Comparative evaluation of antimicrobial efficacy of chlorhexidine digluconate and propolis when used as an intracanal medicament: ex vivo study.

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Abstract:

Objective: The aim of this study was to evaluate the antimicrobial efficacy of propolis when used as an intracanal medicament against Candida albicans ATCC 10231 (C. albicans) and Enterococcus faecalis ATCC 51299 (E. faecalis) and compare this efficacy to that of Chlorhexidine digluconate (CHX). Methods: This study utilised 70 freshly extracted single rooted human teeth. The crowns were sectioned at the cemento-enamel junction and the root lengths were standardized to 15-17mm. After root canal preparation and sterilization, the canals were contaminated with a mixture of suspension containing C. albicans ATCC 10231 and E. faecalis ATCC 51299. Teeth were randomly divided into 3 groups including one control group. The canals were then filled with intracanal medicament, sealed coronally and incubated at 37 degree celsius for an experimental time period of 48 hrs and 10 days. Microbiological sampling was carried out at the end of 48 hrs and 10th day to determine the Colony Forming Units per ml (CFU/ml). Statistical Analysis used: One way Analyses of Variance and post hoc test of Least Square Distance were used to assess the statistical significance between groups. In the above test p value less than 0.05 was taken to be statistically significant. The data was analyzed using Statistical Package for Social Science (SPSS, V 10.5). Results: When the activity against E. faecalis ATCC 51299 was evaluated, Ethanolic extract of propolis (Group 2) was significantly more effective than CHX (Group 1) for both the experimental time periods. Ethanolic extract of propolis (Group 2)

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was less effective than CHX (Group 1) at the end of 48 hrs against C. albicans ATCC 10231 and this difference was statistically significant. At the end of 10 days this difference was not significant. Conclusion: Ethanolic extract of propolis is effective as an intracanal medicament ex vivo.

Key words: Intracanal medicament, Chlorhexidine digluconate, Propolis.

Introduction:

Endodontic infections have been found to have a polymicrobial nature. Development of certain microbial combinations contributes to persistent clinical signs and symptoms. The most common species isolated from the root canals with secondary apical periodontitis are *Candida albicans* and *Enterococcus faecalis.*[1] The goal of endodontic treatment whether surgical or nonsurgical essentially is debridement i.e to disrupt and remove the microbial ecosystem associated with the disease process and to neutralize any antigen that may be left in the canal after elimination of the microorganisms. Therefore, the infected root canal is subjected to combined chemo-mechanical treatment involving instrumentation plus copious irrigation with antimicrobial agents or disinfectants. Calcium hydroxide as intracanal medicament has been widely used in the past because of its consistent antibacterial activity and minimal cytotoxicity. But the role of calcium hydroxide to eliminate *C. albicans* and *E. faecalis* is questionable.[2-4]

The search for an effective antimicrobial agent led to use of Chlorhexidine digluconate (CHX) within the root canals. CHX when used as intracanal medicament has shown potent results against common endodontic pathogens especially *E. faecalis.*[5,6] Its action against *C. albicans* is also well documented.[2]

An antimicrobial agent, propolis has gained the attention of researchers in recent times because of its antibacterial, antiviral, antifungal, antiprotozoal, anti-inflammatory and antioxidant effects.[7] Propolis is a resinous hive product. The antibacterial activity of propolis is reportedly due to flavonoids, aromatic acids and esters present in the resin.

In dentistry, propolis has been used for surgical wound repair, root canal irrigation [8], direct and indirect pulp capping[9] reduction of dentin hypersensitivity[10] in caries prevention against *Streptococcus mutan*[11] and as a storage media for avulsed teeth.[12,13] Ethanolic extract of propolis has proved to be an effective intracanal medicament in teeth infected with *E.faecalis.*[14-19] However, there is limited information available on the use of propolis as intracanal medicament against *C. albicans* and *E. Faecalis.* Its efficacy compared to that of CHX is unknown.

Thus the present ex vivo study was undertaken to explore the antimicrobial effectiveness of propolis when used as an intracanal medicament against these microorganisms and also to compare its efficacy to CHX.

Materials and method:

This study utilized 70 freshly extracted intact caries free human permanent incisors, canine, premolars with straight single canal and mature apices. They were stored in physiologic saline solution until use. The crowns of the teeth were sectioned with diamond disk with water cooling and root lengths were standardized to approximately 15-17mm.

Root canals were instrumented .5mm beyond the apical foramen up to size 25 K File. Roots having apical diameter greater than a size 25 K File were discarded. The orifice was enlarged with Endoacess bur No1 (cutting head FG1, Dentsply). Gates Glidden drill No1-4 was used for coronal preflaring. Root canals were instrumented 1mm short of length where the file exited the main apical foramen, upto size 50 K File. Canals were irrigated with 2ml physiologic saline solution. The external surfaces of the roots were coated with epoxy resin (Fevilite) except the cervical access and apical foramen. After setting of the epoxy resin the root canals were filled with 17% EDTA and left for 3mins in order to remove the smear layer. This was followed by irrigation with 5ml of physiologic saline solution. Subsequently, all the roots were sterilized by autoclaving at 121^0C for 20 minutes. *C .albicans* ATCC 10231 and *E. faecalis* ATCC 51299 used in this study, was standardized to 1.5 x10^8 microorganisms/ml. The tooth apices were sealed with Cavit^{TM} G temporary cement. Ten microliters of the inoculum was then injected into the prepared canal with the help of an automatic micropipette. Sterile cotton soaked with the inoculum were placed in the cervical access of the canals and then sealed with Cavit^{TM} G temporary cement. The roots were then placed on a gauze pad in sterile petri plates and incubated at 37+_1^0C for 7 days.

Microbiological sampling was carried out to establish the level of contamination prior to application of medicaments. The Colony Forming Units/ml of *C. albicans* ATCC 10231 and *E .faecalis* ATCC 51299 was 50000 and 100000 respectively.

The canals were dried using paper points. Teeth were then randomly divided into two experimental groups

Figure 1: Mean CFU/ml of *Candida albicans* at the end of 48 hrs and 10 days when CHX and Ethanolic extract of propolis were used as intracanal medicament.

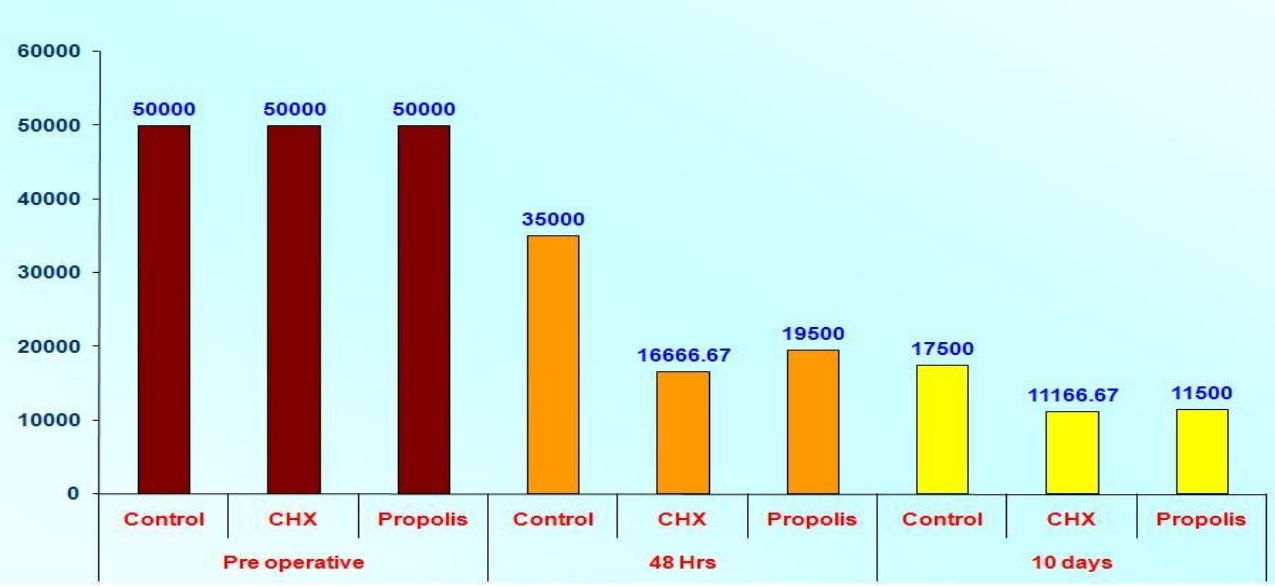
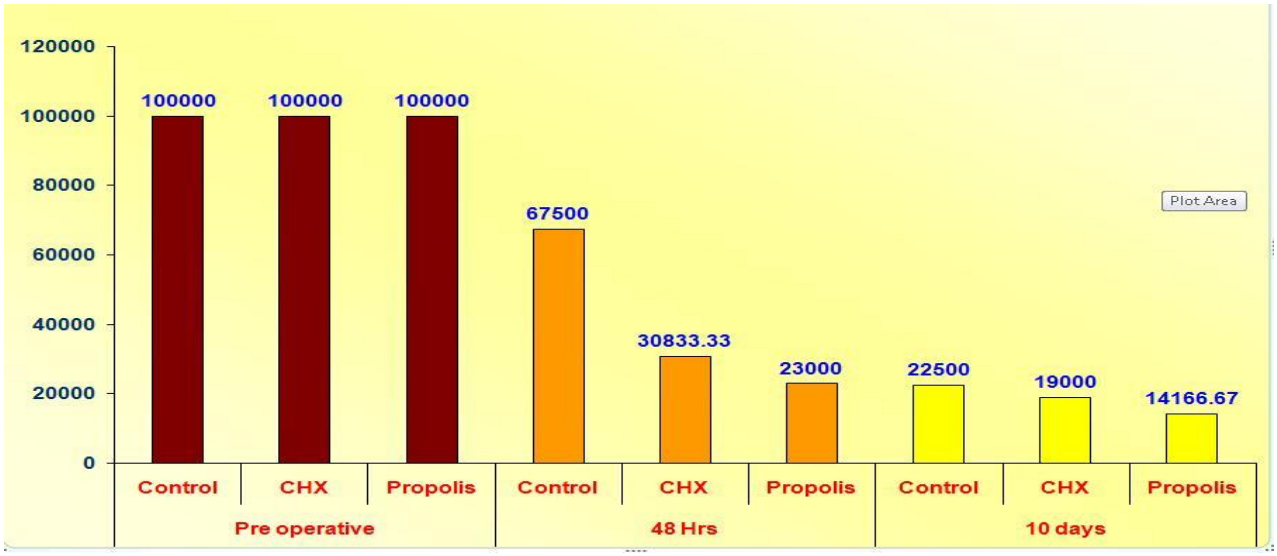


Figure 2 : Mean CFU/ml of *Enterococcus faecalis* at the end of 48 hrs and 10 days when CHX and Ethanolic extract of propolis were used as intracanal medicament.



depending on the intracanal medicament used and one control group.

Group1: (n=30) R4 (Septodont Ltd.co.uk)

Group2: (n=30) 30% Ethanolic extract of propolis. (Brazilian Green propolis; Apiario Silvestre)

Control: (n=10) No medicaments applied.

The test medicaments CHX and ethanolic extract of propolis were injected in to the canals using a 26 gauge needle. A sterile cotton pledget was adapted at the orifice and the specimens were coronally sealed with Cavit^{TM} G temporary cement. The specimens were then incubated at 37^0C for the two experimental time periods of 48 hrs and 10 days under anaerobic conditions.

To determine the microbiological count at the end of 48 hrs the coronal seal of 35 teeth (15 each from Group1 and Group2 and 5 from Control) were

randomly selected. Canals were irrigated with sterile physiologic saline. They were then instrumented with size 50 H file to create dentinal shavings and filled with physiologic sterile solution. Sterile paper points were placed in to the canal for 60 seconds to collect samples for microbial testing. Paper points were then placed in test tubes containing 1ml of sterile physiologic saline solution. Test tubes containing microbiological samples were incubated for 30 min at 37^0C and shaken vigoursly for 60 secs in a vortex mixer. Doubling dilution was carried out prior to determining the CFU/ml.

To determine the microbiological count at the end of 10^{th} day, coronal seal of remaining 35 teeth were opened and microbiologic sampling and doubling dilution were carried out as explained above and the Colony Forming Units/ml were determined.

Method of Statistical Analysis:

One way Analyses of Variance and post hoc test of Least Square Distance were used to assess the statistical significance between groups. In the above test p value less than 0.05 was taken to be statistically significant. The data was analyzed using Statistical Package for Social Science (SPSS, V 10.5)

Results:

Figure 1: Mean CFU/ml of *Candida albicans* at the end of 48 hrs and 10 days when CHX and Ethanolic extract of propolis were used as intracanal medicament.

At 48 hrs following application of the intracanal medicament, the control group had the Colony Forming Count of 35000 per ml of *C.albicans* ATCC 10231. Chlorhexidine digluconate reduced the growth to 16666.67 CFU/ml mean value, while propolis reduced the count to 19500 CFU/ml mean value.

At 10^{th} day, the Control group showed the presence of 17500 CFU/ml mean value of *C. albicans* ATCC 10231 in the canal. Chlorhexidine digluconate reduced the growth to 11166.67 CFU/ml mean value and propolis reduced the count to 11500 CFU/ml mean value.

At 48 hrs there is statistically significant difference between all groups. No statistical significant difference was found between Chlorhexidine digluconate (Group1) and propolis (Group2) at 10^{th} day.

Figure 2 : Mean CFU/ml of *Enterococcus faecalis* at the end of 48 hrs and 10 days when CHX and Ethanolic extract of propolis were used as intracanal medicament.

At 48 hrs, the Control group showed up to 67500 CFU/ml of *E.faecalis* ATCC 51299 in the canal. The Chlorhexidine digluconate group reduced the growth of microorganisms to 30833.33 CFU/ml mean value. Propolis reduced the growth to 23000 CFU/ml.

At the 10^{th} day, the control group showed presence of 22500 CFU/ml of *E faecalis* ATCC 51299 in the canal. Chlorhexidine digluconate reduced the growth to 19000 CFU/ml. Propolis reduced the growth further to 14166.67 CFU/ml. The differences between all groups were statistically significant.

Discussion:

The most common microorganism within root canals of teeth with persistent periapical lesions is *C. albican* and it is associated with other bacteria mainly *E. faecalis.*[1]

There is mounting evidence, however, that obtaining sterility of the infected root canal by presently available treatment methods might be more difficult than once thought.[20,21] Thus intracanal medication may be a valuable adjunct to chemomechanical preparation in disinfection of the root canal system.[22]

Calcium hydroxide has been widely used in the past as an intracanal medicament for its consistent antimicrobial efficacy. Recent researchers have found it to be ineffective against *E. faecalis.* [3,4,23,24]It has been shown that *C. albicans* can tolerate high alkalinity even longer than *E. faecalis. C. albicans* seemed to be highly resistant to calcium hydroxide [2]

Recently, interest has been focussed on the effectiveness of Chlorhexidine digluconate (CHX) as an intracanal medicament. It has been found to be effective against *E. fecalis* and *C. albicans*.[24-30] CHX in a liquid form is a stronger disinfectant than CHX in gel form at various concentrations.[6] Hence in this study R4 Chlorhexidine digluconate (20% solution- 0.2 g) by Septodont Ltd. was used.

This study was performed to investigate the beneficial use of a Brazillian Propolis as an endodontic intracanal medicament against both *E. fecalis* and *C. albicans*. The flavonoid content was expressed as quercetin (7.3%) by the manufacturers. A 30% soloution of propolis was very effective in eliminating the tested microorganism after both 48hrs and 10 days of application. Our results are similar to some of those published studies in which propolis were effective.[7,15-19]

The methodology and the sampling technique followed here is similar to other studies.[15] Size 50 H file was used to abrade the canal producing dentine debris, the canal was filled with sterile physiologic saline and the sample was collected using a sterile paper point. This method produces best results compared to

disc immersion and paper point method.[16] Bacteriological samples were taken at 48 hrs and at the end of 10 days. These two time periods were selected to evaluate the short and long term effects of the medicaments.

It was observed in this study that after 7 days of incubation of the inocula containing 1.5x 10^8 of each microorganism in the canal, the count of *E. faecalis* ATCC 51299 was 10^5 CFU/ml while that of *C. albicans* ATCC 10231 was 50,000 CFU/ml. This may be attributed to that fact that *E. faecalis* has the ability to form biofilms which may explain why the growth of bacteria superseded the growth of *C. albicans* during the incubation period even though equal amounts of innoclum were introduced into all samples. Besides, this observation may also be attributed to the fact that *E. faecalis* competes with other cells for survival. At 48 hrs, the control group maintained the viability of the organisms. For comparison with other experimental groups doubling dilution was performed for the control group too. On the 10^{th} day, the overall viability of the control group reduced. This may be due to the unfavourable in vitro environment that the microorganisms were exposed to, compared to the in vivo conditions in the mouth.

Antifungal and antibacterial properties of propolis when used as an intracanal medicament were evaluated in this study. At 48 hrs and 10 days, propolis reduced the growth of *C.albicans* ATCC10231. It also inhibited the growth of *E. faecalis* ATCC 51299 at the two experimental time periods. Thus it was observed in this study that propolis when used as an intracanal medicament had both antifungal and antibacterial property against *C. albicans* and *E. faecalis* respectively.

Further, the efficacy of propolis as an intracanal medicament was compared to that of Chlorhexidine digluconate. Even though CHX inhibited the growth of *C. albicans* ATCC 10231 at 48 hrs better than that of propolis, the difference in their efficacy at the end of 10^{th} day was not statistically significant. This could mean that the activity of propolis against C. *albicans* over time had increased.

The biological activity of propolis is usually attributed to the flavanoids. The possible mechanism of the antifungal action of propolis was studied by Mello et al. It was suggested that effect of propolis on fungi was attributed to its interaction with cellular sulphydryl compounds thus having a deleterious effect on integrity of the cell wall.[31]

In this study propolis proved to have superior antibacterial property against *E. faecalis* in both the experimental time periods (48 hrs and 10 days). Mirzoeva et al. suggested that the effect of propolis on membrane permeability and membrane potential may contribute enormously to its overall antibacterial activity and may decrease the resistance of cells to other antibacterial agents.[32]

Limiting factor with propolis, as with some other hive products, is that its composition varies with the flora of a given area, the time of collection and the inclusion of wax contaminants. This could vary the clinical effectiveness of propolis on the intracanal microflora. Propolis is a potent sensitizer and is known to cause of occupational allergic eczematous contact dermatitis in apiarists. There also have been certain reports on allergic eczematous contact dermatitis because of non occupational exposure to propolis in natural products and bio cosmetics.

Ex vivo studies have limitations. Clinical trials using propolis as an intracanal medicament on failed RCT's or persistent non healing lesions would give us a better understanding on the actual effectiveness of propolis.

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