

Research & Reviews: Journal of Dental Sciences

Comparison of Antimicrobial Efficacy of 0.3% Propolis, 10% Neem, 10% Triphala and 5% Sodium hypochlorite on *Candida albicans* and *E. faecalis* Biofilm formed on Root Dentin: An *in-Vitro* Study

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Research Article

Received date: 23/05/2016

Accepted date: 09/07/2016

Published date: 15/07/2016

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Keywords: *Candida albicans*, *Enterococcus faecalis*, Sodium hypochlorite, Propolis, Triphala, Neem.

ABSTRACT

Introduction: *Candida albicans* and *Enterococcus faecalis* are the most common organisms isolated from failed endodontic cases. The constant increase in antibiotic resistance strains and side-effect caused by synthetic drugs used for canal disinfection has prompted researches to look for herbal or other natural alternatives.

Objective: To assess and compare the antimicrobial efficacy of 0.3% propolis, 10% neem, 10% triphala and 5% sodium hypochlorite as root canal irrigants against *Candida albicans* and *Enterococcus faecalis* biofilm.

Materials and Methods: 50 Extracted human mandibular premolars were biomechanically prepared, vertically sectioned, placed in tissue culture wells exposing the root canal surface to *Candida albicans* and *Enterococcus faecalis* grown on Sabouraud Dextrose Agar and Brain Heart Infusion broth respectively to form a biofilm. At the end of 2 weeks the specimens were randomly distributed to 4 groups with 10 specimens in each experimental group and 5 specimens in control groups. The specimens in the experimental groups were treated with 5 ml of test solutions for 10 minutes and then the dentin on the root canal portion was scraped and inoculated on respective culture media and incubated for 24 hours at 37 °C. The number of colonies formed was counted with digital method and the data obtained was subjected to statistical analysis using one way ANOVA followed by post hoc test.

Results: Significant reduction in the microbial count was observed with the experimental groups compared to the control group with p value <0.001. Group wise comparison showed no difference between Neem, Triphala and Sodium hypochlorite against *C. albicans* whereas Propolis showed less antimicrobial activity.

For *E. faecalis* there was no statically significant difference between Triphala & Sodium hypochlorite whereas Propolis was less effective and Neem was not effective.

Conclusion: Triphala and NaOCl show best antimicrobial activity against both *Candida albicans* and *Enterococcus faecalis*, whereas Neem is effective against *C. albicans* and Propolis against *E. faecalis*.

INTRODUCTION

The microorganisms most commonly seen in long standing periapical infection or retreatment cases are *Candida albicans* and *Enterococcus faecalis*. Both these microorganisms can survive harsh conditions due to biofilm formation and their physicochemical properties which can modify their prevalence according to the environment and nutritional conditions^[1].

Sodium hypochlorite has been widely used as an irrigant for the elimination of these microorganisms, but requires careful handling as several factors are associated with its safety concerns. Its main disadvantages are unpleasant taste, high toxicity, corrosive to instruments, [2] inability to remove smear layer and reduction in elastic modulus and flexural strength of dentin [3]. These disadvantages have prompted researches to look for other alternatives.

Propolis is a brownish resinous substance collected by bees mainly from plants, which is used to reinforce their hives and keep the environment aseptic. It is a potent antimicrobial, antioxidant and anti-inflammatory agent due to the presence of flavonoids, phenolics and other aromatic compounds [4].

Neem juice has a broad range of therapeutic effect. Neem elaborates a vast array of biologically active compounds that are chemically diverse and structurally complex. Neem leaf and its constituents have been demonstrated to exhibit immunomodulatory, anti-inflammatory, antifungal, antibacterial, antiviral, anti-oxidant, antimutagenic and anticarcinogenic properties [5].

Triphala is an Indian ayurvedic herbal formulation consisting of dried and powdered fruits of 3 medicinal plants (*Terminalia bellerica*, *Terminalia chebula* and *Emblica officinalis*). It has a potential antibacterial activity and anti-inflammatory activity [6].

The purpose of the present in-vitro study is to evaluate the antimicrobial efficacy of commercially available products of 0.3% Propolis, 10% Neem, 10% Triphala and compare with 5% sodium hypochlorite as root canal irrigants on *E. faecalis* and *C. albicans* biofilm formed on the root canal dentin of extracted human teeth.

The Null hypothesis tested is that there is no difference in the antimicrobial efficacy of the tested agents.

MATERIALS AND METHODS

***C. albicans* and *E. faecalis* culture preparation**

A pure culture of *C. albicans* [ATCC 10231] was grown in Sabouraud Dextrose Agar, adjusted to an optimal density of one with sterile Brain Heart Infusion Broth and *E. faecalis* [ATCC 29212] was grown in Muller – Hilton Agar, adjusted to an optimal density of one with sterile Muller – Hilton broth and incubated at 37 °C overnight respectively.

Test solutions

Commercially available following products are tested in the study:

1. Neem Ras 10% (Biogreen Health Care, Mumbai)
2. Triphala Ras 10% (Biogreen Health Care, Mumbai)
3. Propolis 0.3% (Herb Pharma, U.S.A.)
4. Sodium hypochlorite 5%. (Nice Chemicals, Kochin).

Tooth sample preparation

50 Single rooted human premolar teeth were sectioned below the cemento-enamel junction to obtain a standardized tooth length of 8 mm. The teeth were cleaned of superficial debris, calculus, tissue tags and stored in normal saline.

The root canals were then instrumented using the crown down technique with rotary instruments to an apical size of ProTaper F3. EDTA and saline were used between each instrument during the cleaning and shaping procedure.

All teeth were vertically sectioned along the mid-sagittal plane into two halves and autoclaved twice for 30 min at 121 °C to ensure complete sterilization. The concave tooth surface was minimally grinded to achieve flat surface to enable placement in tissue culture wells exposing the root canal surface to *C. albicans* and *E. faecalis* to form a biofilm. To provide an enriched environment in the dentinal tubules for bacterial growth, Sabouraud Dextrose Agar and Brain Heart Infusion (BHI) culture medium were used. Thereafter the samples were incubated at 37 °C for 2 weeks.

Grouping and assessment protocol

The samples were divided into FOUR experimental groups with TEN samples each and an additional positive and negative control groups with FIVE samples each:

1. Group 1 – 0.3% Propolis
2. Group 2 – 10% Neem
3. Group 3 – 10% Triphala
4. Group 4 – 5% Sodium hypochlorite
5. Group 5 – Positive control
6. Group 6 – Negative control

Five samples contaminated with the microorganisms but not exposed to antimicrobial agents serve as positive control whereas 5 samples which are not contaminated with the microbial agents serve as negative control.

At the end of the second week, all groups were immersed in 5 ml of the test solutions for 10 minutes.

The dentin on the root canal portion was scraped and inoculated on Mueller – Hinton and BHI plates and incubated for 24 hours at 37 °C which was then analyzed digitally for colony count.

The data was then subjected to statistical analysis using one way ANOVA followed by post hoc test for comparison.

RESULTS

There is significant reduction in the microbial count with experimental group to control group with p value <0.001 (**Tables 1 and 2**).

Table 1. Inter group comparison of microbial count for *Candida albicans*.

CFU/ml			
Groups	Median	IQR	p value*
Propolis	1700	2900.00	<0.001
Neem	425	1462.5	
Triphala	500	362.5	
5% NaOCl	400	437.5	
Positive control	18,800	6100.00	

*Kruskall Wallis ANOVA

Table 2. Inter group comparison of microbial count for *Enterococcus faecalis*.

CFU/ml			
Groups	Median	IQR	p value*
Propolis	14400.00	8500.00	<0.001
Neem	46000.00	32400.00	
Triphala	8600.00	5250.00	
5% NaOCl	9900.00	12825.00	
Positive control	39800.00	23200.00	

*Kruskall Wallis ANOVA

Sodium hypochlorite, Neem and Triphala showed no statistically significant difference, whereas Propolis showed less antimicrobial activity against *C. albicans*.

The order of efficacy of different groups is as follows (**Table 3**).

Table 3. Group wise comparison of microbial count for *C. albicans*.

Post hoc analysis**				
Groups	Neem	Triphala	5%NaOCl	+ve control
Propolis	0.02	<0.001	0.001	0.001
Neem		0.85	0.63	0.001
Triphala			0.58	0.001
5% NaOCl				0.001

**Mann Whitney u test

NaOCl ~_ Triphala ~_ Neem > Propolis

Triphala and Sodium hypochlorite showed no statistically significant difference in the antimicrobial activity against *E. faecalis* whereas Propolis was less effective and Neem was not effective.

The order of efficacy of different groups is as follows (**Table 4**).

Table 4. Group wise comparison of microbial count for *E. faecalis*.

Post hoc analysis**				
Groups	Neem	Triphala	5%NaOCl	+ve control
Propolis	0.004	0.004	0.39	0.005
Neem		<0.001	0.002	0.86
Triphala			0.22	0.001
5% NaOCl				0.001

**Mann Whitney u test

Triphala ~_ NaOCl > Propolis > Neem

DISCUSSION

The present study is conducted on *C. albicans* and *E. faecalis* biofilm because these microorganisms are commonly encountered in endodontic infections or retreatment of apical periodontitis. It is established that the biofilm forming capacity and the structural organization are influenced by the chemical nature of the substrate. Biofilm experiments conducted on polycarbonate or glass substrate will not provide a true indication of the bacteria- substrate interaction. Hence biofilm formed on the tooth substrate is used in the study in accordance with the methodology by Kishen et al. [7] and Prabhakar et al. [8].

There is significant reduction in the microbial count with all the antimicrobial agents tested in the study and there is difference in the antimicrobial efficacy of these agents. Hence the Null Hypothesis tested in the study is rejected.

Sodium hypochlorite is an age old, time tested, effective root canal irrigant which is considered a gold standard. Hence the antimicrobial efficacies of Triphala, Neem and Propolis are compared with Sodium hypochlorite.

Triphala is equally effective as Sodium hypochlorite in the present study against both *C. albicans* and *E. faecalis* biofilm. The studies conducted by Pujar et al. [6] and Prahalakar et al. [8] have also proved the efficacy of Triphala against *E. faecalis* biofilm. However there are no studies in the literature where Triphala is tested on *C. albicans*. Antimicrobial activity of Triphala is attributed to its formulation which contains three different medicinal plants in equal proportions – *Terminalia bellerica*, *Terminalia chebula*, *Emblica officinalis* resulting in an additive or synergistic positive effect. The strong antioxidant activity may be partially responsible for many of the biological properties [9].

Propolis tested in the study has a good antimicrobial action but less than Sodium hypochlorite and Triphala. This is similar to the observation in the study by Mattigatti et al. [10]. But in a study conducted by Garg et al. [5] and Tyagi et al. [11]. Propolis was equally effective as NaOCl & Triphala. This may be due to the difference in the concentration of Propolis, where 11% alcoholic extract is used, but in the present study commercially 0.3% Propolis in an aqueous solution is considered. The nature of antimicrobial components of Propolis has not been elucidated although they are among the flavonoids & various esters of caffeic acid [12]. The mode of action requires clarification. An unidentified, water soluble, ultraviolet absorbing component of Propolis has been shown to inhibit bacterial DNA dependent RNA polymerases [13]. It is also imperative to note that Propolis of different origin have different compositions & antimicrobial activity. The composition of Propolis is highly variable and its original plant like other medicinal plants requires standardization [14].

Neem is the other herbal extract tested in the present study which showed good antimicrobial effect similar to NaOCl and Triphala against *C. albicans* biofilm, but not effective on *E. faecalis*. This is in accordance with the study by Paridhi et al. [5] and aqueous extract of Neem is used in both the studies. Tyagi et al. [11] also observed good antimicrobial effect of Neem against *C. albicans*. Bohora et al. [15] has observed that neem leaf extract has a significant antimicrobial effect against both *C. albicans* and *E. faecalis*. This may be due to the difference in the methodology and solvents used for Neem as well as the concentration and the preparation method. Neem has antimicrobial properties due to the presence of alkaloids, glycosides, flavonoids, steroids, anthraquinone and tannic acid.

Our general observation after going through the literature is that there is no standardized protocol for extraction of various herbal agents tested against various microorganisms, as well as the solvents & the concentration of the products. Hence in our study we used commercially available products. However a standardized protocol for extraction of herbal agents to make use of their benefits in Endodontics as root canal disinfectants is strongly recommended.

CONCLUSION

Under the limitation of the study it was concluded that, Triphala performed equally well as sodium hypochlorite against *E. faecalis* and *C. albicans* biofilm formed on root dentin.

The use of herbal alternatives such as triphala as root canal irrigant might prove to be advantages considering the several desirable properties. Further research is needed to conclusively recommend herbal solutions as root canal irrigants.

ACKNOWLEDGEMENT

I would like to thank Dr. Rajesh, Biogenix Research Center, Trivandrum, where the microbiological assessment was carried out and Mr. Tendulkar, Biogreen Health Care, Mumbai.

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