

RESEARCH ARTICLE

The Effect of Different Formulations of Calcium Hydroxide on Healing of Intentionally Induced Periapical Lesions in Dogs

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ARTICLE HISTORY

Received: December 22, 2011

Revised: May 22, 2012

Accepted: July 08, 2012

Key words:

Calcium Hydroxide

Chlorohexidine

Dogs

Iodoform

Periapical Tissue

Saline

ABSTRACT

The aim of the present work is to study the effect of different formulations of Ca (OH)₂ on healing of induced periapical lesions in dog. A total of 96 teeth with intentionally induced periapical lesions were classified according to the observation period into three groups; I, II and III (2 dogs each). Each group was subdivided into four subgroups (8 teeth each) namely; A, B, C and D which were dressed with Ca(OH)₂ with saline, Ca(OH)₂ with chlorhexidine, Ca(OH)₂ with iodoform and control respectively. Histopathological findings showed that the apical and periapical repair were better in subgroup A than in other subgroups in all groups. Total inflammatory cell count was significantly different between the four subgroups in group I. In both groups II and III, there was no significant difference between subgroups B and C. In conclusion, the use of saline as a vehicle for Ca (OH)₂ has a favorable action on periapical tissue healing in endodontically treated dogs.

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To Cite This Article: El-Ashry S, A. Abu-Seida, H. Al-Boghdady, K. El-Batouty and M Abdel-Fattah, 2013. The effect of different formulations of calcium hydroxide on healing of intentionally induced periapical lesions in dogs. Pak Vet J, 33(1): 48-52.

INTRODUCTION

Calcium hydroxide is considered to be the most frequent intra canal medicament. It gains its action through its chemical dissociation into calcium ions and hydroxyl ions. Many vehicles were used for mixing of calcium hydroxide producing different degrees and rates of chemical dissociation. Also some vehicles have direct antibacterial and anti-inflammatory effects which synergist the action of calcium hydroxide such as iodine, electrophoretically activated copper (Fuss *et al.*, 2002) and chlorhexidine (Gomes *et al.*, 2003; Podbielski *et al.*, 2003; Schafer and Bossmann, 2005)

The released hydroxyl ions from calcium hydroxide raise the pH of the surrounding media (Esberard *et al.*, 1996) producing unsuitable environment for bacterial growth. Calcium hydroxide is considered a potent anti-inflammatory compound through decreasing the adhesion capacity of the macrophages (Segura *et al.*, 1997), neutralizing the endotoxins and deactivation of many inflammatory mediators (Khan *et al.*, 2008).

Ca(OH)₂ based materials had a favorable action on periapical tissue healing and repair of orthodontic root

resorption in dogs' teeth (Leonardo *et al.*, 1997; Filho *et al.*, 2002; De Souza *et al.*, 2009; Yildirim *et al.*, 2011). Borlina *et al.* (2010) added that apical foramen widening and calcium hydroxide-containing sealer were more favorable to the healing of chronic periapical lesions in dogs. All these advantages of Ca (OH)₂ are opposed by a critical disadvantage which is the weakening effect of the root canal dentin especially with the prolonged use of calcium hydroxide. Ca (OH)₂ produces denaturation of the dentinal protein and affects on the mineral contents of the teeth by its strong alkalinity (Rosenberg *et al.*, 2007; Andreasen and Farik, 2002). This extra weakening of the endodontically treated teeth makes it mandatory to determine the ideal combination and time for the use of calcium hydroxide to gain the maximum advantages of its use with minimizing of its disadvantages. Therefore the aim of the present work is to study the effect of different formulations of Ca (OH)₂ on healing of induced periapical lesions in dogs.

MATERIALS AND METHODS

Animals: In the present study, six healthy mongrel dogs were used. The age and weight of the dogs were 10-18

months and 15-25 kg, respectively. In each dog, sixteen teeth were used (four teeth in each quadrant) including the second, third, fourth premolar and first molar teeth. The dogs were classified according to the observation period into three groups. Groups I, II and III (2 dogs each) were sacrificed after two, four and six weeks. Each group was subdivided into four subgroups according to the dressing material. In subgroup A, 8 teeth were dressed with Ca (OH)₂ mixed with saline. Subgroup B had 8 teeth which were dressed with Ca (OH)₂ mixed with chlorhexidine. In subgroup C, 8 teeth were dressed with Ca (OH)₂ mixed with iodoform. Eight teeth were used as control in subgroup D.

Induction and dressing of periapical lesions: General anesthesia for each dog was performed using atropine sulphate at a dose of 0.05mg/kg subcutaneously and xylazine HCl at a dose of 1.1mg/kg intramuscularly as premedication. The anesthesia was induced by using ketamine HCl at a dose of 5mg/kg intravenously using the cephalic vein. The anesthesia was maintained by 25mg/kg incremental doses of 2.5% solution of thiopental sodium (Torad, 2000). Access cavities were opened in the desired teeth using a round bur size 2 at the speed of 40000 rpm. A k-file # 15 was introduced into the root canals for traumatization of the pulp. The accesses were left open for three weeks to induce periapical lesions (Thibodeau *et al.*, 2007).

K-files # 30, 35 and 40 were used for pulp extirpation together with irrigation by saline solution until there were no pulp tissues in the root canal. The dressing materials were applied to the root canals using metapex plastic application tips. The access cavities were closed using zinc phosphate cement. Euthanasia of the dogs was done at the end of each experimental period using 20 ml of 5% Thiopental sodium rapidly injected in cephalic vein. Block sections including each tooth and its surrounding bone were obtained.

Histopathological examination: The apical third of the root was carefully removed with water cooled diamond wheel stone. Teeth were placed in 10% neutral buffered formalin for 72 hours then decalcified in 17% ethylene diamine tetra acetic acid (EDTA). A volume of 150 times that of the tissue was renewed every 5 to 7 days during decalcification process. A fine needle was used to perforate the specimens to allow the penetration of EDTA. The specimens were examined weekly for decalcification. After decalcification, specimens were dehydrated in 70% ethanol, embedded in paraffin blocks and serially sectioned in a buccolingual plane to the tooth main vertical axis into sections of 6 microns thickness.

The specimens were evaluated by histopathological examination and the total inflammatory cells count. For each slide, four representative fields were analyzed at the magnification power of x400. The total number of the inflammatory cells (plasma cell and lymphocytes) of each microscopic field was counted using the image analysis software (Image j, 1.41, NIH, USA). Images were converted into 8 bit grey scale type after being corrected for brightness and contrast followed by automatic color thresholding. The threshold for inflammatory cells was according to the area of these cells ranged from 170-260 pixels to exclude counting of other cells such as endothelial cells and fibroblasts.

The data of inflammatory cell count were subjected to statistical analysis using ANOVA and T-test to test the statistical difference of each group and subgroups.

RESULTS

Histopathological evaluation: In group I, microscopic examination of subgroup A revealed the presence of generalized edema in the periapical region, numerous blood vessels, cementum resorption (Fig. 1A) and inflammatory infiltrates composed of mononuclear cells. While in subgroup B; periapical generalized edema and inflammatory infiltrate mainly lymphocytes and plasma cells together with fibrillar dissociation were seen (Fig. 1B).

In subgroup C; periapical granulation tissue infiltrated with a large number of inflammatory cells mainly lymphocytes and surrounded by delicate connective tissue fibrils, high vascularity and edema (Fig. 1C) was seen. Microscopic examination of subgroup D revealed numerous dilated blood vessels with periapical granulation tissue. There was active alveolar bone and cementum resorption (Fig. 1D). Osteoclasts among mononuclear inflammatory cells and delicate connective tissue fibrils were also noticed.

In group II, subgroup A showed generalized edema in the periapical area, cementum resorption, minimal inflammatory cells, fibrillar dissociation, degenerated connective tissue and almost complete lack of inflammatory cells and blood vessels (Fig. 2A). While in subgroup B; periapical granulation tissue surrounded by generalized edema, areas of congested blood vessels, mononuclear inflammatory cell infiltrate and areas of fibrillar dissociation were observed (Fig. 2B).

Microscopically, subgroup C showed periapical granulation tissue with intense inflammatory cells (lymphocytes, plasma cells and macrophages), areas of fibrillar dissociation and edema (Fig. 2C). In subgroup D; a central area of hyaline degeneration surrounded by vascular granulation tissue, mononuclear inflammatory cells and edema were noticed (Fig. 2D).

In group III, subgroup A showed absence of any periapical lesion in the periodontal membrane area, direct contact of the periodontal ligament tissues with the surrounding bone, congested blood vessels and nearly complete absence of inflammatory cellular infiltrate (Fig. 3A). Subgroup B microscopically showed; cementum resorption, a mass of degenerated connective tissue in the periapical area surrounded by edematous connective tissue and mononuclear inflammatory cell infiltrate (Fig. 3B).

In subgroup C; Microscopic examination revealed large periapical edematous area containing necrotic tissue together with fibrillar dissociation, mononuclear inflammatory cells and congested blood vessels (Fig. 3C). While in subgroup D; a central area of degenerated connective tissue surrounded by vascular granulation tissue and mononuclear inflammatory cells was seen (Fig. 3D).

Total inflammatory cell count: The data was collected and statistically analyzed in Table 1. In group I, there was statistically significant difference between the four subgroups. In both groups II and III, there was no statistically significant difference between subgroup B and C. However, the difference was significant when comparing subgroup

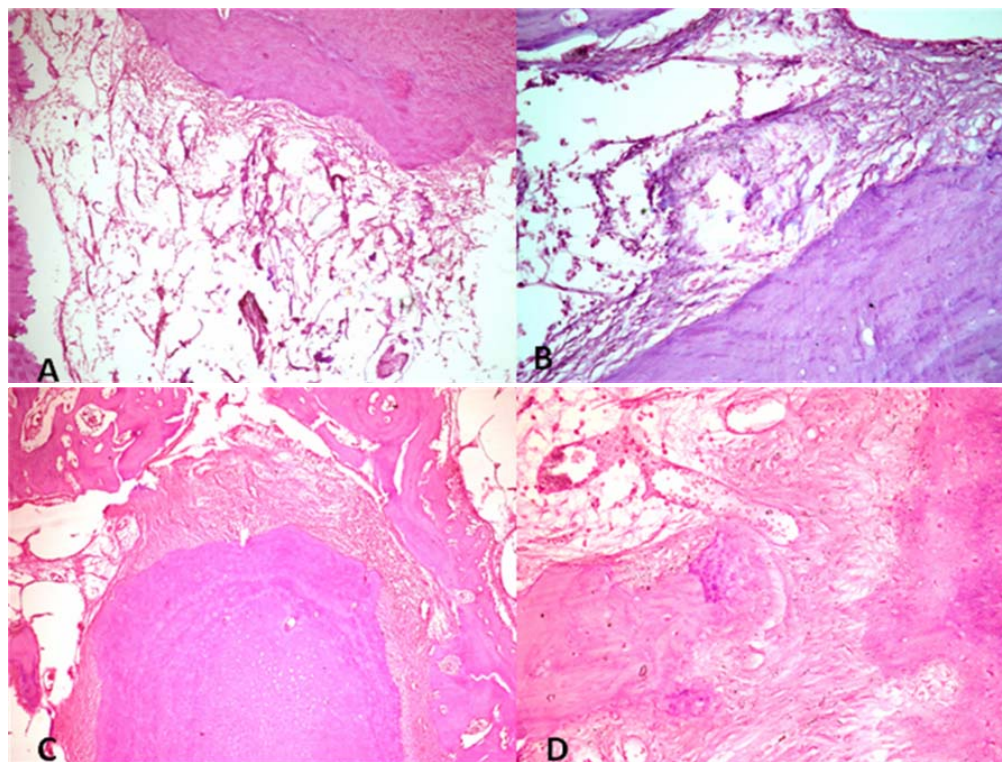


Fig. 1: Photomicrographs of group (I) (2 weeks): (A) $\text{Ca}(\text{OH})_2$ + Saline Subgroup showing generalized edema with connective tissue fibrils (H & E X10). (B) $\text{Ca}(\text{OH})_2$ + CHX subgroup showing chronic inflammatory cells (lymphocytes and plasma cells) surrounded by edematous connective tissue. Fibrillar dissociation was seen in the periodontal ligament (H & E X40). (C) $\text{Ca}(\text{OH})_2$ + Iodoform subgroup showing periapical granulation tissue surrounded by edema (H & E X10). (D) Control subgroup showing dilated and congested blood vessels among mononuclear inflammatory cells and delicate connective tissue fibrils. Notice the presence of osteoclasts (H & E X40).

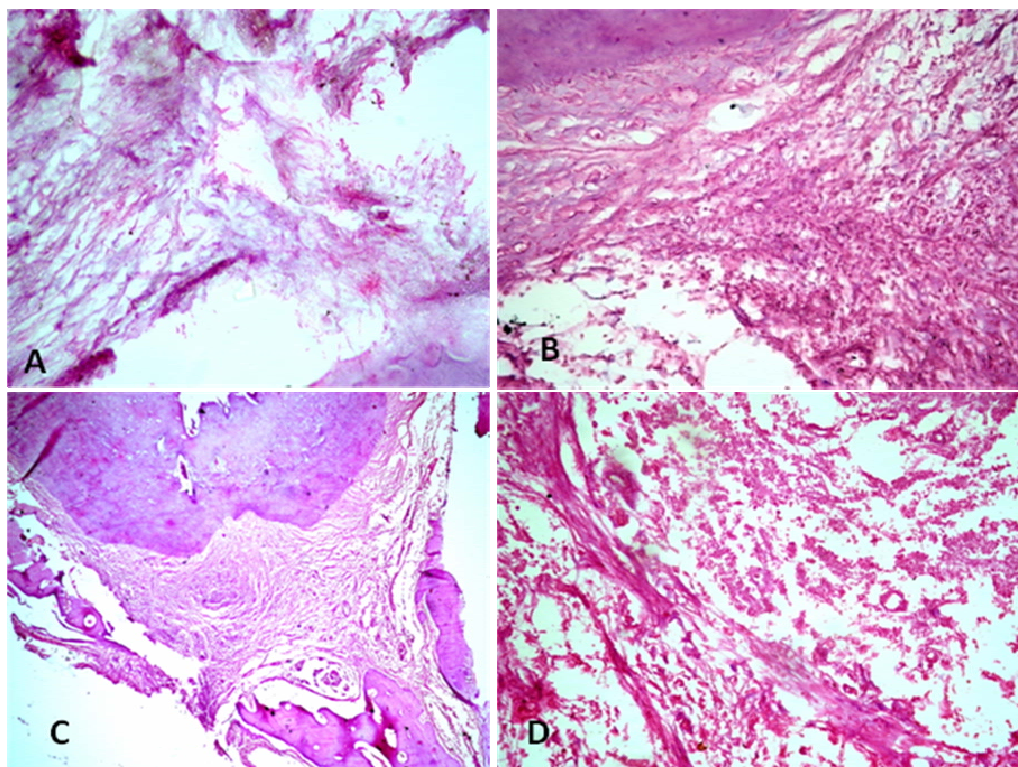


Fig. 2: Photomicrographs of group (II) (4 weeks): (A) $\text{Ca}(\text{OH})_2$ + Saline Subgroup showing fibrillar dissociation with minimal cellularity and vascularity in degenerated connective tissue (H & E X40). (B) $\text{Ca}(\text{OH})_2$ + CHX subgroup showing mononuclear inflammatory cell infiltrate, edema and fibrillar dissociation within the periodontal ligament (H & E X40). (C) $\text{Ca}(\text{OH})_2$ + Iodoform subgroup showing periapical granulation tissue with areas of edema (H & E X10). (D) Control subgroup showing mononuclear inflammatory cells, blood vessels, hyaline degeneration and areas of edema. (H & E X40).

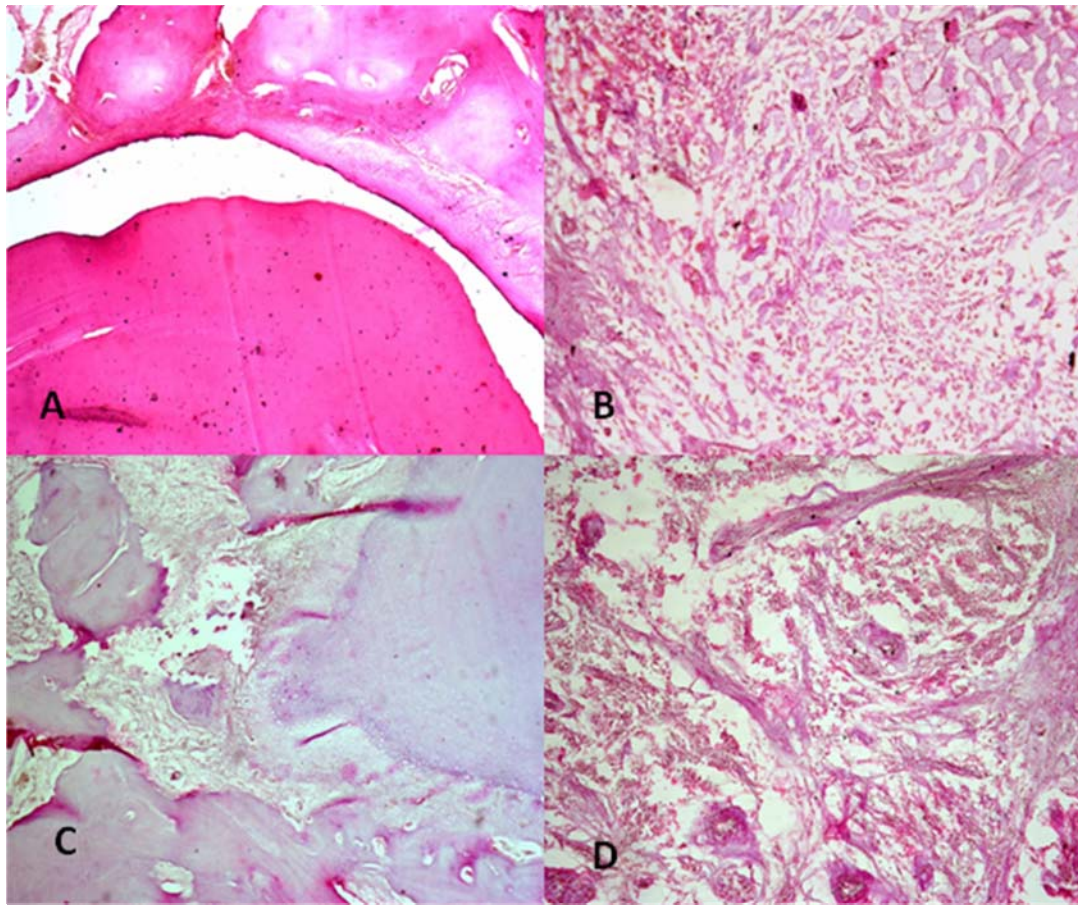


Fig. 3: Photomicrographs of group (III) (6 weeks): (A) $\text{Ca}(\text{OH})_2$ + Saline Subgroup revealing absence of any periapical lesion and direct contact of the periodontal ligament with the adjacent bone (H&E X10). (B) $\text{Ca}(\text{OH})_2$ + CHX subgroup showing mononuclear inflammatory cells, areas of hyaline degeneration and edema (H & E X40). (C) $\text{Ca}(\text{OH})_2$ + Iodoform subgroup showing a large edematous area in the periapical tissue (H & E X10). (D) Control subgroup showing mononuclear inflammatory cells, areas of hyaline degeneration, fibrillar dissociation and numerous blood vessels (H&E X40).

Table 1: The main total inflammatory cell count in the different groups and subgroups

Subgroup	A $\text{Ca}(\text{OH})_2$ + Saline	B $\text{Ca}(\text{OH})_2$ + CHX	C $\text{Ca}(\text{OH})_2$ + Iodoform	D Control
Group I	19±5a	49±8b	32±5c	89±12d
Group II	13±3b	23±3d	21±3.6d	63±9c
Group III	2±1.5c	13±4.8a	13±5.3a	36±4b

Different small letters indicate significant ($P \leq 0.05$) difference between subgroups within the same group

A or D with the other subgroups. It was found that there was statistically significant difference between the three groups for all subgroups.

DISCUSSION

Intracanal medication in pulpectomy therapy is used between appointments with the objective of reducing pain and inflammatory processes in pulp and periapical tissues (Ramos *et al.*, 2012). The main aim for the use of the calcium hydroxide as an intra-canal medicament is its biological action through the inhibitory effect on the bacterial viability and against most of the bacterial byproducts and the activation of the healing potentiality of the periapical tissues. Cleaning and shaping of the root canal of the dogs' teeth with normal saline was done to

avoid the antibacterial effect of any other irrigation's solution and also to standardize the effect of the tested materials.

Results showed that the use of any formulation of calcium hydroxide accelerates the healing potentiality of the periapical tissue. This is related to the antibacterial effect of calcium hydroxide (Podbielski *et al.*, 2003), the inactivation of bacterial byproducts and prevention of macrophage adherence capacitance so it decreases the inflammatory reaction of the periapical tissue (Segura *et al.*, 1997; Khan *et al.*, 2008). All these actions with the alkaline medium created by calcium hydroxide allow the inhibition of the osteoclastic activity which is the main cells for bone resorption (Sazak *et al.*, 1996; Ingle *et al.*, 2008) in addition to the activation of the osteoblasts to form new collagen fibers (Leonardo *et al.*, 1997).

The histopathological analysis showed that there was better apical and periapical repair in the teeth in subgroup A than in other subgroups. This could be explained by high rate of ionic dissociation of the calcium hydroxide with saline. Subgroup A showed the least inflammatory cells count compared with other subgroups in all groups. In group III, no inflammation was seen in subgroup A. This means that the using of saline as a vehicle for $\text{Ca}(\text{OH})_2$ potentiates the anti inflammatory action of $\text{Ca}(\text{OH})_2$.

It was noticed that there was statistically significant difference in total inflammatory cell count between the three time intervals (2, 4 and 6 weeks). This could be attributed to the continuous release of hydroxyl and calcium ions with the continuous ionic dissociation of the tested calcium hydroxide formulations and its anti-inflammatory action. These findings were in agreement with the results of a previous study (Khan *et al.*, 2008) and in disagreement with another study (Filho *et al.*, 2002) which concluded that all calcium formulations causing an inflammatory response.

Conclusions: The use of saline as a vehicle for Ca (OH)₂ has a favorable action on periapical tissue healing in endodontically treated dogs' teeth.

REFERENCES

- Andreasen J and B Farik, 2002. Long-term calcium hydroxide as a root canal dressing may increase risk of root fracture. *Dent Traumatol*, 18: 134-137.
- Borlina SC, V De Souza, R Holland, S Murata, JE Gomes-Filho, E Dezan Junior, JJ Marion and A Neto Ddos, 2010. Influence of apical foramen widening and sealer on the healing of chronic periapical lesions induced in dogs' teeth. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 109: 932-940.
- De Souza R, V De Souza, V Holland, J Filho, S Murata and C Sonoda, 2009. Effect of calcium hydroxide-based materials on periapical tissue healing and orthodontic root resorption of endodontically treated teeth in dogs. *Dent Traumatol*, 25: 213-218.
- Esberard E, D Carnes and C Del Rio, 1996. Changes in pH at the dentin surface in root obturated with calcium hydroxide pastes. *J Endod*, 22: 402-405.
- Filho PN, MR Leonardo, L Silva and L Assed, 2002. Radiographic evaluation of the effect of endotoxin (LPS) plus calcium hydroxide on apical and periapical tissues in dogs. *J Endod*, 28: 694-696.
- Fuss Z, A Mizrahi, S Lin and O Chirniak, 2002. A laboratory study of the effect of calcium hydroxide mixed with iodine or electrophoretically activated copper on bacterial viability in dentinal tubules. *Int Endod J*, 35: 522-526.
- Gomes B, S Souza and C Ferraz, 2003. Effectiveness of 2% chlorhexidine gel and calcium hydroxide against *Enterococcus faecalis* bovine root dentin in vitro. *Int Endod J*, 36: 267-275.
- Ingle JL, LK Bakland and J Baumgartner, 2008. *Ingle's Endodontics*. 6th Ed, Ajanta Offset and Packaging Limited, city, India, pp: 502-504.
- Khan A, X Sun and K Hargreaves, 2008. Effect of calcium hydroxide on proinflammatory cytokines and neuropeptides. *J Endod*, 34: 1360-1363.
- Leonardo M, L Silva, L Utrilla, S Assed and L Ether, 1997. Calcium hydroxide root canal sealers- Histopathologic evaluation of apical and periapical repair after endodontic treatment. *J Endod*, 23: 428-432.
- Podbielski A, A Spahr and B Haller, 2003. Additive antimicrobial activity of calcium hydroxide and chlorhexidine on common endodontic bacterial pathogens. *J Endod*, 29: 340-345.
- Ramos IF, MT Biz, N Paulino, A Scremin, A Della Bona, FB Barletta and JA Figueiredo, 2012. Histopathological analysis of corticosteroid-antibiotic preparation and propolis paste formulation as intracanal medication after pulpectomy: an in vivo study. *J Appl Oral Sci*, 20: 50-56.
- Rosenberg B, P Murray and K Namerow, 2007. The effect of calcium hydroxide root filling on dentin fracture strength. *Dent Traumatol*, 23: 26-29.
- Sazak H, M Gunday and C Alaty, 1996. Effect of calcium hydroxide and combinations of Idermix and calcium hydroxide on inflamed pulp of dogs teeth. *J Endod*, 22: 447-449.
- Schäfer E and K Bossmann, 2005. Antimicrobial efficacy of chlorhexidine and two calcium hydroxide formulations against *Enterococcus faecalis*. *J Endod*, 31: 53-56.
- Segura J, R Llamas and A Manzanaris, 1997. Calcium hydroxide inhibits substrate adhesion capacity of macrophages. *J Endod*, 32: 444-447.
- Thibodeau B, F Teixeira, J Yamauchi, J Daniel, D Caplan and M Trope, 2007. Pulp revascularization of immature dog teeth with apical periodontitis. *J Endod*, 33: 680-689.
- Torad, FA, 2000. Evaluation of some anesthetic techniques in pit animals under local circumstances. M.V.Sc. thesis, Faculty of Veterinary Medicine, Cairo University.
- Yildirim S, A Can, M Arican and MC Embree and JJ Mao, 2011. Characterization of dental pulp defect and repair in a canine model. *Am J Dent*, 24: 331-335.