"COMPARATIVE EVALUATION OF EFFECT OF N-ACETYL CYSTEINE, MALEIC ACID AND EDTA ON THE DEPTH OF DENTINAL TUBULE PENETRATION OF AN EPOXY RESIN-BASED ROOT CANAL SEALER: A CONFOCAL LASER SCANNING MICROSCOPY STUDY"

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Preeti K Doddwad

Head of Department

Dr. Preeti Doddwad M.D.S
Professor & Head,
Department of Conservative Dentistry and Endodontics,
KAHER V.K. Institute of Dental Sciences,
Belagavi.

Date: 27.12.2022

Place: Belagavi

Professor and Head
Dept. of Conservative Dentistry
KLE'S V. K. Institute of Dental Sciences,
Belagavi

Principal

Dr. Alka Kale M.D.S
Principal,
KAHER V. K. Institute of Dental Sciences,
Belagavi

Date: 28/12/22

Place: Belagavi

PRINCIPAL
KLE V.K. Institute of Dental Sciences
Nehru Nagar, BELAGAVI-590010.

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LIST OF ABBREVIATIONS

SR.NO	ABBREVIATIONS	FULL FORM
1	EDTA	Ethylene diamine tetra acetic acid
2	NAC	N- acetyl cysteine
3	MA	Maleic acid
4	CA	Citric acid
5	PAA	Peracetic acid
6	CEJ	Cemento-enamel junction
7	CLSM	Confocal laser scanning microscope
8	SEM	Scanning Electron Microscope
9	LM	Light Microscope
10	NaOCl	Sodium Hypochlorite
11	CHX	Chlorxhexidine
12	MTAD	Mixture of Tetracycline, citric acid and detergent
13	µm	Micrometres
14	et al	Additional persons involved in the same study
15	mL	Milliliter
16	° C	Degree Celsius
17	mm	Millimeter
18	min	Minutes

19	n	Number of specimens
20	p-value	Probability of obtaining a test statistic at least as extreme as the one that was actually observed
21	i.e.	That is
22	gm	Gram
23	mg/mL	Milligram/millilitre
24	PUI	Passive ultrasonic irrigation
25	WL	Working Length
26	SC	Single Cone
27	Ca2+	Calcium ions
28	Fe3+	Ferric ions
29	NCPs	Non collagenous proteins
30	ANOVA	Analysis of variance

ABSTRACT

AIM: To evaluate and compare the effect of N-acetyl cysteine, maleic acid and EDTA as final irrigating solution on the maximum depth of penetration of an epoxy resin-based sealer (AH Plus) into the dentinal tubules at the apical, middle, and coronal areas of root canals using confocal laser scanning microscopy.

METHODOLOGY: Sixty-six extracted human mandibular premolar teeth were used for this study. All the samples were decoronated up to the level of CEJ with the help of a diamond disc to produce a standardized root length of 14 mm. Working length was evaluated using a size 10 K file and the root canal was enlarged up to size 20 K file with hand instrument. Samples were further prepared with ProTaper Universal rotary system up to size F3. The root canals of the teeth were irrigated with 1 ml of 2.5% NaOCl between consecutive instrumentation.

The teeth were randomly divided into 3 groups according to the final irrigation protocol:

1. Group 1: 20% N-acetyl cysteine
2. Group 2: 7% Maleic Acid
3. Group 3: 17% EDTA

NAC Irrigating Solution Preparation: NAC solution was prepared by dissolution of 0.2 gm NAC in 1 ml of distilled water to obtain a concentration of 200 mg/ml.

Maleic Acid Irrigating Solution Preparation: MA solution was preparaed by dissolution of 0.07 gm maleic acid in 1 ml of distilled water to obtain a concentration of 70 mg/ml.

Commercially available 17% EDTA solution (Canalarge) was used in the present study.

All irrigation solutions were introduced into the canal using a 5 ml, 30-gauge side vented disposable plastic syringe**.** Final flush with 5 ml of experimental irrigating solution for a period of 1 minute was performed. The root canals were irrigated copiously with 10 ml of distilled water for 1 minute and then dried with paper points.

For evaluation of samples using confocal laser scanning microscopy, Rhodamine B isothiocyanate dye was used to promote fluorescence. It was added to AH plus sealer during manipulation at an approximate ratio of 0.1% by weight. Obturation was done with ProTaper Universal F3 single gutta-percha cone. Excess gutta-percha was removed using a heated plugger and the access cavity sealed with cavit. The samples were stored in an incubator at 37°C and 100% humidity for 7 days and later the samples were sectioned perpendicular to its long axis in 1-mm-thick sections, at levels 2, 5, and 8 mm from the root apex using a diamond disc and examined with confocal laser scanning microscope. Digital images were analysed using ImageJ software.

RESULTS: The results showed a statistically significant difference between the penetration of all the three experimental irrigants at the coronal, middle and apical third. Greater penetration of sealer was observed at the coronal third, followed by middle and apical third. The depth of peneteration of sealer in coronal and middle third among all the three groups were similar and were statistically insignificant. In the apical third, statistically significant higher penetration of sealer was observed with 7% MA, as compared to 20% NAC and 17% EDTA.

CONCLUSION: Within the limitations of this study, it was concluded that all the three irrigants exhibited penetration of sealer inside the dentinal tubules. Sealer penetration at coronal and middle third were similar among all three groups. Higher penetration of sealer at the apical region was observed with 7% MA as compared to 20% NAC and 17% EDTA. Also, maximum sealer penetration was noted in the coronal third, followed by middle and least in apical third for all tested groups.

KEY WORDS: N-acetyl cysteine, Maleic acid, EDTA, AH Plus, Confocal laser scanning microscope, Rhodamine B

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INTRODUCTION

The foremost objective of an endodontic therapy is to optimize root canal disinfection by eradicating microorganisms from the root canal.[1,2] Mechanical instrumentation is inadequate to thoroughly disinfect the root canals due to its complex anatomy, which includes lateral or accessory canals, apical ramifications, deltas and fins.[2,3] Irrigation is critical and must be accompanied with bio-mechanical preparation to aid in removal of bacteria and their by-products, debris and non-vital tissues present in the smear layer.[3]

Smear layer is produced during bio-mechanical preparation of the root canal and it contains organic and inorganic materials. Smear layer prevents optimum disinfection of the root canals as it acts as a substrate for bacteria. It also hinders in the penetration of disinfecting agents.[4] Furthermore, it may also limit the penetration of root canal sealer by forming a physical barrier between the obturating core material and the dentinal tubules, thus inhibiting the formation of an adequate seal.[5] Hence, removal of smear layer is of utmost importance to achieve an impervious seal of the root canals.[6]

Chelating agents aid in the removal of smear layer and the most widely used chelating solution in endodontic therapy is EDTA.[4] Disodium salt of EDTA is commonly used at 17% concentration and neutral pH. EDTA effectively dissolves the inorganic debris in the root canals.[7] The dissolution of the inorganic material is due to the reaction of EDTA with the calcium ions in the dentin. This results in decalcification of dentin up to 20-30 µm depth.[8]

However, EDTA has a deleterious effect on the periapical tissues, causes moderate degree of irritation and also excessively erodes the dentin, thus causing a change in the microhardness of root dentin. [5,9]

A recent study has demonstrated that, the chelating property of N-acetyl cysteine (NAC) is comparable to EDTA in removing the smear layer from the root canal. It also caused a significantly lesser reduction in the microhardness of dentin of the root, compared with EDTA.[9]

N-acetyl cysteine is derived from amino acid -L-cysteine and a glutathione precursor.[10] It has been used as a chelating agent in clinical medicine for detoxification in case of heavy metal poisoning, such as mercury and lead.[9] NAC is a potent thiol containing antioxidant and possesses antimicrobial properties.[11] It is effective against E. Faecalis and also reduces biofilm formation.[12]

Maleic acid is a mild organic acid which possesses chelating properties and can effectively remove smear layer.[5] It is also used as an acid etchant to prepare the tooth surface for application of an adhesive.[8] Maleic acid can be used at 5% and 7% concentration for smear layer removal.[5] It has been reported that maleic acid at 7% concentration is more effective than 17% EDTA for smear layer removal when used as a final irrigating solution for 1 minute.[13]

A three-dimensional hermetic seal is of utmost importance for a successful endodontic therapy and it is achieved by root canal obturation.[14] The sealer penetration into the dentinal tubules during obturation is advantageous as it entombs the bacteria and debris in the tubules and cuts off the nutrient source of the microorganism. This prevents microbial colonization and reinfection.[1,14]

Furthermore, infiltration of root canal sealers also increases the gutta percha and root dentin interface which greatly improves the retention by mechanical locking.[15] Hence, sealer penetration may indicate the extent of smear layer removal after final irrigation using different chelating solution.[1]

AH Plus sealer is a widely used epoxy resin-based sealer. It is considered the "Gold Standard" sealer due to its superior qualities with adequate sealing ability. It has good adherence to dentin and has low solubility.[16,17]

Confocal laser scanning microscopy (CLSM) was used to assess the penetration of sealer into the dentinal tubule as additional processing is not required for the specimen.[18]

It is essential to label the sealer with 0.1% Rhodamine B dye to check the penetration of the sealer using CLSM, as it promotes fluorescence. 0.1% Rhodamine B dye has reportedly shown no alteration in the flow and physicochemical properties of the sealer.[19]

Though N-acetyl cysteine and maleic acid when used as irrigating agents have been proven to be effective in smear layer removal, no previous studies have been conducted comparing the effect of N-acetyl cysteine, maleic acid and EDTA on the depth of penetration of root canal sealer.

Hence the aim of this study is to evaluate and compare the effect of N-acetyl cysteine, maleic acid and EDTA as final irrigating solution on the maximum depth of penetration of an epoxy resin-based sealer (AH Plus) into the dentinal tubules at the apical, middle, and coronal areas of root canals using confocal laser scanning microscopy.

OBJECTIVES OF THE STUDY

AIM

To evaluate and compare the effect of N-acetyl cysteine, maleic acid and EDTA as final irrigating solution on the maximum depth of penetration of an epoxy resin-based sealer (AH Plus) into the dentinal tubules at the apical, middle, and coronal areas of root canals using confocal laser scanning microscopy.

OBJECTIVES

- To evaluate the dentinal tubule penetration of an epoxy resin-based sealer after using different final irrigating solutions (N- acetyl cysteine, maleic acid and EDTA) at various sections of the tooth, apical, middle, and coronal third by using confocal laser scanning microscopy.
- To compare the dentinal penetration of an epoxy resin-based sealer after using different final irrigating solutions (N- acetyl cysteine, maleic acid and EDTA) at various sections of the tooth, apical, middle, and coronal third by using confocal laser scanning microscopy.

HYPOTHESIS

NULL HYPOTHESIS: -

There will be no difference in the effect of N- acetyl cysteine, maleic acid and EDTA on the dentinal tubule penetration of an epoxy resin-based root canal sealer at apical, middle and coronal third of the root canal.

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ALTERNATE HYPOTHESIS: -

There will be a difference in the effect of N- acetyl cysteine, maleic acid and EDTA on the dentinal tubule penetration of an epoxy resin-based root canal sealer at apical, middle and coronal third of the root canal.

REVIEW OF LITERATURE

1. An examination was conducted by Tuncer et al, to assess the effects of final irrigation with 17% EDTA, 7% MA and 10% CA on penetration of sealer into dentinal tubules using CLSM and the results concluded that, the dentinal tubule penetration of sealer were comparable in all the three groups.[3]

2. An examination was done by Kaushal et al, to evaluate the smear layer removal from the root canals irrigated with 17% EDTA, 10% Citric acid, 7% Maleic acid and normal saline using SEM and the investigation concluded that, all the three irrigating solutions removed comparable smear layer from coronal and middle thirds and least from the apical third. The smear layer removal from coronal and middle third of the root canal were comparable among all the groups. However, for smear layer removal in the apical third, 7% maleic acid was the most effective irrigating solution. [5]

3. An examination was conducted by Jaiswal et al, to evaluate the ability to remove smear layer by 17% EDTA, 7% maleic acid and 0.7% fumaric acid using SEM. The results concluded that, both fumaric acid and maleic acid were equally effective at coronal and apical third and no significant difference was observed between them. Smear layer removal in the middle third was better removed by fumaric acid, compared to maleic acid and EDTA and this difference was statistically significant. Compared to EDTA, both fumaric acid and maleic acid showed significantly better results.[6]

4. An examination was done by Rajakumaran et al, to compare the smear layer removing ability of N-acetyl cysteine and EDTA and also to evaluate their effect on the microhardness of dentin of the root canal. The results concluded that both

EDTA and NAC were comparable in removing smear layer from the canal walls. Both the irrigants removed more smear layer from coronal and middle thirds and least from apical third of the root. EDTA showed greater reduction in percentage of dentin microhardness and this difference was significantly higher compared to NAC.[9]

5. An examination was done by Ballal et al, to evaluate the smear layer removal with 17% EDTA and 7% maleic acid after irrigation with 2.5% NaOCl solution using SEM. The results concluded that, the final irrigation with 7% maleic acid was more effective in removing smear layer at apical third compared to 17% EDTA. At coronal and middle thirds, both the groups showed comparable results in the removal of smear layer.[13]
6. An examination was conducted by Butala et al, to evaluate the removal of smear layer from the root canal by 7% maleic acid, 0.5% peracetic acid (PAA), and 17% EDTA using SEM. The study concluded that, in the middle and apical third of the root canal, 7% maleic acid was more effective in smear layer removal than 17% EDTA and 0.5% PAA.[20]
7. An examination was conducted by Kuruvilla et al, to evaluate and compare the smear layer removal ability of 17% EDTA, 18% etidronic acid, and 7% maleic acid using SEM. The study concluded that, all three irrigating solutions were comparable and removed smear layer effectively from coronal and middle third. Furthermore 7% maleic acid showed better smear layer removal ability compared to 17% EDTA and 18% etidronic acid from the apical third of root canal.[21]
8. An examination was conducted by Ballal et al, to evaluate smear layer removal using SmearOFF, 7% maleic acid and EDTA at two different concentrations, 17% and 18%. The specimen were analysed using SEM and it was concluded

that, all the three groups showed comparable results in the coronal and middle thirds of the root canal. However, in the apical third, 7% MA was most effective in removing smear layer.[22]

9. An examination was conducted by Attur et al, to evaluate and compare the efficiency of irrigants in the removal of smear layer with 17% EDTA irrigation, 7% maleic acid irrigation and 2% chlorhexidine irrigation and analysed through SEM. The study concluded that, 17% EDTA was most efficacious at removing smear layer compared to 7% maleic acid and 2% chlorhexidine. The least reduction in smear layer was seen by 2% chlorhexidine.[23]
10. An examination was conducted by Shetty et al, to evaluate and compare the penetration of sealers into the dentinal tubules after using different final irrigation solutions, 17% EDTA, 10% citric acid, 5% maleic acid and 5.25% NaOCl and the specimen were evaluated using CLSM. For activation of final irrigant, passive ultrasonic irrigation was used. The result concluded that, most effective sealer penetration into the dentinal tubule was seen with a combination of 17% EDTA and PUI compared to other groups.[24]
11. An examination was conducted by Osazir et al, to evaluate and compare the effects of different final irrigation regimens such as 17% EDTA, 17% EDTA and 2% CHX, 7% MA, 7% MA and 2% CHX, 5.25% NaOCl on the dentin tubule penetration of three different root canal sealers, AH Plus, EndoREZ, and Tech BioSealer Endo using confocal laser scanning microscopy. Greatest tubular penetration was shown by AH Plus sealer. Sealer penetration between 17% EDTA and 7% MA was comparable. Higher penetration of sealer was seen when final irrigation solutions were used in combination with chlorhexidine. [25]

12. An examination was conducted by Gupta et al, to compare the effect of different irrigating solutions such as 17% EDTA, MTAD, 7% MA and 10% MA for smear layer removal and to evaluate dentin microhardness. Smear layer removal in coronal and middle thirds were comparable among all the experimental groups. However, in the apical region, highest smear layer removal was seen with MTAD and 10% maleic acid, followed by 7% maleic acid and least by 17% EDTA. MTAD and 10% maleic acid showed the greatest reduction in dentin microhardness, followed by 17% EDTA and 7% maleic acid.[26]
13. An examination was conducted by Machado et al, to compare and evaluate the smear layer removal and sealer penetration in dentinal tubules using final irrigating solutions, 17% EDTA and 10% citric acid. The results concluded that, smear layer removal of both the final irrigants were comparable at all the three levels. Sealer penetration into dentinal tubules were comparable for both the groups and no statistical difference was observed between them.[27]
14. An examination was done by Mozayeni et al, to assess the smear layer removal ability of MTAD and 17% EDTA. The results concluded, that smear layer removal in the coronal and middle third for both the groups were comparable. However, in the apical third, MTAD was more effective in smear layer removal compared to 17% EDTA.[28]
15. An examination was done by Zivanovic et al, to compare and evaluate the removal of smear layer using different final irrigation solutions, MTAD, QMix, and 17% EDTA. Among the three experimental groups, the smear layer removal in the middle and coronal thirds were comparable and no significant difference was seen between them. In the apical third, greater smear layer removal was seen with QMix and MTAD, as compared to 17% EDTA.[29]

MATERIALS AND METHODS

SOURCE OF DATA:

- The study was conducted in the Department of Conservative Dentistry and Endodontics, KLE Academy of Higher Education & Research, KLE V.K Institute of Dental Sciences, Belagavi.
- The laboratory procedures were undertaken in KLE Academy of Higher Education & Research, Dr. Prabhakar Kore's Basic Science Research Laboratory, Belagavi.
- N-acetyl cysteine and maleic acid irrigation solutions were prepared in KLE Academy of Higher Education & Research, KLE College of Pharmacy, Belagavi.
- Specimens were evaluated under the confocal laser scanning microscope at Birla Institute of Technology and Science- Pilani, Goa.
- Extracted human mandibular premolar teeth were collected from Department of Oral and Maxillofacial Surgery, KLE Academy of Higher Education & Research, KLE V.K Institute of Dental Sciences, Belagavi.

INCLUSION CRITERIA

- Extracted human mandibular premolar teeth with single root & single straight canal with closed apex.
- Teeth with apical width corresponding to 20 K-file or less.

EXCLUSION CRITERIA

- Teeth with apical width more than 20 K-file size.
- Teeth with calcified canals.

- Teeth with root caries.
- Teeth with fracture/crack.
- Teeth with internal and external root resorption.
- Teeth with presence of anatomic variations.
- Teeth with severe curvature.
- Teeth with endodontic treatment.
- Teeth with multiple canals.

MATERIALS USED FOR THE STUDY:

- Human mandibular premolars
- 0.1% thymol solution
- Radiographs
- 2.5% NaOCl (Vishal Dentocare, Ahmedabad)
- 0.9% Saline solution

  17% EDTA (Canalarge)
- 20% N- acetyl cysteine
- 7% Maleic acid
- Distilled water
- Paper points (Diadent Group)
- Rhodamine B dye (Sigma Aldrich)
- AH Plus sealer (Dentsply, Germany)
- Gutta-percha points
- Cavit (3M, ESPE, USA)

ARMAMENTARIUM USED FOR THE STUDY

- K Files (Mani Inc, Japan)
- ProTaper Universal nickel-titanium files (Dentsply Maillefer, Switzerland)
- Airotor (Being, China)
- Endomotor (X- Smart, Dentsply)
- Lentulospiral
- Micromotor (NSK Pana Air)
- 5 ml, 30-gauge side vented needle
- Incubator
- Diamond disc
- Silicon carbide abrasive paper
- Glass Slide
- Confocal laser scanning microscope

SAMPLE SIZE ESTIMATION:

$S = \frac{S_1 + S_2}{2} = 9.06$

S1 = 8.65

S2 = 9.47

d = 8.32

Z_α= 1.96 at 5% αerror.

Z_β= 1.037 at 15% β error.

$$n = \frac{2S^2 (Z_\alpha + Z_\beta)^2}{d^2} = 22 \text{ samples in each group}$$

METHODOLOGY

Sixty-six extracted human mandibular premolar teeth cleaned of calculus and soft tissue were stored in 0.1% thymol solution and maintained hydrated till the time of use. All teeth were radiographed and were selected as per the inclusion and exclusion criteria. Teeth were decoronated up to the level of CEJ with the help of a diamond disc to produce a standardized root length of 14 mm.

Working length was evaluated using a size 10 K file till it was just seen at apex and reducing 1 mm from the recorded length. The root canal was enlarged up to size 20 K file with hand instrument.

Samples were further prepared with ProTaper Universal rotary system using crown down technique to prepare the canals to full working length with an endomotor. Shaping file Sx, was used for coronal enlargement, S1 for expanding the glide path, and S2 for middle third enlargement. Finishing files, F1, F2 and F3 which corresponds to apical size 30 were used for finishing apical third. The root canals were irrigated with 1 ml of 2.5% NaOCl between instrumentation.

The teeth were randomly divided into 3 groups according to the final irrigation protocol:

1. Group 1: 20% N-acetyl cysteine
2. Group 2: 7% Maleic Acid
3. Group 3: 17% EDTA

NAC Irrigating Solution Preparation: NAC solution was prepared by dissolution of 0.2 gm NAC in 1 ml of distilled water to obtain a concentration of 200 mg/ml.

Maleic Acid Irrigating Solution Preparation: MA solution was preparaed by dissolution of 0.07 gm maleic acid in 1 ml of distilled water to obtain a concentration of 70 mg/ml.

All irrigation solutions were introduced into the canal using a 5 ml, 30-gauge side vented disposable plastic syringe, inserted to a depth that is 1 mm less than the working length**.** 5 ml of final irrigating solution was used as a final flush for a duration of 1 minute for each sample. The samples were irrigated copiously for a duration of 1 minute with 10 ml of distilled water. The samples were later dried with paper points.

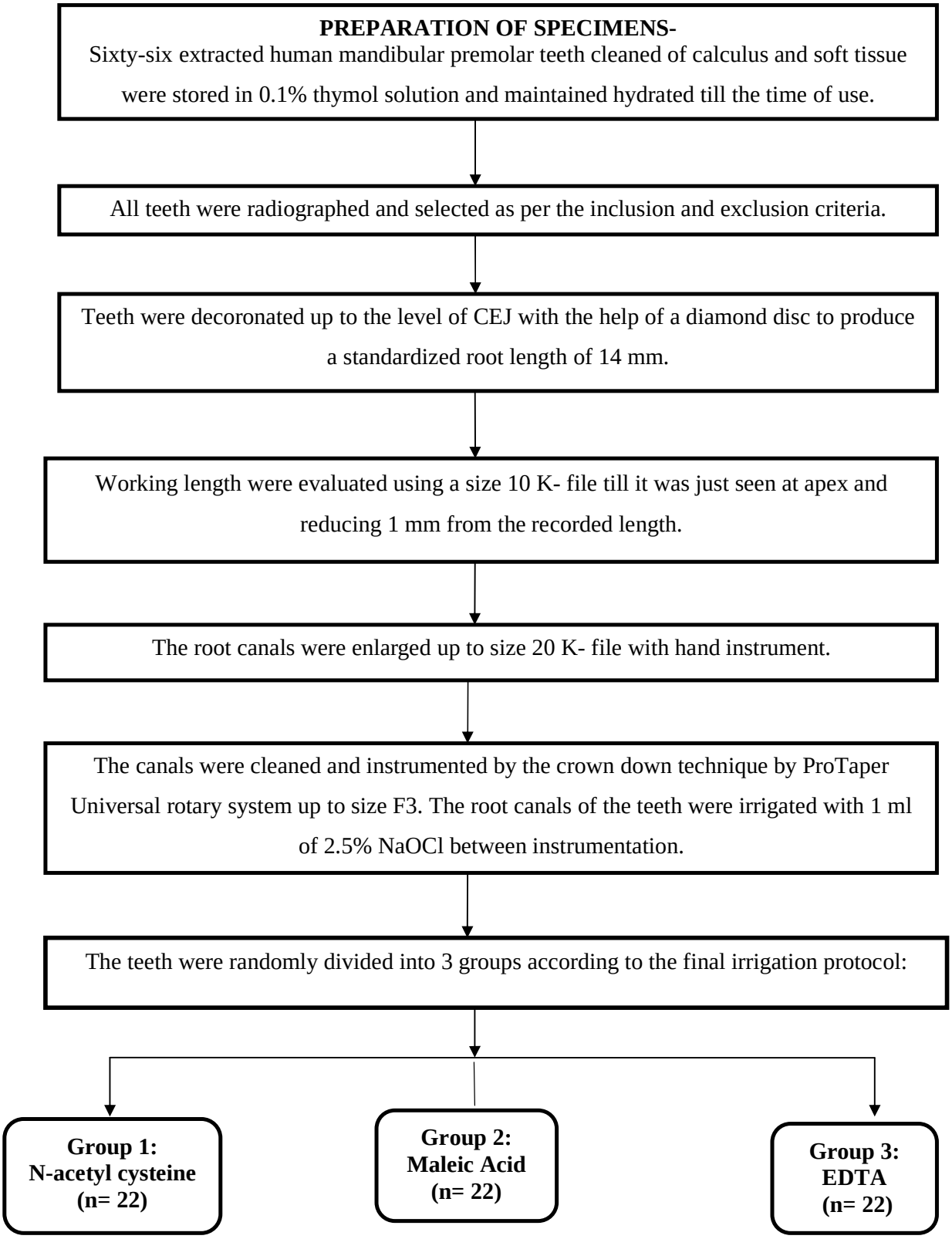
For evaluation of samples using confocal laser scanning microscopy, Rhodamine B isothiocyanate dye was used to promote fluorescence. It was added to AH plus sealer during manipulation at an approximate ratio of 0.1% by weight. The labelled root canal sealer was placed into the canal to 1 mm short of the working length using a no. 25 lentulospiral. A ProTaper Universal F3 single gutta-percha cone, was slightly coated with labelled epoxy resin-based sealer, AH Plus sealer and placed in the root canal to the working length. Excess gutta-percha was removed using a heated plugger and the access cavity sealed with cavit. For the sealer to set completely, the samples were stored in an incubator at 37°C and 100% humidity for 7 days.

After the sealer was set completely, each sample was sectioned perpendicular to its long axis. The sections were made at three different levels, i.e. 2,5, and 8 mm from the root apex using a diamond disc to obtain 1 mm thick sections. Silicon carbide abrasive paper was used to polish the tooth sections and the sections were

mounted onto the glass slides. Following which the examination was done using confocal laser scanning microscope.

ImageJ software was used to analyse the dentinal tubule penetration of the sealer. A measuring tool was used to measure the depth of penetration from the canal wall to the point of maximum sealer penetration, which was measured in micrometers.

FLOWCHART DEPICTING THE STUDY DESIGN



NAC Irrigating Solution Preparation: NAC solution was prepared by dissolution of 0.2 gm NAC in 1 ml of distilled water to obtain a concentration of 200 mg/ml

Maleic Acid Irrigating Solution Preparation: MA solution was prepared by dissolution of 0.07 gm maleic acid in 1 ml of distilled water to obtain a concentration of 70 mg/ml.

↓

All irrigation solutions were introduced into the canal using a 5 ml, 30-gauge side vented disposable plastic syringe inserted to a depth that is 1 mm less than the working length.

↓

Final irrigation of specimen was done with 5 ml of irrigating solution for a duration of 1 minute.

↓

The root canals were irrigated copiously with 10 ml of distilled water for 1 minute and then dried with paper points.

↓

To promote fluorescence under confocal laser scanning microscopy, Rhodamine B isothiocyanate dye was added to AH plus sealer during manipulation at an approximate ratio of 0.1% by weight.

↓

The endodontic sealer was applied in the canal 1-mm shorter than the working length using no. 25 lentulospiral and single cone obturation was done with ProTaper Universal F3 gutta-percha cone.

↓

Excess gutta-percha was removed using a heated plugger and the access cavity sealed with cavit.

↓

Each tooth was sectioned perpendicular to its long axis in 1-mm-thick sections, at levels 2, 5, and 8 mm from the root apex using a diamond disc.

The teeth were stored in an incubator at 37°C and 100% humidity for 7 days to allow the sealer to set.

↓

All sections were polished with silicon carbide abrasive paper.

↓

All specimens were mounted onto glass slides and examined with a confocal laser scanning microscope and dentinal tubule penetration were measured in micrometers with ImageJ software.

FIGURE 1: Human mandibular premolar selected for study

FIGURE 2: Debris removal with ultrasonic scaler

FIGURE 3: Materials used for the study

FIGURE 4: Armamentarium used for the study

FIGURE 5: Decoronation of the samples with diamond disk

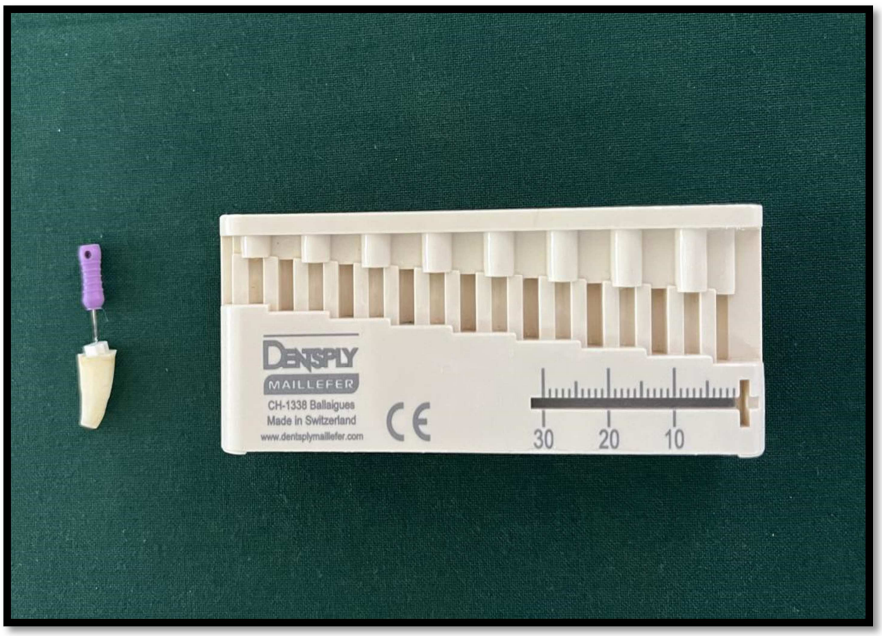


FIGURE 6: WL Determination

FIGURE 7: Biomechanical Preparation

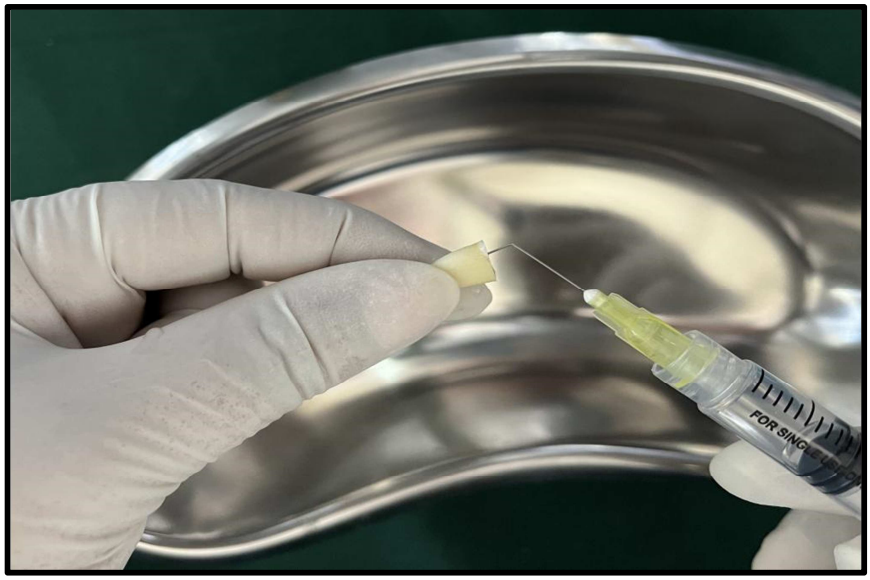


FIGURE 8: Irrigation of samples

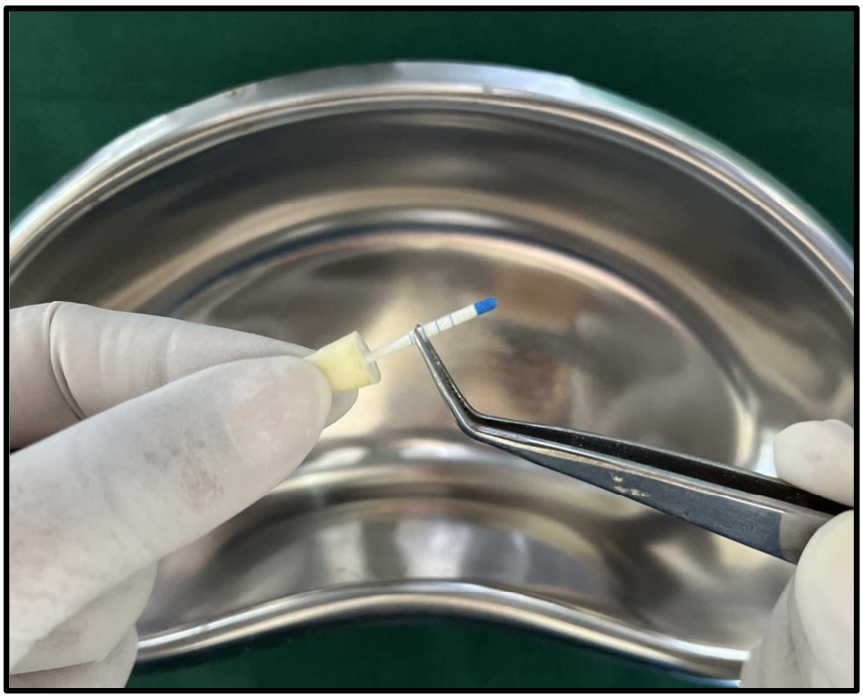


FIGURE 9: Drying canals with paper point

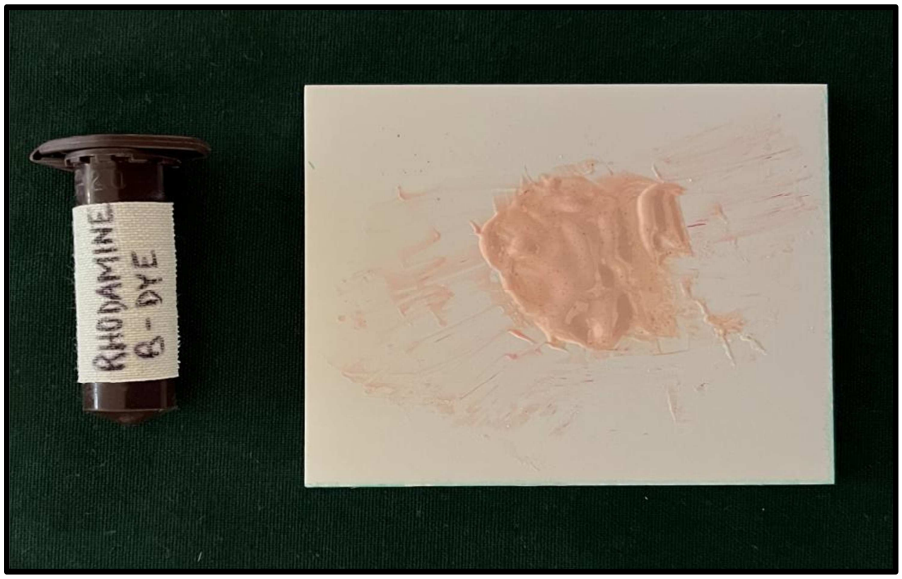


FIGURE 10: 0.1% Rhodamine B Dye incorporation in sealers

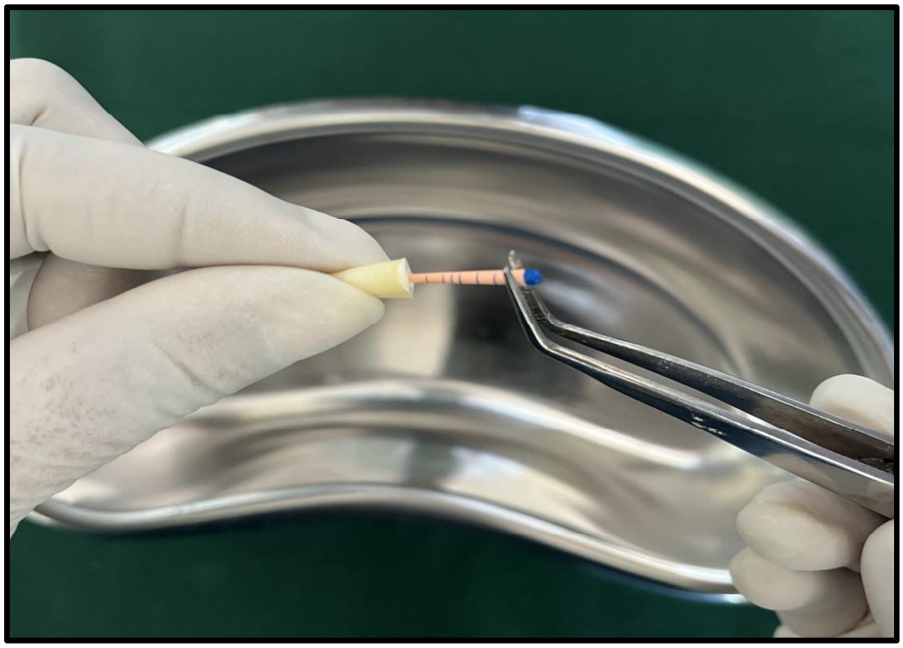


FIGURE 11: Obturation

FIGURE 12: Incubator

FIGURE 13: Sectioning

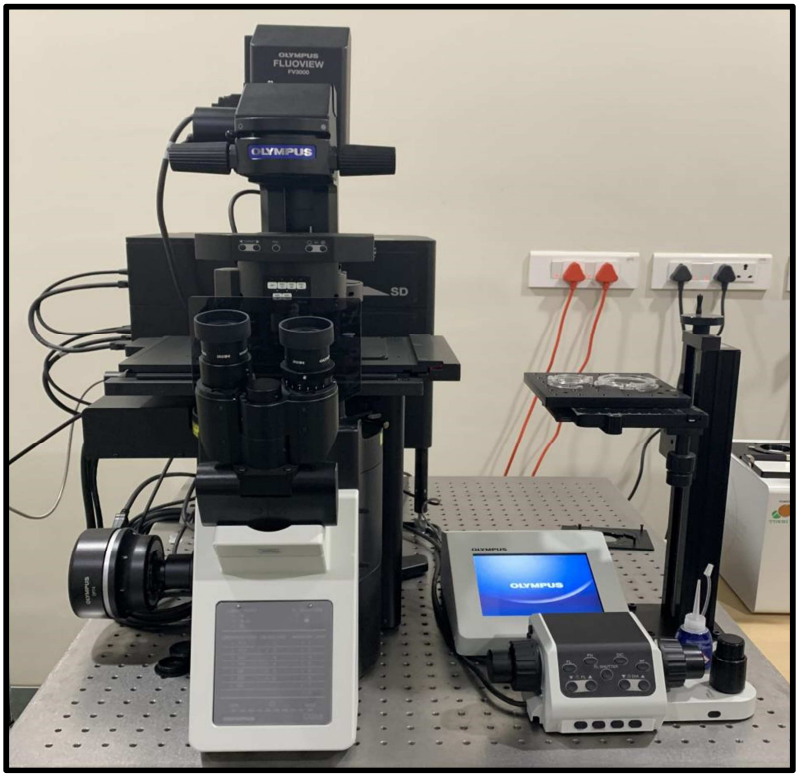


FIGURE 14: Confocal laser scanning microscope

RESULTS

Table 1: Shows mean depth of sealer penetration, standard deviation, and standard error in three groups of final irrigating solutions containing 22 samples each at three levels.

Factors	Level of factors	N	Mean	SD	SE	95%CI for mean	
						Lower	Upper
Group	N-Acetyl cysteine	66	514.28	261.99	32.25	449.88	578.69
	Maleic Acid	66	566.34	205.47	25.29	515.83	616.85
	EDTA	66	499.01	241.59	29.74	439.62	558.40
Region	Coronal region	66	811.43	83.68	10.30	790.86	832.00
	Middle region	66	497.08	99.00	12.19	472.74	521.41
	Apical region	66	271.13	73.59	9.06	253.04	289.22
Interactions	N-Acetyl cysteine with Coronal	22	829.35	85.36	18.20	791.50	867.20
	N-Acetyl cysteine with Middle	22	495.46	92.25	19.67	454.56	536.36
	N-Acetyl cysteine with Apical	22	218.03	18.45	3.93	209.85	226.21
	Maleic Acid with Coronal	22	814.35	73.46	15.66	781.78	846.91
	Maleic Acid with Middle	22	522.92	112.32	23.95	473.12	572.72
	Maleic Acid with Apical	22	361.76	49.03	10.45	340.03	383.50
	EDTA with Coronal	22	790.59	90.51	19.30	750.46	830.72
	EDTA with Middle	22	472.85	88.85	18.94	433.46	512.24
	EDTA with Apical	22	233.59	31.37	6.69	219.68	247.50

Graph 1: Shows graphical representation of mean for depth of penetration in each group at each level. Group 1 exhibited greatest penetration of sealer inside tubules at coronal level i.e., 829.35 µm. Group 2 exhibited greatest penetration of sealer at middle level i.e., 522.92 µm and apical level i.e., 361.76 µm.

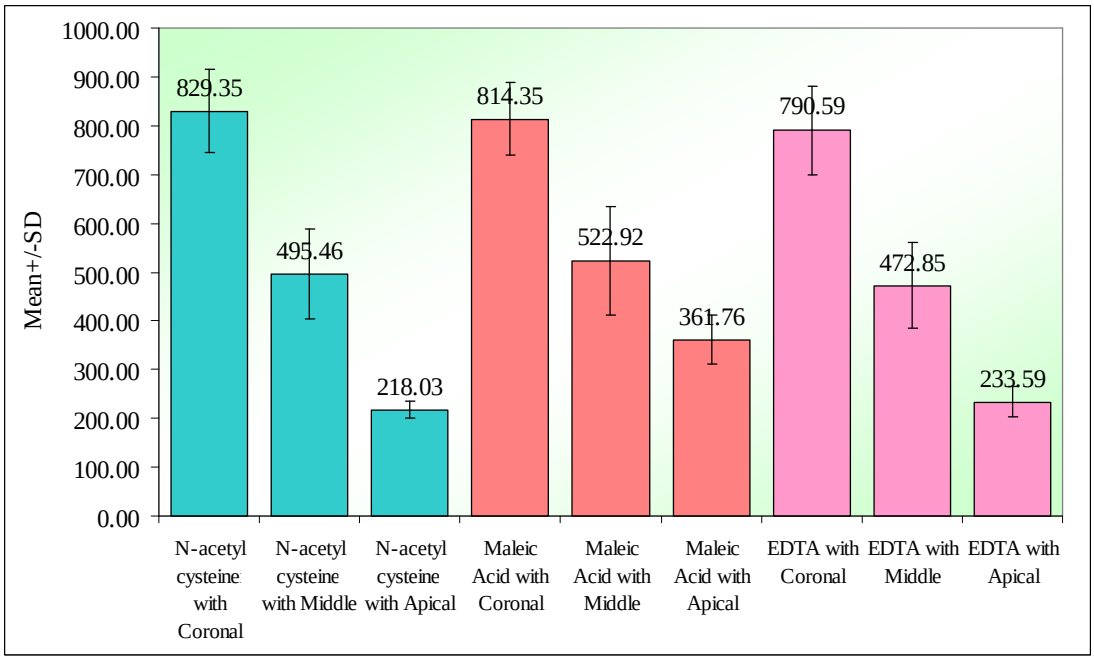


Table 2: Shows that according to Two-way ANOVA test, variation of depth of penetration of sealer after final irrigation between 3 groups at 3 levels was statistically significant.

Sources of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F-value	p-value
Main effects					
Groups	164507.00	2	82253.50	13.8052	0.0001*
Regions	9719455.13	2	4859727.57	815.6443	0.0001*
2-way interaction effects					
Groups*Regions	153718.56	4	38429.64	6.4499	0.0001*
Error	1126089.56	189	5958.15		
Total	11163770.25	197			

*$p<0.05$ indicates significant

Table 3: Shows details of Tukey's multiple post hoc analysis which illustrates a statistically significant difference in mean depth of infiltration of group 2 compared with group 1 and 3.

Groups	N-Acetyl cysteine	Maleic Acid	EDTA
Mean	514.28	566.34	499.01
SD	261.99	205.47	241.59
N-Acetyl cysteine	-		
Maleic Acid	P=0.0003*	-	
EDTA	P=0.4914	P=0.0001*	-

*p<0.05 indicates significant

Graph 2: Graphical representation of mean depth of infiltration of sealers in three groups.

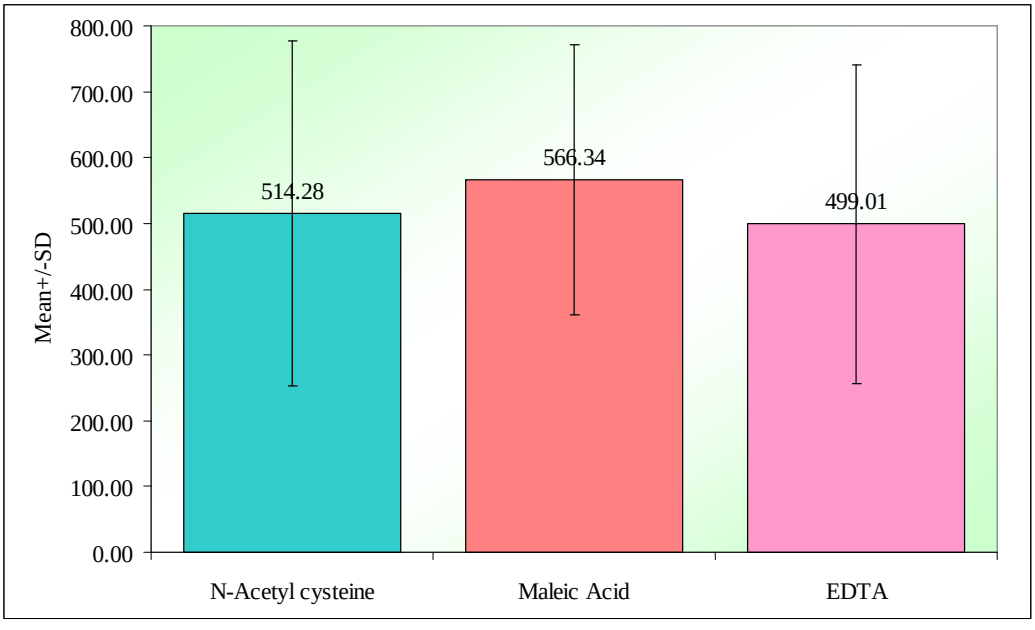
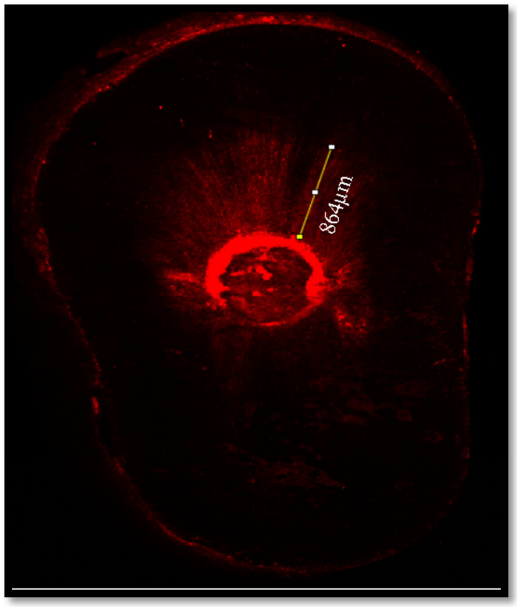


Table 4: Shows pair wise comparisons of mean depth of dentinal tubule penetration in three groups at three levels by Tukey's multiple post hoc analysis

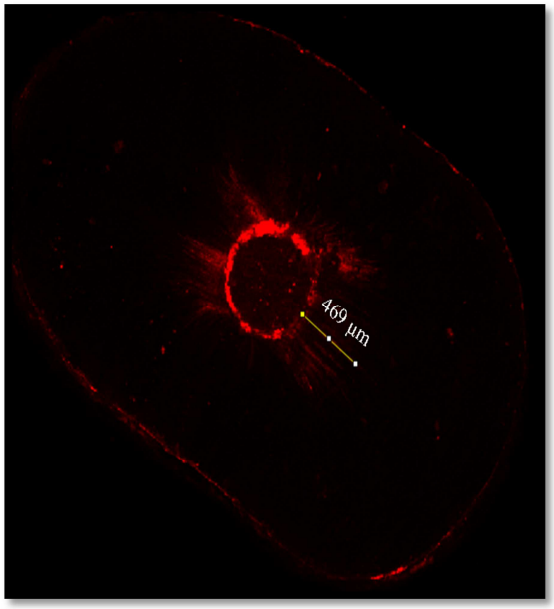
Interactions	N-acetyl cysteine with Coronal	N-acetyl cysteine with Middle	N-acetyl cysteine with Apical	Maleic Acid with Coronal	Maleic Acid with Middle	Maleic Acid with Apical	EDTA with Coronal	EDTA with Middle	EDTA with Apical
Mean	829.35	495.46	218.03	814.35	522.92	361.76	790.59	472.85	233.59
SD	85.36	92.25	18.45	73.46	112.32	49.03	90.51	88.85	31.37
N-acetyl cysteine with Coronal	-								
N-acetyl cysteine with Middle	**P=0.0001***	-							
N-acetyl cysteine with Apical	**P=0.0001***	**P=0.0001***	-						
Maleic Acid with Coronal	P=0.9993	P=0.0001*	P=0.0001*	-					
Maleic Acid with Middle	P=0.0001*	P=0.9609	P=0.0001*	**P=0.0001***	-				
Maleic Acid with Apical	P=0.0001*	P=0.0001*	P=0.0001*	**P=0.0001***	**P=0.0001***	-			
EDTA with Coronal	P=0.7677	P=0.0001*	P=0.0001*	P=0.9840	P=0.0001*	P=0.0001*	-		
EDTA with Middle	P=0.0001*	P=0.9884	P=0.0001*	P=0.0001*	P=0.4381	P=0.0001*	**P=0.0001***	-	
EDTA with Apical	P=0.0001*	P=0.0001*	P=0.9991	P=0.0001*	P=0.0001*	P=0.0001*	**P=0.0001***	**P=0.0001***	-

*p<0.05 indicates significant

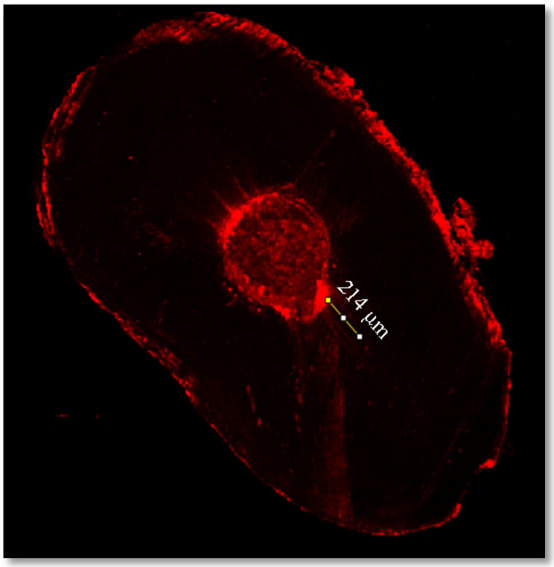
Figure 15: Shows dentinal tubule penetration of AH Plus sealer after final irrigation with Group 1: 20% N- acetyl cysteine at coronal, middle, and apical level respectively. Maximum infiltration was exhibited in coronal area followed by middle and least in apical part.



CORONAL

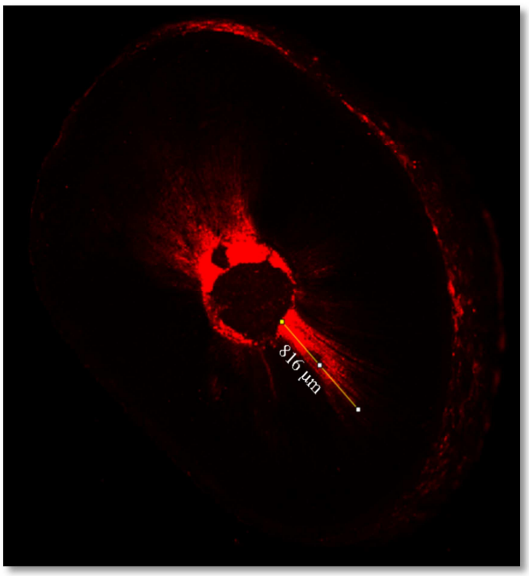


MIDDLE

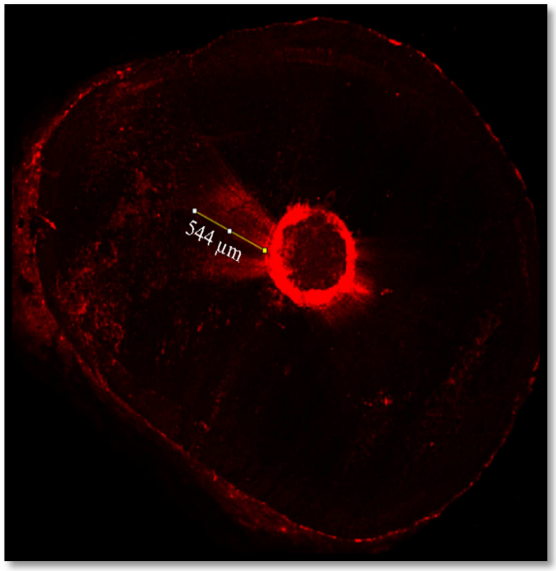


APICAL

Figure 16: Shows dentinal tubule penetration of AH Plus sealer after final irrigation with Group 2: 7% Maleic acid at coronal, middle, and apical level respectively. Maximum infiltration was exhibited in coronal area followed by middle and least in apical part



CORONAL



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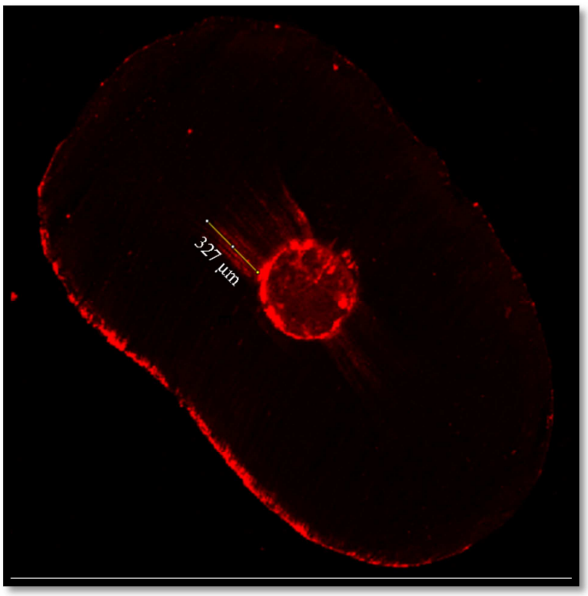
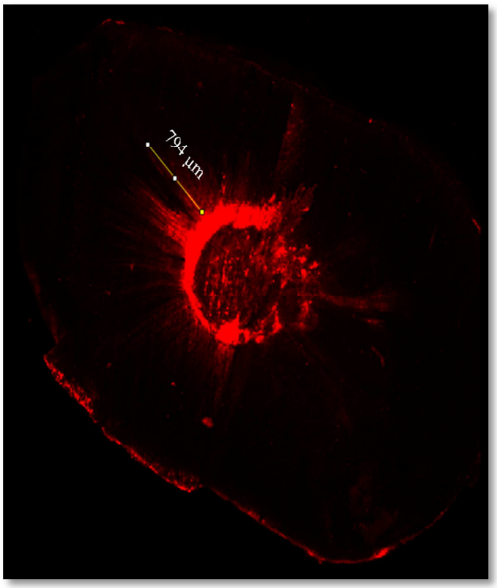
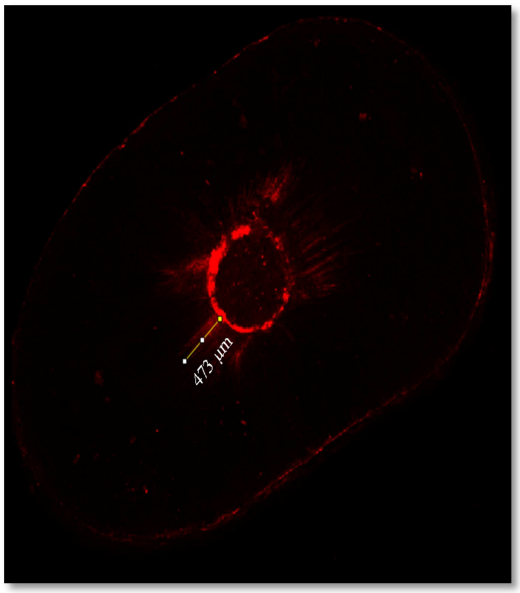


APICAL

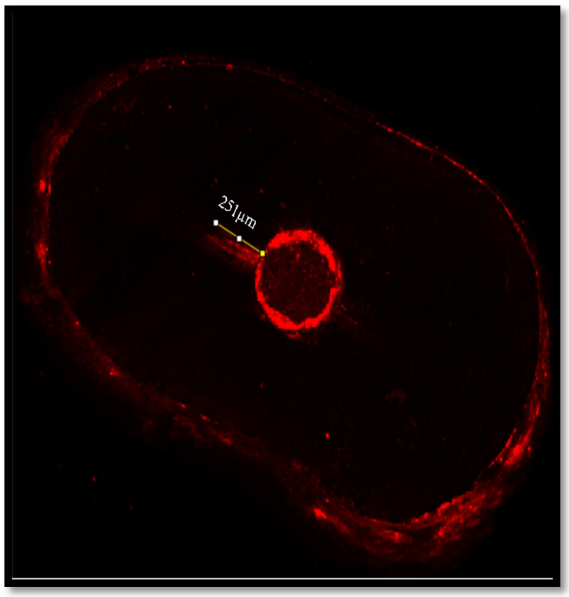
Figure 17: Shows dentinal tubule penetration of AH Plus sealer after final irrigation with Group 3: 17% EDTA at coronal, middle, and apical level respectively. Maximum infiltration was exhibited in coronal area followed by middle and least in apical part.



CORONAL



MIDDLE



APICAL

DISCUSSION

Endodontic therapy is focused on eliminating the microorganisms from the root canal system since the presence of microorganism may cause persistent infection. Hence, penetration of final irrigant is crucial for antibacterial activity.[30] The use of irrigating agents also aid in the removal of smear layer which assists in optimum penetration of root canal sealer.[5] Sealer penetration into the dentinal tubule is of great significance as it occludes the dentinal tubules by increasing the contact interface between the gutta percha and canal wall.[25] The penetration of sealer into the dentinal tubules not only creates a physical barrier but also has antibacterial action.[31]

Various chelating agents have been used in endodontic therapy and EDTA is one of the most commonly used chelators in dentistry.[4] Disodium salt of Ethylenediaminetetraacetic acid (EDTA) is a polyaminocarboxylic acid and possesses the property to sequesterate di- and trivalent metal ions such as Ca^{2+} and Fe^{3+} and forms a stable ring structure.[32,33] The reaction of EDTA with calcium ions in the hydroxyapatite crystals causes the dissolution of the inorganic material present in the root dentin.[8] EDTA is mostly used as a neutral solution (pH 7) and can be used at different concentrations such as 5%, 10% and 17% for smear layer removal. However, it is commonly used at 17% concentration and has been reported to efficiently remove smear layer when used for 1 minute.[7,33,34]

17% EDTA has exhibited a few drawbacks such as, its reduced efficacy in smear layer removal from the apical section as compared to the coronal section and middle section of the root canal.[5,6] Application of EDTA also has an erosive effect on the root dentin and causes erosion of peritubular, as well as the intertubular dentin which reduces the microhardness of the root dentin.[34,35] A study done by Saleh and

Ettman have also demonstrated that the chelating action of EDTA causes significant depreciation in microhardness of the root dentin.[36] Furthermore, EDTA causes certain amount of irritation since it has a deleterious effect on the periapical tissues.[9]

N-acetyl cysteine is a glutathione precursor and a cysteine prodrug. It is a derivative of amino acid-L-cysteine.[10] NAC has been used as a chelating agent for detoxification in case of heavy metal toxicity such as lead and mercury, by binding metal ions into complexes. NAC is also a potent thiol containing antioxidant that scavenges free radicals.[10,37] NAC also possesses excellent anti-microbial and anti-biofilm activity and is effective against endodontic pathogens, thus eradicating residual bacterial infection within the root canals.[37,38] Rajakumaran et al has proved N-acetyl cysteine to be as effective as EDTA in removing smear layer from the root canal.[9]

There are some disadvantages of NAC, such as lower concentrations of NAC at 12.5-50 mg/ml were inefficient at completely removing mature multispecies biofilm and hence, warrants the use of NAC at higher concentrations.[38] It has also shown to alter the hardness of root dentin and causes a reduction in the dentin microhardness. However, it has proven to cause lesser reduction in microhardness as compared to EDTA.[9] There are no current studies evaluating the effect of N-acetyl cysteine on the penetration depth of root canal sealer.

Maleic acid is a mild organic acid, which is used as a chelating agent in endodontic therapy. It is also used as an acid etchant to prepare the tooth surface for application of an adhesive.[23] Maleic acid can be used at 5% and 7% concentrations.[5] According to Ballal et al, maleic acid at 7% concentration has shown to be more effective than 17% EDTA for smear layer removal when used as a final irrigating

solution for 1 minute.[13] Also various studies evaluating the dentinal tubule penetration of the sealer after using maleic acid as final irrigating agent has shown comparable dentinal tubule penetration as EDTA.[3,25] Maleic acid has few disadvantages, such as its high acidity (pH 1.05) which causes a significant reduction in root dentin microhardness.[5,39] MA has a substantial demineralizing effect on the dentin and also causes damage to the intertubular dentin.[32]

There are a few documented studies on the effect of final irrigation on the penetration of sealer into the dentinal tubules. However, according to current literarture there are no studies comparing the three irrigants, i.e., 20% NAC, 7% MA, and 17% EDTA.

Thus, the current study investigated the effect of N-acetyl cysteine, maleic acid and EDTA on the depth of dentinal tubule penetration of an epoxy resin-based root canal sealer using confocal laser scanning microscopy.

Human mandibular premolars were used in the present study, due to their easy availability, since they are often extracted for orthodontic reasons. Teeth were stored in 0.1% thymol solution to inhibit fungal growth. Teeth were radiographed and sixty-six teeth were selected based on inclusion and exclusion criteria. Teeth were decoronated to produce a standardized root length of 14 mm using a diamond disc. Standardization of root length was done to eliminate any dissimilarity in access cavity preparation. It also establishes an even surface which would provide a stable and uniform reference point for tooth preparation. Working length was evaluated using a size 10-K file till it was just seen at apex and reducing 1 mm from the recorded length. The root canals were enlarged up to size 20-K file with hand instrument. Van der Vyer had stated that, 15 or 20-K hand file should reach the working length easily

to establish a glide path. K- files offer certain advantages over rotary Ni-Ti files for glide path preparation such as better tactile sensation and less potential for instrument separation. The stiffness of the stainless-steel instrument also aids in negotiation of calcified canals.[40] ProTaper Universal Ni-Ti rotary files were utilized for biomechanical preparation of the canals according to the recommended protocol given by the manufacturer. ProTaper instruments provide an advantage over stainless steel instruments by using lesser files for biomechanical preparation of canals and reducing the preparation time. Furthermore, Ni-Ti instruments have superior properties such as superelasticity and enhanced cutting efficiency.[41]

Irrigation plays a pivotal role in the disinfection of root canal system. In the current study, 1 ml of 2.5% NaOCl solution was used between consecutive instrumentation due to its strong antimicrobial activity, pulp dissolution abilty and lubricating action.[42]

According to the final irrigation protocol, the teeth were randomly divided into 3 groups, i.e N-acetyl cysteine, maleic acid, EDTA respectively. The process of randomization allows an equal opportunity for every specimen to be allocated into any of the groups. It reduces bias and allows for comparability.

NAC Irrigating Solution was prepared according to Quah et al, by dissolution of 0.2 gm NAC in 1 ml of distilled water to obtain a concentration of 200mg/ml.[11]

Maleic Acid Irrigating Solution was prepared according to Ballal et al, by dissolution of 0.07 gm maleic acid in 1 ml of distilled water to obtain a concentration of 70 mg/ml.[13]

For the present study, commercially available 17% EDTA solution (Canalarge) was used.

Final irrigation of specimen was done with the experimental irrigating solution since smear layer obstructs the penetration of sealer into the dentinal tubules. All irrigation solutions were introduced into the canal using a 5 ml, 30-gauge side vented disposable plastic syringe inserted to a depth that is 1 mm less than the working length to effectively clean the apical third of the canal. Needle is positioned close to the working length since irrigating solutions rarely flow 1-mm beyond the needle tip.[43]

30-gauge side vented needles were used for the current study and is the finest needle currently used in endodontic treatment. Side vented needles reduce the extrusion of irrigants, thereby minimizing tissue damage. Furthermore, Kahn et al has reported side vented closed end needles to be more effective than conventional needles.[44] The specimens were copiously irrigated using distilled water to terminate the chelating action of the final irrigating agent.

In the current study AH Plus sealer was used. AH Plus sealer is an epoxy resin-based sealer and is widely used in endodontic therapy .[16] AH Plus sealer is considered 'Gold Standard' due to its excellent properties such as good handling characteristics and superior physicochemical properties such as high bond strength and minimal polymerization shrinkage.[17,45]

Labelling of sealer with a fluorescent dye is necessary to evaluate the specimen with confocal laser scanning microscope. Rhodamine B (SigmaAldrich) was used as dye for CLSM to promote optimal fluorescence in the endodontic sealer.

The use of Rhodamine dye does not influence the physicochemical properties of the sealer.[19]

The endodontic sealer was applied in the canal using lentulospiral, since it has shown better sealer penetration into the dentinal tubule due to the centrifugal action of lentulospiral which pushes the sealer against the root canal walls.[46]

Obturation was done using single cone (SC) technique, which is widely used in endodontic therapy. Obturation aims at elimination of canal space and sealing of apical foramen to acquire greater area of GP and lesser area of sealer.[47] According to Rodrigues et al, SC has shown a higher percentage of GP in the apical area compared to lateral condensation, thus providing a viable alternative.[48]

Excess GP was sheared off using heated hand plugger and the access cavity was sealed with cavit for all the specimen. Incubation was done at 37º C and 100% humidity for 7 days. This was done to allow the sealer to set completely and to simulate oral conditions.

Sectioning of the teeth were done to obtain 1-mm-thick sections from all three levels using a diamond disc and used for CLSM scanning. Sectioning of each sample was done perpendicular to its long axis at three different levels, 2,5 and 8 mm from the root apex which represents the apical, middle, and coronal regions respectively.

The sections were then polished using silicon carbide abrasive paper. This was done to obtain an even surface and to remove debris produced during the sectioning of specimen with diamond disc.[49]

In the present study, CLSM was used to check tubular penetration. Other evaluation techniques utilized to assess the sealer penetration are light microscope[50,51] and scanning electron microscope.[52,53] The drawback of LM is the inability to differentiate between sealer and the radicular dentin.[54] The disadvantage of SEM is that the procedure is cumbersome and time-consuming, since SEM works under vacuum and must undergo additional processing such as gold sputtering.[55]

The advantage that CLSM provides over the other techniques is that it does not need additional sample processing and produces fewer artifacts. CLSM has additional advantages over SEM, such as ability to control depth of field. It also reduces image degradation due to elimination of background information from the focal plane.[54]

Current research data was analysed using ImageJ software. Statistical analysis was done using Two-way ANOVA and Tukey's multiple post hoc test. Intragroup comparisons were made with Two-way ANOVA while intergroup comparisons were carried out by utilizing Tukey's multiple post hoc test.

Result of the current study shows that on intragroup comparison, maximum sealer penetration had occurred in the coronal third, followed by middle third and least penetration in apical third in each group i.e, 17% EDTA, 20% NAC and 7% MA. This difference in sealer penetration was statistically significant among all the three sections i.e., coronal, middle and apical section among all the three groups.

This could be attributed to the fact that apical third of the root canal has fewer dentinal tubules compared to the rest of the canal and some regions in the apical area may even be devoid of tubules.[56] The apical third of the root canal shows variation in

anatomy, such as presence of irregular secondary dentin and cementum like tissue, which may hinder penetration of the sealer in the apical region.[57] The diameter of dentinal tubule at DEJ is 1.2 µm. The diameter decreases towards the apex and measures 0.4 µm at the CEJ.[58] Due to the morphological and histological variations, there is a reduction in the effectiveness of smear layer removal in the apical region. Furthermore, the lesser diameter of the apex also makes it difficult to access this part of the canal.[54]

The intragroup results observed in this study for 17% EDTA is in accordance with previous studies by Giardino et al, Kamin et al, and Matos et al wherein 17% EDTA showed a significant increase in the sealer penetration depth from apical to coronal sections.[59,60,61] However in a study conducted by Machado et al sealer penetration at apical third had similar values of the coronal and middle thirds. These controversial results might be explained by the obturation technique used in the study. The increased penetration of the sealer could be attributed to the greater force applied during lateral and vertical condensation which presses the sealer against the canal walls.[27]

NAC has also shown significantly greater dentinal penetration of sealer in coronal third, followed by middle and apical third. This could be due to the greater smear layer removal in coronal third compared to middle and apical third, as demonstrated in a study by Rajakumaran et al.[9] However, in the current literature, no previous studies exist which evaluates the effect of NAC on the dentinal tubule penetration of sealer.

Tuncer et al, Shetty et al, and Osazir et al, had also reported increased dentinal tubule penetration in coronal third, followed by middle and apical third with maleic acid as final irrigating agent.[3,24,25] In these studies, a statistical significance has been noted among the three levels which are in accordance to the results of the current investigation.

Intergroup comparison done by Tukey's post hoc analysis revealed significant difference in the apical third, wherein 7% MA has shown significantly increased sealer penetration compared to 17% EDTA and 20% NAC.

Thus, the null hypothesis has been rejected that there will be no difference in the effect of N-acetyl cysteine, maleic acid and EDTA on the dentinal tubule penetration of an epoxy resin-based root canal sealer at apical, middle, and coronal third of the root canal.

According to Osazir et al and Tuncer et al, there was no statistical difference in the depth of sealer penetration in the apical sections comparing 7% MA and 17% EDTA.[3,25] However, Osazir et al has shown a marginal increase in the depth of sealer penetration with 7% MA acid as compared to 17% EDTA.[25] In the current study, the increased sealer penetration in the apical region could be attributed to the effectiveness of maleic acid in removal of smear layer from the apical region compared to other irrigants as seen in previous studies.[5,6,13] Ballal et al, has reported that final irrigation for the removal of smear layer with 7% MA for 1 minute was more effective than 17% EDTA in the apical third of the root canal. This may be due to the higher surface tension of 17% EDTA as compared to 7% MA. Also, maleic acid is a more acidic chelator compared to EDTA. It has shown to have a better demineralizing effect on dentin.[13]

Decreased sealer penetration with EDTA could be attributed to the ability of EDTA to remove calcium ions from dentin, as well as calcium bonded to non-collagenous protein component (NCPs) of dentin and there is a reduction in the number of NCPs in the apical region. Hence, lesser decalcification is seen in this region.[8] Jaiswal et al, conducted a study comparing smear layer removal between 7% MA and 17% EDTA. They have stated that the higher molecular size of EDTA than that of maleic acid may be the reason for lesser demineralization by EDTA, since larger molecules bind to lesser number of calcium ions.[6]

However, in a study conducted by Shetty et al, comparing the sealer penetration between 17% EDTA and 7% MA, the depth of sealer penetration with 17% EDTA was shown to be more in apical third as compared to 7% MA, which is contradictory to the results obtained in the current investigation. The greater depth of sealer penetration with 17% EDTA was attributed to the ability of EDTA to react with calcium ions in the dentin. Interaction of EDTA with root dentin forms soluble calcium chelates.[24]

In the current study, dentinal penetration of sealer at the apical region with 20% NAC and 17% EDTA has shown to be comparable and there is no statistical significance between them. This could be attributed to the effectiveness of smear layer removal in the apical third by 20% NAC to be comparable to 17% EDTA, according to the investigation conducted by Rajakumaran et al.[9] Since N-acetyl cysteine is a novel endodontic irrigant which has been introduced in dentistry due to its antioxidant and chelating properties, no previous studies have been done yet on the effect of NAC on tubular penetration of the sealer.

Intergroup comparisons of the middle and coronal sections showed no statistical difference between the three groups. This finding is in accordance with previous studies by Tuncer et al, Osazir et al comparing the effect of 7% MA and 17% EDTA for sealer penetration, where no statistical difference was seen in the tubular penetration of sealer in the middle third and coronal third. [1,25]

However, Shetty et al has reported increased depth of sealer penetration with 17% EDTA as compared to 7% MA in middle third and coronal third, which is contradictory to the results obtained in the current investigation. The greater depth of sealer penetration with 17% EDTA compared to 7% MA was due to the ability of EDTA to interact with the root dentin and form soluble calcium chelates.[24]

Comparable sealer penetration between 17% EDTA and 7% MA could be attributed to the smear layer removal efficacy of maleic acid. According to Ballal et al and Kaushal et al, 7% MA was equally effective in smear layer removal as 17% EDTA in middle third and coronal third.[5,13] According to Rajakumaran et al, 20% NAC has also shown to be equally effective in smear layer removal in middle third and coronal third compared to 17% EDTA.[9]

In the current study, optimal sealer penetration has been observed with 7% MA and 20% NAC and can be used as alternatives to 17% EDTA for final irrigation of root canal. Further studies are needed to evaluate sealer penetration with other final irrigation protocol and obturation methods. The major limitation of the present study is that the experiment was an in-vitro laboratory study and has yet to be clinically tested. Clinical studies are required to evaluate the effect of the final irrigants on the clinical success of endodontic treatment.

CONCLUSION

Within the limitations of this study, the following conclusions can be drawn:

- All the three final irrigants exhibited penetration of sealer inside dentinal tubules.
- The sealer penetration at coronal and middle third were similar among all three groups.
- Higher penetration of sealer at the apical region was observed with 7% MA as compared to 20% NAC and 17% EDTA.
- Also, maximum sealer penetration was noted in the coronal third, followed by middle and least in apical third for all tested groups.

Hence, 7% MA and 20% NAC are good alternatives to 17% EDTA as final irrigating agents and can be recommended for endodontic use.

SUMMARY

A successful endodontic treatment consists of biomechanical preparation, disinfection and obturation of the root canal system. Formation of an impervious three-dimensional seal is of utmost importance to form a microbiological barrier. Endodontic sealers are used to occlude the voids and irregularities between the obturating material and the canal wall. Tubular penetration of sealer enhances the root-dentin bond. Endodontic irrigants are crucial for the penetration of sealer into the dentinal tubules for a fluid tight seal. Endodontic irrigants eliminates microorganisms and also removes smear layer which assists in optimum penetration of root canal sealer.

In the current investigation, evaluation and comparison of the effect of final irrigating solutions i.e, N-acetyl cysteine, maleic acid and EDTA on the depth of penetration of an epoxy resin-based sealer in the dentinal tubules was done.

Human mandibular premolar teeth were used for the present study. Diamond disc was used to decoronate the samples up to the level of CEJ to obtain a standardized working length of 14 mm. Working length determination was done using 10-K file. Biomechanical preparation of the canals were done using ProTaper Universal rotary system up to size F3 and samples were irrigated with 2.5% NaOCl between consecutive instrumentation. The samples were randomly divided into three groups according to final irrigation used i.e, 20% N- acetyl cysteine, 7% maleic acid, 17% EDTA. Samples were copiously irrigated using distilled water. The specimen were later dried with paper points. AH plus sealer was mixed with Rhodamine B dye to promote fluorescence under confocal laser scanning microscope. Teeth were

incubated for 7 days and later sectioned at coronal, middle and apical thirds with diamond disc for CLSM evaluation.

Results obtained indicated that, all the three final irrigants exhibited penetration of sealer inside dentinal tubules. The sealer penetration at coronal and middle third were similar among all the three groups and no statistical difference was observed between them. Higher penetration of sealer at the apical region was observed with 7% MA as compared to 20% NAC and 17% EDTA. 7% MA showed statistically significant higher sealer penetration compared to 20% NAC and 17% EDTA in the apical section.

Therefore the null hypothesis has been rejected that there will be no difference in the effect of N-acetyl cysteine, maleic acid and EDTA on the dentinal tubule penetration of an epoxy resin-based root canal sealer at apical, middle and coronal third of the root canal.

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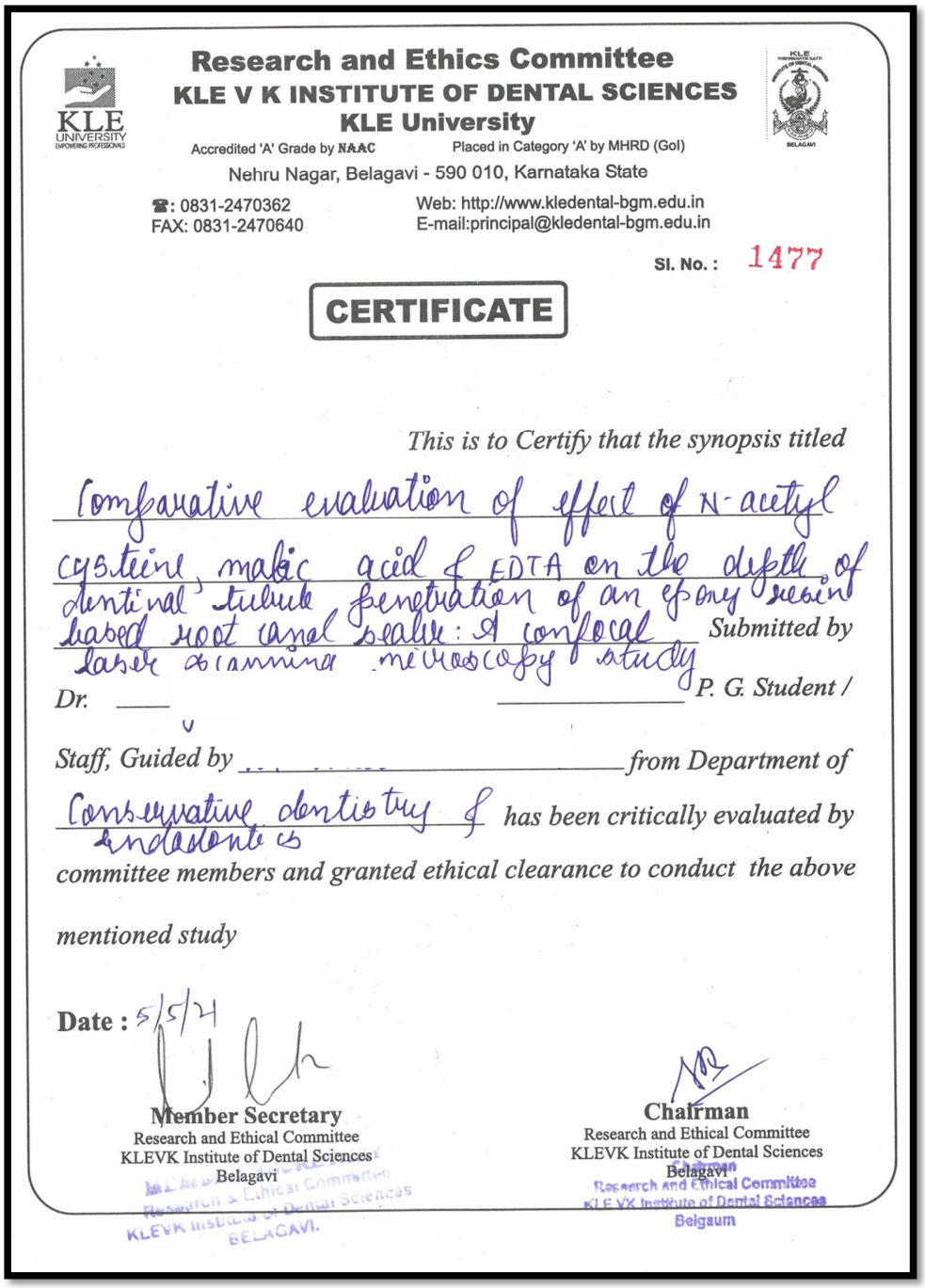
ANNEXURE I

ETHICAL CLEARANCE CERTIFICATE



Research and Ethics Committee
KLE V K INSTITUTE OF DENTAL SCIENCES
KLE University
Accredited 'A' Grade by NAAC Placed in Category 'A' by MHRD (GoI)
Nehru Nagar, Belagavi - 590 010, Karnataka State
☎: 0831-2470362
FAX: 0831-2470640
Web: http://www.kledental-bgm.edu.in
E-mail:principal@kledental-bgm.edu.in

Sl. No. : 1477

CERTIFICATE

This is to Certify that the synopsis titled

Comparative evaluation of effect of N-acetyl cysteine, malic acid & EDTA on the depth of dentinal tubule penetration of an epoxy resin based root canal sealer: A confocal laser scanning microscopy study

Submitted by Dr. ____ P. G. Student / Staff, Guided by ____ from Department of Conservative dentistry & endodontics *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date : 5/5/24

Member Secretary
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

KLEVK Institute of Dental Sciences BELAGAVI.

Chairman Research and Ethical Committee KLE VK Institute of Dental Sciences Belgaum

ANNEXURE II

BIOSTATISTICS CLEARANCE CERTIFICATE

KLE V.K. Institute of Dental Sciences
(A Constituent unit of KLE Academy of Higher Education & Research
Deemed-to-be-University u/s 3 of the UGC Act, 1956)
Nehru Nagar, Belagavi-590 010 INDIA
Re-Accredited 'A' grade by NAAC (2nd Cycle) & Placed in Category 'A' by MHRD (GoI)
: 0831-2470362
FAX: 0831-2470640
Web: http://www.kledental-bgm.edu.in
E-mail: principal@kledental-bgm.edu.in

Biostatistics Clearance Certificate

This is to certify that the Biostatistics aspect of the Dissertation / Research work of **Post Graduate Student**, under the guidance of ,M.D.S, **Professor and HOD, Department of Conservative Dentistry and Endodontics**, entitled **"Comparative evaluation of effect of N- acetyl cysteine, maleic acid and EDTA on the depth of Dentinal tubule penetration of an epoxy resin-based root canal sealer: A confocal laser scanning Microscopy study"** has been done under my guidance and considered satisfactory.

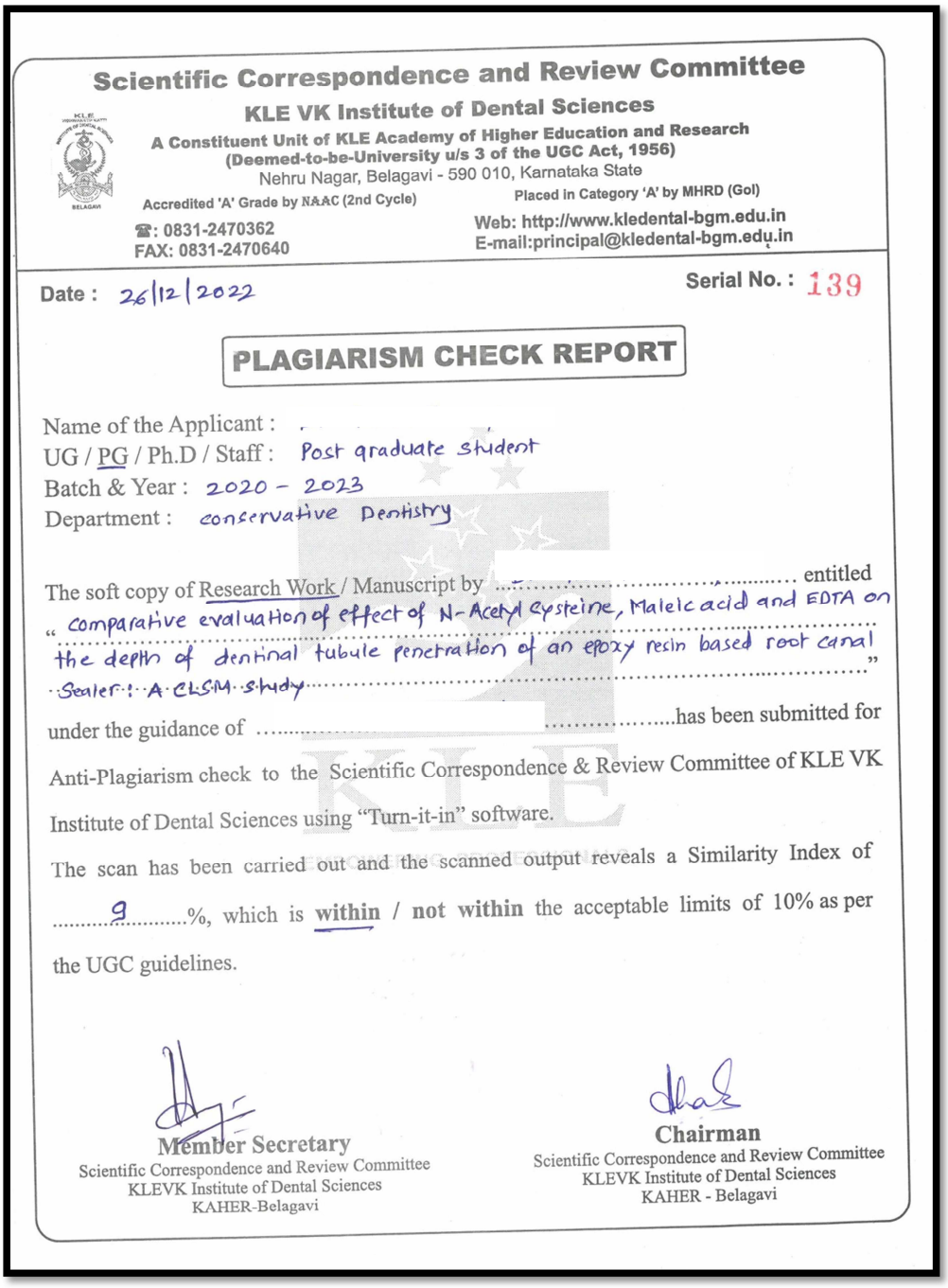
Place: Belagavi
Date: 14/11/2022

Name & Signature of Biostatistician

(Dr. S. B. Javali)
Sr. Asso. prof. in Statistics
USM KLE SMP, Belagavi

ANNEXURE III

PLAGIARISM CHECK CERTIFICATE



Scientific Correspondence and Review Committee

KLE VK Institute of Dental Sciences

A Constituent Unit of KLE Academy of Higher Education and Research
(Deemed-to-be-University u/s 3 of the UGC Act, 1956)
Nehru Nagar, Belagavi - 590 010, Karnataka State

Accredited 'A' Grade by NAAC (2nd Cycle) — Placed in Category 'A' by MHRD (GoI)

☎: 0831-2470362
FAX: 0831-2470640

Web: http://www.kledental-bgm.edu.in
E-mail:principal@kledental-bgm.edu.in

Date : 26/12/2022 Serial No. : 139

PLAGIARISM CHECK REPORT

Name of the Applicant :
UG / PG / Ph.D / Staff : Post graduate student
Batch & Year : 2020 - 2023
Department : conservative Dentistry

The soft copy of Research Work / Manuscript by ..... entitled
" comparative evaluation of effect of N-Acetyl cysteine, Maleic acid and EDTA on the depth of dentinal tubule penetration of an epoxy resin based root canal Sealer : A CLSM study "
under the guidance of ..... has been submitted for Anti-Plagiarism check to the Scientific Correspondence & Review Committee of KLE VK Institute of Dental Sciences using "Turn-it-in" software.

The scan has been carried out and the scanned output reveals a Similarity Index of 9 %, which is **within** / **not within** the acceptable limits of 10% as per the UGC guidelines.

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