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Evaluation of antimicrobial efficacy of sodium hypochlorite, propolis, octenidine dihydrochloride and chlorhexidine on microorganisms

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ABSTRACT

Objectives: The aim of this present study was to evaluate the antimicrobial effect of 2.5% sodium hypochlorite (NaOCl), 12.5% propolis, 25% propolis, octenidine dihydrochloride (OCT) and 2% chlorhexidine (CHX) on microorganisms with different structural characteristics.

Materials and Methods: *S. aureus*, *E. faecalis*, *E. coli* and *C. albicans* were included in the study. Pre-sterilized paper discs (6 mm in diameter) were soaked with the test solutions and placed on the plates, following Muller-Hinton agar plates were inoculated with the microbial suspensions. Then zones of inhibition were recorded and the results were analysed statistically. 2.5% NaOCl, 2% CHX and OCT produced inhibitory zones against all microorganisms tested. Statistical analysis was carried out with analyses of variance (ANOVA). Differences were identified by post-hoc Bonferroni test. The level of significance was set at $p=0.05$.

Results: NaCl was ineffective against all microorganisms; however, 2.5% NaOCl, 2% CHX and OCT produced inhibitory zones against all microorganisms tested. 2.5% NaOCl and 2% CHX showed significantly larger average zones of inhibition compared to the other experimental irrigants ($p<0.05$). While 12.5% propolis extract produced only slight inhibition on *S. aureus,* 25% propolis extract was effective on *S. aureus*, *E. faecalis* and *C. albicans*.

Conclusions: The present study showed that 2.5% NaOCl and 2% chlorhexidine had superior antimicrobial effects than other irrigants used.

Key words: Chlorhexidine, microorganisms, sodium hypochlorite, octenidine dihydrochloride, propolis.

INTRODUCTION

Bacteria or their products are considered to be the primary aetiological agents of pulpal necrosis and periapical lesions.[1] Failure to effectively eliminate them and their by-products might result in persistent irritation and impaired healing.[2] Therefore, their elimination is one of the most important steps in root-canal treatment.

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The irrigation solutions are very important during root canal preparation because they aid in the cleaning of the root canal, lubricate the files, flush out debris, and have an antimicrobial effect and tissue dissolution, without damage to periapical tissues.[3] To date, sodium hypochlorite (NaOCl) is the most commonly employed root canal irrigant, but no general agreement exists regarding its optimal concentration, which ranges from 0.5% to 5.25%.[4] NaOCl provides good tissue solvent action,[5] has a broad spectrum of antimicrobial activity,[6] acts as a lubricant for instrumentation, and can flush loose debris from root canals.[7] Spangberg et al.[8] reported that 5.25% NaOCl was considerably stronger than

necessary to kill the bacteria commonly present in the root canal. However, the major disadvantages of NaOCl are its cytotoxic effect if injected into the periapical tissues,[9] its foul smell and taste, its ability to bleach clothes, and its potential for causing corrosion.[10] It is also known to produce allergic reactions.[11]

Natural products have been used for several years in folk medicine. Apitherapy, or therapy with bee products (e.g. honey, pollen, propolis, fortified honey, herb honey, etc), is an old tradition that has been revitalized in recent research.[12] Propolis (bee glue) is a resinous hive substance produced by honeybees from products collected from plants. In general, it is composed of 50% balsams and resins, 30% wax, 10% essential oils, 5% pollen and 5% of various other substances like sugars, vitamins, etc.[13] Bees modify propolis by β-glucodiases, enzymes from hypopharyngeal glands, during collection and processing. Results of this enzymatic modification are hydrolyzation of phenolic compounds like flavonoid heterosides to free flavonoid aglycones and sugars and enhancement of the pharmacological action of the resulting products. Chemically, flavonoid aglycones from propolis are flavones, flavonols, flavanones, dihydroflavonols and chalcones. Other phenolic compounds are phenolic aldehydes and polyphenolic derivates of cinnamic and benzoic acid, including caffeic acid esters, terpenes, β-steroids, sesquiterpenes, naphthalene and stilbene derivatives.[14] It is known to possess valuable antimicrobial, antiviral, fungicidal, local anesthetic, antiulcer, immunostimulating, hypotensive and cytostatic properties.[15,16]

Octenidine dihydrochloride (OCT) was developed at the Sterling-Winthrop Research Institute as a potential topical antimicrobial agent.[17] OCT is an antiseptic for skin burns, wound disinfection and mouth rinses consisting of octenidine hydrochloride and phenoxyethanol. OCT belongs to the bipyridines carrying two cationic active centres per molecule and demonstrates broad spectrum antimicrobial effects covering both Gram-positive and Gram-negative bacteria, fungi and several viral species.[18]

Chlorhexidine (CHX) is probably the most widely used biocide in antiseptic products in general. It is able to permeate the cell wall or outer membrane and attacks the bacterial cytoplasmic or inner membrane or the yeast plasma membrane. High concentrations of CHX cause coagulation of intracellular constituents.[19] As a root canal irrigant and intracanal medicament, CHX has lower cytotoxicity than NaOCl and an antibacterial efficacy comparable to that of NaOCl.[6] Chlorhexidine has been shown to have long-term antimicrobial properties because of its unique ability to bind to hydroxyapatite.[20] At low concentrations, it has a bacteriostatic effect. At higher concentrations, this agent has a bactericidal effect due to precipitation and/or coagulation of the cytoplasm, probably caused by protein cross-linking.[21]

The purpose of this study was to evaluate antimicrobial effect of 2.5% NaOCl, OCT, 12.5% propolis, 25% propolis, 2% CHX on selected microroganisms (*E. faecalis*, *S. aureus*, *E. coli*, *C. albicans*) often found in infected root canals.

MATERIALS AND METHODS

The intracanal irrigants tested in this experimental study were: 2.5% NaOCl, OCT (Octenisept, Schülke & Mayr, Norderstedt, Germany), 12.5% propolis, 25% propolis, 2% chlorhexidine gluconate (Drogsan, Ankara, Turkey) and sterile physiological saline solution (NaCl). NaCl was used as the negative control and 2.5% NaOCl was used as the positive control.

Propolis sample was produced by honeybees in the region of Yomra,

Trabzon, Turkey. Propolis was provided by Trabzon Agricultural Development Cooperative. Hand collected propolis were kept desiccated and in the dark up to their processing. The extract of propolis was prepared at two different concentrations: (1) 12.5% w/v propolis, 25% v/v of 70% ethanol, 10% v/v propylene glycol and deionized water; (2) 25% w/v propolis, 25% v/v of 70% ethanol, 10% v/v propylene glycol and deionized water.

The species of microorganisms were: *S. aureus* (ATCC 25923), *E. faecalis* (ATCC 29212), *E. coli* (ATCC 25922) and one yeast, *C. albicans* (ATCC 14053). McFarland standard number 0.5 was used to evaluate the broth to ensure that the number of bacteria was 1.5×10^8 colony-forming units (CFU) ml^{-1}. Muller-Hinton agar plates were inoculated with the microbial suspensions, using sterile swabs that were spread for the disc diffusion test. Paper discs (6 mm in diameter) were soaked with 20 µl of the test solutions and placed on the plates, which were incubated at 37 °C for 24 hours. Zones of inhibition were measured across the diameter and recorded. The tests were repeated five times for all strains. Statistical analysis was carried out with analyses of variance (ANOVA). Differences were identified by post-hoc Bonferroni test. The level of significance was set at p=0.05.

Table 1. Means of the diameters (in mm) of the inhibition zones provided by tested materials

	n	2.5% NaOCl	2% CHX	OCT	25% propolis	12.5% propolis	NaCl
S. aureus	5	18.75	20.75	8.25	12.25	7.5	0
E. faecalis	5	11.75	17.75	7.25	8	0	0
E. coli	5	25.25	17.25	7	0	0	0
C. albicans	**5**	**48.50**	**22**	**8.75**	**7.25**	**0**	0

2.5% NaOCl: 2.5% sodium hypochlorite; 2% CHX: 2% chlorhexidine; OCT: Octenidine dihydrochloride, NaCl; saline solution

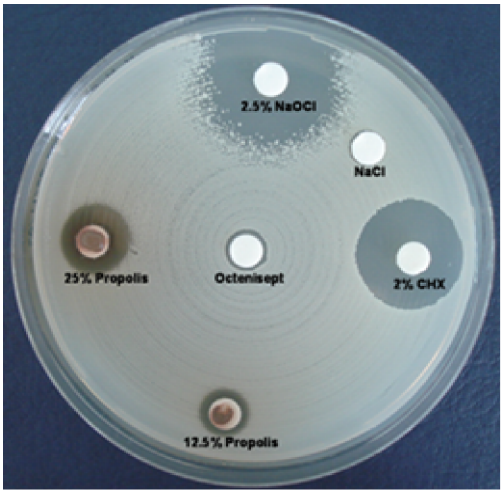

Figure 1. Inhibition zones against *S. aureus.*

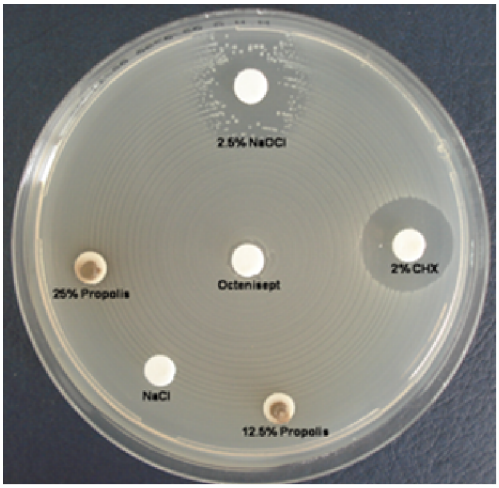

Figure 2. Inhibition zones against *E. faecalis*

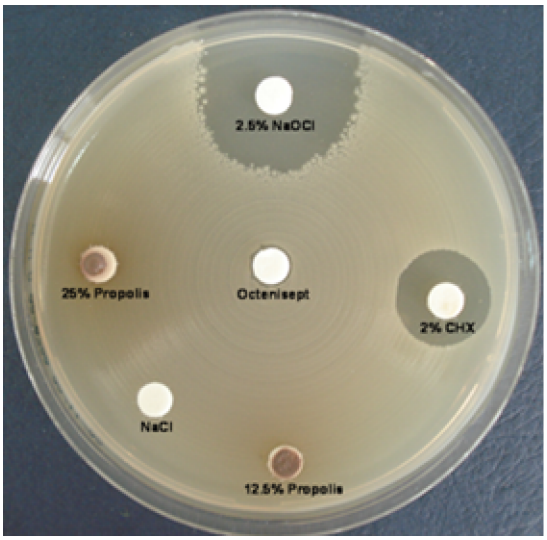

Figure 3. Inhibition zones against *E. coli*

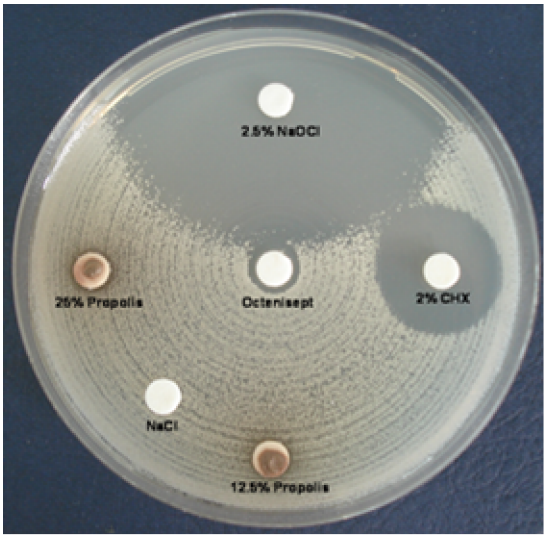

Figure 4. Inhibition zones against C. *albicans*

RESULTS

The results of the agar diffusion test are shown in Table 1. Although, NaCl was ineffective against all microorganisms, 2.5% NaOCl, 2% CHX and OCT produced inhibitory zones against all microorganisms tested (Figure 1-4).

While 2.5% NaOCl was more effective on *E. coli* and *C. albicans*, 2% CHX was more effective on *E. faecalis* and *S. aureus*. 2.5% NaOCl and 2% CHX showed significantly larger average zones of inhibition compared to the other experimental irrigants ($p<0.05$). Also there were statistically significant differences between 2.5% NaOCl and 2% CHX against all microorganisms ($p<0.05$).

OCT showed slight inhibition against all microorganisms tested. Differences between OCT and 12.5% propolis extract against *S. aureus*, OCT and 25% propolis extract against *E. faecalis* and *C. albicans* were not statistically significant ($p>0.05$). 25% propolis extract was effective against *S. aureus*, *E. faecalis* and *C. albicans* except *E. coli*. Although 12.5% propolis extract produced only slight inhibition against *S. aureus*, it was ineffective against other test microorganisms. There was statistically significant difference between 25% propolis extract and 12.5% propolis extract against *S. aureus* ($p<0.05$).

DISCUSSION

Amongst the procedures involved in the control of endodontic infection, instrumentation and irrigation are important agents in eliminating the micro-organisms from the root canal system. Mechanical debridement alone does not result in total or permanent reduction of bacteria.[22] Therefore, the use of antimicrobial agents has been recommended as an adjunct to mechanical instrumentation to reduce the numbers of microorganisms.[23]

In the present study, agar diffusion test was used and we found that NaOCl, CHX and OCT were effective on all microorganisms. 2% CHX showed larger zone of inhibition than 2.5% NaOCl against *S. auerus* and *E. faecalis*. However, NaOCl was more effective than CHX against *E. coli* and *C. albicans*. The results of the present study were similar to some previous studies. Estrela et al.[3] evaluated the antimicrobial effect of 2% NaOCl and %2 CHX on *S. aureus*, *E. faecalis*, *P. aeruginosa*, *B. subtilis* and *C. albicans* by agar diffusion and direct exposure tests. They found that 2% CHX was more effective than 2% NaOCl in the agar diffusion test, while 2% NaOCl showed better performance in the direct exposure

test. Vahdaty et al.[24] analyzed the efficacy of 2% CHX and 2% NaOCl on dentinal tubules infected with *E. faecalis*. The results indicated that CHX and NaOCl were equally effective antibacterial agents at similar concentrations against *E. faecalis*. Vianna et al.[4] showed that 1% and 2% CHX eliminated *S. aureus*, *E. faecalis* and *C. albicans* in 15 seconds. However, 0.5%, 1%, 2.5% and 4% NaOCl produced negative cultures for facultative microorganism and aerobic microorganisms between 5 minutes and 30 minutes.

Although, in vitro studies have demonstrated the antibacterial effect of CHX against microorganisms, CHX can not be recommended as the main irrigant for standard endodontic cases. Because CHX is unable to dissolve necrotic tissue remnants, which is one of the obvious benefits of NaOCl,[25] and less effective on Gram-negative than on Gram-positive bacteria.[26,27]

OCT was originally developed as a potential broadspectrum topical antimicrobial agent.[16] The mode of action is bactericidal/fungicidal by interfering with cell walls and membranes. Phenoxyethanol, an ethanol derivate, serves as a preservative component in OCT which is also supposed to improve the antibacterial activity of octenidine synergistically. Previous studies showed the efficacy of OCT against dental plaque-associated bacteria, such as *S. mutans* and *A. viscosus* comparable to CHX.[22,28] In the present study, OCT was effective against all microorganisms. However, the antibacterial activity of 2.5% NaOCl, 2% CHX and 25% propolis (except *C. albicans*) was superior than OCT. In contrast, Sedlock and Bailey[18] showed that antimicrobial activity of OCT was superior to that of CHX. This discrepancy might be due to the fact that higher concentrations (0.5%, 1.5%, 2.0%) of OCT were used in this previous study[18] and increasing concentrations of OCT may cause more effective antibacterial activity.

Antibacterial activity of propolis is reported to be due to flavonoids, aromatic acids, and its esters.[29] Flavonoids are well-known plant compounds that have antioxidant, antibacterial, antifungal, antiviral, and anti-inflammatory properties.[30] Caffeic acid (3,4-dihydroxycinnamic acid) and its esters, volatile fractions with phenols and/or terpenoids[31] and chrysin (5,7-dihydroxyflavone) possess notable antimicrobial activities as well.[29] But, it is still not known whether antibacterial and antifungal activities of ethanolic extracts of propolis depend on the concentration of galangin, pinocembrin and caffeic acid derivates or on synergism of these or other compounds.[32,33] In this study, we found that *S. aureus* was susceptible to both propolis concentrations (25% and 12.5%). However, 25% propolis was not effective against only *E. coli,* while 12.5% propolis was not effective against other test microorganisms. Our results were in agreement with previous studies. Davey and Grange[34] and Dobrowalski et al.[35] reported that propolis was effective against Gram-positive bacteria, yet showed limited activity against Gram-negative bacteria. In another study,[36] propolis showed high antibacterial activity against Gram-positive cocci (*S. aureus*), but had weak activity against Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and yeasts (*C. albicans*). However, Özan et al.[37] showed that 10% propolis was effective on *E. coli* and *C. albicans*. Koo et al.[30] evaluated in vitro the antimicrobial activity of Arnica montana (10%, w/v) and propolis (10%, w/v) against fifteen oral pathogens. They found that the propolis extract significantly inhibited all the microorganisms tested and showed only a slight inhibitory zone against *C. albicans*. We think that differences between the results could be related to the medical preparation of propolis and its heterogeneous chemical composition, which may differ from area to area and from season to season.

CONCLUSIONS

In conclusion, NaOCl, CHX and octenisept have antibacterial effect on all microorganisms tested, with the NaOCl, CHX being more effective than the OCT. 25% propolis extract was more effective on microorganisms compared with 12.5% propolis extract. Also, the results of the present study suggests that combining 2% CHX with 2.5% NaOCl may be more effective against root canal pathogens and 25% propolis and OCT can be aid to NaOCI and CHX in endodontic treatment.

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