Comparative Evaluation of Antimicrobial Efficacy of Three Different Endodontic Irrigants against *Candida albicans*: An *In Vitro* Study

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Abstract

Aim: To compare and assess the antimicrobial effectiveness of three different endodontic irrigants against *Candida albicans* by measuring the colony-forming units using a light microscope.

Materials and methods: Sixty central incisors of human maxillary teeth extracted for periodontal concerns were collected, stored, and decoronated to a standardized working length of 14 mm. Followed by the preparation of the canal, the teeth have been contaminated with *C. albicans* (ATCC-10231) and cultured at 37°C for 21 days. The teeth were randomly divided into four experimental groups based on the different endodontic irrigants employed namely group I: distilled water ($n = 15$), group II: 5.25% sodium hypochlorite (NaOCl) ($n = 15$), group III: 5.25% calcium hypochlorite $(CaOCl)_2$ ($n = 15$), group IV: mixture of tetracycline isomer, citric acid, and detergent (MTAD), and 5 mL of irrigant was used for 2 minutes. Microbiological samples were collected by introducing a sterile paper tip into the canal for 60 seconds and then shaking for 30 seconds on a vortex in a micro test tube containing 0.5 mL of sterile distilled water. Then, 0.1 mL aliquot of the microbial suspension was plated on a Sabouraud dextrose broth agar plate. Microbial growth was confirmed and a number of colony-forming units (CFUs/mL) of *C. albicans* were recorded and validated by Gram stain under a light microscope at 400× magnification.

Results: Significantly reduced number of microbiological colonies were observed on the experimental group III $(CaOCl)_2$ followed by group II (NaOCl), and group IV (MTAD).

Conclusion: Calcium hypochlorite was significantly better in its antimicrobial efficacy against *C. albicans* for 21 days.

Keywords: Antimicrobial efficacy, Calcium hypochlorite, *Candida albicans*, Colony-forming units.

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Introduction

The initiation and maintenance of the periapical disease are dependent on the bacterial invasion of the root canal system. As a result, endodontic treatment's goal is to eliminate microorganisms from the root canal system. Despite the fact that biomechanical preparation and root canal shaping significantly reduce bacteria from the lateral canals, accessory root canals, isthmus, and apical deltas.[1] However, some microorganisms still remain or proliferate following chemomechanical preparation, such as *Candida albicans*.[2] *C. albicans* is taken to be the versatile, dentinophilic, and dimorphic microorganism since it grows both as yeast and filamentous cells. Due to collagenolytic activity, it has the potential to invade into the dentinal tubules and use dentin and smear layer as sources of nutrition and promote colonization in the root canal.[3] Therefore, endodontic irrigants are employed to get rid of microorganism from the infected root canal.

The use of the endodontic irrigation is crucial to the effectiveness of root canal therapy.[4] The flushing mechanism of the irrigant during chemomechanical preparation helps in removal of debris, tissue remnants, and dentin fragments from the root canal.[5] According to recent studies, about 35–50% of the canal space remains uninstrumented following mechanical instrumentation. As a result, using an endodontic irrigant during instrumentation is critical for cleaning all parts of the root canal system, especially those that are difficult to reach with instrumentation.[6]

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Sodium hypochlorite (NaOCl) is one of the most often utilized irrigants in endodontics because of its antibacterial and tissue dissolving properties. Proteolysis and tissue dissolution are the mechanisms through which it works. However, inadvertent periapical extrusion of this irrigant is well known to induce tissue irritation, discomfort, and swelling,[6] and it also interferes with the bonding of restorative materials to dentine and chemical instability as active chlorine content diminishes from storage.[7] Newer alternatives have been discovered to be successful as adjuvant irrigants to aid in the biomechanical preparation of root canals, despite of its limitations.

Calcium hypochlorite ($CaOCl)_2$ is often utilized in the purification of water (35%) and disinfection procedures. In contrast, NaOCl has a nearly constant pH and higher availability of chlorine (65%) and is biocompatible.[6]

Torabinejad et al. described the creation of Biopure MTAD (Dentsply, Tulsa, Oklahoma), a mixture of a tetracycline isomer (doxycycline), an acid (citric acid), and a detergent (Tween 80), which can be utilized for canal sterilization and smear layer eradication. The doxycycline in MTAD has a high dentine bonding affinity, allowing it to have a longer antibacterial property and less cytotoxic (Fig. 1).[8]

The aim of this study was to compare and assess the antimicrobial efficacy of three distinct endodontic irrigants in root canals infected with *C. albicans*.

Materials and Methods

A detailed protocol explaining the purpose and procedures of the study was submitted, and ethical clearance was obtained from the institution.

Sixty intact human permanent maxillary central incisors freshly extracted for periodontal concerns were collected. All the collected teeth were examined under microscope for craze lines and analyzed with periapical radiographs for the absence of calcification, internal resorption, open apex, or an additional canal. Methodology planned was through the following steps:

- Biomechanical preparation of a tooth
- Microbiological procedure (contamination with *C. albicans*)
- Irrigation regimen
- Microbiological analysis

Biomechanical Preparation of Teeth Specimen

The extracted teeth were collected and immediately stored in deionized water to which 0.1% thymol solution was added to prevent dehydration. The cervical portion of the tooth was sectioned using double faced cylindrical diamond saw so that all the roots were standardized to a uniform length of 14 mm.[8] The working length of the canal was determined by inserting a #10 K-file (Dentsply–Maillefer) into the canal until the tip was visible at the apical foramen. To standardize its working length, 1 mm was deducted from this measurement. The patency of each canal was confirmed with 15 size K-file. Gates Glidden Drills No. 1, 2, and 3 (Dentsply/Maillefer, Switzerland) were used to flare the coronal aspect of each canal.[8] Wave one primary (25/0.07) was used for the root canal preparation. During instrumentation, irrigation was performed with 5.25% NaOCl (Denta Pro, Chandigarh, India) using side-vented needles after each and every file.

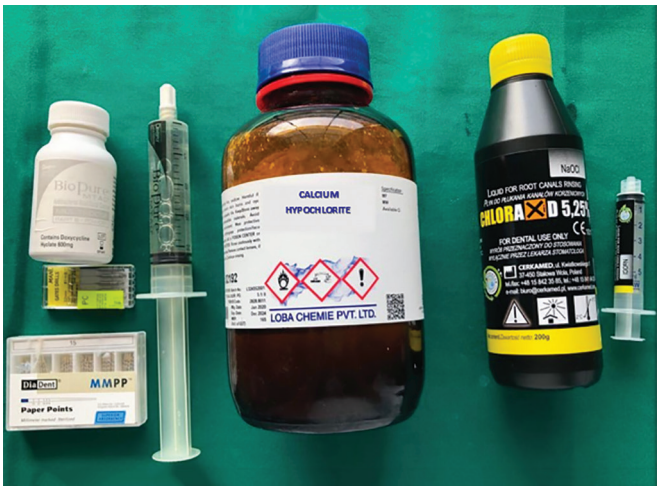


Fig. 1: Armamentarium

One milliliter of 17% ethylenediaminetetraacetic acid (EDTA) (prima dental product) was used to irrigate the root canals after instrumentation for 3 minutes to get rid of smear layer. After that, canal was filled for 3 minutes with 1 mL of 5.25% NaOCl.[9] To get rid of the residual irrigants, the canals were rinsed with 10 mL of sterile distilled water. The roots were secured in plastic microtubes using Putty-C Silicone to ensure that they remained upright with the cervical section facing upwards. The root canals were dried with paper tips before being autoclaved for 15 minutes at 121°C with 15 pounds of pressure. To prevent irrigants from getting into contact with the external surface, the apex of the root was sealed with acrylic resins and the surface of the specimen was covered with two layers of nail varnish.

Microbial Procedure (Contamination with *C. albicans*)

Clinical isolates of *C. albicans* were used as the test microorganism. A suspension of *C. albicans* (ATCC 10231) was cultured for 48 hours in 1 mL sterile Sabouraud dextrose agar (SDA) at 37°C.[8]
The 60 root specimens were transferred into sterile cell culture plates under sterile conditions. Roots were mounted within the well plates containing 2% sterile agar media that was allowed to solidify so that root specimens can be stabilized.

Ten milliliters of 0.5 McFarland solution (1.5×10^8 fungi/mL) of the fungal suspension were transferred into each canal of the laminar flow hood under sterile conditions using micropipettes. Samples were incubated at 37°C for 21 days (Fig. 2). The inoculated broth was renewed every 2–3 days, and 10 mL of fresh Sabouraud dextrose broth was added to ensure the viability of the fungi. After 21 days of incubation, each tooth was rinsed with sterile distilled water and blotted dry.

Irrigation Regimen

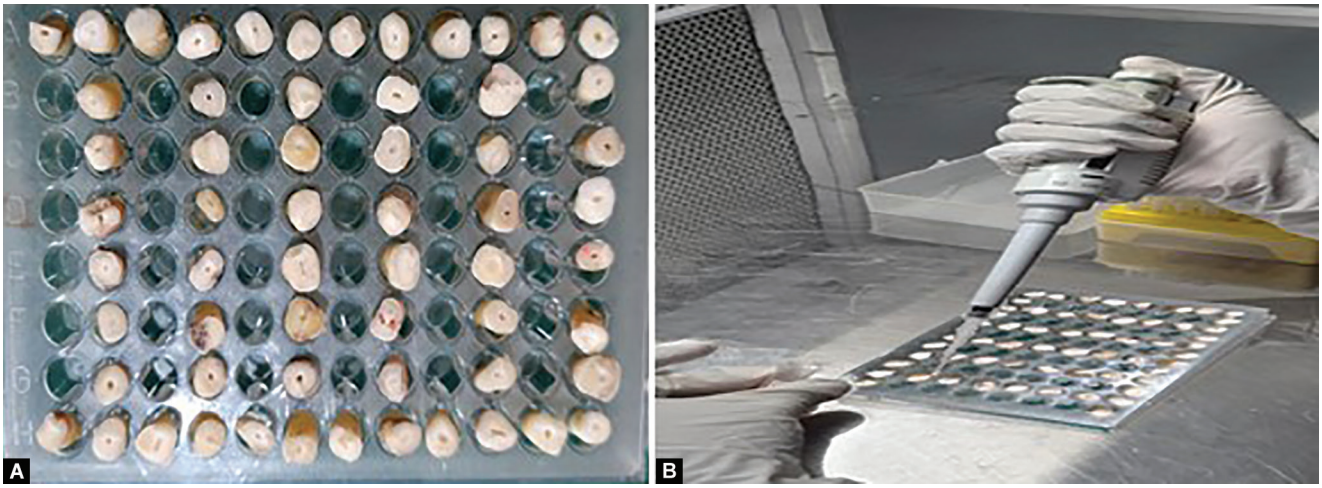
Following incubation, the samples were split into four groups, each consisting of 15 samples.

- Group I—distilled water
- Group II—5.25% of NaOCl
- Group III—5.25% of $Ca(OCl)_2$
- Group IV—MTAD

Each group was irrigated with 5 mL of irrigating solution using side vent needle for 2 minutes. The depth of the needle was 2 mm short from obtained working length. The canals were agitated with K-file approximately 1 mm short of the working length after final irrigation with 10 mL of sterile distilled water.[10]

Microbiological Analysis

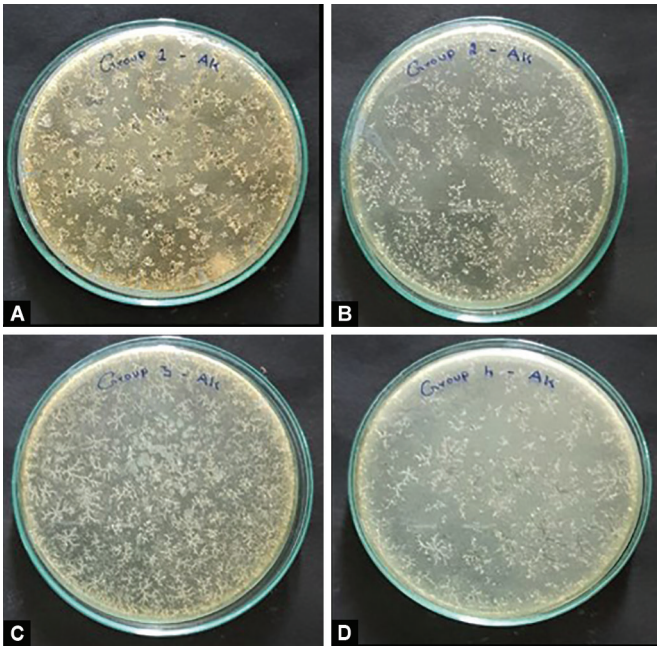
Microbiological samples were collected by inserting a sterile absorbent paper tip into the canal for 1 minute, then transferring the paper point into a test tube containing 0.5 mL of sterile distilled water and vortex shaking it for 30 seconds. The microbial suspension was then plated on SDA broth in a 0.1 mL aliquot. The SDA plates were then incubated aerobically for 48 hours at 37°C. Microbial growth was confirmed, and the total number of the colony-forming units (CFUs/mL) of *C. albicans* was recorded and analyzed using Gram stain under a 400× magnification light microscope[11] (Fig. 3).



Figs 2A and B: Fungal suspension transferred into laminar flow hood using micropipette

Table 1: Descriptive statistics of four groups at 21 days

					95% confidence interval for mean			
Group	*N*	*Mean (CFU/mL)*	*Std. deviation*	*Std. error*	*Lower bound*	*Upper bound*	*Minimum*	*Maximum*
Group I	15	21.1533	0.85094	0.21971	20.6821	21.6246	19.40	22.30
Group II	15	4.9153	0.63777	0.16467	4.5621	5.2685	3.52	5.75
Group III	15	2.0807	0.16395	0.04233	1.9899	2.1715	1.82	2.43
Group IV	15	5.2940	0.30907	0.07980	5.1228	5.4652	4.83	5.92
Total	60	8.3608	7.57228	0.97758	6.4047	10.3170	1.82	22.30



Figs 3A to D: *Candida* CFUs

Statistical Analysis

SPSS version 23 was used to statistically analyze the results. For the microbiological examination, a one-way analysis of variance (ANOVA) test was used, followed by Tukey's post hoc test to determine significant value.

Results

Descriptive statistics for the four groups at 21 days are given in Table 1. A highly significant value (<0.01) was found in the antimicrobial efficacy of different endodontic irrigants against *C. albicans* at 21 days are given in (Table 2).

A significantly reduced number of microbiological colonies was observed on the experimental plates in group III $(CaOCl)_2$ when compared with group I (control), group II $(NaOCl)_2$, and group IV (MTAD) at 21 days interval are given in Fig. 4.

Multiple comparisons among all the four groups were given in Table 3, i.e., group III < group II < group IV < group I.

Discussion

The fundamental goal of root canal treatment is to get rid of bacteria from root canal and forestall infections. Endodontic infections are polymicrobial in nature with prevalence toward anaerobic species.[12] Among the microorganisms, fungi may play a pivotal role within the failed endodontic treatment. The most important fungi belong to genus *Candida* with *C. albicans* being the foremost predominant in persons who are both healthy and medically challenged.

C. albicans was chosen due to its capacity to enter into the dentinal tubules and populate in the root canal. In the present study, the specimen was infected with *C. albicans* for 21 days considering this microorganism invade into the dentinal tubules after 21 days of incubation, with the expectation that this would be sufficient for the formation of a structured biofilm, assuming that the decontamination methods could be properly verified, better simulating the clinical scenario.[13] In this investigation,

microbiological examination was carried out by counting CFUs since it provides for accurate bacterial quantification from the root canals.

The transport media, the culture conditions, and the culturing media selection are all important aspects to consider while doing a microbiological study. The bacteriological sample in this investigation was done with a sterile paper tip that absorbed the root canal contents. After that, the paper points are transferred to tubes containing saline solution, which are subsequently plated on SDA agar plates. The use of paper points has the advantage of limiting microbiological sampling, since only bacteria found within the root canals may be sampled with them.[11] Because of the lack of osmolarity, which would break the bacterial cellular wall, sterile saline solution was employed to collect this transport medium.[14]

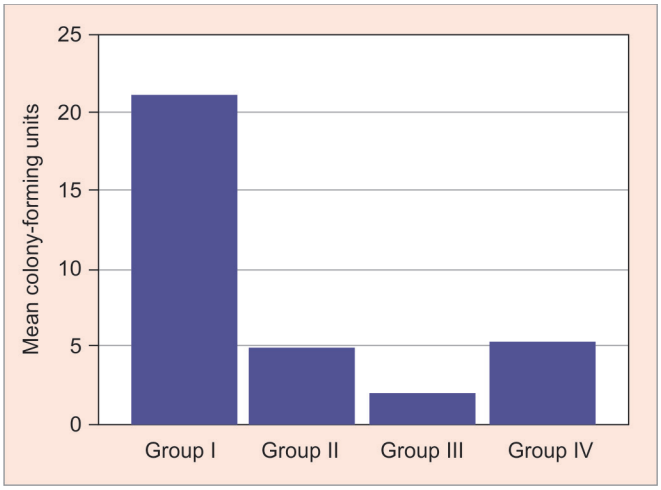


Fig. 4: Mean of CFUs

Table 2: One-way ANOVA for comparison among groups

	Sum of squares	*Df*	*Mean square*	*F*[#]	*p*
Between groups	3365.482	3	1121.827	3580.526	0.000[*]
Within groups	17.546	56	0.313		
Total	3383.028	59			

[*]Statistically significant; [#]One-way ANOVA test

C. albicans is a dentinophilic microorganism that needs moderate temperatures to thrive and reproduce. As a result, a bacteriological incubator with a continuous temperature of 37°C was utilized to provide appropriate development conditions for the growth of this microorganism.[15]

In endodontic therapy, a variety of irrigation solutions are used to help in cleansing the root canal system. Sodium hypochlorite is now the most widely used irrigant.[16] It contains an antibacterial agent with a broad spectrum of activity against bacteria, bacteriophages, spores, yeasts, and viruses. *C. albicans*, *Enterococcus faecalis*, and *Bacillus* species were shown to be completely killed by 5.25% NaOCl.[17] The capacity of NaOCl to oxidize and hydrolyze cell proteins, as well as osmotically pull fluids out of cells due to its hypertonicity, contributes to its antibacterial effectiveness.[18] The pH of NaOCl lies between 11 and 12. When hypochlorite reacts with tissue proteins, formaldehyde, nitrogen, and acetaldehyde are produced in a short amount of time, and peptide bonds are disrupted, resulting in protein disintegration.[19] The hydrogen within the amino groups is changed by chlorine throughout the process, resulting in chloramine, which is important for antibacterial efficacy.[17]

NaOCl, on the contrary, is cytotoxic, chemically unstable, and interferes with restorative material adherence to the dentinal surface. Furthermore, NaOCl causes considerable changes in the dentinal structure as well as a loss in dentin's mechanical characteristics.[8] As a result, the use of NaOCl solutions may reduce the success of endodontic treatment.

Fidalgo et al. revealed that 2.5 and 5.25% of NaOCl were more efficient against *C. albicans* than 17% EDTA.[20] Valera et al. demonstrated a high antifungal activity of NaOCl in *C. albicans*.[21]

Calcium hypochlorite is a chemical substance that is commonly used during industrial disinfection and purification of water.[16]

Calcium hypochlorite is accessible in granules, and when dissolved in water, it releases two molecules of hypochlorous acid. As a result, as compared to NaOCl, where just one molecule of hypochlorous acid is produced, a greater quantity of chlorine is released.[7]

However, the current study's findings revealed no significant differences in depletion of *C. albicans* between groups that used NaOCl and $Ca(OCl)_2$ solutions. These results are in consistent with the results of the study by Almeida et al. $Ca(OCl)_2$ has a greater

Table 3: Tukey's post hoc test for multiple comparisons among groups

					95% confidence interval	
I	*J*	*Mean difference (I–J)*	*Std. error*	*p*	*Lower bound*	*Upper bound*
Group I	Group II	16.23800[*]	0.20439	0.000[*]	15.6968	16.7792
	Group III	19.07267[*]	0.20439	0.000[*]	18.5315	19.6139
	Group IV	15.85933[*]	0.20439	0.000[*]	15.3181	16.4005
Group II	Group II	−16.23800[*]	0.20439	0.000[*]	−16.7792	−15.6968
	Group III	2.83467[*]	0.20439	0.000[*]	2.2935	3.3759
	Group IV	−0.37867	0.20439	0.260	−0.9199	0.1625
Group III	Group II	−19.07267[*]	0.20439	0.000[*]	−19.6139	−18.5315
	Group III	−2.83467[*]	0.20439	0.000[*]	−3.3759	−2.2935
	Group IV	−3.21333[*]	0.20439	0.000[*]	−3.7545	−2.6721
Group IV	Group II	−15.85933[*]	0.20439	0.000[*]	−16.4005	−15.3181
	Group III	0.37867	0.20439	0.260	−0.1625	0.9199
	Group IV	3.21333[*]	0.20439	0.000[*]	2.6721	3.7545

[*]Statistically significant

surface tension, which might influence the entry of substances into root canal walls. Gomez et al. reported that both NaOCl and $Ca(OCl)_2$ have similar antimicrobial efficacy with no statistically significant differences between them.[22] Dal Bello et al. conducted a study comparing NaOCl and $Ca(OCl)_2$ and revealed that both were equally effective.[7] Reddy et al. showed that NaOCl reduced the mechanical properties of root dentin compared to $Ca(OCl)_2$. Paula et al. showed that better antimicrobial activity is seen in $Ca(OCl)_2$ compared to NaOCl.[23]

Biopure MTAD is another root canal irrigant with antibacterial properties (Dentsply, Tulsa, Oklahoma). It is a combination of a tetracycline isomer (doxycycline), an acid (citric acid), and a detergent (Tween 80).[13] Doxycycline's antimicrobial characteristic is its capacity to eliminate organic and inorganic contaminants from root surfaces, which is enhanced by the presence of citric acid and a detergent (Tween 80) that improves its ability to penetrate into the root canal and dentinal tubules. Detergents have been found to increase the antibacterial effects of medications in the root canal by lowering its surface tension.[24] Because of its antibacterial characteristics and capacity to remove the smear layer, Biopure MTAD has been recommended as a final rinse irrigant.[8] Biopure MTAD is less toxic than NaOCl at a concentration of 5.25%. Under standard *in vitro* microbiological techniques, MTAD was contrasted with NaOCl as well as EDTA for its antimicrobial efficacy and concluded that MTAD was proved to be more effective.

However, a number of researchers have lately challenged these findings. When compared to MTAD, Ruff et al. found that 6% NaOCl and 2% chlorhexidine had a superior antifungal impact.[25] Giardino et al. examined the effectiveness of 5.25% NaOCl and MTAD and found that at all times 5.25% NaOCl can disaggregate and eliminate the biofilm.[26]

Asna Ashari et al. found that NaOCl was considered to be more efficient against *C. albicans* compared to MTAD,[27] this is in line with the results of the current study. On the contrary, Mattigatti et al. revealed that MTAD was more effective against *C. albicans* compared to 2% NaOCl.[28]

This study has reported that a significantly reduced number of microbiological colonies were observed on the experimental plates in group III ($CaOCl_2$) when compared with group I (Control), group II (NaoCl), and group IV (MTAD) at 21 days.

Conclusion

Within the limitations of the study, all four groups were found to exhibit antimicrobial action. But the $Ca(OCl)_2$ was significantly better in its antimicrobial efficacy against *C. albicans* over the period of 21 days. More studies are required to validate the findings of this approach.

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