

Antimicrobial activity of a calcium hydroxide and *Ricinus communis* oil paste against microorganisms commonly found in endodontic infections

Atividade antimicrobiana de uma pasta medicamentosa a base de hidróxido de cálcio e óleo de *Ricinus communis* sobre microrganismos comumente encontrados nas infecções endodônticas

Abstract

Purpose: To evaluate the antimicrobial activity of two pastes used as root canal dressing: a calcium hydroxide and *Ricinus communis* oil paste (Paste A) and a calcium hydroxide and propylene glycol paste (Paste B).

Methods: The Agar-well diffusion test was used to evaluate the antimicrobial activity of pastes A and B against the following microorganisms, commonly found in the oral cavity, specifically in endodontic infections: *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, *Streptococcus sanguinis*, and *Candida albicans*. The diameter (mm) of the inhibition halos was measured using a digital pachimeter, and the data were statistically analyzed using 1-way ANOVA and Tukey test ($\alpha = 0.05$).

Results: Paste A displayed larger inhibition halos than Paste B and the control group ($P < 0.05$).

Conclusion: It was concluded that Paste A had greater antimicrobial activity than Paste B.

Key words: Products with antimicrobial action; calcium hydroxide; *Ricinus communis*

Resumo

Objetivo: Avaliar a atividade antimicrobiana de duas pastas medicamentosas para utilização intracanal: pasta de hidróxido de cálcio e óleo de *Ricinus communis* (Pasta A) e pasta de hidróxido de cálcio e propilenoglicol (Pasta B).

Metodologia: O teste de difusão em Ágar foi utilizado para avaliar a atividade antimicrobiana das pastas em relação aos microrganismos *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, *Streptococcus sanguinis* e *Candida albicans*, comuns na cavidade bucal, especificamente em infecções endodônticas. O diâmetro (mm) dos halos de inibição foi medido utilizando um paquímetro digital e os valores obtidos foram submetidos à análise estatística (ANOVA e teste de Tukey – $\alpha = 0.05$).

Resultados: A Pasta A apresentou maiores halos de inibição que a Pasta B e o grupo controle, com diferença estatística significativa entre eles ($P < 0.05$).

Conclusões: Conclui-se que a Pasta A teve maior atividade antimicrobiana que a Pasta B.

Palavras-chave: Produtos com ação antimicrobiana; hidróxido de cálcio; *Ricinus communis*

Lucas da Fonseca Roberti Garcia^a
Gabriela Lemos de Almeida^b
Fernanda de C. P. Pires-de-Souza^c
Simonides Consani^d

^a Graduate Program in Dental Materials, Piracicaba School of Dentistry, State University of Campinas, Piracicaba, SP, Brazil

^b Department of Social Dentistry – Public Health and Preventive Dentistry, Piracicaba School of Dentistry, State University of Campinas, Piracicaba, SP, Brazil

^c Department of Dental Materials and Prosthodontics, Ribeirão Preto School of Dentistry, University of São Paulo, Ribeirão Preto, SP, Brazil

^d Department of Restorative Dentistry, Dental Materials Area, Piracicaba School of Dentistry, State University of Campinas, Piracicaba, SP, Brazil

Correspondence:

Lucas da Fonseca Roberti Garcia
Rua Bernardino de Campos, 30 - apto. 1002 -
Higienópolis
Ribeirão Preto, SP – Brazil
14015-130
E-mail: drlucas.garcia@gmail.com

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Introduction

Several factors may interfere with the success of the endodontic treatment in the daily clinics. According to Trope et al. (1), there is a direct relationship between the presence of microorganisms before the root canal obturation and the failure of the endodontic therapy. The comprehensive disinfection in Endodontics aims to eliminate the microorganisms present in the root canal system. The disinfection procedure begins with the biomechanical action of files and other instruments combined with the chemical action of irrigant solutions (2,3). However, this step does not completely eliminate the microorganisms present in long infectious processes, which disseminate the infection and toxins to the dentin tubules, accessory canals, lateral and collateral ramifications, and apical delta (4). Flatten canals pose a complex situation because the access for mechanical instruments and irrigant solutions is restricted (2,3). Thus, an effective microbial control requires the use of intracanal medication (5).

Many substances have been used as intracanal medication. Among them, the calcium hydroxide is a common clinical choice due to its antimicrobial properties (5-7), solvent action for organic matter (8), and induction of mineralized tissue formation (9). On the other hand, the development of medical products using components from plants has mobilized researchers in the health area in recent years (10). In Endodontics several phytochemicals with antimicrobial activity may have a potential use as intracanal medication (11), such as the *Ricinus communis*, a tropical plant. The ricinoleic acid, also called castor oil acid, is an unsaturated fatty acid and the main bioproduct obtained from the *Ricinus communis*. This acid can be found as a polymer or a detergent, and the latter form could be used as an irrigating solution for the disinfection of root canals according to Ferreira et al. (12). Endoquil, a 3.3% *Ricinus communis* detergent (Poliquil, Polímeros Químicos Ltda, Araraquara, SP, Brazil) has produced good results as an endodontic irrigant, and has shown antimicrobial activity similar to 0.5% NaOCl solution when used in the treatment of root canals with pulpal necrosis (13). Endoquil was effective against Gram-positive microorganisms, and 0.5% NaOCl solution was effective only against *S. aureus* (14). It also shows high repairing and osteogenic potential (15,16) and anti-inflammatory action (17). Therefore, the association of ricinoleic oil with calcium hydroxide could be a viable alternative intracanal medication.

The aim of this study was to evaluate the antimicrobial activity of pastes used as root canal dressing containing calcium

hydroxide and *Ricinus communis* oil (RCO) or calcium hydroxide and propylene glycol, against microorganisms commonly found in endodontic infections.

Methods

Inoculum preparation

Each *inoculum* was prepared in test tubes, using 1 mL of Brain Heart Infusion (BHI – Oxoid Ltda, Basingstoke, Hampshire, Inglaterra) and 10 µL of the following microorganisms: *Enterococcus faecalis* (ATCC-29210), *Streptococcus mutans* (ATCC-25175), *Candida albicans* (ATCC-29210), *Staphylococcus aureus* (ATCC-6538), *Pseudomonas aeruginosa* (ATCC-27853) and *Streptococcus sanguinis* (ATCC-10556), from the American Type Culture Collection. After preparation, the *inocula* were incubated at 37°C for 24 hours. The cultures were then diluted in a 0.5 MacFarland scale, adding 10 µL into 90 µL of BHI to result in a concentration of approximately 1×10^6 colony forming units (CFU)/mL.

Pastes preparation

Two pastes were prepared: a calcium hydroxide and *Ricinus communis* oil paste (Paste A) and a calcium hydroxide and propylene glycol paste (Paste B). The tested pastes were manipulated according the formula described in Table 1.

Agar-well diffusion test

The antimicrobial activity of the two tested pastes against the selected microorganisms was determined in triplicate by using the agar-well diffusion test. First, 0.5 mL of each *inoculum* was transferred to thioglycolate+0.2% agar-agar and then placed in an anaerobic jar (GasPack Jar, BD, Franklin Lakes, NJ, USA). After 24 h, 10 mL of BHI+5.0% of yeast extract+0.1% hemin were poured into 12 Petri dishes and left to set. Subsequently, 5 mL of BHI was inoculated with 2 mL of the *inoculum* poured on top and then placed again in an anaerobic jar (18,19). Three equidistant wells (5 mm-diameter and 4 mm-depth) were bored into the agar using a sterile cork borer and were completely filled with the tested pastes. One of them received propylene glycol (negative control) only. The plates were left at room temperature for 2 h and then incubated at 37°C for 24 h in anaerobiosis (BBL GasPak Plus, BD, Franklin Lakes, NJ, USA) (20). Antimicrobial activity was determined by measuring the diameters of the microbial growth inhibition zones with a digital pachimeter (Digimess, Shinko Precision, Gaging, China) (21).

Table 1. Technical information of the tested pastes.

Paste	Components	Proportion	Manufacturer
A	Calcium hydroxide	3.0g	Hidroxil, Inodon, Porto Alegre, RS, Brazil
	RCO	1.75 mL	Polímeros Químicos Ltda., Araraquara, SP, Brazil
B	Calcium hydroxide	3.0g	Hidroxil, Inodon, Porto Alegre, RS, Brazil
	Propylene glycol 400	1.75 mL	Alfa Aesar GmbH & Co. KG, Karlsruhe, Germany

Statistical analysis was performed using 1-way ANOVA and Tukey test ($\alpha=0.05$) to compare the growth inhibition zones values.

Results

Table 2 displays the size of the inhibition halos in the Petri dishes. Paste A showed larger inhibition halos than Paste B and the control group, with statistically significant difference among them ($P<0.05$) (Fig. 1).

Table 2. Average diameter (mm) of inhibition halos produced by the tested pastes in the agar-well diffusion test.

	Paste A ^a	Paste B ^b	Propylene glycol ^c
<i>E. faecalis</i>	1.5	1.2	0
<i>S. mutans</i>	1.3	1.1	0
<i>C. albicans</i>	1.2	1.1	0
<i>S. aureus</i>	1.3	1.0	0
<i>P. aeruginosa</i>	1.1	1.1	0
<i>S. sanguinis</i>	1.4	1.2	0

* Different lowercase letters in columns means statistically significant difference (1-way ANOVA, Tukey test – $P<0.05$).

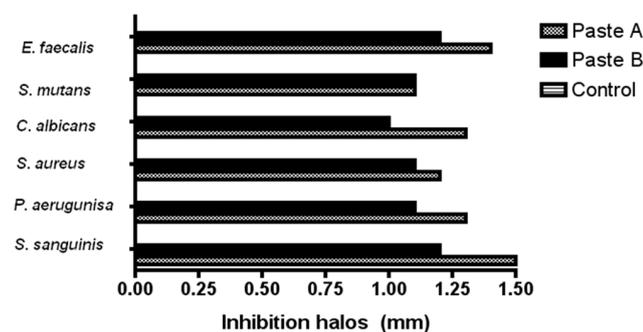


Fig. 1. Inhibition halos diameter (mm) produced by the tested pastes in the agar-well diffusion test.

Discussion

The sanitation process of the root canal system consists in the reduction of microorganisms to enable local response and tissue repair (1,4,5). However, the complex anatomy of this system offers the opportunity and conditions for microorganisms growth, multiplication, and interaction in the pulp infection process (2,3). The selection of microorganisms for the present research was based on the fact that some

microorganisms have been often related to endodontic infections, such as *Enterococcus faecalis* (22), *Pseudomonas aeruginosa* (5,23), *Staphylococcus aureus* (5,23), and *Candida albicans* (23). According to Sunde et al. (23) these microorganisms are present in 75% of refractory periapical lesions.

Intracanal medication is often a necessary procedure, and the calcium hydroxide has many chemical and biological properties that turn it the material of choice among endodontists (5-9). The antimicrobial effectiveness of calcium hydroxide is related to the release of hydroxyl ions. The propylene glycol used as vehicle to calcium hydroxide paste, allows controlled release of OH⁻ and Ca₂ (24). However, this vehicle is innocuous and does not show any antimicrobial action, as seen in this study, which would not happen with the RCO. Recent studies have shown that components of the *Ricinus communis*, such as its oil, present antimicrobial activity against microorganisms frequently found in root canals and periapical lesions. The oil could be used as a vehicle to a paste based on calcium hydroxide aiming to combine the biochemical action of the two products in association (12-17).

Such association of calcium hydroxide and RCO would optimize the spectrum of action of the resulting combined paste, as seen in the present study. In the Agar-well diffusion test, Paste A produced larger inhibition halos than Paste B and the control group, proving its higher antimicrobial activity. Several studies in Endodontics have demonstrated that the RCO has high antimicrobial capacity, especially when used as an irrigant solution (12-14). The present results showed that the use of this phytochemical as a component of intracanal medication seems to be promising. Considering the inherent properties of RCO, the association of calcium hydroxide and RCO may be a viable option in the endodontic sanitation. However, additional antimicrobial tests are necessary to address different time intervals than the ones tested in the present study. In addition, further studies should continue to identify the components of the *Ricinus communis* with antimicrobial activity, followed by tests for the preparation of an ideal formulation to be used with safety and efficacy in the routine endodontic treatment.

Conclusions

Within the limitations of this study, the results suggest that Paste A had greater antimicrobial activity than Paste B.

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