



15th FDI-IDA Continuing Dental Education

Allia Hotel Solo Indonesia
13 - 16 November 2019

Proceeding of The 15th FDI-IDA Continuing Dental Education Programme

"Updates in Current Scientific and Technological Innovations in Dentistry"



Alila Hotel, Solo November 15-16, 2019

online version available at
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LSKI Lembaga Studi Kesehatan Indonesia
Bandung 2020

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First published by Lembaga Studi Kesehatan Indonesia (LSKI) Bandung 2020
for 15th FDI-IDA Continuing Dental Education Programme Committee

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Setting, Layout Cover Design Preprinted Copyright ISBN	Heryanto Ary Kurniawan Agus Sono © 2020 15 th FDI-IDA Continuing Dental Education Programme Committee 978-602-60959-6-1

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FOREWORD

Science and technology in the field of dentistry are developing rapidly with the passage of time. These developments require practitioners in dentistry to be constantly up to date with the newest trends. One of the ways in which dentists can improve their knowledge and skills in maintaining the quality of services for society is to attend scientific conferences. In light of this, the FDI World Dental Federation together with the Indonesian Dental Association (PDGI) organize an international scientific conference and exhibition on the latest and most advanced medications, tools and materials in the practice of dentistry, taking place in different cities in Indonesia every year.

This year, the Solo Regional Branch of the Indonesian Dental Association has been honored to receive the opportunity to organize the 15th FDI-IDA Continuing Dental Education Programme 2019, 15-16 November, in Alila Hotel Solo. Through this forum, themed "*Updates in Current Scientific and Technological Innovations in Dentistry*", we are trying to provide space for a very comprehensive and excellent exchange of knowledge and experience by organizing main and short lectures, full paper and e-poster presentations, oral presentations and competition, Pepsodent FDI Award, as well as a dental equipment exhibition. The presented papers are compiled in this proceeding in the form of research papers, case reports, and literature reviews.

This year's seminar also brings something new to the experience since various organizations from different disciplines have joined us to enrich and broaden our discussion. These renowned organizations are the Military Dentistry, International Military Dental Forum (IMDF), International College of Dentists (ICD Region 38), Indonesian Conservative Dentistry Society / ICDS (IKORGI) and Indonesian Society of Periodontology (IPERI).

I would like to extend my heartfelt thanks and gratitude to these esteemed organizations, reputable invited speakers, notable dental suppliers, sponsors, as well as all participants for the strong support and enthusiasm in making this event a success. Most importantly, I would like to extend my deepest gratitude towards the team of reviewers who have meticulously chosen the selected papers and made the publication of this proceeding a success. I would also like to express my gratitude to the steering and organizing committee for their tireless effort and commitment.

Sincerely yours,
Sherly Indratno, drg., Sp. Ort
Chairperson of the Organizing

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The Irrigation Effect of Mangosteen Rind (*Garcinia mangostana* Linn.) Extract in The Root Canal of Wistar Rat (*Rattus norvegicus*) on the Number of Neutrophil Periapical Tissue

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ABSTRACT

Root canal treatment has three stages that called triad endodontic treatment, there are preparation, sterilization, and root canal obturation. Sterilization is the most important stage as a determinant of the success of root canal treatment. The irrigation material commonly used in root canal treatment is sodium hypochlorite 2.5%. The irrigation material when passing through apical foramen, it can trigger an inflammatory response and it can cause tissue damage. Based on this situation, an alternative material is needed for root canal irrigation especially the material comes from nature. The alternative material that can be used as root canal irrigation material is mangosteen peel extract (*Garcinia mangostana* L.) because of its various contents when the extract is penetrated into periapical tissue it does not have an effect on the number of neutrophil cells. This study used a laboratory experimental post-test only control group design. In this study, 48 male wistar rats were given treatment. The sample were divided into 4 groups. The control group consisted of positive controls (sodium hypochlorite 2.5%) and negative controls (sterile aquades). The treatment group (mangosteen peel extract) with concentrations 80% and 100%. The sample were divided again into different days, the first day, third day, and seventh day. Results: The results of this study showed 100% mangosteen peel extract showed the least number of neutrophil cells compared to the other groups. Mangosteen rind extract can be used as root canal irrigation material and it doesn't have any effect on the number of neutrophil cells.

Keywords: Root Canal Treatment, Irrigation Material, Sodium Hypochlorite, Mangosteen Rind Extract

INTRODUCTION

The damaged teeth either due to caries or trauma if not treated can cause pulp death or pulp necrosis. Root canal treatment is needed for teeth with necrosis. Endodontic treatment includes three stages known as triad endodontic treatment. The triad endodontic treatment includes three phases, namely root canal biomechanical preparation, disinfection, and root canal obturation¹. Root canal irrigation has an important role in debris removal and disinfection of the root canal, this procedure cannot be separated from the root canal preparation procedure. Stages of root canal preparation are cleansing and forming of the root canal, which is done by honing the root canal wall. This action results in debris and smear layers.

The smear layer can contain organic substances, inorganic substances, and microorganisms, thus the walls of the root canals needs to be cleaned². The purpose of doing root canal irrigation is to dissolve debris, especially organic and non-organic which is contained in the root canal and hidden areas where bacteria breed, clean dentin flakes so as to prevent the closing of the root canal, an instrument lubricant inserted into the root canal, as a bleaching agent of coronal and radiks³.

Irrigation materials that are often used include sodium hypochlorite (NaOCl), kelator solution or Ethylene Diamine Tetraacetic Acid (EDTA), Mixture of Tetracycline, an Acid, and a Detergent (MTAD), Iodine Potassium Iodide (IPI), and chlorhexidine. Irrigation materials can also come from a combination of several materials, but sodium hypochlorite remains as the main irrigation solution. Root canal irrigation which is often used is sodium hypochlorite. Deficiencies of sodium hypochlorite include toxic effects on vital tissues, damage to the skin, eyes and can cause tissue necrosis if the irrigation material passes through the apical foramen and causes neurological complications⁴. Strong pressure when irrigating with sodium hypochlorite can cause the material to pass through the apical foramen so that it hits the periapical tissue. This is because the apical foramen is still wide, apical constriction is damaged during root canal preparation, and the presence of root resorption⁴. Sodium hypochlorite irrigation material that passes through the apical foramen can cause an inflammatory response that is the occurrence of vasodilation and an increase in vascular permeability so that neutrophyl cells which are the first cells chemically will go to the inflammatory area⁵.

Based on the lack of sodium hypochlorite irrigation materials that are often used in dental practice, researchers wanted to find an alternative natural irrigation material to replace that material. An alternative irrigation material that can be used is the mangosteen (*Garcinia mangostana* L.) rind extract. Previous studies had shown that mangosteen rind had benefits as anti-inflammatory, antihistamine, antibacterial, antifungal, anticancer, antihypertensive, antistroke, HIV therapy, and high levels of complex antioxidants, especially phenolic or polyphenol compounds including xanthone compounds. Xanthone compounds have a derivative compounds that are alpha-mangostin and gamma-mangostin which play as an antibacterial, the alpha-mangostin is known as well as antibiotics such as ampicillin and minocycline. Besides xanthenes, another content contained in mangosteen rind is flavonoids which can reduce the inflammatory response by preventing the activity of the cyclooxygenase (COX) enzyme which is an inflammatory marker enzyme in the body⁶.

Mangosteen rind extract was expected to be used as an alternative material for root canal irrigation; therefore another biocompatibility test was needed to determine the effect of the material if it was pushed into the periapical tissue. The in vivo biocompatibility test was implemented in male Wistar rats (*Rattus norvegicus*). The test was to see the inflammatory effect of mangosteen rind extract material on rats' periapical tissue by looking at the presence of inflammatory cells, which is neutrophyl cells. Based on the background above, the researchers wanted to conduct a research on the effects of mangosteen rind extract with a concentration of 80% and 100% as an irrigation agent seen from the number of neutrophyl cells in perapical tissue.

METHOD

This research was a laboratory experimental research with the posttest only control group design. The research was carried out in the Laboratory of Botany and Tissue Culture, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Jember to certify mangosteen (*Garcinia mangostana* Linn.) Species, Laboratory of Pharmacy Biology, Faculty of Pharmacy, University of Jember for manufacturing mangosteen rind extract, Laboratory of Biomedics, Faculty of Dentistry, University of Jember for the maintenance and treatment of wistar rats, Anatomical Pathology Laboratory of the Faculty of Dentistry, University of Jember for the manufacture and observation of tissue preparations in September 2018 to April 2019.

This research was a laboratory experimental by using 48 rats which were divided into 4 groups, positive control group (NaOCl 2.5%), negative control group (Aquadex), treatment 1 of mangosteen rind extract 80%, and treatment 2 of mangosteen rind extract 100%. The ingredients were irrigated into root canal of the molar teeth of male wistar rats that has been prepared its root canal by using file until apical foramen perforation was obtained to facilitate the penetration of the irrigation material into periapical tissue. The Teeth tissue and bone around were cut and

used as histology preparation and was given color by using hematoxylin-eosin. The preparation was observed for its neutrophil cells that appeared in pericapical tissue under the prepared teeth by using microscope with 400x magnification in the 1st, 3rd, and 7th days.

RESULTS

Based on the research, the average number of neutrophil cells is presented in Table 1.

Observed	Day 1 (X ± DS)	Day 3 (X ± DS)	Day 7 (X ± DS)
K-	3.25 ± 0.25	2.50 ± 0.28	1.25 ± 0.47
K+	2.75 ± 0.25	3.75 ± 0.85	2.50 ± 0.50
P1	3.50 ± 0.28	2.75 ± 0.25	2.75 ± 0.25
P2	2.50 ± 0.28	2.25 ± 0.25	1.25 ± 0.47

Descriptions:

X : average of lymphocyte

DS : standard deviation

The above table showed the number of neutrophil cells was mostly high in mangosteen rind extract 80% in the first day and the lowest number of neutrophil cells was in aquades and mangosteen rind extract in the seventh day.

The data obtained were then tested for its normality and homogeneity through Shapiro-Wilk and Levene test. The significance values of normality test was $p < 0.05$ and homogeneity test was more than $p > 0.05$, it means that the data were abnormal and homogeneous, it was then followed by statistical test in the form of nonparametric test. Nonparametric test performed was Kruskal-Wallis test. The significance value of Kruskal-Wallis was $p < 0.05$, it points out that there was a difference between the time and duration of exposure on all treatment groups, then Mann-Whitney test was done on it. The test showed that there was no significant difference ($p > 0.05$) between all treatment groups, except the aquadest group on first day with the aquadest group on seventh day; they revealed that there were significant differences ($p < 0.05$), the aquadest group on seventh day was carried out with 80% of mangosteen rind extract on the seventh day, and 80% mangosteen rind extract on the seventh day was with 100% mangosteen rind extract on the seventh day.

DISCUSSION

Concerning this research, after irrigation material with 2.5% NaOCl were penetrated into the periapical region, it did not trigger an acute inflammation after being observed from day 1 to day 7. 80% and 100% irrigation material of mangosteen rind extract did not trigger any inflammations in the apical area of the teeth during day 1 to day 7. Likely, it occurred as the concentration of NaOCl and mangosteen rind extract was not irritative to the apical region. The concentrations of NaOCl used as irrigation material ranges from 0.5%, 1%, 2.5%, 5.25%, and 6% 7. NaOCl concentration that is considered the most appropriate for root canal treatment is 2.5% since even though it has low cytotoxicity but it still shows good bactericidal action and dissolution time compared to 0.5% and 1% concentration⁸. NaOCl concentration which has a cytotoxic effect on the contact with vital tissue and is able to cause hemolysis was 5.25% concentration⁹.

The cytotoxic effect of the irrigation content is due to the formation of hypochlorous acid (HOCl-) and hypochlorite ion (OCI-); if they are in contact with organic tissue, it releases chlorine. Chlorine is capable to damage the metabolism of bacterial cell. Chlorine refers to a strong oxidizing agent, the absence of antioxidant in irrigation material creates oxidative free radical with reactive oxygen, due to its reactive characteristic the free radical will oxidize the substances that are beneficial to the body and cause tissue damages. At the end, it makes the irrigation material have high toxicity at high concentration and tend to irritate the tissue when it comes into contact^{10,5,5}.

The influence of NaOCl on the tissue is also influenced by pH, in which the irrigation material with pH of 11-12.5 is able to cause the damages due to protein oxidation⁹, that was why this research used 2.5% NaOCl that was in contact with the periapical tissue of the teeth was still tolerated by the body as there was no sign of inflammatory response increase from day 1 to day 7. The natural alternative substitute of NaOCl for root canal irrigation that was investigated in this research used mangosteen rind extract with the concentrations of 80% and 100%. The extract also did not trigger an inflammatory response when they were in contact with the apical tissue from day 1 to day 7, there was no increase in the number of acute inflammatory cells (neutrophils); it happened due the report that the compound of mangosteen rind contains xanthenes which are included in flavonoids, mangostin, garsinon, anthocyanins, and tannin¹¹.

Xanthenes are included in flavonoids in which the content plays a role in suppressing the inflammatory response. The inflammatory process causes many substances released endogenously, it is known as an inflammatory mediator. One of the inflammatory mediators is arachidonic acid because arachidonic acid plays a role in the biosynthesis of prostaglandins through the cyclooxygenase way. Xanthenes contained in the mangosteen rind have been proven to have anti-inflammatory characteristic *in vitro* and *in vivo*, able to prevent the activity of the cyclooxygenase (COX) enzyme where the enzyme is an enzyme marking inflammation in the body¹². Cyclooxygenase 2 is the main enzyme whose work of inflammatory process is inhibited by xanthenes, so that if an inflammatory response occurs, it can reduce the inflammatory process marked by a decrease in neutrophil cell levels^{13,14}.

The content of xanthenes in extract of mangosteen rind also has the ability as an antioxidant. The existence of free radicals can disrupt stable molecules in the tissue, thus binding to the electrons, so that the molecule becomes unstable. Increased free radicals cause the level of Reactive Oxygen Species (ROS) through the antioxidant defenses in the body called oxidative stress. This causes lipid peroxidation in cells, which can damage these cells. Mangosteen rind that contains a lot of antioxidants, can inhibit lipid peroxidation, so that it will prevent cell damage^{10,15}.

Irrigation material in teeth root canal preparation must have antibacterial ability such as NaOCl irrigation material, in low concentrations it can kill bacteria very quickly, but if the material is in high concentration and pH, it can be very toxic to tissue, that is why the recommended NaOCl concentration as irrigation material is the concentration of 2.5%^{16,17}. The use of extract of mangosteen rind as the irrigation material also has antibacterial characteristic because the xanthone mangosteen has alpha-mangostin derivative which has antibacterial activity¹⁸. Besides, the tannin content in the mangosteen can also be antibacterial.

The advantage of using extract of mangosteen rind as a root canal irrigation material compared to NaOCl aside from being anti-inflammatory and antibacterial, extract of mangosteen rind contains emulgator or detergent saponins which has the ability to dissolve smear layer which has an organic component consisting of pulp tissue and bacterial debris and dissolving inorganic components which is debris dentin¹⁹, whereas NaOCl cannot dissolve inorganic component²⁰. Therefore, mangosteen peel extract was able to use as an alternative of potential irrigation material to replace the existing irrigation material such as NaOCl (sodium hypochlorite). From the observation, it was known that 80% and 100% concentrations of extract of mangosteen rind were found not to trigger an inflammatory response if the irrigation material was pushed into the periapical area, so that extract of mangosteen rind of 80% and 100% was able to use as an alternative to root canal irrigation.

CONCLUSION AND SUGGESTION

From the results of the research that had been conducted, it can be concluded that the extract of mangosteen rind as an irrigation material of 80% and 100% concentrations did not trigger an inflammatory response so that it had no effect on the number of neutrophil cells in

periapical tissue of teeth, so that it was able to use as a natural alternative material for root canal irrigation.

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