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In Vitro Evaluation of Effectiveness of Chlorhexidine, *Curcuma Longa*, Calcium Hydroxide as Intracanal Medicaments in *Enterococcus Faecalis* Infected Dentinal Tubules

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ABSTRACT

Thorough disinfection of the root canal system is essential for the success of root canal therapy. This study evaluates the disinfection of dentinal tubules using 1% Chlorhexidine gel, *Curcuma Longa*, in comparison with Calcium hydroxide paste. The antibacterial efficacy of the three medicaments against *E. faecalis* is assessed in vitro, by using extracted single rooted tooth at the depths of 200µm and 400µm. The inhibition of bacterial growth was observed after placement of medicaments for 7 days. The data were statistically analyzed with one-way analysis of variance followed by Tukey multiple comparison means [P<.01]. There is highly statistically significant difference between the three groups. The inhibition of growth of *Enterococcus faecalis* at 200 µm and 400 µm was not statistically significant. Overall percentage inhibition at two depths was 80% for 1% Chlorhexidine, 65% for *Curcuma Longa* and 30% for Calcium hydroxide.Under the limitations of present study, 1% Chlorhexidine is most effective against *E. faecalis* as compared to *Curcumin Longa* and Calcium hydroxide.

Keywords: *Enterococcus faecalis*, Chlorhexidine, *Curcuma Longa*, Calcium hydroxide and Dentinal tubules.

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INTRODUCTION

One of the important objectives of root canal treatment is the elimination of the microorganisms from the root canal [1]. *Enterococcus faecalis* is a major component of the flora of previously roots filled teeth with chronic apical periodontitis. This microorganism can even survive in an environment with scant available nutrients. [2,3,8]. Cleaning and shaping of the root canal reduce the bacterial population but do not completely eliminate them. One possible reason for persistent endodontic infection might be due to the retention of microorganisms in the dentinal tubules of the root canal [4,9]. Hence, the use of an intracanal medicament helps in the elimination of bacteria, thereby providing an environment conducive for periapical tissue repair [5,6].

Since its introduction in 1920, calcium hydroxide has been widely used in endodontics. It is a strong alkaline substance, which has a pH of 12.5. Various biological properties have been attributed to this substance, such as antimicrobial activity, tissue-dissolving ability, inhibition of tooth resorption [7].

Chlorhexidine is a broad spectrum antibacterial agent whose antimicrobial efficacy equals that of the other root canal irrigants and medicaments. The positively charged molecules of Chlorhexidine can adsorb onto the dentin and prevent microbial colonization on the dentin surface [10].

Turmeric has been used for thousands of years as a dye, a flavoring agent and a medicinal herb. In India it has been used traditionally as a remedy for stomach and liver ailments, as well as topically to heal sores. Turmeric with its antimicrobial, antioxidant, astringent and other useful properties can be quite useful in dentistry also [11].

The aim and objective of study is to evaluate of effectiveness of Chlorhexidine, *Curcuma Longa*, Calcium Hydroxide as intracanal medicaments in *Enterococcus faecalis* infected dentinal tubules.

MATERIALS AND METHODS

Extracted single rooted human teeth were selected and the model proposed by Haapsalo and Orstavik [12] was modified in this study.

Preparation of the Blocks

A rotary diamond disk was used to decoronate the teeth 5 mm below the cemento-enamel junction. The remaining root was then sectioned such that 6 mm of the middle third of the root was obtained. Cementum was removed from the root surface to standardize the external diameter to 4 mm [13]. The internal diameter was standardized to Gates Glidden drill no.3 [Mani Inc., Tachigi- ken, Japan] in a slow speed handpiece [NSK,Japan] [6]. Organic and inorganic debris was removed by treating the blocks in an ultrasonic bath of 17% EDTA for 5

minutes followed by 3% sodium hypochlorite for 5 minutes [10]. The blocks were immersed in an ultrasonic bath of distilled water for 5 minutes to remove all traces of the chemicals used and sterilized in an autoclave at 121°C. The blocks were then subjected to a second cycle of sterilization, with the blocks immersed in 1 mL of Brain heart infusion [BHI] broth in individual test tubes. This allows better penetration of the broth into the dentinal tubules [6,10].

Contamination of the Blocks

Isolated 24-hour colonies of pure culture of *Enterococcus faecalis* grown on BHI agar were suspended in 5 mL of BHI broth and incubated for 4 hours at 37°C. Fifty microliters of the inoculum was transferred to presterilized individual test tubes containing 1 mL of the BHI broth and dentin block. The dentin blocks were transferred to fresh broth containing *Enterococcus faecalis* every second day. All the procedures were carried out under laminar flow. The dentin blocks were contaminated during a period of 21 days. [6,14]

Antimicrobial Assessment

After the incubation period, the blocks were irrigated with 5 mL of distilled water to remove the incubation broth. The dentin blocks were assigned to the following groups: Group 1, Saline [negative control]; Group 2, Calcium hydroxide; Group 3, 10% *Curcuma Longa* paste; Group 4, 1% Chlorhexidine gel[6,14] All blocks after medication were sealed above and below with sticky wax and incubated in an aerobic environment at 37°C. Antibacterial assessment was performed at the end of 7 days. The blocks were washed with 5 mL of sterile saline to remove the medicament. Two samples of root dentin were then obtained by enlarging the canal with sterile Gates-Glidden drills : the “inner” sample was obtained with drill #4 to a depth of 0.2 mm from the root canal surface, and the “outer” sample was then obtained with drill #5 to a depth of 0.4 mm from the root canal surface as shown in **Figure 1**. Each dentin sample was collected into a separate sterile vial containing 3 mL of sterile fresh BHI broth and was incubated at 37°C for 72 hours to allow the growth of any bacteria.[6,10,14]

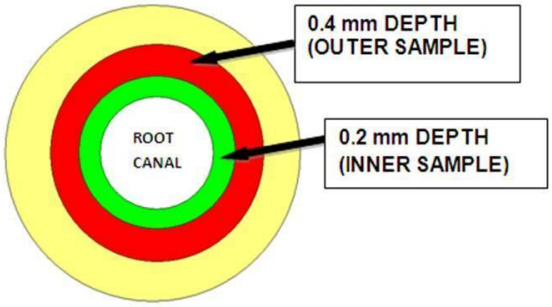

Figure 1: Schematic representation of circumferential sampling of dentin. Root canals initially standardized with #3 Gates Glidden drill were enlarged with #4 [Inner sample] and #5 [Outer sample], and the dentin shavings are collected for analysis of bacterial growth.

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After the incubation period, the content of each test tube was serially diluted, 100µL of broth in 100µL of normal saline for 5 times. Five microliters of this diluted sample was plated on BHI agar plates and incubated for 24hours. Colonies were counted, and readings were tabulated. [6]

Statistical Analysis

The data were statistically analyzed with one-way analysis of variance followed by Tukey multiple comparison means to check the differences in bacterial inhibition between groups [P <.01].

RESULTS

Current study shows that all 3 medicaments exerted antibacterial activity at 7 day interval at two depths as shown in **Table 1**. The inhibition of growth is statistically significant in comparison to control group. Chlorhexidine [1%] is more effective against *Enteroccoccus faecalis* to the depth of 0.4mm. There is highly statistically significant difference [P<.001] between the three groups. The inhibition of growth of *Enteroccoccus faecalis* at 200 µm and 400 µm was uniform with no statistically significant differences.

Table 1: Mean colony count [Cfu/ml [10^6] of *Enterococcus faecalis*

Groups	D=0.2mm (200µm)	D=0.4mm (400 µm)
	Cfu/ml (10^6)	
Group 1: Control	16	13.9
Group 2: Calcium hydroxide	11.2	12.6
Group 3: chlorhexidine	3.16	3.6
Group 4: *Curcuma Longa*	5.6	6.3

DISCUSSION

The persistence of bacteria in the root canal system often leads to the failure of treatment. Several studies have attempted to demonstrate the possible ways in which bacteria invade the tubules [15,16]. Experimental model used in this study was adapted from that proposed by Haapasalo and Orstavik [12]. Human permanent teeth were used instead of the bovine teeth as suggested by Basrani et al [10], as studies with human dentin blocks would definitely be more suitable to simulate the clinical scenario.

Enterococcus faecalis was chosen as a test organism in this study because it is among few facultative organisms associated with persistent apical periodontitis [10,17] and it may be difficult to eliminate from root canals, Moreover it is considerably resistant to the common intracanal medicament with calcium hydroxide[18].

Calcium hydroxide was tested in the study because of its prevalent clinical application as intracanal medication. To be effective against bacteria located inside the dentinal tubules, the

hydroxyl ions from calcium hydroxide should diffuse into dentin at sufficient concentrations and should exceed the dentine buffering capacity, reaching pH levels sufficient to destroy bacteria [19, 20, 21]. Because of buffering action of dentin [17, 20] due to the presence of proton donors, it is unlikely that high pH of calcium hydroxide [>11.5] is attained within dentinal tubules[10,22,23].

Siqueira et al [24] demonstrated that calcium hydroxide associated with saline solution was ineffective in eliminating *Enterococcus faecalis* and Fusobacterium nucleatum cells inside dentinal tubules even after 1week of contact. This is in accordance with the present study which shows less bacterial reduction by calcium hydroxide at both depths.

Another factor can also help to explain the inefficacy of calcium hydroxide in disinfecting dentinal tubules. The arrangement of bacterial cells colonizing the root canal walls can reduce the antibacterial effects of calcium hydroxide, since cells located at the periphery of colonies can protect those located more deeply inside the tubules [25].

In the present study, 1% Chlorhexidine gel provided 80% inhibition of *E.faecalis* at the both depths at an interval of 7 days. The plausible reason is good antibacterial action and increased diffusibilty of medicament into tubules.

Chlorhexidine gluconate has inhibitory effects on bacteria commonly found in endodontic infections acting against gram positive and gram negative microorganisms. One of the mechanisms that explains its efficacy is based on the interaction between positive and negatively charged phosphate groups on the bacterial cell wall, which allows Chlorhexidine molecule to penetrate into the bacteria with toxic effects [18,26].

The results in present study is in accordance with studies performed by others [Krithikadatta et al 2007 [6], Gomes et al 2003 [17], Gomes et al 2001 [26], Basrani et al 2002 [10], Siqueira and Uzeda 1997 [24], Haapsalo et al 2000 [20].

Portenier et al [27] have shown that dentin matrix and collagen type I have inhibitory effects on Chlorhexidine. The inhibitory effects of dentin on Chlorhexidine can be overcome by increasing the concentration [20]. This might have vital role in deciding clinical effectiveness of antibacterial agents [28, 29].

Also, Chlorhexidine has “carry over effect” because of which it gives too positive picture of antibacterial effectiveness of medication. Various studies showed the need for more attention to the properties of Chlorhexidine due to scavenging and generation of reactive oxygen species, potential Genotoxicity and tissue damage, when extruded periapically and systemic risk due to decomposition of Chlorhexidine.

In endodontics because of the cytotoxic reactions of the most of the commercial intracanal medicaments used and their inability to eliminate bacteria from dentinal tubules,

trend of recent medicine attends to use biologic medication extracted from natural plants [11, 30].

Development of bacterial resistance to the available antibiotics and increasing popularity of traditional medicine has led researchers to investigate the antibacterial compounds in plants. Curcumin longa, commonly known as 'turmeric', is well known for its medicinal properties [30]. Components of turmeric contain a mixture of powerful antioxidant phytonutrients known as curcuminoids. Curcumin is the most important fraction which is responsible for the biological activities of turmeric. It is a potent antioxidant is believed to be the most bioactive and soothing portion of the herb turmeric and posses the properties like antioxidant, anti-inflammatory, anti-platelet, cholesterol-lowering antibacterial and anti-fungal effects. It also inhibits inhibits cancer at initiation, promotion and progression stages of tumor development.

Curcuma Longa oil inhibits the growth of Staphylococcus albus and Staphylococus aureus in concentrations up to 1 to 5,000. In the present study *Curcuma Longa* with distilled water was used as an intracanal medicament. *Curcuma Longa* showed 60% reduction in bacterial cell count of *Enterococcus faecalis* over a period of seven days.

CONCLUSIONS

Under the limitations of present study, 1% Chlorhexidine is most effective against *Enterococcus faecalis* as compared to Curcumin Longa and Calcium hydroxide. However, due to various local or systemic risk associated with Chlorhexidine, there is need for better and more biocompatible intracanal medicaments. Keeping in view the important role of *Curcuma Longa* in inhibition of different cultures of bacteria and its role as antioxidant, it can be used as an alternative intracanal medicament. However, there is a great need for further studies to appraise the efficacy of *Curcuma Longa* as an intracanal medicament.

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