EFFECT OF DIFFERENT CONCENTRATION OF CHITOSAN ON CALCIUM LOSS AND MICROHARDNESS IN ROOT DENTINE – AN INVITRO STUDY

Dissertation submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

in partial fulfillment for the degree of

MASTER OF DENTAL SURGERY

BRANCH – IV

CONSERVATIVE DENTISTRY AND ENDODONTICS

2017 – 2020

ENDORSEMENT BY THE H.O.D, PRINCIPAL / HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled **"EFFECT OF DIFFERENT CONCENTRATION OF CHITOSAN ON CALCIUM LOSS AND MICROHARDNESS IN ROOT DENTINE – AN INVITRO STUDY"** done by **Dr. M. MYTHILI,** post graduate student(M.D.S),**CONSERVATIVE DENTISTRY AND ENDODONTICS** (Branch – IV), KSR Institute of Dental Science and Research, Tiruchengode, submitted to the Tamil Nadu Dr. M.G.R. Medical University in partial fulfillment for the M.D.S. degree examination (May 2020) is a bonafide research work carried out by her under my supervision and guidance.

Seal & signature of H.O.D.

Dr. SEBEENA MATHEW, M.D.S.,

PROFESSOR & HEAD

PROFESSOR & HOD,
DEPT OF CONSERVATIVE
DENTISTRY & ENDODONTICS
K. S. R. Institute of Dental
Science & Research,
K. S. R. KalviNagar,
Thiruchengode, Tamil Nadu

24/1/2020

Seal & signature of Principal

Dr. G.S. KUMAR, M.D.S.,

PRINCIPAL

PRINCIPAL,
K.S.R. INSTITUTE OF DENTAL
SCIENCE & RESEARCH,
K.S.R. KALVI NAGAR,
THOKKAVADI POST,
TIRUCHENGODE - 637 215

CERTIFICATE BY GUIDE

This is to certify that the dissertation titled **"EFFECT OF DIFFERENT CONCENTRATION OF CHITOSAN ON CALCIUM LOSS AND MICROHARDNESS IN ROOT DENTINE – AN INVITRO STUDY"** is a bonafide research work done by **Dr. M. MYTHILI** in partial fulfillment of the requirements for the degree of **MASTER OF DENTAL SURGERY** in the specialty of **CONSERVATIVE DENTISTRY AND ENDODONTICS.**

Date: 24-1-2020

Place: Tiruchengode

Deepa M 24/1/2020

Signature of the guide

Dr. DEEPA.N.T, M.D.S.,

PROFESSOR

DECLARATION BY THE CANDIDATE

TITLE OF DISSERTATION	**EFFECT OF DIFFERENT CONCENTRATION OF CHITOSAN ON CALCIUM LOSS AND MICROHARDNESS IN ROOT DENTINE – AN INVITRO STUDY.**
PLACE OF STUDY	K.S.R. Institute of Dental Science and Research
DURATION OF COURSE	2017- 2020(3 years)
NAME OF THE GUIDE	Dr. DEEPA.N.T, M.D.S.,
HEAD OF THE DEPARTMENT	Dr. SEBEENA MATHEW, M.D.S.,

I hereby declare that no part of the dissertation will be utilized for gaining financial assistance for research or other promotions without obtaining prior permission from the Principal, K.S.R Institute of Dental Science and Research, Tiruchengode. In addition, I declare that no part of this work will be published either in print or electronic form without the guide who has been actively involved in this dissertation. The author solely has the rights reserved for publishing the work solely with prior permission of the Principal, K.S.R Institute of Dental Science and Research, Tiruchengode.

24/1/2020

Head of the Department

24/1/20

Signature of candidate

ACKNOWLEDGEMENT

First, I want to thank **God, the Almighty** for giving me what i love. Learned a lot in these three years professionally as well as personally which is to be cherished.

With overwhelming gratitude I thank my Professor **Dr. Deepa.N.T,M.D.S.,** my guide and mentor, for her immense support and valuable guidance for my dissertation as well as for my clinical and academic works

I want to pay my special regards to my **Head of the department, Dr.Sebeena Mathew M.D.S.,** for her caring and friendly guidance for my dissertion as well as for my clinical works.

I would also like to engrave here, my heartfelt respects and thanks to all my teachers in the department, **Dr.K.Karthick M.D.S, Dr.T.Boopathi M.D.S,** for their constant encouragement in all aspects of my career as an MDS student.

My Special and sincere thanks to well wisher, professor **Dr.M.Chandra mohan M.D.S,** with tremendous respect and deepest gratitude for his valuable suggestions in my Under graduation and post graduation.

I would like to thank my back bones, What I am Today is only because of my dad **M.Manoharan**, for being in my ups and downs. More than a dad, he has been my good friend. Nothing can equal for this but I try to do something to make him proud. my mom **Rajalakshmi Manoharan,** and my brother **M.Dinesh kumar,** for their love, care, support and prayers. Love you all. Nothing is possible without them.

I want to thank my seniors **Dr.Poojitha Viswanath** and **Dr.Abitha Banu** for their good guidance, support and help.

I extend my heartfelt thanks to my copg **Dr.Kanimozhi, Dr.Myilanandam** and my seniors and other friends for their help, advice and support.

Once again, I thank **God, the Almighty**, for blessing me abundantly for giving me the time and inclination to write this dissertation.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF GRAPHS

INTRODUCTION

INTRODUCTION

The main objective of endodontic treatment is complete disinfection of root canal system by thorough cleaning and shaping[1]. Irrigation of the root canal system plays a very important role in the removal of pulpal remnants, necrotic debris and smear layer which contains organic and inorganic portion[2]. Endodontic treatment success depends on chemomechanical debridement which can be achieved by mechanical instrumentation of the root canal and irrigating solutions. There is no single irrigant which can eliminate this necrotic debris and smear layer. Combination of irrigants are needed for complete removal of necrotic debris and smear layer[3]. Irrigants can change the physical and chemical properties of dentine[4].

Human dentine is composed of approximately 70% of inorganic material, 20% of organic material and 10% of water. In that 20% of organic material, 90% is collagen, which plays a major mechanical role in dentine[3,4]. Depletion of this organic phase, i.e collagen after root canal irrigation may cause changes in the mechanical properties, including microhardness, permeability and solubility of dentine[5].

The purpose of using root canal irrigating solutions while dealing with smear layer is twofold i.e, to remove/ dissolve its organic and inorganic components[6].

As there is no single irrigating solution has the ability to do so, the sequential use of organic and inorganic solvents has been recommended. 17% EDTA and 5.25% NaOCl currently are the gold standard endodontic irrigants for effective removal of smear layer. These endodontic irrigants proved dentine surface free from smear layer & provide a decrease in bacterial count[3,7].

Sodium hypochlorite (NaOCl), because of its tissue-dissolving properties and broadspectrum antimicrobial action, considered as the gold standard root canal irrigant[3]. Sodium Hypochlorite (NaOCl) is a non specific proteolytic agent, which will effectively remove the organic components of smear layer[8]. For complete removal of smear layer, NaOCl should be mixed with chelating agents that can remove the inorganic phase of smear layer [9]. Concentration of NaOCl ranging from 1% to 5.25% are used in endodontics[6].

Østby (1957) proposed the use of EDTA (ethylene diamine tetra acetic acid) solution in Endodontics initially[10]. EDTA is polyaminocarboxylic acid, water soluble solid and colorless. Its chelating properties are due to its ability to sequester metal ion such as Ca2+ and Fe3+. After being bound by EDTA, metal ions remain in solution, but exhibit diminished reactivity. EDTA is produced as several salts, notably disodium EDTA and calcium disodium EDTA. It has detrimental effect on periapical tissues[11,12]. On the other hand, it results in excessive erosion of peritubular and intertubular dentine that decreases microhardness of root dentine[13].It demineralizes the inorganic components of smear layer via calcium chelation. EDTA reacts with calcium ions in hydroxyapatite crystals and removes them from the dentine by forming stable water soluble complexes[11].

Chelating agents like EDTA, citric acid, maleic acid, MTAD (Mixture of tetracycline, acid & detergent), chitosan, Tetracycline isomer and etidronate which is also known as bisphosphonate, etidronic acid or HEBP (1-Hydroxyethylidene-1, 1-Bisphosphonate) have the ability to remove the inorganic phase of smear layer. Among these 17% EDTA is generally accepted as the most common chelating agent with outstanding lubricant properties and is commonly used in endodontic therapy[14].

Chelating agents induce changes in the structure of dental tissues and alter the calcium phosphorous ratio (Ca/P) which in turn changes the microhardness, permeability, and solubility characteristics of dentine[10].

The decalcifying efficacy of solutions such as EDTA, citric acid or phosphoric acid is known to depend on the concentration, pH, and time of application[15].

Loss of Calcium ions of the hydroxyapatite crystals results in micro-structural changes of dentine by changing the Ca2+: PO4 3- ratio. This in turn, results in reduction of the microhardness, changes the permeability and solubility of dentine which adversely affecting the sealing ability of resin-based cements and sealers to root canal dentine[14].

EDTA has erosive effect on dentine at various concentrations, application time and it creates rough surface[12].

Irrigating solution should eliminate the smear layer completely with less erosive effect on dentine. Panighi and G'Sell reported a positive correlation between hardness and the mineral content of the tooth. It has been indicated that microhardness determination can provide indirect evidence of mineral loss or gain in dental hard tissues[10].

Chitosan, a natural polysaccharide, derived from deacylation of chitin. It is bio-based polymer. Chitin is obtained from shells of crabs and shrimp. They are biocompatible, biodegradable, bioadhesive, non-toxic. Also has high bioactivity, selective permeability, antimicrobial activity, adsorption capacity and chelation ability. Molecular weight of polysaccharide ranges from 1000000 to 3000000. At its acidic pH, it has remarkable chelation capacity to various metal ions. It is used in many sectors of industries. Chitin is ecologically most abundantly available substance and its economically viable[12,16].

Chitosan is differed from chitin by the presence of amino groups. The amino group of the D-glucosamine residues might be protonated and providing solubility in diluted acid (pH < 6) which opens prospects to wide range of application. Due to the amino groups, chitosan efficiently complex various species[17].

Applications of chitosan mainly seen in the areas of medicine and pharmaceuticals as antibacterial and antitumour agent, drug carrier, wound healing accelerator, in biotechnology as enzyme and cell carrier, chromatography resin, in environment as water treatment, in agriculture as seed preparation, in cosmetics and in food products as iron and calcium absorption accelerator, fibre source[16].

Application of chitosan in dentistry observed in different specialties as a modulator of inflammation, assistant in the periodontal regeneration, in intraosseous defects, in intracanal medication and as an antimicrobial agent associated with bonding agents and composite resin[18].

Some authors evaluated the smear layer removing properties of chitosan[16] and its time dependent effect on dentine[12] and it also has anti-bacterial and anti- fungal property[19].But the calcium loss by chitosan as chelating agent and its correlation with microhardness of root dentine was not yet evaluated. So, in this study different concentration of 0.2% and 0.5% chitosan was used to analyse its effect on calcium loss and microhardness of root dentine.

The aim of the study was to evaluate the effect of different concentration of chitosan on calcium loss and its effect on microhardness of root dentine.

AIM AND OBJECTIVES

Aim

To compare the effect of 17% EDTA, different concentration of chitosan (0.2% and 0.5%) on calcium loss and microhardness in root dentine.

Objectives

The main objectives was to

- Evaluate the effect of 17% EDTA, 0.2% and 0.5% chitosan on Calcium loss using **Integrated plasma mass spectrometry (ICP-MS).**
- Evaluate the effect of 17% EDTA, 0.2% and 0.5% chitosan on Microhardness of root dentine using **Vickers Hardness Tester.**

REVIEW OF LITERATURE

REVIEW OF LITERATURE

DEMINERALIZATION

- **Brenna Magdalena Lima Nogueira et al (2018)**, Evaluated the effect of irrigating solutions on mineral content and ultrastructure of root canal dentine. Thirty single rooted teeth were taken and they were divided into different groups : G1, saline solution (0.9% NaCl); G2, 2.5% NaOCl + 17% EDTA + 2.5% NaOCl; G3, 2.5% NaOCl + 9% Etidronate (HEBP) + 2.5% NaOCl; G4, mixture of 5% NaOCl + 18% HEBP; G5, 2.5% NaOCl + 17% EDTA + 0.9% NaCl, and G6, 2.5% NaOCl + 9% HEBP + 0.9% NaOCl. The chemical composition like calcium, phosphorus, magnesium and potassium and Ca/P ratio were determined after respective irrigation. Ultrastructural changes of dentine was evaluated using scanning electron microscopy and crystalline phase were analysed using X-ray diffraction and concluded that irrigating solutions showed changes in the morphology, physical and chemical composition of the dentine. Significant change in Ca/P ratio was seen in 2.5% NaOCl + 17% EDTA + 2.5% NaOCl and 2.5% NaOCl + 17% EDTA + 0.9% NaCl and maximum volume of Ca and P was observed in 2.5% NaOCl + 9% Etidronate (HEBP) + 2.5% NaOCl, 5% NaOCl + 18% HEBP, 2.5% NaOCl + 9% HEBP + 0.9% NaCl. However, no significant differences were observed crystallographically[20].

- **Paôla Caroline da Silva Mira et al (2017)**,evaluated the chelating effect of chitosan solubilised in different acids. Cervical region of Maxillary central incisors were taken in the study. Chitosan were solubilised in different acids like acetic acid, hydrochloric acid, nitric acid and citric acid.GI – 0.2% chitosan solubilized in 1% acetic acid; GII – 0.2% chitosan solubilized in 3.3% citric acid; GIII – 0.2% chitosan solubilized in

0.00145% hydrochloric acid; and GIV – 0.2% chitosan solubilized in 0.00112% nitric acid. A control was made from the chelating properties of the following acids: GV – 3.3% citric acid, GVI – 0.00145% hydrochloric acid, GVII – 0.00112% nitric acid, and GVIII – control (distilled water). After the preparation of solution, their chelating property and volume needed for chelating calcium ions were evaluated using colorimeter and they standardized the volume as 50µL and application time for 5mins and concluded that chelating ability of chitosan solubilised in acetic acid was higher when compared with other acids[18].

- **Reem Adel Abd-Elgawad et al (2017)**, Evaluated the Smear Layer Removal, Calcium Ion Loss and Dentine Microhardness after Different Final Irrigation Solutions with 5.25% NaOCl, 17% EDTA, QMix 2in1 and 0.2% Chitosan. The specimens were longitudinally divided into two equal halves and one halves for smear layer removal determination using SEM, another halves for calcium ion loss using atomic absorption spectrometry and microhardness evaluated using vicker's microhardness tester. They concluded that NaOCl not able to remove the smear layer, it has more microhardness reduction than other final rinse with irrigating solutions like 17%EDTA, Chitosan and QMix. These irrigants significantly removed the smear layer and they had more calcium ion loss compared to other irrigants[21].

- **Hagar A. El Naby Bastawy et al (2016)**, assessed the impact of chitosan on microhardness and mineral content of intraradicular dentine. 60 single rooted teeth were taken in the study, longitudinally segemented into 120 segments. The specimens were divided based on the irrigating solution used. G1: 0.2% chitosan, G2: 2%

chitosan, G3: 17% EDTA and G4: saline (control group). 80 segments were analysed for Vickers microhardness tester to determine the microhardness reduction. 40 segments were analysed for mineral content loss (calcium, phosphorus and magnesium) using inductively coupled plasma atomic emission spectrometry (ICP-AES). The results concluded that Chitosan 0.2% solution was equally effective to 17% EDTA in removing Ca2+ ions from root canal dentin without much altering its microhardness[13].

- **Gusiyska A et al (2016)**, evaluated the effect of chitosan- citrate solution on smear layer removal. Single rooted human teeth were taken in the study and the teeth were decoronated and instrumentation was done with Protaper universal upto F4 and irrigation solutions used were, Group I (n=5) 3 ml 5.25% NaOCl, Group II (n=5) 17% EDTA, Group III (n=5) 5.25% NaOCl and 17% EDTA, Group IV (n=5) 0.6 % chitosan-citrate (0.6 mg of the chitosan powder were dissolved in 100 ml of 1% citric acid) was used between the files. In a control Group V (n=1) distilled water was used for irrigation and in a negative Group VI (n=1) the root was instrumented without irrigation. Then the Specimens were bisected longitudinally into two halves and subjected to scanning electron microscope to evaluate the smear layer removal and concluded that smear layer removal of 0.6% chitosan-citrate was similar to that of 17% EDTA and 5.25% NaOCl and in combination. Chitosan citrate solution showed less dentinal erosion whereas17%EDTA showed significant dentine erosion[17].

- **HM Bayram et al (2016)**, evaluated the calcium ion release from different calcium silicate-based endodontic materials like white MTA, bioaggregate (BA) and biodentine after immersion with new irrigants like 0.2% chitosan, 10% propolis, 1% acetic acid, 17% EDTA and distilled water.150 silicone tubes were prepared and randomly divided into three groups based on calcium silicate-based endodontic materials. Each groups was subdivided into five subgroups based on immersion in new irrigants. The irrigation solutions were subjected to Atomic adsorption photospectrometry. They concluded that calcium release was more in EDTA and less in distilled water and no statistically significant difference between propolis, chitosan and distilled water,and also between 17% EDTA and 1% acetic acid. Natural irrigants like chitosan and propolis can be preferred when used with MTA, BA and Biodentine [22].

- **Atul Jain et al (2016)**, Comparatively Evaluated the Calcium Ion Loss and Microhardness reduction using 5.25% sodium hypochlorite+ distilled water, 5.25% sodium hypochlorite +18% Hydroxyethylidene bisphosphonate (HEBP), 5.25% sodium hypochlorite+15% Citric acid. Calcium ion loss was determined using atomic adsorption spectrophotometry and microhardness using Vickers microhardness test. They concluded that all the specimens treated with irrigants results in calcium loss during first 5mins. While comparing all these irrigants 5.25% NaOCl had less calcium loss, citric acid had more calcium loss[23].

- **Keyur Pankaj Chande et al (2014)**, evaluated and compared the decalcifying effect of 17% ethylenediaminetetraacetic acid (EDTA), 15% citric acid, 37% phosphoric acid and 5.25% sodium hypochlorite on root canal dentine at 5, 10, and 15 minutes by immersing in the 20 ml of repective solution for stipulated time period. The calcium loss were evaluated using mass spectrometry and concluded that 17% EDTA and 15% citric acid extracted significantly largest amount of calcium followed by 5% phosphoric acid at each time period. 5.25% NaOCl solution extracted small amount of calcium initially[24].

- **Sonali Taneja et al (2014)**, compared the effect of Q Mix, peracetic acid and 17% ethylenediaminetetraacetic acid on calcium loss and its effect on microhardness of root dentine. Lower premolars was taken and they were decoronated, transverse section of 2mm were obtained from coronal third of root and divided into four parts, each in one group. The samples were immersed in the following irrigating regimen. Group 1 (Control): 5% Sodium hypochlorite (NaOCl) for 5 min + distilled water for 5 min; Group 2: 5% NaOCl for 5 min + 17% ethylenediaminetetraacetic acid (EDTA) for 5 min; Group 3: 5% NaOCl for 5 min + 2.25% Peracetic acid (PAA) for 5 min and Group 4: 5% NaOCl for 5 min + QMix for 5 min. Then the irrigating solutions were subjected to atomic absorption spectrophotometer to evaluate the calcium loss and the samples were subjected to Vickers microhardness for microhardness reduction. The results concluded sodium hypochlorite + 2.25% peracetic acid had maximum calcium loss and minimum microhardness reduction followed by 17% EDTA, QMiX[1].

- **P. V. Silva et al (2013)**, evaluated the efficacy of smear layer removal and calcium loss using chelating agents as final irrigating solutions like 15% EDTA, 0.2% chitosan, 10% citric acid, 1% acetic acid after preparing the root canal with crown-down technique and irrigated with 1% sodium hypochlorite. The irrigating solution were collected and subjected to atomic adsorption photospectrometry to determine the calcium loss and the specimens were split longitudinally and examined under SEM to determine the smear layer removal. They concluded that 15% EDTA, 0.2% chitosan and 10% citric acid similarly removed the smear layer and they were significant from 1% acetic acid from middle and apical third. Root dentine demineralization was high in 15% EDTA and 0.2% chitosan followed by 10% citric acid and 1% acetic acid[16].

- **Carmen-María Ferrer-Luque et al (2013)**, assessed the Decalcifying effects of antimicrobial irrigating solutions on root canal dentine. The specimens were prepared from 2mm thick slice of cervical root dentine and divided into four equal halves, each halves in each group were distributed and evaluated for decalcifying efficacy of 7% maleic acid (MA), 2% chlorhexidine (CHX), and combinations of 7% MA + 0.2% cetrimide (CTR) and 2% CHX + 0.2% CTR, in 1min, 2mins, 3mins and 5mins time periods. The irrigating solutions were collected and subjected to calcium loss determination using atomic absorption spectrometry. They concluded that calcium loss was more in 7% maleic acid followed by 7% MA + 0.2% cetrimide, 2% CHX and 2% CHX + 0.2% cetrimide. Stastistically significant difference was seen in all time period[15].

- **Polliana Vilaça Silva et al (2012)**, assessed the time dependent effect of chitosan on Dentine. Maxillary canine were taken in the study, instrumented using nickel titanium instruments four times greater than the apical diameter and the specimens were subjected to different irrigating solution. G1: 0.1% chitosan for 3 min; G2: 0.2% chitosan for 3 min; G3: 0.37% chitosan for 3 min; G4: 0.1% chitosan for 5 min; G5: 0.2% chitosan for 5 min; G6: 0.37% chitosan for 5 min. The specimens were bisected longitudinally and evaluated in the scanning electron microscope and concluded that G1 exhibited removal of the smear layer, but not the smear plugs. G2 showed visible and open tubules with slight erosion of the peritubular dentine. Cleaning in G3 was similar to that of G2, however, the erosive effect was greater. There was expansion of the diameter of the tubules in G4, G5 and G6, with severe erosion and deterioration of dentin surface. And clinically, 0.2% chitosan for 3 mins were efficient in smear layer removal with little dentine erosion[12].

- **Lora Mishra et al (2012)**, evaluated the Calcium loss from root canal dentine following irrigation with distilled water, 2.5% NaOCl, 17% EDTA, 1% tetracycline HCl, 17% EDTA + 2.5% NaoCl, 1% tetracycline HCl + 2.5% NaOCl and its effect on microhardness. The specimens were longitudinally divided into two equal halves and one halves was subjected to ICP-AES (inductively coupled plasma- atomic emission spectrometry) for determining calcium loss and other halves was subjected to vicker's microhardness tester for determining microhardness. They concluded that maximum calcium loss was in 17% EDTA + 2.5% NaOCl followed by 17% EDTA, 1% Tetracycline HCl + 2.5% NaOCl and 1% Tetracycline HCl. Negative correlation between calcium loss and microhardness was observed in all the groups[25].

- **L. F. Machado-Silveiro et al (2004)**, evaluated the demineralization capability of 1% and 10% citric acid, 10% sodium citrate, 17% EDTA and distilled water at 5, 10 and 15 min time interval on root canal dentine. The specimens were prepared by obtaining 3mm thick cross sectional cervical root dentine and divided into four halves. That four halves are distributed as one in each group. After the irrigation, irrigating solutions were collected and lanthanum oxide were added and the specimens were subjected to spectrophotometry. They concluded that 10% citric acid had more decalcifying effect than 1% citric acid, 17% EDTA and 10% sodium citrate. Citric acid at both concentration had decreased effectiveness in calcium removal with time and EDTA had decreased effectiveness in calcium removal with time and sodium citrate had removed only less calcium and small significant increased effectiveness with time[26].

MICROHARDNESS

- **Srinidhi surya raghavendra et al (2018)**, assessed the effect of different irrigating solution on microhardness of root canal dentine. 47 single rooted teeth were taken, decoronated, canals were prepared using protaper upto F3 size. Grooves were placed on long axis of roots and cleaved with a chisel and a mallet. The specimens were embedded in the dental stone. The specimens were divided based on the irrigating solution used. Group I- Etidronic acid (n=15), Group II- 17% EDTA (n=15), Group III – 0.2% chitosan solution. This was prepared by mixing Chitosan nanoparticles with 1% acetic acid. (n=15). The specimens were subjected to Vickers microhardness tester at 1000µ, 1200µ and 1400µ from the canal lumen to determine microhardness reduction. The result showed that 17% EDTA had maximum reduction in microhardness than 0.2% chitosan and etridonate[27].

- **Tenzin Rapgay et al (2018)**, evaluated and compared the microhardness of root dentine using QMix, Tea tree oil, Tamarindus indica, Green tea extract and 17% EDTA. Sixty Single rooted premolar were taken and the roots were decoronated, the root canals were enlarged till 40 K file and they were divided into two halves and embedded in the acrylic resin. The specimens were divided into 6 groups based on different irrigants, Group 1: Qmix for 5 minutes, Group 2: Tea tree oil for 5 minutes, Group 3: 5% Tamarindus indica for 5 minutes, Group 4: 5% Green tea extract for 5 minutes, Group 5: 17% EDTA for 5 minutes, Group 6: Control group: Saline for 5 minutes. Then the specimens were subjected to Vickers microhardness test and concluded that microhardness reduction was more in 17% EDTA group followed by

Qmix and Tamarindus indica groups. No significant reduction in microhardness in Tea tree oil group and Green tea group was observed[28].

- **Suparna gangulysaha et al (2017)**, evaluated the effect of various endodontic irrigants on the microhardness of root canal dentine. 80 single rooted mandibular premolar were taken, decoronated and roots were longitudinally sectioned into two equal halves. The specimens were embedded in the autopolymerizable resin, divided based on the different irrigating solution. 3% Sodium Hypochlorite (3% NaOCl), 17% Ethylene Diamine Tetra Acetic Acid (17% EDTA), 0.2% Chitosan and 6% Morindacitrifolia Juice (MCJ) for 15 minutes each. The specimens were subjected to Vickers microhardness tester to determine the microhardness reduction. The results showed that 17% EDTA and 0.2% Chitosan, significantly decreased the microhardness of root dentine whereas 6% MCJ and 3% NaOCl had no significant effect on the microhardness[29].

- **Soha F. Massoud et al (2017)**, Compared the different irrigation protocols on the microhardness of root canal dentine after irrigation with 2.5% NaOCl, 17% EDTA, 2% CHX. Forty single rooted lower premolar were taken in the study and stainless steel K- files were used to instrument the canal and split longitudinally. The samples were divided into four groups, Group I: 10 ml of 2.5% Sodium Hypochlorite (NaOCl), Group II: 10 ml of 17% ethylene diamine tetra-acetic acid (EDTA) followed by 10 ml of 2.5% NaOCl, Group III: 10 ml of 2.5% NaOCl followed by 10 ml of 2% chlorhexidine digluconate (CHX), Group IV: 10 ml of 2.5% NaOCl followed by 10 ml of distilled water then they were immersed in 10ml of 2% CHX. The specimens

were subjected to Vickers microhardness test to evaluate the microhardness reduction and concluded that all the groups showed reduction in microhardness.17% EDTA followed by 2.5% NaOCl had maximum reduction in microhardness. Coronal third had maximum reduction in microhardness when compared with middle and apical third in all the irrigation protocols[3].

- **Vineeta Nikhil et al (2016)**, evaluated the effect of different irrigating solution on the microhardness of the human radicular dentine. 30 dentine specimens were divided into three groups of 10 specimens each according to the irrigant used. G1 — 1% phytic acid, G2 — 17% EDTA, and G3 — 0.2% chitosan. Each chelating solution was used for 3 min. The specimens were subjected to Vickers microhardness tester before and after application of the irrigants at the cervical, middle, and apical levels. The results showed that all chelating solutions reduced microhardness of the radicular dentine layer at all the levels. However, reduction was least at the apical level. 17% EDTA caused more reduction in dentin microhardness than chitosan while phytic acid reduced the least[30].

- **Flavia Emi Razera Baldasso et al (2016)**, Evaluated the effect of final irrigation protocols with QMIX, 17% EDTA, 10% citric acid, 1% peracetic acid on root canal dentin. All the groups were finally flushed with 2.5% NaOCl for 5 mins and rinsed with 10 ml of distilled water. The specimens were subjected to knoop indenter before and after irrigation at 100µm and 500µm from the lumen of root. After microhardness evaluation, specimens were split longitudinally and dentin erosion were examined by scanning electron microscope. The results showed that dentinal erosions were more in

citric acid followed by 1% peracetic acid and 17% EDTA. QMIX doesn't show dentine erosion. Microhardness was reduced at greater depth in QMIX and 17% EDTA than 10% CA and 1% PA[31].

- **Bhavana Gandhi et al (2016)**, Evaluated the effect of CPP-ACP as remineralizing agent in improving the microhardness after irrigation protocol and its influence on the bond strength of self etch resin sealer. Maxillary incisors were taken and the samples were divided based on the irrigation protocol. Group 1-normal saline, Group 2-17% EDTA (Ethylene Diamine Tetraacetic Acid) + 5.25% NaOCl (Sodium Hypochlorite), Group 3 - 17% EDTA + 5.25% NaOCl + CPP-ACP. They were divided into two groups for determining microhardness and push out bond strength . one group was evaluated for microhardness using Vickers microhardness and another group was obturated with Real seal SE and 6% gutta percha cones and subjected to universal testing machine to evaluate the pushout bond strength and concluded that CPP-ACP increases the microhardness of root dentine due to its remineralization property and also it doesn't effect the bond strength[32].

- **Vasundhara shivanna et al (2016)**, Compared the 15% EDTA solution, 15% EDTA gel, 10% citric acid , 5% maleic acid and saline on microhardness reduction. The specimens were subjected to Vickers microhardness to evaluate the microhardness reduction and concluded that EDTA and citric acid showed greater reduction in dentine microhardness but there was no significant difference. Maleic acid showed less reduction in dentine microhardness[6].

- **Anushree Das et al (2014)**, evaluated the Dentine microhardness changes following Conventional irrigation regimen with 5 ml of 5% NaOCl for 5 minutes followed by 5 ml of 17% EDTA for 5 minutes and finally with 5 ml of 2% CHX for 5 minutes, Morinda Citrifolia Juice (MCJ) regimen : 5 ml of 6% MCJ for 5 minutes followed by rinsing with 5 ml of 17% EDTA for 5 minutes. Q Mix regimen: 5 ml of 5% NaOCl for 5 minutes followed by 5 ml of Q Mix and Control : 5 ml of distilled water for total 5 minutes. The specimens were prepared from maxillary central incisors, roots were longitudinally sectioned from cervical to apical region. After respective irrigation regimen, the specimens were subjected to vicker's microhardness tester to determine the microhardness. They concluded that QMix had less reduction in microhardness than other regimens[33].

- **Kamakshi G et al (2014)**, evaluated the Relation between Calcium Loss and Microhardness of Root Canal Dentine Following Treatment With 17% Ethylene Diamine Tetraacetic acid at Different Time Intervals. Single rooted premolar teeth were taken and the teeth were decoronated, splited longitudinally and divided into different groups. Group 1: 17% EDTA Solution for 1 min, Group 2: 17% EDTA Solution for 3 min, Group 3: 17% EDTA Solution for 5 min, Group 4: 17% EDTA Solution for 7 min, Group 5: 17% EDTA Solution for 10 min, Group 6: 17% EDTA Solution for 12 min, Group 7: 17% EDTA Solution for 15 min, Group 8: 0.9% Saline (control). Then the irrigating solutions were subjected to atomic absorption spectrophotometer to evaluate the calcium loss and specimens were subjected to Vickers microhardness for microhardness reduction. They concluded that calcium loss

and reduction in microhardness was increased by increasing the immersion time with 17% EDTA[14].

- **Eda E. Aslantas et al (2014)**, evaluated the effect of EDTA, Sodium Hypochlorite, and Chlorhexidine Gluconate with or without Surface Modifiers on Dentine Microhardness . Root halves were prepared from distal root of mandibular third molar. Irrigating solutions used in the study were 17% EDTA (Vista Dental, Racine, WI), REDTA (17% EDTA containing 0.84 g cetrimide) (Sigma-Aldrich, Munich, Germany), 6% NaOCl (ACE, Proctor & Gamble, Gebze, Turkey), 6% NaOCl with surface modifiers (Chlor-XTRA) (Vista Dental), 2% CHX (Klorhex, Drogsan, Turkey), or CHX-Plus (Vista Dental). The samples were irrigated with 5ml of irrigating solutions for 5 minutes and the specimens were subjected to Vickers microhardness tester to evaluate the microhardness reduction at the mid-root level. The results showed that surface modifier had no effect in microhardness of root dentine. EDTA had maximum reduction in microhardness of root dentine[5].

- **Alexandre Correa Ghisi et al (2014)**, assessed the effect of super-oxidized water, NaOCl and 17% EDTA on microhardness of root dentine. Bovine incisors were taken in the study. Irrigations were done using 2% sodium hypochlorite (NaOCl), 5% sodium hypochlorite (NaOCl), super-oxidized water (400 ppm Sterilox - Sx) and 17% EDTA. Cervical third of the root were cut from the specimen and they were subjected to Vickers microhardness tester for the evaluation of microhardness 500µm-1000µm from the root canal lumen (Distance 1) and 500µm-1000µm from the external root surface (Distance 2) and concluded that statistically significant difference was seen between distance 1 and distance 2 expect 5% sodium hypochlorite (NaOCl) and 5% sodium hypochlorite (NaOCl) + 17% EDTA. No statistically significant difference

was seen between all the groups at distance 1. At distance 2, statistically significant difference was seen between 17% EDTA and Super-Oxidized water (Sx) only[9].

- **Hakan Arslan et al (2013)**, evaluated the effect of agitation of EDTA with 808-nm diode laser on dentine microhardness. Maxillary anterior teeth were taken in the study. The roots were sectioned longitudinally and subjected to different treatments. Group 1: distilled water, Group 2: 17 % EDTA, Group 3: EDTA with 60 s ultrasonic agitation, Group 4: EDTA with 10 s laser agitation, Group 5: EDTAwith 20 s laser agitation, Group 6: EDTAwith 30 s laser agitation, and Group 7: EDTAwith 40 s laser agitation. After that the specimens were irrigated with 5 % NaOCl and distilled water except the distilled water group. The specimens were subjected to Vickers microhardness tester to evaluate the microhardness before and after treatments and concluded that all the treatments had reduced the microhardness but statistically higher reduction in 17% EDTA with 40s agitation with diode laser. Ultrasonic agitation had no reduction in microhardness reduction[34].

- **Marta Barón et al (2013)**, assessed the Nanostructural changes in dentine caused by endodontic irrigants. Mandibular premolar were taken in the study and dentine disk were taken and divided into different groups. 5.25% NaOCl for 1 minute and 17% EDTA for 1 minute. Nanoindentations was placed on peritubular(PD) and intratubular(ID) dentine using NanoScope Illa version 5.30r2 atomic force microscope. Stiffness and adhesion force before and after treatment were evaluated using atomic force microscope and concluded that reduction in stiffness and adhesion forces were maximum in 17% EDTA and in 5.25% NaOCl, stiffness reduced in ID,

increased in PD and adhesion force increased in both ID and PD. Further research were needed on different concentration and application time[35].

- **Talita Tartari et al (2013)**, evaluated the effect of sodium hypochlorite (NaOCl), ethylenediaminetetraacetic (EDTA), etidronic (HEBP), and citric acid (CA) associated with different irrigation regimens. The samples were then randomly distributed into groups as follows: G1 (n=9): saline solution (control) for 30 min; G2 (n=9): 5% NaOCl + 18% HEBP, mixed in equal parts for 30 min; and G3 (n = 27): 2.5% NaOCl for 30 min. After the microhardness measurements, the G3 samples were divided to form G4, G5, and G6 (n=9), which received the following chelating agents to remove the smear layer: 17% EDTA for 3 min, 10% CA for 3 min, and 9% HEBP for 5 min, respectively. Following the new microhardness measurements, the samples in Groups G4, G5, and G6 received a final flush with 2.5% NaOCl for 3 min to remove the exposed collagen matrix by chelation, resulted in Groups G7, G8, and G9 and they were subjected to knoop indenter and concluded that all the regimens reduced the microhardness of dentine lumen. In initial microhardness, no statistically significant difference between coronal, middle and apical third is seen[36].

- **Chetan R Patil et al (2011)**, assessed the microhardness and surface roughness of root canal dentine. Incisor teeth were taken in this study and they were decoronated. The specimen were bisected longitudinally and divided into different groups. Group 1: 5 ml of 5.0% NaOCl for 15 min, Group 2: 5 ml of 2.5% NaOCl for 15 min, Group 3: 5 ml of 3% Hydrogen peroxide for 15 min, Group 4: 5 ml of 17% EDTA solution for 15 min, Group 5: 5 ml of 0.2% Chlorhexidine gluconate for 15 min, Group 6: 5 ml

of Distilled water for 15 min (control). The specimens were subjected to Vickers microhardness tester to evaluate the microhardness reduction and surface roughness tester to evaluate the surface roughness and concluded that all the irrigating solution reduced the microhardness and 0.2% CHX showed no significant reduction in microhardness. Significant increase in surface roughness in 2.5%, 5% NaOCl and 17% EDTA. No significant reduction in surface roughness in 3% Hydrogen peroxide and 0.2% Chitosan[37].

- **Deepa Natesan Thangaraj et al (2009)**, Determined the calcium loss and its effect on microhardness of root canal dentine following treatment with 17% ethylenediaminetetraacetic acid solution at different time intervals. Canine teeth were taken in the study and splited longitudinally, divided into different groups. Group 1: 17% EDTA Solution for 1 min, Group 2: 17% EDTA Solution for 2 min, Group 3: 17% EDTA Solution for 3 min, Group 4: 17% EDTA Solution for 4 min, Group 5: 17% EDTA Solution for 5 min, Group 6: 17% EDTA Solution for 6 min, Group 7: 17% EDTA Solution for 7 min, Group 8: 0.9% Saline (control). The irrigating solutions were subjected to atomic adsorption spectrophotometer to evaluate the calcium loss and specimens were subjected to Vickers microhardness tester to evaluate the microhardness and concluded that by increasing the time of immersion results in increased in the calcium loss and reduction in microhardness of root canal dentine[38].

- **Sandeep Singh et al (2009)**, Evaluated the effect of 17% EDTA, EDTAC, RC-Prep for 1 minutes and BioPure MTAD for 2 and 5 minutes respectively on the microhardness of coronal, middle and apical root canal dentine using Vicker's microhardness testing machine and concluded that no statistically significant difference in the microhardness of root dentine in the coronal, middle and apical third when treated with 17% EDTA, EDTAC, RC-Prep and BioPure MTAD. While comparing the irrigating solution, Microhardness reduction was more in 17% EDTA and microhardness reduction was less in biopure MTAD[10].

- **Taner Cem Sayin et al (2007)**, evaluated the effect of EDTA, EGTA, EDTAC, and tetracycline-HCl with and without subsequent NaOCl treatment on the microhardness of root canal dentine. Single rooted teeth were taken in the study, bisected longitudinally and they were divided into different groups. Group 1: 2.5% NaOCl; Group 2: 17% EDTA; Group 3: 17% EGTA; Group 4: 15% EDTAC; Group 5: 1% tetracycline-HCl; and group 6:distilled water (negative control). The specimens were subjected to the Vickers microhardness tester and concluded that significant decrease in Microhardness only for EDTA and EDTA+NaOCl in the coronal region and for EDTAC and EDTAC+NaOCl in the apical and middle regions of the root canal[2].

- **Luciane Dias Oliveira et al (2007)**, assessed the microhardness of root dentine after irrigation with sodium hypochlorite and chlorhexidine. Single rooted teeth were taken in the study, decoronated and divided into cervical, middle and apical segment and mounted in the acrylic resin. The samples were divided into three groups based on irrigating solutions. Group 1: control (saline solution); Group 2: 2% chlorhexidine

gluconate solution and Group 3: 1% sodium hypochlorite (NaOCl). The specimens were irrigated for 15 mins and subjected to Vickers microhardness tester at 500µm and 1000µm from the root canal lumen. The results concluded that 2% chlorhexidine gluconate solution and 1% sodium hypochlorite (NaOCl), significantly reduced the microhardness at 1000µm and no significant difference at 500µm[4].

- **Charu Dayal et al (2007)**, evaluated the microhardness of root dentine prepared with different file types like stainless steel k files and protaper nickel titanium rotary files with 2.5% sodium hypochlorite. The specimens were prepared and microhardness reduction was determined at the distance of 500 mm and 1000 mm from pulp dentine interface by vicker's microhardness tester and concluded that protaper nickel titanium rotary with irrigation had significantly less reduction in microhardness when compared with nickel titanium K-files and stainless steel K-files. The irrigation with 2.5% sodium hypochlorite had changed the biomechanical properties of root dentine[8].

MATERIALS AND METHODS

ARMAMENTARIUM USED

1. 0.5% Thymol solution
2. Diamond disc and mandrel
3. Straight handpiece
4. Self cure acrylic resin
5. 5.25% sodium hypochlorite
6. 17% EDTA
7. 0.2% chitosan
8. 0.5% chitosan
9. Distilled water
10. Pipette
11. Centrifuge tubes
12. Centrifuge machine
13. Polyprophylene tubes with lid
14. Integrated plasma mass spectrometry (ICP-MS)
15. Vickers microhardness tester

INCLUSION CRITERIA

- Single rooted teeth with single root canal

EXCLUSION CRITERIA

- Teeth with multiple canals
- Teeth with root caries
- Teeth with anomalies like taurodontism
- Teeth with root resorption

Thirty single rooted teeth were used for this study. Teeth were stored in 0.5% thymol until the experimental procedure . The soft-tissue covering of the root surface was removed with curettes. The teeth were decoronated at the cementoenamel junction using a high speed carbide bur under copious water irrigation. Thick transverse sections of 2 mm with a maximum and minimum width of 3 mm and 2 mm respectively were obtained from the coronal third of each root using a low speed safe sided diamond disc. Each section was further divided into 2 halves. The Specimens were horizontally embedded in auto polymerizing resin so as to expose the canal part of the dentine. The specimens were ground flat on a circular wet grinding machine with ascending grades of SiC abrasive papers (320, 600, 1000, 1200 and 1500 grit) under constant water irrigation using Leco grinder polisher. A total of 60 samples were prepared, 15 specimens for each group, based on treatment groups were divided. The samples were immersed in beaker with respective irrigants as follows.

TREATMENT GROUPS:

Group 1 (n=15) : 5% NaOCl for 5 mins & saline for 5 mins

Group 2 (n=15) : 5% NaOCl for 5mins &17% EDTA for 5mins

Group 3 (n=15) : 5% NaOCl for 5mins & 0.2% chitosan for 5mins

Group 4 (n=15) : 5%NaOCl for 5mins & 0.5%chitosan for 5mins

Figure 1 : Irrigants used in the study

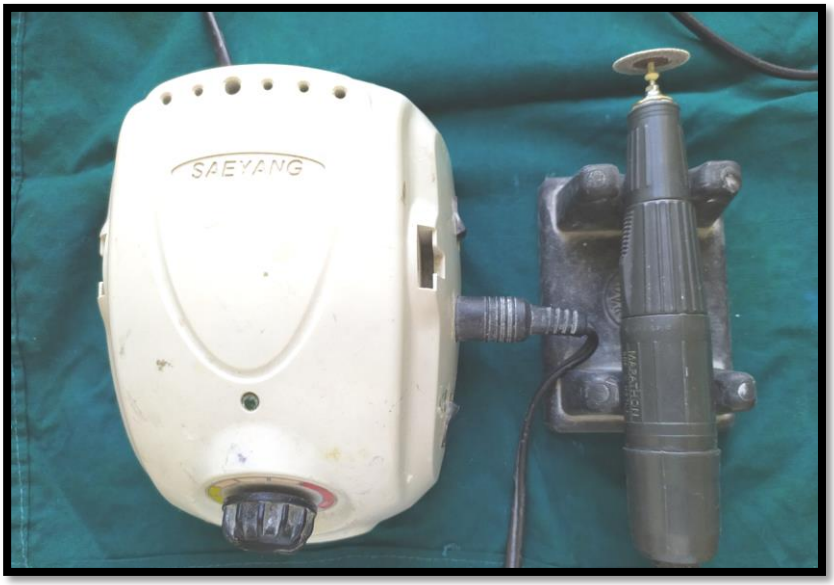


Figure 2 : Micromotor and Straight handpiece

CALCIUM LOSS DETERMINATION

All the specimens were immersed in 10 ml of the first test solution for 5 min. Then the specimens were rinsed thoroughly with saline. They were then immersed in 10 ml of the second test solution of the respective group for another 5 min. Each time after irrigation of one specimen per group, the elutes were centrifuged at 4000 g for 10 min. The 20 ml of total elute per specimen were collected in individual glass vials. Subsequently, 10 ml of the supernatant was transferred to a polypropylene tube with a lid until further analysis. Once all the elutes for all the samples had been collected, they were subjected to analyze calcium content using **Integrated plasma mass spectrometry (ICP-MS)**. Results were expressed as ppb (parts per billion) Ca^{2+} in the elute.

MICROHARDNESS MEASUREMENT

For each specimen after the combined treatment, surface hardness of the root dentine was measured with a **Vickers Hardness Tester (High wood micro vicker's hardness tester)**. Hardness was measured under the load of 300 g with duration of 15 s. In each sample, three indentations were made. The representative hardness value for each sample was obtained as the average of the three indentation values.

STATISTICAL ANALYSIS

Statistical analyses were performed using Statistical Package for the Social Sciences [SPSS, ver. 18.0; SPSS Inc., Chicago, IL, USA]. According to Shapiro Wilks test of normality the data was found to be in normal distribution. and $P < 0.05$ was considered as statistically significant. Mean values were compared among study groups by using one – way ANOVA followed by *post hoc* Tukey test. Pearson's correlation was done to compare the relation between calcium loss and microhardness of root dentine.

Figure 3: Thirty single rooted teeth were taken

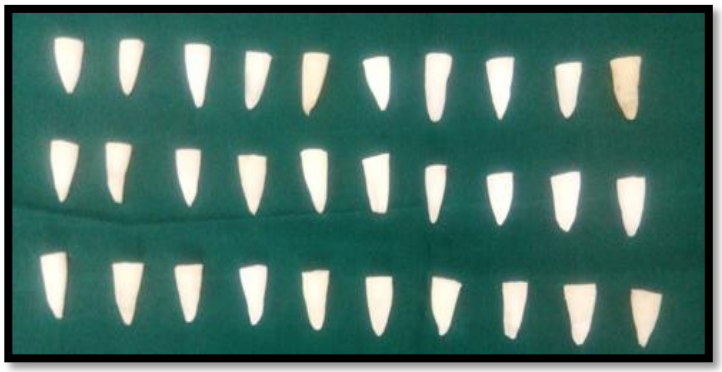


Figure 4: Teeth were decoronated

Figure 5: Coronal third of root were taken

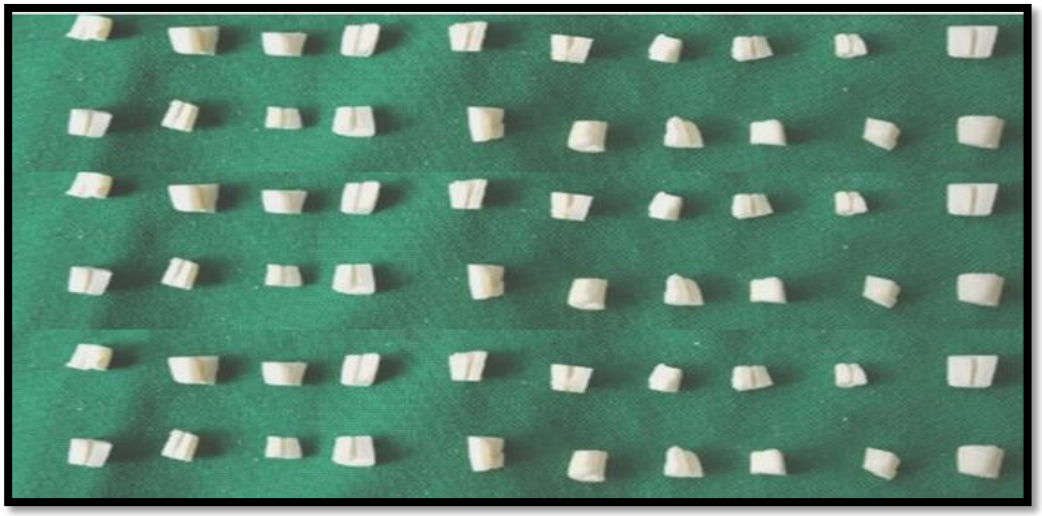


Figure 6: Coronal third of the roots were divided into two equal halves

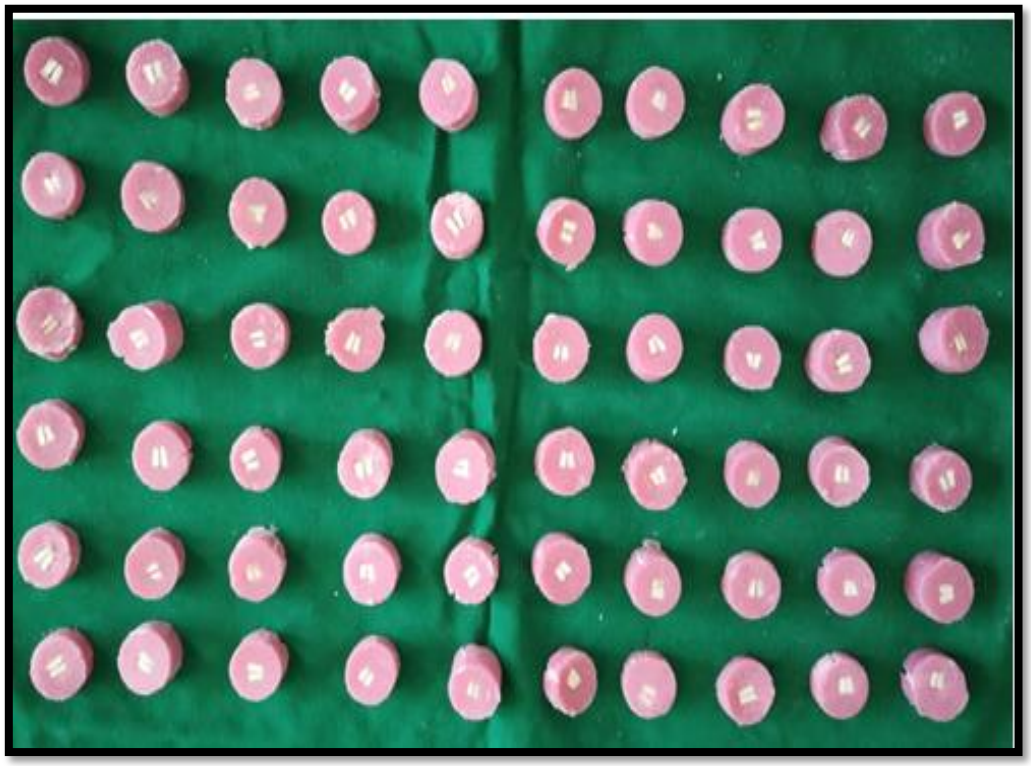


Figure 7: Sectioned specimens were embedded in the self cure acrylic resin

Figure 8: 10 ml of test solutions were pipetted

Figure 9 : Immersing in the first test solution for 5 minutes

Figure 10 : Rinsing after immersion in the first test solution with saline

Figure 11 : Immersing in the second test solution for 5 minutes

Figure 12 : Centrifuge Machine

Figure 13 : After centrifuging

Figure 14 : Pipetting 10 ml of total elute after centrifuging irrigants

Figure 15 : Integrated coupled plasma- mass spectroscopy (ICP-MS)

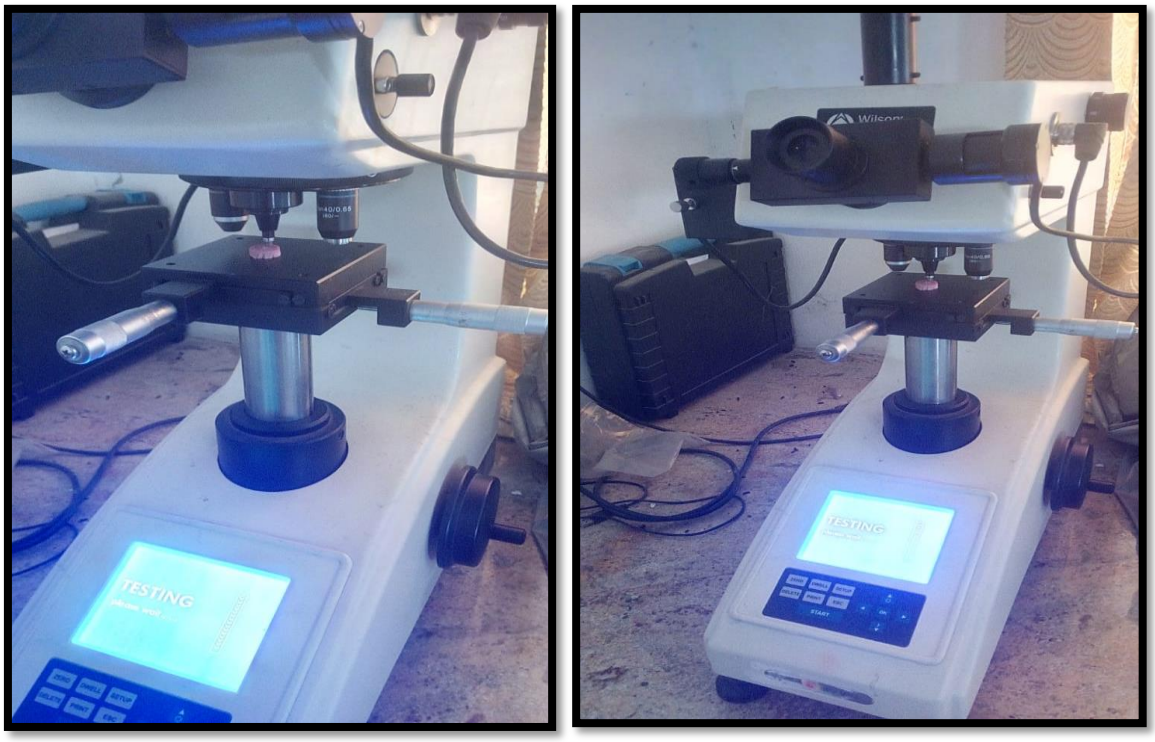


Figure 16 : Vickers microhardness tester

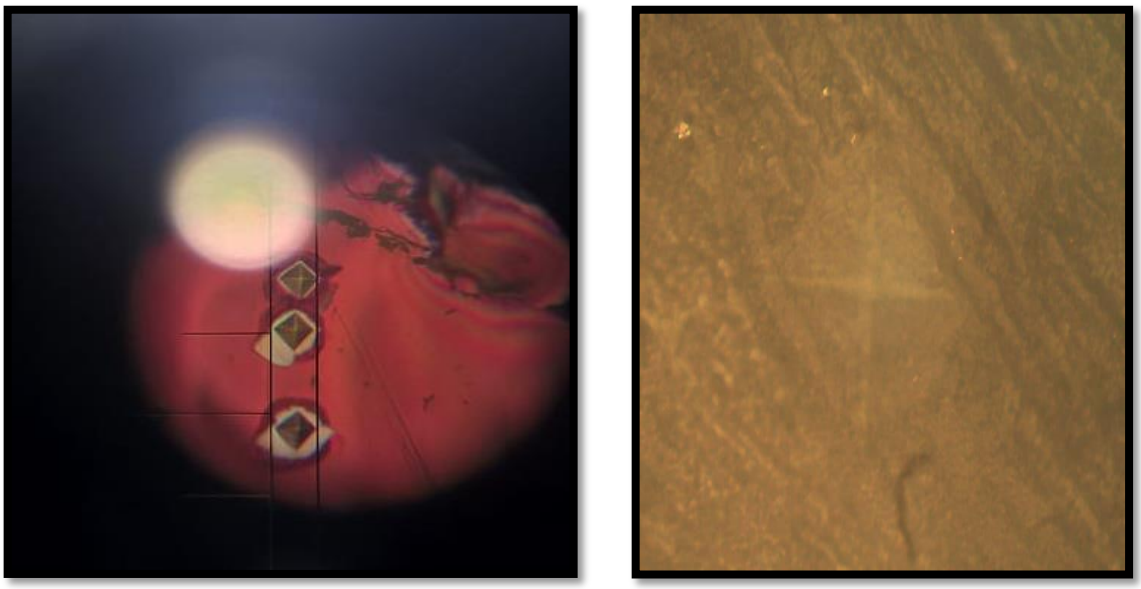


Figure 17 : Indentation in the specimens

RESULTS

Calcium content evaluation by Inductively coupled plasma mass spectrometry (ICP-MS)

Total samples : 60

Saline (GROUP I)	Calcium content (ppb)
1	0.486
2	0.374
3	0.160
4	0.294
5	0.345
6	0.268
7	0.360
8	0.421
9	0.156
10	0.157
11	0.223
12	0.453
13	0.473
14	0.231
15	0.334

17% EDTA (GROUP II)	Calcium content (ppb)
1	3.380
2	2.637
3	3.670
4	1.457
5	1.675
6	2.578
7	2.225
8	3.890
9	2.876
10	2.874
11	2.876
12	2.678
13	1.843
14	1.564
15	2.698

0.2% chitosan (GROUP III)	Calcium content (ppb)
1	0.637
2	0.841
3	0.645
4	0.278
5	0.447
6	0.548
7	0.378
8	0.798
9	0.476
10	0.378
11	0.467
12	0.267
13	0.478
14	0.470
15	0.567

0.5% chitosan (GROUP IV)	Calcium content (ppb)
1	0.814
2	0.780
3	0.980
4	0.678
5	0.879
6	0.783
7	0.853
8	0.768
9	0.823
10	0.701
11	0.456
12	0.750
13	0.793
14	0.675
15	0.774

Microhardness evaluation by Vickers microhardness test

Saline (GROUP I)	Microhardness (VHN)	Mean
1	64.5, 64.3, 63.4	64.06
2	63.6,62.8,64.6	63.6
3	62.7,65.3,65.2	64.4
4	63.6,62.1, 62.4	62.7
5	64.3,63.2,61.4	62.96
6	63.5,63.4,63.6	63.5
7	64.5,64.6,64.0	64.36
8	65.7,65.4,64.9	65.33
9	61.4,62.0,61.9	61.76
10	62.4,61.7,60.9	61.66
11	64.3,65.6,63.2	64.36
12	65.7,65.5,64.2	65.13
13	65.9,64.8,63.7	64.8
14	63.5,62.6,61.9	62.66
15	63.4,62.6,60.2	62.06

17% EDTA (GROUP II)	Microhardness (VHN)	Mean
1	45.7,45.8,45.5	45.66
2	48.6,48.8,48.9	48.76
3	40.5,40.8,40.7	40.66
4	41.5,41.7,41.6	41.6
5	42.5,42.6,42.9	42.66
6	43.6,43.7,42.1	43.13
7	40.1,40.6,40.7	40.46
8	42.4,42.1,42.6	42.36
9	43.4,43.2,43.2	43.26
10	41.2,41.3,41.0	41.16
11	42.1,42.2,42.3	42.2
12	40.5,40.6,40.7	40.6
13	42.5,42.7,42.8	42.66
14	41.6,41.5,41.4	41.5
15	43.5,43.6,43.6	42.13

0.2% chitosan (GROUP III)	Microhardness (VHN)	Mean
1	56.7,57.6,58.7	57.66
2	57.4,58.7,60.4	58.83
3	57.8,59.8,59.2	58.93
4	58.5,58.5,58.4	58.46
5	57.4,56.9,58.8	57.7
6	57.8,58.6,59.8	58.73
7	56.8,58.6,57.9	57.76
8	58.9,59.1,59.3	59.1
9	57.8,57.5,57.7	57.66
10	58.6,58.7,58.4	58.56
11	55.9,55.8,55.5	55.73
12	58.6,58.4,58.3	58.43
13	57.3,57.4,57.2	57.3
14	56.4,57.6,58.4	57.46
15	57.5,57.8,57.4	57.56

0.5% chitosan (GROUP IV)	Microhardness (VHN)	Mean
1	55.6,55.7,55.4	55.56
2	54.6,54.4,54.5	54.5
3	56.4,56.3,56.2	56.3
4	55.1,55.3,55.5	55.3
5	54.8,54.9,54.0	54.56
6	53.5,53.6,53.2	53.43
7	52.5,52.6,52.7	52.6
8	55.6,55.8,55.7	55.7
9	54.7,54.3,54.2	54.4
10	52.1,51.3,51.5	51.63
11	53.5,53.4,53.6	53.5
12	52.1,52.3,51.5	51.9
13	53.5,53.6,53.7	53.6
14	52.4,52.5,54.6	53.1
15	53.4,53.6,53.7	53.5

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	p value
						Lower Bound	Upper Bound			
Calcium loss	Grp I	15	.32	.115	.030	.25	.38	0	0	
	Grp II	15	2.59	.737	.190	2.19	3.00	1	4	0.000*
	Grp III	15	.51	.167	.043	.42	.60	0	1	
	Grp IV	15	.77	.116	.030	.70	.83	0	1	
	Total	60	1.05	.990	.128	.79	1.30	0	4	

One-way ANOVA * shows (P <0.05 considered as significant).

Table 1:One-Way ANOVA for the Comparison of different irrigation on calcium loss

Dependent Variable	(I) grps	(J) grps	Mean Difference (I-J)	Std. Error	p value	95% Confidence Interval	
						Lower Bound	Upper Bound
Calcium loss	Grp I	Grp II	-2.279*	.141	.000	-2.65	-1.91
		Grp III	-.196	.141	.512	-.57	.18
		Grp IV	-.451*	.141	.012	-.83	-.08
	2	1	2.279*	.141	.000	1.91	2.65
		3	2.083*	.141	.000	1.71	2.46
		4	1.828*	.141	.000	1.45	2.20
	3	1	.196	.141	.512	-.18	.57
		2	-2.083*	.141	.000	-2.46	-1.71
		4	-.255	.141	.280	-.63	.12
	4	1	.451*	.141	.012	.08	.83
		2	-1.828*	.141	.000	-2.20	-1.45
		3	.255	.141	.280	-.12	.63

Post-hoc Tukey test *. The mean difference is significant at the 0.05 level.

Table 1a : *Post-hoc* Tukey test for the Intergroup comparison for calcium loss

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	p value
						Lower Bound	Upper Bound			
microhardness	Grp I	15	63.56	1.207	.312	62.89	64.22	62	65	
	Grp II	15	42.59	2.156	.557	41.39	43.78	40	49	0.000*
	Grp III	15	57.99	.867	.224	57.51	58.47	56	59	
	Grp IV	15	53.97	1.384	.357	53.21	54.74	52	56	
	Total	60	54.53	7.886	1.018	52.49	56.56	40	65	

One-way ANOVA * shows (P <0.05 considered as significant).

Table 2: One-way ANOVA for the Comparison of different irrigation on the micro-hardness of the root dentine.

Dependent Variable	(I) grps	(J) grps	Mean Difference (I-J)	Std. Error	p value	95% Confidence Interval	
						Lower Bound	Upper Bound
Microhardness	Grp I	Grp II	20.969*	.541	.000	19.54	22.40
		Grp III	5.565*	.541	.000	4.13	7.00
		Grp IV	9.584*	.541	.000	8.15	11.02
	2	1	-20.969*	.541	.000	-22.40	-19.54
		3	-15.405*	.541	.000	-16.84	-13.97
		4	-11.385*	.541	.000	-12.82	-9.95
	3	1	-5.565*	.541	.000	-7.00	-4.13
		2	15.405*	.541	.000	13.97	16.84
		4	4.019*	.541	.000	2.59	5.45
	4	1	-9.584*	.541	.000	-11.02	-8.15
		2	11.385*	.541	.000	9.95	12.82
		3	-4.019*	.541	.000	-5.45	-2.59

Post-hoc Tukey test *. The mean difference is significant at the 0.05 level

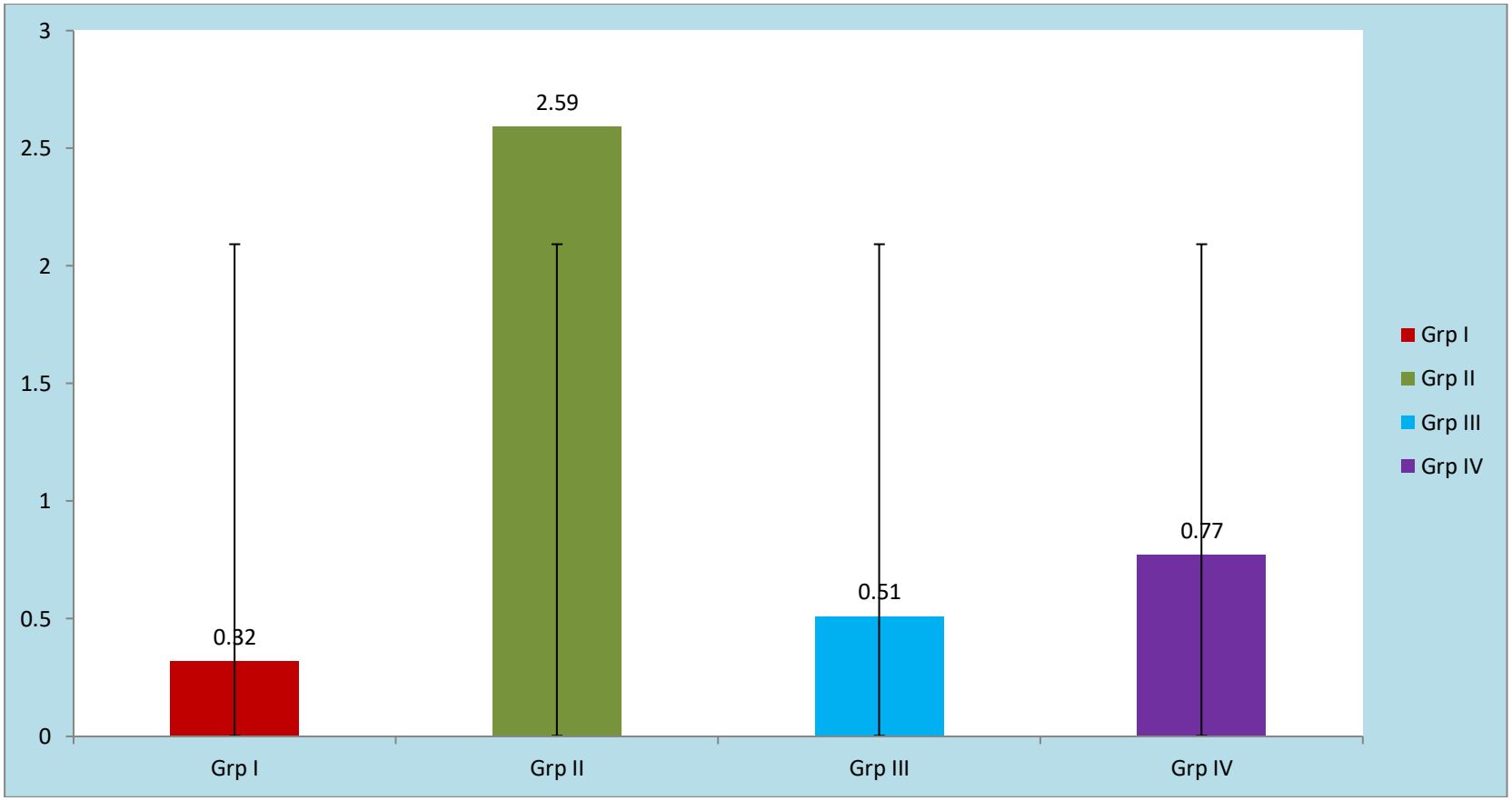
Table 2a: *Post-hoc* Tukey test for the Intergroup comparison for microhardness

		Calcium content	microhardness
calcium_content	Pearson Correlation	1	-.861**
	Sig. (2-tailed)		.000
	N	60	60
microhardness	Pearson Correlation	-.861**	1
	Sig. (2-tailed)	.000	
	N	60	60

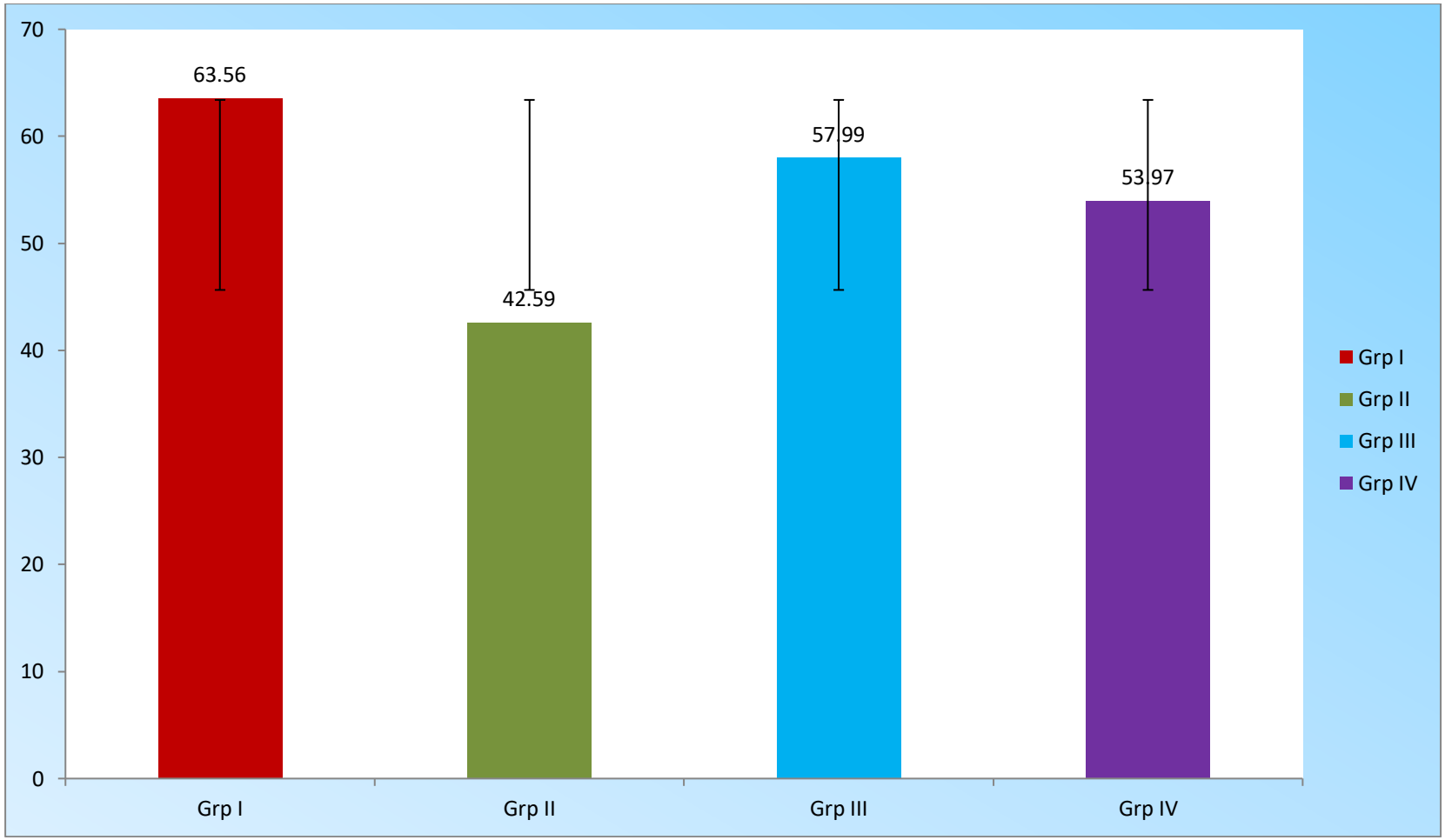
**. Correlation is significant at the 0.01 level (2-tailed).

A strong negative correlation existed between the calcium loss and reduction in the microhardness of root dentin (r = -0.861) which was found to be highly
significant ($p < 0.001$)

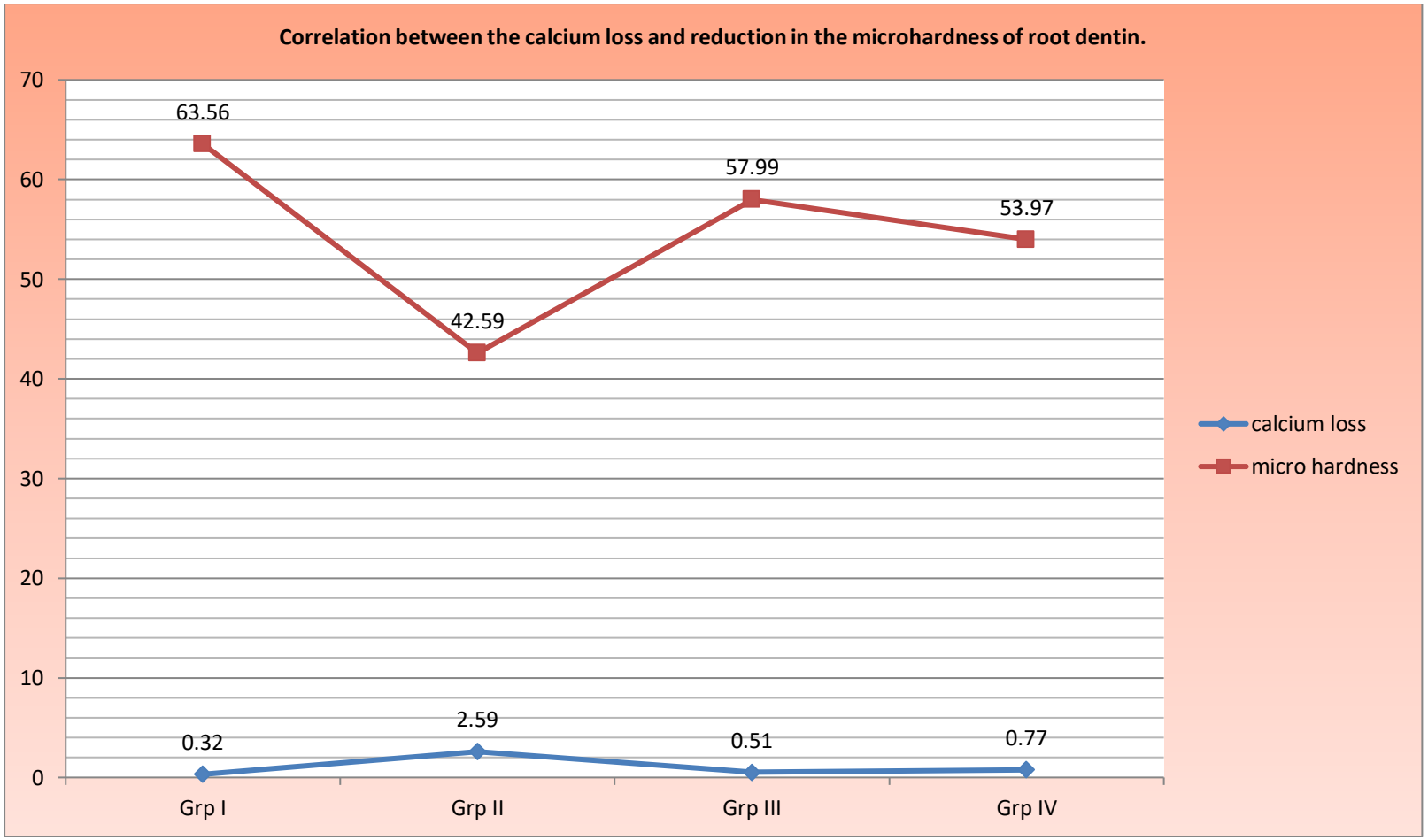
Table 3: Pearson correlation between calcium loss and microhardness



Graph 1: Comparison of different irrigation on calcium loss



Graph 2: Comparison of different irrigation on microhardness of the root dentine



Graph **3 : Correlation between the calcium loss and microhardness of root dentine**

Comparsion of different irrigation on calcium loss was done by one way ANOVA as shown in Table 1 & Graph 1. (P <0.05 considered as significant). Statistically significant difference was seen between all the groups.

Intergroup comparison for calcium loss was done by *Post-hoc* Tukey test as shown in Table 1a. (The mean difference is significant at the 0.05 level).

While comparing the groups,

1. Group I (control), with group II, III, IV/ Experimental groups (17% EDTA, 0.2% chitosan and 0.5% chitosan), the results showed stastistically significant difference between group II and IV (17% EDTA and 0.5% chitosan) but not stastistically significant difference was seen in group III (0.2% chitosan).
2. Group II (17% EDTA) with group I, III, IV (control, 0.2% chitosan and 0.5% chitosan), stastistically significant difference was seen between group I, III, IV (17% EDTA, 0.2% chitosan and 0.5% chitosan).
3. Group III (0.2% chitosan) with remaining groups (control, 17% EDTA and 0.5% chitosan), stastistically significant difference with 17% EDTA and control group but not stastistically significant difference with 0.5% chitosan.
4. Group IV (0.5% chitosan) with remaining groups (control, 17% EDTA and 0.2% chitosan), stastistically significant difference was seen between control and 17% EDTA but not stastistically significant difference with 0.2% chitosan.

Comparison of effect of different irrigation on the micro-hardness of the root dentine was done by one way ANOVA as shown in Table 2 & Graph 2. (*P* <0.05 considered as significant).Statistically significant difference was seen between all the groups.

Intergroup comparison for micro-hardness of root dentin using *Post-hoc* Tukey test were shown in Table 2a (The mean difference is significant at the 0.05 level).

While comparing the groups,

1. Group I (control) with Experimental groups (17% EDTA, 0.2% chitosan and 0.5% chitosan), the results showed stastistically significant difference between all the groups.
2. Group 2 (17% EDTA) with remaining groups (control, 0.2% chitosan and 0.5% chitosan), stastistically significant difference was seen between all the groups
3. Group 3 (0.2% chitosan) with remaining groups (control, 17% EDTA and 0.5% chitosan),stastistically significant difference between all the groups was noticed.
4. Group 4 (0.5% chitosan) with remaining groups (control, 17% EDTA and 0.2% chitosan), they showed stastistically significant difference between all the groups.

Pearson correlation between calcium loss and microhardness shown in Table 3 & Graph 3. [.** correlation is significant at the 0.001 level (2-tailed)]. A strong negative correlation existed between the calcium loss and reduction in the microhardness of root dentine ($r = -0.861$) which was found to be highly significant ($p < 0.001$).

DISCUSSION

DISCUSSION

Dentine consists of organic and inorganic contents. The inorganic content consists of hydroxyapatite crystals. These hydroxyapatite crystals consists of calcium and phosphorus and its ratio about approximately 1.67. Calcium and phosphorus ratio in hydroxyappatite crystals are determined by many factors like crystals type, mineralization level, tissue age and its anatomic site [1].

In root canal treatment, chemicomechanical preparation plays an important role in removing the necrotic debris, pulpal remnants and also for the removal of smear layer. Instruments were used to remove the contents physically where as the, irrigating solutions helps in flushing of loosened debris and also dissolves these contents from inaccessible areas of complex root canal system [39].

Sodium hypochlorite acts upon the organic component and chelating agents acts on inorganic component of dentine. These irrigating solutions may causes changes in the surface morphology of dentin which in turn affects the physical, chemical and mechanical properties of dentine. Changes in Ca/P ratio after the chemical alteration by using the irrigating solutions change the composition of organic and inorganic part of dentine which in turn leads to reduction in microhardness and also changes permeability and solubility of dentine [2].

There is a positive correlation exists between mineral content and microhardness of dentine. So microhardness evaluation helps us to determine the mineral gain or loss in dentin which is the indirect evidence [10].

As we known from previous studies, it has been concluded that 17% EDTA and 15% citric acid had extracted maximum amount of calcium when compared with 5.25% NaOCl [24]. On comparing the effect of 17% EDTA with QMIX , tamarindus indica, tea tree oil, 17% EDTA

had more microhardness reduction[28]. In comparing the solution and gel form of 15% EDTA with 10% citric acid and 5% maleic acid, 10% citric acid showed more microhardness reduction than 5% maleic acid [6]. On comparing the different irrigating agents like 17% EDTA, Bio Pure MTAD, NaOCl and CHX , it has been proven that 17% EDTA had more microhardness reduction [3,10].

Even the chelating agents when used with lasers and surface modifiers, 17% EDTA with laser had more reduction in microhardness when compared with ultrasonic agitation, surface modifiers doesn't have any effect on dentine microhardness reduction[5,34]. In contradictory study, by Flavia emi razera et al, more microhardness reduction in citric acid and 1% peracetic acid than 17% EDTA [31].

From the previous studies, it has been concluded that 17% EDTA had maximum calcium loss and maximum microhardness reduction by increasing the time of immersion.It has been proved that EDTA most commonly used chelating agents had more microhardness reduction and more calcium loss from root dentin and also causes dentin erosion [12,38] .

Chitosan is naturally available polysaccharide, used at the lower concentration of 0.2% and 0.5% , removes the smear layer as effectively as 17% EDTA. Some authors suggested that it causes less dentinal erosion and its more biocompatible with less alteration of dentine microhardness at the 0.2% concentration when compared with 17% EDTA [40]. Its used in the many fields of dentistry because of its anti-bacterial and anti- fungal properties [19].

While comparing the effect of 17% EDTA, Etidonic acid, phytic acid and 0.2% chitosan on dentine microhardness,17% EDTA showed maximum reduction in dentin microhardness when compared with 0.2% chitosan [27,30].

Gusiyska A et al, compared the smear layer removal and dentine erosion of 0.6% chitosan-citrate and 17% EDTA, showed that smear layer removal between 0.6% chitosan-citrate and 17% EDTA were similar but dentinal erosion by 17% EDTA were significantly higher when compared with 0.6% chitosan- citrate [17].

Some of the contradictory studies, stated that 17% EDTA and 0.2% chitosan showed more microhardness reduction in root dentine when compared with 3% NaOCl [29]. Root canal demineralization were higher in 15% EDTA and 0.2% Chitosan when compared with 10% Citric acid and 1% acetic acid [16].

There are different methods in evaluation of demineralization by different irrigating solutions like flame photometry, atomic adsorption spectrometry, complexometric titration with EDTA, energy dispersive spectrometer, scanning electron microscope, Fourier Transform Infrared (FTIR), Inductively coupled plasma-atomic emission spectroscopy (ICP-AES) [23].

In this study, we have used Inductively Coupled Plasma - Mass Spectroscopy (ICP-MS) to evaluated the demineralization effect of different chelating agents and to measure calcium concentration in the irrigating solution after irrigation with samples. This techniques expressed the calcium content in parts per billion (ppb). It is a type of emission spectroscopy that uses the inductively coupled plasma, produces excited atoms and ions which emit electromagnetic radiation at wavelengths that is characteristic of a particular element. This is more advanced technique than Atomic absorption spectroscopy [25].

In our study, Microhardness reduction of root dentine after irrigation with treatment groups were evaluated by Vickers Microhardness tester. This gives the evidence of loss of minerals indirectly.

About to our knowledge, there is no study compared the 17% EDTA, commonly used chelating agent with different concentration of Chitosan on calcium loss and microhardness of root dentine.

In comparing the different concentration of chitosan i.e, 0.2% and 0.5% chitosan on smear layer removal and surface roughness with 17% EDTA , it has been proven that no significant difference in different concentration of chitosan for smear layer removal but the surface alteration was more in 17% EDTA [40]. In our study, the different concentration of chitosan on calcium loss and its effect on microhardness with 17% EDTA was evaluated.

Our results showed that calcium loss by 17% EDTA group was more when compared with NaOCl,0.2% chitosan and 0.5% chitosan. There was no significant difference between different concentration of chitosan in calcium loss. In Microhardness reduction,17% EDTA showed more reduction in comparing with different concentration of chitosan.

Negative correlation exists between calcium loss and microhardness of root dentine. When the calcium loss increased, microhardness of root dentine was reduced. Results of present study stated that, there is no significant difference in calcium loss between different concentration of chitosan but there is significant difference in microhardness reduction between different concentration of chitosan. This may be due to other mineral ion loss like phosphorus and magnesium which is also the part of the inorganic portion of dentin [20].

In present study, we have evaluated only the calcium loss and it's effect on dentine microhardness by the different chelating agents. Further studies are needed to evaluate the other mineral ion loss and its correlation with properties of root dentine.

SUMMARY

SUMMARY

The present study was conducted in the Department of Conservative Dentistry and Endodontics, KSRIDSR which has been approved from Instiututional review board. Thirty single rooted teeth were taken. They were decoronated, coronal third of the root were taken and divided longitudinally, so that totally sixty samples. They were divided based on the irrigating solutions used (n=15). Irrigating solutions were 17% EDTA, 0.2% and 0.5% chitosan. After immersion in the respective irrigating solution for particular period of time, the irrigated solutions were collected and centrifuged, 10ml of total elute were subjected to Integrated plasma mass spectrometry (ICP-MS) to evaluated the calcium loss. The same teeth samples were subjected to Vickers Hardness tester to evaluate the microhardness.

The findings of the present study was summarized as follows

1. There was statistically significant difference in calcium loss between 17% EDTA and different concentration of chitosan
2. There was no statistically significant difference in calcium loss between the concentration of chitosan
3. There was statistically significant difference between different concentration of chitosan in microhardness reduction.
4. Negative correlation exists between calcium loss and microhardness of root dentine.

CONCLUSION

CONCLUSION

The following inference has been derived from this study.

- 17% EDTA showed more calcium loss and more microhardness reduction when compared with 0.2% and 0.5% chitosan.
- In calcium loss, no significant difference between different concentration of chitosan, whereas in microhardness reduction, the difference exists in the concentrations.
- Chitosan is better alternative chelating agent with less calcium loss and microhardness reduction when compared with most commonly used chelating agent 17% EDTA.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Sonali Taneja, Manju Kumari, Surbhi Anand. Effect of QMix, peracetic acid and ethylenediaminetetraacetic acid on calcium loss and microhardness of root dentine. Journal of Conservative Dentistry. 2014;17(2):155-158.
2. Taner Cem Sayin, DDS, PhD, Ahmet Serper, DDS, PhD, Zafer C. Cehreli, DDS, PhD, and Harika G. Otlu, BSc. The effect of EDTA, EGTA, EDTAC, and Tetracycline-HCL with and without subsequent NaOCl treatment on the microhardness of root canal dentin. OOOOE 2007;104:418-424.
3. Soha F. Massoud BDS, Sybel M. Moussa PhD, Seham A. Hanafy PhD, Rania M. El Backly PhD. Evaluation of the microhardness of root canal dentin after different irrigation protocols (in vitro study). Alexandria Dental Journal. 2017;42:73-79.
4. Luciane Dias Oliverira, MD, PhD, Claudio Antonio Talge Carvalho, MD, PhD,Willian Nunes, Marcia Carneiro Valera, MD, PhD, Carlos Henrique Ribeiro Camargo, MD, PhD, and Antonio Olavo Cardoso Jorge, MD, PhD. Effect of chlorhexidine and sodium hypochlorite on the microhardness of root canal dentin. OOOOE. 2007;104:125-128.
5. Eda E. Aslantas, DDS, Hatice Dogan Buzoglu, DDS, PhD, Emre Altundasar, DDS, PhD, and Ahmet Serper, DDS, PhD. Effect of EDTA, Sodium Hypochlorite, and Chlorhexidine Gluconate with or without Surface Modifiers on Dentin Microhardness. J Endod. 2014;40(6):876-9.
6. Vasundhara Shivanna, Sharil Pottanchaliyil. Microhardness test to evaluate the effect of chelating agents on the superficial layer of root canal lumen dentin. CODS Journal of dentistry. 2016; 8(2):104-107.
7. M. Haapasalo, Y. Shen,1 Z. Wang and Y. Gao. Irrigation in endodontics. British Dental Journal. 2014; 216:299-303.

8. Charu Dayal, Girija S. Sajjan, T.S. Subhash. An in-vitro evaluation of microhardness of root dentin prepared with different file types with 2.5% sodium hypochlorite. Endodontology. 2007;19(1):23-29.
9. Alexandre Corrêa Ghisi , Patrícia Maria Poli Kopper, Flávia E. R. Baldasso, Caroline P. Stürmer, Giampiero Rossi-Fedele, Liviu Steier, José Antonio Poli de Figueiredo, Renata Dornelles Morgental, Fabiana Vieira VierPelisser. Effect of Super-Oxidized Water, Sodium Hypochlorite and EDTA on Dentin Microhardness. Brazilian Dental Journal. 2014;25(5):420-424.
10. Sandeep Singh, Shashi Rashmi Acharya, Vasudev Ballal, Rijesh M. "Evaluation of the effect of EDTA, EDTAC, RC-Prep and BioPure MTAD on the microhardness of root canal dentine An in vitro study". Endodontology. 2009;21(2):33-39.
11. Zahed Mohammadi, Sousan Shalavi, Hamid Jafarzadeh. Ethylenediaminetetraacetic acid in endodontics. European Journal of Dentistry. 2013;7:135-142.
12. Polliana Vilaça Silva, Débora Fernandes Costa Guedes, Jesus Djalma Pécora, Antonio Miranda Da Cruz-Filho. Time-Dependent Effects of Chitosan on Dentin Structures. Braz Dent J. 2012;23(4): 357-361.
13. Hagar A. El Naby Bastawy and Rania Ezzat. Impact of Chitosan as Chelating Agent on Microhardness and Mineral Content of Intraradicular Dentin. Al-Azhar Dental Journal. 2016;3(1):1-10.
14. Kamakshi G , Nithin Suvarna, Harish Kumar Shetty, Jaishri Khed. Relation between Calcium loss and its effect on Microhardness of Root canal dentin following treatment with 17% Ethylene Diamine Tetraacetic acid (EDTA) at Different Time Intervals: An Ex-Vivo Study. Journal of International Medicine and Dentistry. 2014;1(2):75-85.
15. Carmen-María Ferrer-Luque , Mercedes Perez-Heredia , Pilar Baca , María-Teresa Arias-Moliz, MaríaPaloma González-Rodríguez. Decalcifying effects of antimicrobial

irrigating solutions on root canal dentin. Med Oral Patol Oral Cir Bucal. 2013;18 (1):e158-61.

16. P. V. Silva , D. F. C. Guede , F. V. Nakadi, J. D. Pe´ cora & A. M. Cruz-Filho. Chitosan: a new solution for removal of smear layer after root canal instrumentation. International Endodontic Journal. 2013;(46):332–338.
17. Gusiyska A, Dyulgerova E , Vassileva R , Gyulbenkiyan E. The Effectiveness of a Chitosan-Citrate Solution to Remove the Smear Layer in Root Canal Treatment An in-vitro study. International Journal of Science and Research (IJSR). 2016;5:1169-1174.
18. Paôla Caroline da Silva Mira, Luis Eduardo Souza-Flamini and Antonio Miranda Da Cruz-Filho. Evaluation of the chelating effect of chitosan solubilized in different acids. Journal of Conservative Dentistry. 2017;20(5):297-301.
19. Natasha jaiswal, Dakshita-Joy Sinha, Udai-Pratap Singh, Kanwardeep Singh, Urja-Ahuja Jandial, Shivika Goel. Evaluation of antibacterial efficacy of chitosan, chlorhexidine, propolis and sodium hypochlorite on Enterococcus faecalis bioflim: An invitro study. J Clin Exp Dent. 2017;9(9):1066-1074.
20. Brenna Magdalena Lima Nogueira , Thais Isabele da Costa Pereira , Victor Feliz Pedrinha , Patrícia de Almeida Rodrigues. Effects of Different Irrigation Solutions and Protocols on Mineral Content and Ultrastructure of Root Canal Dentine. Iranian Endodontic Journal. 2018;13(2):209-215.
21. Reem Adel Abd-Elgawad and Dalia Mukhtar Fayyad. Comparative Evaluation of Smear Layer Removal, Calcium Ions Loss and Dentin Microhardness after Different Final Irrigation Solutions. Egyptian Dental Journal. 2017;63. (https://www.researchgate.net/publication/327824965).

22. HM Bayram, E Bayram , O Atakol. Atomic absorption spectrometry evaluation of calcium ion release from different calcium silicate-based endodontic materials used with new irrigants. Biomed Res- India. 2016:S333-S336.
23. Atul Jain , Pallavi Waghmare , Padma.Gandi , Neeraj Nigam. Comparative Evaluation of Calcium Ion Loss and Microhardness using Different Irrigants - An In Vitro Study. International Journal of Oral Health and Medical Research. 2016;(3)2:13-16.
24. Keyur Pankaj Chande, Narendra U Manwar, Manoj G Chandak, Jyoti Lokade, Sneha R Chandak. Effect of Chelating Agents and Irrigants on Mineral Content of Root Canal Dentin: An In Vitro Study. International Journal of Clinical Preventive Dentistry. 2014;(10)3:135-138.
25. Dr. Lora Mishra , Dr. Manoj Kumar , Dr. C.V. Subba Rao. Calcium loss from root canal dentin following EDTA and Tetracycline HCl Treatment with or without subsequent NaOCl irrigation and evaluation of microhardness of dentine. International Journal of Advancements in Research & Technology. 2012;1(2):45-50
26. L. F. Machado-Silveiro , S. Gonza´lez-Lo´ pez & M. P. Gonza´lez-Rodrı´guez. Decalcification of root canal dentine by citric acid, EDTA and sodium citrate. International Endodontic Journal. 2004;(37):365–369.
27. Srinidhi surya raghavendra, Ajit hindlehar, Nishant vyavahare, Effect of etridronic acid, chitosan and EDTA on microhardness of root canal dentin. Saudi J. Oral. Dent. Res. 2018;3(4):118-121.
28. Tenzin Rapgay, Preeti Gupta, Hitesh Gupta, Arvinder Singh and Anjula Jain. Effect of Different Irrigant Solutions on Root Dentine Microhardness: An In Vitro Study. Acta Scientific Dental Sciences. 2018;(2)8:3-8.
29. Suparna Ganguly SAHA , Vini Sharma , Anuj Bharadwaj , Poorvi Shrivastava , Mainak Kanti Saha , Sandeep Dubey , Shubham Kala , Shubhra Gupta. Effectiveness

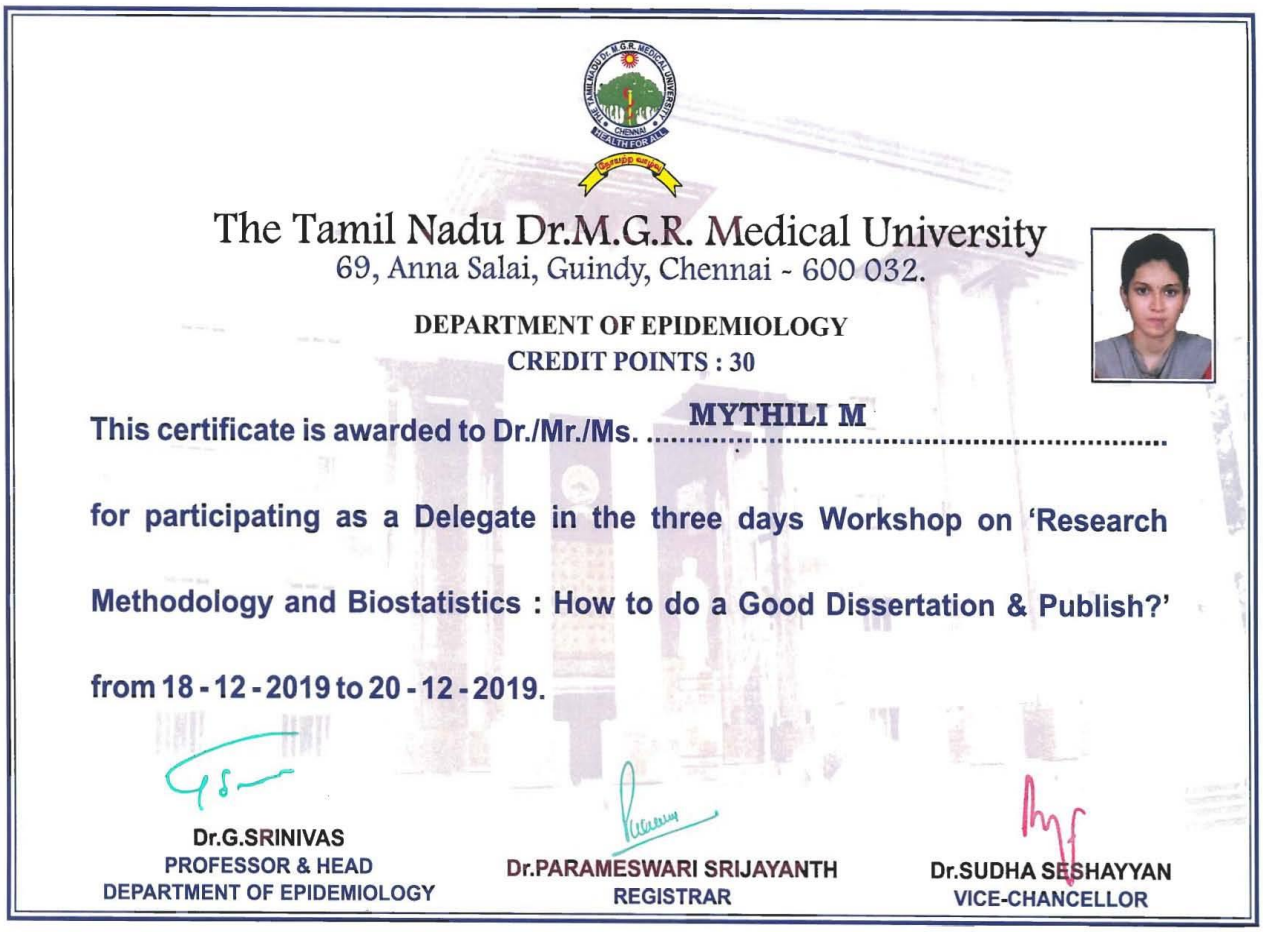
of Various Endodontic Irrigants on the Micro-Hardness of the Root Canal Dentin: An in vitro Study. Journal of Clinical and Diagnostic Research. 2017;11(4): ZC01-ZC04.

30. Vineeta Nikhil, Shikha Jaiswal, Parul Bansal, Rohit Arora, Shalya Raj, Pulkit Malhotra. Effect of phytic acid, ethylenediaminetetraacetic acid, and chitosan solutions on microhardness of the human radicular dentin. Journal of Conservative Dentistry. 2016;19(2):179-183.
31. Flávia Emi Razera Baldasso, Luana Roleto, Vinicius Duval Da Silva, Renata Dornelles Morgentan, Patrícia Maria Poli Kopper. Effect of final irrigation protocols on microhardness reduction and erosion of root canal dentin. Braz. Oral Res. 2017;31:e40.
32. Bhavana Gandhi, Swetha Bollineni, Ravi Kumar Janga, Disha Saraswati, Mandava Ramesh Babu. Evaluating the Effect of CPP-ACP as a Final Irrigant in Improving the MicroHardness of Erosive Root Dentin and its influence on the Bond Strength of Self Etch Resin Sealer – An In-vitro Study. Journal of Clinical and Diagnostic Research. 2016;10(8): ZC53-ZC56.
33. Anusree Das, Jojo Kottoor, Joy Mathew, Sanjana Kumar, and Saira George. Dentine microhardness changes following conventional and alternate irrigation regimens: An in vitro study. Journal of Conservative Dentistry. 2014;17(6):546-549.
34. Hakan Arslan, Kübra Y. Yeter, Ertugrul Karatas, Cenk B. Yilmaz, Leyla B. Ayranci, Damla Ozsu. Effect of agitation of EDTA with 808-nm diode laser on dentin microhardness. Lasers Med Sci. 2015;30(2):599-604.
35. Marta Barón, Carmen Llena, Leopoldo Forner, María Palomares, Cristina González-García, Manuel Salmerón-Sánchez. Nanostructural changes in dentine caused by endodontic irrigants. Med Oral Patol Oral Cir Bucal. 2013;18(4):e733-6.

36. Talita Tartari, Patrícia de Almeida Rodrigues Silva e Souza, Bruno Vila Nova de Almeida, José Otávio Carrera Silva Júnior, Oscar Facíola Pessoa and Mario Honorato Silva e Souza Junior. A New Weak Chelator in Endodontics: Effects of Different Irrigation Regimens with Etidronate on Root Dentin Microhardness. International Journal of Dentistry.2013. DOI :10.1155/2013/743018.
37. Chetan R Patil, Veerendra Uppin. Effect of endodontic irrigating solutions on the microhardness and roughness of root canal dentin: An in vitro study. Indian Journal of Dental Research. 2011;22(1):22-27.
38. Deepa Natesan Thangaraj, Vasudev Ballal, Shashi Rashmi Acharya. Determination of calcium loss and its effect on microhardness of root canal dentin following treatment with 17% ethylenediaminetetraacetic acid solution at different time intervals - An in vitro study. Endodontology. 2009;21(1):7-13.
39. Li-sha Gu, DDS, MS, Jong Ryul Kim, DMD, PhD, Junqi Ling, DDS, PhD, Kyung Kyu Choi, DMD, PhD, David H. Pashley, DMD, PhD and Franklin R. Tay, BDSc (Hons), PhD. Review of Contemporary Irrigant Agitation Techniques and Devices. J Endod. 2009;35(6):791-804.
40. Sijo Pazhayahottathil Mathew, Veena suresh Pai, G. Usha, Roopa R. Nadig. Comparative evaluation of smear layer removal by chitosan and ethylenediaminetetraacetic acid when used as irrigant and its effect on root dentine: An in vitro atomic force microscopic and energy-dispersive X-ray analysis. Journal of Conservative Dentistry. 2017;20(4):245251.

ANNEXURE

APPENDIX-I



The Tamil Nadu Dr.M.G.R. Medical University
69, Anna Salai, Guindy, Chennai - 600 032.

DEPARTMENT OF EPIDEMIOLOGY
CREDIT POINTS : 30

This certificate is awarded to Dr./Mr./Ms. **MYTHILI M**

for participating as a Delegate in the three days Workshop on 'Research Methodology and Biostatistics : How to do a Good Dissertation & Publish?'

from 18 - 12 - 2019 to 20 - 12 - 2019.

Dr.G.SRINIVAS
PROFESSOR & HEAD
DEPARTMENT OF EPIDEMIOLOGY

Dr.PARAMESWARI SRIJAYANTH
REGISTRAR

Dr.SUDHA SESHAYYAN
VICE-CHANCELLOR

APPENDIX-II

INSTITUTIONAL ETHICAL COMMITTEE

KSR INSTITUTE OF DENTAL SCIENCE & RESEARCH

KSR Kalvi Nagar, Tiruchengode-637 215, Tamilnadu.
Phone : 04288-274981, Fax : 04288-274761,
email : ksrdentalcollege@yahoo.com

Chairman
Dr. PHILIP ROBINSON, Ph.D
Prof. & Head Dept. of Biotechnology
KSR College of Technology,
KSR Kalvi Nagar, Tiruchengode.

Member Secretary
Dr. G.S. KUMAR, MDS.,
Principal,
KSR Institute of Dental Science & Research,
KSR Kalvi Nagar, Tiruchengode.

Members

Dr.G.Ayppadasan,Ph.D.,
Biotechnologist

Mr.A.Thirumoorthi,M.A.B.L.,
Human Activist

Dr.R.Renuka,M.D.S.,(Perio),M.Sc.,
Family Counsellor

Dr.M.Rajmohan, MDS,(Oral Path)

Dr.R.Prakash, MDS,(PHD)

Dr.Suman, M.D.S.,(OMDR)

Dr.Sharath Ashokan,MDS.,(Pedo)

Dr.G.Rajeswari,Ph.D.,(Biochemistry)

Dr.K.Karthick,MDS.,(Cons.Dent.)

Mr.V.Mohan,M.Sc.,M.Phil.,(Physicist)

Mr.A.P.S.Raja,B.A.,
(Layperson)

Ref.: 187 /KSRIDSR/EC/2017 **Date : 18.12.2017**

To

Dr.M.Mythili,
Postgraduate Student,
Dept. of Conservative Dentistry & Endodontics,
KSR Institute of Dental Science & Research,

*****

Your dissertational study titled "EFFECT OF DIFFERENT CONCENTRATION OF CHITOSAN ON CALCIUM LOSS AND MICROHARDNESS IN ROOT DENTINE – AN INVITRO STUDY" presented before the ethical committee on 14th Dec. 2017 has been discussed by the committee members and has been approved.

You are requested to adhere to the ICMR guidelines on Biomedical Research and follow good clinical practice. You are requested to inform the progress of work from time to time and submit a final report on the completion of study.

Signature of the Chairman
(Dr. Philip Robinson)

APPENDIX-III

URKUND

Urkund Analysis Result

Analysed Document: thesis plagiarism checking.docx (D61994348)
Submitted: 08/01/2020 05:37:00
Submitted By: mythilim4740@gmail.com
Significance: 18 %

Sources included in the report:

plagiarism.docx (D40507841)
corrected urkund thesis.docx (D45657051)
article cons..docx (D58362992)
9431 - Smear Layer Removal And Microhardness Alteration Potential Of A Naturally Occ urring Antioxidant – An Invitro Study.docx (D54488201)
Dissertation-Dr.Gorakh Beble-Dental.pdf (D44721670)
FINAL THESIS.pdf (D34337635)
plagarism.docx (D46366255)
ELANGO THESIS plagiarism.docx (D47679079)
https://www.researchgate.net/publication/328890927_The_Effects_of_Two_Root_Canal_Irrigants_and_Different_Instruments_on_Dentin_Microhardness_In_Vitro_Study
https://www.researchgate.net/publication/267103176_Effect_of_QMix_irrigant_on_the_microhardness_of_root_canal_dentine
https://www.researchgate.net/publication/322763378_Impact_of_Chitosan_as_Chelating_Agent_on_Microhardness_and_Mineral_Content_of_Intraradicular_Dentin
https://www.researchgate.net/publication/261957132_Effect_of_QMix_peracetic_acid_and_ethylenediaminetetraacetic_acid_on_calcium_loss_and_microhardness_of_root_dentine
https://go.gale.com/ps/i.do?id=GALE%7CA447033872&sid=googleScholar&v=2.1&it=r&linkaccess=abs&issn=09720707&p=HRCA&sw=w
https://selfdecode.com/chemical/sodium-calcium-edetate/
https://go.gale.com/ps/i.do?id=GALE%7CA360538606&sid=googleScholar&v=2.1&it=r&linkaccess=abs&issn=09720707&p=AONE&sw=w
https://www.researchgate.net/publication/269694146_Dentine_microhardness_changes_following_conventional_and_alternate_irrigation_regimens_An_in_vitro_study

URKUND

https://www.researchgate.net/publication/325296458_Effect_of_Etidronic_Acid_Chitosan_and_EDTA_on_Microhardness_of_Root_Canal_Dentin
https://www.researchgate.net/publication/301278277_Comparison_of_the_effect_of_ethylenediamine_tetra-acetic_acid_chlorhexidine_etidronic_acid_and_propolis_as_an_irrigant_on_the_microhardness_of_root_dentin_An_in_vitro_study
https://www.researchgate.net/publication/49844224_Effect_of_Chelating_Solutions_on_the_Microhardness_of_Root_Canal_Lumen_Dentin
https://www.researchgate.net/publication/24396905_Atomic_Absorption_Spectrometry_and_Scanning_Electron_Microscopy_Evaluation_of_Concentration_of_Calcium_Ions_and_Smear_Layer_Removal_With_Root_Canal_Chelators
https://www.researchgate.net/publication/327824965_Comparative_Evaluation_of_Smear_Layer_Removal_Calcium_Ions_Loss_and_Dentin_Microhardness_after_Different_Final_Irrigation_Solutions
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4001273/
https://www.researchgate.net/publication/10568880_Efficacy_of_various_concentrations_of_citric_acid_at_different_pH_values_for_smear_layer_removal
https://www.researchgate.net/publication/10826868_A_New_Solution_for_the_Removal_of_the_Smear_Layer

Instances where selected sources appear:

62

APPENDIX-IV

CERTIFICATE-II

This is to certify that this dissertation work titled **EFFECT OF DIFFERENT CONCENTRATION OF CHITOSAN ON CALCIUM LOSS AND MICROHARDNESS IN ROOT DENTINE – AN INVITRO STUDY** of the candidate **Dr.MYTHILI.M** with registration number **241717403** for the award of **Master of Dental Surgery** in the branch of **Conservative Dentistry and Endodontics** has been checked for plagiarism. I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion pages and result show **18%** percentage of plagiarism in the dissertation.

Deepa N7. 24/1/2020

Guide & supervisor sign with seal.

DEPARTMENT OF CONSERVATIVE
AND ENDODONTICS,
K.S.R. INSTITUTE OF DENTAL
SCIENCE & RESEARCH,
K.S.R. KALVI NAGAR,
THOKKAVADI POST
TIRUCHENGODE-637209