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Full Length Research Paper

Effect of intracanal administration of association between calcium hydroxide paste and capsaicin in the periapical region of dog's teeth

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The aim of this study was to histologically examine the periapical region of dog teeth with regard to the inflammatory and resorptive effects of 0.025 and 0.05% capsaicin when introduced into the root canal. Fifty-four root canals were prepared and filled with the following: Group I (calcium hydroxide paste; control group), Group II (0.025% capsaicin and calcium hydroxide paste), and Group III (0.05% capsaicin and calcium hydroxide paste). Serial sections of the teeth were prepared for histomorphological descriptive analysis of the periapical region at 7, 14, and 30 days. The results presented a favorable diagnosis, in which resorption and inflammation were found in all groups, followed by tissue repair.

Key words: Capsaicin, calcium hydroxide, inflammation.

INTRODUCTION

The periapical region is a complex structure that surrounds the tooth apex with distinct cementum, periodontal ligaments, and alveolar bone. This region has a high capacity for repair, because clinical procedures maintain the tissue integrity (Leonardo, 2005). Tissue repair is a process by which the modified periapical tissues are replaced by original tissue to restore its function and architecture. Inflammation and repair are independent entities that form part of the tissue injury response process (Trowbridge and Emiling, 1997). The material used to seal the root canal is an important factor for prognosis and may directly interfere with repair. Calcium hydroxide is a white, fine, and odorless powder. It is the most widely used intracanal dressing, because of its biological and antimicrobial effects, and its use in endodontic procedures has been well established (Maekawa et al., 2011), in addition to having excellent biological qualities (Binnie and Rowe, 1973). Capsaicin is a pungent ingredient found in a wide variety of red peppers of the genus *Capsicum*. It has been used topically on the oral mucosa for the treatment of neuropathic pain (Vickers et al., 1998). Its mechanism of action consists of nociceptor binding, with initial neuronal excitation and increased local sensitivity. At this stage, capsaicin application is associated with burning and stinging sensations and cutaneous vasodilation (Flores et al., 2012).The adverse effects of topical capsaicin, the intensity of which depends on the concentration of the formulation, are mainly derived from the necessity for repeated applications that compromise patient adherence to treatment (Flores et al., 2012) The hypothesis of this study was that the introduction of intracanal capsaicin may eliminate the burning sensation and reduce the need for repeated applications. However, the dental and periodontal tissue response to contact with this substance is unknown. Therefore, the aim of this study

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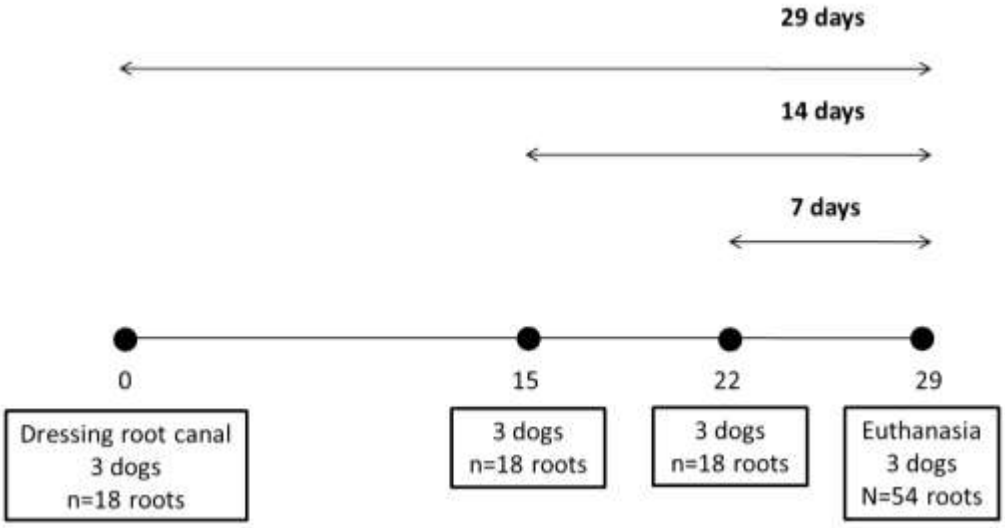

Figure 1. Time course for the entire experiment.

was to histopathologically evaluate the effects of intracanal capsaicin administration on periapical tissue in dog teeth.

MATERIALS AND METHODS

Three adult beagle breed dogs, weighing an average of 15 kg, were used in this study. The research was approved by the Ethics in Animal Research Committee of the State University of Maringá in compliance with the Ethical Guidelines of Animal Experimentation (protocol no. 013/2011-CEEA/UEM). The analyzed teeth included the central and lateral incisors, second and third upper premolars, and second, third, and fourth lower premolars, totaling 54 root canals.

Endodontic procedure

The animals were intravenously anesthetized with 0.03 mg/kg of 0.2% acepromazine and 3 µg/kg fentanyl citrate, and Ringer's lactate solution was administered to maintain venous access. Anesthesia was induced with intravenous 3 mg/kg propofol and 2 mg ketamine to 10% (8 mg/kg ketamine; Agener Union Chemical Pharmaceutical National, Brazil). Anesthesia was maintained with isoflurane, and 0.5% bupivacaine was used for local anesthetic block.

The endodontic procedure began with absolute isolation of the tooth, and then the pulp chamber was accessed with a 1090 cylindrical diamond bur. The pulp tissue was extirpated with #15 K-files.

The teeth were instrumented over #50 K-files using the crown-down technique at the canal dentin-cement limit determined by touch. During instrumentation, the root canals were irrigated with 2.5% sodium hypochlorite. The apical foramen was cross-sectioned with Gates Glidden n1 (Dentsply/Maillefer Instruments SA) and was radiographically confirmed. Ethylenediaminetetraacetic acid was used for 3 min for smear layer removal, followed by final irrigation with 2.5% sodium hypochlorite. Afterward, the root canals were filled with the following: Group I (control group; calcium hydroxide + propylene glycol + topical anesthetic), Group II (calcium hydroxide + propylene glycol l + topical anesthetic + 0.025% capsaicin), and Group III (calcium hydroxide + propylene glycol + topical anesthetic + 0.05% capsaicin).

The cavities were sealed with mineral trioxide aggregate (MTA) (Ângelus, Ind. de Produtos Odont. Ltda, Londrina, Brazil), Cimpat (Septodont, Brasil Ltda, Barueri, Brazil), and composite resin (Charisma, Heraeus Kulze, São Paulo, Brazil).

Experimental time course

The experimental procedure was split into three periods, each involving 18 root canals, with six canals from each dog. Fifteen days after the first experiment, the second experiment was conducted, followed 7 days later by the third experiment (Figure 1). Analyses were performed at intervals of 7, 14, and 29 days.

Finally, the dogs were euthanized with an overdose of anesthetic 7 days after the last experimental period. The maxillaries were cut, fixed in 10% formalin for 10 days, and decalcified in sodium citrate and formic acid for 25 days. Each tooth with sustention tissue was then processed for embedding in paraffin. Sections (6 µm) were cut and stained with hematoxylin and eosin.

The histomorphological parameters were based on Holland et al. (2007). Chronic or acute inflammatory infiltrate was subjectively classified as absent, mild, moderate, or severe. Fibrosis and mineral tissue resorption of the dentin, cementum, and bone were classified as being present or absent.

RESULTS AND DISCUSSION

Inflammation is a defense mechanism that promotes the repair of damaged tissue. When successful, the inflammatory process promotes the destruction and elimination of the injurious agent, followed by repair.In this study, 7 days after the first root canal dressing in the control group and 0.025 and 0.05% capsaicin groups, acute moderate cellular infiltrate was observed in the periapical region with neutrophilic predominance. The surrounding area of the root apex, previously occupied by the alveolar bone,

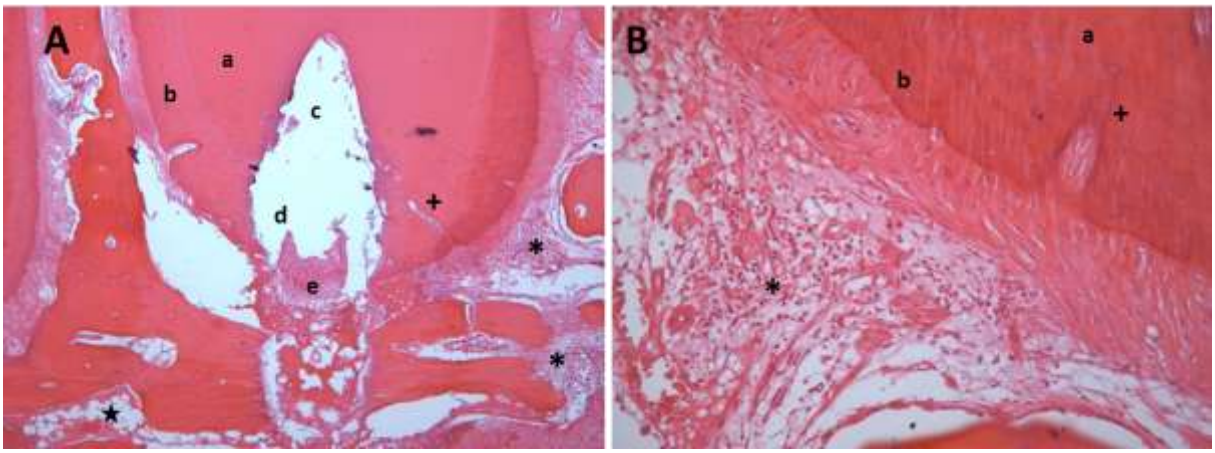

Figure 2. Control teeth's section after 7 days of root canal filling at 4X (A) and 20X (B), showing dentin (a), cementum (b), root canal (c), apical overinstrumentation (d), calcium hydroxide paste overfilling (e), cementum resorption (+), bone resorption (star) and presence of moderate inflammation (*).

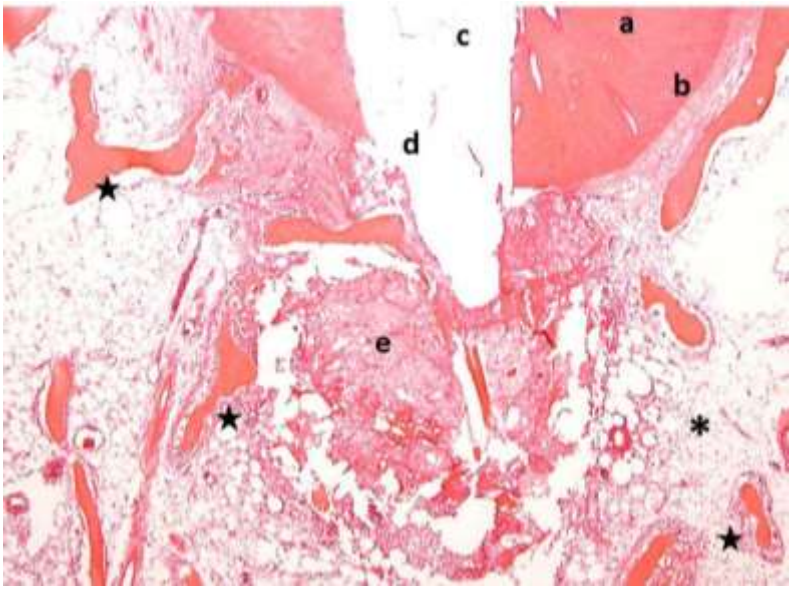

Figure 3. Teeth's section after 7 days of root canal filling with capsaicin paste 0.025% at 4X, showing dentin (a), cementum (b), root canal (c), apical overinstrumentation (d), paste overfilling (e), bone resorption (star) and moderate inflammation (*).

was filled with loose connective tissue and richly vascularized. Granules of calcium hydroxide were visualized inside the macrophages and dispersed in loose connective tissue. The interaction between calcium hydroxide and capsaicin did not promote this qualitative change in the infiltrate (Figures 2, 3, and 4). These data indicate that capsaicin, regardless of the concentration, did not demonstrate an irritating potential during the 7 days period.

On the 14th day evaluation of the cellular and tissue response, the morphology of the periradicular region was similar to the morphology observed on the 7th day. The process of granulated tissue formation that corresponded with a subchronic inflammatory process and the initiation of repair was visualized (Figures 5, 6, and 7). The association with capsaicin did not promote a differential response when compared with the control group, demonstrating that capsaicin did not have a synergistic effect in the formation of this tissue (that is, it did not interfere with subchronic inflammatory mechanisms).

On day 30, a moderate inflammatory process was still observed, with a predominance of macrophages (Figures

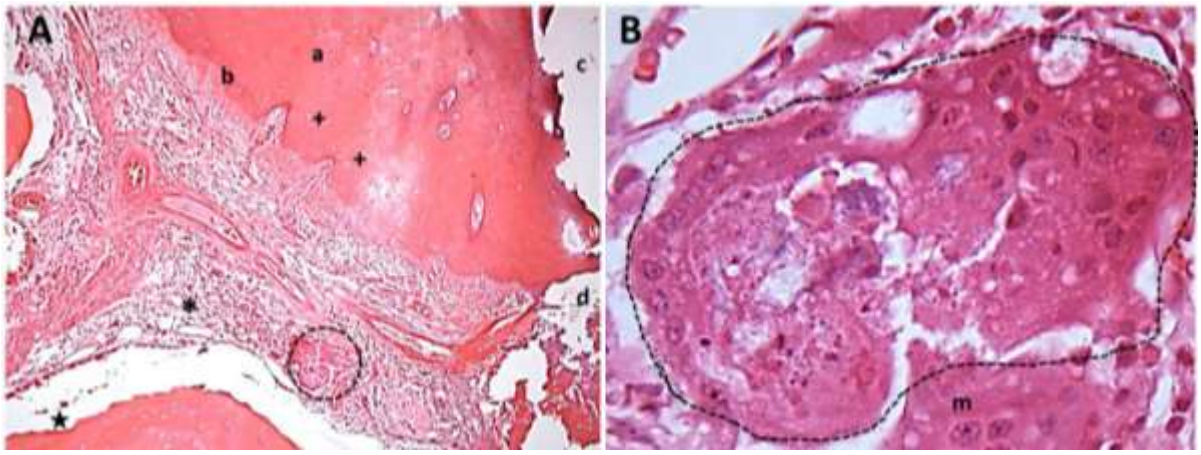

Figure 4. Teeth's section after 7 days of root canal filling with capsaicin paste 0.05% at 10X (A) and 100X (B), showing dentin (a), cementum (b), root canal (c), apical overinstrumentation (d), cementum and dentin resorption (+), bone resorption (star), giant cell (circle), alone macrophages (m) and moderate inflammation (*).

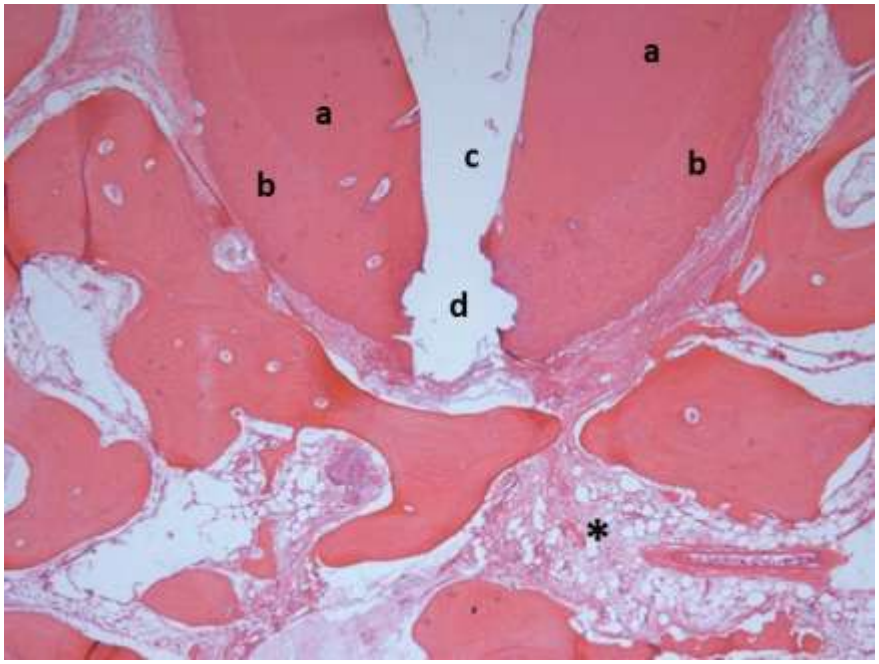

Figure 5. Control teeth's section after 14 days of root canal filling at 4X, showing dentin (a), cementum (b), root canal (c), apical overinstrumentation (d), and presence of moderate inflammation (*).

8, 9, and 10). Dense connective tissue circumscribed the infiltrate (Figures 8B and 10B). The resorption of hydroxyapatite granules was detected, reflected by its presence inside the vessels and macrophages (Figure 11), giant cells that phagocytized the extravasation material, and a reduction of blood vessels (Figure 4B).

Dogs have several foramens, rather than a single apex foramen (Masson et al., 1992). For this reason, creating a central foramen is necessary in experimental dog models, which is achieved by overinstrumentation of the apex to cause injury (Holland et al., 1979). This trauma induces an inflammatory response that results in the release of cytokines and the promotion of hard tissue resorption. Therefore, the inflammatory process and resorption observed in all of the groups in the present study are related to the methodology.

Cementum, dentin, and bone resorption was present in all of the groups at 7, 14, and 30 days. At these times, clasts was observed in the hard tissue surface associated with mononuclear cells located peripherally, generating

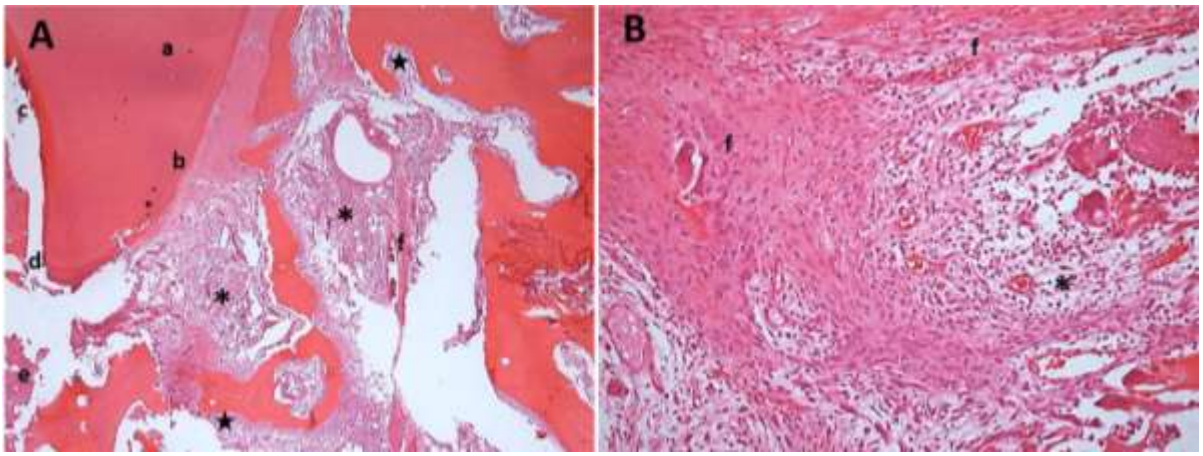

Figure 6. Teeth's section after 14 days of root canal filling with capsaicin paste 0.025% at 10X (A) and 20X (B), showing dentin (a), cementum (b), root canal (c), apical overinstrumentation (d), paste overfilling (e), fibrosis area (f), bone resorption (star) and moderate inflammation (*).

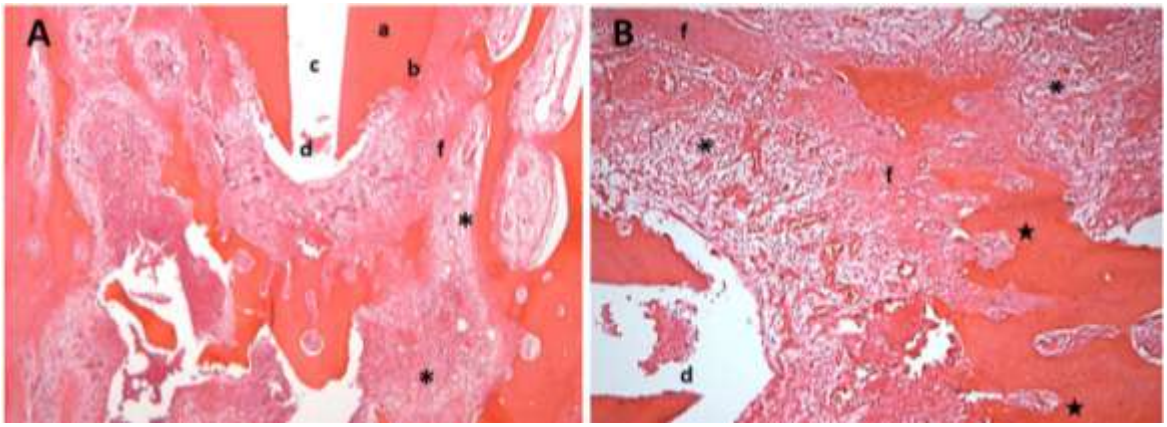

Figure 7. Teeth's section after 14 days of root canal filling with capsaicin paste 0.05% at 4X (A) and 10X (B) showing dentin (a), cementum (b) root canal (c), apical overinstrumentation (d), fibrosis area (f) , bone resorption (star) and moderate inflammation (*).

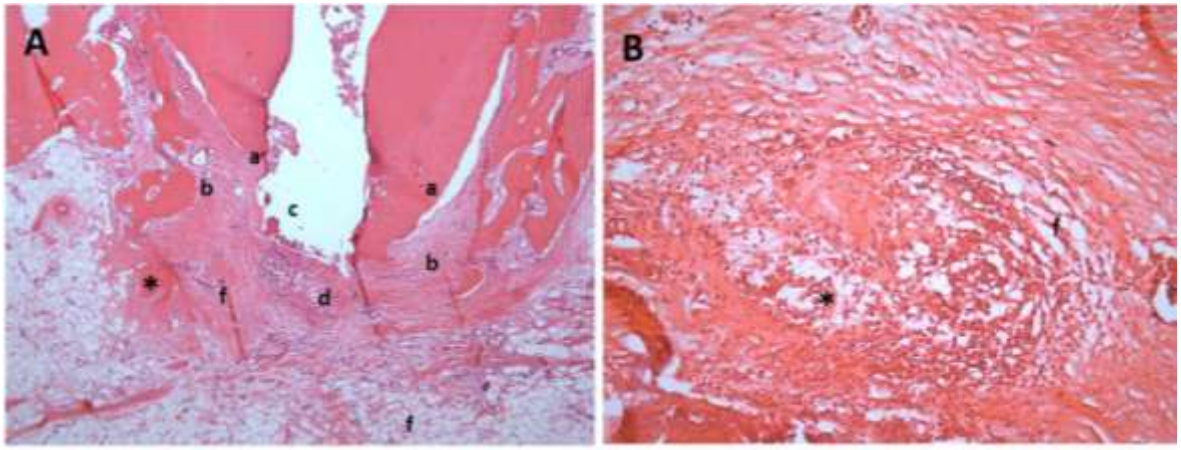

Figure 8. Control teeth's section after 30 days of root canal filling at 4X (A) and 100X (B), showing dentin (a), cementum (b), root canal (c), apical overinstrumentation (d), presence of fibrosis area (f), bone resorption (star) and mild to moderate inflammation (*).

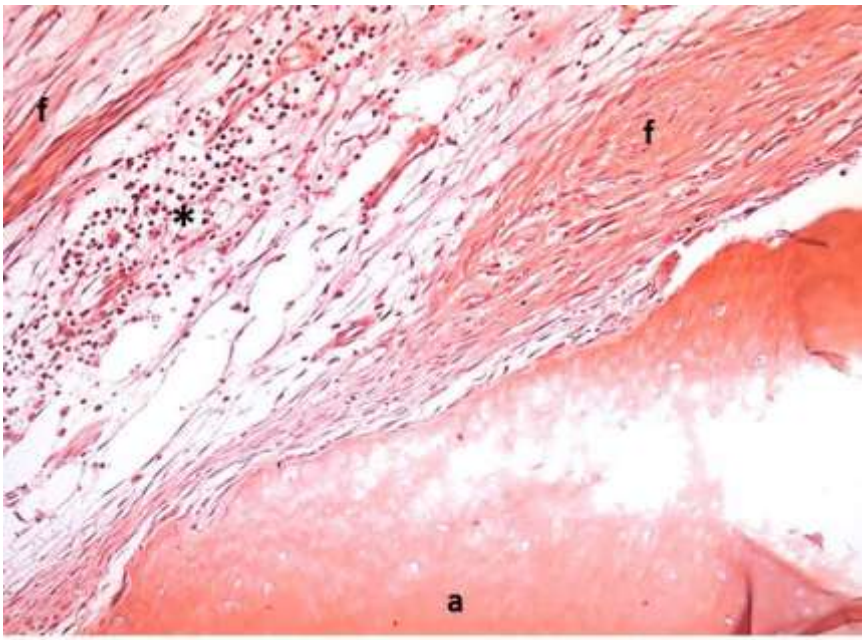

Figure 9. Teeth's section after 30 days of root canal filling with capsaicin paste 0.025% at 10X showing dentin (a), fibrosis area (f) and moderate inflammation (*).

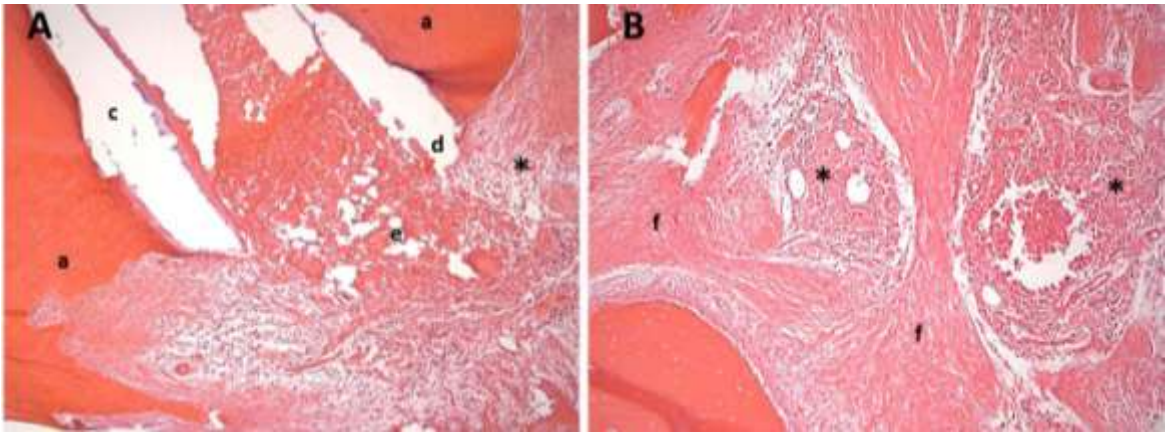

Figure 10. Teeth's section after 30 days of root canal filling with capsaicin paste 0.05% at 10X (A and B) showing dentin (a), root canal (c), apical overinstrumentation (d), paste overfilling (e), fibrosis area (f) and moderate inflammation (*).

an irregular surface (Figure 12). Apical resorption (that is, cementum and dentin) results in a shortening of the tooth long axis and decreased periodontal support. In the three analyzed periods, apical resorption was superficial (Figure 2B) and did not affect the function and tooth support in the alveolus. In these kind of experiments, a resorption process greater than 3 mm is necessary (Kalkwarf et al., 1986).Introducing calcium hydroxide in the root canal influences the resorption area, preventing osteoclast activity and stimulating the repair process. This occurs because of the presence of calcium ions that activate adenosine triphosphate-dependent calcium, which is associated with hard tissue formation (Tronstad et al., 1981) and alkaline phosphatase because of its high pH and may initiate or promote mineralization (Torneck and Howley, 1983). In this study, calcium hydroxide only had an alkalization effect on the dentinal structure that was insufficient to stop inflammation, because the primary causal factor, the extravasation material, was still present. The association between calcium hydroxide and capsaicin did not affect the resorption profile observed at any of the experimental periods. Capsaicin is a substance that relieves pain (Marino et al., 2010) by acting on sensory nerve endings and temporarily inhibiting substance P and other mediators involved in analgesia. The topical application of capsaicin is widely used in

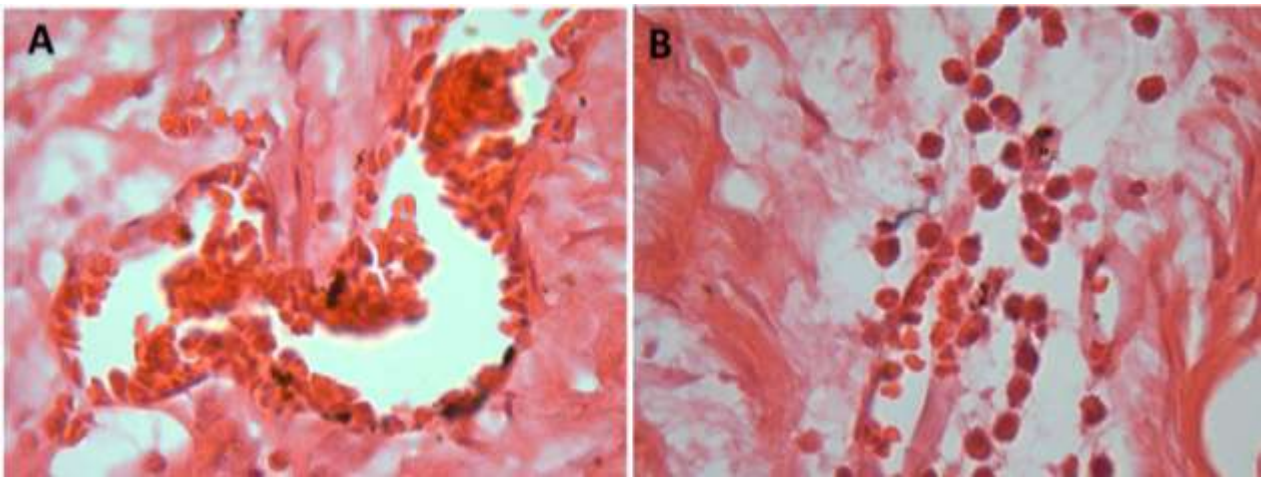

Figure 11. Control teeth's section after 30 days of root canal filling at 100X (A) and (B), showing hydroxyapatite granules inside the vessels (A) and hydroxyapatite granules inside the macrophages (B).

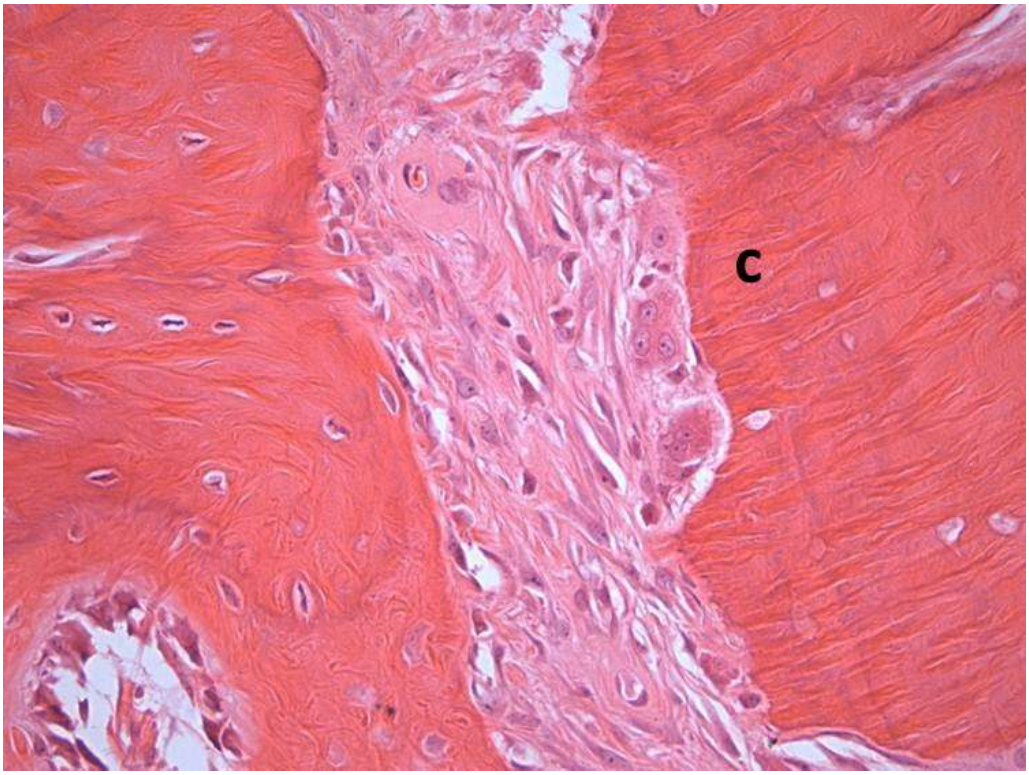

Figure 12. Teeth's section after 30 days of root canal filling with capsaicin paste 0.05% at 40X (c) Clasts cells on the surface.

experimental pain models to stimulate receptors on sensory nerve fibers (Frot et al., 2004). It is topically applied on the nasal mucosa in animals (Lundblad, 1984) and humans (Geppetti et al., 1988), oral mucosa (Baad-Hansen et al., 2003), skin of primates and rodents (Alber et al., 1989), and tongue in humans (Szolcsanyi, 1977). However, from a morphological perspective, no reports have described the reaction of periapical tissues after intracanal capsaicin administration.Capsaicin is an irritant and it is painful. These factors may interfere with the inflammatory process and repair. Our data showed that capsaicin administered by the intracanal route did not affect the inflammatory response or resorption. Overall, the present results provide support for the future clinical

use of capsaicin for intracanal dressing in dental patients who suffer from neuropathic pain, such as atypical toothache.

REFERENCES

Alber G, Scheuber PH, Reck B, Sailer-Kramer B, Hartmann A, Hammer DK (1989). Role of substance P in immediate-type skin reactions induced by staphylococcal enterotoxin B in unsensitized monkeys. J. Allergy Clin. Imniunol. 84:880-885.

Baad-Hansen L, Jensen TS, Svensson P (2003). A human model of intraoral pain and heat hyperalgesia. J. Orofac. Pain 17(4):333-340.

Binnie WH, Rowe AH (1973). A histological study of the periapiacal tissues of incompletely formed pulpless teeth filled with calcium hydroxide. J. Dent. Res. 52(5):1110-1116.

Flores MP, Castro AP, Nascimento JS (2012). Topical analgesics. Rev. Bras. Anestesiol. 62(2):244-252.

Frot M, Feine JS, Bushnell MC (2004). Sex differences in pain perception and anxiety. A psychophysical study with topical capsaicin. Pain 108(3):230-236.

Geppetti P, Fusco BM, Marabini S, Maggi CA, Fanciullacci M, Sicuteri F (1988). Secretion, pain and sneezing induced by the application of capsaicin to the nasal mucosa in man. Br. J. Pharmacol. 93(3): 509-514.

Holland R, Souza V, Nery MJ, Bernabe PFE, Otonobi Filho JA (1979). A histological study of the effect of calcium hydroxide in the treatment of pulpless teeth of dogs. J. Br. Endo. Soc. 12(1):15-23.

Holland R, Mazuqueli L, de Souza V, Murata SS, Dezan Júnior E, Suzuki P (2007). Influence of the type of vehicle and limit of obturation on apical and periapical tissue response in dogs' teeth after root canal filling with mineral trioxide aggregate. J. Endod. 33(6):693-697.

Kalkwarf KL, Krejci RF, Pao YC (1986). Effect of apical root resorption on periodontal support. J. Prosthet. Dent. 56(3):317-319.

Leonardo MR (2005). Endodontia: Tratamento de canais radiculares, princípios técnicos e biológicos. São Paulo, Artes Médicas p.1491.

Lundblad L (1984). Protective reflexes and vascular effects in the nasal mucosa elicited by activation of capsaicin-sensitive substance P-immunoreactive trigeminal neurons. Acta Physiol. Scand. Suppl. 529:1-42.

Maekawa LE, Valera MC, Oliveira LD, Carvalho CA, Koga-Ito CY, Jorge AO (2011). *In vitro* evaluation of the action of irrigating solutions associated with intracanal medications on *Escherichia coli* and its endotoxin in root canals. J. Appl. Oral Sci. 19(2):106-112.

Marino R, Torretta S, Capaccio P, Pignataro L, Spadari F (2010). Different therapeutic strategies for burning mouth syndrome: preliminary data. J. Oral Pathol. Med. 39(8):611-616.

Masson E, Hennet PR, Calas PL (1992). Apical root canal anatomy in the dog. Endod. Dental. Traumatol. 8(3):109-112.

Szolcsanyi J (1977). A pharmacological approach to elucidation of the role of different nerve fibres and receptor endings in mediation of pain. J. Physiol. 73(3):251-259.

Torneck CD, Moe H, Howley TP (1983). The effect of calcium hydroxide on porcine pulp fibroblasts *in vitro*. J. Endod. 9(4):131-136.

Tronstad L, Andreassen JO, Hasselgren G, Kristerson L, Riis I (1981). pH changes in dental tissues after root canal filling with calcium hydroxide. J. Endod. 7(1):17-21.

Trowbridge HO, Emiling RC (1997). Inflammation: A review of the process. 5th ed. Chicago, Illinois: Quintessence.

Vickers ER, Cousins MJ, Walker S, Chisholm K (1998). Analysis of 50 patients with atypical odontalgia. A preliminary report on pharmacological procedures for diagnosis and treatment. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 85(1):24-32.