Comparative evaluation of antimicrobial efficacy of mushroom, Aloe vera, and Curcuma longa with calcium hydroxide as an intracanal medicament against Enterococcus faecalis: An in vitro study

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Abstract

Background: The success of endodontic treatment mainly depends on the eradication of microorganisms from the root canal system. The use of intracanal medicaments plays a crucial role in eliminating resistant bacteria such as Enterococcus faecalis. Intracanal medicaments similar to herbal compounds can be used as a substitute for conventional calcium hydroxide (CaOH2) to prevent toxicity. The existing study aimed to compare and evaluate the antimicrobial activity of four different intracanal medicaments against E. faecalis.

Objectives: The objective of this study is to compare and evaluate the antimicrobial efficacy of CaOH2, extracts of mushroom, aloe vera, and Curcuma longa as intracanal medicaments against E. faecalis.

Materials and Methods: A total of 120 extracted human permanent premolars were decoronated, and chemomechanical preparation of the root canal was performed. After sterilization of the samples, pure cultures of E. faecalis were inoculated and incubated. Then, samples were separated randomly into five groups (n = 24). The antibacterial efficacy of the different intracanal medicaments was recorded at the end of days 1, 7, and 14 by determining the % reduction colony count. Data were statistically analyzed using a one-way analysis of variance, a Chi-square test for association, and a comparison of means using a t-test.

Results: Curcuma longa exhibited an increased percentage reduction in colony counts compared to other herbal extracts against E. faecalis.

Conclusion: Antibacterial action of the C. longa extract was uppermost followed by CaOH2, A. vera, and mushroom against E. faecalis.

Keywords: Aloe vera; calcium hydroxide; Curcuma longa and mushroom; Enterococcus faecalis; intracanal medicament

INTRODUCTION

The purpose of nonsurgical endodontic therapy is to eliminate pathogenic microorganisms from the root canal system, shape the canal system, and obturate it with an appropriate material.[1] Root canal infection may still persevere even after rigorous procedures, due to the complexity of the canal system, which is tough to the instrument. As a result, using intracanal medications in such circumstances eliminate infections that persist even after cleaning and shaping, creating an environment favorable for periapical tissue restoration.[2]

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Numerous chemicals have been tried as intracanal medicaments. The majority of these preparations excluding calcium hydroxide (CaOH2) are not used regularly in contemporary endodontic practice due to stated toxicity, allergic reactions, and resistance. The growing number of antibiotic-resistant bacteria, as well as the adverse effects of synthetic medications, has prompted experts to hunt for natural alternatives.

Mushrooms contain low-molecular-weight (LMW) and high-molecular-weight active compounds which hold medicinal properties such as immunomodulatory, antiinflammatory, antiviral, antioxidant, and antimicrobial properties with increased antimicrobial activity against Gram-positive bacteria.

Aloe barbadensis Miller (Aloe vera) is a part of the Liliaceae family. It contains active components such as curcumin, nimbidin, myristic acid, tannin, and anthraquinone that have antiinflammatory, antibacterial, antifungal, and antiviral properties.

Turmeric (Curcuma longa) is a traditional herbal medication that is used to cure a variety of ailments. Curcumin (diferuloylmethane), the major yellow bioactive component of turmeric, has been demonstrated to have a wide range of biological functions, including antibacterial, anti-inflammatory, and antioxidant properties.

The aim of this study was to evaluate the antimicrobial efficacy of extracts of mushroom, A. vera, and C. longa against Enterococcus faecalis and then compare it with CaOH2.

MATERIALS AND METHODS

Preparation of tooth specimens
In this study, 120 recently extracted intact human mandibular premolars with a single straight canal and mature apices were collected from the department of oral and maxillofacial surgery and stored in distilled water until use. Decoronation of all the specimens was done up to 2–3 mm below the cementoenamel junction with a safe-sided diamond disk (SHOFU) and standardizing root length to 10 mm. Rotary Pro Taper files were used to prepare canals up to F3 (Dentsply India). To remove inorganic and organic debris, all the samples were placed in 17% ethylenediaminetetraacetic acid for 5 min followed by 5.25% NaOCl for 5 min.

Contamination of the samples
The test organism E. faecalis (ATCC 29212) was used. The colonies of bacteria were isolated for 24 h, and then put in 5 ml of brain/heart infusion broth and incubated for 4 h at 37°C. All samples were contaminated with a 10-ml inoculum of E. faecalis and then incubated for 21 days at 37°C. After incubation, a 30 no. H file was used to scrape the dentin and the canals were flooded with sterile saline. A paper point was placed in the canal for 60 s and then put into test tubes containing 1.0 ml of saline solution and shaken in a vortex mixer. A 10-fold dilution was prepared (up to 1:10^4), from which 0.1 ml was transferred and plated on HiCrome™ Mueller Hinton Agar medium (HiMedia Laboratories, Mumbai, Maharashtra, India). An aerobic chamber was used to incubate the plates for 24 h at 37°C. A colony count was performed to detect the growth of bacteria and tabulated for all 120 samples to determine the level of contamination before the application of medicament and the first microbiological sampling was performed.

Antimicrobial assessment
The samples were divided into five groups (n = 24): group 1, CaOH2; Group 2, mushroom; Group 3, A. vera; Group 4, C. longa; and Group 5, saline (negative control) [Figure 1]. To obtain paste-like consistency, CaOH2 powder was mixed with sterile saline in a ratio of 1.5:1 (wt/vol). The paste was carried with lentulo spirals and condensed using hand pluggers into the canal. Mushroom (Agaricus bisporus) and A. vera after washing thoroughly were powdered with the help of a blender. Fifty gram of powder was boiled in 500 ml of distilled water and allowed to boil to a final volume of 10–20 ml. Hydroxyethylcellulose was used as a thickening agent in the ratio of 2:1 (volume/weight) for Group 2 (mushroom), Group 3 (A. Vera), and Group 4 (C. longa) to obtain paste-like consistency. The intracanal medicaments were injected into the canal with a syringe, respectively. Teeth were placed in Eppendorf tube and incubated at 37°C for 1 week.

After placing the medicaments, all the groups were divided into three subgroups of eight samples (n = 8) and incubated for a 1, 7, and 14 day time period. After incubation for 24 h at 37°C, flooding the canal with sterile saline intracanal medicament.
was removed followed by scraping the dentin with 30 no. H file and transferred into test tubes containing 1 ml of physiological saline. A 10-fold dilution was prepared (up to $10^5$), and 0.1 ml was transferred and plated on the Hicrome Mueller Hinton Agar medium. The colony count was performed for these samples. Similarly, the colony count was performed for the samples on the 7th day and 14th day of incubation. The antibacterial efficacy was calculated by comparing the percentage reduction in colony counts (%RCC) before and after intracanal medication at three-time intervals (1, 7, and 14 days) [Figure 2]. The reduction in the percentage of colony count was calculated using the following formula:

$$\text{Percentage reduction in colony} = \frac{(\text{initial colony count} - \text{final colony count})}{\text{initial colony count}} \times 100$$

**RESULTS**

There was statistically significant difference in the mean colony-forming unit (CFU) among the groups ($P < 0.001$). C. longa showed the highest reduction in CFUs followed by CaOH2, A. vera, and mushroom [Table 1].

Graph 1*Higher mean (CFU/ml) * $(10^4)$ value recorded for the 1st-day results was seen in the control group followed by A. vera, mushroom group, CaOH2 group, and C. longa group, respectively. The variance in mean (CFU/ml) * $(10^4)$ value among the groups was found to be statistically significant ($P < 0.001$).

Graph 1**Higher mean (CFU/ml) * $(10^4)$ value recorded for the 7th-day results was seen in the control group followed by mushroom, A. vera group, C. longa group, and CaOH2 group, respectively. The variance in mean (CFU/ml) * $(10^4)$ value among the groups was found to be statistically significant ($P < 0.001$).

Graph 1***Higher mean (CFU/ml) * $(10^4)$ value recorded for the 14th-day results was seen in the control group followed by the mushroom group, A. vera extract group, CaOH2 group, and C. longa, respectively. The variance in mean (CFU/ml) * $(10^4)$ value among the groups was found to be statistically significant ($P < 0.001$).

**DISCUSSION**

Endodontic treatment mainly depends on the proper eradication of bacterial growth from the root canal space. The failure of root canal treatment is closely related to the presence of anaerobic, facultative bacteria, and majorly *E. faecalis*.[6]

At present, most of the commercial products used as intracanal medicaments are cytotoxic and are not able to completely eliminate bacteria from the dentinal tubules and leading to the usage of biologic medication derived from natural plants. Benefits of utilizing herbal medications are their low cost, low toxicity, easy availability, increased shelf life, and decreased microbial resistance.[7]

The present study used mushroom, A. vera, and C. longa extract as an intracanal medicaments and compare their antibacterial efficacy with CaOH2 against *E. faecalis*.

In the current study, mandibular first premolars were used as they are easily available due to orthodontic extractions and have usually a single and straight canal, help ease in the placement and removal of the medicament, three-time periods were taken to know the disinfection antimicrobial action, as some of the tested intracanal medicaments increases after 48 h. Therefore, time periods chosen to evaluate the antimicrobial assessment included the 1st, 7th, and 14th day.

**Table 1: Mean CFU/ml values distribution of samples with different groups**

| Groups | 1st day | 7th day | 14th day |
|--------|---------|---------|----------|
| Group I | 818.75±15.52 | 329.38±21.28 | 263.75±15.52 |
| Group II | 847.50±21.38 | 449.38±21.28 | 354.38±27.08 |
| Group III | 876.88±24.61 | 436.25±14.52 | 336.25±20.42 |
| Group IV | 720.00±18.89 | 406.25±17.67 | 227.50±20.87 |
| Group V | 981.88±12.48 | 958.13±12.36 | 961.25±15.36 |

SD: Standard deviation, CFU: Colony-forming unit
In this study, paste form was chosen as it helps ease of placement inside the lumen and the material remains better within the canal walls. For the antimicrobial assessment, the mean CFU/ml values obtained were subjected to statistical analysis which declares antimicrobial efficacy among the five experimental groups.

The results discovered that all the medicaments tested reduced *E. faecalis*, the mean CFUs of each group decreased over time.

In the current study, CaOH2 showed maximum reduction in CFUs on day 7 against *E. faecalis* (59.7%) when compared to other herbal intracanal medicaments. Siqueira and Lopes showed that CaOH2 was ineffective in eradicating *E. faecalis* after 1 week. The cause could be the restricted action of CaOH2 against facultative anaerobes, buffering action of dentin, and the prearrangement of bacterial cells colonizing the root canal walls.[9]

*Curcuma longa* showed maximum reduction in the CFUs against *E. faecalis* on the 14th day (68.40%). In a study by Hemanshi Kumar,[9] *C. longa* demonstrated good results in the elimination of *E. faecalis* and the author concluded that *C. longa* can also be used as an intracanal medicament. A study by Sinha et al.[14] showed that *C. longa* with distilled water showed 60% reduction of *E. faecalis* over 7 days.

In this study, compared to *C. longa* and CaOH2, *A. vera* has shown less reduction in CFUs. These results are inconsistent with those reported in a study by Bhardwaj et al.[10] which stated that *A. vera* and CaOH2 inhibited bacterial growth by 78.9% and 64.3%, respectively. Alterations might be due to the different preparations of *A. vera* used in both studies.

In the current study, the mushroom extract has also shown the inhibition of bacterial growth. A study was done by Signoretto et al.[11] concluded that LMW mass fraction of mushroom extract had antimicrobial activity against most oral pathogens. A study conducted by Kurian et al.[2] demonstrated that mushroom extract (MIC 40 mg/ml) showed the highest antibacterial activity against *E. faecalis*. However, compared to other herbal intracanal medicaments used in the present study, mushroom is considered the least effective. A study by Panchal V et al.[12] cinnamon extract showed better antimicrobial efficacy against *E. faecalis* as intracanal medicament as compared to calcium hydroxide. Whereas a study by Adam SH et al.[13] showed Ca(OH)2 had the highest significant cytotoxicity compared to Neem oil.

A study by Sofiani E et al.[14] showed that calcium hydroxide with propolis 25% more effective than the mixture of calcium hydroxide with 2% CHX digluconate as root canal medicament against *E. faecalis* bacteria. While another study by Love RM et al.[15] postulated that a virulence factor of *E. faecalis* in failed endodontically treated teeth may be related to the ability of *E. faecalis* cells to maintain the capability to invade dentinal tubules and adhere to collagen in the presence of human serum. Among all the groups, *C. longa* showed the highest inhibition of growth of *E. faecalis* at all the time periods with not much statistical difference. This was in accordance with the study by Vasudeva et al.,[11] Kurian et al.[2] and Neelakanta et al.[9]

Further long-term *in vivo* studies must be conducted with the herbal extracts to know their toxicity and allergic potential and also effectiveness as intracanal medicaments against *E. faecalis*.

**CONCLUSION**

Under the limitations of the present study, it can be concluded that the decrease in the percentage of colony count was highest with *C. longa* followed by CaOH2, *A. vera*, and mushroom. Among the herbal extracts, *C. longa* holds a favorable future as intracanal medicament but further clinical trials and long-term studies are to be conducted.

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**Conflicts of interest**

There are no conflicts of interest.

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