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 *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Enterococcus 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.

**Keywords:** Microbiology; Endodontics; Phytotherapy; Phytotherapeutic drugs; *Aloe vera*
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 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 antimicrobial 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 Staphylococcus aureus, Streptococcus pyogenes, and Escherichia coli 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 Piaui. 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 $3 \times 10^8$ 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, calcium hydroxide P.A. and 2% chlorhexidine gluconate, only 2% chlorhexidine gluconate, calcium hydroxide P.A. in saline and Aloe vera 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>
<thead>
<tr>
<th>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</th>
<th>Test material / bacteria</th>
<th>S. aureus</th>
<th>S. pyogenes</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium hydroxide P.A. + Aloe vera</td>
<td>27,17</td>
<td>26,21</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Calcium hydroxide P.A. + Saline</td>
<td>23,25</td>
<td>26,21</td>
<td>21,5</td>
<td></td>
</tr>
<tr>
<td>Calcium hydroxide P.A. + Chlorhexidine digluconate (2%)</td>
<td>25,25</td>
<td>25,67</td>
<td>22,75</td>
<td></td>
</tr>
<tr>
<td>Aloe vera</td>
<td>*</td>
<td>20,45</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Chlorhexidine digluconate (2%)</td>
<td>30,78</td>
<td>29,37</td>
<td>26,04</td>
<td></td>
</tr>
</tbody>
</table>

* There was no halo formation.

Source: Laboratory of Microbiology, Federal University of Piaui.
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 holes for residual microorganisms that survived the antimicrobial action of an intracanal medication | Beserra et al.
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.

**References**