Role of Honey in Prevention and Management of Cancer

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Contents

Abstract

Cancer is one of the major causes of death across the globe. Some of the major hurdles in the cancer management are augmented drug resistance, cancer stemness, metastasis, and serious side effects of the drugs used for chemotherapy.

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These factors forced the popularity of alternative or natural medicines which show comparatively reduced side effects than the synthetic drugs. Honey, as a natural healer, is in public domain since ancient times. Among all the natural antioxidants, honey is extensively studied for its beneficiary action against both acute as well as chronic ailments. There is now sizeable evidence that showed honey is a natural agent as immune booster, anti-inflammation, antimicrobial, anticancer, and promoter for healing chronic ulcers and wounds. Chemically, these natural healers comprise of various polyphenols and flavonoids which are known for their health-beneficial effects. Although recent scientific evidences support the mechanism of honey in inducing cell death, the full-fledged mechanisms are yet to be explored. Here in this chapter, we reviewed different aspects of honey, i.e., the bioactive components and various mechanisms of honey in inducing cancer cell death.

Keywords

Honey · Cancer · Oxidative stress · Cell signaling · Apoptosis · Drug resistance

Introduction

Since the beginning of human civilization, honey bees are of great importance to mankind. They are believed to be the hidden treasure of nature as their importance in the present day is stretched from maintaining ecology to enhancing economy of the human race. Various bee products like honey, bee venom, royal jelly, propolis, and bee pollen are presently consumed widely due to their therapeutic values. Among all these products of the honey bee, honey is quite popular since ancient times. Although different honey bees are engaged in pollination as well as providing bee products, the most important species for producing honey are *Apis dorsata, Apis cerana, Apis floria, Apis mellifera, Apis koschevnikov, Apis laborisa,* and *Apis andreniformis.* Most of the ancient civilizations, including the Egyptians, Greek, Mayans, Chinese, Babylonians, and Romans, used honeys both for therapeutic as well as nutritional requirements (Adebolu 2005). However, some communities across globe also use honey for their traditional and religious significance. In Hinduism, honey is considered as one of the elixirs of immortality, i.e., *panchamrit*. Similarly, in Islam, honey is strongly recommended for wound healing. Traditionally, honey is used by Buddhists across globe to celebrate the "*Madhu purnima*" in which monks are offered honey. As honey has wide applications and vivid values, present-day researches are mainly focused on its scientific validation in terms of improvising human health standards. Evidences suggest that honey has lot of health beneficial effects including antidiabetic (Ali et al. 2020), antibacterial (Shamala et al. 2002), antioxidants (Zhang et al. 2021), wound healing (El-Senduny et al. 2021), and anticancer (Farooq et al. 2020) activites to name a few. Besides, therapeutic applications of honey on the cardiovascular system, respiratory system, and immunity building are also studied, but their evidences are limited in comparison to the aforesaid diseases.

Cancer, as a global concern, claims millions of lives daily. Active drug resistance, serious physiological side effects of chemotherapy drugs, and recurrence of the disease have been a matter of concern globally. Natural healing through various nutraceuticals as well as plant-based products is in practice to increase life expectancy of the affected populations. These products are either used singly or in combination to alleviate the physiological side effects. Honey, as a nutraceutical, is vividly studied for its health-beneficial effects against cancer. Presence of various polyphenols is the crucial factor which imparts antioxidant property to this natural healer (Meo et al. 2017). In vitro cytotoxic assessment of honey has been studied in diverse groups of cancer cell lines obtained from breast, prostate, oral, cervical, and colorectal cancers (Hakim et al. 2014; Yaacob et al. 2013; Samarghandian et al. 2011a; Ghashm et al. 2010). As per our literature survey from PubMed and Google scholar with the key word “honey and cancer,” maximum studies have been reported in colon cancer (25% of total reports) followed by breast (18%) and oral (14%) cancer (Fig. 1). This information provides a clear evidence on the inclined researches carried out in colon cancer and the effectiveness of honey in combating various modules of colon carcinoma. These studies not only focused on the antiproliferative effect of honey but also on various possible mechanisms underlying honey-mediated cell death.

Apart from these, honey plays a wide role in combating angiogenesis, metastasis, and inflammation by various molecular mechanisms (Fig. 2). Recent evidences suggest a boom in the scientific validation of honey against various cancer conditions as well as their role in oxidative stress. In this chapter, we reviewed the role of honey in combating various hallmarks of cancer in selected cancer types and their probable mechanism of actions.

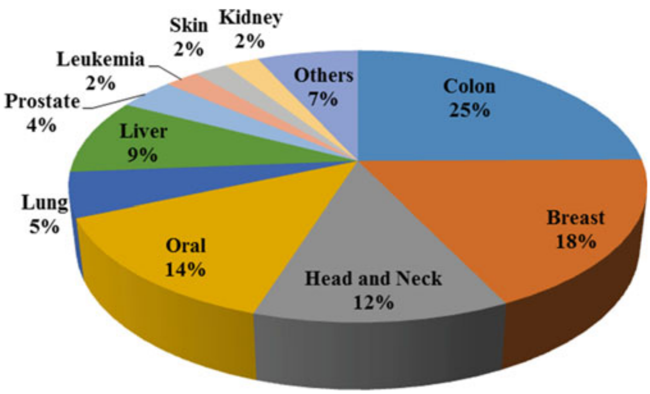

Fig. 1 Percent of existing reports on the effect of honey against various cancers

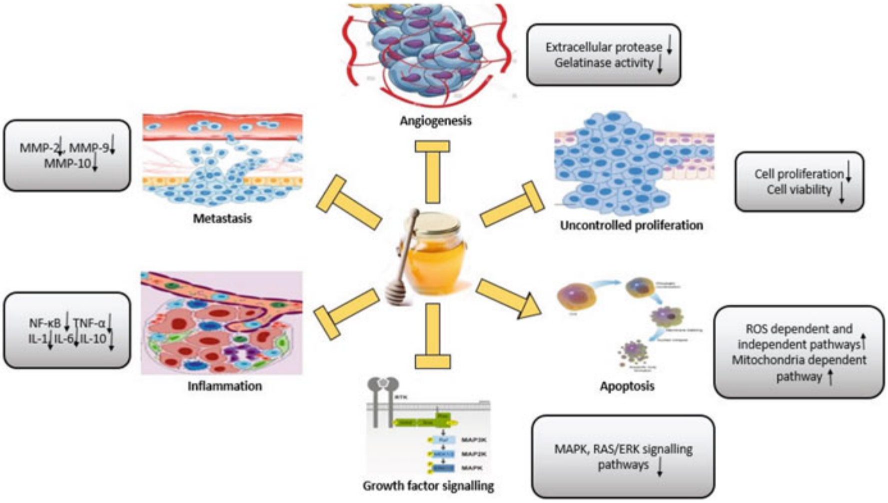

Fig. 2 Role of honey in management of cancer

Bioactive Components of Honey

From ancient Egypt and the classical civilizations, like Greeks and Romans, to the Arab people of the Middle ages, honey has not only been considered as a food or a sweetener but also a natural medicinal alternative throughout the history of the human race. The diverse roles of honey come from its richness of miscellaneous compositions which eventually make it a good source of natural medicinal remedy. The therapeutic effects of honey depend on the bioavailability of different phytoconstituents, their method of absorption, and their metabolization (Cianciosi et al. 2018).

It is reported that natural honey is composed of more than 200 compounds, among which, although sugars are the predominant compounds, mostly mono- and disaccharides, other compounds like amino acids, polyphenols, proteins, enzymes, and vitamins are also present (Nugraheni and Ersam 2017). Bureau of Indian Standards (BIS) has set a basic standard applicable for pure natural honey. Any commercial honey in order to be considered as pure should fulfill all the essential criteria of the standards of natural honey as summarized in Table 1 (Bureau of Indian Standards (BIS) 1994).

Sugars

Sugars are the most dominant components of honey, comprising more than 95% of its dry weight. Honey naturally comprises three types of sugars: (i) fruit sugar, that is, fructose, the highest sugar among the others (41%); (ii) grape sugar, that is, glucose,

Table 1 Standards for honey as per Bureau of Indian Standards, India

Sr No.	Characteristics	Special grade	A grade	Standard grade
1.	Specific gravity at 27^0 C (min)	1.37	1.37	1.37
2.	Moisture, % by mass (max)	20	22	25
3.	Total reducing sugar, % by mass (min)	70	65	65
4.	Sucrose, % by mass (max)	5.0	5.0	5.0
5.	Fructose-glucose ratio (min)	1.0	1.0	1.0
6.	Ash, % by mass (max)	0.5	0.5	0.5
7.	Acidity (expressed as formic acid), % by mass (max)	0.2	0.2	0.2
8.	Fiehe's test[a]	Negative		
9.	Hydroxymethyl furfural (HMF), mg/kg (max)	80	80	80
10.	Total counts of pollens and plant elements/g of honey (max)	50,000	50,000	50,000
11.	Optical density at 660 nm, % (max)	0.3	0.3	0.3

[a]{If Fiehe's test is positive, carry out the determination of the hydroxymethyl furfural (HMF) content. If it is more than 80 mg/kg, then fructose-glucose ratio should be more than **1.00**}

comprises 34% of the total sugar contents; and (iii) ordinary sugar – it ranges between 1 and 2%. The ratio of these sugar types in honey depends upon the source, time of collection, and the species of plant nectars. According to Codex Alimentarius Commission (CAC 2001) and International Honey Commission (IHC 2002), the fructose-glucose ratio (F/G) of the pure honey should not be less than 0.95%. Any commercial products, which contain sucrose or maltose more than 5%, must not be labeled as pure honey (Bureau of Indian Standards (BIS) 1994). It is correct to state that the two prime monosaccharides, D-fructose and D-glucose, constitute about 95% of the honey solids, in the free and combined form. Recent studies, using paper chromatography and thin-layer chromatography, reported that, besides monosaccharides, honey also contains disaccharides (sucrose, maltose, lactose, melibiose, etc.) and higher oligosaccharides (raffinose, melezitose, and erlose) to some extent (Böttcher and Monks n.d.).

Polyphenols

Polyphenols, the most crucial secondary metabolites of plants, comprise a variety of chemical structures from simple phenolic molecules to complex polymeric structure. These are mainly the products of plant shikimate and acetate pathways and possess at least one hydroxyl group attached to one aromatic ring of the molecule. Mainly, these polyphenols contribute for the different biological activities of honey like antimicrobial, antiviral, anticancer, and antidiabetic activities. Since honey is a food product which is derived from plant nectars or different plant parts, it is rich in different naturally occurring polyphenols. Among the 10 different classes of polyphenols, honey mainly contains 2 important classes – phenolics and flavonoids (Tedesco et al. 2020).

Among the phenolics, gallic acid is the most important and abundant one. A previous study on Australian honey reported that the content of gallic acid in it ranges from 13.9 to 45.2 μg/g of honey sample (Uthurry et al. 2011). Another study on Acacia honey revealed the same statement by showing the gallic acid content varies from 36.9 to 40.8 μg/g of sample (Yaoa et al. 2005). Apart from gallic acid, honey is also rich in different other phenolics like chlorogenic acid, caffeic acid, vanillic acid, and p-hydroxybenzoic acid. Chlorogenic acid, p-hydroxybenzoic acid, and caffeic acid are found in very high percentage in Manuka honey, which is well known for its antibacterial and anticancer activities. Likewise, vanillic acid is a good chemical marker for the thyme honey from Spain (Kečkeš et al. 2013).

Flavonoids are found to be an important part of the honey. Different scientific findings, over the years, have reported several flavonoids that are present in different honey samples based on their geographical location and source of the nectar. Among all the flavonoids, kaempferol, quercetin, chrysin, pinocembrin, pinobanksin, and myricetin are the most abundant flavonoids. The highest total flavonoid content has been reported to be present in raspberry honey (33.58 mg/g of honey sample), whereas thyme honey possesses the lowest content of flavonoids (17.45 to 20.21 mg/g of honey sample) (Cheung et al. 2019).

Moreover, polyphenols are the most important components of honey for its biological activity. Based on the geographical location, time of collection, and most importantly source of the nectar, the phenolic and the flavonoid content varies in different types of honey, which also specifies them to their respective biological activities. Table 2 summaries the polyphenol components of different honey.

Amino Acids

Amino acids comprise 1% of the total honey weight, which ranges from 390 mg/kg to 633.50 mg/kg. The main source of amino acids in honey (mostly found in bound form) is pollen, and based on that the characteristics of botanical origin can be determined. However, the amino acid profile in honey can also be of animal origin as free amino acids are added by bees themselves (Pauliuc et al. 2020). There are 27 amino acids found in honey, and among them proline is the major one that comprises almost 60-80% of total free amino acids. The proline content also indicates about the ripeness of honey as it is constantly decreased with time during storage. The amount of proline in honey is also being used to check for honey adulteration. According to guideline, any commercial honey with proline amount less than 180 mg/kg should be treated as adulterated. Among the essential amino acids, threonine, phenylalanine, and leucine are highest in honey. Next to proline, phenylalanine is the second most abundant amino acid, generally found in all honeys (Janiszewska et al. 2012; Szeles et al. 2008).

Table 2 Polyphenol compositions of different honey

Type of honey	Polyphenols		References
	Phenolics	Flavonoids	
Manuka honey	Gallic acid, caffeic acid, ferulic acid, syringic acid, and p-Coumaric acid	Chrysin, galangin, kaempferol, quercetin, pinobanksin, pinocembrin, and luteolin	Cianciosi et al. (2018) and Kečkeš et al. (2013)
Acacia honey	Gallic acid, caffeic acid, chlorogenic acid, p-Coumaric acid, syingic acid, vanillic acid, and ferulic acid	Apigenin, chrysin, galangin, kaempferol, quercetin, pinobanksin, pinocembrin, myricetin, luteolin, and genistein	
Tualang honey	2-Hydroxycinnamic acid, caffeic acid, cinnamic acid, gallic acid, p-Coumaric acid, and syringic acid	Apigenin, catechin, chrysin, kaempferol, and luteolin	
Strawberry honey	2-cis,4-transabscisic acid, cinnamic acid, and syringic acid	Apigenin, galangin, kaempferol, luteolin, rutin, pinobanksin, and pinocembrin	
Clover honey	Cinnamic acid, p-hydroxybenzoic acid	Quercetin	
Heather honey	Chlorogenic acid, cinnamic acid, ellagic acid, ferulic acid, gallic acid, p-Coumaric acid, p-hydroxybenzoic acid, vanillic acid, syringic acid, protocatechuic acid, and sinapic acid	Chrysin, galangin, and myricetin	
Thyme honey	Caffeic acid, chlorogenic acid, cinnamic acid, ferulic acid, gallic acid, p-Coumaric acid, and syringic acid	Chrysin, kaempferol, luteolin, myricetin, and quercetin	
Rosemary honey	p-Coumaric acid	Chrysin, kaepmferol, luteolin, pinobanksin, and pinocembrin	
Pine honey	Gallic acid, p-Coumaric acid, and Protocatechuic acid	Catechin, isorhamnetin, and Luteolin	
Mustard honey	Gallic acid, caffeic acid, and benzoic acid	Kaempferol, naringenin, and chrysin	

Proteins and Enzymes

Honey possesses different proteins and enzymes that comprise about 0.5% of its total weight. These proteins can be derived from the nectar or the pollen of different flowers, although generally they originate from the secretions of the cephalic glands of honey bees. Among different proteins, nine Major Royal Jelly Proteins (MRJPs) are most important for their abundance in honey as well as biological activities they possess. Of these, MRJP1 is the most abundant protein family among nine MRJP families and is also called Royalactin. It has been reported to show potent cytoprotective as well as regenerative effects on hepatocytes while MRJP3 demonstrated immunoregulatory effects (Hermosín et al. 2003).

Like different proteins, occurrence of different enzymes in honey is well known for many years. The total protein content of honey generally ranges from 1000 to 2000 μg/g of honey sample although it may vary depending upon the source of honey. Some of these enzymes are found in the pollen/nectar while others are introduced by bees. Enzymes that are commonly found in honey are diastase, invertase, glucose oxidase, catalase, and maltase. Diastase and invertase are of major interests among different enzymes. They are introduced in honey by bees. It is responsible for the conversion of starch into dextrin and eventually simple sugars. Invertase, on the other hand, converts sucrose into glucose and fructose, and its action is shown very early in the life of honey as very less amount of invertase is present in ripe honey. Glucose oxidase, another important enzyme in honey, is mainly known for its excellent antibacterial activity by catalyzing glucose to form gluconic acid and hydrogen peroxide (H_2O_2). Glucose oxidase is thought to be incorporated by the bees. The production of H_2O_2 by the action of glucose oxidase in honey is normalized and catalyzed into water and oxygen by another enzyme in honey, i.e., catalase which is sourced from the nectar. Thus, this enzyme has a very important role in protecting the normal cells of the body from the damaging effects of different reactive oxygen species like H_2O_2. Apart from these, some honey have also been reported to have α- and β-glucosidase activity which breaks the complex polysaccharide starch into simple form of sugars (Hermosín et al. 2003; Okamoto et al. 2003).

Vitamins and Minerals

Honey contains small amounts of different vitamins and minerals. Although, in this aspect, the contribution of honey to the recommended daily intake (RDI) is marginal, these substances possess important nutraceutical and health benefits. The commonly found vitamins in honey include ascorbic acid (vitamin C), niacin (vitamin B3), riboflavin (vitamin B2), and pantothenic acid (vitamin B5). Among various minerals, calcium, copper, iron, magnesium, manganese, zinc, and potassium are abundantly found in honey (Rossano et al. 2012).

Mechanistic Perspective of Honey in Combating Cancer

As discussed above, honey is composed of diverse groups of polyphenols and other essential vitamins and minerals. These constituents are mainly responsible for enhancing the therapeutic potential of the nutraceuticals against acute as well as chronic diseases. However, previous reports confirmed the variation in metabolite composition of honey samples with change in geographical locations (Kavanagh et al. 2019). Hence, some honey varieties have superior efficacy in comparison to others. Honeys like Manuka and Tualang have been extensively studied for their effect against various cancer cells and tissues (Cianciosi et al. 2020a; Ahmed and Othman 2017). Several groups have studied the anticancer mechanisms of these honey and their role in alleviating cancer progression as well as related hallmarks of cancer. These reports confirm the involvement of allied pathways and proteins in response to honey. Hence, the mechanistic role of honey in activating different forms of cell death by targeting various proteins and pathways is discussed below.

Honey Induces Apoptotic Cell Death

Apoptosis is a gene-mediated program, which has profound implications for our understanding of developmental biology and tissue homeostasis. It is an important event in controlling cancer cell proliferation. Most of the modern chemotherapy drugs are designed to achieve cellular apoptosis during their prophylaxis. Recently, various reports claimed and established the anticancer effect of honey in different carcinoma conditions. As per a study by Samargandhian et al. (2011), honey reduced the renal carcinoma cell (ACNH cells) proliferation by inducing apoptosis (Samarghandian et al. 2011b). Similarly, another study by Ghaffari et al. (2012) showed the effect of honey in human gastric mucosa. They confirmed by histopathology and TUNEL assays about the induction of apoptosis in gastric cancer tissues in honey-treated subjects (Ghaffari et al. 2012). A study by Cheng et al. (2019) established the anticancer effect of Jujube honey in hepatocarcinoma cells. Jujube honey, a very popular honey in China, induced apoptosis by imparting DNA damage, upregulating the expression of p53 protein and subsequently affecting both proapoptotic (Bax and Bad) and antiapoptotic proteins (Bcl-2 and Bcl-XL) by increasing and decreasing their expressions, respectively (Cheng et al. 2019). Furthermore, another study on Acacia honey revealed the anticancer potential of this natural healer. As per the study, Acacia honey showed profound effect on breast carcinoma cells, i.e., MCF-7 cells. Honey helped in retarding the cancer cell proliferation by inducing apoptosis which was clearly observed by cell shrinkage and apoptotic body formation (Salleh et al. 2017). Manuka honey is one of the vividly studied honey types for their effect against carcinoma. As per a study by Afrin et al. (2018a, b, c), Manuka honey induced apoptosis in human colon carcinoma cells, i.e., HCT-116 and LOVO cells by inducing apoptosis and cell cycle arrest (Afrin et al. 2018a). As per the report, apoptosis event was achieved by upregulating expression of p53 followed by activation of both intrinsic and extrinsic apoptotic pathways. Manuka honey also helped in arresting the cells at G2/M phase through the modulation of cell cycle-regulator genes, i.e., cyclin E, cyclinD1, p21, p27, CDK2, and CDK4. The studies on the therapeutic effectiveness of honey and apoptotic cell death were not confined to in vitro only. Various in vivo studies were also conducted to support the above evidences in animal models. As per a study by Ahmed and Othman (2017), Tualang honey modulated the hematological parameters, estrogen levels, and the expressions of estrogen receptors (ER1), and pro- and antiapoptotic proteins in breast cancer-induced rats (Ahmed and Othman 2017). Another report by Fernandez-Cabezudo et al. (2013) showed that intravenous administration of Manuka honey inhibited tumor growth and improved host survival when used in combination with chemotherapy in a melanoma mouse model (Fernandez-Cabezudo et al. 2013). The same report showed the treatment with Manuka honey alone resulted in about 33% inhibition of tumor growth, which correlated with histologically observable increase in tumor apoptosis. Similarly, another report by Swellam et al. (2003) also demonstrated the antineoplastic efficacy of honey in experimental bladder cancer murine model (Swellam et al. 2003).

From above studies, it is quite evident that honey can be a potential nutraceutical for inducing apoptosis in cancer cells. This may provide some clue on using this gift of nature for preventing and managing cancer. However, further detailed investigations are needed to fully recognize the mechanisms involved in honey-induced cellular damage by which honey can be considered as a potential nutraceutical for cancer management.

Honey Regulates the Mitochondrial Pathways and Related Proteins

The rate of malignancy in cancer cells, their recurrence, and stemness depend on various factors. Of them, mitochondrial membrane potential (Δψm), which reflects the physiological status of the mitochondrion in the cells, is well connected to cancer malignancy. Besides, mitochondrial pathway activation is equally an important mechanism required to induce cellular death either by chemotherapy or radiotherapy. Intrinsic mitochondrial pathway involves a series of interactions following various stimuli including physical stress, chemical interventions, oxidative stress, and nutrients. During such events, cytochrome-C located in inner membrane space (IMS) of mitochondria is released and in turn causes cellular apoptosis. Therefore, honey rich in flavonoids shows the ability to release cytochrome-C and induce apoptosis in colon carcinoma cells (HCT-15 and HT-29 cells) (Jaganathan and Mandal 2010). Furthermore, induction of mitochondrial outer membrane permeabilization (MOMP) results in the leakage of mitochondrial proteins in the cytosol that makes the common mechanism of inducing cell death with mediators having anticancer properties. Several reports show the involvement of mitochondria in oxidative stress and in regulating malignancy (Kowaltowski and Vercesi 1999). As per a study by Fauzi et al. (2011), Tualang honey induces apoptosis and disrupts mitochondrial membrane potential of both human breast (MCF-7 and MDA-MB-231 cells) as well as cervical cancer cell lines (HeLa cells) (Fauzi et al. 2011). Similarly, in another recent study by Afrin et al. (2018a, b, c), in human colon cancer cells (HCT1 and LoVo cells), Manuka honey was reported to show profound effect in inducing the oxidative stress and alternation of mitochondrial respiration and glycolysis (Afrin et al. 2018b). There are several other reports involving the active ingredients of honey which were shown to cause MOMP in different cell lines and thus destabilizing mitochondrial functions (Yaacob et al. 2013; Canonico et al. 2014).

Based on these data, it could be very easily conceived that honey and its flavonoid moieties attribute cell death by the involvement of MOMP.

Honey Induces ROS, and This Arrests Cell Cycle

The role of oxidative stress and reactive oxygen species (ROS) on cancer cell is controversial. Previous evidences have shown that ROS performs a dual role in cancer, i.e., stimulatory as well as inhibitory (Ajibola et al. 2012; Erejuwa et al. 2014). According to these studies, ROS behaves like both friend and foe to a cancer cell, because lower levels of ROS are beneficial for a cancerous cell as they promotes cell proliferation. On the other hand, increased levels of ROS contribute to the development of oxidative stress and thus stop cancer cell progression by either arresting cell cycle or activating apoptotic pathways. Therefore, considering its decisive role, the development of ROS and oxidative stress is a new add-on technique to combat cancer cell progression (Ajibola et al. 2012). Honey has been used as a natural food that possesses potential inhibitory activity toward the development of cancer. Its different biologically active components, namely caffeic acid and caffeic acid phenethyl esters, make honey highly cytotoxic against cancerous cells, whereas it bears no cytotoxicity for the normal cells. This may be explained by one of the many molecular observations, e.g., upon treatment of honey to cancerous and normal cells, honey-treated cancerous cells showed increased apoptotic activity as evidenced by augmented enzymatic activity of caspase-3 in respect to the control (untreated) cancerous cells as well as normal cells. But a detailed understanding is required to unleash the exact mechanistic way behind the cytotoxicity of honey in cancerous cells but not in normal cells (Erejuwa et al. 2014).

The cell cycle possesses different phases (G0, G1, S, G2, and M) through which cell passes during its growth and division. These phases have several checkpoints which allow a cell to cross-through based on its metabolic activity. There are several protein markers in these checkpoints which confirm the cell's current state in the cell cycle. The novel nuclear protein Ki-67 is one of those excellent operational markers. It is highly expressed in all the actively proliferating phases (G1, S, G2, and M) and only absent in the resting phase (G0). Treating with honey has shown a drastic decrease in the Ki-67 expression levels in cancer cells of rats thus exerting the antiproliferative effect of honey by arresting the cell cycle of the cancer cells (Jaganathan and Mandal 2010). A study with Acacia honey also showed its anti-proliferative effect on mouse melanoma (B16) and human melanoma (A375) cell lines. In this study, using the flow cytometric analysis, they observed an increased accumulation of cells in the G0/G1 phase after 72 hours of Acacia honey treatment, depicting the efficiency of Acacia honey in cell cycle arrest of cancer cells in a time- and dose-dependent manner. According to the authors of this study, the anti-proliferative effect of Acacia honey may be due to presence of chrysin, the most abundant flavonoid present in Acacia honey (Ahmed and Othman 2013). Another study on human colon carcinoma cell lines (HCT-15 and HT-29) treated with Indian multifloral honey depicted its cell cycle arrest and apoptotic activity by inducing

ROS production in the treated cells. The antiproliferative activity of the multifloral honey was determined by monitoring the increased accumulation of cells at sub-G1 phase as compared to the control cells. They showed that the accumulation of cells at sub-G1 phase was quite significantly high in both HCT-15 [1.95% (control) to 40.65% (treated)] and HT-29 cells [4.05% (control) to 32.56% (treated)] as tested by them (Uthurry et al. 2011). Effects of Manuka honey from New Zealand have also been studied for its cell cycle inhibition activities. Manuka honey-treated HCT-116 (human colon adenocarcinoma) and LoVo (Dukes' type C, grade IV, and colon metastasis) cell lines showed significant cell cycle arrest although the mechanism in those cells were different. Manuka honey suppressed the HCT-116 cell proliferation at the S-phase by downregulating CDK-2, CDK-4, Cyclin-D1, Cyclin-E, and p-RB and increasing the p21 and p27 expressions. In the case of LoVo cells, Manuka honey exerted the cell cycle arrest at a different point, i.e., the G2/M phase, through the activation of p21 and p27 and suppressing cyclin-D1 expression (Pichichero et al. 2010).

Form all the previous works, it is now clear that honey possesses antiproliferative effect on cancer cells by arresting the cell cycle, although the mechanisms responsible for it may vary with different honey and cancer types. But all these studies have confirmed one common thing that is the development of significant ROS upon honey treatment that ultimately leads to the activation of different cell cycle-regulating proteins and restriction of cell cycle progression in cancer cells.

Anti-inflammatory and Immune-Modulatory Effects of Honey

Immunomodulation is the development of a set of altering immune system in a beneficial or damaging manner. Since this process is linked to immunostimulation system, the overexpression of related proteins and factors can impart both beneficial and adverse effects. Immunomodulatory cytokines like TNF-α, IL-1, IL-6, and IL-10 possess the ability to stimulate the phagocytic and lymphocytic activity of the blood cells that ultimately induce an immunomodulatory response. Many natural products, like honey, have the ability to activate and modulate the immune system. It has been found that honey encourages the B-cells, T-cells, and macrophages to exert their anticancer activity (Afrin et al. 2018c). Different articles have revealed that the immunomodulatory activity of honey may also be due to the production of short chain fatty acids during the digestion of honey. Moreover, a sugar present in honey, nigero-oligasaccharides (NOS), has been reported to induce significant immunomodulatory responses (Ahmed et al. 2018; Samarghandian et al. 2017).

Chronic inflammation and continuous swelling are associated with malignant cell formation by elevating various proinflammatory cytokines and oxidizing agents. Inflammation also causes release of specific cytokines that can trigger angiogenesis and cause tumor initiating mutations. Thus, from initiation and progression to invasion and metastasis, this process is directly linked with the development of carcinogenesis and has been called as the seventh hallmark of cancer. The phenolic compounds of honey show their anti-inflammatory effects by suppressing the proinflammatory events raised by cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), enzymes that speed up the metabolic process in the tissues with chronic inflammation by inducing the breakdown of arachidonic acid into prostaglandin which is involved in the development of carcinogenesis. Apart from this, honey and its moieties can also hinder the progressive carcinogenesis process (Samarghandian et al. 2017).

Chrysin, one of the major flavonoids preset in honey, has been found to reduce the cytosolic levels of NF-κB and thus check the intensity of diethylnitrosamine-induced hepatocarcinogenesis when administered at 250 mg/kg body weight dosage three times daily in Wister rats. Honey has also been shown to regulate MAPK and NF-κB pathways, necessary for inflammatory responses, by modifying the expression levels of few inflammatory cytokines and related transcription factors in various cancer models (Erejuwa et al. 2014). Gelam honey has been found to possess anti-tumorigenesis activity when administered with crude ginger extract through the modulation of ERK/RAS pathways, the main contributing pathway for the development of carcinogenesis (Waheed et al. 2019). Arabic honey and thyme honey have been found to reduce proinflammatory cytokine IL-6 and NF-κB levels in the prostate cancer cell lines, which are significantly upregulated in different kinds of malignancy (Ranneh et al. 2021).

Finally, based on these data, it could be concluded that the phenolics and flavonoid components of honey exert anti-inflammatory effects on various cancerous cell lines. The process is mainly mediated through the attenuation of different proinflammatory factors (IL-1, IL-6, and TNF-α) and the suppression of MAPK and NF-κB-signaling pathways.

Role of Honey in Combating Metastasis and Stem Cell Populations

One of the major issues in the cancer management is the recurrence of the cells in spite of radiotherapy, chemotherapy, as well as surgical removal of the affected tissue. Two major issues are actively involved in the same and aggravate cancer cell regeneration: first, heterogeneity of the tumor microenvironment, which provides a niche for the existence of the cancer stem cells, and second, metastasis and invasion of the primary tumor cells, which helps in regrowth of tumor at various distant sites. Metastasis-related disease recurrence is responsible for over 90% death and involves series of events, i.e., “invasion-metastasis-cascade” (Steeg 2006). Series of recent evidences show the role of honey, a flavonoid-rich nutraceutical, in restricting the metastatic capability of the cancer cells. As per a recent study by Afrin et al. (2019), strawberry honey inhibited cellular metastasis in HCT-116 and LoVo cells by downregulating the expression of MMP-2, MMP-9, N-cadherin, and β-catenin (Afrin et al. 2019). A very similar pattern of cell death and inhibition of metastasis was also observed in the case of Manuka honey where the expression of E-cadherin and N-cadherin were up- and downregulated, respectively, in honey-treated colon carcinoma cells indicating its antimetastatic properties (Ahmed et al. 2018). Another very recent report by Cianciosi et al. (2020a, b) claimed the involvement of Manuka honey polyphenols in managing the colorectal cancer stem cells. As per the study, Manuka honey reduced the volume of the entire culture of spheroids, affecting their morphological parameters and induced apoptosis and intracellular accumulation of ROS in cancer stem cell-like cells (Cianciosi et al. 2020b). Chrysin is one of the abundantly present metabolites in various honey and bee venoms. As per another report, chrysin suppresses hypoxic survival and metastatic growth of mouse breast cancer cells (4 T1 cells). The same report also established the reduction of metastatic colonies in Balb/c mice fed with chrysin orally (Lirdprapamongkol et al. 2013). These findings confirm the potential of various honey in inhibiting metastasis and stem cell populations. However, detailed clinical investigation using honey may provide some new insights and thus establish it as a potential therapy for targeting cancer stem cell populations.

Honey as a Combinatorial Approach for the Management of Cancer

Development of chemoresistance to a particular drug and severe side effects of the chemotherapy drugs are some of the major hurdles in cancer management. Hence, the use of natural products or nutraceuticals has become an essential part of cancer chemoprevention. Recently, combinatorial drug therapy (with nutraceuticals) is gaining momentum across the globe due to its greater success rate in terms of enhanced life expectancy and physiological safety. Combinatorial therapy usually involves combination of two different therapies like radiotherapy / immune therapy or combination of drugs targeting different pathways, i.e., mTOR/ β-catenin. In many instances, combinatorial therapy also includes the use of nutraceuticals or alternative medicines along with chemotherapy to reduce the active chemotherapy drug load. Nutraceuticals are known to have very promising effect in detoxifying and inhibiting inflammatory and cell growth-signaling pathways. Along with their therapeutic efficacy, they are promptly accepted due to their biocompatibility and safety features. Series of evidences suggest the efficacy of the combinational approach in alleviating the associated problems due to chemotherapy and also in reducing cancer cell growth (Suganuma et al. 2011; Qiu et al. 2006). Several reports claim the therapeutic benefits of this synergism in cancer cells. Isothiocyanate derivative, indole-3-ethyl isothiocyanate (ITC), a popular ingredient found in cruciferous vegetables, works synergistically with chemotherapy drug cisplatin to induce apoptosis in human ovarian carcinoma cells (Shamala et al. 2002). Similarly, milk thistle (*Silybum marianum*) has been used by humans since a long time and is believed to be used for the treatment of liver disease. One of the reports by Tyagi et al. (2002) claimed the synergistic effect of silibinin (an active phytoconstituent of milk thistle) along with doxorubicin in inducing apoptosis in prostate cancer cells (Tyagi et al. 2002). Hence, the above facts show that synergistic approach not only helps in balancing the ailments but also decreases the load of drugs which in turn yields low side effects.

Recently, there are several reports on synergistic effect of honey in the management of the cancer progression. As per a report by Tahire et al. (2015), ginger extracts and gelam honey synergistically inhibit colon cancer (HT-29) cell proliferation by modulating the RAS/ERK and PI3K/AKT pathways (Tahire et al. 2015). Similarly, the same combinatorial approach has also been found to potentiate the anticancer effect of 5-FU against HCT-116 cells (Hakim et al. 2014). Furthermore, the same combination, i.e., ginger extracts and gelam honey was also found to be effective in reducing HT-29 cell proliferation by regulating PI3K-Akt-mTOR, Wnt/β-catenin, and apoptosis-signaling pathways (Wee et al. 2015). Another study by Afrin et al. (2018a, b, c) showed the synergistic effect of Manuka honey in increasing the 5-Fu sensitivity in human colon cancer cells (Afrin et al. 2018c). All the abovementioned facts indicated that honey in combination with other drugs or phytoconstituents has the potential to act synergistically and impart better effects as compared to their individual usage.

Cautions for the Use of Honey

Honey, being a multidimensional medicinal alternative, has drawn the attention for its universal usage in the cure and management of various ailments. There are two major bottle necks in the use of honey as nutraceutical product. First, its high rate of variation in composition due to change in geographical locations, season, climatic condition, and of course the type of bees being used for producing honey. Naturally, at times, it becomes difficult to exactly spell out with conformity that a particular type of honey could be used for a particular ailment. Therefore, there is a need for easy identification of its constituents which will be universally accepted and mentioned in the labels of products. Second, continuously growing demand of this pricey food commodity in medical field makes it more vulnerable to all fraudulent individuals/manufacturers by incorporating cheaper sweeteners into natural honey.

Different commercial adulterants are being used depending upon the region, availability, and the price of these sweeteners. The most common and cheap adulterant is high fructose corn syrup (HFCS), an isomerized product of corn syrup. Different types of HFCS are commercially available based on the degree of isomerization, 20HFCS (20%), 40HFCS (40%), and 80HFCS (80%). Corn sugar syrup is the second most widely used artificial sweetener for the adulteration of natural honey. It is made up of glucose (45%), maltose (30%), maltotriose (13%), and fructose (10%). Apart from these, inverted sugar syrup and cane sugar syrup are also being used for this lucrative business purpose. Inverted sugar syrup is the product of acid hydrolysis of different disaccharides, sourced from beat sugars, mimicking closely the natural sugar profile of honey due to its C3 plant origin as the natural honey and hence making it more difficult to be detected (Se et al. 2019). Recently, two newly emerged adulterants are attracting the market attention, the rice syrup and maltose syrup, for their C3 plant origin and easy availability. Rice syrup consists of maltotriose, maltose, and glucose. Since, all of these sugars are made of glucose (maltotriose- 3 molecules of glucose, maltose- 2 molecules of glucose), it acts exactly like glucose in the body. Further, having the C3 plant origin makes it more difficult to be detected which in turn affects the quality assurance and food safety (Fakhlaei et al. 2020).

Consumption of adulterated honey can be a gateway of multiple disorders. Long-term feeding of adulterated honey has shown to develop several abnormal conditions like obesity, diabetes, stomach disorder, and liver and kidney damage in rats. In this study, rats supplemented with adulterated honey showed development of fibrosis with necrosis of hepatocytes, shrunken and deformed Bowmann's capsule in kidney, and abnormal blood glucose level while rats feeding natural honey did not develop any such symptoms (Samat et al. 2018). The harmful effects of honey adulteration have also been studied in human. According to previous studies, various sugar adulterants (corn syrup, cane sugar, palm sugar, invert sugar, and rice syrup) have different health disadvantages in human, mainly kidney and liver failure. Apart from these, diabetes, obesity, and hypercholesterolemia are some direct consequences of consuming adulterated honey. Table 3 summarizes effects of adulterated honey consumption in internal organs as determined by different in vivo studies (Fakhlaei et al. 2020).

Different detection techniques are now being used to analyze the sugar profile of various commercial honey and identify the adulterants to keep the importance of natural honey intact. Among them, various chromatographic techniques (thin layer chromatography, gas chromatography, liquid chromatography, and high-performance anion exchange chromatography) and spectroscopic techniques (NMR spectroscopy, Infrared spectroscopy, and Raman spectroscopy) are of

Table 3 Effects of different adulterated honey in different organs

Type of adulterant	Animal type	Affected organs	References
Inverted sugar	Human	Stomach damage	Fakhlaei et al. (2020) and Samat et al. (2018)
Sucrose	Rat	Increased creatinine and urea level	
Sugar cane vinasse	Tilapia fish	Liver damage	
Fructose	Rat	Renal failure	
Sugar syrup	Rat	Hypertriglyceridemia and hyperinsulinemia	
Commercial honey	Rat	Increased serum lipid, body weight, and liver and kidney damage	
HFCS (high fructose corn syrup)	Rat	Kidney failure	
Commercial honey	Rat	Obesity, diabetes, and hypercholesterolemia	
Sugar cane extract	Broiler chicken	Hypertrophied intestinal villi and epithelial cells	

special interests (Se et al. 2019; Zábrodská and Vorlová 2015). Thin layer chromatography (TLC) was first used to detect HFSC in honey in the late 1970s. Various honey samples are loaded and subjected to run along the silica gel, and the respective Rf value determined the purity of the samples (pure honey should have the Rf value of 0.35). Recently, a more advanced and simple method of detection, high-performance thin-layer chromatography, (HPTLC) is being used to detect the adulterated honey based on the fructose/glucose (F/G) ratio and the sucrose content (Qiu et al. 2006). Like TLC and HPTLC, gas chromatography (GC) has also gained attention in the detection of adulterated honey, with a relatively high resolution and sensitivity. GC was mainly used to detect the HFSC, maltose, and isomaltose in the impure honey samples. But what makes GC important to the detection of adulterants is its capability to detect difructose anhydrides (DFAs). DFAs are pseudodisaccharides containing two fructose units present in HFSC and inverted sugar syrup, which can be exclusively detected by GC (Wu et al. 2017). High-performance anion exchange chromatography (HPAEC) is also being used to detect the impurities in honey by virtue of its capability to separate amino-sugars, alditols, mono-, di-, and polysaccharides based on their size, composition, and linkage isomerism. NMR spectroscopy, besides the chromatographic techniques, is now deliberately being used to separate the adulterants because of its exclusive capability to detect all the organic materials quantitatively without any prior separation, while other chromatographic methods require some kinds of separation and modification before detection (Wu et al. 2017).

Conclusions

Honey, a natural healer, has health-beneficial effects in terms of alleviating chronic disorders as well as providing nutritional support. Since ancient times, this gift of nature is used in various aspects of life. Recent scientific validation through various research publications sheds some light on the mechanism of action of honey in managing cancer cell proliferation. The therapeutic effect of honey not only is limited to cancer but also in alleviating various other disorders including diabetes, ulcers, wound healing, microbial, and inflammation to name a few. However, the full mechanism is yet to be understood. Some other mysteries behind the mechanism of honey are still unanswered: (i) How sugars in honey can be cytotoxic to cancer cells? (ii) Are effects of honey caused by only sugars or are other micronutrients present in this natural healer? Most of the evidences we discussed in this chapter are based on the studies on cancer cells or in animal models. However, there is still need for organized clinical trials to delineate its impact with conformity on human subjects as well. Hence, all these reports warrant a detailed systematic study involving in vitro, in vivo, preclinical, and ultimately patient-centric broad clinical trials so as to appreciate the nutraceutical potential of honey.

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