



COMPARATIVE EVALUATION OF SIX INTERMEDIATE ROOT CANAL IRRIGANTS TO PREVENT ORANGE-BROWN PRECIPITATE FORMATION DUE TO INTERACTION OF SODIUM HYPOCHLORITE AND CHLORHEXIDINE GLUCONATE SOLUTIONS DURING ENDODONTIC TREATMENT. AN IN-VITRO STEREOMICROSCOPE ANALYSIS.

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ABSTRACT

Aim: In-vitro comparative evaluation of Six Intermediate root canal irrigants to prevent Orange-Brown colour precipitate formation due to Interaction of Sodium hypochlorite and Chlorhexidine gluconate solutions during endodontic treatment using Stereomicroscope analysis.

Materials & Method: Seventy human extracted permanent mandibular single rooted premolar teeth were collected. Access cavity preparation done, working length was determined and biomechanical preparation was done using ProTaper Next rotary files upto size X3 file. All specimens were irrigated with 2ml of 3% NaOCl solution and equally divided into Seven groups with 10 specimens each, based on type of Intermediate canal irrigants used. Group 1: 0.9% Normal Saline, Group 2: Distilled water at room temperature, Group 3: Distilled water at 65°C, Group 4: 20% Citric acid, Group 5: 70% Isopropyl alcohol, Group 6: 0.2% Chitosan solution, Group 7: Control group (No intermediate canal irrigants used). All specimens were then finally irrigated with 2ml of 2% Chlorhexine gluconate solution. All specimens were then longitudinally sectioned into two equal halves and each half was examined at 9mm (coronal), 6mm (middle) & 3mm (apical) third's of root canals with Stereomicroscope for presence or absence of Orange-Brown colour Precipitate formation.

Statistical plan: OneWay ANOVA and Tukey Post-hoc tests were used for statistical analysis. $P \leq 0.05$ was considered statistically significant.

Results: Statistically highly significant difference ($P < 0.05$) was seen in inter-group comparison among the groups. Group 5 (70% Isopropyl alcohol) showed maximum efficiency in the prevention of orange-brown precipitate formation followed by Group 6 (0.2% Chitosan solution) both at coronal, middle and apical-third's of root canals.

Conclusion: 70% Isopropyl alcohol used as Intermediate canal irrigant between the use of sodium hypochlorite and Chlorhexidine solutions showed maximum efficiency in preventing the formation of orange-brown precipitate compared to other Intermediate canal irrigants used at all 3-levels of root canals.

Keywords: Chlorhexidine, Sodium hypochlorite, Orange-Brown precipitate, Stereomicroscope, Root canal irrigants.

INTRODUCTION

Residual infected pulpal tissue, infected dentin and

bacterial remnants in the root canal can result in the root canal treatment failure.¹ Microorganisms present in the

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root canals can lead to the development of periapical inflammatory lesions.² The goal of root canal therapy is to remove the inflamed and necrotic pulp tissue from the root canal system using both the chemical and mechanical debridement.³

Root canal disinfection during chemomechanical preparation relies heavily on the use of root canal irrigants. Although mechanical instrumentation can reduce the microbial load, but it alone cannot completely eliminate microbes from the root canal system due to complex internal anatomy of the root canals. Root canal irrigants are recommended along with instrumentation to completely disinfect the root canal system because of the anatomic complexities of the root canal system.^{4,5}

The purpose of using root canal irrigating solutions during root canal treatment is to enhance the debridement, cleaning and disinfection of the root canal system.⁶ These irrigants should ideally have both antimicrobial and tissue-dissolution actions as well as they should provide lubrication, demineralization and the ability to remove both organic and inorganic debris and smear layer, thus preventing failure of root canal therapy.⁴ Since no single root canal irrigant possesses all ideal characteristics, the combination of various root canal irrigants in irrigation protocol during root canal therapy is recommended to reduce root canal necrotic tissue and microorganisms.⁶

Sodium hypochlorite (NaOCl) is the most commonly used root canal irrigant during endodontic therapy, due to its potent tissue-dissolving and antimicrobial properties.⁷ Its germicidal action is linked to the formation of hypochlorous acid when it comes into contact with organic debris in the root canal. While NaOCl at high concentrations (5.25% and 3%) is bactericidal, However it was found to be ineffective at low concentrations (0.5% to 1%). However, the effect of NaOCl is short-lived and it does not provide sustained antimicrobial activity.^{8,9}

Chlorhexidine Gluconate (CHX) solution was proposed as an alternative to Sodium hypochlorite as root canal irrigant or to use in combination with it due to its lower toxicity.^{10,11} Its broad-spectrum antimicrobial agent effective against various strains of microorganisms and provides potent antimicrobial substantivity in root canal. However, it should be noted that chlorhexidine does not possess tissue-dissolving properties.¹² A combination of sodium hypochlorite and chlorhexidine gluconate solutions was advocated to enhance both their anti-microbial properties during root canal treatment. However, when more than one root canal irrigant is used in

sequence, the 1st irrigant used can still remain in root canal and come into contact with next irrigant used within the root canal system.¹³ When sodium hypochlorite remains within the root canal, the use of chlorhexidine gluconate as the next irrigant can lead to the formation of an orange-brown colour precipitate called Parachloroaniline. This precipitate occludes the dentinal tubules, potentially compromising complete root canal disinfection and 3-dimensional seal of root canal obturation. Additionally, the precipitate formed, Parachloroaniline is cytotoxic, raising concerns about it leaching out of the root canal into periapical tissues. Therefore, it is essential to prevent the formation of this precipitate effectively.^{14,15}

Various root canal irrigating solutions such as normal saline, distilled water, chitosan solution, citric acid, isopropyl alcohol can be used as Intermediate root canal irrigating solutions to prevent the interaction between NaOCl and CHX root canal irrigants; thus preventing formation of Parachloroaniline (orange-brown precipitate). So, the aim of our in-vitro study was comparative evaluation of Six Intermediate root canal irrigants in the prevention of orange-brown precipitate formation by inhibiting any interaction of sodium hypochlorite with chlorhexidine gluconate solution during endodontic treatment.

MATERIALS AND METHOD

Seventy freshly extracted human permanent mandibular single-rooted premolar teeth were collected. Teeth extracted either for orthodontic reasons or periodontally compromised within the age group of 18-30 years were only included in the study following strict inclusion criteria. All teeth were examined under Stereomicroscope (Olympus SZ61, Olympus Optical Co., Tokyo, Japan) at 10X magnification to ensure that they were intact and without any caries or non-caries lesions, devoid of restorations, clinically detectable fractures or cracks on root, open root apices, root caries, resorptive defects, calcifications. Previously endodontically treated teeth were excluded from the study.

The collected teeth were cleaned of superficial debris, calculus, and residual tissue tags using ultrasonic instruments. Occupational Safety and Health Administration (OSHA), Center for Disease Control (CDC), and Prevention recommendations and guidelines were strictly followed during the collection, sterilization, and handling of extracted teeth to prevent any biohazard transmission. All teeth were then stored in 0.5% thymol at room temperature until used.

All specimens were then decoronated using a diamond disc (Novo Dental products, Mumbai) attached to a straight micromotor handpiece (NSK, Japan) at low-speed under water coolant at the level of cement-enamel junction perpendicular to the long axis of teeth to standardize root length of all specimens to 14mm measured using Digital Vernier calliper (Hetvi Trend, Surat, India). A no.4 Round bur (Mani, Japan) was attached to a contra-angle high-speed airtor handpiece (NSK, Japan) to gain access into root canal orifices of all specimens. A no.10K(Kerr)-file (Dentsply, Maillefer, Switzerland) was placed into the root canals to establish the canal patency, with the tip of the trial file visible at the apical foramen, the working length was determined by subtracting 0.5mm from the length in all specimens. The apical foramen in all specimens was sealed using Sticky wax (Pyrex polymers. Pvt Ltd. India) and teeth were mounted in Vinyl Polysiloxane Putty impression material blocks (Addition silicone elastomer, Affinis, Coltene Whaledent AG Altstatten/Switzerland) for ease of handling of specimens during canal instrumentation. In all specimens, root canal instrumentation was done in crown-down technique using ProTaper Next rotary file system (Dentsply, Maillefer, Switzerland) in file sequence of X1, X2 and X3, with X3 as Master Apical File (MAF) (Size 0.30, Taper 0.07) at 300 rpm, 2.8 Ncm of torque attached to torque-controlled Endomotor handpiece (Eighteenth **E-xtrème Cordless Endomotor**, Orikam Healthcare India Pvt Ltd, Delhi, India) following manufacturer's instructions. Each rotary file was used for instrumentation in five root canals and were later discarded, followed by the use of new files. In each root canal, 15% EthyleneDiamineTetraacetic Acid (EDTA), (Glyde prep canal, Dentsply, Maillefer, Switzerland) was used during root canal during instrumentation. Upon each file change, the canals were irrigated with 0.9% normal saline solution (NIVY Remedies Pvt Ltd, Hatharia, West Bengal, India) using 30 Gauge side-vented needle (RC Twents Irrigation Needle, Prime Dental Products Pvt. Ltd, Bhiwandi, India) placed 2mm short of the root apex. After the completion of biomechanical preparation, each specimen was flushed thoroughly with 2ml of 3% NaOCl solution (Neelkaanth Health Care Pvt Ltd, Ahmedabad, India) for 60 seconds using 30 Gauge side-vented needle. All specimens (n=70) were then randomly allocated into six experimental groups (n=60), based on the type of Intermediate root canal irrigant solution used; 2ml of intermediate root canal irrigant was used for 60 seconds as per the Group assigned; Group 1 (n=10): 0.9% Normal saline solution (Nivy Remedies Pvt Ltd, Hatharia, India) at room temperature. Group 2 (n=10): Distilled water (Agrawal Pharmaceuticals, New Delhi, India) at room

temperature. Group 3 (n=10): Distilled water (Agrawal Pharmaceuticals, New Delhi, India) Pre-heated upto 65°C. Group 4 (n=10): 20% Citric acid (Citra Wash, Prime Dental Products Pvt Ltd, Mumbai, India) at room temperature. Group 5 (n=10): 70 % Isopropyl Alcohol (Medwin Pharmatech, Indore, India) at room temperature. Group 6 (n=10): 0.2% Chitosan Solution (Vedayukt India Pvt Ltd, Ghatsila, India) at room temperature (Figure no.1). Group 7 (n=10): Control Group (No intermediate root canal irrigant solution used).



Figure 1. Intermediate root canal irrigants used in the study: 0.9% Normal saline, Distilled water, Citric acid, 70% Isopropyl alcohol, 0.2% Chitosan solution.

All specimens were then irrigated with 2% Chlorhexidine gluconate (Neelkaanth Health Care Pvt Ltd, Ahmedabad, India) solution for 60 seconds using 30Gauge side-vented needle (Neoendo Endo irrigation needles, Orikam Healthcare Pvt Ltd, Gurugram, India). Sterile Paper points (Dia Dent, Korea) were used to dry the root canals. All specimens were then removed from the Putty impression material blocks. Two vertical grooves were then made along the buccal and lingual surfaces of all specimens using a diamond disc (DZ, Munchen, Germany) attached to micromotor straight handpiece (NSK, Japan) at low-speed 20,000 RPM (Revolutions Per Minute) without invading into the root canal space and were then longitudinally sectioned into two equal halves using a hand chisel and mallet. Each root half was marked at 3mm (apical-third), 6mm (middle-third), 9mm (coronal-third) from the predetermined working length of specimens and were then evaluated under Stereomicroscope (Olympus SZ61, Olympus Optical Co., Tokyo, Japan) at $\times 10$ magnification, based on quantum of Orange-Brown colour precipitate formation on the dentinal walls of root canal space and scored as per criteria suggested by Arslan H et al.¹⁶ **Score 0:** The root canal surface was completely free of orange-brown precipitate. **Score 1:** Orange-brown precipitate seen in less than half of the root canal surface. **Score 2:** Orange-brown precipitate seen in more than half of the root canal surface. **Score 3:** Root canal surface completely covered with orange-brown precipitate.

RESULTS

The Tabulated data in Microsoft excel was statistically analyzed with SPSS (Statistical Package for the Social Sciences) V.24 software. OneWay ANOVA and Tukey Post-hoc tests were used for the statistical analysis. P value ≤ 0.05 was considered as statistically significant. One-way

ANOVA test showed statistically significant difference among all the groups, $P \leq 0.05$ considered as statistically significant. Table no.1 showed the comparison of presence of orange-brown precipitate at coronal-thirds of root canals among all groups, Group 7 exhibited the highest mean of 3.00 and Groups 5 & 6 showed the lowest mean of 0.00

Table 1. Comparison in prevention of orange-brown Precipitate formation among the 7 groups at Coronal-thirds of root canals using OneWay ANOVA.

Groups (n=70)	Mean $\pm$ SD	P value
Group 1:0.9% Normal Saline solution (n=10)	2.70 $\pm$ 0.47	<0.001(HS)
Group 2: Distilled water at room temperature (n=10)	2.10 $\pm$ 0.55	
Group 3: Distilled water Pre-heated upto 65°C (n=10)	1.70 $\pm$ 0.47	
Group 4:20% Citric acid (n=10)	1.70 $\pm$ 0.47	
Group 5:70 % Isopropyl alcohol (n=10)	0.00 $\pm$ 0.00	
Group 6:0.2 % Chitosan solution (n=10)	0.00 $\pm$ 0.00	
Group 7: Control group (n=10)	3.00 $\pm$ 0.00	

P: Probability SD: Standard Deviation HS: Highly Significant

Table no.2 showed the comparison of presence of orange-brown precipitate at middle-thirds of root canals among all groups, Group 7 showed the highest mean at 2.50 and Group 5 the lowest at 0.20.

Table 2. Comparison in prevention of orange-brown Precipitate formation among the 7 groups at Middle-thirds of root canals using OneWay ANOVA.

Groups (n=70)	Mean $\pm$ SD	P value
Group 1:0.9% Normal Saline solution (n=10)	2.40 $\pm$ 0.50	<0.001(HS)
Group 2: Distilled water at room temperature (n=10)	1.70 $\pm$ 0.47	
Group 3: Distilled water Pre-heated upto 65°C (n=10)	1.20 $\pm$ 0.62	
Group 4:20% Citric acid (n=10)	1.20 $\pm$ 0.41	
Group 5:70 % Isopropyl alcohol (n=10)	0.20 $\pm$ 0.41	
Group 6:0.2 % Chitosan solution (n=10)	0.30 $\pm$ 0.47	
Group 7: Control group (n=10)	2.50 $\pm$ 0.51	

Table no.3 showed the comparison of presence of orange-brown precipitate at apical-thirds of root canals among all groups, Group 7 showed the highest mean at 2.60 and Group 5 the lowest at 0.10

Table.3 Comparison in prevention of orange-brown Precipitate formation among the 7 groups at Apical-thirds of root canals using One way ANOVA.

Groups (n=70)	Mean $\pm$ SD	P value
Group 1:0.9% Normal Saline solution (n=10)	2.50 $\pm$ 0.51	<0.001(HS)
Group 2: Distilled water at room temperature (n=10)	1.30 $\pm$ 0.47	
Group 3: Distilled water Pre-heated upto 65°C (n=10)	1.10 $\pm$ 0.31	
Group 4:20% Citric acid (n=10)	1.00 $\pm$ 0.00	
Group 5:70 % Isopropyl alcohol (n=10)	0.10 $\pm$ 0.31	
Group 6:0.2 % Chitosan solution (n=10)	0.20 $\pm$ 0.41	
Group 7: Control group (n=10)	2.60 $\pm$ 0.50	

OneWay ANOVA test showed; Group 5 (70% Isopropyl alcohol) and Group 6 (0.2% Chitosan solution) as Intermediate canal irrigants exhibited No orange-brown precipitate formation in the coronal-third's and minimal (or) least precipitate formation at middle and apical-third's of root canals. Group 1 (0.9% Saline solution) & Group 7 (Control group) showed maximum orange-brown precipitate formation both at coronal, middle and apical-third's of root canals among the tested specimens. Tukey Post-hoc test was used for inter-group pair-wise comparison of the orange-brown precipitate formation at coronal, middle and apical-third's of root canals among all the tested specimens. The test showed; At coronal-thirds of root canals (Table no.4 and Graph no.1), significant difference was seen between Group 2 Vs Group 3 & Group 2 Vs Group 4 in the amount of precipitate formation. At middle-thirds of root canals (Table no.5 and Graph no.2), statistically significant difference was seen between Group 2 Vs Group 3 & Group 2 Vs Group 4 and at apical-thirds of root canals (Table no.6 and Graph no.3), no statistically significant difference was seen among tested specimens in all groups.

Table.4. Pairwise Inter-group comparison between the 7 groups at coronal-thirds of root canals using Tukey post-hoc test.

Inter-group Pairwise Comparison	P value
Group1 Vs Group2	<0.001 (HS)
Group1 Vs Group3	<0.001 (HS)
Group1 Vs Group4	<0.001 (HS)
Group1 Vs Group5	<0.001 (HS)
Group1 Vs Group6	<0.001 (HS)
Group1 Vs Group7	0.150 (NS)
Group2 Vs Group3	0.015 (S)
Group2 Vs Group4	0.015 (S)
Group2 Vs Group5	<0.001 (HS)
Group2 Vs Group6	<0.001 (HS)
Group2 Vs Group7	<0.001 (HS)
Group3 Vs Group4	1.000 (NS)
Group3 Vs Group5	<0.001 (HS)
Group3 Vs Group6	<0.001 (HS)
Group3 Vs Group7	<0.001 (HS)
Group4 Vs Group 5	<0.001 (HS)
Group4 Vs Group6	<0.001 (HS)
Group4 Vs Group7	<0.001 (HS)
Group5 Vs Group6	0.00
Group5 Vs Group7	<0.001 (HS)
Group6 Vs Group7	<0.001 (HS)

S: Significant

NS: Non-Significant

HS: Highly Significant

Table 5. Pairwise Inter-group comparison between the 7 groups at middle-thirds of root canals using Tukey post-hoc test.

Inter-group Pairwise Comparison	P value
Group1 Vs Group2	<0.001(HS)
Group1 Vs Group3	<0.001 (HS)
Group1 Vs Group4	<0.001 (HS)

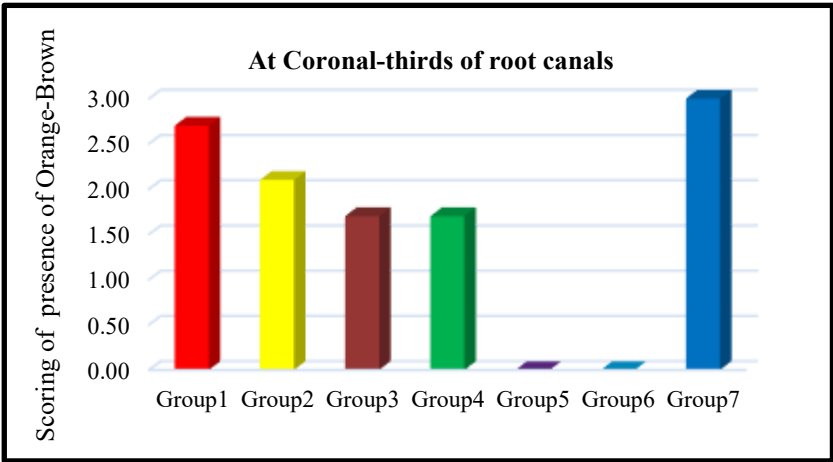
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Group1 Vs Group5	<0.001 (HS)
Group1 Vs Group6	<0.001 (HS)
Group1 Vs Group7	0.995 (NS)
Group2 Vs Group3	0.025 (S)
Group2 Vs Group4	0.025 (S)
Group2 Vs Group5	<0.001 (HS)
Group2 Vs Group6	<0.001 (HS)
Group2 Vs Group7	<0.001 (HS)
Group3 Vs Group4	1.000 (NS)
Group3 Vs Group5	<0.001 (HS)
Group3 Vs Group6	<0.001 (HS)
Group3 Vs Group7	<0.001 (HS)
Group4 VsGroup5	<0.001 (HS)
Group4 Vs Group6	<0.001 (HS)
Group4 Vs Group7	<0.001 (HS)
Group5Vs Group6	0.995 (NS)
Group5 Vs Group7	<0.001 (HS)
Group6 VsGroup7	<0.001 (HS)

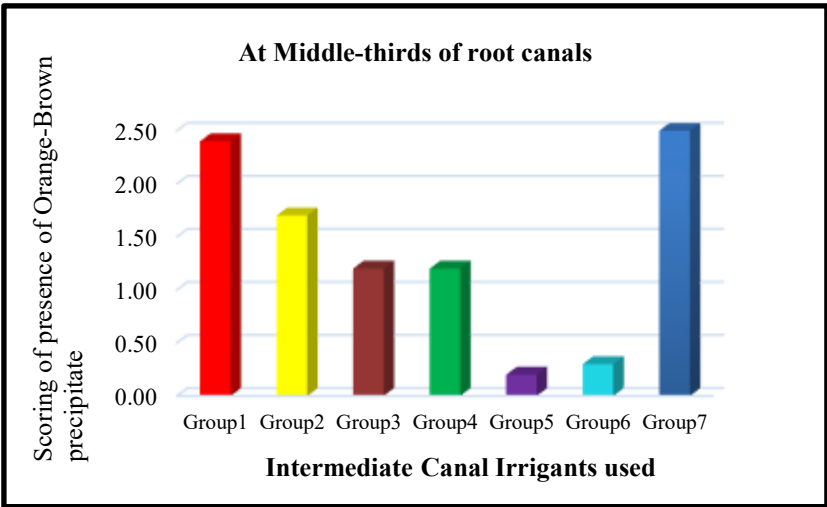
Table 6. Pairwise Inter-group comparison between the 7 groups at apical-thirds of root canals using Tukey post-hoc test.

Inter-group Pairwise Comparison	P value
Group1 Vs Group2	<0.001 (HS)
Group1 Vs Group3	<0.001 (HS)
Group1 Vs Group4	<0.001 (HS)
Group1 Vs Group5	<0.001 (HS)
Group1 Vs Group6	<0.001 (HS)
Group1 Vs Group7	0.985 (NS)
Group2 Vs Group3	0.683 (NS)
Group2 Vs Group4	0.207 (NS)
Group2 Vs Group5	<0.001 (HS)
Group2 Vs Group6	<0.001 (HS)
Group2 Vs Group7	<0.001 (HS)
Group3 Vs Group4	0.985 (NS)
Group3 Vs Group5	<0.001 (HS)
Group3 Vs Group6	<0.001 (HS)
Group3 Vs Group7	<0.001 (HS)
Group4 Vs Group5	<0.001 (HS)
Group4 Vs Group6	<0.001 (HS)
Group4 Vs Group7	<0.001 (HS)
Group5 Vs Group6	0.985 (NS)
Group5 Vs Group7	<0.001 (HS)
Group6 Vs Group7	<0.001 (HS)

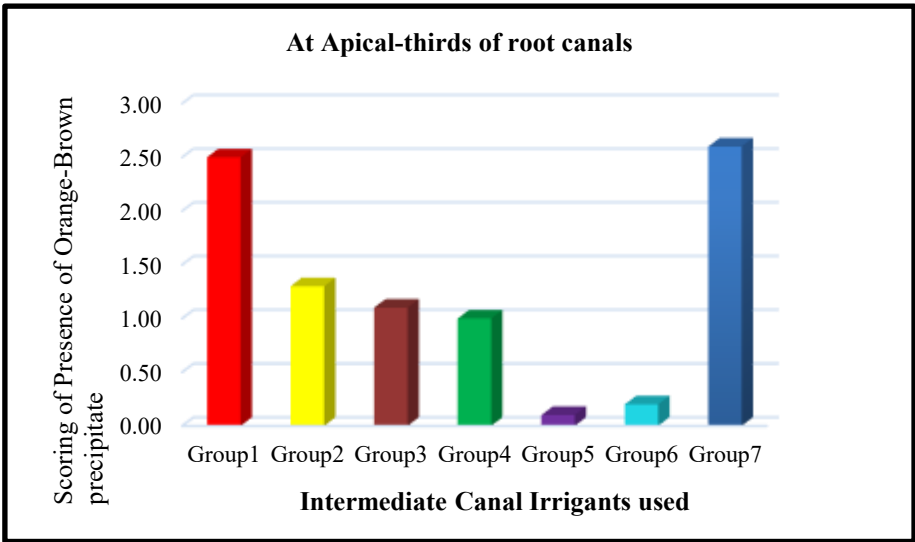
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Graph 1. Vertical Bar graph; Extent of Orange-Brown precipitate at Coronal-thirds of root canals in all specimens



Graph 2. Vertical Bar graph; Extent of Orange-Brown precipitate at Middle-thirds of root canals in all specimens



Graph 3. Vertical Bar graph; Extent of Orange-Brown precipitate at Apical-thirds of root canals in all specimens

Group 5 (70% Isoprophyl alcohol) showed maximum efficiency in the prevention of orange-brown colour precipitate formation at all 3-levels (coronal, middle and apical-third's) of root canals (Figure no.2), followed by Group 6 (0.2% Chitosan solution). Whereas, Group 1 (0.9% Normal saline), Group 2 and Group 3 (Distilled water at room temperature and preheated) exhibited least efficiency in preventing orange-brown precipitate formation. Group 7 (Control group) (Figure no.3) showed greater amount of orange-brown colour precipitate formation upon direct interaction between NaOCl and CHX solutions, as no intermediate canal irrigant was used.



Figure 2. Group 5 (70% Isoprophyl alcohol as Intermediate canal irrigant)

Figure 3. Group 7 (Control group): No Intermediate root canal irrigant used.

DISCUSSION

Endodontic microflora is polymicrobial in nature. Although biomechanical preparation of the root canals is able to reduce the number of microorganisms, complete canal disinfection is difficult because of the complexity of the internal root canal anatomy.⁵ Root canal irrigants plays an

important role in eliminating microorganisms from root canals which otherwise are difficult to remove only with root canal instrumentation alone.^{17,18} Hence, irrigants are either used alone or in combination with other irrigants along with canal instrumentation to achieve optimal antimicrobial effect. The need for root canal irrigation is of paramount importance during chemo-mechanical preparation of root canals due to its role in the eradication of microorganisms, tissue dissolution, removal of debris and smear layer, especially in areas untouched during mechanical instrumentation of root canals.

Bue TB et al¹⁴ suggested a clinical protocol for root canal irrigation and it consists of irrigation with NaOCl to dissolve the organic components, EDTA to eliminate the smear layer and CHX to impart substantive antimicrobial activity. Although such combination of root canal irrigants enhance their antimicrobial properties, but possible negative chemical interactions between them should also be considered.¹⁴ The beneficial effects of using NaOCl and CHX alternatively is due to their exclusive

properties of tissue dissolution and antimicrobial effectiveness known to be superior rather than the use of these root canal irrigants individually.¹² However, Kuruvilla JR et al,¹⁰ Zehender M et al¹⁹ and Gupta H et al²⁰ reported that the combination or alternate use of NaOCl and CHX as root canal irrigants resulted in negative effect on the success of root canal therapy due to the formation of by-products causing poor adaptation of endodontic sealers to dentinal walls of root canals. Direct interaction of CHX with NaOCl result in the formation of orange-brown colour precipitate called Para-ChloroAniline (PCA) due to acid-base reaction between them. Chlorhexidine gluconate as a dicationic acid (pH 5.5–6.0) has the ability to donate protons and the alkaline Sodium hypochlorite is able to accept protons from the dicationic CHX, this proton exchange results in the formation of a neutral and insoluble precipitate containing ParaChloroAniline (PCA) or 1-Amino-4-chlorobenzene and is found to be cytotoxic. When 2% CHX solution directly comes in contact with any concentration of NaOCl, the precipitate colour changes depending upon the concentration of sodium hypochlorite (0.19% to 6%) used, ranging from peach to dark-brown.^{15,22} In the our study, emphasis is given to the interaction of 3% NaOCl with 2% CHX solutions. In our study, Biomechanical preparation was done in all specimens using Protaper Next files upto size X3 to enhance penetration of root canal irrigants till apical-third of root canals. 70% Isoprophyl alcohol as intermediate canal irrigant showed minimal or least

orange-brown precipitate formation, followed by 0.2% Chitosan solution. The findings of our study are in agreement with Krishnamurthy S et al⁵ reported Isopropyl alcohol as intermediate canal irrigant completely prevented precipitate formation. Isopropyl alcohol is a volatile, tensioactive, highly electronegative, penetrates deeper in root canal dentin to remove any residual NaOCl from the root canal dentin. whereas the use of saline or distilled water as intermediate canal irrigant showed remnants of orange-brown precipitate mainly in the coronal and middle-third's of root canals.

In our study, 20% Citric acid solution as intermediate irrigant showed moderate effect in prevention of precipitate formation. However, Matthias Zehnder et al¹⁹ and Maira Prado et al²³ stated that an exothermic reaction (oxidising reaction) takes place when citric acid, an organic acid comes in contact with sodium hypochlorite solution leading to formation of bubbles releasing Chlorine gas (Cl₂) rendering sodium hypochlorite solution ineffective.²⁴ In our study, 0.2% Chitosan solution as intermediate irrigant also showed minimal orange-brown precipitate formation. It selectively gets oxidized (on its C-6 primary alcohol groups by 2,2,6,6-tetramethyl-1-piperidine oxoammonium ion) upon contact with sodium hypochlorite solution.²⁵ It shows chelation reaction and is responsible for the elimination of dentin calcium ions from root canal dentin and showed optimal prevention of orange-brown precipitate formation.^{26,27} Pre-heated (65°C) distilled water showed to reduce its surface tension, viscosity, improves its reach, dispersion and flow especially in the apical-thirds anatomical complexities of the root canal system.²⁸ The findings of our study showed, Preheating (65°C) has increased the distilled water ability as intermediate irrigant to remove the residual NaOCl deposited on root canal walls compared to use of distilled water at room temperature. MC Dos Santos²⁹, D. Sonntag³⁰, RG. Macedo³¹, D. Sonntag et al³⁰ observed that any preheated solutions used as canal irrigant returns to normal body temperature (37°C) within average time of 60 seconds, thus makes it safe to use in endodontic treatment. However, the findings of our study showed either the use of distilled water at room temperature or preheated as intermediate canal irrigants were not highly effective in completely preventing the formation of orange-brown precipitate and these findings are in accordance with studies of Maiola EC et al³², M. Do Prado et al.³³

In our study, the deposition of orange-brown precipitate in the control group specimens was more concentrated

in coronal and middle-third's of root canals and it is due to larger diameter of dentinal tubules in these areas supposedly served as reservoir for NaOCl, thus resulted in a greater amount of precipitate formation upon its direct interaction with CHX, as no intermediate canal irrigant was used in the control group.

Previous studies used Stereomicroscope to evaluate the presence (or) absence of orange-brown precipitate, because it allowed an accurate examination of colour changes on dentin walls of root canals without modifying or damaging the specimens.^{5,33} The limitation of our study was, we used only Stereomicroscope for evaluation of orange-brown precipitate, however an SEM (Scanning Electron Microscope) examination along could have shown the extent in the depth of precipitate formation inside the radicular dentinal tubules.

Clinicians need to have a thorough understanding of the potential antagonistic interactions of NaOCl and CHX solutions upon their direct contact as root canal irrigants during routine endodontic treatment, as it can adversely affect success of root canal treatment by negatively altering tissue dissolution, antimicrobial effectiveness, cleaning efficacy and sealing ability of obturation. Additionally, there is a risk of tooth discoloration and most importantly potential adverse effects on a patient's overall health due to the leaching of chemicals (PCA) into the periradicular tissues.³⁴

CONCLUSION

Both Normal saline and Distilled water exhibited least efficiency in preventing orange-brown precipitate formation. 70% Isoprophyl alcohol as Intermediate root canal irrigant showed maximum efficiency in the prevention of orange-brown colour precipitate formation at all 3-levels (coronal, middle and apical-third's) of root canals followed by 0.2% Chitosan solution, as they completely neutralized the effect of NaOCl on the walls of root canals, thus preventing any interaction with CHX gluconate used as root canal irrigants.

DECLARATION

Conflict of Interest

There are no conflicts of interest.

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Competing Interests

The authors have no competing interests to declare.

1. Moorer WR, Wesselink PR. Factors promoting the tissue dissolving capability of sodium hypochlorite. *Int Endod J.* 1982;15:187–196.
2. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. *Oral Surg Oral Med Oral Pathol.* 1965;20:340–349.
3. John E. Thomas, Daniel S. An in-vitro spectroscopic analysis to determine whether para-chloroaniline is produced from mixing sodium hypochlorite and chlorhexidine. *J Endod.* 2010;36;2:315-317.
4. Gutmann JL, Dumsha TC, Lovdahl PE. Problem Solving in Endodontics. Chapter-11. Problem solving in Cleaning and Disinfecting the root canal system. Page no.209-217.4th edition. St Louis, MO: Elsevier; 2006.
5. Krishnamurthy S, Sudhakaran S. Evaluation and prevention of the precipitate formed on interaction between sodium hypochlorite and chlorhexidine. *J Endod.* 2010;36;7:1154-1157.
6. M. Haapasalo, Y. Shen, Z. Wang, Y. Gao. Irrigation in endodontics. *British Dental Journal.* 2014;216;6:299–303.
7. Estrela C, Estrela CRA, Barbin EL, Sapno JC, Marchesan MA, Pecora JD. Mechanism of action of sodium hypochlorite. *Braz Dent J.* 2002;13;2:113–117.
8. Holland R, Soares IJ, Soares IM. Influence of irrigation and intracanal dressing on the healing process of dog's teeth with apical periodontitis. *Endod Dent Traumatol.* 1992;8;6:223–229.
9. White RR, Hays GI, Janer IR. Residual antimicrobial activity after canal irrigation with chlorhexidine. *J Endod.* 1997;23;4:229–231.
10. Kuruvilla JR, Kamath MP. Antibacterial activity of 2.5% sodium hypochlorite and 0.2% chlorhexidine gluconate separately and combined as endodontic irrigants. *J Endod.* 1998;24;7:472–476.
11. Leonardo MR, Tanomaru Filho M, Silva LA, Nelson Filho P, Bonifacio KC, Ito IY. In vivo antimicrobial activity of 2% chlorhexidine used as a root canal irrigating solution. *J Endod.* 1999;25;3:167-171.
12. Okino LA, Siqueira EL, Santos M, Bombana AC, Figueiredo JA. Dissolution of pulp tissue by aqueous solution of chlorhexidine digluconate and chlorhexidine digluconate gel. *Int Endod J.* 2004;37;1:38-41.
13. PP Wright, B. Kahler, LJ Walsh. Alkaline sodium hypochlorite irrigant and its chemical interactions. *Materials.* 2017;29;10:1147:1-8.
14. Bui TB, Baumgartner JC, Mitchell JC. Evaluation of the interaction between sodium hypochlorite and chlorhexidine gluconate and its effect on root dentin. *J Endod.* 2008;34;2:181-185.
15. Basrani BR, Manek S, Sodhi RNS, Fillery E, Manzur A. Interaction between sodium hypochlorite and chlorhexidine gluconate. *J Endod.* 2007;33;8:966-969.
16. Arslan H, Uygun AD, Keskin A, Karatas E, Seckin F, Yildirim A, et al. Evaluation of orange-brown precipitate formed in root canals after irrigation with chlorhexidine and QMix and spectroscopic analysis of precipitates produced by a mixture of chlorhexidine/NaOCl and QMix/NaOCl. *Int Endod J.* 2015;48:1199-1203.
17. Bystrom A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. *Scand J Dent Res.* 1981;89;4:321-328.
18. Metzger Z, Zary R, Cohen R, Teperovich E, Paque F. The quality of root canal preparation and root canal obturation in canals treated with rotary versus self-adjusting files: A three-dimensional micro-computed tomographic study. *J Endod.* 2010;36;9:1569-1573.
19. Zehender M. Root canal irrigants. *J Endod.* 2006;32;5:389-398.
20. Gupta H, Kandaswamy D, Manchanda SK, Shourie S. Evaluation of the sealing ability of two sealers after using chlorhexidine as a final irrigant: An in vitro study. *J Conserv Dent.* 2013;16;1:75-78.
21. Akisue E, Tomita VS, Gavini G, de Figueiredo JAP. Effect of the combination of sodium hypochlorite and chlorhexidine on dentinal permeability and scanning electron microscopy precipitate observation. *J Endod.* 2010;36;5:847-850.
22. Rossi-Fedele G, Dogramaci EJ, Guastalli AR, de Figueiredo JAP. Antagonistic interactions between sodium hypochlorite, chlorhexidine, EDTA, and citric acid. *J Endod.* 2012;38;4:426-431.
23. Prado M, Santos Junior HM, Rezende CM, Pinto AC, Faria RB, Simao RA, Gomes BP. Interactions between irrigants commonly used in Endodontics practice: A chemical analysis. *J Endod.* 2013;39;4:505-510.
24. Barbin LE, Saquy PC, Guedes DF, Sousa-Neto MD, Estrela C, Pecora JD. Determination of para-chloroaniline and reactive oxygen species in chlorhexidine and chlorhexidine associated with calcium hydroxide. *J Endod.* 2008;34;12:1508-1514.
25. Yoo SH, Lee JS, Park SY, Kim YS, Chang PS, Lee HG. Effects of selective oxidation of chitosan on

- physical and biological properties. *Int J Biol Macromol.* 2005;35;1-2:27-31.
26. Silva PV, Guedes DF, Nakadi FV, Pecora JD, Cruz-Filho AM. Chitosan: a new solution for removal of smear layer after root canal instrumentation. *Int Endod J.* 2013;46;4:332-338.
 27. M Rhazi, J. Desbrieres, A. Tolaimate, M. Rinaudo, P. Vottero, A. Alagui, et al. Influence of the nature of the metal ions on the complexation with chitosan. Application to the treatment of liquid waste. *European Polymer Journal.* 2002;38;8:1523-1530.
 28. C. Poggio, M. Ceci, R. Beltrami, M. Colombo, A. Dagna. Viscosity of endodontic irrigants: influence of temperature. *Dental Research Journal.* 2015;12;5:425-430
 29. MC Dos Santos, C da Silveira Teixeira, LD Garcia, B Henriques, FR Tay, EA Bortoluzzi. Heated distilled water with or without continuous ultrasonic irrigation improves final irrigation efficacy and reduces dentine erosion. *Journal of Dentistry.* 2020;103:103-507
 30. D. Sonntag, W. H.M. Raab, E. Martin, R. Keppel. Intracanal use of heated rinsing solutions: A pilot study. *Quintessence International.* 2017;48;4:281-285.
 31. RG. Macedo, B Verhaagen, M. Versluis, L. Van der Sluis. Temperature evolution of preheated irrigant injected into a root canal ex-vivo. *Clinical Oral Investigations.* 2017;21;9:2841-2850.
 32. Maiola EC, Boppre LM, Savaris JM, Dias Junior LCL, Garcia LDFR, Teixeira CDS, et al. Did in-between rinsing and agitating with distilled water prevents precipitate formation by the interaction between sodium hypochlorite and chlorhexidine canal irrigants. *Microsc Res Tech.* 2024;87;2:315-325.
 33. M. Do Prado, R A Simao, BPFA Gomes. Evaluation of different irrigation protocols concerning the formation of chemical smear layer. *Microscopy Research and Technique.* 2013;76;2:196-200.
 34. Rossi-Fedele G, Dogramaci EJ, Guastalli AR, Steier L, de Figueiredo JA. Antagonistic interactions between sodium hypochlorite, chlorhexidine, EDTA and citric acid. *J Endod.* 2012;38;4:426-431.