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Comparison of Antimicrobial Efficacy of Calcium Hydroxide, 10% Bamboo Salt and Cerium Oxide Against Enterococcus Faecalis and Candida Albicans- An In Vitro Study

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KEYWORDS

Bamboo Salt; C. albicans; Calcium Hydroxide; Cerium Oxide; E. faecalis; Intracanal Medicaments.

ABSTRACT:

Background:

Persistent microorganisms like Enterococcus faecalis and Candida albicans challenge complete disinfection in endodontic therapy due to complex root canal anatomy. Traditional medicaments, such as calcium hydroxide, show limited efficacy.

Aim:

To evaluate and compare the antimicrobial efficacy of calcium hydroxide, 10% bamboo salt, and cerium oxide against E. faecalis and C. albicans.

Methods and Material:

Seventy-two decoronated, single-rooted premolars were instrumented and inoculated with E. faecalis (n=36) and C. albicans (n=36) for 21 days. They were divided into three groups based on the medicament used: calcium hydroxide, 10% bamboo salt, and cerium oxide. Medicaments were placed using lentulospirals and sealed with resin-modified glass ionomer cement. CFUs were evaluated on the 2nd and 7th days post-treatment.

Statistical Analysis Used:

Data were analyzed using Shapiro-Wilk and Levene's tests in SPSS version 22.0.

Results:

10% bamboo salt showed the highest efficacy against E. faecalis, while calcium hydroxide was most effective against C. albicans. All medicaments significantly reduced microbial counts.

Conclusion:

Therefore, Bamboo Salt could be considered as a promising intracanal medicament for cases resistant to Calcium Hydroxide, though further in vivo research and clinical evaluations are essential for its validation.

INTRODUCTION

During endodontic therapy, eliminating microbes and necrotic tissue from the endodontic canal system is crucial for treatment success. Persistent infections, often caused by resilient pathogens like *Enterococcus faecalis* and *Candida albicans*, are major contributors to endodontic failure. The Gram-positive, facultative

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anaerobic microorganism, *E. faecalis*, thrives in harsh environment, resists many antimicrobials, and permeates through the dentinal tubules to greater depths. Similarly, *C. albicans*, the most prevalent oral fungal species, exhibits strong virulence through biofilm formation, pH tolerance, and tissue invasion, especially in retreatment cases. Due to the complex root canal anatomy, mechanical instrumentation alone cannot fully eradicate these microbes, necessitating the use of effective intracanal medicaments.[1]

Calcium hydroxide, introduced in 1920, is widely used for its excellent antimicrobial action and ability to neutralize bacterial endotoxins. However, its limited penetration depth and potential to decrease dentin microhardness when used for longer period of time are the factors which raise concerns.[2]

Natural and nanomaterial-based agents are gaining attention for their therapeutic potential. Bamboo salt has shown antibacterial effects against several pathogens, including *E. faecalis*, although its role as an intracanal medicament remains underexplored.[3] Similarly, cerium oxide nanoparticles, with their redox activity and biofilm inhibition capabilities, hold potential as an intracanal medicament due to their ability to penetrate intricate anatomical spaces. However, their efficacy against common endodontic pathogens has not been thoroughly evaluated.[4]

Therefore, this study aimed to compare the antimicrobial efficacy of calcium hydroxide, 10% bamboo salt, and cerium oxide against *E. faecalis* and *C. albicans*, in an effort to identify a more effective intracanal medicament for persistent endodontic infections.

MATERIALS AND METHODS

Preparation of Intracanal Medicaments

Three medicaments were used: calcium hydroxide, 10% bamboo salt, and cerium oxide nanoparticles. Each was mixed with methylcellulose to obtain a paste-like consistency for intracanal application.

Sample Preparation

Seventy-two single-rooted premolars were cleaned, scaled, and examined radiographically. Teeth were autoclaved at 121°C and stored in distilled water. All specimens were decoronated using a rotary diamond disk to a standardized length of 16 mm (fig.1).

Fig1. Decoronation of the specimen

Working length was established with a size 15 K-file and confirmed radiographically (fig.2).

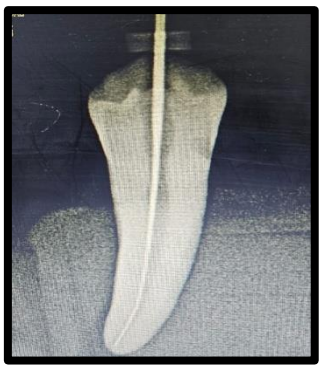

Fig 2. Working length determination

Biomechanical preparation was done using ProTaper Gold files up to size F3 (30/0.09) (fig.3), followed by irrigation with 3 ml of 3% NaOCl and 3 ml of 17% EDTA for 3 minutes. Samples were then rinsed in distilled water for 5 minutes and dried.

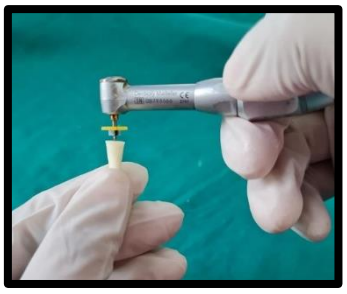

Fig 3. Biomechanical preparation

Inoculation of Bacterial Suspension

E. faecalis and *C. albicans* were cultured on Brain Heart Infusion (BHI) and Sabouraud's Dextrose Agar (SDA) media, respectively, and incubated at 37°C for 24 hours.

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Thirty-six samples were inoculated with *E. faecalis*, and the remaining 36 with *C. albicans* (fig.4). The inoculated samples were incubated for 21 days at 37°C.

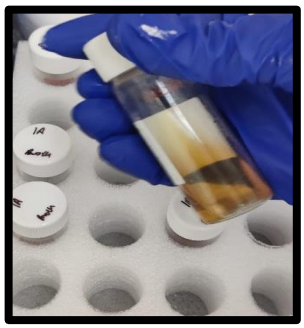

Fig 4. Inoculation of bacterial suspension

Placement of Intracanal Medicaments

Post-incubation, canals were flushed with sterile saline (fig.5), and samples were divided into three groups (n=12 per microorganism per group) depending on the medicament placement- Group I: Calcium Hydroxide; Group II: 10% Bamboo Salt; Group III: Cerium Oxide. The intracanal medicaments were introduced using lentulospirals and sealed with resin-modified glass ionomer cement (fig.6). Samples were incubated at 37°C in an oxygen-rich environment.

Fig 5. Canals flushed with normal saline

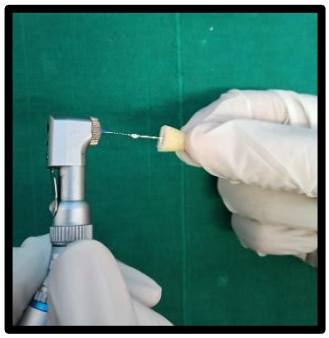

Fig 6. Medicament placement using lentulospirals

Bacterial Counting

On the 2nd and 7th days, medicaments were flushed out with sterile saline. Dentinal debris were collected using GG drill no. 4 into 1 ml of phosphate buffer saline solution (fig.7). Diluted solution were then transferred to their respective medium for culture, ie. Brain Heart Infusion (BHI) for E. faecalis and Sabouraud's Dextrose Agar (SDA) for C. albicans. Colony-forming units (CFUs) were counted semi-quantitatively and statistically analyzed.

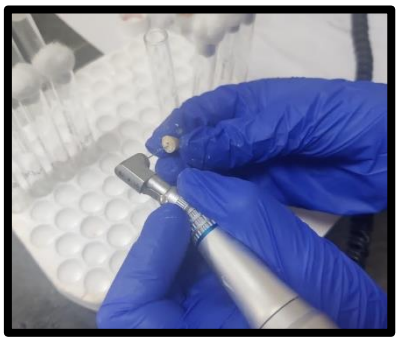

Fig 7. Dentinal debris collected

STATISTICAL ANALYSIS

All the data were investigated using SPSS software (Windows version 22.0). The data were summarised in Mean ± SE (standard error of the mean). Groups were compared by two factor (group x period) analysis of variance (ANOVA) and the significance of mean difference within (intra) and between (inter) the groups was done by Tukey's HSD (honestly significant difference) post hoc test after ascertaining normality by Shapiro-Wilk's test and homogeneity of variance between groups by Levene's test. A two-tailed (α=2) $P <$ 0.05 was considered statistically significant.

RESULTS

The study's outcome measure was the colony-forming unit (CFU).

I. E. faecalis

The E. faecalis CFU count for three groups (Group I, Group II, and Group III) on the 2nd and 7th day is summarized in Table 1 and illustrated in Graph1. Across all three groups, the mean E. faecalis CFU count showed a significant decline over time, with the lowest count observed in Group II, followed by Group I, and the

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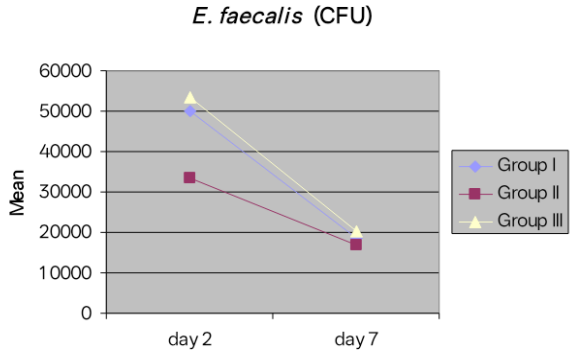
highest in Group III on both the days (Group II < Group I < Group III).

Group	day 2 (n= 6)	day 7 (n= 6)
Group I	50050 ± 22338	18550 ± 16367
Group II	33400 ± 21061	16750 ± 16650
Group III	53350 ± 20915	20200 ± 16076

Table 1: *E. faecalis* CFU count of three groups at two time intervals



Graph 1. Line graph showing the mean E. faecalis CFU count of three groups

On comparison, the Tukey test indicated statistically significant difference (P > 0.05) in the mean *E. faecalis* CFU count between the groups. The CFU count was the least for Group II (Bamboo Salt) with a mean of 33400 ± 21061 on 2nd day and 16750 ± 16650 on the 7th day followed by Group I (Calcium Hydroxide) with a mean of 50050 ± 22338 on 2nd day and 18550 ± 16367 on the 7th day.

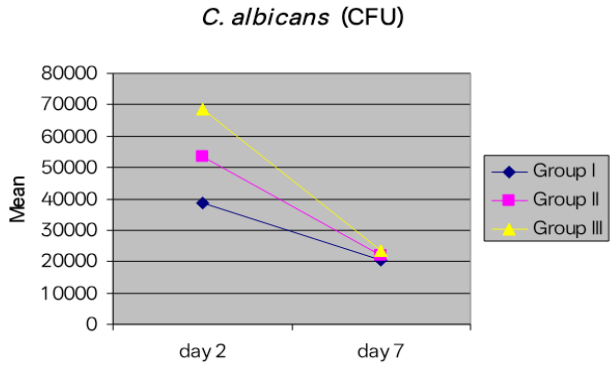
The highest CFU count was showed by Group III (Cerium Oxide) with a mean of 53350 ± 20915 on the 2nd day and 20200 ± 16076 on the 7th day.

II. C. albicans

The *C. albicans* CFU count for the three groups (Group I, Group II, and Group III) on the 2nd and 7th day is summarized in Table 2 and illustrated in Graph 2. Across all three groups, the mean *C. albicans* CFU count showed a significant decline over time with the lowest count observed in Group I, followed by Group II, and highest in Group III on both the days. (Group I < Group II < Group III).

Group	day 2 (n=6)	day 7 (n=6)
Group I	38500 ± 19500	20350 ± 16039
Group II	53500 ± 20839	22000 ± 15704
Group III	68500 ± 19956	23350 ± 15415

Table 2: *C. albicans* CFU count of three groups at two time intervals



Graph 2. Line graph showing the mean *C. albicans* CFU count of three groups

On comparison, the Tukey test revealed statistically significant difference (P > 0.05) in the mean *C. albicans* CFU count between the groups. The CFU count was the least for Group I (Calcium Hydroxide) with a mean of 38500 ± 19500 on 2nd day and 20350 ± 16039 on the 7th day followed by Group II (Bamboo Salt) with a mean of 53500 ± 20839 on the 2nd and 22000 ± 15704 on the 7th day.

The highest CFU count was shown by Group III (Cerium oxide) with a mean of 68500 ± 19956 on the 2nd and 23350 ± 15415 on the 7th day.

DISCUSSION

The main objective of endodontic treatment is the eradication of microorganisms and their byproducts from the pulpal canal system to prevent infection or reinfection of the tooth and surrounding tissues.

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Complete debridement and disinfection are essential for long-term success, typically achieved through adequate bio-mechanical preparation. However, due to the complex anatomy of root canals, mechanical instrumentation alone often fails to reach all canal surfaces, leaving behind necrotic tissue and microbes, which can cause persistent inflammation.[5]

As per studies microorganisms, particularly *Enterococcus faecalis*, can survive in these inaccessible areas. *E. faecalis* is a resilient gram-positive facultative anaerobe, found in 24%–77% of failed treatments. It can withstand extreme conditions, such as high pH and temperature, making it difficult to eradicate.[6]

Fungi, although less prevalent, also pose a challenge. *Candida albicans*, the most common fungal species found in persistent infections, adheres to canal walls, invades dentinal tubules, and forms biofilms within 48 hours. It can survive in harsh conditions, resisting standard disinfection methods.[7]

Research indicates that microorganisms can survive within the intricate anatomy of root canals, even after comprehensive cleaning and shaping, due to the limited accessibility of instruments and irrigants.[8] Applying an intracanal medicament between treatment appointments has been proven to efficiently inhibit microbial resurgence and lower the microbial count, particularly in cases of severe infection. An ideal intracanal medicament should be non-irritating to periradicular tissues, stable in solution, exhibit prolonged antimicrobial activity, and remain effective in the presence of blood, serum, and tissue protein derivatives.[9] ***Chong and Pitt Ford*** (1992) highlighted the critical role of medicaments in multi-visit treatments to inhibit residual bacteria and prevent reinfection.[10]

Antibacterial efficacy

The antibacterial effectiveness of the intracanal medicaments was assessed using the Semi Quantitative Method of counting the Colony Forming Units (CFU's). This technique enables a quick evaluation of the viable microorganism count, comparable to traditional plating, utilizing standard laboratory apparatus. It is more rapid and cost-effective than conventional plating techniques.[11]

The results of this study indicate that while all experimental agents demonstrated antimicrobial activity, their effectiveness varied. During the overall assessment, the CFU's were lower on the 7th day compared to that of 2nd day for both the microorganisms. This observation was consistent with a research done by ***Kumar et al (2021)*** where he concluded that the antimicrobial efficacy increases with the increase in contact period of these medicaments with the root canal wall.[12]

Bamboo Salt demonstrated superior antimicrobial efficacy compared to conventional Calcium Hydroxide against E. faecalis. Bamboo salt exhibits potent antibacterial, antioxidative, and anti-inflammatory effects. It comprises of ions like Zinc, Manganese, Calcium, Phosphorus, Potassium, Magnesium and Iron. These metallic ions, together with its strongly alkaline pH (12.4), initiate oxidative damage, interfere with protein activity, and lead to membrane disruption in bacterial cells.[13]

Certain metal ions in bamboo salt, especially iron (Fe^{2+}/Fe^{3+}), can participate in Fenton reactions, leading to the production of hydroxyl radicals (-OH). These radicals causes structural changes in the cell wall, such as pits which increases cell permeability, leading to distortion and finally lysis of the bacterial cells.[14]

The reduced effectiveness of Calcium Hydroxide may be due to E. faecalis' capacity to survive in highly alkaline conditions (up to pH 11.5), enabled by its proton pump activity and internal pH homeostasis mechanisms. In contrast, Calcium Hydroxide can only achieve an alkalinity of approximately pH 10.3 within the root dentin because of the buffering capacity of dentin which limits its ability to achieve and sustain a highly alkaline pH. E. faecalis regulates pH levels in both acidic and alkaline conditions, and thereby maintains an internal pH within a narrow range, ensuring the proper functioning of enzymes and proteins.[15]

CeO_2 nanoparticles exhibited markedly lower antimicrobial efficacy than the other experimental groups on both days. The lower efficacy of CeO_2 nanoparticles against Enterococcus faecalis is likely due to a combination of bacterial resistance mechanisms, limited Reactive Oxygen Species production, weak nanoparticle-membrane interaction. The antimicrobial activity of CeO_2 is mainly due to its capability to produce reactive oxygen species (ROS), including hydroxyl radicals and superoxide ions.[16] However, CeO_2 has a redox-switching ability between Ce^{3+} and Ce^{4+}, which

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can act as both a pro-oxidant and an antioxidant. If the CeNPs predominantly exhibit antioxidant properties (Ce^{3+} dominant state), they may scavenge ROS rather than produce them, thereby reducing their antibacterial effectiveness.[17]

The antibacterial efficacy of cerium oxide nanoparticles against Enterococcus faecalis can be enhanced by optimizing particle size, surface charge, and doping with antimicrobial agents or metals such as silver or zinc.[18]

Calcium Hydroxide showed the least mean CFU count against C. albicans. The main action of calcium hydroxide come from the ionic dissociation of Ca2+and OH- ions, and the action of these ions on vital tissue and microbes generates the induction of hard tissue deposition and the antimicrobial effect. The yeasts become completely inviable after 48h of contact with calcium hydroxide. The higher efficacy of Calcium Hydroxide may also be due to the limited penetration depth of C. albicans, which ranges from approximately 10 to 150 µm into the dentinal tubules comparable to the penetration depth of Calcium Hydroxide, which extends up to 200 µm.[19]

The antifungal effect of Bamboo Salt can be due to the osmotic stress caused by it to the fungal cell wall. Osmotic stress occurs when a high concentration of solutes (such as salt) creates a hypertonic environment, leading to water loss from fungal cells.[20]

The antifungal efficacy of Cerium Oxide is thought to result from its interaction with fungal cell wall components, leading to irreversible changes, including the inhibition of fungal enzymatic activity. However, no studies have been conducted so far regarding its efficacy against C. albicans. C. albicans has an efficient antioxidant defense system (e.g., catalase and superoxide dismutase), which neutralizes ROS, thereby reducing CeNPs' effectiveness. CeO_2 nanoparticles often exhibit a negative or neutral surface charge, which may lead to weak electrostatic interactions with the negatively charged fungal membrane. This weak binding results in limited uptake of CeNPs into fungal cells, reducing their antifungal potency.[21]

CONCLUSION

Based on the results, it can be inferred that Bamboo Salt has the potential to serve as an effective intracanal agent in managing persistent periapical infections unresponsive to Calcium Hydroxide. However, further researches and clinical studies with larger sample size and in-vivo conditions are necessary to determine this study's results.

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