Dentinal tubule disinfection with 2% Chlorhexidine Gel, Aloe Vera, Propolis, Septilin, and Calcium Hydroxide

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Abstract

Aim: To investigate the antimicrobial activity 2% Chlorhexidine gel (CHX), Aloe vera, Propolis, Septilin and Calcium hydroxide [Ca (OH) 2] on Enterococcus faecalis infected root canal dentine at two different depths (200 µm and 400 µm) and three time intervals (day 1, 3 & 5).

Methodology: One hundred and eighty extracted human teeth were infected for 21 days with E. faecalis. Samples were divided into six groups. Group I (Saline) (Negative control), Group II (Propolis), Group III (2% CHX), Group IV (Aloe vera), Group V (Calcium hydroxide), Group VI (Septilin). At the end of 1, 3, and 5 days, the remaining vital bacterial population was assessed. Dentine shavings were collected at two depths (200 µm and 400 µm), and total numbers of colony forming units were determined. The values were analysed statistically with one-way analysis of variance followed by Tukey multiple comparison test. The paired t-test was used to check for differences in growth at different time intervals within groups and for differences at the two depths (P < 0.01). Results: The number of colony-forming units was statistically significant in all groups compared to the control group (Saline). Group III (CHX) and Group VI (septilin) (100%) produced better antimicrobial efficacy followed by aloe vera (78.94%), propolis (66.7%), calcium hydroxide (58.5%). There was significant difference between aloe vera and propolis and no significant difference between data at 200 µm and 400 µm. Conclusion: Septilin and Aloe vera were effective against E. faecalis in dentine of extracted teeth.

Key words: Dentine tubule disinfection, E. faecalis, calcium hydroxide, chlorhexidine gel, Propolis, Septilin, Aloe vera

Introduction

One of the most important objectives of root canal treatment is the elimination of microorganisms in the root canals. Anaerobic bacteria, especially black-pigmented Gram negative species, have been linked to the signs and symptoms of Periapical disease [1]. Failure of root canal treatment may be related to facultative bacteria such as Enterococcus faecalis which is isolated from infected root canals [2, 3].

Although chemo-mechanical preparation of root canals is able to reduce the number of bacteria, an intracanal medicament with antibacterial action is required to maximize the disinfection of the root canal system in infected cases [4]. The need for medication increases, especially in those cases where an infection is resistant to regular treatment and the therapy cannot be successfully completed due to presence of pain or continuing exudates [5]. For this reason, a wide variety of intracanal medicaments have been used, such as calcium hydroxide pastes and Chlorhexidine gels.

Calcium hydroxide was introduced in 1920 [6] it is widely used in endodontics as an intracanal medicament. It has pH (of about 12.5) in an aqueous solution. Various biological properties have been attributed to this substance, such as antimicrobial activity [7], tissue dissolving ability [8], inhibition of tooth resorption [9] and induction of repair by hard tissue formation [10] (Foreman et al. 1990). However,
calcium hydroxide is not effective in eliminating bacteria from dentinal tubules [11] reported that E. faecalis present in the dentinal tubules was resistant to calcium hydroxide over 10 days.

Chlorhexidine gluconate has been widely used in periodontics due to its antibacterial activity [12]. Its use in endodontics has been proposed both as irrigant and intracanal medicament [13,14]. Chlorhexidine has inhibitory effects on bacteria commonly found in endodontic infections [15], acting against Gram-positive and Gram-negative microorganisms [16]. One of the mechanisms that explains its efficacy is based on the interaction between the positive charge of the molecule and negatively charged phosphate groups on the bacterial cell wall, which allows the Chlorhexidine molecule to penetrate into the bacteria with toxic effects [17]. Septilin (The Himalaya Drug Company, Bangalore, India) is a herbal preparation containing powders of Balsamodendron mukul and Shankha bhasma, Maharasndi quath, and extracts of Tinospora cordifolia, Rubia cordifolia, Emblica officinalis, Moringa pterygosperma and Glycyrrhiza glabra [18]. It has been reported to possess antibacterial (Ross et al.1984), anti-inflammatory [20] and wound healing properties [21]. It is said to be helpful in treating Gram-positive as well as Gram-negative infections [22,23]. There are reports that septilin is effective in chronic stubborn upper respiratory tract infections [24], tonsillitis [22], tropical eosinophilia [25] and infective dermatoses [23].

Propolis (bee glue) is a resinous substance that honeybees collect from various plants and use in the hive to cover hive walls, fill cracks or gaps, and embalm dead invaders. The chemical composition of propolis is very complex and includes organic compounds such as phenolic compounds and esters, flavonoids in all their forms (flavonols, flavones, flavonones, dihydroflavonols, and chalcones), terpenes, beta-steroids, aromatic aldehydes and alcohols, sesquiterpenes, and stilbene terpenes [26]. Propolis has been shown to possess antibacterial, antifungal, antiviral, antiinflammatory, hepatoprotective, antioxidant, antitumor, and immunomodulatory effects [26,27]. Antibacterial activity has been linked mainly to the flavonoid content among these various functional properties [28]. Aloe barbadensis miller (Aloe vera) belongs to the liliaceae family, of which there are about 360 species. It is a cactus like plant with green, dragger shaped leaves that are fleshy, tapering, spiny margined and filled with a clear gel that grows readily in hot, dry climates [29]. Aloe vera contains over 75 nutrients and 200 active compounds, including vitamins, enzymes, minerals, sugars, linin, antraquinones, saponins, salicylic acid and amino acids [30,31]. Numerous scientific studies on Aloe vera are demonstrating its analgesic, anti-inflammatory, wound healing, immune modulating, anti-tumor activities as well as antiviral, antibacterial and antifungal properties [36]. This study was undertaken to evaluate the disinfection of dentinal tubules when contaminated with E. faecalis using septilin, aloe vera, propolis, 2% CHX gel when compared to calcium hydroxide.

Materials and Methods

Preparation of dentine specimens- The model proposed by Haapasalo & Orstavik (1978) was modified. One hundred and eighty single-rooted human mandibular premolar teeth freshly extracted for orthodontic reasons from 90 individuals were selected. A rotary diamond disc was used to decoronate the teeth below the cement to enamel 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.,Tachigi-ken, Japan) in a slow-speed hand piece was used to standardize the internal diameter of the root canals. The specimens were placed in an ultrasonic bath containing distilled water for 5 min. All the specimens were sterilized in an autoclave for two cycles. The first cycle was at 121 oC and the second was with the specimens immersed in 1 mL of tryptose 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) was grown in tryptose soya agar for 24 h. The culture was suspended in 5 ml of TS broth and incubated for 4 h at 37 oC and its turbidity adjusted to 0.5 McFarland standards. Each dentine block was placed in pre-sterilized microcentrifuge tubes containing 1 ml of the TS broth. Fifty microlitres of the inoculum containing the E. faecalis was transferred into each of the microcentrifuge tubes. At the end of 24 h, the dentine specimens were transferred into fresh broth containing E. faecalis. All procedures were...
carried out under laminar flow. Purity of the culture was checked by subculturing 5μL of the broth from the incubated dentine specimen’s in TS broth on tryptose 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 seven groups (n = 30 dentine blocks). Group 1, saline (negative control); Group 2, propolis; Group 3, 2% CHX gel; Group 4, Aloe Vera; Group 5, calcium hydroxide; Group 6, septilin. Calcium hydroxide (Sigma-Aldrich, Mumbai, India) was mixed with sterile saline in a ratio of 1.5: 1 (wt/vol) to obtain a paste-like consistency (Krithika data et al. 2007). Methyl cellulose was used as a thickening agent for Groups 2, 4, and 6. The medicaments were placed inside the canals and sealed at both ends with paraffin wax. They were incubated in an anaerobic environment for 37ºC. At the end of 1, 3, and 5 days an assessment of microbial cells was carried out with 10 specimens at each time interval.

Harvesting of dentine was carried out at two depths (200 and 400 lm) with Gates Glidden drills no 4 and 5, respectively. The collected dentine shavings were transferred into 1 mL of sterile TS broth and incubated in an anaerobic environment at 37ºC for 24 h. After 24 h, the contents of each tube was serially diluted, 100 μL of the broth in 100 μL of sterile saline five times. Fifty microlitres of the dilution was then plated on TS agar plates and incubated for 24 h. Colonies were counted and readings were tabulated.

**Statistical analysis** - The data were statistically analyzed with one-way analysis of variance followed by Tukey multiple comparison means to check the difference in bacterial inhibition between groups (P < 0.01). The paired t-test was used to check for differences in growth at different time intervals within groups and for differences at the two depths (P < 0.01).

**Results**

The number of colony-forming units in all the experimental groups was significantly lower in comparison with the control group (Saline). Group III (CHX) and VI (Septilin) (100%) demonstrated better antimicrobial efficacy followed by Aloe vera (78.94%), Propolis (66.7%), and calcium hydroxide (58.5%).

**Table I: Mean, standard deviation, median, and interquartile ranges for various intracanal medicaments at Log Day 1 at 200 & 400 μm**

<table>
<thead>
<tr>
<th>Medicament</th>
<th>N</th>
<th>Mean 200 μm</th>
<th>Mean 400 μm</th>
<th>Standard deviation 200 μm</th>
<th>Standard deviation 400 μm</th>
<th>Median 200 μm</th>
<th>Median 400 μm</th>
<th>Interquartile ranges 200 μm</th>
<th>Interquartile ranges 400 μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aloevera gel</td>
<td>10</td>
<td>0.61</td>
<td>0.71</td>
<td>0.02</td>
<td>0.02</td>
<td>0.61</td>
<td>0.71</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Septilin</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Calcium</td>
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<td>1.25</td>
<td>1.35</td>
<td>0.04</td>
<td>0.04</td>
<td>1.26</td>
<td>1.35</td>
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<tr>
<td>Hydroxide</td>
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<td>0.02</td>
<td>0.02</td>
<td>1.02</td>
<td>1.12</td>
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<tr>
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<td>0</td>
<td>0</td>
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<td>3.74</td>
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<td>0.05</td>
<td>3.75</td>
<td>3.86</td>
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**Table II: Mean, standard deviation, median and interquartile ranges for intracanal medicaments at Log day 3 at 200 & 400 μm.**

<table>
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<tr>
<th>Medicament</th>
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<th>Mean 400 μm</th>
<th>Standard deviation 200 μm</th>
<th>Standard deviation 400 μm</th>
<th>Median 200 μm</th>
<th>Median 400 μm</th>
<th>Interquartile ranges 200 μm</th>
<th>Interquartile ranges 400 μm</th>
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<tbody>
<tr>
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<td>0.69</td>
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<td>0.69</td>
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<td>0</td>
<td>0</td>
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<td>Calcium</td>
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<td>0</td>
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<td>0</td>
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<tr>
<td>Chlorhexidine gel</td>
<td>10</td>
<td>3.61</td>
<td>3.69</td>
<td>0.05</td>
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<td>3.61</td>
<td>3.71</td>
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</tbody>
</table>
Table III: Mean, standard deviation, median and interquartile ranges for intracanal medicaments at Log day 5 at 200 & 400 µm.

<table>
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<tr>
<th></th>
<th>N</th>
<th>Mean 200 µm</th>
<th>Standard deviation 200 µm</th>
<th>Mean 400 µm</th>
<th>Standard deviation 400 µm</th>
<th>Median 200 µm</th>
<th>Median 400 µm</th>
<th>Interquartile ranges 200 µm</th>
<th>Interquartile ranges 400 µm</th>
</tr>
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<td>0.95</td>
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<td>0.81</td>
<td>0.95</td>
<td>0.02</td>
<td>0.04</td>
</tr>
<tr>
<td>Septilin</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Calcium hydroxide</td>
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<td>1.40</td>
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<td>1.40</td>
<td>0.03</td>
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<tr>
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<td>10</td>
<td>1.31</td>
<td>0.02</td>
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<td>0.02</td>
<td>1.32</td>
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<tr>
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<td>3.28</td>
<td>0.04</td>
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<td>0.07</td>
<td>3.29</td>
<td>3.50</td>
<td>0.06</td>
<td>0.12</td>
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</tbody>
</table>

**Discussion**

In the present study from day 1 to day 5, 2% CHX gel showed 100% inhibition of E. faecalis at depths of 200 µm and 400 µm. The reason may be attributed to the bactericidal dosage of 2% and increased diffusion of the medicament into the dentinal tubules [32]. Basrani et al. (2003) observed that 2% CHX gel was a better antimicrobial when compared to 0.2% CHX gel or calcium hydroxide mixed with 0.2% chlorhexidine [33]. The result of the present study was similar to that of Kriithikadatta et al. (2007), Gomes et al. (2003).

Septilin produced 100% inhibition of E. faecalis at depths of 200 µm and 400 µm from day 1 to day 5. The possible reason for antimicrobial action of septilin might be due its formulation and synergistic action of ingredients. Emblica officinalis [34], Rubia cordifolia [35] and Moringa pterygosperma [36] has antibacterial properties. The antimicrobial activity has been investigated in previous studies and had significant antimicrobial activities Emblica officinalis was active against Escherichia coli, Klebsiella pneumoniae, Klebsiella ozaeae, Proteus mirabilis, Serratia marcescens Salmonella paratyphi A and B, Salmonella typhi, and Pseudomonas aeruginosa [39,40]. Antibacterial and antiviral properties of Moringa pterygosperma inhibit the growth of gram-positive and gram-negative bacteria such as E. coli, S. typhi and S. paratyphi [36]. Aloevera produced 80.3% and 77.4% inhibition of E. faecalis at depths of 200 µm and 400 µm from day 1 to day 5. The antimicrobial effects of aloevera have been attributed to the plants natural anthraquinones. Anthraquinones contains phenolic compounds like aloe emodin, aloetic acid, aloin, anthracine, anthranol, ethereal oil, barbaloin, ester of cinnamonic acid, chrysophanic acid, isobarbaloin, and resistanol [37]. These anthraquinones provide analgesic, antibacterial, antifungal, and antiviral activity [29]. Saponins, which contain glycosides, are soapy substances that have both cleansing and antiseptic properties [38]. In previous studies it was indicated that microbes like S. aureus, P. aeruginosa [39,40], K. pneumoniae [41], E. coli, M. luteus [42], E. faecalis [40,41] (Robson et al. 1982) and C. albicans were shown to inhibited by the isolates from aloevera [39,42]. Propolis produced 67.6% and 66.3% inhibition of E. faecalis at depths of 200 µm and 400 µm from day 1 to day 5. Flavanoid content was probably the most possible reason for the antimicrobial action of propolis [26]. Gram-positive and gram-negative organisms were inhibited by good antibacterial ability of Propolis [43]. The results of the present study was similar to the study of Awawdeh et al. [44] who compared the antimicrobial activity ofpropolis with calcium hydroxide as intracanal medicament against E. faecalis and that Propolis was effective in eliminating the microorganism. According to the findings of the study presented here, it is concluded that the 2% chlorhexidine and septilin demonstrated significant inhibition against E. faecalis followed by aloevera, propolis and Ca (OH)2.

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**Permission from IRB:** Yes

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