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EFFICACY IN DEBRIS AND SMEAR LAYER REMOVAL OF TWO DIFFERENT IRRIGATING SOLUTIONS- AN INVITRO STUDY

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ABSTRACT

Aim: The purpose of this invitro study was compare the smear layer removing capability of sodium hypochlorie and super oxidised water with or with out EDTA. **Materials and Methods:** 80 mandibular first premolar were decoronated apical to the cemento enamel junction to standardize the canal length to 16 mm and working length of 15 mm. The apical orifice was then covered with inlay wax to achieve a close model of irrigation.

The samples were divided into five groups(n=10), based on the final irrigation to be used.

- Group 1: Instrumentation with Hflex CM (25\06) and irrigation with saline (Control group)
- Group 2: Instrumentation with Hyflex CM (25\06) and irrigation with sodium hypochlorite
- Group 3: Instrumentation with Hyflex CM (25\06) and irrigation with sodium hypochlorite and EDTA
- Group 4: Instrumentation with Hyflex CM (25\06) and irrigation with super oxidised water
- Group 5: Instrumentation with Hyflex CM (25\06) and irrigation with super oxidised water and EDTA.

The irrigation needle size used is 30 gauge with closed end and side vented. Finally, the specimens were irrigated with 5 mL sterile distilled water, dried, temporarily sealed, and stored separately in labeled bottles and were evaluated under scanning electron microscope after sectioning.

Inferential statistics – Friedman's test followed by Wilcoxon Signed Rank Post hoc test will be used to compare the mean debris removal scores between different study groups. The level of significance will be set at $P<0.05$. **Results:** According to the comparison, irrigation with superoxide and EDTA showed higher debris removal. **Conclusion:** Super-oxidized water along with EDTA proved to be significantly equal in smear layer removal when compared to sodium hypochlorite and EDTA. Considering the side effects and cytotoxicity of NAOCL, super oxidised water can be considered as a final irrigant after biomechanical preparation.

KEYWORDS: EDTA, NAOCL, Superoxidised water, Hyflex CM.

INTRODUCTION

- During the chemomechanical preparation process of the root canal system, an amorphous and irregular layer is produced on the root canal wall (Torabinejad et al., 2002).
- The removal of smear layer, including organic pulpal remnants, inorganic dentinal debris, microorganisms and their by-products, has been recommended to improve antibacterial activity of irrigation solution and penetration of endodontic sealers into the dentinal tubules (Abarajithan et al., 2011; Torabinejad et al., 2002).
- Currently, Sodium hypochlorite (NaOCl) (0.5–6%) and ethylenediaminetetraacetic acid (EDTA) (15–17%) are the commonly used intracanal irrigants in endodontic practice.
- Many in vitro and in vivo studies have reported moderate to **severe cytotoxicity** when sodium hypochlorite solution in clinically recommended concentrations is extruded through the apex. Moreover, EDTA associated with NaOCl has proved

to induce dentin erosion and adversely affect the dentin microstructure by altering the primary ratio of organic and inorganic components.

- All these deleterious effects, leading to a significant decrease in dentin's elastic modulus and flexural strength, as well as potential cellular damage, may jeopardize the long-term prognosis of the tooth.
- Therefore, super-oxidized, or electrochemically activated, water has been previously suggested as one of the potential alternatives for the conventional application of NaOCl and EDTA.

MATERIALS AND METHODS

PROCEDURE

All the required armamentarium and the restorative materials for the study were procured.

STEP 1: Eighty mandibular first premolar were decoronated apical to the cemento enamel junction to standardize the canal length to 16 mm with a diamond disc under water coolant mounted on a straight micro motor handpiece.

STEP 2: The canal patency was determined by passing a no. 8 size kfile in narrow canals and 10 k-file in medium sized root canals until the tip of the file was visible at the apical foramen.

STEP 3: Those teeth with wider apical patency were discarded and replaced with appropriate teeth. Working lengths were established by subtracting 1mm from the measurement obtained when a size 10 file was placed into the canal until its tip was visible at the apex.

STEP 4: The working length of the samples was 15 mm . Initial negotiation of root canal space was performed using a size 15 manual K-file used in a watch-winding motion to assure the presence of a glide path .In all the experimental groups , the apical orifice was then covered with inlay wax. This was done to achieve a close model of irrigation.

STEP 5: 2ml of 0.9% saline was used as an irrigant during instrumentation of all canals. Based on the final irrigant used for irrigation, the samples were divided into five groups (n=10)

- **Group 1:** Instrumentation with Hflex CM (25\06) and irrigation with saline (Control group)
- **Group 2:** Instrumentation with Hyflex CM (25\06) and irrigation with sodium hypochlorite
- **Group 3:** Instrumentation with Hyflex CM (25\06) and irrigation with sodium hypochlorite and EDTA
- **Group 4:** Instrumentation with Hyflex CM (25\06) and irrigation with super oxidised water
- **Group 5:** Instrumentation with Hyflex CM (25\06) and irrigation with super oxidised water and EDTA.

Each irrigant was used in the following manner.

i) 30 sec conventional syringe irrigation
ii) 20 sec passive ultrasonic irrigation
iii) 20 sec conventional syringe irrigation
iv) 20 sec passive ultrasonic irrigation
v) 30 sec syringe irrigation.

- 1.5mL of irrigant was used in the first 30 s of irrigation, 1 mL in between the two periods of passive ultrasonic irrigation and then 1.5 mL of irrigant in the final stage of irrigation

Step 6: The irrigation needle size used is 30 gauge with closed end and side vented. Finally, the specimens were irrigated with 5 mL sterile distilled water, dried, temporarily sealed, and stored separately in labeled bottles.

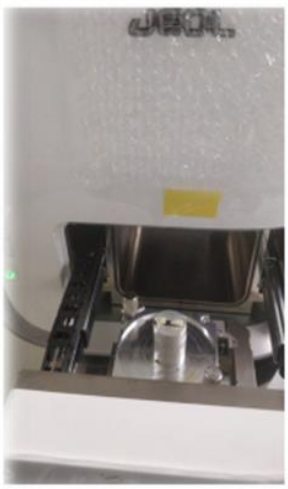
Step 7: The canals were dried with absorbent paper points, and the entrance to each of the canals was protected with a cotton pellet.

Step 8: Using carborundun discs, deep grooves were cut on the buccal and palatal surfaces of the roots, without perforating the root canal.

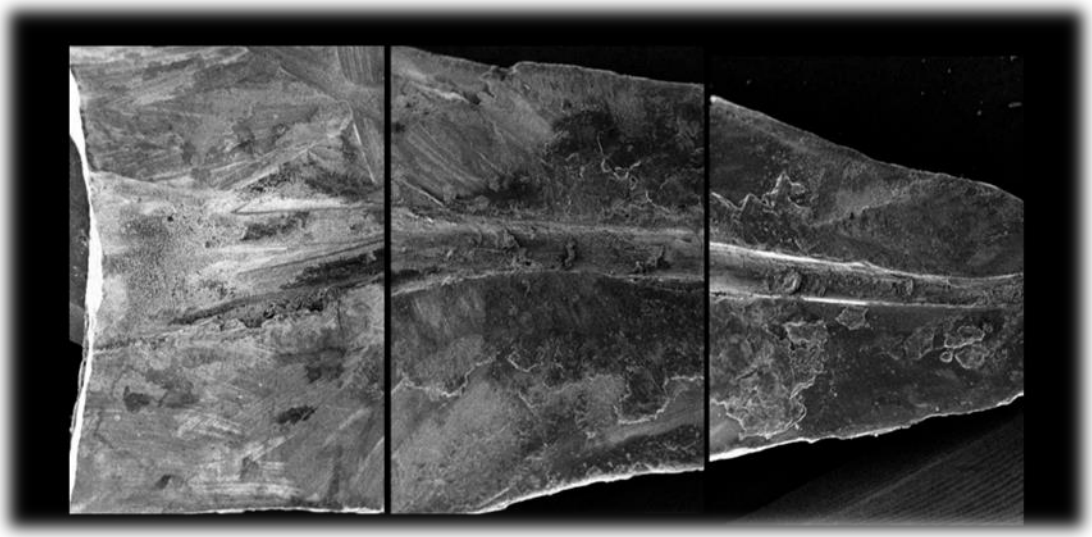
Step 9: The roots were then split with a chisel and a hammer. One half of each tooth was selected and prepared for SEM examination.

Step 10: After assembly on coded stubs, the specimens were placed in a vacuum chamber and sputter-coated with a 300 A˚ gold layer (BalTec SCD 005; Bal-Tec Co., Balzers, Liechtenstein).

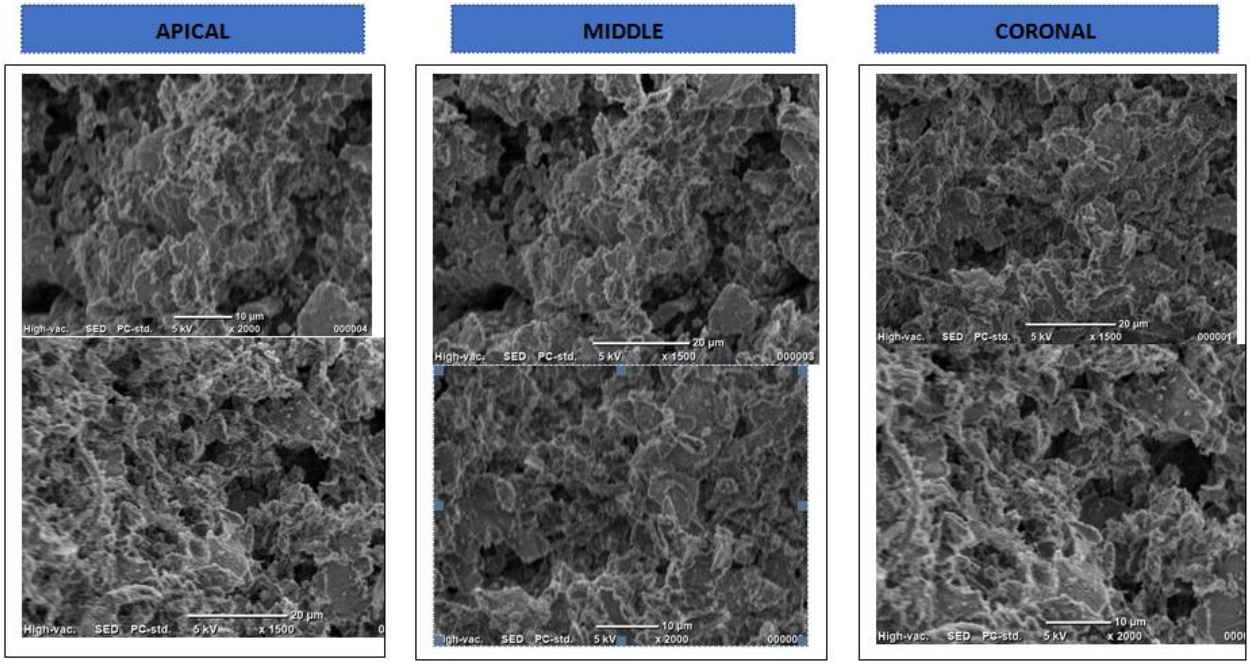
Step 11: The specimens were then analysed using a Zeiss EVO SEM XL 30 (Carl Zeiss Microscopy).



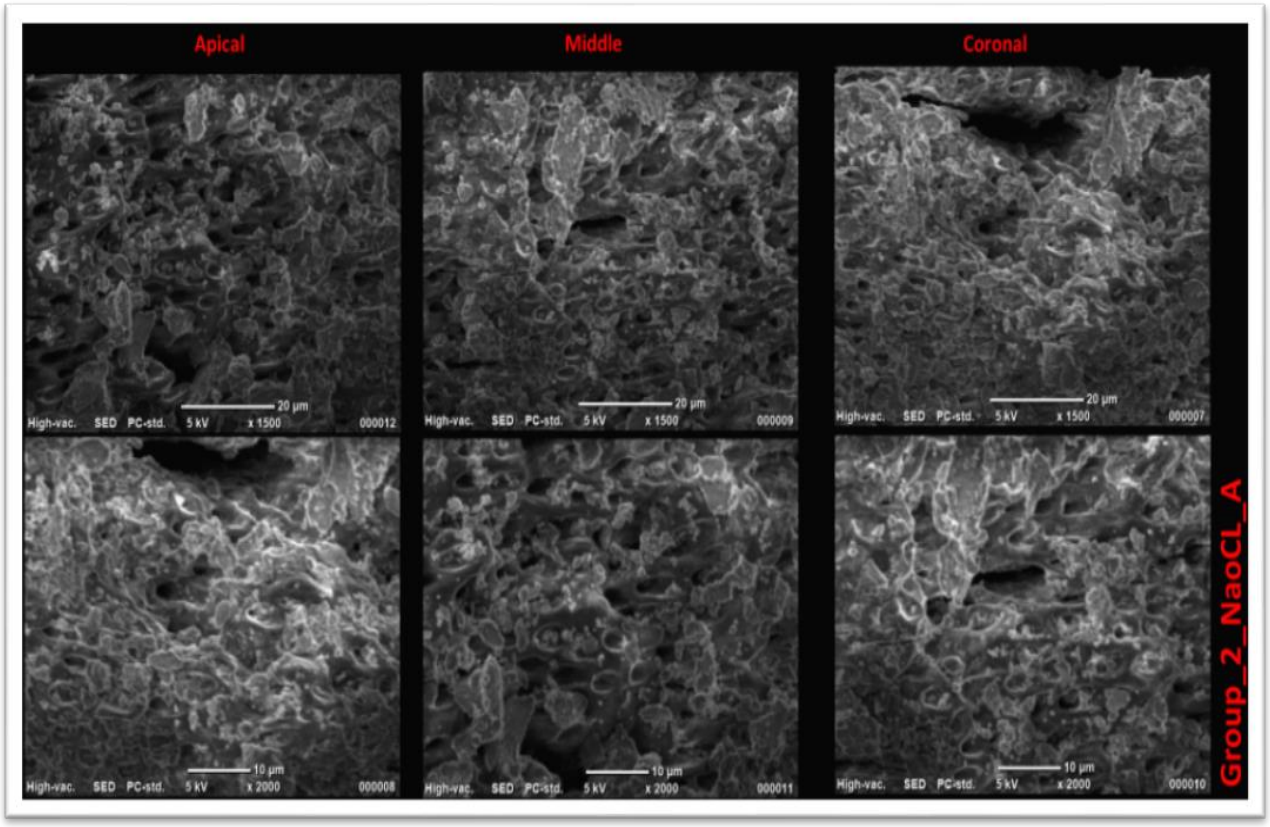
SCANNING ELECTRON MICROSCOPY IMAGES



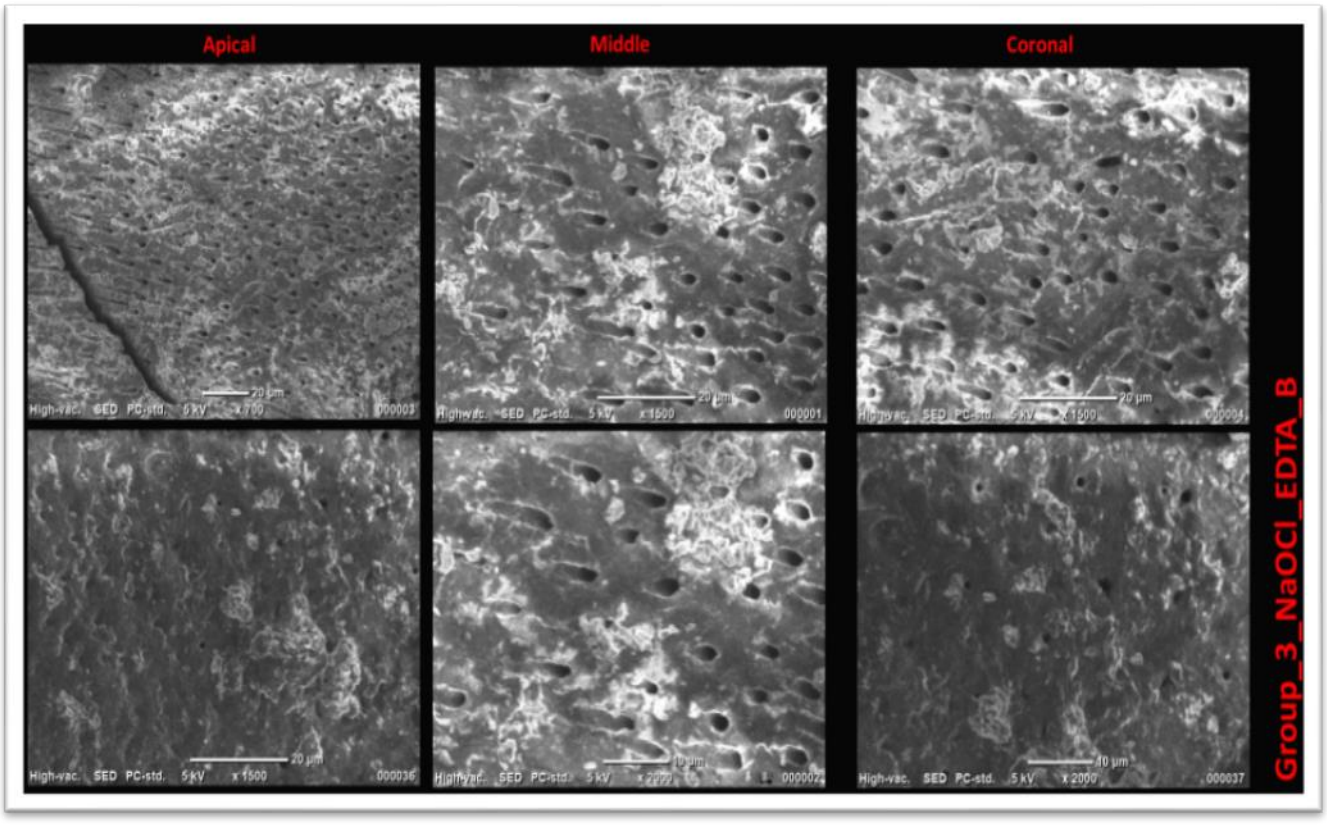
Group 1: Saline.



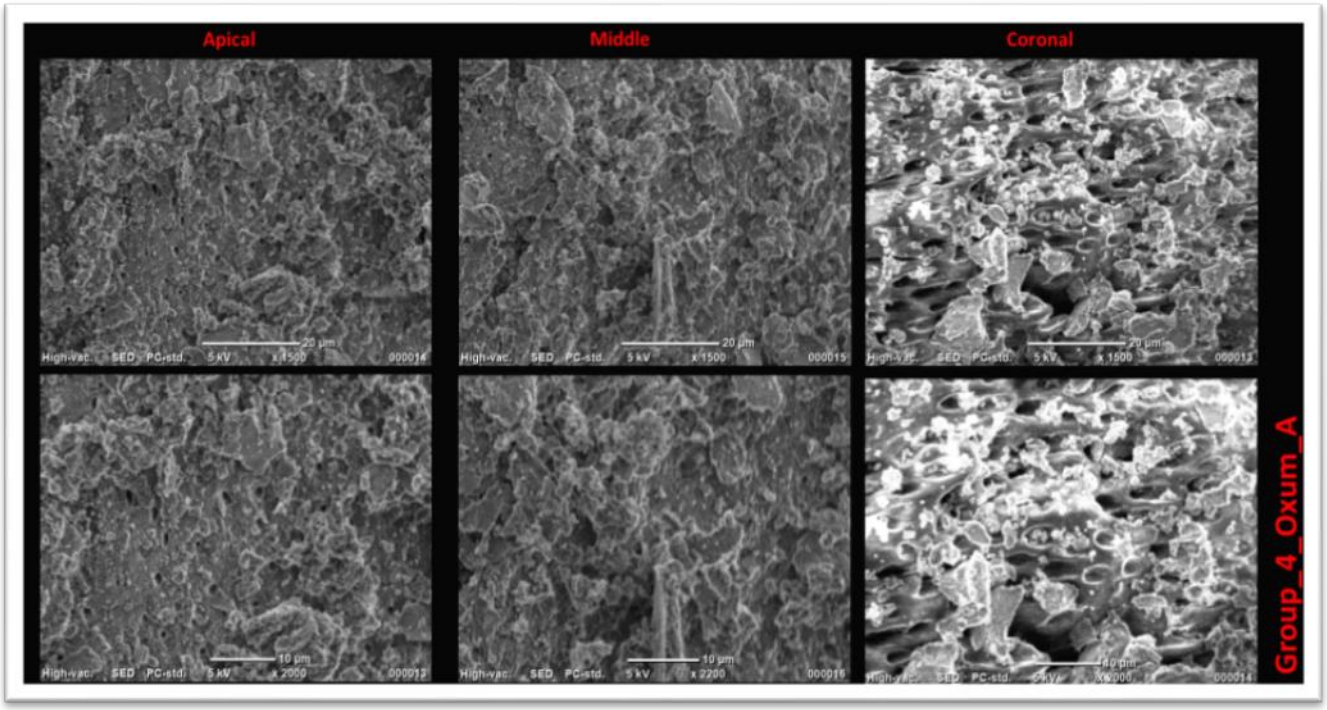
GROUP 2 : NaOCl.



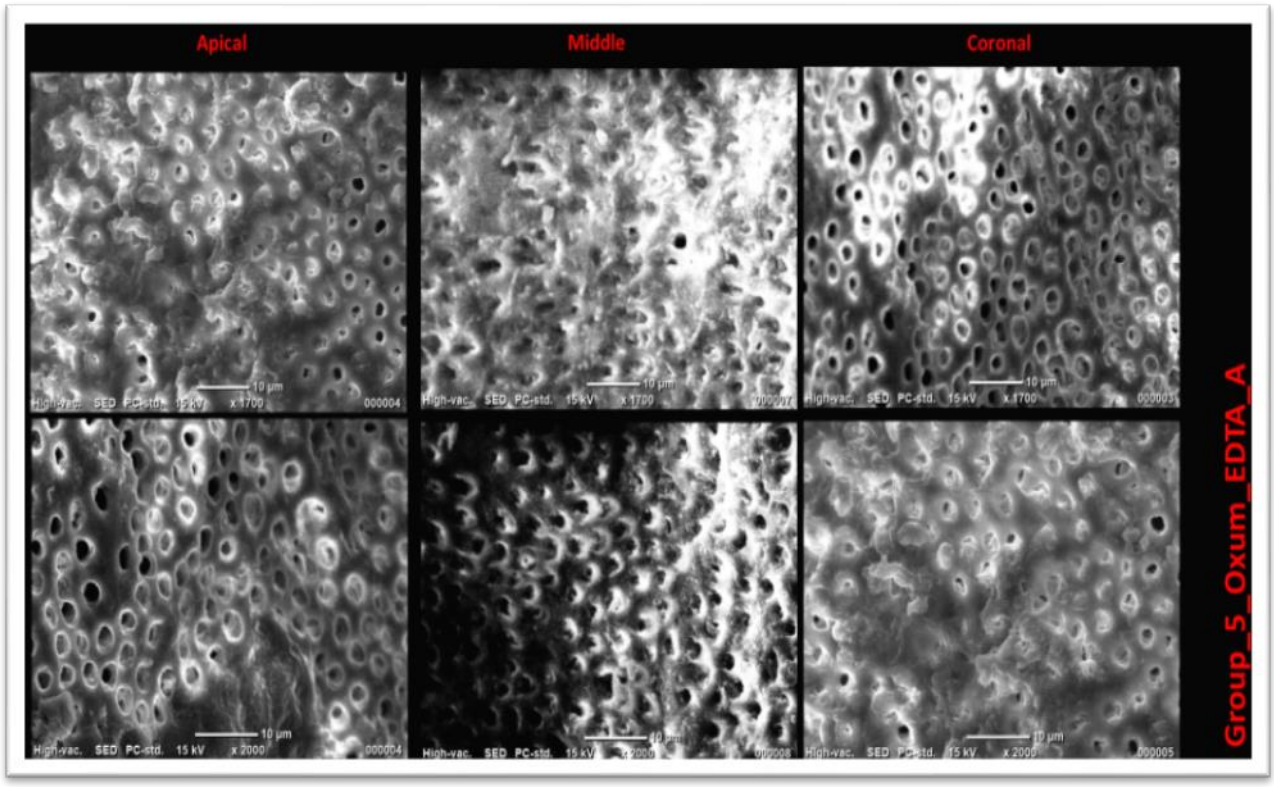
GROUP 3: NaOCl EDTA.



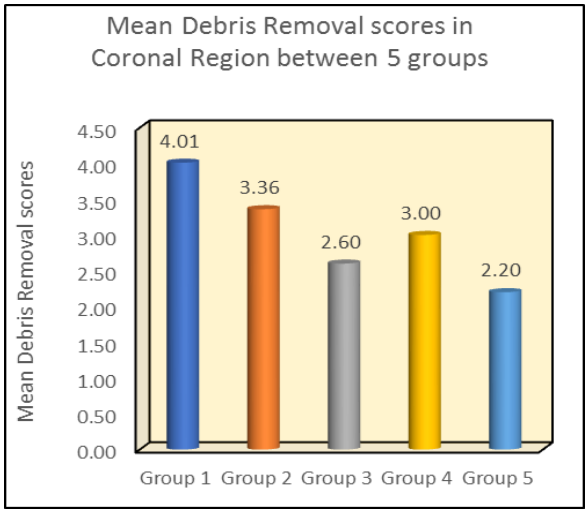
GROUP 4: Oxum.

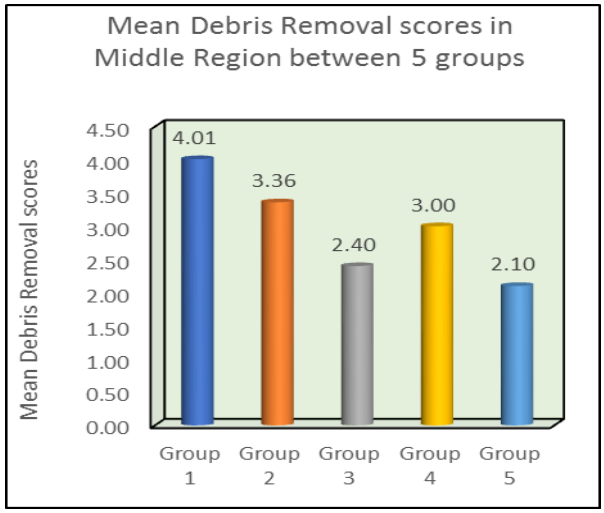


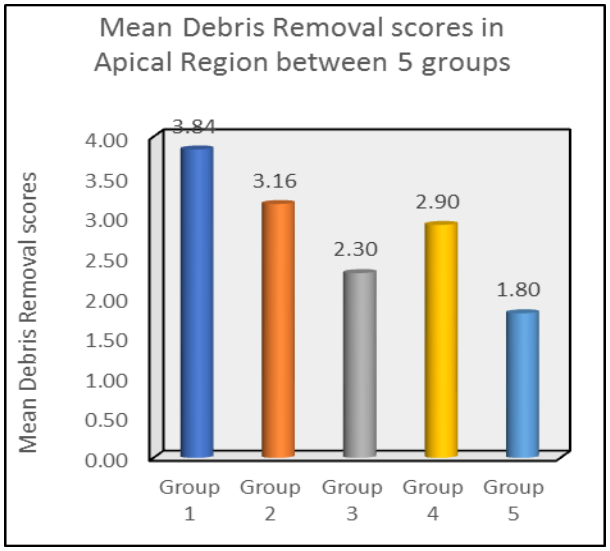
GROUP 5: Oxum EDTA.



The smear layer was scored according to the criteria given by Hulssman et al .
Score 1: No debris.
Score 2: Clumps of debris covering
Score 3: Clumps of debris covering 25–50% of the canal wall.
Score 4: Clumps of debris covering more than 50–75% of the canal wall.
Score 5: More than 75% of canal wall covered by debris







Multiple comparison of mean difference in Debris Removal scores b/w groups in Coronal Region using Dunn's Post hoc Test

(I) Groups	(J) Groups	Mean Diff. (I-J)	95% CI for the Diff.		p-value
			Lower	Upper	
Group 1	Group 2	0.66	-0.05	1.36	0.10
	Group 3	1.41	0.71	2.11	<0.001*
	Group 4	1.01	0.31	1.71	0.007*
	Group 5	1.81	1.11	2.51	<0.001*
Group 2	Group 3	0.76	0.06	1.46	0.03*
	Group 4	0.36	-0.34	1.06	0.31
	Group 5	1.16	0.46	1.86	0.001*
Group 3	Group 4	-0.40	-1.10	0.30	0.27
	Group 5	0.40	-0.30	1.10	0.24
Group 4	Group 5	0.80	0.10	1.50	0.02*

STATISTICAL ANALYSIS

Friedman's test followed by Wilcoxon Signed Rank Post hoc test was used to compare the mean debris removal scores between different study groups. The level of significance will be set at P<0.05.

RESULTS

Multiple comparison between groups revealed that Group 5 showed significantly higher mean debris removal scores as compared to Group 1, 2 and 4 at $p<0.001$, $p=0.002$ & $p=0.02$ respectively. This was then followed next by Group 3 showing significantly higher mean scores as compared to Group 1 and 2 at $p<0.001$ & $p=0.03$ respectively. Finally, Group 4 also showed significantly lesser mean debris removal scores as compared to Group 1 and it was statistically significant at $p=0.007$.

However, no significant differences were noted between other groups at coronal region.

DISCUSSION

- Root canal treatment usually involves the chemo mechanical removal of bacteria and infected dentine from within the root canals. The process is often followed by an intracanal dressing and a root filling. Amongst important factors affecting the prognosis of root canal treatment is the seal created by the filling against the walls of the canal. Goldberg et al did considerable effort to understand the effect of the smear layer on the apical and coronal seal.
- American Association of Endodontists defined smear layer as a "surface film of debris retained on dentin or other surfaces after instrumentation with either rotary instruments or endodontic files; consists of dentin particles, remnants of vital or necrotic pulp tissue, bacterial components & retained irrigants" The thicknessof smear layer depends on the type and sharpness of cutting instrument and also whether the cutting was carried out on dry or wet dentin.
- Although sodium hypochlorite appears to be the most desirable single endodontic irrigant . it cannot dissolve inorganic dentin particles. Demineralizing agents such as ethylenediamine tetraacetic acid (EDTA) have therefore been recommended as adjuvants in root canal therapy according to Garberoglio et al .
- EDTA is a chelator, which is used after NaOCl as the final irrigant. EDTA solution is neutral or slightly alkaline; it is usually used as a 15% or 17% solution, Complete cleaning of the root-canal system requires the use of irrigants that dissolve organic and inorganic material.
- As hypochlorite is active only against the organic debris, other substances must be used to complete the removal of the smear layer and dentin debris. EDTA effectively dissolve inorganic material, including hydroxyapatite. They have little or no effect on organic tissue and alone they do not have antibacterial activity, despite some conflicting reports on EDTA.
- According to studies, the smear layer removal ability of EDTA is two minutes, but thick layers may require longer times of exposure. The smear layer should be removed as it contains microbes and microbial antigens baked into it during instrumentation of the necrotic, infected root canal .
- The complete removal of the smear layer can only be achieved when NaOCl has been used before the final rinse with EDTA.
- sodium hypochlorite has various undesirable characteristics such as tissue toxicity, risk of emphysema when overfilling, allergic potential, a disagreeable smell and taste. Moreover, it might not completely cleanse the surfaces of the root canal walls. The long-term exposure to high concentrations of hypochlorite can lead to considerable reduction in the flexural strength and elastic modus of dentin. Even short-term irrigation with hypochlorite after EDTA or Citric Acid at the end of chemomechanical preparation causes strong erosion of the canal-wall surface dentin.
- Because of these drawbacks of NAOCL and EDTA alternative irrigant solution with less side effects are in search.
- Super-oxidized water is a powerful anti-microbial agent against bacteria, fungi, protozoa and viruses. It is rich in reactive oxygen with a neutral pH and studies show its ability to remove the smear layer. Super-oxidised water, is stable & has longer shelf life.
- In this study smear layer removing ability of NAOCL and super oxidised water with or without EDTA were evaluated at coronal, middle and apical third of the root.
- During the conventional needle irrigation, replenishment and fluid exchange do not extend much beyond the tip of the irrigating needle.
- The trapped air in the apical third of root canals causing a vapour lock effect may obstruct the exchange of irrigants and hamper the debridement effectiveness of irrigants.
- Ahmad et al reported that the use of ultrasonics has been suggested to improve irrigation in the root canal. Less debris and smear layer have been observed in the apical region of the canal than its coronal aspects with the use of ultrasonics; this effect is thought to be generated by acoustic streaming.
- In this study 4 ml of each irrigating solutions are irrigated for about 2 minutes under with ultrasonic irrigation. This is in accordance with Bhuva et al which states that an irrigation time of 2 min shown to eradicate the biofilm reliably within this time period along with the removal of smear layer.
- Previous studies have confirmed that electrochemically activated water exhibits broad-spectrum antimicrobial activity, effectively prevents biofilm formation and leads to a considerable reduction in microbial load, with no significant differences to NaOCl or chlorhexidine gluconate (CHX).
- super-oxidized water has no significant differences in debris and smear layer removal as compared to the conventional root canal irrigation protocol using

NaOCl and EDTA. The exact mechanism by which super-oxidized water dissolves tissues is not fully understood. Evidence suggests that the tissue-dissolving properties of chlorine-based solutions are mainly determined by OCl^- ions, which begin to be released in low amounts from HOCl at pH values above 5.5 and become the completely dominant form at a pH of 9

- However, the OCl^- concentration in super-oxidized water is generally assumed to remain low due to its pH range of 5 to 6.5. Theoretically, these pH values could be increased by the buffering effect of dentin, leading to the higher amount of OCl^- ions and thus the more efficient removal of debris and the smear layer.
- Super-oxidized water is a powerful anti-microbial agent against bacteria, fungi, protozoa and viruses. It is rich in reactive oxygen with a neutral pH. The main advantage of this super-oxidized water is that it is stable and has a longer shelf life. It mainly contains oxidized solution (H2 O), sodium hypochlorite, hypochlorous acid, hydrogen peroxide, ozone, chlorine dioxide, sodium hydroxide, sodium carbonate and sodium chloride. The molecules are broken into ions and free radicals, which rapidly react and denature protein of bacterial cell wall. It produces an environment of unbalanced osmolarity that damages the cell wall of single cell organisms. The low pH in super oxidized water may sensitize the outer membrane of bacterial cell, thereby enabling oxygen anion radicals to attack the bacterial cell more efficiently.
- The damage is due to the difference in osmolarity between the concentrations of ions in solution versus the concentration of same ions in the cell. Multicellular organisms are not prone to such changes so host tissues are spared. For these reasons it is referred to as a well suited alternative irrigating agent. Based on the present study, it has shown that superoxidized water when used as an irrigant, cleans the root canal surfaces in a clinically significant manner and removed the smear layer in large areas leaving the collagen fibers intact and completely exposed with less erosion.

CONCLUSION

- Within the limitation of the present study, super-oxidized water along with EDTA proved to be significantly equal in smear layer removal when compared to sodium hypochlorite and EDTA. Considering the side effects and cytotoxicity of NAOCL, super oxidised water can be considered as a final irrigant after biomechanical preparation.
- However it may be worthwhile to investigate further, the effect of super oxidized water alone as a root canal irrigant to evaluate its effect on smear layer and on dentine.

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