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The Effect of Propolis on Cytokines during Dental Pulp Inflammation

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Abstract

Propolis has been used empirically for centuries because of its several biological and pharmacological properties. It was always mentioned as an anti-inflammatory agent. When dental pulp has inflammation, several mediators will be produced by innate immune cells. Cytokines are proteins released from cells in inflammatory process. In recent years, *in vitro* and in vivo assays provided information concerning about propolis and cytokines, thus a review dealing with the effect of propolis on cytokines during dental pulp inflammation became imperative. This review complied data from our study as well as from other researchers, focusing on its chemical composition on inflammatory process. Its anti-inflammatory property, considering its effect on interleukin, tumour necrosis factor, transforming growth factor as well as other cytokines during dental pulp inflammation are discussed. *In vitro* and *in vivo* assays demonstrated that propolis could inhibit inflammation process in dental pulp. Since humans have used propolis for many inflammation diseases and propolis-containing products have been marketed, the knowledge of its properties with scientific basis is not only of academic interest but also of those who use propolis as well. This review opens a new prespective on the investigation of propolis, mainly with respect to the immune system.

Key words: Propolis, Cytokines, Inflammation, Dental pulp.

INTRODUCTION

Inflammation of dental pulp is similiar to that in other connective tissue in that it is mediated by cellular and molecular factors (Fouad, 2002). The inflammatory response to dental pulp injury or infection has major clinical significance. Injury may be caused by dental caries, dental restorative procedures (iatrogenic), tooth fracture or attrition (Trowbridge, 2002).

Cytokines are proteins that provide commucation between cells and play a critical role in a wide veriety of processes including inflammation. Cytokines released from cells in an inflammatory process that activate, mediate or potentiate actions of other cells or tissues. Their actions may be effected in an autocrine, paracrine or endocrine manner (Seymour *et al*., 1995).

Propolis is an adhesive substance produced by honeybees from the bud and exudates of certain trees and plants (Bankova *et al*., 2000). Propolis has a long history as a general tonic promoting health, due to its several biological properties, such as anti-inflamatory (Hu *et al*., 2005), antibacterial (Sforcin *et al*., 2000) and immunomodulatory (Sforcin, 2007).

Therefore the aim of this report is to review the effect of propolis on cytokines during dental pulp inflammation, so could opens a new prespective on the investigation of propolis mainly with respect to the immune system.

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INFLAMMATION PROCESS

The concept of inflammation has evolved since the discovery of cells in the 19th century. By this time, inflammation was seen to be preceded by cell and tissue injuries, and that vascular changes including leukocyte emigration were secondary events (Trowbridge, 2002).

During the 1920s, the idea that the vascular system facilitated quick accumulation of great quantities of phagocytes and antibodies was reviewed. The first physical-chemical analysis of inflammation, cell stress and local tissue changes, promoted by an increasing concen-tration of oxidants and osmotic pressure, were also made at this time (Mitchell and Cotran, 2003). Therefore, modern investigators have considered inflammation a primary event of the host defense system.

Inflammation can be represented by capillary dilatation with fluid accumulation (oedema) and by phagocyte emigration and accumulation (neutrophils, monocytes, macrophages), which also contribute to hyperalgesia generation and loss of tissue function (Abbas *et al*., 2007). Other characteristics, such as erithema and fever, can also be observed during inflammatory events. The last feature occurs after cytokine release (Interleukin-1 [IL-1], Tumor Necrosis Factor-alpha [TNF-α]) by activated macrophages, leading to a vessel dilatation resulting from smooth muscular relaxation and followed by an increase in local blood flow (hypothermia) (Fouad, 2002). Inflammatory events also involve micro-vascular changes with increased vascular permeability, flow exudation, including plasmatic protein and amplification of endogenous chemical mediators (Cirino, 1998).

The acute-phase response involves serous, fibrinous, supurative or exudative events as well as micro-vascular and cell events; this response to pathogen occurs within 72 hours. The chronic-phase response includes proliferative events and histological alterations, different from those in the acute phase, characterized by cell emigration and intensive mitosis. In addition, inflammation may be physiological or pathological, depending mainly on histological aspects (Mitchell and Cotran, 2003).

PROPOLIS AND ITS CHARACTERISTICS

In a recent years the researchers were searching for natural products with medicinal properties, particularly those from plants and bees to found a natural anti-inflammatory agent. Several plants produce resinous exudates with strong anti-microbial and anti-necrotic properties, in addition to impermeability provided by populus a substance from *Populus* sp. (Greenaway *et al*., 1990). Bees collect resin exudates from certain plants and add their secretion, wood fragments, pollen, and wax; this product from bees and plants is called propolis. The word propolis comes from the Greek *pro* meaning in defense of and *polis* city, representing defense of bee cities (or beehives). Propolis has been used in folk medicine since primeval times. Nowadays, propolis is still used in home made remedies and cosmetics. Two characteristics of propolis are its smell and its various colors from dark green to brown (Marcucci *et al*., 1998).

Propolis chemical composition has been correlated with plant diversity around the beehive (Sforcin *et al*., 2000). In general, raw propolis contains about: 50~55% resins and balsams (phenols, phenolic acids, esters, flavanons {quercetin, galangin, pinocembrin}, dihydroflavanons, flavons, flavonols, chalkones, phenolic glycerides, cinnamic acid, coumaric acid, prenylated compounds and artepillin C), 25~30% waxes, 10% volatile oils, 5% pollen and 5% organic and mineral substances. The components are rich in vitamins such as B1, B2, B6, C, E and mineral elements like Mg, Ca, I, K, Na, Cu, Zn, Mn and Fe. It also contains number of fatty acids and enzymes as succinic dehydrogenase, glucose-6-phosphatase, adenosine triphosphatase and acid phosphatase (Parolia *et al*., 2000). Considering that propolis is a complex mixture, synergistic interactions between its compounds must also be considered as an important factor in its anti-inflammatory activity.

Ethanol, the most commonly used solvent for propolis preparations, and other solvents such as ethylic ether, water, methanol, petroleum ether, and chloroform are used for extracting and identifying many propolis compounds. Moreover, glycerin, propylene glycol and some solutions have been used in propolis preparations by the pharmace-

utical and cosmetic industry (Castaldo and Capasso, 2002).

Propolis compounds have recently become the subject of investigation in order to determine its therapeutic application in dentistry (Sabir, 2012); flavonoids are consider as the most biologically active substance in propolis (Havsteen *et al*., 2002). In the last 20 years, there has been increased commercial interest in propolis use due to its therapeutic properties to treat many diseases (Parolia *et al*., 2010). Nowadays, we can found propolis is commercially in sprays, ointments, capsules, capillary lotions and toothpastes because of its bacteriostatic activity and pharmacological properties.

EFFECT OF PROPOLIS ON CYTOKINES IN INFLAMMATION

The knowledge of propolis mechanisms of action on the immune system has advanced in the last years. However, only a few reports were published about the effect of propolis on cytokines during dental pulp inflammation since it was known that inflammation of dental pulp is similiar to that in other connective tissue. Many *in vitro* and *in vivo* experiments are performed with Ethanolic Extracts Propolis (EEP) and Aqueous Extracts Propolis (AEP) to confirm the anti-inflammatory activity of propolis. EEP anti-inflammatory effects was observed in inhibit both platelet aggregation and cytokines (Hu *et al*., 2005; Missima *et al*., 2009; Bachiega *et al*., 2012). Our previous study using immunohistochemistry method showed that propolis could delay TNFα expression on inflammed rat dental pulp tissue (Sabir, 2015). This antiinflammatory activity of propolis can be explained by the presence of active flavonoids and cinnamic acid derivatives (Chirumbolo, 2011). The former includes acacetin, quercetin, and naringenin (terpenoid constituents may exert an addictive effect); the latter includes Caffeic Acid Phenyl Ester (CAPE) (Tolba *et al*., 2013; Zhang *et al*., 2014) .

Experiment by Orsi *et al*. (2000), showed that after propolis treatment (2.5 and 5mg/kg) of mice for 3 days, peritoneal macrophages were activated *in vitro* with Interferon-gamma (IFN-γ). This fact suggests that propolis treatment leads macrophages to a higher responsiveness to stimuli IFN-γ. Another indicative of macrophages activation is Nitric Oxide (NO). NO is synthesized via L-arginine oxidation by a family of NO synthases (NOSs) and several cofactors. Nitric Oxide production by cells in response to cytokines can destroy host tissue and impair discrete cellular responses (Clancy *et al*., 1998). Law *et al*. (1999) investigated NO activity in the inflammed pulp of rat molars. The results showed that there was evidence of a dramatic increase in NO activity at the site of pulp irritation. Propolis (50 and 100μg/ml) inhibited NO generation by peritoneal macrophages (Orsi *et al*., 2000). Study by Moriyasu *et al*. (1994) also observed that propolis (0.2~1.0mg/ml) inhibited NO production by lipopolysaccharides (LPS)-stimulated macrohages. Krol *et al*. (1996) stated that this effect to flavonoid. Meanwhile, Hu *et al*. (2005) evaluated the action of EEP and WEP in a murine model of acute inflammation, verifying that both extracts inhibited NO generation. Transforming Growth Factors (TGF)-β was known as an endogenous suppressor of NOS expression in murine macrophages which destabilized NOS2 mRNA, retarded the synthesis of NOS2 protein and accelerated its degradation (Macmicking *et al*., 1997). This was supported by Ansorge *et al*. (2003), who found that the concentration of TGF-β was increased in the supernatant of T cell or peripheral mononuclear blood cell culture, after incubation with propolis, and this may be a possible explanation for propolis inhibit NO production. These effects mediated by some of propolis constituents such as CAPE and flavonoids (quercetin and hesperidin).

Study by Bachiega *et al*. (2012) found that CAPE, cinnamic acid and coumaric acid in propolis may be involved in the action of propolis inhibit both IL-6 and IL-10 but stimulated IL-1β production by macrophages. They were also observed that CAPE and cinnamic acid are strong Lipoxygenase (LOX) inhibitors, suppressing leukotriene production by peritoneal macrophages. Their action on Leukotriene (LTC)-4 was smaller *in vivo*. Quercetin inhibits LOX, and at high concentrations blocks COX. Naringenin only inhibited LTC4 causing weakness. All these data have demonstrated the strong and different inhibitory action of several propolis preparations or its isolated constituents on inflammation events. However, its anti-inflammatory effects depends mainly on the admini-

stration route and its potency (Mirzoeva and Calder, 1996).

CONCLUSION

In vitro and *in vivo* assays as well as animal model experiments demonstrated that propolis and its component has strong anti-inflammatory activity thereby could be effective to treat dental pulp inflammation. One of the anti-inflammatory mechanism of propolis is by inhibited cytokines production, although the other mechanisms are not fully understand. Human have been using propolis for a long time, so scientific-based information has important role for further studies on the investigation of propolis to opening the new prespective, mainly with respect to the immune system.

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