In vivo Evaluation of Antimicrobial Effect of Propolis, Miswak, Green Tea Compared to Sodium Hypochlorite and Chlorhexidine as Root Canal Irrigants in Necrotic Infected Single Rooted Teeth

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Abstract

Aim: To assess clinically, radiographically and laboratory the antimicrobial effect of propolis, miswak, green tea, sodium hypochlorite and chlorhexidine on necrotic teeth with apical periodontitis. Materials and Methods: A total of 50 patients with chronic apical periodontitis in a permanent mature single-rooted tooth with a necrotic pulp were included. Root canals were instrumented using Revo S NiTi files and different irrigants: Sodium hypochlorite 3% (NaOCl), 2% chlorhexidine (CHX), 20% ethanolic extract of Egyptian propolis, 20% ethanolic extract of Miswak and 20% ethanolic extract of Green tea. Root canals were sampled before (S1) and immediately after the chemomechanical preparation (S2). The samples (S1&S2) were transferred for culturing and incubation (both aerobically and anaerobically). The patients were re-evaluated both clinically and radiographically after 1, 3 and 6 months. Results: CHX group showed the highest mean % reduction in Log₁₀ of anaerobic bacterial counts (95.5±8.0). CHX group showed non-statistically significant difference from NaOCl (86.2±16.7) and Propolis (84.4±9.7). Miswak group showed statistically significantly lower mean % reduction (75.6±19.1) than CHX group and NaOCl group, but non-statistically significant difference from Propolis group. Green tea group showed the lowest statistically significant mean % reduction in Log₁₀ of anaerobic bacterial counts (50.10±10.35). Conclusion: Propolis, Salvadora Perisca and green tea alcoholic extracts at 20% concentration showed considerable antimicrobial effect against chronic apical periodontitis microbes generally and E. faecalis definitely.

Keywords: E.faecalis, herbal irrigants, chronic apical periodontitis.

Introduction

Complete elimination of microflora from the root canal system is of utmost importance for the success of root canal therapy, which is dependent on mechanical preparation, irrigation, microbial control and complete obturation of root canals. When endodontic treatment is performed under aseptic conditions and according
to accepted clinical principles, the success rate is generally high\(^{(1)}\). The most popular endodontic irrigant is sodium hypochlorite (NaOCl), which has been used for well over four decades. Although it is an effective antimicrobial agent and an excellent organic solvent, it is known to be highly irritant to the periapical tissues, mainly at high concentrations. For this reason the search for another irrigant with a lower potential to induce adverse effects is desirable\(^{(2)}\). Chlorhexidine gluconate (CHX) has also been recommended as a root canal irrigant and many studies have demonstrated its broad spectrum antimicrobial action, substantivity, and low grade of toxicity. However the inability of chlorhexidine to dissolve pulp tissue has been a problem, some attempts were made to solve this deficiency by the combined use of NaOCl and CHX\(^{(3)}\). Propolis is a natural non-toxic beehive product, which is used for building and restoration of the honey comb. In the hive, propolis act as a biocide, being active against the invasive bacteria, fungi and even invading larvae. Other biological activities have also been depicted for propolis, including antibacterial, antifungal, antiviral, antitumor, immunemodulation. Anti-bacterial activity of propolis ethanolic extract of different geographic origin against oral pathogens has been studied by several authors\(^{(4)}\). Miswak is mainly used to describe the stick, which is used for cleansing the teeth. Arak is the plant from which Miswak is derived (Salvadora persica). Many studies have been carried out on different types of chewing sticks focused mainly on antimicrobial activity of these sticks\(^{(5)}\). Green tea is a non-fermented tea, and contains more Catechins, than black tea or oolong tea. Catechins are strong antioxidants. In addition, its content of certain minerals and vitamins increases the antioxidant potential of this type of tea. Green tea has been consumed throughout the ages in India, China, Japan and Thailand. Recent human studies suggested that green tea contributes to overall oral health. It has been used in dentistry and has a promising role in future\(^{(6)}\).

### Materials and Methods

Total of fifty patients-males and females- with chronic apical periodontitis in a permanent mature single-rooted tooth with a necrotic pulp were included in this study, their ages ranged from 18–60 years. Each of them was diagnosed for being healthy, not suffering from any systemic illness. The 50 subjects were randomly divided into 5 groups of 10 patients according to the type of irrigant that was used as follow: Group 1: 3% sodium hypochlorite, Group 2: 2% chlorhexidine, Group 3: 20% ethanolic extract of miswak, Group 4: 20% ethanolic extract of propolis, Group 5: 20% ethanolic extract of green tea. After rubber dam isolation and access opening, the first microbiological samples (S1) (baseline) were collected by sterile paper points for 1 minute. The paper points were placed into transport media (Thioglycollate broth) for microbial culturing. The root canals were instrumented with Revo S NiTi rotary files with a with crowndown technique. The root canals were irrigated after each file size with 3 ml of the respective solution related to each group of patients for about 30 seconds. Then the canals were finally irrigated with 10 ml of the respective irrigant solution, dried with
sterile paper points, and a sample of its contents was taken again (S2). The root canals of all groups were obturated with gutta percha points and resin based sealer (AH plus) at the same visit then a periapical x-ray film was taken immediately post obturation (base line image). The samples (S1&S2) were transferred for culturing and incubation. A 0.1 ml of thioglycollate broth will be inoculated on two plate count agar plates. One Petri plate was incubated aerobically for 24 hours and the other Petri plate was incubated anaerobically for 48 hours. After the incubation period, the plates were examined and the colonies were counted with digital colony counter. The patients were returned to the clinic for evaluation both clinically and radiographically after 1 month, 3 months and 6 months.

**Results**

There was a statistically significant decrease in mean Log_{10} CFU of anaerobic bacterial counts after chemomechanical preparation for all tested irrigation solutions. There was a statistically significant decrease in mean Log_{10} CFU of anaerobic bacterial counts after chemomechanical preparation for all tested irrigation solutions.

<table>
<thead>
<tr>
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<th>Before preparation</th>
<th>After preparation</th>
<th>P-value</th>
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<tr>
<td>Mean Log_{10}</td>
<td>SD</td>
<td>Mean Log_{10}</td>
<td>SD</td>
</tr>
<tr>
<td>NaOCl</td>
<td>5.34 0.45</td>
<td>2.55 2.80</td>
<td>0.028*</td>
</tr>
<tr>
<td>CHX</td>
<td>5.49 0.61</td>
<td>1.43 2.22</td>
<td>0.027*</td>
</tr>
<tr>
<td>Propolis</td>
<td>4.91 0.49</td>
<td>3.56 1.78</td>
<td>0.028*</td>
</tr>
<tr>
<td>Miswak</td>
<td>5.51 0.29</td>
<td>2.66 2.91</td>
<td>0.027*</td>
</tr>
<tr>
<td>Green tea</td>
<td>5.25 0.26</td>
<td>4.23 2.10</td>
<td>0.028*</td>
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* : Significant at P ≤ 0.05

**Discussion**

In our clinical study, CHX group showed the best antimicrobial activity among all groups against both aerobic as well as anaerobic endodontic pathogens. This comes in accordance with Ohara et al. (1993) who reported that antibacterial effect of CHX was the best among six irrigants against anaerobic bacteria(9). Moreover, Jeansonne and White (1994) who reported that 2% CHX was more effective in reducing the number of positive cultures and CFU than 5.25% sodium hypochlorite. The findings of our study were also parallel with Ayhan et al. (1999) who concluded that CHX was effective in endodontic infections(9), and Zamany et al. (2003) who found that at the end of the first appointment, 2% CHX was significantly more effective than the saline control in providing a bacteria-free root canal(6). Moreover, Zamany et al. (2003) showed that a 2% CHX solution, when used as a final irrigant, significantly decreased bacterial loads in root canals that had been irrigated with NaOCl during canal preparation(10). Again, Ercan et al. (2004) stated that CHX and NaOCl
were significantly effective in reduction of the microorganisms in the teeth with necrotic pulp, periapical pathologies, or both, and could be used successfully as a root canal irrigant\(^{(11)}\). Also, Siqueira et al. (2007) showed that CHX based protocol was highly effective in reducing the bacterial populations within the infected root canals rendering most canals free of cultivable bacteria\(^{(12)}\). Besides, Ferraz et al. (2007) concluded that 2% CHX in comparison with all concentrations of NaOCl had more antibacterial effect on gram-negative anaerobic bacteria\(^{(13)}\). This finding may be explained by the fact that CHX has a wide antimicrobial spectrum and it is effective against both Gram-positive and Gram-negative bacteria as well as yeasts\(^{(14)}\). 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 and results in cell death\(^{(15)}\).

Moreover, CHX was used in the present study in 2% concentration which could be a possible reason of great inhibition of the microorganisms because Krithikadatta et al\(^{(16)}\) demonstrated that this dosage may increase diffusion of the irrigant into the dentinal tubules. Furthermore, CHX can bind to surrounding tissues and then be released again slowly at therapeutic levels over extended periods of time in a phenomenon known as substantivity\(^{(12)}\). On the other hand, a randomized clinical trial by Ringel et al\(^{(17)}\) (1982) concluded that NaOCl was found to be significantly more efficient than CHX in obtaining negative cultures. Also a study conducted by Yama-shita et al. (2003)\(^{(18)}\) demonstrated that root canal cleaning by CHX was inferior compared with the cleaning by NaOCl with and without EDTA, which was explained by the inability of CHX to remove smear layer \(^{(18)}\). The results of this study also indicated that 2% CHX is more effective than propolis as an antimicrobial endodontic irrigant. The results also indicated that propolis have antibacterial effect on the growth of aerobic and anaerobic bacteria when used as an endodontic irrigant but, it is significantly more effective in aerobic than anaerobic condition. The antimicrobial effect
of propolis in this study were similar to those obtained by others who have evaluated the inhibitory effect of propolis solution on bacterial growth (Ahangari et al., 2009)\(^{(19)}\); Mohammadzadeh et al., 2007\(^{(20)}\). Soley et al. (2011) also concluded that propolis has antimicrobial activity against *E. faecalis* and *C. albicans* and is an effective intracanal irrigant in eradicating *E. faecalis* and *C. albicans*\(^{(21)}\). In another study focused on the chemical composition, oral toxicity and antibacterial activity of Iranian propolis on male rats, Mohammadzadeh et al. (2007) stated that this substance has no significant clinical toxicity and is capable of preventing the growth of all the tested microorganisms including bacteria and fungi\(^{(20)}\). Bruschi et al. (2006) found that propolis has an inhibitory effect on microorganism of oral importance (*E. faecalis*, *Streptococcus salivarius*, *Streptococcus sanguinis*, *Streptococcus mitis*, *Streptococcus mutans*, *Streptococcus sobrinus*, *C. albicans* and *Lactobacillus casei*)\(^{(22)}\). Ferreira et al. (2007) also found that propolis had an antibacterial effect on selected endodontic anaerobic bacteria (*Prevotella nigrescens*, *Fusobacterium nucleatum*, *Actinomyces israelii*, *Clostridium perfringens* and *E. faecalis*)\(^{(23)}\). Also our results were in agreement with Kayaoglu et al (2011)\(^{(24)}\) who concluded that propolis samples were antimicrobially effective; however, their activity did not exceed CHX. Miswak group exhibited significant antimicrobial activity against both aerobic as well as anaerobic bacteria. Miswak group showed statistically significantly lower mean % reduction (75.67±19.16 -anaerobic bacterial counts) than CHX group and NaOCl group, but non-statistically significant difference from Propolis group. Previous studies have reported that Miswak extracts were effective against *S. mutans* and *E. faecalis*, even using low extract concentrations. Almas and Stakiw reported that the aqueous extract (50% v/v) of the chewing sticks Miswak inhibited the growth of *E. faecalis*, with 2 mm as a diameter of inhibition zone\(^{(25)}\). In 2003, Alali and Al-Lafi reported that the volatile oil of Jordanian Miswak stems exhibited potent antibacterial activity against both Gram-positive and Gram-negative bacteria\(^{(26)}\). Recently, Al-Bayati and Sulaiman tested the activity of aqueous and methanol extracts of Iraqi Miswak against seven isolated oral pathogens. The strongest antibacterial activity was observed using the aqueous extract against *E. faecalis*\(^{(27)}\). As reported by Sher et al. the extract of Miswak was found to be effective against *S. pyogenes*, *E. faecalis*, *P. aeruginosa* and *Lactobacillus acidophilus*\(^{(28)}\).

**Table 2:** Mean Log\(_{10}\), standard deviation (SD) values and results of comparison between Log\(_{10}\) CFU of aerobic bacterial counts before and after preparation within each group (in vivo study)

<table>
<thead>
<tr>
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<th>P-value</th>
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<tbody>
<tr>
<td></td>
<td>Mean Log(_{10})</td>
<td>SD</td>
<td>Mean Log(_{10})</td>
</tr>
<tr>
<td>NaOCl</td>
<td>5.59</td>
<td>0.29</td>
<td>0.67</td>
</tr>
<tr>
<td>CHX</td>
<td>5.66</td>
<td>0.37</td>
<td>0.00</td>
</tr>
<tr>
<td>Propolis</td>
<td>5.83</td>
<td>0.27</td>
<td>2.05</td>
</tr>
<tr>
<td>Miswak</td>
<td>6.05</td>
<td>0.33</td>
<td>2.93</td>
</tr>
<tr>
<td>Green tea</td>
<td>5.64</td>
<td>0.38</td>
<td>3.05</td>
</tr>
</tbody>
</table>

*:* Significant at \(P \leq 0.05\)
On the contrary Mehdi et al. (2010) compared the antimicrobial effects of Persica® and CHX with NaOCl on *E. faecalis* and Candida albicans. The results showed that the microorganisms were very sensitive to NaOCl but there was not any sensitivity to Persica, totally used concentrations had less effect than Sodium hypochlorite (29). The present study showed that green tea hydroalcoholic extract have antibacterial effect against both aerobic as well as anaerobic bacteria. Green tea group showed the lowest % reduction in Log_{10} of aerobic and anaerobic bacterial counts than the other groups. Green tea group showed a statistically significant difference from NaOCl and CHX. This was in agreement with other studies; Garg et al. investigated the antibacterial effect of green tea polyphenols compared with 5.25% NaOCl. According to the obtained results, GTPs exhibited significant antimicrobial activity but this activity was statistically significant difference from NaOCl(30). Trilaksana & Saraswati investigated the efficacy of green tea leaf extract with NaOCl 2.5% as an alternative solution for root canal irrigation; they also stated that NaOCl 2.5% have superior antibacterial effect compared to green tea leaf extract(31). Unlike our study, Martina et al concluded that green tea extract showed antibacterial activity which was similar to that of the 2% CHX Martina et al. 2013(32). Such weak antibacterial action of green tea migh be due to use of low concentration, Noormandi and Dabaghzadeh showed that not only does antimicrobial activity of green tea increased by increasing the concentration, but also that at the same concentrations with an increase in the amount of the substance, antimicrobial activity of the compound increases as well(33).

**Conclusion**

Propolis, Salvadora Perisca and green tea alcoholic extracts at 20% concentration showed considerable antimicrobial effect against chronic apical periodontitis microbes generally and *E. faecalis* definitely. Thus, they offer a promising natural antimicrobial alternative and may serve as a new endodontic irrigants.
References


17. Ringel AM, Patterson SS, Newton CW, Miller CH, Mulhern JM. In


32. Martina LP, Ebenezar AV, Ghani MF, Narayanan A, Sundaram M,
