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Original article

Interleukin-6 expression on inflamed rat dental pulp tissue after capped with *Trigona* sp. propolis from south Sulawesi, Indonesia

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ABSTRACT

Background: Propolis is a natural product of plant resins collected by honeybees from various plant sources. It is used as a remedy in folk medicine since ancient times because of its several biological and pharmacological properties. Recently, propolis has been used by dentist to treat various oral diseases. It was always mentioned as an anti-inflammatory agent. Cytokines are proteins that provide communication between cells and play a critical role in a wide variety of processes. It released from cells in an inflammatory process that active, mediate or potential actions of other cells or tissues. When dental pulp has inflammation, several pro-inflammatory cytokines including Interleukin-6 (IL-6) was released by innate immune cells. *Objective:* To analyse the expression of IL-6 on inflamed rat dental pulp tissue following application of propolis. *Material and methods: Trigona* sp. propolis was obtained from Luwu Regency, south Sulawesi Province, Indonesia. Flavonoid and non-flavonoid extracts were purified from propolis using thin layer chromatography. The study was applied on 80 male *Sprague Dawley* rats, 10–12 weeks of age, divided randomly and equally into 5 groups. Group I, as negative control group was not conducted any treatment. At group II, III, IV and V. A Class I cavity (Black Classification) were made on the occlusal surface of right maxillary first molar. The dental pulp was perforated using dental explorer and allowed in the oral environment for 1 h, after that, Ethanolic Extract Propolis (EEP) (Group II), Extract Flavonoid-Propolis (EFP) (Group III), Extract Non-Flavonoid Propolis (ENFP) (Group IV), or Calcium Hydroxide ($Ca(OH)_2$) (Group V) were applied on dental pulp. All cavities were then filled with Glass Ionomer Cement as permanent filling. The rats being sacrificed in 6 h, 2 days, 4 days and 7 days. Sample biopsy were obtained, IL-6 expression was detected by using immunohistochemistry method. Data was analyzed statistically using Freidman and Kruskal Wallis tests with significance level of $P < 0.05$. *Results:* All agent showed IL-6 expression in inflamed rat dental pulp tissue, and this expression was decreased with the longer of observation time periods. EEP more stronger to decreased IL-6 expression on inflamed rat dental pulp tissue than other agent. There is significant difference ($P < 0.05$) of IL-6 expression between group I and other groups in 6 h and 2 days but not in 4 and 7 days time periods. *Conclusion: Trigona* sp. propolis from south Sulawesi, Indonesia could suppressed the expression of IL-6 on inflamed rat dental pulp tissue.

1. Introduction

Dental pulp is a connective tissue uniquely situated within the rigid encasemet of mineralized dentin. Inflammation of dental pulp is similar to that in other connective tissue in that it is mediated by cellular and molecular factors (Okiji, 2002; 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 soluble proteins that play an important role in the initiation and maintenance of inflammatory and immune responses as well as intercellular crosstalking. Interleukin-6 (IL-6) is a multifunctional cytokine synthesized in response to stimuli such as inflammation and trauma (Abbas et al., 2007) by a variety of cells such as macrophages, neutrophils, keratinocytes, fibrob-

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lasts, and endothelial cells (Nibali et al., 2012). Interleukin-6 cell signals are transmitted through a receptor expressed in a wide range of target cell types. In addition to this, a soluble IL-6 receptor (sIL-6R) enables to widen the repertoire of cells responsive to IL-6 (Jones et al., 2001). Interluekin-6 is able to stimulate a number of biologic processes including antibody-producing cells, activation of T cells, B cell differentiation, synthesis of acute-phase proteins B cells, hematopoiesis, induction of angiogenesis, vascular permeability, and osteoclast differentiation (Abbas et al., 2007). Interleukin-6 activity in inflammation is considered double-edged, acting both as anti-inflammatory (e.g., down regulation of neutrophil recruitment and proinflammatory cytokine expression) but also as proinflammatory in chronic diseases (Fouad, 2002).

Propolis is a resinous material bee honey product which collect from various plant mainly buds and leafs. Honey bee used propolis as antibiotic, seal hole or cracks of its combs, and also protect it from insects (Shakespeare and Henry, 2011). Propolis has been used since long time ago as traditional medicine such as antibacterial and anti-inflammatory drugs. The chemical composition of propolis is very complex, depends on the collecting location, time, and plant source (Toreti et al., 2013). However, the composition of propolis primarily consists of resinous (50%), wax (30%), essential and aromatics oils (10%), bee pollen (5%), and other substances (5%) (Bankova, 2009).

In recent years, propolis has been used in dentistry including in Conservative Dentistry and Endodontics to treat many tooth and pulp diseases such as a cariostatic agent to prevent caries (Libério et al., 2009), as a desensitizing agent to treat hypersensitivity dentin (Purra et al., 2014), intracanal irrigant (Bhardwaj et al., 2013) and medicament (Awawdeh et al., 2009) during root canal treatment and also as direct pulp capping agent to stimulate reparative dentin barrier (Parolia et al., 2010). Previous studies have demonstrated that propolis is toxic to dental pulp fibroblasts at 2 mg or above (Al-Shaher et al., 2004) and not reduced the viability of dental pulp fibroblasts at 1 mg/mL (Jahromi et al., 2014).

One of honeybee species that we can found in south Sulawesi province, Indonesia was *Trigona sp*. This honeybee species is stingless and can produce a lot of propolis. Therefore, the aim of the present study was to analyse the expression of IL-6 on inflamed rat dental pulp tissue following application of *Trigona* sp. propolis from south Sulawesi province, Indonesia.

2. Material and methods

Propolis (*Trigona* sp.) was collected from honeycomb in Luwu Regency, south Sulawesi province, Indonesia in the early monsoon season. Dry propolis was subjected to exhaustive maceration, filtered using aqueous ethanol, and concentrated using a rotary evaporator. The residue was separated using toluene solution to yield flavonoid and non-flavonoid fractions, which were then subjected to silica gel thin layer chromatography. Examination under ultraviolet light showed that the flavonoids group from propolis contain flavones, flavonols, flavanols, and chalcone (Sabir et al., 2015). The study was conducted at The Animal Research Development Center, Faculty of Veterinary and Department of Pathology, Faculty of Medicine, Gadjah Mada University, Yogyakarta.

Eighty male 10–12-week-old *Sprague-Dawley* rats (weight 200–250 g) were divided into 5 groups, each consisting of 16 animals. Group I, as negative control group was not conducted any treatment. At group II, III, IV and V. The rats were anesthetized intramuscularly with ketamine (Ketalar, Warner Lambert, Ireland) (65 mg kg^{-1} body weight) and xylazine-HCl (Xyla, Interchemie, Netherlands) (7 mg kg^{-1} body weight), and then Class I cavities (Black Classification) were prepared on the occlusal surface of right maxillary first molar using a low-speed tapered round diamond bur (Intensiv, Switzerland) (0.84 mm in diameter). The pulp was then exposed at the cavity floor using a dental explorer (Martin, Germany) (0.35 mm in tip diameter) and allowed in the oral environment for 1 h, after that, the pulp directly capped with Ethanolic Extract Propolis (EEP) (0.5 mg) (Group II), Extract Flavonoid-Propolis (EFP) (0.5 mg) (Group III), Extract Non-Flavonoid Propolis (ENFP) (0.5 mg) (Group IV), or Calcium Hydroxide ($Ca(OH)_2$) (0.5 mg) (Group V). Each cavity was then air-dried and filled with Glass Ionomer Cement (HS Posterior Extra, GC, Tokyo, Japan) as permanent filling. The experimental protocol was approved by the ethical committee of Faculty of Medicine, Hasanuddin University.

Four rats were sacrificed at 6 h, 2 days, 4 days and 7 days respectively. The teeth and surrounding bone were resected, fixed in Bouin's fixative for 24 h, decalcified with acetic acid/formal saline for 7 days, embedded in paraffin and sectioned serially at 6 μm thickness. The sections were stained with IL-6 monoclonal antibody (Neuromics, USA) using immunohistochemistry method and viewed by light microscopy. Immunohistochemistry evaluation was carried out as described previously (Faleiro-Rodrigues et al., 2004). Data were statistically analyzed by Freidman and Kruskal Wallis non-parametric tests. Data were analyzed using the SPSS 20.0.1 software (SPSS Inc, Chicago, IL, USA). Significance was established at $P < 0.05$ level.

3. Results

Except group I as negative control (no treatment), all treatment group showed IL-6 expression on inflamed rats dental pulp tissue after 6 h, 2 days, 4 days and 7 days application, but the expression was decreased with the longer of observation time periods. However, EEP was looks more stronger than other material test in inhibit IL-6 expression on inflamed rat dental pulp tissue (Fig. 1). No evidences of necrotic pulp tissues in all groups of animals were found throughout the study. For the sake of clarity and brevity, the photomicrograph of IL-6 expression is presented here in only by the section from all groups at 6 h and 7 days (Fig. 2).

The results of Freidman test revealed that there was no significant difference of IL-6 expression among time periods for each group (Table 1). Meanwhile, Kruskal Wallis test showed that there was significant difference ($P < 0.05$) of IL-6 expression between group I and other groups in 6 h and 2 days but not in 4 days and 7 days time periods (Table 2).

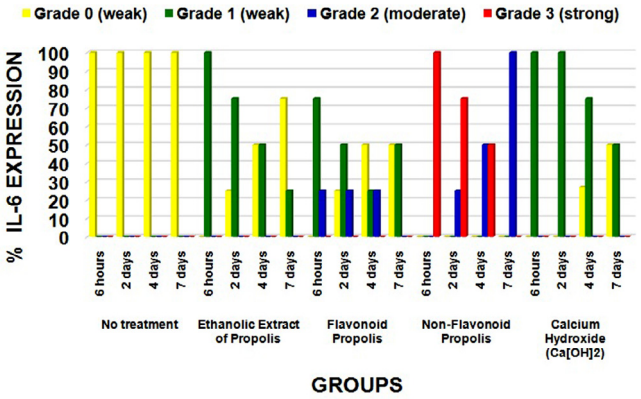

Figure 1. Histogram of percentage of IL-6 expression at group I (normal dental pulp) and inflamed rats dental pulp tissue group II, III, IV, V was capped with Ethanolic Extract Propolis (EEP), Extract Flavonoid-Propolis (EFP), Extract Non-Flavonoid Propolis (ENFP), and Calcium Hydroxide ($Ca(OH)_2$), respectively.