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Biological Effect of Bee Propolis: A Review

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Abstract: For centuries bee propolis (bee resin) has been used in traditional medicine. It is a natural product derived from plant resins composed by bees. It has anticancer, anti-tumor antioxidant, antimicrobial, anti-ulcer and antifungal properties. It is also contains hepatoprotective, cardioprotective and antihypertensive activities, as well as some properties that helps in controlling blood glucose and modulates the metabolism of blood lipids. In recent years, propolis has attracted much attention as a valuable or potential substance used in medicine and cosmetic products. Therefore, it is now widely used in food and beverages with the assertion that it can preserve or improve human health. The chemical components of propolis are quite complex. More than 300 compounds including polyphenols, phenolic aldehydes, sequiterpene quinines, coumarins, amino acids, steroids and inorganic compounds have been identified in propolis samples. The content depends on the collecting time, location and plant source. As a result, biological activities of propolis gathered at various times and from different phytogeographical areas which vary greatly. In this review, the therapeutic efficacy of propolis in treating diseases caused by microorganisms is described. Some concepts about propolis and its use in medicine is presented.

Key words: Bee propolis · Anticancer · Antioxidant · Antibacterial · Anti-inflammatory · Hepatoprotective · Cardioprotective

INTRODUCTION

Propolis is a resinous blend composed from plants by the *Apis mellifera* bee, which is utilized as a glueor as a building filling material in the beehive as well as for keeping it in excellent health [1]. It is one of the oldest known therapeutic agents that are used even today in traditional medicines. It has a significant pharmacological properties and it can be used for a variety of purposes such as an anticancer, anti-inflammatory anti-hypotensive agent, antifungal, bacteriostatic and bactericidal agent. It is also works as an immune system stimulant among several other uses which broadened its pharmaceutical demands [2]. Propolis is a complex chemical substance that includes phenols, polysaccharides, tannins, terpenes, aromatic acids and aldehydes etc. [3]. National Food Institute identified propolis as a dietary supplement in Argentina [4]. Similarly, it was used in Egypt and Greece since ancient times, for its efficient use on wound healing [5].

Chemical Components of Propolis: Propolis is a resin, brown or dark green in color, with a pleasing flavor of poplar buds, honey, wax and vanilla but it can have a bitter taste too. When scalded, propolis reveals an aromatic smell of resins which adds to its great value [6]. The chemical composition of propolis and aroma differ according to the geographical zones. Propolis is hard and brittle when cold, but it becomes soft and very sticky when warm. The composition and chemical properties of propolis were investigated [7]. It contains â–amylase [8], many polyphenolic compounds, flavones, flavonones, phenolic acid, esters and fatty acids [9-12].

Twelve different flavonoids were found in propolis: pinocembrin, acacetin, chrysin, rutin, catechin, naringenin, galangin, luteolin, kaempferol, apigenin, myricetin and quercetin. In addition to that two phenolic acids were found, which are cinnamic acid and caffeic acid. The levels of chemical compounds in three different propolis extracts, ethanolic, aqueous-ethanolic and aqueous-glycolic were determined. The aqueous-ethanolic propolis

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extract showed a great percentage of caffeic acid, galangin, quercetin and chrysin, whilst the ethanolic preparation was composed of a great amount of resveratrol, chrysin and caffeic acid. While the aqueous-glycolic extract was composed of approximately 11% of caffeic acid and a low amount of the other identified flavonoids due to the presence of approximately 85% of unidentified compounds. Investigators concluded that the Capillary Zone Electrophoresis (CZE) represents a valuable method for the qualitative and quantitative analysis of the most relevant polyphenol components of propolis, it contains phenolic acid esters (72.7%), phenolic acids (1.1%), aliphatic acids (2.4%), dihydrochalcones (6.5%), chalcones (1.7 %), flavanones (1.9%), flavones (4.6 %) and tetrahydrofuran derivatives (0.7%) [13-14].

Titerpenoids and diterpenoids represent the major components of the biologically active fraction of propolis (72% and 8% of total extract, respectively). In addition to that, High-Speed Countercurrent Chromatograph (HSCCC) with pre-fractionation and successive purification steps resulted in the isolation and characterization of various bioactive components from the highly complex propolis fraction. (12*E*)- and (12*Z*)-communic acid, sandaracopimaric acid, (+)-ferruginol, (+)-totarol, 3*β*-acetoxy-19(29)-taraxasten-20*a*-ol, cycloartenol and 24-methylene-cycloartenol, as well as five triterpene acetates. Free fatty acids and two labdane fatty acid esters, the 15-O-oleoyl- and 15-O-palmitoyl-isocupressic acid were also identified [15, 16].

Anticancer Effects of Propolis: The main chemicals constituents of propolis have been identified as Caffeic Acid Phenethyl Ester (CAPE) andchrysin that possess antiproliferative property. The anti-proliferative (anticancer) effects of CAPE or chrysin in cancer cells are the result of the suppression of complexes of cyclins, as well as cell cycle arrest [17]. The results of *in vitro* and *in vivo* studies suggest that CAPE and chrysin may inhibit tumor cell progression and may be useful as potential chemotherapeutic or chemopreventive anti-cancer drugs. Oral cancer represents 3rd most common malignancy of all cancers in Saudi Arabia, after lymphoma and leukaemia. In addition to high rates of the recurrence of head and neck squamous cell carcinoma because of the frequent formation of the second primary tumor in 3% to 7% per year, this is considered the highest rates for any malignancy [18].

Chemopreventive agents can serve as an appropriate therapy for patients with a premalignant lesion or patients who have had head and neck squamous cell carcinoma. Also, Squamous Cell Carcinoma (SCC) of the oral mucosa is an excellent cancer candidate for the assessment of chemoprevention because lesions are amenable to oral delivery of chemopreventive agents. Dietary administration or intraregional injection of "propolis" could inhibit the occurrence and progression of malignant oral lesions. The effects can be visually monitored during treatment and modulation or inhibition of genes or gene products involved in SCC constitute molecular targets against which chemopreventive approaches can be tested and validated. Apoptosis induction is one of the mechanisms proposed for the therapeutic effects of propolis [19, 20]. The mechanism of apoptosis induced by propolis seems dependent on the kind of compounds and the concentration of the propolis extract. Recent studies suggested that both the astaxanthin and flavonoids in propolis can protect the cells from beta-Amyloid which induced apoptotic death [20- 23]. Bee propolis has many other biological activities including the immunostimulant activity. It has antimutagenic effect against different environmental mutagens such as 4-Nitro-O-phenylenediamine, 1-nitropyrene, 2-amino-3-methylimidazo [4, 5-f] quinoline and benzo[a]pyrene [24].

Antioxidant Effect of Propolis: The antioxidant capacity of propolis may be related to some of its biological effects, including chemoprevention. The flavonoids in propolis are powerful antioxidants and are capable of scavenging free radicals and thereby protecting the cell membrane against lipid peroxidation [25]. Moreover, Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS), together with other factors, are involved in cellular ageing and death in some conditions. Examples of the some disease caused by ROS and RNS are cardiovascular diseases, arthritis, cancers, diabetes, Parkinson's disease and Alzheimer's disease [26-29]. Propolis can reduce cellular levels of H_2O_2 and NO, which may be involved in its anti-inflammatory effects [30]. Diverse compounds from propolis have been described as potent inhibitors of oxidative stress. One of its major components, Caffeic Acid Phenethyl Ester (CAPE), blocks ROS production in several systems [31]. CAPE has also been identified as anti-cancer. *In vitro*, propolis inhibits peroxidation of Low density Lipo-Protein (LDL) and nitration of proteins. In *vivo*, propolis can increase antioxidant capacity in animals [32] and humans [33], leading to decreased lipid peroxidation [34, 35]. Turkish propolis inhibited hydrogen peroxide (H_2O_2) induced damage to DNA in cultured fibroblasts [36].

Krol *et al.* [37] described the remarkable medical property of the Ethanolic Extract of Propolis (EEP) that is the protection against gamma radiation. They performed their experiment on mice and found that the anti-oxidative effect could be attributed to its radical scavenging ability. They also demonstrated the ability of increasing amounts of EEP to inhibit luminol H_2O_2 chemiluminescence *in vitro* and suggested that its anti-oxidative capacity was partly due to its high content of flavonoids. In addition, another study investigated the antioxidant activity of a propolis extract deprived of CAPE. Propolis extract (with and without CAPE) and its active components showed a dose-dependent free radical scavenging effect, a significant inhibition of xanthine oxidase activity and an antilipoperoxidative capacity. Propolis extract with CAPE together were more active than propolis extract without CAPE. The experimental evidence, therefore, suggests that CAPE plays an important role in the antioxidant activity of propolis [38]. In addition, the antioxidant activities of tectochrysin, a major compound of propolis, were investigated. Tectochrysin exhibited a significant decrease in serum transaminase activities elevated by hepatic damage induced by CCl_4-intoxication in rats. Furthermore, tectochrysin increased the antioxidant enzymes activity such as hepatic cytosolic superoxide dismutase, catalase and glutathione peroxidase in CCl_4-intoxicated rats as well as a significant decrease in the Malonaldehyde (MDA) production [39].

Antibacterial and Antiviral Activities of Propolis: Propolis is a flavonoid-rich product, exhibits antibacterial and anti-inflammatory properties [40] which are very powerful natural antibiotic [41]. It is very useful in fighting upper respiratory infections, such as common cold and influenza viruses [42]. Propolis contains a variety of potent polyphenols which may enhance the antistaph activity of some pharmaceutical antibiotics including streptomycin [43]. The chemical composition and antibacterial activity of propolis have been reported by Velikova *et al*. [44, 45] and Marcucci *et al*. [46]. Natural antibiotics such as propolis may be effective to prevent *S. aureus* and *E. coli* contamination [47]. Moreover, propolis was found to have antibacterial activity against a wide range of Gram-positive rods, but it only demonstrated limited activity against Gram-negative *bacilli* [48, 49]. According to tests done by Ugur and Arslan [50] the antimicrobial activity of propolis varied depending on the propolis sample, the dosage of propolis and the extraction solvents for all the tested propolis samples. At 125-500 µg/ml propolis inhibited the growth of *B. cereus* and *S. aureus* [51]. Antimicrobial activity was related to polyphenols content (above 59%) in the alcoholic extracts of propolis [52]. Propolis may inhibit bacterial growth by preventing cell division which results in forming pseudo-multicellur bacteria. Additionally, propolis inhibits protein synthesis, causes partial bacteriolysis and disorganizes the cytoplasmic membrane [53].

In vitro activity of 3-methyl-but-2-enyl caffeate against Herpes simplex virus type 1 was investigated. This compound, which is a minor component of propolis was found to effectively reduce the virus titer and viral DNA synthesis effectively [54]. Isopentlyferulated isolated from propolis was found to inhibit the infectious activity of influenza virus *in vitro* [55]. Furthermore, the aqueous extract of propolis was correlated to a decrease in mortality and an increase in the average survival length in mice infected with influenza virus A/PR8/34 (HONI) [56]. Several compounds were isolated from Brazilian propolis and tested for anti-HIV activity in H9 lymphocytes including: melliferone, three known triterpenoids, moronic acid, anwuweizonic acid and betulonic acid and four known aromatic compounds. Significant anti-HIV activity was shown by moronic acid [57].

Antifungal Activity of Propolis: The antifungal activity of propolis was studied through sensitivity tests conducted on 80 strains of Candida yeasts, 20 strains of *Candida albicans*, 20 strains of *Candida tropicalis*, 20 strains of *Candida krusei* and 15 strains of *Candida guilliermondii* [58]. The sensitivity tests demonstrated clear antifungal activity in the following order*: C. albicans>C. tropicalis>C. krusei>C. guilliermondii*. Twelve patients suffering from chronic sinusitis, caused by *Candida albicans* were investigated by Kovalik [59]. In 8 cases it was found that the fungus in *vitro* was sensitive to propolis, while in two cases the fungus was resistant to propolis. Two more cases exhibited weak sensitivity to propolis. The patients were treated with an alcohol-oil emulsion of propolis. After irrigation with isotonic saline, the emulsion (2-4 ml) was introduced into the sinuses (everyday or every second day). There was an improvement in the condition of some patients after 1-2 treatments with propolis. A clinical recovery occurred in nine patients after 5-8 treatments and an improvement was observed in the other three patients. After 10-17 days, recovery occurred in all patients.

Pure propolis extracts, at a concentration of 15-30 mg/ml inhibit the growth of *Candida albicans, Aspergillus flavus, A. ochraceus, Penicillium viridicatum*

and *P. notatum*. In addition, it was found that propolis concentrations at 0.25-2.0 mg/ml repressed the growth of *A. sulphureus* for ten days [60]. Ethanolic Extract of Propolis (EEP) prohibited 38 strains of fungi and 60 strains of yeasts [61],and *Aspergillus parasiticus* strain NRRL 2998 [62].The ethanolic and Dimethyl-sulphoxide Extracts of Propolis (DEP), were active against Trypanosomacruzi [63] and lethal to *Trichomonas vaginalis* [64].

Anti-Inflammatory Activity of Propolis: Caffeic acid phenethylester which is derived from the propolis of honeybee hives has been shown to reveal anti-inflammatory properties. Since T-cells play a keyrole in the onset of several inflammatory diseases. Márquez *et al.* [65] evaluated the immunosuppressive activity of CAPE in human T-cells, discovering that this phenolic compound is a potent inhibitor of early and late events in T-cell receptor-mediated T-cell activation. Moreover, they found that CAPE specifically inhibited both interleukin (IL)-2 gene transcription and IL-2 synthesis in stimulated T-cells. To further characterize the inhibitory mechanisms of CAPE at the transcriptional level. They examined the DNA binding and transcriptional activities of Nuclear Factor (NF)-B, Nuclear factor of activated cells (NFAT) and Activator Protein-1(AP-1) transcription factors in Jurkat cells. They found that CAPE inhibited NF-B-dependent transcriptional activity without affecting the degradation of the cytoplasmic NF-B inhibitory protein. However, both NF-Bbinding to DNA and transcriptional activity of a Gal4-p65 hybrid protein were clearly prevented in CAPE-treated Jurkat cells [66].

Anti-Agents Causing Ulcers: The inhibitory effect of propolis on *Helicobacter pylori* growth *in vitro* was investigated by Boyanova *et al.* [67]. Activity of 30 % Ethanolic Extract of Propolis (EEP) against 38 clinical isolates of *H. pylori* was evaluated by using the agar-well diffusion method. In this study Ethanol was used as controlling mechanism. In addition, the effect of propolis on the growth of 26 *H. pylori* and 18 *Campylobacter* strains was tested by the disc diffusion method. Mean diameters of *H. pylori* growth inhibition by the agar-well diffusion method. Dried propolis discs exhibited antibacterial effect against 73.1% of *H. pylori* isolates, with a considerable zone of growth inhibition (15 mm) in 36.4% of isolates. Using dried propolis discs resulted in mean diameters of growth inhibition of 12.4mm for *H. pylori* and 11.6mm for *Campylobacter* spp. They concluded that the Bulgarian propolis possesses considerable antibacterial activity against *H. pylori* and can inhibit the growth of *Campylobacter jejuni* and *Campylobacter coli* [68]. Moreover, propolis was used to manage chronic skin ulcers which were found to be effective as reported by Tossoun *et al.* [69].

Hepatoprotective Effect of Propolis: Aqueous propolis Extract (APE) was found to protect the liver in rats against carbon tetrachloride (CCl_4) injury. APE afforded its protection as manifested by a decrease in the leakage of the cytosolic enzyme Lactate Dehydrogenase (LDH), decreased generation of lipid peroxide and maintenance of cellular reduced glutathione (GSH) content [70]. The protective effects of propolis (PP) on hepatotoxicity induced by acetaminophen (AA, Paracetamol) and the mechanism of its hepatoprotective effect were investigated. In rat hepatocyte culture, pretreatment with PP (1, 10, 100, 200 and 400 µ/ml, 24 h) significantly decreased the cytotoxicity of AA (0.5 mm) in a dose-dependent manner. In mice, pre-treatment with PP (10 and 25 mg/kg, P.O., 7 days) also decreased the mortality and the incidence and severity of hepatic necrosis induced by AA (400 mg/kg, i.p.). After the treatment with PP for 7 days, the hepatic enzyme activities of cytochrome P450 monooxygenases (P450s), UDP-glucuronyl transferase, Phenolsulpho transferase (PST), glutathione S-transferase (GST) were measured in both rats and mice. In rats, PP (50 and 100 mg/kg, P.O.) decreased the activity of P4502E1, but significantly increased the activities of GST and PST. On the other hand, in mice treated with PP (10 and 25 mg/kg, P.O.), the activities of P4501A2, 2B1, 3A4 and 2E1were dramatically inhibited and the activity of PST was significantly enhanced. These results suggested that PP has a protective effect on hepatic injury and that its effect may be explained by the inhibition of phase I enzymes and the induction of phase II enzymes [71].

Cardioprotective Effect of Propolis: Propolis showed an antihypertensive effect in rats [72]. In diabetic rats, administration of bee propolis extracts led to decreased levels of fasting blood glucose (FBG), malonaldehyde (MDA), nitric Oxide (NO), Total Cholesterol (TC), Triglyceride (TG), Low-Density Lipoprotein Cholesterol (LDLC), Very Low-Density Lipoprotein Cholesterol (VLDL-C) in serum of fasting rats; and to increased serum levels of High Density Lipoprotein Cholesterol (HDL-C) and Superoxide Dismutase (SOD). This suggests that propolis can control blood glucose and modulate the metabolism of glucose and blood lipid, leading to decreased outputs of lipid peroxidation and scavenge the free radicals in rats with diabetes mellitus [73].

Doxorubicin (DXR)-induced myocardiopathy is the consequence of oxidative stress through the mediation after radicals. The effect of intraperitoneal administration of propolis (50 and 100 mg/kg) was studied on cardiomyopathy produced by doxorubicin (10 mg/kg, i.v.) in rats. An elevation of the levels of Creatine Phosphokinase (CK), Aspartate Aminotransferase (AST), blood and tissue glutathione (GSH) and Thiobarbituric Acid Reactive Substances (TBARS) were observed following doxorubicin treatment. Parallel experiments with a pre-treatment of propolis significantly showed cardio-protective effect as evidenced by reducing the levels of this parameter [74]. Flavonoids scavenging activity of propolis has been exploited to obtain protection against the peroxidative damage in rat heart mitochondria which was induced by the administration of an acute dose of doxorubicin (20 mg kg^{-1}, i.p), 24 H after DXR administration. Pre-treatment of rats with propolis extract, given per os (100 mg/kg/day) during four days prior to DXR injection, substantially reduced the peroxidative damage in the heart mitochondria: reduced mitochondrial MDA formation and production of superoxide anion and reducing of rate and the amplitude of mitochondrial swelling [75].

CONCLUSION

Propolis is one of the few natural remedies that have maintained its popularity over a long period of time. The pharmacologically active molecules are flavonoids, phenolic acids and their esters. These components have multiple effects on bacteria, fungi and viruses. In addition, propolis and its components have anti-inflammatory, immunomodulatory activities and antitumor activities. Moreover, propolis has been shown to lower blood pressure and cholesterol levels as well as lowering fasting blood glucose levels. Besides that propolis has antioxidant effect in specific the flavonoids in propolis have very powerful antioxidants and are capable of scavenging free radicals and thereby protecting the cell membrane against lipid peroxidation. Not only that but the hepatoprotective effect of propolis was found to protect the liver against CCl_4. Therefore, propolis is a natural medication with a promising future but further studies should be conducted to investigate its merit and demerits in clinical medicine.

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