EVALUATION OF ANTIMICROBIAL EFFICACY OF AYURVEDIC AND HOMEOPATHIC DRUGS IN COMPARISON WITH 5.25% SODIUM HYPOCHLORITE AGAINST ENTEROCOCCUS FAECALIS AS ROOT CANAL IRRIGANT : AN IN VITRO STUDY.

DISSERTATION

Submitted to

BABU BANARASI DAS UNIVERSITY, LUCKNOW, UTTAR PRADESH

In the partial fulfilment of the requirement for the degree

of

MASTER OF DENTAL SURGERY

In the subject of

CONSERVATIVE DENTISTRY & ENDODONTICS

Submitted by

DR. DIVYA

Under the guidance of

DR. B. RAJKUMAR

DEPARTMENT OF CONSERVATIVE DENTISTRY & ENDODONTICS

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES, LUCKNOW

Batch: 2019-22 **Enrolment No.: 11903222218**

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DEPARTMENT OF CONSERVATIVE DENTISTRY & ENDODONTICS

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"EVALUATION OF ANTIMICROBIAL EFFICACY OF AYURVEDIC AND HOMEOPATHIC DRUGS IN COMPARISON WITH 5.25% SODIUM HYPOCHLORITE AGAINST ENTEROCOCCUS FAECALIS AS ROOT CANAL IRRIGANT : AN IN VITRO STUDY"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. B. Rajkumar**, Professor and Head, Department of Conservative Dentistry & Endodontics, Babu Banarasi Das College of Dental Sciences, Babu Banarasi Das University, Lucknow, Uttar Pradesh.

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This is to certify that the dissertation entitled **"Evaluation of antimicrobial efficacy of ayurvedic and homeopathic drugs in comparison with 5.25% sodium hypochlorite against enterococcus faecalis as root canal irrigant : an in vitro study."** is a bonafide work done by **Dr. Divya**, under our direct supervision & guidance in partial fulfillment of the requirement for the degree of **Master of Dental Surgery (M.D.S.)** in the speciality of Conservative Dentistry and Endodontics.

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This is to certify that this dissertation entitled **"Evaluation of antimicrobial efficacy of Ayurvedic and Homeopathic drugs in comparison with 5.25% sodium hypochloride against Enterococcus Faecalis as root canal irrigant : An in-vitro study"** is a bonafide work done by **Dr. Divya**, under the direct supervision & guidance of **Dr. B. Rajkumar**, Professor and Head, Department of Conservative Dentistry & Endodontics, Babu Banarasi Das College of Dental Sciences, Babu Banarasi Das University, Lucknow, Uttar Pradesh.

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I hereby declare that **Babu Banarasi Das University** shall have the right to preserve, use and disseminate this dissertation in print or electronic format for academic/research purpose.

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Best Regards

Dr Divya

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LIST OF ABBREVIATIONS

Abbreviations	Full Form
E. faecalis	Enterococcus faecalis
NaOCl	Sodium Hypochlorite
CHX	Chlorhexidine
EDTA	Ethylene Diamine Tetra Acetic Acid
$Ca(OH)_2$	Calcium Hydroxide
%	Percentage
&	And
mL	Millilitre
CFU	Colony Forming Units
GIC	Glass Ionomer Cement
pH	Potential of hydrogen
WHO	World Health Organization
NiTi	Nickel titanium
PCR	Polymerase chain reaction
DH	Dentinal hypersensitivity
CSPS	Calcium sodium phosphosilicate
PBS	Phosphate buffered saline
SEM	Scanning electron microscopy
NSPT	Non-surgical periodontal therapy
CG	Control group
TG	Test group
CM	Controlled memory
NaCl	Normal Saline
mg	Milligram
C	Celsius
HOCL	Hypochlorous acid
mm	millimetre
H2O2	Hydrogen Peroxide

ABSTRACT

The main aim of endodontic treatment is to clean the root canal system and make it free from all vital and necrotic tissues, microorganisms, and their by-products. But because of diverse and complex anatomy of the root canal system, it becomes difficult to clean, debride and shape the canal effectively only with the help of biomechanical preparation. Hence, irrigation plays the foremost role in successful root canal therapy. Sodium hypochlorite has been considered as gold standard irrigant but it has some limitations. Thus, the aim of this study is to evaluate and compare the antimicrobial efficacy of different ayurvedic and homeopathic agents as a root canal irrigant against E. faecalis.

Ninety permanent extracted human premolar teeth were taken and decoronated in order to standardize the canal length. After biomechanical preparation, teeth were inoculated with E.faecalis & randomly divided into five groups and the final irrigation was carried out with tested irrigants. Group I (*n* = 15): Hiora mouthwash; Group II (*n* = 15): Hepar Sulpharis; Group III (*n* = 15): Hekla Lava; Group IV (*n* = 15): Mercurius Solubilis; Group V(n=5): 5**.**25% Sodium hypochlorite; and Group VI(*n*=15): Normal Saline (control group).

The obtained constituents were cultured on agar plates and the number of CFUs (Colony forming units) per plate were determined using a digital colony counter, and statistically analysed using student t-test.

The results were statistically significant among the tested groups with least number of CFUs count in Sodium hypochlorite followed by hepar sulphur, hekla lava, hiora, mercurius solubilis when compared with control group i.e., normal saline.

Hence, this present in-vitro study indicates that both ayurvedic and homeopathic drugs exhibit antimicrobial efficacy against E. Faecalis. Thus, more in-vivo studies are suggested to co-relate clinically and evaluate which drugs are more appropriate as root canal irrigants, not just against E. faecalis, but also other persistent endodontic pathogens.

Keywords: Enterococcus faecalis, Root Canal Irrigation, Root canal irrigant, Ayurvedic drugs, Homeopathic drugs.

INTRODUCTION

Root canal infections are polymicrobial in nature which means that they are caused by combination of different microbes that have penetrated the dental pulp and colonized the root canal system[1]. This is typically dominated by obligatory anaerobic bacteria. *E. faecalis* has been one of the microbes which is repeatedly identified as the species most commonly recovered from infected root canals and the ones which are undergoing retreatment, in cases of failed endodontic therapy and canals with persistent infections.[2]

E. faecalis are gram positive cocci and facultative anaerobes. They are normal intestinal organisms which may inhabit the oral cavity and gingival sulcus. When this bacterium is present in small numbers, it is easily eliminated; but if it is in large numbers, it is difficult to eradicate it completely [2,3]. *E. faecalis* has many distinct features which results in making it an exceptional survivor in the root canal. It can survive in extreme environmental conditions such as low pH, high salinity and high temperatures. It is also capable of persisting in poor nutrient environment. It generally invades and metabolize fluids within the dentinal tubules and binds to collagen fibres.[4] In order to eliminate E. faecalis from root canal space, numerous measures have been described, including the use of various instrumentation techniques, irrigation regimens, and intracanal medicaments.[5]

Biomechanical preparation is one of the ways that aids in reducing the bacterial load but there are areas which remain untouched by the files, such as fins, isthmuses and large lateral canals. Hence, irrigation is a key part of successful root canal treatment as it fulfils several important mechanical, chemical and microbiological functions.[6] It is also the only way to impact these inaccessible areas of the root canal wall that are not touched by mechanical instrumentation. During and following instrumentation, irrigating solutions facilitate the killing and removal of microorganisms, necrotic and inflamed tissue and dentin debris. Irrigants also helps in reducing friction between the instrument and dentin, improves the cutting effectiveness of the files, dissolves tissue, and cools the file and tooth especially during the use of ultrasonic energy. Thus, irrigation dynamics plays a crucial role in the success of root canal treatment[7].

Ideally, the root canal irrigants should have a broad antimicrobial spectrum and high efficacy against anaerobic and facultative microorganisms organized in biofilms. They should be capable of dissolving necrotic pulp tissue remnants and endotoxin inactivation. Alongwith this, it should also prevent the formation of a smear layer during instrumentation or dissolve the latter once it has formed. Moreover, an irrigant must be systemically nontoxic and non-caustic to periodontal tissues. In addition to these properties, they must have little potential to cause an anaphylactic reaction [(8)].

The most widely used endodontic irrigant is Sodium hypochlorite (NaOCl).[(9)] Sodium hypochlorite was first recommended as an antiseptic solution by Henry Dakin in 1915 during the 1st world war. Dakin's solution, i.e., 0.5% of NaOCl buffered with $NaHCO_3$ was used for dressing of wounds. In 1920, Crane was the first who described its use for root canal debridement and sterilization. Since then, it has gained popularity as an effective intracanal irrigant. It is used in concentrations ranging between 0.5-6% [(10)]. At these concentrations, it is highly hypertonic in nature and strongly alkaline with pH 11 to 13. At present, it is the only used solution that can dissolve organic matter in the canal. It has strong proteolytic and oxidative properties. It can dissolve both necrotic and vital pulp tissue and can kill broad range of pathogens like Gram +ve, Gram -ve bacteria, fungi and viruses[(12)] Generally, it is instilled into the canals during and after mechanical preparation with a disposable plastic syringe with a fine needle attached.[(13)] But there are several drawbacks associated with the extrusion of sodium hypochlorite. It can cause soft tissue inflammation if passed outside the confines of root canals. When sodium hypochlorite comes into contact with vital tissue, it results in acute inflammation followed by necrosis results.[(14)] The severity of the complication depends on the concentration of solution, its pH and its duration of exposure. Sodium hypochlorite has a pH of 11-12.5 which causes injury by oxidation of proteins. Higher concentrations have some irritating effects on the periodontal ligament. Even if minute quantities extrude, they cause vaccular probabilities in blood vessels due to the damage to the vessels as well as release of chemical mediators such as histamine for the involved tissue. This causes immediate swelling and often profuse bleeding through the root canal. The inadvertent penetration of the irrigant can occur in the event of wide apical foramina or

the destruction of apical constriction during canal preparation or due to external resorption which can lead to swelling and hemorrhage. [15]

CHX has also been used effectively to irrigate the canals due to its broad-spectrum antimicrobial activity, biocompatibility and ability to disinfect the dentinal tubules against Enterococcus faecalis. [16,17] However, its use as an irrigant is generally restricted because it can discolor the teeth and tongue, can cause loss of taste, burning sensation of the oral mucosa and subjective dryness of oral cavity. Due to these side effects, there has been a shift in the paradigm and new alternatives are coming into existence. [18] In the search for novel irrigants and intracanal medicaments with good biocompatibility and antimicrobial activity, researchers have been exploring a number of potential agents of natural origin.

Nowadays there has been an increasing trend to seek herbal alternatives for endodontic treatment. This way of use of herbals for curing various diseases is known as "Phytotherapy or Phytomedicine or Ethnopharmacology".[19][20]Herbs contains different components such as essential oils, flavonoids, tanins and alcohols through which it attains varying degree of medicinal values which have potential use in endodontics. They contain high antimicrobial activity, biocompatibility, anti-inflammatory and anti-oxidant properties. The major advantages of these agents are safety, easy availability, increased shelf life, cost effectiveness and lack of microbial resistance so far. [21,22]

The World Health Organization (WHO) is also now encouraging and supporting local governments to increase research efforts, training facilities and exchange of information relating to traditional medicines. [23] Homeopathy is one of the Complementary Alternative Medicine. which has been widely used since 200 years. Samuel Hahnemann was the pioneer of homeopathy which came into existence in Europe (1755- 1843). The term Homeopathy is originated from the Greek words homeo, meaning similar, and pathos, meaning suffering or disease. The main principle of homeopathy is "like cures like" or the "principle of similar".[24][25]

Homeopathic medications are available in four forms of alcoholic solution (medication is dissolved in a combination of water and ethyl alcohol in different ratios; the percentage of alcohol is often <40%), tablets, globules (most common form, made of lactose ranging in size from 1mm to 10mm, they are wetted with homeopathic medication), and ointment or crème (effective for treatment of topical and cutaneous conditions). In manufacture of homeopathic medications, a process called potentization is performed. If the primary ingredient is diluted once in 1:100, it is referred to as C1 (centesimal). [26,27,28]

In the present study, an herbal mouthwash, Hiora (Himalaya, Herbal Healthcare) is used which properties. Other 3 homeopathic drugs which are tested in this study are heparsulphuris, mercursolubilis and hekla lava at concentration 30c. All these drugs have anti-inflammatory, anti-oxidant and anti-microbial properties.

Thus, the objective of this study is to evaluate and compare the antimicrobial properties of ayurvedic (hiora) and homeopathic drugs (Heparsulphur, Merc solubilis and hekla lava) as new irrigating agents against endodontic pathogen and to find out which irrigant is more appropriate for use as a root canal irrigating solution against Enterococcus faecalis.

AIM AND OBJECTIVES

AIM

The aim of this study was to compare and evaluate antimicrobial efficacy of ayurvedic and homeopathic drugs in comparison with 5.25% sodium hypochlorite against Enterococcus faecalis as root canal irrigant: An in vitro study

OBJECTIVES

1. To evaluate the antimicrobial efficacy of ayurvedic drugs.
2. To evaluate the antimicrobial efficacy of homeopathic drugs.
3. Intercomparison of each experimental Root canal irrigants used in the study.
4. To conclude which is the best material to provide highest antibacterial effect against E.faecalis in Root canal irrigation.

REVIEW OF LITERATURE

1. **S V Barbosa , K E Safavi, S W Spångberg(1994)[9]-**has investigated the effect of 5% sodium hypochlorite and 35% hydrogen peroxide on dentine. They concluded that the use of high concentrations of sodium hypochlorite during root canal treatment may negatively affect the integrity of the root canal wall thereby allowing increased access of caustic bleaching fluids to cervical vital tissues.
2. **Sundqvist G., Figdor D., Persson S. et al. (1998)[4] -** determined which microbial flora were present in teeth after failed root canal therapy and established the outcome of conservative re-treatment. Fifty-four root-filled teeth with persisting periapical lesions were selected for re-treatment. After removal of the root filling, canals were sampled by means of advanced microbiologic techniques. The teeth were then re-treated and followed for up to 5 years. The microbial flora was mainly single species of predominantly gram-positive organisms. The isolates most commonly recovered were bacteria of the species Enterococcus faecalis. The overall success rate of re-treatment was 74%. The microbial flora in canals after failed endodontic therapy differed markedly from the flora in untreated teeth. Infection at the time of root filling and size of the periapical lesion were factors that had a negative influence on the prognosis.Three of four endodontic failures were successfully managed by re-treatment.
3. **Dahlén G, Samuelsson W, Molander A, and Reit C(2000)[30]-** Enterococci are occurring in opportunistic infections involving the oral cavity. This study has identified enterococcal species in 29 endodontic infections undergoing treatment with Ca $(OH)_2$ dressings. The *in vitro* antimicrobial susceptibility of 29 isolated enterococcal strains was determined. *Enterococcus faecalis* was speciated for 26 isolates and *Enterococcus faecium* for three isolates. *In vitro* antimicrobial susceptibility testing revealed enterococcal isolates resistant to benzylpenicillin, ampicillin, clindamycin, metronidazole and tetracycline but sensitive to erythromycin and vancomycin. Due to low sensitivity to antimicrobial agents, enterococci may be selected in root canals undergoing standard endodontic treatment and significantly contribute to endodontic treatment failures.

4. **Love RM (2001)[31]** -identified a possible mechanism that would explain how E. faecalis could survive and grow within dentinal tubules and reinfect an obturated root canal. Cells of Streptococcus gordonii, Streptococcus mutans, or E. faecalis were grown in brain heart infusion broth containing various amounts of human serum for 56 days. The ability of the three species to invade dentine and bind to immobilized type 1 collagen in the presence of human serum was assessed by dentine invasion and microtitre well experiments. All three species remained viable over the period of the experiment when grown in human serum. Cells of all three bacteria were able to invade dentine and bind to immobilized collagen. Both of these properties were inhibited by the presence of collagen in the cell solution. Human serum inhibited dentine invasion and collagen adhesion by S. gordonii and S. mutans, whilst dentine invasion by E. faecalis was reduced in the presence of serum, but not inhibited, and binding to collagen was enhanced. It was postulated that a virulence factor of E. faecalis in failed endodontically treated teeth is related to the ability of E. faecalis cells to maintain the capability to invade dentinal tubules and adhere to collagen in the presence of human serum.
5. **Pinheiro E, Gomes B. y col.(2003)[32]-** has performed a study in which they found that isolated bacteria and their prevalence in endodontic failure were Enterococcus spp. (36.7%), Enterococcus faecalis (45.8%), Streptococcusspp. (30%), Peptostreptococcus spp. (23.3%), Actinomices (13.3%), Prevotella spp. (10%), Stafilococcus (10%), Gemella (10%), Fusobacterium (6.7%), Veionella (6.7%), Lactobacillus (6.7%), Propionibacterium (3.3%), Haemofilus (3.3%).
6. **Peters et al(2003)[33]- investigate** physical parameters for ProTaper nickel-titanium (NiTi) rotary instruments whilst preparing curved canals in maxillary molars in vitro. Whilst high forces were used in some cases, no ProTaper instrument fractured when a patent glide path was present. There were significant positive correlations between canal geometry and physical parameters during shaping.
7. **Siqueira J, Rocas I (2004)[34]-** has conducted an in vivo study by isolating bacteria, with PCR, and their prevalence in endodontic failure are: E. faecalis (77%), Pseudoramibacter alactolyticus (52%), Propionibacterium propionicum (52%), Dialister pneumosintes (48%), Filifactor alocis (48%), Candida albicans (9%)
8. **Vianna ME et al (2004)[35]-** conducted a study to investigate in vitro the antimicrobialactivity of 0.2%, 1%, and 2% chlorhexidine gluconate (CHX gel and

CHX liquid), against endodontic pathogens and compare the results with the ones achieved by 0.5%, 1%, 2.5%, 4%, and 5.25% sodium hypochlorite (NaOCl) using broth dilution test. They found that all the testing irrigants eliminated Porphyromonas endodontalis, Porphyromonas gingivalis, and Prevotella intermedia in 15 seconds.

9. **Christine Sedgley, Gwendolyn Buck, Oliver Appelbe,(2006)[36]-** investigated the prevalence of E. faecalis in multi-site oral samples (n = 136) from 41 endodontic patients using culture and polymerase chain reaction (PCR). Additionally, culturable strains were investigated for virulence traits. Overall, E. faecalis was detected in at least one tongue, oral rinse, or gingival sulcus sample in 68% of patients and in the root canals only in 5% of patients. In 21 patients from whom samples were obtained from all four sites, E. faecalis was detected in more tongue than gingival sulcus, oral rinse, and root canal samples (43, 14, 10, and 10%, respectively; $p = 0.0148$, $\chi 2$), and in proportionally greater numbers of patients with gingivitis/periodontitis compared to healthy periodontium (73% versus 20%; $p = 0.03$, Fisher's exact test). PCR was more sensitive than culture in detecting E. faecalis in oral samples (32% and 4%, respectively; $p < 0.0001$, McNemar's test). Multiple virulence traits were identified in culturable strains.
10. **Sharifian MR et al(2009)[37]-** found in their study thatNaOCl group demonstrated the most efficient antibacterial action ($P<0.05$) at all experimental times. Also, the substantivity of NaOCl group was significantly greater than Carvacrol at all time intervals; beingpresent in root dentin for at least 28 days
11. **Prabhakar J, Senthilkumar M, Priya M, Mahalakshmi K, Sehgal P, and Sukumaran V(2010)[38] –** stated that 5% sodium hypochlorite showed maximum antibacterial activity against *E. Faecalis* biofilm formed on tooth substrate. Triphala, green tea polyphenols and MTAD showed statistically significant antibacterial activity. The use of herbal alternatives as a root canal irrigant might prove to be advantageous considering the several undesirable characteristics of NaOCl
12. **Shahani M N, Subba Reddy V V (2011)[39]-** Disinfection of the root canal system is one of the primary aims of root canal treatment. This can be achieved through the use of various antimicrobial agents in the form of irrigants and medicaments. The antimicrobial substantivity of 2% chlorhexidine gluconate, 1% povidone iodine, 2.5% hydrogen peroxide followed by 2% sodium hypochlorite, and 2% sodium hypochlorite alone as irrigants was assessed in instrumented root canals. 2% chlorhexidine showed

antimicrobial substantivity lasting up to 72 h, followed by 1% povidone iodine, and 2% sodium hypochlorite. Thus 2% chlorhexidine should be used as a final rinse irrigant in endodontic treatment protocols.

13. **Santhini Gopalakrishnan, Rajesh S., Jotish Ravi(2014)[40]-** evaluated the antimicrobial efficacy of Cinnamon and Garlic as endodontic irrigants against Enterococcus faecalis with comparison of 5.25% NaOCl and 2% CHX. They stated that 5.25% NaOCl and 2% CHX showed complete inhibition in all the samples. Complete inhibition was observed in 2 samples of Garlic and 1 sample of Cinnamon. Saline showed innumerable colonies in the same dilution. They concluded that 5.25% NaOCl and 2% CHX showed complete inhibition and remain as the standard irrigants.
14. **Rasouli Ghahroudi Amir Alireza et al(2014)[41]-** assessed the antimicrobial activities of methanolic extract of Salvadora persica (miswak) on isolated strains from the oral fluid. They included 50 female university students (21.4 ± 1 year) participated in the study. Based on examination by a periodontist, they were grouped into (Group I, n = 21) and (Group II, n = 29) i.e., with and without periodontitis respectively. Their un-stimulated saliva samples were obtained in sterile tubes. While three bacterial genera, Staphylococcus, Streptococcus and Lactobacillus were identified in all subjects, Enterococcus and Escherichia were only detected in Group I. They concluded that effective substances are present in S. persica extracts because of which they exhibit a broad range of antibacterial activity and affect almost all bacterial species regardless of the Gram-staining nature
15. **Bhalla V., Bhoiwala V., Rajkumar B. et al.(2015)[42]–** compared the antimicrobial efficacy amongst 2% Tea Tree Oil, 2% Chlorhexidine Digluconate, 3% Sodium Hypochlorite and the control (Distilled Water) using the Minimum Inhibitory Concentration (MIC) Test. The MIC test was performed using 10-fold dilution in ninety-six U-Well Micro Test plates. The results were tabulated and statistically analyzed using binary statistics. It was seen that Tea Tree Oil was the most effective in inhibiting E. fecalis, followed by sodium hypochlorite, and chlorhexidine digluconate was the least successful. Distilled water showed no effect on the gram-positive organisms.
16. **Bagchi S, Saha S, Jagannath GV, Reddy VK, Sinha P.(2015)[43]-** evaluated the effectiveness of commercially available available herbal mouthwash, as an anti-plaque and antigingivitis agent when compared with chlorhexidine. They concluded that

chlorhexidine group proved to be the best anti-plaque and antigingivitis agent, it was found that HiOra group also showed gradual improvement from baseline to 21 days. Whereas no improvement was seen in the Group C using distilled water over 21 days.

17. **Swathi.A et a(2015)[44]-** they have found that the tooth powder containing hekla lava and calendula have considerable antimicobial effects, which are compared with chlorhexidine and calcium hydroxide, which are already in practice. They have also stated that these have a good anti-microbial effect against mutans and enterococcus
18. **Seelan RG, Kumar A, Jonathan R, Maheswari U, Raja J, Chelliah P(2015)[45]-**did comparative evaluation of effect of different irrigation solutions against Enterococcus faecalis. Twenty single rooted premolars were taken were taken stored in 0.1% thymol solution at 4°C decoronated to obtain 12 mm length, teeth were autoclaved at 121°C, canals were instrumented up to 35k file (International Organization for Standardization). The samples were randomly divided into three groups Group I- 5.25% sodium hypochlorite (NaOCL) and 17% ethylenediaminetetraacetic acid (EDTA), Group - II 5.25% NaOCL and 2% chlorhexidine (CHX), Group III - 5.25% NaOCL and 17% EDTA and 2% CHX. The results showed that Group III which is 5% NaOCl followed by 17% EDTA and followed by 2% CHX showed maximum antimicrobial activity in all the three different time intervals.
19. **Babaji P, Jagtap K, Lau H, Bansal N, Thajuraj S, Sondhi P(2016)[46]** – conducted a study which was aimed to evaluate the antimicrobial effect of herbal root canal irrigants (*Morinda citrifolia*, *Azadirachta indica* extract, *Aloe vera*) with sodium hypochlorite (NaOCl).Tested herbal medicine (*A. indica* extract, *M. citrifolia*, *A. vera*) showed inhibitory zone against *E. faecalis*. Hence, these irrigants can be used as root canal irrigating solutions.
20. **Diksha Sayal et al(2016)[47]-** carried out a study which aimed to aim was to compare the effect of three different mouthwashes cranberry, hiora and chlorhexidine on the management of localized periodontitis. They concluded that herbal products like Cranberry can prove to be effective or better alternatives to Chlorhexidine in improving the oral health with added systemic benefits and minimal side effects.
21. **El-Latif Hesham, Abd and Alrumman, Sulaiman A(2016)[48]-** conducted a study to evaluate the antimicrobial effects of ethanol, methanol, and ethanol/methanol extracts of Miswak against three bacterial pathogens of the oral cavity. Based upon the significant effects of the Miswak extracts, against the oral cavity pathogens in our

study, we recommend that Miswak could be used as a dental hygiene method to prevent tooth caries.

22. **Majji P, Murthy K R(2016)[49]-** conducted a study to compare the efficacy of four commercially available toothpastes in the reduction of dentinal hypersensitivity (DH), based on the hypothesis that calcium sodium phosphosilicate (CSPS) group had a better efficacy of the four. The four desensitizing kinds of toothpaste containing different active agents were effective in relieving dentinal hypersensitivity. However, CSPS group showed a better clinical response at the end of 2 months.
23. **Siqueira J, Antunes H y col. (2016)[50] –** performed an in-vivo study in which they observed that **t**he mo. isolated in teeth with posttreatment apical periodontitis are: Proteobacteria (46%), Firmicutes (18%), Fusobacteria (15%), Actinobacteria (8%), E. faecalis (1.9%).
24. **Akash Agrawal et al(2017)[51] -** evaluated the antibacterial efficacy of ayurvedic and homeopathic drugs against E. fecalis with different concentrations of sodium hypochlorite. They have compared 3% sodium hypochlorite, triphala, myrestica sebifera. They found that among the testing irrigants, triphala was more effective on cultures.
25. **Gupta R, Yadav OP, Khan M, et al(2017)[52]** - Evaluate the relative effectiveness of Chlorhexidine, Hiora, and T. chebula mouth rinses on plaque reduction. They found that among the tested groups Chlorhexidine shown better results compared to its herbal alternative but Hiora and Terminalia chebula mouthwash due to its natural ingredients has no reported side-effects and further studies with varied concentrations, different parameters and larger sample size are to be carried out to assess their efficacy of herbal mouthwash.
26. **Su-Hee Go(2017)[53] -** This study evaluated the use of antibiotics as canal irrigants for removal of susceptible intracanal bacteria, using an Enterococcus faecalis biofilm model. Enterococcus faecalis biofilm was developed in the root canal and test tube. For the experiments, sodium hypochlorite (NaOCl), augmentin and erythromycin were used as intracanal irrigants. The test tubes and canals were prepared and irrigated with phosphate buffered saline (PBS), NaOCl, augmentin and erythromycin. Bacterial samples were collected after irrigation in the test tube models on day 1, and from the tooth model on days 1, 4 and 7. The surface of each sample and attached pattern of the bacteria was also analyzed by examining under scanning electron microscopy (SEM).

The antibacterial study using 10 ml test tubes (n=10) revealed that 5% NaOCl, augmentin and erythromycin inhibited bacterial growth relative to PBS. In the tooth model, NaOCl, augmentin and erythromycin inhibited bacterial growth significantly (all $p < 0.05$) at days 4 and 7, relative to PBS. Compared to day 1, bacterial density of all groups reduced at day 7, and changes in cell morphology were observed in all experimental groups. Our studies revealed evidence of significant differences in the antimicrobial efficacy at days 4 and 7, upon irrigation with augmentin and erythromycin versus PBS, in root canals infected with E. faecalis.

27. **Bansal Disha, Mahajan Mrinalini and Raina Dimple(2018)[54]-** assessed the antimicrobial activity of the herbal solutions and its comparison with 2% chlorhexidine solution against E. faecalis. Hiora and 2% Chlorhexidine were used to assess the antimicrobial activity against E. Faecalis using Agar well diffusion test. They concluded that Hiora doesn't show any antimicrobial activity when compared to 2% chlorhexidine.
28. **Jaiganesh Ramamurthy, Visha MG(2018)[55]-** evaluated the effect of Hiora mouthwash versus Chlorhexidine mouthwash for the treatment of gingivitis. They found that both hiora and chlorhexidine can be effectively used as an adjunct in the plaque control. There was no significant difference found in the efficacy of different mouthwashes.
29. **Divia AR, Nair MG, Varughese JM, Kurien S(2018)[56]-** has done comparative evaluation of antimicrobial efficacy ofMorinda citrifolia (MC), green tea polyphenols and Triphala was compared with 5% NaOCl against Enterococcus faecalis. after irrigation with the testing samples, the CFUs count was analysed and they found that NaOCl was the most effective irrigant the elimination of E. faecalis reinforcing its role as the best irrigant available currently and a gold standard for comparison of the experimental groups. Its antibacterial effect was comparable to Triphala.
30. **Almaguer A., Gonzalez P.(2018)[57]-** conducted an in vitro study s to evaluate the in vitro antibacterial activity of two homeopathic tinctures Arsenicum album (mineral extract) and Lycopodium clavatum (plant extract) on the periodontal bacteria Actinomyces israelii, Streptococcus sanguinis, Prevotella intermedia, Aggregatibacter actinomycetemcomitans and Phorphyromonas gingivalis (P. gingivalis). They have used equal numbers of bacteria were seeded on agar plates containing enriched media with the homeopathic solutions at 1dH and 1cH dilutions. After 7 days of incubation

under anaerobic conditions, colony forming units (CFUs) were counted. The antibacterial effect was calculated based on the total number of CFUs observed on non-tincture containing agar, and on the tincture containing plates. There was no visible growth of any of the strains was observed on the plates containing Arsenicum album at any of the dilutions tested. In contrast, when Lycopodium clavatum at 1cH dilution was tested, only P. gingivalis was susceptible to this compound. The results suggested that the mineral extract tincture had a greater antibacterial activity than the plant extract tincture, also Lycopodium clavatum preparation could be an effective inhibitor of periodontal pathogens bacteria such as P. gingivalis.

31. **María Elena Monterde-Coronel(2018)[58]-** they had evaluated the efficacy of *Mercurius solubilise* which is a homeopathic medication, for the treatment of periodontal disease. No adverse effects have been reported during treatment with *Mercurius solubilis.* A decrease was found in the depth of the periodontal pockets of both treatment groups. MMP-8 expression decreased in both groups after 3-week treatments.
32. **Aishwarya, Dilip Pasalkar et.al. (2019)[59]-** aimed to screen Homoeopathic Medicines such as Antimonium crudum, Arsenic album, Hepar sulphur, Silicea, and Kali bichromicum with potenciesof 6C, 12C,30C, 200C, 1M, 10M for antibacterial activity against Staphylococcus epidermidis. Medicines werescreened by Agar well diffusion method, MIC assay and Bactericidal activity. Where in Arsenic album 10M (1.06 ± 0.05)cm, Hepar Sulphur 30C (1.1 ±0.1)cm, 200C (1.03±0.20)cm, 10M(1±0.26)cm, Silicea 10M (0.93±0.28)cm, 1M (0.9±0.17)cm, Kali bichromicum 1M (1.06±0.25)cm shows the Zone of inhibition in Agar well diffusion assay. Where in MIC assay in each potency of four Homoeopathic Medicines among the five Homoeopathic Medicines such as in Arsenic album 6C, Antimonium crudum 200C, Hepar sulphur 200C, Silicea 12. n Bactericidal study there were more dead cells as compared to live cells.
33. **Yalgi, Viraj & Bhat, Kishor. (2019)[60]-** carried out a study to investigate the antimicrobial efficacy of 4 homeopathic drugs namely Hypericum perforatum, Arnica Montana, Echinacea angustifolia and Calendula officinalis against two strains of bacteria namely Streptococcus mutans, Enterococcusfaecalis. s concluded that all the homoeopathic medicines used in the form of tinctures exhibited good antibacterial activity against both strains of bacteria, except for Calendula officinalis for disc

diffusion against S.mutans but with Hypericum perforatum the highest activity was observed. The use of homoeopathic tinctures is safe and may provide a good alternative for use as antibacterial agents.

34. **Grando et al. (2019)[61] -** in this vitro study they showed that there was no significant difference between PA and NaOCl-EDTA irrigation regimens regarding either antimicrobial action against E. faecalis or removal of the smear layer, except for greater removal in the middle third by the NaOCl-EDTA group.
35. **Sistla Datta Prasad. et al.(2019)[62]** – has determined the antimicrobial efficacy of alcoholic neem and Aloe vera leaf extracts against Enterococcus faecalis and Candida albicans in comparison with 3% sodium hypochlorite (3% NaOCl) and 2% chlorhexidine (2% CHX). They concluded that antimicrobial efficacy of extracts was well demonstrated from the present in vitro observation of the agar-well diffusion method, and hence it may be advantageous if we use these extracts as irrigating solutions in endodontics. However, it requires further preclinical and clinical evaluation.
36. **MLeila Mourão1, Romeu Carillo Jr, Sabrina Martins Linares, Antonio Canabarro1, Ricardo Guimarães Fische(2019)[63]-** conducted a 1-Year Randomized Clinical Trial which aimed to evaluate the effects of homeopathy (H) as an adjunct for non-surgical periodontal therapy (NSPT) in individuals with DMII and CP. The study comprised of two groups: control group (CG) and the test group (TG), and both groups received the NSPT. TG also received homeopathic therapy, including Berberis, Mercurius solubilis/Belladonna/Hepar sulphur and Pyrogenium, while CG received placebo. They found that there was a significantly further reduction of these parameters in TG, as compared to CG**.**
37. **Alghamdi F, Shakir M (2020)[64]-** conducted a systemic review which aimed to compile all the current studies concerning Enterococcus faecalis as a dental root canal pathogen that causes endodontic failure. They observed that in most of the studies Enterococcus faecalis was the primary pathogen associated with endodontic treatment which has characteristic proprieties that make it capable of escaping disinfection means. They added onto this those clinical trials are required to examine E. faecalis and may provide valuable information about novel microbial detection methods to decrease the number of E. faecalis within the root canal system.

38. **Dutta SD, Maria RD.(2020)[65]-** was conducted with an aim to compare the antibacterial efficacy of two commonly used homeopathic medicaments acid benzoicum 30C and silicea 6C with that of calcium hydroxide as intracanal medicament against *Enterococcus faecalis* (ATCC-29212). They found that antimicrobial activity of the acid benzoicum extract was the highest followed by silicea extract and then calcium hydroxide.
39. **Mohan, U. Vamsi. (2020)[66]-** has checked the antimicrobial study of Dental Caries Using Different Delusions of Hecla Lava and Plantago Major. The caries sample was collected by an expert dental physician using with sterilized cotton swab in the site of caries and preserved in a sterilized test tube in normal temperature. Then gram staining technique was performed to identify the gram positive and gram-negative organisms. After identification of organisms, the biochemical characterisation of bacterial isolates was performed by doing citrate test, methyl red test, oxidase test, catalase test. The antimicrobial activity was evaluated by well diffusion method. Homoeopathic medicines show maximum zone of inhibition in different potencies and mother tinctures. Homoeopathic medicines are more effective than allopathic medicines. Based on the antimicrobial activity against oral pathogens, homoeopathic drugs showed good inhibition and acted on most of the oral microbes.
40. **Gupta D, Kamat S, Hugar S, Nanjannawar G, Kulkarni R(2020)[67]-** they conductedcomparative evaluation of the antibacterial efficacy of *Thymus vulgaris, Salvadora persica, Acacia nilotica, Calendula arvensis*, and 5% sodium hypochlorite against *Enterococcus faecalis*.5% NaOCl showed the maximum antibacterial activity, and herbal products demonstrated significant antibacterial activity against *E. faecalis* and can be employed as an alternative to NaOCl.
41. **Taísa Gomes Amaral, Lívia Guimarães Zina, and Janice Simpson de Paula(2021)[68]-** The clinical trials identified were published from 1965 to 2019, using homeopathy in several dental specialties: in Endodontics, Periodontics, Orofacial Pain, Surgery, Pediatric Dentistry, and Stomatology, as well as in cases of dental anxiety. There is still a scarcity of studies about homeopathy and dentistry. The clinical trials selected showed positive effects on oral health; however, when they were critically evaluated, it was possible to recognize qualitative failures, mainly relative to double-blinding.

42. Nahla Ayoub et al(2021)[69]- aimed to assess the effectiveness of *S. persica* petroleum ether extract (SPE) as an intracanal bactericidal for endodontic treatment against *Enterococcus faecalis* using microdilution method. They found that high content of bactericidal BITC in synergism with other antimicrobial components, representing 70.71% of SPE. Thus, SPE is a good candidate as an intracanal medicament, which warrants further investigation.

MATERIALS & METHODS

The present in-vitro study is conducted in the Department of Conservative Dentistry and Endodontics, in collaboration with MRD Life Sciences, Lucknow.

A total of 90 human premolar teeth were collected following the inclusion and exclusion criteria. The collected teeth were cleaned using ultrasonic scaler and then stored in 0.9% normal saline until further used.

On the basis of radiographic and clinical examination, inclusion & exclusion criteria were set to select the teeth which is as follows:

INCLUSION CRITERIA

Completely developed human premolar teeth with single root, having one canal and minimal curvature.

EXCLUSION CRITERIA

1. Teeth with any crack, caries or calcification.
2. Teeth with any developmental anomaly.
3. Teeth with any previous restoration.
4. Endodontically treated teeth.

Following materials and armamentarium were used:

1) For Sample preparation:

S. No.	Material & Armamentarium	Manufacturer
1.	Ultrasonic Scaler with tips	Coltene, Switzerland
2.	Straight hand piece	Marathon, Korea
3.	Micro motor (Slow Speed)	Unicorn Denmart, India
4.	Diamond disc & Mandrel	Horico, Germany
5.	K-files (ISO #6,8,10,15,20)	Dentsply, U.S.A.
6.	Rotary HyFlex CM files (4% 20 & 4% 25)	Coltene, Switzerland
7.	17% Ethylenedieamine tetra acetic acid (EDTA) solution	SDFCL, India
8.	5.25% Sodium hypochlorite (NaOCl)	Chloraxid 5.25%, CERKAMED Medical, Poland
9.	Normal Saline (0.9%w/v NaCl)	Beryl Drugs Ltd., India
10.	Disposable syringe of 5ml	Dispo Van, India
11.	30-gauge needle	Oro, India
12.	Endo block	Dentsply, U.S.A.
13.	Autoclave	Confident, India
14.	Glass ionomer Cement	Prevest DenPro, India
15.	Curing Light	Woodpecker, China
16.	Composite filling instrument	GDC, India
17.	Modelling Wax	Pyrex, India

2) Irrigating solutions to be tested:

- Hiora (Himalaya, herbal healthcare, India)
- Hepar sulphur CH (Schwabe, India)
- Hekla lava LATT (Schwabe, India)
- Mercurius solubilis (Schwabe, India)
- 5.25% Sodium Hypochlorite (Pyrax, India)
- Normal Saline (0.9%w/v NaCl) (KRPL, India)

3) For Microbiology

- Enterococcus Faecalis
- Absorbent Paper Points #30
- Sterile Tweezer
- Appendorf tubes containing 1 mL of sterile saline
- BHI agar plates
- Digital Colony Counter

Hiora (Himalaya, Herbal Healthcare) is an herbal mouthwash which is composed of Pilu (Salvadora persica) 5 mg, Bibhitaka (Terminalia bellerica) 10 mg, Nagavalli (Piper betle) 10 mg. Along with these it also contains essential oils of Gandhapura taila (Gaultheria fragrantissima) 1.2 mg, Oil extracted from Ela (Elettaria cardamomum) 0.2 mg and Yavani satva (Trachyspermum ammi) 0.4 mg. These agents have anti- inflammatory, anti-oxidant and anti-microbial properties.

Hepar sulphuris- It is a homeopathic remedy that was created by Samuel Hahnemann. He combined the inner layer of oyster shells (*Calcium carbonica*) with flowers of sulfur and burned them to create *Hepar sulphuris calcareum*, or *Hepar sulph.* as it is commonly called. It is also known as calcium sulfide or Hahnemann's calcium sulfide. *Hepar* is the Latin word for liver, and as certain compounds of sulfur had the color of liver, the remedy

was so named. It is mainly used to prevent the formation of pus and hastens healing of abscesses.

Mercur solubilis- It is a known, accepted homoeopathic medicine for the treatment of various mouth, throat, eye, and ear infections. It is composed of precipitated black oxide of Mercury, with varying (according to temperature) amounts of Nitric acid and Ammonia. It is prescribed during bad breath, excessive salivation and oral herpes.

Hekla lava- Hekla lava is a tooth powder which is a fine ash from mount hekla, an Iceland volcano. It offers support in issues of dental sensitivity, gum abscess, caries of the bone, tooth decay. It can be used as intracanal medicament in root canal treatment, since it contains large amount of sulphur, silica, lime, magnesia, ferrous oxide and fluoride. It has anti-inflammatory effect which helps in repair the loose sockets, gingivitis, chronic periodontitis etc. Hekla' s ash is not really ash at all, but tephra, “a lightweight material containing silica, like a very small grain of sand containing bubble cavities which have dissolved acidic salts attached to the surface as a precipitation”. This tephra is known to have great healing properties.

In the human body, chlorine compounds are part of the nonspecific immune system. They are generated by neutrophils via the myeloperoxidase-mediated chlorination of a nitrogenous compound or set of compounds. Potassium hypochlorite was first chemically produced in France by Claude Louis Berthollet as an aqueous chlorine solution. Subsequently, sodium hypochlorite was recommended by Labarraque to prevent childbed fever and other infectious diseases. Based on the controlled laboratory studies by Koch and Pasteur, hypochlorite then gained wide acceptance as a disinfectant by the end of the 19th century. In World War I, chemist Henry Drysdale Dakin and the surgeon Alexis Carrel extended the use of a buffered 0.5% sodium hypochlorite solution to the irrigation of infected wounds, based on Dakin’ s meticulous studies on the efficacy of different solutions on infected necrotic tissue. Beside their wide-spectrum, non-specific killing effects on all microbes, hypochlorite preparations are sporicidal, viricidal and show far greater tissue dissolving effects on necrotic than on vital tissues. These features prompted

the use of aqueous sodium hypochlorite in endodontics as the main irrigant as early as 1919 as recommended by Coolidge.

METHODOLOGY

SAMPLE COLLECTION

Ninety human mandibular premolars extracted for orthodontic/periodontal reasons were obtained from Department of Oral and Maxillofacial Surgery, BBDCODS, Lucknow. Teeth collected were examined and cleaned mechanically for any tissue remnants, plaque and calculus on the root with ultrasonic scaler. Digital Radiographs from different angulations i.e., mesially and distally, were taken to confirm the presence of single canal in collected sample teeth in order to fulfil for inclusion and exclusion criteria.

SAMPLE CALCULATION

Formulae used

$N = (Z_{\alpha/2})^2 \, 2s^2/d^2$

Where N denotes sample size, s is the standard deviation obtained from previous study, and d is the accuracy of estimate or how close to the true mean. Zα/2 is normal deviate for two-tailed alternative hypothesis at a level of significance. Power design is assumed as 80%.

Statistical methods:

The quantitative variables will be evaluated using unpaired t-test. The qualitative variables will be compared using Chi-square test. A p-value < 0.05 will be assumed statistically significant. Statistical Package for Social Sciences (SPSS) version 22.0 will be used for analysis.

Calculations:

S– Standard deviation = 0.18

$Z_{@/2} = Z_{0.05/2} = Z_{0.025}$ = 1.96 at type 1 error of 5%

d = 0.15

$N= (1.96)^2 2*0.18^2/0.15^2$

$= 12.5$

Considering the error and drop out, the sample size will be increased to 15 per group.

SAMPLE DISTRIBUTION

GROUPS	SAMPLE SIZE	IRRIGANT
Group A	**15**	**Mercurius Solubilis**
Group B	**15**	**Hiora**
Group C	**15**	**Hekla Lava**
Group D	**15**	**Hepar Sulphur**
Group E	**15**	**5.25% Sodium hypochlorite**
Group F	**15**	**Normal Saline**

SPECIMEN PREPARATION

Standardization of teeth

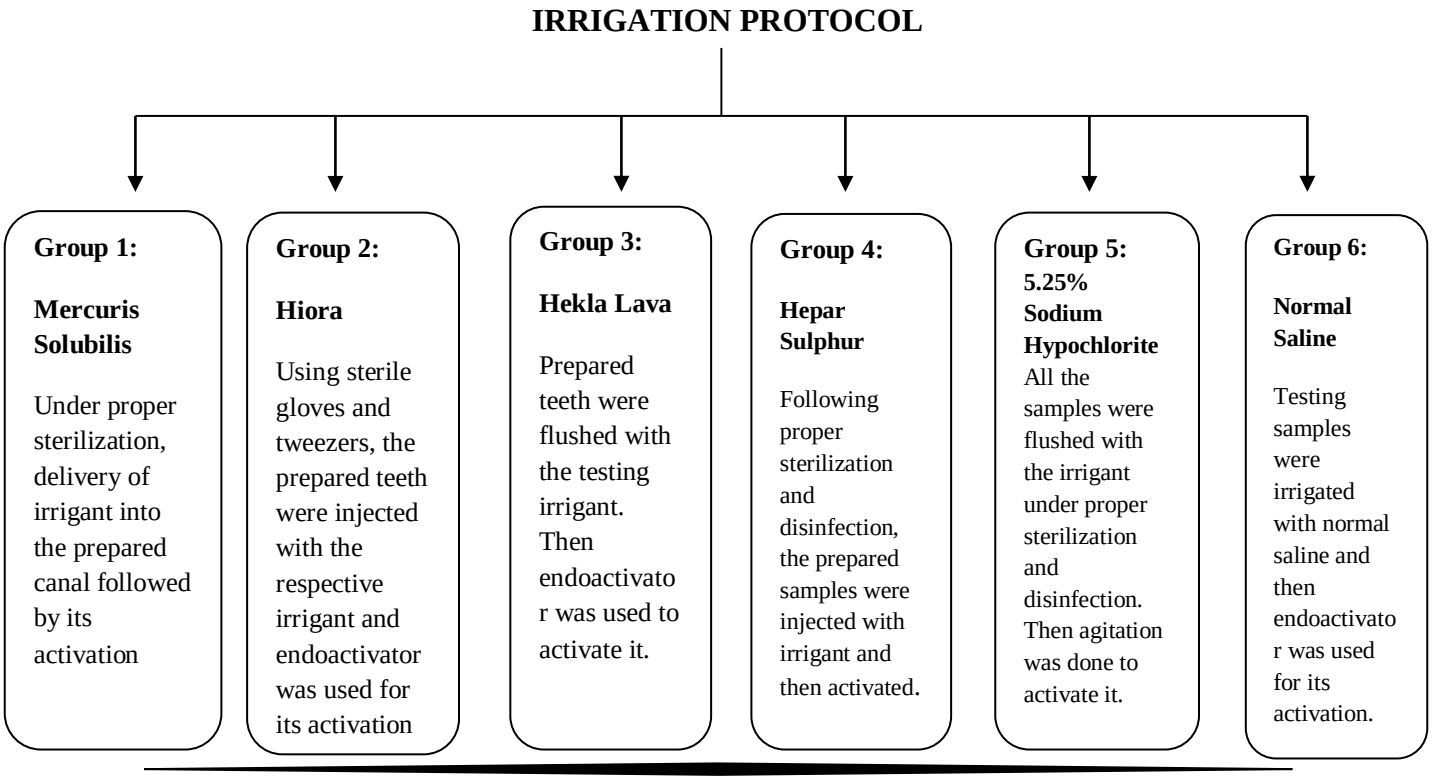
All the selected teeth were standardized at 14mm from the apex towards the cementoenamel junction using a measuring scale. These teeth were then marked and sectioned perpendicular to the long axis of the tooth, with a diamond disc fitted into mandrel held in straight handpiece of low speed micromotor hand piece with copious amount of water spray.

Root canal preparation

Working length was determined by passively inserting a size 10 K file into the root canal & measuring on endoblock and was radiographically confirmed. Root canals were prepared till #30k hand files followed by using HyFlex CM files (4% 20 & 4% 25), with the help of crown-down technique. During shaping, each canal was irrigated with 5.25% sodium hypochlorite with the help of 30-gauge needle in increments of 1ml over 15seconds, followed by irrigation with 17% EDTA solution. To avoid irrigant carry-over, all experimental teeth were then flushed with distilled water.

Inoculation & Irrigation

After root canal preparation, all the samples were autoclaved under steam at temperature 121° C, 15 lbs pressure for 15 minutes. After sterilization, the samples root canal apex was sealed with light-cure Composite filling instrument and mounted on wax. Then the samples were inoculated with Enterococcus faecalis strain ATCC 29212 and thereafter the teeth were divided into 6 experimental groups of 15 samples each. The irrigation protocol which was followed for each group is listed below:



For Group 1- In group 1, the irrigant to be tested was Mercuris Solubilis. In order to avoid contamination, all the teeth were handled with sterile gloves and tweezers. A 5ml syringe with a 30-gauge needle was used to deliver the irrigant into canal. 0.5ml of it was injected for a period of 3 minutes which was then agitated for 10 seconds with endoactivator.

For Group 2- This group contains Hiora which is a homeopathic drug. 0.5ml of it is used as an irrigant with the help of 5ml syringe into the prepared canal under proper sterilization. The irrigant is kept in the canal for 3minutes. All the samples are then agitated with the help of endoactivator for a duration of 10 seconds.

For Group 3- Group 3 comprises of Hekla lava. The protocol for the delivery of this irrigant is also same i.e., under proper sterilization, the samples are injected with the testing solution whose quantity is 0.5ml for 3mins. It is then allowed to activate with endoactivator for a period of 10seconds.

For Group 4- Hepar Sulphur is the testing solution which comprises group 4. This irrigant is filled in the sterile canal of the testing samples for 3minutes whit the help of 5ml syringe and 30-gauge needle. After the delivery of the irrigant, it is activated for 10 seconds so as to show its maximum action using an endoactivator.

For Group 5- This group comprises of 5.25% Sodium hypochlorite. With a sterile 5ml syringe with a 30-gauge needle, 0.5 ml of irrigant was delivered into the prepared canal of each sample of the group for a period of 3 minutes, which was then agitated for 10 seconds with endoactivator.

For Group 6- This is a control group which consists of Normal saline. In this group also all the samples were irrigated with the solution for 3minutes under proper sterilization. Then they were agitated with endoactivator for 10 seconds.

Microbial Sampling

A 4%, 20# paper point was then placed into the canal till the working length for 30 seconds each to soak up the canal contents. Paper points were then transferred to the eppendorf tubes containing 1 mL saline and agitated in vortex for 1 minute. Aliquots of 500µl

dilutions were cultured into Blood agar plates in laminar air flow in order to avoid contamination. All plates were cultured at 37°C in a microaerophilic environment for 24hours. After that, a Digital Colony Counter was used to determine the number of CFUs (Colony Forming Units) per plate. It is designed for rapid and accurate counting of bacterial and mould colonies. It consists of an illuminated pad on which Petridish is placed. A marking pen is provided along-with it which is used to touch the dish to mark each colony in turn. In this way, account to be registered on the digital display and audible tone confirms each count made.

Analysis of the sample

- The number of colony-forming units (CFU) per millilitre of sample was calculated as per the following formula:

  $$\text{COLONY FORMING UNIT /ml} = \frac{\text{Number of colonies obtained X Dilution Factor}}{\text{Volume of sample inoculated}}$$

Colour plates

Figure 1: Teeth to be prepared

Figure 2: Armamentarium for Sample preparation

Figure 3: Decoronation of teeth

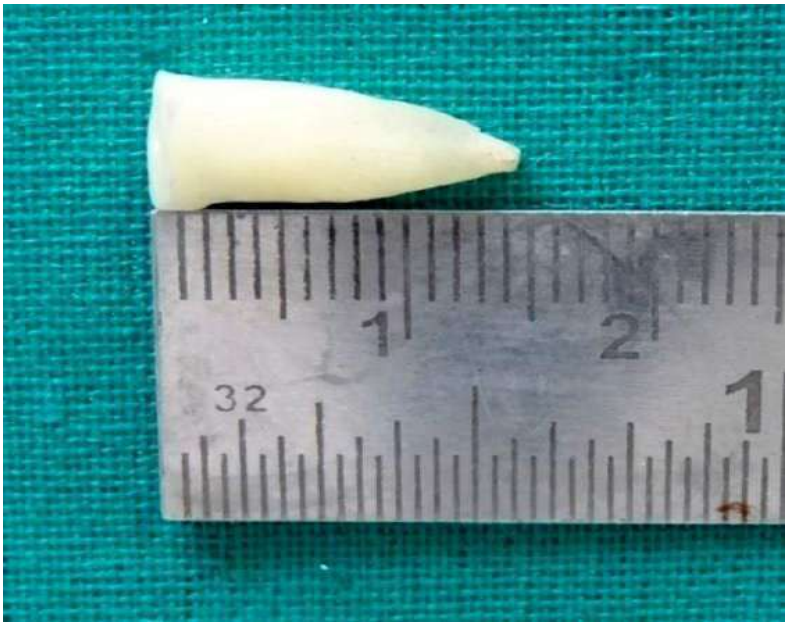


Figure 4: Standardization of length

Figure 5: Armamentarium for Cleaning & Shaping

Figure 6: Cleaning and shaping of canal

Figure 7: Autoclave

Figure 8: Sterilized samples

Figure 9: Armamentarium for apex sealing

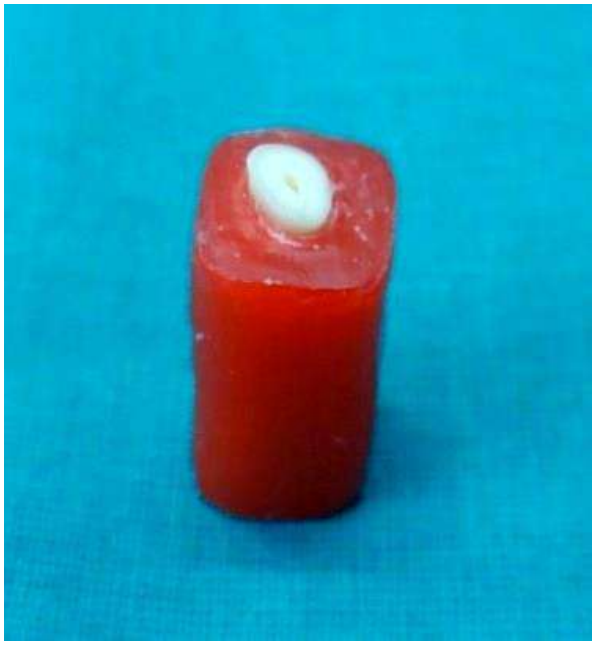


Figure 10: Sample mounted in wax

Figure 11: Enterococcus faecalis (ATCC 29212)

Figure 12: Micropipette used to carry E. faecalis

Figure 13: Armamentarium for Irrigation

Figure 14: Endoactivator for agitation

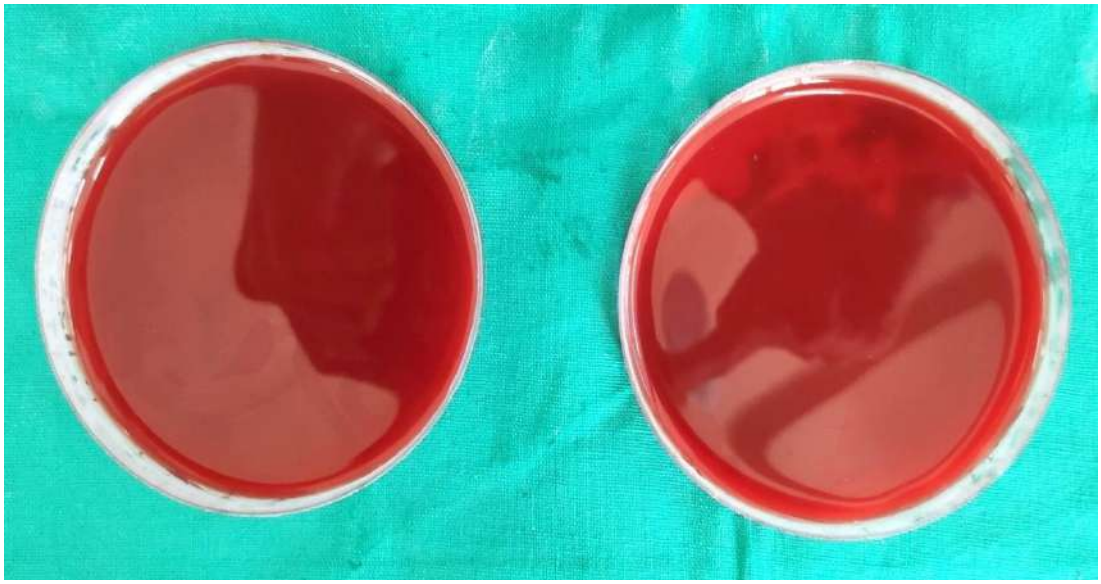


Figure 15: Prepared Blood agar plates

Figure 16: CO2 incubator

Figure 17: Incubating culture plates

Figure 18: Group I showed E- faecalis growth after using mercurius solubilis

Figure 19: Group II showed E- faecalis growth after using hiora

Figure 20: Group III showed E- faecalis growth after using Hekla lava

Figure 21: Group IV showed E- faecalis growth after using Hepar sulphur

Figure 22: Group V showed E- faecalis growth after using sodium hypochlorite

Figure 23: Group VI showed E- faecalis growth after using normal saline.

RESULTS AND OBSERVATIONS

The present was conducted to evaluate and compare the efficacy of six different irrigant containing saline, mercurius, hiora, hekla, hepar and hypochlorite respectively the check the efficacy against E-faecelis. Samples were broadly divided into two groups, Control group & Test group. Control group comprised 15 samples where CFUs were observed by using Saline against E-faecalis. Test group comprised of 75 samples which were sub-divided equally into five groups, Group I (Mercurius solubilis), Group II (Hiora), Group III (Hekla lava), Group IV (Hepar Sulphur) and Group V (Sodium hypochlorite). Intragroup comparison was done to observe the efficacy of all the groups for the amount of CFUs.

Table 1: Descriptive analysis of various groups

Group	**N**	**Minimum**	**Maximum**	**Mean X10^5**	**SD X10^5**
Normal Saline	15	79	92	84.87	4.086
Mercurius solubilis	15	65	86	75.87	7.386
Hiora	15	50	81	63.13	10.134
Hekla lava	15	39	72	51.67	10.581
Hepar Sulphur	15	30	68	42.53	10.582
Sodium Hypochlorite	15	18	50	27.07	9.565

Graph 1: Descriptive analysis of various groups

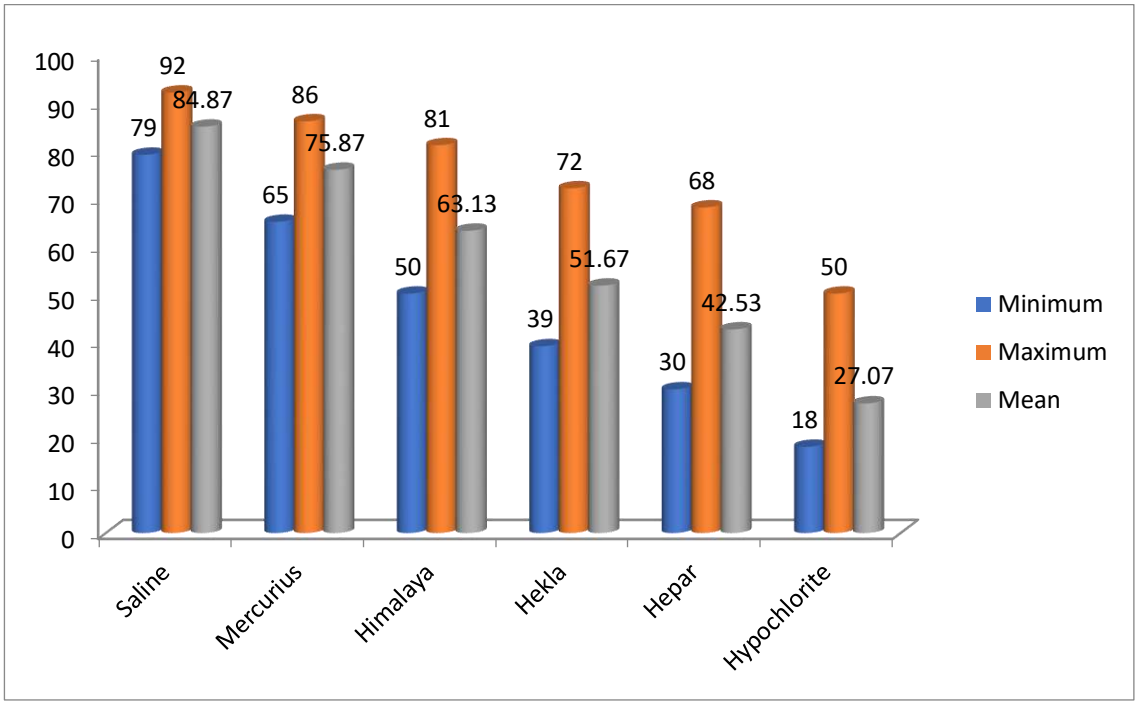


Table 1 and Graph 1: shows the data for different groups that is

Control Group (Normal saline): all the 15 samples were prepared and irrigated with normal saline and means CFUs was counted after 24hr and was found to be 84.87 X 10^5 ± 4.086 X 10^5which was highest among the all.

Group I (Mercurius Solubilis): All the samples were irrigated with Mercurius and after the bacterial inoculation the mean CFUs was counted after 24hr which was found to be 75.87 X 10^5 ± 7.386 X 10^5.This was the second highest finding among the all groups.

Group II (Hiora): the samples which were irrigated with hiora were cultured and mean CFUs was counted after 24hr and was found to be 63.13 X 10^5 ± 10.134 X 10^5.

Group III (Hekla lava): in this group, the mean CFUs count after 24hrs was found to be 51.67 X 10^5 ± 10.581 X 10^5.

Group IV (Hepar Sulphur): 15 samples were irrigated with the Hepar sulphur, inoculated and cultured. After culturing, the mean CFUs was counted after 24hr which was 42.53 X 10^5 ± 10.582 X 10^5.

Group V (Sodium hypochlorite): This group has least number of CFUs count after 24hrs which 27.07 X 10^5 ± 9.565 X 10^5.

Table 2: Comparison of Hypochlorite with other groups

		Group	t test	p value
Sodium Hypochlorite	**vs**	Normal Saline	23.34	<0.01*
		Mercurius Solubilis	23.11	<0.01*
		Hiora	20.15	<0.01*
		Hekla lava	14.32	<0.01*
		Hepar Sulphur	9.51	<0.01*

*: statistically significant, P value (P≤0.05)

Graph 2: Comparison of Hypochlorite with other groups

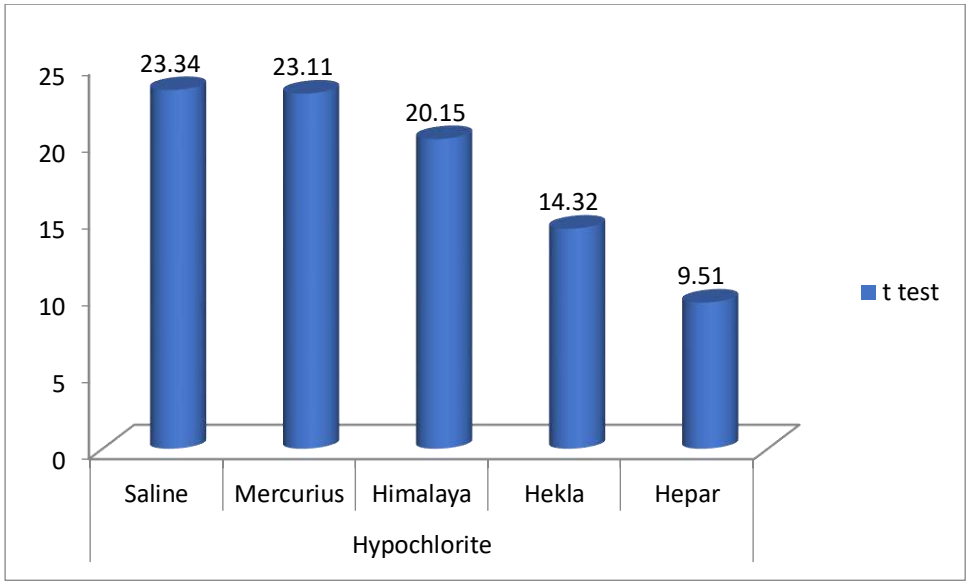


Table 2 and Graph 2 shows the comparison of Sodium hypochlorite (group V) with other groups. The CFU reduction was compared with statistic using student t-test among the Sodium hypochlorite and other groups.

When Sodium hypochlorite (Group V) was compared with Normal saline (control group) using t-test which showed significant difference of $p \leq 0.01$.

When Sodium hypochlorite (Group V) was compared with mercurius solubilis (group I) with t- test, result showed the statistically significant difference of $p \leq 0.01$.

When Sodium hypochlorite (Group V) was compared with Hiora (group II) using t-test, it showed statistically significant difference of $p \leq 0.01$.

When Sodium hypochlorite (Group V) was compared with Hekla lava (group III) with t-test, showed the statistically significant difference of $p \leq 0.01$.

When Sodium hypochlorite (Group V) was compared with Hepar sulphur (group IV) using the student t-test, showed the statistically significant difference of $p \leq 0.01$.

Table 3: Intergroup analysis of other groups

Group	t test	p value
Normal Saline vs Mercurius Solubilis	4.33	0.001*
Normal Saline vs Hiora	8.58	<0.01*
Normal Saline vs Hekla lava	13.27	<0.01*
Normal Saline vs Hepar sulphur	17.13	<0.01*
Mercurius solubilis vs Hiora	9.38	<0.01*
Mercurius solubilis vs Hekla lava	13.71	<0.01*
Mercurius solubilis vs Hepar sulphur	18.53	<0.01*
Hiora vs Hekla lava	7.22	<0.01*
Hiora vs Hepar Sulphur	14.14	<0.01*
Hekla lava vs Hepar Sulphur	8.85	<0.01*

*: statistically significant, P value (P≤0.05)

Graph 3: Intergroup analysis of other groups

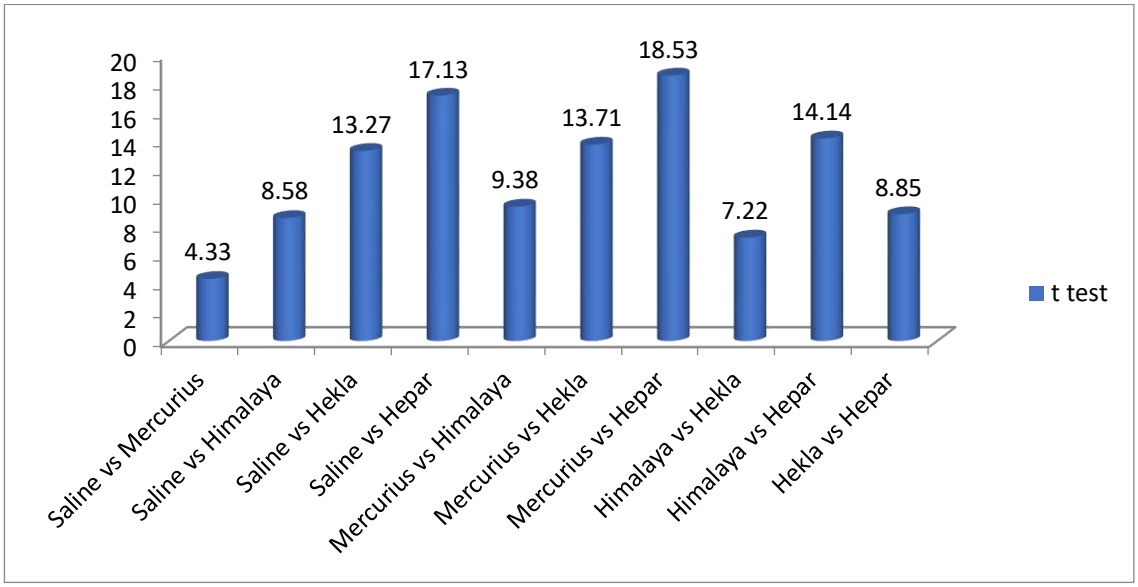


Table 3 and Graph 3 shows the intergroups comparisons of different irrigant for the efficacy of CFUs reduction against E-faecalis using the statistical formula student t-test.

When Normal saline (control Group) was compared with test group mercurius solubilis (group I) it showed a statistically significant difference of $p \leq 0.01$.

When Normal saline was compared with other test group Hiora (group II), result showed a statistically significant difference of $p \leq 0.01$.

When Normal saline was compared with another test group Hekla lava (group III) using t-test, result showed statistically significant difference of $p \leq 0.01$.

When Normal saline was compared with last test group Hepar sulphur (group IV) by using t-test, it showed statistically significant difference of $p \leq 0.01$.

When test group Mercurius solubilis (group I) was compared with another test group Hiora (group II) using the formula t-test result showed t- test value 9.38 which was statistically significant difference of $p \leq 0.01$.

When Mercurius solubilis (group) was compared with hekla lava (group III) using t-test, it showed statistically significant difference of $p \leq 0.01$.

Lastly when Mercurius solubilis was compared to test group Hepar (group IV), using t-test, it showed statistically significant difference of $p \leq 0.01$.

When test group Hiora was compared to other test groups Hekla lava (group III) and Hepar sulphur (group IV) using the student t-test, statistically significant difference of $p \leq 0.01$ was seen.

At last, when test group Hekla lava (group III) was compared with another test group Hepar sulphur (group IV) using the student t-test, result showed the statistically significant difference of $p \leq 0.01$.

DISCUSSION

This experimental study was conducted in the Department of Conservative Dentistry and Endodontics, Babu Banarasi Das College of Dental Sciences, Lucknow in collaboration with MRD Life Sciences, Lucknow.

The foremost aim of an endodontic treatment is to prevent or completely debride the root canal system of bacterial contamination.[29] This may be achieved through biomechanical preparation of the root canal. In many cases, the anatomy of a root canal system is highly complex and variable. This can hinder the ability of clinician to completely disinfect and clean the root canal. Biomechanical preparation of root canals is done using hand and rotary instrumentation techniques.[70] Peters et al. examined root canals before and after instrumentation under micro-CT scan. They observed that regardless of the instrumentation technique, 35% or more of the root canal surfaces (including canal fins, isthmi and cul-de-sacs) remained un-instrumented which may become habitat of micro-organisms.[33] Persistence or survival of the microorganisms in the complex root canal system will ultimately lead to the failure of endodontic treatment. Therefore, irrigation is an essential part of root canal debridement because it allows for cleaning beyond what might be achieved by root canal instrumentation alone.[29]

The endodontic infections are polymicrobic in nature that means the number of bacterial species in an infected root canal may vary from one to more than twelve. Among these, the most common micro-organism is E. Faecalis. Pinheiro ET et al. has stated that E. faecalis was the most frequently isolated bacteria from the root canal systems (45.8%) in previously treated cases.[32] Similarly, Siqueira et al. and Sedgley et al observed the prevalence of E. faecalis was 77% and 79.5%, respectively, using the polymerase chain reaction (PCR).[33][34]

E. faecalis are gram positive cocci and are facultative anaerobes. They are normally present in intestine and may inhabit the oral cavity and gingival sulcus. Jhajharia K et al in 2015 stated that E faecalis possesses many survival mechanisms to live in unfavorable conditions, such as to grow an environment with low oxygen, at high pH, at a wide range

of temperatures between 10° and 60°, at high salinity or in a poorly nutrient environment. It can penetrate dentinal tubules and can also use fluid in periodontal ligament as nourishment. This results in formation of biofilms as protection against host resistance and disinfecting agents.[71] Adding to this mechanisms, E. faecalis has also developed antibiotic resistance, especially against erythromycin and azithromycin. Moreover, it can survive in up to 6.5% concentrated sodium hypochlorite (NaOCl), sodium dodecylsulfate, hydrogen peroxide, heat, hyperosmolarity, ethanol, and both acidity and alkalinity.[64]

As E. faecalis is considered as the most common and resistant organism to the canal disinfecting agents when compared to other organisms in the root canal so it was taken up for this study.

Application of irrigants during endodontic treatment with antimicrobial property can facilitate the removal of microorganisms, tissue remnants and dentin chips from root canals. Irrigants also help in preventing clogging of hard or soft tissue in the apical third of the root and extrusion of infected debris in the periapical area.[72] Various popular irrigants being used are namely Sodium Hypochlorite, Chlorhexidine, EDTA, Hydrogen Peroxide and Normal Saline. In modern era, NaOCl is the most commonly used intracanal irrigation solution at various concentrations.[73] It has a broad antimicrobial activity against endodontic microorganisms and biofilms as it ionizes in water into Na^{+}& hypochlorite ions, establishing equilibrium with hypochlorous acid (HOCl). NaOCl in higher concentrations has a better tissue-dissolving ability, but even in lower concentrations when used in high volumes it can be equally effective. It has the unique capacity to dissolve necrotic tissue. This property is dependent on its concentration, temperature & time of application. It is a strong base (pH>11) and acts as an organic solvent, causing amino acid degradation and hydrolysis. When NaOCl is used as the firstirrigant, the hydroxyapatite coating on collagen seems to protect the collagen fibers and the effect of the NaOCl on dentin is limited.[74]

In a study by Sharifian MR et al, 5.25% NaOCl displayed the most efficient antibacterial action and had significantly greater substantively at different time intervals.[37] Studies evaluating cytotoxicity of NaOCl have shown higher cytotoxicity and caustic effects of

5.25% NaOCl compared to its 3% and 1% concentrations on healthy tissues. In many countries concerns about the chemical and toxic effects of the solution have resulted in the use of 0.5 and 1% concentrations of NaOCl as an intracanal irrigation solution instead of 5.25% concentration.[75]

In the present study, human mandibular premolars were taken into consideration. According to Slowey et al (1979) mandibular premolars are often considered "endodontist's enigma" and may present the greatest difficulty of all teeth to perform successful endodontic treatment and thus most prone to endodontic failure. Mandibular premolars have even shown high flare-up and highest failure rates because of extreme variations in root canal morphology.

As the incidence of increased resistance by pathogenic bacteria to currently used antibiotics and chemotherapeutics agents is more, the researchers are developing interest towards alternative treatment options and products for oral diseases. Hence, the natural phytochemicals isolated from plants used in traditional medicine are considered as good alternatives to synthetic chemicals. Herbs have been scientifically proven to be safe and effective medicine against various oral health problems without any side effect till date due to their high antimicrobial activity, biocompatibility, anti-inflammatory and anti-oxidant properties.[76] In the present study, hiora which is an herbal mouthwash is used as an irrigant. Previous studies have demonstrated its antimicrobial efficacy therefore in present study it was tested. It contains antimicrobial properties so tested in this study against E. Faecalis.[77]

Similar to phytomedicine, Homeopathy is an alternative system of medicine that has been in the use for more than thousands of years. Homeopathic remedies have had an impact on the medical and dental practice because of the antimicrobial activity, biocompatibility, anti-inflammatory properties, easy availability, cost-effectiveness, increased shelf life, and lack of microbial resistance. The probable mechanism of action of homeopathic solution or drug depends on their chemical composition. The particles of the homeopathic medicaments attach to the membrane of the bacterial cell by electrostatic interaction and disrupt the integrity of the cell membrane, this in turn is responsible for their antibacterial

effect.[78]The urge to combat bacterial resistance toward antibiotics and the ineffectiveness of other currently available irrigants to completely disinfect the root canal has inspired us to conduct this study with the help of phytochemicals and homeopathic drugs.

In the present study, antibacterial efficacy of three homoepathic solutions was compared which are namely, Hepar sulpharis, Hekla lava and Mercurius solubilis. Ghosh *et al*. has found that the higher potency of the homeopathic drug shows a bacteriostatic effect. Thats why 30C is chosen as a standard concentration for irrigation in the present study as these drugs has better effects and least toxic at this concentration.[79] Moreover, these combination remedies are in use by many homoeopaths worldwide, and through observation of their beneficial therapeutic effects have been accepted as an essential means of treatment. (Gaier, 1991: 98)

In a study conducted by Tânia Aguiar Passeti found the action of some Homeopathic Medicine on the Growth of Methicillin-Resistant Staphylococcus aureus (MRSA). The MRSA treated with Silicea and Heparsulfur reduced in vitro growth.[80] Similarly, Pankaj Goel et al stated that heparsulphuris in higher doses like 30C-200C can be helpful to abort the pus formed in dental abscesses. They have also stated that Mercuris solubilis can be use for acute ulcerative tissue with a coated tongue and metallic taste in mouth. It is also useful for treatment of pregnancy gingivitis.[81]

Hekla Lava acts upon the jaw and is an important remedy for dental sensitivity, gum abscess, and tooth decay. Hekla Lava is also used to relieve abscess with facial swelling. Hekla Lava lessens swollen glands. Hekla lava is considered a small remedy in the Materia Medica. Swathi.A et al carried out a study using hekla lava and calendula against Enteroccous and mutans. They found the tooth powder has anti-microbial effect which can be used in treating various oral problems.[44] Moreover, R.T. Mathie et al has conducted a study using homeopathic drugs and found strong positive outcomes in the frequently treated conditions of pericoronitis, periodontal abscess, periodontal infection, reversible pulpitis, sensitive cementum, and toothache with decay.[82] Hence, these drugs are used in the present study.

In this study, all the teeth were marked and then sectioned 14 mm from the apex with a carborundum disc using a low-speed straight hand piece, so as to standardize roots of all the teeth approximately to the same length. In a study, Saunders E has concluded that hand files are strongly recommended for initial negotiation of canal and preparation. So the canals were prepared initially with the hand files till No 25 k file followed by rotary files. Literature states that the crown-down technique is superior to the step-back technique in the preparation of curved root canals ranging from 10 to 35 degrees of curvature. The crown-down technique prepares the root canal by starting from the coronal part of the root canal system and then progressing to the apical region.(83)

Hence, in the current study, hyflex CM files are used for the mechanical preparation with the help of crown down technique. HyFlex CM instrument was reported to have the ability to prepare curved canals without causing any significant shaping error or instrument fracture during instrumentation. (84) Thus, it is used in the current study.

A 30-gauge side-vented close-end irrigating needle was used to deliver irrigants to the root canal of the prepared tooth samples. It has been suggested that the side-vented needle is more efficient than the beveled and notched ones in removing bacteria. The external diameters of 30-gauge needle are 0.32 mm which facilitates better penetration of irrigant into the canals.(85)

For the CFU count, in present study digital colony counter is used because can provide a faster and more reproducible means to capture microbial test results. A key practical advantage is with automatically transferring results to a computerized system Central to this the validation, and microbiologists need to be confident that the automated colony counting technology is as accurate (or better) than a manual counting method and that the data is secure and cannot be manipulated.(86)

The bacterial strain ATCC 29212 is the most common strain used in most of the in vitro studies. Therefore, it is used in the present study.

Same study design was used by Sharma DK. et al in order to evaluate the antimicrobial efficacy of Graphene Silver Composite Nanoparticles against E. faecalis as Root Canal Irrigant.[87]

In this study, 5.25% sodium hypochlorite has shown lesser CFUs as compared to other groups. This can be because of its broad antimicrobial activity against endodontic microorganisms and biofilms. Moreover, studies have shown 5.25% NaOCl displayed the most efficient antibacterial action and had significantly greater substantively at different time intervals.[88]

After sodium hypochlorite, hepar sulphur has demonstrated second lowest CFUs count because it contains sulphur which is the third most abundant mineral in the human body. Because of its high sulphur content, it shows good antimicrobial efficacy. Leila Mourão et al has conducted a study using homeopathic drugs and concluded that it acts by reduction of cellular oxidative stress and, especially, in the reduction of TNFa, regulating thereby the stimulation of immune activity.[36]

Hekla lava has also shown decrease in CFUs count. It contains large amount of sulphur, silica, lime, magnesia, ferrous oxide and fluoride. Alongwith this it has anti-microbial activity against various micro-organisms. In a study conducted by Swathi.A et al have that the Hekla lava powder has got the definite antimicrobial activity against Streptococcus mutans and Enterococci.[34]

Similarly, hiora which is herbal mouthwash has antimicrobial properties against E. Faecalis. Its antimicrobial efficacy is due to its key ingredient salvadora persica. S persica comprises of components as chlorides, tannins, trimethylamine, salvadorine, nitrate, thiocynate and sulphur. Their anionic components act as a substrate for lactoperoxide to generate hypothiocyanite (OSCN-) in the presence of hydrogen peroxide (H2O2). OSCN− has been showed to react with sulfhydryl groups in bacterial enzymes, which finally leads to bacterial death. Al- subawi NAK has stated in their study that salvadora persica has a significant effect on both aerobic and anaerobic bacteria isolated from necrotic pulps of teeth. Another key constituent of the tested component is Terminalia bellerica which

includes gallic acid, tannic acid, and glycosides., possesses antioxidant, anti-bacterial, properties.[89]

Mercurius solubilis has also shown reduction in the CFU count. It acts by either inhibiting release of the inflammatory enzymes or by stabilizing lysosomal membrane.[90]

Highest CFUs count were observed in Group 6 which comprises of normal saline. It was the control group as safe substance is used alone, administered in the same way in which it will be used with the experimental compound.

Thus, according to the present experimental study, 5.25% sodium hypochlorite is the most effective antimicrobial agent against E. Faecalis. But, hepar sulphur has also shown impressive reduction in the bacterial load followed by hekla lava,hiora, and merc solubilis.

CONCLUSION

Within the limitations of this study, we conclude that all the testing irrigants were effective against E. faecalis when compared to the control 0.9% Normal Saline, thus can be used as an effective root canal irrigant. 5.25% Sodium hypochlorite has shown maximum antimicrobial activity against E. faecalis when compared with hepar sulphur, hekla lava, hiora and mercuric solubilis.

Hence, this present in-vitro study indicates that both ayurvedic and homeopathic drugs exhibit antimicrobial efficacy against E. Faecalis. Thus, more in-vivo studies are suggested to co-relate clinically and evaluate which drugs are more appropriate as root canal irrigants, not just against E. faecalis, but also other persistent endodontic pathogens.

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ANNEXURES 1

Babu Banarasi Das University
Babu Banarasi Das College of Dental Sciences,
BBD City, Faizabad Road, Lucknow – 226028 (INDIA)

Dr. Lakshmi Bala
Professor and Head Biochemistry and
Member-Secretary, Institutional Ethics Committee

Communication of the Decision of the VIII[th] Institutional Ethics Sub-Committee

IEC Code: 04 **BBDCODS/03/2020**

Title of the Project: Evaluation of Antimicrobial Efficacy of Ayurvedic and Homeopathic Drugs in Comparison with 5.25% Sodium Hypochlorite against Enterococcus Faecalis as Root Canal Irrigant: An In-Vitro Study.

Principal Investigator: Dr. Divya **Department:** Conservative Dentistry & Endodontics

Name and Address of the Institution: BBD College of Dental Sciences Lucknow.

Type of Submission: New. MDS Project Protocol

Dear Dr. Divya,

The Institutional Ethics Sub-Committee meeting comprising following four members was held on 18[th] March, 2020.

1.	Dr. Lakshmi Bala Member Secretary	Prof. and Head, Department of Biochemistry, BBDCODS, Lucknow
2.	Dr. Amrit Tandan Member	Prof. & Head, Department of Prosthodontics and Crown & Bridge, BBDCODS, Lucknow
3.	Dr. Sahana S. Member	Reader, Department of Public Health Dentistry, BBDCODS, Lucknow
4.	Dr. Sumalatha M.N. Member	Reader, Department of Oral Medicine & Radiology, BBDCODS, Lucknow

The committee reviewed and discussed your submitted documents of the current MDS Project Protocol in the meeting.

The comments were communicated to PI thereafter it was revised.

Decisions: The committee approved the above protocol from ethics point of view.

Forwarded by:

Lakshmi Bala 18/03/20

(Dr. Lakshmi Bala)
Member-Secretary
IEC

Member-Secretary
Institutional Ethic Committee
BBD College of Dental Sciences
BBD University
Faizabad Road, Lucknow-226028

(Dr. B. Rajkumar)
Principal
BBDCODS

PRINCIPAL
Babu Banarasi Das College of Dental Sciences
(Babu Banarasi Das University)
BBD City, Faizabad Road, Lucknow-226028

ANNEXURES 2

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES (FACULTY OF BBD UNIVERSITY), LUCKNOW

INSTITUTIONAL RESEARCH COMMITTEE APPROVAL

The project titled **"Evaluation of Antimicrobial Efficacy of Ayurvedic and Homeopathic Drugs in Comparison with 5.25% Sodium Hypochlorite against Enterococcus Faecalis as Root Canal Irrigant: An In-Vitro Study"** submitted by **Dr Divya** Post graduate student from the **Department of Conservative Dentistry and Endodontics** as part of MDS Curriculum for the academic year 2019-2022 with the accompanying proforma was reviewed by the Institutional Research Committee present on **19th December 2019** at BBDCODS.

The Committee has granted approval on the scientific content of the project. The proposal may now be reviewed by the Institutional Ethics Committee for granting ethical approval.

Prof. Vandana A Pant
Co-Chairperson

Prof. B. Rajkumar
Chairperson

ANNEXURES 3

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To Whom It May Concern

This is to certify that ***Dr. Divya****, final year MDS,* ***Babu Banarasi Das College of Dental Sciences,*** *has done her Comparison of Antimicrobial efficacy of different Ayurvedic and Homeopathic drugs in comparison with 5.25% Sodium hypochlorite against Enterococcus faecalis as Root Canal Irrigant (using Digital Colony Counter) in Microbiology Department MRD LifeSciecnes Pvt. Ltd. Lab.*

Dr. Pallavi Sharma
Research Scientist
MRD LifeSciences Pvt.
Ltd.Lucknow

MRD LifeSciences Pvt. Ltd. Lucknow

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ANNEXURES 4

Data Entry

Sr. No.	Saline	Mercurius	Himalaya	Hekla	Hepar	Hypochlorite
1	$83X\ 10^5$	$80X10^5$	$60X10^5$	$55X10^5$	$40X10^5$	$20X10^5$
2	$80X10^5$	$74X10^5$	$56X10^5$	$42X10^5$	$38X10^5$	$18X10^5$
3	$79X10^5$	$85X10^5$	$68X10^5$	$50X10^5$	$42X10^5$	$24X10^5$
4	$92X10^5$	$82X10^5$	$70X10^5$	$68X10^5$	$54X10^5$	$30X10^5$
5	$91X10^5$	$83X10^5$	$74X10^5$	$60X10^5$	$53X10^5$	$28X10^5$
6	$84X10^5$	$68X10^5$	$51X10^5$	$40X10^5$	$32X10^5$	$27X10^5$
7	$88X10^5$	$65X10^5$	$50X10^5$	$44X10^5$	$38X10^5$	$24X10^5$
8	$91X10^5$	$73X10^5$	$68X10^5$	$54X10^5$	$47X10^5$	$31X10^5$
9	$83X10^5$	$86X10^5$	$78X10^5$	$65X10^5$	$51X10^5$	$47X10^5$
10	$84X10^5$	$84X10^5$	$81X10^5$	$72X10^5$	$68X10^5$	$50X10^5$
11	$81X10^5$	$65X10^5$	$50X10^5$	$47X10^5$	$30X10^5$	$21X10^5$
12	$82X10^5$	$68X10^5$	$52X10^5$	$40X10^5$	$32X10^5$	$20X10^5$
13	$85X10^5$	$75X10^5$	$61X10^5$	$51X10^5$	$42X10^5$	$22X10^5$
14	$87X10^5$	$79X10^5$	$63X10^5$	$48X10^5$	$41X10^5$	$25X10^5$
15	$83X10^5$	$71X10^5$	$65X10^5$	$39X10^5$	$30X10^5$	$19X10^5$

ANNEXURE 5

Statistical analysis: Data so collected was tabulated in an excel sheet, under the guidance of statistician. The means and standard deviations of the measurements per group were used for statistical analysis (SPSS 22.00 for windows; SPSS inc, Chicago, USA). Difference between two groups was determined using student t-test and the level of significance was set at $p < 0.05$.

The statistical analysis for the present study was done by applying the following formulae:

1. **Mean**: The mean (or average) is the most popular and well known measure of central tendency. It can be used with both discrete and continuous data, although its use is most often with continuous data. The mean is equal to the sum of all the values in the data set divided by the number of values in the data set. So, if we have n values in a data set and they have values x_1, x_2, ..., x_n, the sample mean, usually denoted by $\bar{x}$ (pronounced x bar), is:

$$\bar{x} = \frac{(x_1 + x_2 + \cdots + x_n)}{n}$$

This formula is usually written in a slightly different manner using the Greek capitol i.e.:

Sample Mean	Population Mean
$\bar{x} = \frac{\Sigma x}{n}$	$\mu = \frac{\Sigma x}{N}$

where Σx is sum of all data values

N is number of data items in population

n is number of data items in sample

2. **Standard deviation**: the standard deviation (SD, also represented by the lower case Greek letter sigma σ or the Latin letter s) is a measure that is used to quantify the amount of variation or dispersion of a set of data values. A low standard deviation indicates that the data points tend to be close to the mean (also called the expected value) of the set, while a high standard deviation indicates that the data points are spread out over a wider range of values.

$$\sigma = \sqrt{\frac{\Sigma\ (x - \bar{x})^2}{n}}$$

σ = **lower case sigma**
Σ = **capital sigma**
$\bar{x}$ = **x bar**

3. **Student T test**: A student *t*-test is any statistical hypothesis test in which the test statistic follows a student *t*-distribution under the null hypothesis. It can be used to determine if two sets of data are significantly different from each other. It is most commonly applied when the test statistic would follow a normal distribution if the value of a scaling term in the test statistic were known. When the scaling term is

unknown and is replaced by an estimate based on the data, the test statistics (under certain conditions) follow a Student's *t* distribution.

ANNEXURE 6

Curiginal

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