Scientific Article

Pulp Repair after Pulpotomy Using Different Pulp Capping Agents: A Comparative Histologic Analysis

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Abstract: ***Purpose:*** *This study's purpose was to histologically evaluate the repair of rat tissue after pulpotomy and covering the pulp tissue with Copaifera langsdorffi oil resin, green propolis extract, fibrin sponge and iodoform-based paste.* ***Methods:*** *Pulpotomies were performed in the maxillary and mandibular first molars of 21 Wistar rats (84 total teeth). The access cavities were sealed with Coltosol, and histological evaluations were performed at 24 hours, 15 days, and 30 days postoperatively.* ***Results:*** *For all experimental periods and materials, an inflammatory response constituted predominantly by neutrophils was observed, being of milder intensity for the Copaifera langsdorffii oil-resin group and more severe for the fibrin sponge group, which exhibited periapical microabscesses. Necrosis was observed in all groups, and its extension increased with time, except for teeth protected with Copaifera langsdorffii oil-resin. Formation of a mineralized tissue barrier in the pulp exposure area occurred only in the Copaifera langsdorffii oil-resin group. Other findings, such as vascular congestion, edema, and hemorrhage, were observed in all cases.* ***Conclusions:*** *The inflammatory response was less severe, the area of pulp necrosis was smaller, and more frequent formation of a mineralized tissue barrier was noted after pulpotomy was performed with Copaifera langsdorffii oil-resin compared to the other materials tested. (Pediatr Dent 2011;33:14-8) Received July 16, 2009 / Last Revision October 26, 2009 / Accepted October 28, 2009*

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The use of biocompatible substances has become a major interest in modern dentistry, especially when direct contact with the dental tissues is necessary.[1] In this sense, the field of phytotherapy, which is the use of plants or plant extracts for medicinal purposes, has experienced a remarkable advance in recent years. This has stimulated the investigation of different herbal products with potential therapeutic properties for dental applications.[2-5] Phytomedicines, simply defined, are a special category of plant drugs. They are standardized, which means that certain compounds in the plant material are quantified and elucidated so as to have a replicable final product.[6]

The idea of protecting and preserving the pulp tissue's vitality is not new. The first attempted vital capping was conducted in 1756 by Philipp Pfaff, dentist to Frederick the Great of Prussia, with a small piece of gold foil carefully adapted to the cavity's base.[7] In more recent days, several materials have been investigated as pulp capping materials, such as calcium hydroxide, mineral trioxide aggregate, formocresol, ferric sulfate, enamel matrix derivative, propolis, *Copaifera langsdorffii* oleo-resin, and recombinant human bone morphogenetic protein-7. Until the present date, however, there is no scientific evidence that irrefutably supports the superiority of one material over the others, which has increased interest in studies in this area.[5,8-18]

Propolis is a resinous hive substance produced by honeybees from products collected from plants. It is known to possess valuable antimicrobial, antiviral, fungicidal, local anesthetic, antiulcer, immunostimulating, hypotensive, and cytostatic properties.[8,19] *Copaifera langsdorffii* oleo-resin comes from the *Copaifera langsdorffii* trees, which are largely distributed in northern South America, mainly in the Amazon Rainforest. The purified resin oil, extracted from the trunk of these trees as a transparent shiny liquid ranging in color from yellow to brown. It is one of the most popular and promising phytomedicines used in Brazil by Amazonian traditional medical practitioners due to its recognized anti-inflammatory, analgesic, and wound-healing properties, as demonstrated in pharmacological studies.[9,20] More recently, the good results in traditional medicine have motivated studies with *Copaifera langsdorffii* oil in different fields of dentistry.[21,22]

There is a lack of research-based evidence that establishes the superiority of one type of vital pulp treatment for the primary dentition. The rationale for developing the current investigation was to seek new pulpal medications with minimal side effects and low cost. The purpose of this study was to evaluate histologically the repair of rat pulp tissue

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after pulpotomy and covering the pulp with *Copaifera langsdorffii* oil-resin, green propolis extract, iodoform-based paste, and a fibrin sponge.

Methods

Twenty-two approximately 90-day-old female Wistar rats (*Rattus norvegicus albinus*), weighing between 250 and 300 g, were obtained from the vivarium of Potiguar University, Natal, Rio Grande do Norte, Brazil, after the research protocol was approved by the local Animal Care and Research Use Committee. All guidelines regarding the care of animal research subjects were strictly followed in this study.

The animals were acclimated to the housing conditions over 1 week. Seven rats were housed in each cage and maintained under climate-controlled conditions (12-hour light/dark cycles; temperature between 22°C and 24°C). The cages were kept clean, and the sawdust beds were changed daily. During the experimental period, the animals were fed solid rat chow and water ad libitum, except for the 12 postoperative hours.

In 21 animals, caries-free maxillary and mandibular first molars were pulpotomized (84 total teeth) and randomly assigned to 4 groups of 21 teeth each, according to the material used for covering the remaining vital radicular pulp tissue: *Copaifera langsdorffii* oil-resin (Institute of Scientific and Technological Research of the State of Amapá, IEPA, Amapa, Brazil); an aqueous solution of green propolis extract containing 12% of the active substance (Propomax, Apis Flora, Ribeirão Preto, São Paulo, Brazil); a fibrin sponge (Hemospon, Technew, Rio de Janeiro, Brazil); and an iodoform-based paste [Guedes-Pinto paste[23], which has three components: iodoform, Rifocort® (5 mg prednisolone acetate, 1.5 mg rifamycin sodium salt and 0.25 mg propylene glycol; Merrel Lepetit, Santo Amaro, SP, Brazil) and camphorated paramonochlorophenol].

All rats received all materials because each first molar in every rat was randomly assigned to 1 of the 4 groups. The other animal did not receive pulpotomies and served as a parameter to evaluate the normal pulp's histological aspect.

In preparation for the clinical procedures, the animals were anesthetized with an intramuscular injection of tiletamine hydrochloride: zolazepam hydrochloride (Zoletil-50, Virbac do Brasil, Indústria e Comércio Ltda, São Paulo; 50 mg/kg body weight) diluted at a ratio of 1 g anesthetic powder to 5 mL sterile water.

After anesthesia, antisepsis of the oral cavity was performed with a polyvinylpyrrolidone-iodine solution (Asteriodine-Aster, Sorocaba, São Paulo), and the animals were immobilized in dorsal decubitus in a surgical table. The mouth was kept open with the aid of a holding device, and the pulp access cavities were prepared in the maxillary and mandibular first molars with a sterile one quarter spherical bur (KG Sorensen, São Paulo) mounted in a high-speed handpiece with coolant. After pulpotomy, the pulp chambers were irrigated with sterile saline and gently dried with sterile absorbent paper points. The pulp-capping materials were applied, and the access cavities were sealed with a temporary coronal filling material (Coltosol, Coltene-Whaledent, Cuyahoga Falls, Ohio)[24] under relative isolation with the use of cotton rolls and saliva ejectors. The green propolis extract solution and *Copaifera langsdorffii* oil-resin were applied to the pulp chamber with a 1-mm fragment of Hemospon fibrin sponge, which remained in the cavity.

At 24 hours, 15 days, and 30 days after the operative procedures, the animals were sacrificed in a carbon dioxide chamber and decapitated. Their maxillas and mandibles were dissected for gross and histological examinations. For the gross examination, the pieces were observed by 3 calibrated examiners blinded to the groups with the aid of an operative clinical microscope (DF Vasconcelos, São Paulo) at 16X magnification to determine whether the restoration was intact or not. After that, the pieces were: individually fixed in neutral buffered 10% formalin for at least 24 hours; washed in running water to remove traces of the solution; and demineralized in 7.5% nitric acid for 24 to 36 hours. Demineralization was considered complete when the piece was perforated with an insulin needle without offering resistance.

The specimens were then: washed in running water; dehydrated in a series of increasing ethanol concentrations; cleared in xylol; embedded in paraffin; and cut longitudinally to obtain semiserial 3- to 4-µm-thick sections totaling 2 microscopic glass slides containing 3 sections for each tooth. The sections were stained with hematoxylin & eosin and examined blindly by the 3 calibrated examiners with a light microscope (Olympus CX31, Olympus, Tokyo, Japan) coupled to an Olympus digital camera at magnifications of 40X, 100X, and 400X. The characteristics of the inflammatory infiltrate (cell types and their distribution), formation of mineralized tissue barrier, and presence of pulp necrosis and fibrosis were evaluated.

The severity of the inflammatory infiltrate and the extension of pulp necrosis were determined using a 4-point scoring system according to the following criteria: absence or insignificant presence of inflammatory infiltrate/necrosis; inflammatory infiltrate/necrosis close to the pulp medicament, reaching up to one third of the radicular pulp; inflammatory infiltrate/necrosis involving up to two thirds of the radicular pulp; and inflammatory infiltrate/necrosis involving more than two thirds of the radicular pulp.

The deposition of mineralized tissue was determined based on the absence or presence of mineralized tissue in the dental pulp, according to the following 3-point scoring system: 0=absence of mineralized tissue; 1=presence of mineralized tissue far from the pulp medicament; and 2=presence of mineralized tissue close to the pulp medicament.

Results

From the 84 pulpotomized teeth, some teeth were excluded from the study due to loss of the restoration, impossibility of using the histological sections (eg, absence of a clearly identifiable material/pulp interface); or furcal perforation during preparation of the access cavities. After discarding the teeth that met any of these exclusion criteria, other teeth were randomly eliminated in order to have all groups with the same number of specimens, thus totalizing 24 exclusions and a final sample size of 60 teeth distributed in the four groups (N=15).

In the 3 evaluation periods (24 hours, 15 days, and 30 days), the inflammatory response consisted primarily of a neutrophil infiltrate, being of milder intensity in the *Copaifera langsdorffii* oil-resin-treated group and more severe in the fibrin sponge-treated group (Table 1), in which periapical microabscesses were observed. Coagulation or liquefaction necrosis was observed in all cases, and the extension of the necrotic pulp area increased over time (Figure 2), except for the group in which the remaining vital radicular pulp tissue was covered with *Copaifera langsdorffii* oil-resin (Table 2).

Only the teeth treated with *Copaifera langsdorffii* oil-resin presented deposition of mineralized tissue subjacent to the capping material, starting from the 15th day (Figure 1). In the teeth capped with the other materials, deposition of mineralized tissue occurred far from the pulp exposure area (Figure 2; Table 3).

The teeth that were not pulpotomized were rated as 1 (no inflammation), indicating normal conditions. Edema and vascular congestion were commonly observed in all groups without important morphological differences. In several cases, vascular congestion was observed in regions far from the necrotic pulp tissue.

Table 1. DISTRIBUTION OF THE TEETH ACCORDING TO THE PULP MEDICAMENTS, EVALUATION PERIOD, AND INTENSITY OF THE INFLAMMATORY INFILTRATE*

Substances	24 hrs				15 days				30 days			
	Score				Score				Score			
	1	2	3	4	1	2	3	4	1	2	3	4
Copaifera langsdorffii oil-resin	-	5	-	-	-	3	2	-	2	3	-	-
Green propolis extract	-	4	1	-	2	2	1	-	5	-	-	-
Iodoform-based paste	-	5	-	-	-	-	2	3	-	4	1	-
Fibrin sponge	-	4	1	-	2	-	3	-	2	1	2	-

* From the 84 pulpotomized teeth, 24 were excluded from the study, reducing the sample size to 60 teeth (N=5 teeth per material at each evaluation period). Score 1=absence or insignificant presence of inflammatory infiltrate; score 2=inflammatory infiltrate close to the pulp medicament, reaching up to one third of the radicular pulp; score 3=inflammatory infiltrate involving up to two thirds of the radicular pulp; and score 4=inflammatory infiltrate involving more than two thirds of the radicular pulp.

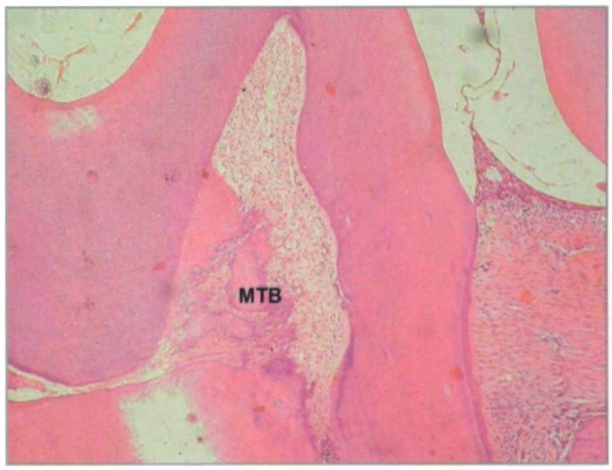

Figure 1. Photomicrograph of a rat tooth, taken 30 days after pulpotomy done with *Copaifera langsdorffii* oil-resin, showing the formation of a mineralized tissue barrier and the presence of subjacent pulp tissue with normal aspect (100X magnification).

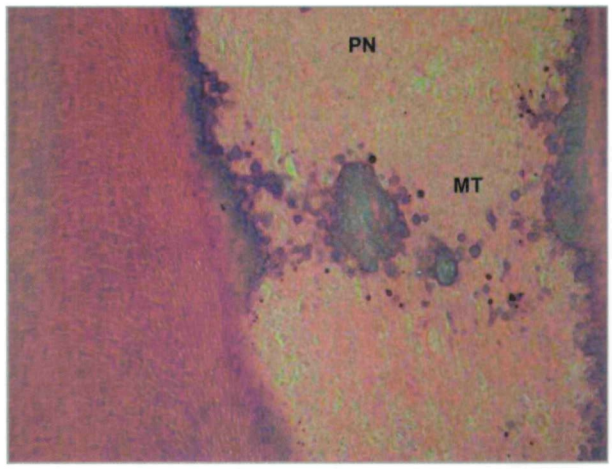

Figure 2. Photomicrograph of a rat tooth, taken 30 days after pulpotomy done with iodoform-based paste, showing an extensive area of pulp necrosis and formation of mineralized tissue (dystrophic calcification) far from the area of pulp exposure (100X magnification).

Discussion

The search for natural substances for use in endodontics has been the aim of several investiagtions.[2,3,5,10,25] Pulp medicaments should induce the regeneration of the remaining pulp tissue, and any potential inflammatory response caused by their application must not cause harm to the pulp. Based on these prerogatives, the present study evaluated the use of green propolis extract and *Copaifera langsdorffii* oil-resin as pulp medicaments, since these substances are considered to be biocompatible and present therapeutic properties that are widespread in folk medicine and supported by the research-based evidence.[10,19,26-29] The iodoform-based (Guedes-Pinto) paste was included in the materials that the authors compared to the *Copaifera langsdorffii* oil-resin, because it has excellent biocompatibility to pulp fibroblasts, produces a mild inflammatory reaction, and is well tolerated by periapical tissues.[23]

In the histological evaluation, the entire interface between the material and the pulp tissue was considered for analysis. One of the difficulties encountered during the laboratory processing in the present study was due to the fact that the roots in rat teeth are not arranged in the same longitudinal plane. This lack of alignment of the roots made it difficult to include 2 or more roots in the same histological section.

Coltosol is one of the best temporary coronal filling materials used in endodontics.[24,30] In human teeth, it needs to have a minimum of 2 mm thickness to prevent microleakage[30] and ideally should not be left in place longer than 2 weeks. In the present study, the material layer had to be thinner because of the rat teeth's small size, and the experimental design required the maintenance of the coronal seal for a longer time.

It has been demonstrated that, in addition to having a low irritating potential,[3] propolis induces the healing process in epithelial tissue[31] and pulp tissue,[2] with formation of

Table 2. DISTRIBUTION OF THE TEETH ACCORDING TO THE PULP MEDICAMENTS, EVALUATION PERIOD, AND AREA OF PULP NECROSIS*

Pulp medicaments	24 hrs				15 days				30 days			
	Score				Score				Score			
	0	1	2	3	0	1	2	3	0	1	2	3
Copaifera langsdorffii oil-resin	-	5	-	-	1	4	-	-	1	3	1	-
Green propolis extract	-	5	-	-	-	1	2	2	-	-	-	5
Iodoform-based paste	3	2	-	-	-	-	3	2	-	1	2	2
Fibrin sponge	-	3	2	-	-	-	1	4	-	-	1	4

* From the 84 pulpotomized teeth, 24 were excluded from the study, reducing the sample size to 60 teeth (N=5 teeth per material at each evaluation period). Score 1=absence or insignificant presence of necrosis; score 2=necrosis close to the pulp medicament, reaching up to one third of the radicular pulp; score 3=necrosis involving up to two thirds of the radicular pulp; and score 4=necrosis involving more than two thirds of the radicular pulp.

Table 3. DISTRIBUTION OF THE TEETH ACCORDING TO THE PULP MEDICAMENTS, EVALUATION PERIOD, AND DEPOSITION OF MINERALIZED TISSUE (MINERALIZED TISSUE BARRIER)*

Substances	24 hrs			15 days			30 days		
	Score			Score			Score		
	0	1	2	0	1	2	0	1	2
Copaifera langsdorffii oil-resin	5	-	-	1	-	4	2	-	3
Green propolis extract	5	-	-	3	2	-	-	5	-
Iodoform-based paste	5	-	-	1	4	-	-	5	-
Fibrin sponge	5	-	-	5	-	-	1	4	-

* From the 84 pulpotomized teeth, 24 were excluded from the study, reducing the sample size to 60 teeth (N=5 teeth per material at each evaluation period). Score 0=absence of mineralized tissue; score 1=presence of mineralized tissue far from the pulp medicament; and score 2=presence of mineralized tissue close to the pulp medicament.

collagen and dentin bridges.[5,32] In the present study, however, in which an aqueous propolis extract was used, the formation of a mineralized tissue barrier subjacent to the region of pulp capping was not observed within the 30-day experimental period. Deposition of mineralized tissue occurred only along the roots, which was characterized as a dystrophic calcification. On the other hand, there was an increase in the area of necrotic pulp over time due to replacement of normal pulp tissue with totally necrotic tissue. It is worth mentioning that propolis products have been implicated in adverse reactions in humans (with dermatological or respiratory symptoms) and that there are at least 16 reports of allergic reactions.[33] Further studies with other substances based on aqueous or alcoholic propolis extracts should be developed to compare the pulp reaction. Considering the possible adverse reactions of propolis to human health, it seems advisable to refrain from using the product in humans until more animal testing is done.

Considering that materials causing tissue irritation lead to a delay in the repair process, the results obtained in the present study demonstrated the biocompatibility of *Copaifera langsdorffii* oil-resin, as confirmed by the deposition of mineralized tissue subjacent to the material. *Copaifera langsdorffii* oil-resin's biocompatibility has been demonstrated by Paiva et al,[20] who described an acceleration in the healing of experimentally induced wounds in rat skin.

In the first 24 hours, the iodoform-based paste induced a mild inflammatory response in all cases. This result can be attributed to the fact that this paste has the medication Rifocort in its composition,which is a corticosteroid (prednisolone) associated with an antibiotic (rifamycin). Prednisolone has the capacity to inhibit vasodilatation and leukocyte migration. The teeth capped with this paste presented an increase in the intensity of the inflammatory infiltrate with time, with subsequent progression of the area of necrosis, which ranged from moderate to large. This finding may be attributed to the following actions of prednisolone contained in the paste: reduces the exudative vascular phenomena due to the inhibition of cellular production of histamine; neutralizes serotonin; decreases the adherence of leukocytes to the endothelial walls; and, consequently, reduces the host's natural defenses, delaying the healing mechanisms and leading to the occurrence of a severe inflammatory reaction.

Analyzing and grading the tissue phenomena (inflammation and repair) in the different groups, it is possible to state that, among the different substances placed in direct contact with the pulp tissue, the fibrin sponge produced the worst healing outcome. Since this material was used as a vehicle for application of the aqueous green propolis extract and *Copaifera langsdorffii* oil-resin in the pulpotomized teeth, it is expected that the replacement of this material by another that does not interfere in the progression of tissue repair might result in an even better healing outcome when those herbal products are used as experimental pulp capping agents. Further research is needed to reach more conclusive results about their possible application in human patients.

Conclusion

The findings of this study showed that, when compared to the other materials tested (propolis extract, fibrin sponge and iodoform-based paste), the inflammatory response to the *Copaifera langsdorffii* oil-resin was less severe; the extension of pulp necrosis increased over time, except when the remaining vital radicular pulp was covered with *Copaifera langsdorffii* oil-resin; and formation of a mineralized tissue barrier subjacent to the capping material was observed only in the teeth treated with *Copaifera langsdorffii* oil-resin.

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References

1. Wataha JC. Biocompatibility of dental materials. In: Anusavice KJ. *Phillips' Science of Dental Materials.* 11th ed. São Paulo, Brazil: Elsevier; 2005:161-90.
2. Bretz W, Chiego Júnior DJ, Cunhai MMC, Custódio A, Schneider LG. Preliminary report on the effects of propolis on wound healing in the dental pulp. Z Naturforsch C 1998;53:1045-8.

3. Silva FB da, Almeida JM, Sousa SMG de. Natural medicaments in endodontics: A comparative study of anti-inflammatory action. Braz Oral Res 2004;2:174-9.
4. Gentil M, Pereira JV, Sousa YT, et al. In vitro evaluation of the antibacterial activity of Arctium lappa as a phytotherapeutic agent used in intracanal dressings. Phytother Res 2006;20:184-6.
5. Sabir A, Tabbu CR, Agustiono P, Sosroseno W. Histological analysis of rat dental pulp tissue capped with propolis. J Oral Sci 2005;47:135-8.
6. Israelsen LD. Phytomedicines as a new crop opportunity. In: Janick J, Simon JE, eds. *New Crops*. New York, NY: Wiley; 1993.
7. Glass RL, Zander HA. Pulp healing. J Dent Res 1949; 28:97-107.
8. Burdock GA. Review of the biological properties and toxicity of bee propolis. Food Chem Toxicol 1998;36:347-63.
9. Basile AC, Sertié JAA, Freitas PCD, Zanini AC. Anti-inflammatory activity of oleoresin from Brazilian Copaifera. J Ethnopharmacol 1988;22:101-9.
10. Bandeira MFCL, Oliveira MRB, Benatti Neto C, Comelli Lia RC. Comparative study of the biological compatibility of *Copaifera langsdorffii* essential oil and oil-resin associated with calcium hydroxide. J Bras Clin Estetica Odontol 1999;3:42-9.
11. Estrela C, Holland R. Calcium hydroxide: Study basic on scientific evidences. J Appl Oral Sci 2003;11:269-82.
12. Salako N, Joseph B, Ritwik P, Salonen J, John P, Junaid TA. Comparison of bioactive gloss, mineral trioxide aggregate, ferric sulfate, and formocresol as pulpotomy agents in rat molar. Dent Traumatol 2003;19:324-20.
13. Qudeimat MA, Barrieshi-Nusair KM, Owais AI. Calcium hydroxide vs mineral trioxide aggregates for partial pul-potomy of permanent molars with deep caries. Eur Arch Paediatr Dent 2007;8:99-104.
14. Silva LA, De Paula e Silva FW, Leonardo MR, Assed S. Pulpal and periapical response of dogs' teeth after pulpotomy and use of recombinant human bone morphogenetic protein-7 as a capping agent. J Dent Child 2007;74:79-84.
15. Sabbarini J, Mohamed A, Wahba N, El-Meligy O, Dean J. Comparison of enamel matrix derivative versus formocresol as pulpotomy agents in the primary dentition. J Endod 2008;34:284-7.
16. Silva LA, De Paula e Silva FW, Leonardo MR, Assed S. Radiographic evaluation of pulpal and periapical response of dogs' teeth after pulpotomy and use of recombinant human bone morphogenetic protein-7 as a capping agent. J Dent Child 2008;5:14-9.
17. Sonmez D, Sari S, Cetinbaş TA. Comparison of four pulpotomy techniques in primary molars: A long-term follow-up. J Endod 2008;34:950-5.
18. Zurn D, Seale NS. Light-cured calcium hydroxide vs formocresol in human primary molar pulpotomies: A randomized controlled trial. Pediatr Dent 2008;30:34-41.
19. Park YK, Koo MH, Abreu JA, Ikegaki M, Curuy JA, Rosalin PL. Antimicrobial activity of propolis on oral micro-organisms. Curr Microbiol 1998;36:24-8.
20. Paiva LA, de Alencar Cunha KM, Santos FA, Gramosa NV, Silveira ER, Rao VS. Investigation of the wound healing activity of oleo-resin from *Copaifera langsdorffii* in rats. Phytother Res 2002;16:737-9.
21. Garrido AD, Lia RC, França SC, da Silva JF, Astolfi-Filho S, Sousa-Neto MD. Laboratory evaluation of the physicochemical properties of a new root canal sealer based on Copaifera multijuga oil-resin. Int Endod J 2010;43: 283-91.
22. Rosa PCF, Mancini MNG, Camargo SEA, Garrido ADB, Camargo CHR, Rode SM. Dimensional alterations and solubility of new endodontic sealers. Braz Dent J 2010; 21:301-4.
23. Cerqueira DF, Mello-Moura AC, Santos EM, Guedes-Pinto AC. Cytotoxicity, histopathological and microbiological, and clinical aspects of an endodontic iodoform-based paste used in pediatric dentistry: A review. J Clin Pediatr Dent 2008;32:105-10.
24. Madarati A, Rekab MS, Watts DC, Qualtrough A. Time-dependence of coronal seal of temporary materials used in endodontics Aust Endod J 2008;34:89-93.
25. Ferreira FB, Torres SA, Rosa OP, et al. Antimicrobial effect of propolis and other substances against elected endodontic pathogens. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;104:709-16.
26. Burdock GA. Review of the biological properties and toxicity of bee propolis. Food Chem Toxicol 1998;36: 347-63.
27. Dobrowolski JW, Vohora SS, Sharma K, Shah SA, Naqui SAH, Dandiya PC. Antibacterial, antifungal, anti-amoebic, anti-inflammatory, and antipyretic studies on propolis bee products. J Ethnopharmacol 1991;35:77-82.
28. Paiva LA, Gurgel LA, De Sousa ET, et al. Protective effect of *Copaifera langsdorffii* oleo-resin against acetic acid-induced colitis in rats. J Ethnopharmacol 2004;93:51-6.
29. Steinberg D, Kaine G, Gedalia I. Antibacterial effect of propolis and honey on oral bacteria. Am J Dent 1996;9: 236-9.
30. Zaia AA, Nakagawa R, De Quadros I, et al. An in vitro evaluation of four materials as barriers to coronal microleakage in root-filled teeth. Int Endod J 2002;35:729-34.
31. Magro Filho O, Carvalho AC. Application of propolis to dental sockets and skin wounds. J Nihon Univ Sch Dent 1990;32:4-13.
32. Scheller S, Ilewicz L, Luciak M, Skrobidurska D, Stojko A, Matuga W. Biological properties and clinical application of propolis. Experimental observation on the influence of ethanol extract of propolis (EEP) on dental pulp regeneration. Arzneimittelforschung 1978;28: 289-91.
33. Menniti-Ippolito F, Mazzanti G, Vitalone A, Firenzuoli F, Santuccio C. Surveillance of suspected adverse reactions to natural health products: The case of propolis. Drug Saf 2008;31:419-23.