

## Histological analysis of rat dental pulp tissue capped with propolis

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**Abstract:** The aim of the present study was to assess the response of rat dental pulp to direct pulp capping with propolis. Flavonoid and non-flavonoid materials were purified from an ethanol extract of propolis obtained from South Sulawesi, Indonesia. A Class I cavity was prepared on the occlusal surface of the right maxillary first molar in Sprague Dawley rats. The dental pulp was exposed and then capped with a zinc oxide-based filler as a control (group I), or with propolis flavonoids (group II) or non-flavonoids (group III). The animals were sacrificed at week 1, 2 or 4, biopsy samples were obtained, and these were stained and viewed by light microscopy. The results showed that pulp inflammation occurred in groups I and III as early as week 1. No dentin bridge formation was seen in these groups. In contrast, there was no evident inflammatory response in group II at week 1. Mild and moderate pulp inflammation in this group occurred at 2 and 4 weeks after treatment, respectively. Partial dentinal bridge formation was seen in group II at week 4. Therefore, the present results suggest that direct pulp capping with propolis flavonoids in rats may delay dental pulp inflammation and stimulate reparative dentin. (*J. Oral Sci.* 47, 135-138, 2005)

**Keywords:** flavonoid; non-flavonoid; propolis; pulp capping; rat.

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### Introduction

Propolis, a resinous material collected by honey bees from various plants, has been used as a traditional anti-inflammatory and anti-bacterial medicine for many centuries. Among its constituents, flavonoids regulate the immune response, reduce the release of free radicals, and inhibit bacterial and fungal growth, suggesting that this component has natural anti-inflammatory and immunoregulatory properties (1).

Direct pulp capping with materials such as adhesive resins is a technique used to seal the dental pulp, which may become exposed due to mechanical and/or chemical factors as well as bacterial activity, and this stimulates reparative dentin formation (2). Previous studies have demonstrated that propolis is toxic to dental pulp fibroblasts at 2 mg or above (3). Scheller and colleagues indicated that propolis also stimulates dental pulp collagen formation and reduces both pulp inflammation and degeneration (4). Therefore, the aim of the present study was to examine the response of rat dental pulp after capping with flavonoid and non-flavonoid extracts from propolis.

### Materials and Methods

Propolis (*Trigona sp.*) was collected from honeycombs in Bulukumba regency, South Sulawesi, Indonesia, in the early monsoon season. Dried propolis was subjected to

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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 chromatography. Examination under ultraviolet light showed that the flavonoids from propolis contain flavones, flavonoles, flavanols and chalcone (5).

Male 8–16-week-old Sprague-Dawley rats (weight 200–250 g) were divided into three groups, each consisting of 9 animals. Group I as a control underwent direct pulp capping with a zinc oxide-based filler (Dentorit®, Dentoria, France). The dental pulp in groups II and III was directly capped with propolis-derived flavonoids and non-flavonoids, respectively. The use of zinc oxide as a control was based on the fact that the rate of dental pulp inflammation is not altered by this material (6). The rats were anesthetized intramuscularly with ketamine (Ketalar, Warner Lambert, Ireland) (65 mg kg<sup>-1</sup> body weight) and xylazine-HCl (Rompun, Bayer, Leverkusen, Germany) (7 mg kg<sup>-1</sup> body weight), and then Class I cavities were prepared on the occlusal surface of the 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 directly capped with Dentorit (0.5 mg), propolis flavonoid (0.5 mg) or non-flavonoid (0.5 mg). Each cavity was then air-dried and filled with glass ionomer cement (Fuji IX, GC, Tokyo, Japan). The experimental protocol was approved by the ethical committee of Gadjah Mada University School of Medicine.

Three rats were sacrificed at week 1, 2 and 4 respectively. The teeth and the surrounding bone were resected, fixed in 10% neutral buffered formalin, decalcified with 10% EDTA for 30 days, embedded in paraffin and sectioned serially at 6 µm thickness. The sections were stained with hematoxylin-eosin and viewed by light microscopy. Histological evaluation was carried out as described previously (7).

## Results

In group I, mild inflammation was evident in the root canal from week 1 (Fig. 1A). However, increased levels of inflammatory response were detected at week 2 and 4 (Fig. 1B and C). No dentinal bridge formation was evident in the control group at week 4. At week 1, no inflammation was evident in the propolis flavonoid-treated animals (group II). However, at week 2 and 4, mild and moderate inflammation was seen in the pulp chamber of 2 animals from this group, respectively (Fig. 1D, E and F). Interestingly, partial dentinal bridge formation was detected

beneath the pulp-capping material at week 4 in group II. Furthermore, direct pulp capping with propolis-derived non-flavonoid materials (group III) induced a mild inflammatory response at week 1 and moderate inflammation in 2 out of 3 animals at week 2 and 4 (Fig. 1G, H and I). No evidence of necrotic pulp tissue was seen in any of the groups throughout the study.

## Discussion

The present study showed that direct pulp capping with flavonoid materials extracted from propolis delayed the inflammatory response. In contrast, direct pulp capping with propolis-derived non-flavonoids or zinc-oxide stimulated an inflammatory response as early as 1 week after treatment. Suppression of dental pulp inflammation by propolis has also been reported previously in a study carried out by Scheller and colleagues (4). The present results are not surprising, since propolis flavonoids are known to have anti-inflammatory properties, perhaps via suppression of immune cell activation, macrophage-derived nitric oxide and cytokine production, and neutrophil activation (1). Alternatively, flavonoids from propolis might inhibit bacterial growth in the pulp chamber, thereby reducing the host response to bacterial antigens (1). However, the exact mechanism responsible for the persistent inflammatory reaction evident at week 4 in the dental pulp capped directly with propolis flavonoids needs to be further clarified. Several possible explanations can be considered, including oral bacterial microleakage (8) that might not have been completely eliminated by propolis-derived flavonoid. Alternatively, both the anti-inflammatory and anti-bacterial properties of propolis flavonoids might have been considerably reduced at week 4 due to metabolism of these materials (9).

One interesting observation was the presence of partial dentinal bridge formation in one of the experimental animals 4 weeks after direct pulp capping with propolis-derived flavonoids. This histological finding suggests that flavonoids from propolis may stimulate reparative dentinogenesis. Dentin formation following pulp capping is known to involve differentiation of odontoblast-like cells that form reparative dentin and biosynthetic activity by surrounding primary odontoblasts. Both phenomena require interaction between extracellular matrix molecules and growth factors such as transforming growth factor (TGF)-β1, a growth factor known to be important for odontoblast-like cell differentiation (10). Indeed, propolis is also capable of stimulating the production of (TGF)-β1 (11), and the synthesis of collagen by dental pulp cells (4). It remains to be further clarified whether or not the stimulatory effects of propolis-derived flavonoids on dentin

bridge formation observed in the present study might occur through this pathway.

In conclusion, the present study has shown that direct pulp capping with propolis-derived flavonoids inhibits the dental pulp inflammatory response in rats at week 1. However, pulp inflammation was evident at week 2 and 4. Dentinal bridge formation was detected in teeth capped

with propolis-derived flavonoids. In contrast, after direct pulp capping with a zinc oxide-based filler or propolis-derived non-flavonoids, there was slight inflammation at week 1 but increased inflammation at week 4 without detectable dentin bridge formation. Therefore, the present results obtained in rats suggest that direct pulp capping with propolis flavonoids may delay pulp inflammation and

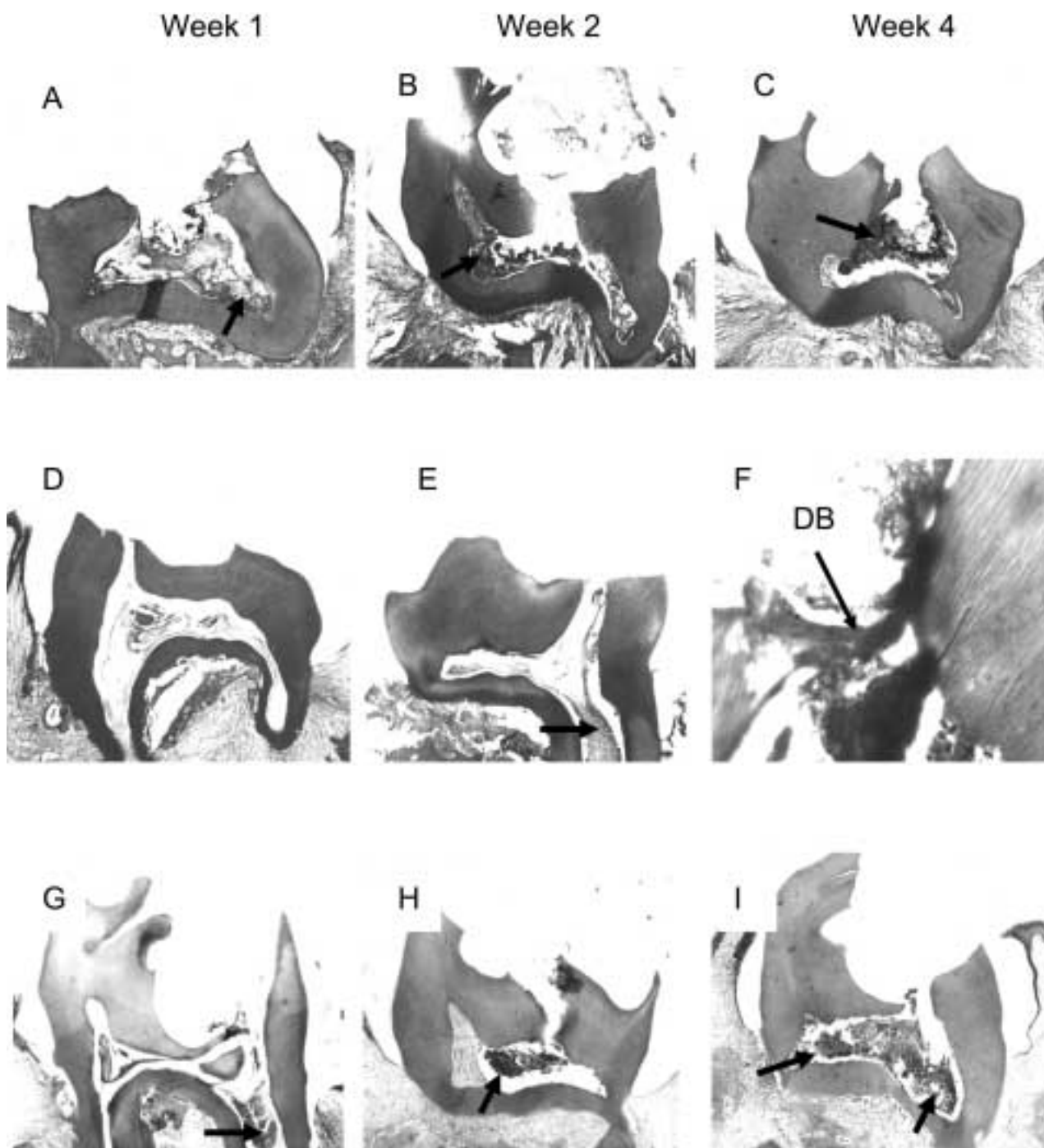


Fig. 1 Rat dental pulp response after direct pulp capping with propolis. Exposed dental pulp in groups I (A - C), II (D - F) and III (G - I) was capped with zinc-oxide-based filler, propolis-derived flavonoids and non-flavonoids, respectively. Arrows show inflammatory cells. DB = dentin bridge. Hematoxylin-eosin stain, original magnification  $\times 40$  (Fig. 1A - E and Fig. 1G - I) and  $\times 400$  (Fig. 1F).

stimulate reparative dentin.

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