Hindawi Publishing Corporation
Journal of Biomedicine and Biotechnology
Volume 2009, Article ID 830616, 13 pages
doi:10.1155/2009/830616

Review Article

Antiproliferative Effects of Honey and of Its Polyphenols: A Review

Saravana Kumar Jaganathan and Mahitosh Mandal

School of Medical Science and Technology, Indian Institute of Technology, West-Bengal, Kharagpur 721 302, India

Correspondence should be addressed to Mahitosh Mandal, mahitosh@smst.iitkgp.ernet.in

Received 5 February 2009; Revised 16 April 2009; Accepted 13 May 2009

Recommended by Kapil Mehta

Honey has been used since long time both in medical and domestic needs, but only recently the antioxidant property of it came to limelight. The fact that antioxidants have several preventative effects against different diseases, such as cancer, coronary diseases, inflammatory disorders, neurological degeneration, and aging, led to search for food rich in antioxidants. Chemoprevention uses various dietary agents rich in phytochemicals which serve as antioxidants. With increasing demand for antioxidant supply in the food, honey had gained vitality since it is rich in phenolic compounds and other antioxidants like ascorbic acid, amino acids, and proteins. Some simple and polyphenols found in honey, namely, caffeic acid (CA), caffeic acid phenyl esters (CAPE), Chrysin (CR), Galangin (GA), Quercetin (QU), Kaempferol (KP), Acacetin (AC), Pinocembrin (PC), Pinobanksin (PB), and Apigenin (AP), have evolved as promising pharmacological agents in treatment of cancer. In this review, we reviewed the antiproliferative and molecular mechanisms of honey and above-mentioned polyphenols in various cancer cell lines.

1. Introduction

Prevention is better than cure and this is very true in case of cancer. Chemoprevention was defined as the administration of agents to prevent induction, to inhibit or to delay the progression of cancer [1], or as the inhibition or reversal of carcinogenesis at a premalignant stage [2]. Chemoprevention utilizes appropriate pharmacological agents [3, 4] or of dietary agents, consumed in diverse forms like macronutrients, micronutrients, or nonnutritive phytochemicals [5–7]. Consumption of antioxidants has been related to the several preventative effects against different diseases such as cancer, coronary diseases, inflammatory disorders, neurological degeneration, and aging [8, 9] led to search for natural foods rich in antioxidants. Although honey has been used since long time, only recently its antioxidant property came to limelight [10]. Honey has some minor constituents compared to its major sugar level, which is believed to have antioxidant properties [11, 12]. Some to mention were flavonoids and phenolic acids [13, 14], certain enzymes (glucose oxidase, catalase), ascorbic acid [15], carotenoid-like substances [16], organic acids [13], amino acids, and proteins [17].

Phytochemicals are one wide class of nutraceuticals found in plants which are extensively researched by scientists for their health-promoting potential. Honey has a wide range of phytochemicals including polyphenols which act as antioxidants. Polyphenols and phenolic acids found in the honey vary according to the geographical and climatic conditions. Some of them were reported as a specific marker for the botanical origin of the honey. Considerable differences in both composition and content of phenolic compounds have been found in different unifloral honeys [18]. Terpenes, benzyl alcohol, 3, 5-dimethoxy-4-hydroxybenzoic acid (syringic acid), methyl 3, 5-dimethoxy-4-hydroxybenzoate (methyl syringate), 3, 4, 5-trimethoxybenzoic acid, 2-hydroxy-3-phenylpropionic acid, 2-hydroxybenzoic acid and 1, 4-dihydroxybenzene are some of the phytochemicals ascribed for the antimicrobial activity of honey [19]. Among these phytochemicals, polyphenols were reported to have antiproliferative potential. In this review, we summarized the

compositional chemistry and antiproliferative potential of crude honey and some of its important polyphenols in various cancer cells.

2. Source and Compositional Chemistry of Honey

Honey bees collect the nectar from various floral sources and store it as honey which serves as food for bees during winter. Honey bees make a journey of nearly 55,000 miles to gather nectar from approximately 2 million flowers for accumulating one pound of honey. In the bee-hive, we can find thee types of bees namely the queen, drone and worker bees. Among them, only worker bees collect and regurgitate the nectar number of times, in order to partially digest the nectar, before storing in the honey comb. During the collection of nectar, pollen can be included into the honey though variety of ways. As the honeybee visits the flower in hunt of nectar, some of the flower's pollen falls into the nectar collected by the bee and stored in the stomach which will be regurgitated along with nectar. Moreover some pollen grains often attach themselves to the various parts of the honey bee body like legs, antenna, hairs, and also in the eyes of visiting bees which will get entangled in the hive and thereby paving entry into the honey. Airborne pollen is also another route of entry for pollen into the honey which got transferred though wind currents. Honey bees use its wings to fan the honey comb, to evaporate most of the water from nectar thereby avoiding the fermentation of honey. The color of the honey collected by the bees varies according to the floral source and its mineral content, which usually ranges from water white to dark amber. Flavor of the honey depends upon the color, generally the darker the honey the stronger the flavor and quality (Figure 1). It has been reported more than 300 unique varieties of honey depending upon the floral sources from United States alone. Honey mainly composed of sugars and water which accounts roughly 79.6% and 17.2%, respectively, (Figure 2). Major sugars of honey are levulose and dextrose which constitutes 38.19% and 31.28% correspondingly, remaining is the sucrose 1.3% and maltose 7.3%. Honey minor constituents include acids (0.57%), protein (0.266%), nitrogen (0.043%), amino acids (0.1%), a little amount of minerals (0.17%), and a number of other minute quantities of components like pigments, flavor and aroma substances, phenolics compounds, colloids, sugar alcohols and vitamins which all together accounts for the 2.1% of whole honey composition [20, 21].

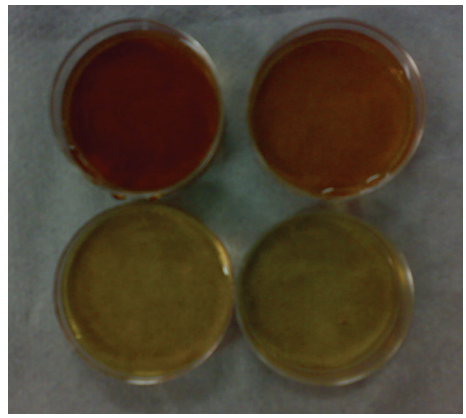


Figure 1: Color variation of honey samples from dark amber (top-left dish) to whitish yellow (bottom-right dish). Flavor of the honey depends upon the color, generally the darker the honey the stronger the flavor and quality.

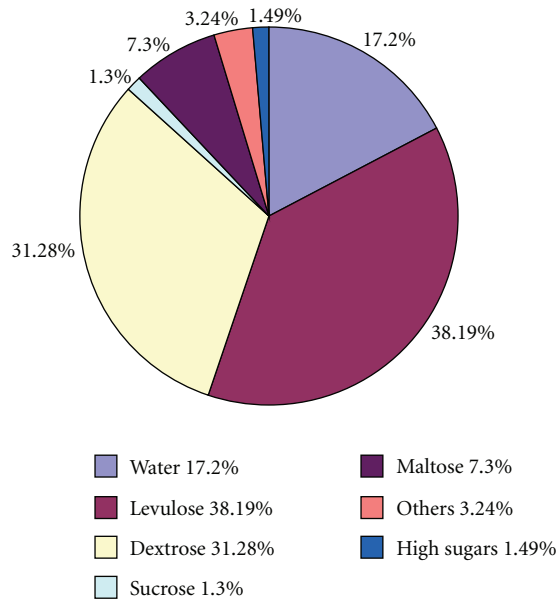


Figure 2: Pie-chart of Honey composition indicating the percentage share of various sugars, water and other minor constituents.

3. Anticancerous Property of Crude Honey

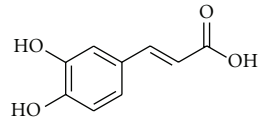
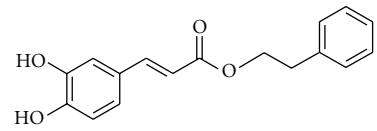
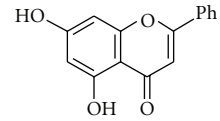
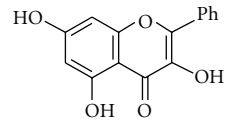
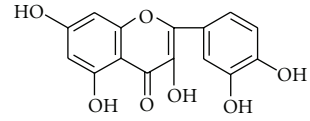
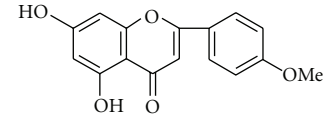
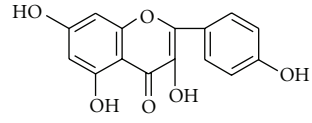
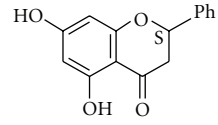
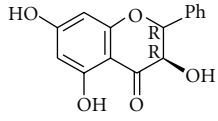
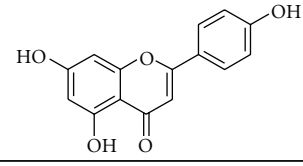
Few researchers studied the effect of crude honey in cancer. In a recent research conducted by Jaganathan et al. illustrated the apoptosis inducing ability of the honey. They showed honey induced apoptosis in human colon cancer cells by arresting the cells at subG1 phase. Honey possessing higher phenolic and tryptophan content was more potent in inhibiting the colon cancer cell proliferation. Finally they demonstrated that honey induced apoptosis was associated with Caspase-3 activation and PARP cleavage. DNA fragmentation assay in HT 29 cells displayed typical ladder pattern confirming apoptosis [22]. Research by Orsolic et al. showed that water soluble derivative of propolis and its associated phenolic compounds have antimetastatic effect on the tumor mice models before and after the injection of tumor cells. Further they showed that honey could exert antimetastatic effect when given before tumor cell injection [23]. In the study conducted by Tarek et al. honey was proven to be a very effective agent for repressing the growth of bladder cancer cell lines (T24, RT4, 253J, and MBT-2) in vitro. Further honey was found to be effective when administered intralesionally or orally in the MBT-2 bladder cancer implantation models. There was also a significant

difference between the final tumor volume ($P < .05$) in the Intra lesion (IL) honey-treated groups (IL 6% honey) compared to the IL saline group. The difference between the final tumor volume or weight in the IL saline group and the control group was not significant [24]. Research conducted by Gribel and Pashinskii indicated that honey exhibited moderate antitumor and significant antimetastatic effects in five different strains of rat and mouse tumors. Moreover, the antitumor activity of certain chemotherapeutic drugs such as 5-fluorouracil and cyclophosphamide was also facilitated by the honey [25]. It has been elucidated that polyphenols are anticarcinogenic, antiinflammatory, antiatherogenic, antithombotic, immune modulating and also act as antioxidants [26–31]. Hence antitumor properties of honey could be attributed to the polyphenols found in the honey. Moreover, with the evolution of extraction procedure for various polyphenols, which had been attributed with anticancerous property of honey, researchers concentrated on the polyphenolic compounds extracted from the honey rather than crude honey itself.

Phenolic compounds or polyphenols are the important groups of compounds occurring in plants, where they are widely distributed, comprising at least 8000 different known structures [32]. It is also produced by plants as a secondary metabolite. Some of these phenolic compounds were also available in the honey. In general, phenolic compounds can be divided into at least 10 types depending upon their basic structure: simple phenols, phenolic acids, coumarins and isocoumarins, naphthoquinones, xanthones, stilbenes, anthaquinones, flavonoids and lignins. Flavonoids constitute the most important polyphenolic class, with more than 5000 compounds already described. Flavonoids are the natural antioxidants exhibiting a wide range of biological effects including antibacterial, antiinflammatory, antiallergic, antithombotic and vasodilatory actions [26].

Various polyphenols were reported in honey. Polyphenols found in the honey was used as a marker for particular type of honey, for example, flavanol kaempferol as an indicator for rosemary honey [33, 34] and quercetin for sunflower honey [35]. The hydroxy-cinnamates like caffeic acid, ferulic acid and p-coumaric acid have been found in the chest-nut honey [36]. Characteristic flavonoids of propolis like pinocembrin, pinobanksin and chrysin were also found in the most European honey samples [35]. In this review, we concentrated on the some major polyphenols available in the honey which exhibited antiproliferative effect on various cancer cell lines. The list of compounds (refer Table 1 for figures) reviewed for their anticancerous activity is Caffeic acid (CA), Caffeic acid phenyl ester (CAPEs), Chrysin (CR), Galangin (GA), Quercetin (QU), Acacetin (AC), Kaempferol (KF), Pinocembrin (PC), Pinobanksin (PB), and Apigenin (AP).

Table 1: Molecular representation of Polyphenols found in the honey.

Polyphenols	Descriptive figures
Caffeic acid	
Caffeic acid phenyl ester	
Chrysin	
Galangin	
Quercetin	
Acacetin	
Kaempferol	
Pinocembrin	
Pinobanksin	
Apigenin	

Ph- phenyl; Me- methyl

3.1. Role of Individual Polyphenols in Cancer

3.1.1. Effect of Caffeic Acid and Its Esters in Animal Model and Cancer Cell Lines. Caffeic acid is a naturally occurring phenolic compound present in the honey. Research conducted by Hirose et al. studied the carcinogenicity of low dietary levels of the antioxidants like butylated hydroxyanisole (BHA), caffeic acid, sesamol, 4-methoxyphenol (4-MP) and catechol. These antioxidants were eminent target of the forestomach or glandular stomach and these were examined for their predominant effects in alone or in combinations, for

a two-year period experiment. Carcinogenicity study was undertaken in groups of 30-31 male F344 rats, by treating with 0.4% BHA, 0.4% caffeic acid, 0.4% sesamol, 0.4% 4-MP and 0.16% catechol either alone or in combination for up to 104 weeks and then killed. The ultimate average body weights of rats having basal diets were higher than those treated with antioxidants alone, and were the lowest in the combinational groups. Moreover the relative liver and/or kidney weights were greater than before in the BHA, sesamol, catechol and combination groups. It led to the conclusion, that the occurrences and frequencies of fore-stomach histopathological lesion were increased by exposing to antioxidants except in the case of BHA. The incidences and/or multiplicities of forestomach papillary or nodular (PN) hyperplasia were appreciably increased in the groups treated with 4-methoxyphenol, caffeic acid and the antioxidants in combination, as compared with the basal diet group. Studies on medium-term multiorgan carcinogenesis model, suggested an increase in the occurrence of fore-stomach papillomas in each high-dose group and no synergistic effect was observed in combinations. In the low dose case, the incidence of fore-stomach papillomas was significantly increased only in the combination group. The effect on the other organs particularly colon tumors, was significantly decreased only in the high-dose combination group. Hence it can be inferred that at low dose levels, the phenolic compounds can exhibit additive/synergistic effect on carcinogenesis [37]. From these early experiments, caffeic acid is still listed under older Hazard Data sheets as a potential carcinogen.

Rao et al. performed a detailed study by synthesizing thee caffeic acid esters namely methyl caffeate (MC), phenylethyl caffeate (PEC) and phenylethyl dimethylcaffeate (PEDMC) and examined them against the 3, 2′-dimethyl-4-aminobiphenyl (DMAB, a colon and mammary carcinogen) induced mutagenecity in *Salmonella typhimurium* strains TA 98 and TA 100. Both the strains of *Salmonella* subsisted (survival rate >98%) concentration of about 2,500 μM CA, 150 μM MC, 70 μM PEC and 80 μM PEDMC/plate. Moreover 150 μM MC, 40–80 μM of PEDMC, 40–60 μM of PEC significantly inhibited the DMAB-induced mutagenecity in both strains. The outcome of these experiments placed MC at a concentration greater than 225 μM and PEC and PEDMC at a level greater than 60 μM was toxic. CA exhibited significant toxicity only at above 2500 μM concentration. In colon cancer cell line (HT-29), cytotoxicity effect of CA, PEC, PEDMC and MC was evaluated. The growth inhibitory effect of these compounds was measured after exposing cells for a period of 48 hours. CA was found to be the least effective in inhibiting the growth of HT-29 cells when compared to its ester analogs. To further corroborate the growth inhibitory effects, synthesis of polynucleotide and protein synthesis after incubating the HT-29 cells with these agents for 48 hours were investigated. It has been observed that at the concentration of 175 μM of MC, 40 μM of PEC and 60 μM of PEDMC blocked the DNA, RNA and the protein synthesis. Moreover ornithine decarboxylase (ODC) activity was inhibited at concentrations of 150 μM MC, 40 μM PEC and 20 μM PEDMC. Tyrosine protein kinase (TPK) activity was also inhibited at concentrations of 100 μM of MC, 30 μM of PEC and 20 μM of PEDMC [38]. In their follow-up studies made by them, reported the inhibitory effects of methyl caffeate (MC) and phenylethyl caffeate (PEC) on azoxymethane-(AOM-)induced ornithine decarboxylase (ODC), tyrosine protein kinase (TPK) and arachidonic acid metabolism in liver and colonic mucosa of male F344 rats. They depicted the inhibitory effects of caffeic acid, MC, PEC, phenylethyl-3-methylcaffeate (PEMC), and phenylethyl dimethyl caffeate (PEDMC) on in vitro arachidonic acid metabolism in liver and colonic mucosa. Finally they investigated the effects of PEC, PEMC, and PEDMC on AOM-induced aberrant crypt foci (ACF) formation in the colon of F344 rats. For a period of five weeks, groups of F334 rats were fed with diets containing 600 ppm of MC or PEC for biochemical studies and 500 ppm of PEC, PEMC or PEDMC for ACF studies. After two weeks, subcutaneous injection of AOM was given once in a week for two consecutive weeks, for all animals except the vehicle-treated groups. Biochemical studies were performed by sacrificing the animal after 5 days. In case of ACF study, F334 rats were sacrificed after 9 weeks latter for analyzing ACF in colon. The colonic mucosa and liver of the rats were analyzed for the orinithine decarboxylase activity, tyrosine protein kinase activity (TPK), lipoxygenase and cyclooxygenase metabolites. PEC diet significantly inhibited AOM-induced ODC and TPK activities in liver and colon. It had been observed that PEC diet significantly repressed the AOM-induced lipoxygenase metabolites 8(*S*)- and 12(*S*)-hydroxyeicosatetraenoic acid (HETE). The animals fed the MC diet exhibited a moderate inhibitory effect on ODC and 5(*S*)-, 8(*S*)-, 12(*S*)-, and 15(*S*)-HETEs and a significant effect on colonic TPK activity. However, both the MC and PEC diets showed no significant inhibitory effects on cyclooxygenase metabolism. ACF were significantly inhibited in the animals fed with PEC (55%), PEMC (82%), or PEDMC (81%). The results of the study indicated that PEC, PEMC, and PEDMC present in the honey, inhibited AOM-induced colonic preneoplastic lesions, ODC, TPK, and lipoxygenase activity, which were relevant to the colon carcinogenesis [39].

Huang et al. showed the strong repressive effect of CAPE application on 12-0-tetradecanoylphorbol-13-acetate-(TPA-)induced tumor promotion and production of 5-hydroxymethyl-2′-deoxyuridine (HMdU) in the deoxyribonucleic acid (DNA) of the mouse skin. They established the inhibitory effect of CAPE on TPA-induced tumor promotion by topical application of CAPE in CD-I mice previously treated with 7, 12-dimethylbenz[a]anthracene (DMBA). They applied CAPE in concentration ranging from 1, 10, 100, or 3000 nmol together with 5 nmol of TPA twice a week for 20 weeks. At the above concentrations, CAPE inhibited the number of skin papillomas by 24, 30, 45 and 70% and tumor size per mouse was decreased by 42%, 66%, 53%, and 74%, respectively. Moreover topical application of 5 nmol of TPA twice weekly for 20 weeks to mice produced an average of 12.6 HMdU residues per 104 normal bases in epidermal DNA. Topical application of 1, 10, 100, or 3000 nmol of CAPE along with 5 nmol of TPA twice weekly for 20 weeks to DMBA-initiated mice decreased the levels

of HMdU in epidermal DNA by 40–93%. CAPE at 1.25, 2.5, 5, 10, or 20 μM inhibited the incorporation of [^{3}H]-thymidine into DNA in cultured HeLa cells by 32%, 44%, 66%, 79%, and 95% respectively. Similarly incorporation of [^{3}H]-uridine into RNA was inhibited by 39%, 43%, 58%, 64%, and 75% whereas incorporation of [^{3}H]-leucine into protein was inhibited by 29%, 30%, 37%, 32%, or 47%, respectively. These results indicated that CAPE is a potent inhibitor of DNA synthesis but it is somewhat less effective in inhibiting RNA synthesis and it is least effective in inhibiting the protein synthesis [40].

The molecular basis of CAPE action was elucidated by Natrajan et al. Since NF-κB has a role in these activities, they examined the effect of CAPE on this transcription factor in an exhaustive manner. They preincubated the U-937 cells with CAPE with various concentrations for 2 hours before treating with TNF (0.1 nM) for 15 minutes. CAPE inhibited the TNF-dependent activation of NF-κB in a dose-dependent manner with maximum effect occurring at 25 μg/mL. NF-κB activation induced by the phorbol ester, phorbol-12-myristate 13-acetate (PMA), ceramide, okadaic acid and hydrogen peroxide was also inhibited by CAPE. It prevented the translocation of p-65 subunit of NF-κB to the nucleus without affecting the TNF-induced IκBα degradation. It does not showed any inhibitory effect on the other transcription factors like AP-1, TFIID and oct-1. To study further precisely about the role of CAPE in inhibiting NF-κB various structural analogues of CAPE were examined. It has been configured that a bicyclic, rotationally constrained, 5, 6-dihydroxy form showed supremacy, whereas 6, 7-dihydroxy variant was the least active in inhibiting the NF-κB. With these findings they concluded that CAPE is a potent and a specific inhibitor of NF-κB activation and this may provide the molecular basis for its multiple immunomodulatory and antiinflammatory activities of CAPE [41].

In another study initiated by Lee et al. investigated the cytotoxicity potential of CAPE and the molecular mechanism of its action in C6 glioma cells. The results of the experiments indicated C6 glioma cells underwent internucleosomal DNA fragmentation after 24 hours treatment with CAPE (50 μM). FACS analysis of CAPE-treated C6 glioma cells showed increasing accumulation of hypodiploid nuclei (24% at 36 hour) in time-dependent fashion. Further results showed that CAPE induced the release of cytochome-c from mitochondria into the cytosol after 3 hours of treatment resulting in the activation of caspase-3 (CPP32) from the beginning of 3 hours. Moreover the cleavage of PARP (substrate of CPP32) started within 12 hours after CAPE treatment. CAPE enhanced the serine phosphoryaltion of p53 after 0.5 hours and the protein level of p53 was increased after 3 hours. CAPE treatment also enhanced the expression of Bax and Bak and resulted in the reduced level of B-cell lymphoma/leukemia-2 gene (Bcl_2) protein (after 36 hours). Moreover they reported that CAPE application activates the extracellular signal-regulated kinase (ERKs) and p38 mitogen-activated protein kinase (p38 MAPK) in the C6 glioma cells. Further they showed that expression of p53, phospho-serine 15 of p53, Bax and inactivate form of CPP32 were suppressed by a pretreatment of a specific p38 MAPK inhibitor, SB203580. Hence they concluded p53 dependent apoptosis in C-6 glioma cells were mediated by p38 MAPK [42].

Chung et al. showed both CA and CAPE selectively inhibited Matrix Metalloproteinases-2 (MMP-2) and MMP-9. CAPE inhibited strongly with IC_{50} of 2–5 μM whereas CA requires 10–20 μM. But MMP-1, 3, 7 and Cathepsin-K were not completely inhibited by both of them. CA and CAPE had a dose-dependent inhibitory effect on the proliferation of HEPG2 cells. In HepG2 cells, CA at the concentration of 200 μg/mL reduced the cell viability to 61% compared to the control, and the treatment with CAPE (at low concentration of 20 μg/mL) reduced the viability to 72% compared of the control. CAPE and CA suppressed the MMP-9 expression, exposed to phorbol 12-myristate 13-acetate (PMA), by blocking the NF-κB activity in HEPG2 cells. They also confirmed that CA (20 mg/kg) and CAPE (5 mg/kg) repressed the growth of HepG2 tumor xenografts in nude mice as well as liver metastasis when administered subcutaneous or orally. Finally they concluded their observation that CA and its derivative CAPE: (1) inhibited the enzymatic activity of MMP-9 that plays an important role in cancer invasion and metastasis, (2) blocked the invasive potential through the suppression of MMP-9 gene transcription by inhibiting NF-κB function in PMA-stimulated HepG2 cells and (3) suppressed the growth of HepG2 cell xenografts in nude mice. Therefore, these two drugs were reported as strong candidates for treatment of cancer and metastasis via dual mechanisms (dual inhibition of metastasis-specific enzyme activity and gene transcription) [43].

Further in a recent study initiated by Hwang et al. investigated the effect of CAPE on tumor invasion and metastasis in HT 1080 fibrosarcoma cells by determining the regulation of matrix metalloproteinase's (MMPs). HT 1080 cells were treated with increasing concentration of CAPE and the m-RNA transcripts of MMP-2 and MMP-9 were analyzed using semi-quantitative RT-PCR. Both MMP-2 and 9 proteins levels were significantly suppressed at dose dependent manner. Gelatin zymography also indicated constitutively expressed MMP-2 and 9 proteins in HT 1080 cells which gradually reduced after treating with CAPE. To further corroborate the downregulation of MMP-2, activation studies of pro-MMP2 were performed using organomercuric compound, 4-aminophenylmercuric acetate (APMA), and the result indicated the down regulation of MMP-2 by CAPE. It has been shown that m-RNA levels of Tissue inhibitor of matrix metalloproteinase's (TIMPs) and Membrane type-Matrix Metalloproteinase's (MT-1 MMPs) were also reduced significantly. CAPE also inhibited the cell invasion, cell migration and colony formation of tumor cells. Thus CAPE acts as a vital antimetastatic agent, by inhibiting the metastatic and invasive potential of malignant cells [44].

Moreover some researchers investigated the possible UVC (280–100 nm) protective properties of caffeic acid in human diploid fibroblast and A-431 epidermoid cancer cell lines. The UVC safeguarding effect of CA in two different concentrations (55.5 μM and 166.5 μM) was clearly illustrated both in transformed and normal cells. A marked difference in the proliferation of normal and transformed

cells when irradiated to UVC radiation was observed when cell was grown in DMEM media containing CA. CA's protective effect was distinct in the transformed cells compared to normal cells [45]. In a sequential study by Vanisree et al. explained protective effect of CA against UVB (280–320 nm) radiation-induced IL-10 expression and the activation of the Mitogen-activated Protein Kinases (MAPKs) in mouse skin. CA inhibited the IL-10 promoter transcription, measured using in vivo transgenic IL-10 promoter-luciferase reporter gene base assay. IL-10 mRNA expression and protein production in the mouse skin were significantly repressed by CA. There have also been shown the upstream regulators like extracellular regulated protein kinase (ERK), c-Jun N-Terminal protein, p-38 mitogen-activated protein kinase (p38 MAPK) and the downstream transcription factors like activator protein (AP-1) and nuclear factor kappa B (NF-κB) were also inhibited by CA in mouse skin. From these experiments it was inferred that CA could be used as a topical agent against harmful UVB irradiation [46].

3.1.2. Effect of Chrysin and Its Derivatives in Cancer Cell Lines. Chrysin (5, 7-dihydroxyflavone) is a natural and biologically active compound extracted from honey, plants and propolis. It possesses potent antiinflammatory, antioxidant properties and promotes cell death by perturbing cell cycle progression. In a recent study conducted by Weng et al. illustrated the molecular mechanism of action of chrysin against C6-glioma cells. In an antiproliferation assay performed on C6 glioma cells, chrysin inhibited the cell proliferation after 24, 48 and 72 hours. After 72 hours of incubation with 50 μM of chrysin, 90% of cell proliferation was inhibited. Flow cytometry analysis reported that by 30 and 50 μM treatments after 24 hours, chrysin increased the proportion of cells in the G1 phase of the cell cycle from 69 to 79% and 83% and decreased the proportion of S phase cells from 11.4 to 6.1% and 2.8%, respectively. The proportion of G2/M phase cells changed from 17.9 to 12.2% and 9.2%, after 30 and 50 μM treatments. It has been found that levels of phosphorylation of Retinoblastoma (Rb) protein in C6 glioma cells decreased after treating with 30 μM of chrysin. Moreover in chrysin-treated cells, it has been demonstrated that cyclin dependent kinase inhibitor ($p21^{Waf1/Cip1}$) levels are increased significantly without the change in p53 protein level. To depict the role of p38 in chrysin-mediated $p21^{Waf1/Cip1}$ induction, they used p38 specific inhibitor which resulted in the lowering of $p21^{Waf1/Cip1}$ level. Furthermore they showed that proteosome activity, cyclin dependent kinase 2 (CDK2) and 4 (CDK4) were also inhibited by chrysin. These results suggested that chrysin exerts its growth-inhibitory effects either through activating p38-MAPK leading to the accumulation of $p21^{Waf1/Cip1}$ protein or through mediating the inhibition of proteosome activity [47].

In another study by Woo et al. reported the chrysin-mediated apoptosis in U-937 cancer cell lines. DNA fragmentation assay of chrysin-treated cells after 12 hours showed typical inter-nucleosomal fragmentation of DNA. FACS analysis of treated cells showed marked increase of accumulation of subG1 cells after 12 hours. Decreased proenzyme level of caspase-3 after chrysin treatment indicated the importance of activated caspase-3 in apoptosis. Further the activation of phospho-lipase C-γ (PLC-γ), a down stream target of caspase-3 in chrysin treated cells confirmed the role of caspase-3 in chrysin treated U937 cells. Western blotting analysis of chrysin treated cells indicated the reduction in the level of XIAP (a member of Inhibitor of Apoptosis Proteins) and cytochrome c induction in dose dependent manner. Mitogen activated protein kinase (MAPK) does not have any role in the signaling pathway as shown by western blot analysis, whereas Akt-signaling played significant role in chrysin mediated apoptosis of U937 cells. It has been shown that inhibition of Akt phosphorylation in U937 cells by the specific PI3K inhibitor, LY294002, significantly enhanced the apoptosis. Overexpression of a constitutively active Akt (myr-Akt) in U937 cells inhibited the induction of apoptosis, activation of caspase 3 and PLC-γ1 cleavage by chrysin [48].

Further Zheng et al. synthesized 13 derivatives of chrysin and tested it for anticancer effect against human gastric adenocarcinoma cell line (SGC-7901) and colorectal adenocarcinoma (HT-29) cells. These derivatives were formed mainly by alkylation, halogenation, nitration, methylation, acetylation and trifluoromethylation. MTT assay revealed that 5, 7-dimethoxy-8-iodochrysin and 8-bromo-5-hydroxy-7-methoxychrysin have the strongest activities against SGC-7901 and HT-29 cells respectively. The compound 5, 7-Dihydroxy-8-nitrochrysin was found to have strong activities against both SGC-7901 and HT-29 cells [48]. Zhang et al. tried to improve the biological properties of chrysin by synthesizing diethyl chrysin-7-yl phosphate (CPE: C19H19O7P) and tetraethyl bis-phosphoric ester of chrysin (CP: C23H28O10P2) though a simplified Atheron-Todd reaction. In Mass spectroscopy analysis, CPE formed complexes with lysozyme and hence phosphate esters of chrysin enhanced the interaction with proteins compared to unmodified chrysin. Cultured human (HeLa) cell lines were treated by CR, CP and CPE with 10 μM for 24, 48, and 72 hours. The Cell viability markedly declined in time-dependent fashion. Moreover methyl green-pyronin staining, PCNA immunohistochemistry and TUNEL techniques were also employed to study the effect of CR, CPE and CP in the cultured HeLa cell lines. It favored their hypothesis that all CR, CP and CPE could inhibit proliferation and induce apoptosis in the following order of inhibition potency CP > CPE > CR. Hence they suggested CP and CPE as a new potential candidate for human cervical cancer [49].

3.1.3. Effect of Galangin in Leukemia Cancer Cell Line. Charles et al. described the antiproliferative effect of galangin on human leukemia (HL-60) cell line. Trypan blue exclusion method indicated the remarkable decrease in the cell viability after treating with 100 μM for 24 hours. Galangin of 1–10 μM exerted antiproliferative effect which is evident after 48 hours of incubation. Early and late apoptosis were detected using annexin-V-FITC and PI staining using 100 μM galangin and these results correlated with the results of trypan-blue method reported already. Active caspase-3, a hallmark of apoptosis process, was detected after 24 hours and

72 hours of incubation with 50 and 10 μM of galangin respectively. Cell cycle analysis indicated the increase in the subG1 phase of galangin (>10 μM) treated cells. This was illustrated further in DNA fragmentation assay, in which they could observe typical ladder pattern after 24 hours of 100 μM galangin exposure. Forward and side scatter changes were predominantly observed after 24 hours and 72 hours incubation with 100 μM galangin. Galangin treated cells displayed reduced forward scatter indicative of decreased relative size, and enhanced side scatter indicative of increased internal complexity. Rhodamine median florescence intensity measured as an indicator of ROS levels, showed no evidence for intracellular oxidative stress as a key-player of cytotoxicity and significant phagocyte-like differentiation was not detected [50].

3.1.4. Effect of Quercetin in Cancer Cell Lines. Kang et al. investigated the role of quercetin as an anticancer agent in HL-60 cells. From their experiments they inferred the concentration dependent inhibition of HL-60 cell proliferation between the ranges of 10 to 80 μM. They showed cells incubated with 10 μM displayed inhibition on the growth of HL-60 cells. It was 17.1%, 27.3%, 40.1%, and 52.7% after 24, 48, 72, and 96 hours of treatment. Cell cycle analysis indicated that quercetin (20, 40, and 60 μM) increased the number of cells in the G_2/M phase from 7.6% to 12.4%, 19.1%, and 23.5% correspondingly, and decreased the population of G_0/G_1, cells from 46.2% to 40.2%, 32.1%, and 34.5%, respectively, without significant changes in the S-phase cell population after 24 hours of treatment. Quercetin showed remarkable inhibitory effect on the activities of cytosolic Protein Kinase C (PKC) and membrane TPK of HL-60 cells in vitro, with IC_{50} values of about 30.9 and 20.1 μM, respectively, but did not have the effect on membrane PKC or cytosolic TPK activity. It has also repressed the complete activity of phosphoinositides like phosphatidylinositol (PI), phosphatidylinositol 4-phosphate (PIP), and Phosphatidylinositol 4, 5-bisphosphate (PIP2) at the concentration of 80 μM. Hence they concluded that the inhibitory effect of quercetin on the growth of HL-60 cells may be related to its inhibitory effects on PKC and/or TPK in vitro and/or on the production of phosphoinositides [51]. Csokay et al. studied the effect of quercetin in K562 human leukemia cells. Treatment with quercetin (5.5 μM) activated both apoptosis and differentiation programs. After 1 hour exposure to the drug it resulted in apoptosis of the leukemia cells. Differentiation of K562 cells was observed atleast after 12 hours of exposure. They attributed these effects to the early downregulation of c-myc and Ki-ras oncogenes and rapid reduction of Inositol-1, 4, 5-triphosphate (IPs) concentrations [52].

Robaszkiewicz et al. illustrated the effect of quercetin in A-549 cells. They found quercetin exerted both antioxidant and pro-oxidant properties depending upon the concentration used. Quercetin in low concentration (1–20 μM) promoted the cell proliferation whereas higher concentration (50–200 μM) showed the concentration dependent cytotoxicity. The lower concentration (10 μM) of quercetin produced increased number of live cells, repressing the number of cells in the apoptotic and necrotic portions. On the other hand if the concentration was above 50 μM, it reduced the number of live cells by increasing the apoptotic/necrotic fractions. Quercetin decreased production of reactive oxygen species in the cells producing peroxides in the medium. They also found incubating with low concentrations of quercetin led to a small increase in Total Antioxidant Capacity (TAC) of cell extracts but higher concentrations of the quercetin led to a progressive decrease in the TAC of cell extracts. Total thiol content of the cells followed a pattern similar to TAC. Hence they suggested that cellular effects of quercetin are complex and include both antioxidant effects and induction of oxidative stress due to formation of reactive oxygen species in the extracellular medium [53]. In another study made by Elizandra et al. substantiated that quercetin may act differently on cancer and normal neuronal tissue. Quercetin decreased the cell viability in glioma cell cultures resulting in necrotic and apoptotic cell death. It also arrested the glioma cells in the G_2 checkpoint of the cell cycle, and decreased the mitotic index. Furthermore, they demonstrated quercetin was able to protect the hippocampal organotypic cultures from ischemic damage. These results showed that although it induced growth inhibition and cell death in the U138MG human glioma cell line, still it has a cytoprotective effect in normal cell cultures [54].

Indap et al. examined the antiproliferative effect of quercetin both in vitro and in vivo. They showed quercetin could exert antiproliferative effect against MCF-7 cell line in a dose and time dependent manner with IC_{50} value of 10 μg/mL. Further quercetin was found to arrest the MCF-7 cell growth in G2/M phase of cell cycle. Moreover it was shown that quercetin inhibited the tumor growth by more than 58% in mice grafted with mammary carcinoma and it extended the survival ability of sarcoma 180 bearing mice by 2.3 times. Further quercetin enhanced the inhibitory effect of mitomycin C in mammary adenocarcinoma. Finally they concluded that these effects were mediated in part by the often poorly vascularised and hypoxic regions of tumors [55]. In a recent study initiated by Choi et al. studied the anticancer effect of quercetin against breast cancer cell (MDA-MB-435). MTT assay revealed that quercetin showed inhibitory effect on MDA-MB-435 cell growth in a time and dose dependent manner. Further cell cycle analysis of quercetin treated cells showed significant increase in the accumulation of cells at subG1 phase. Further quercetin treatment increased Bax expression but decreased the Bcl_2 levels. Cleaved caspase-3 and PARP expression were also increased by quercetin [56].

3.1.5. Effect of Acacetin in Liver and Lung Cancer Cell Lines. Hsu et al. investigated the antiproliferative effect of acacetin in human liver cancer cell line (HepG2). The maximum inhibitory effect (nearly 72%) was observed at a concentration 20 μg/mL after 48 hours. The IC_{50} value was observed to be 10.44 μg/mL for the HepG2 cells. Flow cytometry results indicated an increase in the G1 phase of cells from 31.1 to 61.6 and 76.5% at a concentration of 10 and 20 μg/mL,

respectively. DNA fragmentation assay of cells treated with acacetin indicated the number of cells undergoing apoptosis increased to about 4-fold at 10 μg/mL and 8-fold at 20 μg/mL after 48 hours. Further it has been demonstrated that acacetin increased the induction of p53 and its downstream target, p21/WAF1 as assayed by Enzyme linked Immunosorbent assay (ELISA). Fas ligand assay indicated that FasL, mFasL and sFasL increased in a dose-dependent manner. Pro-apoptotic Bax protein level also increased due to acacetin treatment at 24 and 48 hours. In their continuity study, they examined the role of acacetin in human nonsmall cell lung cancer A549 cells. They reported the antiproliferative effect was significant in dose-dependent manner and the IC_{50} value was found to be 9.46 μM. Cell cycle analysis of A-549 cells treated with 5 and 10 μM of acacetin indicated an increase in G_1 phase from 34.7% to 42.6% and 61.2%, respectively. Similarly DNA fragmentation assay indicated the number of cells undergoing apoptosis increased from about 3.2-fold to 8.1-fold at 5 and 10 μM of acacetin, respectively after 48 hours. Similar to the observation in HepG2 cells, acacetin increased the induction of p53 and its downstream target, p21/WAF1 as assayed by Enzyme linked Immunosorbent assay (ELISA). Fas ligand assay indicated that FasL, mFasL, and sFasL increased in a dose-dependent manner. They concluded that p53 and Fas/FasL apoptotic system may participate in the antiproliferative activity of acacetin in HepG2 and A549 cells [57, 58].

3.1.6. Effect of Kaempferol in Lung and Leukemia Cells. Henry et al. explored the significance of Kaempferol induced apoptosis in human lung nonsmall carcinoma cells (H460). Trypan blue exclusion assay, demonstrated the varying concentration of kaempferol reduced the cell viability in the dose-dependent manner with an IC_{50} value of 50 μM. Lactate dehydrogenase (LDH) assay indicated cell death is due to apoptosis since there is no release of LDH enzyme with the cells treated with kaempferol. ROS production is not the cause for the cytotoxicity observed, since the oxidant-sensitive fluorescent probe, CM-H2DCFDA signal does not showed any change after kaempferol treatment. Mitochondrial membrane potential measured using fluorescent probe 3, 3′-dihexyl-oxacarbocyanine (DiOC6), a mitochondrion-specific and voltage-dependent dye, indicated no change after treating with kaempferol at varying concentrations for 16 hours. Kaempferol (50 μM) induced Apoptosis inducing factor (AIF) from mitochondria to nucleus and elicited DNA fragmentation and condensation in H460 cells. The levels of pro-caspase 3 were decreasing after exposing to kaempferol for 8 hours. Moreover protein levels of Mn SOD and Cu/Zn SOD increased during treatment with 50 μM kaempferol for 24 hours [59].

Bestwick et al. reported the kaempferol antiproliferative effect in the pro-myelocytic leukemia cells (HL60). Dose-dependent inhibition of HL-60 was observed over 72 hours exposure to kaempferol. FACS analysis reported that treatment of cells with Kaempferol (10 μM) decreased the cell growth. After 5 hours of treatment the proportion of cells in S-phase increased compared to decrease in the G1 phase. 100 μM of kaempferol induced an initial accumulation in S-phase and then G2/M as the time course progressed from 48 to 96 hours. Phosphodityl serine exposure without membrane damage as indicated by annexin V-FITC binding, a feature of the early prenecrotic phase of apoptosis, was only observed for a minor proportion of cells treated with $\geq$20 μM kaempferol following either 24 hours or 72 hours treatments. After exposing the cells with kaempferol for 24 and 72 hours, a decrease in the mitochondrial potential followed by enhanced expression of active caspase-3 was observed. Retinoic acid treatment results nearly 5% differentiation of the cell population indicated by phorbol 12-myristate 13-acetate stimulated nitro blue tetrazolium NBT reduction over 72 hours of treatment with 100 μM of kaempferol. Multiparametric flow cytometric analysis revealed distinct subpopulations of cells with decreased size, typical of apoptosis and necrosis, possessing heightened caspase-3 activity followed by decreased antiapoptotic Bcl_2 expression and changes in the membrane asymmetry and integrity. The remaining population had elevated active caspase-3 but no change or a moderate increase in Bcl_2 expression and no plasma membrane alterations. Hence kaempferol growth inhibitory effect on HL-60 leukemia cells is due to heterogeneous response mainly dominated by cell cycle alternation although some degree of cytotoxicity results from apoptotic as well as nonapoptotic process [60].

3.1.7. Role of Pinocembrin and Pinobanksin in Cancer Cell Lines. Suresh Kumar et al. showed cytotoxicity of pinocembrin against a variety of cancer cells including normal lung fibroblasts with relative non toxicity to human umbilical cord endothelial cells. Pinocembrin induced loss of mitochondrial membrane potential (MMP) with subsequent release of cytochome *c* and processing of caspase-9 and -3 in colon cancer cell line HCT 116. The initial trigger for mitochondrial apoptosis appears to be by the translocation of cytosolic Bax protein to mitochondria [61]. Pinobanksin has been reported to exert antioxidant activity by lowering the Fe (II) induced lipid peroxidation as well as inhibiting the mitochondria membrane permeability transmission (MMPT) [62].

3.1.8. Effect of Apigenin in Cancer Cells. Apigenin belongs to the flavonoid family and it is widely reported for its antitumor effects in various cell lines. It had exerted antiproliferative effect against colon, breast, cervical, neuroblastoma and liver cancer cell lines. Wang et al. studied the effect of apigenin on the cell growth and cell cycle in the colon carcinoma cell lines like SW480, HT 29 and Caco-2. Cell count and protein content of the apigenin treated cells showed reduction compared to the control. Apigenin inhibited the cell growth with the IC_{50} values of 40, 50, and 70 μM for the SW480, HT-29, and Caco-2 cells respectively. Flow cytometric analysis of apigenin (80 μM) treated cells resulted in G2/M arrest of 64%, 42%, and 26% in SW480, HT-29, and Caco-2 cells respectively. They had also reported the inhibition of $p34^{cdc2}$ kinase and cyclin B1 proteins in the apigenin

Table 2: Summary of in vitro studies of honey polyphenols.

Compound	Cell line tested	Observation/result	Reference no.
CA, MC, PEDMC, PEC	HT -29	Toxicity: CA >2500 μM PEC >60 μM PEDMC >60 μM MC >225 μM Inhibition of DNA/RNA: 150 μM MC, 40 μM PEC and 20 μM PEDMC TPK activity downregulation: 100 μM of MC, 30 μM of PEC and 20 μM of PEDMC ODC activity downregulation: 150 μM MC, 40 μM PEC and 20 μM PEDMC	[38]
CAPE	HeLa	Substance Inhibition percentage Concentration DNA 95% 20 μM RNA 75% 20 μM Protein 47% 20 μM	[40]
CAPE	U-937	(a) Maximum inhibition of NFκB at 25 μg/mL after TNF treatment (b) No inhibitory effect on AP-1, TFIID, and Oct-1 (c) Structural analogue 5, 6 dihydroxy strongly inhibited the NF-κB	[41]
CAPE	C6 glioma	(a) DNA fragmentation at 50 μM after 24 hours (b) p-p53 ↑, active Caspase 3↑, Bak and Bax ↑, Bcl_2 ↓	[42]
CA, CAPE	HepG2	(a) CA and CAPE inhibited MMP-2 and 9 with IC_{50} of 10–20 μM and 2–5 μM (b) CA at the concentration of 200 μg/mL reduced the cell viability to 61% viability compared to the controls, and the treatment with CAPE (20 μg/mL) in HepG2 cells reduced the viability to 72% of the controls	[43]
CAPE	HT 1080	(a) m-RNA levels of MMP-2 and MMP-9 were inhibited ↓ (b) m-RNA levels of TIMP-1 and MT-1 MMP level decreased ↓	[44]
Chrysin	C-6 glioma	(a) 72 hours of incubation with 50 μM of chrysin, inhibited ↓ 90% of cell-proliferation (b) $p21^{Waf1/Cip1}$ levels increased ↑, CDK2 and CDK4 were inhibited ↓	[47]
Chrysin	U-937	(a) PLC-γ and active Caspase-3 level increased ↑ (b) XIAP level decreased ↓ whereas cytochome-C level ↑	[48]
Galangin	HL-60	(a) Galangin of 1–10 μM also promoted antiproliferative effect which is evident after 48 hours of incubation (b) Active Caspase 3 ↑, a hallmark of apoptosis process, was detected after 24 hours and 72 hours of incubation with 50 and 10 μM of galangin respectively (c) Cell cycle analysis indicated the increase in the subG1 phase ↑ of galangin (>10 μM) treated cells	[50]
Quercetin	HL-60	(a) Quercetin had a remarkable inhibitory effect ↓ on the activities of cytosolic PKC and membrane TPK from HL-60 cells in vitro, with IC_{50} values of about 30.9 and 20.1 μM, respectively (b) Quercetin repressed ↓ the complete activity of phosphoinositides like PI, PIP, and PIP2 at the concentration of 80 μM	[51]
Quercetin	A-549	(a) Quercetin in low concentration (1–20 μM) promoted the cell proliferation ↑ whereas higher concentration (50–200 μM) showed the concentration dependent cyotoxicity ↓ (b) Increase in TAC ↑ of cell extracts but higher concentrations of the quercetin led to a progressive decrease in the TAC ↓	[53]

Table 2: Continued.

Compound	Cell line tested	Observation/result	Reference no.
Quercetin	K562	(a) reduction of c-myc and Ki-ras oncogenes ↓ (b) fall in Inositol-1,4,5-triphosphate (IPs) concentrations ↓	[52]
Quercetin	Glioma cell	(a) arrested the glioma cells in the G_2 checkpoint of the cell cycle (b) decreased the mitotic index	[54]
Quercetin	MCF-7	(a) IC_{50} value of 10 μg/mL (b) cell cycle arrest at G2/M phase (c) inhibited the tumor growth by more than 58% in mice grafted with mammary carcinoma	[55]
Acacetin	HEPG2	(a) IC_{50} value = 10.44 μg/mL (b) p53 ↑, $p21^{Waf1}$ ↑, FasL ↑, mFasL ↑, sFasL ↑ and Bax ↑	[58]
Acacetin	A-549	(a) IC_{50} value = 9.46 μM (b) p53 ↑, $p21^{Waf1}$ ↑, FasL ↑, mFasL ↑, sFasL ↑ and Bax ↑	[57]
Kaempferol	HL-60	(a) Mitochondrial potential decreased ↓ caspase-3 level increased ↑ (b) Kaempferol growth inhibitory effect on HL-60 leukemia cells is due to heterogeneous response mainly dominated by cell cycle alternation although some degree of cytotoxicity results from apoptotic as well as nonapoptotic process	[60]
Pinocembrin	HCT116	(a) Mitochondrial potential decreased ↓ (b) BAX translocates in to mitochondria (c) Cyt-C release (d) Caspase-3 and Caspase 9 level increased (↑)	[61]
Pinobanksin	Rat liver Mitochondria	(a) Inhibits the mitochondria membrane permeability transition ↓ (b) Lowers the lipid peroxidation ↓	[62]
Apigenin	Colon cancer	(a) inhibition of $p34^{cdc2}$ kinase (b) cyclin B1 ↓ (c) IC_{50} values: HT 29 = 50 μM SW480 = 40 μM Cac0-2 = 70 μM	[63]
Apigenin	Breast Cancer	(a) inhibiting the HER2/neu-overexpressing cells (MDA-MB-453 cells) compared to basal level HER2/neu-expressing cells (MCF-7)	[64]
Apigenin	Hela	(a) IC_{50} = 35.89 μM (b) p21/WAF1 ↑ (c) p53 and caspase-3 increased ↑ (d) Bcl_2 decreased	[65]
Apigenin	Neuroblastoma	(a) EC_{50} = 35 μmol/L in NUB-7 (b) EC_{50} = 22 μmol/L in LAN-5 (c) p53 and $p21^{WAF1/CIP1}$ ↑ (d) Bax ↑	[66]
Apigenin	Liver cancer	(a) IC_{50} was observed to be 8.02 μg/mL for HepG2, 2.16 μg/mL for Hep3B and 22.73 μg/mL for PLC/PRF/5 (b) G2/M cell cycle arrest (c) Increase of p53 and p21/WAF1 ↑	[67]

treated cells [63]. Way et al. demonstrated the antiproliferative nature of apigenin against breast cancer cells. They reported that apigenin was found to be more potent in inhibiting the HER2/neu-overexpressing cells (MDA-MB-453 cells) compared to basal level HER2/neu-expressing cells (MCF-7). For instance, 40 μM of apigenin resulted in 48% inhibitory effect in MDA-MB-435 whereas in MCF-7 it caused only 31% growth inhibition. They examined the role of HER2/HER3-PI3K/Akt pathway in the apigenin induced apoptosis and showed that it inhibited directly the

PI3K activity first, consequently inhibiting the Akt kinase activity. Moreover they demonstrated the inhibition of HER2/neu autophosphorylation and transphosphorylation resulting from depleting HER2/neu protein in vivo [64]. In another study by Zheng et al. elucidated the apoptosis induced by apigenin in human cervical cancer cell HeLa. It was found that apigenin could decrease the cell viability with an IC_{50} of 35.89 μM. DNA fragmentation assay and flow cytometric analysis of apigenin treated cells confirmed the apoptosis induction. They had observed increased expression of p21/WAF1 and p53. Further Fas/APO-1 and caspase-3 increase and Bcl_2reduction in the apigenin treated HeLa cells confirmed the apoptosis induction [65]. Torkin et al. reported that apigenin could induce apoptosis in neuroblastoma cells like NUB-7 and LAN-5. Apigenin repressed the cell viability in a dose-dependent manner in human neuroblastoma cell lines with an EC_{50} = 35 μmol/L in NUB-7, and EC_{50} = 22 μmol/L in LAN-5 after 24 hours. Moreover it was found to inhibit the colony forming ability and NUB-7 xenograft tumor growth in nonobese diabetic mouse model. They had shown that apigenin induced apoptosis was mediated though p53 as it enhanced the expression of p53 and p53 induced gene products like $p21^{WAF1/CIP1}$ and Bax [66]. Recent research by Chiang et al. suggested the antiproliferative effect of apigenin in HepG2, Hep3B and PLC/PRF/5 cells. It was found that apigenin could inhibit the cell growth of the above reported liver cancer cells but not the normal murine liver BNL.CL2 cells. IC_{50} was observed to be 8.02 μg/mL for HepG2, 2.16 μg/mL for Hep3B and 22.73 μg/mL for PLC/PRF/5. In addition, DNA ladder and flow cytometric analysis indicated apoptosis in the HepG2 cells. Apigenin treated cells were arrested at G2/M phase of the cell cycle. Further they observed increasing accumulation of p53 and p21/WAF1 in the treated cells [67].

4. Summary

Chemoprevention utilizes appropriate pharmacological agents [3, 4] or of dietary agents, consumed in diverse forms like macronutrients, micronutrients, or nonnutritive phytochemicals. Various polyphenols are reported in honey. Some of the polyphenols of honey like Caffeic acid (CA), Caffeic acid phenyl ester (CAPE), Chrysin (CR), Galangin (GA), Quercetin (QU), Acacetin (AC), Kaempferol (KF), Pinocembrin (PC), Pinobanksin (PB) and Apigenin (AP) have evolved as promising pharmacological agents in treatment of cancer. The summaries of individual polyphenols were tabulated under Table 2.

Caffeic acid has been reported as a carcinogen in initial studies, but the same caffeic acid along with combination of other antioxidant has been shown to suppress colon tumors in rats. Chung et al. showed that oral administration of Caffeic acid and Caffeic acid phenyl esters (CAPE) reduced liver metastasis, mediated by the dual inhibition of NF-κB and MMP-9 enzyme activity [43]. Natarajan et al. demonstrated that CAPE is known to have antimitogenic, anticarcinogenic, antiinflammatory and immunomodulatory properties [41]. CAPE's antiinflammatory and anticancer property has also been shown to protect skin cells when exposed to ultra-violet radiation and UVB radiation [46].

Weng et al. showed that the growth inhibitory effect of chrysin in C6 glioma cells was either though activating p38-MAPK which leads to the accumulation of $p21^{Waf1/Cip1}$ protein or mediating the inhibition of proteasome activity [47]. In another study by Woo et al. it has been elucidated that chrysin induces apoptosis in association with the activation of caspase-3 and Akt signal pathway, that plays a crucial role in chrysin-induced apoptosis in U937 cells [48]. Galangin expressed antiproliferative effect on HL-60 cells on dose dependent manner and also induced DNA fragmentation without loss of membrane integrity [50]. Quercetin also inhibited the HL-60 cell proliferation in association with the inhibition of cytosolic Protein Kinase C (PKC) and membrane Tyrosine Protein Kinase (TPK) in vitro [51]. It has been reported that quercetin in low concentration promoted cell proliferation of A-549 cells, whereas in higher concentration it inhibited cell proliferation and survival [53]. Further quercetin exerted antiproliferative effect against glioma and breast cancer cells [54–56].

Acacetin, another important flavanoid inhibited the proliferation of A549 cells, induced apoptosis and blocked the cell cycle progression at G1 phase. It also improved the expression of p53 and Fas ligands [57]. In another study, it has been shown to inhibit HepG2 cell proliferation and provoke apoptosis by enhancing the p53 and Fas ligands as in the case of A549 cells [58]. Kaempferol induced apoptosis in H460 cells which was accompanied by significant DNA condensation and increasing ATP levels. It also changed the expression of Caspase 3 and Apoptosis Inducing Factor (AIF) levels [59]. Bestwick et al. reported recently that kaempferol growth inhibitory effect on HL-60 leukemia cells is due to heterogeneous response mainly dominated by cell cycle alternation although some degree of cytotoxicity results from apoptotic as well as nonapoptotic process [60]. Pinocembrin induced loss of mitochondrial membrane potential (MMP) with subsequent release of cytochrome c and processing of caspase-9 and -3 in colon cancer cell line HCT 116 [61]. Apigenin exerted antiproliferative effect against colon, breast, cervical, neuroblastoma and liver cancer cell lines [63–67].

Our review has clearly demonstrated certain honey polyphenols tested in laboratorial setups showed to be a promising pharmacological agent for inhibiting cancer cell proliferation. After generating more in-depth and exhaustive information of these compounds jointly in in vitro and in vivo studies, clinical trials have to be initiated to further validate these compounds in medical applications.

References

[1] M. B. Sporn and D. L. Newton, "Chemoprevention of cancer with retinoids," *Federation Proceedings*, vol. 38, no. 11, pp. 2528–2534, 1979.

[2] G. J. Kelloff, "Perspectives on cancer chemoprevention research and drug development," *Advances in Cancer Research*, vol. 78, pp. 320–334, 1999.

[3] G. J. Kelloff and C. W. Boone, Eds., "Cancer chemopreventive agents: drug development status and future prospects," *Journal of Cellular Biochemistry*, vol. 20, pp. 1–303, 1994.

[4] G. J. Kelloff, E. T. Hawk, and C. C. Sigman, Eds., *Cancer Chemoprevention: Promising Cancer Chemopreventive Agents*, vol. 1, Humana Press, Totowa, NJ, USA, 2004.

[5] L. R. Ferguson, "Antimutagens as cancer chemopreventive agents in the diet," *Mutation Research*, vol. 307, no. 1, pp. 395–410, 1994.

[6] L. R. Ferguson, M. Philpott, and N. Karunasinghe, "Dietary cancer and prevention using antimutagens," *Toxicology*, vol. 198, no. 1–3, pp. 147–159, 2004.

[7] Y.-J. Surh, "Cancer chemoprevention with dietary phytochemicals," *Nature Reviews Cancer*, vol. 3, no. 10, pp. 768–780, 2003.

[8] J. Wollgast and E. Anklam, "Review on polyphenols in Theobroma cacao: changes in composition during the manufacture of chocolate and methodology for identification and quantification," *Food Research International*, vol. 33, no. 6, pp. 423–447, 2000.

[9] D. V. Madhavi, S. S. Despande, D. K. Salunkhe, D. L. Madhavi, S. S. Deshpande, and D. K. Salunkhe, Eds., *Food Antioxidants*, Marcel Dekker, New York, NY, USA, 1996.

[10] Food and Agriculture Organization, "Agricultural Services Bulletin," Food and Agriculture Organization, Rome, Italy, 1996.

[11] S. M. Antony, I. Y. Han, J. R. Rieck, and P. L. Dawson, "Antioxidative effect of maillard reaction products formed from honey at different reaction times," *Journal of Agricultural and Food Chemistry*, vol. 48, no. 9, pp. 3985–3989, 2000.

[12] P. Vit, C. Soler, and F. A. Tomás-Barberán, "Profiles of phenolic compounds of Apis mellifera and Melipona spp. honeys from Venezuela," *Zeitschrift fur Lebensmittel -Untersuchung und -Forschung*, vol. 204, no. 1, pp. 43–47, 1997.

[13] A. Cherchi, L. Spanedda, C. Tuberoso, and P. Cabras, "Solid-phase extraction and HPLC determination of organic acid in honey," *Journal of Chromatography*, vol. 669, pp. 59–64, 1994.

[14] A. M. C. Davies and R. G. Harris, "Free amino acid analysis of honeys from England and of the geographical origin of honeys," *Journal of Apicultural Research*, vol. 21, pp. 168–173, 1982.

[15] J. W. White and E. Crane, Eds., *Honey a Comprehensive Survey*, Heinemann, London, UK, 1975.

[16] S. T. Tan, A. L. Wilkins, P. T. Holland, and T. K. McGhie, "Geographical discrimination of honeys though the employment of sugar patterns and common chemical quality parameters," *Journal of Agricultural and Food Chemistry*, vol. 37, pp. 1217–1221, 1989.

[17] J. W. White, "The protein content of honey," *Journal of Apicultural Research*, vol. 17, pp. 234–238, 1978.

[18] M. J. Amiot, S. Aubert, M. Gonnet, and M. Tacchini, "Les composés phénoliques des miels: étude préliminaire sur l'identifi cation et la quantifi cation par familles," *Apidologie*, vol. 20, pp. 115–125, 1989.

[19] World Wide Wounds, "Honey as a topical antibacterial agent for treatment of infected wounds," February 2002, http://www.worldwidewounds.com/2001/november/Molan/honey-as-topical-agent.html.

[20] National Honey Board, "Honey and Bees," April 2007, http://www.honey.com/consumers/kids/beefacts.asp.

[21] F. E. Todd and G. H. Vansell, "Pollen grains in nectar and honey," *The Journal of Economic Entomology*, vol. 35, pp. 728–731, 1942.

[22] S. K. Jaganathan and M. Mandal, "Honey constituents and its apoptotic effect in colon cancer cells," *Journal of Apiproduct and Apimedical Science*, vol. 1, pp. 29–36, 2009.

[23] N. Orsolic, A. Knezevi, L. Sver, S. Terzi, B. K. Hackenberger, and I. Basi, "Influence of honey bee products on transplantable tumours," *Journal of Veterinary and Comparative Oncology*, vol. 1, no. 4, pp. 216–226, 2004.

[24] S. Tarek, M. Naoto, O. Mizuki, et al., "Antineoplastic activity of honey in an experimental bladder cancer implantation model: in vivo and in vitro studies," *International Journal of Urology*, vol. 10, pp. 213–219, 2003.

[25] N. V. Gribel and V. G. Pashinsky, "Antitumor properties of honey," *The Voprosy Onkologii*, vol. 36, no. 6, pp. 704–709, 1990.

[26] N. C. Cook and S. Samman, "Flavonoids—chemistry, metabolism, cardioprotective effects, and dietary sources," *Journal of Nutritional Biochemistry*, vol. 7, no. 2, pp. 66–76, 1996.

[27] A. L. Catapano, "Antioxidant effect of flavonoids," *Angiology*, vol. 48, no. 1, pp. 39–44, 1997.

[28] K. Loku, T. Tsushida, Y. Takei, N. Nakatani, and J. Terao, "Antioxidant activity of quercitin monoglucosides in solution and phospholipid bilayers," *Biochimica et Biophysica Acta*, vol. 1234, pp. 99–104, 1995.

[29] N. Salah, N. J. Miller, G. Paganga, L. Tijburg, G. P. Bolwell, and C. Rice-Evans, "Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants," *Archives of Biochemistry and Biophysics*, vol. 322, no. 2, pp. 339–346, 1995.

[30] M. Serafini, A. Ghiselli, and A. Ferro-Luzzi, "In vivo antioxidant effect of green and black tea in man," *European Journal of Clinical Nutrition*, vol. 50, no. 1, pp. 28–32, 1996.

[31] J. A. Vinson, Y. Hao, X. Su, and L. Zubik, "Phenol antioxidant quantity and quality in foods: vegetables," *Journal of Agricultural and Food Chemistry*, vol. 46, no. 9, pp. 3630–3634, 1998.

[32] L. Bravo, "Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance," *Nutrition Reviews*, vol. 56, no. 11, pp. 317–333, 1998.

[33] F. Ferreres, M. A. Blazquez, M. I. Gil, and F. A. Tomás-Barberan, "Separation of honey flavonoids by micellar electrokinetic capillary chromatography," *Journal of Chromatography A*, vol. 669, no. 1-2, pp. 268–274, 1994.

[34] F. Ferreres, T. Juan, C. Perez-Arquillue, A. Herrera-Marteache, C. Garcia-Viguera, and F. A. Tomás-Barberán, "Evaluation of pollen as a source of kaempferol in rosemary honey," *Journal of the Science of Food and Agriculture*, vol. 77, no. 4, pp. 506–510, 1998.

[35] F. A. Tomás-Barberán, I. Martos, F. Ferreres, B. S. Radovic, and E. Anklam, "HPLC flavonoid profiles as markers for the botanical origin of European unifloral honeys," *Journal of the Science of Food and Agriculture*, vol. 81, no. 5, pp. 485–496, 2001.

[36] A. Cherchi, L. Spanedda, C. Tuberoso, and P. Cabras, "Solid-phase extraction and HPLC determination of organic acid in honey," *Journal of Chromatography*, vol. 669, pp. 59–64, 1994.

[37] M. Hirose, Y. Takesada, H. Tanaka, S. Tamano, T. Kato, and T. Shirai, "Carcinogenicity of antioxidants BHA, caffeic acid, sesamol, 4-methoxyphenol and catechol at low doses, either alone or in combination, and modulation of their effects in a rat medium-term multi-organ carcinogenesis model," *Carcinogenesis*, vol. 19, no. 1, pp. 207–212, 1998.

[38] C. V. Rao, D. Desai, B. Simi, N. Kulkarni, S. Amin, and B. S. Reddy, "Inhibitory effect of caffeic acid esters on azoxymethane-induced biochemical changes and aberrant

crypt foci formation in rat colon," *Cancer Research*, vol. 53, no. 18, pp. 4182–4188, 1993.

[39] C. V. Rao, D. Desai, and B. Kaul, "Effect of caffeic acid esterson carcinogen-induced mutagenecity and human colon adenocarcinoma cell growth," *Chemico-Biological Interactions*, vol. 84, pp. 277–290, 1992.

[40] M.-T. Huang, W. Ma, P. Yen, et al., "Inhibitory effects of caffeic acid phenethyl ester (CAPE) on 12-O-tetradecanoylphorbol-13-acetate-induced tumor promotion in mouse skin and the synthesis of DNA, RNA and protein in HeLa cells," *Carcinogenesis*, vol. 17, no. 4, pp. 761–765, 1996.

[41] K. Natarajan, S. Singh, T. R. Burke Jr., D. Grunberger, and B. B. Aggarwal, "Caffeic acid phenethyl ester is a potent and specific inhibitor of activation of nuclear transcription factor NF-κB," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 17, pp. 9090–9095, 1996.

[42] Y.-J. Lee, H.-C. Kuo, C.-Y. Chu, C.-J. Wang, W.-C. Lin, and T.-H. Tseng, "Involvement of tumor suppressor protein p53 and p38 MAPK in caffeic acid phenethyl ester-induced apoptosis of C6 glioma cells," *Biochemical Pharmacology*, vol. 66, no. 12, pp. 2281–2289, 2003.

[43] T.-W. Chung, S.-K. Moon, Y.-C. Chang, et al., "Novel and therapeutic effect of caffeic acid and caffeic acid phenyl ester on hepatocarcinoma cells: complete regression of hepatoma growth and metastasis by dual mechanism," *The FASEB Journal*, vol. 18, no. 14, pp. 1670–1681, 2004.

[44] H. J. Hwang, H. J. Park, H.-J. Chung, et al., "Inhibitory effects of caffeic acid phenethyl ester on cancer cell metastasis mediated by the down-regulation of matrix metalloproteinase expression in human HT1080 fibrosarcoma cells," *Journal of Nutritional Biochemistry*, vol. 17, no. 5, pp. 356–362, 2006.

[45] J. Neradil, R. Veselska, and J. Slanina, "UVC-protective effect of caffeic acid on normal and transformed human skin cells in vitro," *Folia Biologica*, vol. 49, no. 5, pp. 197–202, 2003.

[46] V. Staniforth, L.-T. Chiu, and N.-S. Yang, "Caffeic acid suppresses UVB radiation-induced expression of interleukin-10 and activation of mitogen-activated protein kinases in mouse," *Carcinogenesis*, vol. 27, no. 9, pp. 1803–1811, 2006.

[47] M.-S. Weng, Y.-S. Ho, and J.-K. Lin, "Chrysin induces G1 phase cell cycle arrest in C6 glioma cells through inducing p21Waf1/Cip1 expression: involvement of p38 mitogen-activated protein kinase," *Biochemical Pharmacology*, vol. 69, no. 12, pp. 1815–1827, 2005.

[48] K. J. Woo, Y.-J. Jeong, J.-W. Park, and T. K. Kwon, "Chrysin-induced apoptosis is mediated through caspase activation and Akt inactivation in U937 leukemia cells," *Biochemical and Biophysical Research Communications*, vol. 325, no. 4, pp. 1215–1222, 2004.

[49] T. Zhang, X. Chen, L. Qu, J. Wu, R. Cui, and Y. Zhao, "Chrysin and its phosphate ester inhibit cell proliferation and induce apoptosis in Hela cells," *Bioorganic & Medicinal Chemistry*, vol. 12, no. 23, pp. 6097–6105, 2004.

[50] C. S. Bestwick and L. Milne, "Influence of galangin on HL-60 cell proliferation and survival," *Cancer Letters*, vol. 243, no. 1, pp. 80–89, 2006.

[51] T. Kang and M. Liang, "Studies on the inhibitory effects of quercetin on the growth of HL460 leukemia cells," *Biochemical Pharmacology*, vol. 54, pp. 1013–1018, 1997.

[52] B. Csokay, N. Prajda, G. Weber, and E. Olah, "Molecular mechanisms in the antiproliferative action of quercetin," *Life Sciences*, vol. 60, no. 24, pp. 2157–2163, 1997.

[53] A. Robaszkiewicz, A. Balcerczyk, and G. Bartosz, "Antioxidative and prooxidative effects of quercetin on A549 cells," *Cell Biology International*, vol. 31, no. 10, pp. 1245–1250, 2007.

[54] E. Braganhol, L. L. Zamin, D. Canedo, et al., "Antiproliferative effect of quercetin in the human U138MG glioma cell line," *Anti-Cancer Drugs*, vol. 17, no. 6, pp. 663–671, 2006.

[55] M. A. Indap, S. Radhika, L. Motiwale, and K. V. K. Rao, "Quercetin: antitumor activity and pharmacological manipulations for increased therapeutic gains," *Indian Journal of Pharmaceutical Sciences*, vol. 68, no. 4, pp. 465–469, 2006.

[56] E. J. Choi, S. M. Bae, and W. S. Ahn, "Antiproliferative effects of quercetin through cell cycle arrest and apoptosis in human breast cancer MDA-MB-453 cells," *Archives of Pharmacal Research*, vol. 31, no. 10, pp. 1281–1285, 2008.

[57] Y.-L. Hsu, P.-L. Kuo, C.-F. Liu, and C.-C. Lin, "Acacetin-induced cell cycle arrest and apoptosis in human non-small cell lung cancer A549 cells," *Cancer Letters*, vol. 212, no. 1, pp. 53–60, 2004.

[58] Y.-L. Hsu, P.-L. Kuo, and C.-C. Lin, "Acacetin inhibits the proliferation of Hep G2 by blocking cell cycle progression and inducing apoptosis," *Biochemical Pharmacology*, vol. 67, no. 5, pp. 823–829, 2004.

[59] H. W.-C. Leung, C.-J. Lin, M.-J. Hour, W.-H. Yang, M.-Y. Wang, and H.-Z. Lee, "Kaempferol induces apoptosis in human lung non-small carcinoma cells accompanied by an induction of antioxidant enzymes," *Food and Chemical Toxicology*, vol. 45, no. 10, pp. 2005–2013, 2007.

[60] C. S. Bestwick, L. Milne, and S. J. Duthie, "Kaempferol induced inhibition of HL-60 cell growth results from a heterogeneous response, dominated by cell cycle alterations," *Chemico-Biological Interactions*, vol. 170, no. 2, pp. 76–85, 2007.

[61] M. A. S. Kumar, M. Nair, P. S. Hema, J. Mohan, and T. R. Santhoshkumar, "Pinocembrin triggers Bax-dependent mitochondrial apoptosis in colon cancer cells," *Molecular Carcinogenesis*, vol. 46, no. 3, pp. 231–241, 2007.

[62] A. C. Santos, S. A. Uyemura, J. L. C. Lopes, J. N. Bazon, F. E. Mingatto, and C. Curti, "Effect of naturally occurring flavonoids on lipid peroxidation and membrane permeability transition in mitochondria," *Free Radical Biology and Medicine*, vol. 24, no. 9, pp. 1455–1461, 1998.

[63] W. Wang, L. Heideman, C. S. Chung, J. C. Pelling, K. J. Koehler, and D. F. Birt, "Cell-cycle arrest at G2/M and growth inhibition by apigenin in human colon carcinoma cell lines," *Molecular Carcinogenesis*, vol. 28, no. 2, pp. 102–110, 2000.

[64] T.-D. Way, M.-C. Kao, and J.-K. Lin, "Apigenin induces apoptosis though proteasomal degradation of HER2/neu in HER2/neu-overexpressing breast cancer cells via the phosphatidylinositol-3′- kinase/Akt-dependent pathway," *The Journal of Biological Chemistry*, vol. 279, pp. 4479–4489, 2004.

[65] P.-W. Zheng, L.-C. Chiang, and C.-C. Lin, "Apigenin induced apoptosis through p53-dependent pathway in human cervical carcinoma cells," *Life Sciences*, vol. 76, no. 12, pp. 1367–1379, 2005.

[66] R. Torkin, J.-F. Lavoie, D. R. Kaplan, and H. Yeger, "Induction of caspase-dependent, p53-mediated apoptosis by apigenin in human neuroblastoma," *Molecular Cancer Therapeutics*, vol. 4, no. 1, pp. 1–11, 2005.

[67] L.-C. Chiang, L. T. Ng, I.-C. Lin, P.-L. Kuo, and C.-C. Lin, "Antiproliferative effect of apigenin and its apoptotic induction in human Hep G2 cells," *Cancer Letters*, vol. 237, no. 2, pp. 207–214, 2006.