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Antimicrobial action of an intracanal medication trial using *Aloe vera*

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Abstract

Objective: This study aimed to test the calcium hydroxide associated with vehicles *Aloe vera*, chlorhexidine digluconate (2%) and saline, in inhibiting bacterial growth on Mueller-Hinton agar plates.

Methods: The species *Staphiloccocus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Enterecoccus faecalis* were isolated and inoculated in 3 mL of BHI (Brain Heart Infusion). Holes (5 mm diameter) were made in the plates and filled with the test materials. After incubation, readings were taken with a hand lens and a caliper with 0.1 mm accuracy to determine the diameter of the inhibition zone, after 24 and 48 hours. Each experiment was repeated six times, and the average values were obtained.

Results: Chlorhexidine digluconate without calcium hydroxide resulted in better inhibition of bacterial growth, followed by the hydroxide pastes evaluated. *E. coli* strains were the most resistant to the tested compounds, followed by *S. pyogenes* and *S. aureus*. There was no statistically significant interaction between the variables.

Conclusion: Chlorhexidine digluconate (2%) alone showed the best antimicrobial effectiveness. *Aloe vera* is a promising vehicle for the calcium hydroxide but more studies should be conducted on herbal medicines in dentistry.

Key words: Microbiology; Endodontics; Phytotherapy; Phytotherapeutic drugs; *Aloe vera*

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Ação antimicrobiana de uma medicação intracanal experimental utilizando-se *Aloe vera*

Resumo

Objetivo: Este estudo teve como objetivo testar o hidróxido de cálcio associado com os veículos *Aloe vera*, digluconato de clorexidina (2%) e solução salina, na inibição do crescimento bacteriano em placas de ágar Mueller-Hinton.

Métodos: As espécies *Staphiloccocus aureus*, *Streptococcus pyogenes*, *Escherichia coli* e *Enterecoccus faecalis* foram isoladas e inoculadas em 3 mL de BHI (Brain Heart Infusion). Orifícios de 5 mm de diâmetro foram feitos nas placas e as substâncias a serem testadas foram inseridas. Após a incubação, as leituras dos halos de inibição foram aferidas com lente de mão e cursor com precisão de 0,1 mm a fim de determinar o diâmetro da zona de inibição, após 24 e 48 horas. Cada experimento foi repetido seis vezes, e os valores médios foram obtidos.

Resultados: Digluconato de clorexidina (2%) isolado apresentou melhor inibição do crescimento bacteriano, seguido pelas pastas de hidróxido de cálcio avaliadas. Bactérias *E. coli* foram as mais resistentes aos compostos testados, seguidas por *S. pyogenes* e *S. aureus*. Não houve interação significativa entre as variáveis.

Conclusão: Digluconato de clorexidina (2%) isolado apresentou melhor efetividade antimicrobiana. *Aloe vera* é um veículo promissor para o hidróxido de cálcio, entretanto mais estudos devem ser realizados com medicamentos fitoterápicos na Odontologia.

Palavras-chave: Microbiologia; Endodontia; Fitoterapia; Medicamentos fitoterápicos; *Aloe vera*

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Introduction

Teeth with radiographically visible periapical lesions have variations in their internal anatomy that can interfere with the success of the cleaning and disinfection of the root canals. The number of bacteria remaining after chemical-mechanical instrumentation can be reduced by a temporary dressing, providing an environment conducive to periapical repair. Thus, intracanal dressings are crucial for the success of endodontic treatments [1,2].

Calcium hydroxide has been widely used for this purpose, as its high alkalinity (pH 12.5) destroys bacterial cell membranes, denatures proteins and damages DNA [3]. Due to its anti-inflammatory and antibacterial properties – including induction of mineralization and biocompatibility – this material has an extremely important role in therapies affecting periodontal and apical dentin-pulp complexes [4].

Different vehicles have been added to calcium hydroxide to facilitate and improve its action. Several substances have been proposed for this purpose, and there is no total consensus regarding the ideal substance [5,6]. The chosen vehicle is important during this process due to its chemical and physical properties and, thus, its application. Moreover, the vehicle has a significant effect on the antimicrobial activity of calcium hydroxide [7].

As global interest in herbal medicine has grown, plant extracts have been evaluated for their application to preventive and curative programs. Among herbal plants, species of the genus Aloe (Liliaceae) have been extensively studied due to their medicinal properties; *Aloe vera*, for example, contains many biologically active substances [8] that provide important anti-inflammatory, anti-bacterial, antifungal, hypoglycemic and antidiabetic, immunomodulatory, healing and regenerative functions [9,10,11]. The pharmacodynamic properties of anti-inflammatory and immunoregulatory natural products have been tested in several tissues for their contribution to tissue repair [12].

Chlorhexidine gluconate (2%) is an effective anti-microbial agent that has a relative lack of toxicity [13] and broad spectrum of action [14,15]. Furthermore, it is a stable substance with minimal absorption into the skin and mucosa, and it has substantivity, meaning that it is largely liberated as its concentration decreases, thereby enabling an extended operating time. Thus, this substance maintains its effects for a long period of time [13].

This study analyzed the resistance of the bacteria *Staphiloccocus aureus*, *Streptococcus pyogenes, Escherichia coli* and *Enterecoccus faecalis* to calcium hydroxide delivered with different vehicles.

Methods

To test antimicrobial activity, *Staphylococcus aureus, Enterococcus faecalis, Streptococcus pyogenes* and *Escherichia coli* cultures were isolated and maintained in the Laboratory of Microbiology, Department of Microbiology and Parasitology, Federal University of Piauí. The microorganisms were inoculated in 3 mL of BHI (Brain Heart Infusion) and incubated at a constant temperature of 37 °C for 24 hours. Bacteria were diluted in saline solution to an approximate final concentration of 3×10^8 cells/mL with turbidity similar to a tube 0.5 of the McFarland scale (0.1 mL of barium chloride and 1.0 mL +9.9% sulfuric acid 1.0%). Mueller-Hinton agar plates were inoculated with sterile swabs that were saturated with the standardized bacterial suspensions. The full length of the plates was spread uniformly.

Holes (5 mm diameter) were made in the plates with a punch sterile and Pasteur pipettes connected to a vacuum pump. The holes were filled with the following test materials: calcium hydroxide P.A. with *Aloe vera* (Aloe Alpha®, Jungconsult Brazil), only *Aloe vera*, calcium hydroxide P.A. with 2% chlorhexidine digluconate, only 2% chlorhexidine digluconate, calcium hydroxide P.A. in saline and saline alone (control group).

The plates were incubated at 37 °C for 48 to 72 hours. After incubation, readings were taken with a hand lens and a caliper with 0.1-mm accuracy to determine the diameter of the inhibition zone in mm. Each experiment was repeated six times, and the average values were obtained.

The data were analyzed using the software Statistical Analysis System (SAS) 6.11 version. The mean inhibition zones were subjected to analysis of variance and the Student-Newman-Keuls (SNK) multiple comparison test with a significance level of 5%.

Results

Tables 1, 2 and 3 summarize the results. Calcium hydroxide delivered with different vehicles (*Aloe vera*, saline and 2% chlorhexidine gluconate) resulted in inhibition zones that were characteristic of bacterial sensitivity. All results were similar, and there were no statistically significant differences between the different calcium hydroxide pastes.

Table 1. Mean of inhibition zones of bacterial growth in millimeters (mm) after 24 hours growth of bacteria in the presence of the test material. Teresina, 2013

Test material / bacteria	*S. aureus*	*S. pyogenes*	*E. coli*
Calcium hydroxide P.A.+ *Aloe vera*	27,17	26,21	22
Calcium hydroxide P.A.+ Saline	23,25	26,21	21,5
Calcium hydroxide P.A.+ Chlorhexidine digluconate (2%)	25,25	25,67	22,75
Aloe vera	*	20,45	15
Saline	*	*	*
Chlorhexidine digluconate (2%)	30,78	29,37	26,04

* There was no halo formation.
Source: Laboratory of Microbiology, Federal University of Piauí.

Table 2. Mean of inhibition zones of bacterial growth in millimeters (mm) after 48 hours growth of bacteria in the presence of the compounds tested. Teresina, 2013.

Test material / bacteria	*S. aureus*	*S. pyogenes*	*E. coli*
Calcium hydroxide P.A + *Aloe vera*	26,08	26,25	21,46
Calcium hydroxide P.A + Saline	22,58	26,12	21,04
Calcium hydroxide P.A + Chlorhexidine digluconate (2%)	24,46	24,92	22,29
Aloe vera	*	20,6	15,42
Saline	*	*	*
Chlorhexidine digluconate (2%)	31,04	31,12	26,25

* There was no halo formation.
Source: Laboratory of Microbiology, Federal University of Piauí.

Table 3. Mean values of halos of growth inhibition in millimeters (mm) according to the test material, bacteria and maturation time. Teresina, 2013.

Test material	Mean (mm)
Calcium hydroxide P.A + *Aloe vera*	24,93 ^{B}
Calcium hydroxide P.A + Saline	23,52^{B}
Calcium hydroxide P.A + Chlorhexidine digluconate (2%)	24,25^{B}
Aloe vera	18,88^{C}
Saline	–
Chlorhexidine digluconate (2%)	28,85^{A}
Bacteria	
Staphylococcus aureus	26,01^{A}
Streptococcus pyogenes	26,78^{A}
Escherichia coli	21,90^{B}
Time (Hour)	
24	24,69^{A}
48	24,42^{A}
Coeficiente de variância 16,27%	

A, B and C are paired groups, i.e., the media listed with the same letter had similar values. The SNK test showed no statistically significant differences at the 5% significance level.
Source: Laboratory of Microbiology, Federal University of Piauí.

The results from *Enterecoccus faecalis* were not determined because there were defined inhibition zones only on the plates containing 2% chlorhexidine digluconate alone.

Discussion

The antimicrobial activity of calcium hydroxide, which is characterized by high pH, is associated with the release of hydroxyl ions in aqueous solution at concentrations sufficient to destroy bacteria [5]. The time necessary for calcium hydroxide to thoroughly disinfect the root canal system is unknown, but it may depend on the presence of exudate in the root canal; the type of organism involved and its location in the root canal system; and the presence of smear layer [3].

Intracanal medications disturb established nutritional interrelationships by eliminating bacteria that are either essential for the growth of other bacteria or that limit the growth of other bacteria [3]. In the short term, calcium hydroxide appears to function as an intracanal barrier, preventing the seepage of apex tissue fluids and eliminating the substrates for residual microorganisms that survived the chemomechanical preparation [13].

Calcium hydroxide associated with different vehicles (*Aloe vera*, saline and 2% chlorhexidine gluconate) produced satisfactory inhibition zones that were characteristic of bacterial sensitivity (Tables 1 and 2). However, all results were similar, and there was no significant difference between different vehicles delivering calcium hydroxide (Table 3). However, the bactericidal activity of 2% chlorhexidine digluconate was reduced when it was combined with calcium hydroxide for all strains of bacteria tested, suggesting that the combination of these compounds reduces their known properties and thus it is a vehicle biologically active [3,16].

At the times tested, 2% chlorhexidine digluconate without calcium hydroxide resulted in better inhibition of bacterial growth (Tables 1 and 2). This antiseptic compound contains positively charged molecules, which result in a broad spectrum of antimicrobial activity [14,17]. The apparent mechanism of action of chlorhexidine digluconate is that it is adsorbed into the cell wall of microorganisms, causing leakage of their intracellular components. At low concentrations, it has a bacteriostatic effect, causing low molecular weight substances to leach from microorganisms; at higher concentrations, chlorhexidine has a bactericidal effect due to the precipitation of cytoplasmic components and/or coagulation [17].

The use of *Aloe vera* alone showed no better inhibition zone compared with the other groups (Tables 1 and 2). Generally, alternative therapies with herbal medicines are not subject to further research and experiments that clarify or determine, with some degree of security, how they should be used [7]. The antibacterial effect of *Aloe vera*, including its effect against resistant microorganisms found in the pulp chamber, has been shown in several studies, and this effect has been attributed to the presence of polysaccharides found in plant leaves [19,20,21].

E. coli strains were the most resistant to the tested compounds, followed by *S. pyogenes* and *S. aureus* (Table 3). Compared with the other bacteria, *S. aureus* showed decreased resistance halos in calcium hydroxide associated with *Aloe vera*, suggesting that this combination enhanced the antibacterial properties of the two substances on this species.

Notably, bacteria grew more rapidly in areas distal to the punch holes, suggesting that diffusion of the material was not sufficient to kill bacteria, but this result can be explained by the fact that the culture media contained buffering substances. Thus, slow diffusion of material from the punch

hole did not modulate the pH dramatically enough to kill the bacteria. This effect was observed in tests with *E. faecalis*, which showed resistance in an alkaline medium compared with the other bacteria.

There was no statistically significant interaction between the variables (Table 3). The coefficient of variance (16.27%) shows that the size of the halos diverged. In other words, the behavior of the variables was different. There was influence of the same variables on the different results.

Conclusions

In conclusion, 2% chlorhexidine digluconate alone showed the best antimicrobial effectiveness. However, *Aloe vera* is a promising vehicle for calcium hydroxide. More studies should be conducted on herbal medicines in dentistry so that these compounds can be used as alternatives in endodontic treatment. In the future, these compounds may play an important role in dentistry.

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