Original Article

A comparative evaluation of the antimicrobial efficacy of calcium hydroxide, chlorhexidine gel, and a curcumin-based formulation against *Enterococcus faecalis*

ABSTRACT

Aim: This study aimed for a comparative evaluation of the antimicrobial efficacy of calcium hydroxide, chlorhexidine gel, and a curcumin-based formulation against *Enterococcus faecalis*.

Methods: Thirty single-rooted teeth were taken. Access preparation was done. Biomechanical preparation was done using Protaper universal file till F3. Teeth were decoronated to a standardized root length of 14 mm, and the apical end from outside was sealed using paraffin wax. Teeth were autoclaved under standard conditions and after autoclaving, Phosphate-buffered saline solution was introduced into the roots and was incubated for 24 h to check for the disinfection of roots. Once the disinfection of roots was achieved, the wild strain of *E. faecalis*, i.e., ATC 29212 strain, was introduced into the teeth and allowed to incubate for 24 h. Following bacterial colonization inside the roots, antimicrobials were introduced and efficacy was checked. The data obtained were subjected to statistical analysis using paired *t*-test for significance.

Results: Significant difference was observed between all the test groups. However, chlorhexidine gel showed the best value of mean difference, indicating it as the best antibacterial medicament.

Conclusion: Within the limitations of this study, it can be concluded that chlorhexidine gel showed better antimicrobial properties against *E. faecalis* than other medicaments.

Keywords: Calcium hydroxide, chlorhexidine, curcumin, *E. faecalis*

INTRODUCTION

Complete elimination of microorganisms and sources of nutrient supply from the root canal system is the primary objective of root canal treatment.[1] The presence of these microorganisms within the root canal system induces pathological changes in the dental pulp. These pathological changes produce numerous irritants within the root canal system. Formation of peri-radicular lesion occurs when these irritants progress from the infected canals into the surrounding tissues, the response to which is manifested as an immune inflammatory reaction.[2]

Various studies have clearly established the role of microorganisms and their by-products in the pathogenesis of pulpal and peri-radicular diseases.[1,3,4] Anatomical complexity of the root canal system makes chemomechanical instrumentation insufficient for complete debridement.

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Byström and Sundqvist[5-7] performed a series of studies to evaluate the antibacterial effectiveness of chemomechanical instrumentation. Although a 100 to 1000-fold reduction in bacterial counts was observed after chemomechanical instrumentation (irrigant used was saline), all teeth still had a positive culture after the first appointment.[7] The combined use of sodium hypochlorite and ethylenediaminetetraacetate as irrigants significantly improved bacterial elimination, but approximately 50% of teeth still harbored detectable bacteria after instrumentation.[5,6] Bacterial counts usually decrease after the appointment, but leaving the canal empty between appointments results in bacterial counts to original levels.[5,7] Therefore, intracanal medication is important as it can complement the work of chemomechanical instrumentation. It can reduce the counts of remaining bacteria, thus supporting the healing of periapical tissues.[8]

Enterococcus faecalis is a Gram-positive and facultative anaerobe. Its prevalence ranges from 24% to 77% in persistent and secondary infections.[9] A search of an intracanal medicament which can totally eliminate *E. faecalis* still continues. Calcium hydroxide is the most commonly used intracanal medicament. Due to its high pH, it destroys and alters the bacterial polysaccharides in the cell wall. This high pH is not maintained in the presence of *E. faecalis*, thus ineffective in killing it.[10] Chlorhexidine in gel or liquid form has shown high efficacy against *E. faecalis*.[11] Positively charged chlorhexidine molecule interacts with the negatively charged phosphate groups on the bacterial cell wall, thus killing the bacteria.[12]

However, there are several disadvantages associated with chemical medicaments. Disadvantages are the side effects of medicaments, overuse and misuse leading to toxicity, and cytotoxic reaction. Considering these side effects in mind, herbal and natural products have become more popular as they are associated with low toxicity and fewer side effects when compared to chemical medicaments.[13] Curcumin, a polyphenolic compound derived from dietary spice turmeric, has a wide spectrum of biological and pharmacological activities. It exhibits anti-inflammatory, antioxidant, anticarcinogenic, antiviral, and antimicrobial activities.[14] Therefore, a study was conducted with the aim to comparatively evaluate the antimicrobial efficacy of calcium hydroxide, chlorhexidine gel, and a curcumin-based formulation against *E. faecalis*.

METHODS

Thirty single-rooted teeth extracted [Figure 1] for orthodontic and periodontal reasons with patent root canals were collected from the Department of Oral and Maxillofacial Surgery, Faculty of Dental Sciences, King George's Medical University, Lucknow. The collected samples were stored in normal saline during the study. A diamond disc was used to decoronate the teeth to a standardized root length of 14 mm. Access preparation and biomechanical preparation were done using Protaper universal file till F3. All the specimens were sterilized in an autoclave at 121°C for 15 min.

Figure 1: Mean colonies of *Enterococcus faecalis* before and after antibiotic introduction

Each tooth was placed in a sterile Eppendorf tube. 0.5 Mcfarland solution of *E. faecalis* was made. Each of the sterilized sample was inoculated with 10 µl inoculums of bacteria using a micropipette and was incubated at 37°C for 24 h. The solution was taken from each tooth using a sterile straight wire and cultured on blue agar plates which were then incubated at 37°C for 24 h. After 24 h, the plates were checked for the colonies of *E. faecalis*.

Following bacterial colonization, specimens were divided into the following groups based on the antimicrobial used [Figure 2]:

- Group I: *Curcuma longa* extract (Curenext, Abbott, India)
- Group II: 1% chlorhexidine gel (HEXIGEL, ICPA Health Products Limited, Maharashtra, India)
- Group III: Calcium hydroxide paste (Cal Excel, AM DENT, New Delhi, India).

These teeth with antibiotic solutions were again incubated at 37°C for 24 h. After 24 h, a sample was taken from each tooth using a sterile straight wire and cultured on blue agar plates which were then incubated at 37°C for 24 h.

Colony count was done from the plates before and after antibiotic solution. The values were calculated and tabulated.

Data analysis

Statistical analysis was done using SPSS (Statistical Package for the Social Sciences) Version 20.0 statistical analysis

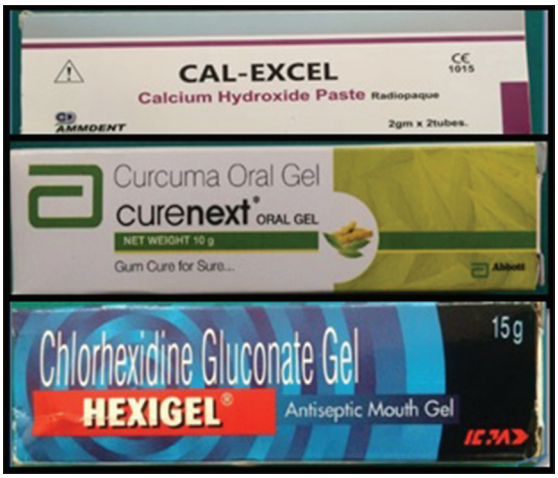

Figure 2: Different types of gels

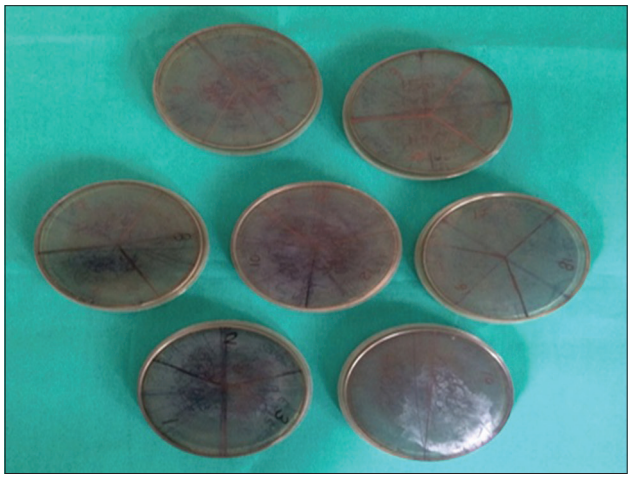

Figure 3: Antimicrobial activity of different gels

software), Chicago, IL, USA. The data obtained were subjected to statistical analysis using paired *t*-test for significance. The statistical analysis was carried out accepting a level of significance of 5%.

RESULTS

A significant difference was observed between all the test groups. However, chlorhexidine gel showed the best value of mean difference, indicating it as the best antibacterial medicament [Table 1]. Chlorhexidine gel was followed by *C. longa* extract, while calcium hydroxide paste was the least efficient in eliminating *E. faecalis* [Figure 3].

DISCUSSION

Eliminating bacteria and its by-products is the main objective of root canal treatment. Along with chemomechanical instrumentation, using biocompatible intracanal medicaments between appointments may facilitate this cause. Furthermore, its need increases in persistent and secondary infections.

Although *E. faecalis* is not the most common bacteria in the root canal, it is the most common (90%) when it comes to secondary infections.[15,16] This is the reason of *E. faecalis* being the focus of interest in endodontics in the recent years. Eradication of *E. faecalis* is difficult because of its ability to tolerate high pH levels, high salt concentration, and long periods of starvation.[17,18] Moreover, biofilm formation and resistance to antibiotics has made it more challenging.

In this study, we found that 1% chlorhexidine gel is more effective in eliminating *E. faecalis* when compared to calcium hydroxide paste and Curenext gel. This finding may be explained by the fact that chlorhexidine has a wide antimicrobial spectrum and it is effective against both Gram-positive and Gram-negative bacteria as well as yeasts.[19] Chlorhexidine acts 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] Furthermore, chlorhexidine has a property of substantivity, i.e., releasing slowly at therapeutic levels over extended periods of time.[22] The results of the present study were in agreement with several authors who found that the medicaments containing chlorhexidine were effective against *E. faecalis*.[23-25] The results are in accordance with the previous studies, though the concentration of chlorhexidine used in this study is 1%. It could be attributed to the fact that the property of substantivity remains the same irrespective of the concentration.

Curcumin, an essential component of *C. longa,* possesses antibacterial, antithrombotic, antiangiogenic, antineoplastic, antiapoptotic, immunomodulatory, and wound healing properties.[26] The stability and assembly of FtsZ protofilaments as a crucial factor for bacterial cytokinesis are introduced as a possible drug target for antibacterial agents. Curcumin suppresses the bacterial cytokinesis through induction of filamentation. It also markedly suppressed the cytokinetic Z-ring formation in bacteria without significantly affecting the segregation and organization of the nucleoids.[27] It suppresses bacterial cell proliferation due to the inhibition of assembly dynamics of FtsZ in the Z-ring,[27] thus proving its potent antibacterial activity against *E. faecalis*.

Calcium hydroxide showed the least antibacterial efficacy. 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.

Table 1: Mean colonies of *Enterococcus faecalis* before and after antibiotic introduction

Groups	Mean±SD		*P*	Mean difference	95% CI
	Colony count before antibiotic introduction	Colonies of *Enterococcus faecalis* after antibiotic introduction			
Group I (*Curcuma longa* extract)	84.50±19.254	22.60±22.623	<0.001	61.9	44.406-79.394
Group II (1% chlorhexidine gel)	116.50±47.058	13.50±16.675	<0.001	103	70.615-135.39
Group III (calcium hydroxide paste)	119.00±37.041	68.70±25.140	0.004	50.3	20.231-80.369

SD: Standard deviation, CI: Confidence interval

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 *E. faecalis*.[28,29]

CONCLUSION

Within the limitations of this study, it can be concluded that chlorhexidine gel showed better antimicrobial properties against *E. faecalis* than other medicaments.

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Conflicts of interest

There are no conflicts of interest.

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