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by Tamara Yuanita

Submission date: 01-Jul-2019 12:06PM (UTC+0800)
Submission ID: 1148311755
File name: 3573-12501-4-PB.pdf (664.32K)
Word count: 3157
Character count: 16407

Dental Journal
Majalah Kedokteran Gigi

Dental Journal
(Majalah Kedokteran Gigi)
2017 March; 50(1): 6–9

Research Report

The cleanliness differences of root canal walls after irrigated with East Java propolis extract and sodium hypoclorite solutions

Tamara Yuanita
Department of Conservative Dentistry,
Faculty of Dental Medicine, Universitas Airlangga
Surabaya - Indonesia

ABSTRACT

Background: *Root canal instrumentation produces smear layer that covers dentine tubules of the root canal surface. Smear layer is organic and inorganic particles that have to be removed. East Java propolis extracts contais of saponin components used as a natural surfactant. 2.5% NaOCl and 5% NaOCl solutions have been widely used for irrigation in root canal treatment.* ***Purpose:*** *The purpose of this study was to analyze the cleanliness of the root canal walls, irrigated with aquadest, 8% East Java propolis extract, 2.5% NaOCl and 5% NaOCl.* ***Method:*** *Forty extracted teeth with straight single root canals were randomly divided into four groups (n=10). The specimens were prepared with ProTaper. During instrumentation, the root canals were irrigated with different solutions: Control Group irrigated with aquadest; Group 1 irrigated with 8% East Java propolis extract; Group 2 irrigated with 2.5% NaOCl and Group 3 irrigated with 5% NaOCl. The root canals were cut at apical third and SEM scores were tested by using Mann-Whitney test at the significance level of p=0.05 and Median Control test.* ***Result:*** *The results of Mann-Whitney Test, there were significant differences between control group with Group 1, 2 and 3 (p<0.05). Based on the Median Control test, the value of 8% East Java propolis extracts was 1,000, which was the best value compared to 2.5% NaOCl, 5% NaOCl and aquadest.* ***Conclusion:*** *It can be concluded that 8% East Java propolis extract is the most effective solution for cleaning root canal walls compared with 2.5% NaOCl and 5% NaOCl.*

Keywords: *propolis extracts; NaOCl; root canal walls*

Correspondence: Tamara Yuanita, Department of Conservative Dentistry, Faculty of Dental Medicine, Universitas Airlangga. Jl. Mayjend. Prof. Dr. Moestopo no. 47 Surabaya 60132, Indonesia. E-mail: tamara25jun@yahoo.com; HP: 081703490329/08155130747

INTRODUCTION

Root canal treatment is one type of dental conservation treatments that aims to maintain teeth function in oral cavity. Root canal treatment consists of several stages, namely root canal preparation including cleaning and shaping (biomechanical preparation), disinfection, and root canal filling. The main principle of root canal cleaning is the preparation process should reach and clean the entire surface of the root canal walls.[1] However, root canal preparation has some risks, one of which is that instrumentation can cause the formation of smear layers composed of organic and inorganic materials, such as dentin powders and necrotic pulp tissue remnants. The smear layers formed would make colonizes of bacteria form biofilms on the root canal walls.[2] Smear layers can also reduce the adaptation of filler materials with canal walls that can cause leakage between the obturation materials and the root canal walls leading to treatment failure.[3]

The key role of root canal irrigants is to clean the canal during the enlarging and shaping process. Consequently, one or more irrigants must be used for the complete elimination of smear layer and debris from the root canal system. The ideal irrigation materials are materials which have antimicrobial properties, ability to dissolve soft tissue or smear layers, low surface tension, and low toxicity. Various materials are commonly used for irrigation, such as citric acid, EDTA, chlorhexidine, and sodium hypochlorite.[4]

Sodium hypochlorite (NaOCl) can be classified as halogenated groups that are oxygenating. Chemical reactions in NaOCl showed that NaOCl acts as an organic

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solvent and degrades fats into fatty acids and glycerine which serve to reduce the surface tension of the liquid.[5] NaOCl in solution form hypochlorus acid (HOCl) and oxychloride (OCL). These disinfectants contain chlorine-based solution (Cl 2). These solutions are considered as high level disinfectants because they are very active in all bacteria, viruses, fungi, parasites, and some spores. The ingredients work fast or fast acting. NaOCl has specific properties when in contact with vital tissue since these substances can be cytotoxic and destructive.[6] NaOCl nevertheless, is not able to eliminate smear layers because it can dissolve inorganic materials perfectly and smells unpleasant.[7]

Propolis is a natural product, which is interesting in pharmaceutical application, a mixture of resin and wax bees collected from important parts of plants.[8] Antimicrobial activities of propolis against a variety of bacteria, fungi and viruses have been studied since the late 1940s, and have showed different variable activities from microorganisms.[9] The antimicrobial effects of propolis can resist more than 100 types of bacteria, fungi and viruses, including agents causing tuberculosis, syphilis, diphtheria, and influenza.[10]

Saponins in propolis, moreover, act as surfactants, which resemble the properties of detergent. Saponins, thus, are often referred to as 'natural detergents', a foaming solution classified by aglykon complex structure into triterpenoid and steroid saponins. Saponins are characterized by their ability as surfactants that can reduce surface tension to wet root canal walls optimally [11]

This study used 8% East Java propolis extract based on Minimal Concentration of East Java Propolis to inhibit *Enterococcus faecalis* (*E. faecalis)* bacteria.[12] The study aimed to compare the effectiveness among 8% East Java propolis extract, 2.5% NaOCl and 5% NaOCl on the cleanliness of root canal wall.

MATERIALS AND METHODS

East Java propolis extraction was conducted by maceration method in Balai Penelitian dan Konsultasi Industri, Surabaya, East Java. 350 grams of raw East Java propolis was macerated with 650 grams of 70% ethanol in a sealed container. Propolis and ethanol were shaken by using a shaker at a speed of 80 rpm in Balai Penelitian dan Konsultasi Industri, Surabaya, East Java, Indonesia. After 7 days, the maceration process was stopped and filtered. The maceration process was repeated for 7 days until the color of ethanol was stable. It was evaporated until the substance free from ethanol and then was diluted with aquadest to obtain 8% propolis extracts.

Each first permanent mandibular premolar with matured apices in 21 mm length (n=40) was placed in an acrylic container that has a hole resembling a tooth socket for having a treatment. Root canal preparation was conducted using Pro Tapper with hand instrumentation and crown down technique. During instrumentation, the canal of each samples were irigated with 25 gauge open ended needle just 2 mm before working length. To remove the smear layer, the root canals in every each sample were irrigated with aquadest as control group and group I: irrigated with 8% propolis extract, in group II : irrigated with 2.5% NaOCl (Kimia Farma, Surabaya, Indonesia) and group III: irrigated with 5% NaOCl (Kimia Farma, Surabaya, Indonesia) and aquadest as final rinse in every each sample

Irrigation was conducted by using the same pressure (1 atm). Irrigation solution in each group was used every change of instrument as much as 3 ml for 30 sec, and aquadest as final rinse then dried with sterile paper points 3 times. The samples were stored in a desiccator to keep them dry. Those samples were cut with a low speed diamond disc in a horizontal plane along the apical third of 4 mm from the apex tip then cut longitudinally in bucco-lingual.

Samples that have been cut were attached to the coated holders. After coating with paladium and aurum, one by one of the sample was inserted into scanning electron microscope (SEM) and photographed with a magnification of 1000x. Cleanliness assessment then was carried out by using a transparent plastic tool (13.5x8.5 cm). It then was divided into nine squares of the same size. The observation was conducted by three observers. Finally, assessment was conducted by placing a transparent plastic on each photo, then a score for each box was taken as follows : score 0: 95-100% of dentin tubulesare open; score 1: 50-95% of dentin tubules, score 2: less than 50% of dentin tubules are open, score 3: dentin tubules are not open. [13]

RESULTS

This study was conducted to know the effectiveness of 8% East Java propolis extract, 2,5% NaOCl and 5% NaOCL in cleaning root canals. This study had four treatment groups, the group irrigated with aquadest as a control group and three treatment group irrigated with 8% East Java propolis extracts, group irrigated with 2,5% NaOCl, and group irrigated with 5% NaOCl. (Figure 1).

The assessment of SEM results was conducted by three dentist as observers, and then its validity was tested by using Friedmann Test. The data showed that there was no difference among the three observers as shown in Table 1.

The data was tested by using a non-parametric test, Kruskal-Wallis test, to know the difference among all groups. The results then showed that the significance value obtained was 0.001, smaller than 0.05 ($p<0.05$). It means that there was a significant difference among all treatment groups.

Mann-Whitney test was conducted to know the differences in each treatment group (Table 2). The results showed that the treatment group irrigated with 8% propolis extract compared with the group irrigated with aquadest had 0.001 score, while the treatment group irrigated with 2,5%

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NaOCl compared with the group irrigated with aquadest had 0.007 less than 0.05 ($p<0.05$). It means that there was a significant difference between the treatment group and the control group. Furthermore, the treatment group irrigated with 8% propolis extract compared with the treatment group irrigated with 2.5% NaOCl had 0.015 and 5% NaOCl had score less than 0.05 ($p<0.05$). It indicates that there was a significant difference between the treatment group irrigated with 8% propolis extract and the treatment group irrigated with 2.5% NaOCl and 5% NaOCl. The median value of each group was measured. Based on the results, it is known that 8% propolis extract had the smallest value compared to the other groups.

DISCUSSION

During root canal preparation, endodontic instruments usually cause friction leading to smear layer formation. Smear layer is defined as a surface film of debris that is retained on the dentin or other surfaces after instrumentation with either rotary instruments or endodontic files, which composed of organic and inorganic particles of calcified tissue, necrotic tissue and microorganisms.[14]

This study was conducted to determine the effectiveness of 8% East Java propolis extract, 2.5% NaOCl and 5% NaOCl as irrigation materials in the cleanliness of root canal walls. The indicators of the cleanliness can be observed from the covered areas of smear layer on the surface of root canal as seen on photo.

The effectiveness of 8% East Java propolis extract, 2.5% NaOCl, and 5% NaOCl, moreover, can be determined by using SEM. SEM can display images of the cleanliness of root canal surface because it can show the topography of the surface of the root canal walls with high resolution. Thus, it can be said that the less smear layers cover the dentinal tubules, the cleaner the root canals are.[15]

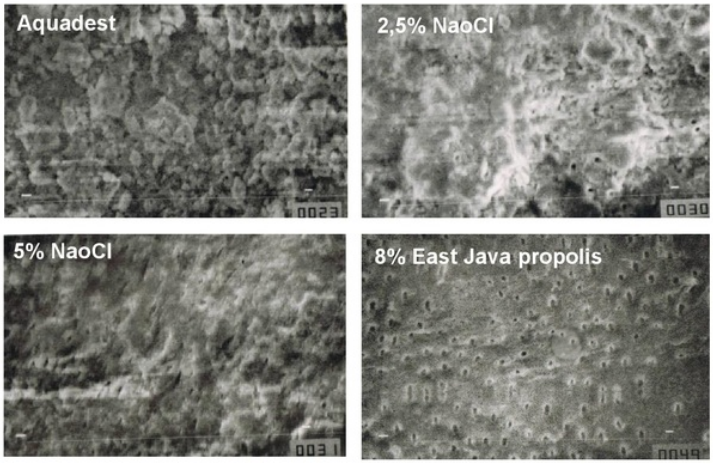

Figure 1. The results of the cleanliness irigating with aquadest, 8% propolis extract, 2.5% NaOCl and 5% NaOCl with 1000x magnification.

Table 1. Friedman Test

Treatment Groups	Friedman Test
Aquadest	p = 0.368
8% East Java propolis	p = 0.060
2,5% NaOCl	p = 0.091
5% NaOCl	P = 0.083

Table 3. Median value

Treatment group	Median
Aquadest	3
2,5% NaOCl	2
5% NaOCl	2
8% East Java propolis	1

Table 2. The result of Man-Whitney test

Treatment groups	Aquadest	8%East Java propolis	2,5%NaOCl	5%NaOCl
Aquadest	-	P= 0,001	0,007	0,009
8% East Java propolis	P = 0,001	-	0,015	0,025
2,5% NaOCl	P = 0,007	P= 0,015	-	0.003
5% NaOCl	P = 0,009	P= 0,025	0,003	-

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Based on the SEM images assessment, the covered areas of smear layer in the group irrigated with 8% East Java propolis extract was less and almost none. It was indicted by the dentine tubules were opened and clean. It means that saponins contained in 8% East Java propolis extract were effective in cleaning the root canals from smear layers. Meanwhile, there were still smear layers covering the dentinal tubules in the groups irrigated with 2.5% NaOCl and 5% NaOCl.

NaOCl furthermore, is able to dissolve organic tissues through several stages, namely saponification reaction, neutralization reaction of amino acids and chlorination reaction. Saponification process of NaOCl acts as an organic solvent that can degrade fats into glycerin (alcohol) and fatty acids, containing -OH which makes fatty acids polar (hydrophilic), as a result, it can reduce the surface tension of the liquid.[15] NaOCl does not have an ability to clean smear layers, one of which consists of inorganic dentin debris. NaOCl can only clean organic materials and does not have an ability to clean inorganic materials so that its power to clean smear layers cannot be optimal.[1]

8% East Java propolis extract, moreover, contains active substances, such as saponins. Saponins have the same characteristics as detergent, often referred to "natural detergents". Saponins can also be considered as glycosides found in many plants, characterized as surfactants. Surfactants serve as active compounds that can be used to lower energy barrier limiting two non-mutually soluble liquids. Surfactants will lower cohesion force (cohesion force will make two substances not stick together when mixed). On the other hand, surfactants can improve adhesion force (adhesion force will make two substances stick together when mixed) so they can reduce the surface tension. This ability is due to the hydrophilic and hydrophobic groups owned by surfactants.[11]

Saponins actually have a long hydrocarbon chain with the tip of the ion group consisting of non-polar (hydrophobic) and polar (hydrophilic). Non-polar groups interact with grease/ oil/ dirt (in this study, the dirt in the form of smear layer). Saponin molecules move around smear layers and then form a ring called a micelle or micelles. The tip containing hydrophilic group will attract water molecules, while the other tip containing hydrophobic group will bind dirt. Cleaning process will occur, in which smear layers will be absorbed into the center of micelles making them change into substances easily dispersed and dissolved in water. Meanwhile, the polar group will dissolve in water to form foam and bind smear layer particles to form an emulsion. When there is maxi probe turbulence movement, smear layers will be carried out. Therefore, saponins can be characterized as surfactants because they can lower surface tension so that smear layers can be dissolved and carried out as irrigation repeated at every Protaper/file substitution.[11,14]

In addition, the smear layers in the control group irrigated with aquadest covered almost the entire dentin tubules. This is because aquadest only serves to moisten the root canal alone and does not have an ability as a surfactant to be able to dissolve smear layers.[13]

Based on the results of Kruskal-Wallis test followed by Mann-Whitney test, there were significant differences among the groups. The median value also showed that aquadest had the biggest value. It indicates that aquadest was the least effective. Meanwhile, 8% East Java propolis extract had the smallest value that indicates propolis extract as the most effective. It means that the group irrigated with 8% propolis extract showed the cleanliness of the root canals. This is because an active substance, saponin, contained in propolis extract acts as a surfactant that can reduce the surface tension of the root canals, so smear layers can be dissolved.[13,14] Finally, it can be concluded that 8% East Java propolis extract is the most effective material for cleaning smear layers in root canal compared to 2.5% NaOCl and 5% NaOCl.

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