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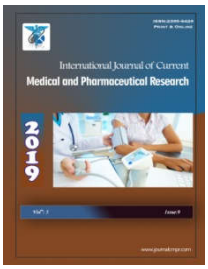

Research Article

A COMPARATIVE STUDY OF ANTIMICROBIAL EFFICACY OF NISIN, GUDUCHI, CHLORHEXIDINE GEL, AND CALCIUM HYDROXIDEAS INTRACANAL MEDICAMENT AGAINST *ENTEROCOCCUS FAECALIS*

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ABSTRACT

Aim: The aim of the study is to compare the antimicrobial efficacy of chlorhexidine gel, calcium hydroxide, Nisin and Guduchi against *Enterococcus faecalis.*
Methods: Sixty single rooted sixty lower premolar teeth were collected which were extracted for orthodontic purposes. Sixty samples were segregated into four groups with 15 samples in each: Group I: Guduchi extract, Group II: nisin, Group III: chlorhexidine gel, and Group IV: calcium hydroxide.Access cavity preparation was done. Cleaning and shaping were done using Protaper universal file till F3. Decoronation of the teeth was done with diamond saw to obtain a standardized root length of 14 mm, and the apical end from outside was sealed using paraffin wax. under standard conditions teeth were autoclaved. After autoclaving, Phosphate-buffered saline solution was introduced into the roots followed by incubation for 24 h to check for the disinfection of roots. After the disinfection of roots, inoculation of E. faecaliswas done and the apex was sealed. Following bacterial colonization inside the roots, antimicrobials were introduced. Antibacterial efficacy was evaluated for these different antimicrobials. Statistical analysis was done
Results: Significant antibacterial effect against E. faecalis was observed with chlorhexidine gel followed nisin, calcium hydroxide Guduchi
Conclusion: Within studies limitation, it can be concluded that chlorhexidine gel followed by nisin had better antimicrobial properties against *E. faecalis* than other medicaments

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INTRODUCTION

Complete elimination of microorganisms and the nutrient supply to the root canals is the major objective of the root canal treatment.[1]Various studies have clearly established the role of microorganisms and their by-products in the pathogenesis of pulpal and peri-radicular diseases [2]

Due to complex root canal configuration, complete debridement through mechanical instrumentation alone cannot remove entire bacterial load.[3]Although a reduction in bacterial counts were seen after chemomechanical instrumentation, still all teeth had a positive culture after the initial appointment. The combined use of sodium hypochlorite and ethylenediaminetetraacetate considerably improved bacterial eradication, but almost 50% of teeth still held measurable bacteria after instrumentation.[4][5] Bacterial counts usually decrease after the appointment, but leaving the canal void in between appointments leads in bacterial counts to initial levels. Hence, intracanal medication is important as it can complement the work of chemomechanical instrumentation. So irrigation and intracanal medicaments play an important role in debriding the infected tissue and to eliminate the microorganisms.[3]

In continuedperiradicular infections, *Enterococcus faecalis* had been isolated in about 24%–77% cases which resulted in failure of root canal treatment. It could be because of the capability of *E. faecalis* to endure at high alkaline environment and deeper tubular invasion. It grows through attaching on biofilm and colonizes on the surface.[6]

A search of an intracanal medicament that can totally eradicate *E. faecalis* still prolongs. Calcium hydroxide is the most universally used intracanal medicament. becauseof its high pH, it destroys and modifies the bacterial polysaccharides in the cell wall. In the presence of *E. faecalis* this high pH is not sustained thus ineffectual in destroying it.[7] Chlorhexidine in gel or liquid form has revealed high efficacy against *E. faecalis*. negatively charged phosphate groups on the bacterial

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cell wall interacts with the positively charged chlorhexidine thus destroying the bacteria.[8][9]

On the other hand, there are many disadvantages related with chemical medicaments. In root canal therapy, due to the cytotoxicity of most of the commercial products used as intracanal medicaments and their incapacity to eradicate bacteria from dentinal tubules, recent trend of holistic approach to use biologic medication extracted from natural plants has swiftly increased. The foremost advantages of using herbal alternatives are easy availability, cost-effectiveness, increased shelf life, low toxicity and decreased microbial resistance. as a result herbal and natural products have become more common in research as they are with low toxicity and lesser side effects when compared to chemical medicaments.[10]

Tinosporacordifolia also known as *Guduchi* had been reported to havetenosporin, coloumbin, and tinosporic acid. It is popular in ayurvedic medicine to treat different ailments such as fever, urinary problems, dermal infection, and inflammation[11]

Nisin is A natural occurring peptide isolated from *Lactococcus lactis* strains .it has been recently introduced. This antibiotic peptide is a Class I bacteriocin. It is efficient against Gram-positive bacteria and spores, including strains of *E. faecalis*. Nisin is commonly used as a food preservative in over fourty countries and is harmless to human beings. Of late studies are suggestive that nisin is effective in elimination of *E. faecalis* from root canals.

MATERIALS AND METHODS

Preparation of teeth

Sixty single-rooted permanent mandibular premolars earlier extracted for orthodontic purpose, non caries and free from developmental defects were incorporated in the study. Using a diamond disc, the crowns were sectioned from the root at cementoenamel junction under saline irrigation. The specimens were then at random divided into four groups (n = 15). The working length estimation was done as measuring file length 1 mm less than apical foramen .standardization was done by cutting the rootsfor the working length15 mm.

The cleaning and shaping of root canals was done using 6% protaper F2 (No. 25) in crown down technique to standardize apical size .Then copious intracanal irrigation was done with 3% sodium hypochlorite using 3mL luer lock syringe and then by irrigating with normal saline .

Then distilled water was used to clean the specimens for 30 min in an ultrasonic bath. After rinsing, sterile water was used to store the teeth. Then the specimen were dried, coated externally with clear nail varnish, and sterilized in an autoclave for 30 min at a temperature of 121°C and at a pressure of 15 psi.

Root canal infection

Each specimen was inoculated with broths of bacterial solution up till the canal entance using a sterile needle in a microbiological safety cabinet. After inoculation, the samples were kept in cotton plugged test tube and incubated at 37°C for 21 days. Every 3rd day, the canals were reinoculated with fresh bacterial samples.

Root canal medication

After 21 days The canal contents were aspirated, later rinsed with saline and then dried with sterile paper points. Later They were randomly divided into 4 groups (n = 15 each) for intracanal medicaments:

- Group I: guduchi extract
- Group II: nisin in distilled water
- Group III: 2 percent chlorhexidine gel
- Group IV: calcium hydroxide

In all the specimen, the respective medicaments of 5 µL were injected in the root canals and fully filled. Then sticky wax (Pyrax, India) were used to seal the canals and incubated at 37°C for 7 days. After 7 days of incubation, the wax was removed from each of the root canal orifice. Themicrorganism samples from each canal were retrieved with sterile paper points and inoculated on brain-heart infusion broth (HiMedia Laboratories, India) and incubated at 37°C for 24 h.

RESULTS

Medicaments.	Enterococcus faecalis cfu	
	Mean±SD	minimum -maximum
Guduchi	97±1.32	91-99
Nisin	2±1.32	00-04
2%CHX gel	7±1.56	04-09
Ca OH	63±1.67	61-67
Kruskal -wallis test	X2=53.354,P=0.000[<0.001],significant difference	
Mann -whitney U test	Guduchi>CaOH>2%chx gel>nisin	

The means Colony Forming Units count of the present study were showed in table 1and graph1 Nisin and chlorhexidine gel showed least CFUs of *E. faecalis*. Calcium hydroxide showed significantly less CFU count when compared with guduchi which is seen in figure1. Statistically significant difference was present in CFU count when different medicaments used against *E. faecalis* by Kruskal–Wallis test. Mann-Whitney U-test showed that CFU was higher in guduchigroup followed by Ca $(OH)_2$, chlorhexidine gel and nisin. Nisin showed significantly least CFU count among all the medicaments

DISCUSSION

Removing microrganisms and its by-products is the main objective of root canal therapy. Along with chemomechanical instrumentation, using intracanal medicaments in between appointments may help this cause. And also, its need increases in persistent and secondary infections.

Although *Enterococcus faecalis* is not the most common bacteria in the root canal, it is the most common (90%) when it comes to secondary infections and the most common organism isolated in re rerct cases.[12][13]That is why *Enterococcus. faecalis* being the focus of interest in endodontics in the recent years. Elimination of *Enterococcus faecalis* is most difficult because of its capability to tolerate high pH levels, high salt concentration, and long periods of starvation. And also, biofilm formation and resistance to antibiotics has made it more challenging.[14][15]

In this study, *Guduchi* showed least potent antibacterial efficacy when compared with other groups,. When CHX, *Nisin*, and calcium hydroxide groups were compared, antibacterial potency of *Guduchi* was found to be statistically least significant but it showed reduction in the count as in accordance with the study of Jeyachandran *et al*.[16]

Calcium hydroxide showed the least antibacterial efficacy next to guduchi. For it to be effective, hydroxyl ions from calcium hydroxide should diffuse into the dentinal tubules at sufficient

concentration, but due to the proton pump mechanism of *E. faecalis* and buffering capacity of dentine, hydroxyl ions were not able to diffuse into the dentinal tubules at sufficient concentration. This result was in accordance with several studies done by Safavi *et al*., Siqueira and de Uzeda, wherein calcium hydroxide failed to show antibacterial efficacy against *Enterococcus. faecalis*. [17] [18]

In the present study, we realized that 2% chlorhexidine gel is more effective in eliminating *E. faecalis* when compared to calcium hydroxide paste and guduchi. This can be elucidated by the fact that chx has a wide antimicrobial spectrum and it is effectual against both Gram-positive and Gram-negative bacteria as well as yeasts.[19] Chlorhexidine mechanism of action by electrostatic interaction as it is positively charged and the bacterial wall is negatively charged, where interaction will happen and increase the cell wall coating, allowing bacterial cytoplasm coagulation, resulting in cell death.[20][21]. And also, chlorhexidine has a property of substantivity, that is releasing slowly at therapeutic levels over long periods of time.[22] The results of this study were in accordance with many authors who found that the medicament containing chlorhexidine were effective against *Enterococcus. Faecalis*.[23][24][25] The results are in accordance with the previous studies, though the concentration of chlorhexidine used in this study is 2%.

This study showed Nisin as the most effective medicament against *Enterococcus. faecalis*. Nisin, an antimicrobial peptide, naturally occurring produced by *Streptococcus lactis*. It is most commonly used as a food preservative in meat and dairy industry. Earlier, studies had reported the antimicrobial activity of Nisin against *Enteroccus. faecalis* both *in vitro* and *in vivo*.[26] Nisin shows its antibacterial activity through pores formation by interacting with a specific molecule "Lipid II" and inhibit cell wall synthesis. The main component of Gram-positive bacterial cell membrane is Lipid II. in a nanomolecular level, Nisin targets this Lipid II as a "docking molecule" to form pores on the cell membrane surface and effectively kills the bacteria.[27][28]Turner *et al*. showed significantly lower infected dentinal shaving of *E. faecalis* with Nisin when compared to calcium hydroxide.[26] According to Somanath *et al*., when Nisin was compared with chlorhexidine is found to be effective after 1 week interval which was confirmed by Hemadri *et al*. [28]

CONCLUSION

Under the limits of the present study, Nisin and2% Chlorhexidine gel are effective in eliminating the *E. faecalis* when used as intracanal medicaments while Guduchi and calcium hydroxide are not so effective in eliminating the *E. faecalis.*

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