Standardized Propolis Extract and Calcium Hydroxide as Pulpotomy Agents in Primary Pig Teeth

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ABSTRACT

Purpose: The purpose of this study was to evaluate the histological features of the **pulp tissue** in primary pig teeth submitted to pulpotomy and capped with calcium hydroxide-based and standardized propolis extract-based pastes, and the combination of these pastes.

Methods: Nine 4-month-old male pigs were used in this study, which were distributed into 4 groups, according to the studied pastes: Group 1—calcium hydroxide; Group 2—standardized propolis extract; Group 3—combination of pastes 1 and 2 in the proportion 1:1; and Group 4—control. The teeth used for the pulpotomy were the 4 mandibular primary incisors.

Results: After 7, 21, and 42 days, the animals were killed and the teeth were removed for histological analysis. At 42 days, all teeth in Groups 1 to 3 presented a complete hard tissue barrier and the pulp tissue beneath was without inflammation.

Conclusion: According to these findings, calcium hydroxide and standardized propolis extract favored the formation of a hard tissue barrier in primary pig teeth submitted to pulpotomy. (J Dent Child 2012;79(2):53-8)

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Among the pulp dressing agents most frequently used in pulpotomies are formocresol, calcium hydroxide, and mineral trioxide aggregate.[1-5] Calcium hydroxide and mineral trioxide aggregate (**MTA**) have shown the best results, since they favor repair of the injured pulp tissue and stimulate the formation of a hard tissue barrier.[1,3,5-7] Some authors affirm, however, that the high pH generated by calcium hydroxide is potentially toxic to pulp tissue and tends to dissolve soft tissue, causing chronic pulp inflammation and cell necrosis in vivo.

Moreover, the results obtained in pulpotomy using calcium hydroxide or calcium hydroxide-based materials are still doubtful, considering that clinical studies have revealed an increase in failure rates in series with long-term follow-ups.[4,8]

The success rate of calcium hydroxide as a pulpotomy agent in primary teeth is poor vs permanent teeth. Recently, a study revealed that no difference was observed between the clinical and radiographic features of primary molars submitted to pulpotomy with MTA or formocresol after 24 months of follow-up; however, there was significantly less frequent internal resorption in the MTA group.[9]

At present, with increasing studies in the area of phytotherapy, which is the study of the use of extracts from

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natural origin as medicines or health-promoting agents, different natural extracts have been studied, such as propolis, particularly for antimicrobial, anti-inflammatory, and reparative purposes.[10-14] Propolis is a resinous substance of varying color and consistency collected by bees, with antibacterial, antiviral, antifungal, immunostimulation, hypotensive, and cytostatic activity, mainly due to the presence of flavonoids, aromatic acids, and esters.[12,15,16] As an anti-inflammatory agent, propolis has been observed to inhibits prostaglandin synthesis.[10,14]

In view of the related benefits and advantages, propolis has indications for the treatment of several oral diseases. Few studies have been conducted, however, to evaluate its use as an intracanal medication, irrigant solution, or pulpotomy medication.[13,16-18] In addition, propolis had not been studied as a pulpotomy agent in the primary teeth of animals.

Therefore, the purpose of this study was to evaluate the histological features of pulp tissue in primary pig teeth submitted to pulpotomy and capped with calcium hydroxide-based and standardized propolis extract-based pastes, and the combination of these pastes in the proportion of 1:1.

METHODS

This study was previously evaluated and approved by the Animal Research Ethics Committee of the University of Ribeirao Preto, Ribeirao Preto, Sao Paulo, Brazil, and the ethical concepts for use of laboratory animals were complied with in all phases of the experiment.

Three different pastes were used as pulpotomy agents: paste 1–3.0 g calcium hydroxide p.a. (Merck KGaA, Darmstadt, Germany) mixed with 1.5 ml of polyethylene glycol 400 (Merck KGaA); paste 2–1.5 g standardized propolis extract (**SPE–AF**) powder at 80% (Apis Flora, Ribeirao Preto) mixed with 1.75 ml of polyethylene glycol 400; and paste 3–a combination of pastes 1 and 2 in equal volumes (1:1). SPE–AF is a formula developed with the intention of combining defined quantities and qualities of propolis from various regions in Brazil with active principles or markers, such as artepelin-c, bacharina, p-cumaric acid, and drupanin, which are evaluated and monitored qualitatively and quantitatively to maintain their chemical stability. This product is in the process of being patented (no. PI 0405483) by the National Institute of Industrial Property of Brazil. According to the paste used, 4 experimental groups were established: Group 1–paste 1; Group 2–paste 2; Group 3–paste 3; Group 4–pulp removal without medication (control).

Nine 4-month-old male pigs, weighing between 75 and 100 kg, were used in the study. The animals were kept at the facilities of the Swine Health Laboratory, Department of Veterinary Clinic and Surgery, School of Agrarian and Veterinary Sciences of Jaboticabal, State University of Sao Paulo, and individualized by the Australian Marking System that allows the animal to be identified by means of a number. The pigs were followed-up and constantly evaluated during the entire period of the experiments and received balanced ration and water ad libitum.

For anesthesia, the animals initially received an intramuscular injection of 7.8 ml of azaperone (Suicalm, Merian, Campinas, Sao Paulo, Brazil). After 30 minutes, 4.7 ml of intramuscular midazolam (Dormonid, Roche, Rio de Janeiro, Brazil) and 7.5 ml (2.0 ml intravenous and 5.5 ml intramuscular) of ketamine chlorhydrate (Cetamin, Produtos Veterinários JA, Patrocínio Paulista, Sao Paulo, Brazil) were administered.

In each animal, 4 primary mandibular incisors were submitted to the pulpotomy procedure, and each incisor received one of the tested pastes. Therefore, in all animals: the right lateral incisor received paste 1 (Group 1); the left central incisor received paste 2 (Group 2); the right central incisor received paste 3 (Group 3); and the left lateral incisor received no paste at all (Group 4). Before the pulpotomy procedure, the experimental teeth were submitted to prophylaxis, and absolute isolation of the operative field was performed with a rubber dam (Damtex, DFL, Rio de Janeiro, Brazil) including asepsis with 2% chlorhexidine digluconate (FMG, Joinville, Santa Catarina, Brazil).

Surgical access to the pulp chamber was performed on the vestibular face of the teeth with carbide burs

Table 1. Summary of the Data Obtained for Histological Analysis of the Pulp Tissue of Primary Pig Teeth After Pulpotomy With the Studied Pastes

Histological features	7 days				21 days				42 days			
	G1	G2	G3	G4	G1 (N)	G2 (N)	G3 (N)	G4	G1 (N)	G2 (N)	G3 (N)	G4
Inflammatory reaction*	++/+++	+/++	+/++	+++	+/++	+	+	++/+++	-	-	-	-
Superficial necrosis†	+	+	+	+	-	-	-	-	-	-	-	-
Pulp necrosis†	-	-	-	-	-	-	-	-	-	-	-	-
Complete hard tissue formation†	-	-	-	-	+(2)	-	-	-	+(3)	+(3)	+(3)	-
Partial hard tissue formation†	-	-	-	-	+(1)	+(3)	+(3)	-	-	-	-	-

** Inflammation: (–) absent; (+) mild; (++) moderate; (+++) severe.* *† (–) absent; (+) present.*

PM no. 2 (Jet Carbide Burs, Ontario, Canada) driven by a low-speed electric motor (Rotex 780, Dentamerica, Rio de Janeiro, Brazil) under constant irrigation with a physiological solution (Laboratório JP Indústria Farmacêutica, Ribeirao Preto, Brazil), taking care to remove the entire pulp chamber roof. The coronal pulp was carefully removed with curettes no. 2 (Hu-Friedy, Rio de Janeiro, Brazil), and the pulp chamber abundantly irrigated with sterile physiological solution until physiological hemostasis occurred.

The pulpotomy agents were placed in the pulp chamber with a calcium hydroxide applicator (Golgran, Sao Paulo, Brazil) and, after removing the excess, the cavity was restored with resinous glass ionomer cement (Vitremer, 3M ESPE, St. Paul, Minn., USA) (Figure 1). In the left lateral mandibular incisor, used as a control, only a cotton pellet was inserted over the exposed pulp tissue and the cavity was restored, as previously described.

At each experimental period of 7, 21, and 42 days, 3 animals were randomly selected to be killed by anesthetic overdose, providing 3 teeth for each group for each observation period. The teeth were removed, fixed in a 10% neutral-buffered formalin solution (Merck KGaA, Darmstadt, Germany) for 72 hours and decalcified with an aqueous solution of 10% trichloroacetic acid (Merck KGaA) for 10 days. After decalcification, the teeth were sectioned longitudinally, and processed for conventional histologic evaluation. Serial histologic cuts 6-µm thick were obtained of paraffin-embedded tissue blocks, stained with hematoxylin-eosin and evaluated for the presence or absence of neutrophils, lymphocytes, macrophages, necrosis, and hard tissue barrier formation on the pulp tissue. In addition, the intensity of the inflammatory reaction in the pulp tissue was classified as absent (-), mild (+), moderate (++), or severe (+++). All features were evaluated and classified according to Shayegan et al.[7]

RESULTS

AFTER 7 DAYS

In Group 1, a superficial layer of necrotic tissue was observed in close contact with the paste in the area of the pulpal exposure. In the coronal third of the root canal, there was granulation tissue with moderate to severe chronic inflammatory reaction, formed by lymphocytes and macrophages, with hyperemic blood vessels (Figure 2). No hard tissue barrier was observed. The remaining pulp tissue appeared normal.

In Group 2, the exposed pulp tissue adjacent to the pulpotomy agent demonstrated superficial necrosis. Under the necrotic area, in the coronal third, mild to moderate chronic inflammation was observed, predominanted by lymphocytes and hyperemic blood vessels. Similar to Group 1, the remaining pulp tissue was shown to be normal, without a mineralized tissue barrier. In Group 3, histological features were similar to those found in group 2.

In Group 4, in the pulp tissue adjacent to the cavity, necrosis and a severe acute inflammatory reaction

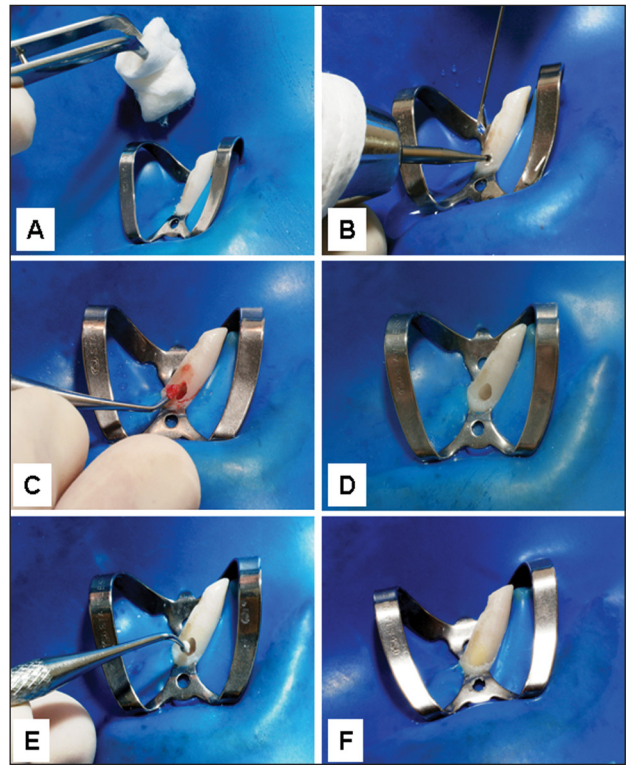

Figure 1. Procedure sequence. (A) Absolute isolation of the operative field with a rubber dam and asepsis with 2% chlorhexidine digluconate. (B) Surgical access of the pulp chamber with carbide burs on the vestibular face of incisor. (C) Resection of pulp tissue. (D) Pulp chamber after pulp tissue removal and hemostasis. (E) Pulpotomy agent placed in the pulp chamber. (F) Cavity restored with resinous glass ionomer cement.

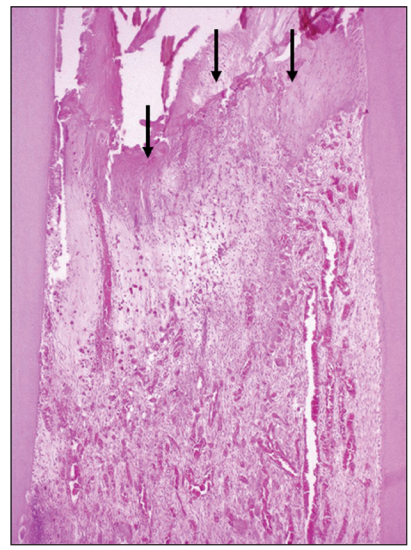

Figure 2. Calcium hydroxide 7 days after the pulpotomy. Superficial necrosis (arrows) and granulation tissue with moderate chronic inflammatory reaction (hematoxylin–eosin, 50X).

(microabscess) were observed. Under this area, there were hyperemic blood vessels and severe chronic inflammation mainly composed of lymphocytes. The remaining pulp tissue in the root canal demonstrated mild chronic inflammation.

AFTER 21 DAYS

In Group 1, a complete hard tissue barrier that fully covered the exposed pulp tissue was found in 2 samples. In another sample, an incomplete mineralized barrier was observed. Under the mineralized barrier, the pulp tissue from the coronal third showed mild to moderate chronic inflammation, predominantly composed of lymphocytes. Macrophages, hyperemic blood vessels and old hemorrhagic areas were also observed. No necrosis, however, was observed. The remaining pulp tissue presented normal features.

In all Group 2 samples, there was incomplete hard tissue formation beneath the exposed pulp tissue. In one specimen, internal resorption was observed (Figure 3). Mild chronic inflammation was observed in the pulp tissue from the coronal third of the root canal. There was no necrosis, and the remaining pulp tissue was normal. In Group 3, histological features were similar to those observed in Group 2.

In Group 4, the exposed pulp tissue situated in the coronal third of the root canal presented moderate to severe chronic inflammation, predominantly formed by lymphocytes. One sample had a thin dentin deposit on the lateral walls of the coronal third of the root canal. Necrosis was absent. The remaining pulp tissue showed normal features.

AFTER 42 DAYS

In Group 1, a complete calcified bridge was observed beneath the exposed pulp tissue (Figure 4). The pulp tissue was more fibrous and without inflammation. In Group 2, histological aspects were similar to those described for Group 1 (Figure 5). In Group 3, morphological features were similar to those observed in Groups 1 and 2.

In Group 4, the exposed pulp tissue showed a mild to moderate chronic inflammatory reaction. One sample presented dentin deposits on the lateral walls of the coronal third of the root canal. No necrosis or hard tissue barrier was found.

DISCUSSION

Several methods and exams have been used to evaluate the clinical and radiographic success of the different pulpotomy agents, such as electrical or thermal tests, presence of pain or swelling, and radiographic signs of periapical pathology.[3,19] However, microscopic analysis is necessary to examine the state of the pulp tissue and hard tissue barrier formation after pulpotomy.[2,5,18,20,21] Some animal study models, mainly using dogs and rats, have been used to evaluate the microscopic characteristics of teeth submitted to pulpotomy with different medicaments.[1,5,18] An alternative animal model that has not been extensively explored for similar studies,[7,20-23] is the use of pigs, as was done in this study. These animals present teeth of adequate size and structure for study purposes, in addition to anatomic and physiologic similarities to humans.[24]

Calcium hydroxide presents antibacterial properties as well as favorable tissue compatibility. Nevertheless,

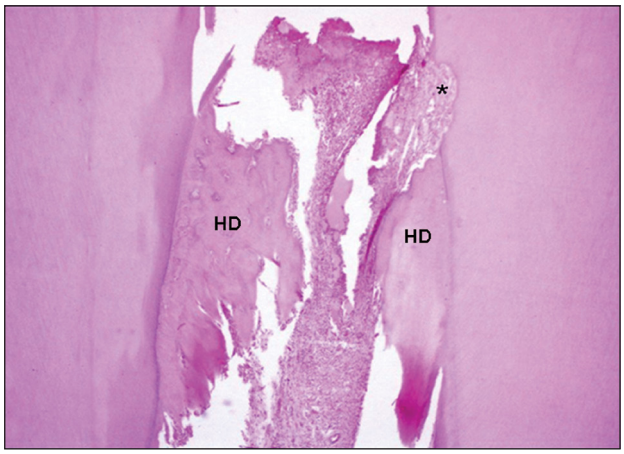

Figure 3. Standardized propolis extract 21 days after the pulpotomy. Partial hard tissue barrier formation and internal resorption (hematoxylin–eosin, 50X).*

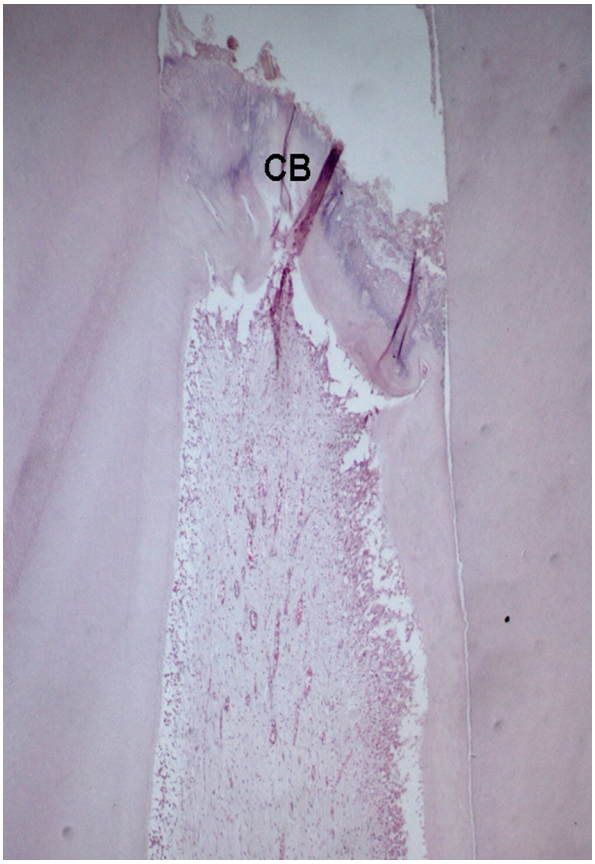

Figure 4. Calcium hydroxide 42 days after the pulpotomy. Complete calcified bridge formation was observed (hematoxylin–eosin, 50X).

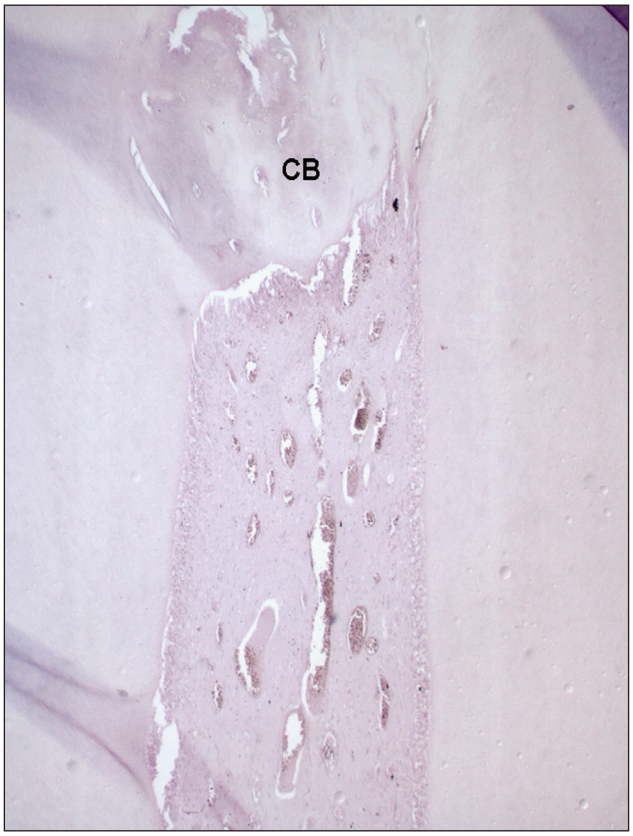

Figure 5. Standardized propolis extract 42 days after the pulpotomy. Complete calcified bridge formation was observed (hematoxylin–eosin, 50X).

there has been an increase in clinical failure after pulpotomy with the use of calcium hydroxide in case series with long-term follow-up.[8] Histological studies have shown that this material presents good performance, with high rates of mineralized material barrier formation covering the exposed pulp tissue, promoting the maintenance of pulp integrity and vitality in primary teeth.[1,5] In the present study, the teeth that were submitted to pulpotomy with calcium hydroxide did not present necrosis of the root pulp, a characteristic that suggests the maintenance of pulp vitality. It is noteworthy, however, that the analysis was performed for only 6 weeks; after this time, the tissue pulp alterations were not available.

Some authors have observed that calcium hydroxide was approximately 10-fold more cytotoxic to pulp fibroblasts than propolis, in culture.[25] In the present study, when the intensity of inflammation among the groups was compared, calcium hydroxide (Group 1) presented a more intense chronic inflammation at 7 and 21 days after pulpotomy. In the subsequent period of time, none of the groups showed inflammation in the pulp tissue. Considering only Group 2 (standardized propolis extract), the findings of the present study revealed a mild to moderate chronic inflammatory reaction after 7 days, which decreased after 21 days to a mild inflammation, similar to the results of another study.[18] After 42 days, there was greater fibrous tissue formation, with absence of inflammatory reaction. As observed for calcium hydroxide, these findings suggest that the teeth treated with propolis-based paste maintained their vitality.

Regarding the formation of a hard tissue barrier, the current study revealed that the application of standardized propolis extract as a pulpotomy medication caused the formation of a partial mineralized tissue barrier after 21 days, and a complete calcified bridge after 42 days. Similar results to those at 21 days were observed by Sabir et al.[18] in rat teeth. These results are probably related to the anti-inflammatory property of propolis, which inhibits prostaglandin synthesis and nitric oxide production, stimulates cell immunity, increases the reparative capacity, and causes less tissue irritation[10,13,14]—provided by the presence of flavonoids, aromatic acids, and esters.[18] Furthermore, propolis also presents an important antimicrobial effect.[17] As previously mentioned, the SPE–AF contains defined quantities of propolis from various regions in Brazil, making it possible to obtain favorable results concerning anti-inflammatory and reparative activity.

Due to the characteristics and properties presented both by propolis and calcium hydroxide, the use of a pulpotomy agent composed of these 2 substances could yield important benefits. Therefore, in this study, the combination of 80% standardized propolis extract and calcium hydroxide was used, which presented a performance similar to that of the substances used in isolation, with the formation of a complete calcified bridge 42 days after the pulpotomy.

A limitation of this study is that there is no pulpotomy technique that currently recommends permanently sealing a cotton pellet in the tooth. An additional control group could have been added, in which the pulp tissue was removed and the restorative material placed directly over the pulp tissue.

Few studies have tested the use of propolis or achieved results comparable to those obtained in this experiment; therefore further studies are necessary to prove propolis' efficiency as a pulpotomy and intracanal medication in order to increase knowledge about the reactions this substance can cause when in contact with pulp tissue. It is important to identify a novel, effective, and natural pulpotomy agent to increase the therapeutic arsenal and successfully perform pulpotomy procedures.

CONCLUSIONS

Based on this study's results, the following conclusions can be made:

1. Calcium hydroxide-based and standardized propolis extract-based pastes, and the combination of these pastes, were effective pulpotomy agents for the formation of a hard tissue barrier.

2. Standardized propolis extract has potential for use as a pulpotomy agent.

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