"DENTINAL TUBULE PENETRATION OF A SILICONE-BASED ENDODONTIC SEALER FOLLOWING N- ACETYL CYSTEINE INTRACANAL MEDICAMENT REMOVAL USING ULTRASONIC AGITATION AND LASER-ACTIVATED IRRIGATION- AN IN- VITRO STUDY."

By

REG.NO. IE0221003

Dissertation

Submitted to the
KLE Academy of Higher Education & Research Belagavi, Karnataka
In partial fulfillment of the requirements for the degree of

MASTER OF DENTAL SURGERY

In

CONSERVATIVE DENTISTRY AND ENDODONTICS

(BRANCH – IV)

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

2021-2024

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 **"DENTINAL TUBULE PENETRATION OF A SILICONE-BASED ENDODONTIC SEALER FOLLOWING N- ACETYL CYSTEINE INTRACANAL MEDICAMENT REMOVAL USING ULTRASONIC AGITATION AND LASER-ACTIVATED IRRIGATION- AN IN- VITRO STUDY"** *is a bonafide research work done by* **REG. NO. IE0221003.**

Head of Department

Dr. SONAL B. JOSHI M.D.S
Dean & Head of Department,
Department of Conservative Dentistry and Endodontics,
KAHER V.K. Institute of Dental Sciences,
Belagavi.

Date: 23rd APRIL 2024
Place: Belagavi

Principal

Dr. ALKA KALE M.D.S
Principal,
KAHER V. K. Institute of Dental Sciences,
Belagavi

Date : 23/4/24
Place : Belagavi

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

UNDERTAKING

I, **REG.NO. IE0221003** hereby declare that the information and data nentioned in my thesis entitled **"DENTINAL TUBULE PENETRATION OF A SILICONE-BASED ENDODONTIC SEALER FOLLOWING N- ACETYL CYSTEINE INTRACANAL MEDICAMENT REMOVAL USING ULTRASONIC AGITATION AND LASER-ACTIVATED IRRIGATION- AN IN- VITRO 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.

Date:

Place:Belagavi **REG.NO. IE0221003**

LIST OF ABBREVIATIONS

SR.NO	ABBREVIATIONS	FULL FORM
1	NaOCl	Sodium Hypochlorite
2	NAC	N-acetyl cysteine
3	EDTA	Ethylene Diamine Tetra-acetic Acid
4	NAC	N- acetyl cysteine
5	PUI	Passive Ultrasonic Irrigation
6	SNI	Syringe Needle Irrigation
7	+	Plus
8	WL	Working Length
9	ANOVA	Analysis of Variance
10	SD	Standard Deviation
11	n	Number of specimens
12	p-value	Probability of obtaining a test statistic at least as extreme as the one that was actually observed
13	<	Less than
14	>	Greater than
15	μm	Micrometers
16	kHz	Kilohertz
17	&	And
18	CH	Calcium Hydroxide
19	CHX	Chlorhexidine
20	Grp	Group
21	ml	Milliliter
22	° C	Degree Celsius
23	mm	Millimeter
24	i.e.	That is
25	SE	Standard error
26	CLSM	Confocal Laser Scanning Mircroscope

ABSTRACT

Aim and Objectives: To evaluate and compare the dentinal tubule penetration of a silicone-based endodontic sealer following N- acetyl cysteine intracanal medicament removal using Ultrasonic Agitation and Laser-Activated Irrigation- an In- vitro study.

Study design: Eighty one extracted human mandibular premolar single rooted teeth were selected, disinfected and decoronated to obtain a standardized root length of 12 mm. The teeth were prepared with ProTaper Universal rotary files upto MAF F3. **PREPARATION OF INTRACANAL MEDICAMENT:** N-acetyl cysteine powder was mixed with propylene glycol in the ratio of 1:1. N- acetyl cysteine intracanal medicament placed using a size #30 Lentulospiral until visible at the apical foramen. The orifice was sealed with Cavit, and specimens stored in an incubator at 37°C with 100% humidity for 14 days. After the incubation period, the specimens were instrumented with #30 Hedström files supplemented with 5 mL of 3% NaOCl to remove the medicament. Then, the specimens were divided into three groups according to irrigant activation techniques for medicament removal:

Group 1: Diode laser activation

Group 2: Passive Ultrasonic agitation

Group 3: No agitation (positive control)

The canals were rinsed with 5ml of saline solution and dried with paper points and were prepared for obturation. GuttaFlow bioseal sealer was with 0.1% Rhodamine B dye. The canals were obturated with gutta-percha cones in combination with the GuttaFlow bioseal sealer using a single cone technique. Teeth were then sealed with

Cavit and incubated at 37°C and 100% humidity for a week to simulate clinical conditions. The specimens were sectioned horizontally using a diamond disc to obtain 1 mm thick sections from 2, 5 and 8-mm levels from the apex. Sections were examined under confocal laser scanning microscope to measure depth of sealer penetration (in µm) into the dentinal tubules using Image J software.

Results: The highest mean depth of penetration of 728.52µm was seen with Diode laser activation group (Group I) when compared with Passive Ultrasonic agitation (Group II) and Positive control (Group III). Coronal third region of 738.85µm when compared to middle and apical third region, showed highest mean depth of penetration. Thus, highest mean depth of penetration of 824.21 µm was seen in Diode laser activation group in the coronal third region.

Conclusion: Within the limitations of the present study, Thus, it can be concluded that Diode laser activation group was most effective in removal of NAC intracanal medicament from the root canals.

Key words: Diode laser activation, Passive Ultrasonic agitation, Syringe Needle Irrigation, GuttaFlow bioseal, N-acetyl cysteine, Confocal Laser Scanning Microscope

TABLE OF CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF FIGURES

ANNEXURE

INTRODUCTION

The primary goal of endodontic therapy is to eradicate micro-organisms and to prevent endodontic reinfection.[1] The basic requirement of endodontic treatment is effective chemo-mechanical preparation and three-dimensional obturation.[2] Hence, obturation is associated with gutta-percha as core substance and endodontic sealer for improved adaptation of obturating material.[3]

Endodontic sealers ought to have good dentin adaptability with radicular dentin and seal the canal apically and laterally. The sealer can flow and fill the dentinal tubules and irregularities present in canal walls unable to fill by gutta-percha.[4] The dentin tubular penetration of sealer is a necessary feature, as it improves the bond between the sealer and radicular dentine.[5]

The use of bioactive sealers, like calcium silicate based sealers, has grown in popularity. A novel material, GuttaFlow bioseal contains calcium silicate and gutta-percha. It is a cold-filling sealer made of bioactive glass and gutta percha powder.[6] The bioceramic sealer's deeper penetration can be explained by its hydrophilicity, low contact angle, and small particle size.[7]

It is challenging to totally eradicate bacteria from the pulp space, it is especially challenging to get rid of sessile bacteria of various species that have accumulated on the surfaces of root canal.[8] It has been suggested to use intracanal medicaments to disrupt biofilms and eliminate any residual bacterial infections to improve the outcome of root canal therapy.[9]

Various studies have been carried by using different materials as intracanal medicaments, like pastes based on calcium hydroxide, chlorhexidine, steroids, triple and double antibiotic pastes.[10]

Calcium hydroxide (CH) has antimicrobial, organic tissue-dissolving, and anti-inflammatory properties, hence it is widely utilized in endodontics. Several microbial species, notably Enterococcus faecalis, are found to be resistant to the effects of CH, despite the medicament's efficacy when used in the canal.[11]

Enteroccocus faecalis, is a prominent factor in development of peri-radicular lesions. E. faecalis can live in the root canal in planktonic form or as a part of biofilm and is present in 22–77% of endodontic failed cases.[12]

Chlorhexidine though is efficacious against E. facalis, it has poor capacity to reach deep biofilm layers and its inactivation by physiological salts restrict its application as an intracanal medication.[13]

Although corticosteroids are present in commercial pastes containing antibiotics, such as Ledermix and Septomixine Forte, none of these pastes are suited for treatment against endodontic microbiota due to their limited range of action.[14]

Another example of an intracanal medicament is triple antibiotic paste (TAP). It is made up of minocycline, metronidazole, and ciprofloxacin. The root canal system was successfully disinfected with this mixture during pulpal regeneration and root canal therapy. The primary side effect of TAP is minocycline-induced tooth discoloration.[15]

Numerous natural extracts, including papain, aloe vera, M. citrifolia, turmeric, propolis have also been studied for their antimicrobial activity against E faecalis. In a

study by Bhardwaj and co-workers, on natural extracts as intracanal medicaments concluded, chlorhexidine gel showed the maximum and calcium hydroxide the least anti-microbial activity towards *E. faecalis,* whereas *M. citrifolia* gel when studied had good antimicrobial properties against E faecalis.[16]

Agrima Vasudeva et al studied herbal extracts as intracanal medicaments, concluded Propolis and turmeric demonstrated good activity against E. faecalis and are hence suitable for use as intracanal medicaments.[17] Due to insufficient in vitro and in vivo studies there is not enough evidence on antimicrobial efficacy of herbal extracts as intracanal medicaments.

N-acetyecysteine, a strong thiol-containing anti-oxidant and mucolytic drug, breaks down di-sulfide bonds in mucous and lowers the viscosity of secretion. It is a derivative of the L-cysteine, an amino acid.[18]

NAC is a potentially effective treatment drug for disorders involving oxidative stress, due to its antioxidant and anti-inflammatory effects.[19] It is still unknown how exactly NAC delivers its cytoprotective and antioxidant benefits.[20] It's commonly assumed that NAC works as a disulfide reductant, a precursor to glutathione (GSH) production, and a scavenger of reactive oxygen species to produce effects it does.[21] A different theory of action was put up that would account for the effects ascribed to NAC. This theory involved NAC being converted into sulfur species known to have cytoprotective and antioxidant qualities.[19]

NAC's main mode of action is to decrease the synthesis of extracellular polysaccharides, which breaks down mature biofilms and lessens bacterial adhesion to surfaces.[22]

The removal of medicament, hard tissue debris or inorganic debris from radicular dentin wall is essential to achieve better adaptation of sealer.[23] Various factors affect the depth of sealer penetration like smear layer cleanliness, permeability of dentin, physio-chemical properties of sealer.[24]

The residual smear layer and medicament on the walls of radicular dentin affect penetration of sealer into dentinal tubules, hence the most effective technique of final irrigation used for medicament removal will allow maximum depth of penetration of sealer into dentinal tubules and can specify cleanliness of canals and the available dentin surface for sealer penetration.[25,26]

Methods such as SNI, CanalBrush, PUI, self-adjusting file, EndoActivator, and laser-activated irrigation are recommended for maximizing the elimination of intracanal medicament. On the other hand, agreement on the most effective approach is still pending.[28]

The elimination of medicament is commonly accomplished by the use of conventional syringe irrigation. It entails either passively or actively agitating the canal by moving the irrigant back and forth. The needle's gauge is inversely correlated with its penetration depth.[29]

Passive-ultrasonic irrigation is among most widely used and proven irrigation techniques. By using cavitation and acoustic micro streaming, PUI might enhance cleaning in intricate canal anatomic locations. The liquid moves in a fast, vortex-like manner due to the acoustic streaming, and cavitation creates spontaneous cavities in the liquid, which helps the irrigants enter the dentinal tubules more effectively.[30] The Passive ultrasonic irrigation was formerly regarded as the gold standard and believed

to be more efficient than self-adjusting file, syringe and needle irrigation, Endo Activator and CanalBrush in removing medicaments from the root canal.[28]

Research has demonstrated that laser-activated irrigation is a viable method for root canal cleaning. High-intensity diode lasers that emits at 980 nm has been explored for its applications in dental field. In endodontics, the Diode Laser has demonstrated encouraging outcomes in terms of smear layer reduction and root canal disinfection.[2] In contrast to other available near-infrared wavelengths the 940nm wavelength and 980 nm wavelength diode laser are significantly better absorbed.[33]

Previously inaccessible areas of root canal are now be more effectively disinfected due to diode lasers.[34] Changes in dentin permeability, apical leakage, and sealer adherence to root canal can all be directly linked to laser therapy.[35] Endodontic sealers may perform better because of the morphological changes in the dentin walls brought on by irradiation with diode laser.[36]

To our knowledge, no research has been done on penetration of GuttaFlow bioseal sealer into dentinal tubules following removal of NAC medicament with different irrigation techniques. This study investigated dentinal tubule penetration of GuttaFlow bioseal by using confocal laser scanning microscope following NAC intracanal medicament removal using three different irrigation techniques namely, laser activated irrigation using high power diode laser, passive ultrasonic agitation and no agitation as positive control.

AIM AND OBJECTIVES

AIM

To evaluate and compare dentinal tubule penetration of GuttaFlow bioseal sealer after the removal of N-acetyl cysteine intracanal medicament with ultrasonic agitation and laser activated irrigation using Confocal Laser Scanning Microscope.

OBJECTIVES

1. To evaluate dentinal tubule penetration of GuttaFlow bioseal sealer after N-acetyl cysteine intracanal medicament removal using ultrasonic agitation.
2. To evaluate sealer dentinal tubules penetration of GuttaFlow bioseal after N-acetyl cysteine intracanal medicament removal using laser activated irrigation.
3. To compare dentinal tubule penetration of GuttaFlow bioseal sealer after N-acetyl cysteine intracanal medicament removal using ultrasonic agitation and laser activated irrigation.

HYPOTHESIS

NULL HYPOTHESIS: -

There will be no difference in dentinal tubule penetration of GuttaFlow bioseal sealer after the removal of N-acetyl cysteine intracanal medicament using ultrasonic agitation and laser activated irrigation.

ALTERNATE HYPOTHESIS: -

There will be a difference in dentinal tubule penetration of GuttaFlow bioseal sealer after the removal of N-acetyl cysteine intracanal medicament using ultrasonic agitation and laser activated irrigation.

REVIEW OF LITERATURE

1. An vitro study by Özlek E, Neelakantan P, et al. evaluated effects of laser-activated irrigation methods or sonic agitation on the elimination of modified triple antibiotic paste and chlorhexidine (CHX) on Guttaflow Bioseal's dislocation resistance and sealer penetration depth.

   The study concluded that Laser activated irrigation had greater depth of sealer penetration compared to other groups.[37]

2. An vitro study by Sunanda Gaddalay et al, evaluated effect of AH Plus- resin-based sealer, Gutta flow Bioseal-silicone based sealer, MTA Fillapex- calcium hydroxide based sealers on dentinal tubule penetration after passive ultrasonic agitation and assessed under confocal laser scan microscope. The study concluded that Guttaflow Bioseal showed a significant difference dentinal tubule penetration and tubule penetration was highest for resin based sealer and is highest in coronal third, least at the apical third after ultrasonic irrigation.[38]

3. An vitro study by Zahid, H. M. and coworkers in their study evaluated penetration depth of GuttaFlow Bioseal-silicone based sealer, AH-Plus- resin based sealer, and Endosequence BC, bioceramic sealer, into artificially created lateral canals. Each group's specimens were cross-sectioned. Penetration depth was assessed by SEM.

   The study concluded that there was no difference in dentinal tubule penetration for filling the lateral canals when GF Bioseal, AH-Plus, and

Endosequence BC were used, with the exception of the middle section, where GF Bioseal demonstrated a noticeably deeper penetration than AH Plus resin-based sealer.[39]

4. An vitro study by Wang X et al., to measure temperature increase of root surfaces while Diode Laser application, to check the difference morphologically of the canal after exposure and to measure the leakage apically post irradiation.

   The study concluded that Diode Laser was helpful in eliminating the smear layer and hard tissue debris from canal walls and decrease leakage.[32]

5. An vitro study by Hmud R et al., to study pressure waves generated by laser and their use for removal of debris and smear layer from canals. Previously, middle infrared erbium laser was used. Presently, examination was done to see if cavitations could be induced in aqueous media with the help of 940 and 980 nm diode lasers. Cavitation formation were observed under a microscope after a capillary tube was exposed to the laser energy using a 200 µm fiber.

   This study concluded that, this phenomenon, of cavitation formation with laser can be used for improving debridement.[33]

6. An invitro study by Abu Hasna A,et al., was conducted to evaluated the efficacy of N-acetylcysteine (NAC), photodynamic therapy (PDT), and NAC combined with PDT in removing microorganisms from Enterococcus faecalis biofilm-infected dentinal tubules. It indicates that regardless of PDT stimulation, NAC showed bactericidal efficacy against E. faecalis biofilms.

Comparable to CH, NAC exhibited antibacterial action. Combined NAC+PDT treatment did not enhance antibacterial efficacy compared to NAC alone.[40]

7. An in vitro study by Quah SY et al evaluated N-acetylcysteine's (NAC) antibacterial and biofilm-eradication efficacy against Enterococcus faecalis. (E. faecalis), a bacterium commonly associated with root canal infections.in this studyUsing pH variation tests, the minimum inhibitory concentration against Enterococcus faecalis were established and effectiveness of N-acetylcysteine against Enterococcus faecalis biofilms was examined.

   The study concluded that NAC demonstrated bactericidal activity against both planktonic phases and and biofilm phases Enterococcus faecalis with potential advantages over traditional medicaments regarding dentin interactions and biofilm eradication.[41]

8. An in vitro study, by Rajakumaran A et al., was conducted to compare NAC's ability to remove smear layer and its effect on dentin microhardness with the conventional chelating agent, EDTA. 84 single-rooted human lower premolars collected and divided into 3 groups namely 17% EDTA, 20% NAC, and Distilled water (control). Analyzed by SEM for smear layer removal, and Vicker's hardness test to evaluate dentin microhardness.

   The study concluded that NAC's chelating property is comparable to EDTA and NAC causes a lower reduction in dentin microhardness compared to EDTA, suggesting its potential as a less damaging alternative in endodontic procedures.[42]

9. An vitro study by Akcay M et al., evaluated penetration of various root canal sealers into dentinal tubules following different irrigation techniques using CLSM. groups based on sealer type: iRoot SP-bioceramic sealer, AH Plus-resin based sealer, MTA Fillapex-calcium hydroxide based sealer, GF Bioseal-silicone based sealer. The study concluded that penetration into dentinal tubule was thought to be affected by canal depth, type of sealer, and irrigation method. iRoot SP demonstrated benefits in dentinal tubule penetration, highlighting the importance of irrigation technique selection in achieving optimal sealer penetration.[43]

10. An in vitro study by Gu Y, Perinpanayagam H et al aimed to compare effectiveness of five irrigant agitation techniques on their that is penetration of irrigant and sealer into radicular dentinal tubules.

    The agitation techniques were conventional needle irrigation , ultrasonic agitation, sonic irrigation, irrigation using Nd:YAP laser, and V-Clean™ system.

    The study concluded that Nd:YAP laser agitation had greater depth of penetration, followed by sonic and ultrasonic agitation. [44]

11. An vitro study by Barbizam JV et al., aimed to assess Epiphany, a resin-based sealer's bond strength to walls of radicular dentin walls after the placing calcium hydroxide dressings into the canals.

    The study concluded that the use of resin based sealer had poor bond strength to radicular dentin as it interferes with the bond of resin and flow of resin into

the tubules thus calcium hydroxide had poor and negative impact on the the adhesion of resin based sealer-Epiphany.[45]

12. An invitro study by Phillips M et al aimed to quantify residual calcium hydroxide left after different agitation methods of root canal using a chemical titration method. The study included SNI, SNI and agitation with file, SNI with 30 s of PUI, or SNI with both file and PUI.

    The use of passive ultrasonic agitation, with or without an file, reduced residual calcium hydroxide significantly.[46]

13. An in vitro study, by Calt S, Serper was conducted to assess tubule penetration of sealers after placement of CH medicament. AH26, CRCS, and Ketac Endo sealers were used for obdurating and evaluated with SEM.

    The study concluded that there was limited penetration into tubules with NaOCl irrigation alone as traces of Ca(OH)2 were remaining . However, maximum removal of Ca(OH)2 and improved penetration was observed with EDTA + NaOCl irrigation.[47]

14. An invitro study, by Shi L et al, aimed to evaluate efficacy of irrigating techniques for removing Ca(OH)2 from S-shaped root canals. CNI, PUI, PIPS, XP and EDDYwere studied. All techniques completely removed Ca(OH)2 from straight portion and coronal curve of S-shaped root canals. In apical curve, Ca(OH)2 showed significant difference CNI, with no significant differences among these four groups.

The study concluded that PUI, EDDY, PIPS, and XP activation of irrigants significantly removed Ca(OH)2 from apical region of S-shaped canals. CNI was least effective than others.[48]

15. An vitro study by Gokturk H et al was conducted which aimed to assess efficacy of LAI, XP, CB, Vibringe, PUI and CNI, in removing CH from simulated root canal irregularities.

    The study concluded that no protocols completely removed entire CH from all three root regions. However, LAI and PUI demonstrated lower remaining CH compared to other protocols in artificial grooves.[49]

16. An vitro study by Devaraj S, et al. evaluated effectiveness of five different intracanal medicaments against mature E.fecalis biofilms in vitro: light-activated curcumin, double antibiotic paste, triple antibiotic paste, calcium hydroxide and chlorhexidine.

    The study concluded light-activated curcumin and TAP completely disrupted biofilm structure, while chlorhexidine and calcium hydroxide were not significantly different when compared to control groups.[50]

17. An vitro study by Akman M, et al. compared efficacy of irrigation activation regimens and conventional syringe irrigation in removing modified triple antibiotic paste from root canal walls. It concluded, irrigation activation regimens significantly enhance mTAP removal from root canals compared to conventional syringe irrigation.[51]

18. An in vitro study, by Sen BH, correlation between dentinal tubule penetration by four different root canal sealers and microleakage of external fluids into canal was investigated using dye leakage test and SEM.

    The study inferred Ketac-Endo demonstrated least penetration into tubules ($P < 0.01$). While a converse relation between tubular penetration and dye leakage was observed, the correlation was not statistically significant.[52]

19. An in vitro study by, Okşan T et al, aimed to assess impact of the smear layer on penetration of four different root canal sealers into dentinal tubules. The results showed that smear layer hindered sealer penetration into tubules. However, experimental groups, Diaket, N2, and SPAD exhibited better penetration compared to Forfenan ($P < 0.01$). The study concluded that sealer penetration may be influenced by presence of smear layer.[53]

20. An in vitro study by Marchesan MA et al, studied effect of 980nm diode laser structure of radicular dentin using SEM at different laser parameter settings. Results showed no difference between between parameters of 1.5 watts in CW mode and 3 watt with frequency of 100 Hz.

    Their study concluded laser irradiation showed structural changes in dentin like smear layer removal and dentin fusion.[54]

21. An vitro study by de Moura-Netto et al., studied effect on radicular dentin by laser irradiation where they analysed changes in morphology of radicular dentin and debris removal in apical third of root canals irradiated with the Nd:YAG laser and diode laser. SEM images showed changes including partial removal of debris and fusion of the dentin surface in laser groups. They

concluded lasers cause changes in dentin. Nd:YAG laser group showed alterations in radicular wall surface whereas uniform changes were observed in diode laser group.[55]

22. An vitro study by Ali Saghiri M et al. evaluated effect of diode laser on structural changes and morphology of canal walls after irrigation with BioPure MTAD, or 17% EDTA. Control group showed closed dentinal tubules while clean walls were evident in laser groups with EDTA groups and with BioPure MTAD. Results of the study confirmed diode laser in combination with BioPure MTAD had good smear layer removal ability. The study concluded that diode laser in combination with Biopure MTAD maybe used final irrigant before obturation.[56]

23. An vitro study by Saraswathi MV et al., aimed to investigate changes in morphology of radicular dentin after 940 nm diode with irrigation using 2.5% NaOCl and 17% EDTA. The results showed smear layer removal was better in middle and apical third in laser group as compared to coronal third. Irradiation with 940 nm diode showed better removal of smear layer combined with NaOCl and EDTA irrigation.[57]

24. An vitro study by Alfredo E, Silva SRC, et al., evaluated bond strength of resin based sealers to radicular dentin after 980 nm diode laser irradiation at various power settings.The irradiated specimens filled with AH Plus sealer exhibited higher bond strength. The study concluded irradiation with 980 nm diode increased the bond strength of AH Plus sealer.[58]

25. In systematic review by ReDent-Nova, et al of in vitro studies aimed to summarise comparison of PUI and other irrigation techniques in their effectiveness in removal of $Ca(OH)_2$ from apical third of canals.The systematic review concluded on basis of available evidence, greater efficacy was seen by PUI compared with SNI and apical negative pressure irrigation.[59]

MATERIALS AND METHODS

Study design

In-vitro study.

Source of data

The study was carried out in Department of Conservative Dentistry and Endodontics, KLE VKIDS, Belagavi, KAHER (KLE University).

The laboratory steps were carried out in Dr. Prabhakar Kore's Basic Research Centre, KAHER, Belagavi.

The CLSM analysis was carried out in BITS PILANI, Goa campus.

Inclusion criteria

- Human permanent mandibular premolar teeth with single and closed apex.

Exclusion criteria

- Calcified canals.
- Fracture or crack
- Presence of anatomic variations/multiple canals
- Curved teeth
- Endodontically treated teeth

Materials used for the study:

1.Human permanent mandibular premolar teeth

2.0.1% thymol

3.3% sodium hypocholorite (NaOCl)

4.17% ethylene diamine tetra acetic acid (EDTA) [GLIDE]

5.Normal saline solution (Amanta Healthcare, Ahmedabad, Gujrat)

6.Paper points (Diadent Group International, Korea)

7.Distilled water (NICE LIFE CARE, NEW DELHI)

8.N- acetyl cysteine (pure form) (Molychem)

9.Propylene glycol

10.Gutta- percha points (Diadent Group International, Korea)

11.GuttaFlow bioseal sealer (Coltene Whaledent, GmBHþCo KG, Langenau, Switzerland)

12.0.1% Rhodamine isothiocynate B dye (Sigma Aldrich, Bangalore)

13.Cavit (3M, ESPE, USA)

14.Ball burnisher (GDC, India)

15.Spirit lamp (GDC, India)

ARMAMENTARIUM USED FOR THE STUDY

1.Micro motor and straight hand piece (NSK PANA AIR)

2.K files (10- 15) (MANI, Japan)

3.30 Hedström files

4.Endomotor (Dentsply X- SMART)

5.Protaper universal rotary files (Dentsply Maillefer, Switzerland)

6.5ml 27 gauge syringe (Dispovan, India)

7.Side vented needle 27 gauge (Dispovan, India)

8.Lentulospiral (30 mm) (Mani, Japan)

9.Diamond disks (Kwality Diamond Tools, Mumbai)

10.Chisel

11.Ultrasonic unit (Ultra X, Eighteeth)

12.Diode laser: (SiroLase Blue Diode Laser, California, USA)

13.Confocal laser Scanning Microscopy (Olympus fluoview FV3000)

SAMPLE SIZE ESTIMATION:

At 95%- Confidence Interval

95%- Power

$$n = \frac{(Z1 - \alpha/2 + Z1 - \beta)^2 (SD_1{}^2 + SD_2{}^2)}{(\overline{x}1 - \overline{x}2)2}$$

Z1- α = 1.96
Z1- β = 0.85
$\bar{x}1$ = 35.26
$\bar{x}2$ = 55.96
SD1 = 28.95
SD2 = 25.37

Estimated sample size for each group, n = 27

Total sample size= 81

METHODOLOGY

Eighty-one extracted human mandibular premolar teeth were selected as per to OSHA guidelines. Calculus was removed and specimens were immersed in 0.1% thymol solution until further use.

For the purpose of meeting the inclusion and exclusion criteria, radiographs and magnification were used to assess every tooth. Decoronation was done, root length of 12 mm was achieved. Working length was determined where 15 K file exits foramen, 1 mm less than root length. Protaper Universal rotary system was used for cleaning and shaping till MAF size F3. Following each change of instruments 2ml of 3% sodium hypochlorite was used as irrigant. Final rinse was done by 5 mL of 17% EDTA followed by 2ml of saline solution for 1min. Then canals were dried using paper points.

PREPARATION OF INTRACANAL MEDICAMENT: N-acetyl cysteine powder was mixed with propylene glycol in the ratio of 1:1 (2mg/ml). N- acetyl cysteine intracanal medicament placed using a size #30 Lentulospiral, orifice was sealed with Cavit, and specimens stored in incubator with 100% humidity at 37°C for 14 days.

After incubation period of 14 days, the samples were instrumented with #30 Hedström files (Dentsply) supplemented with 5ml of 3% NaOCl to eliminate the medicament. Then, according to irrigant activation techniques the specimens were divided into three groups for medicament removal:

Group I: Diode Laser activation

970 nm high power diode laser with peak power 2 watts (CW), was used and a 200μm diameter fiber tip was placed 1mm short of apex and was activated in helical motion for 20 seconds (5 seconds per cycle)

Group II: Passive Ultrasonic agitation

Passive Ultrasonic agitation using no.25 ultrasonic tip (Satellac) was done for 30seconds cycle for 5 times.

Group III: No agitation (positive control)

As a positive control, no agitation was done was done for this group

Canals were then rinsed using 5 mL 17% EDTA, followed by distilled water then dried with paper points.

0.1% Rhodamine B dye was used with GuttaFlow bioseal sealer. Using a single cone approach, obturation was done using gutta-percha cones of size F3 in conjunction with GuttaFlow bioseal sealer. Cavit was used to seal the root canal orifices, and all samples were kept for 7 days at 37 degrees Celsius and 100% humidity in an incubator.

Using a diamond disc, the specimens (n=27) were sectioned horizontally at 2, 5, and 8 mm from the apex to create sections that were 1 mm thick.

Sections were inspected under a confocal laser scanning microscope and analysed using Image J software, for determining the depth of sealer penetration (measured in μm) into dentinal tubules.

MATERIALS USED FOR THE STUDY:

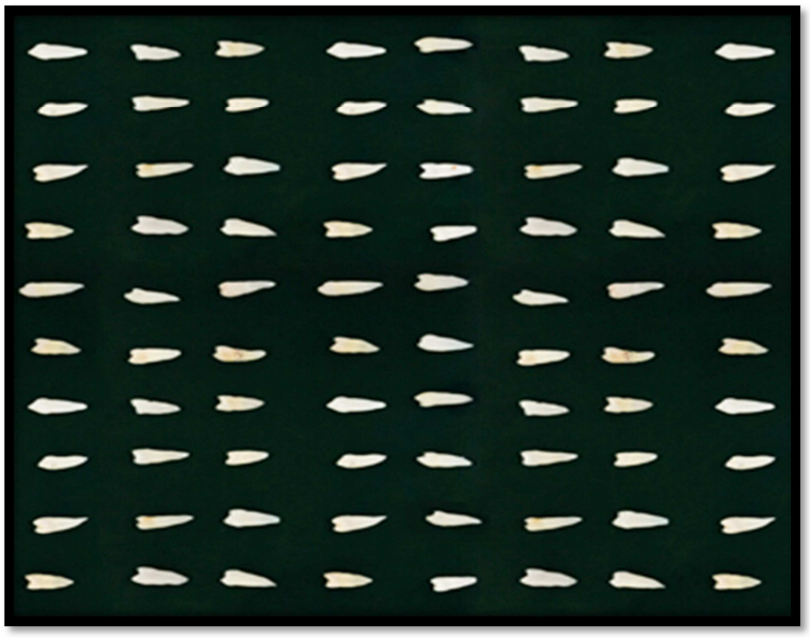


Fig 1: Total Sample Size (n=81)

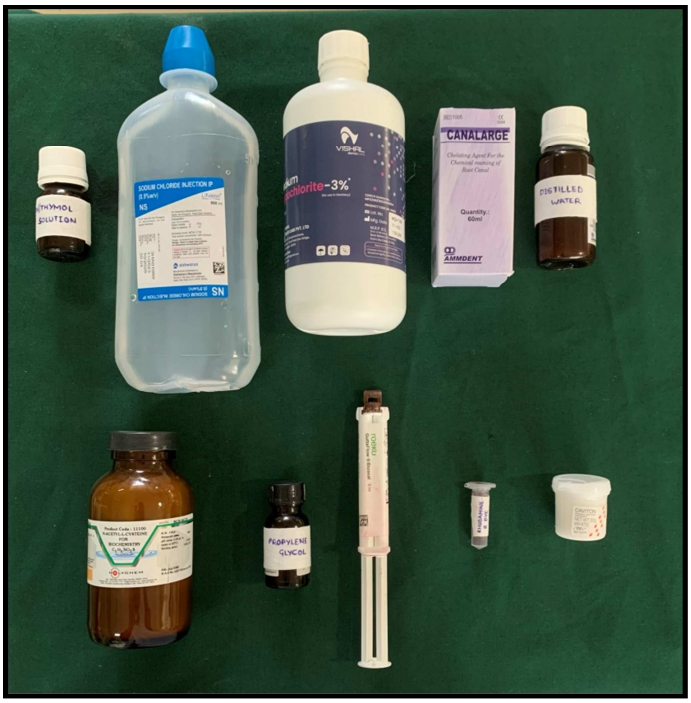


Fig 2: Materials

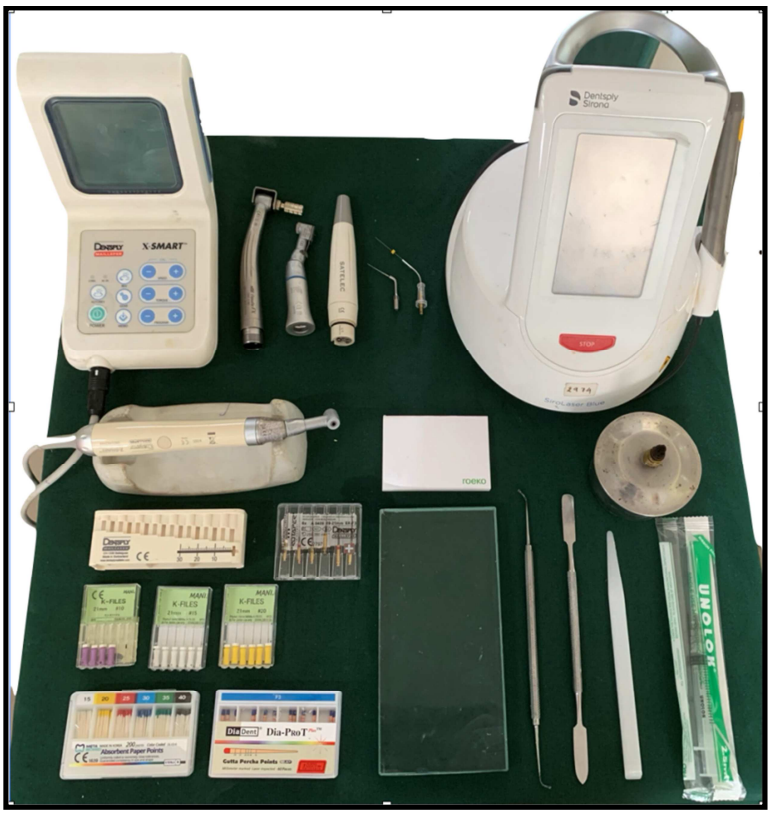


Fig 3: Armamentarium

Fig 4: Debris Removal

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

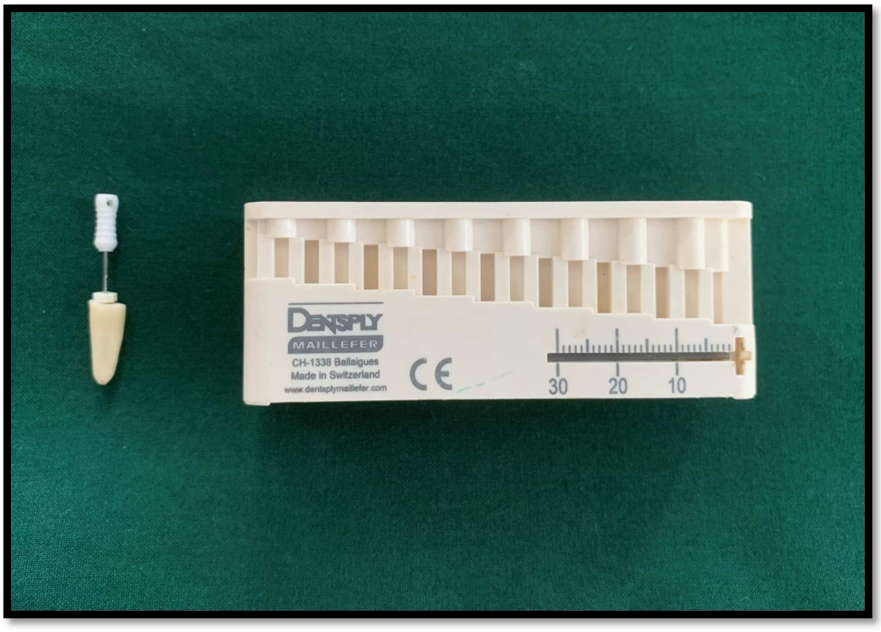


Fig 6: Working Length Determination

Fig 7: Bio-Mechanical Preparation

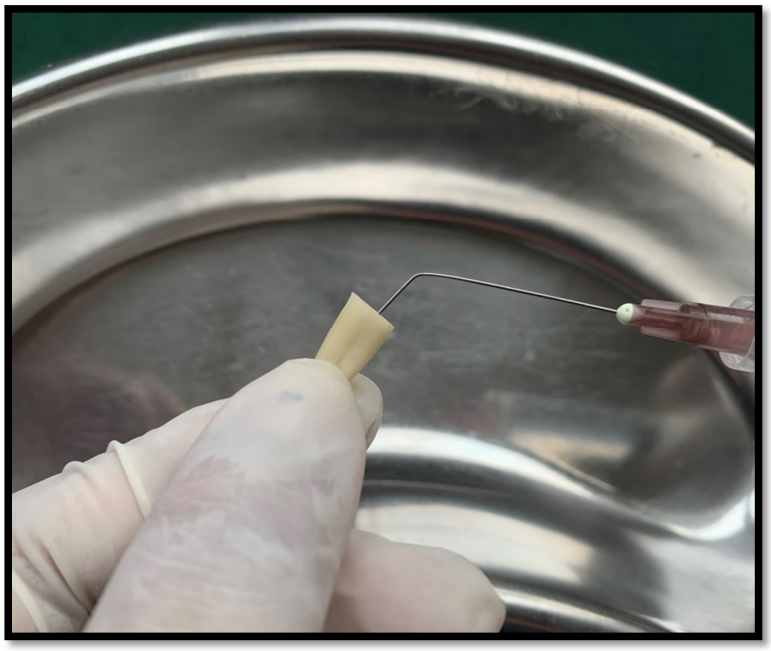


Fig 8: Sodium hypochlorite Irrigation in between instrumentation

Fig 9: NAC medicament preparation

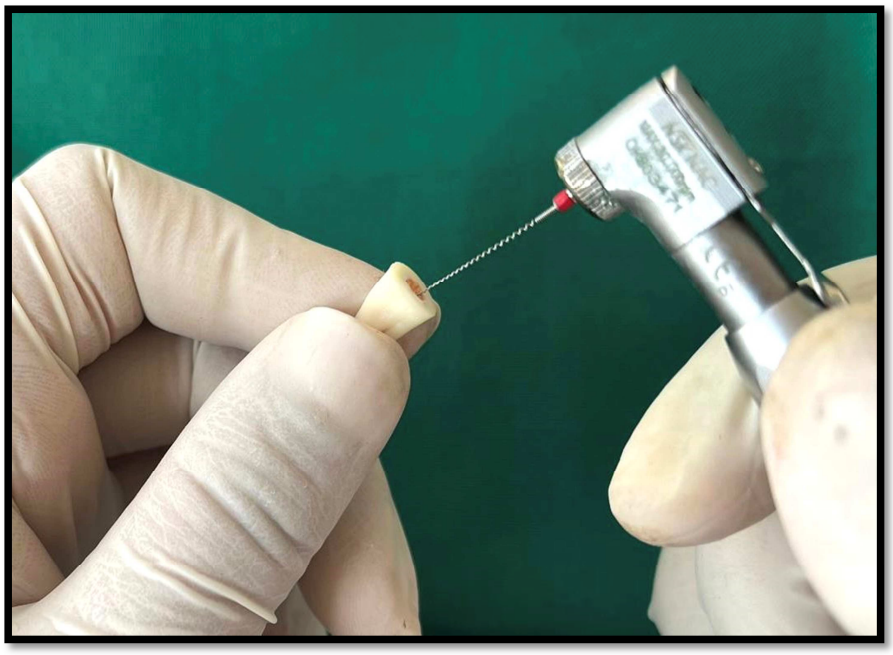


Fig 10: Placement of medicament

Fig 11: Diode Laser (SiroLase Blue)

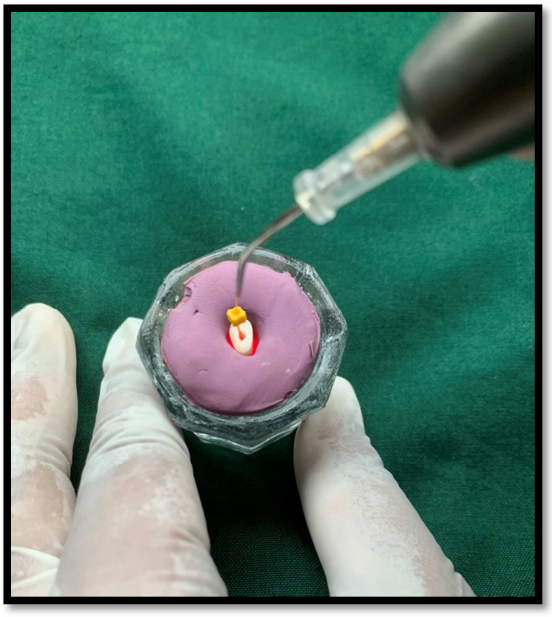


Fig 12: Irrigation activation with Diode Laser

Fig 13: Irrigation activation with Passive Ultrasonic agitation

Fig 14: Drying canals with paper points

Fig 15: Dye Incoroporation

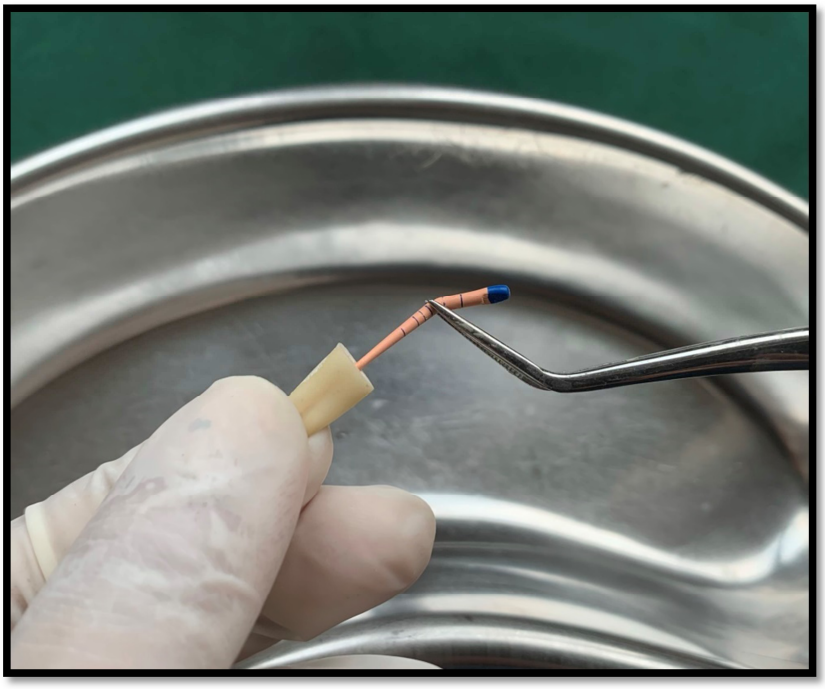


Fig 16: Obturation

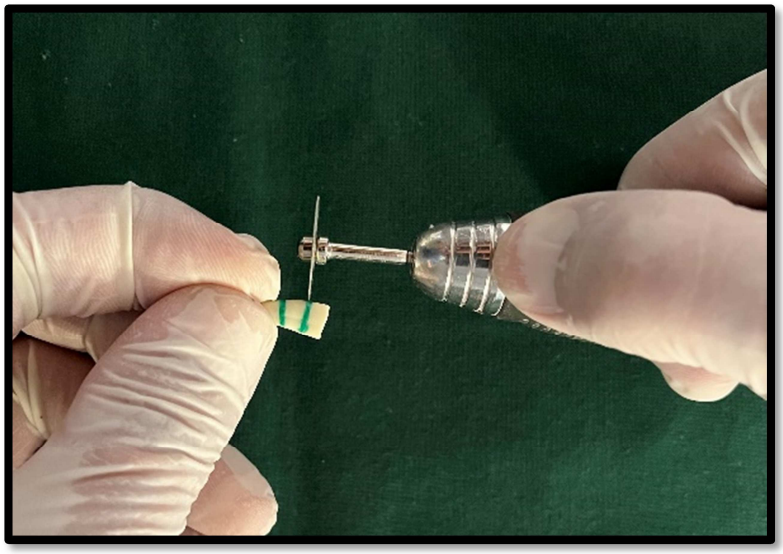


Fig 17: Sectioning

Fig 18: Incubator

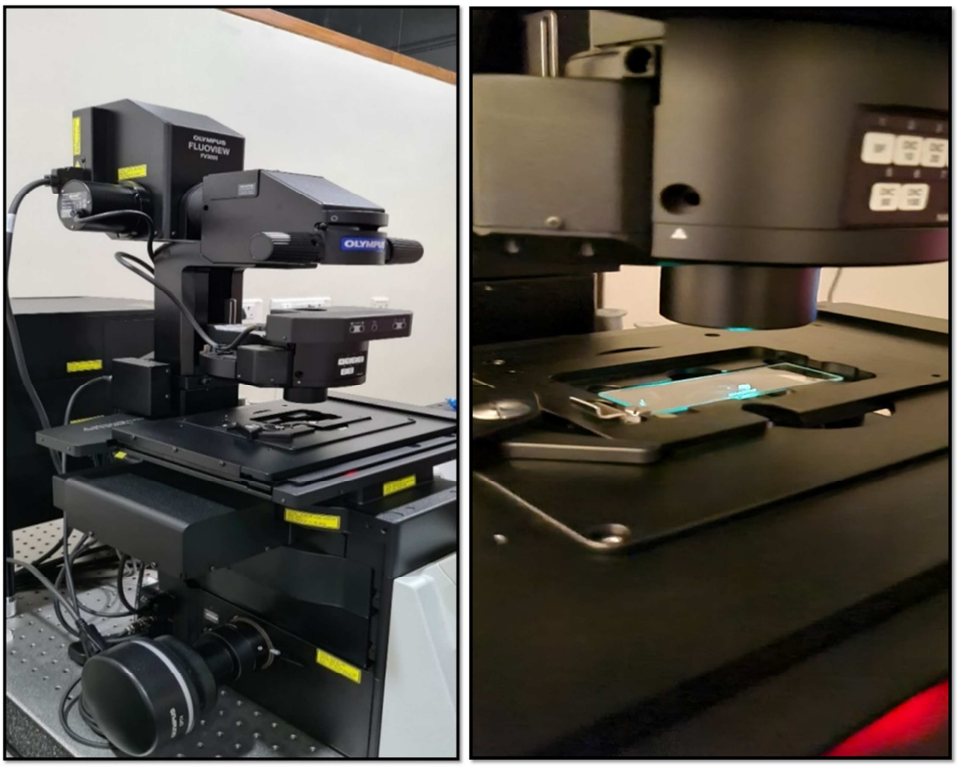


Fig 19: Confocal Laser Scanning Microscopy

Fig 20: CLSM Images depicting penetration of GuttaFlow Bioseal sealer after Group I- Diode laser activation at coronal, middle and apical sections:

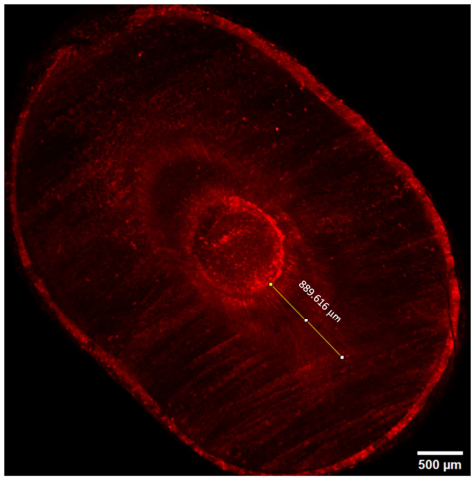


Fig 20 a: CORONAL

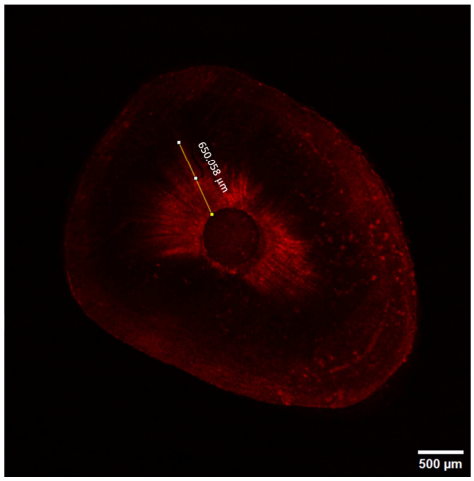


Fig 20 b: MIDDLE

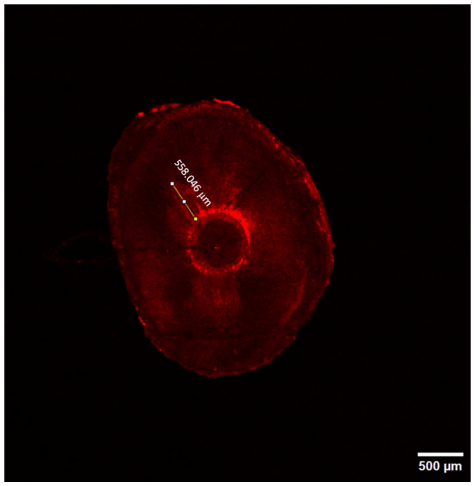


Fig 20 c: APICAL

Fig 21: CLSM Images depicting penetration of GuttaFlow Bioseal sealer after Group II- Passive Ultrasonic agitation at coronal, middle and apical sections:

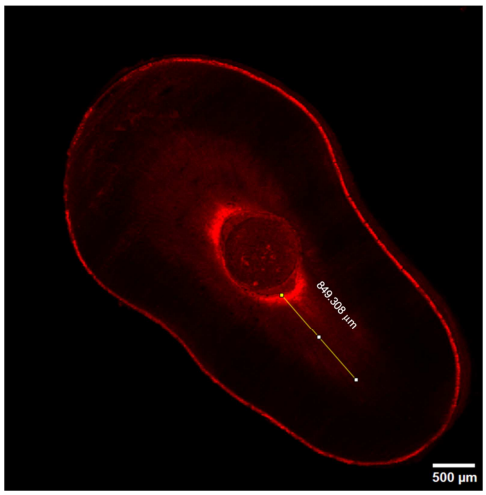


Fig 21 a: CORONAL

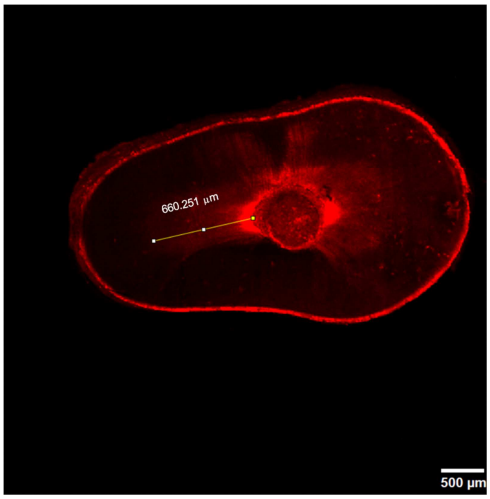


Fig 21 b: MIDDLE

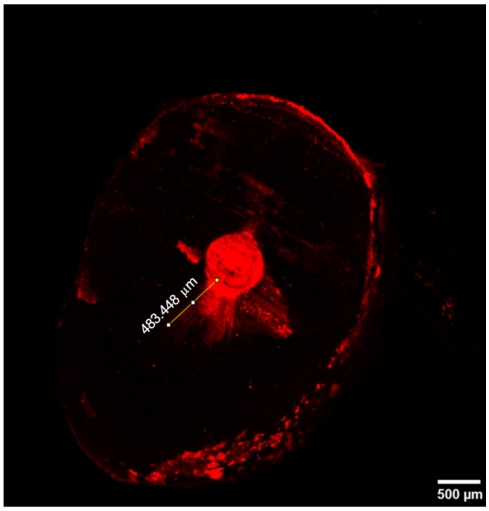


Fig 21 c: APICAL

Fig 22: CLSM Images depicting penetration of GuttaFlow Bioseal sealer after Group III- No agitation (Positive control) at coronal, middle and apical sections:

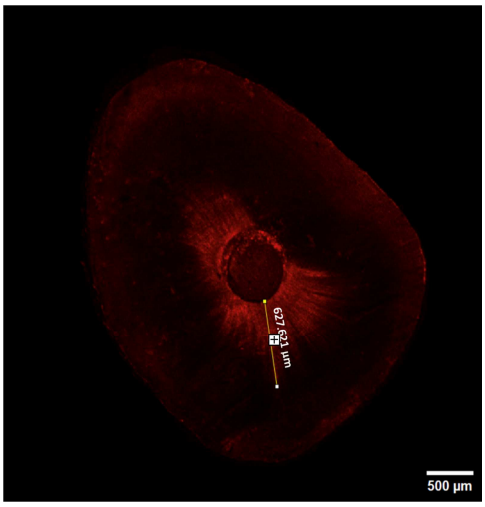


Fig 22 a: CORONAL

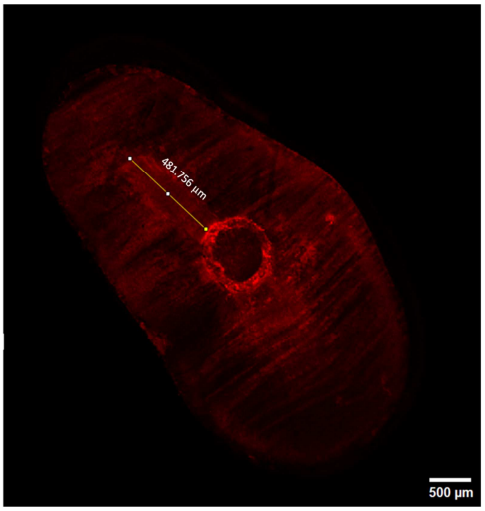


Fig 22 b: MIDDLE

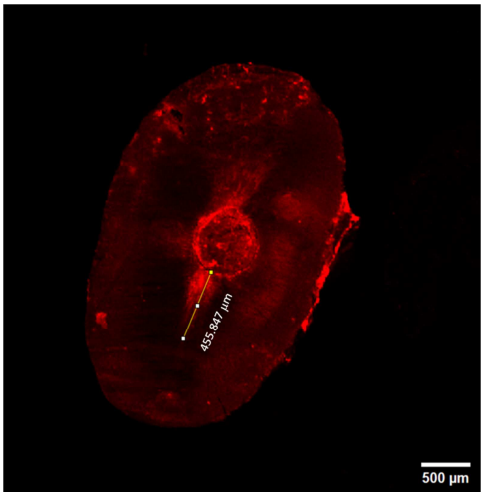


Fig 22 c: APICAL

RESULTS

Group I- Diode laser activation, Group II- Passive Ultrasonic agitation and Group III- No agitation(positive control) (n-27) were examined for penetration of sealer in dentinal tubules in coronal, middle and apical third regions of root canal.

Table:1. Summary of dentinal tubule penetration of sealer in three groups Group I- Diode laser activation, Group II- Passive Ultrasonic agitation and Group III- No agitation(positive control) and three regions coronal, middle and apical third of the root canal.

Factor	Level of factor	N	Mean	SD	SE	95% CI for mean	
						Lower	Upper
Groups	I- Diode laser activation	81	728.52	152.58	16.95	694.79	762.26
	II- Passive Ultrasonic agitation	81	687.97	108.23	12.03	664.03	711.90
	III- No agitation (positive control)	81	489.49	146.72	16.30	457.05	521.93
Regions	Coronal	81	738.85	140.92	15.66	707.69	770.01
	Middle	81	625.84	170.77	18.97	588.08	663.60
	Apical	81	541.29	144.00	16.00	509.45	573.13
Group*region	Diode laser activation with Coronal	27	824.21	149.75	28.82	764.97	883.45
	Diode laser activation with Middle	27	729.27	109.57	21.09	685.93	772.62
	Diode laser activation with Apical	27	632.09	134.29	25.84	578.97	685.21
	Passive Ultrasonic agitation with Coronal	27	787.53	69.44	13.36	760.06	815.00
	Passive Ultrasonic agitation with Middle	27	691.36	72.07	13.87	662.85	719.87

	Passive Ultrasonic agitation with Apical	27	585.01	68.64	13.21	557.86	612.17
	No agitation (positive control) with Coronal	27	604.81	71.89	13.84	576.37	633.25
	No agitation (positive control) with Middle	27	456.89	165.31	31.81	391.50	522.28
	No agitation (positive control) with Apical	27	406.77	108.25	20.83	363.95	449.59

Table 1. depicts, the summary of mean depth of penetration, standard deviation and standard error of the three groups, consisting of 27 specimens each. The highest mean depth of penetration of 728.52µm was seen with Group I- Diode laser activation group when compared with Group II- Passive Ultrasonic agitation and Group III- No agitation (positive control).

The mean depth of penetration in coronal third region irrespective of Group I- Diode laser activation group, Group II- Passive Ultrasonic agitation and Group III- No agitation (positive control) was maximum (738.85) followed middle third (625.84)and least was seen in apical third(541.29).

The maximum mean depth of penetration is seen in Coronal third region of Group I- Diode laser activation group (824.21) and least mean depth of penetration is seen in Apical third region of Group III- No agitation (positive control) (406.77)

Table:2. Comparison of three groups, Group I, Group II, Group III and three regions, coronal, middle, apical third with their mean penetration by two way ANOVA

Sources of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F-value	p-value
Main effects					
Group	2650702.93	2	1325351.46	107.0705	0.0001*
Region	1591676.46	2	795838.23	64.2930	0.0001*

Table.2 depicts comparison of three groups, Group I, Group II and Group III and three regions, coronal, middle, third with their mean penetration of sealer by two way ANOVA.

The mean dentinal tubule penetration was statistically significant (p-value- 0.0001*) among all groups, i.e. between Group I- Diode laser activation, Group II- Passive ultrasonic agitation, between Group I- Diode laser activation and Group III- No agitation (positive control) and Group II- Passive ultrasonic agitation and Group III- No agitation (positive control).

The mean dentinal tubule penetration was statistically significant (p-value- 0.0001*) among three regions i.e., coronal third and middle third, between coronal third and apical third and between middle and apical third.

Table:3. Pair-wise comparison of three groups, Group I, Group II, Group III with their mean dentinal tubule penetration of sealer by Tukeys multiple posthoc procedures

Groups	Diode laser activation	Passive Ultrasonic agitation	No agitation (positive control)
Mean	728.52	687.97	489.49
SD	152.58	108.23	146.72
Diode laser activation	-		
Passive Ultrasonic agitation	0.0500*	-	
No agitation (positive control)	0.0001*	0.0001*	-

Table 3. depicts pair wise comparison of three groups Group I, Group II, Group III with their mean dentinal tubule penetration of sealer by Tukeys multiple posthoc procedures.

Statistically significant difference is seen between Group I- Diode laser activation and Group II- Passive Ultrasonic agitation (p=0.0500*) with better dentinal tubule penetration of sealer in Group I- Diode laser activation. Statistically difference is seen between Group I- Diode laser activation group and Group III- No agitation (positive group) suggesting better penetration of sealer in Group I- Diode laser activation group.

Statistically significant difference (p=0.0001*) between Group II- Passive ultrasonic agitation and Group III- No agitation (positive control) suggesting better dentinal tubule penetration in Group II- Passive ultrasonic agitation group.

Table:4. Pair wise comparison of three regions with their mean dentinal tubule penetration by Tukeys multiple posthoc procedures

Region	Coronal	Middle	Apical
Mean	738.85	625.84	541.29
SD	140.92	170.77	144.00
Coronal	-		
Middle	0.0001*	-	
Apical	0.0001*	0.0001*	-

Table 4. depicts pair wise comparison of three regions that is coronal, middle and apical third with their mean dentinal tubule penetration by Tukeys multiple posthoc procedures.

Statistically significant difference (P=0.0001*) is seen between coronal third and middle third region, between coronal third and apical third region and between middle third and apical third, with maximum mean penetration in coronal third followed by middle third and least in apical third

Table:5. Comparison of interactions of 3 groups and three regions with mean tubule penetration by Tukeys multiple posthoc procedures with their p value(*p<0.05)

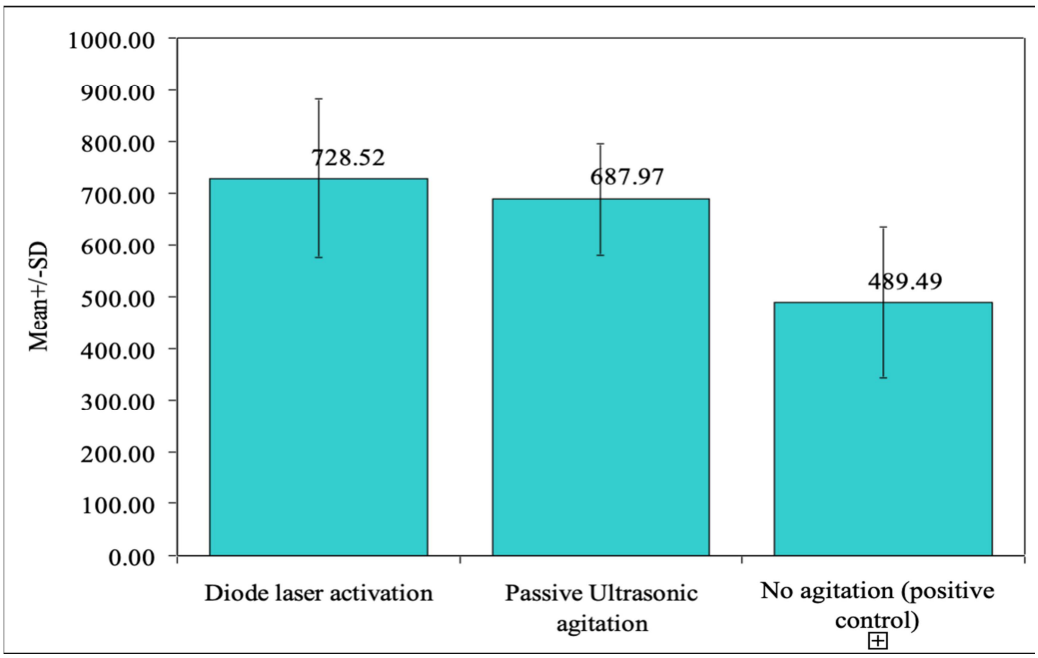
Interactions	Diode laser activation with Coronal	Diode laser activation with Middle	Diode laser activation with Apical	Passive Ultrasonic agitation with Coronal	Passive Ultrasonic agitation with Middle	Passive Ultrasonic agitation with Apical	No agitation (positive control) with Coronal	No agitation (positive control) with Middle	No agitation (positive control) with Apical
Mean	824.21	729.27	632.09	787.53	691.36	585.01	604.81	456.89	406.77
SD	149.75	109.57	134.29	69.44	72.07	68.64	71.89	165.31	108.25
Diode laser activation with Coronal	-								
Diode laser activation with Middle	**0.0452***	-							
Diode laser activation with Apical	**0.0001***	**0.0360***	-						
Passive Ultrasonic agitation with Coronal	0.9543	0.5969	0.0001*	-					

Interactions	Diode laser activation with Coronal	Diode laser activation with Middle	Diode laser activation with Apical	Passive Ultrasonic agitation with Coronal	Passive Ultrasonic agitation with Middle	Passive Ultrasonic agitation with Apical	No agitation (positive control) with Coronal	No agitation (positive control) with Middle	No agitation (positive control) with Apical
Passive Ultrasonic agitation with Middle	0.0004*	0.9447	0.5735	**0.0399***	-				
Passive Ultrasonic agitation with Apical	0.0001*	0.0001*	0.8292	**0.0001***	**0.0132***	-			
No agitation (positive control) with Coronal	0.0001*	0.0013*	0.9930	0.0001*	0.0991	0.9993	-		
No agitation (positive control) with Middle	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0008	**0.0001***	-	
No agitation (positive control) with Apical	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	**0.0001***	**0.7738**	-

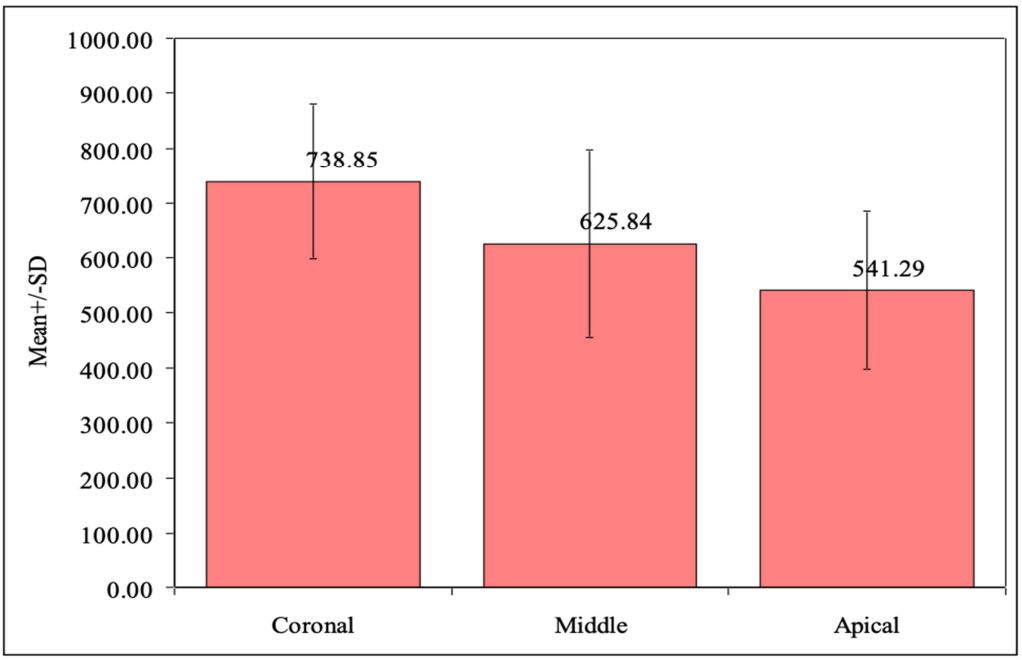
- Coronal third region of Group I- showed no significant difference with coronal third region of Group II
- Middle third region of Group I showed no significant difference with coronal third region of Group II
- Apical third region of Group I showed no significant difference with coronal third region of Group II
- Coronal third region of Group I showed significant difference with coronal third region of Group III
- Middle third region of Group I showed significant difference with coronal third region of Group III
- Apical third region of Group I showed significant difference with coronal third region of Group III
- Coronal third region of Group II showed significant difference with coronal third region of Group III
- Middle third region of Group II showed no significant difference with coronal third region of Group III
- Apical third region of Group II showed no significant difference with coronal third region of Group III

Thus, the table depicts total of 36 interactions of whicn 26 were significant values (*$p<0.05$) with highest mean depth of penetration of sealer in dentinal tubule in Group I- Diode laser activation in coronal third region and lowest mean depth of dentinal tubule penetration of sealer in Group III- No agitation (positive control) in apical third region.

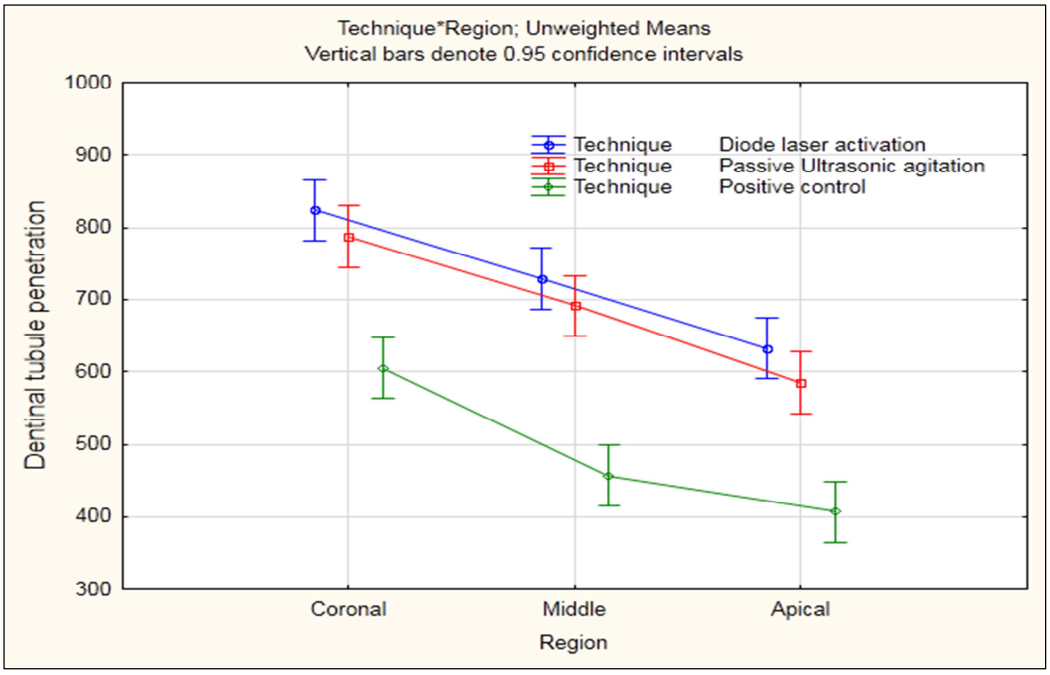
Graph 1. Pair wise comparison of three groups with their mean dentinal tubule penetration



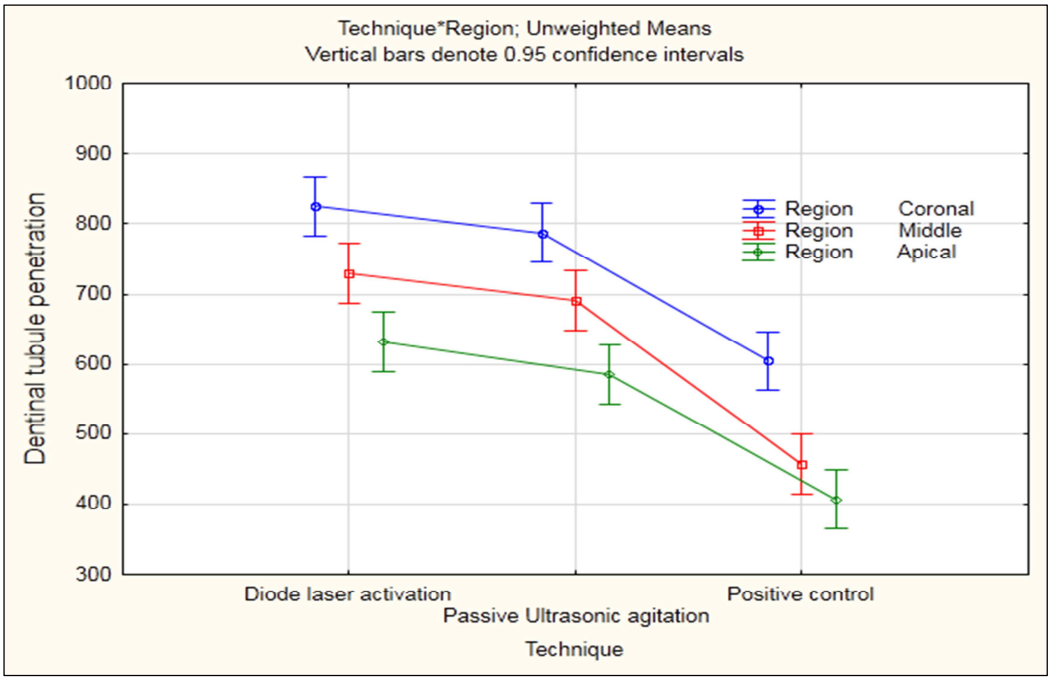
Graph 2. Pair wise comparison of three regions with mean dentinal tubule penetration



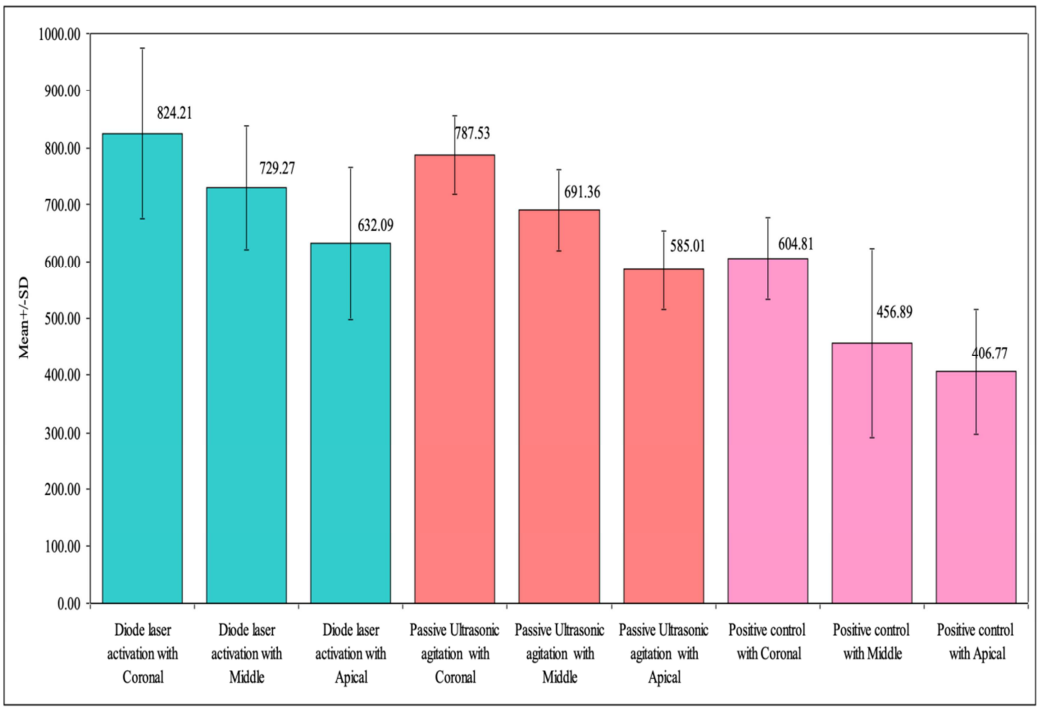
Graph 3. Comparison of interactions of three groups and three regions with mean dentinal tubule penetration



Graph 4. Comparison of interactions of three groups in three regions with mean dentinal tubule penetration



Graph 5. Comparison of interactions of three regions in three groups with mean dentinal tubule penetration



DISCUSSION

Endodontic treatment requires removal of endodontic biofilm from root canal system. The success and longevity of endodontic therapy relies on efficacy of endodontic instrumentation, irrigating solutions, chelating agents and obturating materials for disinfection and filling of root canals. [60]

The removal of endodontic biofilm from root canals is complex task, the failure of which may affect the prognosis of treatment and cause reinfection.[61] To ensure adequate cleaning of canals and rendering them free of microorganisms various measures have been documented such as different techniques and newer armamentarium for chemo-mechanical debridement.[60] Despite the different methods of chemo-mechanical debridement, proper disinfection may not be achieved and some canals may necessitate the use of additional means in order to subside and eradicate microbial load.

The use of intracanal medicament with antimicrobial and anti-biofilm properties is one of the most practiced means to disinfect root canals.[62]

Chlorhexidine (CHX) and calcium hydroxide (CH) due to their remarkable antimicrobial have been widely used as intracanal medicaments.[63] However, the capacity of CHX to enter into deep layer of biofilms is restricted as it is inactivated by physiological salts.[13] Whereas the resistance of Enterococcus faecalis to hydroxyl ions of CH and the buffering action of dentin limit the antimicrobial property of CH.[64]

Triple Antibiotic paste, a mixture of three antibiotics: ciprofloxacin, minocycline and metronidazole has demonstrated the efficacy against endodontic pathogens. However, research has shown that minocycline may result in noticeable

crown discolouration. For this reason, it was eliminated to form Double Antibiotic Paste (DAP) which contains only ciprofloxacin and metronidazole.[65] Due to the strong acidic nature of triple antibiotic paste (pH = 2.9), both double and triple antibiotic pastes have a deleterious influence on the microhardness of radicular dentine.[66]

N-acetyl cysteine (NAC), a powerful anti-oxidant with mucolytic properties, it contains thiols and is commonly used in medicine to treat acetaminophen overdose and chronic bronchitis.[18] NAC is regarded as a non-antibiotic substance with antimicrobial qualities that prevent different bacteria from forming biofilms responsible for systemic diseases including Pseudomonas aeruginosa, Escherichia coli, etc. [19]

Quah SY et al. evaluated NAC's minimum bactericidal concentration and minimum inhibitory concentration against E. faecalis. Antibacterial efficacy against E. faecalis is studied in most in vitro researches as it is a persistent bacterial associated with endodontic treatment failures. The MIC of 1.56 mg/mL and MBC of 12.5 mg/mL NAC were determined. The study concluded that NAC demonstrated bactericidal activity against Enterococcus faecalis They also studied pH variation testing, the ability of dentin powder to neutralize NAC's antibacterial activity and unlike calcium hydroxide, NAC’s antibacterial properties remained uninfluenced by dentin. NAC can so be seen as a promising agent for combating Enterococcus faecalis infection in root canals, with potential advantages over traditional medicaments with respect to its dentin interactions and biofilm eradication.[41]

The minimum bactericidal concentration and minimum inhibitory concentration of NAC against Streptococcus mutans, Actinomyces naeslundii, Lactobacillus

salivarius, and E. faecalis, considered as pathogens associated with endodontic infection was studied by Ji- Hoi Moon et al. their study. They revealed greater NAC has greater biofilm disruption and antimicrobial efficacy than CH or CHX.[21]

One of the main constituents of biofilms is extracellular polymeric substance (EPS), which is mostly made up of polysaccharides but also contains other molecules like lipids, proteins, and extracellular DNA. According to Jachlewski et al. NAC's impact on the formation of exopolysaccharides (EPS) explains its biofilm disruption action.[67]

NAC is thought to interfere with the EPS production in several ways.[68] First, the sulfhydryl group of NAC is considered to have the ability to break disulfide bonds involved in the generation of EPS. Second, NAC's antioxidant properties may indirectly affect the metabolism and EPS synthesis of bacterial cells. Its use in humans is safely advocated as a mucolytic agent.[41] NAC is a potential alternative novel intracanal medicament.

In the present study, the medicament was used in powder form. Propylene glycol was used as a vehicle and mixed in the ratio of 1:1 of 2mg/ml. Propylene glycol vehicle dissolves NAC to form a paste.

In the present study, the medicament was used in powder form. Propylene glycol was used as a vehicle and mixed in the ratio of 1:1 of 2mg/ml. [41] Propylene glycol is an odourless, clear liquid employed as an effective vehicle for pharmacological preparations such as with anti-histamines and barbiturates. It has improved handling qualities owing to its consisitency.[68] In application of NAC as a mucolytic agent when used transdermally, it was desirable to polyalcohols, e.g.

propylene glycol as an enhancing substance in order to increase the amount that may penetrate the skin.[69]

Propylene glycol also displayed antibacterial action against common endodontic pathogens thus contributing to wider application and as a vehicle for intracanal medicaments.[68]

With the use of intracanal medicaments between visits, complete removal of the medicament is important as remaining medicament may stick to the canal wall and affect the bond between dentinal tubules and endodontic sealers.

A bioceramic sealer was used in the study. GuttaFlow bioseal, a silicon-based sealer, has shown outstanding biocompatibility as it resembles hydroxyapatite. Gutta-percha in powder form with particle size less than 30 micrometers and sealer are combined. It is a cold flowable system that comprises a blend of zirconium dioxide, micro-silver, poly-dimethyl siloxane, gutta-percha powder, and platinum catalyst.[43]

When applied in a clinical setting, the bioceramic sealer expands laterally and takes on the shape of the canal by absorbing water from the dentinal tubules in the canal walls. Water absorption causes expansion of the sealer strengthening the bond between the sealer and dentin.[70]

Since the particles for bioceramic sealer have a diameter of less than one millimeter, it may be assumed that they are well suited for tubule penetration.[70] The bioceramic sealer's low contact angle, hydrophilicity, and small particle size account for its deeper penetration.[71]

Obturation technique used in the present study was single cone obturaiton owing to the biominerlisation properties of the sealer and due to its uniformity achieved by this technique. [72]

Despite the properties of bioceramic sealer, Incomplete removal of medicament may lead to poor adaptation of sealers increasing microleakage, which ultimately results in treatment failure.

Different methods utilized for removal of the debris and intracanal medicaments include manual methods like syringe irrigation, agitation using a gutta percha or master apical file, or machine assisted methods like rotary systems, CanalBrush, EndoVac, sonic agitation; EndoActivator, passive ultrasonic irrigation and laser. [73]

Very limited literature on removal of NAC from the root canals, thus, in the present study removal of NAC medicament was studied.

Group I- Diode laser activation, Group II- Passive Ultrasonic agitation were compared to check their efficacy in the removal of NAC. Their removal efficacy was measured by maximum depth of penetration of GuttaFlow bioseal sealer into dentinal tubules.

Newer approaches in disinfecting root canals include use of of high-power diode lasers. After chemo-mechanical instrumentation, laser irradiation can eliminate debris and smear layer and decrease the number of total bacteria within the system.[74]

The results obtained from this study revealed, Group I- Diode laser activation showed highest mean depth of dentinal tubule penetration as compared to Group II- Passive ultrasonic agitation and Group III- No agitation, suggesting clinically significant and better removal of N acetyl cysteine medicament from the entire canal.

The laser settings used in our study were a 970 nm high power diode laser of (SiroLase blue) with power 2 watts (CW). A 200μm diameter fiber tip was introduced 1 mm short of apex and was activated in helical motion for 20 seconds (5 seconds per cycle)

The result of the present study is in accordance with a previous study by Marchesan et al., where they studied effect of diode laser of 980 nm on radicular dentin, ultra-morphological changes were observed which ranged from smear layer removal to fusion of dentinal tubules. [54]

Niemz MH quoted that ultra-morphological changes could be owed to photochemical, photothermal, photodisruption, photoplasmolysis, photodissociation and photoaccoustic effect of the diode laser.[75]

The results obtained from this study are in line with the following earlier studies. Wang et al, concluded smear layer was evaporated and removed, and clean root canal walls were achieved, following use of 980 nm diode laser at 5W. Removal of the smear layer improved adaptation of sealer leading to better seal. [32]

In a study by Moura-Netto C, et al, morphologic changes, smear layer and debris removal was observed following diode laser irradiation. [55]

Diode laser when used in combination with irrigants had minimal effect on chemical properties of radicular dentin and good smear layer removal ability as per Mohammed Ali Saghiri, et al.[56]

Saraswathi et al. quoted significantly better smear layer removal when 940 nm diode laser irradiation combined with NaOCl and EDTA irrigation was studied with minimum additional loss of mineral content.[57]

High power Diode laser reaches the complex anatomical structures as compared to that other techniques.[76] This could be due to the flexible fiberoptic tip of Diode laser which effectively transfer laser light to the radicular dentin and have higher power density at the tip. In our investigation a 200 μm fiber-optic tip was used. The flexible fine diameter tip also allowed easy access in apical third region of canal.[77]

The heat generated by the selective dosage is another aspect contributing to better sealer penetration in the apical third.[78] According to studies, diode lasers are less heat-producing and more efficient while operating at lower power levels. They are better at penetrating the dentinal tubules and have an output power range of 0.5 W to 7 W.[57]

Another method that has been studied in great detail is passive ultrasonic irrigation (PUI) or agitation. PUI is among widely used irrigation techniques. By using cavitation and acoustic microstreaming, PUI could enhance cleaning in intricate canal anatomic locations. [79]

When dentinal tubule penetration in coronal third of Group I- Diode laser activation and Group II- Passive Ultrasonic agitation was compared, statistically no significant difference was obtained. Similarly, the dentinal tubule penetration in middle third and apical third regions of Group I- Diode laser activation when compared with middle and apical third region of Group II- Passive Ultrasonic agitation showed statistically no significant difference. Suggesting comparable removal of medicament in coronal third, middle third and apical third in both Group I- Diode laser activation and Group II- Passive Ultrasonic agitation.

Passive Ultrasonic agitation was done using no.25 ultrasonic tip (Satellac) 5 times for 30seconds cycle. The process of ultrasonic activation depends on the

instrument oscillating inside an irrigant-filled root canal. Ultrasonic activation relies on oscillation of the instrument inside an irrigant- filled root canal. An ultrasonic handpiece operating at a frequency of roughly 25–32 kHz powers the device.[79]

There are two parts to the flow pattern of ultrasonic irrigation, the steady part produces the acoustic streaming. [80,81] Two jets of irrigating solution flow outwards continuously from the instrument along the direction of oscillating tip. The irrigant's streaming flow, which can enter the apical part of root canal at the end of the instrument is a key factor in debriding the apical anatomy.[82]

The rapid component of the ultrasonic instrument, due to the oscillating pressure, causes pressure variations in the irrigant and causes acoustic cavitation, which causes bubbles to form and burst generating large shear stress on surface of canals and improves cleaning. [83]

Acc to Verhaagen B, et al ultrasonic irrigation seemed to outperform sonic agitation [84] whereas a study by Conde AJ et al. concluded no significant difference was seen[85] in terms of removal of hard tissue debris.

In previous studies, by Verstraeten JA et al, laser activated irrigation showed no significant difference when compared to PUI[86] whereas in some studies lasers have proven effective [87]

Group III or no agitation group was preceded by removal of medicament with H file and sodium hypochlorite irrigation with 27 guage side vented needle. In agreement with previous studies, Group III- No agitation or positive control group in our study, showed statistically least mean depth of penetration as compared to Group I- Diode laser activation and Group II- Passive ultrasonic agitation.

Group III- No agitation or positive control group, in coronal third, middle third and apical thirds of root canals when compared to coronal third middle third and apical thirds of Group I- Diode laser activation and Group II- Passive ultrasonic agitation showed statistically significant difference where least depth of penetration was seen in coronal third, middle third and apical third of canal of Group III.

The present results explain that laser activated irrigation and ultrasonic agitation have a positive influence on the removal of NAC medicament from the root canals.

Syringe irrigation acts by flushing action of irrigating solutions which aids in clearing the canal of microbes, organic material, and dentinal debris. It has a somewhat weak flushing activity that depends on the diameter of the needle, the depth of placement, and structure of root canal system.[48] For best results, irrigating solutions should be applied directly to the entire canal, particularly to apical sections of small root canals. In order to guarantee fluid exchange, irrigating needles should be positioned no more than 1 mm from working length.[88]

The results are in accordance to a systematic review on Ca (OH)2 medicament removal, which stated ultrasonic irrigation was superior to syringe needle irrigation and apical negative pressure irrigation .[89]

CLSM was used in our current study as it presents with many advantages. High contrast points are used by CLSM to determine the sealer distribution inside dentinal tubules. In contrast to SEM, which is infamous for creating artifact during processing and has extra drawbacks due to the laborious gold-sputtering and vacuum phases, CLSM operates even with thick sections and produces less artifact.[90]

The study's findings demonstrated that, across all groups, sealer penetrated coronal third the greatest, followed by middle third, and apical third the least in all three groups i.e. Group I- diode laser activation, Group II- passive ultrasonic agitation and Group III - no agitation (positive control).

This is ascribed to differences in the radicular dentin's characteristics, primarily those related to tubule density, tubule size, and metamorphic changes that occur throughout time. The tubule density of dentin is highest in coronal region and falls with depth; the lowest density is seen apically.[91] Additionally, the tubules' mean diameter is highest in coronal third of the root and lowest in apical third which favours high penetration in coronal third. The apical 1/3 of the tubules exhibit obliteration from sclerosis as a result of aging or continuous functional loading. The sealer's inability in apical third to obtain the required space might also be explained by inadequate irrigant supply and inefficient removal of smear layer.[92]

Despite the intricacies of apical third of root canal, Group I- diode laser irrigation showed statistically higher mean depth of penetration when compared to Group II- ultrasonic agitation.

CONCLUSION

Considering the parameters of the study, the following conclusions were made: -

- Group I- Diode laser activation group when compared with Group II- Passive Ultrasonic agitation and with Group III- No agitation (positive control) showed higher mean dentinal tubule penetration of sealer

- Group II- Passive Ultrasonic agitation when compared with Group III- No agitation (positive control) showed higher mean dentinal tubule penetration of sealer .

- The coronal third region of Group I- Diode laser activation group when compared with coronal third region Group II- Passive Ultrasonic agitation showed no significant difference. Similarly, middle third region and apical third region of Group I- Diode laser activation group when compared with middle third region and apical third region Group II- Passive Ultrasonic agitation respectively showed no significant difference.

- The coronal third region of Group I- Diode laser activation group and Group II- Passive Ultrasonic agitation when compared with coronal third region of Group III- No agitation (positive control) showed significant difference where the former two groups showed better penetration. Similar results were obtained for their middle third and apical third regions respectively.

- The coronal third region of Group I- Diode laser activation group, Group II- Passive Ultrasonic agitation and Group III- No agitation (positive control)

showed maximum dentinal tubule penetration of sealer followed by middle third region of Group I- Diode laser activation group, Group II- Passive Ultrasonic agitation and Group III- No agitation (positive control) and apical third region of Group I- Diode laser activation group, Group II- Passive Ultrasonic agitation and Group III- No agitation (positive control) suggesting better penetration in coronal third region, followed by middle third region and least penetration in apical third.

Thus, it can be concluded that Group I- Diode laser activation group was most effective in removal of NAC intracanal medicament from all three regions, coronal, middle, apical third of root canal, Group II- had comparable results to Group I, whereas Group III- No agitation (positive control) was least effective in removal of NAC intracanal medicament.

SUMMARY

Aim of the study was to evaluate and compare dentinal tubule penetration of GuttaFlow bioseal sealer after the removal of N-acetyl cysteine intracanal medicament with ultrasonic agitation and laser activated irrigation using CLSM.

N- acetyl cysteine is new intracanal medicament with antibacterial, anti-biofilm and anti- inflammatory properties. It acts against all endodontic pathogens and has proven to suppress the growth of E. faecalis and to destroy its biofilm[3].

For optimal obturation, all of the medicament should be removed because any leftover medication prevents the sealer from penetrating the dentinal tubules.

Widely used irrigation techniques is passive ultrasonic irrigation (PUI). By cavitation and acoustic microstreaming, PUI application increases the cleaning efficacy in complex root canal anatomy. Nowadays, high-power diode lasers are used to disinfecting the root canals. The laser light is assumed to enter and disinfect areas that are inaccessible with traditional techniques[5.]

A novel silicone-based sealer called GuttaFlow bioseal contains calcium silicate and gutta-percha. Its impact on tubule penetration, bond strength, and the caliber of root filling with this substance has been assessed before.

Hence, this study is for investigating the dentinal tubule penetration of silicone-based sealer, GuttaFlow bioseal, after removal of N- acetyl cysteine intracanal medicament with two different irrigation strategies, ultrasonic agitation and laser activated irrigation.

The study was conducted in the Department of Conservative Dentistry and Endodontics, KLE VKIDS, KAHER Belagavi.

Extracted human mandibular premolar test which met inclusion and exclusion criteria were selected for the study. Decoronation was done to obtain a root length of 12 mm. Working length was calculated 1mm short of where 15 K file exits apical foramen. Protaper Universal rotary system was used for cleaning and shaping till MAF size F3. Following each change of instruments, an irrigant of 2 ml (3% sodium hypochlorite) was used. Final rinse was done with 17% EDTA, followed by 2ml of saline solution for 1min. Then root canals were dried using paper points.

PREPARATION OF INTRACANAL MEDICAMENT: N-acetyl cysteine powder was mixed with propylene glycol in the ratio of 1:1. (2mg/ml)

N- acetyl cysteine intracanal medicament was placed using a size #30 Lentulospiral. The orifice was sealed and specimens stored in an incubator with 100% humidity at 37°C for 14 days. After incubation period, the medicament was removed with #30 Hedström files supplemented with 5ml of 3% NaOCl to eliminate the medication. Then, the specimens were divided according to irrigant activation techniques into three groups for medicament removal:

Group I: Diode laser activation

970 nm Diode Laser (SiroLase blue), peak power 2W (CW), was used and a 200μm diameter fiber tip was introduced 1 mm short of apex and was activated in helical motion for 20 seconds (5 seconds per cycle)

Group II: Passive Ultrasonic agitation

Passive Ultrasonic agitation using no.25 (Satellac) ultrasonic tip was done for 30seconds cycle, 5times.

Group III: No agitation (Positive control)

As a positive control, no agitation was done was done for this group

Canals were rinsed with 17% EDTA and dried with paper points.

0.1% Rhodamine B dye was used with GuttaFlow bioseal sealant. Using a single cone approach, obturation was done with master cone F3 in conjunction with the GuttaFlow bioseal sealer. Cavit was used to seal the root canal orifices, and all samples were kept for seven days at 37 degrees Celsius and 100% humidity in an incubator.

Using a diamond disc, the specimens (n=27) were sectioned to create 1 mm sections at 2mm, 5mm and 8 mm from the apex. Sections were inspected under a CLSM and analyzed using Image J software for measuring depth of sealer penetration(µm) into dentinal tubules.

Statistical analysis was done by Two-way ANOVA and Tukey's Post hoc test.

Mean dentinal tubule penetration was statistically significant amongst all the three groups i.e. between Group I- Diode laser activation and Group II- Passive ultrasonic agitation, Group I- Diode laser activation and Group III- No agitation (positive control) and Group II- Passive ultrasonic agitation and Group III- No agitation (positive control) with highest dentinal tubule penetration in Diode laser

group followed by passive ultrasonic agitation and no agitation (positive control) showing the least dentinal tubule penetration.

Mean dentinal tubule penetration was statistically significant amongst three regions i.e, coronal and middle, coronal and apical, and middle and apical third. The coronal third region exhibited the maximum penetration among the three, followed by the middle third and then apical third.

Highest mean depth of penetration of 824.21µm was seen in Diode laser activation group in the coronal third region.

Therefore, null hypothesis stating there will be no difference in dentinal tubule penetration of GuttaFlow bioseal sealer after the removal of N-acetyl cysteine intracanal medicament using ultrasonic agitation and laser activated irrigation was rejected.

BIBLIOGRAPHY

1. Narayanan LL, Vaishnavi C. Endodontic microbiology. Journal of Conservative Dentistry and Endodontics. 2010 Oct 1;13(4):233-9.
2. Soares JA, de Carvalho MA, Santos SM, Mendonça RM, Ribeiro-Sobrinho AP, Brito-Júnior M, Magalhães PP, Santos MH, de Macêdo Farias L. Effectiveness of chemomechanical preparation with alternating use of sodium hypochlorite and EDTA in eliminating intracanal Enterococcus faecalis biofilm. Journal of endodontics. 2010 May 1;36(5):894-8.
3. Evans JT, Simon JHS. Evaluation of the apical seal produced by injected thermoplas- ticized gutta-percha in the absence of smear layer and root canal sealer. J Endod 1986;12:101–7.
4. Wu MK, Fan B, Wesselink PR. Diminished leakage along root canals filled with gutta-percha without sealer over time: a laboratory study. International Endodontic Journal. 2000 Mar;33(2):121-5.
5. Mamootil K, Messer HH. Penetration of dentinal tubules by endodontic sealer cements in extracted teeth and in vivo. International endodontic journal. 2007 Nov;40(11):873-81.
6. Lee SH, Oh S, Al-Ghamdi AS, Mandorah AO, Kum KY, Chang SW. Sealing ability of ah plus and guttaflow bioseal. Bioinorganic Chemistry and Applications. 2020 Oct;2020.
7. Kossev AD. Ceramics-based sealersas new alternative to currently used endodontic sealers. Roots. 2009;1:42-8.

8. Ricucci D, Siqueira Jr JF. Biofilms and apical periodontitis: study of prevalence and association with clinical and histopathologic findings. Journal of endodontics. 2010 Aug 1;36(8):1277-88.
9. Anders Byström; Rolf Claesson; Göran Sundqvist (1985). The antibacterial effect of camphorated paramonochlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals. , 1(5), 170–175. doi:10.1111/j.1600-9657.1985.tb00652.
10. Moheb H, Zakeer S, Hassan HY. Effect of different intracanal medicaments on eradication of Enterococcus faecalis biofilm–Ex vivo study. Saudi Endodontic Journal. 2023 Sep 1;13(3):254-62.
11. Haapasalo M. rstavik D (1987) In vitro infection and disinfection of dentinal tubules. Journal of Dental Research.;66(13):75-9.
12. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. Journal of endodontics. 2006 Feb 1;32(2):93-8.
13. Portenier I, Haapasalo H, Ørstavik D, Yamauchi M, Haapasalo M. Inactivation of the antibacterial activity of iodine potassium iodide and chlorhexidine digluconate against Enterococcus faecalis by dentin, dentin matrix, type-I collagen, and heat-killed microbial whole cells. Journal of Endodontics. 2002 Sep 1;28(9):634-7.
14. Parhizkar A, Nojehdehian H, Asgary S. Triple antibiotic paste: momentous roles and applications in endodontics: a review. Restorative dentistry & endodontics. 2018 Aug;43(3).

15. Parhizkar A, Nojehdehian H, Asgary S. Triple antibiotic paste: momentous roles and applications in endodontics: a review. Restorative dentistry & endodontics. 2018 Aug;43(3).
16. Bhardwaj A, Ballal S, Velmurugan N. Comparative evaluation of the antimicrobial activity of natural extracts of Morinda citrifolia, papain and aloe vera (all in gel formulation), 2% chlorhexidine gel and calcium hydroxide, against Enterococcus faecalis: An: in vitro: study. Journal of Conservative Dentistry and Endodontics. 2012 Jul 1;15(3):293-7.
17. Vasudeva A, Sinha DJ, Tyagi SP, Singh NN, Garg P, Upadhyay D. Disinfection of dentinal tubules with 2% Chlorhexidine gel, Calcium hydroxide and herbal intracanal medicaments against Enterococcus faecalis: An in-vitro study. Singapore dental journal. 2017 Dec 1;38:39-44.
18. El-Feky MA, El-Rehewy MS, Hassan MA, Abolella HA, Abd El-Baky RM, Gad GF. Effect of ciprofloxacin and N-acetylcysteine on bacterial adherence and biofilm formation on ureteral stent surfaces. Polish journal of microbiology. 2009;58(3):261.
19. Tenório MC, Graciliano NG, Moura FA, Oliveira AC, Goulart MO. N-acetylcysteine (NAC): impacts on human health. Antioxidants. 2021 Jun 16;10(6):967.
20. Tieu S, Charchoglyan A, Paulsen L, Wagter-Lesperance LC, Shandilya UK, Bridle BW, Mallard BA, Karrow NA. N-Acetylcysteine and Its Immunomodulatory Properties in Humans and Domesticated Animals. Antioxidants. 2023 Oct 16;12(10):1867.

21. Choi YS, Kim C, Moon JH, Lee JY. Removal and killing of multispecies endodontic biofilms by N-acetylcysteine. Brazilian journal of microbiology. 2018 Jan;49:184-8.
22. Perez-Giraldo C, Rodriguez-Benito A, Moran FJ, Hurtado C, Blanco MT, Gómez-García AC. Influence of N-acetylcysteine on the formation of biofilm by Staphylococcus epidermidis. The Journal of antimicrobial chemotherapy. 1997 May 1;39(5):643-6.
23. Alamoudi RA. The smear layer in endodontic: To keep or remove–an updated overview. Saudi Endodontic Journal. 2019 May 1;9(2):71-81.
24. .Ørstavik D. Materials used for root canal obturation: Technical, biological and clinical testing. *Endod Topics.* 2005;12:25–38.
25. Blank-Gonçalves LM, Nabeshima CK, Martins GH, de Lima Machado ME. Qualitative analysis of the removal of the smear layer in the apical third of curved roots: conventional irrigation versus activation systems. Journal of endodontics. 2011 Sep 1;37(9):1268-71.
26. Caron G., Nham K., Bronnec F., Machtou P. Effectiveness of different final irrigant activation protocols on smear layer removal in curved canals. *Journal of Endodontia.* 2010;36(8):1361–1366. doi: 10.1016/j.joen.2010.03.037.
27. 27- 28 became 27. Zhou J, Liu T, Guo L. Effectiveness of XP-Endo Finisher and passive ultrasonic irrigation on intracanal medicament removal from root canals: a systematic review and meta-analysis. BMC oral health. 2021 Jun 9;21(1):294.
28. Lee SJ, Wu MK, Wesselink PR. The effectiveness of syringe irrigation and ultrasonics to remove debris from simulated irregularities within prepared root canal walls. International Endodontic Journal 2004;37(10):672-8.

29. Van der Sluis LW, Versluis M, Wu MK, Wesselink PR. Passive ultrasonic irrigation of the root canal: a review of the literature. International endodontic journal. 2007 Jun;40(6):415-26.
30. Capar ID, Ozcan E, Arslan H, Ertas H, Aydinbelge HA. Effect of different final irrigation methods on the removal of calcium hydroxide from an artificial standardized groove in the apical third of root canals. Journal of endodontics. 2014 Mar 1;40(3):451-4.
31. Faria MI, Sousa-Neto MD, Souza-Gabriel AE, Alfredo E, Romeo U, Silva-Sousa YT. Effects of 980-nm diode laser on the ultrastructure and fracture resistance of dentine. Lasers in medical science. 2013 Jan;28:275-80.
32. Wang X, Sun Y, Kimura Y, Kinoshita JI, Ishizaki NT, Matsumoto K. Effects of diode laser irradiation on smear layer removal from root canal walls and apical leakage after obturation. Photomedicine and laser surgery. 2005 Dec 1;23(6):575-81.
33. Hmud R, Kahler WA, George R, Walsh LJ. Cavitational effects in aqueous endodontic irrigants generated by near-infrared lasers. *J Endod.* 2010;36:275–8.
34. Alhadi D, Jaber FM, Agha MT, Saeed MH. The effect of diode laser irradiation on root canal dentin. Journal of International Dental and Medical Research. 2019;12(1):49-53.
35. de Moura-Netto C, de Freitas Carvalho C, de Moura AA, Davidowicz H, Antoniazzi JH. Influence of Nd: YAG and diode laser irradiation on apical sealing when associated with AH plus and EndoREZ endodontic cements. Photomedicine and laser surgery. 2007 Oct 1;25(5):413-7.

36. Alfredo E, Silva SRC, Ozório JEV, Sousa-Neto MD, Brugnera- Junior A, Silva-Sousa YTC. Bond strength of AH Plus and Epiphany sealers on root dentine irradiated with 980 nm diode laser. Int Endod J 2008;41:733-740.
37. Özlek E, Neelakantan P, Akkol E, Gündüz H, Uçar YA, Belli S. Dentinal tubule penetration and dislocation resistance of a new bioactive root canal sealer following root canal medicament removal using sonic agitation or laser-activated irrigation. European Endodontic Journal. 2020;5(3):264.
38. Gaddalay SL, Patil DV, Kabir R. The influence of humidity on bond strength of AH Plus, BioRoot RCS, and Nanoseal-S sealers: An: in vitro: study. Endodontology. 2022 Jul 1;34(3):202-7.
39. Zahid HM, Ghareeb NH. Evaluation of filling ability of Guttaflow Bioseal sealer to the simulated lateral canal by scanning electron microscope: An in vitro study. Erbil Dental Journal (EDJ). 2019 Dec 6;2(2):218-28.
40. Hasna AA, Khoury RD, Toia CC, Gonçalves GB, de Andrade FB, Carvalho CA, Camargo CH, Valera MC. In vitro evaluation of the antimicrobial effect of N-acetylcysteine and photodynamic therapy on root canals infected with enterococcus faecalis. Iranian endodontic journal. 2020;15(4):236.
41. Quah SY, Wu S, Lui JN, Sum CP, Tan KS. N-acetylcysteine inhibits growth and eradicates biofilm of Enterococcus faecalis. Journal of endodontics. 2012 Jan 1;38(1):81-5.
42. Rajakumaran A, Ramesh H, Ashok R, Balaji L, Ganesh A. Smear layer removal and microhardness alteration potential of a naturally occurring antioxidant–An in vitro study. Cureus. 2019 Jul 25;11(7).
43. Akcay M, Arslan H, Durmus N, Mese M, Capar ID. Dentinal tubule penetration of AH Plus, iRoot SP, MTA fillapex, and guttaflow bioseal root

canal sealers after different final irrigation procedures: A confocal microscopic study. Lasers in surgery and medicine. 2016 Jan;48(1):70-6.

44. Gu Y, Perinpanayagam H, Jin DJ, Yoo YJ, Jeong JS, Lim SM, Chang SW, Baek SH, Zhu Q, Kum KY. Effect of different agitation techniques on the penetration of irrigant and sealer into dentinal tubules. Photomedicine and laser surgery. 2017 Feb 1;35(2):71-7.
45. Barbizam JV, Trope M, Tanomaru-Filho M, Teixeira EC, Teixeira FB. Bond strength of different endodontic sealers to dentin: push-out test. Journal of Applied Oral Science. 2011;19:644-7.
46. Phillips M, McCLANAHAN S, Bowles W. A titration model for evaluating calcium hydroxide removal techniques. Journal of applied oral science. 2015 Jan;23:94-100.
47. Çalt S, Serper A. Dentinal tubule penetration of root canal sealers after root canal dressing with calcium hydroxide. Journal of Endodontics. 1999 Jun 1;25(6):431-3.
48. Shi L, Wu S, Yang Y, Wan J. Efficacy of five irrigation techniques in removing calcium hydroxide from simulated S-shaped root canals. Journal of Dental Sciences. 2022 Jan 1;17(1):128-34.
49. Gokturk H, Ozkocak I, Buyukgebiz F, Demir O. Effectiveness of various irrigation protocols for the removal of calcium hydroxide from artificial standardized grooves. Journal of Applied Oral Science. 2017 May;25:290-8.
50. Devaraj S, Jagannathan N, Neelakantan P. Antibiofilm efficacy of photoactivated curcumin, triple and double antibiotic paste, 2% chlorhexidine and calcium hydroxide against Enterococcus fecalis in vitro. Scientific reports. 2016 Apr 21;6(1):24797.

51. Akman M, Akbulut MB, Aydınbelge HA, Belli S. Comparison of different irrigation activation regimens and conventional irrigation techniques for the removal of modified triple antibiotic paste from root canals. Journal of Endodontics. 2015 May 1;41(5):720-4.
52. Şen BH, Pişkin B, Baran N. The effect of tubular penetration of root canal sealers on dye microleakage. International endodontic journal. 1996 Jan;29(1):23-8.
53. Okşan T, Aktener BO, Şen BH, Tezel H. The penetration of root canal sealers into dentinai tubules. A scanning electron microscopic study. International Endodontic Journal. 1993 Sep;26(5):301-5.
54. Marchesan MA, Brugnera-Junior A, Souza-Gabriel AE, Correa-Silva SR, Sousa-Neto MD. Ultrastructural analysis of root canal dentine irradiated with 980-nm diode laser energy at different parameters. Photomed Laser Surg 2008;26:235-40.
55. de Moura-Netto C, de Moura AAM, Davidowicz H, Aun CE, Antonio MPS. Morphologic changes and removal of debris on apical dentin surfaces after Nd:YAG laser and diode laser irradiation. Photomed Laser Surg 2008;26:263–266
56. Ali Saghiri M, Asgar K, Gutmann JL, Garcia-Godoy F, Ahmadi K, Karamifar K, Asatorian A. Effect of laser irradiation on root canal walls after final irrigation with 17% EDTA or BioPure MTAD: X-ray diffraction and SEM analysis. Quintessence International. 2012 Nov 1;43(10).)
57. Saraswathi MV, Ballal NV, Padinjaral I, Bhat S. Ultra morphological changes of root canal dentin induced by 940 nm diode laser: An *in-vitro* study. Saudi Endod J 2012;2:131-5.

58. Alfredo E, Silva SR, Ozório JE, Sousa-Neto MD, Brugnera-Júnior A, Silva-Sousa YT. Bond strength of AH Plus and Epiphany sealers on root dentine irradiated with 980 nm diode laser. International endodontic journal. 2008 Sep;41(9):733-40.
59. ReDent-Nova, Ra'anana, Israel) and the RinsEndo, (DVurr Dental, Bietigheim, Germany (Yaylali IE, Kececi AD, Kaya BU. Ultrasonically activated irrigation to remove calcium hydroxide from apical third of human root canal system: a systematic review of in vitro studies. Journal of Endodontics. 2015 Oct 1;41(10):1589-99.
60. Jaju S, Jaju PP. Newer root canal irrigants in horizon: a review. International journal of dentistry. 2011 Oct;2011.
61. Mohammadi Z. An update on the antibiotic-based root canal irrigation solutions. Iranian endodontic journal. 2008;3(2):1.
62. Siqueira Jr JF, Magalhães KM, Rôças IN. Bacterial reduction in infected root canals treated with 2.5% NaOCl as an irrigant and calcium hydroxide/camphorated paramonochlorophenol paste as an intracanal dressing. Journal of endodontics. 2007 Jun 1;33(6):667-72.
63. Lee JK, Park YJ, Kum KY, Han SH, Chang SW, Kaufman B, Jiang J, Zhu Q, Safavi K, Spångberg L. Antimicrobial efficacy of a human β-defensin-3 peptide using an E nterococcus faecalis dentine infection model. International endodontic journal. 2013 May;46(5):406-12.
64. Haapasalo M, Qian W, Portenier I, Waltimo T. Effects of dentin on the antimicrobial properties of endodontic medicaments. Journal of endodontics. 2007 Aug 1;33(8):917-25.

65. Jagdale S, Bhargava K, Bhosale S, Kumar T, Chawla M, Jagtap P. Comparative evaluation of coronal discoloration induced by two triple antibiotic revascularization protocols when used at varying depths of temporary sealing material at the end of varying time periods. Journal of Conservative Dentistry. 2018 Jul 1;21(4):388-93.
66. Madhukumar M, Geetha P, Nair KR, Unnikrishnan M. The effects of double antibiotic paste and amoxicillin-clavulanate paste used in endodontic regeneration on microhardness of radicular dentine: an in vitro study. Journal of Pharmacy and Bioallied Sciences. 2021 Jun 1;13(Suppl 1):S510-5.
67. Jachlewski S, Jachlewski WD, Linne U, Bräsen C, Wingender J, Siebers B. Isolation of extracellular polymeric substances from biofilms of the thermoacidophilic archaeon Sulfolobus acidocaldarius. Frontiers in bioengineering and biotechnology. 2015 Aug 27;3:123.
68. Fava LR, Saunders WP. Calcium hydroxide pastes: classification and clinical indications. International endodontic journal. 1999 Jul;32(4):257-82.
69. Hoeck U, Kreilgard B, Nathansen C, inventors; Pharmacia AB, assignee. Transdermally administered acetylcysteine as mucolytic agent. United States patent US 6,620,428. 2003 Sep 16.
70. Khullar S, Aggarwal A, Chhina H, Kaur T, Sharma M, Bala D. Sealer penetration in the dentinal tubules: A confocal laser scanning microscopy study. Endodontology. 2021 Apr 1;33(2):92-6.
71. Najafzadeh R, Fazlyab M, Esnaashari E. Comparison of bioceramic and epoxy resin sealers in terms of marginal adaptation and tubular penetration depth with different obturation techniques in premolar teeth: A scanning electron

microscope and confocal laser scanning microscopy study. Journal of Family Medicine and Primary Care. 2022 May 1;11(5):1794-7.

72. Guivarc'h M, Jeanneau C, Giraud T, Pommel L, About I, Azim AA, Bukiet F. An international survey on the use of calcium silicate-based sealers in non-surgical endodontic treatment. Clinical Oral Investigations. 2020 Jan;24:417-24.
73. Khatod S, Ikhar AD, Nikhade PP, Chandak M, Motwani NM, Chandak MS, Rathi CK, Jaiswal AS. Removal techniques for intracanal medicament—A review. J Evol Med Dent Sci. 2020;9:1097-1.
74. Jurič IB, Anić I. The use of lasers in disinfection and cleanliness of root canals: a review. Acta Stomatologica Croatica. 2014 Mar;48(1):6.
75. Niemz MH. Laser-tissue interactions. Springer-Verlag Berlin Heidelberg; 2007.
76. Bago I, Plečko V, Gabrić Pandurić D, Schauperl Z, Baraba A, Anić I. Antimicrobial efficacy of a high-power diode laser, photo-activated disinfection, conventional and sonic activated irrigation during root canal treatment. International endodontic journal. 2013 Apr;46(4):339-47
77. Mathew J, Emil J, Paulaian B, et al. Viability and antibacterial efficacy of four root canal disinfection techniques evaluated using confocal laser scanning microscopy J Conserv Dent 2014;17(5):444–448. DOI: 10.4103/0972-0707.139833.
78. Asnaashari M, Homayuni H, Paymanpour P. The antibacterial effect of additional photodynamic therapy in failed endodontically treated teeth: a pilot study. Journal of lasers in medical sciences. 2016;7(4):238.

79. Van der Sluis LW, Versluis M, Wu MK, Wesselink PR. Passive ultrasonic irrigation of the root canal: a review of the literature. International endodontic journal. 2007 Jun;40(6):415-26
80. Yasui K, Yasui K. Acoustic cavitation. Springer International Publishing; 2018.
81. Yasui K, Yasui K. Bubble dynamics. Acoustic Cavitation and Bubble Dynamics. 2018:37-97.
82. Verhaagen B, Boutsioukis C, Van der Sluis LW, Versluis M. Acoustic streaming induced by an ultrasonically oscillating endodontic file. The Journal of the Acoustical Society of America. 2014 Apr 1;135(4):1717-30.)
83. Macedo RG, Verhaagen B, Rivas DF, Gardeniers JG, Van der Sluis LW, Wesselink PR, Versluis M. Sonochemical and high-speed optical characterization of cavitation generated by an ultrasonically oscillating dental file in root canal models. Ultrasonics sonochemistry. 2014 Jan 1;21(1):324-35
84. Verhaagen B, Boutsioukis C, Van der Sluis LW, Versluis M. Acoustic streaming induced by an ultrasonically oscillating endodontic file. The Journal of the Acoustical Society of America. 2014 Apr 1;135(4):1717-30.
85. Conde AJ, Estevez R, Loroño G, Valencia de Pablo Ó, Rossi-Fedele G, Cisneros R. Effect of sonic and ultrasonic activation on organic tissue dissolution from simulated grooves in root canals using sodium hypochlorite and EDTA. International endodontic journal. 2017 Oct;50(10):976-82.
86. Verstraeten JA, Jacquet W, De Moor RJ, Meire MA. Hard tissue debris removal from the mesial root canal system of mandibular molars with ultrasonically and laser-activated irrigation: a micro-computed tomography study. Lasers in medical science. 2017 Dec;32:1965-70.

87. De Groot SD, Verhaagen B, Versluis M, Wu MK, Wesselink PR, Van Der Sluis LW. Laser-activated irrigation within root canals: cleaning efficacy and flow visualization. International endodontic journal. 2009 Dec;42(12):1077-83.

88. Boutsioukis C, Lambrianidis T, Kastrinakis E. Irrigant flow within a prepared root canal using various flow rates: a computational fluid dynamics study. International Endodontic Journal. 2009 Feb;42(2):144-55.

89. ReDent-Nova, Ra'anana, Israel) and the RinsEndo, (DVurr Dental, Bietigheim, Germany (Yaylali IE, Kececi AD, Kaya BU. Ultrasonically activated irrigation to remove calcium hydroxide from apical third of human root canal system: a systematic review of in vitro studies. Journal of Endodontics. 2015 Oct 1;41(10):1589-99.

90. Tedesco M, Chain MC, Bortoluzzi EA, da Fonseca Roberti Garcia L, Alves AM, Teixeira CS. Comparison of two observational methods, scanning electron and confocal laser scanning microscopies, in the adhesive interface analysis of endodontic sealers to root dentine. Clinical oral investigations. 2018 Jul;22:2353-61.

91. Chadha R, Taneja S, Kumar M, Gupta S. An in vitro comparative evaluation of depth of tubular penetration of three resin-based root canal sealers. Journal of Conservative Dentistry and Endodontics. 2012 Jan 1;15(1):18-21.

92. Paqué F, Luder HU, Sener B, Zehnder M. Tubular sclerosis rather than the smear layer impedes dye penetration into the dentine of endodontically instrumented root canals. International Endodontic Journal. 2006 Jan;39(1):18-25.

ANNEXURE – I – ETHICAL APPROVAL CERTIFICATE

Research and Ethics Committee
KLE VK INSTITUTE OF DENTAL SCIENCES
A Constituent Unit of KLE Academy of Higher Education & Research
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

CERTIFICATE

Sl. No. : 1601

EC/NEW/INST/2021/2435
Research & Ethics Committee

This is to Certify that the synopsis titled

Dentinal tubule penetration of a silicone based endodontic Sealer following N-acetyl cysteine intracanal medicament removal using Ultrasonic Agitation and Laser Activated Irrigation - An In vitro study *Submitted by*

Dr. **REG.NO. IE0221003** *P. G. Student / Staff, Guided by* Dr. *from Department of* Conservative Dentistry and Endodontics *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date : 3/4/24

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

MEMBER SECRETARY
Research and Ethical Committee
KLEVK Institute of Dental Sciences
BELAGAVI.

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

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

ANNEXURE – II – BIOSTATISTICS CLEARANCE LETTER

KLE V.K. Institute of Dental Sciences

(A Constituent unit of KLE Academy of Higher Education & Research Deemed-to-be-University u/s 3 of the UGC Act, 1956)

Nehru Nagar, Belagavi-590 010 INDIA

Re-Accredited 'A' grade by NAAC (2nd Cycle) & Placed in Category 'A' by MHRD (GoI)

☎: 0831-2470362 **Web:** http://www.kledental-bgm.edu.in

FAX: 0831-2470640 **E-mail:** principal@kledental-bgm.edu.in

Biostatistics Clearance Certificate

This is to certify that the Biostatistics aspect of the Dissertation / Research work of **REG.NO. IE0221003** **Post Graduate Student**, under the guidance of **Professor, Department of Conservative Dentistry and Endodontics** entitled "Dentinal tubule penetration of a silicone-based endodontic sealer following N- acetyl cysteine intracanal medicament removal using Ultrasonic Agitation and Laser-Activated Irrigation- an In- vitro study. " has been done under my guidance and considered satisfactory.

Dr. S. B. JAVALI Ph.D.
Sr. Associate Professor in Statistics
Department of Community Medicine
USM KLE International Medical Programme
BELAGAVI-590010.

Place: Belagavi
Date: 29/03/2024

Name & Signature of Biostatistician