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ORIGINAL RESEARCH

Antimicrobial Effect of Conventional Root Canal Medicaments *vs* Propolis against *Enterococcus faecalis*, *Staphylococcus aureus* and *Candida albicans*

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ABSTRACT

Aim: To evaluate and compare antimicrobial effect of various root canal medicaments against *Enterococcus faecalis*, *Staphylococcus aureus* and *Candida albicans*.

Materials and methods: Six root canal medicaments: 2% sodium hypochlorite (NaOCl), 2% chlorhexidine (CHX) Calcium hydroxide ($Ca(OH)_2$), EDTA, MTAD and propolis and three microorganisms: *Staphylococcus aureus*, *Enterococcus faecalis* and *Candida albicans* were used. These strains were inoculated in brain heart infusion (BHI) and incubated at 37 degrees C for 24 hours. For the agar diffusion test (ADT), petri plates with 20 ml of BHI agar were inoculated with 0.1 ml of the microbial suspensions, using sterile swabs that were spread on the medium, obtaining growth injunction. Paper disks were immersed in the experimental solutions for 1 minute. Subsequently, four papers disks containing one of the substances were placed on the BHI agar surface in each agar plate. The plates were incubated at 37°C for 48 hours. The diameter of microbial inhibition was measured around the papers disks containing the substances. One way ANOVA followed by Tukey's post-hoc test were used. p-value <0.05 was considered statistically significant.

Results: Propolis and other irrigants were found to be effective on *C. albicans, S. aureus* and *E. faecalis*. CHX and MTAD were found to be most effective amongst all the materials tested followed by propolis.

Conclusion: Propolis showed antimicrobial activity against *E. faecalis*, *S. aureus, C. albicans*. It appears that propolis is an effective intracanal irrigant in eradicating *E. faecalis* and *C. albicans*.

Clinical significance: Propolis is an effective intracanal irrigant in eradicating *E. faecalis* and *C. albicans*. It could be used as an alternative intracanal medicament.

Keywords: Root canal medicaments, *Enterococcus faecalis*, *Staphylococcus aureus*, *Candida albicans*, Propolis.

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INTRODUCTION

The success of endodontic treatment depends mainly on elimination of infecting microorganisms. This is achieved through chemomechanical preparation of root canals and leaving antimicrobial dressings in the root canal between appointments. However, microorganisms might still survive these challenges.[1] The treatment of apical periodontitis should, therefore, aim at bacterial eradication. Because cleaning and shaping procedures alone do not reliably eliminate bacteria, it seems logical to medicate canals with an antimicrobial agent after canal preparation.[2]

Viable microorganisms remaining after root canal preparation and disinfection contribute significantly to failure in endodontic therapy.[3]

Endodontic infections are considered polymicrobial and more than 150 bacterial species are usually found in combinations of 3 to 6 species in each canal.[4] Also, microorganisms, such as yeasts may be commonly found in root canals with pulp necrosis.[5,6] Najzar-Fleger et al (1992)[7] studying the prevalence of *Candida* genus in different sites of the oral cavity, verified that 55% of the root canals presented these microorganisms. Maekawa et al (2006)[8] analyzed the microbiota from the root canals of teeth with pulp necrosis and showed that in 15.3% of the cases *Candida albicans* was identified. *Enterococcus faecalis* is also frequently isolated from root canals in cases of pulp infection and also recalcitrant infections after endodontic treatment.[9]

Propolis is a natural flavonoid-rich resinous product of honeybees, which is known for its biological properties,

including antimicrobial, antifungal and healing properties.[10] It was suggested that flavonoids from propolis may stimulate reparative dentine formation and may delay pulp inflammation by stimulating production of transforming growth factor (TGF)-b1 and synthesis of collagen by dental pulp cells.[5] Propolis has been used in dentistry as pulp capping agent,[11] as storage media for avulsed teeth,[12] for prevention of caries[13] and dentine hypersensitivity.[14] But its use as a root canal irrigant has not been evaluated yet.

AIM

To evaluate and compare antimicrobial effect of various root canal medicaments against *Enterococcus faecalis*, *Staphylococcus aureus* and *Candida albicans*.

MATERIALS AND METHODS

The materials used in this study are:

1. Test materials used:
   a. 2% sodium hypochlorite (NaOCl),
   b. 2% chlorhexidine (CHX)
   c. Calcium hydroxide ($Ca(OH)_2$),
   d. EDTA
   e. MTAD
   f. Propolis
2. Microorganisms:
   a. *Staphylococcus aureus* (ATCC 25923)
   b. *Enterococcus faecalis* (ATCC 29212)
   c. *Candida albicans* (ATCC 27853)

   The microbial strains, selected for the present study were collected from the American Type Culture Collection (ATCC), USA
3. Brain heart infusion agar
4. Vernier Calliper

Microbial Analysis

Retrieving Viable Growth from Freeze Dried form of Microbes

Nutrient broth was used to get the viable growth of microbes from freeze dried form. Turbidity in test tube, confirmed the growth of microbes. Comparison of this turbidity was made with McFarland 0.5 turbidity standard.

Agar Diffusion Test for Testing the Antimicrobial Properties

Petri plates with 20 ml of BHI agar were inoculated with 0.1 ml of the microbial suspensions, using sterile swabs that were spread on the medium, obtaining growth injunction. Paper disks were immersed in the experimental solutions for 1 minute. Subsequently, four papers disks containing one of the substances were placed on the BHI agar surface in each agar plate. The plates were incubated at 37°C for 48 hours. The diameter of microbial inhibition was measured around the papers disks containing the substances. To ensure the consistency of all findings, the experiment was performed and repeated under strict aseptic conditions. The antimicrobial activity of each extract was expressed in terms of the mean of diameter of zone of inhibition (in mm) produced by each extract at the end of incubation period.

All the measurements of zone of inhibition were carried out by a single examiner. Calibration of examiner was done prior to and during the study by re-examining 5% of the samples, to minimize intraexaminer variability. Intra-examiner agreement was determined using kappa statistics (k). Intraexaminer agreement score (k = 0.94) was almost perfect, according to Landis and Koch, thus meeting the scientific requirement for validity and reliability.

Statistical Analysis

Data collected by experiments were computerized and analyzed using the statistical package for social sciences (SPSS version 16.0).

Since, the data were continuous type, parametric tests were used for analysis. Mean (X) and standard deviation (SD) were calculated. One way analysis of variance (ANOVA) test was used for multiple group comparisons followed by Tukey post-hoc for group-wise comparisons. p-value < 0.05 was considered statistically significant.

RESULTS

At the end of 48 hours, antimicrobial activity was demonstrated by all the test materials against all the three microbes used in this study. Table 1 shows the antimicrobial activity of the test materials against *Enterococcus faecalis*. The maximum inhibitory effect was shown by MTAD followed by chlorhexidine, propolis and NaOCl, EDTA and $Ca(OH)_2$. Table 2 shows the antimicrobial activity of the test materials against *Staphylococcus aureus* at 48 hours. Maximum zone of inhibition was seen in case of chlorhexidine followed by NaOCl while MTAD, propolis and EDTA showed similar antibacterial activity against *S. aureus*. Table 3 shows the antimicrobial activity of the test materials against *Candida albicans* at 48 hours. Maximum antimicrobial activity was shown by chlorhexidine followed by MTAD, NaOCl, propolis. Least antimicrobial activity was seen in case of EDTA and $Ca(OH)_2$.

Table 1: Antimicrobial activity of the test materials against *Enterococcus faecalis* at 48 hours

Sl No	*Sample*	*Mean zone of inhibition (in mm)*	*Standard deviation*	*ANOVA*	*Tukey post-hoc*
1	MTAD	22.05	0.64	F-value	1>2>3 = 4,
2	2% chlorhexidine (CHX)	18.7	0.47	134.051	4 = 5>6,
				p-value < 0.001	3>5>6
3	Propolis	15.8	0.65		
4	2% sodium hypochlorite (NaOCl)	15.1	0.80		
5	EDTA	14.4	0.22		
6	Calcium hydroxide ($Ca(OH)_2$)	12.68	0.56		

Table 2: Antimicrobial activity of the test materials against *Staphylococcus aureus* at 48 hours

Sl No	*Sample*	*Mean zone of inhibition (in mm)*	*Standard deviation*	*ANOVA*	*Tukey post-hoc*
1	MTAD	5.4	1.09	F-value	2>4>1 = 3 =
2	2% chlorhexidine (CHX)	12.05	0.64	110.198	5>6
				p-value < 0.001	
3	Propolis	4.5	0.36		
4	2% sodium hypochlorite (NaOCl)	8.7	0.47		
5	EDTA	4.8	0.54		
6	Calcium hydroxide ($Ca(OH)_2$)	2.7	0.56		

Table 3: Antimicrobial activity of the test materials against *Candida albicans* at 48 hours

Sl No	*Sample*	*Mean zone of inhibition (in mm)*	*Standard deviation*	*ANOVA*	*Tukey post-hoc*
1	MTAD	18.7	0.47	F-value	2>1>4>5 =
2	2% chlorhexidine (CHX)	22.05	0.65	182.998	3 = 6
3	Propolis	14.4	0.22	p-value < 0.001	
4	2% sodium hypochlorite (NaOCl)	15.8	0.65		
5	EDTA	14.5	0.36		
6	Calcium hydroxide ($Ca(OH)_2$)	14.4	0.23		

DISCUSSION

In the present study the maximum inhibitory effect against *Enterococcus faecalis* was shown by MTAD followed by chlorhexidine, propolis and NaOCl, EDTA and $Ca(OH)_2$. Maximum zone of inhibition against *Staphylococcus aureus* at 48 hours was seen in case of chlorhexidine followed by NaOCl while MTAD, propolis and EDTA showed similar antibacterial activity against *S. aureus*. Maximum antimicrobial activity against *Candida albicans* at 48 hours was shown by chlorhexidine followed by MTAD, NaOCl, propolis. Least antimicrobial activity was seen in case of EDTA and $Ca(OH)_2$. Propolis used in this study showed promising antimicrobial activity against common endodontic pathogens.

Chemomechanical procedure plays an important role in reducing microorganisms in the root canal.[15] Even after irrigation of the root canal with an antimicrobial solution, it may not be possible to eliminate all microorganisms from the root canal.[16] The microorganisms may multiply rapidly in 2 to 4 days, almost returning to their original numbers, if the canal is not filled with an antimicrobial substance between visits.[17]

The inclusion of *C. albicans* and *E. faecalis* in this study was based on the literature that relates these microorganisms to pulp infections.[9] The agar diffusion inhibitory test (ADT), or Lawn's technique has been used for long time as the standard test for antibacterial activity of dental materials.[18] The agar diffusion test does not distinguish microbiostatic and microbicidal properties of dental materials neither does it provide any information about the microorganisms viability after the test.[19]

Propolis is a hard, resinous material derived by bees from plant juices and used to seal openings in the hives. It contains pollen, resins and waxes and large amounts of flavonoids which are benzo-7-pyrone derivatives found in all photosynthesizing cells.[20] A study conducted by Grange and Davey showed the antimicrobial activity of propolis against *Enterococcus* species, *Staphylococcus aureus*, *Candida albicans* and several other bacteria. The nature of the antimicrobial components of propolis has not been elucidated although there is evidence that they are to be found amongst the flavonoids and various esters of caffeic acid.[20] Bioautograms, i.e. chromatograms overlaid with bacteria or fungi in agar media, have revealed that propolis

contains more than one agent active against bacteria and *Candida albicans*.[21] The mode of action likewise requires clarification: An unidentified water-soluble, ultraviolet-absorbing component of propolis has been shown to inhibit bacterial DNA-dependant RNA polymerases.[22] In a study conducted by Mora et al in Mexico showed propolis to possess antifungal activity against *Candida albicans*.[23] Studies conducted by Madhubala et al in (2011)[24] and Kayaoglu et al in (2011)[25] have proven the antimicrobial activity of propolis against *E. Faecalis* (endo abstract).

CONCLUSION

Propolis showed antimicrobial activity against *E. faecalis*, *S. aureus*, *C. albicans*. It appears that propolis is an effective intracanal irrigant in eradicating *E. faecalis* and *C. albicans*.

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