Effectiveness Of Combination Aloe Vera Extract With Calcium Hydroxide Against Enterococcus Faecalis Bacteria

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ABSTRACT

Background: *Enterococcus faecalis bacteria is widely regarded as the leading cause of root canal treatment failure due to its ability to survive in extreme environments. The use of herbal plants such as Aloevera can be used as an intracanal medicament because it has been proven to have an antibacterial property. Calcium hydroxide (aqueous) intracanal medication mixed with Aloe vera is able to inhibit the growth of Enterococcus faecalis, to see the antibacterial effectiveness of the combination of Aloe vera extract with calcium hydroxide paste (aqueous) as an intracanal medicament against Enterococcus faecalisbacteria.*

Methods: *This experimental research was conducted in the laboratory with a post-test control design. The number of research samples used was 30 pieces. The maceration method was used to make theAloe vera extract and the Mc Farland turbidity standard was adjusted for Enterococcus faecalis bacterial growth. Then to determine the antibacterial effectiveness, the inhibition zone which is the area around thewell that is clear and smaller in diameter than the well, was measured using a digital caliper.*

Results: *Statistical tests were performed using the Kruskal Wallis test and obtained a significance number of 0,000(p <0.05) then the Mann Whitney test was carried out to obtain a significance number of 0.000 (p <0.05).*

Conclusions: *According to the results of this study, calcium hydroxide paste was less effective at killing Enterococcus faecalis bacteria than a mixture of calcium hydroxide and aloe vera extract.*

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INTRODUCTION

Pulp necrosis is one of the most common diseases encountered in dentistry. Dental and oral issues increased from 25.9% to 57.6%, according to the 2018 Basic Health Research (RISKESDAS).[1] In cases of pulpal necrosis, root canal treatment, which aims to kill bacteria in the root canals, can beperformed to preserve the teeth.[2] Root canal treatment failure due to Enterococcus faecalisbacteria can occur in nearly 90% of cases.[3] Enterococcus faecalis is a facultatively anaerobic gram-positive bacterium.[4] This bacterium is extremely resistant to high pH level.[5]

Enterococcus faecalis bacteria in the root canals can thrive even after cleaning, therefore intracanal antibiotics can be used to reduce the number of these bacteria.[6] One of the antibacterial intracanal medications is calcium hydroxide, which has a pH of 12.5 and serves as a treatment in this situation.[7] The mechanism of calcium hydroxide is based on Ca^{2+} and OH^- ions.[8] Due to the release ofhydroxyl ions causing high alkalization, microorganisms cannot survive in the root canal, making calcium hydroxide the gold standard in intracanal medicaments.[9]

Calcium hydroxide powder adhesionin the root canal can be improved by admixture addition. There are three types of mixing materials that can be used: aqueous, viscous, and oily.[10] Aqueous mixing is the most commonly used mixture because it allows Ca^{2+} and OH^- ions to disintegrate immediately.[11]

In addition, calcium hydroxide has limitations, it can be difficult to remove from the root canal. This causes residue toaccumulate on the surface of the root canalwalls. The use of herbal plant-basedmedicines, such as Aloe vera, provides apossible option for reducing calcium hydroxide deficiency.[9] Aloe vera is widelyused as a root canal sterilizing agent because itcontains active substances such as phenolicsand saponins that have been shown to inhibitbacterial growth.[12] According to the study, calcium hydroxide paste containing Aloe verawas able to diffuse into the dentinal tubules.[13]

Based on the information presented, the researcher wanted to compare the effects of Aloe vera extract with calcium hydroxide alongside calcium hydroxide paste (aqueous) that impact the growth of the Enterococcus faecalis bacteria.

RESEARCH METHOD

This is an experimental laboratorystudy with a post-test-only control design. Thenumber of research samples is 30, as determined by the Federer formula. Three groups of samples were formed: calcium hydroxide (aqueous) paste, Aloe vera extract combined with calcium hydroxide, and sterileaquadest negative control.

The research process begins with thepreparation of tools and materials, followed bythe production of calcium hydroxide paste.To achieve a paste consistency, calcium hydroxide powder is combined with saline ata ratio of 1.5:1 (mg/ml) to create a calcium hydroxide paste. 1.5 mg of calcium hydroxidepowder was mixed with 1 ml of saline on a glass plate with a metal spatula until it reacheda paste consistency, then placed 0.5 ml of the mixture in a syringe with an applicator tip andapplied it to the wells. After that, an experiment combining calcium hydroxidepaste and Aloe vera extract was conducted. Aloe vera extract and calcium hydroxide pasteare combined to create a calcium hydroxide and Aloe vera extract mixture. The maceration method with 96% ethanol solvent was used toobtain Aloe vera extract. To create an Aloe vera extract, 2000 grams of Aloe vera must first be dried in an oven and then ground into a powder. The powder was soaked in

96% ethanol at a ratio of 1:10 for 72 hours at roomtemperature. The residue is then separated through filtering. A rotary evaporator was used to evaporate the extract at 40°C, producing a thick extract as a result. Aloe veraextract and calcium hydroxide paste were blended thoroughly, and the resulting mixturewas then put into a 3 ml syringe with an applicator tip and applied to the wells in amounts of up to 0.5 ml.

The suspension of Enterococcus faecalis was then made. After being collected using sterile loops, the Enterococcus faecalis bacteria were streaked onto a Blood Agar culture medium and incubated at 37°C for 24 hours. The Enterococcus faecalis bacterial culture was extracted using a sterile loop, suspended in a test tube containing 3 ml of 0.9% NaCl solution, and vortexed for 15 minutes to homogenize it and achieve the required standard turbidity of 0.5 McFarland.

The antibacterial effectiveness test was then conducted. Making well holes in a petri dish containing MHA media allowed researchers to conduct an antibacterialeffectiveness test using the diffusion method. A cotton swab was used to collect anEnterococcus faecalis bacteria suspension, which was then streaked on the MHA media'ssurface. In the Bio Safety Cabinets, the mediawas sealed and facilitated to rest for 5 minutes. There were ten 100 mm-diameter petri dishesused in this study. Each petri dish was dividedinto three sections, and wells were created in each section using a perforator that had a 6 mmdiameter and a 4 mm depth. Every well is 40 mm apart from one another. The paste is then dispensed into each hole at a rate of 0.5 ml. Aloe vera extract combined with calcium hydroxide paste was used in holes I and II, andsterile aquadest was used in hole III as a negative control. All Petri dishes were placed in an incubator at 37°C for 24 hours.

The formation of an inhibition zone, which is indicated by the presence of a clear area around the wellbore, can be measured using a digital calliper in millimetres to determine the antibacterial effectiveness. Antibacterial effectiveness was determined bysubtracting the diameter of the inhibition zonefrom the diameter of the wellbore.

RESULTS

This study examined the effectiveness of three different antibacterial treatments on Enterococcus faecalis bacteria: calcium hydroxide (aqueous) paste, Aloe veraextract and calcium hydroxide paste, and sterile aquadest, which was used as the negative control group. The results of the antibacterial test on Enterococcus faecalis in this study were obtained using a millimetre digital calliper, which showed the average value in the three treatment groups, as shown in Table 1.

Table 1. The results of examining the inhibition zone of the bacteria Enterococcus faecalis.

Group	N	Mean
Sterile aquadest	10	0
Calcium hydroxide (aqueous)	10	0,51900
Aloe vera extract with calcium hydroxide combination	10	9,4700

According to Table 1, the averageinhibition zone of Enterococcus faecalis in theAloe vera extract with calcium hydroxide combination group had the highest result, which was 9.4700. The obtained data were subjected to the Shapiro-Wilk test todetermine its normality. Table 2 shows the results of the normality test.

Table 2. The results of the normality test using Shapiro-Wilk test.

Group	Sig. (P)
Sterile aquadest	-
Calcium hydroxide (aqueous) paste	0,000
Aloe vera extract with calcium hydroxide combination	0,005

According to Table 2, the significant number is $p<0.05$, implying that the data for all groups are not normally distributed. When it was determined that the data was not normally distributed, a non-parametric test was performed, Kruskal Wallis, was used to determine whether there were differences in the antibacterial effect on Enterococcus faecalis bacteria in each group. The Kruskal Wallis test results showed a significant number of 0.000 ($p<0.05$), implying that there were significant differences in the three groups of antibacterial tests on Enterococcus faecalis bacteria. The Mann-Whitney test was used to figure out which group had the most significant difference.

The Mann-Whitney test results revealed that there was a significant difference in the value of the antibacterial effect on Enterococcus faecalis bacteria in each group ($p<0.05$). The sterile aquadest group differed significantly from the calcium hydroxide paste (aqueous) group and the combination group of Aloe vera extract and calcium hydroxide. The calcium hydroxide (aqueous) group differed significantly from the combination group of Aloe vera extract and calcium hydroxide.

Figure 1. Inhibition zone of Enterococcus faecalis bacteria in a petri dish.

DISCUSSION

Root canal treatment failure can occur due to the presence of the bacterium Enterococcus faecalis, which is the most common cause of root canal treatment failure.[15] Microorganisms such as Enterococcus faecalis will remain in the root canals, so intracanal antibiotics must be administered to inhibit bacterial growth and improve endodontic therapeutic results.[16]

This study compares the antibacterial effect of calcium hydroxide (aqueous) paste, a combination of Aloe vera extract and calcium hydroxide paste, and sterile aquadest as a negative control on Enterococcus faecalis. Observations were made with a digital vernier calliper in millimetres to see if there was an inhibition zone, i.e. a clear area in the well bore area. In this study, the bacteria were incubated for 24 hours because this is the time when they are in the logarithmic phase, which means they are constantly dividing. The optimal incubation temperature for bacterial growth is 37°C because high temperatures can cause bacteria to die,

while low temperatures can result in poor bacterial growth.[17]

The Kruskal Wallis method test was then performed, revealing a significant difference in the average antibacterial effect on Enterococcus faecalis bacteria in thecalcium hydroxide (aqueous) paste group andthe combination of Aloe vera extract withcalcium hydroxide, namely $p<0.05$. This isbecause the combination of Aloe vera extract and calcium hydroxide contains antibacterial ingredients such as anthraquinones and tannins found in Aloe vera.[18] The data was then tested using the Mann Whitney test, which revealed that each treatment group hada significant difference.

Comparing the combination group ofcalcium hydroxide and Aloe vera extract to the positive control group of calcium hydroxide paste (aqueous), the combination group showed more effective antibacterial results. Compared to calcium hydroxide, aloe vera is more effective against bacteria. This is due tothe presence of active ingredients in Aloe vera such as anthraquinones and tannins, which can inhibit the growth of Enterococcus faecalis bacteria.[18]

Calcium hydroxide paste has anantimicrobial effect by damaging the bacterialcell wall because it provides a highly alkaline neighborhood, whereas Enterococcus faecaliscan survive by balancing the pH level.[18] As a result, calcium hydroxide has the side effect ofmaking bacteria resistant, whereas Aloe vera has no toxicity comparison to calcium hydroxide, Aloe vera extract can be utilized asa substitute for intracanal medications.[19]

CONCLUSION

calcium hydroxide paste was less effective at killing Enterococcus faecalis bacteria than a mixture of calcium hydroxide and aloe vera extract.

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