COMPARATIVE EVALUATION OF EFFICACY OF NISIN AND TRIPLE ANTIBIOTIC MEDICAMENT AS INTRACANAL MEDICAMENTS AGAINST *ENTEROCOCCUS FAECALIS* - AN INVITRO STUDY

Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

In partial fulfilment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH - IV

CONSERVATIVE DENTISTRY AND ENDODONTICS

2017 – 2020

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation titled **"COMPARATIVE EVALUATION OF EFFICACY OF NISIN AND TRIPLE ANTIBIOTIC MEDICAMENT AS INTRACANAL MEDICAMENTS AGAINST *ENTEROCOCCUS FAECALIS* - AN INVITRO STUDY"** is a bonafide work done by **Dr. R. S. ANU RADHA** Post graduate student, during the course of the study for the degree of **MASTER OF DENTAL SURGERY** in the specialty of **BRANCH-IV, CONSERVATIVE DENTISTRY AND ENDODONTICS,** Vivekanandha Dental College for Women, Tiruchengode, during the period of 2017-2020.

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ENDORSEMENT BY THE HEAD OF THE DEPARTMENT AND HEAD OF THE INSTITUTION

This is to certify that **Dr. R. S. ANU RADHA** Post graduate student (2017-2020) in the **DEPARTMENT OF CONSERVATIVE DENTISTRY AND ENDODONTICS,** Vivekanandha Dental College for Women, has done this dissertation titled **"COMPARATIVE EVALUATION OF EFFICACY OF NISIN AND TRIPLE ANTIBIOTIC MEDICAMENT AS INTRACANAL MEDICAMENTS AGAINST *ENTEROCOCCUS FAECALIS* - AN INVITRO STUDY"** under our guidance and supervision in partial fulfilment of the regulations laid down by the Tamilnadu Dr. M.G.R. Medical University, Chennai-600032 for **M.D.S (Branch-IV) CONSERVATIVE DENTISTRY AND ENDODONTICS.**

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DECLARATION

TITLE OF DISSERTATION	**COMPARATIVE EVALUATION OF EFFICACY OF NISIN AND TRIPLE ANTIBIOTIC MEDICAMENT AS INTRACANAL MEDICAMENTS AGAINST *ENTEROCOCCUS FAECALIS* - AN INVITRO STUDY**
PLACE OF STUDY	**VIVEKANANDHA DENTAL COLLEGE FOR WOMEN**
DURATION OF THE COURSE	**3 YEARS (2017-2020)**
HEAD OF THE DEPARTMENT AND GUIDE	**Dr. VAIYAPURI RAVI, M.D.S.,**

I hereby declare that no part of the dissertation will be utilized for gaining financial assistance for research or other promotions without obtaining prior permission of the Principal, Vivekanandha Dental College for Women, Tiruchengode. In addition, I declare that no part of this work will be published either in print or electronic format without the permission of the Guide who has been actively involved in the dissertation. The author has the right to reserve publishing of work solely with prior permission of the Principal, Vivekanandha Dental College for Women, Tiruchengode.

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Signature of the Candidate

Dr. R. S. Anu Radha

CERTIFICATE – II

This is to certify that this dissertation work titled **"COMPARATIVE EVALUATION OF EFFICACY OF NISIN AND TRIPLE ANTIBIOTIC MEDICAMENT AS INTRACANAL MEDICAMENTS AGAINST *ENTEROCOCCUS FAECALIS* - AN INVITRO STUDY"** of the candidate **Dr. R. S. ANU RADHA,** with Registration Number **241717551** for the award of **MASTER OF DENTAL SURGERY** in the branch of **CONSERVATIVE DENTISTRY AND ENDODONTICS.** I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **9%** of plagiarism in the dissertation.

Guide & Supervisor sign with Seal

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First of all, my sincere thanks and deep sense of gratitude to **Dr.Capt.S.Gokulanathan**, **B.Sc**, **M.D.S., (Dean) and Dr. N.Balan, M.D.S., (Principal),** Vivekanandha Dental College for Women, for permitting me to pursue this work.

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AIM AND OBJECTIVES

AIM AND OBJECTIVE

AIM:

The aim of this study was, to compare and evaluate the anti-microbial activity of Nisin and Triple Antibiotic Medicament as intracanal medicaments against *Enterococcus faecalis* using cell culture and Optical Density method (OD_{600}).

OBJECTIVE:

Nisin and Triple Antibiotic Medicament are intracanal medicaments for endodontic therapy which are not been compared in previous studies for its efficacy against *Enterococcus faecalis* which is a common pathogen found in the root canal failures.

The objective of this study was,

- To compare the anti-microbial activity of Nisin and Triple Antibiotic Medicament as intracanal medicaments against *Enterococcus faecalis* using Brain Heart Yeast (BHY) broth and measured under Optical Density $_{600}$(OD $_{600}$) method.
- To select a suitable intracanal medicament against *Enterococcus faecalis* in root canal infection.
- The results will give us the knowledge about the efficacy of naturally occurring medicament VS synthetic medicaments against *Enterococcus faecalis.*

INTRODUCTION

INTRODUCTION

The main aetiological factor for the pulp and the periapical disease are the microorganisms via root canal from the progression of pulpal inflammation by bacterial infection. Due to the sustained bacterial infectious stimuli, the pulpal and periapical inflammation occurs as an immunological self-defence reaction. The strategies for the root canal treatment is directly based upon the removal of these organisms and their toxic bacterial by-products.[1] In the root canal system the infection is considered as a polymicrobial infection, having both aerobic and anaerobic bacteria.[2]

The phases for the endodontic treatment procedure of the root canal include biomechanical preparation, disinfection and obturation. During cleaning and shaping, disinfection of the pulpal space is the very important step.[3]

The instrumentation by chemomechanical method sometimes as not effective at consistently disinfecting the root canal space and the radicular dentine. Intracanal medicaments are used during the treatment procedures as a consequence, in an attempt to eliminate the residual bacterial and their bacterial by-products. This treatment triad of mechanical cleaning, chemical disinfection and antibacterial dressing becomes essential intracanal medicaments are used during the treatment procedures.[4]

For an endodontic treatment to be successful it depends on the proper chemo-mechanical preparation which aids in removal of various microbes, debris and tissue remnants from the root canal and allow the canal to be obturated to achieve a hermetic

seal and prevent future ingress of microbes into the canal and healing of the periapical region can be apreciated.[5, 6]

Apical periodontitis was an inflammatory process including the periradicular tissues causing pain and resorption to the periradicular region. Anaerobic bacteria are the primary aetiological agents for apical periodontitis. One among the factor contributing to the persistent periradicular infection following root canal treatment is the surveillance of microorganism in the peri-apical structure of the root filled teeth. Not as primary endodontic infections, which are polymicrobial in nature and dominated by Gram-negative anaerobic rods, the microorganisms involved in the secondary infections are consisting of one or a few bacterial species in the root canal space.[6]

One among the primary organisms in post-treatment endodontic infection is Enterococcus faecalis.[7] It is a persistent organism and despites making up a small portion of the microbes in non endodontically treated canals, and plays an important role in the aetiology of persistent periradicular lesions after root canal treatment. [6] The most implicated microorganism in asymptomatic persistent infections.

Enterococcus faecalis is a gram positive cocci and a facultative anaerobe, occurring in a singly pairs and short chains as a small proportion of polymicrobial flora in the untreated canals and its predominance in the root canal failures and the persistent endodontic infections. Its presence ranges from 24% to 77% in persistent endodontic infection and 40% in primary endodontic infection.[7]

The endodontically treated tooth with periradicular lesion confirm that Enterococcus faecalis is resisting the antimicrobial activity and the ability to adapt to

changing environment and help to survive in endodontically treated root canals to cause re-infection.[8] And it has the survival ability in hard environment like alkaline pH, salts and concentrations. The resistance to detergents, heavy metals, bile salts, ethanol, azide and desiccation was very evident. The organism can also survive even at 60°C of temperature.[7]

Enterococcus faecalis, as a single organism in the root canal or as a main component of the flora due to its survival ability it can survive to a period of starvation upto 12 months and can prolonged period of nutritional deprivation [6] and multiply by genetic polymorphism, resulting in bone resorption and infection.[5]

Enterococcus faecalis binds to the dentin and proficiently invades the dentinal tubules by altering the host response and suppresses the action of lymphocytes. Enterococcus faecalis possesses lipoteichoic acid, pheromones, cytolysin aggregation substance and lytic enzymes. It forms biofilm in the canals that renders it more resistant to antimicrobial agents, antibodies and phagocytosis. It utilizes serum available as it source of nutrition. [7] Due to its adherence potential to the collagen in the dentin, it acts as a nidus for recurrence of infection in root canal failure treated tooth.[5]

Enterococcus faecalis is known to colonize in the dentinal tubules rami, isthmus, lateral, and accessory canal, and having the ability to penetrate the dentinal tubules to the depth of 1483.33µm that is in nutrient rich aerobic condition, 1166.66µm in nutrient rich anaerobic condition and 620µm in nutrient deprived anaerobic condition by forming micro colonies as a "mushroom shape".[7]

Although the numbers of bacteria are reduced by chemomechanical preparation of the root canal, an intracanal medicament with its antibacterial potential is required to increase the root canal disinfection in the infected conditions. Especially in the cases where the organisms are resistant to regular treatment and the unsuccessful completed therapy due to the presence of pain and continuous exudates, the need for proper intracanal medication increases.

For these strategies, a wide range of antibacterial intracanal medicaments have been used, which are phenolic derivatives, halides, aldehydes, antibiotics, chlorhexidine gels and calcium hydroxide pastes.[6]

In 1928, antibiotics were first discovered but were not used in routine medical practices until the early 1940s after the World War II. After the rapid recovery of wounded military personnel by the use of antibiotics, this was popularized and continued after the end of the war. In different disciplines of medicine and dentistry, the antibiotics have been prescribed for several decades. Addition to the armamentarium, antibiotics gives a valuable support to the health practitioners for management of bacterial infections.

In endodontics and dental traumatology, antibiotics may be applied either locally or systemically via oral and parenteral. In 1951, Grossman used polyantibiotic paste known as PBSC (penicillin, bacitracin, streptomycin, and caprylate), an antibiotic in endodontic treatment. Later caprylate were replaced by nystatin as an anti-fungal agent in a similar medicament as PBSN.[9]

In 1964, Sato et al was first to test the triple-antibiotic regimen. “Lesion sterilization and tissue repair (LSTR) therapy” gave the use of the combination of

antibacterial drugs (metronidazole, ciprofloxacin and minocycline) for the disinfecting the oral infectious lesions in dentinal, pulpal and periradicular lesion.[2]

As the root canal system has complexities in it, the use of single antibiotics alone may not result in effective and proper disinfection of the root canal system. To encounter the diverse microbial flora, a combination of antibiotic might play an important role to eradicate microbial organism. The likelihood of the development of resistant bacterial strains is been decreased and controlled by certain combination of antibiotics. The most promising combination of antibiotics consists of metronidazole, ciprofloxacin and minocycline.[3]

The potential of this mixture to kill bacteria in the deep dentine layer of the root canal was evaluated. From the infected dentinal wall of the root canals, after the application of the combination of antibiotics, they found that there was no evidence of bacteria to be recovered except in few cases.[10]

Although, this combination of antibiotics known as triple antibiotics shows a successful rate in endodontics, the long term use of these drugs showed a disadvantage like crown discoloration. This happens because of the staining property of Minocycline.[11] Hence, in this study minocycline has been replaced by clindamycin.

Nisin is a bacteriocin, naturally occurring antimicrobial peptide, obtained from Gram-positive bacteria from Streptococcus and Lactococcus species. It was first identified in a fermented milk cultures in 1928 and marketed as a antimicrobial agent in England in 1953 The originally described variants of Nisin, which is Nisin A, containing 34 amino acids is obtained from Lactococcus lactis. Nisin is classified as a

Type A (I) lantibiotic that is synthesized from mRNA and the translated peptide containing several unusual amino acid due to post-translational modification.

Nisin has been used widely as a food biopreservative for over past few decades. Since, many genetically modified and natural variants of Nisin which has been identified and observed for their unique antibactericidal properties. Nisin is FDA approved in 1969 and regarded as a safe peptide. Nisin as an oral antimicrobial was first described by Jhonson et al in 1978. Later, Nisin as a antimicrobial mouth rinse was developed by Howell et al in 1993. Then, a study by Tong et al demonstrated that Nisin A can inhibit the growth of cariogenic bacteria, including Streptococcus mutans.

Its biomedical use has reached to a great extend such that it can prevent the growth of drug-resistant bacterial strains like methicillin-resistant Staphylococcus aureus, Streptococcus pneumonia, Enterococci and Clostridium difficile. This antimicrobial activity is against Gram negative disease associated pathogens and a wide range of Gram-positive bacteria and to their spores, even against drug resistant Enterococcus faecalis isolates. Due to its potent and broad spectrum activity, it lowers the likelihood of promoting the bacterial resistance development and lower the cellular cytotoxicity at antimicrobial concentrations. Nisin is also having anti-biofilm properties. Nisin use in dental practice has so far been limited but recently, it was found to be effective in the elimination of Enterococcus faecalis from within the root canal system. Shin et al in 2015, demonstrated that high-purity of Nisin can retard the growth of Gram-negative pathogens like porphyromonas gingivalis, Preventella intermedia, Aggregatibacter actinomycetemcomitans and Ttreponema denticola and exert a anti-biofilm effect without causing cytotoxicity to human oral cells.[12,24]

MATERIALS AND METHODS

MATERIALS AND METHODS

SOURCE OF SAMPLE:

Forty five freshly extracted sound human permanent mandibular premolars were collected from the Department of Oral and Maxillofacial Surgery, Vivekanandha Dental College for Women, Tiruchengode.

MATERIALS USED: (Fig-1,2)

- Enterococcus faecalis of ATCC 29212
- Nisin
- Triple antibiotic medicament
- Distilled water

ARMAMENTARIUM: (Fig- 2)

- Safe-sided diamond disk with mantle.
- Nickel-titanium complete sterile file assortment set Pro-taper universal (DENTSPLY MAILLEFER, SWITZERLAND)
- Nickel-titanium size F3 sterile file Pro-taper universal (DENTSPLY MAILLEFER, SWITZERLAND)
- 3% sodium hypochlorite
- Sterile 2 ml syringe
- 17% Ethylene Diamine Tetra-acetic acid (EDTA)
- Ultrasonic unit, Endo attachment and U- files
- Autoclave
- Clear nail varnish
- Dental wax
- Eppindorf tube

- Brain Heart Yeast (BHY) broth
- Vortex mixer
- Pipette
- Size 10, 15, 20 K-files (MANI, INC. JAPAN)
- Porcelain mortar and pestle
- Cuvette
- Sterile paper points (SURE-ENDO, KOREA)
- Lentulo spiral size 30 (MANI, INC. JAPAN)
- Muller Hinton agar plates
- Universal incubator.
- Optical density(OD_{600})

S.NO	INTRACANAL MEDICAMENTS	MANUFACTURER	COMPOSITION
1.	Nisin	CK's product	Bacteriocin derivative from Lactococcus lactis
2.	Triple antibiotic medicament	a) Cifran® 500mg b) Metrogyl® 400mg c) Dalacin C® 300mg	a)Ciprofloxacin b)Metronidazole c)Clindamycin

PROPORTION OF THE INTRACANAL MEDICAMENT:

a) NISIN:

The medication is prepared by dissolving Nisin powderin sterile distilled water (Fig:1, 16) to a concentration of 100 mg $mL^{-1}$to form a creamy consistency.[4]

b) TRIPLE ANTIBIOTIC MEDICAMENT:

Triple antibiotic medicament was prepared by first removing the enteric coating using a sharp sterile knife and then the tablets were pulverized into fine powder using sterile porcelain mortar and pestle. The powdered drugs were weighed in the proportion of 1:3:3 (Ciproflocaxin : Metronidazole : Cylindamycin) using electronic weighing machine and were mixed with distilled water to form a creamy consistency.[23, 26](Fig: 2, 3, 4)

As Minocycline causes tooth discoloration, it is not used and has been replaced by Clindamycin in this study.

METHOD OF COLLECTION OF SAMPLES:

Forty five mandibular premolars were collected from the Department of Oral and Maxillofacial Surgery, Vivekanandha Dental College for Women, Tiruchengode, that were extracted for therapeutic purpose after obtaining due consentfrom the patients.

INFECTION CONTROL PROTOCOL FOR THE TEETH COLLECTED FOR THIS STUDY:

Collection, storage, sterilization and handling of extracted teeth were followed according to the guidelines and recommendations given by:

Occupational Safety and Health Administration (OSHA) and Centre for Disease Control & Prevention (CDC):

1. Handling of teeth was always done using gloves, mask and protective eyewear.
2. Teeth were cleaned of any visible blood and gross debris.
3. Distilled water was used in wide mouth plastic jars for initial collection.
4. The teeth were stored in separate jars containing distilled water.

5. The initial collection jars, lids and the gloves employed were discarded into biohazard waste receptacles.

6. As and when the teeth were required, they were removed from the jars with sterile tweezer and rinsed in sterile distilled water.

ETHICAL CLEARANCE:

A detailed protocol explaining the purpose and procedures of the study were submitted to the Institutional Review Board, Vivekanandha Dental College for Women and the approval for the study were obtained.

METHODOLOGY:

INCLUSION CRITERIA:

Intact human mandibular single – rooted pre-molar teeth with single root canal were confirmed with radiograph form the proximal aspect. (Fig: 7, 8)

EXCLUSION CRITERIA:

Restored / root canal treated, root caries, root with multiple canals and open apex teeth were excluded for this study.

Tooth Preparation:

Forty- five extracted human permanent mandibular single-rooted pre-molar teeth were collected and stored in distilled water. The specimens were decoronated 1 to 2 mm below the cemento-enamel junction using a safe- sided diamond disk (Fig 5)to standardize the root length at 12 mm.(Fig 9) [4,6]

The samples were divided into 3 groups of 15 roots each. The working length was confirmed using size 10 K-file (MANI, INC. JAPAN) and canal was enlarged using size 15 and 20 K-file (MANI, INC. JAPAN)(Fig: 10), then followed by rotary

files used in a sequential manner size Sx, S1, S2, F1, F2 Pro-taper Universal (DENTSPLY MAILLEFER, SWITZERLAND)[Fig 5,11]. 3% sodium hypochlorite as an irrigating solution was used during instrumentation with a sterile 2ml syringe. Smear layer removal in the root canal was done using 17% Ethylene Diamine Tetra-acetic acid (EDTA) solution which was ultrasonically agitated with U- files size 25.[4,6](fig 6,12)

The specimens were autoclaved at 121^{o}C for 15 minutes. Then three layers of clear nail varnish were applied covering the entire external root surfaces and allowed to dry.[4,6]

Root Canal Infection:

Bacterial samples of *Enterococcus faecalis* of ATCC 29212 strain was used in this study. Each root canal was inoculated with a bacterial solution up to the canal orifice using a sterile syringe(Fig 13).[4,6]

Each canal was then sealed with dental wax and all samples were incubated in a closed container at 37°C (98.6°F) for a period of 21 days. The canal was re-inoculated with fresh bacterial samples every 3 days. To check for cell viability and purity of culture, samples were taken from each canal using a sterile paper point (SURE-ENDO, KOREA) and inoculated into Muller Hilton agar plate and incubated aerobically for 24 hours at 37°C (98.6°F)(Fig 14).[4,6]

Root Canal Medication:

After 21 days, the canal contents were aspirated and each canal was rinsed with 5 ml distilled water using a 5 ml syringe, and then dried with sterile paper points (SURE-ENDO, KOREA) (Fig 15). The test medicaments were applied to the corresponding groups using lentuno spiral of 30 size (Fig 16, 17) [4,6]

Group I (n=15) - Nisin

Group II (n=15) - Triple Antibiotic Medicament

Group III (n=15) - Distilled water

Group I and II are experimental group whereas Group III as control group (Fig 18) .[4,6] The samples were incubated for 7 days at 37°C (98.6ºF) after sealing with dental wax (Fig 19, 20). The degree of infection was check on the 8th day for the root canal was investigated.

Samples Harvesting Method:

The dentin chips from the full length of the radicular dentin were harvested using a sterile rotary nickel-titanium F3 size Pro- taper universal (DENTSPLY MAILLEFER, SWITZERLAND)(Fig 21).[4,6] The dentin chips was removed from the files by placing into a sterile Eppindorf tube containing 1.5 ml of Brain Heart Yeast (BHY) broth in a vortex mixture for 30 seconds (Fig 22, 23, 24). The files were removed and inspected for dentinal debris remaining in the flutes. If, the dentinal debris were present, the files were again placed in a vortex mixer for another 30 seconds for complete removal of dentinal debris.

The samples were incubated for 24 hours at 37°C (98.6ºF) (Fig 25). Following incubation, each sample was mixed in a vortex mixer for 15 seconds (Fig 26) and 1ml of solution was pipetted into a cuvette.[4,6] Optical $Density_{600}$ (OD_{600}) of each sample was measured to estimate the concentration of *Enterococcus faecalis* (Fig 27).[4,6]

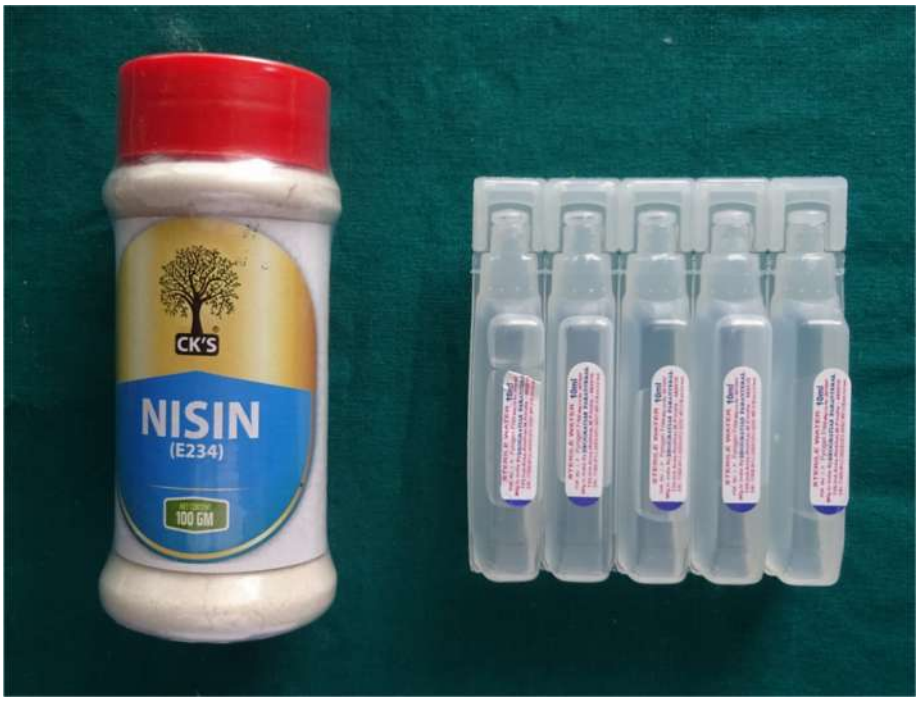

Fig 1: Intracanal medicament – Nisin and distilled water

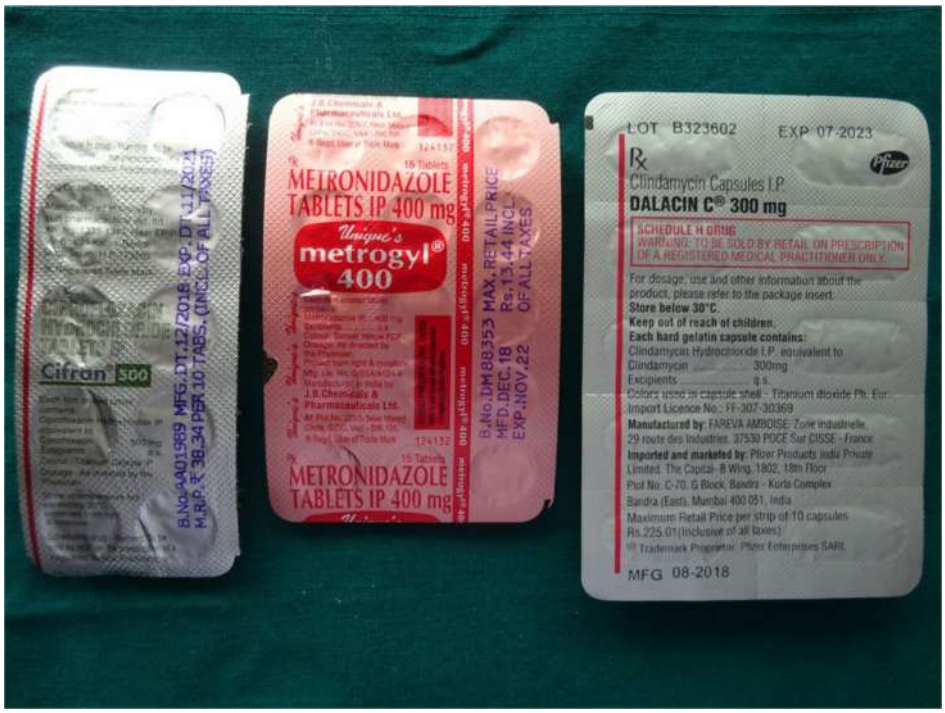

Fig 2: Ciprofloxacin, Metronidazole and Clindamycin as Intracanal medicament.

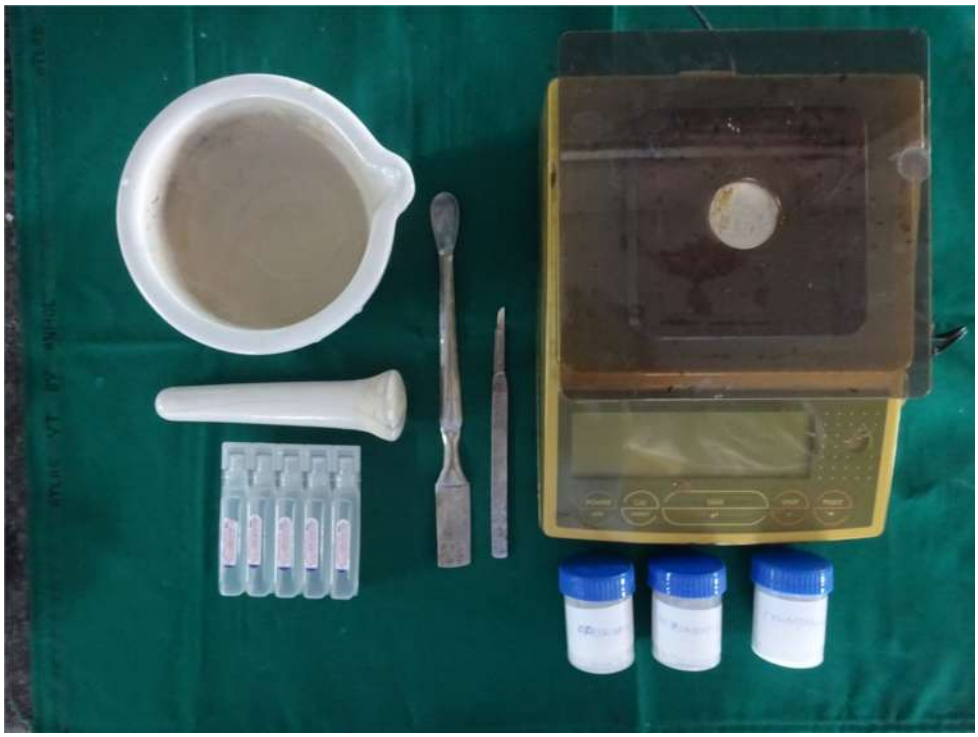

Fig 3: Armamentarium for preparation of triple antibiotic medicament

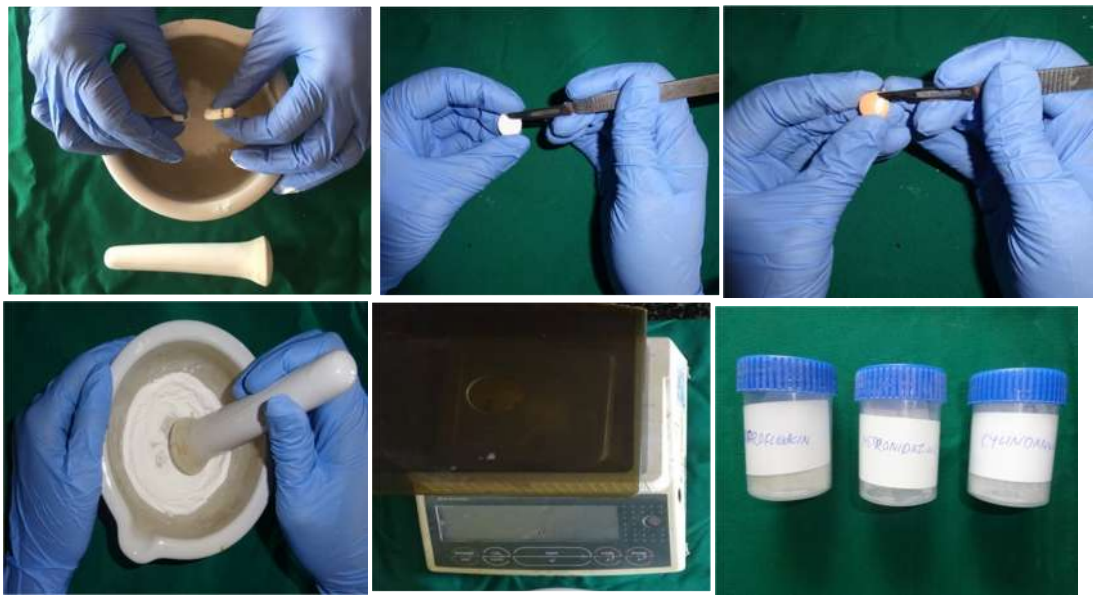

Fig 4: Preparation of triple antibiotic medicament.

Fig 5: Armamentarium for root canal preparation

Fig 6: Ultrasonic unit

Fig 7: Teeth samples

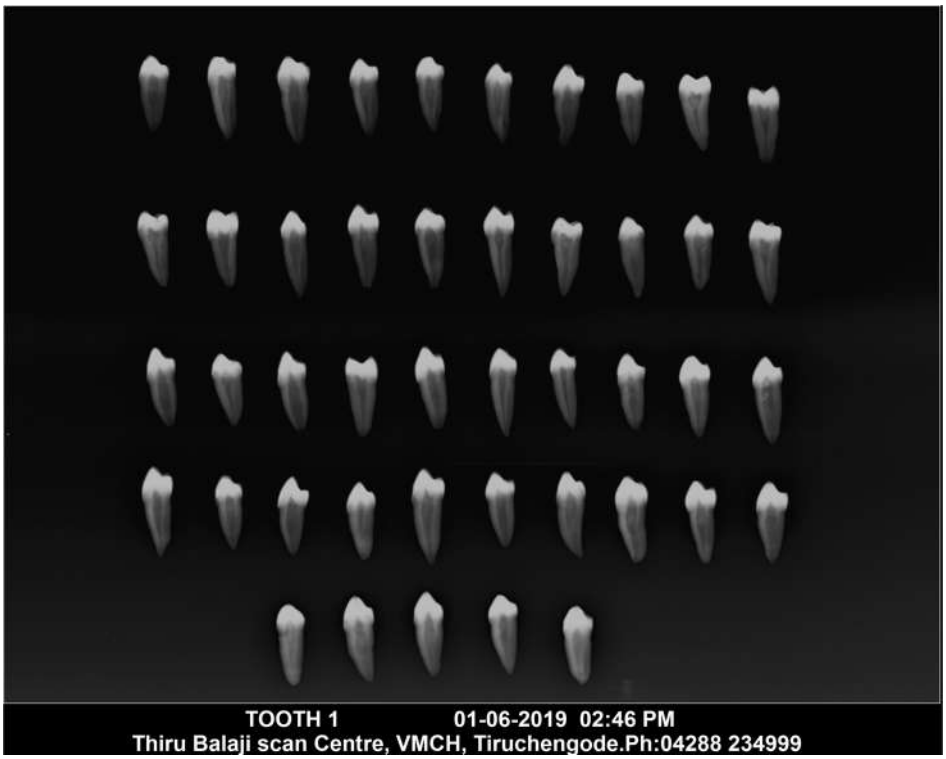

Fig 8: Radiograph of the teeth samples from proximal aspect

Fig 9: Decoronated teeth samples

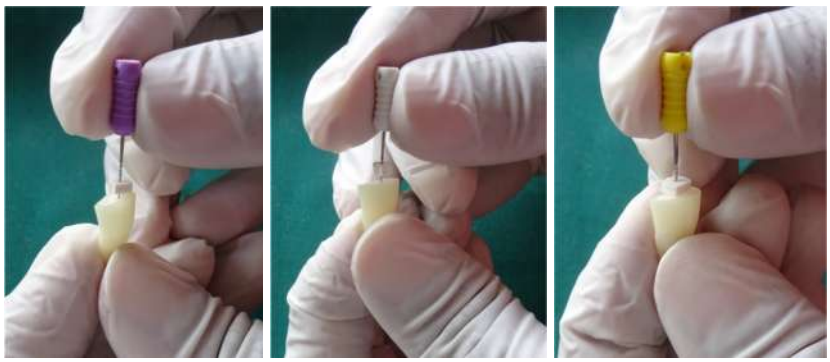

Fig 10: Working length determined using 10 size K file and apical enlargement done with 15 size and 20 size K file.

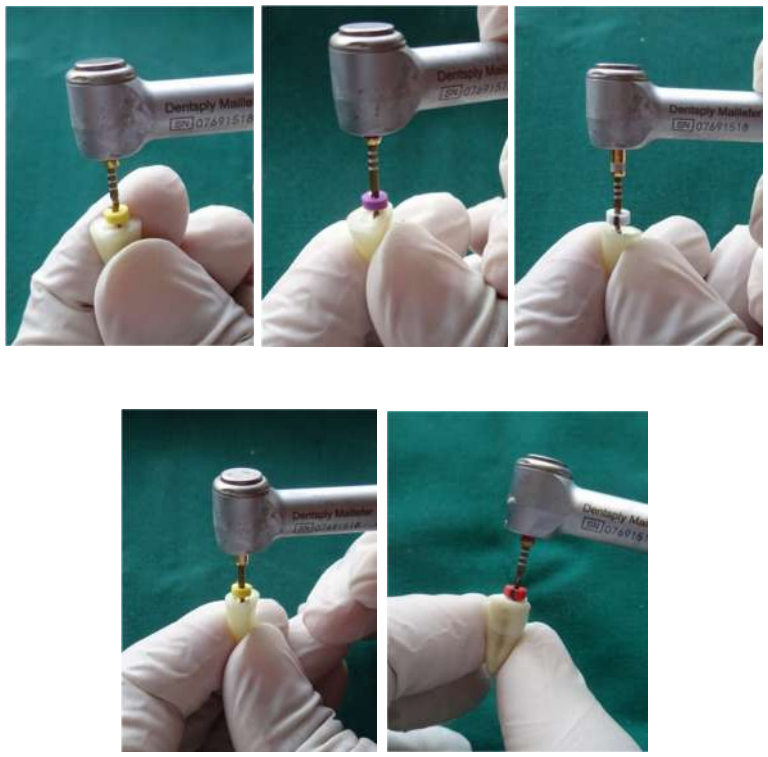

Fig 11: Cleaning and shaping done using Protaper Universal Rotary files

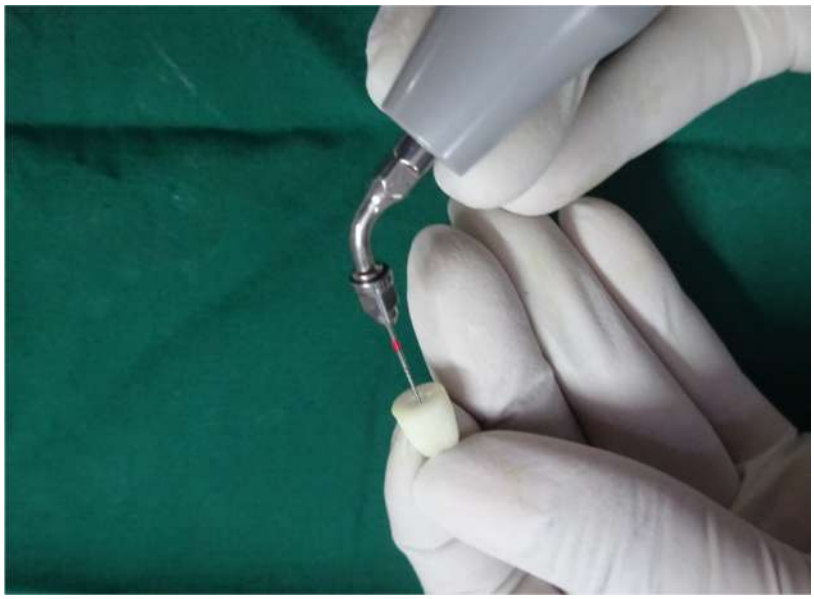

Fig 12: Smear layer removal 17% EDTA solution and ultrasonic agitation

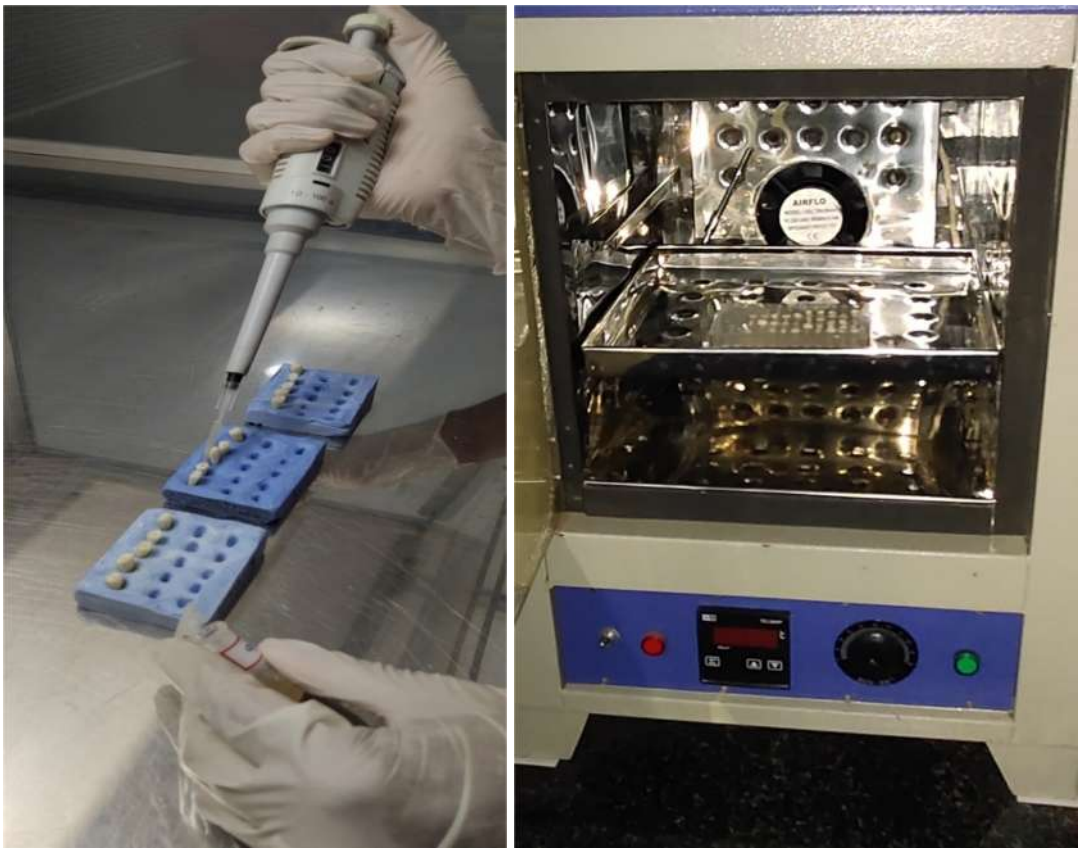

Fig 13: Inoculation of Enterococcus faecalis ATCC 29212 and incubated for 21 days

Fig 14: Growth of Enterococcus faecalis on Muller Hilton agar plate

Fig 15: Teeth samples were rinsed with distilled water and dried using paper points

Fig 16: Preparation of intracanal medicament Nisin and Triple antibiotic medicament with distilled water

A B C

Fig 17: Placement of intracanal medicament : (A) Nisin, (B) Triple antibiotic medicament using lentulo spiral of 25 size and (C) Distilled water.

Fig 18: Placement of intracanal medicament: Nisin, Triple antibiotic medicament and distilled water

Fig 19: Medicament sealed with dental wax.

Fig 20: Incubation of tooth samples after applying test medicaments for 24 hours.

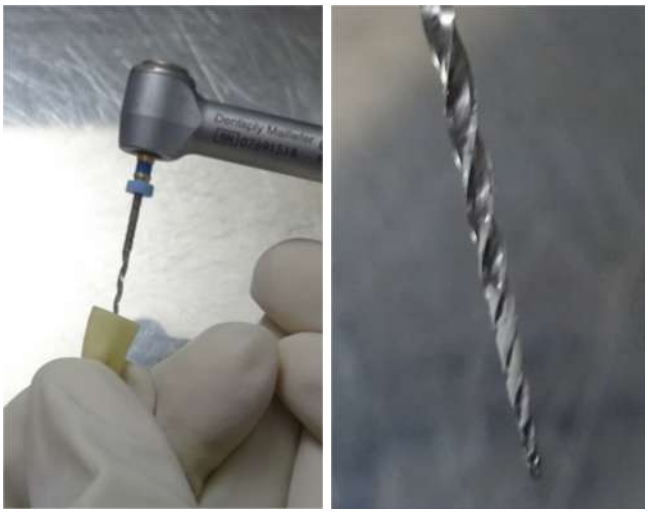

Fig 21: Dentin chips harvest using Protaper universal F3 size

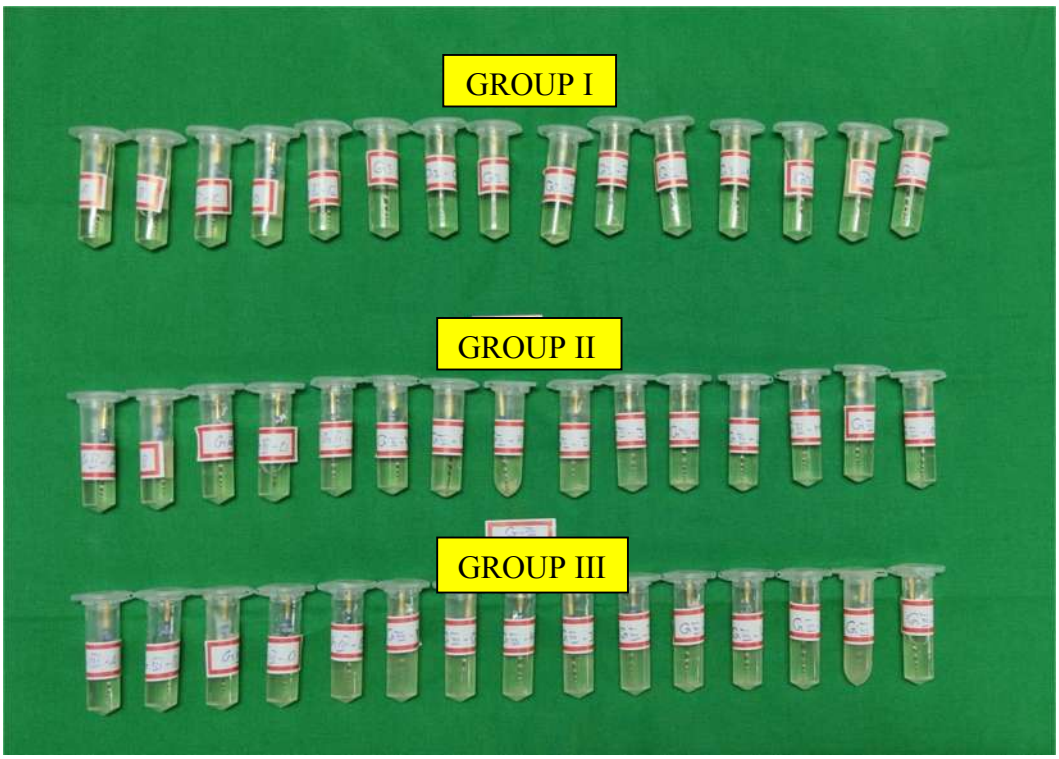

Fig 22: Protaper universal F3 size file with dentin chips are transferred into the eppindrof tube containing Brain Heart Yeast infusion (BHY) broth

Fig 23: Collection dentin chips from the files using a vortex machine

Fig 24: Eppindrof tubes containing the dentin chips with the file are placed in their test tube stands.

Fig 25: Incubation of the samples for 24 hours at 37^{o}C (98.6^{o}F)

Fig 26: Digital Spectrophotometer

REVIEW OF LITERATURE

RESULTS

The results obtained were statistically analysed by One-way analysis of variance (ANOVA) using the SPSS version 2.0, which showed the mean, standard deviation and standard error difference between the group I, group II and group III before and after medication respectively. The values obtained were considered statistically significant as the P value < 0.05.

There was statistically significant difference among all the tested groups after medication. (Table:1,2)

Table:1

		N	Mean	SD	SE	ANOVA	p
Before Medication	Group I	15	0.135	0.002	0.001	0.313	0.733
	Group II	15	0.136	0.003	0.001		
	Group III	15	0.135	0.003	0.001		
	Total	45	0.135	0.003	0.000		
After Medication	Group I	15	0.233	0.012	0.003	136.87	0.001**
	Group II	15	0.313	0.044	0.011		
	Group III	15	0.139	0.020	0.005		
	Total	45	0.228	0.077	0.012		

** Significant at 1 %

Table:2

After Medication

Tukey B Post Hoc Test

Group	N	Subset for alpha = .05		
		1	2	3
Group III	15	0.139		
Group I	15		0.233	
Group II	15			0.313

Before medication, the turbidity of all the samples were more or less same for all the groups when noticed under spectrophotometer for optical density. The higher level of *Enterococcus faecalis* in the solution indicates increase in the turbidity of the sample solution. The photons of light in the 600nm wavelength gets scattered indicating greater turbidity of the sample and then the received photons on the receptor gives the optical density value of that sample.(Fig-27)

When the bacterial growth in the suspended solution decreases, the turbidity of the solution also gets decreased which indicates that the photons of the light can passes through the solution and reaches the receptor of the spectrophotometer. Higher the values in the spectrophotometer indicates lower the bacterial count (ie) higher the efficacy of the medicament used in this study. (Tab-3,Fig-28, 29)

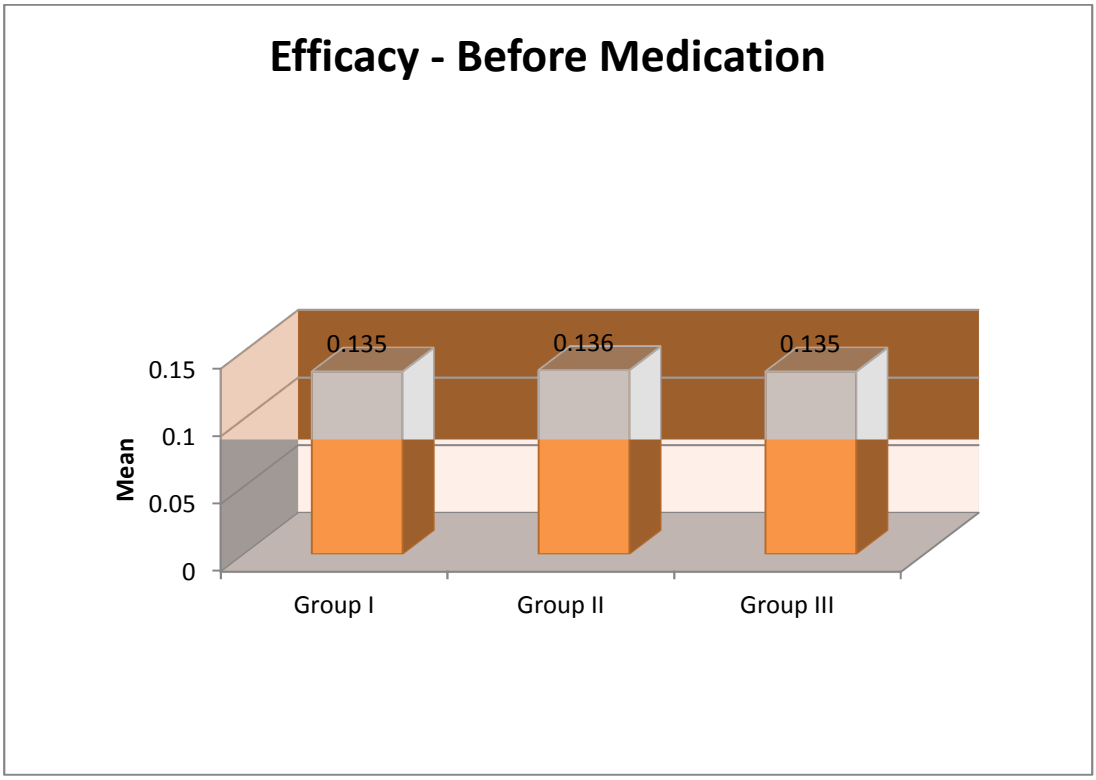

Fig 27: The mean optical density of the bacterial samples in each group before medication

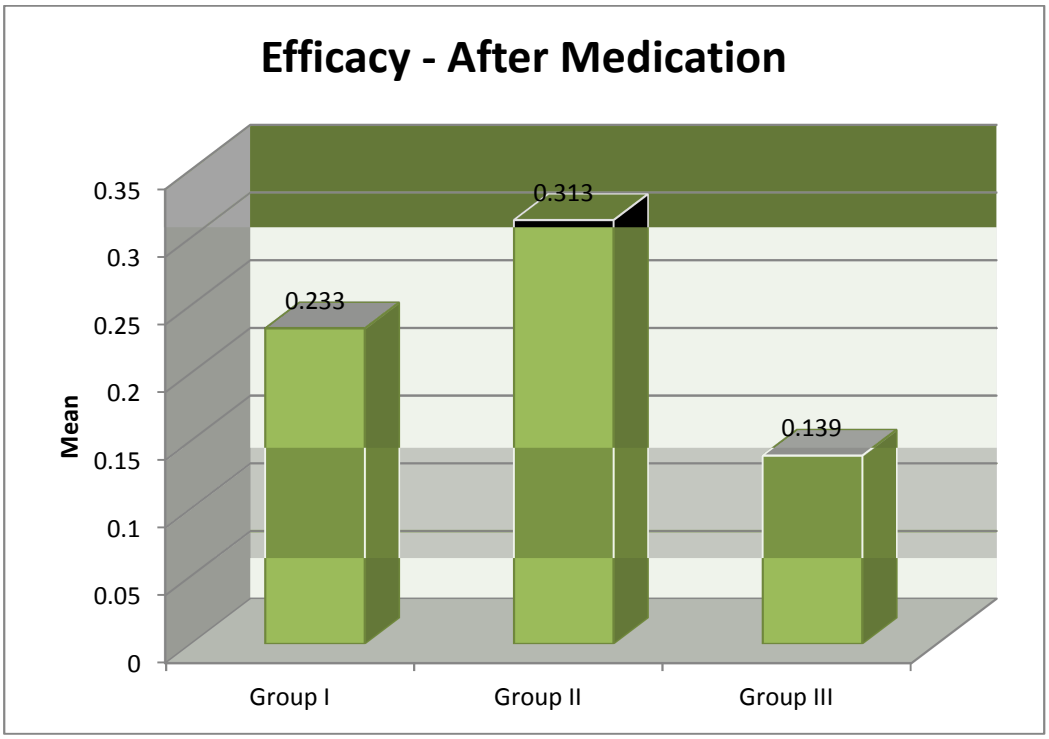

Fig 28: The mean optical density of the bacterial samples in each group after medication

Table: 3							
		N	**Mean**	**SD**	**SE**	**Paired t**	**p**
Group I	Before Medication	15	0.135	0.002	0.001	31.385	0.001**
	After Medication	15	0.233	0.012	0.003		
Group II	Before Medication	15	0.136	0.003	0.001	15.524	0.001**
	After Medication	15	0.313	0.044	0.011		
Group III	Before Medication	15	0.135	0.003	0.001	0.755	0.463
	After Medication	15	0.139	0.020	0.005		

** Significant at 1 %

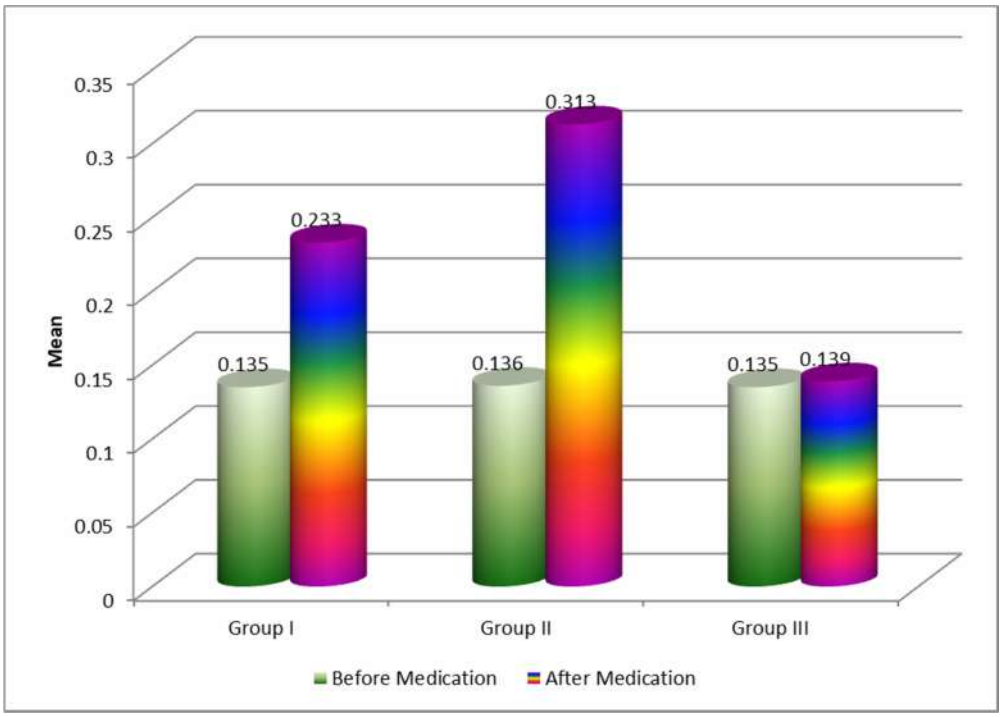

Fig 29: Comparison of the optical density before and after medication for every samples in each groups.

The obtained values showed that Group II (Triple Antibiotic Medicament) is better the other groups: group I (Nisin) and group III (distilled water as control). Thus, Triple antibiotic medicament containing ciprofloxacin, metronidazole and clindamycin is better in eradicating the gram positive organism, *Enterococccus faecalis* when used as an intracanal medicament.

RESULTS

REVIEW OF LITERATURE

T. J. Montville et al[13] in 1998, discussed about the mechanistic action of Pediocin and Nisin. They says that Nisin (34 amino acids) is an amphiphilic peptide. It has both water solubility and membrane-binding ability. Nisin initially binds to the target membrane through some degree of electrostatic interactions leading to membrane binding and pore formation.

L. R. G. Fava et al[14] in 1999, they illustrated about the classification and clinical indications of Calcium hydroxide pastes. The vehicles for Calcium hydroxide are classified into aqueous, viscous and oily, clinical properties of calcium hydroxide changing depending on the vehicle as an antimicrobial and anti-inflammatory agent.

Jennifer Cleveland et al[15] in 2001, denotes that the antimicrobial proteins or peptides produced by bacteria are termed as bacteriocins. Nisin was discovered in 1948 and belongs to Class Ia bacteriocins, consist of cationic and hydrophobic peptides that form pores in the target membrane, depleting the transmembrane potential and the pH gradient, resulting in the leakage of cellular materials.

B. P. F. A. Gomes et al[16] in 2003, have evaluated that the effectiveness of 2% chlorhexidine gluconate gel and calcium hydroxide as intracanal medicaments against Enterococcus faecalis. They concluded, 2% chlorhexidine gel alone was more effective against Enterococcus faecalis than calcium hydroxide. However, its antibacterial activity depended on how long it remained inside the root canal.

Shaul Lin et al[17] in 2003, have evaluated and compared the antibacterial effect of clindamycin and tetracycline in bovine dentinal tubules. Under the experimental conditions used in this study, the commercial preparations of clindamycin were more effective than those of tetracycline (Ledermix) in the agar diffusion test and clindamycin penetrated into dentinal tubules up to 400 μm. Thus, it

has the potential to serve as an effective intracanal medicament in persistent infections when other medicaments fail.

S. R. Turner et al[4] in 2004, made a study to provide a preliminary assessment of the effect of Nisin, a naturally occurring antimicrobial agent, against endodontic pathogens in solution and within the root canal system. They concluded that, Nisin was effective at eradicating *Enterococcus faecalis* and streptococcus gordonii cells in pure culture and was comparable with calcium hydroxide in the elimination of these species from within the root canal system.

Charles H. Stuart et al[18] in 2006, illustrated that *Enterococcus faecalis* is a microorganism commonly detected in asymptomatic, persistent endodontic infections. Its prevalence in such infection ranges from 24% to 77%. This finding can be explained by various survival and virulence factors possessed by *Enterococcus faecalis*, including its ability to compete with other microorganism, invade dentinal tubules, and resist nutritional deprivation. *Enterococcus faecalis* as a persistent organism, despite making up a small proportion of the flora in untreated canals it plays a predominant role in periradicular lesions after root canal treatment. It is commonly found in high percentile of root canal failures and it is able to survive in the root canal as a single organism or as a major component of the flora.

Mohammad Reza Sharifian et al[19] in 2008, evaluated the antimicrobial efficacy of "Consepsis V" and Calcium hydroxide mixed with different vehicles (distilled water or 2% chlorhexidine) on human teeth infected with *Enterococcus faecalis.* They concluded that 2% chlorhexidine and mixture of Calcium hydroxide with distilled water or 2% chlorhexidine were all effective for disinfection of root canal and dentin contaminated with *Enterococcus faecalis*.

Carlos Estrela et al[20] in 2009, mentioned that the invasion of root dentinal tubules by root canal bacteria is a multifactorial event in which a limited number of oral bacteria is a multifactorial event in which a limited number of oral bacterial species have the required properties to participate. He addressed his paper, considering the heterogeneity of guidelines to study antimicrobial strategies for endodontic infections and the high clinical success estimate, adequate disinfection assisted by the intracanal medicaments reduces the bacterial population and favours the prognosis. The antimicrobial efficacy of intracanal medicaments on bacterial biofilm still needs to be confirmed.

Nobuyuki Kawashima et al[1] in 2009, discussed about the root canal medicaments for the ultimate goals of endodontic treatment includes complete removal of bacteria, their by-products and pulpal remnants from infected root canals and the complete seal of disinfected root canals. They concluded that when pharmacological agents are administrated to humans, safety is of critical importance. Calcium hydroxide is the most acceptable medicament for placement in root canals.

Zahed mohammadi[9] in 2009 denotes that antibiotics are an extremely valuable addition to the armamentarium to health practitioners for management of bacterial infection. Lederman, a glucocorticosteroid antibiotic compound, is having anti-inflammatory, antibacterial and antiresorptive properties. Clindamycin alone or in an ethylene vinyl acetate vehicle can reduce the bacterial load inside the root canal system. A triple antibiotic paste consisting of metronidazole, ciprofloxacin, and minocycline are very effective in the disinfection of the root canal system.

Z. Mohammadi et al[10] in 2009, discussed that tetracycline have also been used as part of irrigating solutions but the substantivity is only for 4weeks. Clindamycin and a combination of three antibiotics (metronidazole, ciprofloxacin and

minocycline) have also been reported to be effective at reducing bacterial numbers on the root canal system of infected teeth.

Marcia Carneiro Valera et al[21] in 2009, illustrated that the effect of biomechanical preparation with 1% $NaOCl_2$ irrigation followed by intracanal medicament with Calcium hydroxide paste, 2% chlorhexidine gel and the association of these substances against *Enterococcus faecalis* and *Candida albicans* inoculated in root canals. They concluded that, both 1% $NaOCl_2$ irrigation and the intracanal medicament were effective in eliminating *Enterococcus faecalis* and *Candida albicans* which were inoculated in the root canal.

Lama Awawdeh et al[22] in 2009, investigated the antimicrobial properties of a Jordanian propolis-based intracanal medicament against *Enterococcus faecalis*, to find the minimum time needed to achieve its optimal antibacterial effect using infected dentine models, and to compare its antimicrobial efficacy with that of the non-setting calcium hydroxide paste when used as a short-term medication for 1 and 2 days. Three microbiological sampling methods: paper point, headstrom file and immersion of the dentine disc, were compared as well. They showed that propolis was significantly more effective than non-setting calcium hydroxide against *Enterococcus faecalis* after short-term application, which made comparison from this prospect unlevelled. The most effective microbiological sampling technique was abrading the lumen with headstrom file. Propolis is very effective as intracanal medicament in rapidly eliminating *Enterococcus faecalis* ex vivo.

Jong-Hyun Kim et al[23] in 2010, evaluated that a triple antibiotic mixture of ciprofloxacin, metronidazole, and minocycline was used as an intracanal medicament in an attempt to disinfect the root canal system for revascularization of a tooth with a necrotic pulp. They found that among the components of the triple antibiotic paste,

only minocycline caused the tooth discolouration and the use of dentin bonding agent reduced the intensity of the discolouration but did not prevented it. So, they concluded that, the possible aesthetic problems with the tooth colour should be considered when using minocycline as intracanal medicament. Minocycline should be limited to the root canal because of the potential risk of tooth discoloration, despite the biological success. Suitable techniques for preventing contact with the coronal dentin should be investigated and suggested for the safe use of minocycline.

Suneel Kumar Chinni et al[6] in 2011, determined the efficacy of Nisin against *Enterococcus faecalis* and its efficiency were compared with other intracanal medicaments like calcium hydroxide, chlorhexidine in human radicular dentin. Within the limits for his study he found that Nisin was effective at eradicating *Enterococcus faecalis* cells in pure culture and was comparable with chlorhexidine, positive control Vancomycin in elimination of *Enterococcus faecalis* from within the root canal system.

Hemadri M et al[24] in 2011, purposed a study to evaluate the antimicrobial efficacy of Nisin against Enterococcus faecalis in solution and also to evaluate and compare the effect of Nisin and calcium hydroxide against *Enterococcus faecalis* within the root canal system. They stated that Nisin was effective at eradicating *Enterococcus faecalis* in pure culture and was more effective when compared to calcium hydroxide in the elimination of this species within the root canal system. The ability of Nisin to effectively kill *Enterococcus faecalis* by a mechanism that is not reliant on achieving a high pH would be a valuable addition to endodontic treatment.

Manavalan Madhana Madhubala et al[25] in 2011, compared and evaluated the antimicrobial activity of calcium hydroxide, tri-antibiotic mixture (TAM) and an ethanol extract of propolis as intracanal medicaments on enterococcus faecalis-

infected root canals. In his study he concluded that, propolis was more effective than Tri-antibiotic mixture against Enterococcus faecalis at a 2-day time period, and were equally effective at 7 days.

Rangasamy vijayaraghavan et al[2] in 2012 discussed that the success of the endodontic treatment relies upon the elimination of bacteria from the root canal. Microorganisms in the periapical regions can cause re-infection and failure. Triple antibiotic paste can be effectively used for sterilization of canals and healing of periapical pathology. The effectiveness of Triple antibiotic paste in managing non-vital young permanent tooth was based on the availability of viable stem cells. Development of resistant bacterial strains and tooth discolouration are the possible drawbacks of this technique. Triple antibiotic paste seems to be promising medicament in the sterilization and revascularization.

Zohre Ahangari et al[26] in 2012, aimed at determining the antibacterial efficacy of Propolis in comparison with calcium hydroxide against Enterococcus faecalis in vitro. They concluded that, the antimicrobial activity of Propolis against *Enterococcus faecalis* species was comparable with that of calcium hydroxide at different time intervals. Therefore, it can be used as an alternative natural material for disinfection of canals during endodontic treatment.

Niketa Ramani et al[27] in 2012, evaluated the antimicrobial efficacy of propolis when used as an intracanal medicament against *Candida albicans* ATCC 10231 (C. albicans) and *Enterococcus faecalis* ATCC 51299 (E. faecalis) and compare this efficacy to that of Chlorhexidine digluconate (CHX). They concluded that ethanolic extract of propolis is effective as an intracanal medicament ex vivo.

Dohyun Kim et al[28] in 2012, discussed that the antimicrobial effect of $Ca(OH)_2$ are related to the hydroxyl ions released in an aqueous environment, which

affects cytoplasmic membranes, proteins, and the DNA of microorganisms. $Ca(OH)_2$ has a wide range of antimicrobial effects against common endodontic pathogens, but it is less effective against specific species such as *Enteroccous faecalis* or *Candida albicans*. The addition of vehicles or other agents might contribute to the antimicrobial effect of $Ca(OH)_2$. Although it remains controversial, it seems that by mixing $Ca(OH)_2$ with CHX, the antimicrobial activity of $Ca(OH)_2$ can be increased.

W. H. Chai et al[29] in 2013, they investigated the antimicrobial efficacy of erythromycin, oxytetracycline and calcium hydroxide [$Ca(OH)_2$] against *Enterococcus faecalis* biofilm in dentin. Within the limitation of this study, the antimicrobial efficacy of both the antibiotics was shown to be more effective than the $Ca(OH)_2$, but none were able to completely eradicate *Enterococcus faecalis* biofilm in dentinal tubules.

Manuel Sebastian Thomas et al[11] in 2014, presented a case of coronal discoloration due to the uses of Triple antibiotic paste that is mixture of ciprofloxacin, metronidazole and minocycline as an endodontic intra-canal medicament. They concluded that Triple antibiotic paste as intra-canal can promote healing of large periapical lesion. Despite the biological success, antibiotic medicaments containing minocycline should be used with extra caution especially in teeth present in the aesthetic zone. The effectiveness of various minocycline substitutes and techniques to limit the placement of intra-canal medicament in the root canal without contacting the coronal dentin needs to be evaluated.

Mohammad Ali Mozayeni et al[30] in 2014, evaluated the antimicrobial activity of four intracanal medicaments that is Calcium hydroxide, 2% chlorhexidine gel, Triple antibiotic paste and Nano silver on *Enterococcus faecalis.* They stated, Nano sliver gel was not efficient enough against *Enterococcus faecalis*. However,

Triple antibiotic paste and Chlorhexidine gel showed better antibacterial efficacy than Calcium hydroxide and can be used as an alternative intracanal medicaments in root canal therapies.

Ambikathanaya UK[3] in 2014, discussed about the successful root canal treatment depends on three main phases, biomechanical preparation of the root canal, disinfection and obturation. Disinfection of pulp space is an important step during and after cleaning and shaping. Intracanal medicaments are used for root canal disinfection. It plays a vital role in the success of root canal treatment from the past multivisit to today's single visit technique in various forms. Recent advances in various fields led to development of introducing newer medications as well as modifying the existing ones and their mode of application.

Rashmirekha mallick et al[8] in 2014, states that *Enterococcus faecalis* is commonly detected in endodontic infections that are asymptomatic, persistent and recurrent. They are the microorganisms mainly responsible for persistent periradicular lesions even after root canal treatment. It can survive in the root canal as a single organism or as a major component of the flora.

Swathi Pai et al[31] in 2014, evaluated and compared the effect of antibacterial intracanal medicaments on inter-appointment flare-up in diabetic patients. They stated that, calcium hydroxide and triple antibiotic paste are effective for managing inter-appointment flare-up in diabetic patients. However, Triple antibiotic paste was more effective than Calcium hydroxide in preventing the occurrence of flare-up in diabetic patients.

Belinda Kuan-Jung Chen et al[32] in 2014, examined the extent to which inter-visit corticosteroid-based antibiotic pastes (CAP) medicaments contribute to staining of tooth structure after attempted removal by irrigation techniques using either a

conventional irrigation technique or an Endo Activator. They concluded that, Medicaments that stain teeth may continue to discolour teeth despite best attempts to remove them. This study stresses the importance of material selection and minimising contact of Ledermix within the coronal aspects of teeth.

Kapil Jhajharia et al[33] in 2015, illustrated that *Enterococcus faecalis* is a gram-positive, facultative anaerobic cocci that is strongly associated with endodontic infections. Being as an opportunistic pathogen, it causes nosocomial infections and is frequently isolated from the failed root canals undergoing retreatment. *Enterococcus faecalis* in dentinal tubules can resist intracanal dressings of calcium hydroxide for over 10 days by forming a biofilm that helps it resist destruction by enabling the bacteria to become 1000 times more resistant to phagocytosis, antibodies, and antimicrobials than non-biofilm producing organisms. Calcium hydroxide, a commonly used intracanal medicament, may be ineffective to kill *Enterococcus faecalis* on its own, if a high pH is not maintained but at a pH of 11.5 or greater, *Enterococcus faecalis* is unable to survive.

Sanjay Madhavan et al[34] in 2015, conducted a study to evaluate the antimicrobial activity of the few endodontic sealers in combination with clove oil and antibacterial activity of clove oil with intracanal medicaments against *Enterococcus faecalis.* They concluded that apart from traditional use of clove, antibacterial effect of essential oils like clove oil can also provide an effective intracanal antiseptic medicament against oral pathogens.

Geethu Somanath et al[35] in 2015, evaluated the antimicrobial efficacy of chlorhexidine, Nisin, Linezolid and normal saline against *Enterococcus faecalis*. They concluded that Nisin was found to be the most effective in reducing the bacterial

count of *Enterococcus faecalis* in one week. Its action was found to be short acting with gradual decrease in antibacterial action after 72 hours.

J. M. Shin et al[12] in 2015, discussed about the biomedical applications of Nisin which is a bacteriocin produced by a group of Gram-positive bacteria that belongs to Lactococcus and Streptococcus species. Nisin used widely as a food biopreservative. Nisin can prevent the growth of drug resistant bacterial strains, such as methicillin-resistant *Staphylococcus aureus, Streptococcus pneumonia, Enterococci* and *Clostridium difficile.* Nisin can also inhibit the growth of *Enterococcus faecalis*, which is an opportunistic Gram-positive pathogen frequently recovered form infected root canal of teeth. Nisin successfully eradicates the colonization of *Enterococcus faecalis.*

Jeison B. Carbajal Mejia et al[36] in 2015, to evaluate the viability of *Enterococcus faecalis* after a 14-day exposure to 1% cetrimide (CET), tri-antibiotic paste (TRIA) (i.e., metronidazole, minocycline, and ciprofloxacin), 2% chlorhexidine (CHX) gel, and calcium hydroxide ($Ca[OH]_2$) in an infected dentine model. They have concluded that Both 1% cetrimide (CET) and tri-antibiotic paste (TRIA) significantly reduced the viability of *Enteococcus faecalis* in dentine of extracted teeth in comparison with 2% CHX gel and calcium hydroxide paste. Further laboratory and clinical investigations should be carried out to validate findings of the beneficial use of 1% CET as an intracanal medicament against *Enterococcus faecalis.*

Hévelin Couto Pimenta et al[37] in 2015, evaluated the in vitro antimicrobial activity of Brazilian brown propolis as an intracanal medication against *Enterococcus faecalis.* This study demonstrated that, although medications based on brown propolis with or without calcium hydroxide has limitations inherent to an in vitro study; they are effective against *Enterococcus faecalis*.

Sharmila Devaraj et al[38] in 2016, compared the anti-biofilm activity of photo activated curcumin with triple antibiotic paste, double antibiotic paste, chlorhexidine and calcium hydroxide when used as an intracanal medicament. Then concluded that, photo activated curcumin demonstrated superior anti-biofilm and antibacterial activity against Enterococcus faecalis than triple antibiotic paste, but the difference was not statistically significant. While chlorhexidine killed more *Enterococcus faecalis* cells than calcium hydroxide within the dentinal tubules at 200 microns depth, both these agents were ineffective in disrupting the structure of *Enterococcus faecalis* biofilms.

Triveni M Nalawade et al[39] in 2016, determined the relative antimicrobial effectiveness of various endodontic medicaments that is the double antibiotic paste (DAP), modified DAP, 2% chlorhexidine gluconate and their combination with four vehicles namely Polyethylene glycol 400 (PEG), Propylene glycol (PG), combinations of PG with PEG and Glycirineand using an agar well diffusion assay. In their study they concluded that, 2% chlorhexidine gluconate and modified double antibiotic paste can definitely replace double antibiotic paste and triple antibiotic paste as endodontic medicaments with chlorhexidine having an added advantage of bactericidal action, substantivity, biocompatibility, low toxicity, and lesser chances of developing resistance.

Alagl AS et al[5] in 2016 mentioned that Propolis and *S. persica* have been proven to be effective against *Enterococcus faecalis*.

Marta E. Valverde et al[40] in 2017, compared the antimicrobial activity of different intracanal medications for 2 and 7 days, in simulated open apex root canals contaminated with *Enterococcus faecalis* biofilms. They concluded that, chlorhexidine and/or CTR (Cetrexidin) pastes were as effective as double antibiotic paste and triple antibiotic paste as short-term intracanal medicaments in regenerative

endodontic therapy and Cetrexidin paste could be an alternative once its biocompatibility is proven.

Shibha Mehta et al[41] in 2017, compared the antimicrobial efficacy of triple antibiotic paste (TAP) and a proton pump inhibitor (PPI) (omeprazole) in combination with calcium hydroxide (CH) against *Enterococcus faecalis* and *Candida albicans*. They concluded that, proton pump inhibitor (PPI) enhanced the antibacterial efficacy of calcium hydroxide against *Enterococcus faecalis* and *Candida albicans*. However, Triple antibiotic paste showed the best antibacterial property followed by CH plus and proton pump inhibitor (PPI) against both the selected strains.

Jophie V paikkatt et al[42] in 2017 evaluated and compared the effectiveness of commonly used intracanal medicament against Candida biofilms found in the root canals of human primary teeth with necrotic pulp. They concluded that calcium hydroxide, 1% chlorhexidine and 1% Metronidazole were found to be ineffective in eradicating Candida biofilms when used as intracanal medicament.

G. Anuradha Reddy et al[43] in 2017, evaluated the clinical and radiographic success of pulpectomized primary teeth with chronic infection using a mixture of metronidazole, ciprofloxacin, and minocycline (3MIX)-MP as an intracanal medicament before the obturation. They concluded, all the primary teeth with chronic infection which were treated using 3MIX□MP, followed by the instrumentation and obturation provided excellent success when compared to conventional pulpectomy and noninstrumentational lesion sterilization tissue repair therapy.

Pratibha Ahirwar et al[44] in 2018, evaluated the aerobic and anaerobic antimicrobial efficacy of Ocimum Sanctum (Tulsi) essential oil and compared it with that of triple antibiotic paste (TAP) by collecting microbiological samples from the root canals of primary molars. They concluded that Antibiotic use is often associated

with the adverse effects and development of resistance due to injudicious use. Ocimum sanctum can be used in cases of long□standing infection owing to its antimicrobial efficacy and anti□inflammatory potential as an intracanal medicament in primary teeth.

Ane Poly et al[45] in 2018, quantitatively assessed the ability of two single-step restorative materials to avoid crown darkening caused by the use of minocycline as an intracanal medicament. They stated that, Crown darkening can be minimized by the previous application of RelyX U200 or OptiBond All-In-One to the inner walls of the access cavity before a minocycline-containing paste is applied as an intracanal medication.

Nazanin Zargar et al[46] in 2018, evaluated and compared the antibacterial effect of 2% Clindamycin gel and 2% and 100% tri-antibiotic gel (TAP) and calcium hydroxide in dentine tubular infection with E. faecalis. They concluded that, the antibiofilm effect of clindamycin was comparable with tri-antibiotic gel (TAP), so it may be used instead of Triple antibiotic paste.

Sholeh Ghabraei et al[47] in 2018, they determined the minimum duration of application of Triple antibiotic paste required for elimination of *Enterococcus faecalis* from the root canal system and its Minimum Inhibitory Concentration and Minimum Bactericidal Concentration in an ex-vivo. They concluded, the original concentration of Triple antibiotic paste was found to be 5×104 times its Minimum Inhibitory Concentration. Considering the risk of coronal discoloration of teeth following the use of Triple antibiotic paste, application of its lower concentrations is recommended.

Marcia E. F. Arruda et al[48] in 2018, made a randomized clinical study comparing the antibacterial effectiveness of treatment protocols using either a triple antibiotic solution (1 mg/mL) or calcium hydroxide/chlorhexidine paste as inter-

appointment medication in infected canals of teeth with primary apical periodontitis. They concluded, Inter-appointment medication with a triple antibiotic solution at the concentration of 1 mg/mL significantly improved root canal disinfection, and its effects were at least comparable with the calcium hydroxide/chlorhexidine paste. Effectiveness and easy delivery of the antibiotic solution make it an appropriate medicament as part of a disinfecting protocol for conventional nonsurgical endodontic treatment and possibly regenerative endodontic procedures.

Amrita Rouhani et al[49] in 2018, evaluated the amount of residual materials on canal walls after the use as medicaments within natural open apex teeth. Their result concluded, Propolis is superior to Calcium hydroxide and Triple antibiotic paste in terms of removability from the root canal system within open apex teeth.

Leila Moradi Eslami[50] in 2019, compared the antimicrobial effects calcium hydroxide, Triple antibiotic paste, Photodynamic therapy, Toluidine blue, Light emitting diode and 940nm diode laser on the biofilm of *Enterococcus faecalis* and *Candida albicans* in the root canal system of ex-vivo human teeth. This study showed that the application of Triple antibiotic paste, Photodynamic therapy, and Light emitting diode exposure lead to least biofilm thickness.

Farzaneh Afkhami et al[51] in 2019, they compared tooth discoloration following the application of different intracanal medicaments. Additionally, the effect of the location of intracanal medicament placement on coronal discoloration was evaluated. They concluded, intracanal medicaments may induce tooth discoloration. Use of 3Mix must be short and it must be carefully applied only to the root canals; the access cavity should be thoroughly cleaned afterwards.

DISCUSSION

DISCUSSION

Disinfection of pulp space is an important step during and after cleaning and shaping. It primarily involves cleaning and shaping the root canal space with endodontic instruments along with irrigants. However, in certain clinical conditions the polymicrobial nature of the endodontic infection demands the use of an intracanal medicament in addition to the irrigants.[52]

Leaving the root canal empty between appointment or with an inappropriate intracanal dressing (that is, not adequate antibacterial) is not advisable because the small number of surviving microorganism in the root canal will proliferate rapidly, resulting in the bacterial load returning back to its initial levels within days after chemo mechanical preparation.[53]

Thus, it is necessary to consider that endodontic treatment performed in a single appointment is not the best therapeutic option. Since several steps are required, which include: indicating an intracanal dressing to maintain the sanitization process in difficult situations; to reduce endodontic microbiota in primary and secondary infections; control persistent exudates; neutralize activity of the osteoclasts present in the inflammatory dental resorption; treat large apical periodontitis; favor apical closure in apexification; and to treat root perforations.[54]

Being an opportunistic pathogen, *Enterococci faecalis* causes nosocomial infections and is frequently isolated from the failed root canals undergoing retreatment. Enterococci faecalis is a gram-positive cocci that can occur singly, in pairs, or as short chains. They are facultative anaerobes which have the ability to grow

in the presence or absence of oxygen. They can grow in extremely alkaline pH, salt concentrated environment, in a temperature range of 10–45°C, and survive a temperature of 60°C for 30 min. *Enterococcus faecalis* is able to suppress the action of lymphocytes, potentially contributing to endodontic failure.

Enterococcus faecalis in dentinal tubules can resist intracanal dressings of calcium hydroxide for over 10 days by forming a biofilm that helps it resist destruction by enabling the bacteria to become 1000 times more resistant to phagocytosis, antibodies, and antimicrobials than non-biofilm producing organisms. Calcium hydroxide, a commonly used intracanal medicament, may be ineffective to kill *Enterococcus faecalis* on its own, if a high pH is not maintained.

Enterococcus faecalis has the ability to form biofilm that can resist calcium hydroxide dressing by maintaining pH homeostasis, but at a pH of 11.5 or greater, *Enterococcus faecalis* is unable to survive.[55,56] The frequency of *Enterococcus faecalis* found in persistent periradicular lesions has been shown to be much higher. In fact, failed root canal treatment cases are nine times more likely to contain *Enterococcus faecalis* than primary endodontic infections.[57]

Nisin is a bacteriocin, naturally occurring antimicrobial peptide, obtained from Gram-positive bacteria from Streptococcus and Lactococcus species. The originally described variants of Nisin, which is Nisin A, containing 34 amino acids is obtained from Lactococcus lactis. Nisin is classified as a Type A (I) lantibiotic. Nisin as an oral antimicrobial agent is effective as an antimicrobial mouth rinse and inhibit the growth of cariogenic bacteria, including Streptococcus Mutans.

Its biomedical use has reached to a great extend such that it can prevent the growth of drug-resistant bacterial strains like methicillin-resistant Staphylococcus aureus, Streptococcus pneumonia, Enterococci and Clostridium difficile. This antimicrobial activity is against Gram negative disease associated pathogens and a wide range of Gram-positive bacteria and to their spores, even against drug resistant Enterococcus faecalis isolates. Due to its potent and broad spectrum activity, it lowers the likelihood of promoting the bacterial resistance development and lower the cellular cytotoxicity at antimicrobial concentrations.

Nisin is also having "anti-biofilm" properties that when the biologically relevant human saliva as the multi-species biofilm inoculum and growth media.[58] Its use in dentistry has so far been limited but recently, it was found to be effective in the elimination of Enterococcus faecalis from within the root canal system. The high-purity of Nisin can retard the growth of Gram-negative pathogens like porphyromonas gingivalis, Preventella intermedia, Aggregatibacter actinomycetemcomitans and Ttreponema denticola and exert a anti-biofilm effect without causing cytotoxicity to human oral cells.[12, 24]

The complexity of root canal anatomy and diverse microbial flora in root canal system, the use of single antibiotic to disinfect is not sufficient. More likely, a combination of antibiotics would be needed to address the diverse flora to encountered. The most promising combination in antibiotic that consist of metronidazole, ciprofloxacin and minocycline. This combination is used in "lesion sterilization and tissue repair (LSTR) for disinfecting the oral lesions, including dentinal, pulpal and periapical lesions. The antimicrobial effect of these drugs individually showed none of the drugs resulted in complete elimination of

microorganism. Metronidazole is a nitro-imidazole compound that exhibits a board spectrum of activity against protozoa and anaerobic organism. Ciprofloxacin, a sysnthetic fluoroquinolone, has a bactericidal mode of action and Minocycline is a semisynthetic derivative of tetracycline with a similar spectrum of activity.[2]

Even though, this combination shown an effective results, it is shown to cause discolouration of the coronal tooth structure. Unfortunately, the tooth had a very evident of greenish intrinsic staining as a post-operative effect when the Triple antibiotic (Metronidazole; Ciprofloxacin; and Minocycline) in these combination of drugs when placed for 2 weeks of 2 visits. Minocycline binds with the calcium of dentin forming insoluble complexes, which results in extensive tooth discoloration.[11]

Hence in the study, minocycline is replaced with clindamycin. Clindamycin is less cytotoxic when compared with Minocycline at higher concentrations. Clindamycin is a bacteriostatic lincosamide, which is active against most strains of gram-positive aerobes and most anaerobic organisms responsible for dento-alveolar and endodontic infections and which were able to significantly reduce the growth of endodontic bacteria, such as Enterococcus faecalis and Aggregatibacter actinomycetemcomitans.[59]

The results obtained in this study shows that, Triple antibiotic medicament in combination of metronidazole, ciprofloxacin and clindamycin has more effect than Nisin when used a intracanal medicament against Enterococcus faecalis. As these antibiotics readily permeates bacterial cell membrane and it then binds to DNA in the cell and disrupts its helical structure which leads to rapid cell death. Even though Nisin as a single component it showed a better antimicrobial property and anti-biofilm

property. Nisin interacts with the phospholipid membrane of the target microbial cell and disrupts the cellular membrane inducing leakage of small intracellular content from the bacterial cell.

If the concentration and frequency of the intracanal dressing was considered, the efficacy of Nisin might shows better result than the synthetic antibiotic regimens. When, this combination of Triple antibiotic medicament used in clinical scenario the following conditions should be considered whether the organism involved is drug resistant, patient contra-indications against the drugs to be used. As Nisin is a naturally obtained antimicrobial agent which is economic and cost effective, Nisin can be considered as intracanal medicament for clinical practice. So further clinical trials are needed to support this result which is obtained.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

This study was to compare and evaluate the anti-microbial activity of Nisin and Triple Antibiotic Medicament as intracanal medicaments against *Enterococcus faecalis* using cell culture and Optical Density method (OD_{600}).

Forty- five extracted human permanent mandibular single-rooted pre-molar teeth was collected and stored in distilled water. The specimens were decoronated 1 to 2 mm below the cemento-enamel junction using a safe- sided diamond disk to standardise the root length at 15 mm.

The samples were divided into 3 groups of 15 roots in each group. Then the instrumentation od the canals with sterile rotary nickel-titanium F2 size Pro-taper (DENTSPLY) on a sequential crown down technique. 2.5% sodium hypochlorite was used as an irrigating solution during instrumentation with a sterile 2ml syringe. Smear layer removal in the root canal was done with 17% Ethylene Diamine Tetra- acetic acid (EDTA) solution agitated for 30 seconds using U-files. The roots were autoclaved at 121^{o}C for 15 minutes. Then three layers of clear nail varnish was applied over all external root surfaces and allowed to dry.

Bacterial samples of *Enterococcus faecalis* of ATCC 29212 strain was used in this study. Each root canal was injected with a bacterial solution up to the canal entrance using sterile syringe. Each canal was closed with a dental wax and all samples were incubated in a closed container at 37°C (98.6°F) for a period of 21 days. The canal was re-inoculated with freshly maintained bacterial samples every 3 days. To verify for cell viability and purity of culture, samples made from each canal with a

sterile paper point and inoculated into Muller Hilton agar plate and incubated aerobically for 24 hours at 37°C (98.6°F).

After 21 days, the canal content was aspirated and each canal was rinsed with 5 ml distilled water using a 2 ml syringe, and then was dried with sterile paper points. The test medicaments were applied to the corresponding groups. Group I (n=15) - Nisin for each sample, Group II (n=15) - Triple Antibiotic Medicament for each sample, and Group III (n=15) - Distilled water for each sample. Group I and II as experimental group whereas Group III as control group.

The roots were maintained for 7 days at 37°C (98.6ºF) after sealing with dental wax. The degree of infection of the radicular dentin were investigated on the 8th day, where the specimen of the dentin chips from the full length of the root canal were harvested using a sterile rotary nickel-titanium F3 size pro- taper (DENTSPLY). The dentine debris were removed from the files by placing into a sterile Eppindorf tube containing 1.5 ml of brain heart infused yeast broth in a vortex mixture for 30 seconds. The files will be removed and inspected for dentinal debris remaining in the flutes. The sample will be incubated for 24 hours at 37ºC (98.6ºF). Following incubation, each sample was mixed in a vortex mixer for 15 seconds and 1 ml of solution was pipetted into a cuvette. OD_{600} of each sample was measured to estimate the concentration of *Enterococcus faecalis.*

Under the limitations of the present study, Triple antibiotic medicament in combination of Metronidazole, ciprofloxacin and Clindamycin has higher efficacy in eradicating Enterococcus faecalis as an intracanal medicament. Distilled water had the least efficacy as compared to the other groups throughout the length of the specimen.

Hence from the results obtained from this present study, it can be concluded that using triple antibiotic medicament is effect than Nisin as an intracanal medicament but clinical scenarios like drug resistant, contra-indication for the patient etc., should be taken for consideration. So in vivo studies are need to be carried out to support the results of the present study.

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