# Anti-inflammatory Effect of Trigona spp. Propolis in Restricting Edema Volume

**Wulantika Nurhayati[1], Vycke Yunivita Kusumah Dewi[2], Teguh Marfen Djajakusumah[3]**
[1]Faculty of Medicine, Universitas Padjadjaran, [2]Department of Pharmacology and Therapy, Faculty of Medicine, Universitas Padjadjaran, [3]Department of Surgery, Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital, Bandung, Indonesia

## Abstract

**Background:** Inflammation is a normal response that potentially harmful if it is uncontrolled. On the other hand, it is necessary to find an alternative anti-inflammatory as most anti-inflammatory drugs available nowadays still have adverse effects. Trigona spp. propolis is one of the potential anti-inflammatory alternatives because of its flavonoid, especially caffeic acid phenethyl ester, which is known as an active compound in anti-inflammatory process. This study aimed to understand the anti-inflammatory effect of Trigona spp. prolisin reducing edema volume in rat's paw.
**Methods:** An experimental study was performed on 20 male wistar rats. The rats were divided into control and experimental groups with 10 rats in each group. Control group was treated by propylene glycol 1 ml/day and experimental group was treated by Trigona spp. propolis 200 mg/kg body weight/day. The inflammation was induced by subcutaneous injection of λ-carrageenan 1% at plantar one hour after the treatment. Edema volumes were measured by plethysmometer every hour at 1 to 6 hours and once at 24 hours after induction. The difference in edema volumes was calculated in percentage. This study was conducted during October 2012 at the Pharmacology and Therapy Laboratory, Faculty of Medicine, Universitas Padjadjaran Bandung.
**Results:** The increase of edema volume (in percentage) in control group and Trigona spp. propolis treated group are 100.64±32.22 and 56.46±20.38 respectively (p value=0.000). Multiple comparisons using Dunnet and Duncan post hoc test showed significant differences that were observed at 3, 4, 5, and 6 hours after inflammatory induction.
**Conclusion:** Trigona spp. propolis has an anti-inflammatory effect in reducing edema volume in rat's paw. The most significant effect was observed at 3, 4, 5, and 6 hours after inflammatory induction. Trigona spp. propolis might have a potential to be developed as a future anti-inflammatory drug.

**Keywords:** Edema volume, inflammation, propylene glycol, Trigona spp, propolis

## Introduction

Inflammation is a host response in vascularized tissues that is caused by cell injury by various exogenous and endogenous stimuli. The inflammatory response consists of vascular and cellular reaction. The unique feature of inflammation is reaction of blood vessels leading to accumulation of leucocytes and fluid in extravascular tissues that is called edema.[1]

Physiologically, inflammation is protective response of the body to remove the noxious agent as well as the subsequent harmful events as consequence of the inflammation.[2] It is the body's effort to heal and reconstitute the injured tissues as process of repairing. However, on the other hand, this effort may be potentially harmful if uncontrolled and attack normal tissue.[1] For this reason, anti-inflammatory drugs are being produced, which ideally will control the harmful sequelae of inflammation process without interfering its beneficial effects.[1,3] Eventhough anti-inflammatory drugs that are widely distributed have good enough efficacy, they still have many adverse effects. Insomnia, euphoria, and depression in steroidal use and gastric irritation in NSAIDs (Non-Steroidal Anti-Inflammatory Drugs) use are some examples of acute adverse effects from anti-inflammatory drugs.[3,4]

Propolis (bee glue) is a natural product that is collected by the bees derived from resin or exudates of plants from apices of young leaves and mix with bees' saliva.[5-8] Since early century, propolis has been believed as a product that has a lot of benefits for human.[5,7,9]

**Correspondence:** Wulantika Nurhayati, Faculty of Medicine, Universitas Padjadjaran, Jalan Raya Bandung-Sumedang Km.21, Jatinangor, Sumedang, Indonesia, Phone: +6281394094866 Email: wulantika.nurhayati@gmail.com

Propolis, especially in Brazil and China, is a herbal product that is used as anti-bacterial, anti-fungal, antioxidant, anti-inflammation, and anti-cancer.[10]

Propolis is known having anti-inflammatory effect because its polyphenols (flavonoid, phenolic acidand its esther), terpenoid, steroid and amino acid.[11] Its flavonoid, especially CAPE (caffeic acid phenethyl ester) is known as an active compound in anti-inflammatory process which can inhibit production of cytokines IL-1, IL-6, TNF-α, and TGF-β12 through NF-κB pathway.[13] Its free radical scavenging activity can be mediated by the reduction of arachidonic acid metabolites by inhibiting lipoxygenase and cyclooxygenase.[11] In spite of the fact that anti-inflammatory effect of propolis has been discovered in many researches[11,14,15], Propolis that has been intensely researched is that from bees species *Apis mellifera*, not propolis from the species *Trigona spp.* which are local bees from Asia, especially Indonesia[6], that can produce more propolis than *Apis mellifera*.[6,7]

The aim of this study is to evaluate anti-inflammatory effect of an ethanol extract of Trigona spp .propolis in limiting edema volumes of carrageenan-induced rat paw edema.

## Methods

Twenty male wistar rats bred in Inter-University Centre Laboratory, Bandung Institute of Technology were obtained from Pharmacology and Therapy Laboratory, Faculty of Medicine, Universitas Padjadjaran Bandung. The rats were 2-3 months old, 175±25 grams weight and in healthy condition (clean, has not been injured, and could actively move). Rats were given pellets as standard food and tap water *ad libitum* in a room with good air circulation and illumination. Before the experiment, animals were adapted in laboratory room for seven days.

The ethanol extract of *Trigona spp.* propolis was extracted by Laboratory of Food Processing Technique, Faculty of Agricultural Industry, Universitas Padjadjaran Bandung which then was filled by propylene glycol; the propolis to propylene glycol ratio was 1:12. Propylene glycol that was used as a control was purchased from PT. BRATACO, Bandung. Lambda-carrageenan (λ-carrageenan) with 1% concentration was purchased from PT. SIGMA-ALDRICH, Singapore, which was obtained from Pharmacology and Therapy Laboratory, Faculty of Medicine, Universitas Padjadjaran Bandung.

Equipments that were used are rat's cage with food and drinking water bottle, scale, flannelette, pen, oral tube, syringe 1ml, and plethysmometer with 0.01 ml accuracy.

Rat paw volume was measured from lateral maleolus using plethysmometer. Basal rat paw volume was measured before oral administration of propolis and propylene glycol. Furthermore, propylene glycol 1 ml/day per oral was administered to each rat in the control group and *Trigona spp.* propolis 200 mg/kg body weight/day per oral was

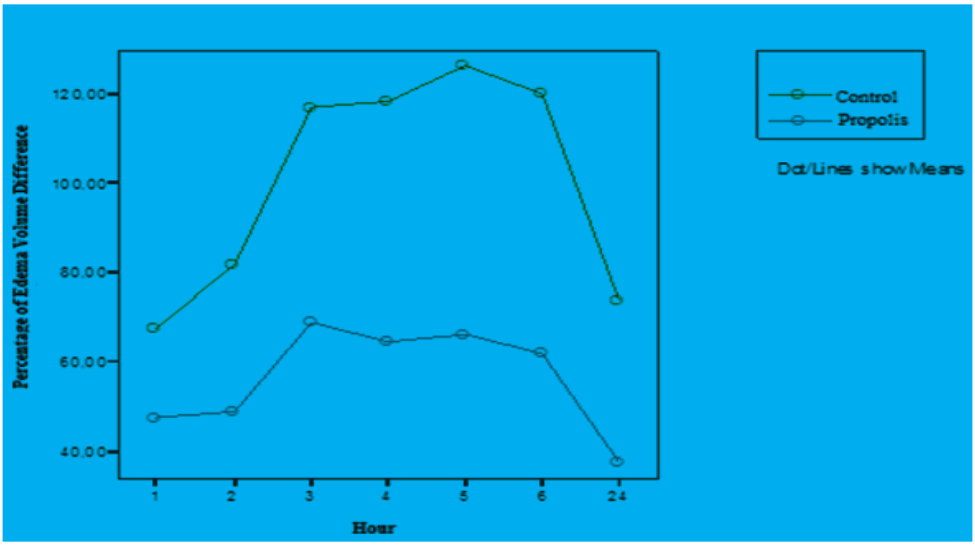


**Figure 1 Percentage of Edema Volume Difference**

administered to the control group through oral tube. Dose of propolis that was given correspond to rat's weight, then was diluted with propylene glycol until 1 ml. One hour later, rat's left plantar was injected using 1% λ-carrageenan as inflammatory inductor. Rat paw edema volume was measured at 1, 2, 3, 4, 5, 6, and 24 hours after carrageenan injection.[16]

This study was conducted during October 2012 in Pharmacology and Therapy Laboratory, Faculty of Medicine, Universitas Padjadjaran Bandung. Data were analyzed using SPSS 15.0 for Windows. Significant differences between groups were determined by Dunnet and Duncan post hoc test for multiple comparisons after analysis of variance (ANOVA). P<0.05 is considered statistically significant.

## Results

To evaluate anti-inflammatory effect, edema volume difference (in percentage) was calculated from the volume measured before carrageenan injection and each hour after induction. The mean percentage from each hour of measurements between control and conduction group was presented in Figure 1.

The increase of edema volume (in percentage) of those in control group and Trigona spp. propolis treated group are 100.64±32.22 and 56.46±20.38 respectively (p value=0.000). Multiple comparisons using Dunnet and Duncan post hoc test showed these significant differences that occurred at 3, 4, 5, and 6 hours after inflammatory induction.

## Discussions

This study showed that *Trigona spp.* propolis 200 mg/kg body weight/day per oral has anti-inflammatory effect in limiting rat paw edema volumes.

Previous study has showed that aqueous extract *Apis mellifera*'s propolis has anti-inflammatory effect with dosage of 500mg/kg body weight/day per oral[15] and in ethanol extract with dosage of 3mg/kg body weight/day in traparenteral[14] as well as 200mg/kg body weight/day per oral.[11] Anti-inflammatory activity of propolis comes from its polyphenols (flavonoid, phenolic acidand its esther), terpenoid, steroid and amino acid.[11] Flavonoid typecaffeic acid phenethyl ester (CAPE) is the most common active compound from propolis tested[11,13] and is believed to act as an active component in mediating anti-inflammatory process.[9] The compound CAPE is believed be able to inhibit production of cytokinesIL-1, IL-6, TNF-α, and TGF-β[12] through NF-κB pathway in nucleus[13], free radical scavenging, and reduce the production of arachidonic acid metabolites by inhibiting lipooxygenase and cyclooxygenase.[11]

In conclusion, *Trigona spp.* propolisis is a potential candidate to be developed as future anti-inflammatory drug.

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