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A COMPARATIVE EVALUATION OF THE ANTIBACTERIAL EFFECTIVENESS OF VARIOUS SURFACTANTS ADDED TO ENDODONTIC IRRIGANTS – AN IN VITRO STUDY

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ABSTRACT
Aim: To evaluate and compare the antibacterial effectiveness of various surfactants added to routine endodontic irrigants against *Enterococcus faecalis*.
Materials and Methods: An in-vitro experimental study was conducted to evaluate the antimicrobial efficacy of sodium hypochlorite alone and in combination with surfactants such as cetrimide and Triton X-100. Antibacterial activity was assessed using the well-diffusion method against *Enterococcus faecalis*. Zones of inhibition were measured after 24 hours of incubation at 37°C. Statistical analysis was performed using one-way ANOVA and Mann–Whitney U test.
Results: All experimental irrigants demonstrated antibacterial activity. Surfactant-modified irrigants showed enhanced antibacterial effectiveness compared to sodium hypochlorite alone. Among the surfactants, cetrimide showed slightly superior antibacterial activity compared to Triton X-100; however, the difference was not statistically significant.
Conclusion: The incorporation of surfactants such as cetrimide and Triton X-100 enhances the antibacterial effectiveness of sodium hypochlorite, indicating a synergistic interaction that may improve root canal disinfection.

KEYWORDS: Sodium hypochlorite, Surfactants, Cetrimide, Triton X-100, *Enterococcus faecalis*, Endodontic irrigants.

INTRODUCTION
The success of endodontic treatment primarily depends on effective cleaning, shaping, and obturation of the root canal system. Among these, disinfection of the root canal system plays a pivotal role, as persistent microorganisms are the main cause of endodontic failure. The complex anatomy of the root canal system, including lateral canals, apical deltas, fins, and isthmuses, limits the effectiveness of mechanical instrumentation alone.
Microorganisms within the root canal system form organized biofilms that protect them from host defense mechanisms and antimicrobial agents. *Enterococcus faecalis* is one of the most frequently isolated microorganisms from failed root canal treatments and persistent apical periodontitis. Its ability to survive under harsh conditions, penetrate dentinal tubules, form biofilms, and resist intracanal medicaments makes it a significant endodontic pathogen.[1]
Chemical irrigation is essential to supplement mechanical preparation. Sodium hypochlorite (NaOCl) is the most commonly used irrigant due to its potent antimicrobial activity and ability to dissolve organic tissue. Despite these advantages, NaOCl exhibits high surface tension, which limits its penetration into dentinal tubules and irregularities of the root canal system.[2]
Surfactants are surface-active agents capable of reducing surface tension and improving the wettability of solutions. The addition of surfactants to endodontic irrigants has been proposed to enhance penetration, increase contact with microorganisms, and improve antimicrobial efficacy. Cetrimide, a cationic surfactant, possesses inherent antibacterial properties, while Triton X-100 is a non-ionic surfactant that enhances membrane permeability and irrigant penetration[3].
The present study was undertaken to evaluate and compare the antibacterial effectiveness of sodium hypochlorite combined with surfactants such as cetrimide and Triton X-100 against *Enterococcus faecalis* using an in-vitro model.

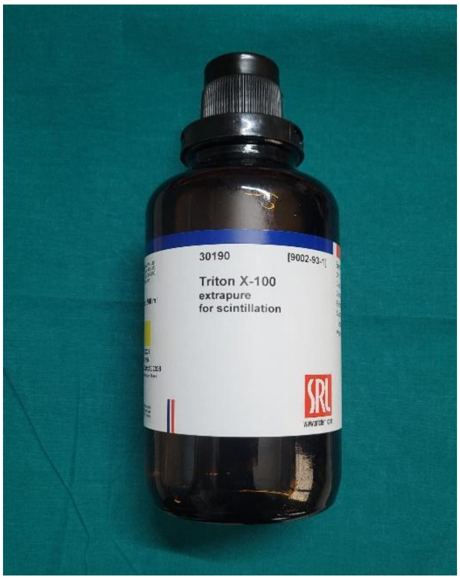
MATERIALS AND METHODS

The present study was designed as an in-vitro experimental laboratory-based investigation to evaluate the antibacterial effectiveness of surfactant-modified endodontic irrigants. A standard strain of *Enterococcus faecalis* (ATCC) was selected as the test microorganism, as it is frequently associated with persistent endodontic infections and post-treatment disease due to its resistance to adverse environmental conditions and intracanal medicaments.[1,2]

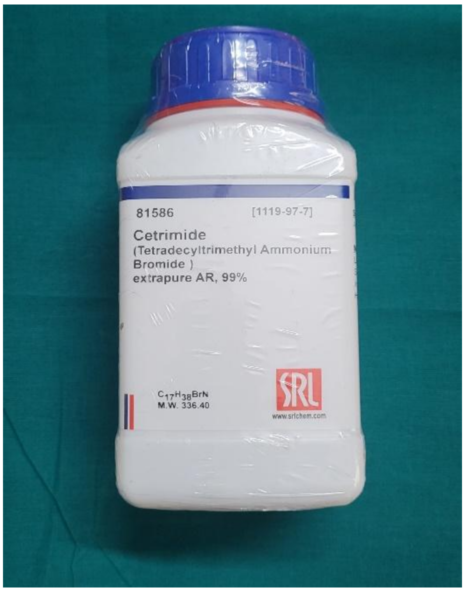
The armamentarium and materials used in the study included sodium hypochlorite, cetrimide, Triton X-100, Mueller–Hinton agar plates, sterile micropipettes, an incubator, and a vernier caliper (Figure 1). The samples were randomly divided into four experimental groups based on the irrigant used. Group I served as the control group and received sodium hypochlorite alone. Group II consisted of sodium hypochlorite combined with Triton X-100, Group III received sodium hypochlorite combined with cetrimide , and Group IV served as the negative control.

GROUP- 1

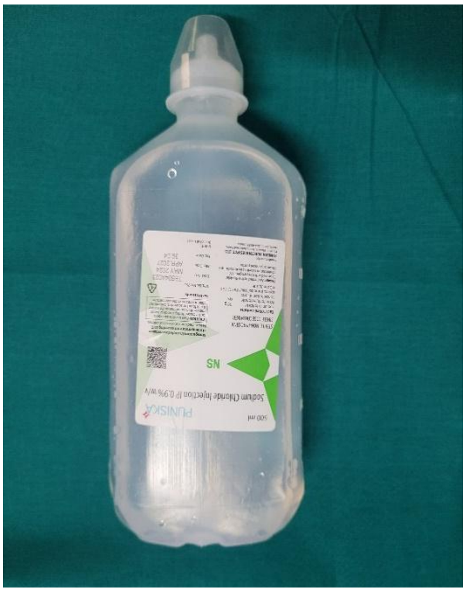
GROUP- 2



GROUP- 3

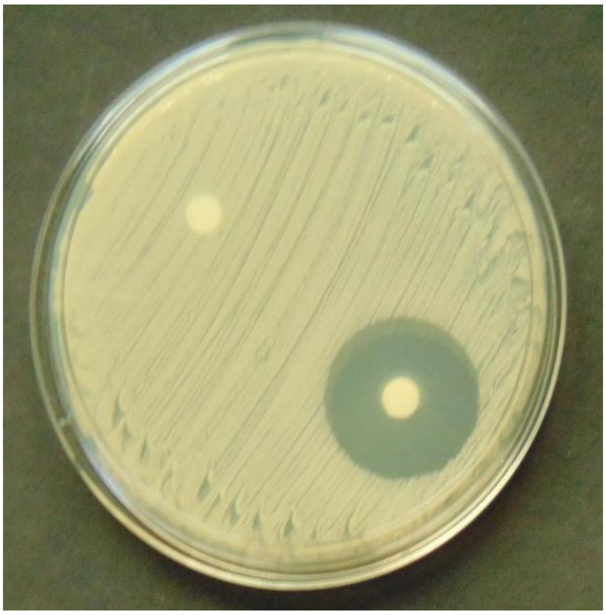


GROUP- 4

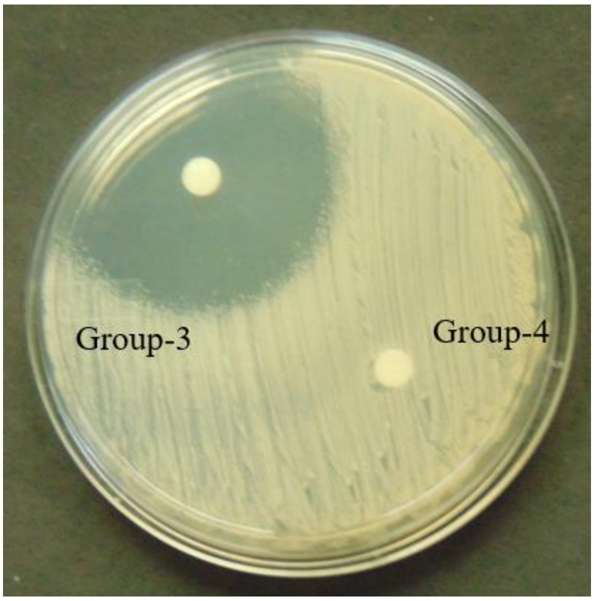


The antibacterial activity of the irrigant solutions was evaluated using the well-diffusion method, which is a widely accepted technique for assessing antimicrobial efficacy of endodontic irrigants.[3] The bacterial suspension of *E. faecalis* was prepared and standardized to a 0.5 McFarland standard, corresponding to approximately 1×10^8 CFU/mL.[4] The standardized inoculum was uniformly spread on Mueller–Hinton agar plates to obtain a confluent lawn of bacterial growth. Wells of 6 mm diameter were prepared on the agar surface using a sterile punch, and the respective test solutions were carefully dispensed into each well.[3,5]

The inoculated plates were incubated at 37°C for 24 hours under aerobic conditions. After incubation, the antibacterial efficacy of the irrigants was assessed by measuring the zones of inhibition around each well in millimeters using a vernier caliper. The presence and size of the clear zones around the wells indicated the antibacterial activity of the tested solutions (Figure 2).[6] The zone of inhibition, expressed in millimeters, was considered the primary outcome measure of the study.



Group-1



Group- 2

STATISTICAL ANALYSIS

The antibacterial efficacy of the experimental irrigants was assessed by determining the number of colony-forming units (CFU) per milliliter of culture. The obtained data were subjected to statistical analysis using one-way analysis of variance (ANOVA) to evaluate intergroup differences. Pairwise comparisons between groups were performed using the Mann–Whitney U-test.

The antibacterial effect of each test irrigant was expressed as the percentage reduction in viable bacterial count when compared with the negative control group. The percentage of bacterial kill was calculated using the following formula:

$$\text{Percentage of kill} = \left[1 - \left(\frac{\text{Mean CFU of test group}}{\text{Mean CFU of negative control group}}\right)\right] \times 100$$

A p-value of less than 0.05 was considered statistically significant.

RESULT

The mean CFU values were recorded for sodium hypochlorite (Group 0), sodium hypochlorite supplemented with cetrimide (Group 1), sodium hypochlorite supplemented with Triton X-100 (Group 2), and the negative control group (Group 4).

All experimental groups demonstrated antibacterial activity against *Enterococcus faecalis* when compared to the negative control group. Sodium hypochlorite exhibited a significant reduction in CFU counts. The surfactant-modified irrigants showed enhanced antibacterial efficacy compared to sodium hypochlorite alone.

Among the experimental groups, the cetrimide-modified irrigant (Group 1) demonstrated slightly higher antibacterial activity than the Triton X-100-modified irrigant (Group 2). However, intergroup comparison revealed that the difference between the surfactant-modified groups was not statistically significant ($p > 0.05$).

SAMPLE CODE	STRAIN	ZONE OF INHIBITION (MM)
Group-1	*E.faecalis*	42
Group-2		48
Group-3		54
Group-4		0

Overall, the results indicate that the addition of surfactants to sodium hypochlorite enhances its antibacterial effectiveness against *Enterococcus faecalis*, although no significant difference was observed between the surfactants tested.

DISCUSSION

Effective elimination of microorganisms from the root canal system is essential for long-term endodontic success. *Enterococcus faecalis* is a persistent pathogen frequently associated with endodontic failures due to its resistance to harsh environmental conditions and intracanal medicaments.

The results of the present study demonstrated that the addition of surfactants significantly enhanced the antibacterial effectiveness of sodium hypochlorite. This enhancement can be attributed to the reduction in surface tension, improved wettability, and increased penetration of the irrigant into microbial niches.

Cetrimide, a cationic surfactant, exerts its antibacterial effect by disrupting bacterial cell membranes, causing protein denaturation and leakage of intracellular contents. Several studies have reported superior antibacterial efficacy of cetrimide when used as an adjunct to conventional irrigants, which corroborates the findings of the present study.

Triton X-100, although lacking inherent antimicrobial activity, enhances membrane permeability and facilitates deeper penetration of sodium hypochlorite. Previous studies evaluating Triton X-100 as a surfactant additive have reported improved antibacterial outcomes, supporting the present observations.

Direct contact and diffusion-based assays are widely used for evaluating antimicrobial efficacy of irrigants. The well-diffusion method employed in the present study provides a reliable assessment of antibacterial activity under standardized conditions.

Despite the promising results, the present study has limitations. Being an in-vitro study, clinical variables such as dentin buffering capacity, smear layer presence, and complex canal anatomy could not be simulated. Further in-vivo and clinical studies are necessary to validate the results.

CONCLUSION

Within the limitations of this in-vitro study, it can be concluded that the addition of surfactants such as cetrimide and Triton X-100 enhances the antibacterial effectiveness of sodium hypochlorite against *Enterococcus faecalis*. Among the surfactants tested, cetrimide demonstrated superior antibacterial activity. Surfactant-modified irrigants may serve as effective adjuncts for improved root canal disinfection.

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