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Disinfection of dentinal tubules with 2% Chlorhexidine gel, Calcium hydroxide and herbal intracanal medicaments against *Enterococcus faecalis*: An in-vitro study

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ABSTRACT

Aim: This in vitro study was conducted to evaluate the disinfection of dentinal tubules using 2% Chlorhexidine gel, Honey, Aloe vera gel, *Curcuma longa*, Propolis gel and Calcium hydroxide against *Enterococcus faecalis*.

Materials and method: Two hundred and ten human mandibular first premolars were infected with *Enterococcus faecalis* for 21 days. Samples were divided into 7 groups. Group I- Saline (negative control), Group II- 2% Chlorhexidine gel(CHX), Group III- honey, Group IV- Aloe vera gel, Group V- 20% *Curcuma longa* gel, Group VI- Propolis gel and Group VII -Calcium hydroxide (CH). At the end of 1, 3 and 5 days, the antimicrobial efficacy of medicaments against *E.faecalis* was assessed at the depths of 200 μm and 400 μm.

Results: 2% Chlorhexidine gel was most effective followed by Propolis and *Curcuma longa*.

Conclusion: 2% Chlorhexidine gel gave the best results. Among the herbal extracts Propolis and *Curcuma longa* hold a promising future but to implement their use as sole intracanal medicaments clinically, further in vivo and long term studies are warranted.

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Introduction

The aim of non-surgical endodontic therapy is to remove pathogenic microorganisms from the root canal system, shape the canal system appropriately and obturate it with a suitable material [1]. Complete disinfection however is not always achievable through instrumentation alone due to the anatomical complexities of the root canal [2]. Retention of microorganisms in dentinal tubules leads to persistent endodontic infection. Thus, the use of intracanal medicaments are required to remove the remaining microbes and to provide an environment conducive for periapical tissue repair [3].

Enteroccocus faecalis is a persistent organism that plays a major role in the etiology of periradicular lesions after root canal treatment. It is found in 22–77% of endodontic failure cases and is able to survive in the root canal as a single organism or as a major component of the flora [4]. *E.faecalis* can survive harsh conditions due to its ability at biofilm formation and making them more resistant to phagocytosis, antibodies and antimicrobial agents [5].

Calcium hydroxide (CH) has been widely used as an intracanal medicament because of its bactericidal properties. Its high pH of about 12.5 has a destructive effect on cell membranes and protein structures [6]. Despite the success of CH as an intracanal medicament, several microbial species, including *Enterococcus faecalis*, are reported to be resistant to its effects [7].

Chlorhexidine (CHX) is a bis-biguanide which has a broad spectrum antimicrobial activity and is active against both gram positive and gram negative microbes. 2% CHX has been used as an intracanal medicament and has shown potent results against common endodontic pathogens especially *E. faecalis* [8].

Natural products are sometimes deemed useful and attractive as replacements of medicaments as they are thought to have fewer side effects and less costly [5].

Honey has a potent broad spectrum antimicrobial activity due to the presence of flavanoid pinocembrim [9].

Aloe barbadensis miller (Aloe leaves) possesses anti-inflammatory,antimicrobial, moisturizing, wound healing and pain relief properties [10]. The antimicrobial effects of aloe vera are due to anthraquinones [11].

Curcuma longa, commonly called as turmeric, contains curcumin (diferuloylmethane) as the main yellow bioactive component and has been shown to have a wide spectrum of actions like anti-inflammatory, antioxidant and antimicrobial activities [12].

Propolis is a resinous material that honeybees collect from various plant species and mix with wax and other substances and it exhibits a wide range of biologic activities, including antimicrobial, anti-inflammatory, antioxidant, anesthetic and cytotoxic properties. It is believed that flavonoids account for much of the biological activities in propolis [13]. In dentistry, propolis has been used in various applications [14].

To date, there is no reported dental literature on the dentinal tubule disinfection by *Curcuma longa* and Aloe vera against *E.faecalis*, therefore an attempt was made to evaluate their disinfecting properties compared with other intracanal medicaments with reported success in dentinal tubule disinfection.

Materials and methods

Preparation of dentine specimens

The model proposed by Haapasalo and Ørstavik (1987) was modified [7]. Two hundred and ten single-rooted human mandibular premolar teeth freshly extracted for orthodontic reasons were selected for the study.

A rotary diamond disc was used to decoronate the teeth below the cementoenamel junction and the apical part of the root to obtain 6 mm of the middle third of the root. Cementum was removed from the root surface. Gates Glidden drills no. 3 (Mani Inc., Japan) in a slow-speed handpiece (NSK, Tokyo,Japan)were used to standardize the internal diameter of the root canals. The specimens were placed in an ultrasonic bath of 17% ethylene diamine tetra acetic acid (Dent Wash;Prime Dental Products PVT. Ltd) for 5 min followed by 3% NaOCl.for 5 min to remove organic and inorganic debris. The traces of chemicals used were removed by immersing the dentine specimens in an ultrasonic bath containing distilled water for 5 min. All the specimens were sterilized in an autoclave for two cycles. The first cycle at 121 °C and the second with the specimens immersed in 1 mL of tryptone soya (TS) broth in individual microcentrifuge tubes.

Contamination of the specimens

The test organism used for this study was *E. faecalis*, which is a gram-positive facultative anaerobic bacterium that is common in root filled teeth with post treatment infection. *E. faecalis* (ATCC 29212) ((Himedia, Mumbai) was grown in tryptone soya agar ((Himedia, Mumbai) for 24 h. The culture was suspended in 5 mL of TS brothand incubated for 4 h at 37 °C and its turbidity adjusted to 0.5 McFarland standard. Each dentine block was placed in pre-sterilized microcentrifuge tubes containing 1 mL of the TS broth. Fifty microlitres of the inoculums containing the *E. faecalis* were transferred into each of the microcentrifuge tubes. At the end of 24 h, the dentine specimens were transferred into fresh broth containing *E. faecalis* in a laminar flow chamber. Purity of the culture was checked by subculturing 5 μL of the broth from the incubated dentine specimens in TS broth on tryptone soya agar plates. Contamination of the dentine specimens was carried out for a period of 21 days.

Antimicrobial assessment

At the end of 21 days, the specimens were irrigated with 5 mL of sterile saline to remove the incubation broth. They were assigned into 7 groups (n=30 dentine blocks).

Group I-Saline (negative control),

Group II:2% CHX gel: 2% solution was made and then gel was prepared with methycellulose as a thickening agent.

Group III: Honey was used at 100% concentration in the available form.(Dabur,India).