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A Scanning Electron Microscope (SEM) Based Evaluation of Effectiveness of Chloroquick, 7% Maleic Acid, and Conventional Multistep Irrigating Solutions with Passive Ultrasonics in Removal of Root Canal Smear Layer

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study was to compare and evaluate the effectiveness of three different irrigation solutions, namely Chloroquick, maleic acid, and a multi-step irrigation solution when used in combination with ultrasonics for the removal of smear layers in endodontic procedures.
Materials and Methods: Sixty single-rooted teeth that had been freshly extracted were gathered, disinfected, and stored in distilled water. To ensure uniform root length, each tooth was coronally removed below the Cementoenamel Junction (CEJ). The working length was meticulously determined, and F3 Protaper rotary files were employed for the biomechanical preparation of the root canals. Subsequently, the teeth were randomly assigned to one of three groups for the irrigation process: Group A (Chloroquick), Group B (Maleic Acid 7%), and Group C (Multistep Irrigation approach). Following the preparation, longitudinal sections of the specimens were obtained and examined under a scanning electron microscope. The obtained results were then subjected to statistical analysis.
Results: Group A demonstrated superior performance in terms of smear layer and debris elimination within all root canal thirds. Statistically, there was no significant distinction between Group C and B, both of which proved equally effective.
Conclusion: Chloroquick emerged as a highly efficient alternative to maleic acid and the traditional multistep irrigants commonly employed in endodontic procedures. The incorporation of ultrasonic agitation as a complementary technique significantly enhanced the efficacy of all three irrigants.

Keywords: Agitation; endodontic irrigation; root canal therapy; smear layer.

1. INTRODUCTION

“Endodontic treatment is primarily focused on achieving a comprehensive cleaning, shaping, and three-dimensional sealing of the root canal system. The principal approach to reducing the bacterial population within the root canal system is through mechanical preparation. However, it's essential to note that a residue, known as the smear layer, remains on the root canal wall during mechanical instrumentation” [1]. “This smear layer has been observed in scanning electron microscopic examinations of the prepared surfaces” [2,3]. Scanning electron microscopic (SEM) evaluations have provided insights into the composition of the smear layer. It consists of two distinct zones: a superficial layer, approximately 1-2µm thick, primarily composed of organic matter. This organic matter includes remnants of odontoblastic processes, pulp tissue, and bacteria. This superficial layer is referred to as the "smear layer." A deeper zone, termed the "smear plug," is primarily composed of inorganic materials [4]. “It predominantly consists of dentin chips, extending up to 40µm into the dentinal tubules. Understanding the composition and presence of the smear layer is critical in the field of endodontics, as it can influence the success of root canal treatments” [5].

“NaOCl has excellent properties of tissue dissolution and antimicrobial activity that make it the irrigant of choice for the treatment of teeth with necrotic pulp, even though it has several undesirable characteristics such as toxicity, allergic potential, and a disagreeable smell and taste” [6,7]. “Sodium hypochlorite is mainly used in concentrations ranging from 0.5% to 5.25%. Free chlorine in NaOCl dissolves vital and necrotic tissue by breaking down proteins into amino acids. The mechanism of action of NaOCl for the removal of the smear layer involves the transformation of organic substances and fatty acids into fatty acid salts and glycerol that reduces the surface tension of the remaining solution, thus facilitating deeper penetration of irrigating solutions” [8].

“Numerous chemicals, like 17% ethylenediamine tetraacetic acid (EDTA), citric acid [7], and tannin, are suggested for smear layer removal, out of which 17% EDTA is the most frequently used” [8,9]. Goldman et al. have also confirmed that “the final flush with NaOCl, followed by 17% EDTA, completely removes the smear layer. The mineral component of dentin is primarily composed of calcium and phosphate, which are easily soluble in water. When disodium salt of EDTA is added to the equilibrium, calcium ions are removed from the solution, which leads to the further dissolution of ions from the dentin to maintain the equilibrium”. “It has been stated that irrigation with 17% EDTA followed by NaOCl could demineralize the dentine and produce

erosion in the coronal as well as the middle part of the root canal" [1].

"Maleic acid is a mild organic acid used as an acid conditioner in adhesive dentistry. It has also been found to possess distinct smear layer-removing qualities when used as an endodontic irrigant. Many concentrations of maleic acid are being postulated and studied. It was found that using 7% concentration is most advisable" [9].

"Till now, no single irrigant is able to fulfill all prerequisites of irrigant; various combinations simultaneously or alternatively have been used until the introduction of Chloroquick® one-step irrigating solution. Chloroquick® is a combination of stabilized sodium hypochlorite solution with buffer and HDEP (hydroxyethane 1,1 diphosphonic acid) with detergent and system activator, along with other excipients. The incorporated surfactant reduces the surface tension, thus increasing the wettability of the prepared surface and improving the efficacy of irrigating solutions" [10,11].

Agitation techniques improve the cleaning efficacy of irrigating solutions by enhancing the irrigation dynamics. Hence, in this study, we have used passive ultrasonic agitation in conjugation with all three irrigants.

The null hypothesis stated that there was no difference in the effectiveness of chloroquick one-step irrigating solutions, 7% maleic acid, and conventional multistep irrigating solutions in conjunction with passive ultrasonics in the removal of root canal smear layers when evaluated under SEM.

2. METHODOLOGY

Sixty Intact human single-rooted teeth extracted due to orthodontic/periodontal reasons were selected. Teeth with caries, fracture, resorption, calcification, coronal restorations, root fillings, severe dilacerations and multirooted teeth were excluded from the study. The study was approved by an institutional ethical committee. Single root canal configuration was confirmed using a Dental operating microscope (16x Magnification) selected for the study. The teeth were stored in distilled water before use to prevent dehydration**.** All the teeth were then disinfected in 0.1% Thymol solution for 24 hours before use. All the teeth were decoronated below the cementoenamel junction using diamond disc to obtain standardized root length of 10 mm. All the samples were randomly divided into 3 groups out of which **Group A** (Chloroquick), **Group B** (Maleic Acid 7 %) and **Group C** (Conventional multistep irrigation system) containing 20 samples respectively. For the closed system, the apical foramen of each root was coated with cyanoacrylate glue before embedding the roots into a clear polyvinylsiloxane impression material (Imprint II; 3M)–filled Plexiglas tube. Working length was established at 0.5mm short of the apical foramen and glide path was established using size 10 and 15 K file (MANI, India) depending upon root constriction; thereafter the root canals were prepared using K-file keeping the apical file size #25. Root canals was prepared till apical size 30, 0.09 (F3) taper using Protaper Next endodontic files (Dentsply, India) in Crown down manner to enlarge the canal**.** During instrumentation each root canal was irrigated using 2 ml of test solution corresponding to its group.

For Group A - Chloroquick irrigating solution had to be mixed fresh before using it. As this was an exothermic reaction slight increase in the temperature of solution was noticed. The solution was then ready to use within 15 minutes. The final rinse irrigation of 5 ml for 3 minutes with side vented needle was done using same Chloroquick Solution**. After** that passive ultrasonic agitation for 10 seconds followed by flushing out the test solution with 3 ml of normal saline for 2 minutes was carried out. The samples were dried using paper point.

For Group B Maleic acid 7% was used as final irrigant solution with flow rate of 5 ml for 3 mins and followed by ultrasonic agitation for 10 sec. The test solution was washed off with 3 ml of normal saline for 2 mins.

For Group C EDTA 17% of 5 ml for 3 minutes was used as final irrigant and ultrasonic agitation for 10 sec followed by 3 ml of normal saline for 2 mins. Each specimen was irrigated using 3 ml of corresponding irrigating solution using 25-G side vent needles (RC Twent, Prime Dental Products) with the flow rate of approximately 5 ml/minute.

Ultrasonic agitation protocol was done in conjugation with the final irrigant with 21mm length stainless steel, non-cutting wire (#20, taper 00) (Irrisafe™; Acteon, France) driven by a piezoelectric unit (P5 Newtron™, Acteon) at power setting of "Blue 5" for 10 seconds.

For all the groups root canal of each specimen was finally rinsed with normal saline and dried using sterile absorbent paper points.

Scanning electron microscopy: Each specimen was mounted in a 'C- clamp' to obtain secure grip, following which vertical grooves were prepared on both the buccal and lingual surfaces of tooth with the help of slow speed diamond disk avoiding penetration into the canal. The roots were split into 2 halves with a chisel and coded. The coded specimens were mounted on metallic stubs, and gold sputtered using a sputter coater, and examined independently by blinded observer using Scanning Electron Microscope. After general evaluation of the canal wall, three SEM photomicrographs was taken at magnification of × 1000 for smear layer and × 200 for debris at the center of the coronal, middle, and apical thirds of each specimen. Cleanliness was evaluated using 5- point scoring system [5,7].

List 1. 5- point scoring system [5,7]

SCORE	DESCRIPTION
1	No smear layer and all dentinal tubules open
2	A small amount of smear layer and some dentinal tubules open
3	A homogenous smear layer covering the root canal wall and only a few dentinal tubules \open
4	Complete root canal wall covered by a homogeneous smear layer and no open dentinal tubules
5	Heavy homogeneous smear layer covering the complete root canal wall.

The presence of debris was defined as dentin chips, pulp remnants, and particles loosely attached to the root canal wall and evaluated from images at ×200 magnification by a 5-score system:

List 2. Scoring system for images

SCORE	DESCRIPTION
1	Clean root canal wall, few small debris particles;
2	Few small agglomerations of debris
3	Many agglomerations of debris covering <50% of the root canal wall
4	>50% of the root canal walls covered with debris
5	Complete or nearly complete root canal wall coverage with debris

One examiner independently examined each image, which was coded and randomly mixed so that the examiner was blinded to the area from which a given sample originated.

The Smear Removal Score (SRS) and Debris Removal Score (DRS) were subjected to Kruskal-Wallis Test and Mann-Whitney test.

3. RESULTS

Smear layer removal:

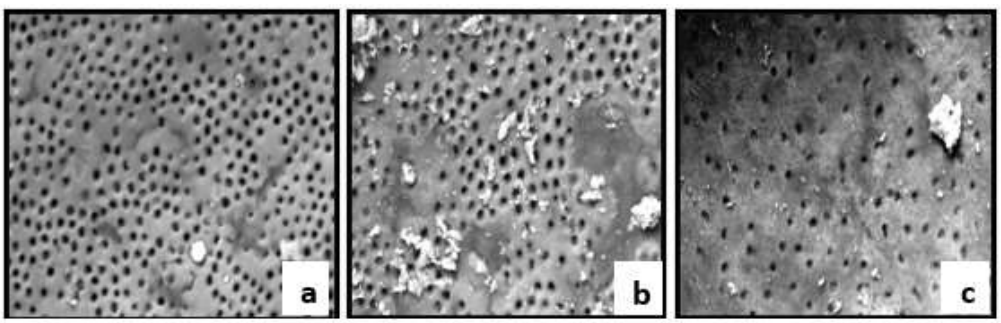

Fig. 1. Group A – Chloroquick Solution with ultrasonic agitation; a) Coronal third; b) Middle third; c) Apical third

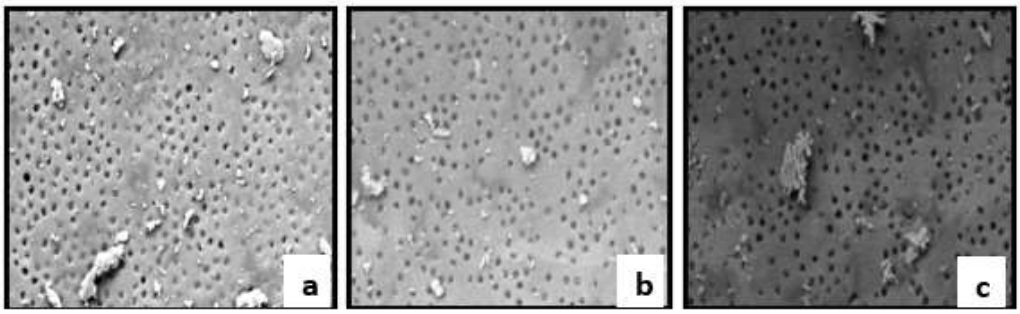

Fig. 2. Group B– Maleic acid 7% with ultrasonic agitation a) Coronal third; b) Middle third; c) Apical third

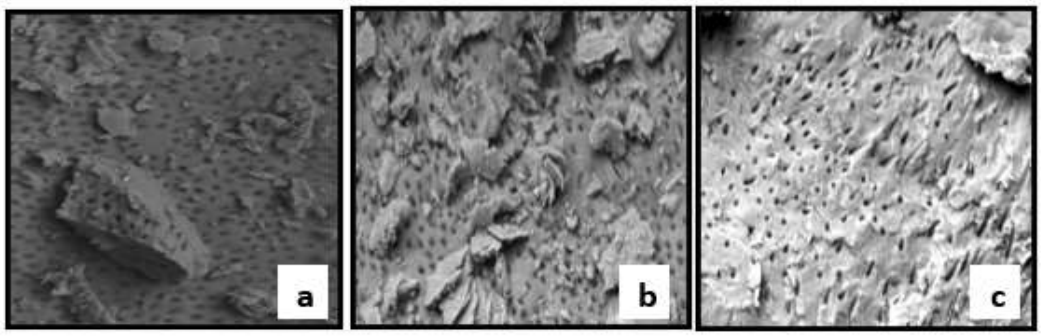

Fig. 3. Group C – Conventional multistep irrigation with ultrasonic agitation; a) Coronal third; b) Middle third; c) Apical third

Debris removal:

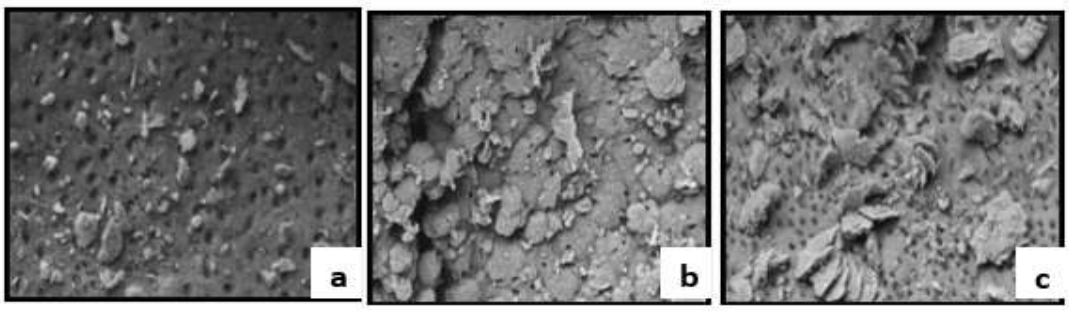

Fig. 4. Group A – Chloroquick irrigation with ultrasonic agitation a) Coronal third; b) Middle third; c) Apical third

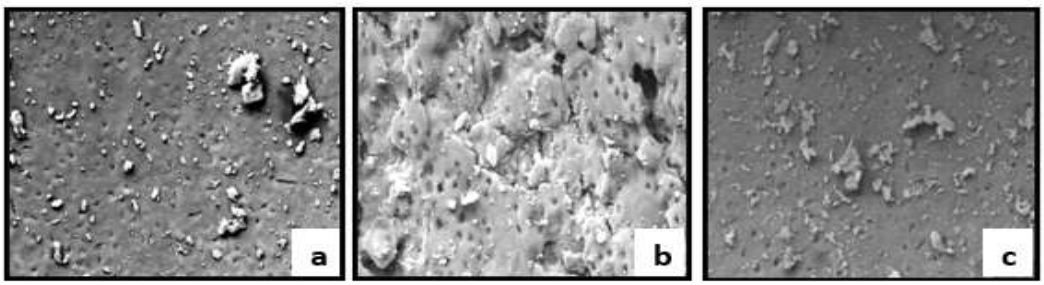

Fig. 5. Group B – Maleic acid 7% with ultrasonic agitation a) Coronal third; b) Middle third; c) Apical third

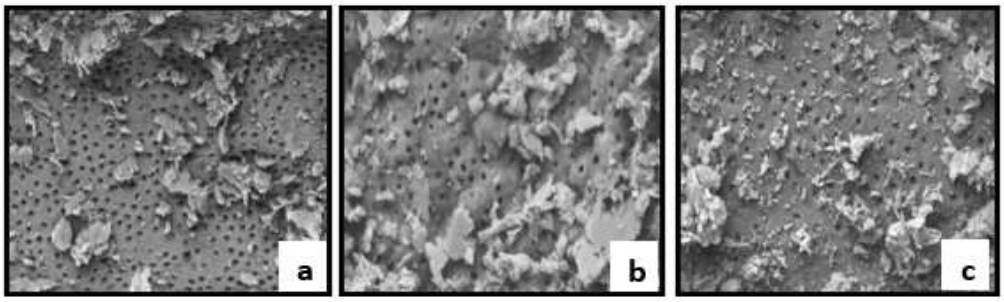

Fig. 6. Group C – Conventional multistep irrigation supplemented with ultrasonic agitation; a) Coronal third; b) Middle third; c) Apical third

Kruskal-Wallis Test:

Table 1. Comparison of Smear Removal Score among three groups at the coronal, middle, and apical thirds of the root canal wall

Smear Removal Score (SRS)	Groups	N	Mean Rank	Chi-square (χ^2)	df	P value
Coronal	Group A	20	17.70	21.12	2	< 0.001*
	Group B	20	36.90			
	Group C	20	36.90			
	Total	60				
Middle	Group A	20	14.90	38.07	2	< 0.001*
	Group B	20	39.58			
	Group C	20	37.03			
	Total	60				
Apical	Group A	20	18.10	20.70	2	< 0.001*
	Group B	20	38.80			
	Group C	20	34.60			
	Total	60				

Table 2. Comparison of Debris Removal Score among three groups at the coronal, middle, and apical thirds of the canal wall

Debris Removal Score (DRS)	Groups	N	Mean Rank	Chi-square (χ^2)	df	P value
Coronal	Group A	20	17.10	22.62	2	< 0.001*
	Group B	20	37.20			
	Group C	20	37.20			
	Total	60				
Middle	Group A	20	13.85	37.27	2	< 0.001*
	Group B	20	40.48			
	Group C	20	37.17			
	Total	60				
Apical	Group A	20	18.20	20.94	2	< 0.001*
	Group B	20	39.35			
	Group C	20	33.95			
	Total	60				

Mann-Whitney Test:

Table 3. Pair wise comparison of Debris Removal Score among groups at the coronal, middle, and apical thirds of the root canal wall

Debris Removal Score (DRS)	Groups	N	Mean Rank	Z	df	P value
Coronal	Group A	20	13.80	-4.08	1	< 0.001*
	Group B	20	27.20			
	Group B	20	20.50	0.00	1	1.000
	Group C	20	20.50			
	Group A	20	13.80	-4.08	1	< 0.001*
	Group C	20	27.20			
Middle	Group A	20	11.45	-5.60	1	< 0.001*
	Group B	20	29.55			
	Group B	20	21.43	-0.81	1	0.420
	Group C	20	19.58			
	Group A	20	12.90	-4.66	1	< 0.001*
	Group C	20	28.10			
Apical	Group A	20	13.65	-4.18	1	< 0.001*
	Group B	20	27.35			
	Group B	20	22.50	-1.26	1	0.209
	Group C	20	18.50			
	Group A	20	15.05	-3.57	1	< 0.001*
	Group C	20	25.95			

** indicates that P value is Highly Significant*

Mann-Whitney Test:

Table 4. Pair-wise comparison of Smear Removal Score among groups at the coronal, middle, and apical thirds of the root canal wall

Smear Removal Score (SRS)	Groups	N	Mean Rank	Z	df	P value
Coronal	Group A	20	14.10	-3.95	1	< 0.001*
	Group B	20	26.90			
	Group B	20	20.50	0.00	1	1.000
	Group C	20	20.50			
	Group A	20	14.10	-3.95	1	< 0.001*
	Group C	20	26.90			
Middle	Group A	20	12.40	-5.07	1	< 0.001*
	Group B	20	28.60			
	Group B	20	21.48	-1.40	1	0.163
	Group C	20	19.52			
	Group A	20	13.00	-4.74	1	< 0.001*
	Group C	20	28.00			
Apical	Group A	20	13.70	-4.23	1	< 0.001*
	Group B	20	27.30			
	Group B	20	22.00	-0.94	1	0.348
	Group C	20	19.00			
	Group A	20	14.90	-3.67	1	< 0.001*
	Group C	20	26.10			

** indicates that P value is Highly Significant*

As per above table, it was found that the mean ranks of Smear Removal Score were less for Group A (Chloroquick – one step irrigation system) as compared to Group B (Maleic Acid 7%) as well as compared to Group C (Conventional multistep irrigation system) at the

coronal, middle, and apical thirds of the root canal wall and this difference found in mean ranks was highly significant statistically.

As per above table, it was found that the mean ranks of Debris Removal Score (DRS) were less for Group A as compared to Group B as well as compared to Group C at the coronal, middle, and apical thirds of the root canal wall and this difference found in mean ranks was highly significant statistically.

While the mean ranks of DRS were comparable for Group B and Group C at all thirds of the canal wall as the difference found was not significant statistically.

So, Group A is more effective in removal of debris as compared to Group B as well as Group C at the coronal, middle, and apical thirds of the root canal wall but Group B was as effective as Group C for removal of debris.

As per above table, it was found that the mean ranks of Smear Removal Score were less for Group A (Chloroquick – one step irrigation system) as compared to Group B (Maleic Acid 7%) as well as compared to Group C (Conventional multistep irrigation system) at the coronal, middle, and apical thirds of the root canal wall and this difference found in mean ranks was highly significant statistically.

While the mean ranks of SRS were comparable for Group B and Group C at all thirds of the canal wall as the difference found was not significant statistically.

So, Group A is more effective in removal of smear layer as compared to Group B as well as Group C at the coronal, middle, and apical thirds of the root canal wall but Group B was as effective as Group C for removal of smear layer.

4. DISCUSSION

Chloroquick solution by Zodenta was one such attempt to incorporate all the requirements of ideal irrigant. As it is a one-step irrigant it comprises of **Vial A**- Sodium Hypochlorite. **Vial B** – HEDP, detergent, Activator, Sodium hydroxide. The combination of NaOCl and HEDP synergises the dissolving and chelating agents of both the constituents respectively. This combination also leads to effervescence formation that leads to the coronal extrusion of the root canal debris rendering the canals clean [12,13].

7% Maleic acid is also amongst the various chemical agents used as endodontic irrigants. Maleic acid is a mild organic acid used as an acid conditioner in adhesive dentistry [10,14]. It has been found to possess the smear layer removing quality when used as an acid etchant in restorative dentistry and is also essential for meticulous eradication of endodontic biofilm.

Available literature suggests that the alternate use of sodium hypochlorite and EDTA as a final rinse is highly effective in complete disinfection of root canal. Therefore, the sequential use of EDTA and NaOCl is most recommended as the final wash during endodontic treatment. Although, the combination provides good disinfection; on the other side, this sequential use utilizes more operator time, it may cause extensive dentinal erosion and bears highly caustic action on the oral soft tissues. Such adverse effects of synthetic chemical irrigants have created the thrust area for the researchers to develop alternatives as irrigants which are more biocompatible as well as less time-consuming [15,16].

Needle and syringe irrigation being the most commonly used irrigation system. 27-gauge needle tips were used in the study as they can penetrate more deeply into the apical one-third because of the small-bore size. RC Twents irrigating needles have a unique anti-obturating end with side venting opening, which increases the contact of the irrigant to the canal walls and prevents the forceful passage of irrigants through the apical foramen and have increased the efficiency of the irrigant in smear layer removal from root canals [15,16].

Limited penetrability especially in the apical third is one of the major hindrances encountered while using various irrigating solutions [14]. Methods are suggested to improve the penetration of irrigating solutions which includes Various agitation techniques ranging from manual to machine assisted are suggested to enhance the irrigation dynamics. These agitation techniques lead to pressure changes and subsequent elimination of the vapour lock which provides replenishment of irrigating solution, thus, improving the cleaning efficacy of the irrigant. Ultrasonic agitation also fall under the category of machine assisted devices. Ultrasonic irrigation can be either simultaneous ultrasonic

instrumentation and irrigation or passive ultrasonic irrigation without instrumentation.

Irrisafe™ files were chosen for the study because they are composed of non-cutting stainless-steel wire, which does not cause any harm to root canal anatomy and is relatively safe. Irrisafe™ tips have special surface treatment which results in better transmission of ultrasonic vibrations and is compatible with a variety of synthetic irrigants used in endodontics [16].

Contact time is one of the important variables for achieving thorough removal of the smear layer, 3 minutes of contact time was selected for all the groups in accordance with the recent research. Recent technologies like digital image analysis are now available for evaluation of smear layer which avoids the occurrence of evaluator bias and requires less time, yet scanning electron microscope has opted in this study as it is one of the most recommended and commonly available tool for evaluation of smear layer and it provides images at much higher magnification. Scanning electron microscopic images at 1000X provided a distinct visualization of the dentinal tubules for evaluation of smear layer and debris at all thirds of the root canal system [17,18].

Null hypothesis was rejected in accordance with the results of this research stating that the experimental irrigant. Chloroquick was equally effective as gold standard NaOCl and 17% EDTA combination and 7% Maleic acid for removal of smear layer when used in conjunction with ultrasonic agitation. Further lineal research shall be carried out in the same perspective that will help to improve the efficacy and minimize adverse effects of the irrigation protocol.

5. CONCLUSION

Within the limitation of the study, it can be concluded that single step Chloroquick solution proves to be an effective alternative to conventional multistep irrigants used in endodontic practice.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standards written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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