Received: March 19, 2007
Accepted: May 7, 2007
Abstract published online: May 8, 2007
Full paper published online: November 30, 2007

J. Venom. Anim. Toxins incl. Trop. Dis.
V.13, n.4, p.697-710, 2007.
Review article.
ISSN 1678-9199.

PROPOLIS: A REVIEW OF ITS ANTI-INFLAMMATORY AND HEALING ACTIONS

RAMOS A. F. N. (1), MIRANDA J. L. (1)

(1) Laboratory of Pathology, Department of Basic Sciences, Federal University of Jequitinhonha and Mucuri Valleys, Minas Gerais State, Brazil.

ABSTRACT: Tissue healing is an adaptive biological response by which the organism repairs damaged tissue. The initial stage of healing is represented by an acute inflammatory reaction, in which inflammatory cells migrate to damaged tissue and phagocyte debris. At a later stage, fibroblasts and endothelial cells proliferate and generate a scar. The occurrence of inflammatory processes and healing imperfections have been a concern for hundreds of years, especially for individuals with healing difficulties, such as diabetics and carriers of peripheral circulation deficiencies. A wide variety of natural products have been used as anti-inflammatory and healing agents, with propolis being a remarkable option. Propolis has been used in popular medicine for a very long time; however, it is not a drug intended for all diseases. Currently, the determination of quality standards of propolis-containing products is a major problem due to their varying pharmacological activities and chemical compositions. The aim of this review is to discuss the use of propolis with emphasis on its anti-inflammatory and healing properties.

KEY WORDS: propolis, inflammation, anti-inflammatory action, healing properties, *Apis mellifera.*

CONFLICTS OF INTEREST: There is no conflict.

CORRESPONDENCE TO:
JOÃO LUIZ DE MIRANDA, Laboratório de Patologia, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Rua da Glória, 187, Centro, 39.100-000, Diamantina, Minas Gerais, Brasil. Email: joaolumi@bol.com.br.

Propolis is a resinous substance with varying colors and consistencies, collected by *Apis mellifera* bees from several vegetal sources. The word propolis comes from the Greek *pro* meaning 'in defense of' and *polis* 'city', i.e. defense of beehives (3). In fact, bees use propolis to protect themselves from insects and microorganisms, employing it as a cement to seal cracks or open spaces in the hive, to sterilize the queen-bee posture site, and to mummify insect invaders. Commonly, small animals or parts of them are found wrapped within propolis in perfect states of conservation (23).

Propolis is a honeybee product with a very complex chemical composition, made by gummy and balsamic material collected by bees from sprouts, flower-buds, trees and other vegetal-tissue resinous exudates. During propolis collection, bees mix the beeswax and the collected propolis with the 13-glicosidase enzyme found in their saliva, hydrolyzing flavonoids glycosides into flavonoid aglycones (34). Afterwards, the collected material is augmented with enzymatic and salivary secretions.

Along with other honeybee products (honey, royal jelly, pollen), propolis has outstanding therapeutic properties (5), being used since 300 years B.C. in popular medicine in various parts of the world. However, interest in the correlation of propolis chemical composition with its pharmacological activities started only forty years ago (23). In Brazil, little is known about propolis, and only few studies have been conducted. Nonetheless, it is widely used in popular medicine. Propolis is one of the few "natural drugs" being used for a long time by different civilizations (7). Currently, several propolis products are being commercialized worldwide, including candies, chocolate bars, shampoos, skin lotions, antiseptic mixtures, and toothpastes (1). The Persians, Greeks, Romans and Incas also used propolis with therapeutic purposes. In ancient Egypt, it was employed to embalm the dead, while in the Balkans States propolis is widely used. In France, the term propolis is found in the general literature since the sixteenth century. In South Africa, during the Anglo-Boer War, more than 90 years ago, it was used with vaseline for ointment preparation to heal war wounds. This helped save the lives of many soldiers, since antibiotics were not yet available (10). In the Second World War, Soviet medical clinics studied propolis with excellent results.

The color of propolis depends on its origin. It varies from dark-brown to reddish-brown, with a greenish tone. It has a typical odor, which can vary from sample to sample, with some being odorless. Flashing point ranges between 60 and 70°C and, in some cases, may reach up to 100°C. Generally, ethanol is the best solvent for

propolis preparation, and other solvents such as ethyl ether, water, methanol and chloroform may also be used for extraction and identification of propolis compounds (24). In addition, glycerin, propylene glycol, and other solutions have been used during propolis preparation for pharmaceutical and cosmetic industries (46). Propolis obtained from beehives, also known as rude propolis, is composed of around 50% balsam resin, 30% wax, 10% essential and aromatic oils, 5% pollen, and 5% other substances, including wood fragments (31).

The resin found in propolis is collected in vegetation nearby beehives, where bees also collect pollen and nectar for feeding. Propolis composition is mainly determined by the phytogeographic characteristics of beehive surroundings (19). However, seasonal variation may occur within the same place (39). Also, variation was observed among samples collected in the same place, but by different *A. mellifera* subspecies (40). Several contaminants close by beehives can be collected and unexpectedly added to propolis, such as asphalt powder, pesticide, iron excess, copper, magnesium, (12) and even lead (2).

It is known that bees are selective when collecting resin from a specific vegetal source, but factors that guide them are not completely understood (37, 45). The nature of aromatic and terpene compounds found in propolis has a biological importance, allowing bees to determine which vegetal species to visit (23).

The pharmacological activities of propolis are more numerous in tropical regions than in temperate climates, reproducing the richer vegetal diversity observed in the former (4, 28).

More than 300 different compounds have been identified so far in propolis, including aliphatic acids, esters, aromatic acids, fatty acids, carbohydrates, aldehydes, amino acids, ketones, chalcones, dihydrochalcones, terpenoids, vitamins, and inorganic substances (23). Of all, flavonoids are the ones which draw greater research interest (15).

Propolis has several therapeutic properties, such as antibacterial, anti-inflammatory, healing, anesthetic (13), anticariogenic (34), antifungal, antiprotozoan and antiviral activities. The *in vitro* antibacterial activity was verified against several Gram-positive and Gram-negative bacteria and results from synergism between propolis compounds, mainly pinocembrin and galangin flavonoids. Other flavonoids, such as chrysin and kaempferol, have shown antiviral activity with reduction of intracellular proliferation of some viruses, such as herpes simplex (23).

Many other biological and pharmacological properties of propolis have been noted: cartilage, bone, and dental pulp regeneration; immunological properties; liver defense and antitoxic activity; antioxidant and immunomodulatory actions. Consequently, there has been increasing interest on propolis, with the chemical industry searching for viable commercial formulations. Also, investigation of isolated compounds, such as flavonoids, has grown. These compounds are biologically more active and are responsible for propolis' spasmodic (quercetin, kaempferol and pectolinarigenin), anti-inflammatory (acacetin) and antiulcerative (apigenin) activities (11).

Propolis is a low-cost potential anti-inflammatory agent for both acute and chronic stages (6). Its properties are used mainly for muscles and articulations, and also other types of inflammations, infections, rheumatisms and torsions. Mice and rabbit studies have shown that hydroalcoholic solutions of propolis possess anti-inflammatory activity following topical, injectable, or even oral administration (23).

Inflammation is the complex biological response of vascular tissues to harmful stimuli such as cell damage by pathogens. It is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. The tissue modifications induced by the causal agent are responsible for the release of inflammatory mediators that lead to subsequent inflammatory events. Cytokine release (IL-1, TNF-α) by activated macrophages leads to vessel dilation and results in smooth muscle relaxation and increased local blood flow (hypothermia). Microvascular changes associated with increased vascular permeability take place, leading to accentuated plasmatic exudation, phagocyte accumulation (neutrophils, monocytes, macrophages), and amplification of endogenous chemical mediators. Simultaneously, mast cells, phagocytic cells and endothelial cells use plasma membrane lipids to generate important inflammatory mediators (21). In an immediately posterior stage, several intra and extracellular phospholipases are activated by cytoplasmic membrane phospholipids and activate other enzymes, such as cyclooxygenase (COX) and lipoxygenase (LOX), which in turn act upon arachidonic acid and eicosanoid metabolism, creating important inflammatory mediators (prostaglandins and leukotrienes). These mediators are responsible for the maintenance of the inflammatory process. The fibrinolytic system, kinins, complement, vasoactive amines (histamine and serotonin), and nitric oxide (NO) may lead to inflammation when physiologically altered (47).

Endothelial-leukocyte cellular adhesion occurs in a sequence of events, and specific molecules are expressed in different stages. Selectins (E, P, and L), integrins (VLA-4 and LFA-1), and members of immunoglobulin super-families (ICAM-1 and ICAM-2) transfer leukocytes from the vascular lumen into the tissues (35). In response to several mediators, the vascular endothelium expresses specific glycoproteins on the cell surface, which mediates blood leukocyte connection and extravasation, an important event in tissue repair (3).

According to the frequency and duration of the injurious agent, the inflammatory process can be classified into acute and chronic. The acute-stage response involves serous, fibrinous, suppurative or exudative events as well as microvascular and cellular events; this response to phlogogen occurs within 72h. The chronic stage response includes proliferative events and histological alterations different from those in the acute stage, being characterized by cell emigration and intensive mitosis. Formation of giant multinuclear cells takes place, and all these events are induced by phlogogen (42).

In certain instances, inflammation must be regulated by using specific drugs, since it may lead to toxic consequences to the organism. The damage to the organism during the inflammatory response is induced by free radicals produced by active macrophages and neutrophils. These molecules degrade lipid acids of the plasma membrane, disrupt membrane proteins, and induce DNA mutations. NO is another potent inflammatory mediator produced by endothelial and inflammatory cells that can induce tissue damage if synthesized in large quantities (21).

Some anti-inflammatory substances found in propolis have been isolated. According to Mirzoeva & Calder (29), these substances are caffeic acid, quercetin, naringenin, and caffeic acid phenethyl ester (CAPE). These compounds contribute to the suppression of prostaglandins and leukotrienes synthesis by macrophages and have inhibitory effects on myeloperoxidase activity, NADPH-oxidase, ornithine decarboxylase and tyrosine-protein-kinase (30). Krol *et al*. (18) attributed propolis anti-inflammatory activity to other compounds, including salicylic acid, apigenin, ferulic acid and galangin.

It is known that macrophages are involved in several body physiology processes, such as phagocytosis, enzyme release, free radical generation, and inflammation. Scheller *et al*. (38) suggested that propolis immunostimulant activity may be associated with macrophage activation and enhancement of macrophage phagocytic

capacity. The results of the study of Orsi *et al*. (33) corroborated the findings of Schelle, which indicated that macrophages produce high amounts of H_2O_2.

While investigating the effects of individual propolis compounds complexes on lysine, Ivanovska *et al*. (17) found that cinnamic acid tends to inhibit H_2O_2 release by peritoneal macrophages, whereas caffeic acid induces increased metabolite production.

The inhibition of NO production by macrophages may also be responsible for propolis anti-inflammatory activity (32). Hu *et al*. (16) evaluated the anti-inflammatory effects of ethanol (EEP) and water (WSD) extracts in ICR mice and Wistar rats on thoracic capillary vessel leakage, carrageenan-induced edema, carrageenan-induced pleurisy, acute lung damage, and Freund's complete adjuvant (FCA)-induced arthritis. In the experiment, EEP and WSD inhibited the increase of PGE_2 and also had a significant inhibitory effect on NO in carrageenan-induced pleurisy exudation. In these models, NO could accelerate an inflammatory reaction by enlarging blood vessels and causing edema. This could increase the expression of inflammatory reactions and accelerate the development of blood poisoning by activating prostaglandin synthesis, as seen in the progression of rheumatism. However, other studies indicated increased NO production by macrophages (32).

Although the mechanism of WSD and EEP on anti-inflammatory performance was apparently similar, there were some differences. In the carrageenan-induced pleurisy and oleic-acid plus LPS-induced acute lung damage studies, WSD not only inhibited the increase of white blood cells (WBC) count, but also inhibited the increase of neutrophils (but not significantly at 5%). This would explain how WSD inhibits WBC and alleviates inflammatory reactions during acute inflammation. The results suggest that additional propolis components, other than flavonoids, possess anti-inflammatory effects. Although EEP did not significantly inhibit WBC, it may possibly alleviate the inflammatory degree synergistically by inhibiting NO. The arthritic rat model induced by FCA is associated with an immune inflammation reaction in these experiments. The main feature of rheumatoid arthritis (RA) is the ongoing damage in arthrosis of cartilage and bone, in addition to a disturbance of the immune function. Cytokines secreted by immune cells (lymphocyte and mononuclear-macrophage), fibroblasts, and endothelial cells play an important role in immune and inflammatory responses *in vivo*. The results of the present experiment show that EEP and WSD had significant inhibitory effects on the levels of IL-6 in FCA-induced arthritis rats, but not on IFN and

IL-2 levels. This could also mean that, in the course of the anti-inflammatory activity of WSD and EEP, the humoral immune system plays an important role inhibiting the activation and differentiation of mononuclear macrophages. This would be a possible mechanism for the anti-inflammatory and immune effects of WSD and EEP (16).

Inflammation can also be related to free radicals increase in the human organism (9). Although oxidative damage is known to be involved in inflammatory-mediated tissue destruction, modulation of oxygen-free radicals production represents a new approach to the treatment of inflammatory diseases (8). Propolis contains polyphenols and a wide range of other compounds capable of removing excessive free radicals from our organism (25). CAPE, a flavonoid-like compound, has been identified as one of the main active ingredients of honeybee propolis and has antioxidant and anti-inflammatory properties (8). For that reason, Celik *et al*. (8) investigated the efficiency of CAPE administration in preventing oxidative damage due to *Escherichia coli*-induced pyelonephritis (PYN) in rats. The main hypothesis of events leading to renal scarring has been that bacterial products (e.g. lipopolysaccharide) stimulate the release of proinflammatory cytokines, which initiate an inflammatory response, including chemotaxis with consequent extravasation of polymorphonuclear leukocytes (PNL) (8). PNL release toxic products (e.g. free-oxygen radicals and lysozymes) that seem to be responsible for tissue damage; inhibition of the PNL-produced free radicals can greatly neutralize tissue damage (14).

The levels of lipid peroxidation and NO production, and the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and xanthine oxidase (XO) in *E. coli*-induced PYN were evaluated in a rat model using kidney homogenates and were significantly increased. However, CAPE administration reduced malondialdehyde (MDA) levels, which is an indicator of free radical generation that increases in final stages of lipid peroxidation. NO levels have been implicated in the mechanisms of cell injury and long-term physiological changes in cellular excitability. This effect may be due to decreased expression of inducible NO (iNOS) (14). In a similar study, Song *et al*. (41) demonstrated that CAPE was able to inhibit iNOS expression and NO production in macrophages. CAPE significantly increased the activities of the antioxidant enzymes SOD and GSH-Px in the kidneys of infected rats. CAPE administration significantly inhibited the activity of XO, a physiological source of superoxide anions in eukaryotic cells. Histopathologic examination showed that

CAPE reduced inflammation induced by *E. coli*. In summary, CAPE administration decreases the oxidative damage occurring in PYN and thus could be used for medical management of bacterial nephropathy.

The effects of CAPE on cyclooxygenase-2 (COX-2) expression in Harvey-*ras*-transformed WB-F344 rat live epithelial cells (H-*ras* WB cells) have also been studied. Several reports have shown that activation of *Ras* signaling pathways is involved in the induction of COX-2 and matrix metalloproteinase expression. COX catalyzes the critical conversion of arachidonic acid into prostaglandins, which are important mediators of the inflammatory process. Improper up-regulation of COX-2 is relevant to the pathophysiology of inflammatory disorders. The eukaryotic transcription factor nuclear factor kB (NF-kB) plays a central role in general inflammatory as well as immune responses. The 5'-flanking region of the COX-2 promoter contains NF-kB binding sites. In agreement with this concept, NF-kB has been shown to be a critical regulator of COX-2 expression in many cell lines (43). CAPE significantly inhibited the constitutive expression of COX-2 and several studies suggest that CAPE is a potent and specific inhibitor of NF-kB activation. In those studies, histopathological examinations showed that CAPE significantly suppressed inflammation. CAPE has been demonstrated to specifically and completely block the activation of NF-kB induced by a wide variety of inflammatory agents, including TNF and H_2O_2. The activation of NF-kB proteins is induced by many factors, such as inflammatory cytokines (IL-1, TNF), bacterial products, and oxidative stress. Others have shown that CAPE not only inhibits transcription factors, but also reduces the production of IL-8 and monocyte chemotactic protein (20).

All the above-mentioned data have demonstrated different mechanisms of inflammatory inhibition of several propolis preparations or its isolated compounds. Nevertheless, the anti-inflammatory effects of propolis depend mainly on the administration mode and dosage (29).

A wide variety of natural products have been used in wounds treatment due to their easy application, innocuity, low cost, and bactericidal/bacteriostatic effect (26). Propolis is remarkably used in dermatology for wounds healing, burn and external ulcers treatment, healing time reduction, wound contraction increase, and tissue repair acceleration. During wound healing, perfect synchronized cellular and molecular interactions occur to repair damaged tissue (22). Healing is a dynamic process involving harmonious biochemical and physiological stages, such as

inflammation, fibroblastic and tissue maturation. The wound healing process can be summarized using the healing of a linear skin wound as a prototype. With any wound, the initial events at the site of injury are hemorrhage and formation of a fibrin-rich clot. Fibronectin stabilizes the clot before dehydration takes place and a scab is formed. Macrophages soon follow neutrophils to the site of tissue injury and wound debridement is aided by the opsonization of tissue by fibronectin. The epidermal response to wounding is initiated very rapidly and within a day or so “tongues” of epidermal cells can be seen cleaving a path between the scab and the viable collagenous tissue underneath; two or three days after injury, the wound floor is covered by a sheet of regenerated epidermal cells. The formation of granulation tissue begins at about the same time with an influx and proliferation of fibroblasts and the beginnings of new capillary formation. Four to five days after wounding, stratification of the wound-floor epidermis is readily apparent, fibroblasts are highly active and secretion of extracellular matrix compounds (especially GAGs and type III collagen) and the process of neovascularization are in full swing. To achieve this invasive process, endothelial cells must secrete a battery of proteinases (e.g. type IV collagenase, interstitial collagenase, elastase, stromelysin, and plasminogen activators) to degrade the basement membrane. The diverse components of the epidermis are restored without the reformation of rete ridges, much of the type III collagen fibers orient parallel to lines of mechanical stress (collagen remodeling), and the once highly vascular granulation tissue undergoes a protracted process of vascularization as it matures into relatively avascular scar tissue (27). Sutta *et al*. (44) used propolis alcoholic solutions at animal wounds treatment in clinical and also experimental cases. Histologically, they observed that propolis treatment induced better healing by reducing the inflammatory response; consequently, epithelial healing was faster with propolis. The authors considered propolis suitable for wound treatment, following elimination of the infection. It is known that healing is directly related to the inflammatory process (36), and if the latter is less pronounced, production of healing molecules and deposition of collagen fiber bundles increase. It is possible that prolonged inflammation unleashes pronounced necrosis, causing more tissue damage and rendering the healing process more difficult. Propolis tissue regeneration properties, including healing, are possibly due to its antioxidant activity. Whenever free radicals are produced, they hamper or even block cells regeneration.

Removal of free radicals by propolis flavonoids would allow regeneration of an ill organ or tissue in an ordinary way (23).

The occurrence of the inflammatory process, along with imperfections in healing, has been a problem for centuries. Despite advances on anti-inflammatory, antibiotic and healing treatment, infections continue to be a major reason of concern, especially for individuals that present difficulties in healing, such as diabetics and peripheral circulation deficiency carriers.

It should be stressed that propolis is not a drug for all diseases. A major challenge currently is the determination of which types of propolis are indicated for which medical use, the appropriate dosages, and what effects propolis has on humans and animals, since product quality varies widely. Several researchers have proposed biological assays as well as quantitative analyses of chemical compounds from different propolis samples. Quality-control problems have been confirmed in countries where propolis is commercialized. Hypersensitivity responses induced by propolis, especially those derived from cinnamic acid, have been reported; in the future, an old question will have to be answered: which propolis is best suited for which disease? Therefore, propolis' precise pharmacological properties must be determined. With further research and knowledge, development of new drugs will become possible.

REFERENCES

1 ACKERMANN T. Fast chromatography study of propolis crudes. *Food Chemistry*, 1991, 42, 135-8.

2 ALCICI NMF. Heavy metals in propolis: practical and simple procedures to reduce the lead in the Brazilian propolis. *Chemical Abstracts*, 1996, 127, 236-7.

3 ALMEIDA EC., MENEZES H. Anti-inflammatory activity of propolis extracts: a review. *J. Venom. Anim. Toxins*, 2002, 8, 191-212.

4 BANKOVA V. Chemical diversity of propolis and the problem of standardization. *J. Ethnopharmacol*., 2005, 100, 114-7.

5 BANKOVA VS., POPOV SS., MAREKOV NL. Isopentenyl cinnamates from poplar buds and propolis. *Phytochemistry*, 1989, 28, 871-3.

6 BORRELLI F., MAFFIA P., PINTO L., IANARO A., RUSSO A., CAPASSO F., IALENTI A. Phytochemical compounds involved in the anti-inflammatory effect of propolis extract. *Fitoterapia*, 2002, 73, 53-63.

7 CASTALDO S., CAPASSO F. Propolis, an old remedy used in modern medicine. *Fitoterapia*, 2002, 73, 1-6.

8 CELIK S., GORUR S., ASLANTAS O., ERDOGAN S., OCAK S., HAKVERDI S. Caffeic acid phenethyl ester suppresses oxidative stress in *Escherichia coli*-induced pyelonephritis in rats. *Mol. Cell. Biochem*., 2007, 297, 131-8.

9 DEVASAGAYAM TP., TILAK JC., BOLOOR KK., SANE KS., GHASKADBI SS., LELE RD. Free radicals and antioxidants in human health: current status and future prospects. *J. Assoc. Physicians India*, 2004, 52, 794-804.

10 DIAZ JCQ., RODRÍGUES OA., VELÁZQUEZ MD., MILIÁ NML. Empleo de la tintura de propóleo en la cura de heridas sépticas faciales. *Rev. Cuba Estomatol*., 1997, 34, 347-51.

11 DIAZ NJ., QUEVEDO AO., LUNA SB. Determination of Fe, Mn, Zn, and Cu in an ethanolic extract of Cuban propolis. Rev. *CENIC.* Ciências Químicas, 1997, 28, 93-5.

12 FERNANDES JUNIOR A., SUGIZAKI MF., FOGO ML., FUNARE SRC., LOPES CAM. *In vitro* activity of propolis against bacterial and yeast pathogens isolated from human infections. *J. Venom. Anim. Toxins*, 1995, 1, 63-9.

13 GHISALBERTI EL. Propolis: a review. *Bee World*, 1979, 60, 59-84.

14 GUPTA R., VERMA I., SHARMA S., GANGULY NK. Prevention of tissue injury in an ascending mouse model of chronic pyelonephritis–role of free radical scavengers. *Comp. Immunol. Microbiol. Infect. Dis*., 2004, 27, 225-34.

15 HAVSTEEN BH. The biochemistry and medical significance of the flavonoids. *Pharmacol. Therapeutics*, 2002, 96, 67-202.

16 HU F., HEPBURN HR., LI Y., CHEN M., RADLOFF SE., DAYA S. Effects of ethanol and water extracts of propolis (bee glue) on acute inflammatory animal models. *J. Ethnopharmacol*., 2005, 100, 276-83.

17 IVANOVSKA N., STEFANO Z., VALEVA V., NEYCHEV H. Immunomodulatory action of propolis: VII. A comparative study on cinnamic and caffeic acid lysine derivates. *Biol. Immun*., 1993, 46, 115-7.

18 KROL W., SCHELLER S., CZUBA Z., MATSUMO T., ZYDOWICZ G., SHANI J., MOS M. Inhibition of neutrophils' chemiluminescence by ethanol extract of propolis (EEP) and its phenolic components. *J. Ethnopharmacol*., 1996, 55, 19-25.

19 KUMAZAWA S., HAMASAKA T., NAKAYAMA T. Antioxidant activity of propolis of various geographic origins. *Food Chemistry*, 2004, 84, 329-39.

20 LEE KW., CHUN KS., LEE JS., KANG KS., SURH YJ., LEE HJ. Inhibition of cyclooxygenase-2 expression and restoration of gap junction intercellular communication in H-ras-transformed rat liver epithelial cells by caffeic acid phenethyl ester. *Ann. N. Y. Acad. Sci*., 2004, 1030, 501-7.

21 LOPES RE., CHAPADEIRO E., RASO P., TAFURI LW. *Patologia*. Rio de Janeiro: Guanabara, 1987: 67-112.

22 MAMDELBAUM SH., SANTIS EP. Cicatrização: conceitos atuais e recursos auxiliares - Parte I. *An. Bras. Dermatol*., 2003, 78, 393-410.

23 MARCUCCI MC. Propolis: Chemical composition, biological properties and therapeutic activity. *Apidologie*, 1995, 26, 83-99.

24 MARCUCCI MC., RODRIGUEZ J., FERREREZ F., BANKOVA V., GROTOR., POPOV S. Chemical composition of Brazilian propolis from São Paulo State. *Z. Neturforsch. Sect. C. Biosc*., 1998, 53, 117-9.

25 MARQUELE FD., DI MAMBRO VM., GEORGETTI SR., CASAGRANDE R., VALIM YM., FONSECA MJ. Assessment of the antioxidant activities of Brazilian extracts of propolis alone and in topical pharmaceutical formulations. *J. Pharm. Biomed. Anal*., 2005, 39, 455-62.

26 MATHEWS KA., BINNINGTON AG. Wound management using honey. *Comp. Cont. Educ. Pract. Vet.*, 2002, 24, 53-60.

27 MCGEE JO., ISAACSON PG., WRIGHT NA. *Principles of Pathology*. Oxford: Oxford University Press, 1994, 1, 378-9.

28 MENEZES H. Própolis: uma revisão dos recentes estudos de suas propriedades farmacológicas. *Arq. Inst. Biol*., 2005, 72, 405-11.

29 MIRZOEVA OK., CALDER PC. The effect of propolis and its components on eicosanoid production during the inflammatory response. *Prostaglandins Leukot. Essent. Fatty Acids*, 1996, 55, 441-9.

30 MIYATAKA H., NISHIKI M., MATSUMOTO H., FIJIMOTO T., MATSUKA M., SATOH T. Evaluation of propolis. I. Evaluation of Brazilian and Chinese propolis by enzymatic and physico-chemical methods. *Biol. Pharm. Bull*., 1997, 20, 496-501.

31 MONTI M., BERTI E., CARMINATI G., CUSINI M. Occupational and cosmetic dermatitis from propolis. *Contact Dermatitis*, 1983, 9, 163.

32 NAGAOKA T., BANSKOTA AH., TEZUKA Y., MIDORIKAWA K., MATSUSHIGE K., KADOTA S. Caffeic acid phenethyl ester (CAPE) analogues: potent nitric oxide inhibitors from the Netherlands propolis. *Biol. Pharm. Bull*., 2003, 26, 487-91.

33 ORSI RO., FUNARI RSC., SOARES AMVC., CALVI AS., OLIVEIRA SL., SFORCIN JM., BANKOVA V. Immunomodulatory action of propolis on macrophage activation. *J. Venom. Anim. Toxins*, 2000, 6, 205-19.

34 PARK YK., IKEGAKI M., ABREU JA., ALCICI NM. Estudo da preparação dos extratos de própolis e suas aplicações. *Ci. Tecnol. Aliment*, 1998, 18, 313-8.

35 PARK YK., KOO MH, IKEGARI M., CONTADO JL. Comparison of the flavonoid aglycone contents of *Apis mellifera* propolis from various regions of Brazil. *Arq. Biol. Tecnol*., 1997, 40, 97-106.

36 PEREZ-TAMAYO R. *Introduccion a la Patologia*. Buenos Aires: Editoria Médica Panamericana, 1987: 670.

37 SALATINO A., TEIXEIRA EW., NEGRI G., MESSAGE D. Origin and chemical variation of Brazilian propolis. *Evid. Based Complement. Alternat. Med*., 2005, 2, 33-8.

38 SCHELLER S., GADZA G., KROL W., CZUBA Z., ZAJUSZ A., GABRYS J., SHANI J. The ability of ethanolic extract of propolis (EEP) to protect mice against gamma irradiation. *Z. Neturforsch. Sect. C. Biosc*., 1989, 44, 1040-52.

39 SFORCIN JM., KANENO R., FUNARI SRC. Absence of seasonal effect on the immunomodulatory action of Brazilian propolis on natural killer activity. *J. Venom. Anim. Toxins*, 2002, 8, 19-29.

40 SILICI S., KUTLUCA S. Chemical composition and antibacterial activity of propolis collected by three different races of honeybees in the same region. *J. Ethnopharmacol*., 2005, 99, 69-73.

41 SONG YS., PARK EH., HUR GM., RYU YS., LEE YS., LEE JY., KIM YM., JIN C. Caffeic acid phenethyl ester inhibits nitric oxide synthase gene expression and enzyme activity. *Cancer Lett*., 2002, 175, 53-61.

42 SOSA S., BARICEVIC D., CINCO M., PADOVAN D., TUBARO A., DELLA LOGGIA R. Preliminary investigation on the anti-inflammatory and anti-microbial activities of propolis. *Pharm. Pharmacol. Lett.*, 1997, 4, 168-71.

43 SURH YJ., CHUN KS., CHA HH., HAN SS., KEUM YS., PARK KK., LEE SS. Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NK-kB activation. *Mutat. Res*., 2001, 480-481, 243-68.

44 SUTTA J., HANKO J., JANDA J., TKAC J. *Experimental and clinical experiences in the treatment of wounds in domestic animals by local application of an alcoholic solution of propolis*. Bratislava: *Folia Veterinaria*, 1974, 18, 143-147.

45 TEIXEIRA EW., NEGRI G., MEIRA RM., MESSAGE D., SALATINO A. Plant origin of green propolis: Bee behavior, plant anatomy and chemistry. *Evid. Based Complement. Alternat. Med.*, 2005, 2, 85-92.

46 TOSI B., DONINI A., ROMAGNOLI C., BRUNI A. Antimicrobial activity of some commercial extracts of propolis prepared with different solvents. *Phytother. Res*., 1996, 1014, 335-6.

47 WU KK-Y. Biochemical pharmacology of nonsteroidal anti-inflammatory drugs. *Biochem. Pharmacol*., 1998, 55, 543-7.