Effect of Extract Propolis on the Adherence of Enterococcus Faecalis as a Candidate Root Canal Irrigation Solution

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ORIGINAL PAPER

Effect of Extract Propolis on the Adherence of Enterococcus Faecalis as a Candidate Root Canal Irrigation Solution

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ABSTRACT

A solidified biofilm E.faecalis in hard environmental circumstances within the root canals. Biomaterial surfaces and biofilm on tissues can lead to biofilm-mediated contaminations, which is hard to treat as a result of the increased antimicrobial resistance of bacteria. The adherence of bacteria to a hard surface forms the original and the most vital step in the biofilm's formation. Propolis contains tt-farnesol and apegenin that have mechanisms for inhibiting adherence of bacterial biofilm. Propolis's effect on the adherence of E.faecalis. Propolis 8-16 µg/ml was extracted, analyzed using the agar diffusion method; the outcome on bacterial adherence to a glass surface was also evaluated. Considerably less bacteria were found adhering to Propolis by using the one-way ANOVA with various assessment. Propolis 8-16 µg/ml reduction in adherence of E. faecalis to smooth surface. Propolis 14µg/ml show. Propolis 14µg/ml displays successful in stopping adhesion of E.faecalis.

INTRODUCTION

Endodontic treatment's failure is frequently related with the existence of persistent microorganism. A calcified biofilm can be formed by Enterococcus faecalis in hard environmental circumstances within the root canals (Jhajharia et al., 2015). Biomaterial surfaces and biofilm formation on tissues can lead to biofilm-mediated contaminations, which is problematic to treat as an outcome of the increased antimicrobial resistance of biofilm bacteria. The adherence of bacteria to a solid surface forms the original and the most significant step in the biofilm's formation (Rosaline et al., 2013).

E.faecalis owns diverse virulence factors that allow them to invade dentinal tubules and adhere to dentin (Kina et al., 2018). Furthermore, enterococci also explicit factors that help their bond to host cells and extracellular matrix. Work of literature has revealed that antifungal and, antioxidant, antimicrobial and anti-inflammatory action therapeutic effects suggesting its ability as an endodontic irrigant.

Lately, a natural product famously knows as Propolis (Russian Penicilin), has shown to posess of potent antimicrobial and inflammatory resistant material. This sticky material varies from yellow-brown to dark-brown colour. Honey bee helps to collect this sticky material from trees and shrubs. In Propolis, there are main chemical categories present, which are phenolics, flavonoids and other numerous aromatic compounds. Flavonoids which is known as the eminent plant compound has antibacterial, antioxidant, antiviral, antifungal and anti-inflammatory properties (Vijay D. Wagh. 2013). The so-called inflammatory resistant agent, Propolis, has shown its capability in detaining synthesis of prostaglandins as well as supporting the immune system. By promoting phagocytic activities, stimulating cellular immunity and augmenting healing effects, it is able to withstand immune system. Propolis has been discovered to be very successful against gram positive bacteria (Seidel et al., 2008). Aside from that Propolis also successes against gram negative bacteria Salmonella (Orsi et al., 2005). Propolis' effect on growth and glucosyltransferase activity of S.mutans and was studied (Elahe et al.,2016; Hyun Koo et al.,2002; Duarte et al., 2003). Numerous types of catechin-based polyphenols have been revealed to interrupt the enzymatic activity of Gtfs (Zhi Ren et al.,2016).

Chemomechanical shaping and cleaning of the root canal can significantly decrease the amount of microorganisms. However, they are not entirely eliminated due to the anatomical complexity and the restriction in accessing the canal system by irrigants and instruments (Plotino et al., 2016). The necessity of medication surges in those occasions where an infection battles regular treatments and the therapy cannot be effectively performed owing to exudation and pain. Consequently one of the most significant aims of endodontic treatment is to decrease the bacterial insult to minimum, permitting host's defence system to conquer and deliver a favourable condition for healing (Prajkta et al., 2014).

In this study, we are assessing Propolis for its function as an intracanal medicament and comparing its antibacterial efficacy with against E faecalis by adherence analysis.

MATERIALS AND METHOD

Each procedure and practice of this research was proposed to Faculty of Dental Medicine Universitas Airlangga Ethic Commission before the research was completed.

Materials

Propolis samples and Fractionation. Crude samples of A. Mellifera propolis were attained from Batu, Malang forest region of East Java state, Indonesia. Propolis contains of resin (50%), wax

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(30%), essential oils (10%), pollen (5%), and organic componen (5%). Resin contains flavonoid, acid, and fenol. One of the phenol is Caffeic Acid Phenethyl Ester (CAPE) (Viuda et al., 2008), which is crucial for antibacterial.

Method

Bacterial E.faecalis

The cultures were stored at 280 °C in brain-heart infusion (BHI) or tryptic soy broth (TSB) containing 20% glycerol. The E.faecalis constructs were a stock of Laboratorium Microbiology Faculty of Dental Medicine Universitas Airlangga Surabaya-Indonesia

Inhibition of Adherence of Growing Cells to a Glass Surface

To testing the bacterial adherence to a smooth surface using lab test tube, bacterial were grow up at 37 °C 10% CO2. To knowing the ability of bacteria to adherence with a smooth surface by positioning medium contain bacteria in the lab test tube with the angle of the slope 30° for 18 h in test lab tubes as detailed in Koo et al., (2000) and Hamada and Torii (1978). The bacteria were grown up in BHI broth plus 1% sucrose (w/v) containing sub-MIC concentrations of the extracts Propolis or control (80% ethanol, v/v). The sub-MIC concentrations were used in this study because these concentrations showed bacterial growth.

After incubation, the adhering cells were washed and resuspended in an ultrasonic bath (Vibracell, Sonics & Material Inc.). The number of adherent cells was measured by spectrophotometry at 550 nm. The concentration for total bacterial adherence inhibition (TBAI) was defined as the lowest concentration that allowed no visible cell adherence on the lab test tube (p 0.05). Six replications were made for each concentration of the test extract.

RESULTS AND DISCUSSION

Table 1 Statistical analysis

Extract Propolis	N	(Mean) (x 10^8)	Standart Deviasi (SD)
16 µg/ml	5	0.0	0.0
14 µg/ml	5	0.025	0.015
12 µg/ml	5	0.097	0.013
10 µg/ml	5	0.135	0.035
8 µg/ml	5	0.115	0.018
control medium	5	0.00	0.00
control Positif	5	1.500	0.069

In order of adherence of E. faecalis, the concentration of Propolis are: 16,14,12,10,8 µg/ml. The medium control group with BHIB without E.faecalis. The positif control with add 1% sucrosa on medium BHIB.

Table 2: Statistical analysis normalitas

concentration	Asymp.Sig(2tailed)	normal
14 µg/ml	0,860	Normal
12 µg/ml	0,702	Normal
10 µg/ml	0,841	Normal
8 µg/ml	0,931	Normal
control (+)	0,542	Normal

All data showen normally with p>0,05

Tabel 3: Statistical analysis of samples showing concentration of propolis

consentration	14 µg/ml	12 µg/ml	10 µg/ml	8 µg/ml	control (+)
14 µg/ml		0.041*	0.001*	0.007*	0.000*
12 µg/ml	0.041*				0.000*
10 µg/ml	0.001*				0.000*
8 µg/ml	0.007*				0.000*
control (+)	0.000*	0.0000*	0.000*	0.000*	

*the mean diffrence is significant at the 0,05 level

Currently, E. Faecalis has become one of the most challenging root canal microorganism to eliminate in the past few decades. Endodontic treatment against E. faecalis by biomechanical cleaning, root canal shaping, and disinfection using root canal medicament including root canal irrigation followed by obturating. The growth of root canal irrigation solution agents targeted at disturbing both colonization of the pulp microorganism pathogens by root canal wall and the subsequent formation of smear layer. This is one of the major tactics to lessen the number of root canal microorganism. The pathogenicity of E. faecalis can be attributed to the various virulence factors on root canal microorganism, including biofilm formation and the expression of surface adhesion components/ Adherence is considered to be the initial phase for E.faecalis colonization of root canal system, including tubule dentin invasion.

In this study, we assessed in vitro the antiadherence activities of Propolis against E.faecalis. The result that Propolis 16% had bactericidal effects against E.faecalis whereas no E.faecalis on a glass surface. Verified, with ranging from 8 to 14 µg/ml (Table 1).

The antiadherence activity of Propolis was also effective against adherent cells of E.faecalis in 14 µg/ml while at least adherence of E.faecalis on glass surface compare with other concentration.

The attendance of active ingredient of Propolis, for instance, apegenin and tt-farnesol that have mechanisms for hindering adherence contribute to the antibacterial action of Propolis. These active elements separate mitochondrial oxidative phosphorylation; thus, preventing the respiratory chain. This caused its anti-adherence activity by changing the microorganism adhesion and the ability of the microorganism to colonize. In so resulting maximum decrease in adherence of E. faecalis to dentin.

One of the most important virulence factors, known as Glucosyltransferase (GTF), are involved in both formation of the smear layer on root canal system and colonization of root canal microorganism. These enzymes are shown in the solvable segment of saliva . Aside from there, in the root canal necrotic formed on root canal system, these enzymes are also presented. The surface-adsorbed GTFs produce glucans in situ, provide compulsory sites for E.faecalis as well as contribute to the smear layer creation (Jhajharia et al., 2015). Therefore, it is favourable to define the potential inhibitors of GTFs' effects, primarily the surface adsorbed enzymes. As potent inhibitor activity of glukosiltransferase enzym, Apigenin, will induces acumulation biofilm *E.faecalis* by restraining the forming of insoluble glucan, and enlarging solvable glucan.

The foremost benefits of using natural as medicamen substitutes such as Propolis are cost-effectiveness, easeness of its availability, low toxicity, increased shelf life, and lack of microbial resistance reported hitherto. Henceforth, the medicamen and antimicrobial properties of the Propolis can be an alternate as a root canal irrigation.

CONCLUSION

Propolis 8-16 µg/ml reduction in adherence of E. faecalis to smooth surface. Propolis 16% had bactericidal effects against E.faecalis. Propolis 14µg/ml show effective in preventing adhesion of E.faecalis.

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