ANTIMICROBIAL POTENTIAL OF CALCIUM HYPOCHLORITE AND CHLORAMINE-T AS ALTERNATIVE ENDODONTIC IRRIGANTS: AN IN VITRO COMPARATIVE STUDY

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Abstract

Background: Effective endodontic therapy can only be achieved by thorough disinfection of the root canal system by removing microorganisms. Some microorganisms, such as Enterococcus faecalis and Staphylococcus aureus, which are not susceptible to many antimicrobial agents, are known to cause endodontic therapy failure. Sodium hypochlorite has been recommended as the gold-standard irrigant due to its potent antimicrobial and tissue-dissolution capabilities. Its cytotoxicity, on the other hand, prompted searches for alternative antimicrobial agents.

Aim: To determine the antimicrobial effect of calcium hypochlorite, chloramine-T, and Septilin compared to sodium hypochlorite on *E. faecalis* and *S. aureus* strains.

Materials and Methods: *E. faecalis* (ATCC 8043) and *S. aureus* (ATCC 6538) strains were cultivated in Brain Heart Infusion broth at 37°C for 24 hours. Lawn cultures were plated onto Mueller-Hinton agar plates. Wells with 5 mm in diameter and 2 mm deep were made and filled with sodium hypochlorite, calcium hypochlorite, chloramine-T, and Septilin. Zone of inhibition values were measured in millimeters after 24 hours of incubation. One-way ANOVA test followed by Bonferroni post hoc test was conducted, and $p < 0.05$ were taken as statistically significant.

Results: Sodium hypochlorite was the most effective agent against both *E. faecalis* and *S. aureus*. Calcium hypochlorite displayed high antibacterial activity, with comparable results with sodium hypochlorite. Moderate antimicrobial effect was found in chloramine-T. Lowest results were shown by Septilin.

Conclusion: All tested irrigating solutions exhibited antibacterial activity against the selected endodontic microorganisms. Sodium hypochlorite remained the most efficacious irrigating solution. Calcium hypochlorite and chloramine-T have shown great potential as possible alternatives for endodontic irrigants.

Key words : Enterococcus faecalis, Staphylococcus aureus, Root Canal Irrigants, Sodium Hypochlorite, Calcium Hypochlorite, Chloramine-T, Endodontics, Antimicrobial Activity.

Introduction

The success of endodontic therapy depends on the cleansing and disinfection of the root canal system. However, mechanical instrumentation is insufficient to eradicate microorganisms from complex structures like lateral canals, fins, isthmuses, and dentinal tubules. Therefore, irrigation is crucial in minimizing the bacterial count, dissolving necrotic tissue, and enhancing the result of root canal treatment [1].

Enterococcus faecalis is one of the organisms that cause recurrent endodontic infection. The organism possesses characteristics such as being able to survive in unfavorable environments, invasion of deep dentinal tubules, biofilm formation, and resistance to intracanal medicament. *Staphylococcus aureus* has also been identified in cases of failure and retreatment infection. The survival of these microorganisms is seen as an essential cause of endodontic treatment failure [2].

Sodium hypochlorite (NaOCl) remains the most popular irrigant for root canals due to its potent antimicrobial and tissue-dissolution properties. Even with its benefits, NaOCl exhibits some demerits, such as cytotoxicity, bitter taste and smell, irritation to the periapical tissues, and changes in dentin composition at higher concentrations [3]. These drawbacks have seen modern-day endodontics explore other options as potential irrigants capable of offering potent antimicrobial actions while being biocompatible [4].

Calcium hypochlorite has become a focus of recent research as a potential substitute for sodium hypochlorite, owing to its high available chlorine content and excellent antibacterial activity. Researches have shown that it has an antimicrobial capability similar to that of sodium hypochlorite, but with comparatively lower cytotoxicity to dentinal collagen [5]. Another compound that releases chlorine ions, chloramine-T, is considered for its oxidizing and disinfection ability. The slow release of active chlorine ions may result in sustained antimicrobial capability; nevertheless, there is insufficient data concerning its efficacy against resistant microorganisms in endodontics [6]. Herbal extracts such as Septilin, owing to their antimicrobial, anti-inflammatory, and immunomodulatory effects, are also under consideration, even though there is no scientific evidence supporting their use as endodontic irrigants [7].

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Despite several types of irrigants having been tested for effectiveness in endodontics, few comparative studies have been performed on calcium hypochlorite, chloramine-T, and herbal preparations, especially against resistant bacteria like E. faecalis and S. aureus. The significance of this research is that resistant bacteria found inside the canals affect the success of the treatment.

Consequently, an in vitro experiment was performed in order to compare the antimicrobial effects of sodium hypochlorite, calcium hypochlorite, chloramine-T, and Septilin against E. faecalis and S. aureus bacteria through the agar diffusion test.

Materials and Methods

Study Design

This is an in vitro experimental study that aimed to comparatively test the efficacy of the

antimicrobial effect of sodium hypochlorite, calcium hypochlorite, chloramine-T, and Septilin on E. faecalis and S. aureus.

Microbial Strains and Growth Conditions

The following microbial strains have been utilized in this study: Enterococcus faecalis (ATCC 8043) and Staphylococcus aureus (ATCC 6538). Bacterial strains have been grown in sterilized Brain Heart Infusion (BHI) broth for 24 hours at 37°C under aerobic conditions. Following incubation, the turbidity of the bacterial suspensions was standardized to achieve concentrations equivalent to 8.6×10 CFU/mL for E. faecalis and 5.9×10 CFU/mL for S. aureus. Standardization was performed to ensure uniform microbial density during antimicrobial assessment.

Preparation of Agar Plates

Antibacterial efficacy testing was performed using the Agar Well Diffusion Method. Sterile Agar Mueller Hinton plates were prepared following the manufacturer's recommendations. The lawn culture was developed by streaking 100 μL of the bacterial standardization broth onto the agar plates with sterile cotton swabs. A total of 20 agar plates per organism were prepared. All microbiological experiments were conducted in a sterile laboratory environment.

Groups Used in Experiment

The following are the groups of experimental irrigating solutions used during the experiment:
Group 1: Sodium Hypochlorite
Group 2: Calcium Hypochlorite
Group 3: Chloramine-T
Group 4: Septilin

Antimicrobial Assessment

With the help of a sterile punch, wells having diameters of 5 mm and depths of 2 mm were created in the inoculated agar plates. The equivalent amounts of different irrigating agents were then introduced into the wells aseptically. Afterwards, the plates were incubated at 37°C for 24 hours. After this period, the effectiveness of each irrigant in terms of antimicrobial properties was evaluated in terms of measuring the zone of inhibition around each well using a measuring scale.

Antimicrobial Activity Against Staphylococcus aureus

Similar results were seen for S. aureus. Sodium hypochlorite proved to be the most effective against microbes, with calcium hypochlorite second. Chloramine-T showed some degree of inhibition, whereas Septilin proved to be the least effective against microbes compared to other irrigating solutions tested (Table 2).

One-way ANOVA analysis revealed a significant difference between groups concerning their zones of inhibition against S. aureus ($p < 0.05$). Post-hoc analysis by Bonferroni test showed that sodium hypochlorite and Septilin, calcium hypochlorite and Septilin, and chloramine-T and Septilin had significant differences. Despite calcium hypochlorite showing slight lower antimicrobial efficacy than sodium hypochlorite, it still remains a potential endodontic irrigation solution.

Comparative Evaluation of Experimental Irrigants

Sodium hypochlorite was the most effective among the studied microorganisms. Calcium hypochlorite exhibited significant antibacterial activity and showed effects similar to those of sodium hypochlorite. Chloramine-T also affected both microorganisms but proved less effective than the hypochlorites used as disinfecting solutions. Septilin exhibited antibacterial activity but produced the smallest inhibitory zones. There were no cases of unexpected microbial

contamination or unidentifiable inhibition zones detected.

Discussion

In the current study, the antimicrobial effectiveness of sodium hypochlorite, calcium hypochlorite, chloramine-T, and Septilin against E. faecalis and S. aureus was assessed. Out of all the irrigation solutions, the maximum antimicrobial action was shown by sodium hypochlorite against the bacteria. The antibacterial action of calcium hypochlorite was found to be similar to sodium hypochlorite while chloramine-T showed moderate action. However, Septilin displayed minimum antimicrobial action among all other solutions.

The outcome of root canal therapy is dependent on the effective eradication of microorganisms within the root canal system. Although mechanical cleaning alone is insufficient in disinfection due to the anatomical complexity of the canal system including the dentinal tubules, fins, and lateral canals [8], persistent microorganisms are common in failed endodontics cases, mainly attributed to their ability to withstand poor nutrition and deep penetration within the dentinal tubules [9]. Another microorganism implicated in persistent intracanal infection is S. aureus [10].

The high antimicrobial efficiency of sodium hypochlorite in the current research can be explained by its powerful oxidative property and the formation of hypochlorous acid, which damages enzyme function, causes membrane destruction, and leads to protein denaturation [11]. Moreover, sodium hypochlorite has a tissue-lysing effect, which is crucial for the debridement process [12]. These results corroborate other existing research findings that indicate the effectiveness of sodium hypochlorite against antibiotic-resistant endodontic bacteria [4,13].

It was seen that calcium hypochlorite had an activity very much similar to that of sodium hypochlorite. It is due to its high chlorine content and the continuous generation of hypochlorous acid upon dissociation [5]. Earlier, studies have suggested that calcium hypochlorite could efficiently reduce the bacterial load but does not cause any significant modification of dentin collagen [14]. These results corroborate those which have revealed that calcium hypochlorite could be considered a potential alternative to the existing endodontic irrigants [15].

Another significant result of this experiment is the similar effect of calcium hypochlorite on E. faecalis and S. aureus. From a clinical perspective, this result may be important since sodium hypochlorite causes cytotoxic effects, a bitter taste, irritation of soft tissues, and extrusion accidents [16]. Consequently, the search for new irrigants that demonstrate acceptable levels of bactericidal activity and increased biocompatibility remains relevant.

Chloramine-T had moderate antimicrobial properties against both microorganisms. The antibacterial effect of Chloramine-T is primarily attributed to the slow release of chlorine, leading to sustained oxidative damage in bacteria [17]. However, the effectiveness of the disinfectant Chloramine-T obtained in the current study was relatively weaker compared to those of NaOCl and Ca(OCl)2. This can be attributed to the slow release rate of chlorine and inability to penetrate bacterial colonies and dentinal tubules. Past investigations involving chlorine-based disinfectants have also found comparable results [6,18].

Among all the irrigants used, Septilin showed the lowest levels of antimicrobial action. Despite showing clear inhibition zones, the action of the irrigant was considerably weaker when compared

to other traditional hypochlorite irrigation solutions. Herbs contain natural substances that have antimicrobial and anti-inflammatory effects, but there is an uneven response in the treatment of antibiotic-resistant bacteria in root canals with these irrigants [19]. One of the reasons for the reduced efficiency of the irrigant in the current experiment could be insufficient diffusion in the agar medium.

Agar well diffusion test was employed in the current investigation due to its simplicity, repeatability, and widespread acceptance in initial antimicrobial testing. This test, however, suffers from certain shortcomings. For instance, the diffusion properties of the irrigants through agar could affect the size of the inhibition zone and would not reflect their antimicrobial effects in vivo [20]. Moreover, the current investigation used planktonic bacterial cultures rather than mature multispecies biofilms, which exhibit higher resistance.

The current investigation also has certain drawbacks in terms of methodology. Evaluation of cytotoxicity, dentin penetration ability, smear layer elimination ability, and tissue solubilization ability was not conducted. Moreover, antimicrobial action was tested under laboratory settings, which cannot completely mirror clinical conditions.

Nevertheless, the study provides useful preliminary data on the potential antimicrobial effects of calcium hypochlorite and chloramine-T solutions as alternatives to conventional endodontic irrigating solutions. In future studies, a multi-species biofilm model, tests using dentin blocks, and the employment of sophisticated imaging tools like confocal laser scanning microscopy and scanning electron microscopy need to be considered. It will be necessary to conduct further studies to assess cytotoxicity, substantivity, smear layer removal efficacy, and overall clinical performance before making recommendations for the use of these irrigants clinically.

Table 1. Mean Zone of Inhibition (mm) Against Enterococcus faecalis

Group	Irrigant	Mean ± SD (mm)	95% CI	p-value
Group 1	Sodium hypochlorite	24.85 ± 1.21	24.01–25.69	<0.001*
Group 2	Calcium hypochlorite	23.74 ± 1.34	22.81–24.67	<0.001*
Group 3	Chloramine-T	18.92 ± 1.18	18.10–19.74	<0.001*
Group 4	Septilin	12.41 ± 0.96	11.74–13.08	<0.001*

One-way ANOVA followed by Bonferroni post hoc test; statistically significant at $p < 0.05$.

Table 2. Mean Zone of Inhibition (mm) Against Staphylococcus aureus

Group	Irrigant	Mean ± SD (mm)	95% CI	p-value
Group 1	Sodium hypochlorite	25.16 ± 1.15	24.36–25.96	<0.001*
Group 2	Calcium hypochlorite	24.08 ± 1.27	23.19–24.97	<0.001*
Group 3	Chloramine-T	19.36 ± 1.09	18.60–20.12	<0.001*
Group 4	Septilin	13.05 ± 0.88	12.44–13.66	<0.001*

One-way ANOVA followed by Bonferroni post hoc test; statistically significant at $p < 0.05$.

Table 3. Intergroup Comparison of Antimicrobial Activity Against E. faecalis (Bonferroni Post Hoc Test)

Comparison	Mean Difference (mm)	p-value
Sodium hypochlorite vs Calcium hypochlorite	1.11	0.084
Sodium hypochlorite vs Chloramine-T	5.93	<0.001*
Sodium hypochlorite vs Septilin	12.44	<0.001*
Calcium hypochlorite vs Chloramine-T	4.82	<0.001*
Calcium hypochlorite vs Septilin	11.33	<0.001*
Chloramine-T vs Septilin	6.51	<0.001*

Statistically significant at $p < 0.05$.

Table 4. Intergroup Comparison of Antimicrobial Activity Against S. aureus (Bonferroni Post Hoc Test)

Comparison	Mean Difference (mm)	p-value
Sodium hypochlorite vs Calcium hypochlorite	1.08	0.092
Sodium hypochlorite vs Chloramine-T	5.80	<0.001*
Sodium hypochlorite vs Septilin	12.11	<0.001*
Calcium hypochlorite vs Chloramine-T	4.72	<0.001*
Calcium hypochlorite vs Septilin	11.03	<0.001*
Chloramine-T vs Septilin	6.31	<0.001*

Statistically significant at $p < 0.05$.

Table 5. One-Way ANOVA Analysis of Antimicrobial Activity

Microorganism	F-value	p-value	Partial Eta Squared (η^2)
Enterococcus faecalis	112.84	<0.001*	0.87
Staphylococcus aureus	118.26	<0.001*	0.89

Statistically significant at $p < 0.05$.

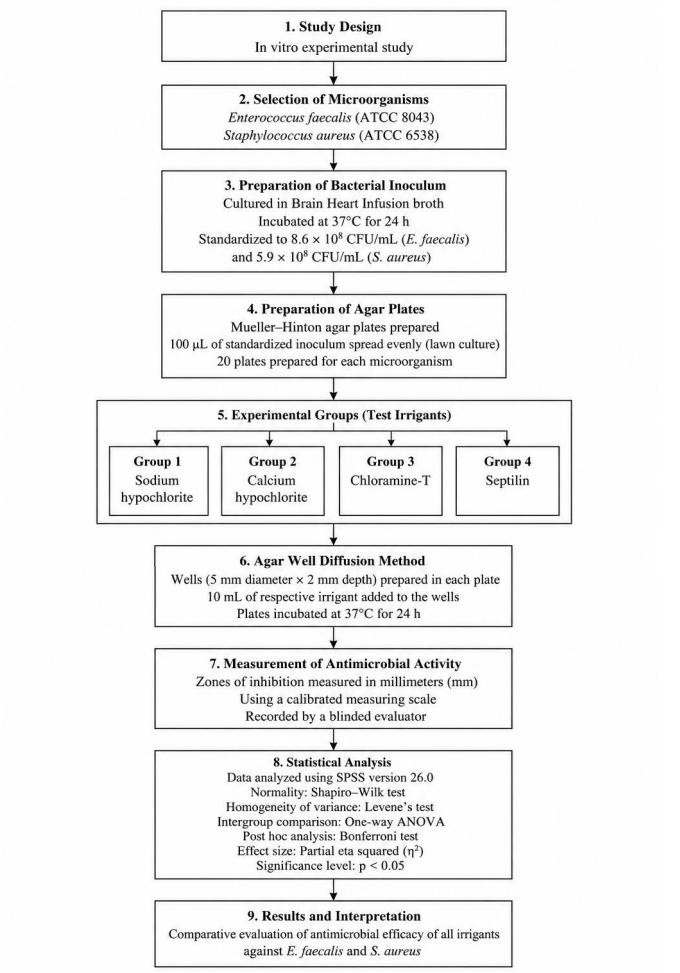


Figure 1: Flowchart illustrating the study design, experimental methodology, antimicrobial assessment, and statistical analysis of sodium hypochlorite, calcium hypochlorite, chloramine-T, and Septilin against Enterococcus faecalis and Staphylococcus aureus.

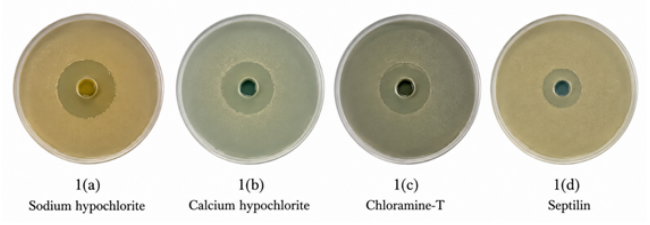


Figure 2: Representative agar well diffusion assay demonstrating the zones of inhibition produced by 1(a) sodium hypochlorite, 1(b) calcium hypochlorite, 1(c) chloramine-T, and 1(d) Septilin against *Enterococcus faecalis* after 24 hours of incubation.

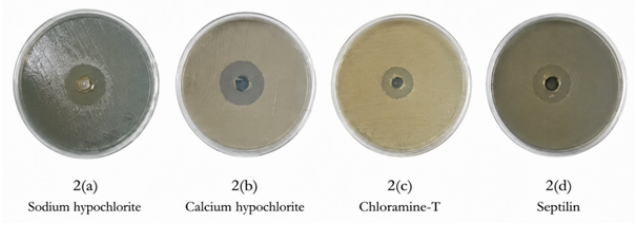


Figure 3: Representative agar well diffusion assay showing the antimicrobial activity of 2(a) sodium hypochlorite, 2(b) calcium hypochlorite, 2(c) chloramine-T, and 2(d) Septilin against Staphylococcus aureus following 24 hours of incubation

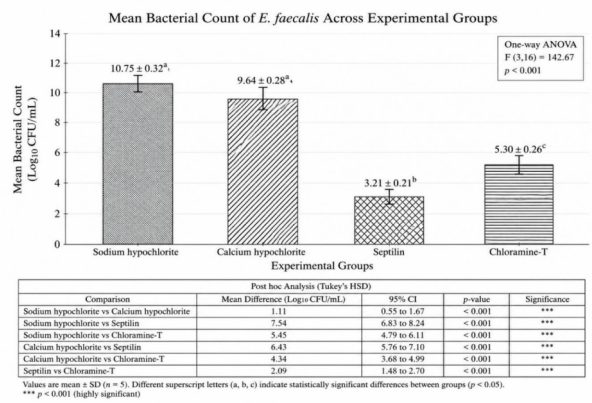
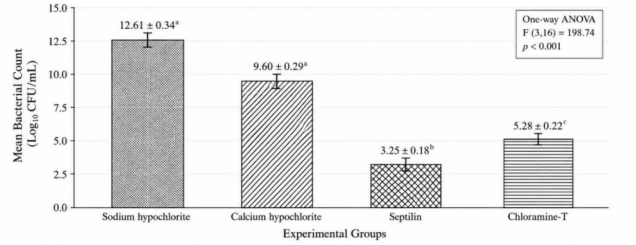


Figure 4: Comparative analysis of mean bacterial counts of Enterococcus faecalis following exposure to sodium hypochlorite, calcium hypochlorite, chloramine-T, and Septilin, demonstrating statistically significant intergroup differences in antimicrobial efficacy.



Post hoc Analysis (Tukey's HSD)				
Comparison	Mean Difference (Log_{10} CFU/mL)	95% CI	*p*-value	Significance
Sodium hypochlorite vs Calcium hypochlorite	3.01	2.35 to 3.66	< 0.001	***
Sodium hypochlorite vs Septilin	9.36	8.59 to 10.13	< 0.001	***
Sodium hypochlorite vs Chloramine-T	7.33	6.59 to 8.06	< 0.001	***
Calcium hypochlorite vs Septilin	6.35	5.67 to 7.02	< 0.001	***
Calcium hypochlorite vs Chloramine-T	4.32	3.64 to 4.99	< 0.001	***
Septilin vs Chloramine-T	2.03	1.30 to 2.77	< 0.001	***

Values are mean ± SD ($n = 5$). Different superscript letters (a, b, c) indicate statistically significant differences between groups ($p < 0.05$).
*** $p < 0.001$ (highly significant)

Figure 5: Comparative evaluation of mean bacterial counts of Staphylococcus aureus following treatment with sodium hypochlorite, calcium hypochlorite, chloramine-T, and Septilin, demonstrating statistically significant differences in antimicrobial efficacy among the experimental groups.

Conclusion

In the current study, all tested irrigants exhibited some degree of antibacterial activity against *E. faecalis* and *S. aureus*. Sodium hypochlorite was found to be the most effective at killing bacteria, thereby establishing its position as the gold standard for endodontic irrigation solutions. The second most efficient agent was calcium hypochlorite, followed by chloramine-T. On the other hand, Septilin showed the lowest antimicrobial activity. The results of this study provide evidence that calcium hypochlorite can be used as an alternative irrigating solution due to its high antimicrobial efficacy against resistant endodontic microorganisms. Nevertheless, there were certain limitations in the current study. For example, the test was conducted in vitro and used the agar diffusion technique. In addition, aspects like biofilm formation, dentin infiltration, cytotoxicity, and tissue dissolution were not investigated. Thus, the findings obtained cannot be generalized to real-life situations. Further research must involve multispecies biofilm models, dentin block tests, cytotoxicity measurements, and microscopic examination to assess the clinical relevance of the studied irrigation solutions. Moreover, long-term in vivo and clinical randomized trials must be carried out to prove their efficiency and safety.

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