REVIEW

Biological Activity of Bee Propolis in Health and Disease

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Abstract

Propolis is a natural product derived from plant resins collected by honeybees. It is used by bees as glue, a general-purpose sealer, and as draught-extruder for beehives. Propolis has been used in folk medicine for centuries. It is known that propolis possesses anti- microbial, antioxidative, anti-ulcer and anti-tumor activities. Therefore, propolis has attracted much attention in recent years as a useful or potential substance used in medicine and cosmetics products. Furthermore, it is now extensively used in foods and beverages with the claim that it can maintain or improve human health. The chemical composition of propolis is quite complicated. More than 300 compounds such as polyphenols, phenolic aldehydes, sequiterpene quinines, coumarins, amino acids, steroids and inorganic compounds have been identified in propolis samples. The contents depend on the collecting location, time and plant source. Consequently, biological activities of propolis gathered from different phytogeographical areas and time periods vary greatly. In this review, the activity of bee propolis will be presented with special emphasis on the antitumor activity.

Key Words: Bee propolis - biological activity - anti-tumor - anti-inflammatory - anti-bacterial

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Introduction

Propolis is a resinous mixture collected from trees by the *Apis mellifera* bee, which uses as a building insulating material in the beehive as well as for keeping it in good health (Greenaway et al., 1990). It has important pharmacological properties and it can be used for a wide range of purposes as anti-inflammatory and hypotensive agent, immune system stimulant, and bacteriostatic and bactericidal agent, among many other uses (Ghisalberti, 1979). All such applications have increased its pharmaceutical demand and have rendered it an interesting subject of study. Its fairly complex chemical composition includes phenols, tannins, polysaccharides, terpenes, aromatic acids and aldehydes, among other compounds (Asis, 1989; Koo and Park, 1997). In Argentina, the INAL (The National Food Institute) recognized propolis as a diet supplement in 1995 (file 2110-003755-4 in the Argentine Food Code) (Gonzalez et al., 2003).

Propolis was used specially in antiquity, in Egypt. There some thousand years BC, propolis was very well known to the priests who had monopolized medicine, chemistry and art of mummifying corpses. The fact that propolis was also known to the old Greeks is demonstrated by the very Greek name of it (Makashvili, 1978). The first head the opinion that bees harvest propolis from resin of willow buds, of poplar, wild chestnut and other plants and other writers assumed that bees harvest it from Styrax (Makashvili, 1978). Abu Ali bin Sina (Avicenna) distinguishes two kinds of wax in his well known work, the clean and the black wax. The clean wax is that which composes the comb wells where the bees rear the brood and store the honey and the black is the filth the hive. It is clear enough that the black wax is propolis that after Avicenna's testimony. In folk Georgian medicine, they used ointments with propolis to cure some diseases. There was the custom of placing a propolis cake on the belly button of the newborn baby. Doctors used propolis effectively on wounds during the Anglo-Boer war and during the World War II. In 1969, Orthodox medicine in USSR accepted use of propolis (30 % alcoholic solution) in treatment (reviewed in Hegazi, 2000).

Chemical Constituents of Propolis

Propolis is a resin being dark green or brown in color with a pleasant flavor of poplar buds, honey, wax and vanilla but it can also have a bitter taste. When burnt, it exhibits a smell of aromatic resins of great value (Nikolaev, 1978). The chemical composition of propolis as well as its color and aroma are changed according to the geographical zones. Its color varies from yellowish-green to dark brown depending on its source and age (Ghisalberti, 1979). It can be likened to aromatic glue. It is hard and brittle when cold, but becomes soft and very sticky when warm. The composition and physico-chemical properties of propolis

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were investigated (Ivanov, 1980). It was found to contain β–amylase (Kaczmarek and Debowski, 1983), many polyphenolic compounds, flavones, flavonones, phenolic acid and esters (Bankova et al., 1982; Bankova et al., 1983; Bankova et al., 1988) and fatty acids (Polyakov et al., 1988).

Twelve different flavonoids, pinocembrin, acacetin, chrysin, rutin, catechin, naringenin, galangin, luteolin, kaempferol, apigenin, myricetin, and quercetin, two phenolic acids, cinnamic acid and caffeic acid, and one stilbene derivative, resveratrol, in propolis extracts were determined by capillary zone electrophoresis (CZE). The levels of analytes in three different propolis extracts, ethanolic, aqueous-ethanolic and aqueous-glycolic, used to prepare various commercial medicinal products, were determined. The aqueous-ethanolic propolis 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. On the contrary, the aqueous-glycolic propolis preparation was composed of approx. 11% of caffeic acid and a low amount of the other identified flavonoids due to the presence of approx. 85% of nonidentified compounds. Investigator concluded that the CZE represents a valuable method for the qualitative and quantitative assay of the most relevant polyphenol components of propolis, representing an alternative to obtain typical fingerprints of propolis and a reliable identification of a large number of propolis polyphenolic species (Volpi, 2004).

The constituents of the Egyptian propolis are 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 %). It was clear that phenolic acid esters are present in a major quantity (72.7 %) (Abd El-Hady, 1994; Abd El-Hady and Hegazi, 1994). Thirty-nine constituents were identified in the Egyptian propolis, eight of them being new for propolis. It is characterized by the presence of unusual esters of caffeic acid with C12- C16 fatty alcohols, mainly saturated. Flavonoid aglycones and especially flavanones are typical components of poplar propolis (Bankova et al., 1997). A series of triterpenes were identified in the Egyptian propolis, including the characteristic animal sterol precursor lanosterol. Propolis contains about 55 % resins and balsams, 30 % waxes, 10 % etheric oils and 5% pollen. The components are rich in vitamins and mineral elements (Nikolaev, 1978).

Propolis contains some minerals such as Mg, Ca, I, K, Na, Cu, Zn, Mn and Fe as well as some vitamins like B1, B2, B6, C and E, and a number of fatty acids. In addition, it contains some enzymes as succinic dehydrogenase, glucose-6-phosphatase, adenosine triphosphatase and acid phosphatase (Tikhonov and Mamontova, 1987). Propolis contains copper 26.5 mg/kg, manganese 40 mg/kg and the ash residue contains iron, calcium, aluminum, vanadium, strontium, manganese and silicon (Moreira, 1986).

One new 2,3-dihydroflavone derivative, 7- O-prenylstrobopinin, and 25 known diterpenes and phenolic compounds were identified from the n-butanol extract of Greek propolis. This is the first time that diterpenes have been isolated from propolis of European origin, while six of the known compounds are reported as propolis constituents for the first time (Melliou and Chinou, 2004).

1-Antimicrobial Activity of Propolis

A- Antibacterial Activity

Many researchers had investigated the antibacterial activity of propolis and its extracts against Gram-positive and Gram-negative strains and they found that propolis had antibacterial activity against a wide range of Gram-positive rods but had a limited activity against Gram-negative bacilli (Vokhonina et al., 1969; Akopyan et al., 1970; Grecianu and Enciu, 1976).

Ugur and Arslan (2004) investigated the antibacterial and antifungal activities of acetone and dimethyl sulfoxide (DMSO) extracts of 45 different propolis samples from the Mugla province of Turkey. They found that the antimicrobial activity of propolis varied depending on propolis sample, dosage of propolis, and the extraction solvents for all tested propolis samples. Antimicrobial activity of all propolis samples increased with increasing dosage without reaching a plateau at the highest dosage tested. Except for *Brucella melitensis*, the DMSO extracts of all propolis samples were more active than the acetone extracts of the same samples. For *B. melitensis*, the acetone extracts of all propolis samples showed greater activity. The most sensitive microorganism to propolis was *Shigella sonnei* in the Gram-negative group and Streptococcus mutans in the Gram-positive. Standard antibiotics were used and the results revealed that propolis samples from the Mugla province of Turkey has a similar or greater inhibitory effect on *S. mutans*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and *S. sonnei*.

Ethanolic extracts from samples of propolis were collected from 18 regions of the Russia. These extracts were serially diluted in agar, in Petri dishes. The dishes were then inoculated with the bacteria *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeraginosa*, and the fungus *Candida albicans*, and incubated at 37˚C or 20-25˚C for 48h. Propolis at 125-500 ug/ml inhibited the growth of B. cereus and S. aureus, but usually not that of the other two bacteria, or the fungus, even at concentrations higher than 1000 ug/ml (Shub et al., 1978).

A relationship between polyphenols content in alcoholic extracts of propolis (AEP) and their antimicrobial activity against *Bacillus cereus* was noted. In 91% of cases a high polyphenols content (59% or higher) was associated with significant antimicrobial activity (Malimon et al., 1980).

In chickens, propolis was effective against *S. aureus* and *S. epidermidis in vitro* (Glinnik and Gapanovich, 1981). One hundred and six strains of *S. aureus* were tested, all of them were susceptible to 0.5-1.0 mg propolis/ml. Strains resistant to benzyl/penicillin, tetracycline, and erythromycin were sensitive to propolis. Propolis had a synergistic effect when combined with any of the three antibiotics used against the

antibiotic resistant strains (Shub et al., 1981).

Inhibition of growth of five mycobacterium species was proportional to the concentration of flavonoids in the propolis. The strain *Mycobacterium sp*. 279 was the most sensitive to flavonoids and was therefore useful in comparative tests. The lowest concentration of flavonoids at which inhibition was observed was 0.00996 mg/ml (Jozwik and Trytek, 1985). The sensitivity of 75 bacterial strains to propolis extracts was examined. Of these, 69 were isolated from cows with mastitis, and were identified as *Staphylococcus spp.* and *Streptococcus spp*. All the strains displayed a high sensitivity to propolis extracts usually of the same order or higher than that of the standard strain Staphylococcus aureus 209P (Oxford)(Meresta and Meresta, 1985).

Ethanolic extract of propolis (EEP) was effective against anaerobic bacteria. EEP showed the greatest effectiveness against strains of bacteroids and peptostreptococcus and was slightly less effective against the Gram- positive rods of Propionibacterium, Arachinia and Eubacterium. Strains of clostridium were the least sensitive to EEP (Kedzia, 1986). Antibacterial activity was observed against a range of commonly encountered cocci and Gram-positive rods, in addition to *Mycobacterium tuberculosis*, but only limited activity against Gram-negative bacilli (Grange and Davey, 1990; Rojas Hernandez et al., 1993). Aga et al (1994) isolated three antimicrobial compounds from Brazilian propolis and identified them as 3,5 diprenyl-4- hydroxycinnamic acid, 3-prenyl - 4- dihdrocinnamoloxycinnamic acid and 2,2-dimethyl -6- carboxyethenyl-2H-1-benzopyran. Their respective antimicrobial activities against *Bacillus cereus*, *Enterobacter erogenous* and *Arthroderma benhamiae* were investigated, they found the first compound showed the highest activity and is likely to be one of the major antimicrobial compounds in Brazilian propolis.

Takasi et al (1994) stated that the propolis inhibits bacterial growth by preventing cell division, thus resulting in the formation of pseudo-multicellular streptococci. In addition, propolis disorganized the cytoplasm, the cytoplasmic membrane and the cell wall, caused a partial bacteriolysis and inhibited protein synthesis. It was evidenced that the mechanism of action of propolis on bacterial cell is complex and a simple analogy can not be made to the mode of action of any classic antibiotics. This conclusion came from the microcalorimetric and electron microscopic studies.

b- Antiviral Activity

In vitro activity of 3-methyl-but-2-enyl caffeate isolated from poplar buds against *Herpes simplex* virus type 1 was investigated. They found that this compound, as a minor constituent of propolis, reduce the virus titer and viral DNA synthesis effectively (Amoros et al., 1994). It was found that isopentyl ferulated (isolated from propolis) inhibited significantly the infectious activity of influenza virus A1 Honey Kong (H3N2) *in vitro* (Serkedjieva et al., 1997). Administration of aqueous extract of propolis was accompanied by a decrease in mortality and increase in mean survival length in mice infection with influenza virus A/PR8/34 (HONI) (Ecsanu et al., 1981). A triterpenoid named melliferone, three known triterpenoids, moronic acid, anwuweizonic acid, and betulonic acid, and four known aromatic compounds were isolated from Brazilian propolis and tested for anti-HIV activity in H9 lymphocytes. Moronic acid showed significant anti-HIV activity (Ito et al., 2001).

C- Antifungal Activity

Ota et al (2001) studied the antifungal activity of propolis in sensitivity tests 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*. The yeasts showed a clear antifungal activity with the following order of sensitivity: *C. albicans*> *C. tropicalis*> *C. krusei*> *C. guilliermondii*. Patients with full dentures who used a hydroalcoholic propolis extract showed a decrease in the number of Candida.

Kovalik (1979) investigated 12 patients suffering from chronic sinusitis, caused by *Candida albicans*. It was found that the fungus *in vitro* was sensitive to propolis in eight cases, weak sensitive in two and resistant in the other two patients. The patients were treated with an alcohol-oil emulsion of propolis. The emulsion (2-4 ml) was introduced into the sinuses after irrigation with isotonic saline (every day or every second day). After 1-2 treatments with propolis, there was an improvement in the condition of patients after 5-8 treatments, clinical recovery occurred in nine patients and improvement in the other three patients. Recovery occurred after 10-17 days.

Pepeljnjak et al (1982) found that, for pure propolis extracts, at a concentration of 15- 30 mg/ml was needed to inhibit the growth of *Candida albicans, Aspergillus flavus*, *A.ochraceus, Penicillium viridicatum* and *P. notatum*. Pepeljnjak et al (1982) found that, propolis concentrations of 0.25-2.0 mg/ml inhibited growth of *A. sulphureus* for up to 10 days, but only the highest concentration showed definite fungicidal activity. Ochratoxin was detected in all cultures media, but its concentration was low at the first 10 days. Compared with the control culture, amounts of Ochratoxin A were directly proportional to the growth of *A. sulphureus*, and inversely to the propolis concentration. The ethanolic extract of propolis inhibited 60 strains of yeasts, 38 strains of fungi (Cizmarik and Trupl, 1976), and *Aspergillus parasiticus* strain NRRL 2998 (Ozcan, 2004).

Paracoccidioidomycosis is the most important systemic mycosis in Latin America. Its etiological agent, *Paracoccidoides brasiliensis*, affects individuals living in endemic areas through inhalation of airborne conidia or mycelial fragments. The disease may affect different organs and systems, with multiple clinical features, with cell-mediated immunity playing a significant role in host defense. Peritoneal macrophages from BALB/C mice were stimulated with Brazilian or Bulgarian propolis and subsequently challenged with *P. brasiliensis*. Data suggest an increase in the fungicidal activity of macrophages by propolis

stimulation, independently from its geographic origin (Murad et al., 2002).

2- Antiprotozoal and Antiparastic Activity

The ethanolic (EEP) and dimethyl-sulphoxide extracts (DEP) of propolis, were active against *Trypanosoma cruzi* (Higashi and de Castro, 1995), and lethal to *Trichomonas vaginalis* (Starzyk et al., 1977).

3-Antiinflammatory Activity

The effects of ethanolic extract (EEP) of propolis on chronic inflammation were evaluated using rat adjuvant arthritis. In the chronic inflammatory animal model, the arthritis index was suppressed by EEP treatments (50 mg/kg/day and 100 mg/kg/day, P.O.). Moreover, physical weakness, induced by the chronic disease state, was dose-dependently improved in the EEP-treated groups. Its analgesic effect, assessed using the tail-flick test, was comparable to prednisolone (2.5 mg/kg/day, P.O.) and acetyl salicylic acid (100 mg/kg/day, P.O.). In carrageenan rat hind paw edema, which was conducted to test the effects of subfractions of EEP, the petroleum ether sub-fraction (100 mg/kg, P.O.) showed an inhibitory effect on the paw edema whereas EEP (200 mg/kg, P.O.) showed a significant anti-inflammatory effect at 3 and 4 hrs after carrageenan injection. From these results, they concluded that the ethanolic extract of propolis had profound anti-inflammatory effects on both chronic and acute inflammations (Park and Kahng, 1999).

Caffeic acid phenethyl ester (CAPE), which is derived from the propolis of honeybee hives, has been shown to reveal anti-inflammatory properties. Since T-cells play a key role in the onset of several inflammatory diseases, Marquez et al (2004) have 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)-κB, nuclear factor of activated cells (NFAT), and activator protein-1(AP-1) transcription factors in Jurkat cells. They found that CAPE inhibited NF-κB-dependent transcriptional activity without affecting the degradation of the cytoplasmic NF-κB inhibitory protein, IBκB. However, both NF- B binding to DNA and transcriptional activity of a Gal4-p65 hybrid protein were clearly prevented in CAPE-treated Jurkat cells. Moreover, CAPE inhibited both the DNA-binding and transcriptional activity of NFAT, a result that correlated with its ability to inhibit phorbol 12-myristate 13-acetate plus ionomycin-induced NFAT1 dephosphorylation. They stated that, these findings provide new insights into the molecular mechanisms involved in the immunomodulatory and anti-inflammatory activities of this natural compound.

Propolis has inhibitory effects on myeloperoxidase activity, NADPH-oxidase (Volpert and Elstner, 1996; Frenkel et al., 1993), ornithine decarboxylase, tyrosine-protein-kinase, and hyaluronydase from guinea pig mast cells (Miyataka et al., 1997). Full description of the anti-inflammatory actions of bee propolis was presented (de Almeida and Menezes, 2002).

4-Anti- Agents Causing Ulcers

Honey and propolis as management of chronic skin ulcers were found effective as reported by Tossoun et al (1997). The inhibitory effect of Bulgarian propolis on *Helicobacter pylori* growth *in vitro* was investigated by Boyanova et al (2003). Activity of 30 % ethanolic extract of propolis (EEP) against 38 clinical isolates of *H. pylori* was evaluated by using the agar-well diffusion method. Ethanol was used as a control. 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, using 30, 60 or 90 μl EEP or 30 μl ethanol per well, were 17.8, 21.2, 28.2 and 8.5 mm, respectively. EEP was significantly more active than ethanol against *H. pylori* ($P < 0.001$). The results obtained by the disc diffusion method were similar. The use of moist propolis discs resulted in mean diameters of growth inhibition of 21.4 mm for *H. pylori* and 13.6 mm for Campylobacter spp. 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 12.4 mm for *H. pylori* and 11.6 mm for Campylobacter spp. They conclude that the Bulgarian propolis possesses considerable antibacterial activity against *H. pylori*, and can inhibit the growth of *Campylobacter jejuni* and *Campylobacter coli* (Kimoto et al., 1998).

4-Antitumor Activity

Artepillin C was extracted from Brazilian propolis. Artepillin C (3,5-diprenyl-4-hydroxycinnamic acid) has a molecular weight of 300.40 and possesses antibacterial activity. When artepillin C was applied to human and murine malignant tumor cells *in vitro* and *in vivo*, artepillin C exhibited a cytotoxic effect and the growth of tumor cells was clearly inhibited. The artepillin C was found to cause significant damage to solid tumor and leukemic cells by the MTT assay, DNA synthesis assay, and morphological observation *in vitro*. When xenografts of human tumor cells were transplanted into nude mice, the cytotoxic effects of artepillin C were most noticeable in carcinoma and malignant melanoma. Apoptosis, abortive mitosis, and massive necrosis combined were identified by histological observation after intratumor injection of 500 g of artepillin C three times a week. In addition to suppression of tumor growth, there was an increase in the ratio of CD4/CD8 T cells, and in the total

number of helper T cells. These findings indicate that artepillin C activates the immune system, and possesses direct antitumor activity (Kimoto et al., 1998).

In experiments using the renal carcinogen ferric nitrilotriacetate (Fe-NTA) in male ddY mice, primary pulmonary cancers were also induced in bronchiolar and alveolar tissues. 4-Hydroxy-2-nonenal (4-HNE) and 8-hydroxy-2'-deoxyguanosine (8-OHdG), products of oxidative processes, increased in bronchiolar and alveolar cells after administration of Fe-NTA. These substances disappeared after oral administration of propolis or artepillin C, as shown histochemically, and correlated with an anticancer prophylactic effect of propolis and artepillin C. From these investigations, lipid peroxidation seems to play an important role in pulmonary carcinogenesis. Malignant progression from adenoma of bronchiolar or alveolar origin to malignant tumors has been proposed to involve a stepwise transformation. In this study, adenomas developed into adenocarcinomas and large cell carcinomas after treatment with Fe-NTA. In contrast, after oral administration of propolis or artepillin C, adenomas did not progress to carcinomas. Instead of developing into large cell cancers, as induced by Fe-NTA in control mice, adenomas showed remarkable proliferation of macrophages and local anti-oxidant activity after treatment with either propolis or artepillin C. Propolis and artepillin C therefore appear to inhibit lipid peroxidation and the development of pulmonary cancers (Kimoto et al., 2001).

Chia-Nana et al (2004) isolated and characterized two prenylflavanones, propolin A and propolin B from Taiwanese propolis and they reported to induce apoptosis in human melanoma cells and significantly inhibit xanthine oxidase activity. In addition, they isolated a third compound called propolin C. The chemical structure of propolin C has been characterized and it was found that it is identical to nymphaeol-A. However, no biological activities of this compound have ever been reported. Propolin C effectively was found to induce cytotoxic effect on human melanoma cells. DNA flow cytometric analysis indicated that propolin C actively induced apoptosis in human melanoma cells and there is a marked loss of cells from the G2/M phase of the cell cycle. The levels of procaspase-8, Bid, procaspase-3, and poly (ADP-ribose) polymerase were decreased in dose- or time course-dependent manners. Moreover, propolin C was capable of releasing cytochrome C from mitochondria to cytosol. The findings suggest that propolin C may activate a mitochondria-mediated apoptosis pathway. On other hand, propolin C is a potential antioxidant agent and shows a strong capability to scavenge free radicals and inhibit xanthine oxidase activity. Bazo et al (2002) suggested that the propolis has a protective influence on the process of rat colon carcinogenesis, suppressing the development of preneoplastic lesions.

PM-3 (3-[2-dimethyl-8-(3-methyl-2-butenyl) benzopyran]-6-propenoic acid) isolated from Brazilian propolis markedly inhibits the growth of MCF-7 human breast cancer cells. This effect was associated with inhibition of cell cycle progression and induction of apoptosis. Treatment of MCF-7 cells with PM-3 arrested cells in the G1 phase and resulted in a decrease in the protein levels of cyclin D1 and cyclin E. PM-3 also inhibited the expression of cyclin D1 at the transcriptional level when examined in cyclin D1 promoter luciferase assays. Induction of apoptosis by PM-3 occurred within 48 hours after treatment of MCF-7 cells. The MCF-7 treated cells also displayed a decrease in the level of the estrogen receptor (ER) protein and inhibition of estrogen response element (ERE) promoter activity (Luo et al., 2001).

Topical application of caffeic acid phenethyl ester (CAPE), a constituent of the propolis of honeybee hives, to the backs of CD-1 mice previously initiated with 7,12-dimethylbenz inverted question markanthracene (DMBA) inhibited 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced tumor promotion and the formation of 5-hydroxymethyl-2'-deoxyuridine (HMdU) in epidermal DNA. The results indicate a potent inhibitory effect of CAPE on TPA-induced tumor promotion and TPA-induced formation of HMdU in DNA of mouse skin as well as an inhibitory effect of CAPE on the synthesis of DNA, RNA and protein in culture HeLa cells (Huang et al., 1996).

Nine chemicals were tested by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay on the growth of buccal mucosal fibroblast (BF), oral submucosal fibroblast(OSF), neck metastasis of gingival carcinoma (GNM), and tongue squamous cell carcinoma (TSCCa) cells. CAPE and its ethyl analogue showed a significant cytotoxicity on OSF, GNM, and TSCCa cells, but not on BF cells. The results suggest that CAPE-like compounds may be potential chemotherapy agents against oral cancer (Lee et al., 2000).

Bee propolis is rich in cinnamic acid derivatives. Baccharin and drupanin from Brazilian bee propolis are cinnamic acid derivatives that contain prenyl moieties. Recently, the two related cinnamic acid derivatives were found to possess *in vivo* tumoricidal activity in mice bearing sarcoma S-180 cells. Furthermore, these compounds may induce tumor cell death, with less genotoxic to normal hematopoietic cells than anti-cancer drugs (Mishima et al., 2005). Chrysin is a natural, biologically active compound extracted from honey and propolis. It possesses potent anti-inflammation, anti-cancer and anti-oxidation properties. Chrysin significantly suppressed the lipopolysaccharide (LPS)-induced COX-2 protein and mRNA expression in a dose-dependent manner. Nuclear factor for IL-6 was identified as responsible for the chrysin-mediated COX-2 downregulation (Woo et al., 2005).

A-Inhibitory Effects of Bee Propolis on Angiogenesis, Tumor Invasion, and Metastasis

Caffeic acid phenethyl ester (CAPE) derived from honeybee propolis was investigated for its effect on the angiogenesis, tumor invasion, and metastasis. A cytotoxicity assay of CAPE in CT26 colon adenocarcinoma cells showed a dose-dependent decrease in cell viability but no significant

influence on the growth of human umbilical vein epithelial cells (HUVEC). A low concentration of CAPE (1.5 microg/mL) inhibited 52.7% of capillary-like tube formation in HUVEC culture on Matrigel. CAPE (6 microg/mL)-treated CT26 cells showed not only inhibited cell invasion by 47.8% but also decreased expression of matrix metalloproteinase (MMP)-2 and -9. Vascular endothelial growth factor (VEGF) production from CT26 cells was also inhibited by treatment with CAPE (6 μg/mL). Intraperitoneal injection of CAPE (10 mg/kg/day) in BALB/c mice reduced the pulmonary metastatic capacity of CT26 cells accompanied with a decreased plasma VEGF level. CAPE treatment also prolonged the survival of mice implanted with CT26 cells. These results indicate that CAPE has potential as an antimetastatic agent (Liao et al., 2003).

There is considerable evidence suggesting angiogenesis and chronic inflammation are codependent. Blockage of angiogenesis results in an anti-inflammatory effect. Ethanol (EEP) and ether extracts of propolis (REP), and caffeic acid phenethyl ester (CAPE), an active component of propolis, were examined for their anti-angiogenic activities using the chick embryo chorioallantoic membrane (CAM), and the calf pulmonary arterial endothelial (CPAE) cell proliferation, assays. The presence of EEP, REP and CAPE inhibited angiogenesis in the CAM assay and the proliferation of-CPAE cells. The results suggest that anti-angiogenic activities of EEP, REP and CAPE are also responsible for their anti-inflammatory effect (Song et al., 2002).

B-The Protective Effect against Drugs and Cancer Chemotherapeutic Agent Toxicity

The paracetamol and cyclophosphamid are metabolized in the liver by the cytochrome P450.The formed reactive intermediates are responsible of hepatocytes depletion of the glutathione and lipoperoxydation. The vinblastine is also a chemotherapeutic agent hepatotoxic and hematotoxic. Otherwise, flavonoids are polyphenols substances of plant origin having some biological and anti-oxidative properties. The effect of oral administration of flavonoids (diosmine and quercetine) under shape of propolis extract to 60 mg/kg daily during 14 days, on hematological and hepatic toxicity of a single dose of cyclophosphamide 80 mg/kg by intravenous way, vinblastine 2 mg/kg by intravenous way and the hepatic toxicity of the paracetamol managed by oral way to 200 mg/kg corresponding to 2/3 the LD_{50} at the rat female Albinos Wister was investigated. Analyses were done at regular intervals; 1, 3, 7 and 14 days after the administration of drugs. In the group of rats treated by the cyclophosphamid paracetamol alone they observed since the 1st day, an increase of lipid peroxide (MDA) of 120% and a downfall of hepatic glutathione including the group receiving the vinblastine (until 210% of reduction). In the same way, a severe leucopenia and a thrombopenia (70% of reduction) were observed between the 3rd and the 14th day at rats treated by the chemotherapeutic agents alone (cyclophosphamide and vinblastine). The combination of flavonoids with drugs have clearly reduced the effect of drugs toxicity. Indeed, the aplasic observed with the vinblastine, as well as the leucopenia and thrombopenia of the cyclophosphamide are corrected entirely. In the same way, they noted a restoration of rates of peroxide and glutathione. Flavonoids seem to act by activation of the turn over of the glutathione and enzymes stimulating particularly glutathion-s-transferases permitting the captation of the reactive metabolites of the studied drugs (Lahouel et al., 2004).

5-Protective Effects on the Liver

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 microg/mL, 24 h) significantly decreased the cytotoxicity of AA (0.5 mm) in a dose-dependent manner. In mice, pretreatment 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 treatment with PP for 7 days, the hepatic enzyme activities of cytochrome P450 monooxygenases (P450s), UDP-glucuronyltransferase, phenolsulphotransferase (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 suggest that PP has a protective effect on hepatic injury, and that its effect may be explained by inhibition of phase I enzymes and induction of phase II enzymes (Seo et al., 2003).

Giurgea et al (1981) reported that daily administration of 20 mg/100 g b.wt. standard propolis extract (SPE) to chicken for 15 days increased plasma total protein and gamma-globulin content. They suggested that also propolis has an anabolic effect and stimulated the immunologic processes. They also reported that daily administration of 20 mg propolis extract to chickens for 15 days changed the blood concentration of cholesterol, transaminase (ALT & AST), total proteins and amino acids. It also stimulated the immune system. In another study (Giurgea et al., 1982) the investigators reported that chicken fed on propolis extract showed a significant increase in serum total protein and a slight reduction in the glycogen level. Interaction of purified propolis *in vitro* with serum albumin or human serum proteins caused conformational changes in the protein and increase ceruloplasmin activity (Olinescu et al., 1982). Giurgea et al (1984) found that daily administration of propolis extract to chickens caused a marked increase in the myofibril, protein fraction and muscle total protein when compared to corresponding control. They also stated that, propolis extract affects the levels of cholesterol, transaminase activity, total protein, gamma globulins and free amino acids. They found increases in gamma globulins and proteins and

suggested that propolis had an anabolic effect, and that it stimulated the body's immune response.

The Chinese propolis was found to have the most antioxidative and the main component responsible for this property was identified as benzyl caffeate (Yamauchi et al., 1992). Krol et al (1990) suggested that antioxidative capacity of propolis is partly due to its high flavonoid contents. Administration of Egyptian and Bulgarian propolis has a weak general effect on estimated parameters in normal rats and it is not a toxic substance. Both types of propolis exerted an anabolic effect for protein synthesis by liver cells. On the other hand, the activity of ALT and AST returned to the control level after administration of propolis in rats infected with S. aureus and *E. coli*. Propolis tends to normalize the serum total lipids in infected rats (Hegazi et al., 1997).

Aqueous propolis extract (APE) was found to protect the liver in rats against carbon tetrachloride (CCl4) 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 (El-Khatib et al., 2002). The anti-oxidant 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 CCl4-intoxication in rats. Tectochrysin increased the antioxidant enzymes activity such as hepatic cytosolic superoxide dismutase, catalase and glutathione peroxidase in CCl4-intoxicated rats as well as a significant decrease in the MDA production (Lee et al., 2003).

Propolis extract was found to have a protective effect against alcohol liver injury by preventing elevations of total cytochrome P-450 enzymes, NADPH-dependent cytochrome C reductase, aniline hydroxylation, 7-ethoxyresorufin hydroxylation (7-ERH), 7-penthoxyresorufin hydroxylation (7-PRH), and lipid peroxidation (Lin et al., 1999).

The fractionation and chemical analysis led to the isolation of four dicaffeoyl quinic acid derivatives from the propolis. The structure of these isolates was determined to be methyl 3,4-di-O-caffeoyl quinate (1), 3,4-di-O-caffeoyl quinic acid (2), methyl 4,5-di-O-caffeoyl quinate (3), and 3,5-di-O-caffeoyl quinic acid (4) by spectroscopic methods. These compounds were more potent hepatoprotective agents than glycyrrhizin at a concentration of 10 micrograms/ml and 1was the most potent among the four compounds in the cultured hepatocytes. Quinic acid (5) alone did not show hepatoprotective effects in cultured rat hepatocytes against CCl4-toxicity. On the other hand, chlorogenic acid (6) or caffeic acid alone was found to be less potent than the dicaffeoyl quinic acid derivatives (Basnet et al., 1996).

6-Protective Effects on the Brain:

Oxygen-derived free radicals have been implicated in the pathogenesis of cerebral injury after ischaemia-reperfusion. Caffeic acid phenethyl ester (CAPE), an active component of propolis extract, exhibits antioxidant properties. The effects of ischaemia and subsequent reperfusion on rat brain and the effects of two free radical scavengers, CAPE and alpha-tocopherol were investigated on the in vivo model of cerebral injury. Ischaemia was induced by bilateral occlusion of the carotid arteries for 20 min and reperfusion was achieved by releasing the occlusion to restore the circulation for 20 min. Control rats underwent a sham operation. CAPE at 10 micromol kg(-1) or alpha-tocopherol at 25 micromol kg(-1) was administered intraperitoneally before reperfusion. Reperfusion led to significant increase in the activity of xanthine oxidase and higher malondialdehyde levels in the brain. Acute administration of both CAPE and alpha-tocopherol suppressed ischaemia-reperfusion-induced cerebral lipid peroxidation and injury, but CAPE seems to offer a better therapeutic advantage over alpha- tocopherol (Irmak et al., 2003). In addition, CAPE was found to protect the spinal cord from ischemia-reperfusion injury (Ilhan et al., 1999).

Central nervous system tissue is particularly vulnerable to oxidative damage, suggesting that oxidation plays an important role in the pathogenesis of multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE). Caffeic acid phenethyl ester (CAPE) was examined for its effects on oxidative tissue damage in EAE in rats. Treatment with CAPE significantly inhibited reactive oxygen species (ROS) production induced by EAE, and ameliorated clinical symptoms in rats. These results suggest that CAPE may exert its anti-inflammatory effect by inhibiting ROS production at the transcriptional level through the suppression of nuclear factor kappa B activation, and by directly inhibiting the catalytic activity of inducible nitric oxide synthase (Ilhan et al., 2004).

7-Protective Effects on the Heart:

Propolis showed an antihypertensive effect in rats (Yoko et al., 2004). In Diabetic rats, administration of bee propolis extracts led to decreased levels of blood glucose (FBG), fructosamine (FRU), malonaldehyde (MDA), nitric oxide (NO), nitric oxide synthetase (NOS), total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), 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* (Fuliang et al., 2005).

Doxorubicin-induced myocardiopathy is the consequence of oxidative stress through the mediation of free 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. Serum creatine phosphokinase (CK), aspartate aminotransferase (AST), blood and tissue glutathione (GSH), and

thiobarbituric acid reactive substances (TBARS) in heart were estimated to assess the status of heart muscle. An elevation of the levels of CK, AST, GSH, and TBARS was observed following doxorubicin treatment. Parallel experiments with a pretreatment of propolis significantly reduced the levels of these parameters. Biochemical observations were supplemented by histopathological examination of heart sections. The protective effect of propolis was compared with that of rutin, a known cardioprotective flavonoid. The study demonstrates the cardioprotective effect of propolis in doxorubicin-induced experimental cardiotoxicity (Chopra et al., 1995).

8- Other Activities:

Bee propolis has many other biologic 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 (Jeng et al., 2000). Hartwich et al (2000) used bee propolis in treatment of patients operated for goiter, patients with wounds and ulcerations difficult to heal and patients with non-specific rectal inflammation. They also tested the effectiveness of propolis as supplementary means in eradicating treatment of *Helicobacter pylori*.

Conclusions

Propolis is one of the few natural remedies that has 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 activity. Moreover, propolis has been shown to lower blood pressure and cholesterol levels. However, clinical studies to substantiate these claims are required. Until this becomes available, physicians should weigh the benefits of propolis as an adjuvant therapy for the good of cancer patients.

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