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Cytotoxic effect of 1% and 3% Sodium hypochlorite on human red blood cells: An in vitro study

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ABSTRACT

Background and Objectives. The endodontic treatment of primary teeth presents significant challenges due to their unique anatomical characteristics. To ensure a successful treatment, it is essential to evaluate a few factors. These factors include accurate diagnosis, disinfection protocol, intracanal medicaments, and irrigation solutions. While Sodium hypochlorite (NaOCl) has gained success as an irrigation agent in primary teeth, it is important to note that higher concentrations of NaOCl can potentially cause toxicity to the periapical environment if they pass through the tooth's apical foramina. Thus, this study compared the cytotoxicity of two different concentrations of sodium hypochlorite at varied volumes on red blood cells (RBC).

Materials and Methods. In order to evaluate the cytotoxicity potential, fresh human blood was collected from a single healthy individual and subjected to testing. For the test, 10 μL, 20 μL, 30 μL, 40 μL and 50 μL of 1% and 3% NaOCl were added to the RBC. The tubes were gently mixed and incubated at 37°C for 30 minutes. A UV-visible spectrophotometer was used to calculate the absorbance of the supernatant at a wavelength of 540 nm.

Results. There was no significant difference in 1% sodium hypochlorite at different volumes with a p value >0.05 using Tukey's post hoc test. However, there was a significant difference between the volumes of 3% sodium hypochlorite at 40 and 50 μg/ml with a p value of 0.004 and 0.001 respectively.

Conclusions: According to the current study's findings, 1% sodium hypochlorite solution exhibited less toxicity than 3% sodium hypochlorite.

Keywords: Cytotoxicity, primary teeth, sodium hypochlorite, red blood cells

Abbreviations: NaOCl – Sodium hypochlorite
RBC – Red Blood Cells
μL - Microlitre

INTRODUCTION

The endodontic treatment of primary teeth can be quite challenging due to the unique anatomical characteristics they possess. These include the presence of supplementary foramina in furcal regions and the atypical morphological structure of their root canal [1,2]. The development of dentinal caries and its subsequent progress towards the pulpal. and periapical areas are primarily attributed to bacterial factors. One frequently encountered species of bacteria within the root canal is Enterococcus faecalis. Based on dental research, it has been observed that E. faecalis exhibits a notable resistance to environments with higher alkalinity [3,4]. In order to achieve successful endodontic treatment, it is crucial to consider various essential factors. Accurate diagnosis, meticulous cleaning, a reliable disinfection protocol, utilization of various intracanal medicaments, and appropriate irrigation solutions are among the key factors that contribute to successful endodontic treatment. Subsequently, the pulp space should be properly filled, and the final restoration should be appropriate [5,6]. Recent imaging techniques have shown that mechanical preparation alone may not be sufficient to treat every single section of the pulp. To ensure thorough removal of debris and proper disinfection of the root canal, it is necessary to perform irrigation and instrumentation [7,8].

There are several irrigation solutions used in primary teeth; however, sodium hypochlorite (NaOCl) has long been the gold standard irrigation solution [9]. The remarkable antibacterial qualities of NaOCl and its capacity to breakdown organic materials make it an extensively utilized irrigant. It's used at different concentrations, usually between 0.5% and 5.25% [10]. Although NaOCl has been effective as an irrigation agent for primary teeth, it is crucial to recognize that higher concentrations of NaOCl have the potential to produce toxic effects to the periapical tissues if it passes through the tooth's apical foramina [7]. The toxicity of endodontic irrigant to periapical tissues is a serious concern, especially when treating children in pediatric dentistry.

Due to the potential for resorption areas, it is essential to be cautious with the excessive flow of irrigating solution through the apical region of primary teeth. This might result in damage to the permanent tooth bud underneath. The cytotoxicity of higher concentrations of NaOCl towards periapical tissues has been mentioned in case reports [11]. Furthermore, it has been regarded those higher concentrations of NaOCl solution cause cytotoxicity to apical tissues through hemolysis, cause inhibition of neutrophil migration, and harm to fibroblast and endothelial cells [12].

Root canal irrigation poses the possibility of irrigant extrusion into the periapical region, which may lead to pain, swelling and irritation to periapical tissue [13]. Apical outflow of an irrigation solution may occur due to high pressure of the irrigating solution or if an irrigating needle becomes lodged in the root canal during shaping. Apical extrusion is more likely to occur in teeth with greater apical diameters and a lack of apical restriction caused by root resorption [14]. In most cases, forcing a concentrated irrigation solution beyond the tooth's apex causes severe irritation of the periradicular tissue. Thus, when selecting an endodontic irrigant for root canal therapy, tissue cytotoxicity is a key consideration. A variety of techniques have been used to assess endodontic irrigants cytotoxicity. The cytotoxicity on red blood corpuscles was assessed by Sawada et al [15], while Chang et al and Karkenhabadi et al [16,17], tested the cytotoxicity on periodontal ligament cells. Additionally, Liu et al.[18], studied the cytotoxicity on human dental stem cells.

An ideal irrigation solution should be non-toxic, have broad antibacterial activity, and break down necrotic tissue in the pulp efficiently. The toxicity of certain substances is often correlated with their concentration levels, where higher concentrations are commonly associated with increased toxicity. However, the impact of lower concentrations remains relatively uncertain in many cases. This ambiguity prompts the critical question of whether low concentrations of an irrigation solution could potentially exhibit similar toxic effects. In the context of NaOCl, its higher concentrations are well-documented for their toxic properties. Yet, the toxicity of lower concentrations remains less explored. Therefore, the primary objective of this research was to investigate the cytotoxic effects of two different concentrations of NaOCl - 1% and 3% by evaluating their potential to induce hemolysis in human red blood cells.

MATERIALS AND METHODS

The in vitro study was carried out in March 2023 at Gold Lab, Saveetha Institute of Medical and Technical Sciences, Chennai.

Preparation of solution

The 3% sodium hypochlorite solution used in this investigation was procured from Prime Dental Solutions, a commercially available product. The 1% sodium hypochlorite solution was obtained by diluting the previously mentioned 3% solution using the formula

V1 = 1%100 ml3% = 33 ml

A calibrated syringe was used for accurately measuring 33 mL of the 3% sodium hypochlorite solution. It was then transferred to a clean and sterile mixing container. The mixing container was filled with distilled water to a total capacity of 100 mL. To achieve complete dilution, the fluid was gently stirred. The 1% sodium hypochlorite solution was labelled suitably with the concentration and date of production. It was kept in a safe place away from direct sunlight and heat.

RBC Suspension Preparation

Fresh human blood was collected from a single healthy individual using a sterile tube with anticoagulants. In order to separate the red blood cells from the other components of the blood, a centrifuge was used at a speed of 1,000 g for a duration of 10 minutes, at the optimal room temperature. The supernatant was removed, and the red blood cells were cleansed three times using phosphate buffered saline. In order to create a 10% (v/v) RBC suspension, the RBCs were resuspended in the tris-HCl buffer.

Assay Methodology

Each centrifuge tube received 1 mL of RBC suspension in total. Each tube was then filled with varying volumes of respective concentrations of 1% and 3% sodium hypochlorite at 10 μg, 20 μg, 30 μg, 40 μg, and 50 μg. (Figure 1,2) The tubes were gently mixed and incubated at 37°C for 30 minutes. Following that, the tubes were centrifuged at 1,000 rpm at room temperature for 10 minutes to pellet the RBCs. A UV-visible spectrophotometer was used to calculate the absorbance of the supernatant at a wavelength of 540 nm.

% inhibition = {(OD control - OD sample)/OD control)}X100 was used to compute the percentage inhibition of haemolysis.

OD control is the absorbance of the RBC suspension without the test component(s), and OD sample is the absorbance of the RBC suspension with the test compound.

STATISTICAL ANALYSIS

SPSS software version 24.0 was used to analyze the data. The cytotoxicity was described using the mean and standard deviation. There were various volumes of control, 1% and 3% sodium hypochlorite, such as 10μg, 20μg, 30μg, 40μg and 50μg. The paired t test was performed to compare mean differences between 1% and 3% sodium hypochlorite before and after incubation. ANOVA was employed as one way to analyze the mean differences between groups within concentration or volume. Following ANOVA, a post hoc test was utilized to analyze individual intergroup differences. The significance threshold was set at less than 0.05.

RESULTS

A paired t test was used to compare the mean difference in hemolysis of RBC before and after the addition of the respective irrigation solution. There was no significant difference between baseline and follow-up in 1% sodium hypochlorite and significant differences were observed at 10μg, 20μg, 30μg, 40μg, and 50μg of 3% sodium hypochlorite solution with a P value of 0.004 (Table 1).

Tukeys post hoc intergroup analysis at specific volumes showed 1% sodium hypochlorite did not have significant differences between their volumes with a P value of >0.05 but a significant difference was observed between the volumes of 3% sodium hypochlorite at 40 μg/ ml and 50 μg/ml with a P value of 0.004 and 0.001 (Table 2).

DISCUSSION

Sodium hypochlorite (NaOCl) is a highly recognized and often utilized endodontic irrigation solution because of its antibacterial properties and capacity to destroy residues of organic and necrotic tissue [19]. The use of NaOCl as an irrigant was initially proposed by Walker [20], however Spano et al. [21] suggest use of high concentrations of NaOCl to degrade protein products found in the root canal. It is essential to note that the periapical tissues may be harmed by these higher concentrations. Endodontic irrigants need to be non-toxic to periapical tissues, especially when treating young patients. Irrigating solution overflowing through the apical portion of primary teeth may cause damage to the permanent tooth bud underneath. Higher concentrations of NaOCl on periapical tissues have been shown to have cytotoxic effects in case reports [11,22]. In consideration of this, we conducted this study to evaluate the cytotoxicity of two different concentrations of sodium hypochlorite in human red blood cells.

Since red blood cells are easily separated using the least intrusive approach, they were selected as the biological model to assess cytotoxic effects. The semipermeable barriers known as red blood cell membranes allow fluid to enter and exit the cells due to the osmotic gradient that is produced on both sides of the membrane. The cells experience a fast osmotic outflow of water when exposed to a hypertonic solution, which causes them to coagulate and ultimately collapse [23]. It is reasonable to use human red blood cells as a cell type for cytotoxicity assessment since it is possible to determine their intracellular hemoglobin. The irrigating agents employed in this investigation were hypertonic; as a result of the oxidizing effect of the solutions on the cell membrane of red blood corpuscles, hemolysis and protein loss were detected.

NaOCl produces hypochlorous acid (HOCl⁻) and hydroxyl ions when it comes into contact with tissue. The hydroxyl ions generated by NaOCl will subsequently undergo an autoxidation reaction with the oxygen generated by the mitochondria in human periodontal ligament fibrous cells, resulting in the formation of hydroxyl radicals. When Reactive Oxygen Species (ROS) generation surpasses antioxidant capture capability, oxidative stress occurs. When oxidative stress is present, lipid peroxidation takes place in both the organelles and plasma membrane. The unstable bond between fatty acids and free radicals subsequently generates lipid radicals, which produce peroxyl radical lipids by reacting with oxygen. Moreover, ROS are capable of causing DNA damage and chain cross-linking. Elevated levels of NaOCl cytotoxicity specify this pathway as the cause of cell death [24].

The current study demonstrated that at a volume of 40μg and 50μg, at a concentration of 3% sodium hypochlorite showed increased cytotoxicity with a statistically significant difference. The concentration of 1% sodium hypochlorite, on the other hand, showed no significant difference in cytotoxicity across all volumes tested. This finding is compatible with the findings of Sirtes et al.'s study [25], which concluded that a lower concentration of NaOCl might potentially serve as an endodontic irrigant due to its low cytotoxicity. Furthermore, Liu et al.'s [18], study, which investigated how sodium hypochlorite affected the proliferation and differentiation of human dental stem cells, concluded that NaOCl has a dose-dependent negative effect on dental stem cell viability, proliferation, and differentiation, particularly at higher concentrations.

Following endodontic treatment, it is crucial for both the lesion and the periodontal connective tissue attachment system to undergo healing. The utilization of toxic antibacterial agents in the root canal has the capacity to impede the periapical tissue healing process [18]. Multiple studies have consistently emphasized the potential increase in toxicity associated with higher concentrations of sodium hypochlorite. This chemical compound demonstrates a direct correlation between its concentration and the severity of its harmful effects. Multiple studies have indicated that the key elements for regeneration include cellular expansion, fibroblast production, and the formation of extracellular matrix. Higher concentrations may have an impact on these components, as these results highlight [16,26]. The ability of periapical tissues to repair and regenerate may be hindered by the adverse effects of increased concentrations of irrigation solutions. According to certain findings, periapical tissue destruction is more likely to occur when the pulp is necrotic or when root canals have large apical foramina. This is due to the increased likelihood of canal materials entering via the apical foramen. According to the findings of this study, higher concentrations of irrigation fluids may have a negative impact on periapical tissues. Nonetheless, further study is needed to identify its clinical significance because the agent's concentration, length of exposure, and exposure surface area all have a substantial influence on the outcome. When determining the most appropriate agent, it is critical to consider each of these factors. However, there are few limitations of the study such as the sample size was limited in the current study. Further research on antimicrobial effectiveness at low concentration of sodium hypochlorite should be considered for effective clinical usage.

CONCLUSION

According to the current study's findings, 1% sodium hypochlorite solution exhibited less toxicity than 3% sodium hypochlorite. Since nontoxic irrigants are essential in dental procedures, particularly in the field of pediatric dentistry, the 1% sodium hypochlorite's lowered toxicity suggests a prospect of fewer adverse effects. According to the results of this study, it was determined that using a 1% sodium hypochlorite solution for treating primary teeth was a much safer alternative.

CONFLICT OF INTEREST

Conflict of interest: No conflict of interest

Financial support: Nil

AUTHOR'S CONTRIBUTIONS

Conceptualization: Divya Mukundan and Maria Anthonet Sruthi

Methodology: Divya Mukundan

Software: Divya Mukundan

Validation: Divya Mukundan and Maria Anthonet Sruthi

Formal analysis: Maria Anthonet Sruthi

Investigation: Divya Mukundan

Resources: Maria Anthonet Sruthi

Data curation: Divya Mukundan

Writing—original draft preparation: Divya Mukundan

Writing—review and editing: Maria Anthonet Sruthi

Visualization: Maria Anthonet Sruthi

Supervision: Maria Anthonet Sruthi

Project administration: Maria Anthonet Sruthi

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All authors have read and agreed to the published version of the manuscript

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TABLES

Table 1. Comparing cytotoxicity between the groups using Paired samples t test

		Sum of squares	Sig
Control	Between Groups	4.00	0.195
	Within Groups	9.00	
	Total	13.00	
1%Sodium hypochlorite	Between Groups	8.000	0.213
	Within Groups	9.000	
	Total	17.000	
3% Sodium hypochlorite	Between Groups	25.270	0.004
	Within Groups	9.000	
	Total	34.270	

Table 2. Tukeys Post hoc comparison between the volumes of 1% and 3% sodium hypochlorite

Dependent variable	(I) Groups according to concentration	(J) Groups according to concentration	Sig
1% SODIUM HYPOCHLORITE	10 µg	20 µg	0.144
		30 µg	0.146
		40 µg	0.148
		50 µg	0.152
	20µg	10 µg	0.144
		30 µg	0.630
		40 µg	1.000
		50 µg	0.630
	30µg	10 µg	0.146
		20 µg	0.630
		40 µg	0.630
		50 µg	0.754
	40µg	10 µg	0.148
		20 µg	1.000
		30 µg	0.630
		50 µg	0.765
	50µg	10 µg	0.152
		20 µg	0.630
		30 µg	0.754
		40 µg	0.765

Dependent variable	(I) Groups according to concentration	(J) Groups according to concentration	Sig
3% SODIUM HYPOCHLORITE	10 µg	20 µg	0.148
		30 µg	0.148
		40 µg	0.004*
		50 µg	0.001*
	20µg	10 µg	0.148
		30 µg	0.630
		40 µg	0.004*
		50 µg	0.001*
	30µg	10 µg	0.148
		20 µg	0.630
		40 µg	0.004*
		50 µg	0.001*
	40µg	10 µg	0.004*
		20 µg	0.004*
		30 µg	0.004*
		50 µg	0.001*
	50µg	10 µg	0.001*
		20 µg	0.001*
		30 µg	0.001*
		40 µg	0.001*

FIGURES

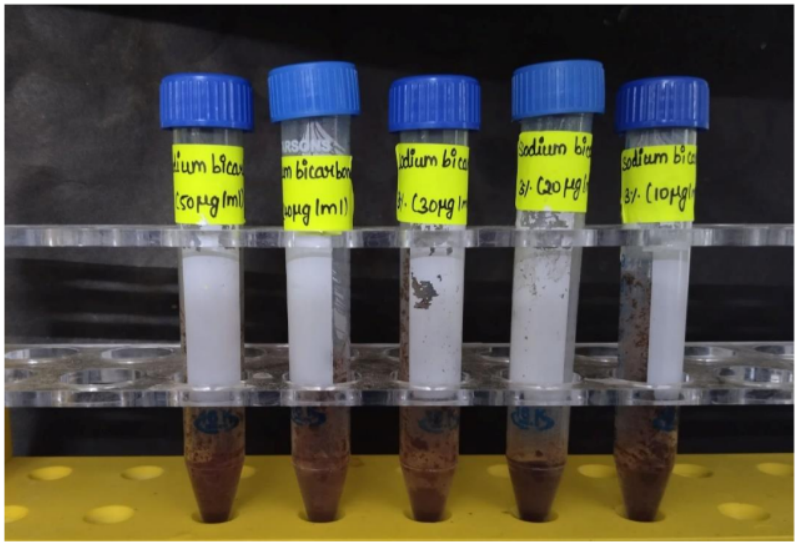

Figure 1. 3% Sodium hypochlorite solution added to 1 ml of RBC suspension at 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, and 50 µg/ml

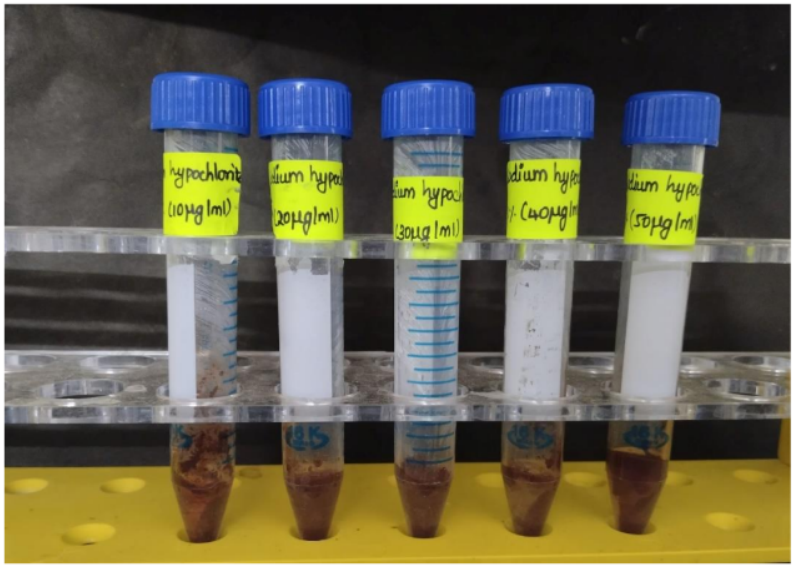

Figure 2. 1% Sodium hypochlorite solution added to 1 ml of RBC suspension at 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, and 50 µg/ml