"COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY OF N- ACETYL CYSTEINE WITH PHOTODYNAMIC THERAPY AND DIODE LASER ON ROOT CANALS INFECTED WITH ENTEROCOCCUS FAECALIS –AN INVITRO STUDY."

By

Dr. NISHI SINGH

REG.NO. IE0222004

Dissertation

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

MASTER OF DENTAL SURGERY

In

CONSERVATIVE DENTISTRY AND ENDODONTICS

(BRANCH – IV)

Under the Guidance of

Dr. SURESH SHENVI M.D.S

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

2022-2025

KLE ACADEMY OF HIGHER EDUCATION & RESEARCH ,

BELAGAVI KARNATAKA

ENDORSEMENT BY THE HEAD OF DEPARTMENT, PRINCIPAL OF THE INSTITUTION

This is to certify that the dissertation entitled **"COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY OF N ACETYL CYSTEINE WITH PHOTODYNAMIC THERAPY AND DIODE LASER INFECTED WITH ENTEROCOCCUS FAECALIS – AN INVITRO STUDY."** iis a bonafide research work done by **REG. NO. IE0222004.**

Head of Department

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

Dr. Sonal B Joshi
Reg No. 27788-A
Consultant
Conservative Dentistry & Endodontics
KLE VK IDS, Belgaum

Date: 19th APRIL 2025
Place: Belagavi

Principal

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

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

Date: 19/4/25
Place: Belagavi

UNDERTAKING

I, **REG. NO. IE0222004**, hereby declare that the information and data mentioned in my thesis entitled **"COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY OF N- ACETYL CYSTEINE WITH PHOTODYNAMIC THERAPY AND DIODE LASER ON ROOT CANALS INFECTED WITH *ENTEROCOCCUS FAECALIS* – AN INVITRO STUDY."** belongs to me and is original.

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

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

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

Place: Belagavi

Date: 19.04.25

REG. NO. IE0222004

LIST OF ABBREVIATIONS

SR.NO	ABBREVIATIONS	FULL FORM
1	AgNP's	Silver nanoparticles
2	ANOVA	Analysis of Variance
3	Apdt	Antimicrobial photodynamic therapy
4	BMP	Biomechanical preparation
5	CAP	Cold Atmospheric Plasma
6	CBCT	Cone Beam Computed Tomography
7	CEJ	Cemento-Enamel Junction
8	CFU	Colony Forming Unit
9	CH	Calcium Hydroxide
10	CHX	Chlorhexidine
11	CUR	Curcumin
12	DL	Diode Laser
13	DOF	Degree Of Freedom
14	*E. faecalis*	*Enterococcus faecalis*
15	EDTA	Ethylene Diamine Tetra-acetic Acid
16	EPS	Extracellular polymeric substances
17	Er:YAG	Erbium-doped yttrium aluminium garnet laser
18	*et al*	Additional persons involved in the same study
19	Gm	Gram

20	Hrs	Hours
21	ICG	Indocyanin Green
22	J	Joule
23	LED	Light Emitting Diode
24	MB	Methylene Blue
25	MG	Malachite Green
26	Mg	Milligram
27	Ml	Millilitre
28	Mm	Millemeter
29	N	Number of specimens
30	NAC	N-Acetylcysteine
31	NaOCl	Sodium Hypochlorite
32	nm	Nanometer
33	OSHA	Occupational Safety and Health Administration
34	PAD	Photo Activated Disinfection
35	PDT	Photodynamic Therapy
36	pH	Power of hydrogen
37	PT	Photothermal
38	p-value	Probability of obtaining a test statistic at least as extreme as the one that was actually observed
39	ROS	Reactive Oxygen Species
40	SD	Standard Deviation

41	Sec	Seconds
42	SEM	Scanning Electron Microscope
43	TAP	Triple Antibiotic Paste
44	TCMD	Traditional conventional mechanical debridememt
45	US scaler	Ultra-Sonic scaler
46	Vs	Versus
47	W	Watt
48	WL	Working Length
49	%	Percent
50	<	Less than
51	>	Greater than
52	µL	Micro litre
53	µm	Micrometer

ABSTRACT

Aim:

Comparative evaluation of antimicrobial efficacy of N-acetyl cysteine with Photodynamic therapy and Diode laser on root canals infected with *Enterococcus faecalis* - an in vitro study

Study design:

Eighty-four single rooted human mandibular premolars were stored in 0.1% thymol solution till use after removing the organic debris. The crowns were sectioned at the cemento- enamel junction (CEJ) to obtain the root canal length of 14mm and were divided into 3 groups (n=28), according to the disinfection protocol used. Group 1-NAC; Group 2-NAC in combination with PDT; Group 3-NAC in combination with diode laser. Antimicrobial efficacy is determined by microbiological culture analysis (CFU/ml). The root canals were contaminated with *E.faecalis* biofilm, instrumented and then irrigated according to the experimental groups. Two samples were collected from the root canals before and after irrigation according to various experimental groups. These samples were plated in specific media cultures, to assess the presence or absence of microbial growth and determine the average reductions of viable microorganisms.

Results:

The mean reduction in CFU values post-treatment relative to pre-treatment was 0.85 ± 0.29 in Group I. Group II showed a higher mean reduction of 1.53 ± 0.551. In contrast, Group III exhibited a mean reduction of 0.91 ± 0.121.

A one-way ANOVA revealed statistically significant differences in CFU reduction among the three groups ($F(2, 81) = 29.69$, $P < 0.0001$). Tukey's post-hoc test indicated that Group II had a significantly greater CFU reduction than Group I ($P < 0.0001$), while Group I and Group III did not differ significantly ($P = 0.8346$). Additionally, Group II showed a significantly greater reduction than Group III ($P < 0.0001$).

Conclusion:

All three disinfection methods were effective for partial elimination of *E. faecalis* biofilm. But PAD was significantly more efficacious as compared to NAC and diode laser assisted irrigation

Key words: *Enterococcus faecalis*, N-acetylcysteine, Photodynamic therapy, Diode laser, irrigation.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF FIGURES

ANNEXURE

INTRODUCTION

Endodontic treatment failure is thought to be primarily caused by persistence of bacteria within the intricate anatomical structure of root canal system. Bacterial presence and their metabolic by-products primarily drive pulpal and periapical diseases.[1] Thus, primary goal of endodontic therapy is to comprehensively disinfect root canal system along with three-dimensional network of dentinal tubules.[2]

Complex anatomy of root canal system, existence of smear layer on canal walls and in dentinal tubules, and development of germ biofilms at different depths of dentin, all result in inadequate cleaning of canal system.[3] Studies show that 40% to 60% of root canal surface remains polluted with bacteria or their by-products, even after traditional chemomechanical debridement (TCMD).[4]

Enterococcus faecalis (*E. faecalis)* is robust microorganism usually found in both primary and chronic endodontic infections.[5] It can persist and proliferate in managed root canals, withstanding extreme conditions such as low pH and high temperatures for extended periods of time.[6] According to an in vitro study by Kishen et al., *E. faecalis* can initiate biofilm formation on dentinal surfaces, which over time may become calcified, forming a protective barrier resistant to antimicrobial agents.[7]

Biofilms generally demonstrate much greater resistance to bactericidal agents than their planktonic forms.[8] *Streptococcus mutans* is another microbial species occasionally found in endodontic infections.[9] It works synergistically with other microbes, further enhancing biofilm formation. Diverse and dynamic nature of microbial communities within infected root canals and their biofilm-forming abilities necessitate effective irrigation strategies.[10]

Reports indicate that even most sophisticated instrumentation methods fail to treat over 35% of root canal surfaces, such as fins, lateral and accessory canals, cul-de-sacs, and isthmuses.[11] Fortunately, irrigating solutions can access these complex areas, aiding in removing or reducing biofilms and enhancing the overall success rate of endodontic therapy.[12]An ideal irrigant should possess multiple desirable characteristics: strong antimicrobial activity, biocompatibility, and favorable physical characteristics. [13]

Sodium hypochlorite (NaOCl) is gold standard for root canal irrigation due to its strong antibacterial characteristics and capability to dissolve organic tissue, preventing bacterial adherence to dentinal walls.[14] Chlorhexidine (CHX), commonly used at a 2% concentration, also exhibits antibacterial effects against Gram-positive & Gram-negative bacteria.[15] Nevertheless, evidence indicates, neither 2% CHX nor 1%–3% NaOCl is entirely effective in eliminating biofilm-associated bacteria such as *E. faecalis*.[16] Berber et al. observed that higher concentrations of NaOCl improved disinfection efficacy; however, such concentrations pose a significant risk of periapical tissue irritation.[17] Additionally, biofilm-embedded bacteria demonstrate heightened resistance to antimicrobials like CHX, highlighting the need for alternative irrigants capable of disrupting biofilms while remaining biocompatible.[18]

N-acetylcysteine (NAC), derivative of amino acid L-cysteine, is acknowledged antioxidant and mucolytic agent, proven effective against *E. faecalis*.[19] Many studies indicate that NAC can inhibit growth and biofilm formation of various microbes, including *E. faecalis.* Its mechanism involves disrupting polysaccharide synthesis, a critical biofilm matrix component, thus preventing bacterial adhesion to dentinal surfaces.[20] By reducing bacterial exopolysaccharides, NAC interferes with

the ability of biofilms to trap and concentrate essential minerals and nutrients, leading to the destabilization of mature biofilms and reduced bacterial viability.[20]

Notably, the antimicrobial effects of NAC are not significantly diminished by the buffering action of dentin, which can otherwise compromise the activity of NaOCl and CHX.[21] Despite the effectiveness of chemical irrigants, their action is largely confined to the superficial dentin layers adjacent to the canal walls, with a maximum penetration depth of approximately 130 µm. In contrast, bacteria can infiltrate dentin up to depths of 1000 µm.[13] To address this limitation and enhance the efficacy of root canal disinfection, adjunctive techniques such as laser irradiation and Photodynamically activated disinfection (PAD) have been proposed.

Laser irradiation has been thoroughly researched as additional method for disinfecting root canals, either on its own or alongside TCMD.[22] Various laser wavelengths, particularly diode and neodymium lasers, have significantly reduced intracanal bacterial load. Diode lasers, in particular, are favored for their compact design and cost-effectiveness.[9, 23] One of key advantages of laser treatment is its ability to penetrate dentin adjacent to the canal, exerting antimicrobial effects through mechanisms such as light scattering, localized intensity enhancement, and attenuation.[24] Absorbed laser energy is converted into thermal energy, inducing tissue alterations.[25] The extent of laser energy absorption is influenced by its wavelength and the optical properties of the target tissue, including its pigmentation and water content.[26]

Photodynamic therapy (PDT) is a promising supplementary method that utilizes a low-level laser alongside a photosensitizing agent to produce antimicrobial effects while avoiding cytotoxicity or thermal harm to dentin and nearby tissues.[26]

PDT operates on principle of activating photosensitizer molecules—whether sourced from within body or introduced externally—using visible light, mainly in red or near-infrared spectrum.[26] This process generates reactive oxygen species (ROS), involving singlet oxygen, that interact with cellular targets, leading to damage and death of the cells.[27] In recent years, the application of PDT has broadened significantly, offering a potential alternative for overcoming antibiotic resistance in endodontic infections.[28]

Although NAC, diode lasers, and PDT individually demonstrate antimicrobial efficacy, combining them may provide synergistic benefits. However, research on such combinations is incomplete.[29] This research seeks to assess and compare effectiveness of NAC combined with Diode laser (DL) therapy and PDT in targeting *Enterococcus faecalis*, which is most resistant and commonly found microorganism in infected dental pulp. The null hypothesis to be tested was that the groups had no difference in antibacterial efficacy.

AIMS AND OBJECTIVES

AIM

To evaluate and compare effectiveness of supplemental Photodynamic therapy and Diode laser irradiation with N-acetyl cysteine in optimising the removal of bacteria from root canals infected with *Enterococcus faecalis.*

OBJECTIVES

1. To evaluate the antibacterial effect of N-acetyl cysteine as root canal irrigant in canals infected with *Enterococcus faecalis.*
2. To evaluate the antibacterial effect of Photodynamic Therapy (PDT) on canals infected with *Enterococcus faecalis.*
3. To evaluate the antibacterial effect of Diode laser on canals infected with *Enterococcus faecalis.*
4. To compare the effectiveness of Photodynamic therapy and Diode laser with N-acetyl cysteine in removing the bacteria from the root canals infected with *Enterococcus faecalis*.

HYPOTHESIS

NULL HYPOTHESIS: There will be no reduction in the microorganisms count when N-acetyl cysteine will be used in combination with PDT and Diode laser.

ALTERNATIVE HYPOTHESIS: There will be reduction in the microorganism count when N-acetyl cysteine will be used in combination with PDT and Diode laser.

REVIEW OF LITERATURE

1. Du et al. studied antibacterial properties of antimicrobial Photodynamic therapy (a-PDT) with 0.01% methylene blue (MB) against *Enterococcus faecalis* biofilms found in bovine and human dentin. a-PDT was compared with 5% NaOCl and saline across different exposure times (3,12, and 30minutes). Researchers employed LIVE/DEAD staining and confocal laser scanning microscopy (CLSM) for determining that both a-PDT and NaOCl notably enhanced bacterial death with extended exposure durations. However, most bacterial killing occurred within the first 3 minutes. NaOCl was more effective than a-PDT at all time points. Additionally, younger biofilms were more susceptible to disinfection methods than mature biofilms. No structural damage to dentin was observed with prolonged aPDT exposure. The results indicate that a-PDT using MB can significantly diminish *E. faecalis* biofilms, especially during their initial stages of formation.[30]

2. In this study, Sardari et al. assessed how well a 980 nm diode laser, combined with 2% chlorhexidine (CHX) and 2.5% NaOCl, can eradicate *Enterococcus faecalis* from root canals. Results showed that DL significantly enhanced the antibacterial efficacy of CHX and NaOCl, with the combination treatments outperforming the irrigants alone.[31]

3. The effects of three disinfection techniques on *E. faecalis* in single-root deciduous teeth were compared by Tehrani et al., using direct high-intensity 810 nm diode laser, PDT with MB and diode laser, and 2.5% sodium hypochlorite. Colony counts (CFUs) following bacterial inoculation were measured after treatment. All treatments examined demonstrated antibacterial activity against

E. faecalis, with sodium hypochlorite emerging as most effective disinfection method, followed by PDT and high-intensity laser treatment.[32]

4. Hoshyari et al. assessed antibacterial efficacy of triple antibiotic paste (TAP), DL irradiation at 970 nm and 445 nm, both alone and in combination with TAP, sodium hypochlorite, and 660 nm Photodynamic treatment using doxycycline as a photosensitizer on *E. faecalis* in this in vitro investigation. Although every evaluated approach demonstrated antibacterial activity, TAP—either by itself or in conjunction with diode lasers—was the most successful in eliminating germs. Diode lasers and PDT with doxycycline also demonstrated significant, but weaker, antibacterial effects.[33]

5. In this study Wang et al, evaluated the effectiveness of three decontamination methods—CHX + Er: YAG laser, CHX + PDT, and CHX alone—on reducing biofilm vitality on titanium surfaces simulating dental implants. Using a custom mouth device worn by eight volunteers, biofilm was allowed to develop on titanium discs over 72 hours. Fluorescence microscopy and image analysis were used to quantify residual vital biofilm. Results showed that CHX combined with Er: YAG laser significantly reduced biofilm on titanium surfaces and appeared superior to both PDT and CHX[34]

6. In this in vitro research, Yavagal et al. evaluated disinfection efficiency of saline, 2.5% NaOCl solution, and 5.25% NaOCl gel, comparing results both with and without DL activation, in root canals of primary teeth contaminated with *E faecalis*. Laser-activated irrigation with 5.25% NaOCl gel proved to be the most effective method for eliminating *E. faecalis*.[35]

7. Afrasiabi et al. assessed effectiveness of hydrogen peroxide (HP) as photosensitiser without irradiation and with 980 nm diode laser-based aPDT targeting *Enterococcus faecalis* in both its planktonic and biofilm states. The results showed HP could improve anti-biofilm efficacy as a photosensitizer in a-PDT[36]

8. The in vitro research carried out by Haghighi et al. assessed antibacterial effectiveness of cold atmospheric plasma (CAP), Photodynamic therapy (PDT) , two distinct Diode laser, 2.5% sodium hypochlorite (NaOCl) and photosensitizers in disinfecting root canals of primary mandibular second molars infected with *E. faecalis*. Research revealed that greatest decrease in bacterial count occurred with 2.5% NaOCl, and that CAP was equally effective, indicating it as a valid alternative. However, the 940 nm DL showed the least effect[37]

9. Goel et al. compared antimicrobial effectiveness of 0.2% chitosan, 3% NaOCl, and 2% CHX against *E. faecalis*, both alone and in conjunction with an 810-nm Diode laser. Teeth have been categorized into experimental groups according to irrigants used and further divided into subgroups based on application of laser. Results showed that chlorhexidine group exhibited the highest antimicrobial efficacy, followed by chitosan group II. Combining irrigants with DL enhanced disinfection compared to irrigants or laser alone.[38]

10. In this invitro study by Sharma et al., antimicrobial effectiveness of photosensitized nanoparticles, DL, and various irrigation combination like - NaOCl alone, NaOCl with EndoActivator, NaOCl with 910 nm diode laser, and a group treated with silver nanoparticles (AgNPs), indocyanine green (ICG),

and 910 nm DL was evaluated against *E. faecalis* in root canals. Results showed that NaOCl + DL group demonstrated highest antimicrobial efficacy (99.93% reduction), followed by the NaOCl + EndoActivator group. Although the AgNPs/ICG/DL group showed promise, its results were not significantly superior to those of NaOCl alone.[39]

11. Jambagi et al. compared antimicrobial effectiveness of DL irradiation, ultrasonic-activated irrigation, and traditional irrigation using 2.5% sodium hypochlorite in single-rooted canals. Microbial samples were collected before and after cleaning, shaping, and disinfection. DL disinfection demonstrated superior antimicrobial efficacy compared to ultrasonic and conventional irrigation methods.[40]

12. Hasna et al. investigated the best ways to remove *E. faecalis* biofilm using CH, NAC, PDT, and their combination NAC + PDT. According to his findings, NAC, NAC+PDT, and calcium hydroxide (CH) significantly reduced bacterial counts compared to saline and PDT alone.Also, NAC alone showed strong antibacterial activity, comparable to CH, and did not require PDT to be effective.[41]

13. Ghorbanzadeh et al. studied effectiveness of three disinfection methods - CCMD, CCMD + light-activated disinfection (LAD) using ICG and an 810 nm DL and CCMD + DL (810 nm, 2 W) alone against *E. faecalis* biofilms in root canals, focusing on both immature(4-day) and mature(4-week) biofilms. Results showed that none of methods eliminated biofilm. CCMD + LAD exhibited highest antibacterial activity, whereas CCMD + DL was least effective.[22]

14. Study by Torres et al. conducted in vitro, antibacterial effects of red laser therapy using various photosensitizers—MB and malachite green (MG)—were evaluated on monoradicular premolars contaminated with *E. faecalis* ATCC 29212. It was concluded that the red laser combined with methylene blue demonstrated the highest antimicrobial potential against *E. faecalis* compared to malachite green.[42]

15. Antimicrobial effectiveness of 5.25%NaOCl, 2%CHX, and 200mg/mL NAC was evaluated and compared against *E. faecalis* and *Streptococcus mutans (S. mutans)* by Bhasin et al. It was found that NAC demonstrated the greatest reduction in planktonic *S. mutans* counts, followed by NaOCl, CHX, and the control. NAC group exhibited lowest count of *E. faecalis*.[43]

16. This study by Eslami et al. compared antimicrobial effects of calcium hydroxide ($Ca(OH)_2$), TAP, light-emitting diode (LED), toluidine blue (TOL), PDT, and 940nm diode laser (DL) against *E. faecalis* and Candida albicans biofilms in ex-vivo human teeth root canals, using scanning electron microscopy (SEM). Research found that TAP, PDT, and LED were most effective methods for decreasing biofilm thickness, with TAP demonstrating greatest reduction.[44]

17. Sarda et al. assessed the antimicrobial effectiveness of DL, PDT, NaOCl, and their combinations on *E. faecalis* and *S. mutans*. Combination of PAD and 3% NaOCl, utilizing laser with lower wavelength, proved to be especially effective. This implies that this combination could offer a better alternative for disinfection of root canals against both *E. faecalis and S mutans*.[29]

18. This study by Singh et al. aimed to compare antimicrobial efficacy of 2%CHX and NAC as root canal irrigants against *S. mutans* and *E. faecalis*. The study

concluded that NAC is highly effective against endodontic pathogens and could be promising alternative irrigant in root canal treatment.[45]

19. In this, in vivo study, Sonarkar et al. compared antibacterial efficacy of PAD, DL, 5% NaOCl, and normal saline in 32 patients and assessed it using microbial culturing techniques. The result showed statistically significant reduction of aerobic and anaerobic microorganisms with PAD and significant reductions with DL, 5% NaOCl.[46]

20. Pourhajibagher et al. studied effectiveness of NaOCl, CHX, and a-PDT using curcumin (CUR) and ICG in eliminating *E. faecalis*. a-PDT demonstrated strong antibacterial and anti-biofilm activity, comparable to traditional irrigants, indicating its potential as an effective adjunctive strategy in endodontic disinfection.[47]

21. This study assessed antibacterial effectiveness of several disinfection protocols: DL (810nm, 2W), LAD with ICG, 0.2% CHX, 0.2% CHX combined with LAD, and 0.2% CHX combined with DL, as reported by Ghorbanzadeh et al. It was observed that none of the methods eliminated biofilm bacteria. However, greatest reduction in biofilm was observed with CHX + LAD method, while DL alone showed least efficacy.[48]

22. Ozkocak et al. assessed effectiveness of various root canal disinfection methods—including 2% chlorhexidine (CHX), 5% sodium hypochlorite (NaOCl), Diode and Er: YAG lasers, and ICG-DL mediated photodynamic therapy (PDT)—against *E. faecalis* infection. All tested methods significantly reduced bacterial counts. Among them, 2% CHX, 5% NaOCl, and PDT using

ICG with a DL were found to be equally effective and superior to diode and Er:YAG lasers alone.[49]

23. In this study, Afkhami et al. evaluated disinfection efficiency of silver nanoparticles(AgNPs), 810-nm DL, conventional PDT utilizing ICG, and modified PDT that combines AgNPs with ICG and DL in their fight against E. faecalis in infected root canals. All treatment methods significantly reduced *E. faecalis* colony counts. The highest bacterial reduction (99.12%) was achieved with the modified PDT (AgNPs + ICG + diode laser).[50]

24. Antibacterial efficacy of PDT and chitosan against *E. faecalis* was evaluated by Camacho-Alonso et al. in vitro using infected root canals of extracted human teeth. Bacterial load was assessed by culturing samples and analyzing CFU/mL, while SEM was used to evaluate contamination. The combination of PDT and chitosan resulted in the lowest bacterial count and contamination area, suggesting enhanced antibacterial effectiveness when used together.[51]

25. In this study, Ridhalaksani et al. evaluated antibacterial potential of NAC at pH2.5 and pH11 against *E. faecalis* biofilms, compared to 2% CHX. NAC at pH 11 showed greatest reduction in bacterial colonies, with statistically significant results compared to NAC pH2.5 and CHX. NAC pH2.5 also reduced bacterial counts, but not significantly.[52]

MATERIALS AND METHODS

SOURCE OF DATA:

Study was carried out at KLE VK Institute of Dental Sciences' Department of Conservative Dentistry and Endodontics, KAHER, Belagavi, while laboratory work was done at Dr. Prabhakar Kore's Basic Science Research Laboratory, also at KAHER, Belagavi. Extracted human mandibular premolar teeth have been obtained from the KLE Academy of Higher Education & Research's Department of Oral and Maxillofacial Surgery at KLE VK Institute of Dental Sciences in Belagavi.

INCLUSION CRITERIA:

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

EXCLUSION CRITERIA:

- Teeth with radicular resorption, cracks or fracture line
- Root canal treated teeth.
- Teeth with calcified canals.
- Teeth with root caries.
- Teeth with multiple canals/Anatomic variation.

MATERIALS USED FOR THE STUDY:

- 84 human mandibular premolar teeth that were removed with a single root
- N-acetyl cysteine (Molychem), distilled water (NICE LIFE CARE)
- 0.85% saline solution, 3% sodium hypochlorite (NaOCl) (Vishal dental care)

- 17% ethylene diamine tetraacetic acid (EDTA) (CANALARGE)
- brain heart infusion agar plate
- brain heart infusion broth
- *E. faecalis* strain
- Indocyanin Green

ARMAMENTARIUM USED FOR THE STUDY:

- Double sided Carborundum discs
- Airotor handpiece (NSK)
- K Files (10,15) (MANI)
- ProTaper universal nickel-titanium files (Dentsply)
- Endomotor (Orikam)
- 27 gauge syringe (Dispovan)
- DL (Indilase) and Optic fibre tip (200µm)
- Paper points (Diadent)
- Eppendorf tubes
- Micropipettes and tips
- Laminar air flow

METHODOLOGY:

Eighty-four extracted human single-rooted, single canal mandibular premolar teeth have been chosen and sustained as per to 'OSHA (Occupational Safety and Health Administration)' rules. Ultrasonic scaler has removed calculus and soft tissue debris. Until it was needed, 0.1 percent thymol was used as storage option. Every tooth was chosen based on inclusion and exclusion criteria after being radiographed

SAMPLE PREPARATION:

Samples were decoronated using a carborundum disc to acquire standardized root length of 14mm, and their apex have been sealed with composite. The working length has been evaluated using 10 size k file until it is observed at apex and was determined by subtracting 1mm from it. Canals have been shaped to an F2 master apical file size using a Dentsply Protaper Universal file, accompanied by abundant irrigation with 3% NaOCl and 17%EDTA. Final irrigation has been done with 3ml of 3%NaOCl for 3 minutes, followed by a final rinse with distilled water. All specimens were sterilized in autoclave (20 min at 121°C). Five teeth from sterilised specimens were placed in sterile BHI broth to serve as a negative control to check for the absence of contamination. The apex of all remaining specimens were sealed using composite.

CONTAMINATION OF SPECIMENS:

MTCC 439 strain of *E. faecalis* will be used in the experiment, isolated colonies of which will be suspended in the BHI broth solution and will be incubated for 24 hrs to adjust turbidity and MC farland unit of solution to 0.5(1× 108 CFUmL−1). Each tooth sample has been placed in pre-sterilized microcentrifuge tube comprising 1mL of BHI broth. 50µL of inoculum containing *E. faecalis* has been transferred into each specimen. All procedures have been carried out under laminar flow. Purity of culture has been checked from subculturing 5µL of broth from incubated dentin specimens in BHI broth on BHI agar plates. Dentin specimens have been contaminated for 21 days at 37± 1ºC.

EXPERIMENTAL GROUPS:

After 21 days, samples have been categorized into three experimental groups based on disinfection protocols utilized.

GROUP 1: Disinfection of root canal with NAC

GROUP 2: Disinfection of root canal with NAC and PAD

GROUP 3: Disinfection of root canal with NAC and DL

DISINFECTION PROTOCOLS:

N-ACETYL CYSTEINE DISINFECTION

200mg/ml of NAC solution was prepared by dissolving 0.2gm in 1ml sterile distilled water with pH adjusted at 11 as per Quah etal.[21] All specimens of Group 1 were treated with 5 ml of this solution followed by final flush with 4 ml of distilled water.

N-ACETYL CYSTEINE (NAC) AND PHOTOACTIVATED DISINFECTION (PAD)

Initially, Group 2 specimens were treated with NAC, then underwent photo-activated disinfection using 1mg/ml Indocyanin Green in canal for 5 minutes. Activation was performed using diode laser with wavelength of 810nm and output power 0.1W, employing an optical fiber with diameter of 200 µm for 60 seconds, with 10-second interval after every 20 seconds.

N-ACETYL CYSTEINE (NAC) AND DIODE LASER DISINFECTION

For group 3, initial disinfection with NAC followed by its activation with 810 nm wavelength Diode laser set at 1.0 W was used. Optical fibre was positioned 1mm below apex and then recessed with helicoidal movements at approx 2mm/ sec^2 for five secs, repeated four times with intervals of 10 seconds in between.

ANTIMICROBIAL ASSESSMENT:

Samples were collected from each specimen both before and after disinfection by placing sterile 20-size paper points in root canal for 1min. Paper points were then shifted to Eppendorf tubes holding 10µl of saline. Using cell spreaders, samples were streaked on BHI agar media plate and incubated at 37°C for 72 hrs.

After this colony forming units will be calculated by following formula-

$$\text{Colony forming unit/mL} = \frac{\text{number of colonies formed}}{\text{Volume plated (mL)} \times \text{Total Dilution used(mL)}}$$

Results obtained was then tabulated.

STATISTICAL ANALYSIS:

Microsoft Excel 2021 was utilized to organize the data in a spreadsheet, and IBM SPSS Statistics for Windows, Version 27.0 (Armonk, NY: IBM Corp.), was used for statistical analysis. GraphPad Prism for Windows, Version 10.1.2 (GraphPad Software, La Jolla, California, USA), created graphs, box plots, and pie diagrams. Data have been transmuted to $\log_{10}$ scale to normalize data distribution and reduce variability, ensuring a more reliable comparison of CFU reductions across groups.

Visual inspection of histograms and Shapiro-Wilk's test, box plots, and normal Q-Q plots demonstrated that gathered data followed normal distribution after log transformation. Descriptive statistics were used to report the quantitative variables in terms of the mean (central tendency) and standard deviations (SD) (measures of dispersion) along with the minimum and maximum values to report range. Parametric tests were carried out to analyze quantitative variables. Intra-group comparisons have been analysed using Paired t-test. Inter-group comparisons have been conducted with One-way analysis of variance (ANOVA) with *post-hoc Tukey HSD test*. *P-value* $\leq$ *0.05* has considered a level of significance.

FLOWCHART OF THE STUDY

Eighty-four extracted human single-rooted single canal mandibular premolar teeth have been chosen and maintained conferring to 'OSHA guidelines'

⇩

Calculus and soft tissue debris have been cleaned utilizing an Ultrasonic scaler

⇩

Samples were decoronated to standardized root length to14 mm.

⇩

Working length has been evaluated using size 10 k file until it is observed at apex and recorded by subtracting 1mm from working length.

⇩

Instrumentation of root canal was completed to master apical file size of F2 by utilizing ProTaper universal, nickel-titanium rotary instruments under copious irrigation with 3%NaOCl and 17%EDTA

⇩

Final irrigation was done with 3ml of 3%NaOCl for 3minutes, followed by final rinse with distilled water

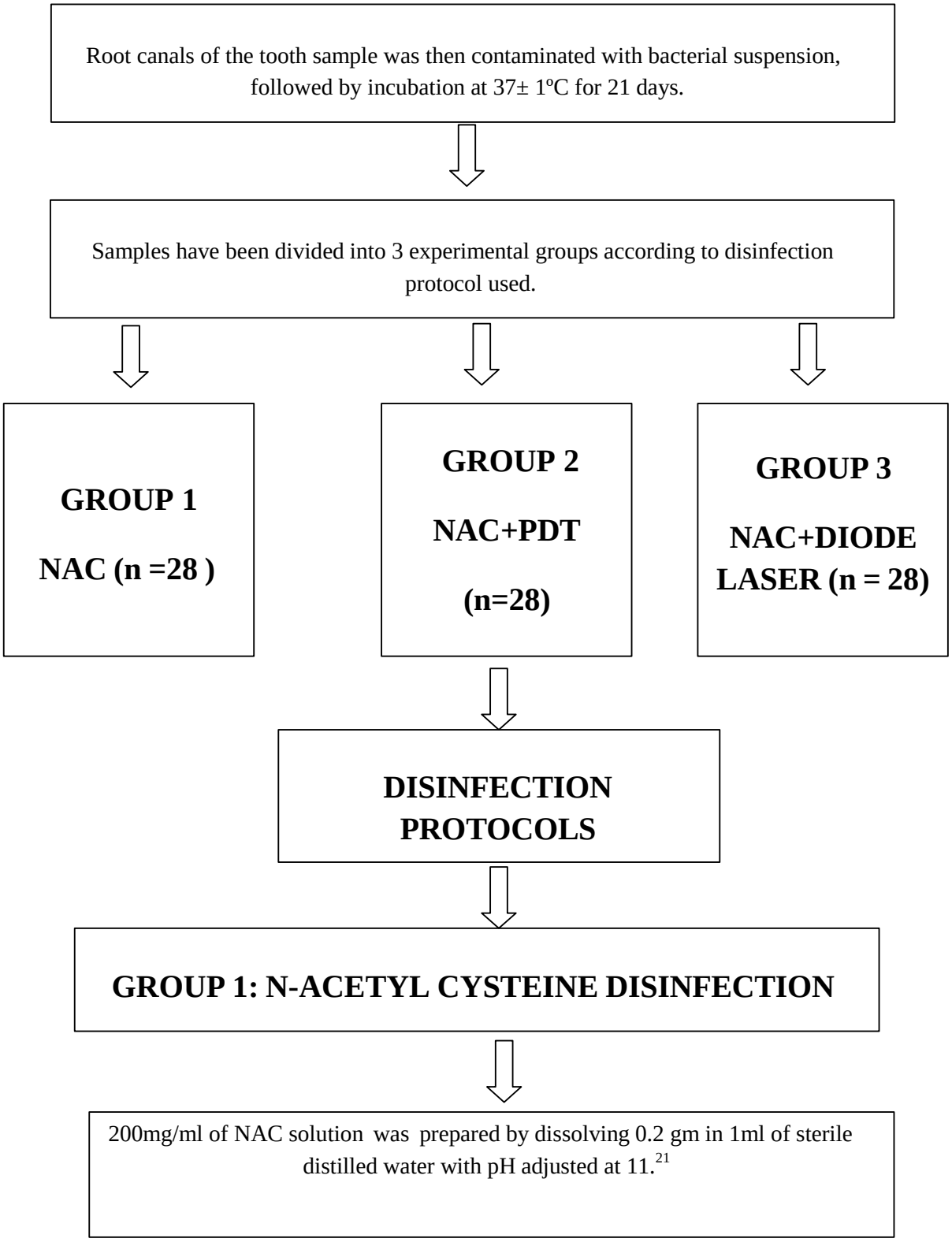
⇩

The apex of all the specimens were sealed with composite

⇩

MTCC 439 strain of *E.faecalis* was used in the experiment, isolated colonies of which was suspended in BHI broth solution and incubated for 24 hrs to adjust turbidity at 0.5 Mcfarland unit.

⇩


Root canals of the tooth sample was then contaminated with bacterial suspension, followed by incubation at 37± 1ºC for 21 days.
Samples have been divided into 3 experimental groups according to disinfection protocol used.
GROUP 1
NAC (n =28)
GROUP 2
NAC+PDT
(n=28)
GROUP 3
NAC+DIODE LASER (n = 28)
DISINFECTION PROTOCOLS
GROUP 1: N-ACETYL CYSTEINE DISINFECTION
200mg/ml of NAC solution was prepared by dissolving 0.2 gm in 1ml of sterile distilled water with pH adjusted at 11.[21]

5 ml of this solution was used for irrigating each sample with final flush with 4 ml of distilled water.

GROUP 2: N-ACETYL CYSTEINE (NAC) AND PHOTODYNAMIC THERAPY (PDT)

Initial disinfection with NAC followed by Photoactivated disinfection was done.

1mg/ml of Indocyanin Green was placed in the canal for 5 minutes.

Source of irradiation has been Diode laser with an output power of 0.1W and wavelength of 810nm, delivering 6J of energy. Light activation was done by using optical fibre of diameter 200 µm for 60 sec with an interval of 10 sec after every 20 secs.

GROUP 3: N-ACETYL CYSTEINE (NAC) AND DIODE LASER DISINFECTION

Initial disinfection with NAC followed by Diode laser disinfection was performed.

Diode laser with energy set at 1.0 W and central wavelength of 810 nm irradiation was used

The optical fibre was introduced 1mm short of the apex and was recessed in helicoidal movements at speed of around 2mm/sec^2 for 5 sec for 4 times at interval of 10 sec.

⇩

BACTERIOLOGICAL ANALYSIS

⇩

For assessment of the microbial growth, sterile 20 size paper points was introduced in the root canal for 1min both before and after the disinfection protocols

⇩

The paperpoints were transferred to the Eppendorf tubes containing I ml of sterile 0.85% saline solution and will be vortexed for 1min

⇩

Using cell spreaders, samples were streaked on BHI agar media plate and incubated at 37 ^{o}C for 72 hrs.

⇩

$$\text{Colony-forming unit/mL} = \frac{\text{number of colonies formed}}{\text{Volume plated (mL)} \times \text{Total Dilution used (mL)}}$$

The readings were then tabulated.

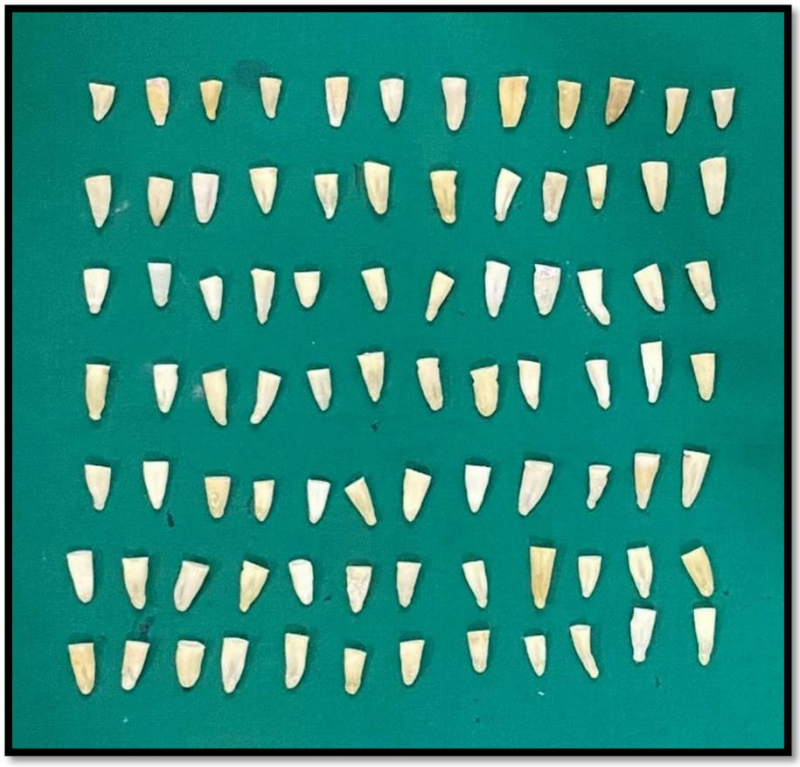


Fig 1: 84 extracted single rooted single canal Premolar teeth.

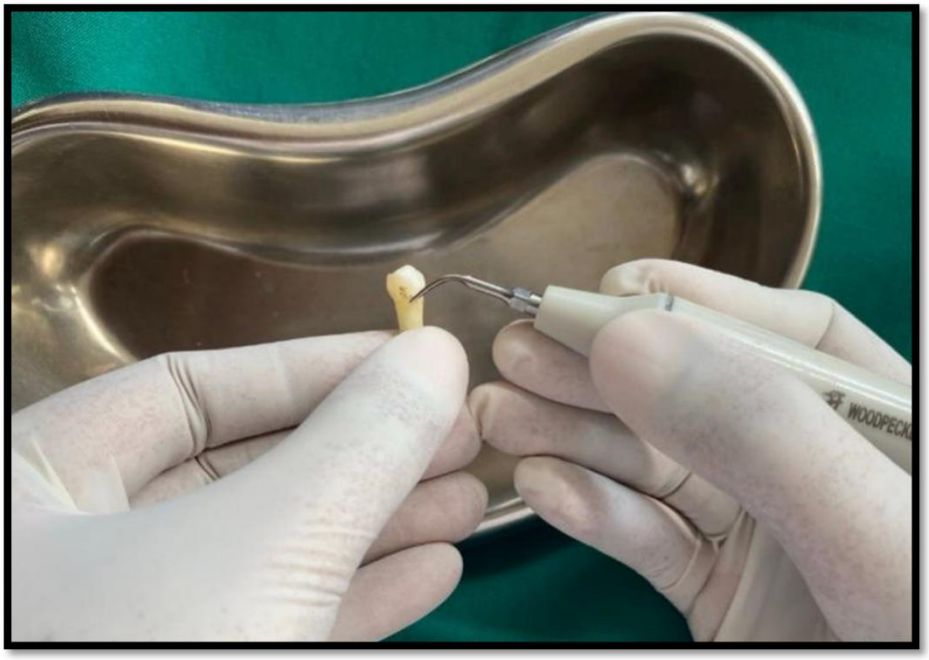


Fig 2: Debris and calculus removed using Ultrasonic scaler

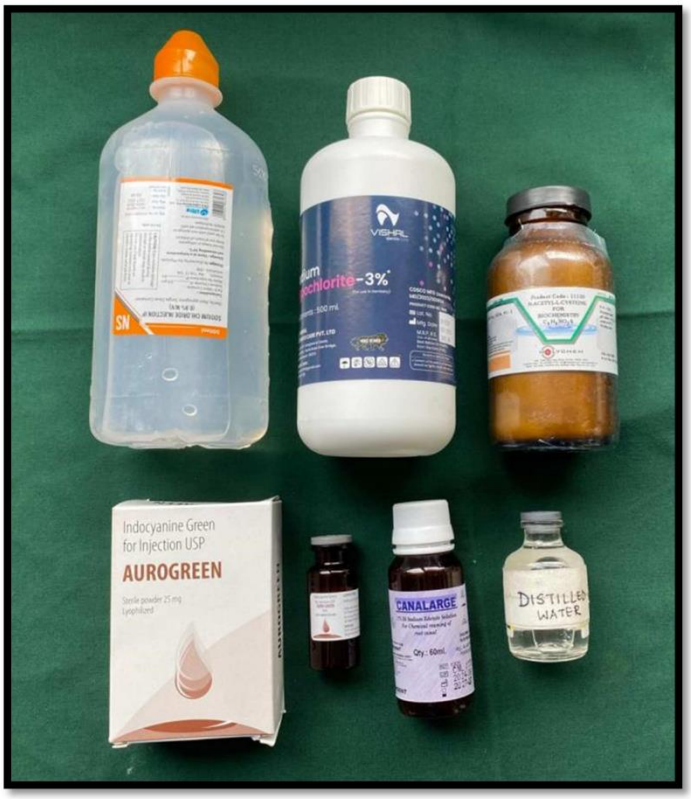


Fig 3: Materials used for the study

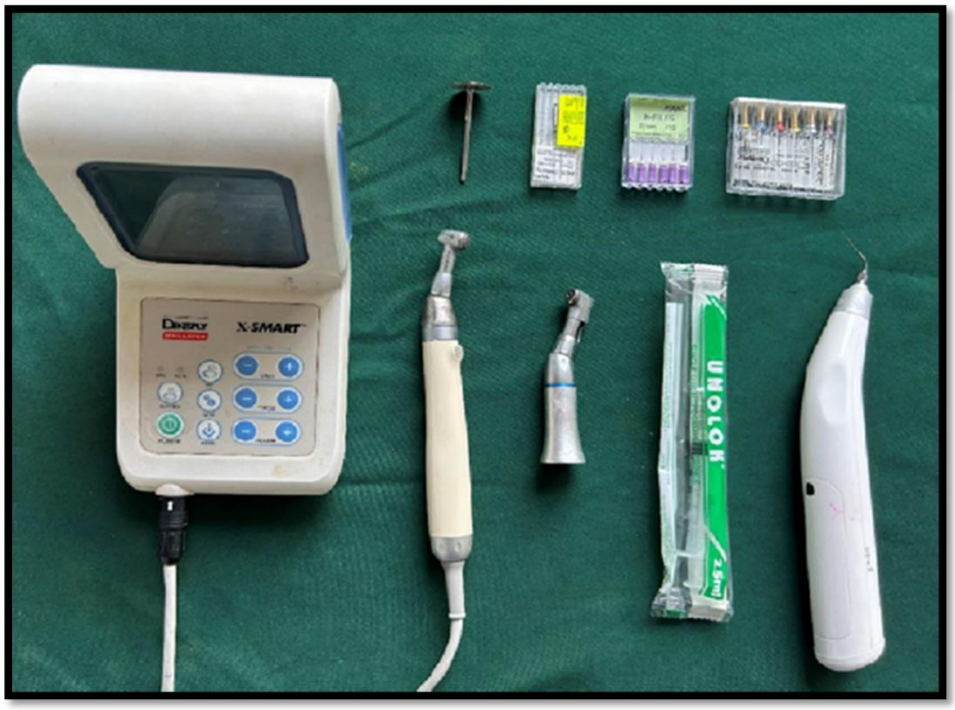


Fig 4 : Armamentarium used for the study

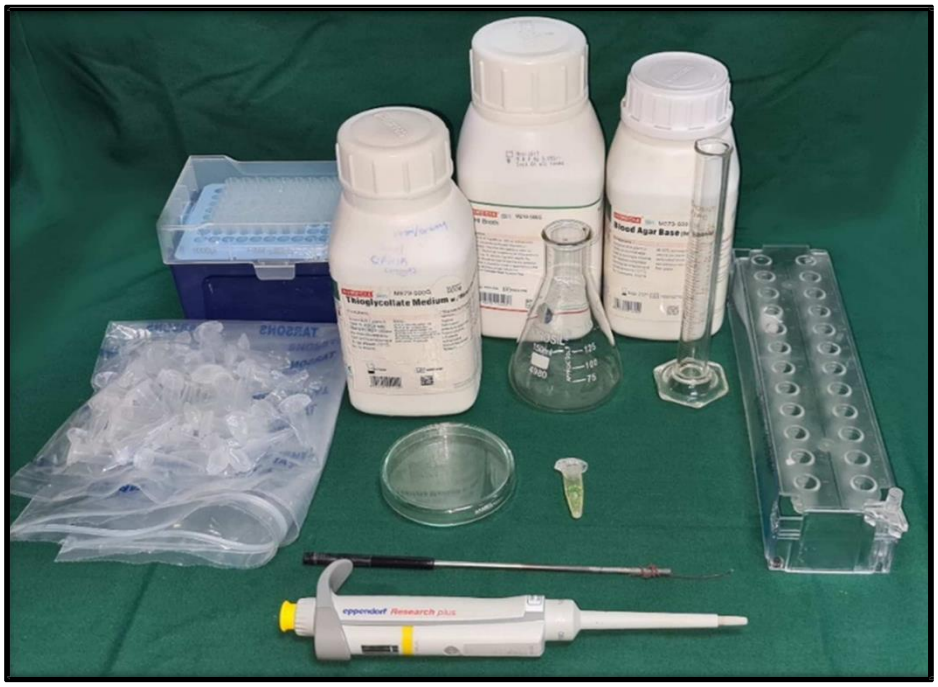


Fig 5: Materials and armamentrium used for microbiological assessment

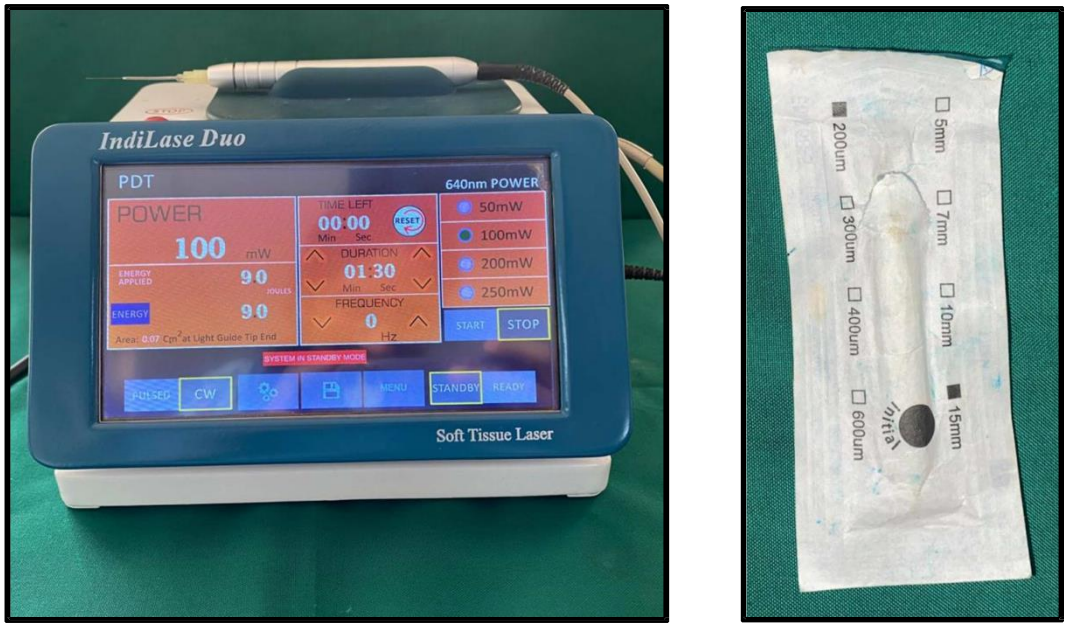


Fig 6: Armamentarium used for disinfection

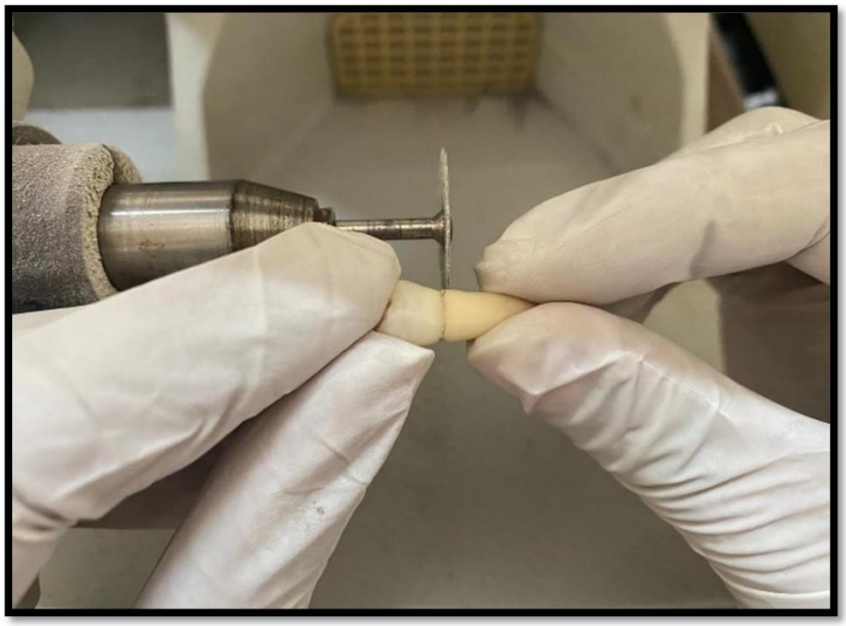


Fig 7: Decoronation of tooth sample

Preparation of tooth samples for disinfection

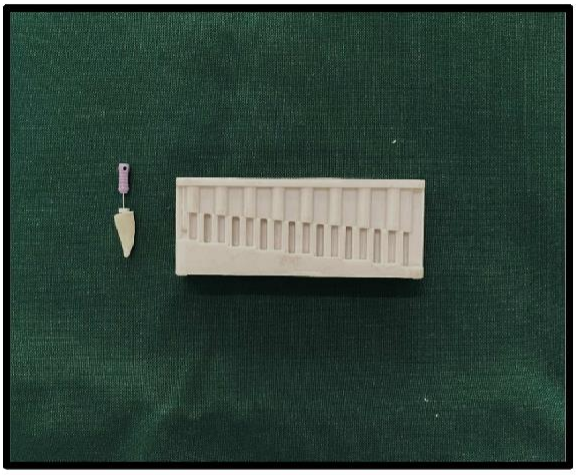


Fig 8: WL Determination of samples

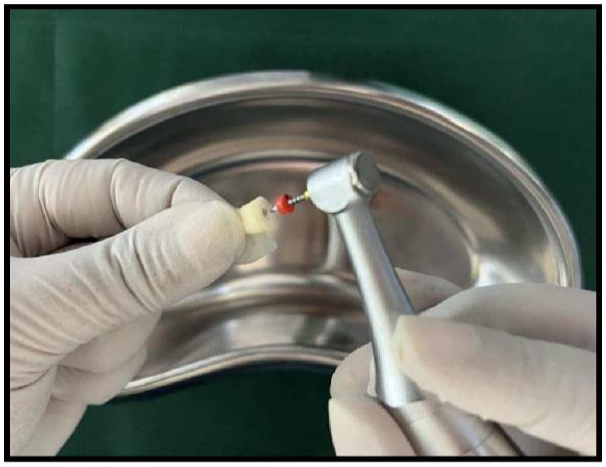


Fig 9: BMP of samples

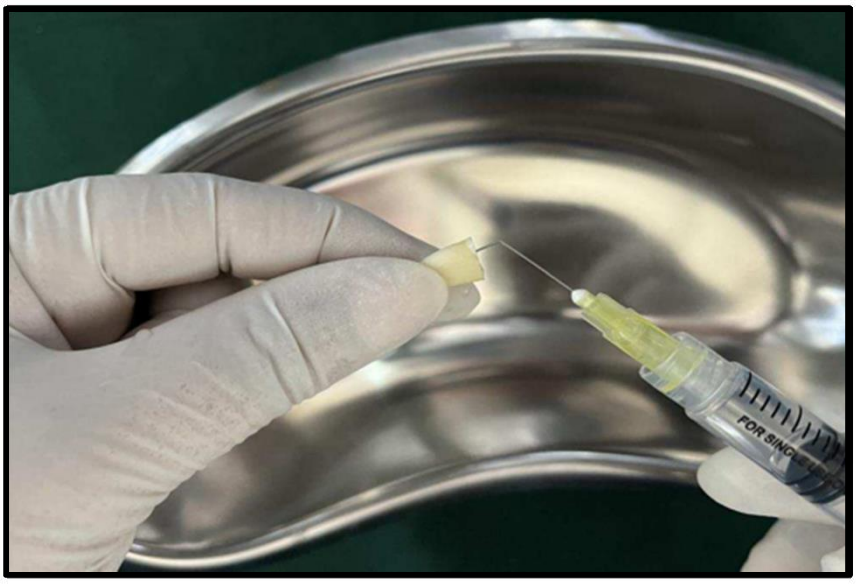


Fig 10: Irrigation of samples with distilled water

Fig 11: Inoculation of test samples with *E faecalis* for 3wk

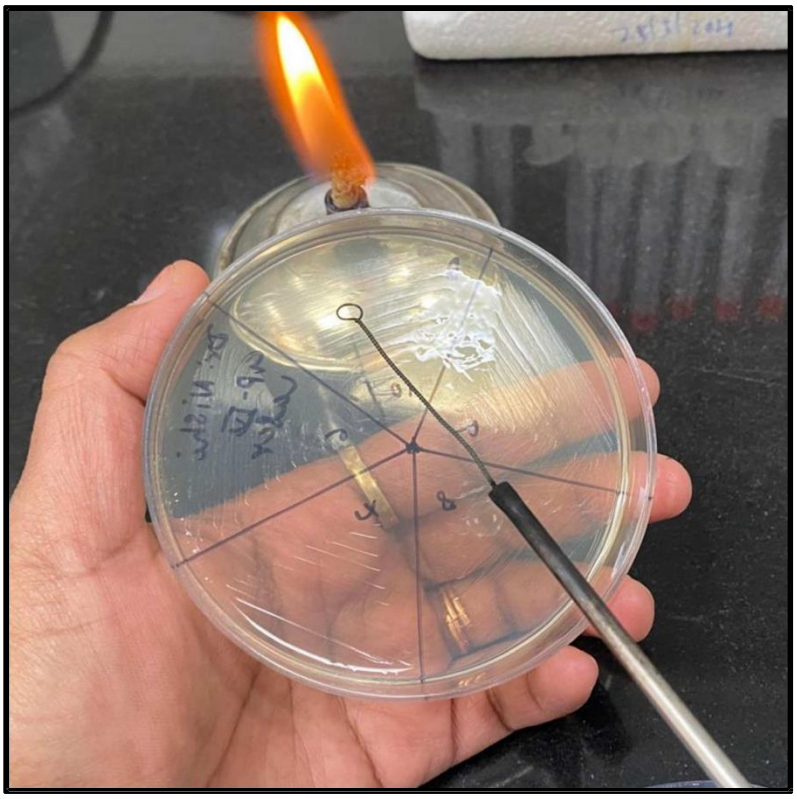


Fig 12: Plating of initial samples in culture plates

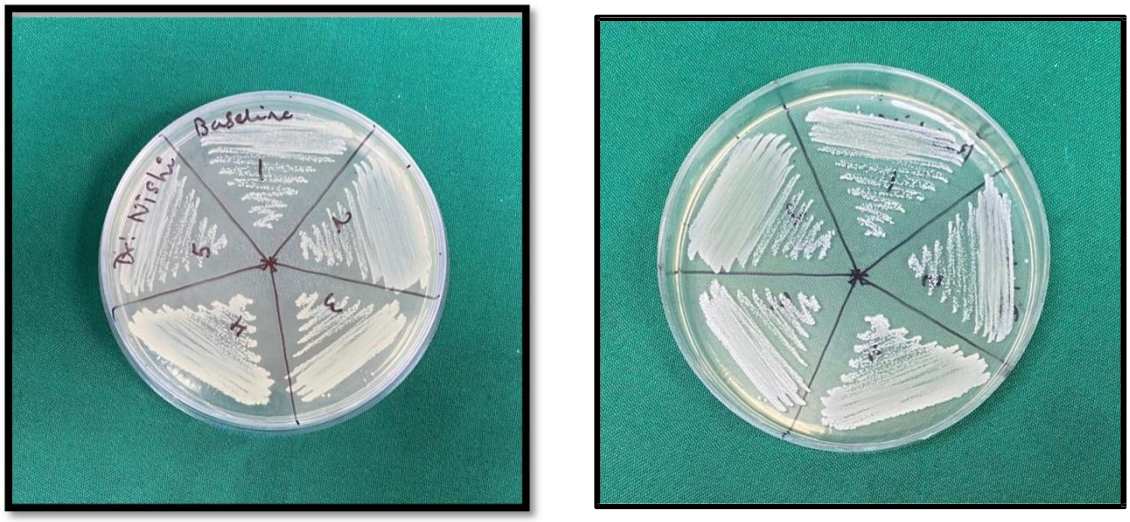


Fig 13: CFU to determine baseline samples

Fig 14: Weighing of required amount of NAC powder for irrigant

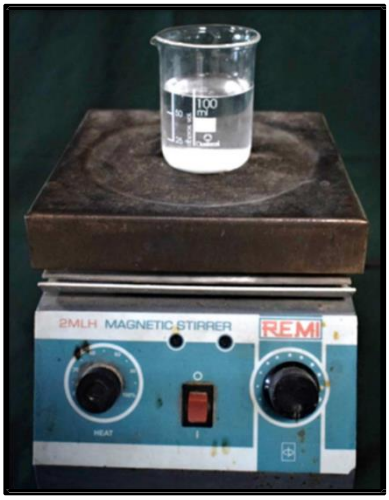


Fig 15: Magnetic Stirrer to get homogeneous NAC solution

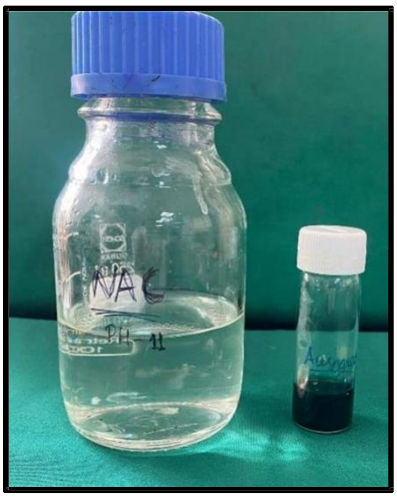


Fig 16: Prepared ICG &NAC solution for disinfection

Disinfection of tooth sample according to different protocols

Fig 17: Irrigation of tooth sample with NAC

Fig 18: Photoactivated disinfection with ICG

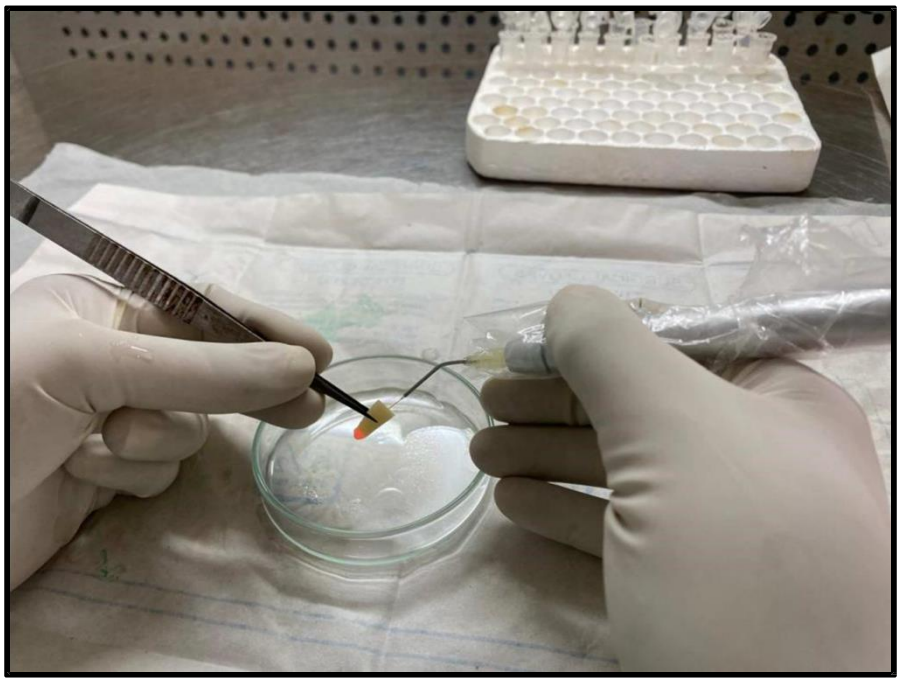


Fig 19: Laser activated irrigation with 810 nm diode laser

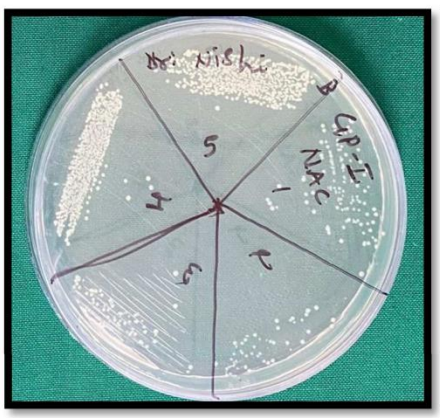


Fig 20: CFU in group I (NAC)

Fig 21: CFU in group II (NAC)

Fig 22: CFU in group III (NAC)

RESULTS

Table 1: Colony forming units (cfu) before and after treatment protocols

SL No	Group 1-NAC		Group 2-NAC+PDT		Group 3-NAC+PTT	
	Initial CFU	Final CFU	Initial CFU	Final CFU	Initial CFU	Final CFU
1	432	103	452	6	715	86
2	424	98	623	12	473	78
3	610	89	501	54	610	120
4	524	176	610	69	453	63
5	542	96	534	43	501	79
6	573	132	580	8	445	44
7	410	146	421	2	672	67
8	610	116	675	37	529	69
9	524	88	390	41	387	76
10	540	46	642	92	540	59
11	575	78	533	7	620	34
12	501	45	478	72	410	57
13	642	50	705	34	524	57
14	372	96	410	13	575	64
15	454	76	590	18	501	69
16	670	67	462	6	642	58
17	529	96	602	5	454	52
18	715	98	550	1	670	76
19	453	46	372	1	529	71
20	662	10	673	46	715	51
21	387	112	529	10	453	62

22	445	79	487	11	662	77
23	664	70	664	55	387	44
24	473	100	425	60	445	65
25	550	96	524	43	664	100
26	672	32	580	10	550	66
27	410	22	670	3	480	89
28	620	88	432	35	690	75

Table 2: Descriptive statistics and comparisons between pre-treatment and post-treatment Colony Forming Units values for Group I

Time Points	**Mean±SD**	**Range**	**Mean Difference**	**t value**	***P* value [a]**
Pre-treatment(*n*=28)	2.72±0.0818	2.57-2.85	0.85	15.61	<0.0001*
Post-treatment(*n*=28)	1.87±0.26	1-1.25			

n:sample size per time point

SD:Standard deviation

a:analyzed by the Paired t-test

*: statistically significant ($P \leq 0.05$)

The mean CFU value before treatment in Group I was 2.72 ± 0.0818, ranging from 2.57 to 2.85. Following treatment, the mean CFU value significantly decreased to 1.87±0.26, with values ranging from 1 to 1.25 ($t=15.61, P < 0.0001$).

Graph 1: Comparisons between pre-treatment and post-treatment Colony Forming Units values for Group I

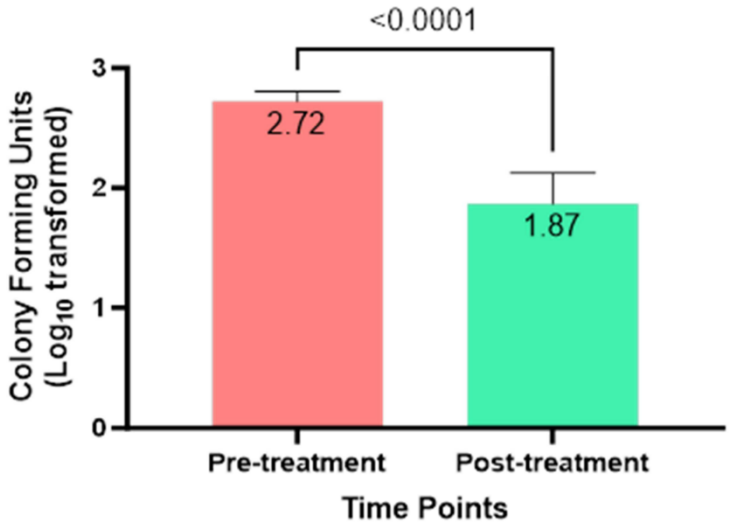


Table 3: Descriptive statistics and comparisons between pre-treatment and post-treatment Colony Forming Units values for Group II

Time Points	Mean±SD	Range	Mean Difference	t value	*P* value [a]
Pre-treatment(*n*=28)	2.73±0.0804	2.57-2.85	1.533	14.73	<0.0001*
Post-treatment(*n*=28)	1.19±0.565	0-1.96			

n:sample size per time point

SD:Standard deviation

a:analyzed by the Paired t-test

*: statistically significant ($P \leq 0.05$)

The mean CFU value before treatment in Group II was 2.73 ± 0.0804, ranging from 2.57 to 2.85. Following treatment, the mean CFU value significantly decreased to 1.19 ± 0.565, with values ranging from 0 to 1.96 ($t = 14.73$, $P < 0.0001$).

Graph 2: Comparisons between pre-treatment and post-treatment Colony Forming Units values for Group II

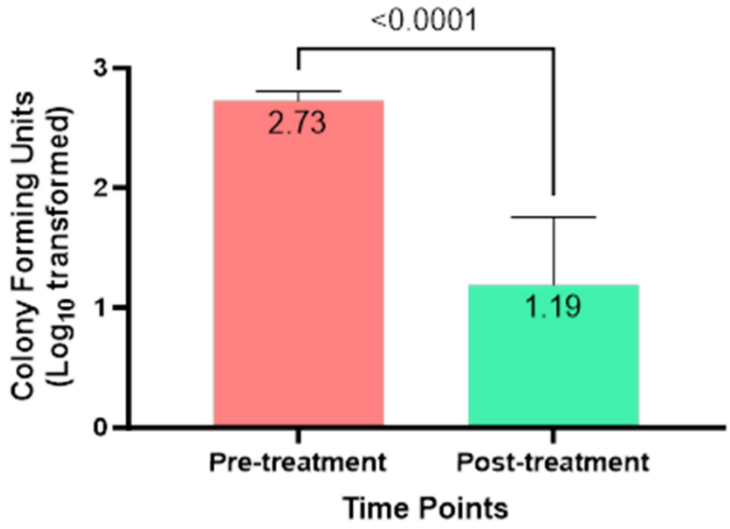


Table 4: Descriptive statistics and comparisons between pre-treatment and post-treatment Colony Forming Units values for Group III

Time Points	Mean±SD	Range	Mean Difference	t value	*P* value [a]
Pre-treatment(*n*=28)	2.73±0.0824	2.59-2.85	0.899	36.26	<0.0001*
Post-treatment(*n*=28)	1.84±0.138	1.53-2.27			

n:sample size per time point

SD:Standard deviation

a:analyzed by the Paired t-test

*: statistically significant ($P \leq 0.05$)

The mean CFU value before treatment in Group III was 2.73 ± 0.0824, ranging from 2.59 to 2.85. Following treatment, the mean CFU value significantly decreased to 1.84±0.138, with values ranging from 1.53 to 2.27 (t =36.26, $P < 0.0001$

Graph 3: Comparisons between pre-treatment and post-treatment Colony Forming Units values for Group III

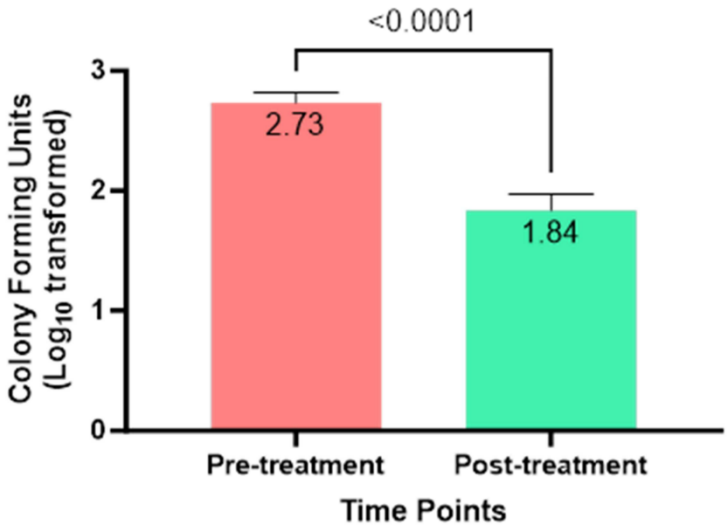


Table 5: Descriptive statistics of the change in Colony Forming Units values post-treatment relative to pre-treatment for all the study groups

Time Points	Mean±SD	Range
Group I (*n*=28)	0.85±0.29	0.448-1.37
Group II (*n*=28)	1.53±0.551	0.822-2.74
Group III (*n*=28)	0.91±0.121	0.706-0.555

n:sample size per time point

SD:Standard deviation

a:analyzed by the Paired t-test

*: statistically significant ($P \leq 0.05$)

Table 6: ANOVA TABLE showing inter-group comparisons

ANOVA table	SS	DF	MS	F (DFn, DFd)	*P* value
Treatment (between columns)	7.960	2	3.980	F (2, 81) = 29.69	<0.0001*
Residual (within columns)	10.86	81	0.1341		
Total	18.82	83			

F: statistic derived from repeated measures ANOVA test

DF: Degrees of freedom

n:numerator, d:denominator

NS:not significant(P>0.05),*: Significant at P≤0.05

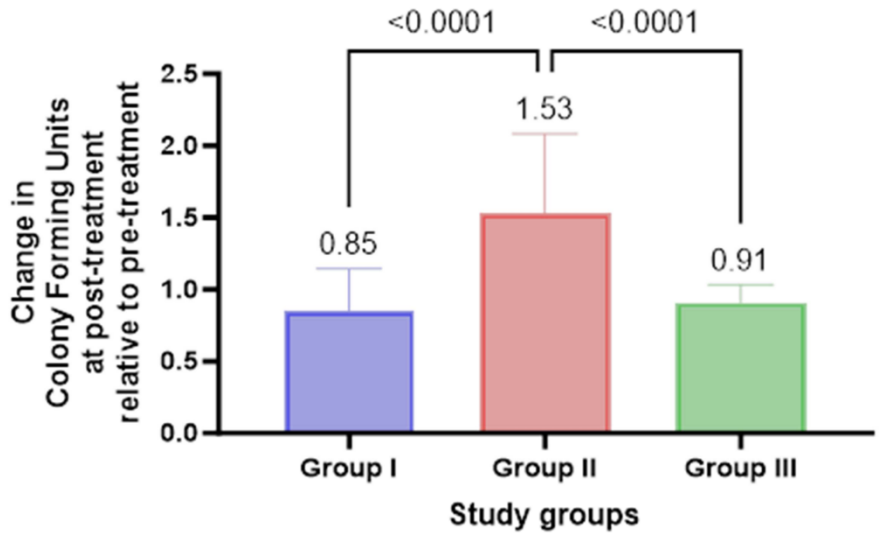
Table 7: *Post-hoc* pairwise comparisons between the Study groups

Tukey's multiple comparisons test	Mean Diff.	Adjusted *P* value[a]
Group I vs. Group II	-0.6793	<0.0001
Group I vs. Group III	-0.05612	0.8346NS
Group II vs. Group III	0.6231	<0.0001

a:Adjusted for alpha error for pairwise comparisons by Tukey HSD test

NS:not significant(P>0.05),*: Significant at P≤0.05

Graph 4: Comparison for the change in Colony Forming Units values post-treatment relative to pre-treatment for all the study groups



The mean reduction in CFU values post-treatment relative to pre-treatment was 0.85 ± 0.29 in Group I, ranging from 0.448 to 1.37. Group II showed a higher mean reduction of 1.53 ± 0.551, with values ranging from 0.822 to 2.74. In contrast, Group III exhibited a mean reduction of 0.91 ± 0.121, ranging from 0.706 to 0.555.

One-way ANOVA revealed statistically significant differences in CFU reduction among three groups ($F(2, 81) = 29.69$, $P < 0.0001$).

Tukey's post-hoc test indicated that Group II had significantly greater CFU reduction than Group I ($P<0.0001$), while Group I and Group III did not differ significantly ($P = 0.8346$). Additionally, Group II showed significantly greater reduction than Group III ($P < 0.0001$).

DISCUSSION

Successful endodontic treatment centers on eliminating microbial infection within root canal system—or at the very least, reducing the microbial load below a level that permits healing of periapical tissues and prevents reinfection.[53] Traditional chemo-mechanical methods, which combine mechanical enlargement of canal space with syringe-based irrigation using antimicrobial and chelating agents, have consistently fallen short of achieving this goal.[7]

Two primary challenges contribute to this shortfall. The first is complex micro-anatomy of root canal system.[54] Conventional techniques focus on the main canals, leaving insufficiently cleaned secondary structures, such as apical deltas, fins, cul-de-sacs, lateral canals, and isthmuses.[55] Second challenge lies in the nature of established infections. Microorganisms within infected root canals are often organized as resilient biofilms, rather than existing solely as free-floating (planktonic) cells.[56] This biofilm organization, with its inherent structural defenses, renders it particularly difficult to eradicate within such a complex anatomy.

E. faecalis is usually an isolated microorganism in infected root canals, detected in 90 percent of secondary and persistent infections, though it's notably less common in primary infections.[57] This stark difference underscores its significant role in treatment failures, as *E. faecalis* demonstrates notable resistance to intracanal medications and can survive independently in harsh conditions while forming biofilms. *Streptococcus mutans* may also be present in these infections.[8]

Given microbial communities diversity and ability to form biofilms, irrigating solutions and intracanal medications must possess strong antimicrobial and anti-inflammatory properties.[13]

With a trend toward single-sitting root canal treatments for greater patient and practitioner convenience, reliance on effective irrigants for disinfection has become increasingly critical. NaOCl and CHX are most frequently employed antimicrobial agents for treatment of root canal infection. However, studies have shown that biofilm-associated bacteria, including *E. faecalis,* are not completely eradicated by 2% CHX or NaOCl concentrations ranging from 1% to 3%.[58]

Clegg et al. claims that high concentrations like 6% NaOCl solution can render bacteria nonviable and remove biofilm[59] but such high concentrations are highly irritating to periapical tissues and can cause dentin deproteination, collagen breakdown, and loss in dentin flexural strength.[21] Moreover, oral bacterial biofilms also show an increased resistance to antimicrobial agents like chlorhexidine.[59]

This scenario necessitates alternative irrigants that combine potent antimicrobial activity with the ability to disrupt biofilms while maintaining biocompatibility.[60] N-acetyl cysteine (NAC) has been shown to effectively combat both biofilm and planktonic forms of *E. faecalis* at pH 11.[21] It's a thiol-containing compound known for its antioxidant and mucolytic properties, along with its antibacterial capabilities.[61]

Its biofilm-disrupting activity is attributed to its interference with the synthesis of extracellular polymeric substances (EPS), where thiol group disrupts disulfide bonds in bacterial proteins, causing irreversible damage to bacterial growth.[21] NAC's robust antioxidant properties further contribute to its anti-inflammatory actions, including preventing the development and release of several pro-inflammatory cytokines.[62] Research conducted by Biswas and de Faria indicates that oxidative stress triggers inflammation, and by scavenging reactive oxygen and nitrogen species

through its free thiol group, NAC prevents inflammatory cascades that can cause permanent cellular damage.[63]

SEM studies show that bacteria can invade dentinal tubules as deep as 1000µm, exceeding penetration depth of conventional irrigants, which usually reach only 60–150 µm.[13] This limitation has spurred the exploration of advanced methods such as high-power lasers and Photodynamic therapy (PDT) to enhance the penetration, distribution, and cleaning action of irrigants. [64]

Photodynamic therapy, also known as photo-activated disinfection (PAD), operates on the principle that a non-toxic photosensitizer, when excited by light, produces highly ROS from the surrounding oxygen.[65] These ROS, which include oxygen ions and radicals, interact with and instantly destroy microorganisms.[66] Wainwright et al have witnessed that effectiveness of PDT hinges on the interaction among the light, the photosensitizer, and oxygen.[67]

Over recent years, various photosensitizers have been applied in endodontics, including hematoporphyrin derivatives, phenothiazines, cyanines, etc. Phenothiazines such as MB and TOL, along with cyanines, are most widely studied for biofilm inactivation.[20] Recently, ICG has garnered attention as new photosensitizer as it doesn't discolor the tooth.[68]As an anionic photosensitizer, ICG primarily operates via photothermal effects rather than photochemical ones, with an absorption peak at 810 nm.[69] Lasers are the most commonly used light sources in clinical PDT, though LEDs and halogen lamps are also employed; however, according to Boucher et al, lasers are favored for their superior penetration depth and efficacy. Hence, ICG is activated with 810nm lasers.[70]

PDT's applications have expanded in recent years, providing a viable substitute for combating antibiotic-resistant bacteria. Because singlet oxygen and free radicals produced by PDT interact with various cellular structures and metabolic pathways, resistance to PDT is improbable.[71]

Moreover, photosensitizers directly impact extracellular molecules. [72] Concerning the disruption of oral biofilm Singlet oxygen is highly reactive, allowing it to damage polysaccharides in the biofilm's extracellular matrix—a dual action absent in antibiotics.[73]

Laser-activated irrigation, also known as Photothermal therapy, has similarly attracted attention due to its robust bactericidal properties, economic viability, and enhanced penetration into dentinal tubules.[74] Research has shown that various laser wavelengths, particularly those from diode and neodymium lasers, effectively reduce intracanal bacterial counts.[75]

The mechanism behind laser-induced bacterial killing involves thermal heating that elevates temperatures beyond lethal thresholds, both externally and within the bacteria.[76] However, due to differences in cell wall structure, Nussbaum et al., found that gram-positive bacteria often require several cycles of laser irradiation for effective eradication, whereas Gram-negative bacteria are more susceptible and are eliminated more quickly.[77]

Among various laser options, Diode lasers are particularly favored due to their compact design, cost-effectiveness, and thin optical fibers flexibility, enabling better access to narrow root canals. This is because diode lasers exhibit minimal affinity for water and hydroxyapatite in hard dental tissues, allowing them to penetrate deeply into dentinal tubules and target bacterial pigments effectively. This results in a strong

bactericidal effect within the deeper layers of dentin.[78] These lasers operate in the red and infrared spectrum, with wavelengths of 810 nm, 940 nm, and 980 nm commonly used.

However, according to a study by Lapari et al., the 980 nm DL has a comparatively higher water absorption coefficient; this characteristic limits its penetration into dentinal tubules.[79]

In contrast, the 810 nm DL demonstrates deeper penetration and more substantial antibacterial effects. Moreover, according to Beer-Lambert law, since there is an inverse relationship between laser wavelength and energy, 810 nm wavelength tends to cause more thermal damage. It is considered more effective and safer in endodontic disinfection.[80] These advancements in lasers and photosensitisers, along with their progressive applications and economic benefits, represent a promising approach for disinfecting previously unreachable areas within the root canal system.

While NAC, Diode lasers, and PDT have each demonstrated individual antimicrobial efficacy, no study has compared the antibacterial effectiveness of PDT and Diode lasers in conjunction with N-acetylcysteine. This research sought to evaluate and compare combined effects of 3 disinfection methods on E. faecalis: using N-acetyl cysteine irrigation, Photodynamic therapy combining 810nm laser with ICG, and Photothermal therapy using 810nm DL.

Using sample size estimation method described by Sarda et al., we chose 84 human permanent mandibular premolars extracted for orthodontic purposes, adhering to specified inclusion and exclusion criteria.[29] These teeth have been stored in 0.1% Thymol solution, chosen for its antifungal properties, until needed.[81] Samples were

decoronated using carborundum disc to standardize root length at 14mm, and their apices were sealed with composite. Working length has been determined using size 10K-file, inserted until visible at apex, and recorded after subtracting 1mm.

Root canal preparation was performed using Dentsply ProTaper Universal files, reaching an F2 master apical file size, with ample irrigation using 3% NaOCl and 17% EDTA. All specimens were sterilized in autoclave at 121°C to eliminate any pre-existing contamination for 20 minutes.[29]

Following this, 50µL of *E. faecalis* (MTCC 439 strain) was inoculated into each tooth sample in Eppendorf tubes, then incubated at 37±1°C for 21days to promote development of robust biofilm. This study examined the susceptibility of 21day-old *E. faecalis* biofilms to NAC and different activation methods, given the growing awareness of biofilms in endodontic infections and *E. faecalis's* capacity to colonize dentinal walls and root canal surfaces, as noted by Distel et al.[82] Culture-based CFU counting method assessed the effectiveness of different treatment protocols and reduction in viable *E. faecalis* capable of forming colonies.[78]

Specimens were divided into 3 experimental groups based on different disinfection protocols.

Group 1: Root canal disinfection using NAC

Group 2: Root canal disinfection using NAC and PAD.

Group 3: Root canal disinfection using NAC and a Diode laser.

Initial bacterial samples were collected from each tooth before treatment by inserting sterile size 20 paper points into root canal for 1min, and transferring them to Eppendorf tubes containing 10µl of saline. BHI agar plates were inoculated with samples using cell spreaders and incubated at 37°C for 72h, followed by counting bacterial colonies.

NAC irrigating solution was prepared following method described by Quah et al., by dissolving 0.2g of NAC in 1ml of distilled water to achieve a concentration of 200mg/ml at pH 11, as NAC exhibits optimal bactericidal activity at this pH.[21] Additionally, research conducted by Ridhalaksani et al. demonstrated that NAC at pH11 was more effective than NAC at pH2.5 and 2%CHX.[83] Based on these findings, all samples in Group 1 were treated with 5 ml of this NAC solution, followed by final flush with 4ml of distilled water.

Group 2 and 3 specimens underwent additional treatment with photodynamic therapy (PDT) using 1 mg/ml of ICG and activation of NAC with an 810nm wavelength DL, respectively, as outlined in the methodology.

Standardization in laser-based disinfection studies is often complicated by variations in key parameters such as energy fluence, power density, and emission duration.[78] To enhance consistency and reproducibility, the present study utilized a 200-µm optic fiber for both photodynamic therapy (PDT) and diode laser activation. The use of a standardized fiber diameter ensures uniform energy delivery within the canal and allows for better control over light distribution, particularly in narrow and curved root canals.[84]

After the disinfection procedure, a final sample was collected from each specimen as previously described, and we calculated Colony-forming units using provided formula.

$$\text{Colony-forming unit/mL} = \frac{\text{number of colonies formed}}{\text{Volume plated (mL)} \times \text{Total Dilution used(mL)}}$$

According to the results of present study, all tested methods—NAC, PDT with ICG, and 810nm diode laser activation— revealed statistically significant differences in CFU reduction. However, compared to other methods and as a complement to conventional irrigation in the final stage, Photodynamic therapy using ICG dye in Group II has shown more significant reduction than Groups I & III. Additionally, Group I & III did not differ significantly. This finding aligns with results of other studies, confirming effectiveness of Photoactivated disinfection.

The superior effect of PDT with ICG in Group II can be attributed to its use of a photosensitizer that binds to bacterial surfaces and, upon activation, generates ROS such as free radicals and singlet oxygen.[85] These ROS effectively eliminate bacteria without causing thermal damage to surrounding tissues, unlike the Diode laser, which primarily relies on heat and limited fluid agitation to disrupt biofilms. Furthermore, PDT targets gram positive and gram negative germs, like antibiotic-resistant strains, because of its non-specific oxidative damage mechanism. This makes PDT particularly advantageous in complex anatomical areas, as ROS can diffuse into deeper regions.[86]The ICG dye used in this study also added to the notable advantage of PAD due to its dual role in increasing temperature and exhibiting antibacterial properties within biofilms. [87]

The suboptimal performance observed in Group III, where 810 nm Diode laser was combined with NAC, may be attributed to the laser's temperature-dependent photothermal mechanism, which could be less effective against the heat-resistant nature of *E. faecalis*, known for its robust cell wall structure.[78]

Furthermore, the reduced efficacy may also be attributed to the differing interactions between lasers and various irrigants. Unlike sodium hypochlorite (NaOCl), which exhibits enhanced activity upon heat activation, NAC may undergo thermal degradation when exposed to laser-induced heat without a corresponding increase in its bactericidal properties. Studies indicate that NAC is susceptible to photodecomposition when exposed to high-energy laser irradiation, potentially reducing its biofilm-disrupting capability. For instance, Primas et al. reported a 24% reduction in NAC content after exposure to 80 °C for 3 hours and a 3% decrease under prolonged light exposure.[88]

In the present study, Group 1 showed less effective outcomes compared to Photodynamic therapy (PDT) of Group-III likely due to the specific mechanism of action of N-acetylcysteine (NAC). Although NAC is known for its biofilm-disrupting properties, it primarily acts by breaking disulfide bonds within the extracellular matrix, rather than directly killing bacteria or producing reactive oxygen species (ROS). As a result, its ability to eliminate free-floating (planktonic) bacteria or those deeply embedded within biofilms may be compromised, contributing to its reduced antibacterial efficacy observed in this study.[89]

Another factor contributing to its reduced effectiveness may be the limited penetration of irrigant into canal system. This limitation is due to highest irrigant flow being concentrated around the needle tip, with minimal distribution beyond this

point.[78] Additionally, dead-water zones or apical vapor lock (air bubble formation in the apical region), along with the narrowing of dentinal tubules toward the apex, restrict the irrigant's diffusion.[90]

The findings of this study are consistent with those reported by Ghorbanzadeh et al.[48], Afkhami et al.[50], Sonarkar et al.[46], Katalinić et al.[9], and Asnaashari et al.[75]. Ghorbanzadeh et al. observed that all three disinfection techniques—conventional chemomechanical debridement (CCMD), laser-activated disinfection (LAD), and 810 nm diode laser irradiation—were effective in eliminating *E. faecalis* biofilms. They found that combining CCMD with LAD significantly reduced both mature and immature biofilms; however, the addition of laser irradiation did not result in a statistically significant improvement in bacterial reduction when used alongside CCMD alone.[48]

Similarly, Afkhami et al. reported the highest bacterial reduction (99.12%) using a combination of silver nanoparticles (AgNPs) and indocyanine green (ICG) activated with an 810 nm Diode laser. Notably, both their study and the current one used ICG at a concentration of 1 mg/mL with a 5-minute incubation period, although their laser parameters differed—300 mW and 200 mW, respectively.[50]

In contrast, a study by Grącka-Mańkowska et al. compared Diode lasers at 1500 mW and 3000 mW (in both continuous and pulsed modes) with photoactivated disinfection using toluidine blue and 5.25% sodium hypochlorite. They found that both diode laser settings resulted in a significant reduction in *E. faecalis* cell counts, whereas the group treated with photoactivated disinfection showed a considerably lower bacterial reduction. This enhanced efficacy of diode lasers was attributed to the repeated application of laser pulses, which likely intensified the bactericidal effect.[90]

Its also important to note that the discrepancies in findings among different studies may stem from variations in laser parameters and the use of bare-end fiber tips. Radial firing tips have been recommended to improve light distribution and ensure uniform coverage of root canal walls.[91]

Research indicates that temperature inside canal may rise by upto 45 °C during laser treatment.[91]In addition to its effectiveness, numerous studies have examined the safety of PDT. Kashef et al. found that PDT with MB, TBO, or ICG does not significantly harm human fibroblasts.[92] George and Kishen also reported that PDT exhibits significantly lower cytotoxicity than conventional antimicrobial rinses. In an in vitro study, *E. faecalis* was eradicated more rapidly than normal fibroblasts, with PDT resulting in 97.7% decrease in bacteria while affecting only 30% of fibroblasts.[93]

Regarding concerns about heat generation during laser application, Photoactivated disinfection (PAD) is considered a safer alternative. Dickers et al. found that PAD led to minimal temperature increase of 0.16 ± 0.08°C on external root surface [94]while Radaelli et al. recorded a maximum temperature change of 7.45°C following an application of an 830-nm Diode laser. [95]One method to reduce heat accumulation is the oscillatory technique with relaxation time created by Gutknecht et al. To provide tissues time to adjust to temperature fluctuations, this approach entails removing fiber from canal in a helicoidal motion at a speed of 2 mm/s.[96]

Based on finding of this study and existing literature, PDT emerges as safe and effective clinical disinfection method. It can potentially reduce bacterial load in a single visit, minimizing the number of treatment sessions, particularly in retreatment cases where microbial resistance is a concern.[97]

One strength of this study was its examination of interventions on a well-developed biofilm model. [98]

However, a limitation was that only a single bacterial species was tested, which does not fully replicate clinical conditions. [99] Another strength was the consideration of CFU differences at baseline, preventing over- or underestimating intervention effectiveness. A notable limitation was using paper points for bacterial collection, which may have restricted bacterial recovery from dentin compared to the scraping method using a file. [100]

Following standard canal preparation, disinfection via N-acetyl cysteine, PDT, or 810nm diode laser activation significantly reduced CFU/ml counts, with PDT demonstrating the greatest bacterial reduction. This reinforces the notion that conventional irrigation alone is insufficient for eliminating persistent bacteria associated with reinfection and treatment failure, highlighting the need for adjunctive therapy.

However, no statistically significant difference was identified between N-acetylcysteine irrigation and laser activation. Given PDT's advantages—such as its non-antibiotic approach and minimal side effects on dental and periodontal tissues- it presents a promising treatment option. Further ex vivo and in vivo studies comparing different photosensitizers and laser parameters are recommended to optimize use of NIR diode lasers and ICG in endodontic disinfection strategies.

CONCLUSION

Considering limitations of current research, it has been concluded that all 3 Groups –

1. N-acetyl cysteine, Photodynamic therapy, and Diode laser activation- effectively reduced bacterial counts.

2. Photodynamic treatment significantly reduced the CFU compared to the N-acetyl cysteine and Diode laser groups.

3. However, efficacy of N-acetyl cysteine showed no significant difference when activated by an 810nm Diode laser.

SUMMARY

Microorganisms are primary etiological agents of pulpal and periapical diseases. Effective endodontic treatment depends on the chemo-mechanical removal of these microorganisms, which can be found as deep as 1100 μm inside the dentinal tubules. Mechanical methods alone cannot adequately clean complex root canal system, highlighting importance of irrigants and activation devices. The most widely utilized irrigants are NaOCl and CHX, which possess potent antibacterial and tissue-dissolving properties. However, the cytotoxicity of NaOCl and the increasing resistance to CHX necessitate the investigation of alternative irrigants.

One such alternative is NAC, a mucolytic agent with antioxidant properties. NAC has demonstrated superior efficacy in biofilm elimination compared to CHX and NaOCl and exhibits strong antibacterial properties. Additionally, it is biocompatible, anti-inflammatory, and non-toxic, making it a promising choice for endodontic irrigation.However, due to the limited penetration of irrigants, various techniques have been explored to enhance their effectiveness, including diode lasers and photodynamic therapy (PDT).

The antibacterial effect of Diode lasers is primarily photothermal (PT) in nature, which is based on a temperature rise. Microbial harm results from energy being transferred both directly to pigmented bacteria and indirectly to transparent bacteria through surrounding microenvironment. On other hand, PDT produces little to no heating and mostly has an indirect effect. PDT needs a photoactivated dye.

After applying, microorganisms absorb the dye. Dye becomes activated upon exposure to laser light and produces ROS within the cells. These highly reactive compounds interfere with cellular metabolism, leading to bacterial death.

This study used ICG dye, which has an absorption peak at 810 nm and exhibits both photothermal and photochemical actions without causing tooth discoloration. Due to the inverse relationship between laser wavelength and energy, the 810 nm Diode laser used in this study offers additional advantages, including deeper penetration and stronger antibacterial effects.

Although various studies have investigated PDT, Diode lasers, and NAC individually, none have compared all three. This study assesses the impact of PDT and diode lasers when used alongside NAC.

The study occurred at Department of Conservative Dentistry and Endodontics and Basic Science Research Centre within Viswanath Katti Institute of Dental Sciences, KAHER, in Belagavi. Total of 84 human single-rooted mandibular premolars have been chosen according to defined inclusion and exclusion criteria. Calculus and soft debris have been removed from teeth, which were then preserved in a 0.1% thymol solution until required.

To achieve standardization, samples were decoronated, and working length was decided using size 10 K file. Canals have been treated using mechanical instruments, with ProTaper Universal instruments up to size F2, and were thoroughly irrigated with 3%NaOCl and 17%EDTA. A final rinse has been performed using 3% NaOCl for three minutes, preceded by distilled water.

All tooth samples were then exposed to a suspension of the MTCC 439 strain of *E. faecalis* and incubated at 37±1ºC for 21days to allow for mature biofilm development. Initial colony-forming units (CFUs) were assessed, after which samples were divided into 3 groups based on disinfection protocols used:

Group 1: Samples were irrigated with 5ml of NAC (pH adjusted to 11), followed by a final flush with distilled water.

Group 2: After initial NAC disinfection, 1 mg/ml of ICG was introduced into the canal for 5 minutes and then activated utilizing an 810 nm wavelength, 0.1 W Diode laser for 60 seconds.

Group 3: NAC was activated with 810nm Diode laser at 1W for 20seconds.

Following disinfection, we assessed final CFU counts and conducted statistical analysis utilizing one way ANOVA, followed by Tukey HSD test. Findings indicated that all tested disinfection methods—NAC, PDT with ICG, and 810nm Diode laser activation—significantly reduced bacterial CFUs. Among these, PDT using ICG as an adjunct was most effective, showing significantly greater bacterial reduction than NAC and Diode laser groups, which did not differ significantly.

High effectiveness of PDT is due to photosensitizer that attaches to bacterial surfaces. When activated, it produces ROS that can reach intricate anatomical areas, effectively destroying gram-positive and gram-negative bacteria, including those that are resistant to antibiotics. In contrast, combining NAC with Diode laser was less effective, likely due to the photothermal mechanism, which may have limit due to the heat resistance of *E. faecalis*. Moreover, NAC may degrade under laser-induced heat, thereby reducing its biofilm-disrupting efficacy.

Although NAC is known for its ability to disrupt biofilms by cleaving disulfide bonds, it lacks direct bactericidal activity and does not generate ROS. Its penetration into root canal system is limited by factors such as apical canal morphology, vapor lock, and the presence of dead-water zones, which further restrict

its antibacterial potential. As a result, null hypothesis is rejected, which states that there would be no reduction in microbial counts when NAC is combined with PDT or Diode laser.

BIBLIOGRAPHY

1. Abdulwahab MA, Almotairi DM, Aldawish BF, Alluqmani SR, Dajam AA, Alzahrani AA, et al. Persistence of bacteria and its role in endodontic treatment failure. *Int J Community Med Public Health*. 2022;9(1):432–6.

2. Elbatouty KM, Nour MS. Evaluation of antibacterial effect ofdiode laser 980 nm, endovac and passive ultrasonic irrigation during final irrigation. Egypt Dent J. 2017;63(2):1617-25.

3. P. Nair, S. Henry, V. Cano, J. Vera, Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after "one-visit" endodontic treatment, Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol. 99 (2) (2005) 231–252.

4. G.B. Shuping, D. Ørstavik, A. Sigurdsson, M. Trope, Reduction of intracanal bacteria using nickel-titanium rotary instrumentation and various medications, J. Endod. 26 (12) (2000) 751–755.

5. S. Paiva, J. Siqueira, I. Rôças, F. Carmo, D. Leite, D. Ferreira, et al., Clinical antimicrobial efficacy of NiTi rotary instrumentation with NaOCl irrigation, final rinse with chlorhexidine and interappointment medication: a molecular study, Int. Endod. J. 46 (3) (2013) 225–233.

6. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. J Endod. 2006 Feb;32(2):93-8.

7. Kishen A, George S, Kumar R. Enterococcus faecalis-mediated biomineralized biofilm formation on root canal dentine in vitro. J Biomed Mater Res A. 2006 May;77(2):406-15.

8. R. Love, Enterococcus faecalis–a mechanism for its role in endodontic failure, Int. Endod. J. 34 (5) (2001) 399–405.

9. Katalinić I, Budimir A, Bošnjak Z, Jakovljević S, Anić I. The photo-activated and photo-thermal effect of the 445/970 nm diode laser on the mixed biofilm inside root canals of human teeth in vitro: A pilot study. Photodiagnosis Photodyn Ther. 2023;41:103348

10. Mohammadi Z, Soltani MK, Shalavi S. An Update on the Management of Endodontic Biofilms Using Root Canal Irrigants and Medicaments. Iran Endod J. 2014;9(2):89–97

11. A.S. Garcez, S.C. Nunez, M.R. Hamblim, H. Suzuki, M.S. Ribeiro, Photodynamic therapy associated with conventional endodontic treatment in patients with antibiotic-resistant microflora: a preliminary report, J. Endod. 36 (9) (2010) 1463–1466

12. Neelakantan P, Romero M, Vera J, Daood U, Khan AU, Yan A, Cheung GSP. Biofilms in Endodontics-Current Status and Future Directions. Int J Mol Sci. 2017 Aug 11;18(8):1748.

13. Haapasalo M, Shen Y, Qian W, Gao Y. Irrigation in endodontics. *Dental Clinics of North America*. 2010;54(2):291-312

14. Teja KV, Janani K, Srivastava KC, Shrivastava D, Jose J, Marya A, Karobari MI. Comparison of Herbal Agents with Sodium Hypochlorite as Root Canal Irrigant: A Systematic Review of *In Vitro* Studies. Evid Based Complement Alternat Med. 2021

Nov 25;2021:8967219.

15. Darrag AM.Antimicrobial efficacy of endodontic irrigation solutions against planktonic microorganisms and dual-species biofilm. *Tanta Dental Journal*. 2014;11(2):93–101.

16. Cai C, Chen X, Li Y, Jiang Q. Advances in the Role of Sodium Hypochlorite Irrigant in Chemical Preparation of Root Canal Treatment. Biomed Res Int. 2023 Jan 13;2023:8858283.

17. Berber VB, Gomes BP, Sena NT, Vianna ME, Ferraz CC, Zaia AA, Souza-Filho FJ. Efficacy of various concentrations of NaOCl and instrumentation techniques in reducing Enterococcus faecalis within root canals and dentinal tubules. Int Endod J. 2006 Jan;39(1):10-7.

18. Thakur D.V., Kaur D.M., Jamwal P., Thakur B. 2% Chlorhexidine in Root Canal Treatment: A Review. Journal of Current Medical Research and Opinion. 2020;770−774.

19. 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 (Basel). 2023 Oct 16;12(10):1867.

20. Abdulrab S, Mostafa N, Al-Maweri SA, Abada H, Halboub E, Alhadainy HA. Antibacterial and anti-inflammatory efficacy of N-acetyl cysteine in endodontic treatment: a scoping review. *BMC Oral Health*. 2022;22(1):398

21. Quah SY, Wu S, Lui JN, Sum CP, Tan KS. N-acetylcysteine inhibits growth and eradicates biofilm of *Enterococcus faecalis*. *J Endod*. 2012;38(1):81–5.

22. Ghorbanzadeh A, Bahador A, Sarraf P, Ayar R, Fekrazad R, Asefi S. Ex vivo comparison of antibacterial efficacy of conventional chemomechanical debridement alone and in combination with light-activated disinfection and laser irradiation against *Enterococcus faecalis* biofilm. *Photodiagnosis Photodyn Ther*. 2020;29:101648.

23. D.J. Coluzzi, Fundamentals of dental lasers: science and instruments, Dent. Clin. North Am. 48 (2004) 751–770.

24. De Souza EB, Cai S, Simionato MR, Lage-Marques JL. High-power diode laser in the disinfection in depth of the root canal dentin. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;106:e68-72.

25. Lapari SS, Jahromi MZ, Tahmourespour A, Shirani AM. Comparison of the *Enterococcus faecalis* colony reduction effect of two wavelengths of diode lasers with three methods of root canal irrigation: an in vitro study. *J Lasers Med Sci*. 2024;15(1):37.

26. Rubilar-Huenchuman M, Ortega-Villanueva C, González IA, Palavecino CE. The Effect of Photodynamic Therapy on Enterococcus spp. and Its Application in Dentistry: A Scoping Review. Pharmaceutics. 2024 Jun 18;16(6):825.

27. R.R. de Oliveira, A.B. Novaes, G.P. Garlet, R.F. de Souza, M. Taba, S. Sato, et al., The effect of a single episode of antimicrobial photodynamic therapy in the treatment of experimental periodontitis. Microbiological profile and cytokine pattern in the dog mandible, Lasers Med. Sci. 26 (3) (2011) 359–367.

28. Alvarez N, Sevilla A. Current Advances in Photodynamic Therapy (PDT) and the Future Potential of PDT-Combinatorial Cancer Therapies. Int J Mol Sci. 2024 Jan 13;25(2):1023

29. R.A. Sarda, R.M. Shetty, A. Tamrakar, S.Y. Shetty,Antimicrobial efficacy of photodynamic therapy, diode laser, and sodium hypochlorite and their combinations on endodontic pathogens,Photodiagnosis and Photodynamic Therapy,Volume 28,2019,Pages 265-272,ISSN 1572-1000

30. Du T, Wang Y, Liu X, et al. Killing effect of antibacterial photodynamic therapy with long-term exposure against young and mature *Enterococcus faecalis* biofilms in dentin. *BMC Oral Health*. 2025;25:287.

31. Sardari F, Tashakori M, Sadeghi M, et al. Assessment of antibacterial activity of diode laser associated with chlorhexidine and sodium hypochlorite against *Enterococcus faecalis* contaminating root canals: an in vitro study. *Lasers Dent Sci*. 2025;9:7.

32. Tehrani NA, Javadinejad S, Shirani AM. Comparison between three methods of diode laser 810 nm, photodynamic therapy with laser 660 nm, and hypochlorite solution for disinfection of pulp canal of primary teeth. Dent Res J (Isfahan). 2024 Mar 26;21:23

33. Hoshyari N, Mesgarani A, Sheikhi MM, Goli H, Nataj AH, Chiniforush N. Comparison of Antimicrobial Effects of 445 and 970 nm Diode Laser Irradiation with Photodynamic Therapy and Triple Antibiotic Paste on *Enterococcus faecalis* in the Root Canal: an *In Vitro* Study. Maedica (Bucur). 2024 Mar;19(1):57-65.

34. Wang J, Geng T, Wang Y, et al. Efficacy of antibacterial agents combined with erbium laser and photodynamic therapy in reducing titanium biofilm vitality: an in vitro study. *BMC Oral Health*. 2023;23:32.

35. Yavagal CM, Subramani SK, Patil VC, Yavagal PC, Talwar RP, Hebbal MI, Saadaldin SA, Eldwakhly E, Abdelhafeez MM, Soliman M. Disinfection Efficacy of Laser Activation on Different Forms and Concentrations of Sodium Hypochlorite Root Canal Irrigant against *Enterococcus faecalis* in Primary Teeth. Children (Basel). 2023 Dec 3;10(12):1887.

36. Afrasiabi S, Chiniforush N. An in vitro study on the efficacy of hydrogen peroxide mediated high-power photodynamic therapy affecting Enterococcus faecalis biofilm formation and dispersal. Photodiagnosis Photodyn Ther. 2023 Mar;41:103310.

37. Haghighi L, Azizi A, Vatanpour M, Ramezani G. Antibacterial Efficacy of Cold Atmospheric Plasma, Photodynamic Therapy with Two Photosensitizers, and Diode Laser on Primary Mandibular Second Molar Root Canals Infected with *Enterococcus faecalis*: An In Vitro Study. Int J Dent. 2023 Apr 21;2023:5514829.

38. Goel P, Galhotra V, Makkar S, Mohan J, Bala N, Kaur T. An *In Vitro* Study Comparing the Antimicrobial Efficacy of 0.2% Chitosan, 3% Sodium Hypochlorite, 2% Chlorhexidine against *Enterococcus faecalis*, Alone and in Conjunction with Diode Laser. Int J Clin Pediatr Dent. 2022 Jan-Feb;15(1):109-114.

39. Sharma, Ananya; Kochhar, Rohit; Kumari, Manju. Antimicrobial efficacy of photosensitized nanoparticles, diode laser, conventional, and sonic irrigation systems against enterococcus faecalis in root canals – An in vitro study. Endodontology 34(2):p 108-114, Apr–Jun 2022.

40. Jambagi N, Kore P, Dhaded NS, Patil SA, Shankar M. Comparison of antimicrobial efficacy of diode laser, ultrasonic activated and conventional irrigation with 2.5% NaOCl during RCT: An interventional study. J Contemp Dent Pract. 2021

Jun 1;22(6):669-73

41. Abu Hasna A, Khoury RD, Toia CC, Gonçalves GB, de Andrade FB, Talge Carvalho CA, Ribeiro Camargo CH, Carneiro Valera M. *In vitro* Evaluation of the Antimicrobial Effect of N-acetylcysteine and Photodynamic Therapy on Root Canals Infected with *Enterococcus faecalis*. Iran Endod J. 2020 Fall;15(4):236-245.

42. Torres F, Mallma Franco AM, Mayta-Tovalino F. Antibacterial effect of red laser therapy on *Enterococcus faecalis* using different photosensitizers: An in vitro study. J Int Soc Prev Community Dent. 2020;10(4):455–60.

43. Bhasin P, Sharma M, Bindal D, Tomar D, Sarin A, Sharma N. An *In Vitro* Evaluation of Antimicrobial Effects of Three Different Root Canal Irrigating Solutions against *Enterococcus faecalis* and *Streptococcus mutans*. J Contemp Dent Pract. 2019 Feb 1;20(2):221-225

44. Moradi Eslami L, Vatanpour M, Aminzadeh N, Mehrvarzfar P, Taheri S. The comparison of intracanal medicaments, diode laser and photodynamic therapy on removing the biofilm of Enterococcus faecalis and Candida albicans in the root canal system Photodiagnosis Photodyn Ther. 2019 Jun;26:157-161.

45. Singh P, Sohi KS. Relative assessment of antimicrobial efects of two root canal irrigating solutions against *E. faecalis* and *S. mutans*: an in vitro study. 2019.

46. Sonarkar SS, Singh S, Podar R, Kulkarni G, Purba R. An *in vivo* comparison of the antibacterial efficacy of photoactivated disinfection, diode laser, and 5% sodium hypochlorite in root canal disinfection. J Conserv Dent. 2018 Mar-Apr;21(2):205-209.

47. Pourhajibagher M, Chiniforush N, Shahabi S, Palizvani M, Bahador A. Antibacterial and Antibiofilm Efficacy of Antimicrobial Photodynamic Therapy

Against Intracanal *Enterococcus faecalis*: An *In Vitro* Comparative Study with Traditional Endodontic Irrigation Solutions. J Dent (Tehran). 2018 Jul;15(4):197-204.

48. Ghorbanzadeh A, Fekrazad R, Bahador A, Ayar R, Tabatabai S, Asefi S. Evaluation of the antibacterial efficacy of various root canal disinfection methods against Enterococcus faecalis biofilm. An ex-vivo study. Photodiagnosis Photodyn Ther. 2018 Dec;24:44-51.

49. Ozkocak, Ismail & Gokturk, Hakan & Şay Coşkun, Umut & Aytac, Fatma. (2018). Antibacterial Efficiency of Different Irrigation Solutions, Lasers and Photodynamic Therapy with Indocyanine Green in Root Canals Infected By Enterococcus Faecalis. Meandros Medical and Dental Journal. 19. 289-295. 10.4274/meandros.08370.

50. Afkhami F, Akbari S, Chiniforush N. Entrococcus faecalis Elimination in Root Canals Using Silver Nanoparticles, Photodynamic Therapy, Diode Laser, or Laser-activated Nanoparticles: An In Vitro Study. J Endod. 2017 Feb;43(2):279-282.

51. Camacho-Alonso F, Julián-Belmonte E, Chiva-García F, Martínez-Beneyto Y. Bactericidal Efficacy of Photodynamic Therapy and Chitosan in Root Canals Experimentally Infected with Enterococcus faecalis: An In Vitro Study. Photomed Laser Surg. 2017 Apr;35(4):184-189

52. Ridhalaksani R, Nazar K, Djauharie NK, Meidyawati R, Npa DA. The antibacterial potential of N-acetylcysteine as an endodontic irrigant on the clinical isolates of the *Enterococcus faecalis* bioflm. Int J Appl Pharm. 2017;9:17–9.

53. Wong J, Manoil D, Näsman P, Belibasakis GN, Neelakantan P. Microbiological aspects of root canal infections and disinfection strategies: an update review on the current knowledge and challenges. Frontiers in Oral Health. 2021 Jun 25;2:672887

54. Meire M, De Moor RJ. Principle and antimicrobial efficacy of laser-activated irrigation: A narrative review. International Endodontic Journal. 2024 Jul;57(7):841-60.

55. Leoni, G.B., Versiani, M.A., Silva-Sousa, Y.T., Bruniera, J.F., Pecora, J.D. & Sousa-Neto, M.D. (2017) Ex vivo evaluation of four final irrigation protocols on the removal of hard-tissue debris from the mesial root canal system of mandibular first molars. *International Endodontic Journal*, 50, 398–406.

56. Siqueira, J.F., Jr. & Rocas, I.N. (2008) Clinical implications and microbiology of bacterial persistence after treatment procedures. *Journal of Endodontics*, 34, 1291–1301.e3

57. K. Nakajo, R. Komori, S. Ishikawa, T. Ueno, Y. Suzuki, Y. Iwami, et al., Resistance to acidic and alkaline environments in the endodontic pathogen Enterococcus faecalis, Oral Microbiol. Immunol. 21 (5) (2006) 283–288.

58. Arias-Moliz MT, Ferrer-Luque CM, Espigares-García M, Baca P. Enterococcus faecalis biofilms eradication by root canal irrigants. J Endod. 2009 May;35(5):711-4

59. Clegg MS, Vertucci FJ, Walker C, Belanger M, Britto LR. The effect of exposure to irrigant solutions on apical dentin biofilms in vitro. Journal of endodontics. 2006 May 1;32(5):434-7.

60. Roy R, Tiwari M, Donelli G, Tiwari V. Strategies for combating bacterial biofilms: A focus on anti-biofilm agents and their mechanisms of action. Virulence. 2018 Dec 31;9(1):522-54**.**

61. Abdulrab S, Mostafa N, Al-Maweri SA, Abada H, Halboub E, Alhadainy HA. Antibacterial and anti-inflammatory efficacy of N-acetyl cysteine in endodontic

treatment: a scoping review. *BMC Oral Health*. 2023;23(1):650.

62. Santus P, Signorello JC, Danzo F, Lazzaroni G, Saad M, Radovanovic D. Anti-inflammatory and anti-oxidant properties of N-acetylcysteine: A fresh perspective. Journal of Clinical Medicine. 2024 Jul 15;13(14):4127**.**

63. Biswas SK. Does the interdependence between oxidative stress and inflammation explain the antioxidant paradox?. Oxidative medicine and cellular longevity. 2016;2016(1):5698931

64. Al-Nazhan S, Al-Sulaiman A, Al-Rasheed F, Alnajjar F, Al-Abdulwahab B, Al-Badah A. Microorganism penetration in dentinal tubules of instrumented and retreated root canal walls. In vitro SEM study. Restorative Dentistry & Endodontics. 2014 Jul 22;39(4):258**.**

65. Klausen M, Ucuncu M, Bradley M. Design of photosensitizing agents for targeted antimicrobial photodynamic therapy. Molecules. 2020 Nov 10;25(22):5239**.**

66. Vatansever F, de Melo WC, Avci P, Vecchio D, Sadasivam M, Gupta A, Chandran R, Karimi M, Parizotto NA, Yin R, Tegos GP. Antimicrobial strategies centered around reactive oxygen species–bactericidal antibiotics, photodynamic therapy, and beyond. FEMS microbiology reviews. 2013 Nov 1;37(6):955-89.

67. Wainwright M. Dyes, flies, and sunny skies: photodynamic therapy and neglected tropical diseases. *Coloration Technology*. 2017;133(1):3–14

68. Pourhajibagher M, Chiniforush N, Ghorbanzadeh R, Bahador A. Antimicrobial photodynamic therapy for inactivation of biofilms formed by oral key pathogens. Photodiagnosis Photodyn Ther. 2019;26:221–769.

69. Vatansever F, de Melo WC, Avci P, Vecchio D, Sadasivam M, Gupta A, Chandran R, Karimi M, Parizotto NA, Yin R, Tegos GP. Antimicrobial strategies centered around reactive oxygen species–bactericidal antibiotics, photodynamic therapy, and beyond. FEMS microbiology reviews. 2013 Nov 1;37(6):955-89.

70. Algorri JF, Ochoa M, Roldan-Varona P, Rodriguez-Cobo L, López-Higuera JM. Light technology for efficient and effective photodynamic therapy: a critical review. Cancers. 2021 Jul 13;13(14):3484.

71. Maharjan PS, Bhattarai HK. Singlet oxygen, photodynamic therapy, and mechanisms of cancer cell death. Journal of oncology. 2022;2022(1):7211485.

72. Afrasiabi S, Partoazar A, Chiniforush N, Goudarzi R. The potential application of natural photosensitizers used in antimicrobial photodynamic therapy against oral infections. Pharmaceuticals. 2022 Jun 20;15(6):767.

73. Xie W, Zhang S, Pan F, Chen S, Zhong L, Wang J, Pei X. Nanomaterial-based ROS-mediated strategies for combating bacteria and biofilms. Journal of Materials Research. 2021 Feb 28;36:822-45.

74. Meire M, De Moor RJ. Principle and antimicrobial efficacy of laser-activated irrigation: A narrative review. International Endodontic Journal. 2024 Jul;57(7):841-60.

75. Asnaashari M, Safavi N. Disinfection of contaminated canals by different laser wavelengths, while performing root canal therapy. Journal of lasers in medical sciences. 2013;4(1):8.

76. Udart M, Stock K, Graser R, Hibst R. Inactivation of bacteria by high-power 940 nm laser irradiation. Medical Laser Application. 2011 Nov 1;26(4):166-71.

77. Nussbaum EL, Lilge L, Mazzulli T. Effects of 810 nm laser irradiation on in vitro growth of bacteria: Comparison of continuous wave and frequency modulated light. *Lasers Surg Med*. 2002;31(4):343–351.

78. Shirani Lapari S, Zare Jahromi M, Tahmourespour A, Shirani AM. Comparison of the *Enterococcus faecalis* colony reduction effect of two wavelengths of diode lasers with three methods of root canal irrigation: an in vitro study. *J Lasers Med Sci*. 2022;13:e25.

79. Purayil TP, Chakravarthy A, Ballal NV, Varghese J. Laser assisted root canal disinfection-a review. J Dent. 2016;4(2):41- 6.

80. Gutknecht N, Vanweersch L, Lampert F. Effects of diode laser (810 nm) irradiation on root canal walls: thermographic and morphological studies. *J Endod*. 2007;33(7):863–867.

81. Amini P, Ghaffari T, Kharazifard MJ, et al. The effect of storage solutions on the microhardness and surface characteristics of human teeth. *J Clin Exp Dent*. 2015;7(1):e58-63.

82. Distel JW, Trope M, Teixeira FB, et al. Effects of calcium hydroxide on Enterococcus faecalis in vitro. *J Endod*. 2002;28(2):68–71

83. Ridhalaksani R, Nazar K, Djauharie NK, Meidyawati R, Npa DA. The antibacterial potential of N-acetylcysteine as an endodontic irrigant on the clinical isolates of the Enterococcus faecalis biofilm. *Int J Appl Pharm*. 2017;9(2):17–19

84. Guerreiro-Tanomaru JM, de Faria-Júnior NB, Duarte MA, Ordinola-Zapata R, Graeff MS, Tanomaru-Filho M. Comparative analysis of Enterococcus faecalis biofilm formation on different substrates. J Endod. 2013 Mar;39(3):346-50.

85. Correia JH, Rodrigues JA, Pimenta S, Dong T, Yang Z. Photodynamic Therapy Review: Principles, Photosensitizers, Applications, and Future Directions. Pharmaceutics. 2021 Aug 25;13(9):1332.

86. Sperandio FF, Huang YY, Hamblin MR. Antimicrobial photodynamic therapy to kill Gram-negative bacteria. Recent Pat Antiinfect Drug Discov. 2013 Aug;8(2):108-20.

87. Ahrari F, Shahabi M, Fekrazad R, Eslami N, Mazhari F, Ghazvini K, Emrani N. Antimicrobial photodynamic therapy of *Lactobacillus acidophilus* by indocyanine green and 810-nm diode laser. *Photodiagnosis Photodyn Ther*. 2018;24:145–149.

88. Primas N, Lano G, Brun D, Curti C, Sallée M, Sampol-Manos E, Lamy E, Bornet C, Burtey S, Vanelle P. Stability Study of Parenteral N-Acetylcysteine, and Chemical Inhibition of Its Dimerization. Pharmaceuticals (Basel). 2023 Jan 3;16(1):72.

89. S. Dinicola, S. De Grazia, G. Carlomagno, J.P. Pintucci *N-acetylcysteine as powerful molecule to destroy bacterial biofilms. A systematic review* Eur Rev Med Pharmacol SciYear: 2014Vol. 18 - N. 19Pages: 2942-2948

90. Jambagi N, Kore P, Dhaded NS, Patil SA, Shankar M. Comparison of antimicrobial efficacy of diode laser, ultrasonic activated and conventional irrigation with 2.5% NaOCl during RCT: an interventional study. J Contemp Dent Pract. 2021;22(6):669-73.

91. Sandini A, Meidyawati R, Npa DA. The antibacterial effect of a diode laser used as an adjunct irrigant on clinical isolate of *Enterococcus faecalis* biofilm (in vitro). Int J Appl Pharm. 2017;9(2):103-6. D

92. Kashef N, Ravaei Sharif Abadi G, Djavid GE. Photodynamic inactivation of primary human fibroblasts by methylene blue and toluidine blue O. *Photodiagnosis Photodyn Ther*. 2012;9(4):355–358.

93. George S, Kishen A. Advanced noninvasive light-activated disinfection: assessment of cytotoxicity on fibroblast versus antimicrobial activity against *Enterococcus faecalis*. *J Endod*. 2007;33(5):599–602

94. Dickers, B., Lamard, L., Peremans, A. *et al.* Temperature rise during photo-activated disinfection of root canals. *Lasers Med Sci* 24, 81–85 (2009).

95. Radaelli E, Zezell DM, Brugnera-Júnior A, Bandéca MC, Merli T, Pinotti M. Effect of a high power diode laser irradiation in root canals contaminated with *Enterococcus faecalis*: In vitro study. *Lasers Surg Med*. 2002;30(5):398–402

96. Gutknecht N, Vanweersch G, Lampert F. Lasers in endodontics. *J Oral Laser Appl*. 2004;4(4):203–206

97. Asnaashari M, Safavi N, Zadsirjan S, et al. Photodynamic therapy in root canal disinfection: a case series and mini-review. *J Lasers Med Sci*. 2022;13:e19

98. Guerreiro-Tanomaru JM, de Faria-Júnior NB, Duarte MA, Ordinola-Zapata R, Graeff MS, Tanomaru-Filho M. Comparative analysis of Enterococcus faecalis biofilm formation on different substrates. J Endod. 2013 Mar;39(3):346-50.

99. .Higuchi N, Hayashi JI, Fujita M, Iwamura Y, Sasaki Y, Goto R, Ohno T, Nishida E, Yamamoto G, Kikuchi T, Mitani A, Fukuda M. Photodynamic Inactivation of an Endodontic Bacteria Using Diode Laser and Indocyanine Green-Loaded Nanosphere. Int J Mol Sci. 2021 Aug 4;22(16):8384.

100. Haghighi L, Azizi A, Vatanpour M, Ramezani G. Antibacterial Efficacy of Cold Atmospheric Plasma, Photodynamic Therapy with Two Photosensitizers, and Diode Laser on Primary Mandibular Second Molar Root Canals Infected with *Enterococcus faecalis*: An In Vitro Study. Int J Dent. 2023 Apr 21;2023:5514829.

ETHICAL CLEARANCE 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

Sl. No. : 1638

CERTIFICATE

This is to Certify that the synopsis titled

Comparative evaluation of antimicrobial efficacy of N-acetyl cysteine with photodynamic therapy and diode laser on root canals infected with Enterococcus faecalis - an in vitro study *Submitted by*

Dr. IE0222004 *P. G. Student /*

Staff, Guided by _____ *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 :

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

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

BIOSTATISTIC CLEARANCE CERTIFICATE

K L E
VISHWANATH KATTI
INSTITUTE OF DENTAL SCIENCES,
Constituent college of
K.L.E. Academy of Higher Education and Research
J.N.M.C. Campus, Nehru Nagar Belagavi -590010 Karnataka, India.
Department of Conservative Dentistry &Endodontics

Biostatistics Clearance Certificate

This is to certify that the Biostatistics aspect of this Dissertation/ Thesis work of **IE0222004** post-graduate student, under the guidance of Reader, **Department of Conservative Dentistry and Endodontics,** entitled **"Comparative evaluation of antimicrobial efficacy of N-acetyl cysteine with photodynamic therapy and diode laser on root canals infected with *Enterococcus faecalis* - an in vitro study "** has been done under my guidance and completed satisfactorily.

Date : 08.04.25

Place: Belagavi, KA

Name and Signature of Biostatistician

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

PLAGIARISM CHECK CERTIFICATE

Scientific Correspondence and Review Committee

KLE VK Institute of Dental Sciences

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

Accredited 'A+' Grade by NAAC (3rd Cycle) | Placed in Category 'A' by MHRD (GoI)

☎: 0831-2470362
FAX: 0831-2470640

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

Date : 16/4/2025 Serial No. : 411

PLAGIARISM CHECK REPORT

Name of the Applicant : **IE0222004**
UG / PG / Ph.D / Staff : PG
Batch & Year : 2022 - 2025
Department : Department of Conservative and endodontic dentistry

The soft copy of Research Work / Manuscript by **IE0222004** ..... entitled
" Comparative evaluation of anti-microbial efficacy of N-acetyl cystein with photodynamic therapy and diode laser on root-canal infected with Enterococcus faecalis - an in vitro study "
under the guidance of .. ..has been submitted for Anti-Plagiarism check to the Scientific Correspondence & Review Committee of KLE VK Institute of Dental Sciences using "Turn-it-in" software.

The scan has been carried out and the scanned output reveals a Similarity Index of 4 %, which is **within / not within** the acceptable limits of 10% as per the UGC guidelines.

16/4/2025
Member Secretary
Scientific Correspondence and Review Committee
KLEVK Institute of Dental Sciences
KAHER-Belagavi

15/4/25
Chairman
Scientific Correspondence and Review Committee
KLEVK Institute of Dental Sciences
KAHER - Belagavi