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Original Article

Dentistry Section

Antimicrobial Efficacy of Different Concentrations of Sodium Hypochlorite in the Elimination of *Enterococcus Faecalis*: An In-vitro Study

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ABSTRACT

Introduction: Primary teeth are equally important as permanent teeth. Endodontic therapy aids in the preservation and maintenance of extremely carious primary teeth. For root canal therapy to be successful, root canals must be properly prepared and effectively irrigated. Sodium Hypochlorite (NaOCl) has been the gold standard for root canal treatment of primary teeth. However, there are a few drawbacks to using higher concentrations of sodium hypochlorite, such as toxic reactions from its apical penetration. In addition, an excess of irrigation fluid via the apical region could harm the permanent tooth underneath. Therefore, it is crucial to select the proper concentration to preserve the delicate balance between effectiveness and safety.

Aim: The present in-vitro study was conducted in the city of Chennai, Tamil Nadu, between March and June 2022. The study aimed to evaluate the effectiveness of lower concentrations of sodium hypochlorite, such as 1%, 0.5%, and 0.25%, against Enterococcus faecalis.

Materials and Methods: The antibacterial activity of different concentrations of sodium hypochlorite (1%, 0.5%, and 0.25%) against Enterococcus faecalis was performed using Muller Hinton agar. Three Mueller Hinton agar plates were prepared and sterilised for 15 minutes for each concentration. The volumes used were 25 µL, 50 µL, and 100 µL, respectively. *E. faecalis* was swabbed and poured into the sterilised plates. The plates were incubated for 24 hours at 37°C. After incubation, the plates were examined, and their zone of Inhibition was determined. ANOVA test was applied with Post-hoc Tukey's analysis.

Results: One-way ANOVA test showed that there was a significant difference with a p-value <0.001 in all three groups. Post-hoc Tukey's analysis was done, and the test showed that the highest zone of inhibition was seen with 1% sodium hypochlorite at 100 µL, with zone diameter of 39 mm, and the least zone of inhibition was seen with 0.25% sodium hypochlorite solution at 25 µL, with zone diamter of 22 mm.

Conclusion: The results of the present study show that a 1% sodium hypochlorite solution can be used to successfully disinfect root canals, indicating its potential as an effective antimicrobial agent in endodontic procedures.

Keywords: Irrigation, Primary teeth, Sodium hypochlorite

INTRODUCTION

Primary teeth are equally important as permanent teeth in terms of jaw growth, arch length maintenance, mastication, and speech. Endodontic therapy aids in the preservation and maintenance of extremely carious primary teeth. Successful root canal therapy relies on the combination of correct instrumentation, irrigation, and obturation of the root canal [1]. Micro-organisms in the root canal are well known to cause pulp and periradicular infections. The purpose of root canal therapy is to eradicate bacteria from the root canal to establish a proper environment for tissue recovery [2,3].

For root canal therapy to be successful, root canals must be properly prepared and effectively filled. Chemico-mechanical preparation involves using endodontic instruments and suitable additional chemical solutions to remove both organic and inorganic components of the Smear Layer (SL). The purpose of irrigation is to enhance intracanal medication's resistance to micro-organisms and promote disinfection, enabling a sufficient seal with filled root material [4]. The bacterial species frequently found in root canals is *Enterococcus faecalis*. According to studies, *E. faecalis* may survive in a highly alkaline environment [2,3,5]. Therefore, it is necessary for the irrigating solution to have antibacterial properties, including the ability to destroy micro-organisms in the root canal system and disinfect parts of the canal that cannot be reached by mechanical instruments [6]. Due to anatomical variations in primary teeth, such as curved and tortuous root canals and their proximity to succedaneous tooth buds, irrigation plays a significant role in paediatric endodontics [7].

Though various irrigation solutions have been used for SL removal, NaOCl has been the gold standard for root canal treatment of primary teeth for many years due to its strong antibacterial action [8]. It has been reported that NaOCl has a strong antibacterial activity that can effectively promote bacterial inactivation even at a low concentration of 0.5%. Hence, it has been suggested that using 1% NaOCl is suitable in primary teeth [9,10].

There are a few drawbacks to using NaOCl, such as toxic reactions from its apical penetration, negative effects on dentin elasticity and flexural strength, a reduction in dentin microhardness, and the probable death of apical stem cells [11,12]. There is a greater risk in the case of primary teeth, as physiological apical resorption can cause direct communication through the apex, and the irrigant may reach beyond the apex. Additionally, an excess of irrigation fluid via the apical region could harm the permanent tooth underneath [13,14]. Therefore, it is crucial to select the proper concentration to preserve the delicate balance between effectiveness and safety.

However, there is a lack of studies reporting lower concentrations of sodium hypochlorite irrigants and their antimicrobial activity [10,12]. The present in-vitro study was conducted to evaluate the antimicrobial activity of lower concentrations of sodium hypochlorite, such as 1%, 0.5%, and 0.25%, against *Enterococcus faecalis*.

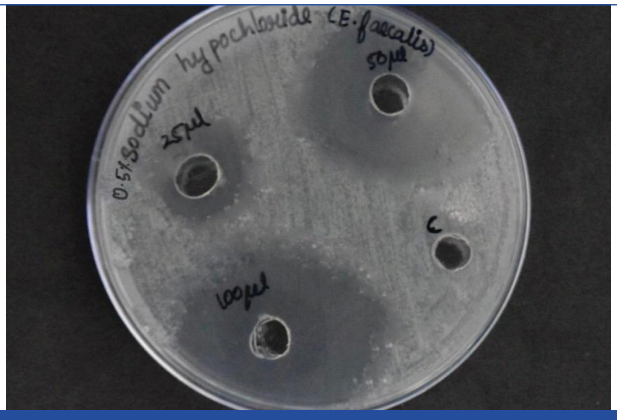
MATERIALS AND METHODS

It was an in-vitro study conducted in the city of Chennai, Tamil Nadu, between March and June 2022. The study was registered with the Saveetha Institute of Medical and Technical Sciences' Institutional Review Board in Chennai, Tamil Nadu, India. The SIMATS

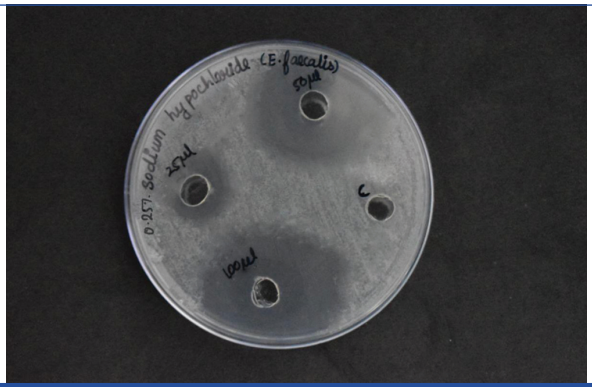
Institutional Review Board granted ethical approval (SRB/SDC/PEDO-2101/22/033).

Antimicrobial analysis: Various concentrations of NaOCl were prepared by diluting 1% sodium hypochlorite solution with an equal proportion of sterile water that did not contain any preservatives. The evaluation of antibacterial activities was conducted using the disc diffusion method. Mueller Hinton agar plates were prepared (n=3 for each concentration), sterilised for a duration of 15 minutes, and subsequently solidified. After solidification, wells measuring 9 mm in diameter were created using a sterile polystyrene tip. Bacterial cultures containing *Enterococcus faecalis* were swabbed onto these plates. Solutions of sodium hypochlorite with three different concentrations, namely 1%, 0.5%, and 0.25%, and saline (control) were loaded into four distinct wells on three agar plates. The volumes used were 25 µL, 50 µL, and 100 µL, respectively. Using different volumes provides a thorough understanding of the substance's antimicrobial properties. The plate was then incubated at a temperature of 37°C for 24 hours. After 24 hours, the zones of inhibition were measured [Table/Fig-1-3].

[Table/Fig-1]: Zone of inhibition seen with 1% sodium hypochloride solution.



[Table/Fig-2]: Zone of inhibition seen with 0.5% sodium hypochloride solution.



[Table/Fig-3]: Zone of inhibition seen with 0.25% sodium hypochloride solution.

STATISTICAL ANALYSIS

The mean and standard deviation were estimated from the sample. The mean values were analysed using one-way ANOVA. Pairwise comparison was done using Tukey's post-hoc analysis. In the present study, the level of significance was set at p≤0.05.

RESULTS

The highest zone of inhibition was observed with a 1% sodium hypochlorite solution at 100 µL, with a zone diameter of 39 mm. The least zone of inhibition was observed with a 0.25% sodium hypochlorite solution at 25 µL, with a zone diameter of 22 mm [Table/Fig-4]. One-way ANOVA test showed a significant difference in all three groups between various volumes (p<0.001) [Table/Fig-5].

Concentrations	Groups	Mean	Std. Deviation	N
1%	25 µL	32.3333	2.51661	3
	50 µL	37.6667	1.52753	3
	100 µL	39	1	3
	Control	9	1	3
	Total	29.5	12.71005	12
0.5%	25 µL	26	1	3
	50 µL	28	1	3
	100 µL	29.6667	1.52753	3
	Control	9.3333	1.52753	3
	Total	23.25	8.57189	12
0.25%	25 µL	22	1.73205	3
	50 µL	31.6667	2.08167	3
	100 µL	38	1	3
	Control	11	1	3
	Total	25.667	10.73934	12

[Table/Fig-4]: Zone of inhibition at various concentrations of sodium hypochlorite.

		Sum of squares	df	Mean square	F	p-value
1%	Between the groups	1755.667	3	585.222	219.458	<0.001
	Within groups	21.333	8	2.667		
	Total	1777	11			
0.5%	Between the groups	794.917	3	264.972	158.983	<0.001
	Within groups	13.333	8	1.667		
	Total	808.25	11			
0.25%	Between the groups	1250	3	416.667	178.571	<0.001
	Within groups	18.667	8	2.333		
	Total	1268.667	11			

[Table/Fig-5]: A one-way ANOVA test was done to determine the differences between and within the groups.

Tukey's post-hoc analysis was performed for pairwise comparison between different volumes. For the 1% sodium hypochlorite concentration, a significant difference was observed between 100 µL and 25 µL (p=0.005). For the 0.5% concentration, a significant difference was observed between 100 µL and 25 µL (p=0.034). However, for the 0.25% concentration, a significant difference was observed between all the groups (p<0.05) [Table/Fig-6].

Tukey's post-hoc analysis was also conducted between the three concentrations at different volumes. In all three volumes that were compared, 1% sodium hypochlorite showed a significantly greater zone of inhibition compared to the 0.5% and 0.25% concentrations (p-value <0.05) [Table/Fig-7].

DISCUSSION

The present study investigated the ability of various NaOCl concentrations to inhibit the growth of *E. faecalis*. The highest zone of inhibition was observed with a 1% sodium hypochlorite solution at 100 µL. Due to branching and the presence of many accessory canals, the root canal system of primary teeth is more challenging than that of permanent teeth. Mechanical instrumentation alone may not

Dependent variable	(I) Groups	(J) Groups	Mean difference (I-J)	Std. Error	p-value
1%	25 µL	50 µL	-5.33333*	1.33333	0.017
		100 µL	-6.66667*	1.33333	0.005
		Control	23.33333*	1.33333	<0.001
	50 µL	25 µL	5.33333*	1.33333	0.017
		100 µL	-1.33333	1.33333	0.754
		Control	28.66667*	1.33333	<0.001
	100 µL	25 µL	6.66667*	1.33333	0.005
		50 µL	1.33333	1.33333	0.754
		Control	30.00000*	1.33333	<0.001
	Control	25 µL	-23.33333*	1.33333	<0.001
		50 µL	-28.66667*	1.33333	<0.001
		100 µL	-30.00000*	1.33333	<0.001
0.5%	25 µL	50 µL	-2.00000	1.05409	0.301
		100 µL	-3.66667*	1.05409	0.034
		Control	16.66667*	1.05409	<0.001
	50 µL	25 µL	2.00000	1.05409	0.301
		100 µL	-1.66667	1.05409	0.439
		Control	18.66667*	1.05409	<0.001
	100 µL	25 µL	3.66667*	1.05409	0.034
		50 µL	1.66667	1.05409	0.439
		Control	20.33333*	1.05409	<0.001
	Control	25 µL	-16.66667*	1.05409	<0.001
		50 µL	-18.66667*	1.05409	<0.001
		100 µL	-20.33333*	1.05409	<0.001
0.25%	25 µL	50 µL	-9.66667*	1.24722	<0.001
		100 µL	-16.00000*	1.24722	<0.001
		Control	11.00000*	1.24722	<0.001
	50 µL	25 µL	9.66667*	1.24722	<0.001
		100 µL	-6.33333*	1.24722	0.004
		Control	20.66667*	1.24722	<0.001
	100 µL	25 µL	16.00000*	1.24722	<0.001
		50 µL	6.33333*	1.24722	0.004
		Control	27.00000*	1.24722	<0.001
	Control	25 µL	-11.00000*	1.24722	<0.001
		50 µL	-20.66667*	1.24722	<0.001
		100 µL	-27.00000*	1.24722	<0.001

[Table/Fig-6]: Tukey's Post-hoc analysis for pairwise comparison between the different volumes of each group.

be sufficient to eliminate pathogens from these microcanals, making root canal treatment for deciduous teeth more challenging [15,16].

E. faecalis was chosen for this investigation because it is a Gram-positive facultative anaerobic coccus that is a well known endodontic pathogen causing root canal infection. Another factor in selecting *E. faecalis* was its ability to survive in harsh environments with limited nutrients and to remain viable in treated root canals for an extended period of time [17,18].

Numerous studies have confirmed the efficacy of sodium hypochlorite solution in eliminating *E. faecalis* [3,17,19]. Although, most dentists recommend and use NaOCl, there have been reports of serious irritations when concentrated solutions have accidentally been forced into the periapical tissues during irrigation or have leaked through the rubber dam [20]. Therefore, the current research was conducted to investigate the effectiveness of sodium hypochlorite in inhibiting *Enterococcus faecalis* at lower concentrations.

According to Retamozo B et al.'s in-vitro investigation, irrigation with 1.3% and 2.5% NaOCl was ineffective in completely eliminating *E. faecalis* [21]. The authors suggested that high concentrations

Dependent variable	(I) Groups	(J) Groups	Mean difference (I-J)	Std. Error	p-value
25 µL	1%	0.5%	6.33333*	1.51535	0.014
		0.25%	10.33333*	1.51535	0.001
	0.5%	1%	-6.33333*	1.51535	0.014
		0.25%	4.00000	1.51535	0.085
	0.25%	1%	-10.33333*	1.51535	0.001
		0.5%	-4.00000	1.51535	0.085
50 µL	1%	0.5%	9.66667*	1.30526	0.001
		0.25%	6.00000*	1.30526	0.009
	0.5%	1%	-9.66667*	1.30526	0.001
		0.25%	-3.66667	1.30526	0.069
	0.25%	1%	-6.00000*	1.30526	0.009
		0.5%	3.66667	1.30526	0.069
100 µL	1%	0.5%	9.33333*	0.98131	<0.001
		0.25%	3.94000*	0.98131	<0.001
	0.5%	1%	-9.3333*	0.98131	<0.001
		0.25%	8.33333	0.98131	0.593
	0.25%	1%	-3.94000*	0.98131	<0.001
		0.5%	-8.33333	0.98131	0.593

[Table/Fig-7]: Tukey's Post-hoc analysis for pairwise comparison of the zone of inhibition between three concentrations.

and prolonged exposure to NaOCl solution may be necessary for effective bacterial reduction. However, contrary to this study, the findings of the present study revealed that 1% sodium hypochlorite was effective in inhibiting *E. faecalis*. This is consistent with the findings of Walia V et al., and Ximenes M et al., who also found that 1% sodium hypochlorite was effective in reducing *E. faecalis* count in root canals [22,23]. The use of higher concentrations of NaOCl solution is still controversial due to their reduced safety, which can result in iatrogenic trauma, especially in paediatric patients. Therefore, it is reasonable to investigate the use of more diluted solutions for the same effectiveness.

Many clinical and in-vitro studies have shown conflicting results, and the main drawback of using sodium hypochlorite in primary teeth, according to researchers, is the apical extrusion of the solution and damage to the succedaneous tooth bud [21,24]. To the best of our knowledge, this is one of the few studies evaluating the effectiveness of a lower concentration of sodium hypochlorite in inhibiting *E. faecalis*.

Further research is needed to investigate the effects of these different concentrations of sodium hypochlorite solution on the anticipated disinfection of infected primary tooth root canals.

Limitation(s)

The study's in-vitro design involves testing the effects of sodium hypochlorite on *Enterococcus faecalis* in a controlled laboratory setting. However, this controlled environment may not fully replicate the complex interactions and challenges encountered in an actual clinical scenario. Additionally, the study only examines *Enterococcus faecalis*, which is a bacterium known to cause endodontic infections. However, root canal infections often involve a variety of micro-organisms with different susceptibilities to disinfectants.

CONCLUSION(S)

The findings of the present study conclude that a 1% sodium hypochlorite solution, at a volume of 100 µL, demonstrated superior efficacy compared to the other concentrations examined. The 1% sodium hypochlorite solution showed a significantly greater inhibition zone compared to lower concentrations, indicating its better ability to inhibit the growth of micro-organisms within the root canal system.

For future research, it is recommended to explore the potential benefits of varying irrigation techniques in combination with the 1% sodium hypochlorite solution. Additionally, investigating the long-term effects of this solution on root canal disinfection and assessing its safety profile and clinical applicability in endodontic practice would be valuable areas of study.

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