

# Radiographic and histopathologic outcomes of immature dog teeth with apical periodontitis after revascularization using propolis. An *in vivo* study

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## Abstract

**Introduction:** This study aimed to assess the radiographic and histopathologic outcomes of immature dog teeth with apical periodontitis after revascularization using propolis.

**Materials and Methods:** Periapical pathosis was induced in 48 double-rooted premolars in six dogs aged 6–9 months. The root canals were irrigated with 10 mL of 2.25% NaOCl per tooth. The teeth were divided into two groups (24 teeth each) according to the disinfection of root canals as follows: Group I (propolis group): disinfected with propolis paste and Group II (control group): without disinfectant. After 3 weeks, bleeding was induced to fill the canal spaces, the pulp chamber of the teeth was plugged with mineral trioxide aggregate, and the access cavities were sealed with glass ionomer cement. Samples were classified into three subgroups depending on the evaluation period as follows: subgroup i: 2 weeks, subgroup ii: 4 weeks, and subgroup iii: 8 weeks. Increase in the root length and root thickness and decrease of apical diameter were assessed by radiography, and new hard tissue, vital tissue, and apical closure scores were assessed by histology. All data were statistically analyzed.

**Results:** There were statistically significant differences between both groups regarding the increase in root length, increase in root thickness, decrease in apical diameter, new hard tissue, and vital tissue in all subgroups ( $P \leq 0.05$ ). There was a statistically significant difference between both groups regarding the apical closure at 8 weeks only ( $P < 0.05$ ). Propolis group showed formation of cementum-like tissue along the inner aspect of root dentin and newly formed dentin layer along the inner aspect of the root with pulp-like tissue and odontoblasts.

**Conclusion:** Propolis is capable of inducing hard-tissue deposition and soft-tissue formation inside the necrotic pulp after revascularization of immature permanent teeth.

**Keywords:** Intracanal medicament, odontoblasts, periapical pathosis, regenerative endodontics, revascularization

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## INTRODUCTION

Revascularization of necrotic immature teeth is a better therapeutic alternative than apexification using calcium hydroxide and mineral trioxide aggregate (MTA) apical plug.<sup>[1]</sup> Revascularization offers stem cells, three-dimensional scaffold, and growth factors for differentiation and proliferation of the stem cells.<sup>[2]</sup>

Disinfection of the necrotic canal is a critical step during the revascularization protocol due to the presence of bacteria that will retard tissue growth inside the canal space. Triple antibiotic paste (TAP) is a very effective intracanal medicament for elimination of the endodontic pathogens.<sup>[3]</sup> However, TAP has some disadvantages such as development of resistant bacteria, allergic reaction, and crown discoloration.<sup>[4]</sup>

Propolis is a natural honeybee's product previously used for the treatment of skin wound and orodental diseases due to its antibacterial, antifungal, antiviral, anti-inflammatory, immunomodulatory, and antioxidant effects.<sup>[5-7]</sup>

In dentistry, propolis has been used for surgical wound repair,<sup>[8]</sup> root canal irrigation,<sup>[4]</sup> direct and indirect pulp capping,<sup>[6]</sup> reduction of dentin hypersensitivity, in caries prevention against *Streptococcus mutans*,<sup>[9]</sup> as a storage media for avulsed teeth,<sup>[10]</sup> and in pulpectomy of deciduous molars with necrotic pulp and periapical pathosis.<sup>[11]</sup> The hypothesis of this research was that propolis may have a beneficial role during revascularization of immature teeth with apical pathosis.

The nature of tissue regenerated in the canals of immature teeth with necrotic pulp/apical periodontitis after successful revascularization has not been widely investigated. Hence, the purpose of this study was to evaluate the radiographic and histopathologic outcomes of immature dog teeth with experimentally induced apical periodontitis after revascularization using propolis as an intracanal medication.

## MATERIALS AND METHODS

This study was approved by the Institutional Animal Care and Use Committee at the Faculty of Dentistry, Suez Canal University, Ismailia, Egypt (protocol no. 15-11-2011). The authors followed up all institutional and international guidelines for animal care and use during this study.

### Preparation of propolis paste

Preparation of propolis powder (Holistic Herbal Solutions, LLC, USA) into a paste form was done according to the

technique described by Hoshino *et al.*<sup>[12]</sup> for the preparation of TAP. The technique in brief was as follows:

- Preparation of the carrier: Equal amounts of macrogol ointment (USP 37) and propylene glycol (Ineos Manufacturing, Deutschland GmbH, Germany) were the carrier used (MP). To prepare 100 g of macrogol ointment, 40 g of polyethylene glycol 3350 was mixed with 60 g of polyethylene glycol 400 (DOW Chemical Company, Michigan, USA); the two ingredients were heated in a water bath at 65°C until complete melting then allowed to cool down to room temperature while stirring until the mixture was congealed
- Preparation of propolis paste: In a suitable clean sterilized glass, 500 mg of propolis was mixed well with 1.2 g of MP to obtain a creamy paste.

### Animal model

Six healthy mongrel dogs aged 6–9 months were selected for the present study. Two double-rooted permanent immature premolars in each quadrant were included in the study summing up a total of 48 teeth (eight teeth/dog). These teeth were classified into two groups (24 teeth each) based on the disinfection modality as follows: Group I (propolis group): root canals were disinfected with propolis and Group II: no intracanal medication was applied in the canals (control group). Both groups were further subdivided according to the post treatment evaluation period into three subgroups (16 teeth/2 dogs each); subgroup i: 2 weeks, subgroup ii: 4 weeks, and subgroup iii: 8 weeks.

### Experimental procedure

Dogs were premedicated with atropine sulfate (Atropine®: Sunways Pvt. Ltd., Mumbai, India) at a dose of 0.05 mg/kg given subcutaneously and xylazine HCl (Xylamed®: Bimeda Animal Health, Dublin, Ireland) at a dose of 1 mg/kg given intramuscularly. General anesthesia was induced by ketamine HCl (Ketalar®: JHP pharmaceuticals, Michigan, USA) at a dose of 5 mg/kg given intravenously and maintained with thiopental sodium 2.5% (Thiopental sodium®: Livealth Biopharma Pvt., Ltd., Mumbai, India) at a dose of 25 mg/kg given intravenously. The pulp chamber of each experimental tooth was mechanically exposed with a #2 Endo access bur in a low-speed hand piece under nonaseptic conditions. A #30 stainless steel endodontic hand file was introduced inside the root canal to disrupt the pulp tissue. Dental calculus was collected from adult dogs and mixed with sterile saline solution to obtain a calculus suspension and then sponges soaked in the prepared suspension were inserted into the pulp chambers of all teeth and sealed with temporary filling. The animals were given carprofen as analgesic (Rimadyl

tablets®: Zoites, USA) at a dose of 4.4 mg/kg given once daily for 7 days postoperatively. The teeth were then monitored by radiography for evidence of development of apical pathosis.

After 2–3 weeks, development of radiographically visible periapical lesion was noticed [Figure 1]. All infected teeth were re-entered under the previously mentioned anesthetic protocol and under aseptic condition (cotton roll isolation and surface disinfection with 0.12% chlorhexidine and tincture of iodine). All the root canals were irrigated using 2.25% NaOCl (10 mL) per tooth. No mechanical instrumentation was performed in any of the canals. In propolis group, propolis paste was administered inside the root canals using a lentulo spiral. Group II did not receive any disinfectant paste. The teeth were temporarily sealed with Cavit (Cavit, 3M™ ESPE™ Dental AG, Seefeld/Oberbay, Germany).

After 3 weeks, the temporary restorations were removed under the same anesthesia and aseptic precautions. Root canals were irrigated with 2.25% NaOCl (10 mL) followed by sterile saline solution (10 mL). A #30 sterile endodontic hand file was introduced inside the root canal beyond the canal terminus to induce bleeding in the canal space. After clotting of the blood, the teeth were double coronally sealed with MTA plug (Angelus, Indústria de Produtos Odontológicos Ltda, Londrina, PR, Brazil) just beyond the cemento-enamel junction to seal the root canal orifices. The access cavities were finally sealed with glass ionomer cement.

## Methods of evaluation

### Radiographic evaluation

Periapical radiographs to verify the establishment of periapical lesions were taken and compared to the follow-up radiographs following treatment at every evaluation period.

Digitization of the radiographs was done using a transparency scanner (HP Scanjet G3110, Hewlett-Packard, Palo Alto, CA, USA). Digital image files were converted to 32-bit TIFF files using Image-J analysis software (Image-J

v1.44, US National Institutes of Health, Bethesda, MD, USA). TurboReg plug-in (Biomedical Imaging Group, Swiss Federal Institute of Technology, Lausanne, Switzerland) was used to transform nonstandardized preoperative and postoperative radiographs into standardized images. The following parameters were evaluated according to Tawfik *et al.*<sup>[1]</sup>

### Increase in root length

Root lengths were measured (mm) as straight line from the cemento-enamel junction to the radiographic apex of the tooth using the Image-J software. Measurements were done pre- and post-operatively and then the difference in root length was calculated.

### Increase in root thickness

Using the preset measurement scale, the root thickness was measured at the same fixed level (5 mm from the cemento-enamel junction). Measurements were done pre- and post-operatively, and then the difference in root thickness was calculated.

### Decrease in the apical diameter

Apical foramen diameter was measured (mm) pre- and post-operatively according to the preset measurement scale, and the difference in apical diameter was calculated.

### Histopathological evaluation

Sacrifice of the animals was done following the designated evaluation period using an overdose of anesthetic solution (20 mL of thiopental sodium 5% solution). Jaws with the involved teeth were resected, and then each tooth with its surrounding bone was separated with a saw and fixed in 10% buffered formalin solution. The fixed tissues were decalcified in 17% ethylenediaminetetraacetic acid (EDTA) solution for 120 days, dehydrated, and embedded in paraffin blocks. The blocks were sectioned and stained with hematoxylin and eosin. The stained sections were examined under Olympus light microscope (BX60, Olympus Corporation, Japan).

For quantitative analysis, presence or absence of new hard tissue on the internal root canal walls and the presence or absence of vital tissues inside the pulp space and apical closure were measured according to Tawfik *et al.*<sup>[1]</sup>

### Qualitative analysis

Criteria for histological identification of hard structure were recorded according to Tawfik *et al.*<sup>[1]</sup>

### Statistical analysis

Numerical data were explored for normality by checking the distribution of data, calculating the mean and



**Figure 1:** Representative photoradiographs of dog's premolars before (a) and after (b) the induction of periapical lesions



median values as well as using the tests of normality (Kolmogorov–Smirnov and Shapiro–Wilk tests). Numerical data were presented as mean and standard deviation values. All data showed nonparametric distribution. Kruskal–Wallis test was used to compare between the different time periods. Mann–Whitney U-test with Bonferroni's adjustment was used for pair-wise comparisons between the time periods when Kruskal–Wallis test was significant. Friedman's test was used to compare between both groups. Wilcoxon signed-rank test with Bonferroni's adjustment was used for pair-wise comparisons between the groups when Friedman's test was significant. The prevalence of apical closure was presented as frequencies (*n*) and percentages (%). Chi-square test was used to compare between the time periods. Cochran's Q-test was used to compare between both groups. The significance level was set at  $P \leq 0.05$ . Statistical analysis was performed with SPSS® Statistics Version 20 for Windows (IBM Corporation, NY, USA).

## RESULTS

No allergic reaction was seen in any of the experimental dogs. Moreover, all dogs survived the procedures without any complications.

### Radiographic findings

There was a statistically significant difference between propolis group and control group regarding the increase in root length and root thickness as well as decrease in apical diameter ( $P \leq 0.05$ ). Control group showed statistically lower mean changes than propolis group [Table 1 and Figures 2 and 3].

Regarding the subgroups of propolis group, there was a statistically significant increase in the root length and root

thickness and degree of closure of the apical diameter from 2 weeks to 4 weeks as well as from 4 weeks to 8 weeks ( $P \leq 0.05$ ). The mean increase in root length was 0.56, 0.89, and 1.74 mm after 2, 4, and 8 weeks, respectively. The mean increase in root thickness was 0.29, 0.52, and 0.69 mm after 2, 4, and 8 weeks, respectively. The mean decrease in apical diameter was 0.64 (29.3%), 0.93 (40.8%), and 1.48 mm (80.1%) after 2, 4, and 8 weeks, respectively.

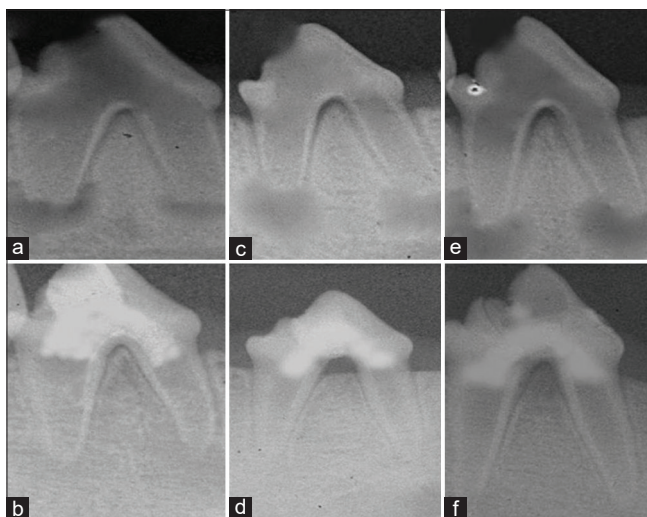
All subgroups in the control group showed no significant differences between the increase in root length (0.01, -0.01, and -0.01 mm at 2, 4, and 8 weeks, respectively) and root thickness (0.00, 0.01, and 0.04 mm at 2, 4, and 8 weeks, respectively) as well as decrease in apical diameter (-0.01, -0.01, and 0.03 mm at 2, 4, and 8 weeks, respectively) through the different time periods ( $P > 0.05$ ).

## Histopathological findings

### Quantitative findings

There were statistically significant differences between propolis group and control group regarding new hard tissue scores and vital tissue scores at all evaluation periods ( $P \leq 0.05$ ). Control group showed statistically lower mean scores than propolis group [Tables 2 and 3]. There was no statistically significant difference between both groups regarding apical closure at 2 and 4 weeks ( $P > 0.05$ ), but there was a statistically significant difference between both groups at 8 weeks ( $P \leq 0.05$ ).

Regarding subgroups of propolis group, there was a statistically significant increase in new hard tissue scores, vital tissue scores, and apical closure from 2 weeks to 4 weeks as well as from 4 weeks to 8 weeks ( $P \leq 0.05$ ). The mean new hard tissue scores were 0.69, 1.31, and

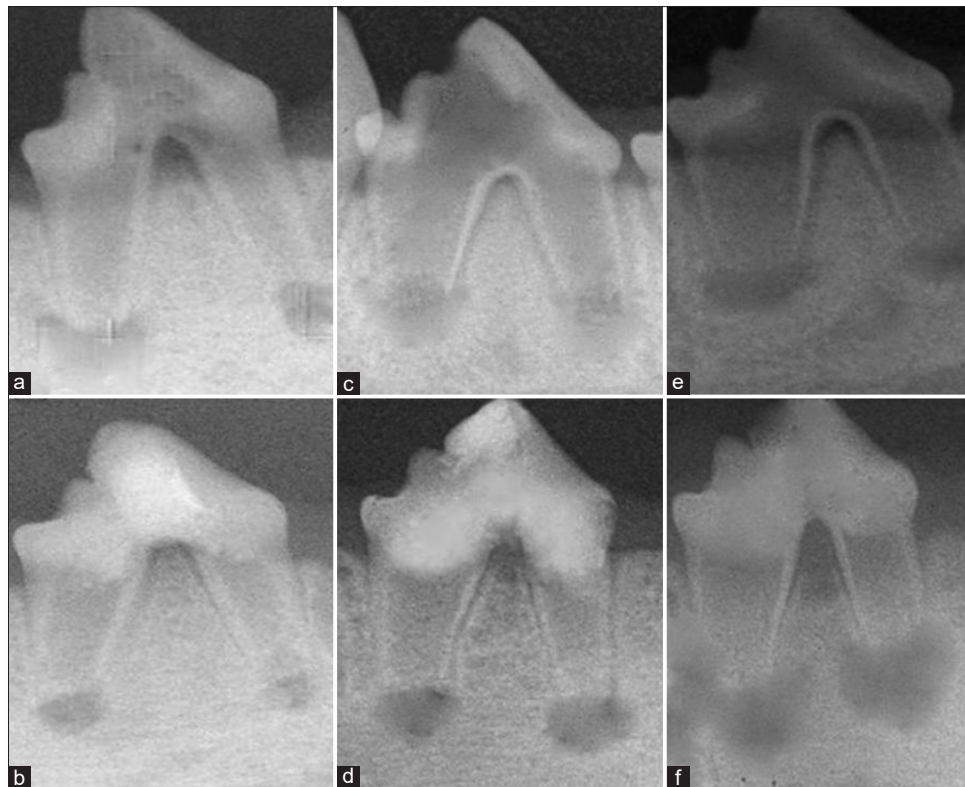


**Figure 2:** Representative photoradiographs of Group I (Propolis group) showing preoperative and postoperative samples after 2 weeks (a and b), 4 weeks (c and d), and 8 weeks of revascularization (e and f)

**Table 1: Changes in root length, root thickness, and apical diameter in all subgroups of both groups: the mean±standard deviation values and results of Friedman's and Wilcoxon signed-rank tests**

Subgroups	Groups		P
	Group I propolis group	Group II control group	
Changes in root length in both groups at each time period			
Subgroup i: 2 weeks	0.56 <sup>a</sup> ±0.20	0.01 <sup>b</sup> ±0.05	<0.001 *
Subgroup ii: 4 weeks	0.89 <sup>a</sup> ±0.36	-0.01 <sup>b</sup> ±0.08	<0.001 *
Subgroup iii: 8 weeks	1.74 <sup>a</sup> ±0.34	-0.01 <sup>b</sup> ±0.12	<0.001 *
Changes in root thickness in both groups at each time period			
Subgroup i: 2 weeks	0.29 <sup>a</sup> ±0.13	0.00 <sup>b</sup> ±0.04	<0.001 *
Subgroup ii: 4 weeks	0.52 <sup>a</sup> ±0.16	0.01 <sup>b</sup> ±0.09	<0.001 *
Subgroup iii: 8 weeks	0.69 <sup>a</sup> ±0.18	0.04 <sup>b</sup> ±0.14	<0.001 *
Changes in apical diameter in both groups at each time period			
Subgroup i: 2 weeks	0.64 <sup>a</sup> ±0.32	-0.01 <sup>b</sup> ±0.07	<0.001 *
Subgroup ii: 4 weeks	0.93 <sup>a</sup> ±0.26	-0.01 <sup>b</sup> ±0.09	<0.001 *
Subgroup iii: 8 weeks	1.48 <sup>a</sup> ±0.31	0.03 <sup>c</sup> ±0.09	<0.001 *

\*Different letters in the same row are statistically significantly different at  $P \leq 0.05$



**Figure 3:** Representative photoradiographs of Group II (control) showing preoperative and postoperative samples after 2 weeks (a and b), 4 weeks (c and d), and 8 weeks of revascularization (e and f)

**Table 2: New hard tissue and vital tissue scores in both groups at each time period: the mean, standard deviation values, and results of Friedman's and Wilcoxon signed-rank tests**

Subgroups	Groups (mean±SD)		P
	Group I propolis group	Group II control group	
Subgroup i: 2 weeks	0.69 <sup>a</sup> ±0.48	0.06 <sup>b</sup> ±0.25	<0.001*
Subgroup ii: 4 weeks	1.31 <sup>a</sup> ±0.70	0.06 <sup>b</sup> ±0.25	<0.001*
Subgroup iii: 8 weeks	1.44 <sup>a</sup> ±0.63	0.06 <sup>b</sup> ±0.25	<0.001*
<b>Vital tissue scores in both groups at each time period</b>			
Subgroup i: 2 weeks	0.69 <sup>a</sup> ±0.48	0.06 <sup>b</sup> ±0.25	<0.001*
Subgroup ii: 4 weeks	1.31 <sup>a</sup> ±0.70	0.06 <sup>b</sup> ±0.25	<0.001*
Subgroup iii: 8 weeks	1.63 <sup>a</sup> ±0.62	0.06 <sup>b</sup> ±0.25	<0.001*

\*Different letters in the same row are statistically significant different at  $P \leq 0.05$ . SD: Standard deviation

**Table 3: The presence of apical closure in both groups at different time periods: The frequencies (n), percentages, and results of Cochran's Q-test**

Subgroups	Groups				P
	Group I propolis group		Group II control group		
	n	Percentage	n	Percentage	
Subgroup i: 2 weeks	2 (16) <sup>a</sup>	12.5	0 (16) <sup>a</sup>	0.0	0.368
Subgroup ii: 4 weeks	4 (16) <sup>a</sup>	25.0	0 (16) <sup>a</sup>	0.0	0.102
Subgroup iii: 8 weeks	13 (16) <sup>a</sup>	81.3	1 (16) <sup>b</sup>	6.3	<0.001*

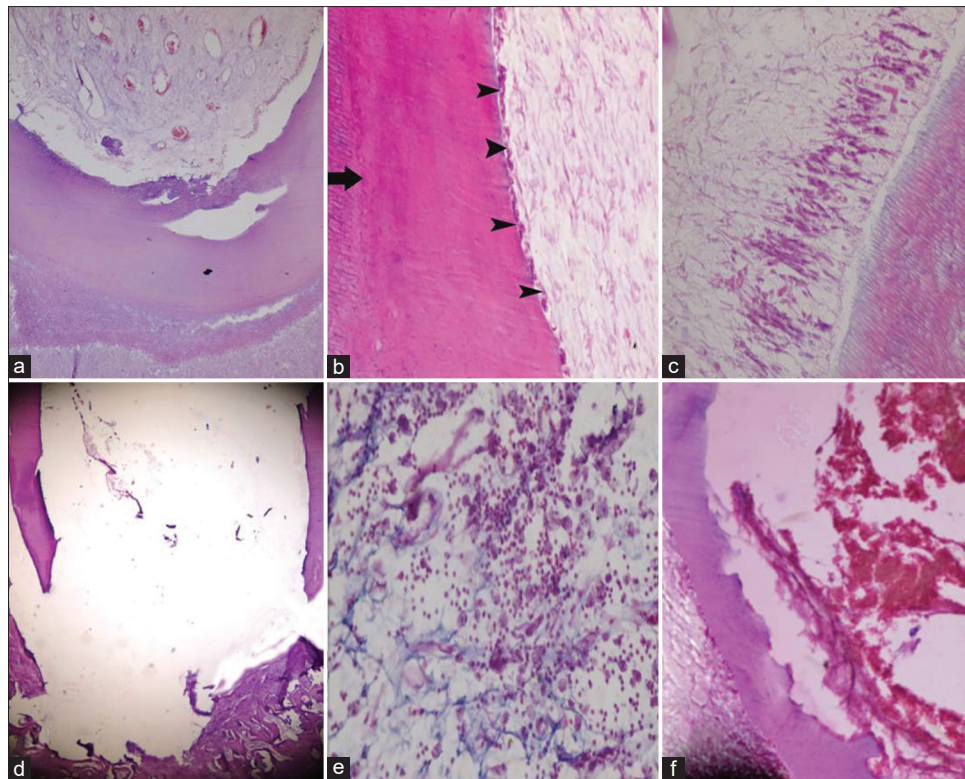
\*Different letters in the same row are statistically significant different at  $P \leq 0.05$

1.63 after 2, 4, and 8 weeks, respectively. The mean vital tissue scores were 0.69, 1.31, and 0.69 mm after 2, 4, and 8 weeks, respectively. The frequency (%) of apical closure was 2 (12.5%), 4 (25%), and 13 (81.3%) after 2, 4, and 8 weeks, respectively.

All subgroups in the control group showed no significant difference between the new hard tissue scores (0.06, 0.06, and 0.06 at 2, 4, and 8 weeks, respectively), vital tissue scores (0.06, 0.06, and 0.06 at 2, 4, and 8 weeks, respectively), and apical closure (0 [0%], 0 [0%], and 1 [6.3%] at 2, 4, and 8 weeks, respectively) through the different time periods ( $P > 0.05$ ).

### Qualitative findings

Histopathological examination of most samples in propolis group at different time periods revealed hard tissue formation along the inner aspect of root canal dentin, resulting in increased dentin thickness and apical closure [Figure 4a]. The newly formed hard tissue resembled cementum-like tissue in some samples, however, in other samples, the new hard tissue resembled dentin with a line of demarcation between the old and new root canal dentin [Figure 4b]. The newly formed dentin exhibited marked decrease in dentinal tubules with the presence of odontoblastic layer [Figure 4c]. Most of the samples



**Figure 4:** Photomicrograph of a sample in propolis group after 4 weeks (a) showing apical closure with dentin formation (H and E,  $\times 100$ ) and a line of demarcation between the dentin of the root and the newly formed dentin (arrow) with marked decrease in its dentinal tubules and the odontoblastic layer (heads of arrows, H and E,  $\times 400$ ) (b). Formation of a new layer of dentin with odontoblastic layer on the inner root wall in propolis group after 8 weeks (H and E,  $\times 400$ ) (c). The control group after 2 weeks (d) showing thin root dentin, open apex, and empty root canal space (H and E,  $\times 100$ ), dense inflammatory cell infiltrate after 4 weeks (e) (H and E,  $\times 200$ ) and internal root resorption after 8 weeks (f) (H and E,  $\times 200$ )

showed inward growth of soft tissue in the pulp space. In some samples, the histological features of the new soft tissue resembled pulp-like fibrous connective tissue devoid of odontoblastic layer. However, there was pulp-like tissue with odontoblastic lining in the samples, showing new dentin formation.

Histopathological evaluation of control group along all evaluation periods revealed thin root canal walls, wide open apex, no formation of new mineralized tissues, either empty root canal space [Figure 4d] or severely inflamed connective tissue [Figure 4e], and resorptive defects on the inner aspect of the root [Figure 4f].

## DISCUSSION

Regenerative endodontics is a recent therapeutic technique allowing root maturation and depends on biologically based procedures.<sup>[13]</sup> Regenerative therapy to save teeth is better than implants.<sup>[14]</sup> Revascularization is a simple protocol of regenerative endodontics, and disinfection of the necrotic root canal space has a crucial role in successful revascularization due to the presence of bacterial growth inside the root canal system.<sup>[2,15]</sup> This study

compared the radiographic and histopathologic progress in root maturation of necrotic immature teeth following disinfection with propolis or just irrigation without application of intracanal medication.

The dogs used in this study were aged 6–9 months because dogs at this age range have immature permanent teeth and can withstand general anesthesia.<sup>[1,3]</sup> Dental calculus was collected to induce pulp necrosis and periapical pathosis because it is easily collected, crushed, and prepared as a suspension. No instrumentation was performed in this study to avoid tooth fracture due to weakening of the root dentin. Furthermore, closure of dentinal tubules could develop due to the formation of smear layer.<sup>[16]</sup> Moreover, avoiding instrumentation is necessary for the preservation of remnant viable stem cells. Thus, disinfection of necrotic immature teeth was mainly depending on the chemical effect of irrigants and intracanal medicaments. Ethylenediaminetetraacetic acid is usually used to release dentin matrix-associated growth factors in the revascularization procedure, however it was not used in this study to exclude any additional effect rather than the effect of the medications on regeneration or repair.



The choice of propolis as an alternative intracanal medication in revascularization was to overcome the complications that may arise from TAP. Moreover, propolis as a natural product is more biocompatible with periapical tissues than the existing intracanal medicaments.<sup>[4,17]</sup> TAP and propolis are equally effective in the eradication of bacteria from the root canal space.<sup>[4]</sup> In addition, propolis maintained PDL cell viability when used as a storage medium of avulsed teeth.<sup>[10]</sup>

In the present study, propolis was able to control the infection through its antimicrobial action. The antimicrobial activity of propolis could be attributed to a synergism between flavonoids (galangin, quercetin, and pinocembrin), hydroxyacids (benzoic, cinnamic, and caffeic acids), and sesquiterpenes.<sup>[18]</sup> Flavonoids inhibit the bacterial RNA-polymerase and destruct the microbial membrane, resulting in structural and functional damages.<sup>[19]</sup> In addition, propolis has immune stimulant effect through stimulating cellular immunity and enhancing phagocytosis.<sup>[20]</sup> Furthermore, propolis has immunomodulatory, antioxidative, and healing effects due to its ability to inhibit free radical formation.<sup>[21]</sup>

One of the detrimental factors for successful regenerative endodontics is the absence of scaffold upon which newly formed tissue could grow. The present study was based on the fact that blood clot acts as a scaffold. Bleeding in the canal was induced by irritation of the periapical tissue with a small sterile file.<sup>[22]</sup> Blood clot is an easy, rapid, and efficient way to produce scaffold that is essential for the population and differentiation of stem cells as well as growth factor release.<sup>[23]</sup>

Radiographic evaluation showed statistically significant increase in dentin thickness and statistically significant decrease in apical diameter ( $P \leq 0.05$ ) during various evaluation periods with the highest mean increase at 8 weeks, which is a logical outcome for healing and root maturation. Similar findings were reported in previous studies.<sup>[1,3,4]</sup>

In the present study, the newly formed hard tissue responsible for the increase in root thickness was cementum-like tissue. These results are in accordance with those of previously published researches.<sup>[24]</sup> In most samples, the nature of the regenerated tissue was pulp-like fibrous connective tissue devoid of odontoblastic layer with calcified bony islands and cementum within the pulp. This is in agreement with several previous studies.<sup>[25-27]</sup> On the other hand, Tawfik *et al.*<sup>[1]</sup> found that the newly formed tissue resembled periodontal tissue in structure.

An interesting finding in the present study was that some specimens from the propolis group showed pulp-like tissue with the presence of odontoblastic layer lining the newly formed dentin. This is in agreement with one case from Saoud *et al.*<sup>[27]</sup> study that rendered this pattern of regeneration to the remnant surviving vital pulp cells. In contrast, Pagliarini *et al.*<sup>[28]</sup> observed absence of pulp-like tissue formation in all specimens treated with propolis. The capability of propolis to preserve vital tissues is well established.<sup>[29]</sup> In addition, the regenerative potential of propolis was documented by Saleh *et al.*<sup>[6]</sup> who found that pulp capping with propolis was associated with the formation of tubular dentin similar to primary dentin with no pores or connective tissue.

The increase in root thickness by cemental deposition on the inner dentinal wall and the presence of intracanal cementum and bone could be attributed to the bleeding induction step as the induced blood is loaded with mesenchymal stem cells from periapical bone. The increase in root thickness can also be explained by the induced blood clot. Furthermore, the formed blood clot serves as a regenerative platform and provides essential growth factors and undifferentiated mesenchymal cells for new tissue growth and development.<sup>[30]</sup> Meanwhile, the presence of newly formed dentin layer on the inner root wall and pulp-like tissue with odontoblastic layer in some specimens of the propolis group may be produced by dental pulp stem cells and periapical stem cells, which are resistant to destruction even in the presence of inflammation<sup>[31]</sup> or remnants of surviving pulp cells at the apical terminus of the canal. These cells may have proliferated into the newly formed matrix (blood clot). The proliferated cells differentiate into odontoblastic cells under the organizing influence of cells of epithelial root sheath of Hertwig.

The control group showed failure of revascularization, which was displayed radiographically in the arrested development of roots in terms of length, thickness, as well as wide apical foramen. Moreover, this failure was displayed histologically in the form of empty pulp space, severe inflammatory reaction, and resorptive defects on the inner and apical sides of the root. These findings were attributed to the persistence of the previously induced infection. The failure of revascularization in control group could be attributed to the failure of NaOCl irrigation alone to achieve disinfection of the root canal. In this regard, NaOCl is not the most effective antimicrobial agent for pulp regeneration because NaOCl has no lasting antibacterial effect in the root canal environment.<sup>[32]</sup>

In contrast, McCabe reported a successful case of single-visit revascularization following irrigation as a

sole disinfectant without instrumentation or intracanal medication.<sup>[33]</sup> This conflict may be due to the difference in the NaOCl concentration used. McCabe used 5% NaOCl aided by ultrasonic agitation versus 2.5% NaOCl used in this study. In addition, the presence of only acute apical periodontitis related to the necrotic pulp as opposed to an infected periapical lesion in this study may be another factor contributing to the difference in outcomes.

The main limitation of this study was the short time of evaluation (8 weeks). Therefore, further studies are recommended for long-term assessment of the newly formed vital tissue inside the root canal space after using propolis as an intracanal medicament during the revascularization of immature permanent teeth with necrotic pulp.

The clinical significance of this study is that propolis can be used as an intracanal medication during regenerative endodontic therapy of necrotic immature permanent teeth.

## CONCLUSION

Propolis, as an intracanal medication, has good outcomes on the regenerative potential of necrotic immature permanent teeth after revascularization, regarding hard-tissue deposition and soft-tissue formation.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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