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Scanning Electron Microscopic evaluation of Smear Layer removal using Ozonated Olive Oil, German Chamomile Oil and Chitosan Nanoparticle Solution as root canal Irrigants.

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Abstract:
Aim:*The purpose of this study is to evaluate and compare the efficacy of Ozonated Olive Oil, German Chamomile Oil and 0.2%Chitosan Nano-particle solution in thesmear layer removal using SEM analysis.*
Methods: *Thirty freshly extracted mandibular premolars were used. After biomechanical preparation, the samples were divided into* ***Group I(OZONATEDOLIVE OIL)****,* ***Group II(GERMAN CHAMOMILE OIL)****, and* ***Group III(0.2% CHITOSAN NANO-PARTICLE SOLUTION)*** *containing 10 samples each. Longitudinal sectioning of the samples was done. The samples were observed under SEM at the coronal, middle and apical levels. The images were scored according to the scoringcriteriagivenbyHulsmann. Statistical analysis was done, with the significance level set at$P < 0.05$, and performed with SPSS 16.0 statistical package for Windows.*
Results:*Smear layer removal at coronal, middle, and apical thirds was more effective when final irrigation was performed using 0.2%Chitosan Nano-particle solution , followed by German Chamomile Oil and Ozonated Olive Oil. At the apical third, all the irrigants showed poor smear layer removing property, but 0.2%Chitosan Nano-particle solutionshowed comparatively better results.*
Conclusion:*A moderate concentration of 0.2% Chitosan Nano-particle solution removes the smear layer with greater efficiency than Ozonated Olive Oil and German Chamomile Oil at the coronal, middle, and apical thirds of the root canals.*
Keywords: *Smear layer removal; SEM analysis;Chitosan nano-particle solution; Ozonated Olive oil; German Chamomile oil; Root canal irrigation; Herbal irrigants; Apical third; Endodontics; Biomechanical preparation.*

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I. INTRODUCTION:

The major role of micro-organisms in the initiation and progression of lesions like apical and peri-radicular has been very well established. An infected root canal caused either due to caries exposure or trauma cannot be removed by using the host defence mechanisms alone or in combination with systemic antibiotic therapy. Therefore, there arises a need to supply therapy in concise way along with preparation which has been referred to as chemo-mechanical preparation: as both components are necessary for successful procedural outcomes and are generally performed together [1] .

Chemo-mechanical debridement plays an important role in the success of root canal treatments. This is performed by utilizing appropriate instruments along with effective irrigating solutions, followed by sealing with suitable materials[2] .

Endodontic instrumentation produces a smear layer that covers the root canal surfaces. This layer harbours micro-organisms, infects dentinal tubules, impedes penetration or diffusion of anti-bacterial irrigants and medicaments into the dentinal tubules and compromises the seal between the filling materials and the dentinal wall. Because of its potential contamination and adverse effects on the outcome of root canal treatment, smear layer removal is recommended (Yamada et al. 1983). Eick et al. (1970) showed that the smear layer was made up of tooth particles ranging from <0.5 to 15 µm . The effectiveness of endodontic space cleaning depends on both instrumentation and irrigation. Irrigation plays a main role in successful debridement and disinfection [3].

Root canal irrigants should have the following attributes: low toxicity; antimicrobial effect; non-caustic to periodontal tissues; non-allergenic; and the ability to dissolve tissue and root canal debris, inactivate endotoxins, lubricate the canal, and remove the smear layer[4] .

Ozone, a powerful oxidizing agent, has strong bactericidal, virucidal, and fungicidal effects making it a potential agent for root canal disinfection. Ozone is non-toxic to oral cells and very efficient against antibiotic‐ resistant strains. Its effect increases in acidic pH unlike NaOCl whose rate of decomposition rapidly decreases from pH 11‐ 7.

Three fundamental forms of ozone application are: – (1) Ozonated water, (2) Ozonated Olive oil, and (3) Oxygen/Ozone gas. Ozonated water and Olive oil have the capacity to entrap and then release oxygen/ozone, constituting an ideal delivery system. Ozone, when dissolved in water, becomes highly unstable and rapidly decomposes, so it cannot be stored. In contrast, when it is dissolved in an oil base, it has a life span that could be measured in years. It chemically reacts with oil and forms long complex molecules.

Hydrolysis of ozonized oil can generate hydrogen peroxide, aldehydes, and acetones. Kishore et al. evaluated the anti-bacterial activity of the ozonized oil, calcium hydroxide, and their combination against Enterococcus faecalis and concluded that ozonized oil was the most effective medicament. Pratyusha et al. evaluated the anti-bacterial activity of the ozonated olive oil and cold pressed neem oil against E. faecalis using the agar well diffusion method and concluded that Ozonated Olive oil was more effective [5] .

Matricariachamomilla is a well-known medicinal plant from the Asteraceae family. The phyto-chemical composition of M.chamomilla essential oil and extracts has been identified as containing more than 120 constituents. M. chamomilla has been shown to have strong anti-bacterial potential against Gram-positive and Gram-negative bacteria. The chamomile plant is known to have anti-bacterial, anti-inflammatory, antiviral, and antioxidant effects, due to the presence of α-bisabolol, luteolin, quercetin, and apigenin. The clinical efficacy of chamomile has also been reported in selectively removing the root canal smear layer [4] .

Chitosan is a natural, cationic amino poly-saccharide co-polymer of glucosamine and N‐ acetyl glucosamine. These compounds are obtained by partial deacetylation of chitin, a substance obtained from the shells of crustaceans and shrimps. It is endowed with many beneficial properties such as bio-compatibility, bio-degradability, bio-adhesion, and anti-microbial activity. Its use is ecologically interesting as it has been found to be the most abundant substance in nature, after cellulose [6] .

The present study evaluates and compares the efficiency of Ozonated Olive oil, German Chamomile Oil (Matricariarecutitia), and 0.2% Chitosan nano-particle solution in their ability to remove smear layer following root canal instrumentation on human extracted teeth using a SEM.

II. MATERIALS and METHODOLOGY:

Sample Selection and preparation :

Thirty freshly extracted single‐ rooted human mandibular premolars with single root canal and closed apex were selected. The selection of teeth was based on their relative dimensions and morphology. Buccal and lingual radiographs of the teeth were taken to ensure that they had only a single canal. The teeth were cleaned of debris and soft tissue remnants, and were then stored in a sterile saline solution. In order to standardize canal

instrumentation, each tooth was decoronated and the length of the root was standardized to 16 mm using a low- speed diamond disk under water as a coolant. The working length of each root canal was established 1 mm short of the apical foramen with #15 K- file after gauging with #10 K- file.

Root canal Preparation :

The canals were instrumented in a standardized crown- down manner with sequentially sized K- files (MANI Inc.,Tochigi, Japan) up to size 40, followed by instrumentation using rotary ProTaper (Dentsply-Maillefer,Switzerland) instruments at 250 rpm upto F3 file. Root canal irrigation was performed with 2 mL of 2.5% NaOCl solution throughout instrumentation and between each file. Finally, the root canals were rinsed with 5 mL of normal saline and randomly divided into threegroups (n = 10) according to the final irrigating solution used for smear layer removal.The samples were divided into Groups I, II, and III containing 10 samples each.

Group I: 5 ml Ozonated Olive Oil solution (Ozonoid ;Adc Inc.DentozoneIndia; Mumbai, India) was used for 3 min;

Group II: 5 mL of German Chamomile Oil solution was used for 3 min;

Group III: 5 mL 0.2% Chitosan nano-particle solution for 3 min. The 0.2% chitosan nano-particle solution was prepared by dissolving 0.2 g of chitosan nano powder (Sisco Research Laboratories Pvt. Ltd, India) in 100 mL of 1% acetic acid. The mixture was agitated using a magnetic agitator for 2 h to obtain a homogenous clear solution. A stopper was placed on the needle such that it was restricted to penetrate only up to 2 mm of the working length. The root canals were then flushed with 5 mL of distilled water, and dried with sterile absorbent paper points.

Scanning electron microscopic evaluation:

Diamond discs were used at a low speed to cut deep grooves on the buccal and lingual surfaces of the roots, without perforating the root canals. The roots were then split into two equal halves with a chisel and mallet.One- half of each tooth was selected and prepared for SEM examination. The specimens were secured on metal stubs, desiccated, sputter coated with gold, and examined under SEM at ×4000 magnification. The dentinal surfaces were observed at cervical,middle, and apical thirds with a magnification of ×4,000 for the presence/absence of smear layer and visualization of the entrance to dentinal tubules. Photomicrographs (×4000) of these areas were taken. The root canal was qualitatively assessed at the coronal, middle, and apical regions of each root half of each specimen using a graded scale from 1 to 5 to assess the quality of smear layer removal according to Hulsmann criteria et al [6] .

SCORE	DESCRIPTION
Score 1	No smear layer, orifices of dentinal tubules open
Score 2	Small amount of smear layer,some dentinal tubules open
Score 3	Homogenous smear layer covering the root canals, only a few dentinal tubules open
Score 4	Complete root canal wall covered by a homogenous smear layer, no open dentinal tubules
Score 5	Heavy, homogenous smear layer covering the entire root canal walls

The degree of evaluation was scored in a blind manner based on a five- grade scale by an examiner who was not privy to the true nature and purpose of this study.

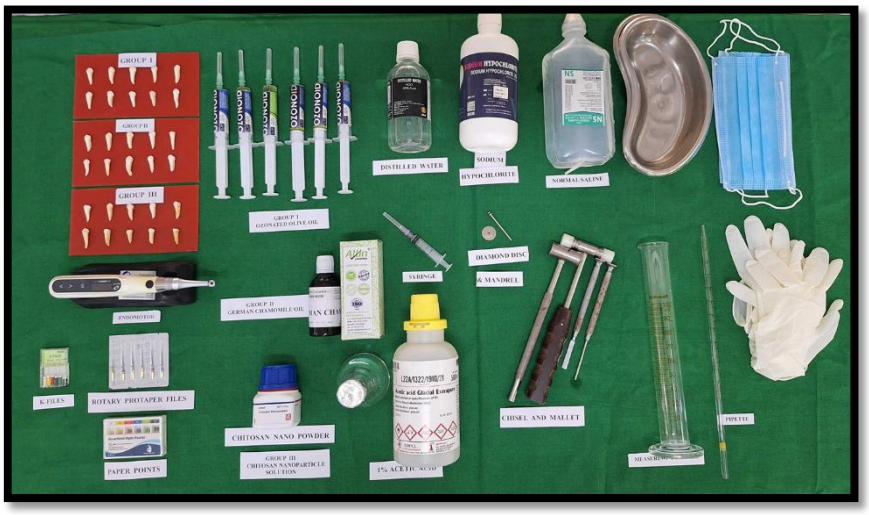

Figure 1 : Materials used in the study

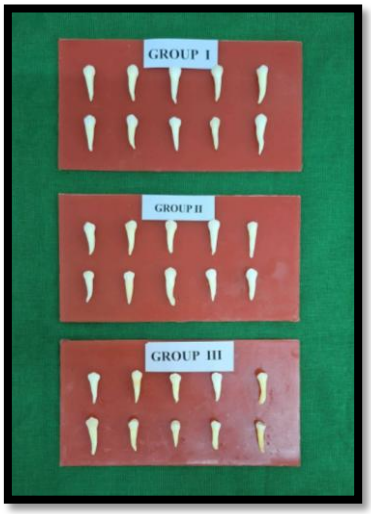

Figure 2 : Extracted Human Mandibular Premolars (n=30)

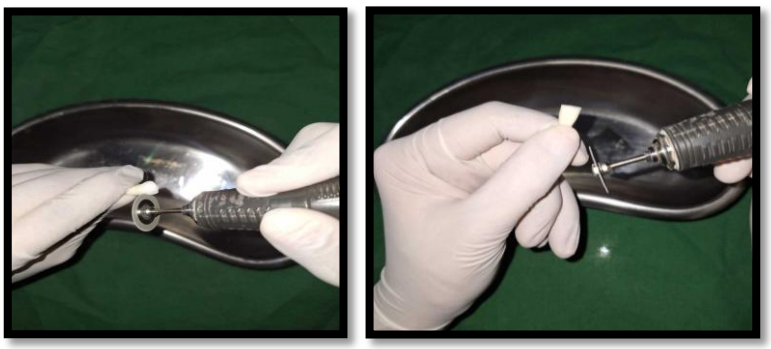

Figure 3 : Decoronation of the tooth sample using a diamond disc

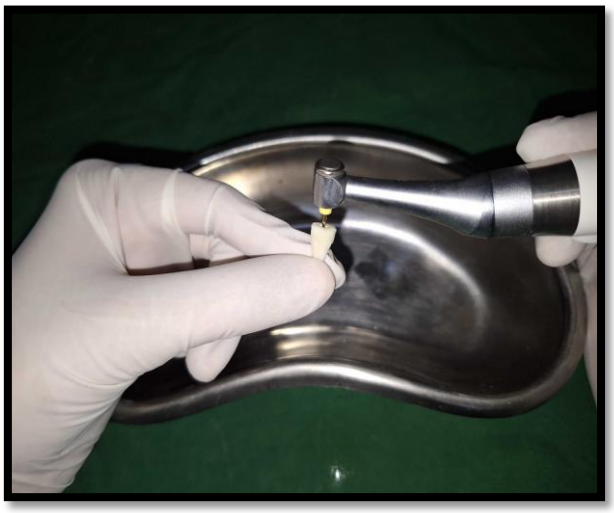

Figure 4 : Bio-mechanical preparation done using PROTAPER rotary files till F3

Figure 5 : 2 ml of 2.5 % NaOClirrigant used throughout instrumentation, between each file.

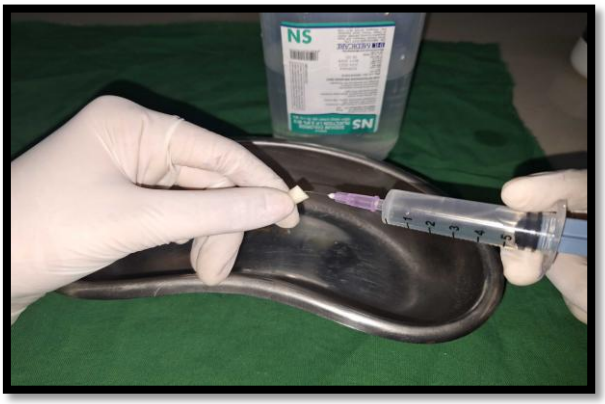

Figure 6 : After 2.5% NaOCl irrigation, the root canal is rinsed with Normal Saline

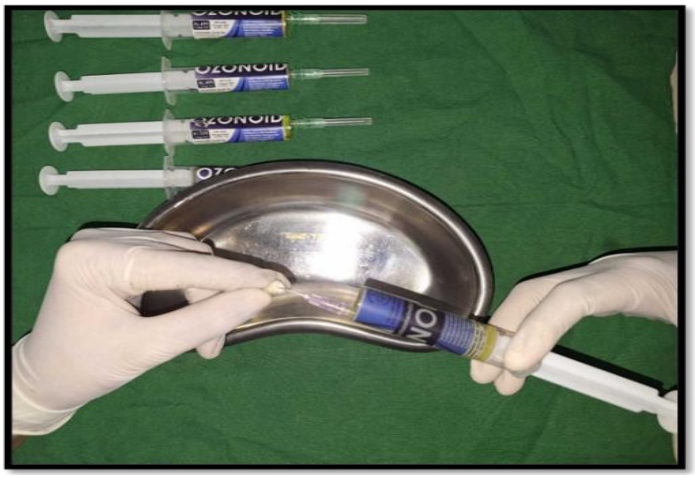

Figure 7: (GROUP 1) 5 ml of Ozonated Olive Oil is used as an Irrigant for 3 mins

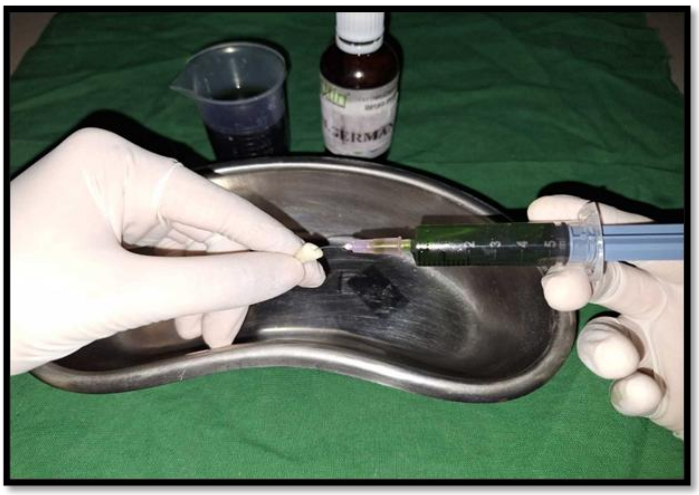

Figure 8: (GROUP 2) 5 ml of German Chamomile Oil is used as anIrrigant for 3 mins

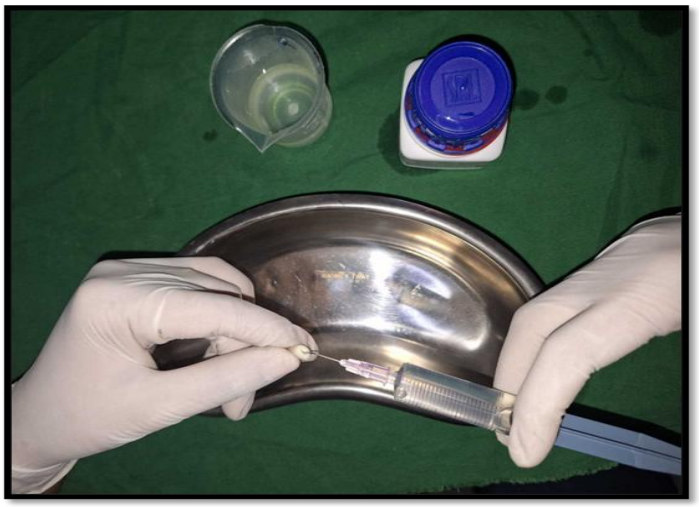

Figure 9: (GROUP 3) 5 ml of 0.2%Chitosan Nano-particle solution is used as an Irrigant for 3 mins

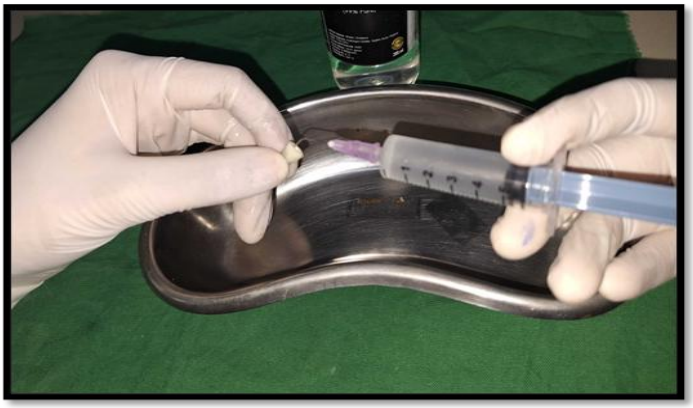

Figure 10: The root canals in all the samples are finally rinsed with Distilled water

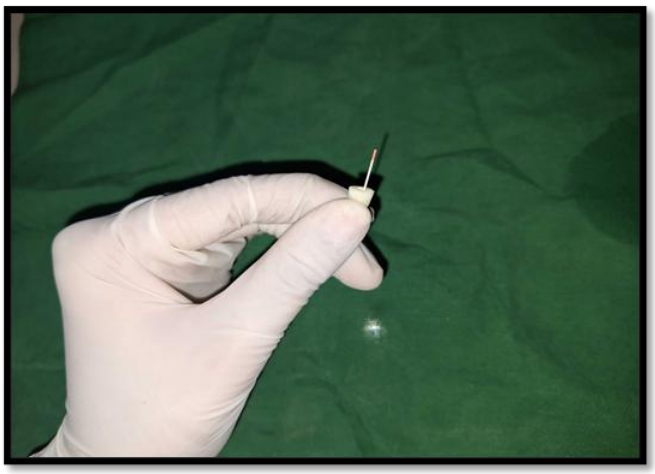

Figure 11: Drying of the root canals using Absorbent Paper points

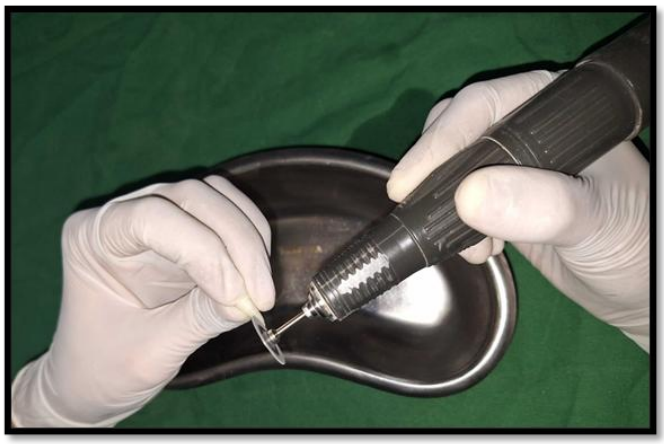

Figure 12: Diamond discs are used at a low speed to cut deep grooves on the buccal and lingual surfaces of the roots

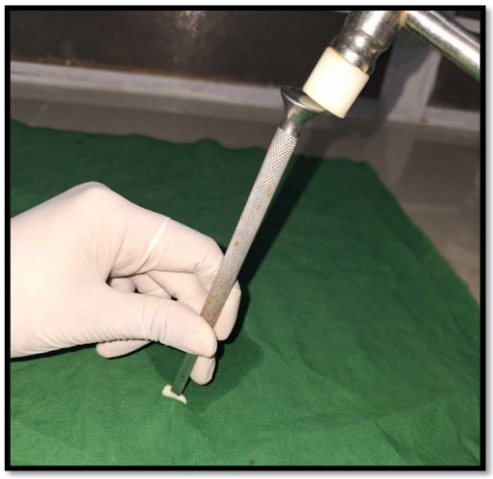

Figure 13: Splitting of the root using Chisel and Mallet

Figure 14: Split halves of the root

Statistical analysis:

Non-parametric data of smear layer scores were presented as percentage distribution and their mean ranks were calculated for each group at each root section. Kruskal–Wallis test was used to compare between final irrigation solutions at each section and Mann–Whitney U- test was used for pair- wise comparisons between the groups whenever indicated. Friedman test was used to compare between root canal thirds at each group followed by Wilcoxon signed- rank test for pair- wise comparisons between root canal thirds if necessary. The significance level was set at P = 0.05. Statistical analysis was performed with IBM SPSS 16.0 software for Windows.

III. RESULTS:

A comparison of smear layer covering the dentinal surfaces at coronal, middle, and apical root canal levels between the groups was performed [Figures 15- 17].

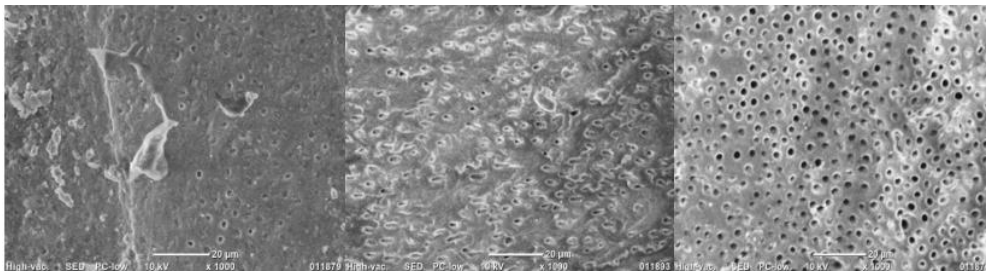

Figure 15: Coronal third (a) Ozonated Olive Oil (b) German Chamomile Oil (c) Chitosan Nano-particle solution

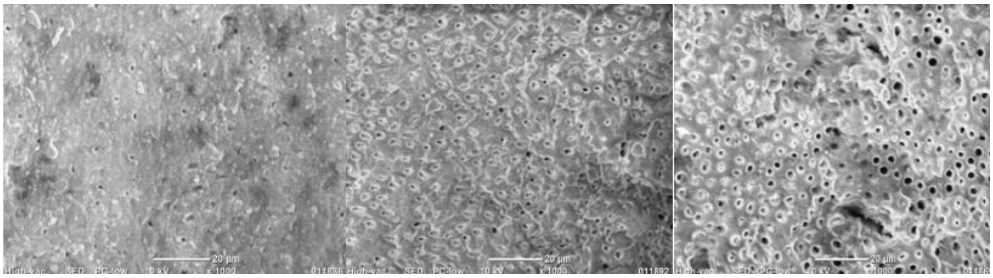

Figure 16: Middle third (a) Ozonated Olive Oil (b) German Chamomile Oil (c) Chitosan Nano-particle solution

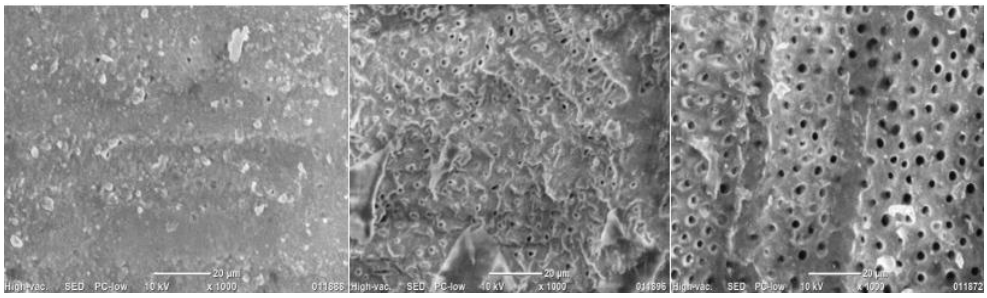

Figure 17: Apical third (a) Ozonated Olive Oil (b) German Chamomile Oil (c) Chitosan Nano-particle solution

Wilcoxon test demonstrated statistically significant differences in the coronal-apical section in the Ozonated olive oil group. Friedman test denoted that there was a statistically significant difference in the coronal root section of the Ozonated olive oil group [Table 1].

Table 1: Comparison of smear score between tooth sections in each study group

Group	N	Mean (SD)	Range	Median (Q1-Q3)	Friedman Test		Wilcoxon Signed Rank Test		
					Chi Square	P value	Coronal - Middle	Middle - Apical	Coronal - Apical
Ozonated Olive Oil									
CORONAL	10	2.30 (0.483)	1	2 (2-3)	8.222	0.016*	0.257	0.059	0.011*
MIDDLE	10	2.60 (0.516)	1	3 (2-3)					
APICAL	10	3.10 (0.316)	1	3 (3-3)					
German Chamomile Oil									
CORONAL	10	2.10 (0.316)	1	2 (2-2)	2.333	0.311	0.564	0.414	0.083
MIDDLE	10	2.20 (0.422)	1	2 (2-2.25)					
APICAL	10	2.40 (0.516)	1	2 (2-3)					
Chitosan Nano-particle Solution									
CORONAL	10	1.10 (0.316)	1	1 (1-1)	1.200	0.549	0.564	0.564	0.317
MIDDLE	10	1.20 (0.422)	1	1 (1-1.25)					
APICAL	10	1.30	1	1(1-2)					

		(0.483)							

*P<0.05 statistically significant, P>0.05 NS. NS: Nonsignificant; SD: Standard deviation

Intragroup comparison showed thatstatistically significant difference exists between the mean smear layer removal scores in the ozonated olive oil group at the coronal, middle and apical thirds ($p<0.05$). Statistically significant difference also exists between the mean smear layer removal scores in the ozonated olive oil group in the coronal versus apical thirds ($p<0.05$).

Inter-group comparison revealed the following:

- Statistically significant difference exists between the mean smear layer removal scores of all three experimental groups at the coronal, middle and apical thirds ($p<0.05$).
- Statistically significant difference exists between the mean smear layer removal scores of group 1 versus group 2 at the apical thirds ($p<0.05$).
- Statistically significant difference exists between the mean smear layer removal scores of group 2 versus group 3 at the coronal, middle and apical thirds ($p<0.05$).
- Statistically significant difference exists between the mean smear layer removal scores of group 1 versus group 3 at the coronal, middle and apical thirds ($p<0.05$).

Smear layer removal at the coronal, middle, and apical thirds was more effective when final irrigation was performed using 0.2% chitosan solution, recording the significantly lowest mean ranks of scores compared to the other groups. This was followed by German Chamomile Oil with the second lowest mean score in relation to all thirds of the root canal [Table 2]. At the Apical third, all the irrigants showed poor smear layer removing property, but 0.2% Chitosan nano-particle solution showed comparatively better results.

Table 2: Comparison of smear score between study groups in each tooth section

Group	n	Mean (SD)	Range	Median (Q1-Q3)	Kruskal Wallis Test		Mann Whitney U Test		
					Chi - Square	P value	1 versus 2	2 versus 3	1 versus 3
CORONAL									
Ozonated olive oil	10	2.30 (0.483)	1	2 (2-3)	21.109	0.000*	0.276	0.000*	0.000*
German Chamomile Oil	10	2.10 (0.316)	1	2 (2-2)					
Chitosan Nano-particle solution	10	1.10 (0.316)	1	1 (1-1)					
MIDDLE									
Ozonated Olive Oil	10	2.6 (0.516)	1	3 (2-3)	18.850	0.000*	0.075	0.001*	0.000*
German Chamomile Oil	10	2.2 (0.422)	1	2 (2-2.25)					
Chitosan Nano-particle solution	10	1.2 (0.422)	1	1 (1-1.25)					
APICAL									
Ozonated Olive Oil	10	3.10 (0.316)	1	3 (3-3)	22.124	0.000*	0.004*	0.001*	0.000*
German Chamomile Oil	10	2.40 (0.516)	1	2 (2-3)					
Chitosan Nano-particle solution	10	1.30 (0.483)	1	1 (1-2)					

*P<0.05 statistically significant, P>0.05 NS. NS: Nonsignificant; SD: Standard deviation

IV. DISCUSSION:

Although most of the contents in the root canal are removed by the instruments, irrigation plays an indispensable role in all areas of the root canal system, particularly in the areas inaccessible for instrumentation[6] . Irrigation is an integral part of chemo-mechanical preparation and it plays a major role in the cleaning and disinfection of the root canal system. Irrigation renders the canal system free of necrotic pulp tissue, bacteria and dentinal debris [5]. Root canal disinfection is one of the important steps in the root canal treatment. Root canal irrigants have been researched often for innovative means to end up with an ideal irrigating solution [1] .

In this study, we compared the efficacy of Ozonated Olive Oil ,German Chamomile Oil and 0.2% Chitosan nano-particle solution as a final irrigant in the removal of the smear layer from the coronal, middle, and apical thirds of the human root canal system.

Ozone is an effective antioxidant associated with the low level of hazards and a high level of biocompatibility [5] . Ozone has numerous beneficial effects, including its antimicrobial activity, the oxidation of bacterial biomolecules and microbial toxins, the ability to remove the smear layer, and opening dentinal tubules to allow the deeper penetration of Ca and fluorine ions into them. Ozonated oils are obtained by the means of chemical reactions that pass pure oxygen and ozone through the oils, and they are potent anti-bacterial irrigants[2] . Ozone has a high oxidation potential, being 1.5 times more effective than chlorine as an antimicrobial agent against several microorganisms.Ozonated oils are obtained from the chemical reaction between ozone and unsaturated fatty acids of vegetable oils. The reaction of ozone with oil occurs exclusively with the carbon-carbon double bonds present in the unsaturated fatty acids and produce different toxic products such as several oxygenated compounds, hyperoxides, ozonides, aldehydes, peroxides, di-peroxides, and poly-peroxides [5] .

M. chamomilla, one of the most popular herbal extracts, is an annual plant from the Asteracea family. Plant extracts are well known as intracanal medicaments and irrigants for disinfection, and as agents for removal of the smear layer. Venkataram et. al. reported that application of a hydroalcoholic extract of chamomile resulted in effective and significant removal of the smear layer when compared with 2.5% sodium hypochlorite and effectively cleaned the coronal and middle thirds of the root canal [4] .

Chitosan is a novel natural biocompatible polysaccharide obtained by the deacetylation of chitin, which is found in crab and shrimp shells. Chitosan has introduced in dental research and gained popularity because of its bio-degradability, bio-adhesion and lack of toxicity. It has a high chelating ability for various metal ions in acidic conditions. The antibacterial nanoparticles having the dimensions in the range of 1-100nm have greater surface area and charge density, which enable them to achieve a higher degree of interaction with the negatively charged surface of bacterial cells and have the ability to disrupt the extracellular polymerase matrix[7].

The effect of smear layer removal of 0.2% Chitosan nano-particlesolution used in this study was better than the other two at coronal, middle, and apical thirds. There are three main factors responsible for the elimination of dentin calcium ions : Adsorption, ionic exchange, and chelation. The chitosan polymer is hydrophilic, and this favours its intimate contact with the root canal dentin, thereby leading to its adsorption to the root canal wall. The ionic interaction between the dentin calcium ions and the chelating agent is due to the presence of a large number of free hydroxyl and amino groups in the polymer, making it cationic in nature.

Previous studies have assessed the chelating capacity of chitosan on root canal dentin, and these showed that the irrigation of the root canals with a chitosan nano-particle solution for 3 min effectively removed the smear layer from the root canals. These results were in accordance with those obtained in the present study, where the final irrigation with chitosan nanoparticle solution for 3 min effectively removed the inorganic contents from the dentin.

In the present research, chitosannano-powder was dissolved in 1% acetic acid to form the solution because it is insoluble in water.Thus, it could be speculated that the chelating effect observed in this study would be due to the acid and not of chitosan. However, previous studies have shown that the capacity of 5% acetic acid for reducing dentin microhardness, removing the smear layer, and chelating calcium ions in the root canal is insignificant.

In this way, it is highly evident that the effect caused by chitosan on dentin microhardness is exclusively due to the substance and not the acid .

Analysis of the dentinal walls of all the specimens demonstrated that cleaning was more effective in the coronal and middle thirds of the root canal than in the apical third. This can possibly be attributed to the increased depth and reduced diameter in that area of the root canal. The flowability and backflow of the fluid were thus found to be poor in the apical third.The presence of more abundant and larger dentinal tubules coronally exposes the dentin to a larger volume of irrigants, thus allowing better flow of the solution.

This was confirmed by several other researchers who concluded that greater amounts of smear layer were found remaining at the apical third of the canal.

Ozonated Olive oil was found to have a lower smear layer removal efficacy in all areas of the root canal when compared to German Chamomile Oil and Chitosan nanoparticle solution.

Aldehydes, ketones, and hydrogen peroxide can be generated as a result of thehydrolysis of ozonized oil. As an oxidant, hydrogen peroxide degrades vital biologicalcomponents such as lipids, proteins, and nucleic acids . Hydrogen peroxide alsohas an effect on the inorganic components of dentin by acidic demineralization[2]. Ozonolysis reaction is meaningless if peroxide released by ozonated oil could not be quantified. Another limitation could be that the known endodontic disinfectants are provided in liquid form, presumably to facilitate penetration into the accessory canals and tubules whereas ozonated olive oil is more viscous in nature thereby limiting the penetration [5] .

The results of the present study demonstrated that Germanchamomile oilshowed better cleaning in the coronal and middle thirds compared with Ozonated olive oil . The chemical analysis of chamomile has revealed its compounds to be chamazulene, alpha-bis alcohol acids such as capric acid, caprylic acid, chlorogenic acid, o-coumaric acid, p-coumaric acid and dihydroxybenzoic acid . It would appear that the cleaning effect of chamomile in this study may be related to these acid components [3] .

V. CONCLUSION:

A moderate concentration of 0.2% chitosan nanoparticle solution removes the smear layer with greater efficiency than Ozonated Olive oil and German Chamomile oil at the coronal, middle, and apical thirds of the root canals.

VI. REFERENCES:

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