Phytochemicals from Honey as MAP-Kinase Inhibitors: Current Therapeutic Standing and Future Prospects

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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.

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