of large amounts of exudates (Boateng et al. 2008).

18.4.3 Water Vapor Transmission Rate (WVTR)

Fluid from the wound that is lost as vapor needs to be measured. The process of fluid evaporation needs to be optimized because an increase in the loss of fluid would reduce body temperature. In contrast, if less fluid is lost, the pressure on the wound increases as the fluid builds up, which makes the wound more painful. It is noted that the dressing's permeability increases over time due to the absorption of fluids. Thus, it is necessary to measure the amount of body fluid that the dressing can transmit. The incorporation of honey in the dressing disrupts the diffusion of fluids, reducing the WVTR significantly (Boateng et al. 2008; Muktar et al. 2018).

18.4.4 Thermal Studies

It is also necessary to determine the thermal stability of the dressing at increasing temperatures through thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). The temperature of the dressing in the analysis is first set at room temperature and then raised gradually at the rate of 10 °C/min. The degradation of the composition is noticed throughout the process including the temperature at which degradation starts, temperature onset T_o, and the temperature when degradation is complete; temperature completion T_c. The addition of honey does not affect T_o significantly; however, it increases the T_c with increasing honey concentration. Thus, the high sugar content adds to the stability of the formulation (Muktar et al. 2018).

18.4.5 Tensile Strength

For use in clinical settings, dressings must have desirable mechanical properties by balancing between flexibility and rigidity. It should be durable, stress resistant, soft, flexible, pliable, and elastic. They should also be easy to apply and remove without causing additional trauma. To determine the brittleness or hardness of the dressing, tensile strength is measured which is the maximum stress applied to a point at which the film breaks. The addition of honey improves the elasticity of the dressing. The tensile strength also changes depending on the degree of hydrogen bonding interaction between the honey and the other components that varies with formulations. It also depends on the type and amount of the polymer and its molecular weight (Boateng et al. 2008; Thenmozhi et al. 2016).

18.4.6 Compressive Tests

Another way to characterize the mechanical properties of dressings is through performing compressive test. It measures the resistance of the formulation to deformation upon the application of compressive forces. This mechanical characterization is carried out using a device called the Instron Universal Mechanical machine at a cross-speed set at 10 nm/min (Boateng et al. 2008; Muktar et al. 2018). Varying results have been reported upon the addition of honey.

18.4.7 Bioadhesive Strength

As dressings are applied on a biological membrane, their adhesive property is to be determined. It is the force required to detach the sample from the surface of excised porcine skin. The determination of the degree of bioadhesion is especially important if the dressing is to be applied on a moist environment. It has been observed that bioadhesion depends on hydrophobicity, level of hydration, and rate of polymer erosion (Boateng et al. 2008).

18.5 Efficiency of Honey in Wound Care

As honey has been used to pack wounds since hundreds of years by our ancestors, it is becoming more crucial to present scientific evidence to support claims of its effectiveness in wound healing. Thus, many studies have been carried out to assess the various biological activities of honey. Following are the observed biological effects of honey that can explain its wound-healing property. The intensity of these effects in honey varies depending on the floral source. One of the most commonly researched types of honey is Manuka honey, which is collected by honey bees from the *Leptospermum scoparium* shrub, which is indigenous to New Zealand. However,

other beneficial types of honey have been proved to exhibit medicinal properties such as jelly bush honey, Acacia honey, Gelam honey, Tualang honey, ...etc.

18.5.1 Antibacterial Effects

After injury, if the wound is colonized with bacteria, it restrains the healing process. Microbes produce various enzymes into the wound including proteases, collagenases, and elastases which destroy the connective tissue and the growth factors that are necessary to rebuild the skin. The oxidative condition produced by the bacteria activates excessive proteases. The endotoxins from the bacteria produce inflammation in the skin constricting the capillaries and cutting off the blood supply which is highly required to commence the repairing process. Honey cleans the wound by killing the bacteria while protecting the connective tissue and, hence, speeds up the healing (Molan 2006).

So, honey is applied onto wounds due to its antibiotic effect. This is attributed to various components and the physical properties of honey.

A summary of these factors is given in Fig. 18.1. High sugar content in honey provides the epithelial cells with glycogen in order to form new skin that will cover the wound. Glucose is also beneficial to the phagocytes whose function is to destroy the bacterial cells and the dead skin cells. It also plays a part in the osmotic action as it drives the fluid away from the wound. This removes the fluids from the wound which is, otherwise, a favorable condition for bacterial growth, provides a moist interface of diluted honey, and avoids the adherence of the dressing to the wound, thus easing its removal. This hyperosmolar effect is due to the high sugar content which can also dehydrate the bacteria. Honey, even though it is moist, does not support any bacterial growth because of its antibacterial properties.

The disinfection properties are also assisted by the production of hydrogen peroxide by peroxidase enzymes which remain below the inflammatory level; this, in turn, stimulates various cells required for cell multiplication and wound healing such as fibroblasts and epithelial cells. Stimulation of protein-digesting enzymes,

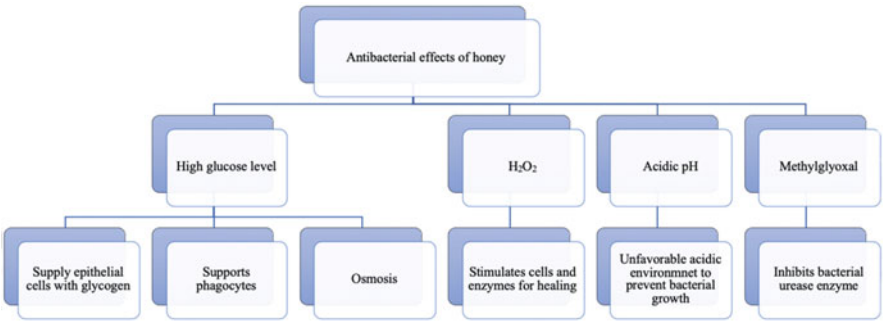

Fig. 18.1 Proposed mechanisms of antibacterial effects of honey

serine proteases and matrix metalloproteases, is also possible due to H_2O_2. The acidity of honey, which is pH 4.4, also contributes to the antibacterial effect and accelerates the healing. One phytochemical present in honey called methylglyoxal contributes to the antibacterial property through inhibition of urease enzyme which usually plays a role in bacterial growth. Bacteria not only invade the wound but also create a biofilm generating a highly resistant infection. Honey has the ability to penetrate the biofilm and inhibits its further growth, thus eradicating the infection. This is achieved by preventing the bacteria from binding with the skin at the wound, specifically the tissue fibronectin. Glucose and fructose play a major role in this activity (Ahmed et al. 2018).

18.5.2 Deodorizing Effects

Wounds are usually accompanied by foul odor. This is usually attributed to the presence of both anaerobic and aerobic bacteria in addition to the necrotic tissue and to a range of different metabolites produced by the bacteria. Hence, odor is frequently considered as an indicator for the occurrence of infection and bacterial colonization. Various potential solutions are used in odor management; ideally the dressings used to pack the wound should show control of wound-associated odors (Akhmetova et al. 2016).

The wound's malodor can be caused by the ammonia, amines, and sulfur compounds produced by the bacteria at the wound due to the metabolism of tissue proteins. Honey has the ability to rapidly neutralize the smell (Molan 2006; Yaghoobi et al. 2013; Akhmetova et al. 2016; Ahmed et al. 2018). This deodorizing activity is possible due to the antimicrobial effect of honey. Additionally, it provides the bacteria with an alternative source of nutrition which on digestion and metabolism produces lactic acid rather than the malodorous compounds. And as honey does not support bacterial proliferation due to its low pH and water content, and high sugar content, the infecting bacteria is eradicated with minimum foul smell with the application of honey (Akhmetova et al. 2016). The mechanism of honey's deodorizing effect is described in Fig. 18.2.

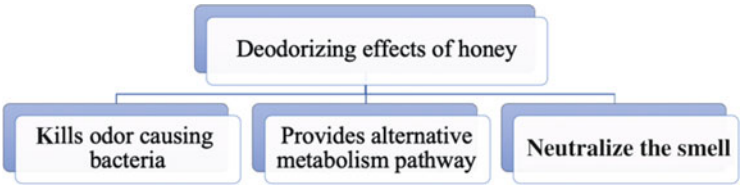

Fig. 18.2 Possible mechanisms of deodorizing effects of honey

18.5.3 Antioxidant Effects

Although oxygen is necessary for all body functions, its metabolism leads to the formation of free radicals. These free radicals can travel through cells and inflict molecular level. Such radicals are associated with various health problems and aging. In wounds, radicals also exacerbate cell damage and hinder tissue healing (Khalil et al. 2010). The process of oxidation caused by free radicals, superoxides, and other oxidants result in cellular damage and deterioration; thus some compounds are used, which can act as a defense against oxidative stress and are known as antioxidants.

Honey is found to have such a property as to be useful in promoting the closure and healing of wounds. The free radicals are formed at the site of injury during the inflammation process in the wound and produced by the respiratory mitochondrial chain. These radicals are known as reactive oxygen species (ROS). Any antioxidant compound implements its effect by producing ROS-reducing oxidative stress. Flavonoids, phenolic acids, and vitamin C in honey exert the antioxidant property which would mainly sequester the free radicals avoiding oxidative damage. It also stimulates the production of carbohydrates, proteins, lipids, and nucleic acids by the cells promoting the antioxidant response. Vitamin C can reduce peroxides. Honey also acts at the cellular level to reduce cellular damage by protecting antioxidant enzymes. Additional proposed mechanisms for the antioxidant behavior of honey include hydrogen donation, metallic ion chelation, flavonoids substrate action for hydroxyl, and superoxide radical actions (Yaghoobi et al. 2013; Ahmed et al. 2018). These are represented in Fig. 18.3.

18.5.4 Anti-inflammatory Effects

As a response to the injury, inflammation occurs in tissues in order to remove the foreign organisms. This is represented by redness, exudate, pain, and itching and if not treated, it can worsen chronic infection. This response is mediated by cytokines, cyclooxygenases, lipoxygenases, macrophages, TNF, ...etc. Thus, honey is

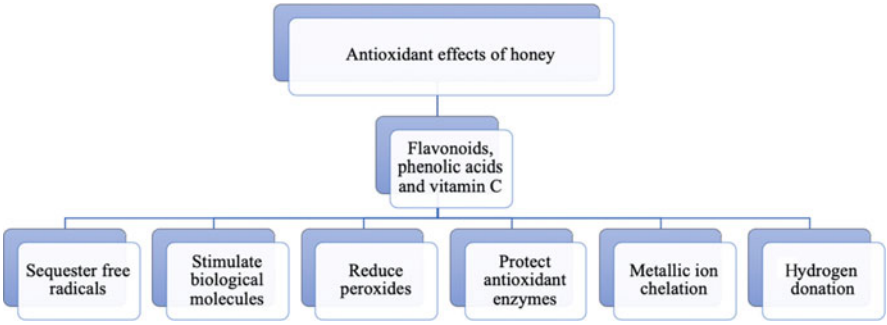

Fig. 18.3 Proposed mechanisms of antioxidant effects of honey

favorable in wound dressings as it can reduce the skin inflammation when applied to the wound. Honey has been shown to actively inhibit the expression of inflammatory mediators like cytokines. It also reduces the reactive oxygen species produced by the macrophages and leukocytes. The polyphenols are responsible for suppressing the lipoxygenases. Production of hydrogen peroxide can stimulate the growth of fibroblasts and epithelial cells. These are all proposed mechanisms for the anti-inflammatory property in honey although the exact mechanism is not completely understood (Ahmed et al. 2018).

Through the microscopic examination of skin, it was proven that inflammation started to subside after application of honey. The edema and exudates have been reduced, in addition to the soothing effect and pain relief produced after a while of applying honey onto the wound. The painfulness is decreased due to the reduction of pro-inflammatory prostaglandins and pressuring of the nerve endings.

Honey promotes the proliferation of new epithelial cells and the formation of connective tissue at the wound site, which may also lead to pain relief. Blistering and scarring of the skin upon injury have also been reduced due to honey. This has been attributed to a series of events including angiogenesis and growth factor formation that add to the fibroblasts and epithelial cells at the wound minimizing scarring. These cells can be destroyed by protease activity; however, the acidic property of honey inhibits this effect as it is not a favorable environment for the protease. Reducing inflammation removes a major obstacle in the healing process and speeds it up (Yaghoobi et al. 2013; Ahmed et al. 2018).

Honey contributes to improved wound healing by increasing collagen synthesis, fibroblast migration, and keratinocyte closure rate. This promotes the wound's ability to close, treating the injury (Minden-Birkenmaier and Bowlin 2018). The different antiinflammatory effects that honey offers are summarized in Fig. 18.4.

18.5.5 Debridement Effects

The persistence of necrotic tissue at the wound increases the risk of infection, especially for bacteria like methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*. So, necrotic tissue removal is a common procedure in the treatment of wounds and promotes wound healing. It consists of the removal of foreign matter, infected tissue, and slough from the wound, leaving the healthy tissue

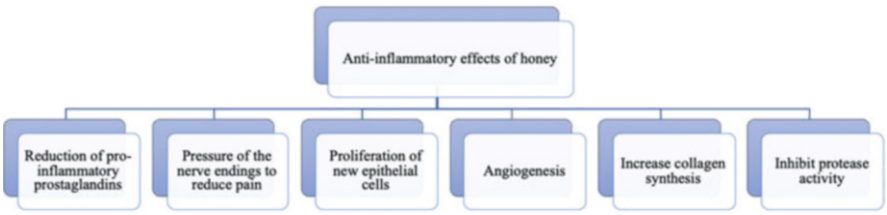

Fig. 18.4 Proposed mechanisms of antiinflammatory effects of honey

exposed. It is especially essential for patients that are frail, elderly, or have a weak immune system, so the first step is to identify any underlying diseases such as diabetes, heart, vascular or lung diseases, poor immune system, ...etc., that may be the reason for delay in wound healing. Then nonviable tissue needs to be removed along with a reduction in edema, exudate, and bacterial bioburden. This can be achieved by applying a dressing containing a molecule with debriding effect.

The debriding effect of honey has been reported in various studies where it has been shown that honey promotes autolytic debridement. It is a process in which the body naturally dissolves its own necrotic tissue by phagocytosis with the help of macrophages and lymphocytes. In order for this process to take place, moisture should be available at the wound interface. Here, honey is capable of pulling moisture out of the wound by its osmotic effect thus rehydrating the tissue. Then the fibrin that connects the necrotic tissue with the skin is digested and broken through the stimulation of plasmin. This process of autolysis makes honey a viable debriding agent (Mitchell 2018).

Many studies have shown the effect of honey on wounds in which patients achieved a faster rate of debridement when compared with other products like hydrogels. This has been achieved without the need for any surgical interventions for the removal of tissue. Detachment of the necrotic tissue from the skin occurred within 5–7 days, attributed to the activation of proteases; it is then removed by forceps (Molan 2009). Another study has shown that with medical grade honey patients achieved complete autolytic debridement in almost a month. It concluded that the use of honey is recommended when wounds contain more than 40% devitalized tissue that needs to be debrided rapidly. However, some pain can be associated with the increase in osmotic pressure causing some discomfort which may not be favorable to all patients (Evans and Mahoney 2013).

18.5.6 Antiviral Effects

Viral infections are ubiquitous around the world, and the search for new antiviral agents has become increasingly important due to the failure of some established agents typically due to the emergence of resistance. Furthermore, some agents are unsuitable for some patients due to immune deficiency, breastfeeding, the use of multiple medications, some serious side effects, high cost of medications, and many other reasons. Many studies have been performed to study the antiviral effect of naturally derived substances to achieve low cost with minimum toxic effects (Hashemipour et al. 2014).

Honey has been a natural compound that is found to demonstrate some antiviral properties, and different mechanisms have been proposed to explain this behavior. Some compounds found in honey are thought to be responsible for its antiviral effect. These include copper which is present in trace amounts, ascorbic acid, flavonoids, and hydrogen peroxide. These inactivate the virus and preventing its growth by inhibiting viral transcription and translation. It has also been found that honeybees have nitric oxide metabolites like nitrite and nitrate in the salivary glands.

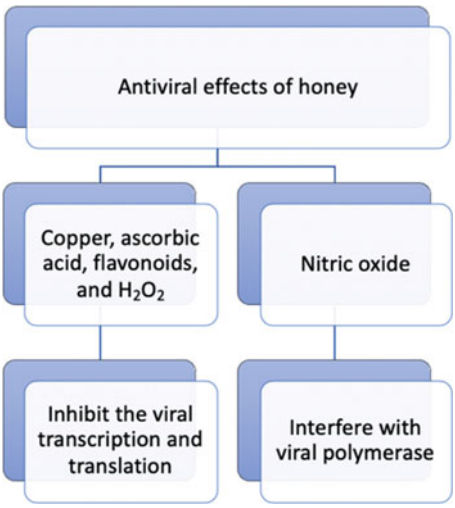

Fig. 18.5 Proposed mechanisms of antiviral effects of honey

The presence of nitric oxide also contributes to the antiviral activity in which it interferes with viral polymerase, nucleic acid, and/or viral capsid proteins, which would also further repress viral replication (Ahmed et al. 2018). A schematic presentation of these processes is given in Fig. 18.5.

The presence of antiviral properties in honey makes it helpful when packing wounds to prevent or eliminate any viral lesions formed or may form. Studies have shown honey to be an effective agent against herpes simplex virus, thus reducing the symptoms and signs of herpetic lesions (Miguel et al. 2017). Another study compared honey with acyclovir, a well-known antiviral, and found it to be effective in the topical treatment of recurrent lesions from genital and labial herpes (Yaghoobi et al. 2013). It was also concluded that honey can be as effective as 5% acyclovir in the management of herpes simplex labialis with regard to time to heal and pain resolution, making it an acceptable alternative to acyclovir cream (Semprini et al. 2019).

18.5.7 Antifungal Effects

The incidence of fungal infections is increasing in both community and hospital environments. Topical infections are a common form of fungal infections. However, due to the emergence of resistance in fungi against antifungal agents, researchers have been performing studies for new antifungals. Honey demonstrated antifungal activity against *Aspergillus niger*, *Aspergillus flavus*, *Penicillium chrysogenum*, *Microsporum gypseum*, *Candida albicans*, *Saccharomyces*, and *Malassezia* species (Irish et al. 2006; Moussa et al. 2012; Ahmed et al. 2018).

Honey can serve as an effective antifungal by disrupting the cellular structure and morphology of the fungi. This occurs by inducing changes to the exopolysaccharide

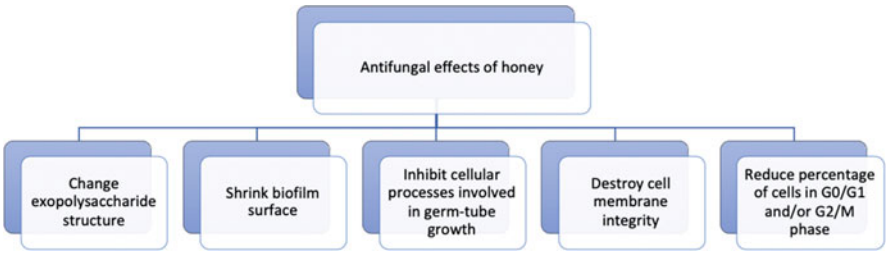

Fig. 18.6 Proposed mechanisms of antifungal effects of honey

structure which causes the disruption of cell membrane integrity and established biofilms, shrinking its surface and eventually leading to its death. It has also shown to have another direct effect on fungi membranes that would lead to the death of fungi and disruption of biofilms; the flavonoids in honey were found to inhibit some cellular processes that are involved in germ-tube growth that would cause poor growth of membranes. Flavonoids were also found to reduce the percentage of cells in G_0/G_1 and/or G_2/M phase (Ahmed et al. 2018). These effects are represented in Fig. 18.6.

A study performed to assess the topical application of honey in the management of seborrheic dermatitis and dandruff showed that patients receiving honey showed relief in scaling and itching within a week, and skin lesions healed within 2 weeks (Al-Waili 2001). When compared with steroid for the treatment of psoriasis and atopic dermatitis, honey proved to be effective and resulted in a 75% decrease in symptoms (Al-Waili 2003). Another study found that honey effectively treated 86% of patients with fungal infections characterized by rash, 78% with fungal infection of the groin, and 75% with fungal infection of arms and legs (Al-Waili 2004).

18.6 Clinical Applications

In addition to the nutritional value of honey, its therapeutic value has been illustrated since ancient cultures. As the biological activity of honey has been explored more rigorously over the years and its antibacterial and antioxidant effects have been proven, honey is being incorporated in various treatment regimens for managing different types of injuries. It is sometimes surpassing other widely used therapeutic agents such as silver sulfadiazine, povidone iodine, hydrogen peroxide, etc., to become the first-line therapy for wound management. Examples of such clinical applications are as follows:

18.6.1 Diabetic Foot Ulcer

An aggravated complication of diabetes is diabetic foot. About 15% of all diabetic patients are at risk of developing diabetic foot. It occurs due to the neuropathy that is

prevalent in most patients who experience a loss of sensory nerve in the periphery along with changes in peripheral blood vessels. With repetitive minor injuries that are left unattended due to loss in sensation, it turns into major ulceration which leaves the foot highly vulnerable to infection. It can be as serious as leading to amputation if neglected (Alexiadou and Doupis 2012).

Applying wound dressing is a part of managing diabetic foot ulcers; various dressings can be applied to accelerate the healing of injuries. This is essential as the healing process in diabetic patients is extremely slow. These wounds are characterized by severe hypoxia and high levels of reactive oxygen species which would only worsen the inflammation of the injury site. Honey, with its broad-spectrum bactericidal properties, is a type of effective dressing that can eradicate infection and promote healing in addition to stimulating the autolytic debridement by hydrogen peroxide production, where old dead tissue is removed allowing new tissue to be formed (Alam et al. 2014; Kateel et al. 2016; Wang et al. 2019).

Multiple studies have been performed to assess the effectiveness of honey dressings in wound healing rates, bacterial clearance, and debridement times in diabetic foot ulcers. Evidence showed removal of exudates and deodorization of the wound within the first week, reduction in inflammation within 10 days, and bacterial eradication within a month. Results also suggest a correlation between the use of honey dressing and the increase in wound healing, bacterial clearance rate, and a shorter debridement time. However, honey is ineffective with more serious injuries such as with exposed bone but can be used after revascularization (Moghazy et al. 2010; Wang et al. 2019).

18.6.2 Postray Amputation

Further complications can occur that is associated with diabetes foot ulceration. If the ulceration is left without attention and treatment, the condition aggravates to diabetic gangrene which in most cases involves the lower limbs. Such a severe injury increases the risk of mortality due to the lack of proper treatment. Usually, the toes are the first to get infected; then the infection spreads further to the joint followed by the metatarsal bones. This extensive spread of infection is untreatable where surgical intervention is the only solution involving the amputation of the affected bones. The amputation of the toes is known as ray amputation, which may include only the distal metatarsal head or the entire metatarsals. The former is known as partial ray amputation while the latter is called complete ray amputations. However, the recovery is a slow process which would increase the social, psychological, and economic burden on the patient. Thus, researchers have been studying different possible agents to be applied onto the wound to improve the healing process following the surgical procedure (Alam et al. 2014; Mohamed et al. 2014).

A study was performed by a group of scientists where honey was applied on the wound after amputation to accelerate the healing process. Initially the wound was macerated and cleaned by application of povidone-iodine in order to reduce the

bacterial burden as much as possible. Then the wound was dressed using honey. As per the results, honey achieved 60% healing rate within the first 2 weeks. The following week the healing rate reached up to 90% and after a month complete wound healing was observed. It was concluded that honey provided an effective alternative for the management of such wounds and is becoming more acceptable by patients as it is efficacious, aesthetically acceptable, and cost effective (Mohamed et al. 2014).

18.6.3 Burns

Burn is a very common skin injury which varies in degree and can result in a substantial loss of skin protection function. This makes the skin at risk of serious infection. If untreated, it can lead to serious deformities and disabilities. Slow healing can leave major scars that would affect the patient psychologically as well. Thus, burns need to be dressed with agents that will clear the infection and speed up the healing leaving minimum irregularities (Al-Waili et al. 2011; Gupta et al. 2011). As honey has been used by the ancient cultures like the Greeks, Romans, Chinese, Egyptians, ...etc., to pack burns, and it is still one of the most commonly used agents used in burn dressings (Smaropoulos et al. 2011).

Several studies performed proved the effectiveness of honey in superficial and partial thickness burns when compared with silver sulfadiazine; a common agent used in burns. This has been attributed to the antioxidant property of honey. Burns have been categorized as oxidative injuries where the free radicals increase at the injury site causing peroxidation, which is the reason for considerable scarring in the burned tissue. The application of honey reduces scarring and pigmentation along with reduction in pain and inflammation and promotes tissue granulation. This would reduce the healing time and the hospital stay, making the use of honey more cost-effective and thus favorable to many patients. However, the effectiveness of honey in full-thickness burns is limited and surpassed by tangential excision and skin grafting (Subrahmanyam 2007). The debriding effect of honey has also been found beneficial in burn treatment by promoting the dead tissue removal and fastening the formation of new ones (Molan and Rhodes 2015).

On assessing its effectiveness in burn patients, it was found that honey outperformed various formulations containing agents like polyurethane film, amniotic membrane, potato peel, and silver sulfadiazine according to studies performed in the UK (Moore et al. 2001), France (Al-Waili et al. 2011), and Netherlands (Postmes et al. 1997).

Another study performed in Pakistan to evaluate microbial eradication found that only 4% of the patients treated with honey returned with positive bacterial culture as opposed to 18% in patients treated with silver sulfadiazine.

In India, studies showed complete control of infection within a week when treated with honey in almost 90% of the study group and the appearance of new healthy tissue within 15 days in the majority of the patients (Al-Waili et al. 2011). However, some patients seemed to dislike the use of honey because of some pain caused on its

application and, therefore, preferred other formulations (Edwards 2013). Honey seemed to be beneficial in burn dressing in pediatrics as well, reducing granulation time and enhancing bacterial eradication (Bangroo et al. 2005).

18.6.4 Nonhealing Wounds

Patients suffering from diseases like diabetes mellitus, chronic venous insufficiency, peripheral artery disease, immobility, and cancer suffer from slow wound healing. The extended period of time for healing is a burden both financially and physically. Thus, a wet therapy is proposed in which the wound is dressed not only with gauze but with an agent that would reduce the long duration of healing. Honey is an inexpensive and favorable choice with its acceptable biological activities and physical properties.

Studies performed by applying honey onto the wounds of patients with concomitant diseases showed higher number of completely healed wounds, faster wound size reduction, less intense wound odor, and reduced wound pain intensity (Vyhlídalová et al. 2018; Zeleníková and Vyhlídalová 2019). It has also proven beneficial in treatment of wounds infected by antibiotic-resistant bacteria like methicillin-resistant *Staphylococcus aureus* (MRSA), where it is now considered one of the first-line treatments of such infections (Visavadia et al. 2008).

18.6.5 Pilonidal Sinus

Pilonidal sinus is a disease that occurs at the sacrococcygeal region, specifically at the hair follicles and more commonly in men than women. The patient suffers from a painful sinus tract along with chronic abscess. It causes severe discomfort and inability to perform normal activity (McCallum et al. 2008). It occurs due to excessive stretching and recurrent trauma to the follicles that enlarge and rupture, ending up with damaged tissues and hair follicles. As the inflamed area gets infected, treatment is required and the use of honey dressing is a plausible option (Woo et al. 2015). Research proves that the use of honey in recurrent or chronic pilonidal sinus is effective, showing early and rapid granulation tissue formations and wound closure as early as 2–5 weeks with complete wound healing within a period of 65 days (Thomas et al. 2011; Woo et al. 2015; Elhorbity et al. 2018).

18.6.6 Venous Leg Ulcers

Ulceration is a loss of the skin integrity, specifically the dermis layer and if it does not heal within the expected time or shows little tendency to heal, it is said to be chronic. A venous leg ulcer is a chronic ulceration in the lower limbs in which the inability of the veins to prevent backflow of the blood results in increasing the blood pressure in the leg veins. This causes the appearance of severe open wounds in the

leg that can become highly infected. Treatment of such ulceration includes compression therapy and the use of agents that can promote wound healing. Compressing on the ulceration accelerates venous flow and reduces venous reflux and edema. The emergence of bacteria in the wounded area causes tissue damage and delays healing. So, the addition of an effective antimicrobial agent in the dressing is also common in order to promote healing and fight infection. Honey wound dressing is found to be effective in these cases (Velasco 2011; Alavi et al. 2016).

Many studies have shown honey dressings are helpful to some degree in the treatment of venous leg ulcers. It has been found that honey dressing did not improve the rate of healing of the wound significantly or cause any reduction in the size of the ulceration after 12 weeks when compared with standard therapy. However, it did help in controlling the infection, thus reducing healing duration to some extent and most importantly reducing the cost of treatment as honey is a relatively inexpensive therapeutic agent. A conclusion is drawn that since honey is a promising wound-healing agent it has the potential to be used in the treatment of such chronic wounds. Nonetheless, research in this field is still limited, and additional trials need to be conducted to reach to more accurate conclusions (Jull et al. 2008; Mayer et al. 2014; Holland and Norris 2015).

18.7 Honey Formulations

18.7.1 Hydrogel

A hydrogel is a matrix of multiple crosslinked insoluble hydrophilic polymers as illustrated in Fig. 18.7. They form a highly absorbent network with a water content of up to 96%. When hydrogels are used to formulate wound dressings, these water molecules are used to maintain a moist environment. However, it is not fully

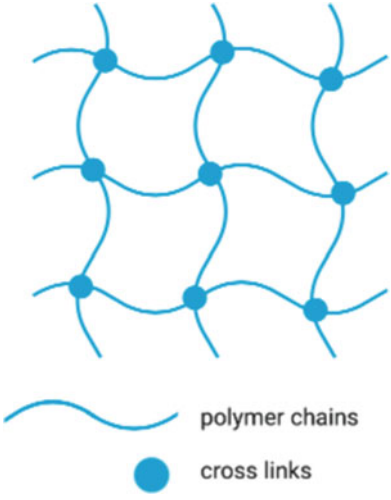

Fig. 18.7 Hydrogel structure

hydrated to make it capable of absorbing the exudate from the wound. Due to the intrinsic porosity of the hydrogel, it allows fluid and gas exchange but the degree of permeability varies with different formulations. It can also promote autolytic debridement as it supports the rehydration of nonviable tissues. Hydrogels may stimulate cell growth, migration, and maturation as it is found to mimic the structural functionality of the extracellular matrix (Jones et al. 2006; Saghazadeh et al. 2018).

Different compositions of hydrogel are available that can be either synthetic or naturally derived. Glycosaminoglycans, for example, enhance cell proliferation as they are the main component of skin tissue. Hyaluronic acid, when included in the hydrogel, improves its resilience along with cell differentiation. Collagen can also be helpful by improving the adherence property of the dressing. Chitosan is highly hydrophilic and improves cell adhesion, migration, growth, and differentiation. Additionally, it is more favorable nowadays due to its discovered bactericidal properties at higher concentrations (Saghazadeh et al. 2018).

Honey-based hydrogels have also been prepared and evaluated for wound dressing by incorporating honey into a mixture of polyvinyl pyrrolidone (PVP) 15% and polyethylene glycol (PEG) 1%, along with a 1% protein free agar solution, poured into a mold and covered by a polyethylene sheet. In order to promote the bactericidal effect of the wound dressing, it needs to be sterilized by gamma irradiation. Honey was found to improve the swelling of the dressing more significantly than hydrogels, supporting exudate absorption due to the honey's high osmolarity. Additionally, honey provided an earlier antiinflammatory and reparative response with a significant acceleration in dermal healing (Yusof et al. 2007; Mohd Zohdi et al. 2012).

It has also been reported that mixing honey with different hydrogel composites promotes the wound healing properties of the dressing. When chitosan and honey are mixed together at significantly lower concentration, a synergistic antibacterial effect is achieved. The presence of protonated positive ions allows the capture of bacteria by the hydrogel, leading to a loss in their physiological function (Wang et al. 2012). Honey added to poly(vinyl alcohol) and carboxymethylate chitosan also showed a significant inhibition in microbial growth (Afshari et al. 2015). Honey also adds to the tensile strength of the dressing; when added to an equal composition of chitosan and alginate, honey shows a good elongation strength property required for wound coverage. A combination with gelatin, although shows improved hydration and swelling property, does not possess desirable mechanical strength (Kurhade et al. 2013). Honey-based alginate hydrogel dressings also showed an acceleration in reepithelization and fibroblast growth making it another productive approach to support dermal wound healing (Nazeri et al. 2015). Many honey dressing products have been formulated using hydrogel techniques and have reached the market. Examples of such products are listed in Table 18.1.

18.7.2 Electrospun Nanofibers

The process of electrospinning produces fibers by passing the polymer solution that is charged using an electrical current through a syringe onto a metallic ground plate

Table 18.1 Marketed products of honey-containing hydrogel dressing

Product name	Type of honey	Description	Manufacturer	References
Manuka Health Wound Dressing with Manuka Honey	Manuka honey	Sheet of cross-linked polyacrylamide hydrogel–containing honey	Manuka Health NZ	Molan (2011)
Manuka Health Breast Pad with Manuka Honey	Manuka honey	Sheet of cross-linked polyacrylamide hydrogel–containing honey	Manuka Health NZ	Molan (2011)
L-Mesitran Hydro	30% medical grade honey	Sheet of acrylic polymer hydrogel–containing honey	Triticum	Halstead et al. (2015)
Gentell Honey Hydrogel	Manuka honey	Hydrogel-containing honey	Gentell	Schoukens (2009)

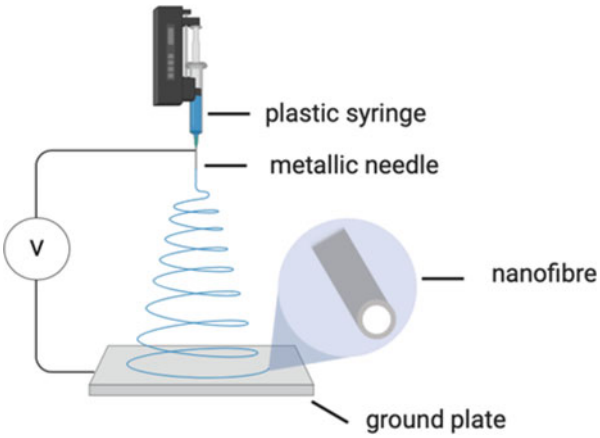

Fig. 18.8 Process of electrospinning

forming thin fibers with micro- to nanoscale diameters. The process is illustrated in Fig. 18.8. Such matrices are known to have a high surface-to-volume ratio with good porosity. These nanofibers are good candidates for wound dressing as they improve wound healing by the reduction of necrosis and enhancement of vascularization. Various polymers are used for the formulation of fibers including polycaprolactone (PCL), poly(L-lactic acid) (PLA), and poly(L-lactic acid-co-glycolic acid) (PLGA). The interconnected web allows better fluid absorption, and its porosity allows for drainage of the wound exudates while permitting atmospheric oxygen to enter (Maleki et al. 2013; Saghazadeh et al. 2018; Fahimirad and Ajalloueian 2019).

Honey can be electrospun with other polymers to produce nanofibers which are applicable for wound healing. Honey with poly(vinyl alcohol) gave a uniform and smooth nanofiber where honey can reach up to 40%. The dressing can be loaded with additional medicinal molecules, and the presence of honey gives an initial burst

release within a short duration of time (Maleki et al. 2013). Honey-loaded alginate/PVA nanofibrous membrane is another formulation that showed enhanced bactericidal effect against both gram-positive and gram-negative bacteria along with antioxidant activity. However, honey led to a decrease in swelling ratio in this formulation (Tang et al. 2019).

Silk fibroin are electrospun to form biocompatible scaffolds known for biocompatibility, mechanical integrity, and permeability to oxygen in addition to facilitating cellular attachment, migration, and proliferation. However, these scaffolds contain hydrophobic regions hindering the process of water retention. The addition of honey into the formulation improves cell adherence and swelling, proving that honey has the capability to augment moisture retention. It has also been shown that honey enhanced the release of growth factors for better wound healing (Kadakia et al. 2018). On studying the antibacterial effect of these silk fibrous matrices, it was proved to be highly efficient against both gram-positive and gram-negative bacteria, being stronger against *E. coli and P. aeruginosa* as compared with *S. aureus* and MRSA. The wound-healing rate also showed improvement with honey incorporation (Yang et al. 2017). Honey electrospun with poly(ε-caprolactone) (PCL) nanofibrous sheets showed similar results where researchers established their usefulness in promoting healing and clearing bacteria from the wound environment (Minden-birkenmaier et al. 2015).

18.7.3 Cryogels

The concept of tissue engineering has emerged as an alternative for skin and bone repair. It is concerned with the regeneration and replacement of these tissues, especially with large critical wounds where there has been a substantial loss in skin. The application of bioengineered scaffolds is now superseding the use of skin grafts for the purpose of skin repair. Cryogels are an example of such tissue-engineered scaffolds that are now widely applied in the field of skin healing and repair. Cryogel is a gel matrix formed by the polymerization and cross-linkage of different polymeric agents. After the polymerization process occurs, temperatures are brought down to below zero. The ice crystals will form pores within the structure due to subsequent thawing, leading to the production of a supermacroporous polymeric material, i.e., the cryogel structure. The process of cryogel formation is demonstrated in Fig. 18.9. The interconnected macropores in the cryogels are beneficial when working with viscous biological fluids, like honey, in which it can be incorporated into the cryogel (Hixon et al. 2017; Bakhshpour et al. 2019).

For the purpose of tissue engineering, cryogels could act as an artificial extracellular matrix. Cryogels can promote healing by providing support to the cells and stimulating cell migration and penetration to the surface including immune cells to initiate wound-healing processes. Cryogels are also known to be both biocompatible and biodegradable, and their characteristic macropores allow the absorption of high volume of tissue fluids (Bakhshpour et al. 2019). In one study, honey has been incorporated into the cryogel structure as it is an antimicrobial agent to form both silk

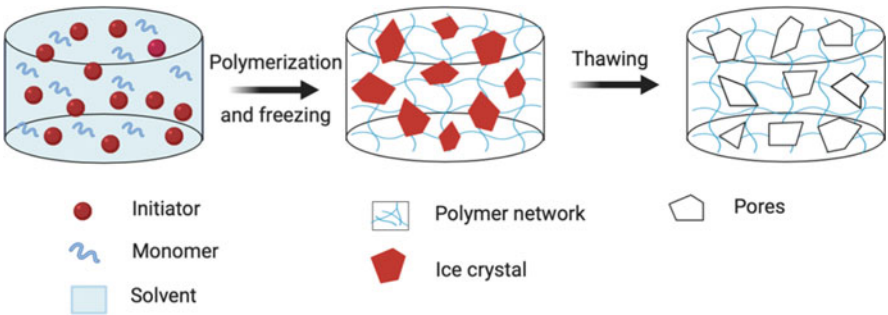

Fig. 18.9 Process of cryogelation

and gelatin cryogels. The silk ones were found to be of highest porosity, enabling better swelling property. Honey also reduced the peak stress in the cryogels making them more durable and resistant to fracture upon compression. The presence of honey, a thick substance, did not affect the cell proliferation and infiltration property of the cryogel while introducing an antimicrobial property against *Staphylococcus aureus*. The antimicrobial property was found to be achieved through the sustained release of glucose over time (Hixon et al. 2018).

Although honey improves cell infiltration and bacterial eradication, it causes a compromise to the mechanical stability of cryogels. This is a major limitation of the addition of honey to cryogels, which needs to be addressed in future studies. Additionally, there is a risk of losing part of the honey content during washing or sterilization, an issue that also needs consideration. One suggested solution is the use of a combination of polymers to achieve the desired mechanical strength (Minden-Birkenmaier and Bowlin 2018).

18.7.4 Honey-Impregnated Dressings

One of the most common traditional ways of wound care is the application of gauze on the wound. Such a conventional dressing can be made of woven or nonwoven gauze which is sterilized; when kept over the wound, it allows absorption of the exudate from the wound. However, this gauze does not provide a good barrier against infection by microbes present in the environment and is, thus, considered permeable to both fluids and bacteria. Furthermore, upon its removal it causes pain and leaves particulate matter in the wound (Khalique et al. 2014; Saghazadeh et al. 2018). The use of medicated gauze is an improved practice in the treatment of wounds, specifically medicated with molecules of antibacterial properties in order to reduce risk of infection. Honey is such a molecule as it exhibits antibacterial activity against a broad spectrum of bacteria including *Staphylococcus aureus* including MRSA, *Pseudomonas aeruginosa*, etc. (Kamaratos et al. 2014).

The honey-impregnated dressing can be prepared by mixing starch, glycerol, and water to form a starch-based gel and then honey is added to the mixture. A standard

Table 18.2 Marketed products of honey-impregnated dressing

Product name	Type of honey	Description	Manufacturer	References
Activon Tulle	Manuka honey	Nonadherent impregnated gauze dressing	Advancis Medical	Honeysett (n.d.); Molan (2011)
Actilite	Manuka honey and Manuka oil	Nonadherent impregnated gauze dressing	Advancis Medical	Morgan (2015)
MelMax	Buckwheat honey	Nonadherent impregnated gauze dressing; honey mixed with polyhydrated ionogens	Dermagenics	Hampton et al. (n.d.); Molan (2011)
MelDra	Buckwheat honey	Impregnated open-weave acetate fabric	Dermagenics	Sarheed et al. (2016); Molan (2011)
Algivon	Manuka honey	Impregnated alginate fiber dressing pad	Advancis Medical	Parker (n.d.); Molan (2011)
HoneySoft	Chilean multifloral honey	Impregnated polyvinylacetate dressing	Taureon	Molan (2011)

sterile gauze is dipped in the solution till saturation and then pressured to remove any excess solution. Finally, it is refrigerated to harden the formulation and then sealed in sterile packages (Zam et al. 2018). The presence of honey proved to be more beneficial than conventional dressings. In infants, it achieved healing of infected wounds within 7–21 days where the conventional dressing failed. In another study, honey achieved debridement within 1 week in almost all the patients, rendering the wounds dehydrated and odorless with new granulation tissue being formed, eliminating the need for surgical debridement (Mathews 2002). A honey-impregnated dressing also achieved healing in burns within almost 15 days (Subrahmanyam 1996). Diabetic patients also benefited from honey-impregnated dressings where almost three-thirds of the population showed complete healing and infection eradication (Kamaratos et al. 2014; Imran et al. 2015). Examples of dressings formulated as honey-impregnated dressings that have reached the market are mentioned in Table 18.2.

18.7.5 Hydrocolloids

A hydrocolloid is a combination of gel-forming agents like carboxymethylcellulose (CMC), pectin, gelatin, or an elastomer. These molecules are hydrophilic colloid particles in which their combination forms a film or a layer that is usually used to dress wounds. A representation of this structure is given in Fig. 18.10. They produce a dressing that is used as a barrier to be placed on the wound for several properties it provides like being absorbent, self-adhesive, and waterproof. When placed on the

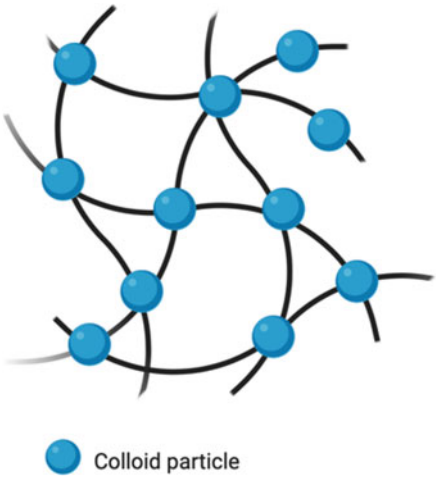

Fig. 18.10 Hydrocolloid structure

wound, the dressing shows a great ability to absorb exudates and swell into a gel-like mass. It offers a protective outer layer that can be either semiocclusive or occlusive against external environment like bacteria, foreign debris, and shearing while providing a moist healing environment. The presence of a hydrocolloid over the wound not only protects the wound but also accelerates the healing process as it stimulates autolytic debridement. It is noted that the wear time for a hydrocolloid dressing is usually up to 7 days, but it depends on the amount of exudates (Weller 2009; Sood et al. 2014).

Japanese researchers combined honey with a hydrocolloid dressing for the purpose of promoting reepithelialization, collagen deposition, and wound contraction as compared to hydrocolloid alone. It was illustrated that the use of honey decreased the wound area ratio but then it gradually increased followed by another decrease. The initial decrease was apparent in the inflammatory phase of the wound-healing process, while the increase was in the proliferative phase and the second decrease was in the remodeling phase. Thus, combining the Japanese honey with the hydrocolloid would avoid these repetitive phases of changes in the wound area; however, the wound healing would not be superior to that of the hydrocolloid dressing alone. A proposed solution was to alternate between the use of honey and hydrocolloid. The application of honey would be to reduce the inflammatory response induced at the wound site. Then this is switched to a hydrocolloid dressing to avoid reexpansion of the wound. Nevertheless, this hypothesis was nullified when it was clear that the reexpansion was unavoidable and the healing process was delayed (Nakajima et al. 2013; Mukai et al. 2015, 2017). More successful products using the hydrocolloid dressing in combination with honey have been formulated and have reached the market. Examples of such products are in Table 18.3.

Table 18.3 Marketed products of honey-containing hydrocolloid dressing

Product name	Type of honey	Description	Manufacturer	References
MANUKAhd	Manuka honey	Super-absorbent polyacrylic fiber coated with dry-touch absorbent hydrocolloid	ManukaMed	Saikaly and Khachemoune (2017)
MANUKAtex	Manuka honey	Nonadherent gauze coated with dry-touch absorbent hydrocolloid	ManukaMed	Saikaly and Khachemoune (2017)
Medihoney Honeycolloid	Manuka honey	Sheet of gelled honey	Dermasciences	Sarheed et al. (2016)

Fig. 18.11 Foam dressing structure

18.7.6 Foam Dressing

A foam dressing is made up of a hydrophilic or hydrophobic material. It forms an outer protective layer on the wound which provides thermal insulation while allowing vapor and oxygen exchange. Usually a foam dressing is composed of polyurethane or silicone foam. The former is generally designed as a multilayer where a hydrophilic layer is kept in contact with the wound surface to facilitate absorption with a hydrophobic backing above it. This facilitates the absorption of large quantities of wound discharge without any leakage to the exterior. The interior of the dressing is composed of small pieces of polyurethane surrounded by a polymeric film. The silicone-based dressing, on the other hand, is a mixture of two different elastomers which when combined produces a film that can expand to mold the wound shape. Foam dressings are characterized by their large interconnected pores as demonstrated in Fig. 18.11, which is supportive of cell infiltration, migration, and signaling. Their high absorptive property makes them good candidates for application on different leg ulcers (Jones et al. 2006; Weller 2009; Dhivya et al. 2015; Saghazadeh et al. 2018).

Honey has been investigated for the removal of necrotic tissues when applied on several cases of challenging wounds with the help of a foam dressing. After application the debridement effect was recorded in multiple patients where it was found that there was almost 75% increase in granulation tissue within 2 weeks of use with no side effects to be noticed. This would contribute to the success in wound healing for better patient care (Gray and Ishii 2015). In another study with less severe conditions, healing and exudate removal was observed after 2 weeks and

Table 18.4 Marketed products of honey foam dressing

Product name	Type of honey	Description	Manufacturer	References
Therahoney foam flex	Manuka honey	Dual-layer dressing; honey as primary layer and foam as secondary layer	Medline Industries	Saikaly and Khachemoune (2017)
Manuka Foam Air	Manuka honey	Non-adherent dressing of absorbent foam-fiber hybrid material	Links Medical Products	Saikaly and Khachemoune (2017)

reepithelization happened after 4 weeks (Robson 2004). Several pharmaceutical companies recognized the benefit of honey foam dressings and designed such products; some examples are listed in Table 18.4.

18.8 Conclusion

Despite the presence of numerous active molecules with desired properties for wound healing, research is now more directed toward benefiting from naturally driven agents. This is due to the increased resistance in all types of microorganisms against the recurrently used agents. It is also believed that utilizing natural products would make the product more cost-effective with fewer side effects. Honey is one of the common natural products that has been historically used by previous generations to treat wounds and injuries. It has reemerged in the clinical field for this application, and countless studies have been performed to explain its benefit and prove its effectiveness in the aspect of wound healing. It has been proven to have antibacterial, antioxidant, antiinflammatory, antiviral, antifungal, and debridement effects, which all contribute to accelerating wound closure and avoiding complications. In the market, many formulations have reached the shelves where honey was incorporated in wound dressings using different techniques. Studies are still on going in this field to optimize new formulations which can improve the healing rate further with lesser application times that can be more convenient and cheaper for the consumer.

References

Afshari MJ, Sheikh N, Afarideh H (2015) PVA/CM-chitosan/honey hydrogels prepared by using the combined technique of irradiation followed by freeze-thawing. Radiat Phys Chem 113:28–35. https://doi.org/10.1016/j.radphyschem.2015.04.023

Agrawal A, Purwar R (2018) Swelling and drug release kinetics of composite wound dressing. Indian J Fibre Text Res 43:104–111

Ahmed S, Sulaiman SA, Baig AA et al (2018) Honey as a potential natural antioxidant medicine: an insight into its molecular mechanisms of action. Oxidative Med Cell Longev 2018:8367846. https://doi.org/10.1155/2018/8367846

Akhmetova A, Saliev T, Allan IU et al (2016) A comprehensive review of topical odor-controlling treatment options for chronic wounds. J Wound Ostomy Cont Nurs 43:598–609. https://doi.org/10.1097/WON.0000000000000273

Alam F, Islam MA, Gan SH, Khalil MI (2014) honey: a potential therapeutic agent for managing diabetic wounds. Evid Based Complement Alternat Med 2014:169130. https://doi.org/10.1155/2014/169130

Alavi A, Sibbald RG, Phillips TJ et al (2016) What's new: management of venous leg ulcers treating venous leg ulcers. J Am Acad Dermatol 74:643–664. https://doi.org/10.1016/j.jaad.2015.03.059

Alexiadou K, Doupis J (2012) Management of diabetic foot ulcers. Diabetes Ther 3:1–15. https://doi.org/10.1007/s13300-012-0004-9

Al-Waili NS (2001) Therapeutic and prophylactic effects of crude honey on chronic seborrheic dermatitis and dandruff. Eur J Med Res 6:306–308

Al-Waili NS (2003) Topical application of natural honey, beeswax and olive oil mixture for atopic dermatitis or psoriasis: partially controlled, single-blinded study. Complement Ther Med 11:226–234. https://doi.org/10.1016/S0965-2299(03)00120-1

Al-Waili N (2004) An alternative treatment for pityriasis versicolor, tinea cruris, tinea corporis and tinea faciei with topical application of honey, olive oil and beeswax mixture: an open pilot study. Complement Ther Med 12:45–47. https://doi.org/10.1016/j.ctim.2004.01.002

Al-Waili NS, Salom K, Al-Ghamdi AA (2011) Honey for wound healing, ulcers, and burns; data supporting its use in clinical practice. Sci World J 11:766–787. https://doi.org/10.1100/tsw.2011.78

Bakhshpour M, Idil N, Perçin I, Denizli A (2019) Biomedical applications of polymeric cryogels. Appl Sci 9:1–22. https://doi.org/10.3390/app9030553

Bangroo AK, Khatri R, Chauhan S (2005) Honey dressing in pediatric burns. J Indian Assoc Pediatr Surg 10:172–175. https://doi.org/10.4103/0971-9261.16970

Boateng JS, Matthews KH, Stevens HNE, Eccleston GM (2008) Wound healing dressings and drug delivery systems: a review. J Pharm Sci 97:2892–2923. https://doi.org/10.1002/jps.21210

Demidova-Rice TN, Hamblin MR, Herman IM (2012) Acute and impaired wound healing: pathophysiology and current methods for drug delivery, part 2: role of growth factors in normal and pathological wound healing: therapeutic potential and methods of delivery. Adv Skin Wound Care 25:304–314. https://doi.org/10.1097/01.ASW.0000416006.55218.d0.Acute

Dhivya S, Padma VV, Santhini E (2015) Wound dressings—a review. Biomedicine 5:22. https://doi.org/10.7603/s40681-015-0022-9

Edwards J (2013) A prospective evaluation of the use of honey dressings to manage burn wounds. Wounds UK 9(4):102–106

Elhorbity MA, Gertallah LM, Mansour MI et al (2018) Food bee honey versus conventional antiseptic in local management of acute infected wounds. J Surg Emerg Med 2:5

Eteraf-Oskouei T, Najafi M (2013) Traditional and modern uses of natural honey in human diseases: a review. Iran J Basic Med Sci 16:731–742. https://doi.org/10.22038/ijbms.2013.988

Evans J, Mahoney K (2013) Efficacy of medical-grade honey as an autolytic debridement agent. Wounds UK 9:30–36

Fahimirad S, Ajalloueian F (2019) Naturally-derived electrospun wound dressings for target delivery of bio-active agents. Int J Pharm 566:307–328. https://doi.org/10.1016/j.ijpharm.2019.05.053

Gray C, Ishii F (2015) Using active leptospermum honey in the debridement process: 6 challenging cases from the inner city. Ostomy Wound Manage 61:63–66

Gupta S, Singh O, Bhagel P et al (2011) Honey dressing versus silver sulfadiazene dressing for wound healing in burn patients: a retrospective study. J Cutan Aesthet Surg 4:183. https://doi.org/10.4103/0974-2077.91249

Halstead FD, Rauf M, Bamford A et al (2015) Antimicrobial dressings: comparison of the ability of a panel of dressings to prevent biofilm formation by key burn wound pathogens. Burns 41:1683–1694. https://doi.org/10.1016/j.burns.2015.06.005

Hampton S, Kerr A, Bree-Aslan C (n.d.) An observational study of a honey impregnated dressing (MelMax®) in the treatment of wounds

Hashemipour MA, Tavakolineghad Z, Arabzadeh SA, Iranmanesh ZNS (2014) Antiviral activities of honey, Royal Jelly, and acyclovir against HSV-1. Wounds 26:47–54

Hixon KR, Lu T, McBride-Gagyi SH et al (2017) A comparison of tissue engineering scaffolds incorporated with Manuka honey of varying UMF. Biomed Res Int 2017:4843065. https://doi.org/10.1155/2017/4843065

Hixon KR, Lu T, Carletta MN et al (2018) A preliminary in vitro evaluation of the bioactive potential of cryogel scaffolds incorporated with Manuka honey for the treatment of chronic bone infections. J Biomed Mater Res-Part B Appl Biomater 106:1918–1933. https://doi.org/10.1002/jbm.b.34002

Holland LC, Norris JM (2015) Medical grade honey in the management of chronic venous leg ulcers. Int J Surg 20:17–20. https://doi.org/10.1016/j.ijsu.2015.05.048

Honeysett J (n.d.) Activon® Tulle in the treatment & prevention of MRSA, an effective treatment for chronic wound infections

Ibrahim N'I, Wong SK, Mohamed IN et al (2018) Wound healing properties of selected natural products. Int J Environ Res Public Health 15:2360. https://doi.org/10.3390/ijerph15112360

Imran M, Hussain MB, Baig M (2015) A randomized, controlled clinical trial of honey-impregnated dressing for treating diabetic foot ulcer. J Coll Physicians Surg Pak 25:721–725. https://doi.org/10.2015/JCPSP.721725

Irish J, Carter DA, Shokohi T, Blair SE (2006) Honey has an antifungal effect against Candida species. Med Mycol 44:289–291. https://doi.org/10.1080/13693780500417037

Jones V, Grey JE, Harding KG (2006) ABC of wound healing: wound dressings. Br Med J 332:777–780

Jull A, Walker N, Parag V et al (2008) Randomized clinical trial of honey-impregnated dressings for venous leg ulcers. Br J Surg 95:175–182. https://doi.org/10.1002/bjs.6059

Kadakia PU, Kalaf EAG, Dunn AJ et al (2018) Comparison of silk fibroin electrospun scaffolds with poloxamer and honey additives for burn wound applications. J Bioact Compat Polym 33 (1):79–94. https://doi.org/10.1177/0883911517710664

Kamaratos AV, Tzirogiannis KN, Iraklianou SA et al (2014) Manuka honey-impregnated dressings in the treatment of neuropathic diabetic foot ulcers. Int Wound J 11:259–263. https://doi.org/10.1111/j.1742-481X.2012.01082.x

Kateel R, Adhikari P, Augustine AJ, Ullal S (2016) Topical honey for the treatment of diabetic foot ulcer: a systematic review. Complement Ther Clin Pract 24:130–133. https://doi.org/10.1016/j.ctcp.2016.06.003

Khalil MI, Sulaiman SA, Boukraa L (2010) Antioxidant properties of honey and its role in preventing health disorder. Open Nutraceuticals J 3:6–16. https://doi.org/10.2174/1876396001003010006

Khalique MS, Khalique AB, Shukr I (2014) Comparison of hydrocolloid with conventional gauze dressing in prevention of wound infection after clean surgical procedures. Pak Armed Forces Med J 64:439–442

Kurhade S, Momin M, Khanekar P, Mhatre S (2013) Novel biocompatible honey hydrogel wound healing sponge for chronic ulcers. Int J Drug Deliv 5:353–361

Maleki H, Gharehaghaji AA, Dijkstra PJ (2013) A novel honey-based nanofibrous scaffold for wound dressing application. J Appl Polym Sci 127:4086–4092. https://doi.org/10.1002/app.37601

Mathews KABA (2002) Wound management using honey. Compendium 24:53–59

Mayer A, Slezak V, Takac P et al (2014) Treatment of non-healing leg ulcers with honeydew honey. J Tissue Viability 23:94–97. https://doi.org/10.1016/j.jtv.2014.08.001

McCallum IJD, King PM, Bruce J (2008) Healing by primary closure versus open healing after surgery for pilonidal sinus: systematic review and meta-analysis. BMJ 336:868–871. https://doi.org/10.1136/bmj.39517.808160.BE

Miguel MG, Antunes MD, Faleiro ML (2017) Honey as a complementary medicine. Integr Med Insights 12:1–15. https://doi.org/10.1177/1178633717702869
Minden-Birkenmaier BA, Bowlin GL (2018) Honey-based templates in wound healing and tissue engineering. Bioengineering 5:46. https://doi.org/10.3390/bioengineering5020046
Minden-Birkenmaier BA, Neuhalfen RM, Janowiak BE, Sell SA (2015) Preliminary investigation and characterization of electrospun polycaprolactone and Manuka honey scaffolds for dermal repair. J Eng Fiber Fab 10:126–138. https://doi.org/10.1177/155892501501000406
Mitchell T (2018) Use of Manuka honey for autolytic debridement in necrotic and sloughy wounds. J Community Nurs 32:38
Moghazy AM, Shams ME, Adly OA et al (2010) The clinical and cost effectiveness of bee honey dressing in the treatment of diabetic foot ulcers. Diabetes Res Clin Pract 89:276–281. https://doi.org/10.1016/j.diabres.2010.05.021
Mohamed H, Abu Salma M, Al Lenjawi B et al (2014) Enhancing primary healing post ray amputation in a diabetic patient: efficacy of natural honey. J Diabet Foot Complicat 6:13–18
Mohd Zohdi R, Abu Bakar Zakaria Z, Yusof N et al (2012) Gelam (Melaleuca spp.) honey-based hydrogel as burn wound dressing. Evid Based Complement Alternat Med 2012:843025. https://doi.org/10.1155/2012/843025
Molan PC (2006) Using honey in wound care. Int J Clin Aromather 3:21–24
Molan PC (2009) Debridement of wounds with honey. J Wound Technol 5:12–15
Molan P (2011) The evidence and the rationale for the use of honey as a wound dressing. Wound Pract Res 19:204–220
Molan P, Rhodes T (2015) Honey: a biologic wound dressing. Wounds 27:141–151
Moore OA, Smith LA, Campbell F et al (2001) Systematic review of the use of honey as a wound dressing. BMC Complement Altern Med 1:2. https://doi.org/10.1186/1472-6882-1-2
Morgan T (2015) The use of Actilite Protect® as an all-round wound care solution. J Community Nurs 29:56–61
Moussa A, Noureddine D, Saad A et al (2012) Antifungal activity of four honeys of different types from Algeria against pathogenic yeast: Candida albicans and Rhodotorula sp. Asian Pac J Trop Biomed 2:554–557. https://doi.org/10.1016/S2221-1691(12)60096-3
Mukai K, Koike M, Nakamura S et al (2015) Evaluation of the effects of a combination of Japanese honey and hydrocolloid dressing on cutaneous wound healing in male mice. Evid Based Complement Alternat Med 2015:910605. https://doi.org/10.1155/2015/910605
Mukai K, Komatsu E, Yamanishi M et al (2017) Effectiveness of changing the application of Japanese honey to a hydrocolloid dressing in between the inflammatory and proliferative phases on cutaneous wound healing in male mice. Wound Compend Clin Res Pract 29:1–9
Muktar MZ, Ismail WIW, Razak SIA et al (2018) Accelerated wound healing of physically cross linked Gellan gum-virgin coconut oil hydrogel containing Manuka honey. ASM Sci J 11:166–182
Nakajima Y, Nakano Y, Fuwano S et al (2013) Effects of three types of Japanese honey on full-thickness wound in mice. Evid Based Complement Alternat Med 2013:1–11. https://doi.org/10.1155/2013/504537
Nazeri S, Ardakani EM, Babavalian H, Latifi AM (2015) Evaluation of effectiveness of honey-based alginate hydrogel on wound healing in rat model. J Appl Biotechnol Rep 2:293–297
Parker J (n.d.) Debridement of chronic leg ulcers with Algivon®
Postmes TJ, Bosch MMC, Dutrieux R et al (1997) Speeding up the healing of burns with honey. In: Bee products. Springer, Boston, MA, pp 57–63
Robson V (2004) Use of Leptospermum honey in chronic wound management. J Community Nurs 18:24–28. https://doi.org/10.1201/9780203494240.ch19
Roy N, Saha N, Kitano T, Saha P (2010) Development and characterization of novel medicated hydrogels for wound dressing. Soft Mater 8:130–148. https://doi.org/10.1080/15394451003756282

Saghazadeh S, Rinoldi C, Schot M et al (2018) Drug delivery systems and materials for wound healing applications. Adv Drug Deliv Rev 127:138–166. https://doi.org/10.1016/j.addr.2018.04.008

Saikaly SK, Khachemoune A (2017) Honey and wound healing: an update. Am J Clin Dermatol 18:237–251. https://doi.org/10.1007/s40257-016-0247-8

Sarheed O, Ahmed A, Shouqair D, Boateng J (2016) Antimicrobial dressings for improving wound healing. In: Wound healing-new insights into ancient challenges. InTech, London, p 13

Schoukens G (2009) Bioactive dressings to promote wound healing. In: Advanced textiles for wound care. Elsevier, pp 114–152

Semprini A, Singer J, Braithwaite I et al (2019) Kanuka honey versus aciclovir for the topical treatment of herpes simplex labialis: a randomised controlled trial. BMJ Open 9:1–9. https://doi.org/10.1136/bmjopen-2018-026201

Smaropoulos E, Romeos S, Dimitriadou C (2011) Honey-based therapy for paediatric burns and dermal trauma compared to standard hospital protocol. Wounds UK 7:33–40

Sood A, Granick MS, Tomaselli NL (2014) Wound dressings and comparative effectiveness data. Adv Wound Care 3:511–529. https://doi.org/10.1089/wound.2012.0401

Subrahmanyam M (1996) Honey dressing versus boiled potato peel in the treatment of burns: a prospective randomized study. Burns 22:491–493. https://doi.org/10.1016/0305-4179(96)00007-1

Subrahmanyam M (2007) Topical application of honey for burn wound treatment—an overview. Ann Burns Fire Disasters 20:137–139

Tang Y, Lan X, Liang C et al (2019) Honey loaded alginate/PVA nanofibrous membrane as potential bioactive wound dressing. Carbohydr Polym 219:113–120. https://doi.org/10.1016/j.carbpol.2019.05.004

Thenmozhi R, Rathinamoorthy R, Thilagavathi G (2016) Optimisation of chitosan-honey composite film for wound dressing application. Indian J Chem Technol 23:279–288

Thomas M, Hamdan M, Hailes S, Walker M (2011) Manuka honey as an effective treatment for chronic pilonidal sinus wounds. J Wound Care 20:528–533. https://doi.org/10.12968/jowc.2011.20.11.528

Velasco M (2011) Diagnostic and treatment of leg ulcers. Actas Dermosifiliogr 102:780–790. https://doi.org/10.1016/j.adengl.2012.01.008

Visavadia BG, Honeysett J, Danford MH (2008) Manuka honey dressing: an effective treatment for chronic wound infections. Br J Oral Maxillofac Surg 46:55–56. https://doi.org/10.1016/j.bjoms.2006.09.013

Vyhlídalová D, Kozáková R, Zeleníková R (2018) Management of non-healing wounds with honey dressings: a literature review. Cent Eur J Nurs Midwifery 9:880–888. https://doi.org/10.15452/CEJNM.2018.09.0019

Wang T, Zhu XK, Xue XT, Wu DY (2012) Hydrogel sheets of chitosan, honey and gelatin as burn wound dressings. Carbohydr Polym 88:75–83. https://doi.org/10.1016/j.carbpol.2011.11.069

Wang C, Guo M, Zhang N, Wang G (2019) Effectiveness of honey dressing in the treatment of diabetic foot ulcers: a systematic review and meta-analysis. Complement Ther Clin Pract 34:123–131. https://doi.org/10.1016/j.ctcp.2018.09.004

Weller C (2009) Interactive dressings and their role in moist wound management. In: Advanced textiles for wound care. Elsevier, Amsteram, pp 97–113

Woo KY, Kwong EWY, Jimenez C, Bishop R (2015) Topical agents and dressings for pilonidal sinus wound healing by secondary intention: a scoping review. Surg Technol Int 26:57–63

Yaghoobi R, Kazerouni A, Kazerouni O (2013) Evidence for clinical use of honey in wound healing as an anti-bacterial, anti-inflammatory anti-oxidant and anti-viral agent: a review. Jundishapur J Nat Pharm Prod 8:100–104. https://doi.org/10.17795/jjnpp-9487

Yang X, Fan L, Ma L et al (2017) Green electrospun Manuka honey/silk fibroin fibrous matrices as potential wound dressing. Mater Des 119:76–84. https://doi.org/10.1016/j.matdes.2017.01.023

Yusof N, Ainul Hafiza AH, Zohdi RM, Bakar MZA (2007) Development of honey hydrogel dressing for enhanced wound healing. Radiat Phys Chem 76:1767–1770. https://doi.org/10.1016/j.radphyschem.2007.02.107

Zam W, Harfouch R, Ali R et al (2018) Natural extracts and honey based impregnated gauze wound dressing preparation and in vitro antibacterial efficacy. Res J Pharmacogn Phytochem 10:1. https://doi.org/10.5958/0975-4385.2018.00001.8

Zeleníková R, Vyhlídalová D (2019) Applying honey dressings to non-healing wounds in elderly persons receiving home care. J Tissue Viability 28:139–143. https://doi.org/10.1016/j.jtv.2019.04.002

Chrysin, an Important Active Ingredient of Honey: Beneficial Pharmacological Activities and Molecular Mechanism of Action

19

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Abstract

In recent years, public and scientific interest in plant flavonoids has tremendously increased because of their postulated health benefits. Flavonoids are found primarily throughout the plant kingdom and are synthesized as secondary phenylalanine metabolites in plants. This chapter was mainly focused on the flavone chrysin (5,7-dihydroxyflavone), which occurs naturally in many plants, honey, and propolis. Chrysin has been known to have various pharmacological activities, including antioxidant, anti-inflammatory, anticancer, and antiviral activities through inhibiting multiple pathways.

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Keywords

Flavonoids · Chrysin · Pharmacological activities · Phenylalanine · 5,7-Dihydroxyflavone · Antioxidant

19.1 Introduction

Prehistoric evidence has demonstrated that humans use natural ingredients to cure different symptoms and even infectious and cancerous diseases (Dias et al. 2012). The use of natural products for medicinal purposes has a rich history. Such practices go back thousands of years from Sumerians, Akkadians, India, and China. Previously thought of as "traditional medicine" used by native or ancient cultures, herbal medicine has arisen as a common supplement or addition to conventional medicine (Rehman et al. 2018). Natural products historically used for treatment that include whole or portions of organisms such as plants (flowers, stems, or roots), animal products (glands or organs), microorganisms, or inorganic salts without thorough processing (Pan et al. 2013). The production and use of natural products for human well-being have developed and grown through the years by trial and error method (Cragg and Newman 2013). Based on the current statistics of the World Health Organization, approximately 70% of the global population are utilizing traditional medicine for certain type of primary health care (Ekor 2014). Botanical dietary supplements alone are projected to surpass $3 billion a year in the United States (Bailey 2020). The rich history of bioactive compounds used in medicine and the confirmation of their structural nature should be viewed as unsurprising because coevolution of flora and fauna has resulted in similarity with the building blocks inherent in both organisms (Hu et al. 2015). Natural products have structural diversity throughout many species in the world and therefore share comparable structural domains with biological targets. Phytochemicals can therefore fit in perfectly throughout the chemical area of target proteins, e.g., interaction with targets in human signal transduction pathways (Yi et al. 2018).

19.2 Chemistry and Occurrence

Flavonoids are secondary metabolites in plants. It has extensive class of polyphenolic compounds having a benzo–γ–pyrone complex with immense biological activities (Pietta 2000; Pasini et al. 2013). They are synthesized by phenylpropanoids pathway and are responsible for many pharmacological activities (Tian et al. 2014). Among the flavonoids, a hydroxylated flavone derivative is known as chrysin. Chrysin is mainly found in honey, propolis, and some plants (Hadjmohammadi et al. 2010; De Vries et al. 1997). Flavonoids are polyphenols with patterns of hydroxylation and when substituted it gives rise to various groups such as flavanones, anthocyanins, flavonols, flavones, isoflavonoids, and chalcones (Fig. 19.1).

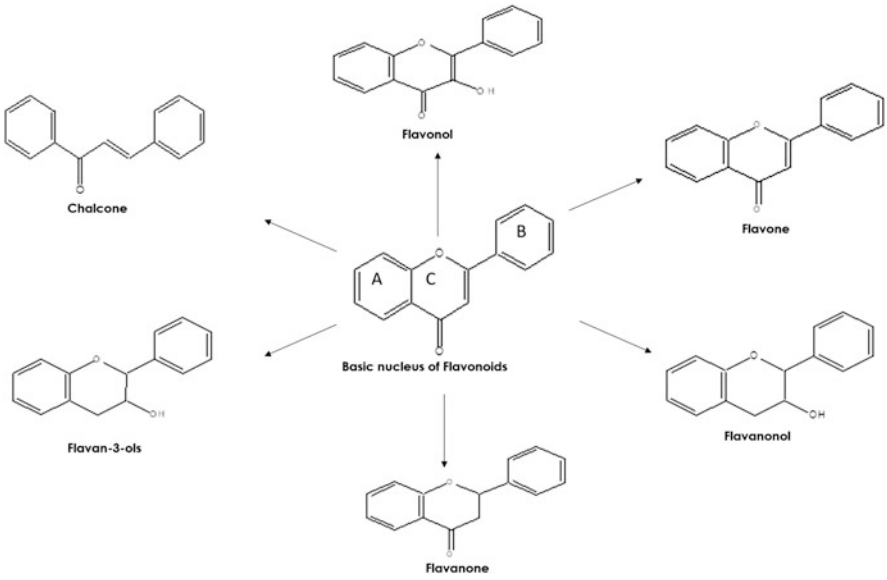

Fig. 19.1 Classification of flavonoids

Flavonoids have varied biological activities including but not limited to antiulcer, antioxidant, anti-atherosclerotic, antibacterial, anti-inflammatory, hepatoprotective, and anticancer, etc. (Beecher 2003; Mercan et al. 2006). They are also inhibitors for many enzymes, for example, cyclooxygenase, xanthine oxidase, phosphoinositide 3-kinase, and lipoxygenase (Walker et al. 2000; Panche et al. 2016). The functions of flavonoids in various bioactivities are shown in Fig. 19.2.

Chemically flavonoids were categorized on the basis of a 15-carbon structure composed of two benzene rings A and B as shown in Fig. 19.3a are attached by a heterocyclic pyrane ring (C) (Kumar and Pandey 2013). Chrysin (5,7-dihydroxyflavone or 5,7-dihydroxy-2-phenyl-4Hchromen-4-one) (Fig. 19.3b) is a naturally occurring flavone, found abundantly in plant extracts, honey, and bee propolis and many plant species. The IUPAC name used for chrysin is 5,7-dihydroxy-2-phenyl-4H-chromen-4-one (3) and with the chemical formula $C_{15}H_{10}O_4$ (Siddiqui et al. 2018). Chrysin has a characteristic feature which is the presence of double bond at C2–C3 in ring C and the lack of oxygenation at C-3. Chrysin, a dihydroxy flavone has a double bond between C-2 and C-3, and the ring B will be coplanar with the rings A and C due to conjugation. The exact mechanism for antioxidants always seemed to be correlated with the A-ring hydroxyl groups, although the influence of the A-ring on antioxidant activity is perhaps not clear. In addition, the C2–C3 double bond in carbonyl containing the C ring and the two hydroxyl groups in positions 3 and 4 in the B-ring are widely known as important structural patterns for the biological activities of flavonoids (Seetharaman et al. 2017; Naz et al. 2019).

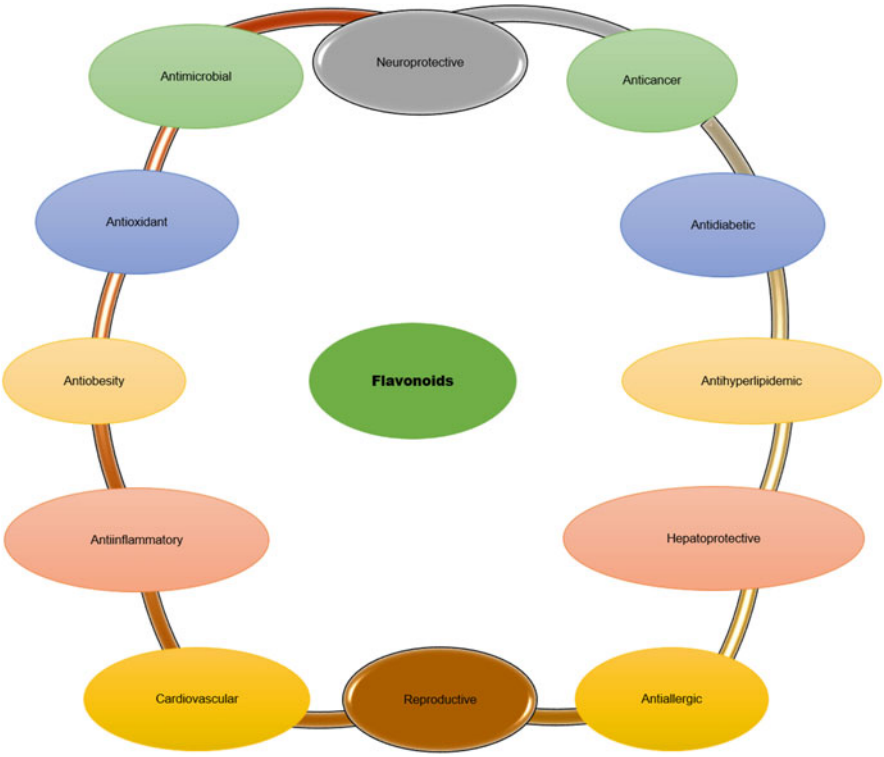

Fig. 19.2 Biological activities of flavonoids

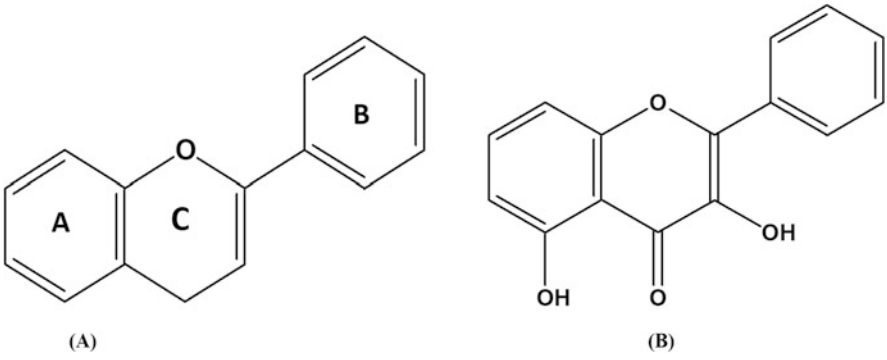

Fig. 19.3 (**a**) Chemical structure of flavonoids and (**b**) chrysin

Chrysin is a natural flavonoid with a wide spectrum of pharmacological behavior including anticancer neuroprotective, antidiabetic antihyperlipidemic anti-inflammatory, hepatoprotective, reproductive, cardiovascular, anti-allergic, anti-obesity, antioxidant as well as its antimicrobial action, they contain extensive

therapeutic activities that were found to be beneficial for the human well-being; therefore, the research conducted on flavonoids has grown immensely in the past few years as they have proven to function through physiological mechanisms as well as multiple significant signaling pathways that take part in numerous diseases implicating on human health (Pietta 2000).

19.3 Pharmacological Activities

19.3.1 Anticancer

Cancer is a real concern for public health around the world, it is a category of diseases identified by unmanageable proliferation as well as maturation of atypical cells that take over and metastasize to different areas of the human body (Salimi and Pourahmad 2018). Chrysin has proven to stimulate apoptosis and suppress proliferation in the majority of cancer cells and an efficient suppressor of tumor cell-induced angiogenesis (Jemal et al. 2011) and it is stronger and more efficacious than alternative flavonoids tested to treat leukemia cells, chrysin is to be expected to function inside the cell by triggering caspases and arresting AKT signaling. Furthermore, the relationships between structure and activity have shown that chrysin chemical structure contains the key structural components required by flavonoids to obtain efficacious cytotoxicity in leukemia cells (Fu et al. 2007).

In carcinoma cells and human tongue squamous cell chrysin causes amplified proline dehydrogenase/proline oxidase and the proline metabolism, therefore, a reduction in proline concentration, prolidase activity, and collagen biosynthesis (Celińska-Janowicz et al. 2018). Chrysin has a combined effect with metformin in which it suppresses the cyclin D1 gene and hTERT expression in breast cancer T47D cell lines (Campos et al. 2018). In addition to its combination effect with metformin, chrysin has a combined effect with cisplatin in where it considerably increases the HepG2 cancer cell apoptosis. The flavonoid has the ability to activate ERK1/2 which in turn steadies p53 gene expression, therefore, enhancing p53 phosphorylation in HepG2 cells (Li et al. 2015) Furthermore, alterations in b-arrestin-2 expression, an adaptable signaling molecule, second to chrysin addition affect multiple signaling pathways (Khan et al. 2011).

Treatment with chrysin decreases the action of NF-κB transcription factor, NF-κB is redox-active, and maintains the antioxidant armory. During renal carcinogenesis, chrysin suppresses biomarkers that encourage tumors and inflammation hence is a possible applicant for the hindrance of renal carcinogenesis (Rehman et al. 2013).

Melanoma tumors significantly showed a decrease in MMP-9, MMP-2, and TERT gene levels were markedly present in C57B16 mice with B16F10 melanoma tumors treated with nanoparticle-based chrysin; however, it exaggerated TIMP-2 and TIMP-1 genes expressions (Celińska-Janowicz et al. 2018; Choi and Yun 2011). Chrysin and curcumin nano-encapsulation enhanced the transmission of these compounds in colorectal cancer cells, Caco-2, and SW480 substantially inhibiting the maturation of cancer cells, as it reduces the expression of hTERT gene by

increasing the solubility as well as bioavailability of these therapeutic agents (Darwish et al. 2014). Among the different types of cancer chrysin has shown, therapeutic effects are against hepatocellular carcinoma (HCC) through multiple pathways. Cell apoptosis was encouraged; the combinations of VDAC-1 and HK-2 on mitochondria were substantially reduced moving Bax to the mitochondria from its original site the cytoplasm. Chrysin-mediated glycolysis suppression and cell apoptosis were severely damaged in exogenous overexpression cells HK-2. Further on it has been documented that tumor growth has been restrained in models of HCC xenograft as well as substantially decreased HK-2 expression with chrysin treatment (Davaran et al. 2018).

Ryu and their colleagues performed a study on PC-3 and DU145 cancer cell lines of the prostate, it was proven that chrysin induced fragmentation of the DNA as well as considerably induced apoptosis moreover inducing sub-G1 phase cell cycle cessation, and declining the extent of elevation of cell nuclear antigen proliferation. It accordingly caused the depletion of MMP conjointly with exaggerating lipid peroxidation and the yield of ROS as per the dose administered. Similarly, endoplasmic reticulum (ER) stress was aided by chrysin through inducing unfolded protein response (UPR) including translation eukaryotic initiation factor 2α (eIF2α), PRKR-like ER kinase (PERK), and 78 kDa glucose-regulated protein (GRP78). The intracellular signaling pathways mediated by chrysin-induced mitogen-activated protein kinases (MAPK), P38, and ERK1/2 proteins activated in association with inhibiting phosphoinositide 3-kinase (PI3K) and the majority of P70S6K, AKT, S6 proteins, and P90RSK (Durak et al. 2016). In conclusion, chrysin is an efficient inhibitor of tumor cell-induced angiogenesis; hence, it has the ability to postpone the formation of the tumor on the contrary to inhibiting the tumor formation (El-Bassossy et al. 2014).

Chrysin-loaded nanoparticles tumors were used in the treatment of breast tumors as it has been reported that the flavonoid enhances the pronouncement of TIMP-1 and 2 and decreases the pronouncement of MMP-2 and 9 therefore prevents the metastasis of the cancer (Eatemadi et al. 2016; Mohammadian et al. 2016). Chrysin provides optimum effects in breast cancer cell (BCC) lines when encapsulated as observed in the expression of the genes hTERT, BRCA1, and FTO. Likewise, in an alternative study, it had been determined that encapsulating chrysin in nanoparticles of PLGA-PEG amplified the expressions of three genes in the human gastric cell line, miR-34a, miR-22, and miR-126 (El-Sisi and Abdelsalam 2017). A concentration of chrysin (25 mg/kg and 50 mg/kg) was found to be very effective in opposition to MCF-7 breast cancer cells, it established extraordinary growth inhibition as well as induction of apoptotic (Mani and Natesan 2018).

Chrysin is a flavonoid with established anticancer activity and is effective on multiple different cell lines including prostate cancer, breast cancer, leukemia, melanoma, squamous cell, and finally colorectal cancer. Its anticancer activity makes it a flavonoid with momentous use therefore obtaining interest for its therapeutic use among scientists (Naz et al. 2019).

19.3.2 Neuroprotective

Chrysin has a wide variety of neuroprotective properties including neuroinflammatory where it showed substantial results in Parkinson's disease. Alzheimer also including antidepressant effects that were tested and proven on mice as well as its antiepileptic effects due to chrysin's containing a ligand for the benzodiazepine receptors (Nabavi et al. 2015). An increase in the production of oxidative stress may be the cause of the deleterious impact on signal transductions RhoA and ERK1/2 that aims for the modified cytoskeleton, most likely by acting on membranes, proteins, as well as DNA by stimulating lipid peroxidation.

Furthermore, astrocytes have shown effects on pro concentrations, they reorder their cytoskeleton, as well as make it across RhoA and ERK-mediated mechanisms. A type of polyvalent cells are astrocytes that are active in most of the processes that take place in the CNS, the effects of chrysin can be appreciated given how fragile the cytoskeleton astrocytes are (Loureiro et al. 2013). Oxidative stress was proven to reduce which therefore elevated the extent of brain damage. Furthermore, a decrease in cell death by the suppression of apoptosis was proven when exposed to chrysin, with lipid peroxidation induction that is associated with toxicity of acrylamide. The free radical scavenging effect of chrysin has been reported to be the most significant molecular mechanism in neuroprotection (Souza et al. 2015; Sathiavelu et al. 2009).

A report has shown oxidative stress and memory impairment is lowered effectively due to the administration of chrysin with reduced levels of brain-derived neurotrophic factor (BDNF) in matured mice's brain hence delegating as an antiaging agent (Zhang et al. 2015). In addition to chrysin acting as an antiaging agent, it contains antioxidant and antiapoptotic potential by inhibiting oxidative impairment, associating with tumor necrosis factor-alpha (TNF-α) and IL-β, and is in association with bcl-2 and caspases, hence elaborating motor as well as sensory functions (Kandhare et al. 2014).

Depression in experimental animals has been correlated with changes in brain neurotrophins in the brain and Na(þ), K(þ)-ATPase (Dubey et al. 2015). Mice that were put through chronic unpredictable mild stress for long periods of time which resulted in a substantial decrease in nerve growth factor (NGF) levels and BDNF, and Na(þ), K(þ)-ATPase activity in the forced swimming test the depressive statues were developed due to CUMS with considerable increase in corticosterone levels. Therapy with oral chrysin came to a decrease in the levels of BDNF and NGF levels, which is proportionate to fluoxetine. Furthermore, chrysin reduced the elevation in glutathione peroxidase (GPx), glutathione reductase (GR), and catalase (CAT) action in the experimental animals (Filho et al. 2015). In vivo, the primary mechanism that explains the antidepressant action of chrysin is the combination with the increase of BDNF and NGF, in addition to chrysin's potent antioxidant action.

Multiple members of flavonoid family contain neuroprotective effects as opposed to the oxidative attack on to the mesencephalic neuron dopamine (DA) after being exposed to *N*-methyl-4-phenyl-1,2,3,6- tetrahydropyridine hydrochloride (MPP). Chrysin can defend mesencephalic cultures from harm by MPP; this was demonstrated by DNA fragmentation studies as well as the use of DA neurons

tyrosine hydroxylase (TH) immunocytochemistry. To identify neuroprotective agents, MPP neurotoxicity is essential in vitro models. Due to the DA results, it has been concluded that chrysin is advantageous for the therapy of Parkinson's disease (Mercer et al. 2005).

A ligand allocated for the benzodiazepine receptors is chrysin, both central (Ki ¼ 3 microM, competitive mechanism) and peripheral (Ki ¼ 13 microM, mixed-type mechanism). The flavonoid has been proven to enhance tonic–clonic seizures expression caused by pentylenetertrazol when administrated intracerebroventricularly with the righting reflex restored to normal in all the mice treated therefore confirming a myorelaxant effect in chrysin (Medina et al. 1990). Another study conducted proved that treatment with extracts of natural sources containing chrysin as the primary component maintained the brain levels of serotonin and noradrenaline (Singh et al. 2012). Therefore, this indicates that chrysin may be used as a substitute of the standard diazepam treatment, which aggravates postictal depression (Sampath et al. 2011).

19.3.3 Antidiabetic

A metabolic disorder known as diabetes mellitus (DM) disrupts the glucose metabolism as well as the general physiology of the body, it results from the action of insulin and defects of its secretion, and diabetes mellitus is characterized by hyperglycemia. Diabetes mellitus decreases the general health of the patient by impacting other organs' functions and physiological process. Glomerular epithelial podocytes are a highly specialized cell that maintains the normality of glomerular filtration barrier although high glucose levels cause apoptosis in those specialized cells, which can be decreased by subjecting them to chrysin mainly by DNA fragmentation. Additionally, by increasing the Bax/Bcl-2 ratio back to its normal state and decreasing the rate of induction of cytochrome c and Apaf-1 in renal podocytes that are under the influence of exceeding glucose concentrations.

Oral administration of chrysin in diabetic mice resulted in substantial management of proteinuria as well as atypical transformation in glomerular ultrastructure. Furthermore, the flavonoid increased the extent of unfolded protein feedback to ER stress as shown by PERK-eIF2α-ATF4-CHOP increase. Inhibition of ER stress feedback by apoptotic episodes in podocytes as well as a major decrease in the synthesis of slit diaphragm is associated with chrysin treatment (Kang et al. 2017). Chrysin was tested on rats and found significant results by reestablishing normal renal function and pathology. TNF-α expression is inhibited due to chrysin which correlates to its nephroprotective action (Ahad et al. 2014).

Diabetes causes vascular problems due to hyperglycemia. HepG2 cells medicated with, positive reference, rosiglitazone, and chrysin, chrysin derivatives were used and monitored to obtain their hypoglycemic effects. An O7-nitrooxyalkyl nitric oxide (NO) donor moiety is connected to the parent moiety chrysin, and its O7-[(nitrooxyl) alkyloxycarbonyl] methyl derivatives were produced. Moreover, a new class of hybrid ester prodrugs was produced. The methyl analog of (nitrooxyl)

ethoxycarbonyl is the prevalent suppressers of AR and AGE, and chrysin is a strong suppresser of AGE. In the presence of L-cysteine, all the hybrid ester prodrugs moderately release NO. The O7-nitrooxyethyl chrysin derivatives O7-[(nitrooxyl) butoxy carbonyl] methyl analog and O7 [(nitrooxyl)hexoxycarbonyl] methyl analog (5c) significantly stimulate the glucose uptake of HepG2 cells. These prodrugs which are a hybrid ester NO donor provide an upcoming drug design idea for the advancement of prophylactic or pharmacological agents for diabetes implicated vascular complications (Zou et al. 2010).

Abnormal retinal function is caused by chronic diabetes known as diabetic retinopathy (DR). Positive effects were obtained when chrysin was administered to human retinal endothelial cells inserted in glucose-bared eyes of diabetic mice, it was reported that the flavonoid stimulated the suppression of VEGF and its receptor-2 and HIF-1α as well as inhibited apoptotic events in retinal endothelial, chrysin also managed apoptosis by increasing Tie-2 and Ang-1 and 2 in the eyes of diabetic mice. An oral administration of chrysin of 10 mg/kg restored the drop of the junction proteins, VE-cadherin, and ZO-1 likely upholding endothelial cells and pericytes interaction (Goes et al. 2018).

Another antidiabetic roles of chrysin inlist the preservation of cognitive descend (DACD) by the decrease of NF-κB p65 unit, TNF-α, IL-1β, IL-6, and caspase-3 in hippocampus and cerebral cortex as proven by multiple studies (He et al. 2012). Liver tissues lipid peroxidation status were substantially decreased as well as the extent of vacuolization in liver tissue and a decline in the number of vacuolated hepatic cells after 7 days of administration of the flavonoid through the intraperitoneal of the diabetic Swiss albino mice that were induced with alloxan (Sirovina et al. 2013).

Varied doses of chrysin produced attenuated the elevated levels of deficient density lipoprotein, triglycerides, malondialdehyde (MDA), cholesterol, and elevated the high-density lipoproteins concentrations, GST, total protein, SOD, and catalase on streptozotocin (STZ)-induced diabetic rats (Samarghandian et al. 2017). Treatment with chrysin was also associated with the hindrance of defective insulin-stimulating molecules as well as the tolerance to glucose. Furthermore, it has been reported that undesirable effects on blood glucose, insulin, and lipid profile were substantially regulated by chrysin treatment diabetic rats subjected to high fat and sucrose diet (Satyanarayana et al. 2015).

19.3.4 Antihyperlipidemic

In women, dyslipidemia upholds a high-risk factor for coronary heart disease. It has been observed that chrysin is commensurate to the standard drug simvastatin. The amplification of triglyceride, non-HDL cholesterol levels, and plasma total cholesterol, can be restricted by chrysin as it contains antihyperlipidemic action. A likely cause of chrysin hypolipidemic effect is its possession of antioxidant action (Zarzecki et al. 2014).

It has been proven that chrysin contains antiatherogenic action in addition to its antioxidant effects. It has been reported that the administration of chrysin resulted in a substantial decrease of lipid profile mean serum levels parameter albeit HDL–cholesterol were found to be elevated. Furthermore, decrease in hepatic marker enzymes was observed in alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and lactate dehydrogenase, in correlation with a contrary substantial increase in mean hepatic levels of lipoprotein lipase (LPL), 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA reductase), enzymatic (catalase, superoxide dismutase, and glutathione peroxidase), as well as nonenzymatic antioxidants (reduced glutathione and vitamins C and E) (Anandhi et al. 2014).

19.3.5 Anti-Inflammatory

Chrysin exhibits impressive anti-inflammatory properties that are well documented, investigated, and characterized. This is backed by deducting studies proposing that chrysin's identified association with the COX-2 binding site which is enough to prove the anti-inflammatory action of this flavonoid. An escalation in levels of proinflammatory cytokines and reduced amount of anti-inflammatory cytokines was observed which majorly mediate neuroinflammatory responses (Xiao et al. 2014). An alternative study proved that the flavonoid suppressed the discharge of proinflammatory cytokines inclusive of tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β) as well as and nitric oxide (NO) in lipopolysaccharide (LPS)-stimulated microglia. In addition to nitric oxide (NO) and proinflammatory cytokines, chrysin demonstrated an ability to inhibit the expressions of inductive NO synthase (iNOS) and cyclooxygenase-2 (COX-2). c-Jun N-terminal kinase (JNK) and nuclear factor-kB (NFkB) are enzymes with a significant impact on neuroinflammation and considered key mediators, both with the potential of being suppressed by chrysin (Ha et al. 2010). Nevertheless, another studied had resulted in alternative results that showed that the treatment with chrysin on mice was not able to regulate constitutively or TNF-α induced NF-κB action in main cultures of mouse cortical astrocytes, hence it has been concluded that chrysin is not as likely that it targets NF-κB signaling in astrocytes (Spilsbury et al. 2012).

Chrysin anti-inflammatory activity is associated with inflammatory cytokine management. The flavonoid activates macrophages through the NF-κB pathway which inhibits the yield of inflammatory cytokine IFN-c and TNF-α (Xiao et al. 2014; Galijatovic et al. 1999). Prostaglandin-E2, COX-2, and NF-κB are three other factors inhibited by chrysin, hence owing this action to its notorious anti-inflammatory action (O'Leary et al. 2004). The pharmacological action of chrysin substantially reduces inflammation and modulates macrophage standards by the inhibition of inflammation. Furthermore, chrysin takes part in the stimulation of PPAR-g functions as well as advancing the PPAR-g effects; it additionally encourages specific gene expressions. When taking all results into consideration, the conclusion this study has reached supports the newly found function of chrysin

as an effective regulator of PPAR-g transcriptional activity and in inflammatory diseases it is used as a therapeutic molecule (Feng et al. 2014).

Chrysin was also found effective in the treatment of osteoarthritis (OA) chondrocytes by stimulating IL-1β. In an alternative report, it was proved that chrysin could decrease IL-1β-induced yield of PGE2, NO; decreases the expression and effect of MMP-3, iNOS, MMP-1, COX-2, ADAMTS-5, ADAMTS-4, MMP-13, as well as diminish collagen-II, aggrecan. With an additional effect on NF-κB and IL-1β-stimulated IκB-α, chrysin blocks the activation of NF-κB as well as causes the deterioration of IL-1β-induced IκB-α (Zheng et al. 2017).

In addition, the flavonoid decreased iNOS-stimulated expression by LPS, the therapy suppressed LPS—caused phosphorylation of JAK-STATs, nuclear translocation of STAT1 and STAT3, suppresses the discharge of TNF-α, IL-6, MCP-1 as well as the yield of ROS in RAW264.7 cells. This is significant as ROS is enforced as an upstream signal to moderate the initiation of JAKSTATs signaling pathway. Hence chrysin acts by inhibiting the action of JAK-STATs moderated by ROS to decrease LPS-stimulated inflammatory feedback in cells of RAW264.7 (Qi et al. 2018).

Inflammatory cytokines are substantially released in human volunteers exposed to cigarette smoke, the inflammatory cytokines include IL-1β, TNF-α, and IL-8 in bronchoalveolar lavage fluid and in the lung tissue MPO expression was observed, when chrysin was administrated in varied doses through the intraperitoneal, the inflammation resulting from cigarette smoke was inhibited as an outcome of inhibiting the inflammatory cytokines TNF-α, IL-8, and IL-1β including the MPO release; in addition, chrysin caused the reduction in the extent of phosphorylation p38 and ERK (Shen et al. 2015; Rauf et al. 2015).

Doxorubicin (DOX) is known to be a chemotherapeutic drug with one of the most efficient and effective however its efficiency relays on the occurrence of cardiotoxicity. As mentioned earlier, chrysin contains many biological activities in the exception of anti-inflammatory including its anticancer as well as its antioxidant effect. The natural flavone has the ability to significantly protect the heart from damages caused by doxorubicin and the induced elevation of the enzymes serum creatine kinase isoenzyme-MB (CK-MB), lactate dehydrogenase (LDH) in addition to, myofibrillar disarray. The risk of oxidative stress was reduced by chrysin treatment. Furthermore, chrysin counteracts doxorubicin's inflammatory response by inhibiting the increase caused by the drug, the inflammatory response is caused by the following components; nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), expression of nuclear factor kappa-B (NF-κB), nitric oxide as well as tumor necrosis factor-alpha (TNF-α) (Shen et al. 2015). Another negative effect caused by doxorubicin is apoptosis induced by DOX it acts by augmenting cytochrome c and caspase-3 activity, Bax expression while reducing Bcl-2 expression therefore damaging the tissues. It has been noted that chrysin substantially reduced such apoptotic actions caused by doxorubicin. Therefore, it has been deducted from the above mentioned that chrysin contains protective action in the defense of doxorubicin-induced acute cardiotoxicity by decreasing apoptotic tissue damage as

well as reducing inflammation, oxidative injury (Rauf et al. 2015; Mantawy et al. 2014).

In conclusion, chrysin contains potent anti-inflammatory effects; it suppresses the releases of inflammatory cytokines in addition to cyclooxygenase action. The flavonoid has the potential to provide a novel pharmacological approach for autoimmune neuropathies as well as documented neuroprotective effects especially when it comes to its neuroinflammatory action.

19.3.6 Hepatoprotective

Chrysin is used in liver as hepatoprotective and antihyperlipidemic effects. Evidences shown in hepatotoxicity of rats that chrysin decreases degrees of triglycerides, cholesterol, free fatty acids, phospholipids, very low and low-density lipoprotein-C, and the degree of high-density lipoprotein-C is increased in the tissues and plasma (Pushpavalli et al. 2010). TNF-α mediates the hepatoprotective activity of chrysin as it lessens soluble TNF-α formation by preventing the activity of TNF-α converting enzyme (Hermenean et al. 2017). In liver damage, Chrysin is additionally seen as viable which is initiated via carbon tetrachloride. The increment degree of centrilobular necrosis, steatosis, serum aspartate–aminotransferase, alanine–aminotransferase, change in hepatocyte ultrastructure, growth of hepatic α-smooth muscle actin protein, tumor necrosis factor-α through intraperitoneal CCl_4 infusion causes liver damage. The outcomes indicate that it diminished serum aspartate–aminotransferase, alanine–aminotransferase and convey assurance against enhancement of steatosis, centrilobular necrosis, and a change of hepatocyte ultrastructure which caused a decrease in hepatic TNF-α interpretation and α-SMA protein. In an investigation, chrysin shielded from the TGF-β1 interfered hepatic stellate cells incitement on fibrogenesis on rats. Chrysin diminished α-SMA, hepatic fibrosis, TGF-β1 immunopositive cells, and TGF-β1 when contrasted with different flavonoids. In addition, these portions significantly diminished mRNA of Smad 2 liver ((Khan et al. 2011; Anandhi et al. 2013). Nephron and hepatotoxicity marker activity degrees were seen in D-GaIN. Albumin serum total protein and A/G proportion are likewise influenced (Tahir and Sultana 2011). In different examination, chrysin diminished hepatic marker enzymatic activities and by-products of lipid peroxidation (Ravishankar et al. 2017; Liu et al. 2016). In addition, chrysin was exposed to albino rats in contrast to the hepatotoxicity lured through dispensation of cisplatin (Lo et al. 2012). The impact of chrysin on methotrexate lured hepatic oxidative stress was watched in other investigation. In rats, cell death is essentially caused by diminish in alanine transaminase, activity of lactate dehydrogenase, aspartate aminotransferase, and malondialdehyde content just as enhanced glutathione reductase, superoxide dismutase, activity of catalase, glutathione peroxidase, and diminished content of glutathione (Ali et al. 2014). Similarly, chrysin in contrast to CCl4 incited toxicity in Wistar male rat's downregulated the mRNA interpretation of the iNOS gene (Lotfi-Attari et al. 2017). To condense chrysin prevents damage in liver in various design of cisplatin, hepatotoxicity, and methotrexate related to

damage in the liver. As a result, it shows that chrysin associated with hepatoprotective, it lessens serum alanine aminotransferase and aspartate aminotransferase, which gives assurance in contrast to expansion of centrilobular necrosis, steatosis, and a change of ultrastructure of hepatocyte. Diminish hepatic α-SMA protein and TNF-α articulation (Hermenean et al. 2013). Diminished hepatic fibrosis, down directed the α-SMA, diminished number of TGF-β1 immunopositive cells, and set apart down controlled the TGF-β1 (Balta et al. 2015).

19.3.7 Reproductive

Campos and collaborators talked in an investigation, they assessed that chrysin has a defensive job against the reproductive abnormities (Campos et al. 2018). Chrysin is supplemented in a male and female gerbils which are 90-day-old displayed prostate gland stromal remodeling and epithelial hyperplasia. It is really the advancement of the organelles, which are engaged with the secretory biosynthetic pathway in epithelial cells. The increase in ovarian follicles and normal testicular morphology is managed by chrysin (Zeinali et al. 2017). It improved the sperm abnormalities, motility, dead rate, diminished cell death, and MDA degrees in paracetamol testicular tissues harm rats (Shen et al. 2001). Ciftci and colleagues made an investigation, they elucidated that chrysin usage indicated a decrease in TBARS, enriched glutathione degree, sperm motility, concentration, and decrease in abnormal sperm rate (Ciftci et al. 2018) in Wistar rats, chrysin and celecoxib separately were administered orally and information discovered that diminished paw edema was practically identical to celecoxib. In addition, through backing down histopathologic and gonadosomatic index by spermatogenesis protection, they also diminished testicular damage. The serum testosterone, interpretation of steroidogenic acute regulation mRNA and FSH is upregulated by the two agents. Through inversion of TNF-α, myeloperoxidase, COX-2 protein interpretation, and enhancing of iNOS and IL-10 chrysin inhibited inflammation. It was accompanied that the mitigation of the testicular damage with restraint of oxidative stress by means of diminishing testicular nitric oxide and lipid peroxides. Caspase-3 and FasL mRNA interpretation was effectively downregulated by the two agents to increase cell survival if there should be an occurrence of apoptosis (Mohammadian et al. 2016). As a result, normal testicular formation and enhanced ovarian follicles are controlled (Campos et al. 2018), additionally, fatty acids proportion (n-6/n-3) and MDA accumulation are reduced and the blood testosterone degree is intensified. Enhanced the sperm abnormalities, motility, dead rate, lowered cell death, and degrees of MDA in paracetamol testicular tissues as well as increases fertility and hatchability (Altawash et al. 2017; Aksu et al. 2016; Testai et al. 2013). Chrysin administration essentially enhances parameters of sperm and securing the reproductive system. Because of that reason, chrysin can be a different remedy for men to enhance the quality of sperm (Missassi et al. 2017). In rats, the induced testosterone benign prostate hyperplasia is essentially protected by chrysin through many processes, for example, prevention from enhancing of lipid peroxidation, glutathione deficiency, prevention of catalase

activities and superoxide dismutase, regaining of separated caspase-3 degree, and regaining of decreased Bax/Bcl-2 proportion and mRNA interpretation of p53 and p21. In addition, it was reported that after chrysin treatment, inhibition from the improvement of attaching of mRNA activity interpretation of like insulin growth factor 1, like insulin growth factor 1 receptor and NF-κB p65 subunit (Shoieb et al. 2018; Russo et al. 2012). In rats, the administration of nitrofurazone induced testicular damage, whereas it has been found out that the combining results of chrysin and mirtazapine lessen the rise of serum acid phosphatase enzyme action and especially inhibited from the decrease of the viability and count of sperm. They also work proficiently to inhibit from the lipid peroxidation, deficiency of glutathione, and in rat testes, raised TNF-α degree and diminished c-kit degree. This connecting reaction impressively diminished the caspase-3 degrees in tissue testicular (Jiang et al. 2014; Tian et al. 2014).

19.3.8 Cardiovascular

In myocardial damage, chrysin is used to improve it and the mechanism of activity is by peroxisome proliferator-initiated receptor-g activation, which thus constricted propelled end product of glycation (AGE)/RAGE-intervened oxidative stress, inflammatory, and cell death reaction. Besides, past examinations gave the essential proof that chrysin considerably alleviates platelet-derived growth factor and H_2O_2 lured prevention of PTP action. It influences glutathione in the reactivation system for PTP reactivation, initiating to dephosphorylation of activated PDGFR and its downstream protein enzymes (Rani et al. 2016). Practically, PDGF lured proliferation and movement in vascular smooth muscle cells is suppressed by chrysin. The prohibition impact of chrysin is definite even when chrysin is included after PDGF induction (Lo et al. 2012; Testai et al. 2013). By restraining p53-dependent apoptotic pathway, oxidative stress, and MAPK and NF-κB pathways, chrysin defend in contrast to doxorubicin lured cardiomyopathy while increasing the vascular endothelial growth factor pathway (Mantawy et al. 2017; De Vries et al. 1997). Chrysin successfully restrains platelet aggregation and granule creation which is actuated by thrombin, collagen, U46619, and ADP. Moreover, it restricts the adhesive platelets and decreases the spread of single platelet on immobilized fibrinogen. In biochemical tests, it showed that the collagen lured activation of PLCγ2, PKC, Syk, along with Akt, and phosphorylation of ERK1/2 is inhibited by chrysin. The phosphorylation of GSK3β, FAK, FcγRIIa, and Akt in platelet permeating on immobilized fibrinogen is also decreased by chrysin (Liu et al. 2016). In an investigation conducted in vitro, the data collected showed that chrysin prevented the development of thrombus and platelet functionality (Ravishankar et al. 2017). In another investigation done by Lo and colleagues, they revealed that chrysin concentration dependently has been formed to prevent the PDGF proliferation and chemotaxis and also reduced PDGF signaling in vascular smooth muscle cell. It effectively reduces NADPH oxidase activation, H_2O_2 signaling, and PDGF lured reactive oxygen species formation, whereas it did not interact with the bounding of PDGF with

VSMCs. The PDGF lured oxidation of protein tyrosine phosphatase active site and support PDGF lured prevention of PTP is prevented by chrysin. The PDGF receptor autophosphorylation that is influenced by vanadate and is also prevented by chrysin (Lo et al. 2012). Action of chrysin rather than antioxidant *N*-acetylcysteine and flavonoid (−)-epigallocatechin-3-gallate on the action of PTP and PDGF signaling was prevented because of intracellular glutathione deficiency which is because of the efficiency of chrysin on glutaredoxin system for reactivation of PTP (Ali et al. 2014). The research showed that in chorioallantoic branch of chicken and human umbilical endothelial cells chrysin prevented lipopolysaccharide lured angiogenesis. Chrysin also decreased LPS lured neovascular density of 58CAM, making downregulation of VEGFR-2, VEGF gene interpretation, but not VEGFR-1. Moreover, the concentration of chrysin independently diminished autoregulation loop of IL-6/IL-6R in LPS conducted HUVEC in humans (Lotfi-Attari et al. 2017). A report made by members of scientists that LNAME-sensitive endothelial NO relief that introduces to cGMP aggregation in aortic rings with endothelium increased by chrysin. It also influenced aortic and endothelium-dependent relaxation. Furthermore, it activated the relief of NO and mediates efficaciously through phosphatidylinositol 3 kinase (Villar et al. 2004). A report made that chrysin improves relaxation of acetylcholine under situations which are under control or incubation with anion superoxide after a specific period generating xanthine oxidase. It also enhanced relaxation, which is influenced by 3 morpholinosydnonimine, 8-bromoguanosine- 3′: 5′-cyclic monophosphates, and sodium nitroprusside (Duarte et al. 2001). Chrysin is beneficial in the prevention from tissue damage because of bioactivation by *S*-adenosyl methionine. An examination has been made that administration of chrysin decreased DNA fragmentation in brain, liver, lipid peroxidation, blood, and protein carbonyls. The micronuclei achieved in bone marrow cells of humans is significantly decreased (Babangida et al. 2018). So the thrombosis and platelet accumulation aortic endothelial damage and cardiac oxidative stress pathways are restrained by chrysin. As a result, chrysin prevents platelet aggregation and granule formation which is influenced by thrombin, collagen, U46619, and ADP prevented the collagen lured activation of Syk, PKC, along with phosphorylation of ERK1/2, PLCγ2, and Akt. The phosphorylation of Akt, FAK, GSK3β, and FcγRIIa in platelet permeating on immobilized fibrinogen is decreased (Liu et al. 2016; Kandemir et al. 2017).

19.3.9 Antiallergic

On BALB/c female mice chrysin was conducted, ovalbumin lured airway hyperresponsiveness shows that it decreased OVA and reduced eosinophils, interleukin-4, IL-13 in bronchoalveolar lavage fluid, and total serum immunoglobulin IgE. Additionally manages the degree of BALF interferon-γ, reduced infiltration of inflammatory cell, goblet cell hyperplasia, and indication of α-SMA over bronchioles. Furthermore, chrysin reduces the extracellular signal monitored kinase and phosphorylation of Akt degree that is correlated to proliferation of ASMC (Yao et al. 2016). In BALB/c mice, it also inhibits ovalbumin lured AHR to acetylcholine

chloride. It merits referencing that chrysin in BALF extensively suppresses the eosinophil counts, IgE degrees in serum, and total inflammatory cell. An investigation of tissue of the lungs reported that chrysin generously reduced eosinophilic inflammation induced by allergen and airway mucus-producing goblet cells. Moreover, by causing the immune response to allergens against GATA-3, T-helper type 1 profile by adjusting the T-bet transcription factors in allergic mice, it activates the immune system (Manzolli et al. 2015; Bae et al. 2011). They also suppress the discharge of systemic hypersensitivity serum histamine and immunoglobulin E-mediated anaphylaxis and decreases discharge of histamine from mast cells. These results are intensive when compared with the known anti-allergic drugs such as cromolyn. Furthermore, pro-inflammatory cytokines gene expression in mast cells and the inhibited reaction was caspase-1 relative and NF-κB is inhibited by chrysin (Khan et al. 2012). Chrysin also caused lessening in infiltration of status of perivascular lung blood vessels, bronchi status, leucocytes, alveolar macrophages activation, inflammation, alveoli stability, and also reduces cellular injury factors in bronchoalveolar hyperresponsiveness rats. Furthermore, it was reported that treatment with chrysin has its anti-asthmatic capacity. It can be because of adjustment of Th1/Th2 polarization by means of prevention of NF-κB, activated protein, and nitric oxide synthase (Mantawy et al. 2014; Wadibhasme et al. 2011).

19.3.10 Anti-obesity

Chrysin is effective in 3T3-L1 adipocytes, it improves interpretation of specific markers of brown fat, protein degrees of proliferator peroxisome stimulated receptor α and receptor 1 *α*, phosphorylated acetyl-CoA carboxylase, PPARδ, phosphorylated AMP-stimulated protein kinase, PPARγ, lipase, perilipin, acyl-coenzyme A oxidase 1, carnitine palmitoyltransferase 1, uncoupling protein 1, fat oxidation, thermogenesis, lipolysis, and also reduces lipogenesis. Improvement of specific markers of brown fat and UCP-1 was because of the chrysin, which activates AMPK based on that inhibition of AMPK by dorsomorphin eliminate interpretation of PR domain-containing 16, UCP-1, and PGC-1α, although 5-aminoimidazole-4-carboxamide ribonucleotide activator enhanced interpretation of brown marker proteins (Vedagiri and Thangarajan 2016; Kang et al. 2015). Chrysin also increased large fat diet lured muscular steatosis, without affecting the weight of hepatic in obese rats. It reduces macrophages permeation into adipose tissue and induces anti-inflammatory M1, M2 phenotype in the obese rat-cultured macrophages, and peritoneal macrophages and as an outcome it changes M1/M2 status. Moreover, chrysin improves transcription of PPARγ activity and its target genes expression as a result it regulates phenotypes of macrophages (Souza et al. 2015).

19.3.11 Antioxidant

Chrysin has likewise been appeared to defend toward hydrogen peroxide lured cell death and constrict neuronal death in many tests conducted in vitro (Souza et al. 2015; Pichichero et al. 2010). An investigation made in mice in vivo, it was shown that chrysin decline age linked improved in oxidative stress and increased cognitive declaring and lessening in derived neurotrophic factor of brain (Souza et al. 2015). Oral administration of chrysin effectively constricted the enhancement in the formation of free radical and prevented the catalase, superoxide dismutase activities, and glutathione peroxidase, and K(þ), Na(þ) ATPase degrees and in the aged mice prevents the activities of GPx, SOD, and CAT. In mice, Chrysin alters the action of K (þ), Na (þ) ATPase, and reduced degrees of BDNF in the hippocampus and prefrontal cortex. Chrysin can also inhibit age-linked declaring in memory because of its strong antioxidant actions and transition production of BDNF (Lirdprapamongkol et al. 2013; Souza et al. 2015). The homeostasis of antioxidant and oxidant status, which is regulated by lipid peroxidation and antioxidants during carcinogenesis, is improved when chrysin is administrated (Li et al. 2011; Karthikeyan et al. 2013). Both in vitro and in vivo, tumor production by the means of apoptosis associated with the activation of Notch1 signaling pathway is inhibited (Yu et al. 2013). Chrysins, primary mechanism of action contains a reduction in proliferation of cell, induction of cell death by apoptosis, and inflammation lessening (Xue et al. 2016; Khan et al. 2012; Kalogeropoulos et al. 2013). The transformers in a versatile signaling molecule, b-arrestin-2 interpretation, subsequent to chrysin supplementation regulate many signaling pathways (Khan et al. 2011). Chrysin and cisplatin on combination are considerably enhancing the death cells of HepG2 cancer cells. The p53 gene interpretation by stimulating ERK1/2 is stabilizing by both of them, which then stimulates p53 phosphorylation in HepG2 cells (Rehman et al. 2013). Treatment with chrysin conserves the armory antioxidant and restrains the redox-active transcription factor NF-κB activation. Chrysin is a prospective for the inhibition of renal carcinogenesis during renal carcinogenesis, it suppresses many biomarkers of tumor promotion and inflammation (Fu et al. 2013). Chrysin can be an efficacious preventor of tumor cell lured angiogenesis (El-Sisi et al. 2017). It is confirmed that chrysin has the prospective to detain tumor development rather than prevent tumor production. As a result, chrysin inhibits lipid peroxidation, deficiency of glutathione, and degrees of tumor necrosis factor-α and decrease c-kit degree (Mantawy et al. 2017). Antioxidant enzymes are increased, interpretation of p53, Bax, Puma, Noxa, caspase-3, and cytochrome c is decreased, interpretation of Bcl-2 is increased, inactivation of JNK, MAPK, and p38 decreases NF-κB, interpretation of PTEN, and enhancement of VEGF/AKT pathway (Izuta et al. 2008). Restraint apoptotic damage to the cells by enhancing the action of cytochrome c and caspase-3 and Bax interpretation at the same time lessening the Bcl-2 interpretation.

19.3.12 Antimicrobial

It is reported that chrysin acquires both antifungal and antibacterial activities. Nevertheless, higher concentration of chrysin is needed to produce the inhibitory action (Mercan et al. 2006). A report made by Chabot et al. that in the fungus *Gigaspora margarita* Becker and Hall, chrysin decreases the spore germination (Chabot et al. 1999).

19.4 Conclusion

The prevention and cure of diseases that use phytochemicals, especially flavonoids, are well known. Fruits and vegetables are really a common source of flavonoids. Wide range of flavonoids present in nature has its own physical, chemical, and physiological properties. Chrysin, a naturally occurring polyphenol, tends to have multiple pharmacological effects, such as anticancer, antidiabetic, neuroprotective, antiallergic, antihyperlipidemic, antimicrobial, anti-obesity, anti-inflammatory, hepatoprotective, cardiovascular, reproductive, and antioxidant activities. The molecular mechanisms influencing the pleiotropic actions of chrysin are complex, including configurations of cell signaling pathways at different stages of numerous diseases. Though, chrysin is plagued by bioavailability issues, close to other polyphenols, which should be resolved prior to clinical trials. The bioavailability of chrysin has to be optimized in order to increase the effectiveness of chrysin as a pharmaceutical molecule.

References

Ahad A, Ganai AA, Mujeeb M, Siddiqui WA (2014) Chrysin, an anti-inflammatory molecule, abrogates renal dysfunction in type 2 diabetic rats. Toxicol Appl Pharmacol 279:1–7

Aksu EM, Ozkaraca F, Kandemir A, Omur E, Eldutar S, Comaklı S (2016) Mitigation of paracetamol-induced reproductive damage by chrysin in male rats via reducing oxidative stress. Andrologia 48:1145–1154

Ali NS, Rashid S, Nafees SK, Hasan SS (2014) Beneficial effects of chrysin against methotrexate-induced hepatotoxicity via attenuation of oxidative stress and apoptosis. Mol Cell Biochem 385:215–223

Altawash ASA, Shahneh H, Ansari H (2017) Chrysin-induced sperm parameters and fatty acid profile changes improve reproductive performance of roosters. Theriogenology 104:72–79

Anandhi R, Annadurai T, Anitha TS, Muralidharan AR, Najmunnisha K, Nachiappan V, Thomas PA, Geraldine P (2013) Antihypercholesterolemic and antioxidative effects of an extract of the oyster mushroom, *Pleurotus ostreatus*, and its major constituent, chrysin, in Triton WR-1339-induced hypercholesterolemic rats. J Physiol Biochem 69:313–323

Anandhi R, Thomas PA, Geraldine P (2014) Evaluation of the antiatherogenic potential of chrysin in Wistar rats. Mol Cell Biochem 385:103–113

Babangida SS, Ibrahim A, Muhammad D, Arthur A, Garba A (2018) The role of molecular modelling strategies in validating the effects of chrysin on sodium arsenite induced chromosomal and DNA damage. Human Exp Toxicol 9:775–777

Bae Y, Lee S, Kim SH (2011) Chrysin suppresses mast cell-mediated allergic inflammation: involvement of calcium caspase-1 and nuclear factor-κB. Toxicol App Pharmacol 254:56–64
Bailey RL (2020) Current regulatory guidelines and resources to support research of dietary supplements in the United States. Crit Rev Food Sci Nutr 60(2):298–309
Balta CH, Herman O, Boldura M, Gasca I, Rosu M, Ardelean A, Hermenean A (2015) Chrysin attenuates liver fibrosis and hepatic stellate cell activation through TGF-β/ Smad signaling pathway. Chem Biol Interact 240:94–101
Beecher GR (2003) Overview of dietary flavonoids: nomenclature, occurrence and intake. J Nutr 133:3248–3254
Campos MS, Ribeiro NC, De Lima MB, Santos PS, Vilamaior LO, Regasini MF, Biancardi SR, Santos FC (2018) Anabolic effects of chrysin on the ventral male prostate and female prostate of adult gerbils (*Meriones unguiculatus*). Reprod Fertil Dev 20:177–189
Celińska-Janowicz K, Zaręba I, Lazarek U, Teul J, Tomczyk M, Pałka J, Miltyk W (2018) Constituents of propolis: chrysin, caffeic acid, p-coumaric acid and ferulic acid induce PRODH/POX-dependent apoptosis in human tongue squamous cell carcinoma cell (Cal-27). Front Pharmacol 9:336
Chabot S, Bel-Rhlid R, Chenevert R, Piche Y (1999) Hyphal growth promotion in vitro of the VA mycorrhizal fungus, Gigaspora margarita Becker & Hall, by the activity of structurally specific flavonoid compounds under CO2-enriched conditions. New Phytol 122(3):461–467
Choi JH, Yun JW (2011) Chrysin induces brown fat-like phenotype and enhances lipid metabolism in 3T3-L1 adipocytes. Nutrition (Burbank, Los Angeles County, Calif) 32(9):1002–1010
Ciftci O, Ozdemir I, Aydin M, Beytur A (2018) Beneficial effects of chrysin on the reproductive system of adult male rats. Andrologia 44(3):181–186
Cragg GM, Newman DJ (2013) Natural products: a continuing source of novel drug leads. Biochim Biophys Acta 1830(6):3670–3695
Darwish HA, Arab HH, Abdelsalam RM (2014) Chrysin alleviates testicular dysfunction in adjuvant arthritic rats via suppression of inflammation and apoptosis: comparison with celecoxib. Toxicol Appl Pharmacol 279(2):129–140
Davaran S, Fazeli H, Ghamkhari A, Rahimi F, Molavi O, Anzabi M, Salehi R (2018) Synthesis and characterization of novel P(HEMA-LAMADQUAT) micelles for co-delivery of methotrexate and Chrysin in combination cancer chemotherapy. J Biomater Sci Polym Ed 29(11):1265–1286
De Vries JH, Janssen PL, Hollman PC, Van Staveren WA, Katan V (1997) Consumption of quercetin and kaempferol in free living subjects eating a variety of diets. Cancer Lett 114:141–144
Dias DA, Urban S, Roessner U (2012) A historical overview of natural products in drug discovery. Meta 2(2):303–336
Duarte JR, Jimenez IC, Villar F, Perez-Vizcaino J, Tamargo J (2001) Vasorelaxant effects of the bioflavonoid chrysin in isolated rat aorta. Planta Med 67:567–569
Dubey VK, Ansari F, Vohora D, Khanam R (2015) Possible involvement of corticosterone and serotonin in antidepressant and antianxiety effects of chromium picolinate in chronic unpredictable mild stress induced depression and anxiety in rats. J Trace Elem Med Biol 29:222–226
Durak MA, Öztanır MN, Başak Türkmen N, Çiftçi O, Taşlıdere A, Tecellioğlu M, Önder A (2016) Chrysin prevents brain damage caused by global cerebralischemia/reperfusion in a C57BL/J6 mouse model. Turkish J Med Sci 46(6):1926–1933
Ekor M (2014) The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol 4:177
El-Bassossy HM, Abo-Warda SM, Fahmy A (2014) Chrysin and luteolin alleviate vascular complications associated with insulin resistance mainly through PPAR-γ activation. Am J Chin Med 42(5):1153–1167
El-Sisi AE, Abdelsalam NM (2017) Protective effects of mirtazapine and chrysin on experimentally induced testicular damage in rats. Biomed Pharmacother 95:1059–1066
El-Sisi AE, El-Sayad ME, Abdelsalam NM (2017) Protective effects of mirtazapine and chrysin on experimentally induced testicular damage in rats. Biomed Pharmacother 95:1059–1066

Eatemadi A, Darabi M, Afraidooni L, Zarghami N, Daraee H, Eskandari L, Mellatyar H, Akbarzadeh A (2016) Comparison, synthesis and evaluation of anticancer drug-loaded polymeric nanoparticles on breast cancer cell lines. Artif Cell Nanomed Biotechnol 44(3):1008–1017

Feng X, Qin H, Shi Q, Zhang Y, Zhou F, Wu H, Ding S, Niu Z, Lu Y, Shen P (2014) Chrysin attenuates inflammation by regulating M1/M2 status via activating PPAR. Biochem Pharmacol 89:503–514

Filho CB, Jesse CR, Donato F, Giacomeli R, Del Fabbro L, da Silva Antunes M, de Gomes MG, Goes AT, Boeira SP, Prigol M, Souza LC (2015) Chronic unpredictable mild stress decreases BDNF and NGF levels and Na (+), K (+)-Atpase activity in the hippocampus and prefrontal cortex of mice: antidepressant effect of chrysin. Neuroscience 19(289):367–380

Fu B, Xue LZ, Shi X, Jiang BH, Fang J (2007) Chrysin inhibits expression of hypoxia-inducible factor-1alpha through reducing hypoxia-inducible factor inhibiting its protein synthesis-1alpha stability inhibiting its protein synthesis. Mol Cancer Ther 6:220–226

Fu XM, Phan T, Patel PN, Jaskula-Sztul R, Chen H (2013) Chrysin activates Notch1 signaling and suppresses tumor growth of anaplastic thyroid carcinoma in vitro and in vivo. Cancer 119 (4):774–781

Galijatovic A, Otake Y, Walle UK, Walle T (1999) Extensive metabolism of the flavonoid chrysin by human Caco-2 and Hep G2 cells. Xenobiotica 29:1241–1256

Goes T, Jesse CR, Antunes MS, Ladd FL, Ladd C, Luchese N, Boeira SP (2018) Protective role of chrysin on 6-hydroxydopamine induced neurodegeneration a mouse model of Parkinson's disease: involvement of neuroinflammation and neurotrophins. Chem Biol Interact 279:111–120

Ha SK, Moon E, Kim SY (2010) Chrysin suppresses LPS-stimulated proinflammatory responses by blocking NF-kappaB and JNK activations in microglia cells. Neurosci Lett 485:143–147

Hadjmohammadi MR, Saman S, Nazari SJ (2010) Separation optimization of quercetin, hesperetin and chrysin in honey by micellar liquid chromatography and experimental design. J Sep Sci 33:3144–3151

He XL, Wang YH, Bi MG, Du GH (2012) Chrysin improves cognitive deficits and brain damage induced by chronic cerebral hypoperfusion in rats. Eur J Pharma 680:41–48

Hermenean A, Mariasiu T, Navarro-González I, Vegara-Meseguer J, Miuţescu E, Chakraborty S, Pérez-Sánchez H (2013) Hepatoprotective activity of chrysin is mediated through TNF-α in chemically-induced acute liver damage: an in vivo study and molecular modeling. Exp Ther Med 13(5):1671–1680. https://doi.org/10.3892/etm.2017.4181

Hermenean A, Mariasiu T, Navarro-Gonzalez I, Vegara-Meseguer J, Miutescu E, Chakraborty S, Perez-Sanchez H (2017) Hepatoprotective activity of chrysin is mediated through TNF-alpha in chemically-induced acute liver damage: an invivo study and molecular modeling. Exp Ther Med 13:1671–1680

Hu H, Barker A, Harcourt-Brown T, Jeffery N (2015) Systematic review of brain tumor treatment in dogs. J Vet Intern Med 29(6):1456–1463

Izuta H, Shimazawa M, Tazawa S, Araki Y, Mishima S, Hara H (2008) Protective effects of Chinese propolis and its component, chrysin, against neuronal cell death via inhibition of mitochondrial apoptosis pathway in SH-SY5Y cells. J Agric Food Chem 56:8944–8953

Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2011) Global cancer statistics. CA Cancer J Clin 61:69–90

Jiang Y, Gong FL, Zhao GB, Li J (2014) Chrysin suppressed inflammatory responses and the inducible nitric oxide synthase pathway after spinal cord injury in rats. Int J Mol Sci 15:12270–12279

Kalogeropoulos N, Yanni AE, Koutrotsios G, Aloupi M (2013) Bioactive microconstituents and antioxidant properties of wild edible mushrooms from the island of Lesvos, Greece. Food Chem Toxicol 55:378–385

Kandemir FM, Kucukler SE, Eldutar C, Gulcin I (2017) Chrysin protects rat kidney from paracetamol-induced oxidative stress inflammation apoptosis and autophagy: a multi-biomarker approach. Sci Pharm 85:4

Kandhare AD, Shivakumar V, Rajmane A, Ghosh P, Bodhankar SL (2014) Evaluation of the neuroprotective effect of chrysin via modulation of endogenous biomarkers in a rat model of spinal cord injury. J Nat Med 68:586–603

Kang MK, Park SH, Choi YJ, Shin D, Kang YH (2015) Chrysin inhibits diabetic renal tubulointerstitial fibrosis through blocking epithelial to mesenchymal transition. J Mol Med 93:759–772

Kang MK, Park SH, Kim YH, Lee EJ, Antika LD, Kim DY, Choi YJ, Kang YH (2017) Chrysin ameliorates podocyte injury and slit diaphragm protein loss via inhibition of the PERK-eIF2-α-ATF-CHOP pathway in diabetic mice. Acta Pharmacol Sin 38:1129

Karthikeyan S, Srinivasan R, AfaqWani S, Manoharan S (2013) Chemopreventive potential of chrysin in 7,12-dimethylbenz(a)anthracene-induced hamster buccal pouch carcinogenesis. Int J Nutr Pharmacol Neurol Dis 3:46–53

Khan MS, Devaraj H, Devaraj N (2011) Chrysin abrogates early hepatocarcinogenesis and induces apoptosis in N-nitrosodiethylamine-induced preneoplastic nodules in rats. Toxicol Appl Pharmacol 251:85–94

Khan R, Khan AQ, Qamar W, Lateef A, Tahir M, Rehman MU, Ali F, Sultana S (2012) Chrysin protects against cisplatin-induced colon. Toxicity via amelioration of oxidative stress and apoptosis: probable role of p38MAPK and p53. Toxicol Appl Pharmacol 258(3):315–329

Kumar S, Pandey AK (2013) Chemistry and biological activities of flavonoids: an overview. Sci World J 2013:162750

Li X, Huang JM, Wang JN, Xiong XK, Yang XF, Zou F (2015) Combination of chrysin and cisplatin promotes the apoptosis of Hep G2 cells by up-regulating P53. Chem Biol Interact 232:12–20

Li X, Wang JN, Huang JM, Xiong XK, Chen MF, Ong CN, Shen HM, Yang XF (2011) Chrysin promotes tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) induced apoptosis in human cancer cell lines. Toxicol In Vitro 25:630–635

Lirdprapamongkol K, Sakurai H, Abdelhamed S, Yokoyama S, Maruyama T, Athikomkulchai S, Viriyaroj A, Awale S, Yagita H, Ruchirawat S (2013) A flavonoid chrysin suppresses hypoxic survival and metastatic growth of mouse breast cancer cells. Oncol Rep 30:2357–2364

Liu GW, Xie AD, He XW, Da ML, Liang GQ, Yao JZ, Xiang CJ, Ming ZY (2016) Antiplatelet activity of chrysin via inhibiting platelet αIIbβ3-mediated signaling pathway. Mol Nut Food Res 60(9):1984–1993

Lo H, Wu MW, Pan SL, Peng CY, Wu PH, Wu WB (2012) Chrysin restores PDGF induced inhibition on protein tyrosine phosphatase and reduces PDGF signaling in cultured VSMCs. J Nut Biochem 23:667–678

Lotfi-Attari JY, Pilehvar-Soltanahmadi M, Dadashpour S, Alipour R, Farajzadeh S, Zarghami N (2017) Co-delivery of curcumin and chrysin by polymeric nanoparticles inhibit synergistically growth and hTERT gene expression in human colorectal cancer cells. Nutr Can 69:1290–1299

Loureiro SO, Heimfarth L, Scherer EB, Da Cunha MJ, De Lima BO, Biasibetti H, Pessoa-Pureur R, Wyse AT (2013) Cytoskeleton of cortical astrocytes as a target to proline through oxidative stress mechanisms. Exp Cell Res 319:89–104

Mani R, Natesan V (2018) Chrysin: sources, beneficial pharmacological activities, and molecular mechanism of action. Phytochemistry 145:187–196

Mantawy EM, El-Bakly WM, Esmat A, Badr AM, El-Demerdash E (2014) Chrysin alleviates acute doxorubicin cardiotoxicity in rats via suppression of oxidative stress inflammation and apoptosis. Eur J Pharmacol 728:107–118

Mantawy EM, Esmat A, El-Bakly WM, Salah ElDin RA, El-Demerdash E (2017) Mechanistic clues to the protective effect of chrysin against doxorubicin induced cardiomyopathy: plausible roles of p53, MAPK and AKT pathways. Sci Rep 7:4795

Manzolli ES, Serpeloni JM, Grotto D, Bastos JK, Antunes LMG, Barbosa F, Barcelos GRM (2015) Protective effects of the flavonoid chrysin against methyl mercury induced genotoxicity and alterations of antioxidant status invivo. Oxid Med Cell Longev 34:85–78

Medina JH, Paladini AC, Wolfman C, Levi de Stein M, Calvo D, Diaz LE, Pena C (1990) Chrysin (5,7-dihydroxy-flavone), a naturally-occurring ligand for benzodiazepine receptors, with anti-convulsant properties. Biochem Pharmacol 40:2227–2231

Mercan N, Kivrak I, Duru ME, Katircioglu H, Gulcan S, Malci S, Acar G, Salih B (2006) Chemical composition effects onto antimicrobial and antioxidant activities of Propolis collected from different regions of Turkey. Ann Microbiol 56:373–378

Mercer LD, Kelly BL, Horne MK, Beart PM (2005) Dietary polyphenols protect dopamine neurons from oxidative insults and apoptosis: investigations in primary rat mesencephalic cultures. Biochem Pharmacol 69:339–345

Missassi GC, Dos Santos BJ, De Lima RP, Villela A, Silva E, Martins FDC, De Grava KW (2017) Chrysin administration protects against oxidative damage in varicocele-induced adult rats. Oxid Med Cell Longev 25:55–76

Mohammadian FA, Abhari H, Dariushnejad A, Nikanfar Y, Zarghami N (2016) Effects of chrysin-PLGA-PEG nanoparticles on proliferation and gene expression of miRNAs in gastric cancer cell line. Iran J Cancer Prev 3:23–29

Nabavi SF, Braidy N, Habtemariam S, Orhan IE, Daglia M, Manayi A, Gortzi O, Nabavi SM (2015) Neuroprotective effects of chrysin: from chemistry to medicine. Neurochem Int 90:224–231

Naz S, Imran M, Rauf A, Orhan IE, Shariati MA, Iahtisham-Ul-Haq, IqraY, Shahbaz M, Qaisrani TB, Shah ZA, Plygun S, Heydari M (2019) Chrysin: pharmacological and therapeutic properties. Life Sci. 235:116797

O'Leary KA, De Pascual-Tereasa S, Needs PW, Bao YP, O'Brien NM (2004) Effect of flavonoids and vitamin E on cyclooxygenase-2 (COX-2) transcription. Mutat Res 551(1–2):245–254

Pan SY, Zhou SF, Gao SH et al (2013) New perspectives on how to discover drugs from herbal medicines: CAM's outstanding contribution to modern therapeutics. Evid Based Complement Alternat Med 2013:627375

Panche AN, Diwan AD, Chandra SR (2016) Flavonoids: an overview. J Nutr Sci 5:e47

Pasini F, Gardini S, Marcazzan GL, Caboni MF (2013) Buckwheat honeys: screening of composition and properties. Food Chem 141:2802–2811

Pichichero E, Cicconi R, Mattei M, Muzi MG, Canini A (2010) Acacia honey and chrysin reduce proliferation of melanoma cells through alterations in cell cycle progression. Int J Oncol 37:973–981

Pietta PG (2000) Flavonoids as antioxidants. J Nat Prod 63:1035–1042

Pushpavalli G, Veeramani C, Pugalendi KV (2010) Influence of chrysin on hepatic marker enzymes and lipid profile against D-galactosamine-induced hepatotoxicity rats. Food Chem Toxicol 48:1654–1659

Qi SM, Li Q, Jiang Q, Qi ZL, Zhang Y (2018) Chrysin inhibits lipopolysaccharide-induced inflammatory responses of macrophages via JAK-STATs signaling pathway. J Southern Med Univ 38:243–250

Rani N, Bharti S, Bhatia J, Nag TC, Ray R, Arya DS (2016) Chrysin, a PPAR-γ agonist improves myocardial injury in diabetic rats through inhibiting age-rage mediated oxidative stress and inflammation. Chem Biol Interact 250:59–67

Rauf A, Khan R, Raza M, Khan H, Pervez S, De Feo V, Maione F, Mascolo N (2015) Suppression of inflammatory response by chrysin a flavone isolated from *Potentilla evestita* Th, wolf, in silico predictive study on its mechanistic effect. Fitoterapia 103:129–135

Ravishankar DM, Salamah A, Attina R, Pothi TM, Vallance M, Javed HF, Williams EM, Alzahrani E, Vaiyapuri R (2017) Ruthenium-conjugated chrysin analogues modulate platelet activity thrombus formation and haemostasis with enhanced efficacy. Sci Rep 7:5738

Rehman MU, Tahir M, Khan AQ, Khan R, Lateef A, Oday-O-Hamiza, Qamar W, Ali F, Sultana S (2013) Chrysin suppresses renal carcinogenesis via amelioration of hyperproliferation, oxidative stress and inflammation: plausible role of NF-kB. Toxicol Lett 216:146–158

Rehman MU, Wali AF, Ahmad A, Shakeel S, Rasool S, Ali R, Rashid SM, Madkhali H, Ganaie MA, Khan R (2018) Neuroprotective strategies for neurological disorders by natural products: an update. Curr Neuropharmacol 17(3):247–267

Russo P, Del Bufalo A, Cesario A (2012) Flavonoids acting on DNA topoisomerases: recent advances and future perspectives in cancer therapy. Curr Med Chem 19:5287–5293

Salimi A, Pourahmad J (2018) Role of natural compounds in prevention and treatment of chronic lymphocytic leukemia polyphenols: prevention and treatment of human disease, 2nd edn. Academic Press, Londan, pp 195–203

Samarghandian S, Farkhondeh T, Azimi-Nezhad M (2017) Protective effects of chrysin against drugs and toxic agents. Dose Response 15:2

Sampath C, Holbik M, Krenn L, Butterweck V (2011) Anxiolytic effects of fractions obtained from *Passiflora incarnata* L. in the elevated plus maze in mice. Phytother Res 25:789–795

Sathiavelu J, Senapathy GJ, Devaraj GJ, Namasivayam N (2009) Hepatoprotective effect of chrysin on prooxidant antioxidant status during ethanol induced toxicity in female albino rats. J Pharm Pharmacol 6:809–817

Satyanarayana KK, Sravanthi IA, Shaker R, Selvaraj J (2015) Role of chrysin on expression of insulin signaling molecules. J Ayurveda Integrative Med 6:248

Seetharaman P, Gnanasekar S, Chandrasekaran R et al (2017) Isolation and characterization of anticancer flavone chrysin (5,7-dihydroxy flavone) producing endophytic fungi from *Passiflora incarnata* L. leaves. Ann Microbiol 67:321–331

Shen Y, Tian P, Li D, Wu Y, Wan C, Yang T, Chen L, Wang T, Wen F (2001) Chrysin suppresses cigarette smoke-induced airway inflammation in mice. Int J Clin Exp Med 8(2):2001–2008

Shen YP, Tian D, Li Y, Wan Yang CT, Chen L, Wang T, Wen F (2015) Chrysin suppresses cigarette smoke-induced airway inflammation in mice. Int J Clin Exp Med 8(2):2001–2008

Shoieb SMA, Esmat AE, Abdel-Naim AB (2018) Chrysin attenuates testosterone-induced benign prostate hyperplasia in rats. Food Chem Toxicol 111:650–659

Siddiqui A, Akhtar J, Uddin MSS, Khan MI, Khalid M, Ahmad M (2018) A naturally occurring flavone (Chrysin): chemistry, occurrence, pharmacokinetic, toxicity, molecular targets and medicinal properties. J Biol Active Prod Nat 8(4):208–227

Singh B, Singh D, Goel RK (2012) Dual protective effect of *Passiflora incarnata* in epilepsy and associated post-ictal depression. J Ethnopharmacol 139:273–279

Sirovina DN, Orsolic MZ, Koncic G, Kovacevic V, Gregorović G (2013) Quercetin vs chrysin: effect on liver histopathology in diabetic mice. Human Exp Toxicol 32:1058–1066

Souza LC, Antunes MS, Borges Filho C, Del Fabbro L, De Gomes MG, Donato M, Prigol SP, Jesse CR (2015) Flavonoid chrysin prevents age-related cognitive decline via attenuation of oxidative stress and modulation of BDNF levels in aged mouse brain. Pharmacol Biochem Behavior 134:22–30

Spilsbury A, Vauzour D, Spencer JPE, Rattray M (2012) Regulation of NF-kappa B activity in astrocytes: effects of flavonoids at dietary-relevant concentrations. Biochem Biophys Res Commun 418:578–583

Tahir M, Sultana S (2011) Chrysin modulates ethanol metabolism in Wistar rats: a promising role against organ toxicities. Alcohol Alcohol (Oxford, Oxfordshire) 46(4):383–392

Testai L, Martelli A, Cristofaro M, Breschi MC, Calderone V (2013) Cardioprotective effects of different flavonoids against myocardial ischaemia/reperfusion injury in Langendorff-perfused rat hearts. J Pharm Pharmacol 65:750–756

Tian SS, Jiang FS, Zhang K, Zhu XX, Jin B, Lu JJ, Ding ZS (2014) Flavonoids from the leaves of *Carya cathayensis* Sarg. Inhibit vascular endothelial growth factor-induced angiogenesis. Fitoterapia 92:34–40

Vedagiri A, Thangarajan S (2016) Mitigating effect of chrysin loaded solid lipid nanoparticles against amyloid β25-35 induced oxidative stress in rat hippocampal region: an efficient formulation approach for Alzheimer's disease. Neuropeptides 58:111–125

Villar IC, Galisteo M, Vera R, O'Valle F, García-Saura MF, Zarzuelo A, Duarte J (2004) Effects of the dietary flavonoid chrysin in isolated rat mesenteric vascular bed. J Vasc Res 41:509–516

Wadibhasme PG, Ghaisas MM, Thakurdesai PA (2011) Anti-asthmatic potential of chrysin on ovalbumin-induced bronchoalveolar hyperresponsiveness in rats. Pharm Biol 49:508–515

Walker E, Pacold M, Perisic O et al (2000) Structural determinations of phosphoinositide 3-kinase inhibition by Wortmannin, Ly294002, Quercetin, Myricetin, and Staurosporine. Mol Cell 6:909–919

Xiao J, Zhai H, Yao Y, Wang C, Jiang W, Zhang C, Simard AR, Zhang R, Hao J (2014) Chrysin attenuates experimental autoimmune neuritis by suppressing immuno-inflammatory responses. Neuroscience 262:156–164

Xue C, Chen Y, Hu DN, Iacob C, Lu C, Huang Z (2016) Chrysin induces cell apoptosis in human uveal melanoma cells via intrinsic apoptosis. Oncol Lett 12:4813–4820

Yao J, Jiang M, Zhang Y, Liu X, Du Q, Feng G (2016) Chrysin alleviates allergic inflammation and airway remodeling in a murine model of chronic asthma. Int Immunopharmacol 32:24–31

Yi P, Huang C, Fu Y, Wang J, Wu Z, Ru J, Zheng C, Guo Z, Chen X, Zhou W, Zhang W, Li Y, Chen J, Lu A, Wang Y (2018) Large-scale exploration and analysis of drug combinations. Bioinformatics (Oxford, England) 31(12):2007–2016

Yu XM, Phan T, Patel PN, Jaskula-Sztul R, Chen H (2013) Chrysin activates Notch1 signaling and suppresses tumour growth of anaplastic thyroid carcinoma invitro and invivo. Cancer 119:774–781

Zarzecki MS, Araujo SM, Bortolotto VC, De Paula MT, Jesse CR, Prigol M (2014) Hypolipidemic action of chrysin on Triton WR-1339-induced hyperlipidemia in female C57BL/6 mice. Toxicol Rep 1:200–208

Zeinali M, Rezaee SA, Hosseinzadeh H (2017) An overview on immunoregulatory and anti-inflammatory properties of chrysin and flavonoids substances. Biomed Pharmacother Biomed Pharmacother 92:998–1009

Zhang Z, Li G, Szeto SS, Chong CM, Quan Q, Huang C, Cui W, Guo B, Wang Y, Han Y, Michael Siu KW, Yuen Lee SM, Chu IK (2015) Examining the neuroprotective effects of protocatechuic acid and chrysin on invitro and invivo models of Parkinson disease. Free Radic Biol Med 84:331–343

Zheng W, Tao Z, Cai CL, Chen C, Zhang Q, Wang X, Ying W, Chen H (2017) Chrysin attenuates IL-1β-induced expression of inflammatory mediators by suppressing NF-κB in human osteoarthritis chondrocytes. Inflammation 40:1143–1154

Zou XQ, Peng SM, Hu CP, Tan LF, Yuan Q, Deng HW, Li YJ (2010) Synthesis, characterization and vasculoprotective effects of nitric oxide-donating derivatives of chrysin. Bioorg Med Chem 18:3020–3025

Honey and Its Potential Antibreast-Cancer Properties: Mechanistic Insights

20

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Abstract

Honey possesses different biological properties such as antioxidant, antiinflammatory, antimutagenic, antiatherogenic, antithrombotic, and wound healing that make it a potential anticarcinogenic agent capable of substituting conventional regimens that cause adverse side effects. Bioactive compounds such as polyphenols and vitamins found in honey are known to induce apoptosis, which serves as the principal mechanism for disposing off unwanted cells in the body. Honey has the potential to inhibit or disrupt initiation, promotion, and metastasis of carcinogenesis, especially in breast cancer which has been demonstrated in in vitro (cell line) and in vivo experimental breast cancer animal studies. Therefore, routine consumption of good quality honey may prevent different cancers, especially breast cancer in women.

Keywords

DMBA-induced breast cancer · MNU- induced breast cancer · Manuka honey · Tualang honey

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20.1 Introduction

Conventional therapies used to treat diseases like cancer are usually associated with adverse side effects that can seriously compromise the patient's quality of life. This necessitates searching for novel and less toxic alternative and complementary methods to treat such diseases. There has been an intensive search going on for chemotherapeutic agents derived from relatively safe foods or natural products. Honey is one such potential food-derived therapeutic candidate. Although the health-boosting effect of honey was known as early as 8000 years back, however, the first documented research into the health-promoting effect of honey was given by Van Ketel in 1892 who discovered its antimicrobial properties. Since then its antioxidant, antiinflammatory, antimutagenic, antiatherogenic, and antithrombotic effects, as well as wound-healing properties have been discovered (Bill 2018). Based on these properties of honey it was anticipated to have anticarcinogenic effects too. The much-anticipated anticarcinogenic effect of honey was finally explored recently by scientists in various cancers including breast cancer. In this chapter we will learn from various mechanistic experimental in vitro (cell lines) and in vivo (animal) studies, the role honey plays as a natural anticancer agent targeting the key hallmark features of breast carcinogenesis. The antiinflammatory and immunomodulatory potential of honey with special reference to the management of breast cancer will be also discussed. The potential of honey in preventing the toxic side effects of conventional cancer therapies and evidences in favor of the synergistic action of honey when used in adjunct with conventional breast cancer therapies will be also elaborated.

20.2 Biology of Breast Cancer

Breast cancer is a neoplastic change in the epithelial cells of the mammary tissue particularly in the epithelium of mammary ducts. It is the second leading cancer among women worldwide. About 1 in 8 (i.e., 12.4%) women are found to develop invasive breast cancer in the world (www.cancer.org/cancer/breast). In the United States alone, an estimated 276,480 new cases of invasive breast cancer and 48,530 new cases of noninvasive (in situ) cancer are expected to be diagnosed, and 42,170 deaths will probably occur in women by 2020 (www.cancer.org/cancer/breast). The incidence and death rate of breast cancer increase with age with two thirds of the breast cancers occurring in postmenopausal women 50 years or older. About 90–95% of the breast cancers are due to environmental or life style factors and only 5–10% have genetic factors. The environmental factors posing a risk for breast cancer include early menarche, late menopause, late-age marriages and nulliparity, hormone replacement therapy (HRT), Epstein Barr virus (EBV) infection, and radiation exposure (Tamimi et al. 2012). The genesis of the disease, however, involves a number of genetically predisposing genes, BRCA (breast cancer associated genes), PALB2 (partner and localizer of BRCA2) genes, ATM (ataxia telangiectasia mutated), CHEK2 (checkpoint kinase 2), tumor suppressors (TP53,

PTEN, p16) and oncogenes of the mitogen-activated protein kinase (MAPK), phosphoinositide-3-kinase (PI3K), and insulin-like growth factor 1 (IGF-1) signaling pathways (Cancer Genome Atlas Network 2012; Turnbull and Rahman 2008; Gabai-Kapara et al. 2014; Antoniou et al. 2014).

Breast cancers are of various histological types, viz., in situ ductal carcinoma (DCIS), in situ lobular carcinoma (LCIS), infiltrating ductal carcinoma (IDC), and inflammatory breast carcinoma (IBC). Traditionally, as per The American Society of Clinical Oncology (ASCO), patient age, TNM (tumor size, lymph node status, metastasis) clinical staging, tumor grade, and histology are the prognostic factors that are generally used, and recently molecular subtyping of breast tumors, viz., luminal A (ER+/HER2-), luminal B (ER+/HER2+), HER2-enriched (ER-/HER2+), triple negative (ER-/HER2-), and gene expression assays like Oncotype DX have been added in medical decision making and for prescribing treatment modalities (American Cancer Society 2020; Giuliano et al. 2017). Currently, surgery (mastectomy), chemo-radiotherapy, and hormone therapy (antiestrogens) remain the predominant treatment options for breast carcinomas especially for luminal A and luminal B subtypes, and trastuzumab (as targeted therapy) has been added to ḤER-2 enriched breast cancers. The triple-negative breast cancer (TNBC) molecular subtype, which does not respond to hormone therapy and for which no targeted therapy is available, still relies on radiotherapy and chemotherapy as the main treatment options. Despite these amazing advancements in treatment, the prognosis for patients with high-grade, locally advanced, and metastatic breast cancers still remains variable, possibly due to intrinsic and evolving heterogeneity responsible for the diverse tumor response to standard therapies. The resistance to chemotherapeutic drugs and relapse after chemotherapy still remain a challenge in breast cancer patients (Giuliano et al. 2017). Added to this, each of these conventional treatment strategies suffers from adverse side effects like nausea, vomiting, fatigue, hair loss, and skin changes. Additionally, long-term consumption of hormone therapy increases a patient's risk of developing uterine cancer and complications like blood clots. Also, immunotherapy used to treat HER-2+ve breast cancers can lead to immune-related adverse events like imbalances in immunological tolerance or autoimmune toxicities in different organs.

20.3 Chemical Constituents of Honey

Natural honey is produced by bees and contains over 200 compounds (Da Silva et al. 2016). The composition of honey varies with floral source, geographical origin, and the method of extraction. The basic makeup of honey consists mainly of water and sugars (75% monosaccharides: glucose and fructose; 10–15% disaccharides: sucrose, maltose, etc.) vitamins (vitamin B6, riboflavin, niacin, thiamine, etc.), minerals, enzymes, phenolics (flavonoids, phenolic acids), and pigments. The phenolic content of honey varies between 86 and 1141 mg/kg. The highest phenolic content is often found in Manuka honey, a monofloral honey derived from New Zealand; in Tualang honey, a multifloral honey from Malaysia; and in

Buckwheat honey, a monofloral honey obtained from several geographical sources. The lowest phenolic content is seen in two monofloral varieties: Gelam honey and Acacia honey. The major phenolic constituents present in honey include phenolic acids such as gallic acid, syringic acid, chlorogenic acid, caffeic acid, ellagic acid, p-coumaric acid, ferulic acid, and flavonoids such as chrysin, quercetin, kaempferol, catechin, galangin, luteolin, pinocembrin, pinobankskin, and myricetin (Wieczorek et al. 2014).

20.4 Bioavailability of Honey

Bioavailability is an important factor to be considered while deliberating a dietary substance for therapy. Studies on the bioavailability of the health boosting constituents, flavonoids and phenolic acids, from honey have shown that supplementation with 1.5 g/kg buckwheat honey produces significant increases in plasma phenolics 2 h after supplementation, and the level remained high for up to 6 h (Schramm et al. 2003). There are a few supporting factors that make the phenolics of honey easily bioavailable. Natural flavonoids occur in a glycoside form that upon hydrolysis to aglycones can be easily absorbed by enterocytes (intestinal absorptive cells). In the case of bee honey, the enzyme glycosidase present in the bee salivary gland makes the flavonoids of honey to be present in the aglycones form. These phenolic aglycones are then easily absorbed through the gut barrier in comparison to their corresponding glycosides. Therefore, it is assumed that honey flavonoids are effortlessly bioavailable than other natural flavonoids (Scalbert and Williamson 2000).

20.5 Honey in Breast Carcinogenesis

Carcinogenesis is a multistep process that grossly has been divided into three phases, viz., initiation, promotion, and metastasis. Generally, cancer-preventive agents may act as antipromoting agents intervening at the initiation or promotion stages of carcinogenesis. Honey, however, has the potential to inhibit or disrupt at each of the three stages of carcinogenesis, at least, in breast cancer. This has been demonstrated in in vitro (cell line) and in vivo experimental breast cancer animal studies. In in vivo studies, the effect of different honey types (Malaysian jungle Tualang honey (TH) and New Zealand Manuka honey(MH)) on breast cancers induced in female Sprague–Dawley rats using two different carcinogens, viz., 1-methyl-1-nitrosourea (MNU) and 7,12-dimethylbenz (alpha) anthracene (DMBA), were demonstrated (Kadir et al. 2013; Ahmed and Othman 2017; Ahmed et al. 2017) (Table 20.1). At varying strengths, honey (TH) decreased both tumor incidences and prolonged tumor latencies. These effects were probably due to the antioxidant and antimutagenic properties of honey which could inhibit carcinogenesis at the initiation stage. Honey is also known to slow down breast cancer development and metastasis as evident from these studies which reported decreased

Table 20.1 The anticarcinogenic effect of honey in breast cancer animal models

Honey type	Carcinogen-induced breast cancer animal model	Mode of action	References
Tualang honey (Malaysia)	MNU-induced breast cancer in Sprague–Dawley rats	• Reduced tumor incidence rate, prolonged tumor latency • Reduced tumor size, tumor weight and tumor volume, better histological grade of tumor, complete disappearance of some tumors • Reduced multiplicity	Ahmed and Othman (2017)
Tualang honey (Malaysia)	DMBA-induced breast cancer in Sprague–Dawley rats	• Reduced tumor size, tumor weight, and tumor volume • Improved histological grade of tumor • Increased apoptotic activity • Reduced level of angiogenesis	Kadir et al. (2013)
Multifloral honey	DMBA-induced breast cancer in rats	• Reduced tumor incidence rate • Reduced tumor size, tumor weight, and tumor volume • Reduced palpable tumor multiplicity	Takruri et al. (2017)
Tualang honey and Manuka honey	MNU-induced breast cancer rats	• Ameliorating hematological and serological parameters • Upregulation of apoptotic factors, caspase-9, apaf-1, p53 • Downregulation of antiapoptotic factor BCL-xL • Upregulation of immunomodulatory factors, INF-gamma INFR1 • Downregulation of inflammatory mediators, TNF-α, Cox-2 • Reduced estrogen signaling via E2 and ESR1	Ahmed et al. (2017)

MNU 1-methyl-1-nitrosourea, *DMBA* 7,12-dimethylbenz (alpha) anthracene

size, weight, and volume of tumor; decreased multiplicity; better histological grading; and complete disappearance of some tumors in the honey-treated group compared to the untreated group. Similar findings of honey modulating tumor latency, incidence, size, weight, volume, and multiplicity were investigated by Takruri et al. (2017) in DMBA-induced breast cancer rats following treatment with multifloral honey.

20.6 Molecular Validation of Honey as a Potential Anticancer Agent in Breast Cancer

The development of cancer is a complex process beginning with the alteration of the genetic material of healthy cells which, if not repaired, can result in development of tumors. According to Hanahan and Weinberg (2011), there are some common hallmark features leading to the progression of a normal cell to a neoplastic state. These include replicative immortality and sustained proliferative signaling, evading growth suppressors, failure of apoptosis, angiogenesis induction, and activating invasion and metastasis. The anticancer potential of honey targeting the key hallmark features of breast carcinogenesis will be deliberated here as demonstrated in various in vivo and in vitro breast cancer studies (Fig. 20.1).

20.7 Honey and Decreased Cell Viability and Cell Proliferation in Breast Cancer

Aberrant proliferation is a defining feature of tumor cells and, therefore, a key target for both conventional and novel chemotherapies. Many chemotherapeutic drugs act usually by inhibiting the cell cycle in S and M phases. However, studies have shown the treatment of cell lines with honey causes arrest of cells in the G0/G1 phase. Multiple honey types have been tested for their in vitro effects on cell proliferation in various cancer cell lines using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay, 5-bromodeoxy uridine (BrDu) labeling index, and flow cytometry analysis. It has been found that the effect of honey on cell proliferation varies with dosage, composition, and the type of cell lines investigated. Generally 6–12% honey has been tested for antiproliferation in various cancer cell lines;

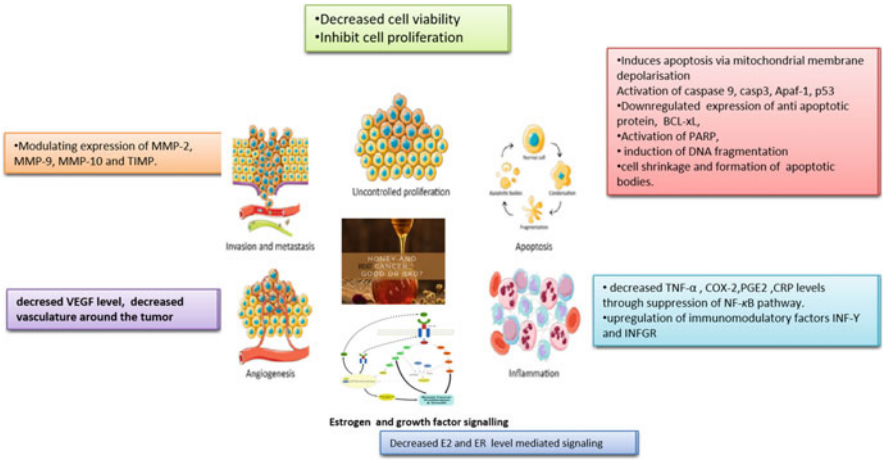

Fig. 20.1 Effect of honey on key hallmark features of breast carcinogenesis

however, maximum antiproliferative effect is reported at 6% honey as evident from flow cytometry analysis which showed an accumulation of a cell population behind the G1 peak and a significant low S phase fraction at this percentage.

Honey from geographically diverse regions with varied compositions has been tested for antiproliferation in varied molecular subtypes of breast cancer (Table 20.2). For example, Manuka honey (UMF 10+) was shown to inhibit cell proliferation in a dose- and time-dependent manner in the human ER+ve breast cancer cell line, MCF-7 (Fernandez-Cabezudo et al. 2013). At 5% final concentration of Manuka honey, incubation of MCF-7 cells was shown to inhibit proliferation by 40% after 24 h and 60% after 72 h (using CellTiter-Glo Luminescent cell viability assay). Tualang honey (TH) was found to have a selective antiproliferative effect in human ER+ve breast cancer cell lines (MCF-7) and TNBC breast cancer cell lines (MDA-MB-231) without any cytotoxic effects on MCF-10A, a normal breast cell line. The effect of TH when used in combination with 4-hydroxytamoxifen was found selectively additive on breast cancer cells without affecting normal cells (Fauzi et al. 2011). According to Fauzi and Yaacob (2016), Tualang honey caused G2M phase arrest in the MCF-7 breast cancer cell line while as in the MDA-MB-231 breast cancer cell line arrest occurred in the S phase. Similarly, Sidr and Wild, two varieties of natural honey, were tested for antiproliferation in the MDA-MB-231 cell line. At 1% concentration (v/v), both honey types were found to reduce cell viability (viable cells were 52.6% and 8.1%, respectively) 48 h posttreatment using the MTT assay (Almeer et al. 2018). Thyme honey when tested for its antiproliferative effect in the breast cancer cell line MCF-7; it was found that at a low concentration of 125 μg/Ml it reduced cell viability by 10% (Tsiapara et al. 2009). At low concentration, Greek honey upon evaluation had shown a weak osteogenic effect and reduced cell viability in MCF-7 cells. The reduced cell proliferation was due to the effect of Greek honey on mitochondrial inducing apoptosis (Anna et al. 2009). Indian honey upon evaluation for cytotoxic activity against MCF-7 breast cancer cells was found to retard cancer cell growth in a dose-dependent manner along with increase in cells at the sub-G1 phase (Jaganathan et al. 2010a). Subsequently, the effect of Indian honey on Ehrlich ascites carcinoma, an impulsive murine mammary adenocarcinoma present in the ascites form, was studied. It was found that the growth of Ehrlich ascites carcinoma was significantly inhibited up to 39.98% by honey containing a higher phenolic content of about 25% (v/v) (Jaganathan et al. 2010b). The antiproliferative effect of crude honey from Egyptian floral sources (*Ziziphus spina*-Christi, *Citrus reticulate*, and *Cassia javanica*) was also demonstrated in HTB-26 breast tumor cell lines (El-Gendy 2010). Fir honey, unlike other honey types, showed an augmentary effect on MCF-7 cell proliferation (Van der Woude et al. 2003). The augmentary effect on cancer cells has been associated with quercetin, a phenolic constituent present in Fir honey.

The in vitro antiproliferative effect of honey was further validated in in vivo experimental breast cancer animal studies (Table 20.1). The anticancer effects in 1-methyl-1-nitrosourea (MNU) (80 mg/kg)-induced breast cancer mice were demonstrated following treatment with Tualang honey (TH) in the treated group versus the nontreated group. The honey treatment which started 1 week prior to

Table 20.2 Effect of honey on key hallmark features of breast carcinogenesis

Honey type	Experimental setting (cell line/ animal)	Mode of action	References
Antiproliferative effect			
Manuka honey (UMF 10+) New Zealand	MCF-7	• Inhibited cell proliferation	Fernandez-Cabezudo et al. (2013)
Tualang honey (Malaysia)	MCF-7 MDA-MB-231 MCA-10A	• Decreased cell viability • Inhibited cell proliferation • Caused G2M phase arrest in MCF-7 • Caused S-phase arrest in MDA-MB-231	Fauzi et al. (2011), Fauzi and Yaacob (2016)
Sidr and wild varieties of natural honey	MDA-MB-231	Decreased cell viability	Almeer et al. (2018)
Thyme honey (Greece)	MCF-7	Decreased cell viability	Tsiapara et al. (2009)
Greek honey	MCF-7	• Decreased cell viability • Inhibited cell proliferation	Anna et al. (2009)
Indian honey	MCF-7 Ehrlich ascites carcinoma	• Decreased cell viability • Inhibited cell proliferation by causing S phase arrest • Caused growth arrest, inhibited cell proliferation	Jaganathan et al. (2010a), Jaganathan et al. (2010b)
Egyptian honey	HTB-26	• Antiproliferation	El-Gendy (2010)
Fir honey	MCF-7	• Promoted cell proliferation	Van der Woude et al. (2003)
Antiapoptotic effect			
Manuka honey (UMF 10+) New Zealand	MCF-7	• Activation of caspase 3, by caspase 9 • Activation of PARP • Induction of DNA fragmentation • Decreased expression of antiapoptotic protein BCL-xL	Fernandez-Cabezudo et al. (2013)
Acacia honey (Malaysia)	MCF-7	• DNA fragmentation • Cell shrinkage and formation of apoptotic bodies	Salleh et al. (2017)
Tualang honey (Malaysia)	MCF-7 MDA-MB-231 HeLa cells	• Induced apoptosis via mitochondrial membrane depolarization • Activation of caspase 3/7, 8, 9 • Upregulation of p53, p21, FADD in MCF-7 • Upregulation of TRADD, FADD, and p21 in MDA-MB-231	Fauzi et al. (2011), Fauzi and Yaacob (2016)

(continued)

Table 20.2 (continued)

Honey type	Experimental setting (cell line/ animal)	Mode of action	References
Tualang honey (Malaysia) and Manuka honey (New Zealand)	MNU-induced breast cancer in Sprague–Dawley rats	• Upregulated caspase-9, apaf-1, p53 • Downregulated expression of antiapoptotic protein BCL-xL	Ahmed et al. (2017), Ahmed and Othman (2017)
Decreased estrogen signaling			
Tualang honey (Malaysia	MNU-induced breast cancer in Sprague–Dawley rats	• Decreased serum E2 • Decreased expression of ER at tissue level	Ahmed and Othman (2017)
Antiinflammatory and immunomodulatory effect			
Tualang honey and Manuka honey Honey	MNU-induced breast cancer in Sprague–Dawley rats	• Downregulated expression of proinflammatory cytokines TNF-α and proinflammatory proteins COX-2 which produce prostaglandins through suppression of NF-κB pathway • Upregulated immunomodulatory factors INF- and INFGR • Digestion of honey produces SCFA which has immunopotentiating activity • Nigero-oligosaccharides in honey has immunomodulatory action	Ahmed et al. (2017), Leong et al. (2012), Hussein et al. (2012)
Natural unprocessed honey	Human subjects (12)	• Decreased plasma PGE2	Al-Waili and Boni (2003)
Natural honey	Human subjects (8)	• Increased blood monocyte count, lymphocytes, and eosinophils • Reduced plasma CRP levels	Al-Waili (2004)
Antiangiogenic effect			
Tualang honey (Malaysia)	DMBA-induced breast cancer in Sprague–Dawley rats	• Decreased VEGF level, decreased vasculature around the tumor	Kadir et al. (2013)
Antiinvasive and antimetastasis effects			
Sidr and wild natural honey	TNBC cell line	• Decreased MMP-2, MMP-9, MMP-10 • Upregulation of TIMP • Decreased PI3K/AKT/ mTOR pathway	Almeer et al. (2018)
Wild flower Crocatia honey	Mammary carcinoma (CBA mice)	• Antimetastatic action when used preventatively	Orsolic and Bašic (2004)

tumor induction and continued for 120 days resulted in decreased incidence (mean 76.6% vs. 100%), multiplicity (mean 2.5 vs. 4 tumor masses per rat), reduced size (mean 0.41 cm vs. 1.47 cm), and weight of the tumor mass (mean 1.22 g vs. 3.23 g) (Ahmed and Othman 2017). In another such study the effect of Malaysian jungle Tualang honey (TH) on the growth of 7,12-dimethylbenz(alpha) anthracene (DMBA)-induced breast cancer in rats was studied. Histological evaluation indicated that honey-treated rats were of better grade (grade 1 and 2) tumors compared to nontreated ones which were of grade 3 (Kadir et al. 2013).

20.8 Honey and Induction of Apoptosis in Breast Cancer

Apoptosis/programmed cell death is recognized as the principal mechanism by which unwanted cells are disposed of in our body. It works as a waste cleaner for unwanted cells in our body. It involves complex mechanisms and numerous molecules especially proteolytic enzymes called caspases. Apoptosis is brought about by two main pathways: (1) mitochondrial-mediated caspase-9-dependent intrinsic apoptotic pathway and (2) FADD or FASLG-activated caspase-8/extrinsic apoptotic pathway. Regulation of apoptosis is important especially in cancer pathogenesis as failure to undergo apoptosis results in an uncontrolled increase in cancerous cells. The regulation is usually brought about by maintaining a critical balance between proapoptotic and antiapoptotic factors/proteins and by regulating the expression or activation of initiator and executive/ effector caspases. Bioactive compounds such as polyphenols and vitamins found in honey are known to induce apoptosis. Presently apoptosis is considered one of the markers in drug design especially in treating cancers.

Multiple honey types of varied compositions have been tested at varied concentrations for studying apoptosis as demonstrated in various breast cancer cell lines and mammary cancer animal model studies. Manuka honey (New Zealand) was shown to induce late apoptotic events in a time- and dose-dependent manner in human breast cancer (MCF-7) cells following incubation with 0.3–5% of Manuka honey (UMF 10+) for 12, 24, and 48 h, respectively. The mechanism of action suggested was the intrinsic apoptotic pathway, which involves activation of the executioner caspase-3 by the initiator caspase-9. In addition, apoptosis was also associated with DNA fragmentation, PARP activation, and decreased expression of the antiapoptotic protein, Bcl-2 (Fernandez-Cabezudo et al. 2013). In another report, the apoptotic effect of Acacia honey (from Malaysia) in breast adenocarcinoma cell line (MCF-7) was demonstrated by the TUNEL assay (for observing DNA fragmentation) and live cell imaging (for observing cell shrinkage and formation of apoptotic bodies). Cell shrinkage, one of the apoptotic features, was shown as early as 2 h followed by formation of apoptotic bodies 6 h posttreatment (Salleh et al. 2017).

Again, Tualang honey (Malaysia) was found to be cytotoxic as it induced cell death in a time- and dose-dependent manner following incubation of breast cancer cell lines, MDA-MB-231, MCF-7, and HeLa cells (Fauzi et al. 2011) with IC50 dose of honey for each cell line (2.8% for MDA-MB-231, 2.4% for MCF-7, and 2.4% for

HeLa cells) for 6, 24, 48 and 72 h. The cells showed maximum percentages of apoptosis after 48 h (51.2% for MDA-MB-231) and 72 h (55.6% for MCF-7 and 56.2% for HeLa cells). This process was analyzed by flow cytometry followed by staining with annexin V fluorescent antibody and propidium iodide. Furthermore, activation of caspase-3/7 and caspase-9 of the mitochondrial apoptotic pathway in all three types of cells was observed by the green fluorescence assay after 6 h incubation with an IC50 dose of honey. The effect was found selective because when a normal breast epithelial cell line (MCF-10A) was incubated with honey at the same dose and for the same time, none of these effects were observed. Furthermore, induction of apoptosis upon treatment with honey in the MCF-7 cell line was associated with upregulation of p53, p21, and FADD, and upregulation of TRADD, FADD, and p21 was associated with the MDA-MB-231 cell line in its mode of action (Fauzi and Yaacob 2016). However, the exact mechanism of apoptosis was further elucidated in in vivo breast cancer studies.

20.9 Mechanism of Apoptosis

According to Ahmed et al. (2017), systemic administration of honey (TH and MH) in in vivo breast cancer models induces apoptosis by increasing the expression of proapoptotic proteins (apaf-1, caspase-9, and p53) and decreasing the expression of antiapoptotic proteins (bcl-xL 1) in its mechanism of action.

20.10 Propapoptotic Proteins (Apaf-1, Caspase-9, and p53)

Apoptotic protease activating factor-1 (apaf-1), of caspase-9 apoptotic pathway, is a tumor suppressor gene. Loss of Apaf-1 expression helps tumor cells in evading immune attack-induced death and/or caspase-9 mediated apoptosis, resulting in metastasis. p53, another well-known tumor suppressor gene has apaf-1 as an essential downstream target to regulate the intrinsic apoptotic pathway. Studies have revealed that honey (TH and MH) as therapeutic agents act against breast cancer by modulating p53 expression which in turn has a potentiating effect on apaf-1 and caspase-9 expression of the intrinsic apoptotic pathway resulting in a slower tumor growth rate. The possible mechanism suggested is that honey (TH and MH) induced apoptosis through multiple signaling pathways that converge on the mitochondria to cause the release of cytochrome c. Cytochrome c then binds to apaf-1, dATP/ATP, and procaspase-9 to form a cytochrome c–apaf-1–caspase-9 complex, called the apoptosome. The apoptosome enables enzymatic self-activation of caspase-9 that subsequently activates procaspase-3 for bringing about apoptosis/cell death.

20.11 Antiapoptotic Proteins (Bcl-xL 1)

Studies have reported the overexpression of the antiapoptotic protein Bcl-xL in breast cancer patients, which has been associated with metastasis and worse prognosis. The decreased bcl-xL expression observed in honey [TH and MH]-treated tumors, therefore, suggests the anticancer effect of honey as evident by lowered tumor cell proliferation and increased apoptosis in the treated group. Bcl-xL exerts its anticancer effect through the loss of the mitochondrial outer membrane integrity by blocking both mitochondrial swelling and membrane hyperpolarization. Bcl-xL as an antiapoptotic protein inhibits the mitochondrial intrinsic apoptotic pathway; therefore, decreased expression of it following treatment with honey promotes apoptosis through the increased expression of mitochondrial pathway proteins, caspase-9, p53, and apaf-1, as observed in vivo studies (Ahmed et al. 2017).

20.12 Honey and Decreased Estrogen Signaling in Breast Cancer

In breast, estrogen (E2) promotes cell proliferation and suppresses apoptosis by directly modulating the genetic expression and, thus, is considered a crucial target in breast cancer treatment. Estrogen receptors (ERs) on binding to estrogens dimerize and then translocate into the nuclei where they bind estrogen-response elements (EREs), resulting in transcription and translation in the targeted tissue. Higher serum estradiol (E2) levels are associated with an increased risk of breast cancer in postmenopausal women. Honey (TH), similar to other chemopreventive drugs, binds to ER and disrupts receptor dimerization and its nuclear localization, hindering this signaling pathway. This was illustrated in experimental honey-treated breast cancer animals in which decreased estrogen E2 at the serum level and decreased ER1 at the tissue level were found. Honey, therefore, behaves as a natural estrogen-lowering agent that shrinks breast cancer masses (Ahmed and Othman 2017). Honey from various floral sources exert estrogen antagonistic effects only at low concentrations (0.2–5 μg/mL) and instead have agonist effects at high concentrations (20–100 μg/mL) (Bill 2018). It is, therefore, postulated that low-dose administration of honey on a long-term basis can have protective effects against the process that converts normal cells into tumor cells, at least, in breast cancers.

20.13 Honey and Its Antiinflammatory (TNF-α, COX-2, PGE2, CRP) and Immunomodulatory Effects (IFN-γ, IFNGR1) in Breast Cancer

Inflammation is a biological response in many pathological processes and is brought about by inflammatory cytokines and inflammatory proteins. Cytokines released from inflammatory cells at the site of damage can trigger angiogenesis or stroma proliferation, while as neutrophils, brought through blood, by releasing reactive oxygen species (ROS) can cause damage to the surrounding tissues and promote

tumor-initiating mutations. Inflammation is, therefore, associated with all stages of carcinogenesis, from initiation and progression, to invasion and metastasis, and has been considered as another hallmark feature of cancer. Tumor necrosis factor alpha (TNF-α) is one such inflammatory cytokine produced by macrophages/monocytes during acute inflammation and is responsible for a diverse range of signaling events within cells, leading to necrosis or apoptosis. Investigations strongly suggest that the expression of TNF-α in breast tumors actually promotes tumor growth. The higher expression of TNF-α in inflammatory breast carcinoma was found to be associated with increasing tumor grade and the metastatic behavior of breast carcinomas. Animal studies have revealed that supplementation of honey (TH and MH) causes lowering of the monocyte levels in blood with consequent decreased TNF-α concentrations at the serum level and decreased expression of it in tumor specimens of treated animal models (Ahmed et al. 2017).

COX-2 is another proinflammatory protein that is over expressed in breast cancer and might be a crucial therapeutic target in breast cancer. It leads to the destruction of the basal membrane and the formation of new blood vessels allowing tumor growth. In vivo breast cancer studies have revealed that honey (TH and MH) treatment may inhibit COX-2 pathway cell proliferation-related transcriptional programs in breast carcinomas. This mechanism was validated by the reduced tumor size and lower tumor multiplicity observed in honey-treated groups. Phenolic acids and polyphenols present in honey have been reported to be responsible for the antiinflammatory activity induced by the COX-2 pathway. The proposed mechanism by which honey may inhibit COX-2-induced inflammation in carcinogenesis is through suppression of the NF-κB pathway. COX-2 expression is regulated by TNF-α and decreased expression of both inflammatory cytokines following treatment with honey validate the hindering of this inflammatory pathway by honey (Ahmed et al. 2017).

Studies have also shown interferon gamma (IFN- γ), an immune-potentiating agent, favors activation of the mitochondrial-mediated apoptotic pathway in breast cancer cell lines by interacting with its receptor, interferon gamma receptor 1 (IFNGR1). The reduced expression of IFNGR1 is associated with poor prognosis and more aggressiveness of breast cancer. This emphasizes the importance of the expression of IFNGR1 as a crucial therapeutic target in breast cancer prevention. According to Ahmed et al. (2017), TH and MH treatments cause a higher expression of IFN-γ at the serum level and its binding receptor IFNGR1 at the tissue level in breast cancer animal models. Honey, therefore, as a novel immune potentiator induces IFN-γ and IFNGR1 expression to potentiate IFN-γ activities that induce apoptosis in breast tumor cells. Furthermore, it has been shown that digestion of honey produces short-chain fatty acids (SCFA) that arouse immunomodulatory actions (Leong et al. 2012). Additionally, nonsugar ingredients and a sugar, nigero-oligosaccharides (NOS), present in honey have been reported to have immune-potentiating effects (Hussein et al. 2012).Human clinical trial studies have demonstrated that natural honey treatment reduces plasma levels of key inflammatory proteins. In one such study on 12 human subjects (9 males, 3 females), consumption of natural unprocessed honey (1.2 g/kg body weight dissolved in

250 mL of water) caused 14% reduction in plasma prostaglandin, PGE2, levels only 1 h postingestion. The participants upon continued consumption of honey at the same doses for 15 days decreased the plasma PGE2 level by 63% (Al-Waili and Boni 2003). In another human study carried out on 8 subjects, the inflammatory protein CRP was shown to be reduced by 7% after 15 days consumption of 75 g of honey dissolved in 250 ml of water (Al-Waili 2004).

20.14 Honey and Its Antiangiogenic Effect in Breast Cancer

Angiogenesis is the process of formation of new blood vessels, which facilitates tissue growth by providing nutrition and oxygen to tissues. This process is important both in wound healing and in the development of malignant tumors, the latter often being described as a wound that does not heal. Honey is well known to promote angiogenesis in normal cells with a varied response at different concentrations. It has been suggested that at low concentrations (0.015–6.2%), honey has proangiogenic effects, which disappears at higher concentrations (>12.5%). Honey has been shown to decrease VEGF formation at high concentrations while PGE_2 can induce VEGF expression, leading to increase in tumor angiogenesis. In the rat air pouch model of inflammation, honey has been shown to inhibit the angiogenic agents PGE_2 and VEGF (Eteraf-Oskouei et al. 2014). In 7,12-dimethylbenz(α) anthracene (DMBA)-induced breast cancer rats, Tualang honey, at concentrations as low as 0.2 g/kg, was shown to significantly reduce VEGF levels and the vasculature around the tumor (Kadir et al. 2013).

20.15 Honey and Its Antiinvasive and Antimetastatic Effect in Breast Cancer

Metastasis is the most destructive feature of cancer and consists of a highly complex process involving a variety of molecules, such as matrix metalloproteinases (MMPs), extracellular-degrading proteases, etc. MMPs are important contributors to the invasive and metastatic properties of cancer cells; their expression (especially MMP-2 and MMP-9) has been elevated in a range of carcinomas. However, MMP activity is inhibited by a group of structurally related, endogenous inhibitors known as tissue inhibitors of metalloproteases (TIMPs). In a study carried out by Almeer et al. (2018), the antimetastatic effect of two varieties of natural honey (Sidr and Wild) was observed possibly by modulating the gene expression of MMPs and TIMPs in triple-negative breast cancer.

In an in vivo experiment carried out in mammary carcinoma CBA mice, treatment with wildflower honey from crocatia was shown to have a significant antimetastatic effect when used preventatively before tumor cell inoculation (Orsolic and Bašic 2004).

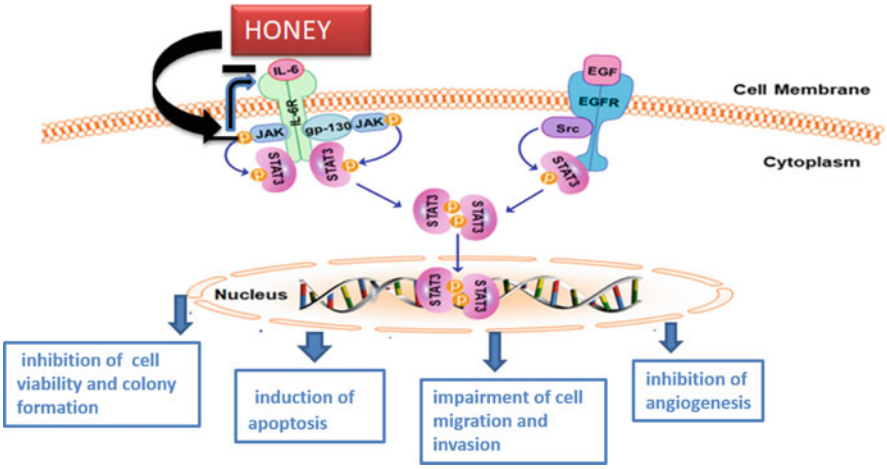

Fig. 20.2 The interleukin-6 (IL-6)/STAT3 signaling pathway. The figure highlights its central role in regulating the expression of multiple genes and the capacity of Manuka honey (MH) to inhibit pY-STAT3, thereby suppressing this signaling pathway

20.16 Honey Targeting IL-6/STAT3 Signaling in Breast Cancer

Although the antitumor properties of honey against several cancers have been demonstrated, the underlying mechanism and molecular targets of this activity were unknown. Studies have demonstrated a pivotal role for signal transducer and activator of transcription 3 (STAT3) in the initiation, progression, metastasis, and immune evasion in breast cancer especially its TNBC molecular subtype in which STAT3 is usually over expressed. According to Aryappalli et al. (2017), the IL-6/STAT3 signaling pathway is one of the earliest potential targets in human breast cancers that are affected by Manuka honey (MH). At low concentrations of MH (0.03–1.25% w/v), a rapid reduction in tyrosine-phosphorylated STAT3 (pY-STAT3) was reported in MDA-MB-231 and MCF-7 breast cancer cell lines. The inhibition of pY-STAT3 coincided with decreased interleukin-6 (IL-6) production. The possible mechanism by which inhibition of the IL-6/STAT3 signaling pathway by Manuka honey exhibits its anticancer effect in MDA-MB-231 breast cancer cells is via inhibition of cell viability and colony formation, induction of apoptosis, impairment of cell migration and invasion, and inhibition of angiogenesis (Fig. 20.2).

20.17 Honey for Chemoprevention and as an Adjunct to Anticancer Drugs in Breast Cancer

The adverse side effects of conventional chemotherapeutic treatments can severely compromise the quality of life for patients. Therefore, novel therapies which can prevent progression to malignancy, reduce the required dosage of conventional

drugs, or lessen the severity of adverse effects are always welcomed as they are of considerable benefit. Honey is one such potential candidate. That honey can be used in breast cancer for chemoprevention was demonstrated in an experimental model of carcinogenesis in which Tualang and Manuka honey administered to rats at a dose of 1 g/kg body weight a week before the induction of breast carcinogenesis with *N*-methyl-*N*-nitrosourea (MNU) and continued for 120 days significantly inhibited tumor development and angiogenesis (Othman et al. 2016).

Chemotherapeutic drugs are known for acting indiscriminately on both cancerous and healthy cells and are, therefore, toxic to the biological system. Honey, as an adjuvant to conventional anticancer drugs, can significantly reduce the dosages of chemotherapies, thereby reducing their toxicities. For example, in cell culture studies, the cytotoxic effect of the anticancer drug 4-hydroxytamoxifen at a final concentration of 10 μM was reduced by consuming 1% Tualang honey. Tualang honey was shown to selectively enhance the tamoxifen-induced anticancer activity in the estrogen receptor–positive (ER+ve) MCF-7 and ER-ve MDA-MB-231 human breast cancer cell lines with less toxic effect of the drug in normal breast cancer cells, MCF-10A. These results suggest that Tualang honey increases the effectiveness of tamoxifen against cancer cells as well as protects normal healthy cells from its toxic effects (Yaacob and Ismail 2014). Honey probably does this by increasing the expression of DNA repair proteins Ku70 and Ku80 in MCF-10A cells that confer normal cells increased protection against the toxic effects of tamoxifen.

Manuka honey, in general, has been shown to reduce the toxicity of the anticancer drug paclitaxel in mice. In a study, in C57BL/6 mice upon administration of biweekly intravenous injections of either 50% Manuka suspension or 10 mg/kg paclitaxel, or a combination of both treatments, the number of caspase-3-positive cells was the highest in the tumors from the mice treated with the combination of Manuka with paclitaxel than either drug alone. The results suggest that administration of Manuka honey together with the standard chemotherapeutic drug may decrease its cytotoxic side effects by increasing efficacy at low doses (Fernandez-Cabezudo et al. 2013). In another study, a mixture of honey bee products (honey, royal jelly, pollen grains) ameliorated the genotoxic effects of 20 mg/kg body weight of the anticancer drug cyclophosphamide in mice (Fahmy et al. 2015). Cisplatin is another common chemotherapeutic drug known for its nephrotoxic side effects. In rats, it has been found that the oral administration of crude honey at 500 mg/kg per day for 1 week prior to and 3 days after the administration of cisplatin reduced the nephrotoxicity of this drug. The possible mechanism suggested is through suppression of NF-kB activation by honey (Hamad et al. 2015).

Honey also has been found protective against radiation-induced mucositis and radiotherapy-induced skin reactions in chemotherapy patients (Bardy et al. 2008). Furthermore, a combination of honey (TH) and anastrozole is known to be more efficacious than anastrozole alone in decreasing breast BPE (background parenchymal enhancement) in breast cancer patients (Hizan et al. 2018).

20.18 Conclusion

Overall, evidence from cell culture and animal studies look promising for honey as a novel chemoprevention drug. The low cost and high availability of honey suggest it to be a good alternative chemopreventive and an adjunct drug to conventional breast cancer drugs keeping in view the healthcare cost containment issues and limited resources especially in developing countries. Based on experimental findings, we propose long-term prophylactic use of honey in low doses as a means for reducing breast cancer incidence, as a complementary and alternative antibreast cancer drug, and as an adjunct to conventional therapies in reducing the adverse effects of conventional chemotherapeutic drugs. Nevertheless, extended research especially human clinical trial studies are still required to provide the best evidence in support of the health-promoting effect of honey in breast cancer patients.

References

Ahmed S, Othman NH (2017) The anti-cancer effects of Tualang honey in modulating breast carcinogenesis: an experimental animal study. BMC Complement Alternat Med 17:208

Ahmed S, Sulaiman SA, Othman NH (2017) Oral administration of Tualang and Manuka honeys modulates breast cancer progression in Sprague-Dawley rats. Evid Based Complement Alternat Med 2017:5904361, 15 pages

Almeer R, Alqarni A, Alqattan S et al (2018) Effect of honey in improving breast cancer treatment and gene expression modulation of MMP and TIMPs in triple negative breast cancer cells. Pak J Zool 50:1999–2007

Al-Waili NS (2004) Natural honey lowers plasma glucose, C-reactive protein, homocysteine, and blood lipids in healthy, diabetic, and hyperlipidemic subjects: comparison with dextrose and sucrose. J Med Food 7:100–107

Al-Waili NS, Boni NS (2003) Natural honey lowers plasma prostaglandin concentrations in normal individuals. J Med Food 6:129–133

American Cancer Society (2020) Breast cancer facts & figures. www.cancer.org/cancer/breast-cancer

Anna VT, Mari J, Ioanna C, Konstadia G, Tiina T, Vesa V, Paraskevi M (2009) Bioactivity of Greek honey extracts on breast cancer (MCF-7), prostate cancer (PC-3) and endometrial cancer (Ishikawa) cells: profile analysis of extracts. Food Chem 116:702–708

Antoniou AC, Casadei S, Heikkinen T et al (2014) Breast-cancer risk in families with mutations in PALB2. N Engl J Med 371:497–506

Aryappalli P, Al-Qubaisi SS, Attob S, George JA et al (2017) The IL-6/STAT3 signaling pathway is an early target of Manuka honey-induced suppression of human breast cancer cells. Front Oncol 7:167

Bardy J, Slevin NJ, Mais KL, Molassiotis AA (2008) Systematic review of honey uses and its potential value within oncology care. J Clin Nurs 17:2604–2623

Bill G (2018). www.foodforbreastcancer.com

Cancer Genome Atlas Network (2012) Comprehensive molecular portraits of human breast tumours. Nature 490:61–70

Da Silva PM, Gauche C, Gonzaga LV, Costa ACO, Fett R (2016) Honey: chemical composition, stability and authenticity. Food Chem 196:309–323

El-Gendy MMA (2010) In vitro, evaluation of medicinal activity of Egyptian honey from different floral sources as anticancer and antimycotic infective agents. J Micro Biochem Technol 2:118–125

Eteraf-Oskouei T, Najafi M, Gharehbagheri A (2014) Natural honey: a new and potent anti-angiogenic agent in the air-pouch model of inflammation. Drug Res (Stuttg) 64:530–536
Fahmy MA, Hassan NHA, El-Fiky SA, Elalfy HGA (2015) Mixture of honey bee products ameliorates the genotoxic side effects of cyclophosphamide. Asian Pac J Trop Dis 5:638–644
Fauzi AN, Norazmi MN, Yaacob NS (2011) Tualang honey induces apoptosis and disrupts the mitochondrial membrane potential of human breast and cervical cancer cell lines. Food Chem Toxicol 49:871–878
Fauzi AN, Yaacob NS (2016) Cell cycle and apoptosis pathway modulation by Tualang honey in ER-dependent and independent breast cancer cell lines. J Apic Res 55:366–374
Fernandez-Cabezudo MJ, El-Kharrag R, Torab F, Bashir G, George JA, El-Taji H, Al-Ramadi BK (2013) Intravenous administration of manuka honey inhibits tumor growth and improves host survival when used in combination with chemotherapy in a melanoma mouse model. PLoS One 8:e55993
Gabai-Kapara E, Lahad A, Kaufman B et al (2014) Population-based screening for breast and ovarian cancer risk due to BRCA1 and BRCA2. Proc Natl Acad Sci U S A 111:14205–14210
Giuliano AE, Connolly JL, Edge SB et al (2017) Breast cancer—major changes in the American Joint Committee on cancer eighth edition cancer staging manual. CA Cancer J Clin 67:290–303
Hamad R, Jayakumar C, Ranganathan P, Mohamed R, El-Hamamy MMI, Dessouki AA, Ibrahim A, Ramesh G (2015) Honey feeding protects kidney against cisplatin nephrotoxicity through suppression of inflammation. Clin Exp Pharmacol Physiol 42:843–848
Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144:646–674
Hizan N, Hassina N, Hassan M et al (2018) Tualang honey adjunct with anastrozole improve parenchyma enhancement of breast tissue in breast cancer patients—a randomized controlled trial. Integ Med Res 7:322–327
Hussein SZ, Mohd Yusoff K, Makpol S (2012) Gelam honey inhibits the production of proinflammatory, mediators NO, PGE2, TNF-ALPHA AND 1L-6 in carrageenan induced acute paw edema in rats. Evid Based Complement Alternat Med 2012:109636
Jaganathan SK, Mandal MS, Saikat KJ, Soumen D, Mandal M (2010a) Studies on the phenolic profiling, anti-oxidant and cytotoxic activity of Indian honey: in vitro evaluation. Nat Prod Res 24:1295–1306
Jaganathan SK, Mondhe D, Wani ZA, Pal HC, Mandal M (2010b) Effect of honey and Eugenol on Ehrlich ascites and solid carcinoma. J Biomed Biotechnol 1–5:51
Kadir EA, Sulaiman SA, Yahya NK, Othman NH (2013) Inhibitory effects of Tualang Honey on experimental breast cancer in rats: a preliminary study. Asian Pac J Cancer Prev 14:2249–2254
Leong AG, Herst PM, Harper JL (2012) Indigenous New Zealand honeys exhibit multiple anti-inflammatory activities. Innate Immun 18:459–466
Orsolic N, Bašic I (2004) Antimetastatic effect of honey. Mellifera 4:38–43
Othman NH, Ahmed S, Sulaiman SA (2016) Inhibitory effects of Malaysian Tualang honey and Australian/New Zealand Manuka honey in modulating experimental breast cancers induced by N-methyl-N-nitrosourea (MNU): a comparative study. Pathology 48(Suppl. S1):S148
Salleh MAM, Eshak Z, Ismail WIW (2017) Acacia honey induces apoptosis in human breast adenocarcinoma cell lines (MCF-7). Jurnal Teknol 79:9–16
Scalbert A, Williamson G (2000) Dietary intake and bioavailability of polyphenols. J Nutr 130:2073–2085
Schramm D, Karim M, Schrader H, Holt R, Cardetti M, Keen C (2003) Honey with high levels of antioxidants can provide protection in healthy human subjects. J Agric Food Chem 51:1732–1735
Takruri HR, Shomaf MS, Shnaight S (2017) Multifloral honey has a protective effect against mammary cancer induced by 7,12-dimethylbenz(alpha) anthracene (DMBA) induced breast cancer in Sprague–Dawley rats. J Agri Sci 9:196–204
Tamimi RM, Colditz GA, Hazra A et al (2012) Traditional breast cancer risk factors in relation to molecular subtypes of breast cancer. Breast Can Res Treat 131:159–167

Tsiapara AV, Jaakkola M, Chinou I, Graikou K, Tolonen T, Virtanen V (2009) Bioactivity of Greek honey extracts on breast cancer (MCF-7), prostate cancer (PC-3) and endometrial cancer (Ishikawa) cells: profile analysis of extracts. Food Chem 116:702–708

Turnbull C, Rahman N (2008) Genetic predisposition to breast cancer: past, present, and future. Ann Rev Genomics Hum Genet 9:321–345

Van der Woude H, Gliszczyńska-Swigło A, Struijs K, Smeets A, Alink GM, Rietjens IMCM (2003) Biphasic modulation of cell proliferation by quercetin at concentrations physiologically relevant in humans. Cancer Lett 200:41–47

Wieczorek J, Pietrzak M, Pomianowski J, Wieczorec Z (2014) Honey as a source of bioactive compounds. Pol J Nat Sci 29:275–285

Yaacob NS, Ismail NF (2014) Comparison of cytotoxicity and genotoxicity of 4-hydroxytamoxifen in combination with Tualang honey in MCF-7 and MCF-10A cells. BMC Complement Altern Med 14:106