



Antibacterial action of red and green propolis extract in infected root canal

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ABSTRACT

OBJECTIVE: This study investigated the antibacterial action of 30% red propolis, 40% green propolis and 2.5% sodium hypochlorite with irrigation protocols in infected root canals.

METHODS: During 60 days, twenty-four root canals were inoculated with *E. faecalis*. In all experimental groups were performed root canal preparation and two irrigation protocols - passive ultrasonic irrigation and conventional irrigation. In the groups 1, 3, 5 and 7 it were made root canal preparation associated to conventional irrigation with 30% propolis, 40% propolis, 2.5% sodium hypochlorite and distilled water, respectively. In groups 2, 4, 6 and 8 it was made root canal preparation associated with passive ultrasonic irrigation with the solutions described above. The groups 9 and 10 were the controls (negative and positive). Samples of the root canals were collected and immersed in 7 mL of BHI, for 48 hours, at 37°C. Bacterial growth was analyzed by turbidity of the culture medium.

RESULTS: Antibacterial action was observed of 30% red propolis and 2.5% sodium hypochlorite when used passive ultrasonic irrigation and conventional irrigation after 20 minutes, but in 72 hours it was not effective.

CONCLUSION: The irrigating agents and protocols were not effective to eliminate the microorganisms of the infected root canals. *Clinical Significance:* Propolis presents potential for clinical application due to its antimicrobial, anti-inflammatory, antioxidant and low toxicity properties.

Key words: Endodontic; Propolis; Sodium hypochlorite

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Ação antimicrobiana do extrato de própolis vermelho e verde em canais radiculares infectados

RESUMO

OBJETIVO: Investigar a ação antibacteriana do própolis vermelhos 30%, do própolis verde 40% e do hipoclorito de sódio 2,5% com protocolos de irrigação em canais radiculares infectados.

MÉTODOS: Durante 60 dias, vinte e quatro canais radiculares foram inoculados com *E. faecalis*. Em todos os grupos experimentais foi realizado o preparo do canal radicular e dois protocolos de irrigação - irrigação ultra-sônica passiva e irrigação convencional. Nos grupos 1, 3, 5 e 7 foi feito o preparo do canal radicular associado à irrigação convencional com própolis 30%, própolis 40%, hipoclorito de sódio 2,5% e água destilada, respectivamente. Nos grupos 2, 4, 6 e 8 foi feito o preparo do canal radicular associado à irrigação ultra-sônica passiva com as soluções descritas acima. Os grupos 9 e 10 foram os controles (negativos e positivos). Amostras dos canais radiculares foram coletadas e imersas em 7 mL de BHI, durante 48 horas, a 37°C. O crescimento bacteriano foi analisado por turvação do meio de cultura.

RESULTADOS: Ação antibacteriana foi observada com própolis vermelho 30% e hipoclorito de sódio 2,5% quando utilizado irrigação ultra-sônica passiva e irrigação convencional após 20 minutos, porém, após 72 horas as substâncias não foram efetivas.

CONCLUSÃO: Os agentes de irrigação e os protocolos não foram efetivos para eliminar os microorganismos dos canais radiculares infectados.

Palavras-chave: Endodontia; Própolis; Hipoclorito de sódio

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INTRODUCTION

Bacteria constitute important etiologic agents for dental pulp and periapical tissues infection [1-3]. Bacterial growth is enhanced by complex anatomy of root canal system, inaccessible areas to endodontic instruments, which represent an ideal environment for bacterial biofilm structuring [2, 3]. Bacterial biofilm is structured from microorganisms attachment on a solid surface embedded in an extracellular matrix, resistant to antimicrobial agents, what favors the maintenance of infection process and represents a special challenge for successful root canal treatment [4, 5].

Enterococcus faecalis is often isolated in teeth with failed root canal treated. The ability to invade the dentinal tubules, be resistant to antimicrobial agents and interfere with host defenses reveals its pathogenic role [6-8]. The use of antibacterial strategies that can promote the disruption of the biofilm is essential to the success of the endodontic therapy [3, 5]. Sodium hypochlorite is extensively used in endodontics as irrigating solution due to its antimicrobial effect. The mechanism of action includes biosynthetic changes; destruction of phospholipids; chloramines formation which interfere in cell metabolism; oxidative action with enzymatic inactivation in bacteria and degradation of fatty acids and lipids⁹. However, extrusion of this irrigating solution can cause intense reactions in the periapical tissues [10].

Antimicrobial activity and biological responses of periapical tissues of irrigating solutions have stimulated studies looking for natural alternatives. In this regard, propolis has been investigated for presenting antimicrobial properties, anti-inflammatory, antioxidant, and others [11-24]. Because of its low toxicity, it has been studied as possible intracanal medication [11, 12].

Maia Filho et al. [13] compared the effectiveness of 5% sodium hypochlorite, 2% chlorhexidine gel and propolis extract against *E. faecalis* by agar diffusion test. Propolis extract showed good antimicrobial activity. Koo et al.¹⁴ evaluated the antimicrobial activity method of 10% propolis extract and 10% arnica montana against fifteen microorganisms of the endodontic microbiota among which *E. faecalis*, by agar diffusion test. Propolis extract significantly inhibited the tested microorganisms with the largest inhibition zones for *Actinomyces spp.* In the analyzed propolis samples, from different regions, an important difference was found in total flavonoids after colorimetric analysis. The results indicated that the chemical composition of propolis, especially in relation to the total flavonoid content is dependent on a variety of factors. These flavonoids in propolis act on bacteria's membrane or cell wall, causing structural and functional damage¹⁹. Alencar et al.¹⁸ determining the minimum inhibitory concentration verified an antimicrobial activity of propolis against *S. aureus* and *S. mutans*.

Considering the potential of propolis as an antibacterial agent, the present study aims to evaluate the antibacterial activity of the alcoholic extract of 30% red propolis, 40%

green propolis and 2.5% sodium hypochlorite, when subjected to passive ultrasonic irrigation in infected root canals.

MATERIALS AND METHODS

This study was approved by in Research Ethics Committee of the Federal University of Goiás. The study design was similar to that previously used⁸. A strain of *E. faecalis* was inoculated into 7 ml of Brain Heart Infusion (BHI; Difco Laboratories, Detroit, MI, USA) and incubated for 24 hours at 37°C. Bacterial cells were suspended in saline solution in order to achieve a concentration of about $3 \times 10^8 \text{ ml}^{-1}$ cells adjusted to #1 McFarland turbidity standard.

The sample included thirty single-rooted human teeth with intact cementum, extracted at the Dental Urgency Service of the School of Dentistry of the Federal University of Goiás, Brazil, for different reasons (periodontal, prosthetic or other). The teeth were stored in a bottle containing 0.2% thymol solution and subsequently immersed in 5% sodium hypochlorite for 30 minutes to remove organic tissue. Periapical radiographs (Eastman Kodak. Comp., NY, USA) in buccolingual and proximal directions were taken to confirm presence of just one root canal and absence of anatomical variations. Teeth with obliteration of the radicular canal and root disruption were excluded.

After initial radiographs, the crowns were removed under continuous jet of air/water, with laminated drill Endo-Z (Maillefer, Ballaigues, Switzerland) at high speed. Radicular lengths were standardized at 16 mm (from the apex to the amelocementarium limit). The teeth were emptied to the apical limit with K-flex instrument # 15 (Maillefer, Ballaigues, Switzerland). The anatomical diameter of the selected teeth was approximately 350-400 micrometers (diameter corresponding to K file-file No. 35/40) to penetrate and maintain just to the working limit. At the following radicular canals were prepared with BioRace system (FKG Dentaire, La Chaux-de Fonds, Switzerland) using the BR5 40/0.04 instrument. Conventional irrigation was performed with 3 mL of 2.5% sodium hypochlorite with a 5 ml syringe and Endo Eze irrigation cannula (Ultradent Products Inc., South Jordan, UT, USA).

Subsequently, the canals were dried and filled with 17% EDTA (pH 7.2 – Biodynamics, Ibiporã, PR, Brazil) for 3 minutes to remove the smear layer and then autoclaved for 30 minutes at 120°C.

Experimental design

A split platform was used during the inoculation period of the bacterial suspension. The coronal portion of the root canal of each tooth was connected to the bottom of a 1.5 mL polypropylene tube Eppendorf (Cral, São Paulo, SP, Brazil). Five milliliters of sterile BHI were mixed with 5 mL of the bacterial inoculum, and the experimental and positive control groups were inoculated with *E. faecalis* using sterile syringes with a sufficient volume to fill the root canal. This procedure was repeated every 72 hours, always using pure

culture with 24 hours of preparation and set the standard #1 McFarland.

During the 60 days of root canal infection, three teeth were left uncontaminated and incubated at 37°C as negative control and as positive control; three teeth were inoculated with *E. faecalis* and incubated at 37°C. The negative control group was used to verify the sterility of the samples and the positive control group was used to check the bacterial viability during the experiment.

After the biofilm formation period, samples were collected from the root canals of all groups to check bacterial viability. The root canals were filled with sterile distilled water and three #40 sterilized paper points (Tanari, Tanariman Industrial Ltda., Manacapuru, Amazonas, Brazil) were introduced into the radicular canal and maintained for 1 minute. The points were individually transported and immersed in 7 mL of BHI (Brain Heart Infusion; Difco Laboratories, Detroit, MI, USA) added with the neutralizers [Tween 80 and sodium thiosulfate (PA Art Laboratory, Campinas, Brazil)] at appropriate concentrations, followed by incubation at 37°C for 48 hours.

The teeth (n=24) were randomly distributed into eight experimental groups (n=3). In all the experimental groups were performed root canal preparation and two irrigation protocols – conventional irrigation and passive ultrasonic irrigation. In groups 1, 3, 5 and 7 was performed root canal preparation associated to conventional irrigation with the tested solutions: 30% red propolis alcoholic extract (Ilha do Porto Apiary, Alagoas, Al, Brazil, Natural Labor Laboratory), 40% green propolis alcoholic extract (Santo Antonio Apiary, São Paulo, SP, Brazil), 2.5% sodium hypochlorite (Fitofarma, Goiânia, GO, Brazil) and sterilized distilled water. In groups 2, 4, 6 and 8 was performed root canal preparation associated to passive ultrasonic irrigation with the same solutions. Each group received the same irrigation solution volume (27 mL). The conventional irrigation process was carried out for groups 1, 3, 5 and 7 throughout the root canal preparation with a 5 mL syringe and Endo-Eze Irrigator Tip irrigation cannula (Ultradent Products Inc., South Jordan, UT USA) with 0.40 mm diameter positioned at 12 mm. Group 9 was the positive control and group 10, the negative control (Table 1).

The groups were prepared with BioRaCe system extension (FKG Dentaire, Swiss Dental Products, La Chaux-de-Fonds, Swiss) following sequence BR6 #50.02, #60.02 and BR7, each one used in only 5 root canals.

For groups 2, 4, 6 and 8 an ultrasonic stirring was performed during the last irrigation with ultrasonic device (EMS MW 200, Swiss), at 20% power in accordance with the manufacturer's instructions. The ultrasonic tip (E1 irrisonic – Heise) was positioned in the radicular canal and activated for 30 sec, performing short shuttle movements, carefully not to touch the walls of the root canal and avoiding damaging it.

Root canals were irrigated with 3 mL of the tested solutions before radicular canal preparation, during and after instrumentation with instrument #BR 50.02 and BR#

60.02. Each group received the same volume of irrigating solution.

After the sanitization process of all experimental groups, each root canal was filled with 3 mL of 17% EDTA (pH 7.2- Biodynamics, Ibioporã, PR, Brazil), which was kept under stirring with a hand file for 3 min to remove of smear layer. An additional irrigation with 5 mL of sterile distilled water was performed with a 5 mL syringe and Endo-Eze Irrigator Tip irrigation cannula (Ultradent Products Inc. South Jordan, UT, USA) to neutralize the effects of irrigating solutions in all experimental groups.

Samples from each tooth were taken from the root canal using three sterilized absorbent paper points # 60 (Tanari, Tanariman Industry Ltda., Manacapuru, Amazonas, Brazil) which were introduced in each experimental group for 1 min. Three absorbent paper points were individually transported and immersed in 7 mL of BHI (Brain Heart Infusion; Difco Laboratories, Detroit, MI, USA) added with neutralizers followed by incubation for 48 hs at 37°C. These procedures were repeated 1 minute after the conclusion of root canal preparation with the use of the tested solutions, after 20 minutes and after 72 hours of the conclusion of root canal preparation.

Bacterial growth was analyzed by turbidity of the culture medium through visual reading. After 48 hours an inoculum of 0.1 ml from obtained medium was transferred to 7 mL of Lethen Broth (Lethen Broth; Difco Laboratories, Detroit, MI, USA) and incubated for 48 hours at 37°C.

Table 1. Experimental group's distribution.

Groups	Antibacterial strategies	Samples (n=30)
1	PCS+RPE 30% (Conventional Irrigation)	3
2	PCS+RPE 30% (Passive Ultrasonic Irrigation)	3
3	PCS+GPE 40% (Conventional Irrigation)	3
4	PCS+GPE 40% (Passive Ultrasonic Irrigation)	3
5	PCS+NaOCl 2,5% (Conventional Irrigation)	3
6	PCS+NaOCl 2,5% (Passive Ultrasonic Irrigation)	3
7	PCS+H ₂ O distilled (Conventional Irrigation)	3
8	PCS+H ₂ O distilled (Passive Ultrasonic Irrigation)	3
9	Positive Control	3
10	Negative Control	3

RCP: Root Canal Preparation; NaOCl: Sodium Hypochlorite 2.5%; RPE: Red Propolis Extract; GPE: Green Propolis Extract.

RESULTS

The antibacterial action of the studied solutions is presented in Table 2. The results showed antibacterial effectiveness of 30% red propolis alcoholic extract and 2.5% sodium hypochlorite when using conventional or passive irrigation only after 20 min. Irrigation protocols and the tested substances were not effective to eliminate *E. faecalis* from the root canals.

Table 2. Antibacterial action of chemical substances in infected root canals.

Groups/ Periods	1 minute	20 minutes	72 hours
RCP+30% RPE (Conventional Irrigation)	+++	---	+++
RCP+30% RPE (Passive Ultrasonic Irrigation)	+++	---	+++
RCP+40% GPE (Conventional Irrigation)	+++	+++	+++
RCP+40% GPE (Passive Ultrasonic Irrigation)	+++	+++	+++
RCP+2.5% NaOCl (Conventional Irrigation)	+++	---	+++
RCP+2.5% NaOCl (Passive Ultrasonic Irrigation)	+++	---	+++
RCP+H ₂ O distilled (Conventional Irrigation)	+++	+++	+++
RCP+H ₂ O distilled (Passive Ultrasonic Irrigation)	+++	+++	+++
Positive Control	+++	+++	+++
Negative Control	---	---	---

RCP: Root canal preparation; RPE: Red Propolis Extract; GPE – Green Propolis Extract.
 +++: presence of bacteria; ---: absence of bacteria.

DISCUSSION

Several sanitization strategies for infected root canal aim to reduce the microorganisms, based on emptying and enlargement, associated with intracanal substances with antimicrobial activities, and finally the endodontic and coronal sealing. Root canal treatment can only be considered complete after the final tooth restoration [5,9].

Bacterial control remains challenging, and new auxiliary sanitization substances and processes has been under investigation. The alcoholic extracts of 30% red propolis, 40% green propolis and 2.5% sodium hypochlorite used as irrigating solutions in conventional and passive ultrasonic irrigation were not effective to decontaminate infected root canals.

Propolis has been studied due several properties, as antimicrobial, anti-inflammatory, antioxidant, and others [11-15, 17-33]. The chemical compounds found in propolis showed antimicrobial and tissue tolerance characteristics. The propolis used in this study presents flavonoids and phenolic compounds [18, 23]. The 30% red propolis alcoholic extract has a chemical composition of 2.4% flavonoids and 13.2% phenolic compounds; while the of 40% green propolis alcoholic extract shows the composition of 3.52% flavonoids and 3.75% phenolic compounds. The antimicrobial property has been attributed much to the flavonoids [14, 26], which are present in propolis with capacity to act on bacteria membrane or cell wall, dissolving the lipophilic part [27, 28], causing structural and functional damage [19].

In the present study, the antibacterial action in the period of 20min showed that conventional or ultrasonic irrigation using 30% red propolis alcoholic extract or 2.5% sodium hypochlorite was better than the 40% green propolis alcoholic extract. Al-Qathami and Al Madi [24] compared the antimicrobial activity of propolis and 2.5% sodium hypochlorite as canal irrigants. Irrigation with propolis showed no significant difference with 2.5% sodium hypochlorite. Ehsani et al. [29] compared the antimicrobial

activity of Aloe Vera gel, 15% and 40% propolis alcoholic extract and 2% chlorhexidine. The 15% and 40% propolis extracts and Aloe Vera showed antibacterial effect on *E. faecalis*. Koo et al. [14] evaluated the antimicrobial activity of 10% propolis extract and 10% Arnica Montana on the endodontic microbiota, including *E. faecalis*. Propolis extract significantly inhibited the tested microorganisms. Gomes et al. [30] verified the antimicrobial efficacy of propolis in various concentrations (5, 10, 15 and 20%) on some microorganisms (*C.albicans*, *S. mutans*, *S. aureus*, *E. faecalis*, *A. israeli*) using agar diffusion test. All tested species were susceptible to propolis. Vargas et al. [31] evaluated an antibacterial action of 50% propolis alcoholic extract. It demonstrated antibacterial activity by inhibiting the growth of Gram-positive in 92.6% and Gram-negative in 42.5%. Propolis achieved a greater effectiveness against Gram-positive bacteria and limited against Gram-negative bacteria [32]. In the present study, it was observed an impregnation and oily darkened pigmentation in the experimental groups constituted by the 30% red propolis alcoholic extract and 40% green propolis alcoholic extract.

Several studies have considered sodium hypochlorite as the substance that agglutinates the largest number of characteristics for its use as irrigant in infected root canals [4, 5, 8, 9].

Bhardwaj et al. [34] reported that passive ultrasonic irrigation with 1% sodium hypochlorite was effective to completely remove *E. faecalis* biofilms compared to natural substances (Aloe Vera, Nona juice and propolis). Whereas, Harrison et al. [35] showed that 1% sodium hypochlorite with passive ultrasonic irrigation was effective, but not completely removed *E. faecalis* from infected radicular canals. Our results also showed that the irrigant solutions (30% red propolis alcoholic extract, 40% green propolis alcoholic extract and 2.5% sodium hypochlorite) and the irrigation protocols (conventional and passive ultrasonic irrigation) were not able to kill *E. faecallis* in infected root canals.



In the current context of endodontics, it must be considered all resources to achieve successful endodontic treatment. Sodium hypochlorite continues to express a wide range of indications as an irrigant for infected root canals.

CONCLUSION

The antibacterial action of 30% red propolis alcoholic extract and 2.5% sodium hypochlorite when used in conventional or passive ultrasonic irrigation was observed only after 20 minutes. The irrigating agents and protocols were not effective to eliminate the test microorganism from the infected root canal.

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