Inflammatory effect of green propolis on dental pulp in rats

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Abstract: Pulpotomy in deciduous teeth is a controversial issue, especially with regard to alternative materials used for the direct pulp capping of the root canal pulp tissue. The aim of the present study was to perform a histological analysis of the initial reaction of the root canal pulp tissue in rats, following pulpotomy and pulp capping with (1) green propolis extract, (2) iodoform paste, (3) green propolis extract + iodoform and (4) calcium hydroxide paste with saline solution. Analyses were performed after 24 hours, 72 hours and 7 days. The substances containing green propolis extract and iodoform led to the production of an intense inflammatory infiltrate and necrosis in the root canal pulp tissue throughout the analyses. In the calcium hydroxide group, inflammatory infiltrate only prevailed at the 72-hour evaluation. Among the substances tested, calcium hydroxide paste induced the lowest intensity of inflammatory response in the root canal pulp tissue. Longer studies should be carried out to analyze the pulp repair process following pulpotomy and pulp capping with the compounds analyzed.

Descriptors: Pulpotomy; Propolis; Dental Materials.

Introduction

Root canal treatment in the deciduous teeth is one of the most widely discussed subjects in pediatric dentistry. The main focus of discussion is the protection of the remaining pulp following pulpotomy.[1] Direct pulp capping is performed to protect the pulp tissue from bacterial agents and induce a local tissue response, thereby maintaining its vitality.[2] A variety of materials have been employed for pulp capping in deciduous teeth, the most common of which is calcium hydroxide, despite its limitations.[3]

The ideal material for the protection of the remaining pulp tissue should be bactericidal and biocompatible with the pulp and adjacent structures. It should also promote a tissue repair process and not interfere with physiological root resorption. However, the best treatment for the deciduous dentition has not yet been defined.[4] In this regard, studies have been carried out on the biocompatibility of different capping materials,[5,6] including the analysis of natural products with therapeutic properties.[7]

Propolis is a natural derivative with anti-inflammatory and antimicrobial properties, and has been used as a pulp capping material in human teeth, demonstrating results that are comparable to those obtained with mineral trioxide aggregate and calcium hydroxide.[2] Green propolis

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is a Brazilian variety that has been widely used owing to its different pharmacological properties,[8] and that has a complex chemical composition including phenolic compounds, terpenes and essential oils.[9]

The aim of the present study was to assess the initial response of the dental pulp in rats, following pulpotomy and pulp capping with substances containing green propolis.

Methodology

An *in vivo* experimental study was carried out with histological evaluations and a descriptive analysis of the data.

Eighteen male Wistar rats (*Rattus norvegicus albinus*) at an approximate age of 90 days and weighing between 250 and 300 g were obtained from the *Universidade Potiguar* animal lodging facility, Natal, RN, Brazil, following approval from the Animal Research Ethics Committee of the institution. Seventy-two teeth (upper and lower first molars) were divided into groups based on capping material and evaluation time (24 hours, 72 hours and 7 days). Each group was initially made up of six teeth.

The animals received general anesthesia through an intramuscular injection of tiletamine and zolazepam (Zoletil-50, Virbac do Brasil, Indústria e Comércio Ltda., São Paulo, Brazil; 50 mg/1 kg of body weight), with 1 g of anesthetic powder diluted in 5 mL of sterile water. The animals were immobilized on an appropriate operating table in dorsal decubitus with their mouth kept open for access to the pulp of the upper and lower teeth.

Surgical access to the pulp chamber was performed on the occlusal surface with a sterile high-speed FG 1/4 round bur (KG Sorensen, São Paulo, Brazil) under water cooling. Irrigation was then performed with saline solution, and the pulp tissue was dried with a sterile absorbent paper cone. Pulp capping was performed with the following materials:

1. aqueous solution of green propolis extract with 12% active ingredient (Propomax®, Apis Flora, Ribeirão Preto, Brazil);
2. iodoform paste: iodoform + camphorated paramonochlorophenol (CPMC) + Rifocort® (Merrel Lepetit, São Paulo, Brazil);
3. green propolis extract paste with iodoform; and
4. calcium hydroxide paste with saline solution.

The chambers were sealed with provisional CAVIT® cement (Espe, Seefeld, Germany). The aqueous green propolis extract was inserted into the pulp chamber with the aid of a fragment of sterile, endodontic absorbent paper cone, which remained within the chamber.

The animals were kept in cages under adequate environmental conditions with free access to balanced, pasty feed and water throughout the experiment, until the day of sacrifice.

The animals were sacrificed in a carbon dioxide chamber and decapitated at the pre-established times following the clinical procedure (24 hours, 72 hours and 7 days), followed by dissection of their maxilla and mandibles. Macroscopic and microscopic analyses were made of the dissected parts with the aid of a light microscope (Olympus CX31, Olympus, Tokyo, Japan) coupled to an Olympus digital camera under 40×, 100× and 400× magnification to ascertain the presence of a coronal seal or lack thereof. Teeth that had lost their seal were discarded. Thus, among the 72 teeth that underwent the procedure, 58 were selected for histological analysis. The parts were fixed in a 10% formalin solution for 24 hours, followed by decalcification in 7.5% nitric acid for 24 to 36 hours. Decalcification was considered satisfactory when the part offered no resistance to perforation with an insulin needle. The material was then cleaved and sent for histological processing following routine laboratory methodology:

- dehydration in alcohol,
- clearing in xylol and
- embedment in paraffin.

Next, 3 μm slices were prepared on a microtome, placed on slides and stained with hematoxylin and eosin. The reading of the slides was performed with the aid of a light microscope. The analysis of tissue phenomena involved inflammatory change and necrosis assessment.

The severity of the inflammatory infiltrate and the extension of pulp necrosis were determined using a four-point scoring system based on the following criteria:

1. absence or insignificant presence of inflammatory infiltrate/necrosis;
2. inflammatory infiltrate/necrosis close to the pulp medication, reaching up to one third of the root canal pulp tissue;
3. inflammatory infiltrate/necrosis involving up to two thirds of the root canal pulp tissue; and
4. inflammatory infiltrate/necrosis involving more than two thirds of the root canal pulp tissue.

Results

Tables 1 and 2 show the findings according to the substances tested, evaluation times and histological alterations. Swelling and vascular congestion were common in all groups, with no significant morphological differences. In many cases, vascular congestion was observed at more distant sites from the compromised pulp.

Discussion

The histological evaluation of the pulp tissue revealed that aqueous green propolis extract led to an increase in the intensity of the inflammatory infiltrate at seven days (Figure 1). Since the teeth in this

Table 1 - Distribution of number of teeth according to substances tested, evaluation time and intensity of inflammatory infiltrate.*

Substance	24 hours				72 hours				7 days			
	Inflammatory infiltrate				Inflammatory infiltrate				Inflammatory infiltrate			
	No. of teeth	score			No. of teeth	score			No. of teeth	score		
		2	3	4		2	3	4		2	3	4
Aqueous green propolis extract	6	2	2	2	5	1	2	2	5	0	1	4
Iodoform paste	6	4	2	0	4	1	2	1	4	1	1	2
Green propolis extract + iodoform	4	1	0	3	5	0	2	3	4	0	1	3
Calcium hydroxide + saline solution	5	1	3	1	6	0	1	5	4	1	2	1

*Among the 72 pulpotomized teeth, 14 were excluded, reducing the sample size to 58 teeth. Score 1 = absence or insignificant presence of inflammatory infiltrate; score 2 = inflammatory infiltrate close to the pulp medication, reaching up to one third of the root canal pulp tissue; score 3 = inflammatory infiltrate involving up to two thirds of the root canal pulp tissue; score 4 = inflammatory infiltrate involving more than two thirds of the root canal pulp tissue.

Table 2 - Distribution of number of teeth according to substances tested, evaluation time and presence of necrosis.*

Substance	24 hours					72 hours					7 days				
	Necrosis					Necrosis					Necrosis				
	No. of teeth	Score				No. of teeth	Score				No. of teeth	Score			
		1	2	3	4		1	2	3	4		1	2	3	4
Aqueous green propolis extract	6	0	2	4	0	5	0	2	2	1	5	0	2	2	1
Iodoform paste	6	0	1	3	2	4	1	2	0	1	4	1	3	0	0
Green propolis extract + iodoform	4	1	2	0	1	5	0	3	2	0	4	0	2	2	0
Calcium hydroxide + saline solution	5	0	3	1	1	6	0	2	3	1	4	1	2	0	1

*Among the 72 pulpotomized teeth, 14 were excluded, reducing the sample size to 58 teeth. Score 1 = absence or insignificant presence of necrosis; score 2 = necrosis close to the pulp medication, reaching up to one third of the root canal pulp tissue; score 3 = necrosis involving up to two thirds of the root canal pulp tissue; score 4 = necrosis involving more than two thirds of the root canal pulp tissue.

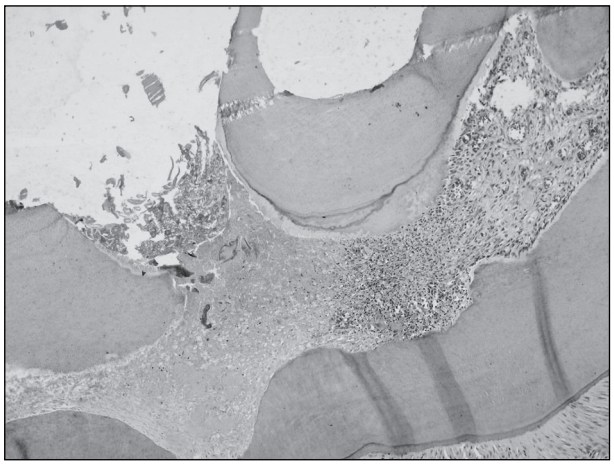

Figure 1 - Photomicrograph of a rat tooth 7 days after pulpotomy and pulp capping with green propolis extract showing significant necrosis and mild inflammatory infiltrate (HE, 100×).

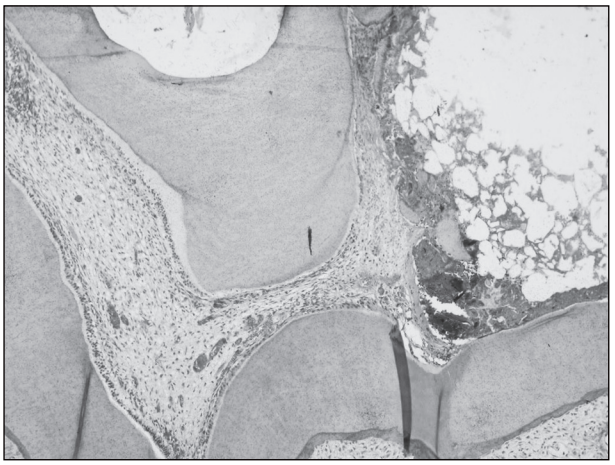

Figure 2 - Photomicrograph of a rat tooth 24 hours after pulpotomy and pulp capping with iodoform paste showing light presence of inflammatory infiltrate (HE, 100×).

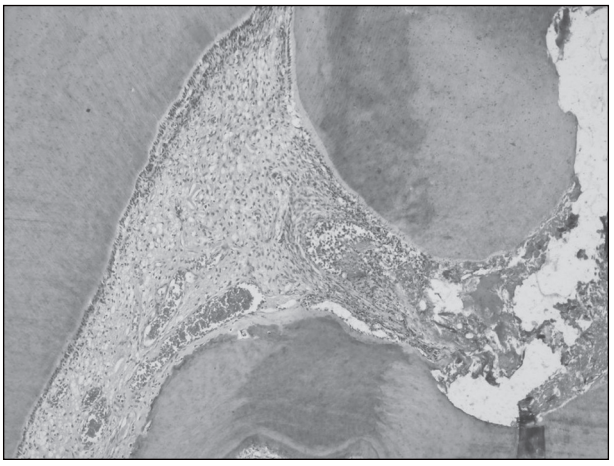

Figure 3 - Photomicrograph of a rat tooth 24 hour after pulpotomy and pulp capping with green propolis extract and iodoform showing mild inflammatory infiltrate (HE, 100×).

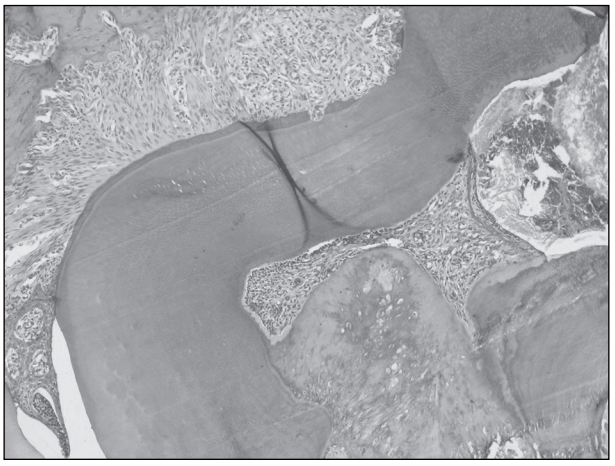

Figure 4 - Photomicrograph of a rat tooth 7 days after pulpotomy and pulp capping with calcium hydroxide showing light inflammatory infiltrate (HE, 100×).

group were protected with a fragment of absorbent paper soaked with the solution, further studies are needed to determine whether the increase in inflammation stemmed from a reaction to the foreign body (absorbent paper) or was a response induced by the propolis itself. On the other hand, the inflammatory reaction may indeed have been caused by the substance, and may indicate a positive tissue response promoting cellular reorganization and repair of the exposed pulp.

Previous studies with a longer evaluation time[10,2] report satisfactory results with green propolis, comparable to those achieved with calcium hydroxide.[11] Moreover, an alcohol extract of propolis was found to induce the formation of collagen bridges and dentin after 28 days.[12] Other studies have demonstrated that propolis has a low irritating potential[13,14] and induces a repair process in both epithelial tissue[15] and pulp tissue.[16] The free-radical- and superoxide-neutralizing components of propolis are believed to be responsible for its main regenerative mechanisms.[17] Longer studies should be carried out to analyze the behavior of the dental pulp exposed to this substance.

The iodoform paste induced a small degree of inflammatory infiltrate in the first 24 hours (Figure 2); however, two of the four teeth analyzed on Day 7 exhibited a significant degree of inflammatory infiltrate, with a predominance of neutrophils. This paste contains Riforcort®, which is a corticoid (prednisolone) associated to an antibiotic (rifampicin). Prednisolone must be the main ingredient responsible for inhibiting the initial inflammatory infiltrate in the conjunctive tissue, insofar as this substance is capable of inhibiting vasodilatation and the inflow of leukocytes.[18]

The paste containing green propolis extract and iodoform induced a significant inflammatory reaction in the pulp tissue at all three evaluation times (Figure 3). Studies found in the literature have reported that iodoform is a tissue irritant; this may have contributed to the histological findings in this group. Despite the substantial presence of inflammatory infiltrate, necrosis ranged from mild to moderate (scores 2 and 3). Iodoform stimulates cell proliferation by producing an initial inflammatory reaction and tissue necrosis and attracting defense cells to the site, especially polymorphonuclear cells, which are rapidly absorbed and replaced with normal conjunctive tissue.[19]

In the calcium hydroxide group, significant inflammatory infiltrate only prevailed in the 72-hour evaluation and was less marked on Day 7 (Figure 4). Necrosis in the specimens ranged from mild to moderate (scores 2 and 3) at the three evaluation times, in most cases. Previous studies have reported that calcium hydroxide induces a lesser degree of inflammatory infiltrate in the initial hours, progressing to a moderate degree after longer periods, and inducing subsequent tissue repair.[20] The necrosis seen in pulp tissue following contact with calcium hydroxide is the result of its alkalinity. This alkalinity actually has a beneficial effect on the injured tissue, insofar as it causes mild irritation and stimulates the conjunctive tissue to defend and repair itself, initiating an inflammatory reaction to control and eliminate the irritating agent.[21]

It was not the intention of the present study to criticize the endodontic pastes used in the treatment of the pulp of deciduous teeth, but rather to provide information on biocompatible materials that may be used as direct pulp capping methods. Longer studies are needed to determine whether the intensity of the inflammatory response observed in the pulp tissue after applying propolis extract is beneficial to the repair process. A number of studies are currently underway to investigate the composition of propolis collected from different regions of Brazil, and analyze its biological activity against oral pathogenic microorganisms. Moreover, further studies are needed to define efficient methods for using propolis in pulp therapy.

Conclusion

Green propolis induced an inflammatory reaction in rat dental pulp following pulpotomy. This reaction was more intensive when the extract was combined with iodoform. Studies conducted with a longer evaluation time are needed to analyze the effect of green propolis on the pulp tissue repair process.

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