"COMPARATIVE EVALUATION OF THE EFFECT OF TRIPLE ANTIBIOTIC PASTE AND DOUBLE ANTIBIOTIC PASTE WITH CHITOSAN AS THE VEHICLE ON THE PUSH OUT BOND STRENGTH OF EPOXY RESIN–BASED SEALERS TO THE RADICULAR DENTIN –AN INVITRO STUDY."

By

Dr. ASHISH RAMAKRISHNAN

REG.NO. IE0218001

Dissertation

Submitted to
KLE Academy of Higher Education and Research
In partial fulfillment
Of the requirements for the degree of

MASTER OF DENTAL SURGERY

In

CONSERVATIVE DENTISTRY AND ENDODONTICS

(BRANCH – IV)

Under the Guidance of

Dr. ANAND C PATIL M.D.S

DEPARTMENT OF CONSERVATIVE DENTISTRY AND ENDODONTICS
KAHER VK INSTITUTE OF DENTAL SCIENCES,
BELAGAVI, KARNATAKA

2018-2021

KLE ACADEMY OF HIGHER EDUCATION & RESEARCH ,

BELAGAVI KARNATAKA

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation/thesis entitled **"COMPARATIVE EVALUATION OF THE EFFECT OF TRIPLE ANTIBIOTIC PASTE AND DOUBLE ANTIBIOTIC PASTE WITH CHITOSAN AS THE VEHICLE ON THE PUSH OUT BOND STRENGTH OF EPOXY RESIN–BASED SEALERS TO THE RADICULAR DENTIN –AN INVITRO STUDY."** is a bonafide and genuine research work carried out by me under the guidance of **Dr. ANAND C PATIL** **M.D.S** Professor, Department of Conservative Dentistry and Endodontics, KAHER Vishwanath Katti Institute of Dental Sciences, Belagavi.

Date :

Place : Belagavi

Dr. ASHISH RAMAKRISHNAN

REG.NO. IE0218001

KLE ACADEMY OF HIGHER EDUCATION & RESEARCH ,

BELAGAVI KARNATAKA

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"COMPARATIVE EVALUATION OF THE EFFECT OF TRIPLE ANTIBIOTIC PASTE AND DOUBLE ANTIBIOTIC PASTE WITH CHITOSAN AS THE VEHICLE ON THE PUSH OUT BOND STRENGTH OF EPOXY RESIN–BASED SEALERS TO THE RADICULAR DENTIN – AN INVITRO STUDY."** is a bonafide research work done by **Dr. ASHISH RAMAKRISHNAN,** post-graduate student in partial fulfillment of the requirement for the degree of **MASTERS IN DENTAL SURGERY** in Conservative Dentistry and Endodontics.

Date:

Place: Belagavi

Dr. ANAND C PATIL **M.D.S**
Professor
Department of Conservative Dentistry
and Endodontics,
KAHER V.K. Institute of Dental Sciences,
Belagavi.

KLE ACADEMY OF HIGHER EDUCATION & RESEARCH ,

BELAGAVI KARNATAKA

ENDORSEMENT BY THE HEAD OF DEPARTMENT, PRINCIPAL OF THE INSTITUTION

This is to certify that the dissertation entitled **"COMPARATIVE EVALUATION OF THE EFFECT OF TRIPLE ANTIBIOTIC PASTE AND DOUBLE ANTIBIOTIC PASTE WITH CHITOSAN AS THE VEHICLE ON THE PUSH OUT BOND STRENGTH OF EPOXY RESIN–BASED SEALERS TO THE RADICULAR DENTIN – AN INVITRO STUDY."** is a bonafide research work done by **Dr. ASHIISH RAMAKRISHNAN**, post graduate student under the guidance of **Dr. ANAND C PATIL** M.D.S Professor, Department of Conservative Dentistry and Endodontics, KAHER Vishwanath Katti Institute of Dental Sciences, Belagavi.

Head of Department

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

Date:
Place: Belagavi

Principal

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

Date:

Place: Belagavi

KLE ACADEMY OF HIGHER EDUCATION & RESEARCH ,

BELAGAVI KARNATAKA

COPYRIGHT

DECLARATION BY THE CANDIDATE

I hereby declare that ***KLE Academy of Higher Education & Research, Belagavi, Karnataka*** *shall have the rights to preserve, use and disseminate this dissertation/thesis in print or electronic format for academic/research purpose.*

Date :

Place : Belagavi

Dr. ASHISH RAMAKRISHNAN

REG.NO. IE0218001

[Established under section 3 of the UGC Act, 1956 vide GOI. Notification No. F.9-19/2000-U.3(A)]

UNDERTAKING

I, **Dr. ASHISH RAMAKRISHNAN**, hereby declare that the information and data mentioned in my thesis entitled **"Comparative evaluation of the effect of Triple antibiotic paste and Double antibiotic paste with Chitosan as the vehicle on the push out bond strength of Epoxy resin–based sealers to the radicular dentin – An Invitro study."** belongs to me and is original.

I am aware of the definition of plagiarism as detailed below:

- An act or instance of using or closely imitating the language and thoughts of another author without authorization and the representation of that author's work as one's own, as by not crediting the original author.
- A piece of writing or other work reflecting such unauthorized use or imitation.
- The deliberate or reckless representation of another's words, thoughts or ideas as one's own without attribution in connection with submission of academic work, whether graded or otherwise.

I hereby declare that the thesis prepared by me is original and does not involve plagiarism anywhere. In case at later stage it is found that I have indulged in plagiarism, then I am solely responsible for the same and the Institution is at liberty to take any disciplinary action against me including cancellation of dissertation or any other penalties imposed by the University.

Place: Belagavi **Dr. ASHISH RAMAKRISHNAN**

Date: _____ **REG. NO. IE0218001**

This dissertation is dedicated to

ALMIGHTY GOD,

My Parents,

&

My Elder Brother

ACKNOWLEDGEMENT

No endeavour can start, continue and complete without the blessings of the Almighty. At the outset I thank the almighty for always being by my side by bestowing strength and patience to complete the task entrusted. With the completion of my dissertation, I am overcome with a sense of satisfaction and even more gratitude towards all my mentors and well-wishers whom I wish to acknowledge.

I would like to express the deepest appreciation to my honourable teacher and guide, ***Dr. Anand C Patil*** ***M.D.S.,*** *Professor, Department of Conservative Dentistry and Endodontics, KAHER Vishwanath Katti Institute of Dental Sciences, Belagavi. His patience, encouragement, and meticulous supervision at every step along with his valuable suggestions for improvement enabled me to complete my dissertation which also helped me tremendously during my postgraduate tenure.*

With deep gratitude, I thank ***Dr. Preeti Doddwad*** ***M.D.S.,*** *Professor and Head of the Department, Department of Conservative Dentistry and Endodontics, KAHER Vishwanath Katti Institute of Dental Sciences, Belagavi for giving enthusiastic encouragement, consistent support and unequivocal help during my post graduation.*

I am grateful to ***Dr. Alka Kale*** ***M.D.S.,*** *Principal, K.L.E. VK Institute of Dental Sciences, Belagavi for extending her help and co-operation towards the completion of this dissertation.*

I would like to express my solemn gratitude, respect & special debt of thanks to my beloved teachers ***Dr. Sonal Joshi*** ***M.D.S.*** *Professor,* ***Dr. Avinash Patil*** ***M.D.S.*** *Reader,* ***Dr. Sunita Shivanand*** ***M.D.S*** *Reader,* ***Dr. Neha Dhaded*** ***M.D.S*** *Reader,* ***Dr. Shavina Patil*** ***M.D.S*** *Reader,* ***Dr. Suresh Shenvi*** ***M.D.S*** *Senior lecturer,* ***Dr. Sneha***

Patil ***M.D.S*** *Senior Lecturer and* ***Dr. Aniket Chavan*** ***M.D.S*** *Senior lecture who selflessly devoted their time to me with patience whenever needed. Their colossal contribution all through these years will never be history.*

My heartfelt thanks to ***Dr. Niraj Godbole*** ***M.D.S*** *Senior lecturer,* ***Dr. Vaidehi Dhopavkar*** ***M.D.S*** *Senior lecturer,* ***Dr. Mateen Peerzade*** ***M.D.S*** *Senior lecturer and* ***Dr. Olivia Banerjee*** ***M.D.S*** *Senior lecturer for their sincere help and support given to me during this dissertation.*

I would also like to extend my thanks to ***Mr. U.B. Bolmal*** ***M.PHARM.*** *Assistant Professor, Department of Pharmaceutics, KAHER College of Pharmacy in helping with the preparation of Chitosan solution.*

My sincere thanks to ***Mr. Javali,*** *the statistician, for his services in carrying out the statistical analysis.*

I am thankful to ***Mr. Bagalkoti,*** *store Incharge,* ***Mr. Kedar****, Technician,* ***Mrs. Geeta,*** *Mr.* ***Mallappa****, Materials Incharge and all* ***non-teaching staff*** *of my Department for their immense help during the course of my study.*

My sincere thanks to my loving seniors ***Dr. Neha, Dr. Ankita, Dr. Akash, Dr. Suraj and Dr. Pratik*** *for having generously shared insight and for their unwavering support.*

I extend my heartfelt gratitude to my colleagues ***Dr. Felbin, Dr. Deepti, Dr. Chaitra and Dr. Shrutika*** *for their constant support and co-operation .*

I am thankful to my juniors ***Dr. Bhavana and Dr. Rohit*** *for always being helpful and proof reading my dissertation.*

I extend my heartfelt thanks to my junior colleagues ***Dr. Mahima, Dr. Priyanka, Dr. Abhijit, Dr. Shefali, Dr. Aishwarya, Dr. Amruta, Dr. Aishika*** *and* ***Dr. Greeta*** *for their help and co-operation.*

I would also like to thank my friends from the batch of ***SHAURIYANS****,* ***BLACK HAWKS KANNUR*** *and all my school mates for always keeping my morale afloat.*

I am thankful to ***Mr. Anand*** *and* ***Mr. Arun*** *of Shri Vigneshwara Associates, Belagavi for formatting, printing and binding of my dissertation.*

I would not have completed this dissertation without the unconditional support of my family, who have always been there for me whenever I needed them, with encouragement and love to empower me all the time. I owe everything of what I am and to my parents, ***Mr. T. Ramakrishnan*** *and* ***Dr. (Mrs.) Amudha Ramakrishnan*** *and my elder brother* ***Adarsh Ramakrishnan*** *who gave form to all my dreams and aspirations.*

I owe every success and joy to them and I humbly acknowledge that everything I am today is because of their love and support.

Thank you, One and all.

Date:

Place: Belagavi **Dr. Ashish Ramakrishnan**

LIST OF ABBREVIATIONS

SR.NO	ABBREVIATIONS	FULL FORM
1	TAP	Triple Antibiotic Paste
2	CH	Calcium hydroxide
3	PUI	Passive ultrasonic irrigation
4	*et al*	Additional persons involved in the same study
5	*E. faecalis*	*Enterococcus faecalis*
6	CHX	Chlorhexidine
7	Ni-Ti	Nickel Titanium
8	CEJ	Cemento-Enamel Junction
9	SEM	Scanning Electron Microscopy
10	° C	Degrees Celsius
11	MPa	Megapascals
12	mm	Millimeter
13	UTM	Universal Testing Machine
14	PEG	Polyethylene Glycol

15	ANOVA	Analysis of Variance
16	SD	Standard Deviation
17	n	Number of specimens
18	p-value	Probability of obtaining a test statistic at least as extreme as the one that was actually observed
19	WL	Working Length
20	DAP	Double Antibiotic Paste
21	NaOCl	Sodium Hypochlorite
22	EDTA	Ethylene Diamine Tetra-acetic Acid
23	<	Less than
24	>	Greater than

ABSTRACT

Aim and Objectives: To evaluate the effect of Triple Antibiotic Paste and Double Antibiotic Paste with Chitosan as the vehicle on the push out bond strength of Epoxy resin–based sealer to radicular dentin.

Study design: Eighty extracted human mandibular premolar teeth were selected, disinfected and decoronated to obtain a standardized root length of 15 mm. The teeth were prepared with ProTaper Universal rotary files upto F4. The samples were divided into 4 groups of 20 samples each based on the intracanal medicament and vehicle used as follows:

Group 1 – Triple antibiotic paste + Distilled water

Group 2 – Triple antibiotic paste + Chitosan

Group 3 – Double antibiotic paste + Distilled water

Group 4 – Double antibiotic paste + Chitosan

The specimens were incubated at 37°C in 100% humidity for 3 weeks to simulate clinical conditions following which the medicament was removed by irrigation. (10 ml 17% EDTA followed by 10ml 3% NaOCl and 5 ml distilled water as the final irrigant). The roots were then obturated with Gutta-percha points and AH Plus sealer and cut into 2mm thick cross sections in the middle third using a disc. The pushout bond strength was then tested using a Universal testing machine.

Results: A statistically significant difference was seen between all the groups ($p<0.01$). The highest mean push-out bond strength value (5.33 MPa) was seen in Group II (TAP + Chitosan)

Conclusion: Under the limitations of present research, it can be established that TAP showed superior performance when compared to DAP when used as an intracanal medicament in terms of bond strength to radicular dentin and Chitosan when used as the vehicle improves the push–out bond strength of Epoxy resin–based sealer and reduces the negative effects of TAP and DAP on radicular dentin ($p<0.01$).

Key words: TAP, DAP, Chitosan, Pushout bond strength.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF FIGURES

ANNEXURE

INTRODUCTION

Bacteria and their products are considered to be the cause of pulp necrosis and periapical lesions. Bacterial proliferation in the root canal system can negatively impact the outcome of root canal treatment.[1] The success of endodontic therapy depends primarily on mechanical preparation, irrigation, microbial control and complete filling of the root canal system.[2]

Chemomechanical preparation is the principal method for eliminating the microorganisms embedded in the root canals. In a majority of clinical situations, this procedure is adequate to attain success and assure periapical tissue repair.[3] However, in rare circumstances, microorganisms may be able to endure this therapy due to varying factors such as complexity of anatomical structures of the root canal and limited access of instruments and irrigation fluids.

To ensure the complete elimination of root canal bacteria, it is necessary to use effective antibacterial agents for a predetermined period of time to predictably eradicate residual bacteria.[2] Therefore, researchers recommend the use of an intracanal medicament to decrease bacterial counts in the root canals. This transpires because the endodontic system can be inhabited by microorganisms, requiring deeper and more definite therapeutic intervention.

In such cases, intracanal medication is crucial to oppose the remaining infection, in addition to the role of the inflammatory reaction moderator. Facultative anaerobic microorganisms are more tolerant to endodontic treatment than anaerobic microorganisms in a majority of cases. This resistance is related to the ability of microorganisms to remain in a latent phase for a long time, with a low metabolic rate.

Environmental changes, such as coronal leakage, can, however, trigger the activation and consequent proliferation of these microorganisms.[3]

Calcium hydroxide (CH) is widely used as an intracanal medicament because of its antibacterial activity and Chlorhexidine (CHX) is utilized for its beneficial antibacterial activity and low toxicity.[1]

Currently, the common antibiotic-containing commercial pastes such as Ledermix and Septomixine Forte have corticosteroids as anti-inflammatory agents. However, due to their insufficient range of action, none of these pastes can be considered as ideal for use against endodontic microbiota.[4]

Triple Antibiotic paste (TAP) is a new material introduced by Hoshino and colleagues, used to disinfect necrotic root canals in immature teeth. It is a mixture of three antibiotics: Ciprofloxacin, Minocycline and Metronidazole. The published literature indicates that when used as an intracanal medicament, it is a suitable disinfectant. Moreover, *in vivo* and *in vitro* tests have shown that this blend of antibiotics is effective in eliminating endodontic pathogens.[1]

Metronidazole is toxic to anaerobes and is considered an antimicrobial agent against anaerobic bacteria. Ciprofloxacin exhibits high antimicrobial activity against gram-negative bacteria. Minocycline has a bacteriostatic effect and is active against both Gram-positive and Gram-negative bacteria.[5]

However, studies have revealed that Minocycline has potential to cause visible crown discoloration and therefore, was removed in a Double Antibiotic Paste (DAP) which consists of only Ciprofloxacin and Metronidazole.[5] Minocycline has been found to chelate calcium and demineralize dental hard tissues.[6] In addition to the

undesired discoloration, another key issue encountered while using antibiotic pastes is that complete removal of the pastes from the root canal is not possible, which could affect the adhesion of endodontic sealers to the root canal dentinal walls.[7] Sealer adhesion to root canal dentin by close contact is essential to resist micromechanical forces during endodontic therapy.[8]

The selection of vehicles related to intracanal medicament is of supreme importance, because they are responsible for the delivery of the drug inside the root canal system and for its diffusion.[9] An optimal vehicle for antibiotic delivery in the root canal should be capable of facilitating a successful transmission of the medication through dentinal tubules, should have its own antimicrobial effects and should not have any adverse effects on the chemical composition of radicular dentin. Various vehicles have been used for placing TAP. Macrogol and Propylene glycol (PEG) were used by Hoshino et al for delivery of TAP. PEG demonstrates antimicrobial effect and hence, the Ciprofloxacin/PEG association had the highest efficacy against the tested bacteria and yeast. However, PEG possesses low order of toxicity.[10,11,12]

Recently, a newer novel vehicle Chitosan has been extensively studied. Chitosan can be used as a drug carrier to increase the stability and insolubility of the intracanal medicament, where it has the additional advantage of slow controlled release of the medicament. Chitosan is a macromolecule produced by the repetition of D-glucosamine, which is extracted from the deacetylation of Chitin, obtained from the shells of marine crustaceans (particularly from crabs and prawns).

Chitosan is a fiber, chemically similar to cellulose and is indigestible. It is a safe, biocompatible, biodegradable, non-toxic polysaccharide with proven antibacterial properties and ability to form film and gel. It has been used in drug delivery, as an absorption enhancer. Chitosan can also form chemical bonds with collagen and is able to reinforce the dentinal collagen.[13,14]

Several *in vitro* experiments have shown that the vehicle used has a strong association with the ionic release concentration and velocity as well as the antibacterial behavior when the paste is taken into a contaminated environment.[15,16] Fava and Saunders concluded that the physical and chemical properties of the intracanal medicament and thus its therapeutic applications are influenced by the vehicle with which it is mixed.[17]

AH-Plus is considered to be the gold standard material for root filling because it has been successfully used for many years, and it has advantages in adaptability and adhesive strength compared to other materials.[18] It is an epoxy-based sealer which is used as a standard in several bond strength studies. Epoxy-based sealers may be accounted for, by the ability of an open epoxide ring to form a covalent bond with exposed amino groups of dentin collagen, as well as by the material's dimensional stability and low polymerization stress.[19]

The push-out test measures the interfacial shear strength formed between different surfaces, thereby providing additional information about the evaluation of adhesion properties. The purpose of this test is to assess the extent to which the sealer and core material are bound together as a solid mass, as well as its bond strength to the root canal wall.[9]

There are very few studies to evaluate the effect TAP with new and novel vehicles on the mechanical properties of radicular dentin. Keeping these concepts in mind, this study aims to evaluate the effect of TAP and DAP with Chitosan as the vehicle on the push out bond strength of Epoxy resin–based sealer to radicular dentin.

AIM

To evaluate the effect of Triple Antibiotic Paste and Double Antibiotic Paste with Chitosan as the vehicle on the push out bond strength of Epoxy resin–based sealer to radicular dentin.

OBJECTIVES

1. To evaluate the effect of TAP and DAP with distilled water as the vehicle on the push out bond strength of Epoxy resin–based sealer to radicular dentin using Universal testing machine (UTM).
2. To evaluate the effect of TAP and DAP with Chitosan as the vehicle on the push out bond strength of Epoxy resin–based sealer to radicular dentin using UTM.
3. To compare the effect of TAP and DAP with Chitosan and Distilled water as the vehicle on the push out bond strength of Epoxy resin–based sealer to radicular dentin using UTM.

HYPOTHESIS:

NULL HYPOTHESIS-

There is no difference in the effect of TAP and DAP with Chitosan as the vehicle on the push out bond strength of Epoxy resin–based sealer to radicular dentin

ALTERNATIVE HYPOTHESIS-

There is a difference in the effect TAP and DAP with Chitosan as the vehicle on the push out bond strength of Epoxy resin–based sealer to radicular dentin.

REVIEW OF LITERATURE:

1. An *in vitro* test was performed to analyse antimicrobial efficacy of Ciprofloxacin, Metronidazole, PEG and Natrosol vehicles with various associations and concentrations. The minimum inhibitory concentration (MIC) was determined by using the agar dilution method. The culture media (Müller-Hinton agar) were prepared containing antimicrobial agents at multiple two-fold dilutions of 0.25 to 16 μg/mL, and with the vehicles at the concentrations of 50, 45, 40, 35, 30 and 25%. The study was assessed and examined with twenty-three selected microbial strains. The conclusion from this study was that Ciprofloxacin presented antimicrobial properties against all tested bacterial strains, and its association with metronidazole was synergic. The vehicle PEG illustrated antimicrobial effects and the Ciprofloxacin/PEG association proved as the most effective combination medium for reducing the tested bacteria and yeasts.[4]
2. An *in vitro* research was performed to evaluate push out bond strength of 2 Mineral Trioxide Aggregate (MTA)–based sealers (Endo-CPM sealer and MTA Fillapex) and of 1 Epoxy resin–based sealer (AH Plus sealer) to root dentin. 45 extracted human teeth with single roots were prepared by using the step-back technique. Following this, irrigation with 2.5% Sodium hypochlorite (NaOCl) and a final rinse with 17% Ethylenediaminetetraacetic acid (EDTA) and distilled water were performed. Canals were filled by using Endo-CPM sealer, MTA Fillapex, or AH Plus sealer by means of the gutta-percha lateral condensation technique. After 7 days, the roots were sectioned perpendicularly to its long axis, and the push-out test was carried out. The data were analysed by using the Kruskal-Wallis and Dunn post hoc tests. The results showed that

Endo-CPM sealer presented advantages when a post preparation was required. MTA Fillapex presented acceptable resistance to dislodgement, which was similar to that observed in samples filled with AH Plus sealer.[2]

3. An *in vitro* research was done to compare the effectiveness of irrigation protocols on removal of TAP from artificial grooves in root canals. Root canals of 72 extracted single rooted teeth were prepared using ProTaper rotary instruments up to size F5. The roots were split longitudinally and a standardized groove was prepared in the apical part of one segment. TAP was filled in the grooves, and the root halves were reassembled. The roots were randomly divided into 6 experimental groups according to the irrigation protocol used: distilled water, 1% NaOCl, 2.5% NaOCl, 100% ethanol, 17% EDTA and passive ultrasonic irrigation (PUI) with 1% NaOCl. The root segments were disassembled, and the amount of TAP remaining was evaluated under a stereomicroscope at 209 magnification using a four-grade scoring system. The data was evaluated statistically using Kruskal–Wallis and Mann–Whitney U-tests with a 95% confidence level (P = 0.05). The study concluded that PUI with 1% NaOCl was more effective in removing TAP from artificial grooves in root canals than other irrigating solutions without ultrasonic agitation. Thus, removing TAP from root canals was not entirely possible.[1]
4. An *in vitro* research was done to analyse the steady release of intracanal medicament with or without a carrier and testing their antimicrobial efficacy in root canal against *Candida albicans* and *Enterococcus faecalis*. A total of 80 single-rooted anterior teeth were selected, root canal preparation was done, and teeth were divided into two halves and contaminated with *C. albicans* and *E. faecalis*, which were further divided into four test groups each according to

intracanal medicaments used. Chitosan was used as vehicle for TAP and CH and antimicrobial assessment was performed on second and seventh day. Dentine samples were collected after each time intervals and the number of colony-forming units (CFUs) was determined. The study concluded that the Combination of TAP + Chitosan and CH + Chitosan produced better results compared with the combination of medicaments with saline.[3]

5. An *in vitro* test was done to analyze the impact of CH, TAP and DAP on bond strength of an Epoxy resin–based sealer (AH Plus Jet; Dentsply) to the root canal dentin. The root canals of 64 mandibular premolars were instrumented and randomized into three treatment groups and an untreated control group. Each treatment group received either TAP, DAP or CH intracanal medicament. Consequently, root canals were obturated with gutta-percha and AH Plus Jet sealer. A push-out test was used to detect the bond strength between the root canal dentin and the sealer. The study concluded that DAP and CH did not affect the bond strength of the epoxy resin– based sealer. Additionally, the TAP improved the bond strength of the epoxy resin–based sealer in the middle and apical thirds.[5]
6. An *in vitro* research was performed to analyze the effect of several endodontic regeneration agents on the microhardness of human root dentin after contact for various time intervals. 35 extracted human maxillary incisors were studied. The canals were enlarged and fixed in acrylic resin blocks. Two sections were obtained from the middle-third of each root (n = 70). The root discs were divided randomly into three groups of 20 and a control group of 10. Baseline microhardness testing was completed using a microhardness tester. The root discs were placed in Petri dishes and then assigned randomly to TAP, DAP,

CH and the control group. The CH and two antibiotic pastes were placed in the Petri dishes, and the discs were covered completely with the mixtures. Microhardness tests were repeated similarly after weeks 1, 2 and 3. The study concluded that applying DAP and TAP for 4 weeks significantly decreased the microhardness values of dentin discs compared with the baseline values.[7]

7. An *in vitro* research was administered to probe into impact on push-out bond strength of Smart-Seal system of intracanal medicaments. The root canals of 64 freshly extracted single-rooted human mandibular premolars were decoronated and prepared using rotary ProTaper system. The specimens were randomly divided into a control group and 3 experimental groups that received an intracanal dressing with either CH, DAP, or TAP (n = 16). The intracanal dressing was extirpated following 3 weeks. The root canals were then obturated with C-points and Endosequence BC sealer. A push-out test was used to measure the bond strength between the root canal dentin and the obturating system. The study concluded The DAP and CH correspondence did not affect the bond strength of the novel hydrophilic obturating system. TAP improved the bond strength of Smart-Seal system in the middle and apical thirds.[8]
8. An *in vitro* analysis was conducted for comparative evaluation of the wetting ability of CH and TAP medicaments when used with Chitosan against the surface of radicular dentin. 40 dentin blocks of thickness 2 mm were prepared and divided into 2 groups of 20 each. Controlled volume (0.1 ml) droplets of CH + 2% Chitosan were placed onto the blocks in group I, and TAP + 2% Chitosan in group II. The contact angle in each case was measured using

Dataphysics OCA Easydrop software on a dynamic contact angle analyzer for 10 mins.[18]

9. An *in vitro* test was done to analyse the impact of different Intracanal medicaments on the push out bond strength of AH26 and MTA Fillapex sealers. A total of 104 one-rooted extracted human teeth were divided into 4 (n=26) experimental groups. After the cleaning and shaping, the root canals were filled with CH, TAP, Metapex or 2% Chlorhexidine gel for two weeks. Then, intracanal medicaments were rinsed away and the samples in the sub-groups were obturated with gutta-percha and AH26 or MTA Fillapex sealers. After two weeks of incubation, 2 mm thick middle section of each root was then subjected to push-out testing. Data were analyzed with two-way ANOVA and LSD test. The study concluded that the bond strengths of sealers to dentin are under the influence of pre-treatment with intracanal medicaments and the effect of TAP on the bond strength of endodontic sealers was not negative.[19]
10. An *in vitro* research was done to analyse the bioactivity of BC Sealer and its micro pushout bond strength to dentin compared to AH-Plus (AH) sealer. 24 root canals of mandibular premolars were instrumented and divided into two groups (n=12). Each root was cut into 4 slices and lumens of the canals were filled with the sealers and submitted to micro push-out test. Failure mode was assessed using scanning electron microscopy (SEM). Bioactivity of BC sealer was investigated with scanning electron microscopy/energy-dispersive X-ray (SEM/EDS) and X-ray diffraction (XRD). Bioactivity assessments were reported descriptively and bond strength data were analysed by parametric *t*-test (α=5%). The study concluded that BC sealer showed indications of bioactivity and lower bond strength to dentine compared to AH.[9]

11. An *in vitro* research was led to analyze the impact of different endodontic regeneration agents on the push-out bond strength of Endosequence Root Repair Material (ERRM) to root-canal dentin. The root canals of 50 single-rooted human teeth were selected and instrumented to obtain a standard internal diameter of 1.5 mm. Specimens were randomly divided into four experimental groups and treated with an intracanal medicament [CH, DAP, TAP], TAP with amoxicillin (mTAP)] and a non-treated control group. Medicaments were removed after three weeks, and ERRM was applied to all specimens. The coronal portion of each root was then sliced into 2mm thick parallel transverse sections (2 slices per tooth, n=20 slices per group), To measure the bond strength of ERRM with dentin, a push-out test was used. The researchers concluded that CH could be used to help enhance ERRM adhesion to dentin.[13]
12. The new observations and notions about TAP and its implementations in dentistry were explored by a review. Despite the problems and pitfalls, research pertaining to this paste unveiled, it has been vastly used in endodontic treatments. The paste's applications vary, from vital pulp therapy to the recently introduced regeneration and revascularization protocol. Studies have presented the paste can eliminate the root canal microorganisms and prepare an appropriate matrix for further treatments. This combination is able to remove diverse groups of obligate and facultative gram-positive and gram-negative bacteria, providing an environment for healing. In regeneration protocol cases, this allows the development, disinfection, and possible sterilization of the root canal system, so that new tissue can infiltrate and grow into the radicular area. Moreover, TAP is capable of creating a discipline in

which other wanted and needed treatments can be successfully performed. In conclusion, TAP, as an antibacterial intracanal medication, has diverse uses in Endodontics.[14]

13. To highlight the new marine developments in the biomedical field, in particular Chitosan in the dental field, a meta-analysis was conducted. Chitosan that is used in different fields of medicine, was analysed in this review with the aim of highlighting its uses and advantages in the dental field. A literature search was conducted in scientific search engines, using keywords in order to achieve the highest possible number of results. A review of randomized controlled trials (RCT) was conducted to evaluate and process all the relevant results for Chitosan and oral health. After a screening and thorough analysis of the literature, there were 12 results highlighted and it was seen that Chitosan performs different functions and utilised in different fields of dentistry in a safe and effective way. Among the uses of Chitosan, the remineralizing property of Chitosan was reported which hardens tissues of the tooth, and therefore it is used as a desensibilizer in toothpastes. According to this systematic review, the use of Chitosan has shown better surgical healing of post-extraction oral wounds and reduction in bacterial biofilm when used in dental cements. In addition, it has antibacterial, antifungal, hemostatic and other systemic properties which aid its use for drug delivering.[15]
14. An *in vitro* research was done to analyze the impact of intracanal treatments with CH or TAP on bond strength of a calcium silicate-based sealer (MTA Fillapex) and an epoxy resin- based sealer (MM Seal). 60 extracted maxillary central incisors were prepared with a rotary system to size 40 and randomly divided into two groups, which received either intracanal CH or TAP. After

rinsing, the teeth in each group were further divided into two additional groups, which were obturated with gutta percha and either the MTA Fillapex or MM Seal root canal sealer. Slices were then taken from three sections of the canal and a push-out test was performed to range and value the bond strength between the root canal dentin and the sealer. Data were statistically analyzed by two-way analyses of variance followed by post-hoc Tukey's tests. The study concluded that the use of TAP rather than CH improves the bond strength of calcium silicate- and epoxy resin-based sealers throughout the root canal.[20]

15. An *in vitro* research was done to analyse the impact of precedent intracanal placement of CH on the bond strength of AH Plus iRoot SP, and MTA Fillapex. The root canals of 90 human incisor teeth were prepared with the ProTaper System up to a master apical file size of F5 and the canals were filled using the single-cone technique either immediately (the control group, n = 30) or after 7-days of CH placement. CH removal was performed either manually using F5 with distilled water irrigation (the CH group, n = 30) or manually using ProTaper F5 followed by passive ultrasonic irrigation with 2.5 % NaOCl with a final flush of 17 % EDTA and then distilled water (the PUI group, n = 30). After obturation, a 2 mm thick middle section of each root was subjected to push-out testing. Statistical analysis was done using 1-way analysis of variance. The study concluded that prior CH placement seemed to improve and augment the dislodgment resistance of iRoot SP but did not affect AH Plus and MTA Fillapex sealers.[21]

MATERIALS AND METHODS

SOURCE OF DATA:

- The study was carried out in the Department of Conservative Dentistry and Endodontics, V.K Institute of Dental sciences, KLE Academy of Higher Education and Research (KAHER), Belagavi, Karnataka.
- Push out bond strength testing was carried out at K.L.E Society's Dr. M.S. Sheshgiri College of Engineering and Technology, Belagavi, Karnataka.

Sample size estimation was done using the formula:

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

Z_α= 1.96 at 5% α error.

Z_β= 0.842 at 80% power.

S1=0.66

S2=0.63

d=0.59

$$n = \frac{2S^2 (Z_\alpha + Z_\beta)^2}{d^2} = 20 \text{ in each group}$$

The teeth used for this study were selected based on the following inclusion and exclusion criteria:

INCLUSION CRITERIA

- Extracted human single canal single rooted mandibular premolar teeth with patent canals.
- Teeth with apical width corresponding to #20 K-file or less.
- Teeth with straight canals.

EXCLUSION CRITERIA:

- Carious teeth.
- Teeth with apical width more than #20 K-file size.
- Teeth with calcified canals.
- Teeth with fracture/crack or a restoration.
- Teeth with internal and external root resorption.
- Teeth with presence of anatomic variations.

MATERIALS USED FOR THE STUDY:

- 80 single rooted premolar teeth
- 0.1% Thymol solution (S D FINE-CHEMICALS LIMITED, MUMBAI)
- 3% Sodium hypochlorite (Thermo Fischer SCIENTIFIC)
- 17% Ethylenediaminetetracetic Acid (EDTA) [GLIDE]
- AH Plus sealer (DENTSPLY, Germany)
- Distilled water (NICE LIFE CARE, NEW DELHI)
- 1% Acetic acid (S.M. CORPORATION, Gujarat)
- Triple antibiotic paste - Metronidazole (500mg) Ciprofloxacin (250mg) Minocycline (100mg)
- Double antibiotic paste - Metronidazole (500mg) Ciprofloxacin (250mg)
- Paper points (DIADENT GROUP INTERNATIONAL, KOREA)
- Chitosan (SIGMA-ALDRICH, BANGALORE)
- Cavit G (3M, ESPE GERMANY)
- Guttapercha (DENTSPLY)

ARMAMENTARIUM USED FOR THE STUDY:

- Airotor (NSK PANA AIR) and Endomotor (DENTSPLY X-MART)
- K files (15-40) (MANI)
- Protaper Universal nickel titanium files (DENTSPLY MAILLEFER, SWITZERLAND)
- Round high-speed endo access diamond bur (DENTSPLY)
- Low speed round diamond bur No.029 (DENTSPLY)
- Micromotor (NSK, JAPAN)
- 5ml 27-gauge syringe (DISPOVAN, HINDUSTAN SYRINGES LTD FARIDABAD)
- Diamond disc (KWALITY DIAMOND TOOLS, Mumbai)
- Mixing pad
- Agate spatula
- Magnetic stirrer (REMI, Mumbai)
- Lentulospiral (No-25) (MANI INC, JAPAN)
- Incubator (BIO TECHNICS, India)
- Universal testing machine (ENKAY ENTERPRISES, New Delhi)

Preparation of Study materials:

Preparation of the Triple antibiotic paste (TAP):

According to Hoshino et al.

- TAP (3 mix): - ratio 1:1:1 [Ciprofloxacin 200 mg, Metronidazole 500 mg, Minocycline 100 mg]
- Equal portions of Metronidazole, Minocycline and Ciprofloxacin were mixed with sterilized distilled water (powder/ liquid ratio of 3:1) in the control group to achieve a paste like consistency.
- 1mg of each medicament was mixed with 1ml of the carrier (2% Chitosan) in the experimental group to achieve a paste like consistency.[22]

Preparation of Double antibiotic paste (DAP):

- According to Yilmaz et al.
- DAP was prepared by mixing antibiotic powders;
- Equal portions of Metronidazole and Ciprofloxacin were mixed with sterilized distilled water (powder/ liquid ratio of 2:1) in control group to achieve a paste like consistency.
- 1mg of each medicament was mixed with 1ml of the vehicle (2% Chitosan) in the experimental group to achieve a paste like consistency.[22]

Preparation of 2% Chitosan:

2% Chitosan was prepared by diluting 2g Chitosan with 100 ml 1% Acetic acid and stirring the solution using a magnetic stirrer for 2 hours.

METHODOLOGY: -

Eighty extracted human mandibular premolar teeth cleaned of soft tissue and calculus were stored in 0.1% Thymol. All teeth were radiographed and selected as per the inclusion and exclusion criteria.

All teeth were then decoronated up to the level of cementoenamel junction (CEJ) with the help of a diamond disc under copious water spray to produce a standardized root length of 15mm. Working length (WL) was established by inserting a size 15 K file into each root canal until it is just visible at the apical foramen and by subtracting 1mm from the recorded length.

The root canals were prepared using ProTaper Universal rotary instruments up to F4 (size 40, 0.06 taper). Irrigation was done with 2ml of 3% sodium hypochlorite (NaOCl) between successive files. Final irrigation was done with 5 ml 17% EDTA acid for 1 minute. The canals were then dried with sterile absorbent paper points.

The teeth were then divided into 4 groups (n=20) depending on the medicament and vehicle used as follows:

Group 1: *TAP + Distilled Water*

Group 2: *TAP + Chitosan*

Group 3: *DAP + Distilled Water*

Group 4: *DAP + Chitosan*

The prepared paste was placed into the root canal using a size #35 lentulospiral. The coronal access was sealed with Cavit G. The specimens were then incubated at 37°C in 100% humidity for 3 weeks to simulate clinical conditions.

After 3 weeks the medicaments were rinsed with 10ml 17% EDTA followed by 10 ml of 3% NaOCl and the final irrigation was done with 5ml of distilled water. The root canals were obturated with Gutta-percha points and AH Plus sealer and stored at 100% humidity at 37^{o}C for one week.

Preparation of samples and measurement of Push out bond strength: -

The root samples were cut into 2mm thick cross sections in the middle third of the roots using a diamond disk.

A push out force was applied with a cylindrical piston measuring 0.8mm in diameter at a cross-head speed of 1mm/min. perpendicular to the sample surface using a UTM.

Calculation of push-out strength:

$$\text{PUSH-OUT BOND STRENGTH} = \frac{\text{FORCE FOR DISLODGEMENT (PUSHLOAD)}}{\text{SURFACE AREA}}$$

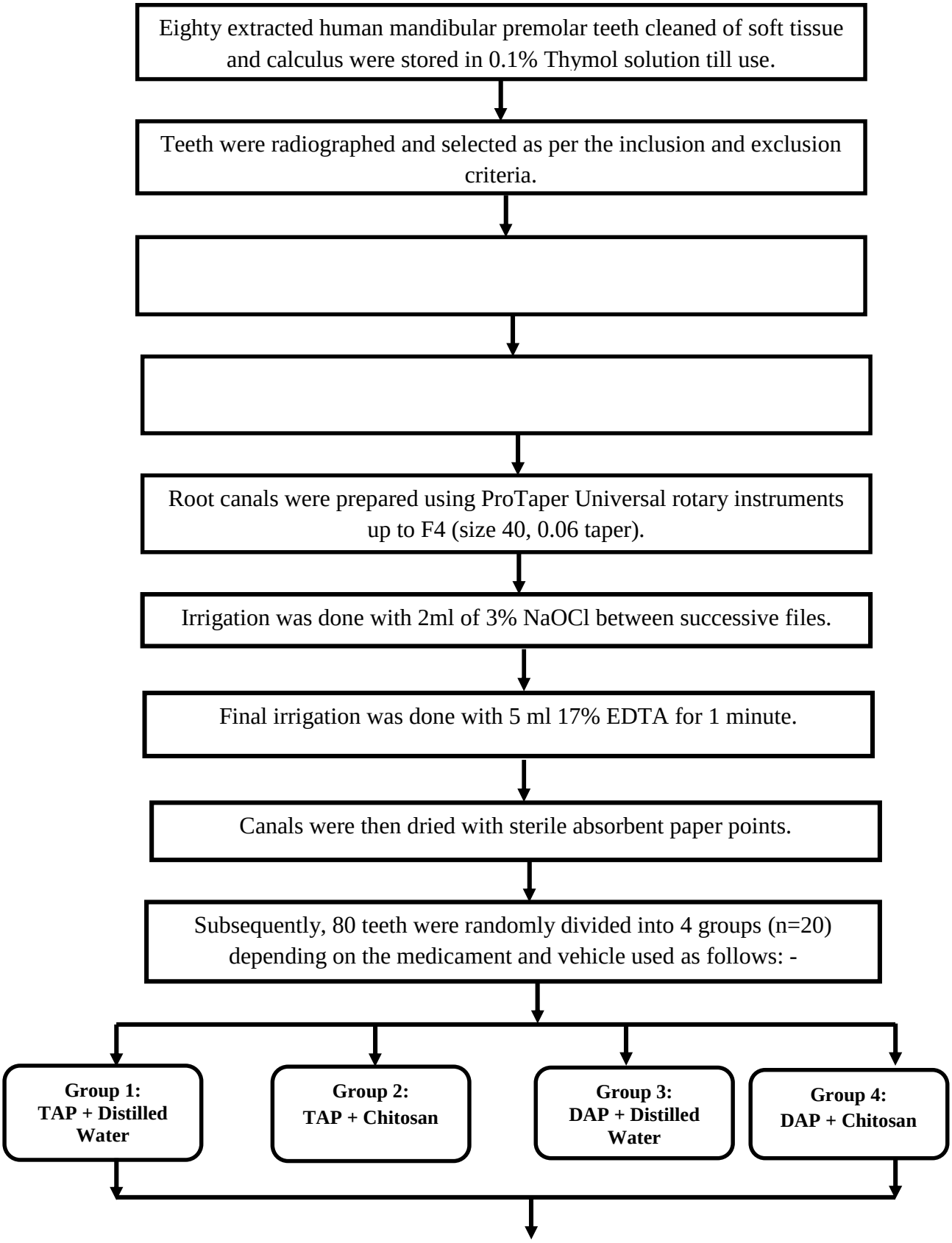
STATISTICAL TEST:

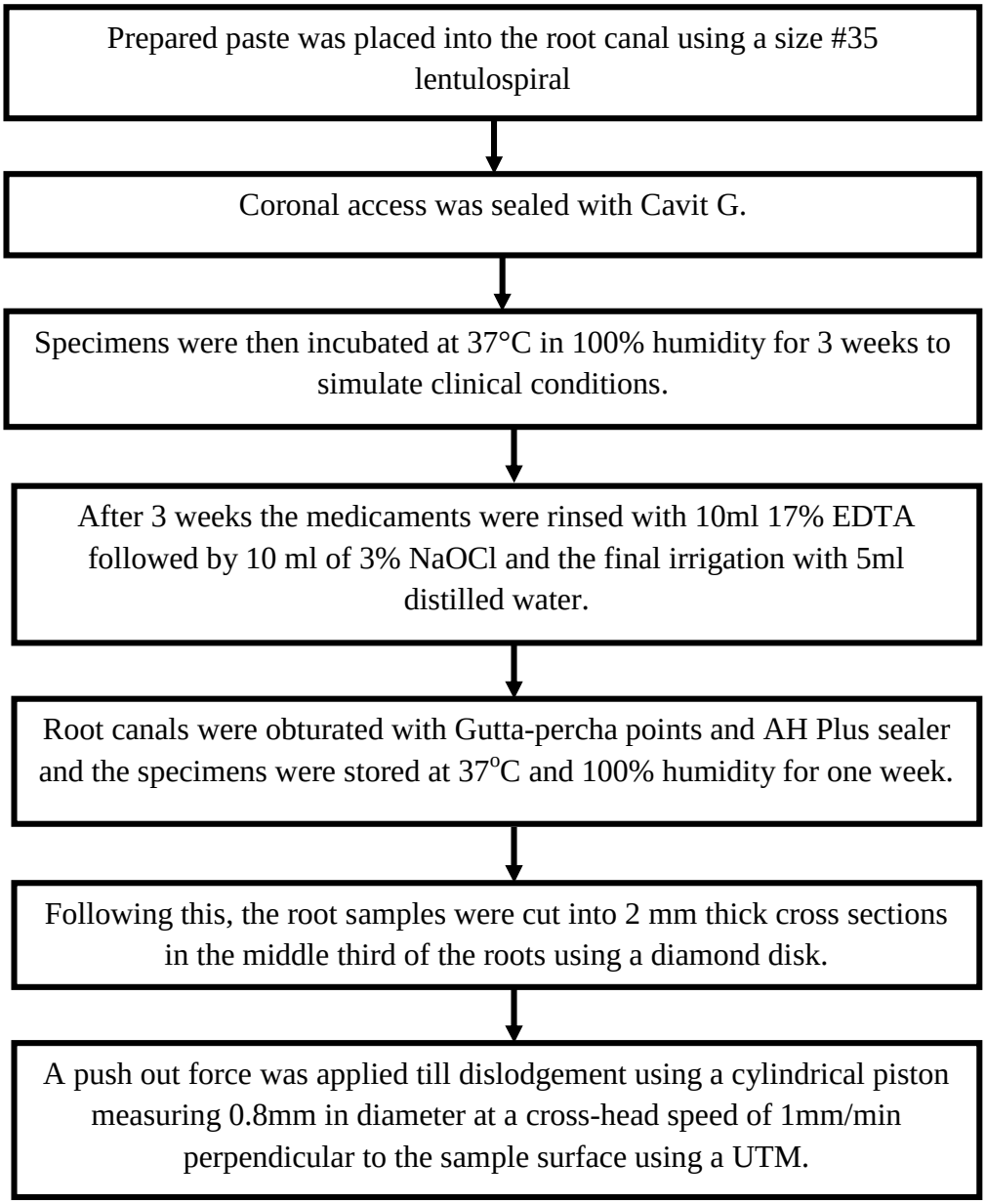
The data obtained were statistically analyzed by-

One-way ANOVA

Newman-Keuls multiple Post Hoc

STUDY DESIGN




Prepared paste was placed into the root canal using a size #35 lentulospiral
Coronal access was sealed with Cavit G.
Specimens were then incubated at 37°C in 100% humidity for 3 weeks to simulate clinical conditions.
After 3 weeks the medicaments were rinsed with 10ml 17% EDTA followed by 10 ml of 3% NaOCl and the final irrigation with 5ml distilled water.
Root canals were obturated with Gutta-percha points and AH Plus sealer and the specimens were stored at 37°C and 100% humidity for one week.
Following this, the root samples were cut into 2 mm thick cross sections in the middle third of the roots using a diamond disk.
A push out force was applied till dislodgement using a cylindrical piston measuring 0.8mm in diameter at a cross-head speed of 1mm/min perpendicular to the sample surface using a UTM.

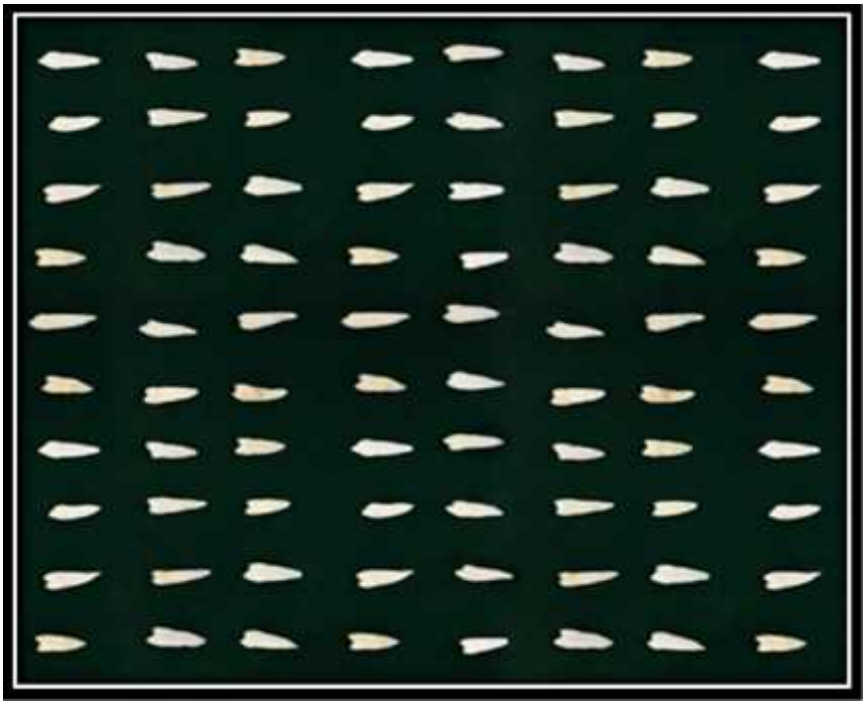


Fig 1: Total Sample Size (n=80)

Fig 2: Debris and calculus removed using Ultrasonic scaler

MATERIALS AND ARMAMENTARIUM

Fig 3: Materials and Armamentarium for access opening, biomechanical preparation and obturation of the tooth samples.

Fig 4: Materials and instruments for preparation of the medicaments and its placement.

Fig 5: Sample decoronated at the level of CEJ using Diamond disc

Fig 6: Cleaning and shaping of the samples done with ProTaper Universal rotary system

Fig 7: Sodium hypochlorite Irrigation in between instrumentation

Fig 8: Powdering of the antibiotics

Fig 9: USP grade powdered antibiotics - 1:1:1 (Ciprofloxacin 200 mg, Metronidazole 500 mg, Minocycline 100 mg)

PREPARATION OF 2% CHITOSAN SOLUTION

Fig 10: 2 g of Chitosan powder weighed by placing it on top of filter paper

Fig 11: Acetic Acid used to dissolve Chitosan powder

Fig 12: 2 g Chitosan Powder dispensed into 100 ml Acetic acid

Fig 13: Magnetic Stirrer used to get a homogeneous 2% Chitosan solution.

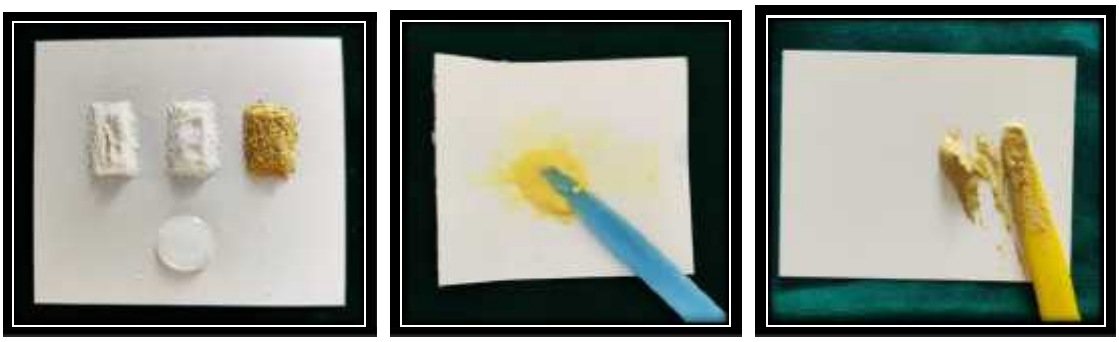


Fig 14: Preparation of TAP with Distilled water and 2% Chitosan as vehicles

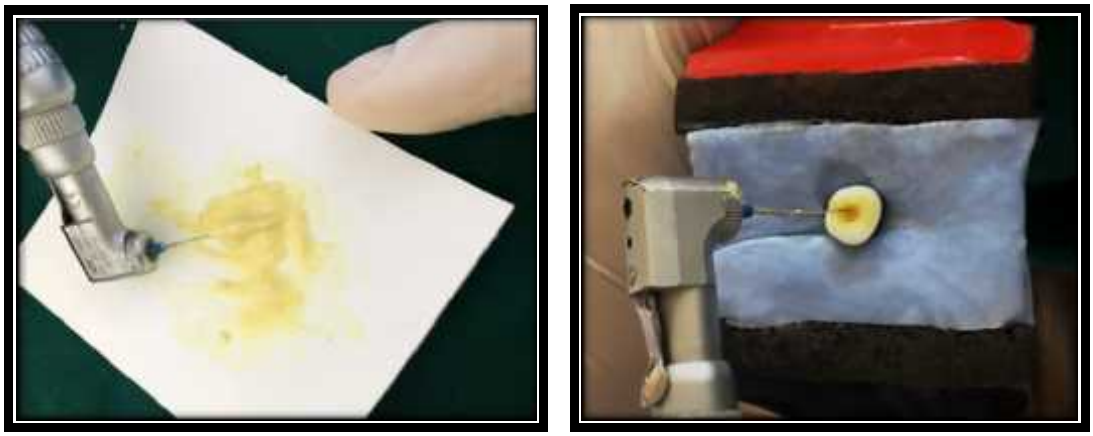


Fig 15: Placement of intracanal medicament using sterile lentulospiral

Fig 16: Access cavity sealed with 4 mm of Cavit G

Fig 17: Storage of teeth in incubator at 100% humidity at and 37î C.

Fig 18: Medicament removed using 17% EDTA

Fig 19: Obturation done using Gutta-Percha and AH Plus sealer

(A) **(B)**

Fig 20: A- Sectioning of tooth specimens; **B –** 2mm thick cross sections taken from the middle third of the root

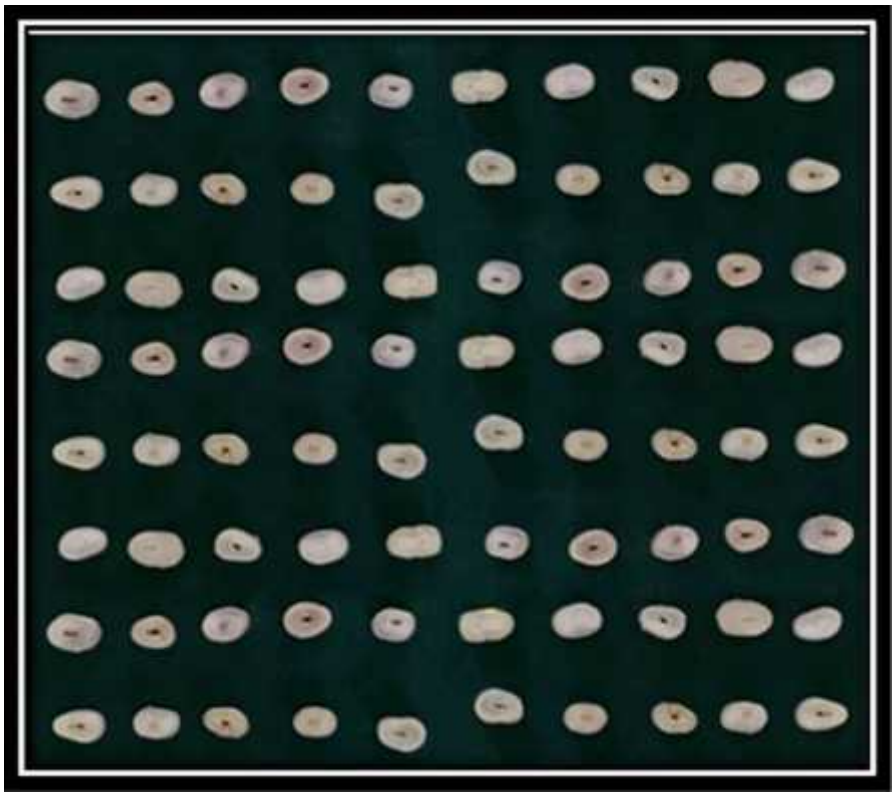


Fig 21: Dentin discs after sectioning

Fig 22: Pushout bond strength testing done using a Universal testing machine at a cross head speed of 1mm/min.

RESULTS

Tables 1, 2, 3 and 4 depict the push-out bond strength value of each sample in the following 4 groups - GROUP I (TAP + Distilled water), GROUP II (TAP + Chitosan), GROUP III (DAP + Distilled water) and GROUP IV (DAP + Chitosan).

TABLE 1: Pushout bond strength values (MPa) of samples in the TAP + Distilled water group.

Group I: TAP + Distilled Water (Control)		
Sample No.	**Maximum Load (N)**	**Pushout Bond Strength (MPa)**
No.1	7.50	2.39
No.2	16.80	5.35
No.3	14.50	4.62
No.4	8.70	2.77
No.5	7.40	2.35
No.6	16.75	5.33
No.7	14.40	4.58
No.8	8.60	2.73
No.9	7.30	2.32
No.10	16.65	5.30
No.11	14.35	4.57
No.12	8.50	2.70
No.13	7.15	2.27
No.14	16.50	5.25
No.15	14.15	4.50
No.16	8.15	2.59
No.17	7.10	2.26
No.18	14.45	4.60
No.19	15.75	5.01
No.20	8.75	2.78
Average		**3.71**

TABLE 2: Pushout bond strength values (MPa) of samples in the TAP + Chitosan group.

Group II: TAP + Chitosan (Experimental)		
Sample No.	**Maximum Load (N)**	**Pushout Bond Strength (MPa)**
No.1	14.30	4.55
No.2	20.70	6.59
No.3	14.75	4.70
No.4	18.50	5.89
No.5	14.55	4.63
No.6	20.10	6.40
No.7	14.70	4.68
No.8	18.35	5.84
No.9	14.35	4.57
No.10	19.90	6.33
No.11	14.60	4.64
No.12	18.10	5.76
No.13	14.15	4.50
No.14	20.25	6.44
No.15	14.15	4.50
No.16	17.95	5.26
No.17	13.90	4.42
No.18	20.65	6.57
No.19	14.45	4.60
No.20	18.15	5.78
Average		**4.785**

TABLE 3: Pushout bond strength values (MPa) of samples in the DAP + Distilled water group.

Group III: DAP + Distilled water (Control)		
Sample No.	**Maximum Load (N)**	**Pushout Bond Strength (MPa)**
No.1	6.00	1.08
No.2	6.50	1.17
No.3	9.30	1.68
No.4	5.65	1.02
No.5	4.35	0.79
No.6	7.20	1.30
No.7	6.80	1.23
No.8	5.10	0.92
No.9	6.15	1.11
No.10	6.45	1.18
No.11	9.15	1.65
No.12	5.50	0.99
No.13	4.40	0.79
No.14	7.10	1.28
No.15	6.25	1.13
No.16	5.00	0.90
No.17	7.35	1.32
No.18	5.60	1.012
No.19	6.80	1.22
No.20	4.95	0.89
Average		**1.17**

TABLE 4: Pushout bond strength values (MPa) of samples in the DAP + Chitosan group.

Group IV: DAP + Chitosan (Experimental)		
Sample No.	**Maximum Load (N)**	**Pushout Bond Strength (MPa)**
No.1	7.65	1.38
No.2	8.25	1.50
No.3	9.50	1.72
No.4	11.20	2.02
No.5	9.30	1.68
No.6	7.95	1.44
No.7	8.60	1.55
No.8	12.80	2.31
No.9	8.10	1.46
No.10	10.90	1.98
No.11	8.75	1.59
No.12	11.15	2.01
No.13	9.15	1.65
No.14	7.75	1.40
No.15	8.50	1.53
No.16	12.50	2.26
No.17	8.75	1.58
No.18	9.45	1.70
No.19	11.75	2.12
No.20	8.95	1.62
Average		**1.73**

Table 5: Mean and Standard deviation values of pushout bond strength (MPa) in the four study groups (I, II, III, IV)

Groups	Min	Max	Mean	SD	SE	95% CI for mean	
						Lower Bound	Upper Bound
Group I	2.26	5.35	3.71	1.26	0.28	3.12	4.30
Group II	4.42	6.59	5.33	0.83	0.19	4.94	5.72
Group III	0.79	1.68	1.13	0.24	0.05	1.02	1.25
Group IV	1.38	2.31	1.73	0.29	0.06	1.59	1.86
Total	0.79	6.59	2.98	1.84	0.21	2.57	3.39

The highest mean push-out bond strength value was seen in group II (5.33), which was significantly higher than that seen in group I (3.71), group IV (1.73) and group III (1.13) respectively.

Figure 1: Graphical representation of mean push out bond strength (MPa) values among the four study groups

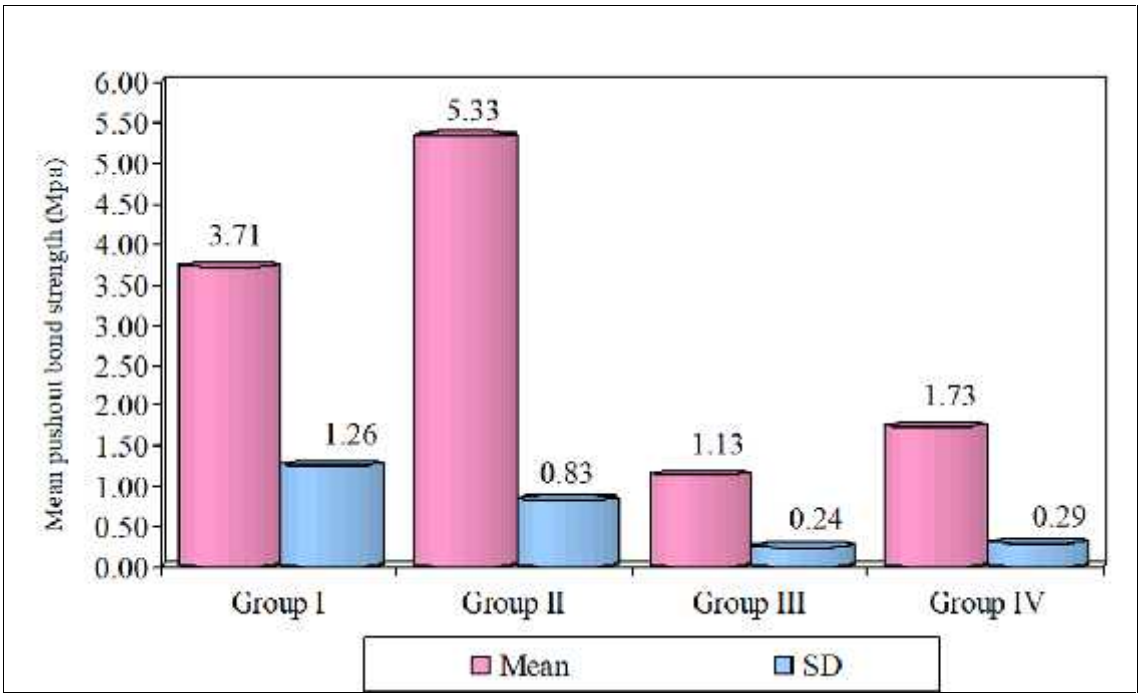


Table 6: Comparison of mean push out bond strength (MPa) among 4 groups by one-way ANOVA

Sources of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value	p-value
Between groups	3	221.1656	73.7219	121.2881	<0.001, HS
Within groups	76	46.1947	0.6078		
Total	79	267.3603			

The four groups were compared by applying One-way ANOVA test (P<0.001). It revealed a significant difference in all four groups GROUP I (TAP + Distilled water), GROUP II (TAP + Chitosan), GROUP 3 (DAP + Distilled water) and GROUP IV (DAP + Chitosan) (**Table 6)**.

Table 7: Pair wise comparison of mean push out bond strength (MPa) by Newman-Keuls multiple Post Hoc procedures among the four study groups

Groups	Group I	Group II	Group III	Group IV
Mean	3.71	5.33	1.13	1.73
SD	1.26	0.83	0.24	0.29
Group I	-			
Group II	P=0.0001*	-		
Group III	P=0.0001*	P=0.0001*	-	
Group IV	P=0.0001*	P=0.0001*	P=0.0856	-

*p<0.01indicates significant

The pairwise comparison of the four experimental groups was done using **Newman-Keuls multiple Post Hoc test** - tabulated in **Table 7**. A significant difference was seen between Group I with Group II, Group III and Group IV. Significant difference was also seen between Group II with Group III and Group IV. But no significant difference was seen between group III and group IV. The same values are depicted in **figure 2**. Level of significance (p<0.01).

Hence, the null hypothesis was rejected.

DISCUSSION

The root canal system is polymicrobial in nature. Complete elimination of pulpal infections alongside the microorganisms from the root canals is the principal and ultimate goal of endodontic therapy. The ideal protocol for root canal disinfection involves a multi-prong approach including: mechanical debridement, irrigation and finally intracanal medication.[20] Root canal complexity and intricacies often make biomechanical preparation inefficient in completely eliminating the microbial load.[23] Thus the placement of intracanal medicament for a 7-day interim period plays an imperative role by hampering growth and obliteration of surviving microorganisms within the root canal system.[24]

Conventionally, the most frequently employed intracanal medicament is Calcium hydroxide (CH) due to the high bactericidal properties, organic tissue dissolution and anti-inflammatory properties.[20] However, studies have shown that *Enterococcus faecalis* is resistant to CH.[25] Moreover, radicular dentine exposed to CH for extended periods are highly susceptible toward decreased fracture resistance.[26] This resulted in the development of Triple antibiotic paste (TAP) which is an amalgam of antimicrobials. It has profound use in revascularization, healing and dissolution of large periapical lesions. A vast range of antimicrobial activity has been witnessed with this paste and it is biocompatible and less noxious. Studies have shown that it is more effective against *Enterococcus faecalis* than CH.[20]

Ciprofloxacin 200 mg, Metronidazole 500 mg and Minocycline 100 mg in a ratio of 1:1:1 was suggested by Hoshino et al. for the use in TAP (3Mix). In various *in vitro* studies, the minimum inhibitory concentrations of Minocycline and

Ciprofloxacin against *Enterococcus faecalis* and *Enterococcus faecium* were 20 and 5 µg respectively, and Metronidazole was found to have no inhibitory effect.[27] However, when used as a combination (100µg each /ml), they have been shown to completely restrain the growth of all strains.[28] TAP (1 mg/mL) showed inhibitory and bactericidal effects against *Enterococcus faecalis* and *Porphyromonas gingivalis.* High concentrations of TAP can cause cytotoxic effects on the stem cells of dental pulp and apical papilla, in regenerative endodontics. Therefore, it is recommended to use lower concentrations, in the range of 0.1 mg/mL to 2 mg/mL. However, various studies have expressed that the minocycline component of TAP causes visible and distinguishable crown discoloration[29,30,31] which led to the development of DAP, comprising of only Ciprofloxacin and Metronidazole. A study by Dall AQ et al concluded that DAP provided better antimicrobial effect with significant decrease in pain and bacterial count in comparison to CH and almost equal efficacy to TAP.[32]

Distilled water as a vehicle does not have its own antibacterial properties, therefore newer novel vehicles which have antibacterial properties have been used for the preparation of the antibiotic pastes.[33,34,35]

Chitosan, a natural and biodegradable polymer, contains copolymers of glucosamine and N acetyl glucosamine, has been examined and studied in detail in the biomedical field and has been found to have antimicrobial and antifungal properties. The use of Chitosan in pharmaceutical formulations as an excipient is a recent development. The ability of Chitosan to retain large amounts of water is a characteristic, which may be of particular value for slow release formulations. It has been tested *in vitro* in hydrocolloids and gels as a drug carrier. An important property of Chitosan for study as an excipient is the ability to hydrate and form gels in a highly

acidic aqueous environment and is thus used to prepare slow release drug delivery systems. Compared with conventional dosage forms, the use of controlled release systems has certain advantages because they can minimize side effects and extend the efficacy of the drug. Drug release rate is regulated, which aids in reducing the frequency of administration, thus assuring better patient compliance. It has been proposed that using Chitosan could extend the survival of formulations at sites of drug action or absorption. It has also been proposed that Chitosan improves the bioavailability of drugs and can be useful for drug distribution to particular regions.[5]

AH plus endodontic sealer is regarded as the gold standard against which other sealers are compared owing to its low solubility, high flow rate, longer setting time and low volumetric polymerization shrinkage.[36] It is suspected that the comparatively strong adhesion of the AH Plus sealer is due to the fact that when the epoxy ring opens, epoxy resin-based sealers will interact with exposed amino groups in collagen to create covalent bonds between the resin and collagen.[37]

The adhesion of the root filling material to dentin is critical to the success of endodontic treatment. Such adhesion is essential to prevent leakage and give the material resistance to displacement forces that develop during condensation of permanent restorative materials. Hence, using mechanical tests to evaluate the bond strength of materials can provide important information for clinical practice. Many factors, including the presence of a smear layer, the intermolecular surface energy of the dentin structure, the surface tension of the sealers, and the wettability, may directly affect the adhesion properties. The smear layer that forms during root canal preparation, may inhibit the penetration of irrigation agents and sealers into the dentinal tubules. For these reasons, it is recommended to remove the smear layer.

EDTA was used as a final flush as it helps in removal of smear layer because of its decalcification action.

Residual intracanal medicament can act as a barrier, preventing the formation of chemical bonds between the sealers and dentin and negatively affect their adaptation with dentinal walls; resulting in decrease in the push out bond strength.[38] These findings are consistent with the results of the studies of Guiotti *et al.*[39] Other tests, however, have suggested that intracanal drugs have no major impact on the sealer's push out bond strength.[8,21,5,40]

The bond strength of the root filling can be affected negatively or positively by Antibiotic pastes. Therefore, the aim of this study was to evaluate the effect of TAP and DAP with Chitosan as the vehicle on the push out bond strength of Epoxy resin–based sealer to radicular dentin.

Adhesion of root canal filling materials is commonly evaluated with the push-out bond strength test which was first designed by Haller for dental researches. It provides both accurate standardization of the samples and determination of the force even in low magnitudes. The push-out test was chosen because it can measure the shear stress at the interface between dentin and cement, which is comparable to the stresses under clinical conditions. Also, it is reproducible, effective and has less sensitivity to small variations in stress distribution during load application. Although Pane et al stated that this approach would not allow perfect standardisation since the natural roots can have structural differences in the whole root, the push-out approach is currently the most accurate way of determining the bond strength of root canal filling materials.[1]

Based on inclusion and exclusion criteria, a total of 80 human permanent mandibular premolars extracted for orthodontic reasons were selected. These teeth were kept in a 0.1% Thymol solution until use, as it has antifungal properties.

All teeth were then decoronated up to the level of CEJ under copious water spray with the help of a diamond disc as it provides more exposure of the diamond surfaces to improve cutting ability and minimize clogging of the instrument to produce a standardized root length of 15 mm. WL was determined by subtracting 1 mm from the standardized root length and ProTaper universal NiTi rotary files were used according to the manufacturer's instructions, for biomechanical preparation of the root canals. Based on the maximum apical constriction diameter of the mandibular premolar, the root canals were enlarged up to F4.

Biomechanical preparation was done with rotary instrumentation in this study because it requires less time than hand ProTaper files and the efficiency of shaping and preparing the canal walls is better in comparison to hand Pro-Taper files, as cited in previous literature. As NiTi ProTaper instruments are proposed to cover the complete range of treatment with fewer files, which integrate high efficiency, superior flexibility, and improved safety, they have been preferred over stainless-steel instruments to prepare the root canals.[41]

Use of antibiotic medicaments at a concentration of 1 mg/mL produces a paste-like consistency, which makes it less technique sensitive. Thus, 1mg each /ml of each medicament was used in the present study. Preparation of the antibiotic pastes and Chitosan were done as per literature, as mentioned in the methodology.

Teeth were then divided into 4 groups based on the medicament and vehicle used (n=20) as follows.

Group 1: *TAP + Distilled Water*

Group 2: *TAP + Chitosan*

Group 3: *DAP + Distilled Water*

Group 4: *DAP + Chitosan*

The prepared paste was placed into the root canal using a size #35 lentulospiral. The coronal access was sealed with Cavit G. The specimens were then incubated at 37°C in 100% humidity for 3 weeks to simulate clinical conditions.

Amin et al showed that prior CH placement did not affect the bond strength of an epoxy resin–based sealer (AH Plus). A research by Akcay et al found that DAP and CH did not impact the epoxy resin-based sealer's bond strength. TAP, however, strengthened the epoxy resin-based sealer's bond strength in the middle and apical thirds.[4]

In a recent review, Arslan et al. compared the efficacy of various irrigation protocols on the removal of TAP from artificial grooves in root canals, finding that it was difficult to clear TAP from root canals using irrigation solutions. In another study, Berkhoff et al. showed that TAP cannot be effectively removed from the root canal system, and no matter what irrigation technique is used, more than 80% of the TAP remains in the root canal system. These findings were credited by investigators to the penetration and binding of the TAP into dentin. Nevertheless, researchers found that the use of 2.5% NaOCl improved the removal of TAP[1]. The efficacy of various

irrigating techniques in eliminating CH from the root canals was examined in another analysis by Rodig et al.[42] According to the findings, chelating agents such as Citric acid and EDTA resulted with the best results. A study by Ustun et al concluded that 17% EDTA was found to remove TAP more efficiently.[40] Based on the results of the above trials, 10 mL of 7% EDTA followed by 10 mL of 3% NaOCl and final irrigation with 5 ml of distilled water were used for the elimination of intracanal medications in the current study.

Subsequent to these procedures, the root canal was dried using paper points. A single gutta-percha cone (F4) was then slightly coated with an epoxy resin–based sealer (AH Plus) and placed in the root canal to the WL. Because the root canals were prepared using rotary instruments up to F4, all specimens were obturated to acquire standard specimens for the push-out test using the single technique with equivalent taper F4 gutta-percha cones.[30] After root filling, the coronal opening was filled with a temporary filling material, and the specimens were stored at 100% humidity at 37^{o}C for 1 week to completely set and simulate clinical conditions. Each specimen was sectioned perpendicular to its long axis using a diamond disk at a slow speed under water cooling.

Evaluating the adhesion of root canal filling materials during the push out test involves collecting slices perpendicular to the long axis of the root. In this study, the roots were cut under water spray cooling system to avoid the heating effect during cutting that might affect the gutta-percha obturation, resulting in errors in the bond strength testing. Slicing of the roots for the push out test was made 2mm thick to permit adequate thickness for obturating materials and to prevent premature debonding during slicing. Using a digital calliper, the thickness of each slice was

determined to eliminate the impact of difference in specimen thickness. The plunger tip was dimensioned and positioned, without touching the canal walls, to touch only the filling material.

Sections were taken from the middle third of the root because dentinal tubules and collagen fibers are more homogeneously distributed in the middle third of the root than in other root thirds. Also, the matching of the canal taper with the used gutta-percha might have proper fitting of the middle third to the prepared canal, leading to the uniform spreading of the sealer and a good bonding.[2]

The push-out test was performed on each specimen with a UTM at a crosshead speed of 1 mm/min using cylindrical pistons measuring 0.8mm. The largest load applied to the filling material before failure was recorded in newtons and converted to megapascals (MPa) according to the formula below:

Push-out bond strength (MPa) = Force for dislodgement (Pushload) (N)/ Surface area (A) (mm^2)

In the present study, the highest bond strength values were observed in the TAP group (Table 5). This may be attributed to multiple reasons, most likely the binding of residual Minocycline through chelation to the calcium ions, which could improve the strength of the bond after TAP was applied.[43] In addition, the increased bond strength of TAP can be correlated with small quantities of irrigation reaching the apex by the extension of a greater residual Minocycline presence. The residual antibiotic paste on the root canal walls was not tested in the present research. Future experiments should be carried out to clarify the mechanisms between the materials and residual Minocycline.

Chitosan has shown to have the properties of a chelating solution and the highest effect was seen when it was solubilized in Acetic acid, similar to the 2% Chitosan used in this study.[44] Chelating solutions have the ability to eliminate smear layer, and expose large number of dentinal tubules[45] which then encourages adhesion due to an increased contact area that would warrant better adaptation amid the sealer and root canal dentin.[46]

Owing to the minimal erosion and chelating effects, and in light of the above information, Chitosan may have increased the bond strength to dentin of the sealers tested in this study in the Antibiotic-Chitosan Groups (Groups II & IV).

The mineral component of dentin adds to the strength of the structure of the tooth and the comparatively long-term exposure to antibiotic pastes of radicular dentin leads to a substantial increase in the susceptibility of the root to fracture.[7] The large decrease in the phosphate/amide I ratio of dentin treated with TAP and DAP indicates the demineralization effect of these pastes and the development of a collagen-rich matrix on the surface of the radicular dentin as evidenced by SEM images.[7] Compared to DAP treated dentin, the substantial drop in the phosphate/amide I ratio of TAP treated dentin may be explained by the lower pH of TAP and the ability of Minocycline found in TAP to chelate calcium and demineralize hard dental tissues.[7] This could have contributed to the difference in the pushout bond strength values among the TAP (Groups I & II) and DAP (Groups III & IV) groups.

According to Deus et al, when the smear layer is removed from the root canal wall, endodontic sealers penetrate to the dentinal tubules and increase adhesion to the root canal dentin. According to Neto et al, the covalent bonding of the epoxy group to the organic part of dentin (the collagen amine group released in dentin) may be the

reason for the chemical bonding of AH Plus to the dentin molecule. Previous studies have emphasized that the high bond strength of AH Plus could be due to the low polymerization stress of the sealer and its long-term dimensional stability.[3]

The results of this study showed that there was a significant increase in the pushout bond strength with the TAP + Chitosan group (5.33 MPa) compared to TAP + Distilled water (3.71 MPa) group. Hence the type of vehicle used had an effect on the pushout bond strength of epoxy-resin based sealer to pretreated dentin. Among the two vehicles tested, Chitosan improved the push out bond strength and reduced the negative effects of TAP on the radicular dentin which could be attributed to its dissociation into free hydroxyl group and amino group. These can interact to form chemical bonds with collagen, which reinforces the dentinal collagen by the cross-linking of collagen and neutralization of matrix metalloproteinases, thereby increasing the resistance to degradation by bacterial collagenase.[8] In addition, Chitosan exhibits electrostatic affinity to collagen that is dependent on local environmental factors, including the relative proximity of the collagen carboxyl groups (-COO-) to the chitosan amino (NH3+) groups and hydrogen bonding mediated chitosan associations.[8]

Distilled water did not have any effect on the detrimental properties of TAP and DAP on root dentin, unlike Chitosan which had a positive effect which might have resulted in increased bond strengths in the Antibiotic-Chitosan groups (Group II & IV) than the control groups (Group I & III).

Therefore, the null hypothesis that there will be no difference in the effect of TAP and DAP with Chitosan as the vehicle on the push out bond strength of Epoxy resin–based sealer to radicular dentin was rejected.

CONCLUSION:

Under the limitations of present research, it can be concluded that:

1) TAP showed superior performance when compared to DAP when used as an intracanal medicament in terms of bond strength to radicular dentin.
2) Chitosan when used as the vehicle improves the push–out bond strength of Epoxy resin–based sealer and reduces the negative effects of TAP and DAP on radicular dentin ($p<0.01$).
3) Combination of TAP with Chitosan (Group II) had the highest bond strength value (5.33 MPa).

SUMMARY

Bacteria and their products are considered to be the cause of pulp necrosis and periapical lesions. To ensure the complete elimination of root canal bacteria, it is necessary to use effective antibacterial agents for a predetermined period of time to predictably eradicate residual bacteria. Thus, biomechanical preparation alongside intracanal medication is essential for complete disinfection of the root canal system. CH is most commonly practiced intracanal medicament, but certain studies have shown its resistance against *E.faecalis* and prolonged exposure to radicular dentin resulted in reduced fracture resistance. Consequently, this led to the development of TAP. Tap has thus been the most widely used intracanal medicinal agent in aiding endodontic revascularization and healing of larger periapical lesions. However, studies have shown that Minocycline can cause visible crown discoloration and therefore, was eliminated in DAP.

The selection of vehicles related to intracanal medicament is of supreme importance, because they are responsible for the delivery of the drug inside the root canal system and for its diffusion. Macrogol and Propylene glycol (PEG) were used by Hoshino et al for delivery of TAP. Recently, a newer novel vehicle Chitosan has been extensively studied. Chitosan can be used as a drug carrier to increase the stability and insolubility of the intracanal medicament, where it has the additional advantage of slow controlled release of the medicament.

There are very few studies to evaluate the effect of TAP with new and novel vehicles on the mechanical properties of radicular dentin. Keeping these rationales in

mind, this study aims to explore the effect of TAP and DAP with Chitosan as the vehicle on the push out bond strength of Epoxy resin–based sealer to radicular dentin.

The study was conducted in the Department of Conservative Dentistry and Endodontics, Viswanath Katti Institute of Dental Sciences, KAHER Belagavi.

80 extracted human mandibular premolar teeth cleaned of calculus and soft tissue were stored in 0.1% Thymol and were radiographed and selected as per the inclusion and exclusion criteria. All teeth were then decoronated up to the level of CEJ with the help of a diamond disc under copious water spray to produce a standardized root length of 15mm. WL was established by subtracting 1mm from the standardized root length.

The root canals were prepared using ProTaper rotary instruments up to F4. Irrigation was done with 2ml of 3% NaOCl between successive files. Final irrigation was done with 5 ml 17% EDTA acid for 1 minute. The canals were then dried with sterile absorbent paper points.

TAP was obtained by mixing with Sterilized Distilled water or Chitosan following the standard protocol in 3:1 ratio. DAP was obtained by mixing with Sterilized Distilled water or Chitosan following the standard protocol in 2:1 ratio. Dilution of 2g Chitosan was done in 100 ml 1% Acetic acid and stirred with magnetic stirrer.

The prepared paste was placed in each root canal using a #35 lentulospiral. The coronal access was sealed with Cavit G. Specimens were then incubated at 37°C in 100% humidity for 3 weeks to simulate clinical conditions.

The teeth were then divided into 4 groups (n=20) depending on the medicament and vehicle used as follows.

Group 1: *TAP + Distilled Water*

Group 2: *TAP + Chitosan*

Group 3: *DAP + Distilled Water*

Group 4: *DAP + Chitosan*

After 3 weeks the medicaments were rinsed with 10ml 17% EDTA followed by 10 ml of 3% NaOCl and the final irrigation was done with 5ml of distilled water. The root canals were obturated with Gutta-percha points and AH Plus sealer and specimens were stored at 100% humidity at 37^{o}C for one week.

The root samples were cut into 2mm thick cross sections in the middle third of the roots using a diamond disk. A push out force was applied with a cylindrical piston measuring 0.8mm in diameter at a cross-head speed of 1mm/min perpendicular to the sample surface using a UTM.

Statistical analysis of the data obtained was done by One-way ANOVA and Newman-Keuls multiple Post Hoc. The results indicated that there is significant difference between all the four groups (p value <0.01).

Among the groups tested, the TAP groups performed better than the DAP groups. This could be because of the large decrease in phosphate/amide I ratio in TAP treated dentin compared with DAP treated dentin, which can be explained by the lower pH of TAP and the ability of Minocycline present in TAP to chelate calcium and demineralize dental hard tissues.

Among all the four groups, Group II (TAP+CHITOSAN) performed best, this could be attributed to its dissociation into free hydroxyl group and amino group which can interact to form chemical bonds with collagen, which reinforces the dentinal collagen by the cross-linking of collagen and neutralization of matrix metalloproteinases, thereby increasing the resistance to degradation by bacterial collagenase.

Chitosan has shown to have the properties of a chelating solution and the highest effect was seen when it was solubilized in Acetic acid, similar to the 2% Chitosan used in this study. Chelating solutions have the ability to eliminate smear layer, and expose large number of dentinal tubules, which then encourages adhesion due to an increased contact area that would warrant better adaptation amid the sealer and root canal dentin.

Distilled water did not have any effect on the detrimental properties of TAP and DAP on root dentin, unlike Chitosan which had a positive effect which might have resulted in increased bond strengths in the Antibiotic-Chitosan groups (Group II & IV) than the control groups (Group I & III).

Therefore, null hypothesis that there is no difference in the effect of TAP and DAP with Chitosan as the vehicle on the push out bond strength of Epoxy resin–based sealer to radicular dentin was rejected.

BIBLIOGRAPHY

1. Arslan H, Capar ID, Saygili G, et al. Efficacy of various irrigation protocols on the removal of triple antibiotic paste. *Int Endod J*. 2014;47(6):594-99.
2. Assmann E, Scarparo RK, Böttcher DE, Grecca FS. Dentin bond strength of two mineral trioxide aggregate-based and one epoxy resin-based sealers. *J Endod*. 2012;38(2):219-21.
3. Shaik J, Garlapati R, Nagesh B, Sujana V, Jayaprakash T, Naidu S. Comparative evaluation of antimicrobial efficacy of triple antibiotic paste and calcium hydroxide using chitosan as carrier against Candida albicans and Enterococcus faecalis: An in vitro study. *J Conserv Dent*. 2014;17(4):335-39.
4. Carreira Cde M, dos Santos SS, Jorge AO, Lage-Marques JL. Antimicrobial effect of intracanal substances. *J Appl Oral Sci*. 2007;15(5):453-58.
5. Akcay M, Arslan H, Topcuoglu HS, Tuncay O. Effect of calcium hydroxide and double and triple antibiotic pastes on the bond strength of epoxy resin-based sealer to root canal dentin. *J Endod*. 2014;40(10):1663-67.
6. Minabe M, Takeuchi K, Kumada H, Umemoto T. The effect of root conditioning with minocycline HCl in removing endotoxin from the roots of periodontally-involved teeth. *J Periodontol*. 1994;65(5):387-92.
7. Yilmaz S, Dumani A, Yoldas O. The effect of antibiotic pastes on microhardness of dentin. *Dent Traumatol*. 2016;32(1):27-31.
8. Hegde V, Arora S. Effect of intracanal medicaments on push-out bond strength of Smart-Seal system. *J Conserv Dent*. 2015;18(5):414-18.
9. Carvalho CN, Grazziotin-Soares R, de Miranda Candeiro GT, et al. Micro Push-out Bond Strength and Bioactivity Analysis of a Bioceramic Root Canal Sealer. *Iran Endod J*. 2017;12(3):343-48.

10. Smyth HF Jr, Carpenter CP, Weil CS. The chronic oral toxicology of the polyethylene glycols. *J Am Pharm Assoc Am Pharm Assoc*. 1955;44(1):27-30.
11. Li BQ, Dong X, Fang SH, Gao JY, Yang GQ, Zhao H. Systemic toxicity and toxicokinetics of a high dose of polyethylene glycol 400 in dogs following intravenous injection. *Drug Chem Toxicol*. 2011;34(2):208-12.
12. Prentice DE, Majeed SK. Oral toxicity of polyethylene glycol (PEG-200) in monkeys and rats. *Toxicol Lett* 1978;2(3):119–22.
13. Oktay EA, Ersahan S, Gokyay S. Effect of intracanal medicaments used in endodontic regeneration on the push-out bond strength of a calcium-phosphate-silicate-based cement to dentin. *Pak J Med Sci*. 2018;34(2):310-15.
14. Parhizkar A, Nojehdehian H, Asgary S. Triple antibiotic paste: momentous roles and applications in endodontics: a review. *Restor Dent Endod*. 2018;43(3):e28.
15. Cicciù M, Fiorillo L, Cervino G. Chitosan Use in Dentistry: A Systematic Review of Recent Clinical Studies. *Mar Drugs*. 2019;17(7):417.
16. Estrela C, Pesce HF. Chemical analysis of the liberation of calcium and hydroxyl ions from calcium hydroxide pastes in connective tissue in the dog--Part I. *Braz Dent J*. 1996;7(1):41-6.
17. Fava LR, Saunders WP. Calcium hydroxide pastes: classification and clinical indications. *Int Endod J*. 1999;32(4):257-82.
18. Kotadia P, Singh S, Raghavendra S, Srinidhi. Comparative evaluation of contact angle of calcium hydroxide and triple antibiotic paste using chitosan as carrier. *Int J Current Research.* 2017;9(5):50685-88.
19. Shakouie S, Shahi S, Samiei M, et al. Effects of different intra canal medicaments on the push out bond strength of endodontic sealers. *J Clin Exp Dent.* 2017;9(3):e443-47.

20. Gündoğar, M. Impact of intracanal calcium hydroxide or triple antibiotic paste on bond strength of root canal sealers: an in vitro study. *Cumhuriyet Dental Journal.* 2017;19: 229-37.
21. Amin SA, Seyam RS, El-Samman MA. The effect of prior calcium hydroxide intracanal placement on the bond strength of two calcium silicate-based and an epoxy resin-based endodontic sealer. *J Endod*. 2012;38(5):696-99.
22. Varalakshmi R, Parasuraman BS, Muljibhai BS. 3Mix-MP in endodontics—an overview. *IOSR-JDMS.* 2012;3(01):36-45.
23. Thakur DA, Patil S, Gade V, Jogad N, Gangrade A, Sinkar R. Comparative scanning electron microscopy evaluation of Canal Brushing technique, sonic activation, and master apical file for the removal of triple antibiotic paste from root canal (in vitro study). *Contemp Clin Dent*. 2015;6(4):517-21.
24. Deepak BS, Sherin JC, Mallikarjun Goud K. Removal of intracanal medicaments – A Review. *UJMDS.* 2015;03(03):14-22.
25. Jasrotia A, Farooq R, Bhagat RK, Bhagat K, Sharma N, Habib ST. Removal efficacy of various calcium hydroxide/chlorhexidine medicaments from the root canal with the aid of biological microscope. *Indian J Dent Sci* 2019;11(1):1-6.
26. Lana PE, Scelza MF, Silva LE, Mattos-Guaraldi AL, Hirata Júnior R. Antimicrobial activity of Calcium Hydroxide pastes on Enterococcus faecalis cultivated in root canal systems. *Braz Dent J*. 2009;20(1):32-6.
27. Krishan S, Gupta S. Comparative histological and morphometric analysis of the effectiveness of manual and different recently introduced rotary instruments: An *in vitro* study. *Indian J Oral Sci* 2016;7(10):83-6.
28. Zehnder M. Root canal irrigants. *J Endod*. 2006;32(5):389-98.
29. Miller EK, Lee JY, Tawil PZ, Teixeira FB, Vann WF Jr. Emerging therapies for the management of traumatized immature permanent incisors. *Pediatr Dent.* 2012;34(1):66-9.

30. Kim JH, Kim Y, Shin SJ, Park JW, Jung IY. Tooth discoloration of immature permanent incisor associated with triple antibiotic therapy: a case report. *J Endod*. 2010;36(6):1086-91.
31. Akcay M, Arslan H, Yasa B, Kavrık F, Yasa E. Spectrophotometric analysis of crown discoloration induced by various antibiotic pastes used in revascularization. *J Endod*. 2014;40(6):845-48.
32. Dall AQK, Tasleem F, Khan MF, Channar KA, Batool S, Bari MA. Reduction of Pain and Bacterial Presence by using Double and Triple Antibiotic Pastes in Symptomatic Apical Periodontitis. *J Liaquat Uni Med Health Sci.* 2019;18(02): 129-35
33. Behnen MJ, West LA, Liewehr FR, Buxton TB, McPherson JC 3rd. Antimicrobial activity of several calcium hydroxide preparations in root canal dentin. *J Endod*. 2001;27(12):765-67.
34. Bystrom A, Claesson R, Sundqvist G. The antibacterial effect of camphorated paramonochlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals. *Endod Dent Traumatol*. 1985;1(5):170-75.
35. Stevens RH, Grossman LI. Evaluation of the antimicrobial potential of calcium hydroxide as an intracanal medicament. *J Endod*. 1983;9(9):372-74.
36. Hashem AA, Ghoneim AG, Lutfy RA, Fouda MY. The effect of different irrigating solutions on bond strength of two root canal-filling systems. *J Endod*. 2009;35(4):537-40.
37. Lee KW, Williams MC, Camps JJ, Pashley DH. Adhesion of endodontic sealers to dentin and gutta-percha. *J Endod*. 2002;28(10):684-88.
38. Ghabraei S, Bolhari B, Yaghoobnejad F, Meraji N. Effect of Intra-Canal Calcium Hydroxide Remnants on the Push-Out Bond Strength of Two Endodontic Sealers. *Iran Endod J*. 2017;12(2):168-72.

39. Guiotti FA, Kuga MC, Duarte MA, Sant'Anna AJ, Faria G. Effect of calcium hydroxide dressing on push-out bond strength of endodontic sealers to root canal dentin. *Braz Oral Res*. 2014;28. doi: 10.1590/S1806-83242014.50000002.
40. Ustun Y, Arslan S, Aslan T. Effects of calcium hydroxide and propolis intracanal medicaments on bond strength of resin-based endodontic sealer as assessed by push-out test. *Dent Mater J*. 2013;32(6):913–9.
41. Galler KM. Clinical procedures for revitalization: current knowledge and considerations. *Int Endod J*. 2016;49(10):926-36.
42. Rödig T, Vogel S, Zapf A, Hülsmann M. Efficacy of different irrigants in the removal of calcium hydroxide from root canals. *Int Endod J*. 2010;43(6):519-27.
43. Tanase S, Tsuchiya H, Yao J, Ohmoto S, Takagi N, Yoshida S. Reversed-phase ion-pair chromatographic analysis of tetracycline antibiotics. Application to discolored teeth. *J Chromatogr B Biomed Sci Appl*. 1998;706(2):279-85.
44. da Silva Mira PC, Souza-Flamini LE, da Costa Guedes DF, Da Cruz-Filho AM. Evaluation of the chelating effect of chitosan solubilized in different acids. *J Conserv Dent*. 2017;20(5):297-301.
45. Torabinejad M, Handysides R, Khademi AA, Bakland LK. Clinical implications of the smear layer in endodontics: a review. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2002;94(6):658-66.
46. Sayin TC, Serper A, Cehreli ZC, Kalayci S. Calcium loss from root canal dentin following EDTA, EGTA, EDTAC, and tetracycline-HCl treatment with or without subsequent NaOCl irrigation. *J Endod*. 2007;33(5):581-84.

ANNEXURE – I

ETHICAL CLEARANCE

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

Sl. No.: 1223

CERTIFICATE

This is to Certify that the synopsis titled

Comparative evaluation of the effect of triple antibiotic paste and double antibiotic paste with chitosan as a vehicle on the push out bond strength of epoxy resin- based sealers to the radicular dentin - An in vitro study *Submitted by*

Dr. Ashish Ramakrishnan *P. G. Student /*

Staff, Guided by Dr Anand C Patil *from Department of* Conservative dentistry & endodontics *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date : 24/06/2019

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

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

ANNEXURE – II

BIOSTATISTIC CLEARANCE CERTIFICATE

KLE V.K. Institute of Dental Sciences
(A Constituent unit of KLE Academy of Higher Education & Research
Deemed-to-be-University u/s 3 of the UGC Act, 1956)
Nehru Nagar, Belagavi-590 010 INDIA
Re-Accredited 'A' grade by NAAC (3rd Cycle) & Placed in Category 'A' by MHRD (GoI)
Ph: 0831 2470352
FAX: 0831-2470640
Web: http://www.kledental-bgm.edu.in
E-mail: principal@kledental-bgm.edu.in

Biostatistics Clearance Certificate

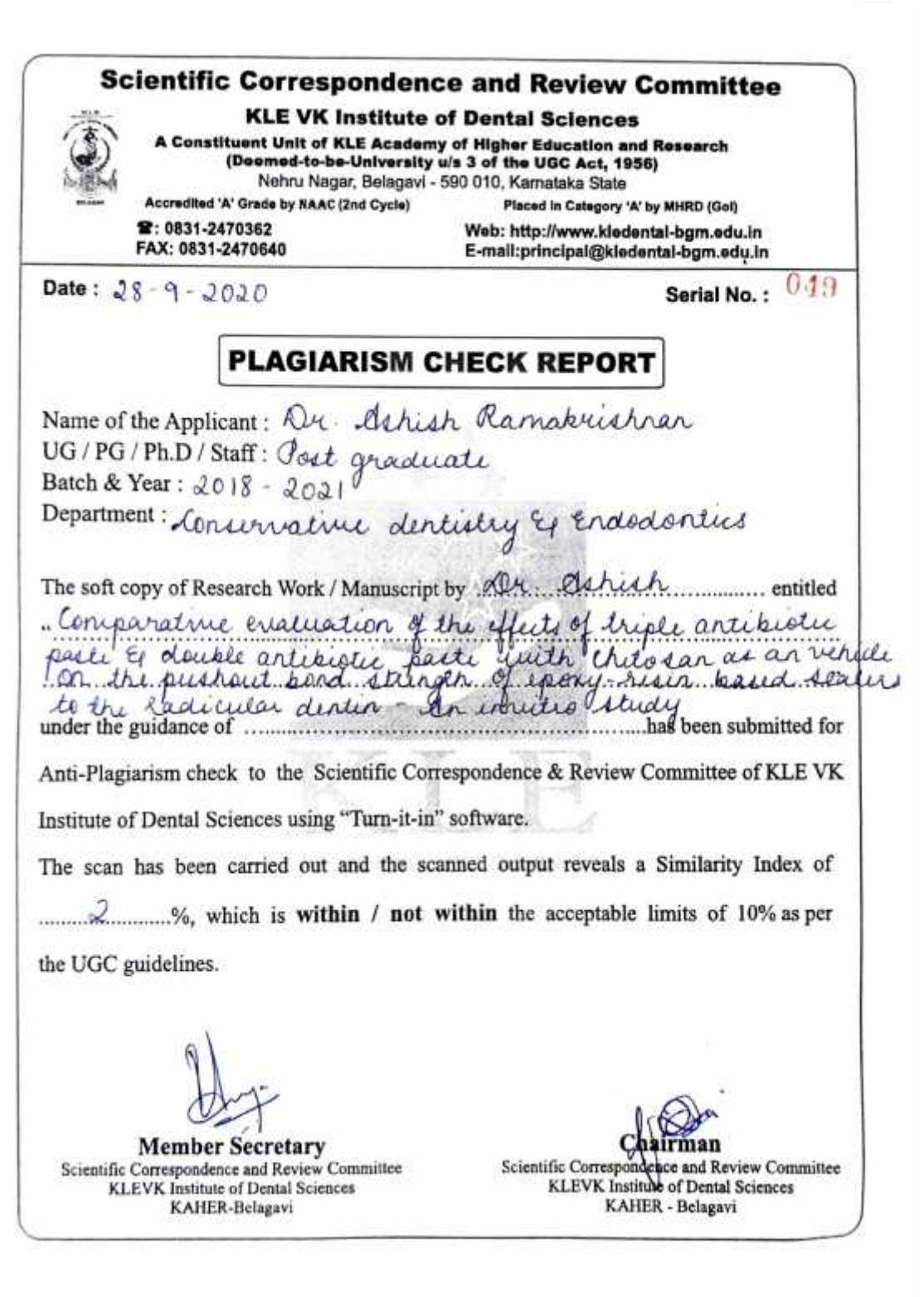
This is to certify that the Biostatistics aspect of the Dissertation / Research work of... Dr. Ashish Ramakrishnan ..... entitled 'Comparative evaluation of the effect of Triple antibiotic paste and Double antibiotic paste with Chitosan as a vehicle on the push out bond strength of epoxy-resin based sealers to the radicular dentin. - An in vitro study.' has been done under my guidance and considered satisfactory.

Place : Belagavi
Date : 22/9/2020

Name & Signature of Biostatistician
(Dr. S. B. Jamadar)

ANNEXURE – III

PLAGIARISM CHECK CERTIFICATE



Scientific Correspondence and Review Committee

KLE VK Institute of Dental Sciences

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

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

☎: 0831-2470362
FAX: 0831-2470640

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

Date : 28-9-2020 Serial No. : 019

PLAGIARISM CHECK REPORT

Name of the Applicant : Dr. Ashish Ramakrishnan
UG / PG / Ph.D / Staff : Post graduate
Batch & Year : 2018 - 2021
Department : Conservative dentistry & Endodontics

The soft copy of Research Work / Manuscript by Dr. Ashish ..... entitled
"Comparative evaluation of the effects of triple antibiotic paste & double antibiotic paste with Chitosan as an vehicle on the pushout bond strength of epoxy resin based sealers to the radicular dentin - An invitro study"
under the guidance of .....has been submitted for

Anti-Plagiarism check to the Scientific Correspondence & Review Committee of KLE VK Institute of Dental Sciences using "Turn-it-in" software.

The scan has been carried out and the scanned output reveals a Similarity Index of .....2.....%, which is **within / not within** the acceptable limits of 10% as per the UGC guidelines.

Member Secretary
Scientific Correspondence and Review Committee
KLEVK Institute of Dental Sciences
KAHER-Belagavi

Chairman
Scientific Correspondence and Review Committee
KLEVK Institute of Dental Sciences
KAHER - Belagavi

Introduction

Aim and Objectives

Hypothesis

Review of Literature

Methodology

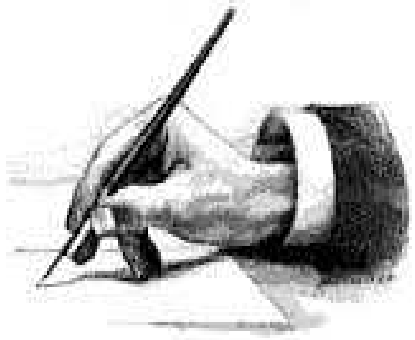
Results

Discussion

Conclusion

Summary

Bibliography



Annexures