

Proliferation of odontoblast-like cells following application of a combination of calcium hydroxide and propolis

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ABSTRACT

Background: One purpose of operative dentistry is the maintenance of healthy pulp by reducing the need for root canal treatment and the possibility of undesirable scenarios such as tooth loss. Propolis is a plant-derived substance that contains a resin produced by honeybees belonging to the *Apis mellifera* species. **Purpose:** This study aimed to investigate the effect of a combination of calcium hydroxide ($\text{Ca}(\text{OH})_2$) and propolis extract on odontoblast-like cell proliferation in Wistar rats (*Rattus norvegicus*). **Methods:** This research constituted a true experimental laboratory-based investigation with post-test control group design. Thirty Wistar rats were randomly divided into six groups. The first molar pulp of each sample was perforated on occlusal surfaces using a low speed round bur. On day 3, the samples were divided into six groups ($n=10$): Group I: control; Group II: $\text{Ca}(\text{OH})_2$ + 11% propolis extract; Group III: $\text{Ca}(\text{OH})_2$ + aquadest, and on day 7: Group IV: control; Group V: $\text{Ca}(\text{OH})_2$ + 11% propolis extract; Group VI: $\text{Ca}(\text{OH})_2$ + aquadest. All samples were filled with restorative material. They were subsequently sacrificed after 3 and 7 days post-pulp capping administration and the afflicted tooth extracted for hematoxylin and eosin (H&E) staining. The resulting data was subjected to statistical analysis to ascertain the proliferation of odontoblast-like cells. The significance of differences between the groups was determined by a one-way ANOVA test followed by a post hoc Tuckey HSD. A p -value <0.05 was considered to be significant. **Results:** On day 3, a significant difference existed between group II ($\text{Ca}(\text{OH})_2$ -propolis) and group I (control group) and group III ($\text{Ca}(\text{OH})_2$ -aquades), whereas $\text{Ca}(\text{OH})_2$ -propolis revealed that the proliferation of odontoblast-like cells was higher. Meanwhile, on day 7, there was a significant difference between all groups whereas, with regard to $\text{Ca}(\text{OH})_2$ -propolis, the proliferation of odontoblast-like cells in group V was higher. **Conclusion:** Application of combination of $\text{Ca}(\text{OH})_2$ -propolis extract can increase the proliferation of odontoblast-like cells in pulp tissue on days 3 and 7.

Keywords: calcium hydroxide; odontoblast like cell; propolis extract

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INTRODUCTION

One objective of operative dentistry is the maintenance of healthy pulp which reduces the need for root canal treatment.¹ Direct pulp capping is a procedure that introduces biocompatible materials and bio-inductors into exposed pulp tissue in order to maintain its vitality,² and induce the differentiation of odontoblast-like cells, in addition to repairing exposed dentin tissue with the formation of reparative dentin.¹ The purpose of this treatment is to seal the pulp, thereby protecting it from

bacterial penetration, in order to induce it to initiate dentin bridge formation and maintain healthy pulp tissue.^{1,2} Dentin bridge is often described as reparative dentin. In fact, the former can be defined as a new matrix deposition located in close proximity to certain material such as that used for pulp capping.³ The success of direct pulp capping depends on biocompatibility with the tissue and an effective physico-chemical composition. The contemporary gold standard for pulp capping material is calcium hydroxide ($\text{Ca}(\text{OH})_2$) which has been employed in a range of therapies, including; direct pulp capping, indirect pulp capping, apexogenesis,

apexification, root resorption, iatrogenic root perforation, root fracture, tooth replantation, and intracanal dressing.⁴ Nevertheless, it also suffers from certain disadvantages. For example, it can induce pulp inflammation for a period of up to three months, render the pulp tissue response unpredictable and cause irregular reparative dentin formation potentially leading to tunnel defects.⁵

Recently, a range of studies have investigated the application of propolis to dentistry because of its numerous positive properties such as; anti-inflammatory, anti-bacterial, and the ability to induce the reorganization of pulp tissue.⁶ Propolis is a substance that contains a plant-based resin produced by honeybees of the *Apis mellifera* species which consists of more than 200 elements, including; phenolic acids, flavonoids, esters, aromatic aldehydes, alcohol, amino acids, fatty acids, vitamins and minerals.^{3,7} In dentistry, propolis has been widely employed because of the protection against caries that it provides to teeth. Moreover, 30% propolis has also been recommended for use in irrigation during root canal treatment, while the resin formation is employed as pulp capping material to protect vital pulps derived from one of the flavonoids with the highest propolis composition.⁶ It can also induce the formation of reparative dentin by stimulating the release of transforming growth factor- β 1 (TGF- β 1) capable of inhibiting pulp inflammation and accelerating collagen synthesis by pulp cells.⁸

The numerous benefits of propolis underlies the authors' support for the integration of natural remedies with modern medicine by combining calcium hydroxide with propolis as a pulp capping material in the hope that the efficacy of each ingredient can compensate for deficiencies in the others. Studies showed that a combination of Ca(OH)₂-propolis used as a pulp capping material produces no toxic reactions and is capable of significantly reducing inflammation.⁶ Therefore, the purpose of this study was to investigate the effect of a combination of Ca(OH)₂ and propolis extract as pulp capping material on odontoblast-like cell proliferation in Wistar rats (*Rattus norvegicus*).

MATERIALS AND METHODS

This research constituted an experimental laboratory study incorporating a post-test control group design. All procedures and treatments which the animal subjects of this research underwent were approved by the Ethical Committee of the Faculty of Dentistry, Universitas Airlangga (Document no. 277/HRECC/FODM/X/2018).

Propolis extract was produced by maceration using 96% ethanol. The research subjects comprised 30 healthy male Wistar rats (aged 6-18 weeks, weighing 200-300 grams) which were anaesthetized using 100mg of ketamine (Ketalar®, Warner Lambert, Irlandia) and 10mg/Kg of xylazine HCl (Rompun®, Bayer, Leverkusen, Jerman) in sterile phosphate buffered saline (PBS). The PBS was then

placed on a fixation board. First, a cavity was created on the occlusal of right maxillary first molar using a low speed handpiece with a round tapered diamond bur (diameter 0.84) until it reached the pulp chamber. The pulp was perforated (diameter 0.46 mm) with a low speed handpiece featuring a round diamond bur. The Wistar rats were divided randomly into six groups, namely: Group I (not treated with any pulp capping, observed on the third day), Group II (treated with a combination of Ca(OH)₂ (EMSURE ACS, Reag. Ph Eur, Germany) and 11% propolis extract, observed on the third day), Group III (treated with a combination of Ca(OH)₂ and aquadest observed on the third day), Group IV (not treated with any pulp capping material, observed on the seventh day), Group V (treated with a combination of Ca(OH)₂ and 11% propolis extract, observed on the seventh day), and Group VI (treated with a combination of Ca(OH)₂ and aquadest, observed on the seventh day).⁹

For all groups, a micro applicator was employed to apply the pulp capping materials. All of the cavities were filled with restoration material (Cention N, Ivoclar Vivadent). A necropsy was performed on the third and seventh days, followed by decapitation and separation of the maxilla. Cutting was performed using a rotary microtome at a thickness of 5 μ m, the resulting tissue being placed on a glass object and stained using haematoxylin and eosin (H&E) and observed through a microscope (Olympus CX 23 Binocular LED, Japan) to enable calculation of the odontoblast-like cells.

The data obtained was analyzed statistically to examine the proliferation of odontoblast-like cells. The significance of differences between groups was determined by means of a one-way ANOVA test followed by a post hoc Tukey HSD. A p-value < 0.05 was considered to be significant.

RESULTS

Figure 1 shows the histological examination of odontoblast-like cells on days 3 and 7. The mean and standard deviation of each sample group used to quantify the proliferation of odontoblast-like cells on days 3 and 7 is shown in the Table 1.

Table 1. Mean and standard deviation of odontoblast-like cells on days 3 and 7

Group	Mean \pm SD
I	6 \pm 1.58
II	12.8 \pm 1.30
III	6.2 \pm 1.30
IV	7 \pm 1.58
V	16.8 \pm 1.30
VI	10.8 \pm 0.83

The conducting of a Tukey HSD indicated a significant difference in the proliferation of odontoblast-like cells between all groups ($p < 0.05$). The contents of Table 2 show that on day 3 there was a significant difference between Group II (Ca(OH)_2 -propolis) and Group I (control groups) and Group III (Ca(OH)_2 -aquades), whereas Group II (Ca(OH)_2 -propolis) presented a proliferation of odontoblast-like cells higher than that in Group I (control) and Group III (Ca(OH)_2 -aquades). Meanwhile, on day 7 a significant difference existed between Group IV (control) and Group V (Ca(OH)_2 -propolis); Group IV (control) and Group VI ($\text{Ca(OH)}_2 + \text{aquadest}$); and Group V (Ca(OH)_2 -propolis) and Group VI ($\text{Ca(OH)}_2 + \text{aquades}$), whereas Group V (Ca(OH)_2 -propolis) experienced a proliferation of odontoblast-like cells higher than that in Group IV (control) and Group VI (Ca(OH)_2 -aquades).

DISCUSSION

Preparation of deep cavity caries can culminate in pulp perforation. The basic principle of operative dentistry is to maintain the health and function of the dentin-pulp complex, especially in cases of exposed pulp.¹⁰ Pulp tissue also possesses the ability to repair itself in the manner of other connective tissue.¹¹ The healing characteristics of exposed pulp tissue include; reorganization of damaged soft tissue, differentiation between sub-odontoblast and odontoblast-like cell and formation of reparative dentin.¹⁰ Pulp tissue damage leads to inflammation. Fibroblasts migrate immediately to the site of destruction, proliferation, and differentiation into odontoblast-like cells, in addition to producing the collagen matrix that subsequently becomes a hard tissue barrier protecting the remaining pulp tissue from irritants.¹²

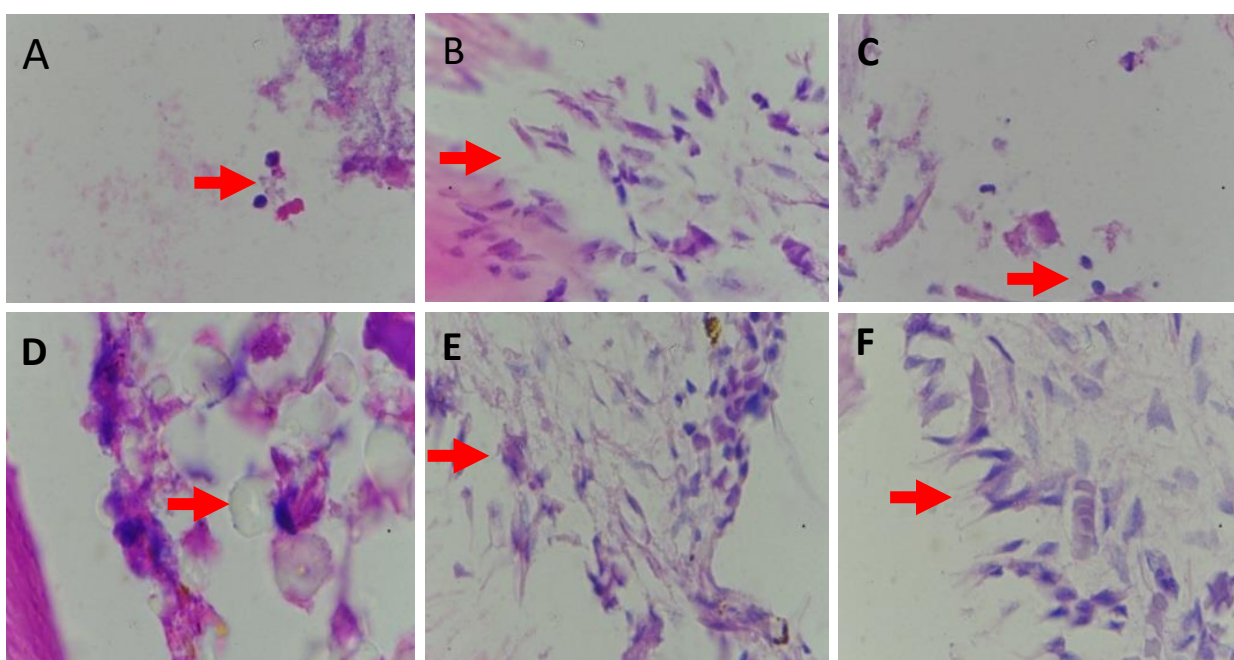


Figure 1. Histological examination of odontoblast-like cells on days 3 and 7. The red arrow indicates the odontoblast-like cells (Magnification 1000X). Day 3 (A) Group I: Control (B) Group II: $\text{Ca(OH)}_2 + 11\%$ propolis (C) Group III: $\text{Ca(OH)}_2 + \text{aquadest}$. Day 7 (D) Group IV: Control (E) Group V: $\text{Ca(OH)}_2 + 11\%$ propolis (F) Group VI: $\text{Ca(OH)}_2 + \text{aquadest}$.

Table 2. Tukey HSD on day 3 and 7 * $p < 0.05$ = significant difference existed

		Day 3			Day 7		
		Group I	Group II	Group III	Group IV	Group V	Group VI
Day 3	Group I						
	Group II	.000*					
	Group III	1.000	.000*				
Day 7	Group IV	.843	.000*	.931			
	Group V	.000*	.001*	.000*	.000*		
	Group VI	.000*	.211	.000*	.002*	.000*	

In this study, enhancement of the significant difference in odontoblast-like cells occurred after application of Ca(OH)₂-11% propolis extract because propolis promotes antibacterial, anti-inflammatory, antioxidant, and immunomodulatory activity in order to prevent infection and increase cell regeneration.^{11,13} It is also known that propolis extract can stimulate TGF-β1 production, while TGF-β1 can induce proliferation of fibroblasts.¹¹

Jahromi *et al.* (2014) stated that Ca(OH)₂ application promotes less cell viability than propolis application.¹⁴ Shaher *et al.* (2004), also reported that Ca(OH)₂ was almost ten times more cytotoxic than the propolis in pulp cells.^{13,14} In another study, the combination of Ca(OH)₂ with propolis produced no toxic reactions and reduced inflammation significantly in the connective tissue of rats.¹⁴

In this study, groups with a Ca(OH)₂ + 11% propolis combination (Group II and Group V) experienced a proliferation of odontoblast-like cells higher than that of the control group and Ca(OH)₂-aquades group. This contrast could be caused by the properties of propolis which are known to have numerous advantages, one of them being immunomodulatory activity which promotes the healing process commencing with the formation of collagen fiber.¹⁰ Two active components of propolis are flavonoids and caffeic acid whose function is to inhibit the arachidonic acid lipoxygenase pathway causing a reduction in the inflammatory response.¹⁵ However, proliferation of odontoblast-like cells on day 3 was less pronounced than that on day 7. This phenomenon was caused by acute inflammation that presented between day 0 and day 3 on which the proliferation of odontoblast-like cells commences, reaching its peak on day 7. This is because on day 7 there was a transition from acute to chronic inflammation in the pulp which involved odontoblast-like cells. Chronic inflammation histologically marked by a form of granulated tissue consists of infiltration of chronic inflammatory cells, proliferation of capillary vessels, and proliferation of fibroblasts.¹⁰ In conclusion, the combination of Ca(OH)₂-propolis as a pulp capping material can increase the proliferation of odontoblast-like cell in pulp tissue on days 3 and 7.

REFERENCES

1. Tziafas D, Smith AJ, Lesot H. Designing new treatment strategies in vital pulp therapy. *J Dent.* 2000; 28(2): 77–92.
2. Parolia A, Kundabala M, Rao NN, Acharya SR, Agrawal P, Mohan M, Thomas M. A comparative histological analysis of human pulp following direct pulp capping with Propolis, mineral trioxide aggregate and Dycal. *Aust Dent J.* 2010; 55(1): 59–64.
3. Nanci A. *Ten Cate's oral histology : development, structure, and function.* 9th ed. St. Louis: Elsevier; 2017. p. 326–7.
4. Farhad A, Mohammadi Z. Calcium hydroxide: A review. Vol. 55, *International Dental Journal.* FDI World Dental Press Ltd; 2005. p. 293–301.
5. Dwiandhono I, Effendy R, Kunarti S. The thickness of odontoblast-like cell layer after induced by propolis extract and calcium hydroxide. *Dent J (Majalah Kedokt Gigi).* 2016; 49(1): 17–21.
6. Montero JC, Mori GG. Assessment of ion diffusion from a calcium hydroxide-propolis paste through dentin. *Braz Oral Res.* 2012; 26(4): 318–22.
7. Mendonça ICG, Medeiros MLBB, Penteado RAPM, Parolia A, Porto ICCM. An overview of the toxic effects and allergic reactions caused by propolis. *Pharmacologyonline.* 2013; 2: 96–105.
8. Ansorge S, Reinhold D, Lendeckel U. Propolis and some of its constituents down-regulate DNA synthesis and inflammatory cytokine production but induce TGF-β1 production of human immune cells. *Zeitschrift fur Naturforsch - Sect C J Biosci.* 2003; 58(7–8): 580–9.
9. Dharsono VA. Aplikasi kombinasi kalsium hidroksida dan ekstrak propolis terhadap ekspresi dmp1 dan dsp pada odontoblas gigi tikus. Thesis. Surabaya: Universitas Airlangga; 2018. p. 26–36.
10. Yu C, Abbott P. An overview of the dental pulp: its functions and responses to injury. *Aust Dent J.* 2007; 52: S4–6.
11. Karube H, Nishitai G, Inageda K, Kurosu H, Matsuoka M. NaF activates MAPKs and induces apoptosis in odontoblast-like cells. *J Dent Res.* 2009; 88(5): 461–5.
12. Sabir A, Tabbu CR, Agustiono P, Sosroseno W. Histological analysis of rat dental pulp tissue capped with propolis. *J Oral Sci.* 2005; 47(3): 135–8.
13. Al-Shaher A, Wallace J, Agarwal S, Bretz W, Baugh D. Effect of propolis on human fibroblasts from the pulp and periodontal ligament. *J Endod.* 2004; 30(5): 359–61.
14. Zare Jahromi M, Ranjbarian P, Shiravi S. Cytotoxicity evaluation of Iranian propolis and calcium hydroxide on dental pulp fibroblasts. *J Dent Res Dent Clin Dent Prospects.* 2014; 8(3): 130–3.
15. Sabir A. Respons inflamasi pada pulpa gigi tikus setelah aplikasi ekstrak etanol propolis (EEP) (The inflammatory response on rat dental pulp following ethanolic extract of propolis (EEP) application). *Dent J (Majalah Kedokt Gigi).* 2005; 38(2): 77–83.