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PHARMACOLOGICAL AND THERAPEUTIC PROPERTIES OF PROPOLIS (BEE GLUE)

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17.1 INTRODUCTION

Propolis has been used by humans for thousands of years and recently has enjoyed a boom in popularity. Bees have used propolis for millions of years, and humans have used it for thousands. Both species find it immensely useful and beneficial. Much of the bees' success in surviving through the ages may be accredited to propolis. The Greek physician Hippocrates prescribed the use of propolis to help heal internal and external sores and ulcers. Ancient Egyptians depicted propolis-making bees on vases and other ornaments and used the resinous substance to alleviate many ailments. Pliny, the Roman scholar, wrote much on the use of resins such as propolis in his massive book, *Natural History*. He touted the abilities of propolis to reduce swelling, soothe pain, and heal sores, to name a few.

In the *History of Plants*, written by John Gerard in 1597, propolis was noted for its ability to provide swift and effective healing for many conditions. During this era, propolis was used in many different healing ointments. Although propolis is vitally important to the colony, there are usually just a few propolis-gathering specialists in the hive. The bees gather propolis, sometimes called "bee glue," and carry it home in their pollen baskets. There they are met by one or two other worker bees that help them unload. These workers take the resinous material and add salivary secretions and wax flakes to it and then use the new product for numerous protective purposes as bee propolis. The bees use it to coat the inside of the hive, including the passageway and the brood chambers. Propolis protects the hive in two ways: First, it reinforces the hive itself; second, it protects the hive from bacterial and viral infection. And it is these latter properties that humans have found so helpful through the centuries.

17.2 PROPOLIS

Propolis is a resinous yellow-brown to dark brown substance collected by worker honeybees from the growing parts of trees and shrubs (e.g., leaf buds, trunk wounds). The bees pack the propolis on their hind legs, and carry it back to their colony, where it is combined with beeswax and used by worker "hive" bees as a sealant and sterilant in the colony nest. These uses take advantage of the antibacterial and antifungal effects of propolis in protecting the colony against disease [1].

Propolis changes consistency with temperature. At temperatures below 15°C it is hard and brittle, but it becomes more pliable and sticky at higher temperatures. Propolis generally melts at 60−70°C, although some samples have been found to have a melting point as high as 100°C [2]. Propolis is collected by commercial beekeepers, either by scraping the substance from wooden hive parts or by using specially constructed collection mats. The raw product undergoes secondary processing to remove beeswax and other impurities before being

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used in a variety of natural health care products (e.g., lozenges, tinctures, ointments, drinks).

17.2.1 History

Propolis, bee glue, a gum gathered by bees from various plants, has been used by humans since early times for various purposes, and especially as a medicine because of its antimicrobial properties [3]. Ancient Greek texts refer to the substance as a "cure for bruises and suppurating sore," and in Rome propolis was used by physicians in making poultices. The Hebrew word for propolis is *tzori*, and the therapeutic properties of *tzori* are mentioned throughout the Old Testament. Records from twelfth-century Europe describe medical preparations using propolis for the treatment of mouth and throat infections and dental care [4].

One of the nonmedical uses of propolis is as a varnish, and it has been suggested that the special properties of Stradivarius violins may be partly due to the type of propolis used, although the claim cannot be substantiated.

17.2.2 Composition

At least 180 different compounds have been identified so far in propolis. The class of compounds present in propolis includes resins, waxes and fatty acids, essential oils, pollen, and minerals. The resins comprise flavonoids, phenolic acids, and esters (45–55%) whereas the essentials oil contain 10% volatiles. There are nearly 12 free amino acids in pollen and 14 trace minerals, iron and zinc being the most common. The other organics present are ketones, lactones, quinines, steroids, benzoic acid, vitamins, and sugars. The most important pharmacologically active constituents in propolis are the flavones, flavonols, and flavanones (collectively called flavonoids) and various phenolics and aromatics. Flavonoids play a major role in plant pigmentation. However, the flavonoids present in propolis are not glycosides (they do not have sugar molecules attached to their chemical structure). The majority of flavonoids found in plants are glycosides.

Flavonoids are thought to account for much of the biological activity in propolis [5], although other phenolic compounds are also involved. At least 38 flavonoids have been found in propolis, including galangin, kaempferol, quercetin, pinocembrin, pinostrobin, and pinobanksin [6]. Other phenolics include cinnamic alcohol, cinnamic acid, vanillin, benzyl alcohol, benzoic acid, and caffeic and ferulic acid. The chemical composition of propolis is highly variable because of the broad range of plants visited by honeybees when collecting the substance [7].

At least 67 plant species have been reported to provide propolis material. Important sources include poplars, alders and birches, chestnut, ash, and willows. Variations in the beeswax content of raw propolis also affect the chemical composition. Studies indicate that the plant resins collected by bees are at least partially altered by honeybees before use in the hive.

17.3 HUMAN NUTRITION

Propolis also has nutritive value, because of the presence of small amounts of proteins, amino acids, minerals, and sugars. Vitamins include small amounts of A, B_1, B_6, C, and E [8]. Dihydroflavonoids, like those found in propolis, have been shown to aid the human body in absorbing vitamin C [9]. Propolis and a number of its components exhibit a wide variety of biological and pharmacological activities [6].

17.4 THERAPEUTIC PROPERTIES

Because of its strong antimicrobial activity, propolis is often known as a "natural antibiotic." Propolis is reported to possess anticancer, apoptotic, wound healing, antiinflammatory, antiulcer, antiviral, anesthetic, and immunomodulatory properties.

17.4.1 Antimicrobial Properties

A large number of studies have shown an inhibitory effect on a variety of microorganisms (Table 17.1). Active components of propolis showing an antibacterial effect include pinocembrin, galangin, caffeic acid, and ferulic acid, whereas antifungal components include pinocembrin, pinobanksin, caffeic acid, benzyl ester, sakuranetin, and pterostilbene and antiviral components include caffeine. Propolis has been found to inhibit the synthesis of protein by bacteria, which may account for at least some of its antimicrobial effects [16].

17.4.2 Synergistic Properties

Most studies on the therapeutic properties of propolis have centered on the phenolic constituents (flavonoids and other phenolic compounds such as caffeic acid esters). Research has tended to isolate and test single substances in propolis. However, it is likely that the presence of a large number of compounds in propolis may produce a synergistic effect greater than the sum of the effects of individual components [31]. Studies have shown that the flavonoids in propolis exert significant antimicrobial activity compared to whole product extracts.

Propolis has also been shown to have a synergistic effect with certain antibiotics and to increase their effectiveness on some bacteria and yeasts, in some cases 100-fold [32]. Antibiotic-resistant strains of *Staphylococcus* were found to become sensitive to antibiotics in combination with propolis [33].

17.4.3 Anticancer Properties

Ethanol extracts of propolis have been found to transform human hepatic and uterine carcinoma cells in vitro and to inhibit their growth [34]. Substances isolated from propolis that produce this cytotoxic effect are quercetin, caffeic acid, and clerodane diterpendoid. Clerodane diterpendoid shows a selective toxicity to tumor cells.

Propolis was also found to have a cytotoxic and cytostatic effect in vitro against hamster ovary cancer cells and sarcoma-type tumors in mice [35].The substance has also displayed cytotoxicity on cultures of human and animal tumor cells, including breast carcinoma, melanoma, colon, and renal carcinoma cell lines [36]. The component producing these effects was identified as caffeic acid, phenethyl ester.

TABLE 17.1 Antimicrobial effects

Microorganisms	Targeted Action	Reference
Bacillus larvae	Destroyed	1
Bacillus subtillis	Destroyed	10
Helicobacter pylori	Inhibited	11
Methicillin-resistant *Staphylococcus aureus*	Inhibited	5
Mycobacterium tuberculosis	Inhibited	12
Bacterioides nodosus	Reduced foot rot	21
Escherichia coli	Inhibited	16
Giardia lambia	Positive effect	20
Klebsiella pneumonia	Positive effect	22
Staphylococcus sp.	Inhibited	13
Staphylococcus aureus	Synergistic effect	14
Streptococcus sp.	Inhibited	15
Streptomyces	Inhibited	16
S. sobrinus, mutans, cricetus	Dental caries	17
Saccharomyces cerevisiae	Brewer's yeast	18
Salmonella	Potentially treated	19
Aspergillus niger	Positive effect	24
Ascosphaera apis	Inhibited	26
Botrytis cinerea	Fungicidal	25
Candida albicans	Synergistic effect	23
Herpes virus	Inhibited	27
Influenza (in mice) virus	Reduced mortality	29
Newcastle disease virus	Affected virus reproduction	30
Potato virus	Effective	28

Caffeic acid esters have been shown to inhibit chemically induced tumor production in mice, as well as having selective toxic effect on cells affected by genes that promote the development of cancerous cells [37].

A substance called artepillin C has been isolated from propolis, and it has been shown to have a cytotoxic effect on human gastric carcinoma cells, human lung cancer cells, and mouse colon carcinoma cells in vitro [38].

17.4.4 Apoptotic Characteristics

It is known that propolis ethanolic extract (100 μg /ml) causes apoptotic-like cell demise. Chemotherapy based on propolis, alone or in combination with vinorelbine, has been suggested to be a useful tool in prostate cancer therapy.

17.4.4.1 Treatment of Breast and Prostate Cancers. There are mainly three types of propolis whose major anticancer ingredients are entirely different: (1) CAPE (caffeic acid phenethyl ester)-based propolis in Europe, the Far East, and New Zealand; (2) artepillin C (ARC)-based Brazilian green propolis; and (3) Brazilian red propolis. Neurofibromatosis (NF)-associated tumors require the kinase PAK1 for their growth, and CAPE-based propolis extracts such as Bio 30 suppress completely the growth of NF tumors in vivo by blocking PAK1 signaling. It was demonstrated that ARC suppresses angiogenesis, suggesting the possibility that ARC also blocks oncogenic PAK1 signaling. The study suggested that both CAPE-based and ARC-based propolis extracts are natural anti-PAK1 remedies and could be among the first effective NF therapeutics available on the market. Since more than 70% of human cancers such as breast and prostate cancers require the kinase PAK1 for their growth, it is quite possible that GPE could be potentially useful for the treatment of these cancers, as is Bio 30 [39].

Vinorelbine bitartrate, a drug widely used in prostate cancer therapy, was utilized as a reference drug because it is known to induce apoptosis [40]. The combined treatments of micronutrients viz. propolis extract and vinorelbine have been studied to test a possible vinorelbine dose reduction, avoiding its side effects without altering its cytotoxic action. Here, SEM and TEM analyses have also been performed to examine the morphological modifications induced; the observations confirmed apoptotic modifications after propolis treatment. They also measured cell cycle progression to study a correlation with p21 and p53, two well-known cell cycle checkpoints. The levels of HSP27 and HSP70, two chaperones exerting antioxidant/antiapoptotic functions, were also analyzed. The data indicated that

propolis modulated cell cycle distribution, increasing p53 levels, without the induced HSPs being able to rescue DU145 from death. Hence chemotherapy based on propolis, alone or in combination with vinorelbine, as a potential useful tool for prostate cancer therapy as it effects increase in cell cycle control and the modulation of HSPs expression, reinforced this suggestion.

17.4.4.2 Propolis-Induced Apoptosis of Carcinoma Cells

The treatment of laryngeal cancer is comprehensive, based on surgery. Chemotherapy is an important component of the treatment for laryngeal cancer, which lacks ideal selectivity. The anticancer active ingredients contained in propolis have been identified as flavonoids, terpenes, sugars, esters, and other compounds, such as a natural combination, their mutual coordination role, given the anticancer effect of propolis [41]. Experimental results show that propolis inhibited chemical carcinogen-induced mutations [42]. Because radiotherapy and chemotherapy kill tumor cells, these would also damage the body's immune and hematopoietic cells, and therefore strengthening the immunity against radiation and chemotherapy-induced side effects, is the important task of cancer prevention and treatment. The results show that propolis on Hep-2 cells significantly inhibited cell proliferation and can induce apoptosis and the cell cycle to a certain extent. It can affect the cell cycle G_1 phase to S phase transition, but also affect the S phase of the transition to the G_2/M leading to tumor cell apoptosis and cell cycle non-specific blocking the whole process. With increase of the concentration of propolis, the killing effect on cancer cells was also enhanced. The study revealed that propolis inhibited the growth of laryngeal cancer cells, and the inhibitory action was through the mechanism of apoptosis [43–45].

In a study two new prenyl flavanones, propolin A and propolin B, isolated and characterized from Taiwanese propolis, induced cytotoxicity in human melanoma A2058 cells and showed a strong capability to scavenge free radicals. In this study, propolin A effectively induced a cytotoxic effect on five different cancer cell lines. The levels of procaspase-8, Bid, procaspase-3, DFF45, and PARP were decreased in dose- and time course-dependent manners. Propolin A and propolin B were also capable of releasing cytochrome *c* from mitochondria to cytosol. The findings suggest that propolin A and propolin B may activate a mitochondria-mediated apoptosis pathway. All these results indicated that propolin A and propolin B may trigger apoptosis of A2058 cells through mitochondria-dependent pathways and also showed that propolin A and propolin B were strong antioxidants [46].

It was demonstrated that the prenyl flavanones propolin A and propolin B, isolated and characterized from Taiwanese propolis, induced apoptosis in human melanoma cells and significantly inhibited xanthine oxidase activity. Furthermore, it the isolation of a third compound called propolin C was reported [47]. The chemical structure of propolin C has been characterized by NMR and HRMS spectra and was identical to nymphaeol-A. Propolin C was found to effectively induce cytotoxic effect on human melanoma cells, with an IC_{50} of about 8.5 μM. In a study to address the mechanism of the apoptosis effect of propolin C, the effect of propolin C on induction of apoptosis-related proteins in human melanoma cells was evaluated. The findings suggested that propolin C may activate a mitochondrion-mediated apoptosis pathway [47].

17.4.5 Antioxidant Properties

The flavonoids concentrated in propolis are powerful antioxidants. Antioxidants have been shown to be capable of scavenging free radicals and thereby protecting lipids and other compounds such as vitamin C from being oxidized or destroyed. The polyphenolic-rich extracts of beeswax have been reported to exhibit antioxidant property in vivo [48–50]. In this study, the extracts were able to reduce CCl_4- and paracetamol-induced oxidative stress in rats, as evidenced by the changes in hepatic antioxidant and detoxifying enzymes.

Propolin C is also a potential antioxidant agent and shows a strong capability to scavenge free radicals and inhibit on xanthine oxidase activity with IC_{50} of about 17.0 μM [47].

17.4.6 Wound Healing Properties

Propolis has been shown to stimulate various enzyme systems, cell metabolism, circulation, and collagen formation, as well as improving the healing of burn wounds [51, 52]. These effects have been shown to be the result of the presence of arginine in propolis [53]. Patients with tibial skin ulcers, aged from 23 to 98 years, were treated with propolis tincture in an ointment. The ointment was applied daily to the ulcerated area, which was also treated on the periphery with antibiotic ointments [54]. The treatment lasted for 4–12 weeks. At the end of treatment, 19 of the 84 treated patients exhibited no clinical signs of the condition, and 19 exhibited an improved condition [54].

Propolis was used in a trial of hospital patients with infected wounds. The propolis improved wound healing rates, while at the same time reducing infection. Over half of infective bacteria were eliminated within 4 days. Propolis did not produce antibiotic-resistant strains of the bacteria [55]. A study of topical application of propolis on wounds, burns, and ulcers showed up to an 80% increase in healing rate compared to control subjects using routine healing regimes.

Patients (229) with burns, clean wounds, infected wounds, or abscesses/ulcers were treated with cream containing propolis at two concentrations (2% and 8%). The higher concentration caused local intolerance in 18% of patients by day 9, whereas the lower concentration caused symptoms in only 1.8% of patients by day 16. Burns and wounds treated with the low-concentration cream healed in 11 days on average, septic wounds in 17.5 days, and 67% of ulcers in 38 days [56].

17.4.7 Antiulcer Properties

In a study to treat ulcerative colitis and Crohn disease by propolis in a double-blind clinical trial in Denmark, improvement was noted in patients with colitis, but no effect was shown against Crohn disease. Propolis has been shown to inhibit the development of externally induced stomach ulcers in rats. Flavonoid components of propolis have also been shown to have this effect. [57].

In a study conducted in patients (138) suffering giardiasis were treated with propolis extracts (10–20%). In children, 52% showed a cure at the lower dose. In adults, the cure rate was the same as for tinidazole, an antiprotozoan drug, at the 20% extract and 60% versus 40% for Undazole at a higher concentration (30% propolis extract) [58]. The efficacy of bee propolis in the treatment of acute and chronic colitis was investigated elsewhere and promising results were reported.

17.4.8 Skin Infection Effects

Propolis has been shown to be effective in inhibiting the growth of yeasts and fungi responsible for such skin infections as ringworm and athlete's foot. Propolis compounds showing activity against these organisms are the flavonoids and caffeic acid derivatives.

Clinical applications of propolis (1–10%) in ether or alcohol were effective against 10 superficial fungi and 9 deep-growing fungi. On oral treatment of 160 psoriasis patients with 0.3 g propolis 3 times daily for 3 months, about one-third were cured or greatly improved. Patients (110) infected with ringworm were treated with 50% propolis as an unguent. In 97 patients it was found to produce excellent results [59].

17.4.9 Antiinflammatory Properties

Studies on mice have shown that extracts of propolis have an antiinflammatory effect similar to that of indomethacin, a common drug used to treat inflammation. Again, flavonoids and caffeic acid are known to play a role in inhibiting the inflammatory response. Injections of an aqueous solution of propolis were used in the treatment of 22 patients with hip joint disease caused by aseptic necrosis of the thigh bone. A further 32 patients with the same condition were given different forms of routine treatment. Significant improvement was observed in the patients given propolis. Patients (90) with cases of vagina and uterine cervix inflammation caused by pyrogens were treated with 3% propolis ethanol extract. Over 50% of the cases responded well to this treatment [60].

17.4.10 Anesthetic Properties

Propolis and some of its components produce anesthesia, which in some studies has been shown to be three times as powerful as cocaine and 52 times as powerful as procaine when tested in rabbit cornea. The anesthetic effect has been shown to be produced by pinostrobin, caffeic acid ester components in propolis [61]. The anesthetic effect may explain why propolis has been used for centuries in the treatment of sore throats and mouth sores. An anesthetizing ointment for dentistry using propolis has been patented in Europe [62].

17.4.11 Immunomodulatory Properties

Propolis has been shown to stimulate an immune response in mice [63]. More recently, Japanese researchers have shown that an extract of propolis produces a macrophage activation phenomenon related to the immune function in humans [64]. Propolis activates immune cells, which produce cytokines. The results help to explain the antitumor effect produced by propolis. Propolis has been shown to stimulate antibody formation in immunized mice. In a joint US-Polish study, spleen cells producing antibodies in mice administered a propolis extract were three times greater than controls. A second dose administered 24 hours later produced an even larger effect, although further doses reduced the effect [65].

Propolis was shown to increase antibody formation between two and three times that of controls in pigs vaccinated with "BUK-628" live Aujeszky's disease vaccine with and without addition of propolis. Antibody formation reached its maximum in 14 days, and antibodies could be detected for up to 330 days. Propolis also enhanced production of plasmacytes in the lymphoidal tissue of the spleen and lymph nodes.

Propolis has been shown to suppress HIV-1 replication and modulate in vitro immune responses and, according to the authors, "May constitute a non-toxic natural product with both anti-HIV-1 and immunoregulatory effects" [66]. In mice, a concentrated extract of propolis has been shown to reduce blood pressure, produce a sedative effect, and maintain serum glucose [67]. Dihydraflavonoids as contained in propolis, have been shown to strengthen capillaries and produce antihyperlipidemic activity.

A strong immune deficiency was found in two patients with alveolitis fibroticans. Treatment with a

combination of the propolis, Esberitox N and calcium-magnesium resulted in good improvements in the state of the immune system and the clinical condition of both patients [68].

17.4.12 Antiviral Properties

A clinical trial has shown a prophylactic effect against influenza infection in humans [69], and another clinical trial showed that infections of the common cold were shorter in duration and completely recovered within 3 days in patients treated with propolis, compared to 5 days for recovery for patients not given propolis [70]. A clinical trial conducted on dermatology patients showed that a propolis cream had significant therapeutic effects against recurrent herpes (Herpes simplex type 1) and Herpes zona zoster (shingles). The propolis cream reduced duration of lesions and pain and increased interval between lesion episodes.

Perhaps the most broadly investigated and widely accepted attribute of bee propolis is its immune-boosting activity. It is a natural, broad-spectrum antibiotic that activates the thymus gland. Bee propolis not only prevents infectious diseases but clears them from the system as well. As demonstrated in numerous experiments, propolis has the ability to directly destroy bacteria, viruses, and fungi, even penicillin-resistant *Staphylococcus*.

17.4.13 Dental Care Properties

In rats inoculated with sobrinus, about half of fissures were carious, while dental caries were significantly less in rats given water containing propolis extract. No toxic effects of propolis on the growth of rats were observed under experimental conditions in this study [17]. Propolis has also been shown to be effective as a subsidiary treatment for gingivitis (gum infections) and plaque [71]. A 50% propolis extract was found to be antiseptic against pulp gangrene [72]. Propolis has also been shown to inhibit the growth of a range of bacterial organisms found in dental caries [73].

The diverse use of propolis in clinical trials showed that its therapeutic efficiency lies mainly in diseases caused by microbial contamination [74]. The results suggest that a propolis preparation can be a useful subsidiary treatment in oral hygiene.

A double-blind clinical trial showed that a propolis mouthwash (10% tincture diluted 1:5 with water) produced significant improvements in patients with gingivitis and periodontal disease. Patients were evaluated for plaque formation and inflammation of the gums. A clinical study used propolis extract and zinc oxide on 160 teeth with indirect capping or deep cavities and teeth with direct capping. The results showed that the paste with propolis exerted effects similar to those of zinc eugenate and were superior for healing compared to pastes based on calcium hydroxide.

A clinical study found propolis useful for the treatment of gum inflammation and oral mucosa and also showed antiscarring effects [75]. Another study showed similar results for periodontitis and suggested that propolis be used in root canal fillings because of its bone regeneration and anesthetic properties.

17.4.14 Respiratory and Ear Infections

A total of 260 steel workers suffering from bronchitis were treated for 24 days by various methods including local and systemic regulation of the immune system and local treatment with an ethanol extract of propolis (EEP) in a physiological salt solution [67]. Promising results were obtained with inhalation of the extract, together with propolis tablets. Propolis has also shown positive effects in other otorhinolaryngologic diseases, such as pharyngitis chronic bronchitis [67], rhinopharyngolaryngitis [76], pharyngolarynitis [77], catarrh [78], and rhinitis.

Patients (126) suffering from external otitis, chronic mesotympanic otitis, and tympan perforation were treated with propolis solutions (5–10%). A positive therapeutic result was reported in most cases. Propolis has also shown positive results in the treatment of acute inflammations of the ear [79].

17.4.15 Effects on Mitosis

Medical researchers (N. Popovici and N. Oita of Rumania) published a report on the effects of bee propolis on mitosis (the process of cell division). They reported that a tissue never becomes entirely malignant; it always contains some normal cells, but the activity of the normal cells is affected and even repressed by malignant cells. Propolis favors the activity of normal cells by repressing malignant cells, which helps the tissue to reestablish its normal condition. Constituents of propolis have a mitodepressive effect (depression of the proliferation of cancerous cells) on cells deranged by malignancy.

17.5 ROLE IN CELLULAR SIGNAL TRANSDUCTION

Signal transduction at the cellular level refers to the movement of signals from outside to inside the cell. The movement of signals can be simple, like that associated with receptor molecules of the acetylcholine class, receptors that constitute channels that, upon ligand interaction, allow signals to be passed in the form of small ion movement either into or out of the cell. These ion movements result in changes in the electrical potential

of the cells that, in turn, propagate the signal along the cell. More complex signal transduction involves the coupling of ligand-receptor interactions to many intracellular events. These events include phosphorylations by tyrosine kinases and/or serine/threonine kinases. Protein phosphorylations change enzyme activities and protein conformations. The eventual outcome is an alteration in cellular activity and changes in the program of genes expressed within the responding cells.

17.5.1 Classifications of Signal Transducing Receptors

Signal transducing receptors are of three general classes:

1. Receptors that penetrate the plasma membrane and have intrinsic enzymatic activity. Receptors that have intrinsic enzymatic activities include tyrosine kinases (e.g., PDGF, insulin, EGF and FGF receptors), tyrosine phosphatases (e.g., CD45 [cluster determinant-45] protein of T cells and macrophages), guanylate cyclases (e.g., natriuretic peptide receptors), and serine/threonine kinases (e.g., activin and TGF-β receptors). Receptors with intrinsic tyrosine kinase activity are capable of autophosphorylation as well as phosphorylation of other substrates. Additionally, several families of receptors lack intrinsic enzyme activity, yet are coupled to intracellular tyrosine kinases by direct protein-protein interactions.
2. Receptors that are coupled, inside the cell, to GTP-binding and hydrolyzing proteins (termed G proteins). Receptors of the class that interact with G proteins all have a structure that is characterized by seven transmembrane spanning domains. These receptors are termed serpentine receptors. Examples of this class are the adrenergic receptors, odorant receptors, and certain hormone receptors (e.g., glucagon, angiotensin, vasopressin and bradykinin).
3. Receptors that are found intracellularly and upon ligand binding migrate to the nucleus, where the ligand-receptor complex directly affects gene transcription. Because this class of receptors are intracellular and function in the nucleus as transcription factors, they are commonly referred to as the nuclear receptors. Receptors of this class include the large family of steroid and thyroid hormone receptors. Receptors in this class have a ligand-binding domain, a DNA-binding domain, and a transcriptional activator domain.

17.5.2 Signaling Molecules

Most signal transduction involves the binding of extracellular signaling molecules (and ligands) to cell surface receptors. While triggering events inside the cell, such receptors typically face outward from the plasma membrane. Intracellular signaling cascades can also be triggered through cell-substratum interactions. One example is integrins, which bind ligands found within the extracellular matrix. Steroids are another example of extracellular signaling molecules that may cross the plasma membrane because of their lipophilic or hydrophobic nature [80]. Many, but not all, steroid hormones have receptors within the cytoplasm, and usually act by stimulating the binding of their receptors to the promoter region of steroid-responsive genes [81]. Within multicellular organisms, numerous small molecules and polypeptides serve to coordinate a cell's individual biological activity within the context of the organism as a whole. These molecules have been functionally classified as:

- Hormones (e.g., melatonin) [82]
- Growth factors (e.g., epidermal growth factor) [83]
- Extracellular matrix components (e.g., fibronectin) [84]
- Cytokines (e.g., interferon γ) [85]
- Chemokines (e.g., RANTES) [86]
- Neurotransmitters (e.g., acetylcholine) [87]
- Neurotrophins (e.g., nerve growth factor) [88]
- Active oxygen species and other electronically-activated compounds (see redox signaling)

Most of these classifications do not take into account the molecular nature of each class member. For example, as a class neurotransmitters consist of neuropeptides such as endorphins [89] and small molecules such as serotonin [90] and dopamine [91]. Hormones, another generic class of molecules capable of initiating signal transduction, include insulin (a polypeptide) [92], testosterone (a steroid) [93], and epinephrine (an amino acid derivative, in essence a small organic molecule).

The classification of a molecule into one class or another is not exact. For example, epinephrine and norepinephrine, secreted by the central nervous system, act as neurotransmitters. However, when secreted by the adrenal medulla, epinephrine acts as a hormone.

17.5.2.1 Artepillin C Derived from Propolis Acts as a Neurotropic-Like Factor. It was investigated whether artepillin C, a major component of Brazilian propolis, acts as a neurotrophic-like factor in rat PC12m3 cells, in which nerve growth factor (NGF)-induced neurite outgrowth is impaired. When cultures of PC12m3 cells were treated with artepillin C at a concentration of 20 μM, the frequency of neurite outgrowth induced by artepillin C was approximately sevenfold greater than that induced

by NGF alone. Artepillin C-induced neurite outgrowth of PC12m3 cells was inhibited by the ERK inhibitor U0126 and by the p38 MAPK inhibitor SB203580. It was proposed that artepillin C-induced activation of p38 MAPK through the ERK signaling pathway is responsible for the neurite outgrowth of PC12m3 cells [94].

17.5.2.2 Brazilian Propolis Suppresses Angiogenesis by Inducing Apoptosis Angiogenesis is defined as the process in which a network of new blood vessels emerges from preexisting vessels. Angiogenesis has been shown to be essential for tumor growth and metastasis, which are two major factors that hinder cancer therapy [95]. Food factors, such as epigallocatechin gallate (EGCg), indole-3-carbinol, resveratrol, and quercetin, possessed antiangiogenic properties [96–99]. Such antiangiogenic food factors could be used to effectively prevent small cancers from progressing.

Investigation of the effects of many angiogenesis inhibitors has revealed that one of the major antiangiogenic mechanisms of these drugs is induction of apoptosis in endothelial cells [100]. Apoptosis is a genetically programmed form of cell death. Angiogenic stimuli such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) are known to activate extracellular signal-regulated kinase 1/2 (ERK1/2) and Akt, which transduce survival signals in endothelial cells and simultaneously prevent apoptosis by inactivating proapoptotic proteins [101–103]. On the other hand, apoptotic stimuli are known to activate a caspase cascade that ultimately leads to the oligonucleosomal fragmentation of DNA and the cleavage of proteins such as poly(ADP-ribose) polymerase (PARP) and lamin A/C [104].

It was reported that ethanol extract of Brazilian propolis (EEBP) suppresses tumor-induced angiogenesis in vivo and tube formation of endothelial cells in vitro [105]. It has also been demonstrated that propolis suppresses angiogenesis through induction of apoptosis in endothelial cells [106].

Propolis suppresses tumor-induced angiogenesis through tube formation inhibition and apoptosis induction in endothelial cells. The schematic diagram of angiogenesis suppression by EEBP is shown in Fig. 17.1. It was also shown that EEBP and U0126 similarly induced activation of caspase-3 and cleavage of PARP and lamin A/C, all of which are molecular markers of apoptosis. These results indicate that inhibition of survival signal ERK1/2, and subsequent induction of apoptosis, is a critical mechanism of angiogenesis suppression by EEBP.

It is known that propolis inhibits tube formation and induces apoptosis in endothelial cells [106]. It was further confirmed that ERK1/2 inactivation was largely

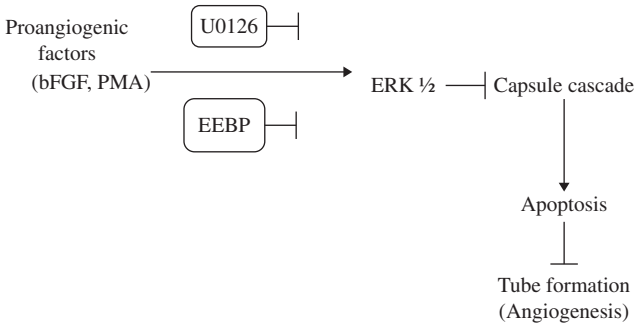

Fig. 17.1 Schematic diagram of angiogenesis suppression by EEBP, bFGF, and PMA (proangiogenic factors) stimulation of ERK1/2 signaling. The survival signal inactivates the caspase pathway, thereby maintaining cell survival facilitating angiogenesis.

responsible for antiangiogenic effects, tube formation inhibition and apoptosis induction, in endothelial cells.

ERK1/2 signaling in endothelial cells has been shown to play an essential role in angiogenesis both in vivo and in vitro. It was reported that bFGF and bone morphogenetic protein-4 induced the formation of capillary-like structures by endothelial cells through ERK1/2 activation [107, 108]. On the other hand, it was demonstrated that several pharmacological inhibitors, dominant negative constructs and siRNA against Raf/MEK/ERK pathway inhibited angiogenesis in vivo and tube formation of endothelial cells without affecting Akt activation [109, 110]. Such inhibitors have also been shown to induce endothelial cell apoptosis in vivo and in vitro [111, 112]. It was further confirmed that ERK1/2 inactivation alone is sufficient to prevent angiogenesis and induce apoptosis in endothelial cells. On this basis, it was suggested that ERK1/2 inactivation was a major mechanism responsible for antiangiogenic action of EEBP.

In this study, it was shown that EEBP inhibited ERK1/2 activation. It was reported that Brazilian propolis, collected from *Baccharis dracunculifolia* DC in Minas Gerais State, was composed mainly of artepillin C, caffeic acid, and *p*-coumaric acid [113]. Several constituents of Brazilian and Uruguayan propolis possessed antiangiogenic activities of varying degrees.

17.6 TOXIC EFFECTS

Propolis has been shown not to be toxic to humans or mammals unless very large quantities are administered [8]. Some of its constituent flavones, for example, quercetin, might be mutagenic by the Ames test, but mutagenicity per se for propolis has not been reported [6].

Contact dermatitis is a well-documented allergic reaction to propolis, with approximately 200 cases

reported in the literature over the last 70 years [114]. Initial reports were made by beekeepers, who came into daily contact with the raw product. Allergic reactions are now also reported in the general population, because of the more widespread use of products containing propolis. Dermatitis can be produced by skin contact with raw propolis as well as propolis extracts, and products containing caffeic acid and its derivatives have been identified as the major allergenic agent [115]. Cinnamic acid derivatives have also been implicated [65].

Dermatitis is relieved once the skin is no longer in contact with the propolis product. It is therefore recommended that with all preparations intended for human use, usage should be ceased whenever there is an allergic reaction. Very few other adverse reactions to propolis have been documented in the literature, and the product is generally considered not to be harmful [6]. Rare cases of oral inflammation and ulceration, mouth edema (swelling), and stomatitis have been reported as a result of oral ingestion of propolis [116, 117].

17.7 COMMERCIAL USE

Raw propolis is collected by beekeepers and sold in bulk to companies that refine the product and turn it into usable extracts. The main commercial uses of propolis are as a dietary supplement and therapeutic. Propolis is sold in tablets (singularly or in combination with other substances such as pollen, royal jelly and nonhive products). In Japan, the use of propolis is permitted as a preservative in frozen fish.

Tinctures and lozenges are popular treatment for sore throats, and tinctures are often used to treat cuts, mouth sores, and skin rashes. For internal use, a 1- to 3-ml dose three times daily of a 1:10 tincture is typical, but higher doses can be used if necessary. Propolis tincture is normally diluted in water, producing a cloudy liquid. For external use, the 1:10 tincture is diluted in water and used as a lotion.

Propolis is a stable product but should nevertheless be stored in airtight containers in the dark, preferably away from excessive and direct heat. Propolis does not lose much of its antibiotic activity, even when stored for 12 months or longer. Propolis and its extract function as a mild preservative because of their antioxidant and antimicrobial activities and thus may actually prolong the shelf life of some products.

17.8 FOOD SAFETY

Because of its antioxidant and antimicrobial activities, microbial contamination is not considered to be a problem with propolis, either in the raw form or as extracts. Concentrations of lead above maximum allowable levels for food products have been found in propolis. Studies have shown that lead levels may be reduced by placing the hives away from areas with heavy air pollution and the use of oil-based paints on hive parts [118]. Propolis destined for commercial use should be routinely tested for lead concentration. Brazilian propolis is of the highest quality available, whereas Chinese propolis has been noted for excessive lead.

17.9 CONCLUSION

Propolis, bee glue gathered by bees from various plants, has been used by humans since early times for various purposes, and especially as a medicine because of its antimicrobial properties. At least 67 plant species have been reported to provide propolis material. Important sources include poplars, alders and birches, chestnut, ash, and willows. The class of compounds present in propolis includes resins, waxes and fatty acids, essential oils, pollen, and minerals. The resins comprise flavonoids, phenolic acids, and esters (45–55%), whereas essentials oils contain 10% volatiles. There are nearly 12 free amino acids in pollen and 14 trace minerals, iron and zinc being the most common. The other organics present are ketones, lactones, quinines, steroids, benzoic acid, vitamins, and sugars. Propolis also has nutritive value because of the presence of small amounts of proteins, amino acids, minerals, and sugars. Vitamins include small amounts of A, B_1, B_6, C, and E. Because of its strong antimicrobial activity, propolis is often known as a "natural antibiotic." Propolis is reported to possess anticancer, apoptotic, wound healing, antiinflammatory, antiulcer, antiviral, anesthetic, and immunomodulatory properties. Artepillin C, a major component of Brazilian propolis, was reported to act as a neurotropic-like factor and to suppress angiogenesis by inducing apoptosis. With regard to toxicity, propolis has been shown not to be toxic to humans or mammals unless very large quantities are administered.

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