A COMPARATIVE EVALUATION OF EFFICACY OF PHYTIC ACID, ETIDRONIC ACID AND EDTA ON SMEAR LAYER REMOVAL AND DENTIN EROSION AT DIFFERENT TIME INTERVALS USING SCANNING ELECTRON MICROSCOPE - AN IN VITRO STUDY

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in partial fulfillment of the requirements
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BRANCH – IV

CONSERVATIVE DENTISTRY AND ENDODONTICS

THE TAMILNADU DR. MGR MEDICAL UNIVERSITY
CHENNAI – 600 032
2014 – 2017

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I hereby declare that this dissertation titled **"A COMPARATIVE EVALUATION OF EFFICACY OF PHYTIC ACID, ETIDRONIC ACID AND EDTA ON SMEAR LAYER REMOVAL AND DENTIN EROSION AT DIFFERENT TIME INTERVALS USING SCANNING ELECTRON MICROSCOPE - AN IN VITRO STUDY"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. B. RAMAPRABHA, Professor,** Department Of Conservative Dentistry and Endodontics, Tamil Nadu Government Dental College and Hospital, Chennai-600003.

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DECLARATION

TITLE OF DISSERTATION	**"A COMPARATIVE EVALUATION OF EFFICACY OF PHYTIC ACID, ETIDRONIC ACID AND EDTA ON SMEAR LAYER REMOVAL AND DENTIN EROSION AT DIFFERENT TIME INTERVALS USING SCANNING ELECTRON MICROSCOPE-AN IN VITRO STUDY"**
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1.

2

ABSTRACT:

Aim: The purpose of this *in vitro* study was to compare the smear layer removal efficacy and dentin erosion of three different irrigating solutions at different time intervals of the root canal under Scanning Electron Microscopy.

Materials and Methods: One hundred extracted human single straight rooted maxillary central incisors were taken and decoronated to standardize the canal length of 15 mm. They were instrumented with ProTaper NEXT rotary system to an apical preparation of file size X5. Prepared teeth were irrigated with 3ml of 3% NaOCl for 5min followed by final rinse of 2ml of 1% phytic acid (Group I), 18% Etidronic acid (Group II) and 17% EDTA (Group III) at 5min, 3min and 1min. The canals of teeth in Control (Group IV) did not receive any final irrigation. The teeth were sectioned longitudinally and prepared for an SEM evaluation. The dentinal wall of cervical, middle and apical thirds were graded according to the amount of smear layer remaining and dentin erosion on the root canal walls. The results were analysed using the Kruskal–Wallis and Mann Whitney U tests with significance set at $P < 0.05$.

Results: Intergroup comparison showed statistically no significant difference (p=1.000) in the smear layer removal efficacy of irrigants tested at 5min, 3min and 1min except for Etidronic acid (Group II) at 1min (p=.000). Control (Group IV) showed statistically high significant difference (p=.000) than other groups. Apical region of all groups showed statistically high significant difference (p=.000) than cervical and middle region. Intergroup comparison of dentin erosion showed EDTA (Group III) had high erosion values (p=.000) than other groups which are statistically significant. Phytic acid (Group I) showed less erosion values (p=.000) than other groups which are highly significant.

Conclusion: Phytic acid showed effective smear layer removal with less erosion of the root canal wall. Increasing the duration of irrigation does not improve the smear layer removal efficacy of irrigants except for Etidronic acid (Group II) but all groups showed more erosion at increased irrigation time. All the groups did not completely remove the smear layer at the apical region.

Keywords: Smear Layer, Dentin Erosion, Phytic Acid, Etidronic Acid, EDTA

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ABBREVIATIONS

EDTA	ETHYLENE DIAMINE TETRA ACETIC ACID
HEDP	1-HYDROXYETHANE 1,1-DIPHOSPHONIC ACID
NaOCl	SODIUM HYPOCHLORITE
SEM	SCANNING ELECTRON MICROSCOPY
Min	MINUTES
NiTi	NICKEL TITANIUM
IP6	INOSITOL HEXAKISPHOSPHATE

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Introduction

INTRODUCTION

For a successful endodontic outcome, root canal system should be devoid of vital and necrotic remnants of pulp tissues, microorganisms and its toxins.[80] However root canal system is highly complex and variable making it difficult to clean and disinfect.

Chemo mechanical preparation plays an important role in success of the endodontic treatment.[18, 11] Pulpal tissue, microorganism and its byproducts are removed by instruments, irrigants and intracanal medicaments which are the main objectives of chemo mechanical phase.[11] Even with the instrumentation, isthmi, canal fins and accessory canals are untouched.[83, 60, 34] Therefore irrigation is an important part of root canal disinfection which cannot be achieved by instrumentation alone.[92]

Most irrigating solutions possess antimicrobial, tissue solvent and lubricant properties to facilitate root canal cleaning.[30] Instrumentation of root canal results in accumulation of organic and inorganic material known as smear layer.[9, 65, 54] During root canal instrumentation, it is almost inevitable for the formation of smear layer.[80]

Smear layer is defined as a surface film of debris retained on dentin or other surface, after instrumentation with either rotary instruments or endodontic files, according to American Association of Endodontists (2000). McComb & Smith (1975) were the first researchers to describe smear layer on root canal instrumented surface. Eick et al in 1970 first reported the use of Scanning Electron Microscopy to identify smear layer.

The amount of smear layer produced is greater in rotary instrumentation than hand instrumentation. Some authors believe that smear layer may block the dentinal tubules and limit bacterial or its toxins penetration by altering dentinal permeability. Alternatively some others believe that smear layer may limit the action of irrigant, intracanal medicament by harboring microorganisms when left in the root canal and it can lead to microleakage acting as a barrier between sealing of root canal wall and the restorative materials. They may interfere with bonding mechanism of resins. There is still a controversy in removing or retaining the smear layer produced. However more evidence favors removal of smear layer rather than its retention.[80]

There are various methods to remove smear layer like chemical, ultrasonic and laser techniques. None of the methods remove smear layer throughout the length of the canal completely.[80] Combination of methods help in achieving higher smear layer removal.

It is a well known fact that none of the currently used irrigating solutions have all the required properties of irrigant. Thus in common endodontic practice, dual irrigants are often used as initial and final rinse to overcome the disadvantages of using single irrigant.[21]

Sodium hypochlorite is the solution of choice during instrumentation in root canal treatment due to its strong antimicrobial properties. It dissolves only organic debris but not inorganic debris.[22]It decreases the micro hardness of the dentin and causes erosion of dentin at all concentrations.[47]They flush out the debris from the root canal completely

with any syringe delivery system. However they are cytotoxic to periapical cells and hence should be used with caution. [6]

Nygaard Ostby was the first to introduce chelating agents in endodontics. Chelating agents decalcify the dentine by combining with calcium ions of the tooth. They effectively removed smear layer at even low concentrations. With respect to chelating agents, effects of the decalcification depend heavily on the type of irrigant used, concentration, and pH of the solution and the application time.[48] Most important aspect of disinfection is that irrigant should be in direct contact with entire root canal for effective action particularly with respect to apical regions of the root canals.

EDTA (ethylene diamine tetra acetic acid) is the most common chelating agent which reacts with dentine to form calcium chelates. [47] It lacks antimicrobial properties. Von der Fehr & Nygaard Ostby (1963) found that in 5 minutes; EDTA can decalcify dentine to the depth of around 20-30µm. Combination of EDTA and NaOCl solutions for effective removal of smear layer were recommended.[47] Whenever NaOCl is used in combination with EDTA, NaOCl is inactivated earlier. This combination results in severe dentin erosion of root canal and dentinal tubules. [47] However the use of EDTA for more than 1 min may result in inadvertent erosion of root dentin. EDTA a synthetic, non-biodegradable material is considered a pollutant in root canal system and reported to be cytotoxic to macrophages. [2]

Therefore the focus was made to use alternative chelating agents that facilitate the complete smear layer removal without being much aggressive on root dentin. Hence an

effort to study two new chelating agents namely Phytic acid and Etidronic acid was taken.

Etidronic acid also known as 1-hydroxyethane 1,1-diphosphonic acid (HEDP) or etidronate. It is a bisphosphonate used in water treatment, cosmetics, detergents and pharmaceutical treatment. It emerged as the substitute for commonly used chelating agents. The advantage of etidronate is that it can be mixed with NaOCl without interfering in its antimicrobial properties. HEDP is a weak chelator, therefore it can be less aggressive on dentin than EDTA.[74] However this solution may need longer time for removal of smear layer. It is biocompatible with periapical tissues

Phytic acid is extracted from plant seeds, rice bran. It is also known as phytate when in salt form or inositol hexakisphosphate, IP6. It is an organic acid and is the major storage form of phosphorous.[45] Phytic acid shows antioxidant action.[27] Phytic acid has been approved as Generally Recognized As Safe (GRAS) in the United States and it is produced by Tsuno food industrial Co. Ltd., Japan. It has affinity to calcium ions because of high negatively charged molecule. It has the pH of around 1.2 and this low pH helps in better calcium extraction. It is the most potent natural mineral chelator. It has anti-fungal, anti-viral and antibiotic properties. [24] Thus the acidity and chelating function of phytic acid can make it an effective agent for smear layer removal. [25] It shows biocompatibility with periapical tissues. Reduction of micro hardness of dentin by phytic acid was less than that of EDTA.[46] It shows good bond strength values and had minimal effects on the pulpal cells when used as etchant.[44]

Scanning Electron Microscopic analysis is used in this study to obtain surface characteristics of dentin erosion and smear layer presence in root canal wall.

There are not many studies done on comparison of phytic acid, a newly available chelating agent, etidronic acid, a weak chelator and EDTA, a strong chelator together. Thus the present in-vitro study is an attempt to compare the efficacy of 1% Phytic acid, 18% Etidronic acid and 17% EDTA solution on the removal of intracanal smear layer and dentin erosion at 5min, 3min, and 1min under Scanning Electron Microscopy.

Aim and Objectives

AIM:

The aim of the study was to compare and evaluate the effectiveness of three chelating solutions namely 1% PHTYIC ACID, 18% ETIDRONIC ACID and 17% EDTA on smear layer removal and dentin erosion at different time intervals after 3% NaOCl irrigation for 5min under Scanning Electron Microscopy.

OBJECTIVES:

- To evaluate the effectiveness of 1% PHYTIC ACID on smear layer removal and dentin erosion at 5 min, 3 min and 1 min.
- To evaluate the effectiveness of 18% ETIDRONIC ACID on smear layer removal and dentin erosion at 5 min, 3 min and 1 min.
- To evaluate the effectiveness of 17% EDTA on smear layer removal and dentin erosion at 5 min, 3 min and 1 min.

Review of Literature

SMEAR LAYER

Shahravan et al (2007) [66] did a systematic review to determine whether smear layer removal reduces leakage of obturated human teeth in vitro. It was concluded that smear layer removal helps in achieving the fluid-tight seal of the root canal system whereas other factors such as the sealer or the obturation technique, did not produce significant effects.

Pintor et al (2016)[52] reviewed whether the smear layer removal procedure influences the outcome of root canal treatment. They concluded that the smear layer removal for root canal treatment of primary teeth with initial clinical signs and symptoms or necrotic status of pulp, could improve the treatment outcome, although further Randomized Control Trial should be performed to achieve evidence.

Likhitkar et al (2016)[36] assessed the effect of the presence/absence of a smear layer on the micro leakage of root canal filled teeth. Elimination of the smear layer enhanced the resistance to micro leakage;

ROTARY INSTRUMENTATION:

Khademi et al. (2006)[8] determined to find the minimum instrumentation size required for the effective penetration of irrigants and elimination of debris and smear layer from the apical third of the root canals. They concluded that minimum instrumentation size needed for penetration of irrigants to the apical third of the root canal is a #30 file.

Yang et al (2008)[87] evaluated the effect of ProTaper and Hero Shaper instruments on the amounts of debris and smear layer remaining on canal walls by NaOCl and EDTA irrigation in curved root canals. They concluded that both instruments in combination with NaOCl and EDTA irrigation produced a clean and debris-free canal surface in the

coronal and middle thirds, but in the apical third, they were unable to produce a canal surface free from debris and smear layer. However, ProTaper instrumented canals in the apical region showed smaller amounts of debris and smear layer.

Wadhwani et al (2011)[82] evaluated the ability of 19% EDTA gel and 17% ethylene diamine tetra acetic acid (EDTA) solution to remove debris, and smear layer produced during root canal istrumentation with two NiTi files systems, Mtwo and Protaper. They concluded that when used with EDTA gel and EDTA solution both the NiTi instruments produced a similar dentin surface on root canal wall.

Reddy et al (2014)[56] evaluated the amount of smear layer and debris removal on canal walls following the using of rotary ProTaper NiTi files compared with manual Nickel Titanium (NiTi) files using a Scanning Electron Microscope in two individual groups. They concluded that both systems of Rotary ProTaperNiTi and manual NiTi files used did not create completely clean root canals. Manual NiTi files produced significantly less smear layer and debris. Manual instruments were more time consuming when compared to rotary instruments.

Suparna et al (2015)[73] compared the cleaning efficacy of two different rotary file systems- WaveOne and ProTaper NEXT, using a Scanning Electron Microscope. They concluded that both the rotary systems ProTaper NEXT and WaveOne resulted in cleaner canals after instrumentation. However, the apical thirds of the root canals demonstrated more residual debris scores when compared to the middle and coronal thirds

Zarei et al (2016)[89] compared the influence of root canal taper (30/0.02 and 30/0.4) on the efficacy of irrigants and chelating agents in smear layer removal. They concluded

that greater smear layer was detected in the apical portion of each group. No statistical difference was found between canals with different tapers.

Kiran et al (2016) [32] evaluated the amount of smear layer and debris on the canal walls prepared with a combination of hand and rotary ProTaper technique using NaOCl and ethylene diamine tetra acetic acid (EDTA) alternately as root canal irrigants using scanning electron microscope (SEM).They concluded that none of the instrumentation techniques could completely eliminate the smear layer and debris from the root canal walls. Instrumentation of the canals with hand files after automated rotary preparation could result in cleaner canal walls. Alternative irrigation with NaOCl and EDTA is ineffective in the apical third.

IRRIGATION:

Kalyoncuoğlu and Demiryürek EÖ (2013) [28] evaluated the efficacy of smear layer removal from teeth following root canals using lasers (Er:YAG and Nd:YAG), NaOCl, 17% EDTA, and MTAD by scanning electron microscopy (SEM). They concluded that although improvement was observed in removal of the smear layer using alternative materials and techniques, application of a combination of EDTA and NaOCl remains an effective technique.

Guo X et al (2014) [19] compared the efficacy of four different irrigation techniques- a sidevented needle group, an EndoActivator group, a NaviTip FX group, a ultrasonic irrigation (UI) group, and a control group (no agitation) combined with 60 °C 3% NaOCl and 17% EDTA in smear layer removal. They concluded that regardless of different types of irrigation technique applied, in the apical third complete removal of the smear layer was not achieved.

Schmidt et al.(2015) [63] evaluated the efficacy of passive ultrasonic irrigation (PUI) with 17% EDTA and 1% NaOCl solutions on smear layer removal. They concluded that when compared with conventional irrigation, PUI did not show higher efficacy in smear layer removal

ROOT CANAL IRRIGANTS:

SODIUM HYPOCHLORITE:

Berber et al (2006) [8] evaluated the efficacy of 0.5%, 2.5% and 5.25% sodium hypochlorite (NaOCl) as intracanal irrigants against Enterococcus faecalis within root canals and dentinal tubule associated with hand and rotary instrumentation techniques. They found that 5.25% NaOCl was shown to be the most effective irrigant solution tested, when dentinal tubules were analysed at all depths and thirds of the root canals and for all techniques used, followed by 2.5% NaOCl. No differences among concentrations in cleaning the canals were found.

Zhang et al (2010) [93] studied the impact on the elastic modulus and flexure strength of standardized human root dentin bars of different irrigation sequences of EDTA (17%; 3 minutes) and NaOCl (2.5% w/v; total exposure time, 24 minutes) .They found that deleterious effects attributed to the use of NaOCl on dentin are time- dependent and concentration-dependent and they are not associated with the demineralization caused by the use of EDTA as the final active irrigant.

Marion et al (2012) [40] evaluated the effectiveness of various concentrations of sodium hypochlorite during endodontic treatment. It was also much toxic to periapical tissues and caused greater irritation when the highest concentration was used. Based on the literature review it can be said that the most suitable irrigant for endodontic treatment

of root canals is the 2.5% sodium hypochlorite concentration, due to its less cytotoxic properties.

Zargar et al (2015) [90] investigated the antibacterial efficacy in the presence and absence of smear layer (SL) by three root canal irrigants. The 2.61% solution of NaOCl was significantly more effective than 0.2% CHX and 0.2% CHX was more efficient than 1% PI for decreasing fungal and microbial infection of dentinal tubules. The presence of smear layer decreased the efficacy of antimicrobial irrigants.

EDTA:

Scelza et al (2004) [62] evaluated smear layer removal from root canal dentin by 17% EDTA, EDTA-T, and 10% citric acid after final irrigation for 3, 10, and 15 min. They concluded that these 3 irrigants were effective at the shortest time tested and with an increase in time, they did not demonstrate an improved effect

Teixeira et al. (2005) [76] verified under the scanning electron microscope (SEM), the influence of irrigation time with sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) on intracanal smear layer removal. They concluded that canal irrigation with EDTA and NaOCl were equally effective in removing the smear layer from the canal walls of straight roots for 1, 3 and 5 min.

Crumpton et al.(2005) [13] quantified the volume of 17% ethylene diamine tetra-acetic acid (EDTA) needed after rotary instrumentation to efficiently remove the smear layer, and to determine if additional irrigation has any effect on debris removal. They concluded that EDTA irrigation volume greater than 1 ml did not improve debris removal. Efficient removal of the smear layer was accomplished with a final rinse of 1 ml of 17% EDTA for 1 min, followed by 3 ml of 5.25% NaOCl.

Sayin et al (2007) [61] evaluated the effect of single and combined use of ethylenediamine tetra acetic acid (EDTA), ethylene glycol bis [b-aminoethylether] N,N,N=,N=-tetra acetic acid (EGTA), EDTA plus Cetavlon (EDTAC), tetracycline-HCl, and NaOCl on the micro hardness of root canal dentin. They concluded that the use of EDTA alone or prior to NaOCl resulted in the maximum decrease in dentin micro hardness. The softening effect of subsequent NaOCl treatment was both region and material dependent.

Dotto et al (2007) [16] compared the efficacy of 17% EDTA solution and 24% ethylene diamine tetra acetic acid (EDTA) gel in cleaning dentine walls after root canal instrumentation. They concluded that both 24% EDTA gel and 17% EDTA solution used in association with 1% sodium hypochlorite were more effective in removing the smear layer compared with sodium hypochlorite alone and that there was no statistical difference between EDTA gel and EDTA solution in smear layer removal.

Khedmat S and Shokouhinejad N (2008) [31] compared the efficacy of SmearClear, 17% EDTA, and 10% citric acid in smear layer removal. They concluded that especially in the apical third, the application of 1 mL of SmearClear, 17% EDTA, and 10% citric acid for 1 minute followed by 3 mL of 5.25%NaOCl was not sufficient to remove the smear layer completely. When compared with EDTA alone, the addition of surfactants to EDTA in SmearClear did not result in better smear layer removal.

Mello et al. (2008) [41] analysed the influence of different volumes (5 mL, 10 mL, 15 mL) of 17% EDTA for final rinse on smear layer removal on the different areas of the root canal. They showed good smear layer removal, with root canal walls free of debris and mostly open dentinal tubules were achieved with the final rinse with 5 mL of 17% EDTA.

Saito et al (2008) [59] evaluated smear layer removal from root canals after rotary instrumentation with irrigation times of 1 minute or less with 1 mL of 17% ethylene diamine tetra-acetic acid (EDTA). They found that the 1-minute EDTA irrigation group had significantly greater smear layer removal than the 30-second or 15-second groups.

Chen et al (2011) [10] evaluated the effect of paste and liquid type EDTA during rotary root-canal instrumentation using an incremental crown-down technique on root-canal debris removal. They concluded that the use of paste/gel-type chelators during rotary nickel titanium instrumentation in the coronal and middle parts of the root canal resulted in improved cleanliness. They recommend using liquid EDTA during root-canal preparation as a final flushing solution because it provides a better smear layer-free condition before 3-dimensional root-canal obturation.

Zaparolli et al (2012) [88] evaluated the effect of irrigation regimens on dentin micro hardness at the furcation area of mandibular molars, using sodium hypochlorite and ethylene diamine tetra acetic acid (EDTA), individually and in alternation. They concluded that the 17% EDTA solution, either alone or in combination with 1% NaOCl reduced significantly dentin micro hardness at the furcation area of mandibular molars.

Wu et al. (2012) [85] compared the efficacy on smear layer removal of 4 decalcifying agents: 20% citric acid, BioPure MTAD, 17% ethylene diamine tetra acetic acid (EDTA), and SmearClear. They concluded that the 4 decalcifying agents especially in the apical third could not completely remove the smear layer. The efficacy of 17% EDTA was better than that of MTAD and SmearClear.

Poudyal S et al (2014) [53] evaluated the effectiveness of solution form of 17% ethylene diamine tetra acetic acid (EDTA) on removing smear layer of root canals at different exposure time periods .When the chelating agent was applied for 7 min, irrigation with

17% EDTA and 2.5% NaOCl could remove the smear layer with no significant alteration in dentinal structure. Partial removal of smear layer was observed at 3 and 5 min of application, and negligible removal of smear layer at 1 min was achieved.

Ashraf et al (2014) [5] evaluated the ability of 17% ethylene diamine tetra acetic acid (EDTA), 18% etidronate and Er: YAG on effective removal of the Smear layer. They concluded that EDTA was more effective in removing Smear layer compared to Er: YAG and etidronate.

Vlad et al (2016) [81] measured the cleaning efficiency of irrigating solutions on smear layer removal from the root canal dentin walls. Ethylene diamino tetraacetic acid (EDTA) 17%, citric acid (CA) 10% and sodium hypochlorite (NaOCl) 2.5 % solutions were tested as final irrigating solutions. They reported that at apical level, final irrigation of the root canal with 10% CA is more efficient than 17% EDTA in smear layer removal, which represents the most important area for disinfection. The chelating agents used, especially EDTA, showed high decalcifying effect, therefore the risk of dentin erosion should be taken into consideration.

PHYTIC ACID:

Nassar et al. (2013) [44] evaluated the effect of phytic acid (IP6), when used as etchant, on resin–dentin bond strength, on smear layer removal, and the viability of pulpal cells. It was concluded that etching of dentin with IP6 enhanced the bond strength of etch-and-rinse adhesive to dentin, efficiently removed the smear layer, and had minimal effects on pulpal cells.

Nassar et al.(2015) [45] investigated the effect of phytic acid, inositol hexakisphosphate (IP6), as a final rinse on the surface of instrumented root canals treated with sodium hypochlorite (NaOCl)and to find its effect on the viability and alkaline phosphatase

activity of osteoblast-like cells (MC3T3-E1). They concluded that IP6 shows the potential to be an effective and biocompatible chelating agent.

Nikhil *et al*. (2016) [46] evaluated the effect of phytic acid, ethylene diamine tetra acetic acid (EDTA), and chitosan solutions on the micro hardness of human radicular dentin. They found that all tested chelating solutions reduced micro hardness of the radicular dentin layer at all the levels. However at the apical level, microhardness reduction was least. Phytic acid caused least microhardness reduction, while EDTA caused more reduction in dentin micro hardness than chitosan.

Kong et al (2016) [33] compared the etching effect of phytic acid (IP6) with phosphoric acid (PA) and ethylene diamine tetra acetic acid (EDTA) on resin–dentin bond strength, the protecting effect against collagen degradation and nanoleakage formation along resin–dentin interfaces. They concluded that phytic acid (IP6) effectively removed the smear layer and provides high bond strength values on etched dentin, and causing minimal nanoleakage and slight collagen degradation

ETIDRONIC ACID:

Arias-Moliz et al.(2002) [4] evaluated the antimicrobial activity on Enterococcus faecalis growing in biofilms and a dentinal tubule infection model of 9% etidronic acid (HEBP) /2.5% sodium hypochlorite (NaOCl) irrigant solution. They concluded that in biofilms and inside dentinal tubules, HEBP did not interfere with the ability of NaOCl to kill E. faecalis.

Paque et al. (2012) [49] investigated short-term compatibility of etidronate with sodium hypochlorite (NaOCl) which could reduce debris accumulation when applied in an all-in-one irrigant during root canal instrumentation. They concluded that a hypochlorite-

compatible chelator – Etidronate can reduce but not completely prevent hard-tissue debris accumulation during rotary root canal instrumentation

Tartari et al. (2013) [74] investigated the effect of sodium hypochlorite (NaOCl), ethylene diamine tetra acetic (EDTA), etidronic (HEBP), and citric acid (CA) on root dentin micro-hardness. They concluded that except saline, all tested irrigation regimens reduced the micro-hardness of human root dentin. Despite being structurally different the root thirds behaved similarly, when subjected to the same irrigation regimen.

Tartari et al (2013) [75] evaluated the effects of sodium hypochlorite (NaOCl), ethylene diamine tetra acetic (EDTA), citric acid (CA), and etidronic (HEBP) on root dentin roughness. They concluded that only the irrigation regimens that used chelating agents altered the roughness of root dentin.

Silva e Souza et al (2014) [67] evaluated the influence of sodium hypochlorite associated with EDTA and etidronate on apical root transportation. They concluded that increased apical transportation in the canals of extracted teeth was seen with the use of NaOCl associated with etidronate

Kuruvilla, *et al* (2015) [35] evaluated and compared the efficacy of 17% EDTA, 7% maleic acid and 18% etidronic acid in smear layer removal using scanning electron microscopic image analysis. They showed that all the three experimental irrigants removed the smear layer from different tooth levels (coronal, middle, and apical). Etidronic acid was found to have smear layer removal efficacy as equal to that of EDTA and maleic acid in coronal and middle third. But in the apical third it showed less smear layer removal when compared with maleic acid.

Yadav, *et al*. (2015) [86] 6evaluated the amount of calcium ions removed from the root canal by etidronic acid (HEBP), SmearClear and BioPure MTAD using atomic

absorption spectrophotometer. They concluded that SmearClear was the most effective agent for the removal of calcium ions from the root canal. A less aggressive calcium complexing agent such as HEBP could be administered during the whole course of root canal preparation to prevent erosive dentinal change

Arias-Moliz et al. (2016) [3] studied the influence of dentin powder on the concentration, pH, and antimicrobial activity of sodium hypochlorite (NaOCl) alone and combined with etidronic acid (HEBP). They concluded that the presence of dentin powder significantly decreased the available chlorine and antimicrobial activity of 1% NaOCl/HEBP irrigating solutions, 1% NaOCl and 2.5% NaOCl. The antimicrobial activity of 2.5% NaOCl/HEBP after a 3-minute contact time against E. faecalis biofilms was not affected by the dentin powder.

Morago et al (2016)[43] evaluated the influence of the antimicrobial activity of a 2.5% sodium hypochlorite (NaOCl) / 9% etidronic acid (HEBP) irrigating solution against bacteria growing inside dentin tubules of the smear layer. They concluded that the presence of the smear layer reduced the antimicrobial activity of 2.5% NaOCl, wheras the smear layer doesnot reduce the antimicrobial activity of the combination of 2.5% NaOCl / 9% HEBP.

DENTINAL EROSION:

Niu et al. (2002)[47] examined dentinal erosion caused by final irrigation with EDTA and NaOCl. They concluded that final irrigation with 6% NaOCl accelerates dentinal erosion following treatment with 15% EDTA.

Spano et al (2009)[71] evaluated the effect of root canal chelators on smear layer and calcium ions removal using flame atomic absorption spectrophotometry and scanning electron microscopy**.** They found that the use of 15% EDTA resulted in the greatest

concentration of calcium ions removal followed by 10% citric acid; Both were the most efficient solutions for removal of smear layer.

Zhang et al (2010)[94] evaluated the effects of different NaOCl concentrations and contact times with and without the adjunctive use of EDTA on removal of the organic phase from mineralized dentin, and the effect of NaOCl concentrations on canal wall erosion after the use of EDTA as the final active irrigant. They concluded that the superficial destructive effect of NaOCl is present irrespective of whether EDTA is subsequently employed as the final active irrigant and it is irreversible.

Mai et al (2010)[39] studied the use of ethylene diamine tetra acetic acid (EDTA) as a final irrigant in causing canal wall erosion only after prolonged use of 5.25% sodium hypochlorite (NaOCl) as the initial irrigant. They concluded that the apparent aggressiveness of EDTA in causing canal wall erosion is attributed to the prolonged use of NaOCl. The associated decline in dentine flexural strength when thin pulp chamber dentine is immersed in NaOCl for lengthy periods during canal instrumentation has potential clinical relevance. This may render root-treated teeth more prone to vertical fracture.

Mahajan et al (2010)[38] evaluated and compared the ability of a mixture of tetracycline isomer, citric acid and detergent (MTAD) and ethylene diamine tetra-acetic acid (EDTA) on smear layer removal and their effects on peritubular and intertubular dentinal structures by scanning electron microscopic (SEM) examination. They concluded that smear layer was removed efficiently by both EDTA and MTAD whereas EDTA shows marked dentinal erosion.

Qian et al (2011)[55] examined the level of erosion on root canal wall dentin caused by immersion in different irrigant solutions in alternative sequences. They concluded that

NaOCl used as a final irrigant solution after demineralization agents causes marked erosion of root canal dentin.

Cruz-Filho et al (2011)[14] evaluated the effect of different chelating solutions (15% EDTA, 10% citric acid, 5% malic acid, 5% acetic acid, apple vinegar, 10% sodium citrate, and control) on the micro hardness of the most superficial dentin layer from the root canal lumen. They concluded that except for sodium citrate, all tested chelating solutions reduced micro hardness of the most superficial root canal dentin layer; EDTA and citric acid were the most efficient.

Wang Z et al (2016)[84] examined the level of erosion in root dentin caused by different irrigation methods which include negative control, syringe needle irigation and GentleWave System following different protocols using Energy-dispersive X-ray Spectroscopy. They stated that NaOCl followed by final EDTA irrigation performed either by syringe needle or the GentleWave System caused minimal dentin erosion. In samples erosion was measured as increased loss of calcium and phosphorus in which additional final irrigation was performed using NaOCl after EDTA.

Materials and Methods

ARMAMENTARIUM: (FIG 10)

One hundred extracted caries free and fracture free, human single rooted maxillary central incisor teeth.

Diamond Disc (MDT Micro Diamond Technologies Ltd)

Micro motor straight hand piece (NSK, Nakanishi Inc., Japan)

Airotor hand piece (NSK, Nakanishi Inc., Japan)

Stainless steel K files (No.10 size) (Mani Inc., Japan)

Endo gauge (Dentsply Maillefer, Switzerland)

Endodontic rotary hand piece (Anthogyr, Dentsply, France).

NiTi Rotary files (ProTaper NEXT X_1-X_5, Dentsply Maillefer).

Endo scale (Dentsply Mallifer, Switzerland)

Tweezer (GDC, India).

5ml syringe (Romsons, India).

29 Gauge needle

Beakers

Chisel and mallet

EQUIPMENTS:

Scanning Electron Microscope (SU 3500, HITACHI, JAPAN) (FIG 19)

MATERIALS:

0.9% Normal saline

3% Sodium Hypochlorite (NaOCl) solution (Septodont, France)

EDTA solution (Canal Pro, Coltene)

Phytic Acid, freshly prepared (TCI CHEMICALS, JAPAN)

Etidronic Acid, freshly prepared (TCI CHEMICALS, JAPAN)

Paper points (Dentsply Maillefer)

TABLE 1: EXPERIMENTAL MATERIALS USED IN STUDY

EXPERIMENTAL MATERIALS	OTHER CHEMICAL NAMES	MANUFACTURER
1% PHYTIC ACID (FIG 1)	• IP6, Inositol Hexakisphosphate • Inositol Hexaphosphate • Phytate	TCI CHEMICALS, JAPAN
18% ETIDRONIC ACID (FIG 2)	• 1-hydroxyethane 1,1-diphosphonic acid (HEDP) • Etidronate	TCI CHEMICALS, JAPAN
17% ETHYLENE DIAMINE TETRA ACETIC ACID (EDTA) (FIG 3)	• *N*,*N*′-Ethane-1,2-diylbis[*N*-(carboxymethyl)glycine][1] • Fiamino ethane-tetra acetic acid • Edetic acid (conjugate base edetate) (INN, USAN) • Ethylene dinitrilo-tetra acetic acid • Versene	CanalPro ,COLTENE

METHODOLOGY:

SAMPLE SELECTION:

One hundred extracted caries-free and visually assessed fracture-free, human single rooted maxillary incisor teeth with mature apices were selected for the study. Remnants of soft tissue debris, calculus and tissue deposit were mechanically removed from tooth surface with ultrasonic scaler. The radiographs were taken to confirm that each tooth had a single straight canal without curvature and resorption. The teeth were stored in 0.9% normal saline solution until use. (FIG 9)

SAMPLE PREPARATION:

The tooth samples were decoronated with a diamond disc (FIG 11) and straight hand piece at the cemento-enamel junction, measuring root samples of 15 mm (FIG 12) in length. The patency of the canal was checked with a No. 10 K file beyond apical foramen (FIG 13). The teeth were grooved on the buccal and lingual surfaces with a diamond disc. They were split longitudinally with chisel and mallet before instrumentation to avoid creating artificial debris, the disc was not allowed to penetrate the canal space

PREPARATION OF IRRIGATING SOLUTION: (FIG 4, 5, 6)

1 % Phytic acid is prepared by adding 1ml of Phytic acid in 100ml of water for injection (FIG 7). 18% Etidronic acid is prepared by adding 18ml of Etidronic acid in 100ml of water for injection (FIG 8).

ROOT CANAL INSTRUMENTATION:

The working length was established by measuring the length at which the # 10 K file was first visible in the apical foramen and subtracting 0.5mm. All teeth were

instrumented in a total time of 4 min each in a crown down manner with Protaper NEXT rotary files upto X5 (ISO size 50) using a 64:1 reduction hand piece (FIG 14) . The irrigation was carried out using 5ml syringe of 29 gauge needle with 14mm length (FIG 15). Samples were irrigated with 3 ml of 3% NaOCl for 5min followed by saline irrigation between every instrument change. The tooth samples were randomly distributed into ten groups of 10 teeth each.

TABLE 2: IRRIGATION PROTOCOL USED IN THE STUDY:

GROUP I: PHYTIC ACID (n-30)	**FINAL IRRIGATION**
SUBGROUP I-A (n-10)	2ml of 1% Phytic Acid for 5min.
SUBGROUP I-B (n-10)	2ml of 1% Phytic Acid for 3min.
SUBGROUP I-C (n-10)	2ml of 1% Phytic Acid for 1min.
GROUP II:ETIDRONIC ACID (n-30)	
SUBGROUP II-A (n-10)	2ml of 18% Etidronic Acid for 5min.
SUBGROUP II-B (n-10)	2ml of 18% Etidronic Acid for 3min.
SUBGROUP II-C (n-10)	2ml of 18% Etidronic Acid for 1min.
GROUP III: EDTA (n-30)	
SUBGROUP III-A (n-10)	2ml of 17% EDTA for 5min.
SUBGROUP III-B (n-10)	2ml of 17% EDTA for 3min.
SUBGROUP III-C (n-10)	2ml of 17% EDTA for 1min.
GROUP IV: CONTROL (n-10)	No final irrigation

The canals were then dried with paper points.

SPECIMEN PREPARATION AND SEM EVALUATION:

The roots were then split longitudinally into two halves with a chisel and mallet. The half with the most visible canal surface of the apex (FIG 16) was used for scanning electron microscopic evaluation. The specimens were air dried, gold sputtered, (FIG 18) and SEM micrographs were obtained at 5000X magnification of the coronal, middle and apical areas of each root canal. The amount of smear layer and degree of dentinal erosion was evaluated using a three step scale given by Torabinajed et al 2003. [78]

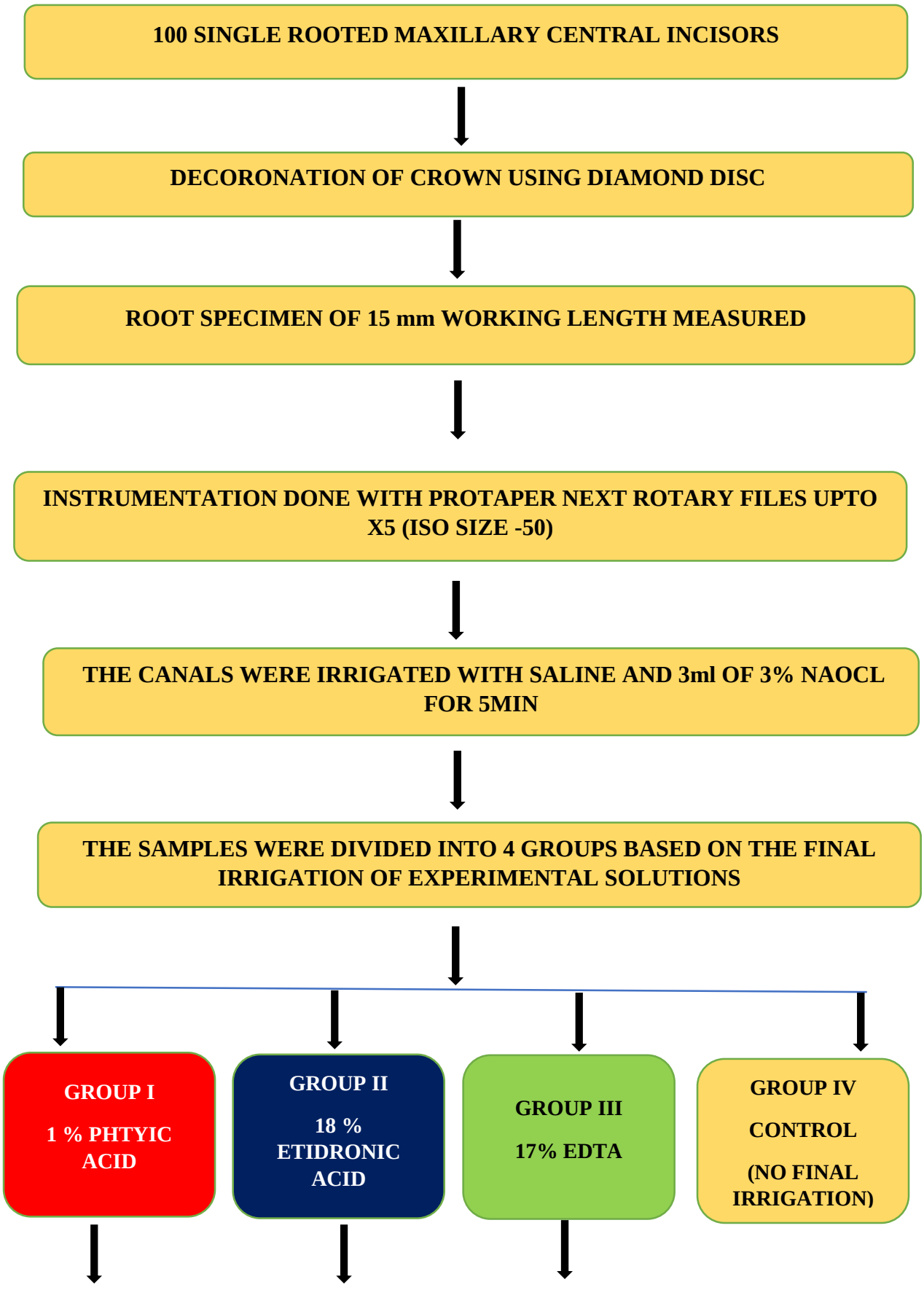
TABLE: 3 Score Rating system developed by Torabinejad *et al*.: [78]

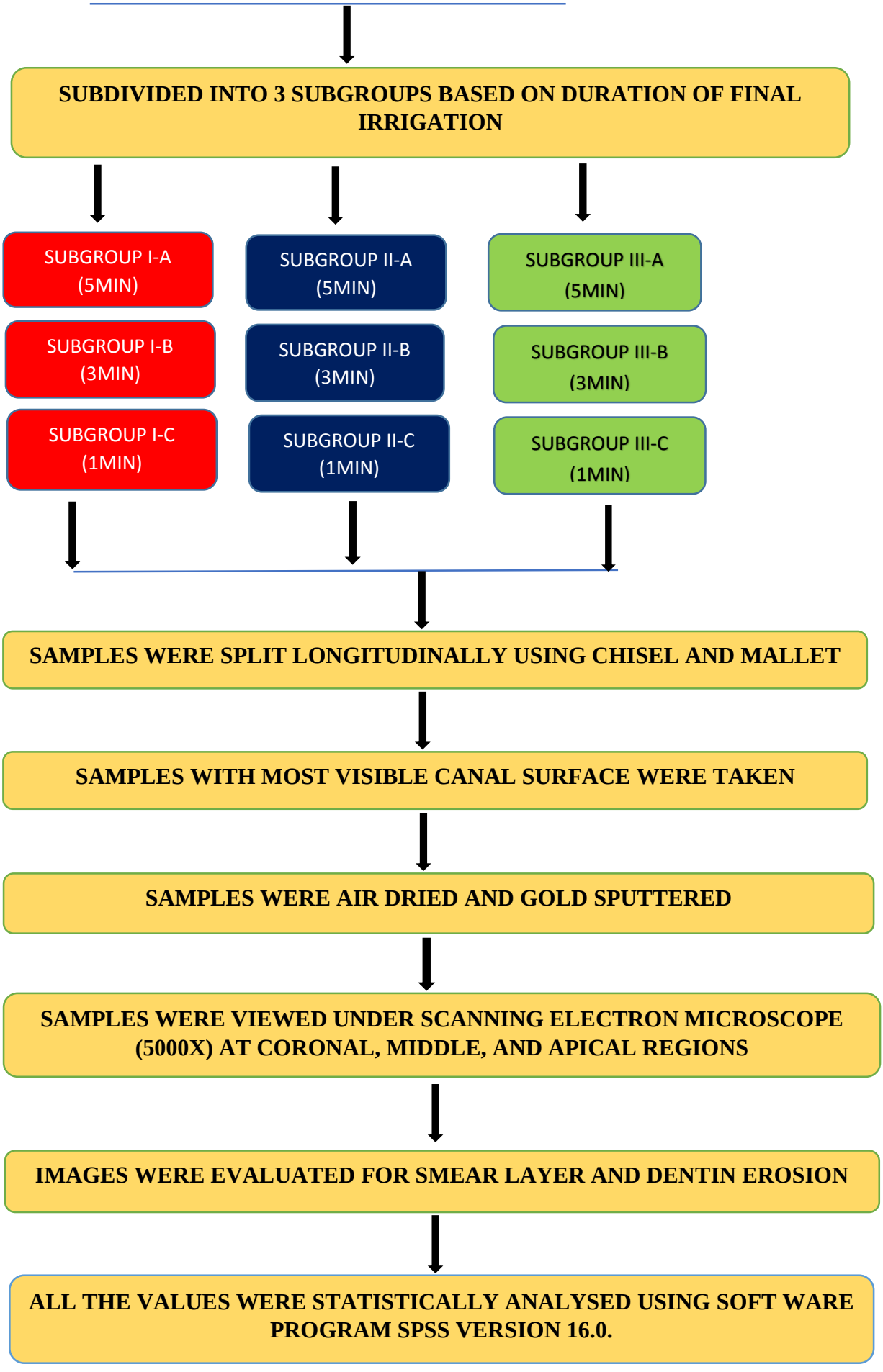
SCORES	**SMEAR LAYER**
1	No smear layer (no smear layer on the surface of the root canal: All tubules were clean and open).
2	Moderate smear layer (no smear layer on the surface of the root canal, but tubules contained debris).
3	Heavy smear layer (smear layer covered the root canal surface and the tubules).
DENTINAL EROSION	
1	No erosion. All tubules looked normal in appearance and size.
2	Moderate erosion. The peritubular dentin was eroded.
3	Severe erosion. The intertubular dentin was destroyed, and tubules were connected with each other.

STATISTICAL ANALYSIS:

The average values of each level, viz. coronal, middle and apical were calculated. The mean, median score for smear layer removal and degree of dentinal erosion were calculated for each tooth, and for each group and were statistically analysed using Mann Whitney and Kruskal Wallis tests. The datas were analysed using software program SPSS version 16.0.

PROCEDURAL FLOW CHART




SUBDIVIDED INTO 3 SUBGROUPS BASED ON DURATION OF FINAL IRRIGATION
SUBGROUP I-A (5MIN)
SUBGROUP I-B (3MIN)
SUBGROUP I-C (1MIN)
SUBGROUP II-A (5MIN)
SUBGROUP II-B (3MIN)
SUBGROUP II-C (1MIN)
SUBGROUP III-A (5MIN)
SUBGROUP III-B (3MIN)
SUBGROUP III-C (1MIN)
SAMPLES WERE SPLIT LONGITUDINALLY USING CHISEL AND MALLET
SAMPLES WITH MOST VISIBLE CANAL SURFACE WERE TAKEN
SAMPLES WERE AIR DRIED AND GOLD SPUTTERED
SAMPLES WERE VIEWED UNDER SCANNING ELECTRON MICROSCOPE (5000X) AT CORONAL, MIDDLE, AND APICAL REGIONS
IMAGES WERE EVALUATED FOR SMEAR LAYER AND DENTIN EROSION
ALL THE VALUES WERE STATISTICALLY ANALYSED USING SOFT WARE PROGRAM SPSS VERSION 16.0.

EXPERIMENTAL MATERIALS

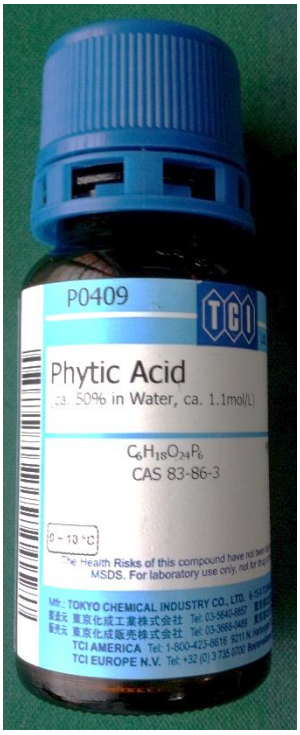


FIG 1 PHYTIC ACID

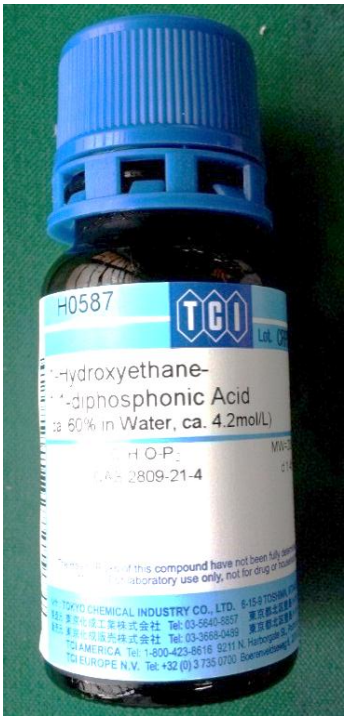


FIG 2 ETIDRONIC ACID

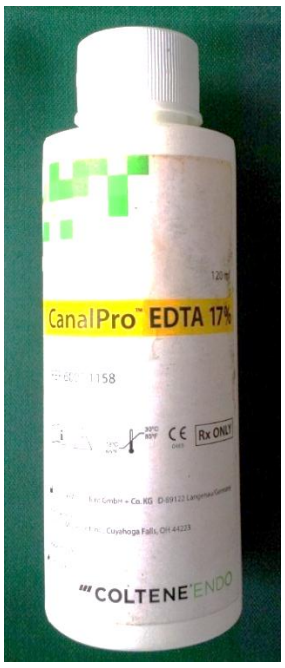


FIG 3 EDTA

PREPARATION OF SOLUTION

FIG 4 WATER FOR INJECTION (WFI)

FIG 5 WFI ADDED TO TEST TUBE

FIG 6 EXPERIMENTAL SOLUTION ADDED TO WFI

PREPARED EXPERIMENTAL SOLUTIONS

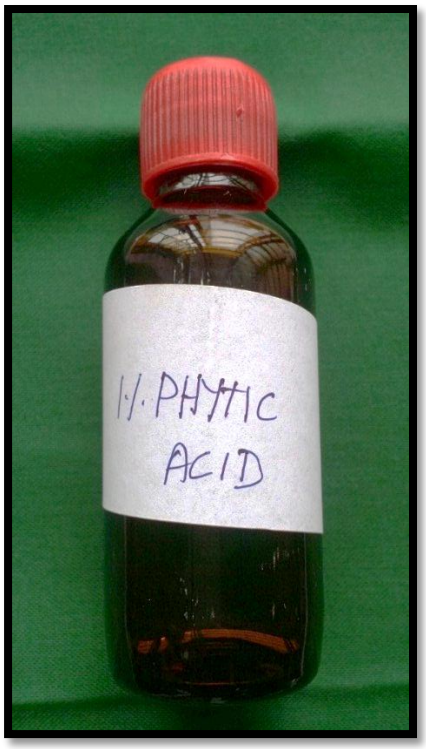


FIG 7

1% PHYTIC ACID

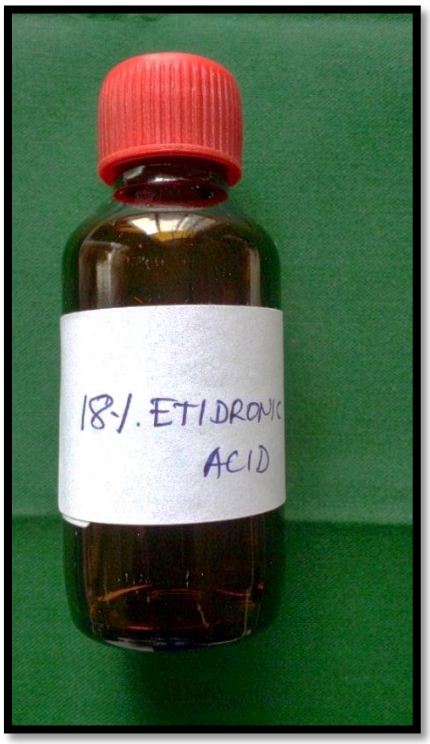


FIG 8

18% ETIDRONIC ACID

STUDY MATERIALS

FIG 9 TOOTH SAMPLES STORED IN SALINE

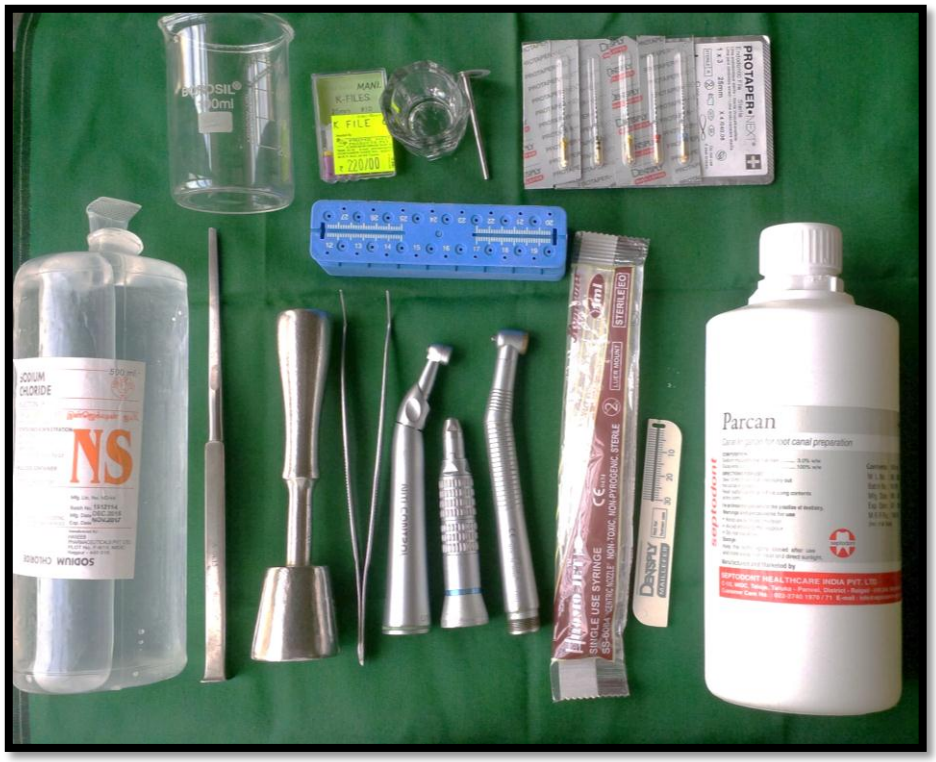


FIG 10 ARMAMENTARIUM

METHODOLOGY

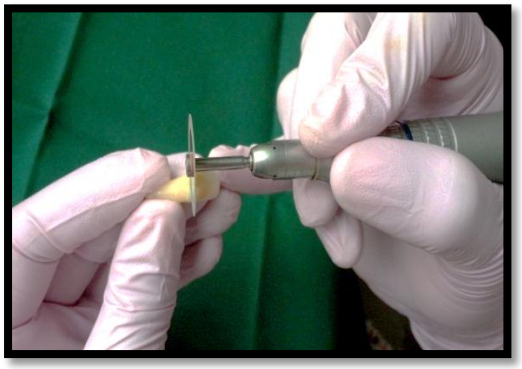


FIG11 DECORONATION

FIG 12 AFTER DECORONATION

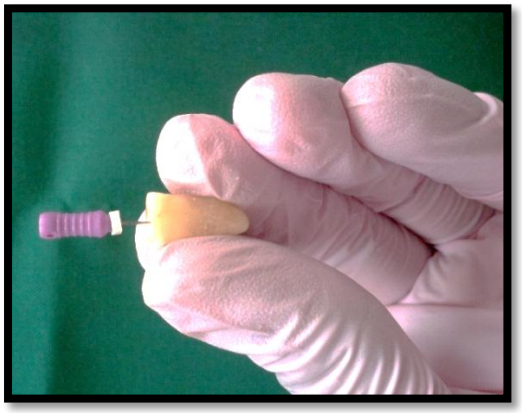


FIG 13 WORKING LENGTH MEASURED WITH 10 K FILE

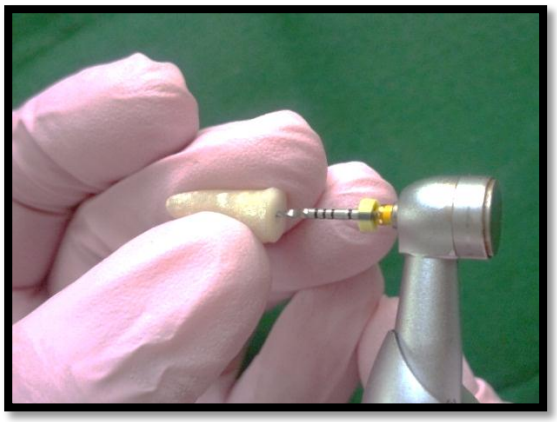


FIG 14 INSTRUMENTATION WITH PROTAPER NEXT

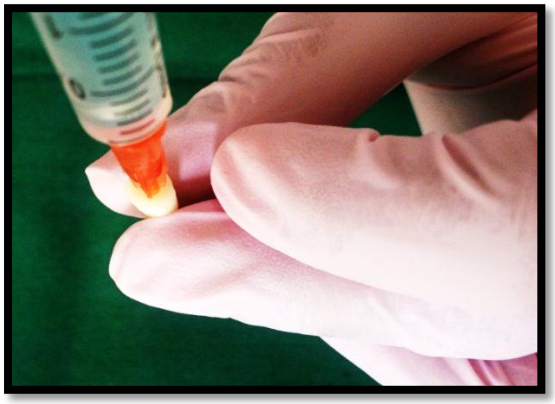


FIG 15 IRRIGATION WITH 29 GAUGE NEEDLE

FIG 16 TOOTH SAMPLE AFTER SPLITING

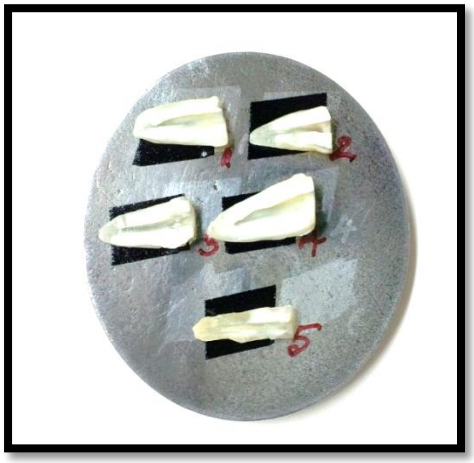


FIG 17 SAMPLES SET FOR AIR DRYING AND GOLD SPUTTERRING

FIG 18 SAMPLES READY FOR SEM EVALUATION

EQUIPMENT

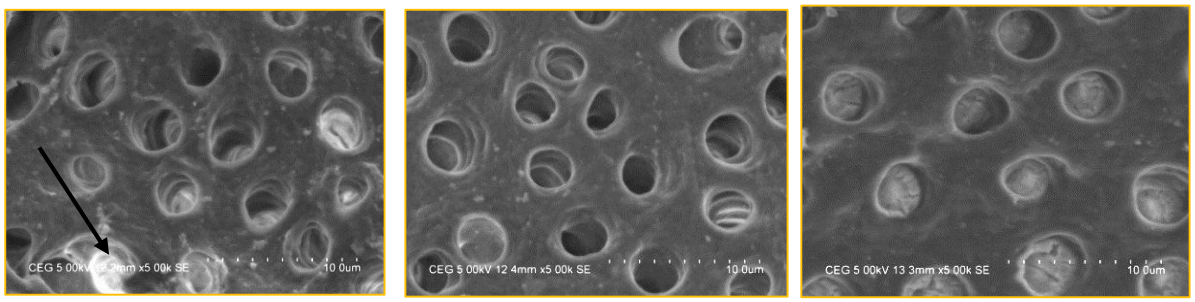
FIG 19 SCANNING ELECTRON MICROSCOPY

Results

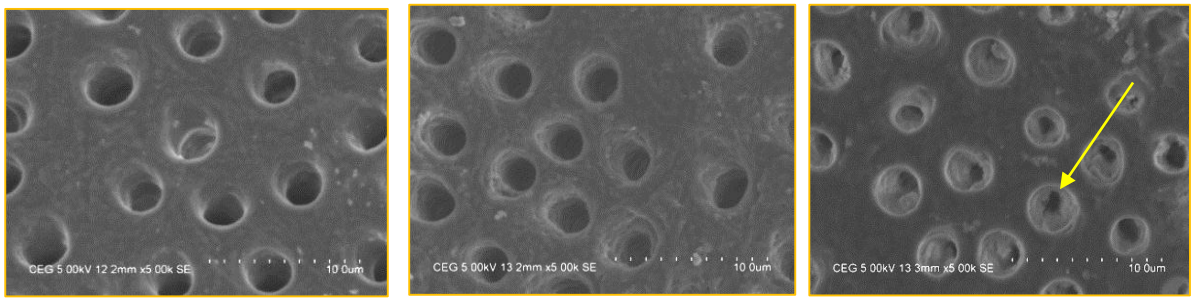
SCANNING ELECTRON MICROSCOPIC IMAGES OF GROUP I: PHYTIC ACID (FIG 20)

CERVICAL **MIDDLE** **APICAL**

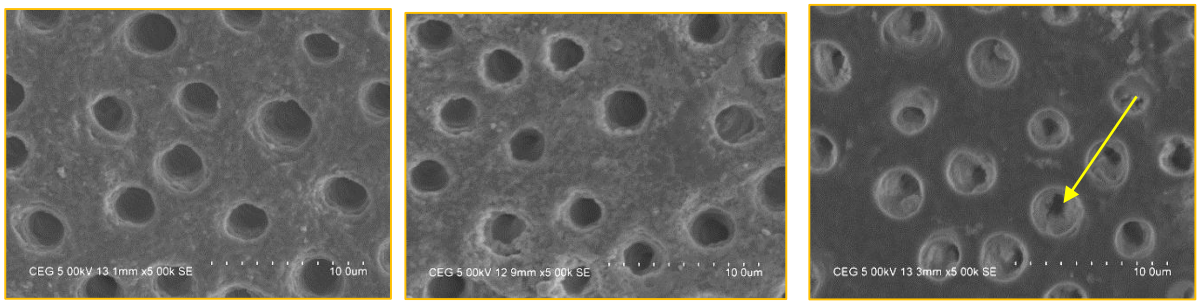
SUBGROUP I-A (5 MIN)



SUBGROUP I-B (3 MIN)



SUBGROUP I-C (1 MIN)

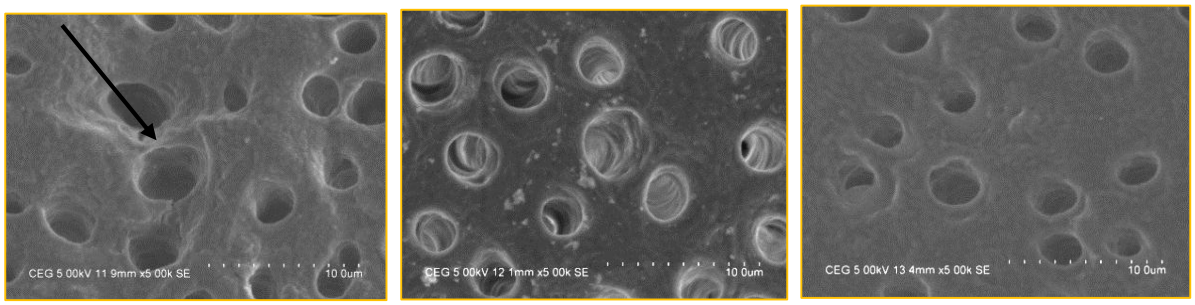


SMEAR PLUGS **DENTIN EROSION**

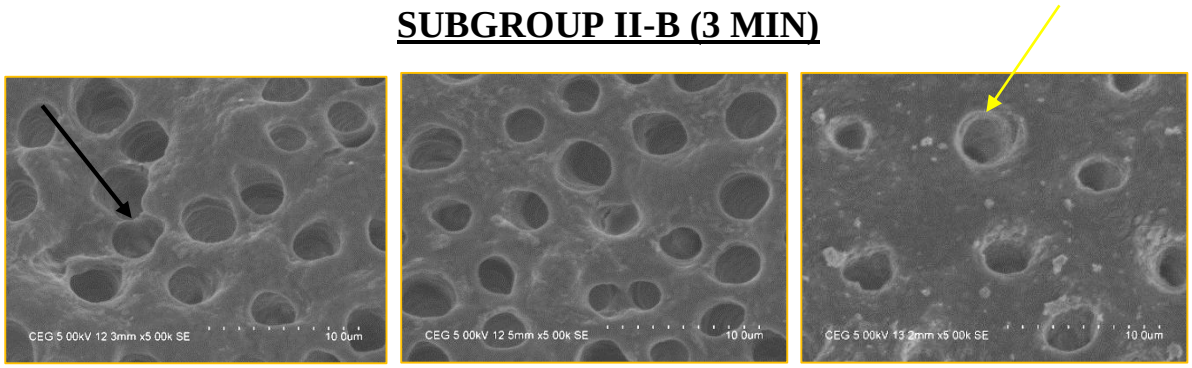
SCANNING ELECTRON MICROSCOPIC IMAGES OF GROUP II: ETIDRONIC ACID (FIG 21)

CERVICAL **MIDDLE** **APICAL**

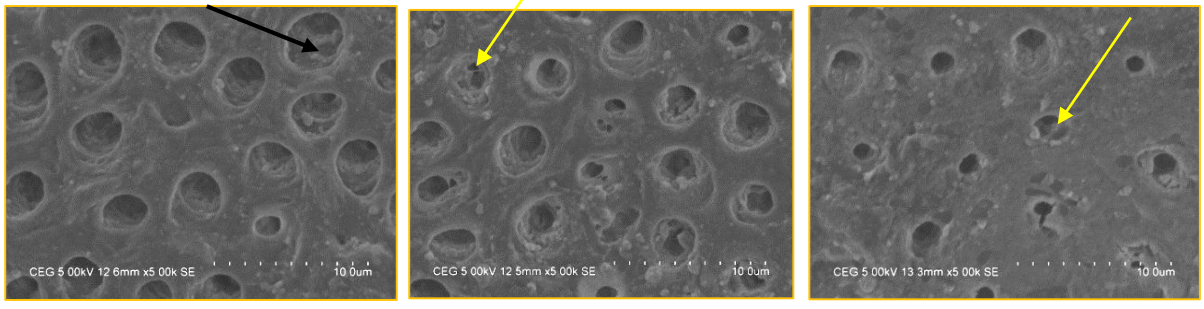
SUBGROUP II-A (5 MIN)



SUBGROUP II-B (3 MIN)



SUBGROUP II-C (1 MIN)

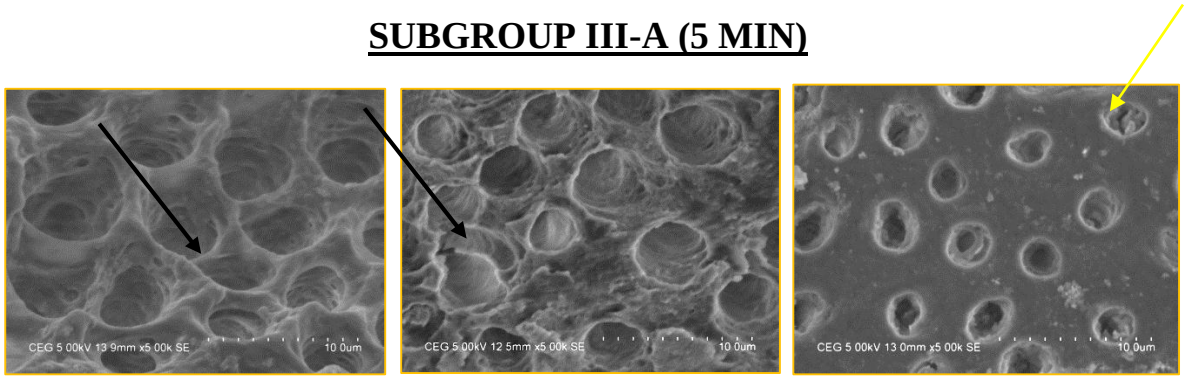


SMEAR PLUGS **DENTIN EROSION**

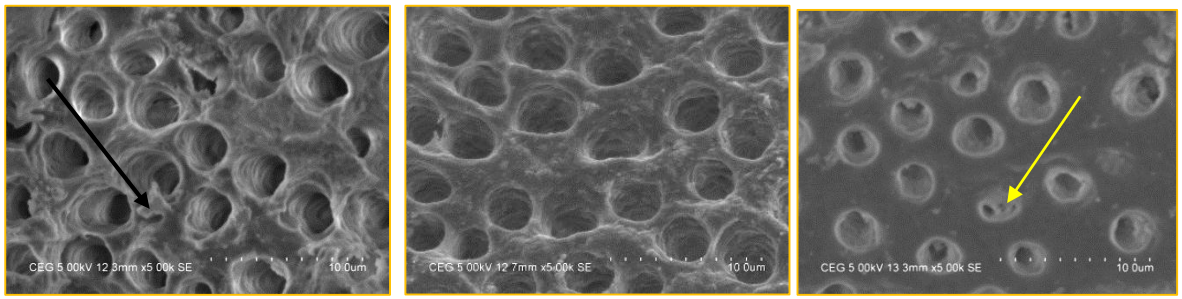
SCANNING ELECTRON MICROSCOPIC IMAGES OF GROUP III: EDTA (FIG 22)

CERVICAL **MIDDLE** **APICAL**

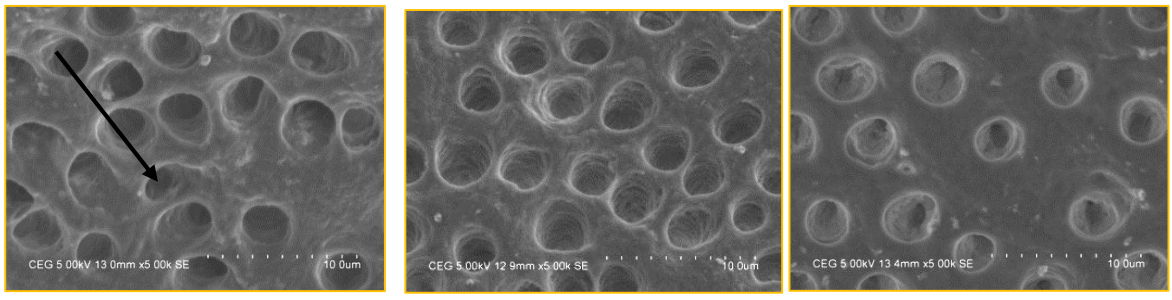
SUBGROUP III-A (5 MIN)



SUBGROUP III-B (3 MIN)



SUBGROUP III-C (1 MIN)

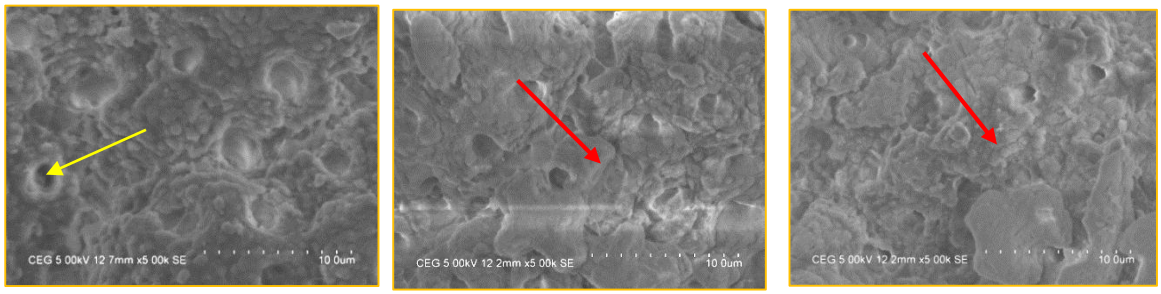


SMEAR PLUGS **DENTIN EROSION**

SCANNING ELECTRON MICROSCOPIC IMAGES OF GROUP IV: CONTROL (FIG 23)

CERVICAL **MIDDLE** **APICAL**

SUBGROUP IV (5 MIN)



SMEAR PLUGS **DENTIN EROSION**

SMEAR LAYER

Table no: 5 SMEAR LAYER SCORES OF ALL GROUPS:

SAMPLES	(5 MIN)			(3 MIN)			(1 MIN)		
	CORONAL	MIDDLE	APICAL	CORONAL	MIDDLE	APICAL	CORONAL	MIDDLE	APICAL
GROUP I-PHYTIC ACID									
1	1	1	2	1	1	2	1	1	2
2	1	1	2	1	1	2	1	1	2
3	1	1	2	1	1	2	1	1	2
4	1	1	2	1	1	2	1	1	2
5	1	1	2	1	1	2	1	1	2
6	1	1	2	1	1	2	1	1	2
7	1	1	2	1	1	2	1	1	2
8	1	1	2	1	1	2	1	1	2
9	1	1	2	1	1	2	1	1	2
10	1	1	2	1	1	2	1	1	2
GROUP II-ETIDRONIC ACID									
1	1	1	2	1	1	2	2	2	3
2	1	1	2	1	1	2	2	2	2
3	1	1	2	1	1	2	2	2	3
4	1	1	2	1	1	2	2	2	2
5	1	1	2	1	1	2	2	2	2
6	1	1	2	1	1	2	2	2	3
7	1	1	2	1	1	2	2	2	2
8	1	1	2	1	1	2	2	2	2
9	1	1	2	1	1	2	2	2	2
10	1	1	2	1	1	2	2	2	3
GROUP III-EDTA									
1	1	1	2	1	1	2	1	1	2
2	1	1	2	1	1	2	1	1	2
3	1	1	2	1	1	2	1	1	2
4	1	1	2	1	1	2	1	1	2
5	1	1	2	1	1	2	1	1	2
6	1	1	2	1	1	2	1	1	2
7	1	1	2	1	1	2	1	1	2
8	1	1	2	1	1	2	1	1	2
9	1	1	2	1	1	2	1	1	2
10	1	1	2	1	1	2	1	1	2
GROUP IV- CONTROL(5MIN)									
1	3	3	3						
2	3	3	3						
3	3	3	3						
4	3	3	3						
5	3	3	3						
6	3	3	3						
7	3	3	3						
8	3	3	3						
9	3	3	3						
10	3	3	3						

Table no: 6 DENTIN EROSION SCORES OF ALL GROUPS:

SAMPLES	(5 MIN)			(3 MIN)			(1 MIN)		
	CORONAL	MIDDLE	APICAL	CORONAL	MIDDLE	APICAL	CORONAL	MIDDLE	APICAL
GROUP I-PHYTIC ACID									
1	2	2	1	1	1	1	1	1	1
2	2	1	1	2	1	1	2	1	1
3	2	2	1	2	2	1	1	1	1
4	2	2	1	1	1	1	2	2	1
5	2	2	1	1	1	1	1	1	1
6	2	2	1	2	1	1	1	1	1
7	2	2	1	1	2	1	1	2	1
8	2	2	1	1	1	1	2	1	1
9	2	2	1	2	1	1	1	1	1
10	2	1	1	1	2	1	1	1	1
GROUP II-ETIDRONIC ACID									
1	3	2	1	3	2	1	2	2	1
2	3	2	1	2	2	1	2	2	1
3	3	2	2	3	2	1	2	2	1
4	3	3	1	3	2	1	2	2	1
5	3	2	1	3	2	1	2	2	1
6	3	2	1	3	3	1	2	2	1
7	2	2	2	3	2	1	2	2	1
8	3	3	1	2	2	1	2	2	1
9	2	2	2	3	2	2	2	2	1
10	3	2	1	3	2	1	2	2	1
GROUP III-EDTA									
1	3	3	1	3	3	1	3	2	1
2	3	3	2	3	2	1	3	2	1
3	3	3	1	3	3	2	3	2	1
4	3	3	1	3	2	1	3	2	1
5	3	3	2	3	3	1	3	3	1
6	3	3	1	3	3	2	3	2	1
7	3	3	1	3	2	1	3	2	1
8	3	3	2	3	3	1	3	3	1
9	3	3	1	3	3	1	3	2	1
10	3	3	1	3	2	1	3	2	1

DENTIN EROSION NOT APPLICABLE FOR GROUP IV -CONTROL

Table: 6 DESCRIPTIVE TABLE SHOWING MEAN, MEDIAN AND STANDARD DEVIATIONS OF SMEAR LAYER AMONG GROUPS

Groups		5MIN			3MIN			1MIN		
		Cervical	Middle	Apical	Cervical	Middle	Apical	Cervical	Middle	Apical
PHYTIC ACID (N-10) Group I	Mean	1.0000	1.0000	2.0000	1.0000	1.0000	2.0000	1.0000	1.0000	2.0000
	Std. Deviation	.00000	.00000	.00000	.00000	.00000	.00000	.00000	.42164	.00000
	Median	1.0000	1.0000	2.0000	1.0000	1.0000	2.0000	1.0000	1.0000	2.0000
HEDP (N-10) Group II	Mean	1.0000	1.0000	2.0000	1.0000	1.0000	2.0000	2.0000	2.0000	2.4000
	Std. Deviation	.00000	.00000	.00000	.00000	.00000	.00000	.00000	.00000	.51640
	Median	1.0000	1.0000	2.0000	1.0000	1.0000	2.0000	2.0000	2.0000	2.0000
EDTA (N-10) Group III	Mean	1.0000	1.0000	2.0000	1.0000	1.0000	2.0000	1.0000	1.0000	2.0000
	Std. Deviation	.00000	.00000	.00000	.00000	.00000	.00000	.00000	.00000	.00000
	Median	1.0000	1.0000	2.0000	1.0000	1.0000	2.0000	1.0000	1.0000	2.0000
Control (N-10) Group IV	Mean	3.0000	3.0000	3.0000						
	Std. Deviation	.00000	.00000	.00000						
	Median	3.0000	3.0000	3.0000						

Table 7: ANALYSIS OF SMEAR LAYER VALUES AMONG DIFFERENT GROUPS USING KRUSKAL WALLIS TEST

Statistical analysis	5min			3min			1min		
	Cervical	Middle	Apical	Cervical	Middle	Apical	Cervical	Middle	Apical
df	3	3	3	2	2	2	2	2	2
Asymp. Sig.	**.000**	**.000**	**.000**	**1.000**	**1.000**	**1.000**	**.000**	**.000**	**.012**

TABLE: 8 INDIVIDUAL COMPARISONS OF SMEAR LAYER VALUES USING MANN WHITNEY U TEST BETWEEN THE GROUPS

GROUPS	5MIN			1MIN		
	Cervical	Middle	Apical	Cervical	Middle	Apical
GROUP I VS II	**1.000**	**1.000**	**1.000**	**.000**	**.000**	**.029**
GROUP I VS III	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**
GROUP I VS IV	**.000**	**.000**	**.000**			
GROUP II VS III	**1.000**	**1.000**	**1.000**	**.000**	**.000**	**.029**
GROUP II VS IV	**.000**	**.000**	**.000**			
GROUP III VS IV	**.000**	**.000**	**.000**			

TABLE 9: DESCRIPTIVE TABLE SHOWING MEAN, MEDIAN AND STANDARD DEVIATIONS OF DENTIN EROSION AMONG GROUPS

GROUPS		5MIN			3MIN			1MIN		
		Cervical	Middle	Apical	Cervical	Middle	Apical	Cervical	Middle	Apical
PHYTIC ACID (N-10) Group I	Mean	2.0000	1.8000	1.0000	1.4000	1.3000	1.0000	1.3000	1.2000	1.0000
	Std. Deviation	.00000	.42164	.00000	.51640	.48305	.00000	.48305	.42164	.00000
	Median	2.0000	2.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
HEDP (N-10) Group II	Mean	2.7000	2.2000	1.3000	3.0000	2.1000	1.1000	2.0000	2.0000	1.0000
	Std. Deviation	.48305	.42164	.48305	.00000	.31623	.31623	.00000	.00000	.00000
	Median	3.0000	2.0000	1.0000	3.0000	2.0000	1.0000	2.0000	2.0000	1.0000
EDTA (N-10) Group III	Mean	3.0000	3.0000	1.3000	3.0000	2.6000	1.2000	3.0000	2.2000	1.0000
	Std. Deviation	.00000	.00000	.48305	.00000	.51640	.42164	.00000	.42164	.00000
	Median	3.0000	3.0000	1.0000	3.0000	3.0000	1.0000	3.0000	2.0000	1.0000

TABLE: 10 ANALYSIS OF DENTIN EROSION VALUES AMONG DIFFERENT GROUPS USING KRUSKAL WALLIS TEST

Statistical analysis	5min			3min			1min		
	Cervical	Middle	Apical	Cervical	Middle	Apical	Cervical	Middle	Apical
df	2	2	2	2	2	2	2	2	2
Asymp. Sig.	**.000**	**.000**	.163	**.000**	**.000**	**.342**	**.000**	**.000**	**1.000**

Table: 11 INDIVIDUAL COMPARISONS OF DENTIN EROSION VALUES USING MANN WHITNEY U TEST BETWEEN THE GROUPS

GROUPS	STATISTICS	5MIN			3MIN			1MIN		
		Cervical	Middle	Apical	Cervical	Middle	Apical	Cervical	Middle	Apical
Group I vs II	Asymp. Sig. (2-tailed)	**.001**	**.051**	**.067**	**.000**	**.001**	**.317**	**.001**	**.000**	**1.000**
Group I VS III	Asymp. Sig. (2-tailed)	**.000**	**.000**	**.067**	**.000**	**.000**	**.146**	**.000**	**.000**	**1.000**
Group II VS III	Asymp. Sig. (2-tailed)	.067	**.000**	1.000	1.000	**.022**	.542	**.000**	.146	1.000

TABLE 12: DESCRIPTIVE TABLE SHOWING MEAN, MEDIAN AND STANDARD DEVIATIONS OF SMEAR LAYER AND DENTIN EROSION OF PHYTIC ACID AT ALL THIRDS

GROUPS		SMEAR LAYER			DENTIN EROSION		
		5MIN	3MIN	1MIN	5MIN	3MIN	1MIN
CERVICAL (N-10) Group 1	Mean	1.0000	1.0000	1.0000	2.0000	1.4000	1.3000
	Std. Deviation	.00000	.00000	.00000	.00000	.51640	.48305
	Median	1.0000	1.0000	1.0000	2.0000	1.0000	1.0000
MIDDLE (N-10) Group 2	Mean	1.0000	1.0000	1.2000	1.8000	1.3000	1.2000
	Std. Deviation	.00000	.00000	.42164	.42164	.48305	.42164
	Median	1.0000	1.0000	1.0000	2.0000	1.0000	1.0000
APICAL (N-10) Group 3	Mean	2.0000	2.0000	2.0000	1.0000	1.0000	1.0000
	Std. Deviation	.00000	.00000	.00000	.00000	.00000	.00000
	Median	2.0000	2.0000	2.0000	1.0000	1.0000	1.0000

TABLE: 13 ANALYSIS OF SMEAR LAYER AND DENTIN EROSION VALUES OF PHYTIC ACID AT ALL THIRDS USING KRUSKAL WALLIS TEST

STATISTICAL ANALYSIS	SMEAR LAYER			DENTIN EROSION		
	5MIN	3MIN	1MIN	5MIN	3MIN	1MIN
df	2	2	2	2	2	2
Asymp. Sig.	**.000**	**.000**	**.000**	**.000**	**.096**	**.197**

TABLE: 14 INDIVIDUAL COMPARISONS OF SMEAR LAYER AND DENTIN EROSION VALUES OF PHYTIC ACID AT ALL THIRDS USING MANN WHITNEY U TEST

GROUPS	STATISTICS	SMEAR LAYER			DENTIN EROSION		
		5MIN	3MIN	1MIN	5MIN	3MIN	1MIN
GROUP 1 VS 2	**Asymp. Sig. (2-tailed)**	**1.000**	**1.000**	**.146**	**.146**	**.648**	**.615**
GROUP 1 VS 3	**Asymp. Sig. (2-tailed)**	**.000**	**.000**	**.000**	**.000**	**.029**	**.067**
GROUP 2 VS 3	**Asymp. Sig. (2-tailed)**	**.000**	**.000**	**.000**	**.000**	**.067**	**.146**

TABLE 15: DESCRIPTIVE TABLE SHOWING MEAN, MEDIAN AND STANDARD DEVIATIONS OF SMEAR LAYER AND DENTIN EROSION OF PHYTIC ACID AT 5, 3 & 1 MIN

GROUPS		SMEAR LAYER			DENTIN EROSION		
		cervical	middle	apical	cervical	middle	apical
5MIN (N-10) Group 1	Mean	1.0000	1.0000	2.0000	2.0000	1.8000	1.0000
	Std. Deviation	.00000	.00000	.00000	.00000	.42164	.00000
	Median	1.0000	1.0000	2.0000	2.0000	2.0000	1.0000
3MIN (N-10) Group 2	Mean	1.0000	1.0000	2.0000	1.4000	1.3000	1.0000
	Std. Deviation	.00000	.00000	.00000	.51640	.48305	.00000
	Median	1.0000	1.0000	2.0000	1.0000	1.0000	1.0000
1MIN (N-10) Group 3	Mean	1.0000	1.2000	2.0000	1.3000	1.2000	1.0000
	Std. Deviation	.00000	.42164	.00000	.48305	.42164	.00000
	Median	1.0000	1.0000	2.0000	1.0000	1.0000	1.0000

TABLE 16: ANALYSIS OF SMEAR LAYER AND DENTIN EROSION VALUES OF PHYTIC ACID AT 5, 3, & 1 MIN USING KRUSKALWALLIS TEST

Statistics	SMEAR LAYER			DENTIN EROSION		
	Cervical	Middle	Apical	Cervical	Middle	Apical
df	2	2	2	2	2	2
Asymp. Sig.	**1.000**	**.126**	**1.000**	**.004**	**.017**	**1.000**

TABLE 17: PAIRWISE COMPARISONS OF SMEAR LAYERAND DENTIN EROSION VALUES OF PHYTIC ACID AT 5, 3 & 1 MIN USING MANN WHITNEY U TEST

GROUPS	STATISTICS	SMEAR LAYER			DENTIN EROSION		
		Cervical	Middle	Apical	Cervical	Middle	Apical
GROUP 1 VS 2	Asymp. Sig. (2-tailed)	**1.000**	**1.000**	**1.000**	**.004**	**.028**	**1.000**
GROUP 1 VS 3	Asymp. Sig. (2-tailed)	1.000	.146	1.000	**.001**	**.009**	1.000
GROUP 2 VS 3	**Asymp. Sig. (2-tailed)**	**1.000**	**.146**	**1.000**	**.648**	**.615**	**1.000**

TABLE 18: DESCRIPTIVE TABLE SHOWING MEAN, MEDIAN AND STANDARD DEVIATIONS OF SMEAR LAYER AND DENTIN EROSION VALUES OF ETIDRONIC ACID AT ALL THIRDS

GROUPS		SMEAR LAYER			DENTIN EROSION		
		5MIN	3MIN	1MIN	5MIN	3MIN	1MIN
CERVICAL (N-10) Group 1	Mean	1.0000	1.0000	2.0000	2.7000	3.0000	2.0000
	Std. Deviation	.00000	.00000	.00000	.48305	.00000	.00000
	Median	1.0000	1.0000	2.0000	3.0000	3.0000	2.0000
MIDDLE (N-10) Group 2	Mean	1.0000	1.0000	2.0000	2.2000	2.1000	2.0000
	Std. Deviation	.00000	.00000	.00000	.42164	.31623	.00000
	Median	1.0000	1.0000	2.0000	2.0000	2.0000	2.0000
APICAL (N-10) Group 3	Mean	2.0000	2.0000	2.4000	1.3000	1.1000	1.0000
	Std. Deviation	.00000	.00000	.51640	.48305	.31623	.00000
	Median	2.0000	2.0000	2.0000	1.0000	1.0000	1.0000

TABLE 19: ANALYSIS OF SMEAR LAYER AND DENTIN EROSION VALUES OF ETIDRONIC ACID AT ALL THIRDS USING KRUSKALWALLIS TEST

STATISTICAL ANALYSIS	SMEAR LAYER			DENTIN EROSION		
	5MIN	3MIN	1MIN	5MIN	3MIN	1MIN
df	2	2	2	2	2	2
Asymp. Sig.	**.000**	**.000**	**.012**	**.000**	**.000**	**.000**

TABLE 20: INDIVIDUAL COMPARISONS OF SMEAR LAYERAND DENTIN EROSION VALUES OF ETIDRONIC ACID AT ALL THIRDS USING MANN WHITNEY U TEST

GROUPS	STATISTICS	SMEAR LAYER			DENTIN EROSION		
		5MIN	3MIN	1MIN	5MIN	3MIN	1MIN
GROUP 1 VS 2	**Asymp. Sig. (2-tailed)**	**1.000**	**1.000**	**1.000**	**.028**	**.000**	**1.000**
GROUP 1 VS 3	**Asymp. Sig. (2-tailed)**	**.000**	**.000**	**.029**	**.000**	**.000**	**.000**
GROUP 2 VS 3	**Asymp. Sig. (2-tailed)**	**.000**	**.000**	**.029**	**.001**	**.000**	**.000**

TABLE 21: DESCRIPTIVE TABLE SHOWING MEAN, MEDIAN AND STANDARD DEVIATIONS OF SMEAR LAYER AND DENTIN EROSION VALUES OF ETIDRONIC ACID AT 5, 3 & 1 MIN

GROUPS		SMEAR LAYER			DENTIN EROSION		
		Cervical	Middle	Apical	Cervical	Middle	Apical
5MIN (N-10) Group 1	Mean	1.0000	1.0000	2.0000	2.7000	2.2000	1.3000
	Std. Deviation	.00000	.00000	.00000	.48305	.42164	.48305
	Median	1.0000	1.0000	2.0000	3.0000	2.0000	1.0000
3MIN (N-10) Group 2	Mean	1.0000	1.0000	2.0000	3.0000	2.1000	1.1000
	Std. Deviation	.00000	.00000	.00000	.00000	.31623	.31623
	Median	1.0000	1.0000	2.0000	3.0000	2.0000	1.0000
1MIN (N-10) Group 3	Mean	2.0000	2.0000	2.4000	2.0000	2.0000	1.0000
	Std. Deviation	.00000	.00000	.51640	.00000	.00000	.00000
	Median	2.0000	2.0000	2.0000	2.0000	2.0000	1.0000

TABLE 22: ANALYSIS OF SMEAR LAYER AND DENTIN EROSION VALUES OF ETIDRONIC ACID AT 5, 3 & 1 MIN USING KRUSKALWALLIS TEST

statistics	SMEAR LAYER			DENTIN EROSION		
	Cervical	Middle	Apical	Cervical	Middle	Apical
df	2	2	2	2	2	2
Asymp. Sig.	**.000**	**.000**	**.012**	**.000**	**.342**	**.142**

Table 23: PAIRWISE COMPARISONS OF SMEAR LAYER AND DENTIN EROSION VALUESOF ETIDRONIC ACID OF 5, 3 & 1 MIN USING MANN WHITNEY U TEST

GROUPS	STATISTICS	SMEAR LAYER			DENTIN EROSION		
		Cervical	MiddlE	Apical	Cervical	MiddlE	Apical
GROUP 1 VS 2	Asymp. Sig. (2-tailed)	**1.000**	**1.000**	**1.000**	**.067**	**.542**	**.276**
GROUP 1 VS 3	Asymp. Sig. (2-tailed)	**.000**	**.000**	**.029**	**.001**	**.146**	**.067**
GROUP 2 VS 3	Asymp. Sig. (2-tailed)	**.000**	**.000**	**.029**	**.000**	**.317**	**.317**

TABLE 24: DESCRIPTIVE TABLE SHOWING MEAN, MEDIAN AND STANDARD DEVIATIONS OF SMEAR LAYER AND DENTIN EROSION VALUES OF EDTA AT ALL THIRDS.

GROUPS		SMEAR LAYER			DENTIN EROSION		
		5MIN	3MIN	1MIN	5MIN	3MIN	1MIN
CERVICAL (N-10) Group 1	Mean	1.0000	1.0000	1.0000	3.0000	3.0000	3.0000
	Std. Deviation	.00000	.00000	.00000	.00000	.00000	.00000
	Median	1.0000	1.0000	1.0000	3.0000	3.0000	3.0000
MIDDLE (N-10) Group 2	Mean	1.0000	1.0000	1.0000	3.0000	2.6000	2.2000
	Std. Deviation	.00000	.00000	.00000	.00000	.51640	.42164
	Median	1.0000	1.0000	1.0000	3.0000	3.0000	2.0000
APICAL (N-10) Group 3	Mean	2.0000	2.0000	2.0000	1.3000	1.2000	1.0000
	Std. Deviation	.00000	.00000	.00000	.48305	.42164	.00000
	Median	2.0000	2.0000	2.0000	1.0000	1.0000	1.0000

TABLE 25: ANALYSIS OF SMEAR LAYER AND DENTIN EROSION VALUES OF EDTA AT ALL THIRDS USING KRUSKALWALLIS TEST

STATISTICAL ANALYSIS	SMEAR LAYER			DENTIN EROSION		
	5MIN	3MIN	1MIN	5MIN	3MIN	1MIN
df	2	2	2	2	2	2
Asymp. Sig.	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**

TABLE 26: INDIVIDUAL COMPARISONS OF SMEAR LAYERAND DENTIN EROSION OF EDTA AT ALL THIRDS USING MANN WHITNEY U TEST

GROUPS	STATISTICS	SMEAR LAYER			DENTIN EROSION		
		5MIN	3MIN	1MIN	5MIN	3MIN	1MIN
GROUP 1 VS 2	**Asymp. Sig. (2-tailed)**	**1.000**	**1.000**	**1.000**	**1.000**	**.029**	**.000**
GROUP 1 VS 3	**Asymp. Sig. (2-tailed)**	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**
GROUP 2 VS 3	**Asymp. Sig. (2-tailed)**	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**

TABLE 27: DESCRIPTIVE TABLE SHOWING MEAN, MEDIAN AND STANDARD DEVIATIONS OF SMEAR LAYER AND DENTIN EROSION VALUES OF EDTA AT 5, 3 & 1 MIN

GROUPS		SMEAR LAYER			DENTIN EROSION		
		Cervical	Middle	Apical	Cervical	Middle	Apical
5MIN (N-10) Group I	Mean	1.0000	1.0000	2.0000	3.0000	3.0000	1.3000
	Std. Deviation	.00000	.00000	.00000	.00000	.00000	.48305
	Median	1.0000	1.0000	2.0000	3.0000	3.0000	1.0000
3MIN (N-10) Group II	Mean	1.0000	1.0000	2.0000	3.0000	2.6000	1.2000
	Std. Deviation	.00000	.00000	.00000	.00000	.51640	.42164
	Median	1.0000	1.0000	2.0000	3.0000	3.0000	1.0000
1MIN (N-10) Group III	Mean	1.0000	1.0000	2.0000	3.0000	2.2000	1.0000
	Std. Deviation	.00000	.00000	.00000	.00000	.42164	.00000
	Median	1.0000	1.0000	2.0000	3.0000	2.0000	1.0000

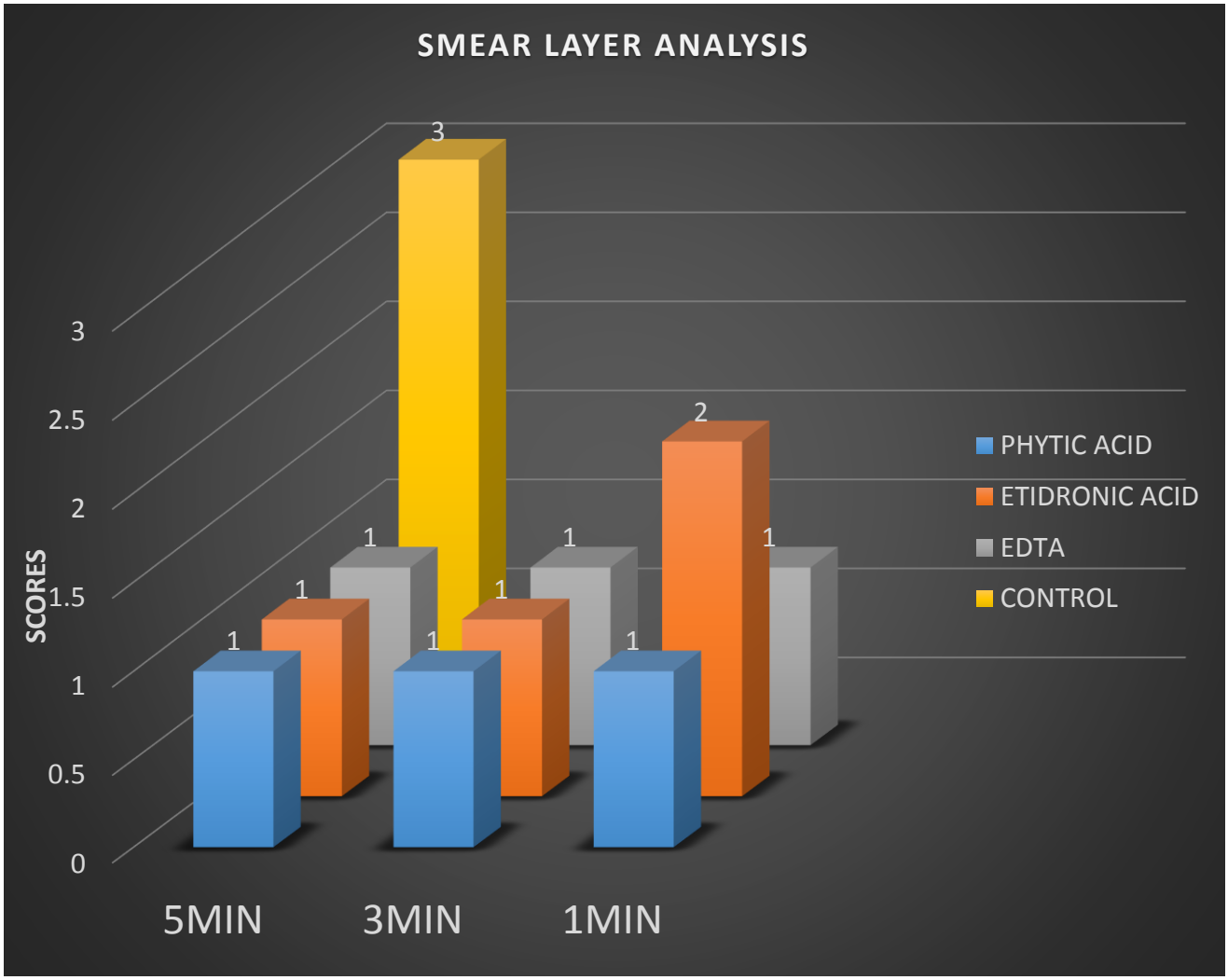
Table 28: ANALYSIS OF SMEAR LAYER AND DENTIN EROSION VALUES OF EDTA AT 5, 3 & 1 MIN USING KRUSKAL WALLIS TEST

statistics	SMEAR LAYER			DENTIN EROSION		
	Cervical	Middle	Apical	Cervical	Middle	Apical
df	2	2	2	2	2	2
Asymp. Sig.	**1.000**	**1.000**	**1.000**	**1.000**	**.002**	**.197**

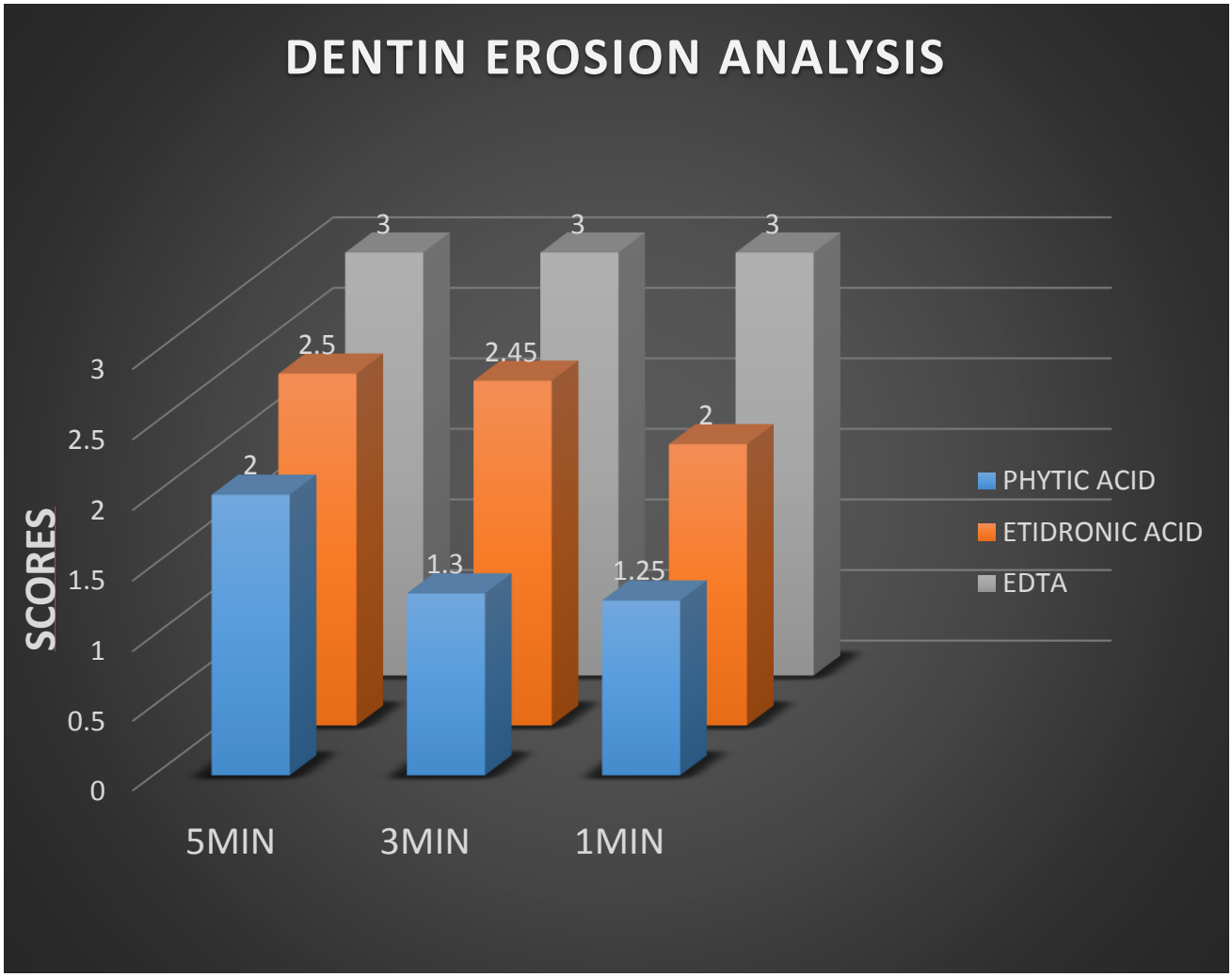
Table 29: PAIRWISE COMPARISONS OF SMEAR LAYER AND DENTIN EROSION OF EDTA AT 5, 3 & 1 MIN USING MANN WHITNEY U TEST

GROUPS	STATISTICS	SMEAR LAYER			DENTIN EROSION		
		Cervical	Middle	Apical	Cervical	Middle	Apical
GROUP 1 VS 2	**Asymp. Sig. (2-tailed)**	**1.000**	**1.000**	**1.000**	**1.000**	**.029**	**.000**
GROUP 1 VS 3	**Asymp. Sig. (2-tailed)**	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**
GROUP 2 VS 3	**Asymp. Sig. (2-tailed)**	**.000**	**.000**	**.000**	**.000**	**.000**	**.000**

GRAPH 1: SMEAR LAYER ANALYSIS AMONG THE GROUPS



GRAPH 2: DENTIN EROSION ANALYSIS AMONG THE GROUPS



INTERPRETATION OF RESULTS OF SMEAR LAYER

The order of smear layer values were as follows

AT 5MIN: EDTA= PHYTIC ACID = ETIDRONIC ACID > CONTROL

AT 3MIN: EDTA =PHYTIC ACID = ETIDRONIC ACID

AT 1MIN: EDTA = PHYTIC ACID > ETIDRONIC ACID

Analysis of mean values of smear layer at 0.05 level significance reveals that

- EDTA (GROUP III) and Phytic acid (GROUP I) showed statistically no significant difference (p= 1.000) at 5min, 3min and 1min.
- Etidronic acid showed statistically no significant difference (p= 1.000) at 5min and 3min with other groups. Whereas it showed statistically high smear layer values (p=.000) at 1min than EDTA (GROUP III) and Phytic acid (GROUP I) which are significant.
- Control (GROUP IV) showed statistically high smear layer values (p=.000) than other groups which are significant
- Final irrigation with EDTA, Phytic acid and Etidronic acid for 1, 3 and 5 min were equally effective in removing the smear layer from the root canal walls except for Etidronic acid at 1min.
- Apical region showed high smear layer values than cervical and middle region which are significant.

INTERPRETATION OF RESULTS OF DENTIN EROSION

The order of dentin erosion values were as follows

AT 5 MIN: EDTA > ETIDRONIC ACID > PHYTIC ACID

AT 3 MIN: EDTA > ETIDRONIC ACID > PHYTIC ACID

AT 1 MIN: EDTA > ETIDRONIC ACID > PHYTIC ACID

Analysis of mean values of dentin erosion at 0.05 level significance reveals that

- All groups showed some degree of erosion.
- Dentin erosion was not applicable for Control (Group IV), since it was covered by smear layer completely
- EDTA (GROUP III) showed statistically high dentin erosion values (p=.000) than other groups which are significant
- Phytic acid (GROUP I) showed statistically low dentin erosion values (p=.000) than other groups which are significant
- Etidronic acid (GROUP II) showed statistically high dentin erosion values than phytic acid and lower than EDTA.
- Increasing the duration of final irrigation showed significantly high dentin erosion values which are significant.
- Cervical region showed statistically high dentin erosion values (p=.000) than middle and apical region which are significant.

Discussion

DISCUSSION:

One of the greatest challenges in endodontic therapy is the procedure of rendering a complex root canal system and its ramifications completely clean of organic and inorganic debris, thereby creating a healthy environment for the tooth to achieve maximal healing. Over these years of technological advancement that has enveloped the practice of endodontics, many new techniques, instruments and materials have been developed for better cleaning and shaping of the radicular spaces.

Chemo mechanical preparation plays an important role in success of the endodontic treatment.[18, 11] However instrumentation of root canal results in accumulation of organic and inorganic material known as smear layer. [9, 65, 54] Pashley found that the smear layer contains organic and inorganic substances that include fragments of odontoblastic process, microorganisms, and necrotic materials.[51] McComb & Smith (1975) were the first researchers to describe smear layer on the instrumented root canal surface.

There was a high controversy regarding the removal of smear layer. Many studies favoured the retention of smear layer which may block the dentinal tubules and limit bacterial or toxin penetration by altering dentinal permeability.[42, 50, 58.] But many studies reported that removal of smear layer prevents apical/coronal micro leakage by a better adherence and penetration of sealer into the dentinal tubules and provides better disinfection by allowing intracanal medicaments to penetrate into the dentinal tubules.[20] It improves the bonding of resins to the tooth structure.

Regarding the chemical composition of smear layer, it can be effectively and totally removed by only agents combining both organic and inorganic solvents.[57]There are various methods to remove smear layer like chemical, ultrasonic and laser

techniques. None of the methods remove smear layer throughout the length of the canal completely.[80] **Kalyoncuoğlu E and Demiryürek EÖ** evaluated the efficacy of smear layer removal from teeth following root canals using lasers (Er:YAG and Nd:YAG), NaOCl, 17% EDTA, and MTAD by scanning electron microscopy (SEM). They concluded that although improvement was observed in removal of the smear layer using alternative materials and techniques, application of a combination of EDTA and NaOCl remains an effective technique.[28] Thus in our study we used NaOCl and EDTA as irrigants.

Since 1920, NaOCl is one of the most commonly used endodontic irrigants. It is known for its antibacterial activity and for its capacity of dissolving organic tissue in root canal.[72] It results in the formation of hypochlorous acid (HOCl) which shows antibacterial properties, when it reacts with organic debris. HOCl disrupts the microbial metabolism by oxidation of sulphydryl groups within bacterial enzyme systems. [68] Strong basic pH and high percentage of free chlorine in solution are its two peculiar actions related to the antibacterial and solvent actions of NaOCl. [1] It has limited activity on the inorganic components of the smear layer and this required the use of chelating agents. [37]

Nygaard Ostby was the first to introduce chelating agents in endodontics. Chelating agents decalcify the dentine by combining with calcium ions of the tooth.[48] Chelating agents and acids have been reported to remove the smear layer from the root canal, because the components of this loosely bound structure are very small particles with a large surface-mass ratio that makes them very soluble in acids[77] Chelating solutions have been used as a part of the final irrigation regimen in various studies.

EDTA is a commonly used irrigation solution because it can chelate and remove the mineralized portion of smear layers. EDTA a colourless, water soluble solid is a widely used acronym for the chemical compound ethylene diamine tetra acetic acid. EDTA is a polyamino carboxylic acid with the formula $[CH_2N\ (CH_2CO_2H)\ _2]_2$. Chelating action occurs by its ability to extract di- and tri-cationic metal ions such as Ca^{2+} and Fe^{3+}.[23] EDTA a synthetic, non-biodegradable material is considered a pollutant in root canal system and reported to be cytotoxic to macrophages. It lacks antimicrobial properties[2]

Even though combination of EDTA and NaOCl appears to be the most effective agent for smear layer removal so far, however this combination cannot be simultaneously used because EDTA solution is able to chemically interact with NaOCl and reduce the amount of free chlorine.[26] This combination allows a synergistic interaction allowing easy penetration of EDTA into the intertubular and peritubular dentine expediting its disintegration and is responsible for a pronounced canal wall erosion.[49]

DENTIN EROSION

Dentin is a molecular complex with calcium ions in its composition. Optimum pH for dentin demineralization is between 5 and 6. Demineralizing effect also acts upon the root canal walls, leaving them almost devoid of mineralized surface which is soft and permeable.[17] Erosion of root canal dentin and dentinal tubules can depend on many factors, such as the type, amount, concentration, pH, and application time of the irrigation agent.[29] When chelating agent is used in excess, 73% of the human dentin powder inorganic component can be chelated after a one hour exposition. This suggests it must not be used inside the canal for a prolonged period of time. Chelating agent not only removes dentin debris, it also begins the erosion of dentin surfaces through the

process of demineralization and excessive opening of the tubules. In this manner, fitting of the filling material to canal walls becomes difficult and decreases sealing, favours bacterial filtration which ultimately leads to the failure of the root canal treatment.[17] The excessive erosion of root dentin eventually leads to weakening and fracture of the tooth structure.

The search for solutions which will not interfere with NaOCl activity, being more biocompatible in an attempt to minimize damage to the periapical tissues, not being erosive on dentin, non pollutant, nontoxic but with effective chelation property has appeared with increasing frequency in the literature. In search of such irrigants, we found phytic acid, a new available chelator and etidronic acid, a weak chelator which can be an effective alternate to EDTA.

Phytic acid (known as inositol hexakisphosphate, IP6), a saturated cyclic acid, is the principal storage form of phosphorus in many plant tissues, especially bran and seeds. Phytic acid has a strong binding affinity to important minerals, such as calcium, iron, and zinc.[45] Low pH of 1.2 helps in better calcium extraction.[24] It is biocompatible with periapical tissues.[24] Studies have shown that reduction of micro hardness of dentin by phytic acid was less than that of EDTA.[46] It shows good bond strength values and had minimal effects on the pulpal cells when used as etchant.[44]

Recently, Etidronic acid also known as Etidronate (HEBP), a substance that prevents bone resorption has been used in medicine for patients suffering from osteoporosis or Paget´s disease, and was suggested as substitute for traditional chelators due to fewer effects observed on dentin structure[15] The advantage of etidronate is that it can be mixed with NaOCl without interfering in its antimicrobial activities.[91] HEDP is a weak chelator, therefore it can be less aggressive on dentin than EDTA.[74]However

these solutions may need longer time for removal of smear layer.[15]It is biocompatible with periapical tissues

1n 1970, Eick et al first reported the use of Scanning Electron Microscopy to identify smear layer. The surface changes caused by dental erosion can be observed through a Scanning Electron Microscope. The SU3500 Scanning Electron Microscope used in this study features innovative electron optics and signal detection systems to provide unparalleled imaging and analytical performance. It is designed with intuitive logic, the new user-friendly graphical user interface provides comprehensive image observation and display functions. It is engineered for a wide range of applications including biological specimens and advanced materials.

Hence in the present study an attempt has been made to compare the effect of 1% phytic acid, a newly available chelating agent (GROUP I), 18% etidronic acid(GROUP II), a weak chelator with 17% EDTA (GROUP III) on smear layer removal and dentin erosion at 5min, 3min and 1min time intervals using SEM analysis. There are no other studies reported in the literature that has compared phytic acid, etidronic acid and EDTA as a chelating agent and its erosive effect on root dentin at different time intervals.

Suparna et al [73] compared the cleaning efficacy of two different rotary file systems- ProTaper NEXT and WaveOne, using a Scanning Electron Microscope. They concluded that both the rotary systems ProTaper NEXT and WaveOne resulted in cleaner canals. Another study by **Yang et al** [87] showed that the canals showed smaller amounts of debris and smear layer remaining in the apical region when prepared with ProTaper instruments. Therefore in this study, samples were instrumented with PROTAPER NEXT rotary files up to X5 (ISO size -50), since minimum

instrumentation size needed for penetration of irrigants to the apical third of the root canal is a #30 file.[30]

Studies of **Siqueira JF Jr et al.** compared 5% NaOCl irrigant to 0.5% during instrumentation and found that even at higher concentration the reduction of intracanal bacteria is not significantly improved. [69] This was probably because of the inability of solutions to physically reach inaccessible areas rather than the concentration of solution. [70] NaOCl at different concentrations 0.5 to 5.25% have shown to be equally efficacious in the disinfection of necrotic root canals as well as removal of loose superficial debris, but ineffective in removal of smear layer. [6] Therefore in our study we used 3ml of 3% NaOCl for 5min during instrumentation along with saline knowing the adverse effects of irritation to periapical tissues and decrease in flexural strength of dentin at higher concentration.

According to the study by **Chen G and Chang YC 2011[10]**, who suggested using liquid EDTA as a final rinse solution during root-canal preparation because it provides a complete smear layer removal before 3-dimensional root-canal obturation. Thus in this study we used EDTA solution instead of gel.

Volume of irrigation and contact time are the most debated elements for smear layer removal. The most effective method according to Ciucchi et al[12] was the use of 2 ml of 15% EDTA as a final rinse compared to the use of 30 ml of 15% EDTA during instrumentation.[7]According to a study by **Saito et al 2008 [59]** who evaluated that after rotary instrumentation; whether irrigation times of 1 minute or less with 1 mL of 17% ethylene diamine tetra-acetic acid (EDTA) effectively removed the smear layer from root canals. They found that significantly greater smear layer removal was found in the 1-minute EDTA irrigation group than the 30-second or 15-second groups. And another

study by **Crumpton et al. 2005** [13] also concluded that EDTA irrigation volume greater than 1 ml did not improve debris removal. A final rinse of 1 ml of 17% EDTA for 1 min, followed by 3 ml of 5.25% NaOCl was the efficient way of removal of the smear layer. Another report by **Calt and Serper** described that effective method of removing the smear layer was by irrigation with 17% EDTA for 1 min, but excessive peritubular and intertubular dentinal erosion was caused by a 10 min application [9]. Increasing contact time and concentration of EDTA from 10 to 17%, as well as using a pH of 7.5 versus pH 9.0 have been shown to increase demineralization of dentin.[81]

Thus we can infer from the above data that effective smear layer removal can be achieved with 1-2ml of irrigating solution at shortest irrigation time and high decalcifying effect when used for increased duration. Hence in our present study, all samples were given final irrigation with 2ml of experimental solution and their effect on smear layer and dentin erosion on root canal wall were investigated at 5min, 3min and 1min duration.

EVALUATION OF SMEAR LAYER ANALYSIS:

GROUP I (PHYTIC ACID): It showed efficient smear layer removal at 5 min, 3min and 1min in cervical and middle region. However it could not completely remove the smear layer at apical region. It showed comparable smear layer values to EDTA and is lower than etidronic acid. This finding is in agreement with the study of Nassar et al in 2015[45], where they investigated the effect of phytic acid, inositol hexakisphosphate (IP6), as a final rinse on the surface of instrumented root canals which are treated with sodium hypochlorite (NaOCl) and to evaluate its effect on the viability and alkaline phosphatase activity of osteoblast-like cells (MC3T3-E1). They concluded that IP6 shows the potential to be an effective and biocompatible chelating agent. There was no

significant difference (p=1.000) in smear layer removal values even at increased duration of irrigation. There was statistically no significant difference (p=1.000) between EDTA and phytic acid.

GROUP II (ETIDRONIC ACID): It showed efficient smear layer removal at 5 min and 3min in cervical and middle region comparable to that of EDTA and phytic acid, whereas it shows less smear layer removal efficiency at 1min in cervical and middle region. These findings are in agreement with the study by De-Deus et al[35] who stated that these solutions need 300 s to completely remove the smear layer, if used for a final flush. None of the groups in the study were completely effective in apical region of the canal .Another study by **Kuruvilla *et al* 2015 [35]** where they evaluated and compared the efficacy of 17% EDTA, 7% maleic acid and 18% etidronic acid, in smear layer removal using SEM; they showed that all the three experimental irrigants removed the smear layer from different tooth levels (coronal, middle, and apical). In coronal and middle third, Etidronic acid was found to have smear layer removal efficacy as equal to that of EDTA and maleic acid. But it showed less smear layer removal in the apical third when compared with maleic acid. They also reported the same findings as that of this study.

These findings are also in agreement with the study done by **Paque et al. [49],** who investigated the extent to which a calcium-complexing agent, etidronate has good short-term compatibility with the irrigant, sodium hypochlorite which could reduce debris accumulation during root canal instrumentation when applied as an all-in-one irrigant. They concluded that a hypochlorite-compatible chelator – Etidronate can reduce but not completely prevent hard-tissue debris accumulation during rotary root canal instrumentation

There was significant difference (p=.000) between 5min and 3min with 1min group of irrigation. There was statistically no significant difference (p=1.000) with EDTA and phytic acid at 5min and 3min.

GROUP III (EDTA): It showed efficient smear layer removal at 5 min, 3min and 1min in cervical and middle region except apical region. The smear layer values were comparable to that of phytic acid and lower than etidronic acid. This finding is in agreement with the study of **Wu et al. (2012)** [85] who compared the efficacy on smear layer removal of 4 decalcifying agents: 17% ethylene diamine tetra acetic acid (EDTA), 20% citric acid, BioPure MTAD, and SmearClear. They concluded that the 4 decalcifying agents could not completely remove the smear layer, especially in the apical third.

However there is a disagreement with the study of Poudyal S et al (2014) [53] where they evaluated the effectiveness of solution form of 17% ethylene diamine tetra acetic acid (EDTA) at different exposure time periods on removing smear layer of root canals. It was concluded that combined irrigation with 17% EDTA and 2.5% NaOCl could remove the smear layer when the chelating agent was applied for 7 min with no significant alteration in dentinal structure. Partial removal of smear layer was observed at 3 and 5 min of application, and negligible removal of smear layer was achieved at 1 min. In our study, final irrigation with EDTA for 1, 3 and 5 min were equally effective in removing the smear layer from the canal walls of straight roots, however they could not completely remove the smear layer, especially in the apical third.

There was statistically no significant difference (p=1.000) in smear layer removal even at increased duration of irrigation. There was statistically no significant

difference (p=1.000) when compared with phytic acid but high significant difference (p=.000) when compared with etidronic acid which were significant.

GROUP IV (CONTROL): It showed heavy smear layer at all region of the canal. It showed highly significant difference (p=.000) when compared with other groups. These results were in agreement with many studies.[79, 37, 6]

The smear layer values of the four groups were in the following order

GROUP III (EDTA) = GROUP I (PHYTIC ACID) > GROUP II (ETIDRONIC ACID) > GROUP IV (CONTROL)

Thus from these results, we can infer that EDTA and Phytic acid showed effective smear layer removal at the shortest time tested, because of their strong chelation property. Etidronic acid showed less smear layer removal than EDTA and Phytic acid at shortest time, due to its weak chelation action. Control group showed least smear layer removal efficiency than other groups because of the absence of chelating agents. All these irrigants could not completely remove the smear layer in the apical third of the root canal. This could be attributed to the use of syringe & needle irrigation rather than any agitation methods. Both Phytic acid and EDTA were effective at the shortest time tested and did not demonstrate an improved effect with an increase in time except for etidronic acid. Phytic acid shows the potential to be an effective and biocompatible chelating agent.

EVALUATION OF DENTIN EROSION ANALYSIS**:**

GROUP I (PHYTIC ACID): It showed less erosion when compared with other groups. It showed more erosion at 5min when compared with 3min and 1min. Cervical region showed more erosion of dentin than middle and apical region. These results can be compared with the study on micro hardness of dentin by Nikhil at el in 2016, who stated

that at the apical level, reduction of micro hardness was least. While phytic acid had least reduction of micro hardness, EDTA caused more reduction in dentin micro hardness than chitosan.[46] There was statistically significant difference (p=.000) in erosion of dentin at increasing duration of irrigation. There was less erosion of dentin when compared with other groups which were statistically (p=.000) significant.

GROUP II (ETIDRONIC ACID): It showed less erosion when compared with EDTA and higher than phytic acid. It showed more erosion at 5min and 3min than 1min. Cervical region showed more erosion of dentin than middle and apical region There was a statistically significant difference (p=.000) in erosion of dentin at increased duration of irrigation. There was a statistically significant difference (p=.000) when compared with other groups. These findings are in agreement with the study of **Tartari et al (2013)** [75] where they evaluated the effects of sodium hypochlorite (NaOCl), ethylene diamine tetra acetic (EDTA), etidronic (HEBP), and citric acid (CA) associated with different irrigation regimens on root dentin roughness. They concluded that only the irrigation regimens that used chelating agents altered the roughness of root dentin.

GROUP III (EDTA): It showed highest erosion than any other groups. It showed severe erosion in 5min, 3min than 1min. Cervical and middle region showed high erosion than apical. There was statistically significant difference (p=.000) in erosion of dentin with increased duration of irrigation. There was statistically high difference (p=.000) in erosion of dentin when compared with other groups. These findings are in agreement with the study of **Zhang et al 2010**[94] who concluded that the EDTA removes the collagen-depleted apatite phase to expose the underlying cause of destruction that is morphologically perceived as canal wall erosion. **Mahajan et al 2010**[38] also evaluated and compared the ability of a mixture of tetracycline isomer, citric acid and ethylene

diamine tetra-acetic acid (EDTA) and detergent (MTAD) on removing the smear layer by scanning electron microscopic (SEM) examination along with their effects on peritubular and intertubular dentinal structures. They concluded that smear layer was removed efficiently by both EDTA and MTAD whereas EDTA shows marked dentinal erosion

GROUP IV (CONTROL): Dentin erosion cannot be applicable since all dentinal tubules were covered by smear layer and smear plugs.

The dentin erosion values of the three groups were in the following order

GROUP III (EDTA) > GROUP II (ETIDRONIC ACID) > GROUP I (PHYTIC ACID)

All groups showed some degree of erosion. Phytic acid showed least erosion when compared to other groups. EDTA showed highest erosion than other groups due to its high decalcifying effect. At increased duration of irrigation, there was an increase in erosion of root dentin. Cervical and middle region showed high erosion than apical region of the root canal. Thus we can infer that phytic acid at 1min showed least erosion of root canal dentin.

Thus within the limitations of this study, we can state that phytic acid has effective smear layer removal comparable to that of EDTA while showing the least erosion of root dentin. Increasing the duration of irrigation does not improve the smear layer removal efficiency except for etidronic acid but causes inadvertent erosion of root dentin.

Phytic acid can be used as an effective alternative to EDTA considering its biocompatible chelation property. In clinical situations, 2ml of this irrigating solution at 1min can be used effectively without causing much erosion of root canal.

However the samples were irrigated and instrumented on bench top with adequate visualisation and easy accessibility without much resistance which may not be the situation in clinical cases. Therefore additional invivo and invitro models resembling that of clinical situation are further needed to confirm these findings of the irrigants in root canal system.

Summary

SUMMARY

The study was done to compare and evaluate the effect of three chelating agents namely 1% phytic acid, 18% etidronic acid and 17% EDTA on smear layer removal and dentin erosion at 5min, 3 min, and 1 min duration after 3% NaOCl irrigation under Scanning Electron Microscopy.

One hundred human single rooted maxillary incisor teeth were selected for the study. Each tooth was decoronated with a diamond disc, 1mm coronal to the cemento-enamel junction measuring root specimens of 15 mm in length. The root canals were instrumented with Protaper NEXT Rotary file upto X5 size. The irrigation was carried out using 5ml syringe of 29 gauge needle. During instrumentation, canals were irrigated with 3 ml of 3% sodium hypochlorite for 5min followed by saline irrigation between every instrument change. The tooth samples were randomly distributed into ten groups of 10 teeth each.

GROUP I –PHYTIC ACID

SUBGROUP I-A: Final rinse of 2ml of 1% Phytic Acid for 5min.

SUBGROUP I-B: Final rinse of 2ml of 1% Phytic Acid for 3min.

SUBGROUP I-C: Final rinse of 2ml of 1% Phytic Acid for 1min

GROUP II –ETIDRONIC ACID

SUBGROUP II-A: Final rinse of 2ml of 18% Etidronic Acid for 5min.

SUBGROUP II-B: Final rinse of 2ml of 18% Etidronic Acid for 3min.

SUBGROUP II-C: Final rinse of 2ml of 18% Etidronic Acid for 1min

GROUP III –EDTA

SUBGROUP III-A: Final rinse of 2ml of 17% EDTA for 5min.

SUBGROUP III-B: Final rinse of 2ml of 17% EDTA for 3min.

SUBGROUP III-C: Final rinse of 2ml of 17% EDTA for 1min

The roots were then split longitudinally into two halves with a chisel and mallet. The specimens were air dried, gold sputtered, and SEM images were obtained at 5000X magnification of the coronal, middle and apical areas of each root canal. The amount of smear layer and degree of dentinal erosion was evaluated using a three step scale given by Torabinejad et al 2003. The results were statistically analysed using Kruskal Wallis Test for intergroup and Mann Whitney test for intragroup. Based on the results obtained and the statistical analysis the following conclusions were drawn.

SMEAR LAYER ANALYSIS:

EDTA = PHYTIC ACID > ETIDRONIC ACID > CONTROL

DENTIN EROSION ANALYSIS:

EDTA > ETIDRONIC ACID > PHYTIC ACID

Results showed that Phytic acid and EDTA have effective smear layer removal efficiency than etidronic acid. All groups caused erosion of the root dentin. None of the groups showed effective smear layer removal at apical region of the root canal.

Phytic acid has smear layer removal efficiency equal to that of EDTA while causing less erosion of the root canal wall. Increasing the duration of irrigation does not improve the smear layer removal efficiency except for etidronic acid but all groups showed more erosion at longer irrigation period.

Conclusion

Within the limitations of this present in vitro study, the following conclusions were drawn:

1. EDTA showed effective smear layer removal but at the expense of severe erosion of the root dentin than Phytic acid and Etidronic acid
2. Phytic acid showed smear layer removal efficiency equal to that of EDTA, higher than etidronic acid and it causes less erosion of root dentin than EDTA and etidronic acid
3. Etidronic acid showed less smear layer removal efficiency when compared with EDTA and phytic acid, whereas it showed more erosion than phytic acid and less erosion than EDTA
4. Control group showed the least smear layer removal efficiency than other groups.
5. Cervical and middle region showed better smear layer removal efficiency but showed more erosion than apical region of the root canal wall.
6. Increasing the duration of irrigation does not increase the smear layer removal efficiency but it causes inadvertent erosion of the root canal wall.

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