

Evaluation of Antifungal Effects of Root Canal Irrigants and Intracanal Medicaments: An *In Vitro* Study

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Abstract

Objective

The aim of this study was to evaluate the antifungal effectiveness of 2.5% sodium hypochlorite and various intracanal medicaments against *Candida albicans* present within the root canal system.

Materials and Methods One hundred and twenty freshly extracted single-rooted human teeth were selected and instrumented. The root canals were contaminated with *Candida albicans* suspension and incubated for 48 hours. The specimens were then divided into six experimental groups based on the intracanal medicament used:

1. Calcium hydroxide mixed with saline
2. Calcium hydroxide mixed with 2% chlorhexidine gluconate
3. Zinc oxide mixed with 2% chlorhexidine gluconate
4. Rezafungin solution prepared in sterile distilled water
5. Irrigation with 2.5% sodium hypochlorite without intracanal medicament
6. Control group with no intracanal medicament

The canals were sealed and incubated at $37 \pm 1^\circ\text{C}$ for 14 days. Microbiological samples were obtained using sterile paper points and cultured on Sabouraud dextrose agar containing chloramphenicol. The plates were incubated at $37 \pm 1^\circ\text{C}$ for 48 hours, and fungal growth was evaluated.

Results

The highest antifungal activity was observed in Group 5, where 2.5% sodium hypochlorite was used as the irrigant, showing elimination of *Candida albicans* in 90% of samples. Rezafungin demonstrated the second highest effectiveness (80% reduction). Calcium hydroxide mixed with saline showed the lowest antifungal activity (50% effectiveness).

Conclusion

Within the limitations of this study, sodium hypochlorite showed the highest antifungal efficacy against *Candida albicans*. Rezafungin also demonstrated promising antifungal activity, suggesting that appropriate intracanal medicaments may enhance microbial elimination from the root canal system.

Keywords: Rezafungin, *Candida albicans*, Calcium hydroxide, Chlorhexidine gluconate, Sodium hypochlorite, Intracanal medicaments

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INTRODUCTION

Persistent endodontic infections remain a significant challenge in root canal therapy, particularly in cases associated with fungal microorganisms such as *Candida albicans*. This opportunistic fungus has frequently been isolated from previously treated root canals and is known for its ability to penetrate dentinal tubules, adhere to dentin surfaces, and survive under harsh environmental conditions^{1,2}. Its presence has been strongly associated with endodontic treatment failures and persistent periapical lesions. Therefore, effective

elimination of *Candida albicans* from the root canal system is essential for successful endodontic outcomes. Intracanal medicaments and irrigating solutions play a crucial role in reducing microbial load within the complex anatomy of the root canal system. Calcium hydroxide has long been considered the gold standard intracanal medicament due to its high alkalinity and antimicrobial properties. However, several studies have reported that *Candida albicans* may demonstrate resistance to calcium hydroxide, which has led to the exploration of alternative or adjunctive antimicrobial agents³. Mixing calcium hydroxide with vehicles such

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as saline or antimicrobial agents like 2% chlorhexidine gluconate may influence its antimicrobial efficacy and diffusion within dentinal tubules.⁴

Chlorhexidine gluconate is a broad-spectrum antimicrobial agent widely used in endodontics due to its substantivity and effectiveness against both gram-positive and gram-negative microorganisms, as well as fungi. The combination of chlorhexidine with intracanal medicaments such as zinc oxide has also been investigated to enhance antifungal activity and improve microbial elimination within the root canal system.⁵

In recent years, newer antifungal agents have been developed to combat resistant fungal infections. Rezafungin, a novel echinocandin antifungal agent with extended stability and potent activity against *Candida* species, has shown promising results in the management of fungal infections. Its potential application in endodontic therapy for the elimination of *Candida albicans* has gained increasing interest.⁴

Additionally, sodium hypochlorite remains the most commonly used root canal irrigant due to its excellent antimicrobial properties and tissue-dissolving capacity. Irrigation with 2.5% sodium hypochlorite is widely employed during root canal instrumentation to reduce microbial load, and its effectiveness against fungal organisms has also been documented.^{4,6}

Considering the persistent nature of *Candida albicans* infections and the limitations of conventional intracanal medicaments, evaluating different antimicrobial combinations is essential. Therefore, this study aims to compare the antifungal effectiveness of calcium hydroxide mixed with saline, calcium hydroxide mixed with 2% chlorhexidine gluconate, zinc oxide mixed with 2% chlorhexidine gluconate, Rezafungin solution prepared in sterile distilled water, and irrigation with 2.5% sodium hypochlorite without intracanal medicament against *Candida albicans* in the root canal system.

Materials and Methods

A total of 120 freshly extracted human single-rooted teeth with non-carious crowns and closed apices were selected for the study. The crowns were sectioned to obtain roots with an approximate length of 16 mm.

Root canal instrumentation was performed up to a #25 file, terminating 0.5 mm short of the apex. The external surfaces of the roots were coated with two layers of nail polish, leaving only the access cavity and apical foramen exposed. The specimens were sterilized in an autoclave at 123°C for 30 minutes.

All procedures were carried out under aseptic conditions in a laminar airflow chamber. The root apices were sealed with Cavit cement, and each canal was inoculated with 0.1 ml of a suspension containing 10⁸ cells of *Candida albicans* using a sterile micropipette.

The access cavities were sealed with sterile cotton pellets and Cavit cement. The specimens were incubated at 37 ± 1°C for 48 hours to allow fungal colonization.

After incubation, the canals were reinstrumented up to a #40 file using 5 ml of sterile saline as the irrigant.

The specimens were randomly divided into six groups:
Group 1: Calcium hydroxide powder mixed with saline

Group 2: Calcium hydroxide powder mixed with 2% chlorhexidine gluconate

Group 3: Zinc oxide powder mixed with 2% chlorhexidine gluconate

Group 4: Rezafungin solution prepared in sterile distilled water

Group 5: Irrigation with 2.5% sodium hypochlorite without intracanal medicament

Group 6: Control group with no intracanal medicament

The canals were sealed with Cavit and incubated at 37 ± 1°C for 14 days.

Following incubation, the canals were reinstrumented using a #25 file and irrigated with sterile saline to remove the intracanal medicaments.

For microbiological evaluation, sterile #25 paper points were inserted into each canal for 1 minute and then transferred into test tubes containing 1 ml sterile saline. The tubes were shaken for 30 seconds, and 0.1 ml of the suspension was inoculated onto Sabouraud dextrose agar supplemented with chloramphenicol.

The culture plates were incubated at 37°C for 48 hours, after which fungal colonies were examined. Identification of *Candida albicans* was confirmed using Gram staining and germ tube tests.

Statistical analysis was performed using Fisher's Exact Test, with a p-value < 0.05 considered statistically significant. Data analysis was conducted using IBM SPSS Software, Version 30.0 (IBM Corp., 2024)

Results

The results of the study demonstrated varying antifungal effectiveness among the tested groups as shown in Graph 1.

Group 5, where 2.5% sodium hypochlorite was used as the irrigant during instrumentation without intracanal medicament, showed the highest antifungal effectiveness, with 90% of samples showing no fungal growth.

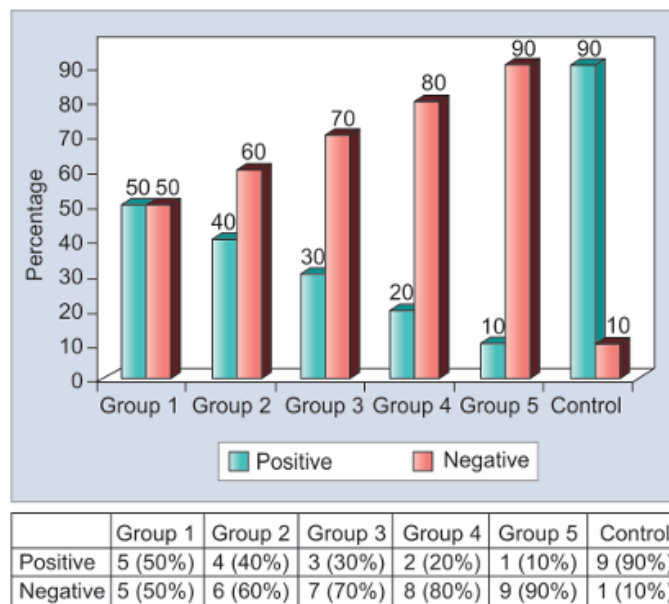
The second most effective treatment was observed in Group 4, where Rezafungin solution prepared in sterile distilled water was used as the intracanal medicament. This group showed 80% reduction in fungal growth, indicating strong antifungal activity against *Candida albicans*.

Group 3, consisting of zinc oxide combined with 2% chlorhexidine, demonstrated 70% effectiveness in reducing fungal growth.

Group 2, where calcium hydroxide was combined with 2% chlorhexidine, showed 60% effectiveness.

Group 1, where calcium hydroxide was mixed with saline, demonstrated the lowest antifungal effectiveness, with only 50% reduction in fungal growth.

The control group (Group 6), which received only saline irrigation without intracanal medicament, showed fungal growth in 90% of the samples, confirming the persistence of *Candida albicans* in untreated canals. Overall, the results indicated that 2.5% sodium hypochlorite exhibited the highest antifungal activity, followed by Rezafungin, while calcium hydroxide mixed with saline showed the least effectiveness.



Graph 1: Positive and negative growth of *C. albicans* in six groups

Discussion

The primary objective of endodontic therapy is the complete elimination of microorganisms from the root canal system and the prevention of reinfection. Microorganisms are the main etiological factors responsible for pulpal and periapical diseases. Although chemomechanical preparation significantly reduces the microbial load, complete elimination of microorganisms from the complex anatomy of the root canal system is often difficult. Microorganisms may survive within dentinal tubules, accessory canals, and anatomical irregularities, thereby contributing to persistent endodontic infections.⁷

Several microbiological studies have demonstrated that fungi, in addition to bacteria, may be present in infected root canals. Among fungal species, *Candida albicans* is the most frequently isolated organism from persistent endodontic infections. This microorganism possesses the ability to adhere to dentin, penetrate deep into dentinal tubules, and survive in harsh environmental conditions, making it difficult to eradicate during routine endodontic procedures. Studies conducted by Waltimo T and Haapasalo M demonstrated that *Candida albicans* isolated from infected root canals may exhibit resistance to commonly used intracanal medicaments such as calcium hydroxide, particularly when it is mixed with inert vehicles^{8,9}.

In the present study, 2.5% sodium hypochlorite (NaOCl) demonstrated the highest antifungal activity against *Candida albicans*, eliminating fungal growth in the majority of the samples. Sodium hypochlorite is widely used as an endodontic irrigant because of its strong antimicrobial activity and its ability to dissolve organic tissue. Similar findings have been reported in previous studies by Sen BH and Safavi KE, who reported that sodium hypochlorite is highly effective against fungal organisms present in infected root canals.¹⁰ The high antifungal activity of sodium hypochlorite can be

attributed to its ability to cause irreversible oxidation of microbial enzymes and disruption of cellular metabolism.

The second most effective medicament observed in the present study was Rezafungin, which demonstrated significant antifungal activity against *Candida albicans*. Rezafungin is a novel echinocandin antifungal agent that acts by inhibiting the synthesis of β-1,3-D-glucan, an essential structural component of the fungal cell wall. Disruption of fungal cell wall synthesis leads to osmotic instability and cell death. Rezafungin belongs to the same class of antifungal drugs as Caspofungin, Micafungin, and Anidulafungin, which are widely used in the management of invasive candidiasis. Recent pharmacological studies have reported that Rezafungin has improved chemical stability and a longer half-life compared with earlier echinocandins, allowing prolonged antifungal activity.^{12,13} Although its application in endodontics has not been widely investigated, the results of the present study suggest that Rezafungin may have potential as an effective intracanal medicament for eliminating fungal infections within the root canal system.

In the present study, the combination of zinc oxide and Chlorhexidine demonstrated better antifungal activity than calcium hydroxide combined with chlorhexidine. Chlorhexidine is a broad-spectrum antimicrobial agent with strong antifungal activity and the ability to bind to dentin, a property known as substantivity. This allows chlorhexidine to exert prolonged antimicrobial effects within the root canal system. Previous studies by Mohammadi Z and Abbott PV have also reported that chlorhexidine exhibits significant antimicrobial activity against *Candida albicans* and may be more effective than calcium hydroxide against certain resistant microorganisms.⁸

Among all the medicaments evaluated in the present study, calcium hydroxide mixed with saline

demonstrated the lowest antifungal activity. This finding is consistent with previous research that reported limited effectiveness of calcium hydroxide against *Candida albicans*.⁶ The resistance of *Candida albicans* to calcium hydroxide may be attributed to its ability to tolerate alkaline environments and maintain intracellular pH homeostasis. Furthermore, the use of inert vehicles such as saline may reduce the diffusion and antimicrobial activity of calcium hydroxide within the dentinal tubules.¹¹

The persistence of fungal growth observed in some samples may also be attributed to the complex anatomy of the root canal system, which may limit the penetration of irrigants and intracanal medicaments into deep dentinal tubules and accessory canals.¹⁴ In addition, the absence of smear layer removal procedures such as the use of EDTA may further reduce the penetration of antimicrobial agents.

Recent molecular studies have also confirmed the presence of *Candida albicans* in persistent and secondary endodontic infections using advanced diagnostic methods such as polymerase chain reaction (PCR).^{2,3} These findings highlight the importance of using effective antifungal agents during endodontic treatment to improve the overall success rate of therapy.

Conclusion

Within the limitations of this study, the tested intracanal medicaments and irrigating solutions demonstrated varying degrees of antifungal activity against *Candida albicans* in infected root canals. Calcium hydroxide mixed with saline showed limited antifungal effectiveness, whereas the combination of calcium hydroxide with 2% chlorhexidine gluconate exhibited enhanced antifungal activity due to the synergistic antimicrobial properties of chlorhexidine. Zinc oxide mixed with 2% chlorhexidine gluconate also demonstrated notable antifungal efficacy. Rezafungin solution prepared in sterile distilled water showed promising antifungal potential against *Candida albicans*, suggesting that newer antifungal agents may be useful in managing persistent fungal infections in endodontic cases. Irrigation with 2.5% sodium hypochlorite without the use of an intracanal medicament showed antimicrobial action but was comparatively less effective in completely eliminating fungal organisms. Therefore, the use of appropriate intracanal medicaments, particularly those combined with chlorhexidine or newer antifungal agents, may improve the elimination of *Candida albicans* from the root canal system and enhance the success of endodontic treatment.

Limitations of the Study

This study has several limitations that should be considered when interpreting the results. Firstly, the study was conducted under in vitro conditions, which may not completely replicate the complex environment of the root canal system in vivo. Factors such as host immune response, tissue fluids, and variations in canal anatomy were not present in the experimental setup and may influence the effectiveness of intracanal medicaments in clinical situations. Secondly, the study

evaluated the antifungal efficacy against a single microorganism, *Candida albicans*, whereas endodontic infections are usually polymicrobial in nature. Thirdly, the sample size and experimental conditions may limit the generalization of the findings to broader clinical scenarios. Additionally, the duration of medicament application and observation period may not fully reflect the long-term effects of these agents within the root canal system. Finally, although promising results were observed with newer antifungal agents such as Rezafungin, further in vivo studies and clinical trials are required to confirm their safety, effectiveness, and practical applicability in endodontic treatment.

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