



Review

Propolis: Is there a potential for the development of new drugs?

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ABSTRACT

Introduction: Propolis has plenty of biological and pharmacological properties and its mechanisms of action have been widely investigated in the last years, using different experimental models *in vitro* and *in vivo*. Researchers have been interested in the investigation of isolated compounds responsible for propolis action; however, there is lack of clinical research on the effects of propolis.

Strategy and objectives: Since propolis-containing products have been marketed and humans have used propolis for different purposes, the goal of this review is to discuss the potential of propolis for the development of new drugs, by comparing data from the literature that suggest candidate areas for the establishment of drugs against tumors, infections, allergy, diabetes, ulcers and with immunomodulatory action.

Conclusions: The efficacy of propolis in different protocols *in vitro* and *in vivo* suggests its therapeutic properties, but before establishing a strategy using this bee product, it is necessary to study: (a) the chemical nature of the propolis sample. (b) Propolis efficacy should be compared to well-established parameters, e.g. positive or negative controls in the experiments. Moreover, possible interactions between propolis and other medicines should be investigated in humans as well. (c) Clinical investigation is needed to evaluate propolis potential in patients or healthy individuals, to understand under which conditions propolis may promote health. Data point out the importance of this research field not only for the readers and researchers in the scientific community waiting for further clarification on the potential of propolis but also for the pharmaceutical industry that looks for new drugs.

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1. Introduction

Natural products are a promising source for the discovery of new pharmaceuticals. In the last decades, several works dealing with propolis' composition and biological properties have been published, revealing the interest of researchers on this bee product

and its potential for the development of new drugs as well (Bankova et al., 1998, 1999; Banskota et al., 2001; Sforcin, 2007; Barros et al., 2008; Libério et al., 2009).

Propolis has been employed extensively since ancient times. Egyptians benefited from the anti-putrefactive properties of propolis in order to embalm their dead. Propolis was used as an antiseptic and cicatrizant agent by the Greek and Roman physicians. Incas employed propolis as an anti-pyretic agent, and the London pharmacopoeias of the 17th century listed propolis as an official drug. Its use continues today as a popular remedy and is available in either

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in pure form or combined with other natural products in cosmetics and as a constituent of health foods. Scientists have been interested in the investigation of its constituents and biological properties in the last decades (Ghisalberti, 1979; Castaldo and Capasso, 2002; Bankova, 2005a; Sforcin, 2007).

Propolis is a resinous material collected by bees from exudates and bud of the plants and mixed with wax and bee enzymes. The word propolis (from the Greek pro=in defense or for, and polis=city) reflects its importance to bees, since they use it to smooth out internal walls, as well as to protect the colony from diseases and to cover carcasses of intruders who died inside the hive, avoiding their decomposition (Bankova et al., 2000).

Propolis presents plenty of biological and pharmacological properties, such as immunomodulatory, antitumor, anti-inflammatory, antioxidant, antibacterial, antiviral, antifungal, antiparasite activities, among others (Sforcin et al., 2000, 2001; Gekker et al., 2005; Orsi et al., 2005, 2006a,b; Freitas et al., 2006; Búfalo et al., 2009b,c).

Heinrich et al. (2008) reported that in vitro methods are useful for preliminary investigation of the possible potential of a natural product. If such in vitro assays yield positive results, further investigation is necessary to produce data with clinical relevance. Moreover, in vitro and in vivo assays do not always include chemically characterized extracts, and one should take into account that pharmacological variability of preparations is expected (Heinrich et al., 2008).

While several authors have been investigating propolis' biological activities, no critical review exists concerning the usefulness of such data in the context of a product's clinical use. On the other hand, new formulations containing propolis or its isolated compounds have been prepared. As an example, Durán et al. (2007) prepared spherical and homogenous microparticles of poly(epsilon-caprolactone) (PCL) containing propolis, with 60% of the substance released in 48 h. Recently, the potential use of beta-cyclodextrin cavity for the incorporation of specific propolis components was investigated, aiming to increase their solubility in water (Kalogeropoulos et al., 2009a). The efficiency of ethanolic and water extracts of Indian propolis towards Ag and Au nanoparticles synthesis was compared with that of naturally occurring hydroxyflavonoids, pinocembrin and galangin isolated from Indian propolis; which were equally efficient in the rapid synthesis and stabilization of Ag and Au nanoparticles (Roy et al., 2010). Thus, the goal of this review is to discuss propolis potential for the development of new drugs in some research fields, such as immunology (e.g. drugs with immunomodulatory action), tumor (tumor cells are a target for propolis or isolated compounds), infections (the potential of propolis or its constituents as cariostatic agents and for the development of biotechnological products to control caries and other infectious diseases), allergy (propolis may be effective in the relief of symptoms of allergic rhinitis), diabetes (propolis seems to possess preventive effect on pancreatic beta-cells destruction) and ulcers (anti-ulcerogenic properties of propolis and its main phenolic acids). Table 1 presents some biological properties of propolis and the experimental approaches used by different authors.

2. Propolis and propolis extracts used in biological experiments: how to obtain scientifically sound results

Propolis chemical composition depends on the phytogeographic characteristics of the site of collection, since bees choose different plants as source of propolis in different habitats (Popova et al., 2010a). This aspect difficult propolis standardization, and different solvents (ethanol, methanol and water) may extract different compounds, influencing its activity (Cunha et al., 2004). Thus, a universal standardization would be impossible, and Bankova (2005a)

proposed that propolis biological properties should be linked to a detailed investigation of its chemical composition and to its botanical sources. Absence of heavy metals and pesticides is required as well, and it has been suggested that propolis might be studied as an environmental contamination indicator (Orsi et al., 2006a).

A vast number of papers dealing with different aspects of the biological properties of propolis have been published during the last decades. However, a considerable part of them are of limited usefulness, although they report "strong", "remarkable" or "significant" activity. The reason is the lack of basis for comparison and scientific evaluation of the results, because they do not refer to the chemical nature of the studied propolis samples. These studies only report that the tests have been performed with extracts of propolis. However, it is important to note that there is no such thing like "just propolis". Although of plant origin, propolis is a bee product and in different ecosystems bees collect it from different source plants, choosing appropriate representatives of the local flora. For example, Brazilian green propolis is derived mainly from alecrim plant (*Baccharis dracunculifolia*). The term "propolis" does not have a chemical connotation unlike the scientific name of a plant species. A plant species is characterized by its genome and this genome eventually determines the secondary metabolites synthesized by the plant enzymes and responsible for its biological activities. Propolis also contains secondary plant metabolites but they are not the same all over the world. There are several chemical types of propolis according to its major plant source(s), as listed in Table 2.

How to select propolis in order to test its biological activity, whatever tests might be planned? A favorable approach is to collect samples from areas where propolis has never been studied before. It is highly probable that in such areas bees have found a plant source of promising activity, having the potential to deliver new biologically active natural compounds. In general, bees choose sticky resinous vegetal material to be used as propolis because of its physical properties. On the other hand, this material is also their chemical defense against microorganisms, based on its chemistry (Ghisalberti, 1979; Bankova, 2005b). Comparative studies have revealed that, although of different chemical composition, propolis always demonstrated a more or less considerable biological activity (Kujumgiev et al., 1999; Seidel et al., 2008). For this reason, propolis chemical diversity has the potential to provide valuable leads (Bankova, 2009).

Raw propolis contains impurities such as wood, wax, pollen and even dead bees, so that it is necessary a macroscopic observation of the sample in order to eliminate and to purify it before preparation of extracts. A critical step in the process of testing is the extraction of the propolis specimens that will be used in the study. The solvents used for extraction are usually alcohols: methanol and ethanol. The most often utilized solvent is ethanol containing different percent of water, 70% ethanol was found to extract most of the active components of propolis but not waxes (Bankova et al., 1992). As propolis might contain up to 20–30% wax, this solvent has been applied in many studies. Water has also been used on many occasions; however, it is important to note that in general, water dissolves a small part of propolis constituents, about 10% of its weight, whereas 70% ethanol may dissolve 50–70%, depending on the wax amount. Propolis extracts are prepared by maceration or in some cases (some procedures with methanol or 96% ethanol) by Soxhlet extraction. Ultrasound assisted extraction appears to give excellent results, spectacularly accelerating the process (Trusheva et al., 2007). Microwave assisted extraction however turned out to be less favorable, especially in case of samples rich in phenolics: MW treatment could lead to decrease of the phenolic content due to oxidation processes (Trusheva et al., 2007).

Obviously, any study of any type of propolis bioactivity must begin with chemical profiling of the extracts to be used in the study.

Table 1

Comparison of the studies investigating propolis biological properties and the experimental approaches used.

Biological property	In vitro/in vivo	Propolis concentration	Authors
Immunomodulatory	In vivo	200 mg/kg	Orsatti et al. (2010a,b)
	In vitro	3–300 µg/100 µl	Orsi et al. (2005)
Anti-tumor	In vivo	50 and 150 mg/kg	Orsolich et al. (2005)
	In vitro	5–100 µg/100 µl	Bassani-Silva et al. (2007)
Antimicrobial			
Antibacterial		0.4–14.0% v/v	Sforcin et al. (2000)
Antifungal	In vitro	0.4–14.0% v/v	Sforcin et al. (2001)
Antiviral		5–100 µg/100 µl	Búfalo et al. (2009c)
Anti-diabetic	In vivo	100 and 300 mg/kg	Zamami et al. (2007)
Anti-ulcer	In vivo	50, 250 and 500 mg/kg	Barros et al. (2007)

In general, the metabolic profile of the extract gives an insight into its plant origin and allows the identification of its major constituents, and also of a number of minor constituents, depending on the technique. It reveals the types of compounds present and gives an idea about the possible activities to be expected. For example, the presence of a significant amount and number of phenolics might lead to the expectation that the extract has the potential to scavenge free radicals, and to demonstrate bioactivities connected with this potential.

Different techniques are appropriate for the purpose of chemical profiling, as demonstrated by numerous papers dealing with propolis analysis; hyphenated techniques are the most appropriate ones: HPLC–DAD, LC–MS, LC–MS–MS, GC–MS, etc. The relatively polar nature of propolis constituents (in general they have several OH groups in their molecules), combined with the advent in 1990s of soft ionization techniques compatible with liquid chromatography, made HPLC–DAD and HPLC–MS the favorite methods for analysis of propolis constituents. Nevertheless, the unprecedented resolving power of capillary GC and the valuable structural information provided by EIMS have proved to be still useful and GC–MS makes recently a remarkable comeback, as demonstrated by several articles in peer reviewed journals (Popova et al., 2005; Campo Fernandez et al., 2008; Kalogeropoulos et al., 2009b; Hernandez et al., 2010).

Of course quantitative data are highly recommendable but obtaining them is not always possible and reasonable. In case of the most popular propolis chemical types, European poplar propolis and Brazilian green Alecrim propolis, the biologically active constituents are largely known and methods have been proposed for standardization and quality control. For poplar type propolis, quantification of total phenolics, total flavons/flavonols and total flavanones/dihydroflavonols are used as a measure for the amount of active principles (Popova et al., 2004), and for green

Brazilian (*Baccharis*) propolis total phenolics and total flavonoids are applied (Mendez da Silva et al., 2006). The methods are proposed by the International Honey Commission. It is important to remember that the characteristic values for phenolic and flavonoid content are different for every propolis type, and the reference compounds used for calibration are also different. Of course there are many other propolis types: Pacific (Macaranga-derived) (Kumazawa et al., 2008), Mediterranean (containing mainly diterpenes) (Popova et al., 2010b), South American (Cuban, Brazilian, Mexican) red propolis (*Dalbergia*-derived) (Piccinelli et al., 2005; Dausgch et al., 2008; Lotti et al., 2010). Among them are such with very low or no phenolic content. For every one of them specific procedures are yet to be developed. This process is going on, e.g. the recently proposed procedure for spectrophotometric quantification of total flavanones in Pacific type (Macaranga) propolis (Popova et al., 2010a). Quantification of individual constituents is not necessary at the initial stages of the studies. As the investigation goes further, this might become meaningful, especially if samples with similar qualitative composition demonstrate significant differences in their activities.

Because of the chemical variability of propolis, the study should not be limited to a single specimen. A reasonable number of samples should be involved, at least three, from different parts of the geographic region where propolis is collected. This will help to avoid irreproducible results originating from local random fluctuations in chemical composition.

3. Propolis immunomodulatory action

Recent articles have provided information of propolis influence on the immune system (Sforcin, 2007; Orsatti et al., 2010a). Immunomodulatory assays have included tests with positive con-

Table 2

Most widespread propolis types: plant origin and major constituents.

Propolis type	Geographic origin	Plant source	Major constituents	Authors
Poplar	Europe, North America, non-tropic regions of Asia, New Zealand	<i>Populus</i> spp. of section Aigeiros, most often <i>P. nigra</i> L.	Flavones, flavanones, cinnamic acids and their esters	Nagy et al. (1986), Greenaway et al. (1988), Markham et al. (1996), Bankova et al. (2000)
Green (alecrim) Brazilian	Brazil	<i>Baccharis</i> spp., predominantly <i>B. dracunculifolia</i> DC.	Prenylated <i>p</i> -coumaric acids, diterpenic acids	Salatino et al. (2005)
Birch	Russia	<i>Betula verrucosa</i> Ehrh.	Flavones and flavonols (not the same as in Poplar type)	Popravko (1978)
Red propolis	Cuba, Brazil, Mexico	<i>Dalbergia</i> spp.	Isoflavonoids (isovlavans, pterocarpanes)	Campo Fernandez et al. (2008), Dausgch et al. (2008), Lotti et al. (2010)
Mediterranean	Sicily, Greece, Crete, Malta,	Cupressaceae (species unidentified)	Diterpenes (mainly acids of labdane type)	Trusheva et al. (2003), Melliou and Chinou (2004), Popova et al. (2010b)
“Clusia”	Cuba, Venezuela	<i>Clusia</i> spp.	Polyprenylated benzophenones	Cuesta-Rubio et al. (2002), Trusheva et al. (2004)
“Pacific”	Pacific region (Okinawa, Taiwan, Indonesia)	<i>Macaranga tanarius</i>	C-Prenyl-flavanones	Chen et al. (2008), Kumazawa et al. (2008), Trusheva et al. (in press)

trols, such as lipopolysaccharide (LPS), concanavalin A (Con A), phorbol miristate acetate (PMA), cytokines (IFN- γ) or others to compare propolis efficiency. Cyclophosphamide is commonly used as an immunosuppressive drug, and it has been used in vivo both as a negative control and also to investigate poplar propolis immunorestorative action (Dimov et al., 1991; Ivanovska et al., 1993).

As to the immunomodulatory action of Brazilian green propolis, the administration of ethanolic extract of propolis (200 mg/kg) to mice for 3 days enhanced the innate immunity, activating the initial steps of the immune response by upregulating TLR-2 and TLR-4 expression and pro-inflammatory cytokines (IL-1 and IL-6) production by macrophages and spleen cells, contributing to the recognition of microorganism and to lymphocytes activation by antigen presenting cells (Orsatti et al., 2010a). Brazilian green propolis (2.5 and 5 mg/kg) also increased hydrogen peroxide (H₂O₂) generation, favoring the microorganisms killing (Orsi et al., 2000).

Propolis capsules (500 mg) were administered for 2 weeks to humans, and their effects on pro-inflammatory cytokines were analyzed, verifying a significant increase of both spontaneous and LPS-induced cytokine (TNF- α , IL-1 β , IL-6 and IL-8) secretion capacity of peripheral blood leukocytes (Bratter et al., 1999).

Brazilian green propolis (2.5, 5 and 10 mg/kg) showed inhibitory effects on splenocyte proliferation (Sá-Nunes et al., 2003), and this immunosuppressor effect on the lymphoproliferative response may be attributed to flavonoids (You et al., 1998). Ansorge et al. (2003) verified that propolis suppresses DNA synthesis of human peripheral blood mononuclear cells (PBMC) and purified T cells, and these effects were at least in part mediated by caffeic acid phenethyl ester (CAPE, an important constituent of poplar type propolis) and by the flavonoids quercetin and hesperidin.

CAPE (1, 5 and 10 μ M) had inhibitory effects on transcription factors NF- κ B and NFAT (Márquez et al., 2004), and, as a consequence, CAPE inhibited IL-2 gene transcription, IL-2R (CD25) expression, and proliferation of human T cells, providing new insights into the molecular mechanisms involved in the anti-inflammatory and immunomodulatory activities of this natural compound.

The anti-inflammatory action of propolis has been reported by several researchers, using different experimental models (Khayyal et al., 1993; Miyataka et al., 1997; Hu et al., 2005; Paulino et al., 2006). Propolis administration (200 mg/kg) over a short-term (3 days) to mice inhibited IFN- γ production in splenocyte cultures (Orsatti et al., 2010b). Moreover, C57BL/6 mice treated with Brazilian green propolis (200 mg/kg) for 14 days showed an inhibition of IL-1 β , IL-6, IFN- γ , IL-2 and IL-10 production by spleen cells, suggesting its anti-inflammatory activity once it is well established that cytokines orchestrate and perpetuate the chronic inflammatory features of several diseases (Missima et al., 2009, 2010).

Brazilian green propolis 10% stimulates antibody production (Sforcin et al., 2005). CAPE administration (5, 10 and 20 mg/kg) to BALB/c mice increased antibody production as well (Park et al., 2004), but besides the effect of individual constituents, synergistic effects of several compounds may be responsible for the different pharmacological activities to propolis. Kujumgiev et al. (1999) suggested that general biological properties of propolis are due to a natural mixture of its components, and a single propolis constituent does not have an activity greater than that of the total extract. These data strongly suggest the adjuvant capacity of propolis in association with vaccines. As an example, Fischer et al. (2007) associated Brazilian propolis (5 mg/dose) to inactivated Suid herpesvirus type 1 (SuHV-1) vaccine, verifying that mice inoculated with SuHV-1 vaccine plus aluminium hydroxide and propolis showed higher antibodies titers. Propolis was also efficient as an adjuvant to the inactivated vaccine against *Aeromonas hydrophila* in carps, since the phagocytic activity of these fishes and their serum antibod-

ies against *A. hydrophila* were higher comparing to non-adjuvant vaccinated fishes (Chu, 2006).

The effects of propolis on immobilization stress-challenged animals were also investigated. In acutely stressed mice, Brazilian green propolis (200 mg/kg for 3 days) restored TLR-2 and TLR-4 expression (Pagliarone et al., 2009b), contributing to the recognition of microorganisms during stressful conditions, and increased IL-4 production, favoring humoral immune response (Pagliarone et al., 2009a). In chronically stressed mice, Brazilian green propolis treatment (200 mg/kg for 7 days) potentiated H₂O₂ generation by macrophages and counteracted the alterations found in the spleen (Missima and Sforcin, 2008). Brazilian green propolis (200 mg/kg for 14 days) also exerted an immunomodulatory activity in melanoma-bearing mice submitted to chronic stress (Missima et al., 2009, 2010).

In humans, CAPE (1, 2 and 4 μ g/ml) showed protective effect against hyperthermal stress in athletes, enhancing the hyperthermal tolerance in immune mononuclear cells of competitive cyclists (Chen et al., 2009). Since the modern life comprises a wide range of stressful conditions, these preliminary results point out the importance of further research in order to understand propolis usefulness during stress and for the development of new medicaments.

4. Propolis antitumoral action

Brazilian green propolis (10, 25, 50 and 100 μ g/100 μ l) showed a markedly activity against different tumor cells in vitro (Bassani-Silva et al., 2007; Búfalo et al., 2009b), and the main mechanisms by which propolis affects tumor cells are related to the inhibition of cell growth and to apoptosis (Sforcin, 2007). CAPE (50–200 μ M) also interferes in cell cycle arrest, and flow cytometric analysis showed cell arrest at G2/M phase (Lee et al., 2005).

In vivo, Brazilian green propolis 10% treatment for 3 days increased the cytotoxic activity of natural killer cells against murine lymphoma (Sforcin et al., 2002a). Poplar propolis (50 and 150 mg/kg) and some isolated polyphenolic compounds (caffeic acid, CAPE and quercetin) decreased the number of tumor nodules in the lung; however, the antimetastatic effectiveness of propolis was higher than that presented by its constituents (Orsolich et al., 2004). Propolis, caffeic acid and CAPE (50 mg/kg) could be useful tools in the control of tumor growth, and Orsolich et al. (2005) reported that poplar propolis antitumor action could be the consequence of synergistic activities of its polyphenolic compounds. Moreover, propolis action should be compared to antitumor drugs or even be tested in association with them, in order to investigate a possible synergistic action.

There have been a great number of publications lately considering the antitumor action of propolis and its constituents, what indicates their potential for the development of new antitumor agents. Kim et al. (2008) have synthesized a polymeric nanoparticle-encapsulated formulation of propolis (propolis nanofood) utilizing micellar aggregates of cross-linked and random copolymers of N-isopropylacrylamide (NIPAAm) with N-vinyl-2-pyrrolidone and poly(ethylene glycol) monoacrylate. These authors reported that propolis nanofood, unlike free propolis, is readily dispersed in aqueous media and demonstrates a therapeutic efficacy comparable in vitro to free propolis against a panel of human pancreatic cancer cell lines, as assessed by cell viability and clonogenicity assays in soft agar. Such findings are promising in pre-clinical in vivo models of cancer and other diseases that might benefit from the effects of propolis. However, an aspect that should be clearly investigated in vivo and further in humans is a possible interaction between propolis or its isolated compounds and other medicines.

5. Propolis antimicrobial action

Propolis antimicrobial activities are well documented against different bacteria (Sforcin et al., 2000), yeasts (Sforcin et al., 2001), virus (Gekker et al., 2005; Búfalo et al., 2009c) and parasites (Freitas et al., 2006). In vitro, propolis may act directly on microorganisms, and in vivo it may stimulate the immune system, activating the mechanisms involved in the microorganisms killing.

Paenibacillus larvae, the agent behind American foulbrood, a key larval pathogen of the honey bee *Apis mellifera*, has become increasingly resistant to conventional antibiotics, and propolis extracts from various states of Brazil significantly inhibited this microorganism (Bastos et al., 2008).

Propolis may also show synergistic effects with antimicrobial drugs, and its association to commercially disposable drugs is a field of interest to the development of new products by the pharmaceutical industry. Oksuz et al. (2005) verified a synergistic activity between ciprofloxacin and propolis in the treatment of experimental *Staphylococcus aureus* keratitis. Orsi et al. (2006b) reported that propolis diminished the resistance of the bacteria wall to antibiotics (amoxicillin, ampicillin and cefalexin) and had synergistic effects with antibiotics acting on the ribosome (chloramphenicol, tetracycline and neomycin) (Orsi et al., in press-b). Nevertheless, propolis does not seem to interact with the antibiotics acting on the DNA (ciprofloxacin and norfloxacin) and folic acid (cotrimoxazole) (Orsi et al., in press-a). These data enables us to compare the action of propolis with antimicrobial drugs.

Libério et al. (2009) published a review dealing with the effects of propolis on *Streptococcus mutans* group, suggesting the potential of propolis or its compounds as cariostatic agents and for the development of biotechnological products to control caries and other infectious diseases. Santos et al. (2008) evaluated the clinical efficacy of a new Brazilian propolis gel formulation in patients diagnosed with denture stomatitis, verifying the complete clinical remission of palatal edema and erythema and suggesting that this gel was efficient and could be an alternative topical choice for the treatment of denture stomatitis.

6. Allergy, rhinitis and asthma

No side effects were related in mice, rats and humans after Brazilian green propolis administration (Sforcin et al., 2002b; Mani et al., 2006, 2008; Sforcin, 2007). Propolis is non-toxic, and the safe concentration for humans would be approximately 1.4 mg/kg and day or 70 mg/day (Burdock, 1998). However, cases of allergy and contact dermatitis to propolis have been always reported (Sforcin, 2007), mainly among beekeepers (Rudeschko et al., 2004; Gulbahar et al., 2005). Rajpara et al. (2009) mentioned that the increased incidence of contact dermatitis over the last two decades is likely to be due to its use in cosmetic and pharmaceutical preparations.

Rhinitis is a symptomatic disorder of the nose, with nasal obstruction, secretion and sneezing, most commonly induced by allergen exposure, bacteria or virus. It is a global health problem, affecting social life, sleep, school and work performance, regardless of gender, age and ethnic background (Hellgren et al., 2010). Shinmei et al. (2009) studied the effect of Brazilian propolis on sneezing and nasal rubbing in experimental allergic rhinitis of mice, concluding that propolis may be effective in the relief of symptoms of allergic rhinitis through inhibition of histamine release. A single administration of propolis caused no significant effect on both antigen-induced nasal rubbing and sneezing at a dose of 1000 mg/kg, but a significant inhibition was observed after repeated administration for 2 weeks at this dose.

Asthma is a chronic inflammatory disorder of the pulmonary airways due to the hyperresponsiveness to inhaled allergens, leading to reversible airflow obstruction and airway inflammation, persis-

tent airway hyperactivity and airway remodeling (Sy et al., 2006). Khayyal et al. (2003) administered an aqueous extract of propolis 13% daily for 2 months to patients with mild to moderate asthma. As a result, propolis-treated patients showed a reduced incidence and severity of nocturnal attacks and improvement of ventilatory functions, what was associated with decreased prostaglandins, leukotrienes, pro-inflammatory cytokines (TNF- α , IL-6, IL-8) and increased IL-10.

CAPE (10 mg/kg/day) attenuated allergic airway inflammation and hyperresponsiveness in a murine model of ovalbumin-induced asthma. Jung et al. (2008) reported that the oxidative stress may have a crucial function in the pathogenesis of bronchial asthma, and CAPE may be useful as an adjuvant therapy for its treatment.

7. Propolis and diabetes

Diabetes mellitus is a disease characterized by metabolic disorders, such as hyperglycemia and glycosuria due to absolute or relative insulin deficiency. Hyperglycemia results of reduced entry of glucose into various tissues and increased liberation of glucose into the circulation from the liver, while glycosuria is resultant of exceeded renal capacity for glucose reabsorption. Diabetes also induces damage to peripheral nerve, culminating in development of peripheral diabetic neuropathy, which occurs as a consequence of complex interactions among multiple hyperglycemia-initiated mechanisms. Oxidative stress may play a key role in the pathogenesis of diabetic nephropathy. Most of the authors used streptozotocin (STZ) as a diabetes inducer.

Matsushige et al. (1996) related that the water extract of propolis (200 mg/kg) showed a preventive effect on pancreatic β -cells destruction by inhibiting IL-1 β generation and NO synthase activity. Fuliang et al. (2005) observed that the administration of water or ethanolic extract of propolis for 7 weeks to STZ-induced diabetic rats may control the glycemia and modulate glucose and lipid metabolism, leading to decreased outputs of lipid peroxidation and scavenging the free radicals in diabetic rats. Zamami et al. (2007) reported that propolis treatment (100 and 300 mg/kg) of 15% fructose-treated rats for 8 weeks significantly decreased the plasma level of insulin and body weight, without affecting blood glucose levels. McLennan et al. (2008) reported that propolis can accelerate wound healing and reepithelization of diabetic wounds in rodents, providing a rationale for studying topical application of this agent in a clinical setting. Abo-Salem et al. (2009) suggested that the strong antioxidant effect of propolis (100, 200 and 300 mg/kg) might ameliorate oxidative stress and delay the occurrence of diabetic nephropathy in diabetes mellitus.

The administration of ethanolic extract of Brazilian green propolis (10 and 90 mg/kg) for 7 days to STZ-induced diabetic rats had no effect after diabetes establishment (Sartori et al., 2009). The long-term administration (28 days) of Brazilian green propolis (200 mg/kg) was also investigated, in order to explore its therapeutic potential in STZ-induced diabetic rats (Búfalo et al., 2009a). Based on these findings from our laboratory, propolis did not seem to counteract STZ effects, even when administered over a short (7 days) or long (28 days) term to animals, while data from literature reveal that propolis administration over a long-term could exert a positive effect in diabetic animals.

Inflammatory cytokines and oxidative stress have a central role in the pathogenesis of acute pancreatitis, and the treatment with ethanolic extract of propolis (300 mg/kg) improved the biochemical and histopathological findings in a rat model of experimental pancreatitis (Buyukberber et al., 2009).

As to isolated compounds, Okutan et al. (2005) reported that the eight-week treatment with CAPE (10 μ mol/kg) reduced the oxidative stress in STZ-induced diabetic rats.

All these data indicate that further research is still needed in order to investigate the optimal concentrations of propolis or its constituents, intake period and the type of extract, exploring its potential use for diabetes treatment in humans.

8. Anti-ulcer activity

Gastroduodenal ulcer may be the result of the imbalance between aggressive and protective factors in the stomach, such as acid-pepsin secretion, mucosal barrier, mucus secretion, cellular regeneration and epidermal growth factors (Lima et al., 2006). The treatment of peptic ulcer is often based on the inhibition of gastric acid secretion by histamine H₂-antagonists, proton pump inhibitors, and antimuscarinics. Acid-independent therapy including sucralfate and bismuth cholinergics is used as well (Bighetti et al., 2005). Omeprazole, indomethacin and cimetidine have been commonly used as a positive control to induce gastric ulcer.

Barros et al. (2007) reported the gastric protective effects of propolis (50, 250 and 500 mg/kg). Barros et al. (2008) described the antiulcerogenic properties of the main phenolic acids from Brazilian propolis (50 and 250 mg/kg), in different models: non-steroidal-anti-inflammatory drug-induced ulcer, ethanol-induced ulcer, and stress-induced ulcer, evidencing that caffeic, ferulic, *p*-coumaric and cinnamic acids displayed antiulcer activity.

Massignani et al. (2009) investigated the effects of the essential oil (50, 250 and 500 mg/kg) obtained from *Baccharis dracunculifolia* – the most important botanical source of Brazilian green propolis, on gastric ulcers, suggesting that it could probably be a good therapeutic agent for the development of new phytotherapeutic medicine for the treatment of gastric ulcer.

9. Perspectives and conclusions

Propolis biological properties have been intensely investigated in the last years, attracting a great interest of consumers in propolis-containing products marketed by health-food stores and pointing out propolis potential for the development of new drugs. However, in order to establish minimum requirements or setting standards to start the investigation of new drugs, some points should be addressed.

First, not all works found in literature investigated propolis chemical composition, and we suggest that the new investigations should include the study of propolis or its constituents. Pharmacological variability of preparations is expected, and although it is not possible to systematically compare the studies since a universal standardization of propolis composition would be impossible, propolis biological properties could be linked to its chemical composition and to its botanical sources.

Second, in the title of this review we posed a challenging question about propolis: is there a potential for the development of new drugs? Concerning the developing of a herbal drug based upon a specific propolis type, we think that it is possible in principle. If it is standardized based on most important active constituents, it can be subjected to clinical trials and eventually be registered. With proper standardization, even a licensed medicine could be produced and registered. It might be not easy but in our opinion it is possible and could be done. There is however a legal problem for the registration of propolis as “herbal drug”, because it is regarded as a bee (meaning animal) product, and not a herbal product. In order to become a new drug, propolis from different regions should not be used as a mixture of all constituents, and we believe that isolated compounds such as phenolics from propolis could become leads for modern medicines. We also believe in the synergistic effects of individual compounds, depending on their concentrations.

Third, propolis efficacy should be always compared to well-established parameters, and articles should include positive or negative controls in the experiments.

Fourth, in vitro assays have provided new insights regarding propolis mechanisms of action, and in vivo experiments have provided information about the biological properties of this bee product. Nevertheless, little information is available concerning propolis efficiency clinically, and a new step to complement the basic research would be the development of clinical investigation, in order to evaluate the potential of propolis in patients or healthy individuals. Possible interactions between propolis or its isolated compounds and other medicines should be investigated as well.

This review indicates that propolis and its isolated compounds may be useful in different pathological conditions such as tumors, infections, allergy, diabetes and ulcers since new formulations containing propolis or its isolated compounds have been prepared lately, but before establishing a strategy using this bee product, it is necessary to understand under which conditions it may promote health.

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