PROCEEDING

INTERNATIONAL SEMINAR

2nd DENTISPHERE

"Current Concept in Dentistry"

Shangri-La Hotel, Surabaya, November 8-9, 2013
WELCOME NOTES

Dear Colleagues,

It’s great honor to welcome you to Surabaya and 2nd International Dentisphere 2013 seminar which held on 8-9th November, 2013 at Shangrilla Hotel. My great appreciation to all the speakers from Japan, Korea, Thailand, Singapura and Indonesia, thank you for the contribution and participation and your willingness to come and share the valuable knowledge and experience. It’s been honor to us that this forum may be apart of strong role as quality control mechanism to ensure sustainability and continuous improvement of dentist.

The theme of this 2nd International Dentisphere is “Current Concept In Dentistry” This is addressed to meet our aims to provide our nation a generation of professional and skillfull dentist with continuously update knowledge. We hope this event will be increase our professionalism to all dentist and participants.

My appreciation to the committee, for aranging this event very well. Hope the seminar will be well done accomplished tomorrow. Also, I would like to thank all sponsors who support this event. For the speaker, thank you for the contribution support of the seminar.

And for all the participants, thank you for joining the 2nd International Dentisphere, please enjoy the seminar and the events. I would like to ask for apologize if maybe in some ways we have some limitations in serving you on the event. Finally, I hope we all could get the benefit and advantage from this seminar to raise our professionalism in dentistry, in each of our ways

Sincere regards,

Dr. Dian Mulawarmanti., drg., M.S

Dean Faculty of Dentistry Hang Tuah University
WELCOME NOTES

Dear Colleagues,

It is a great pleasure for us to be the organizer of the 2nd Dentisphere from faculty of dentistry Hang Tuah University. We extend our warmest welcome to all Participants, Speakers, and Sponsors that make this 2nd Dentisphere to be a successful conference.

Under the theme of “Current Concept of Dentistry”, this meeting will offer a platform to learn and exchange ideas with a host of internationally and national speakers. 2nd Dentisphere will provide participants with unique opportunities to develop their professional knowledge and skills as well as to network with another audience. I also strongly encourage you to take advantages of the presence of dental companies to keep up to date with evolving technologies of equipment and the latest dental materials. We do hope that this seminar will allow all participants to capitalize enough knowledge and experience keep in touch with issues world wide. Dental Health.

Lastly I wish to thank all Participants, Distinguish Speakers, Sponsors and all who contribute for the success of the 2nd Dentisphere in Surabaya. Hope you not only have an event for developin our professionalism, but also you could enjoy a nice stay and have a memorable excursion on Surabaya. Thank you for your kind attention, have a nice, enjoy and fruitful discussion and God Bless You.

Sincere regards,

Aprillia drg.,Sp.KG
Chairperson 2nd Dentisphere
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EFFECT OF Avicennia marina sp LEAF EXTRACT TO RAT GINGIVAL CATALASE LEVEL INDUCED BY MIX PERIODONTOPATHOGEN BACTERIA

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ABSTRACT

Background: Periodontal disease is the second largest oral disease in Indonesia population caused by infection of periodontopathogen bacteria. Most of the bacteria of periodontitis are Gram negative anaerobic bacteria. Avicennia marina sp is a natural product that has some medical potential regarding to its nutritional contents including antioxidant activity.

Objectives: The aim of this study is to investigate the effect of Avicennia marina sp extract on catalase activities in gingival Wistar rats induced mix periodontopathogen bacteria.

Methods: The experiment was held by post test only control group design. Fivety male Wistar rats divided into five group. Group-1 group was negative control group, group-2 group was a positive control group, and the other groups were induced by mixed periodontopathogen bacteria and treated with Avicennia marinaspleaf extract on various concentration. After treatment, the rats were sacrificed. Gingival catalase level (mg/ml) of each group was measured. All of datas were analyzed by one way ANOVA and LSD multiple comparison test at 5% significance level.

Result: This study showed that gingival catalase level was significantly lower in group-2 than group-1. Gingival catalase level in treatment group was significantly higher than control positive group.

Conclusion: Avicennia marina sp leaf extract can increase rat gingival catalase level.

Key words: Avicennia marina sp leaf extract, periodontitis, catalase

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INTRODUCTION

Periodontal infections occur when biofilm microorganisms initiate a host immune response and produce signs of periodontitis, including loss of connective tissue attachment and alveolar bone resorption.\textsuperscript{1,2,3} As a chronic inflammatory disease, periodontitis is the most commonly encountered dental disease, appearing large scale of the population. This disease is the result of an interaction between microbial biofilms and host response in gingival connective tissue, which leads to gingival bleeding, periodontal pocket formation, connective tissue destruction, and alveolar bone resorption, ultimately causing tooth loss.\textsuperscript{4,5,6} Although oral bacteria and their products, such as lipopolysaccharide and proteases, are considered to be the primary cause of periodontitis, host response to the subgingival plaque biofilm seems to be the main factor that contributes to the progression of this disease.\textsuperscript{7}

Bacteriamediated periodontal diseases generally result in polymorphonuclear leukocyte (PMNL) infiltration. PMNLs are an important part of the immune system, and appear to be functionally activated and exhibit increased production of reactive oxygen species (ROS).\textsuperscript{8,9,10} In the tissue, ROS mediated myeloperoxidase (MPO) activity can lead to an increase in inflammation severity.\textsuperscript{11}

The oxygen-dependent production of reactive oxygen species (ROS) is a principal part of normal cellular metabolism as a host defence mechanism against bacterial pathogens.\textsuperscript{12} Because ROS is a considerably toxic agent, production of ROS in large amounts, including hydrogen peroxide, superoxide, hydroxyl radicals, nitric oxide, and peroxynitrite not only serve as antimicrobial agents, but also lead to injury of extracellular structures.\textsuperscript{13} ROS can also stimulate lipid peroxidation (LPO). Previous reports\textsuperscript{5,6} have shown that periodontitis is associated with increased lipid peroxidation in gingival crevicular fluid and saliva. Excessive production of LPO can cause oxidative stress and, ultimately, damage to cell integrity. The studies\textsuperscript{14,15} have also demonstrated that polymorphonuclear leukocytes (PMN) are the primary mediators of host response against proliferating pathogenic microorganisms during periodontal disease, which causes oxidative DNA damage in gingival tissues. This evidence shows that oxidative stress is one of the factors that leads to the progression of periodontal disease.\textsuperscript{5} Oxidative stress develops when the levels of antioxidants are lowered. Thus, activity of antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) is important in cell defence.\textsuperscript{9} In the literature there are a lot of studies that have reported on the effects of antioxidant agents against oxidative stress, and recently researchers have intensely investigated boron (B) as an antioxidant agent.\textsuperscript{16,17,18}
Recent medical and dental research in this area has been geared towards the prevention of free radical mediated diseases by using specific nutrient antioxidants. Other important candidate factors that may modulate periodontitis are pro-inflammatory cytokines such as tumor necrosis factor-a (TNF-a), interleukin (IL)-1b known to be up-regulated early in the course of periodontitis. In addition, recruitment of inflammatory cells from the circulation is an important process to augment the inflammatory response. Pro-inflammatory cytokines production also induces the expression of adhesion molecules in the vascular endothelium, and invasion of inflammatory cells into inflamed tissues subsequently occurs. P-selectin, a member of the selectin family of adhesion molecules, and intercellular adhesion molecule-1 (ICAM-1), both of which are expressed at the surface of the vascular endothelium, are involved in this process. Various mediators contribute to the upregulation of endothelial cell and leukocyte-adhesion molecules in inflammation.

Principles of periodontal therapy is to reduce the supragingival or subgingival plaque and calculus with appropriate action for oral health. However, scaling and root planing fails to demonstrate maximum results to eliminate plaque and bacteria in the long term because can’t eliminate the primary etiologic completed so that the bacteria will experience recolonisation. Antibiotics used to support mechanical periodontal therapy because antibiotics kill the subgingival bacteria remain. Unfortunately, treatment antibiotics with inadequate doses and for a long time contributed greatly to the increase of antibiotic resistance. Bacterial resistance to antibiotics has become a problem in the world.

Antibiotics consisting of natural and synthetic antibiotics. Antibiotic synthesis have adverse effects if used carelessly. While natural antibiotics are generally derived from secondary metabolites derived from the extract of a particular plant, which is expected to have efficacy for the drug. The high level of biodiversity of flora in Indonesia, many of which are used as herbal drug such as Avicennia marina sp. There are many types of Avicennia marina sp which is a mangrove species that can tolerate a wide range of salinity than other types of mangrove lainnya. Parts of Avicennia marina sp containing various active compounds such as flavonoids, tannins, and saponins which are compounds potentially useful as an antioxidant and anti-inflammation. The aim of this study is to investigate the effect of Avicennia marina sp extract on catalase activities in Wistar rats induced gingival mix periodontopathogen bacteria.
MATERIAL AND METHODS

Animals preparation. Fivety male Wistar rats weighing about 220–250 g (8 weeks of age) were housed in an air-conditioned room (23–25°C) with a 12-h light–dark cycle and they received humane care. They were kept in the cage for 1 week for proper acclimatization before starting the experiment under controlled conditions of illumination (12 h light/12 h darkness) and temperature (23±2 °C). The animals were given standard rat pellets and tap water ad libitum. All experiments in this study were approved by the Local Ethics Board of Animal Experiments at Faculty of Dentistry HangTuah University.

Animal periodontitis model. The experiment was held by post test only control group design. Fivety male Wistar rats were randomly divided into five group. Group-1 group was negative control group, group-2 group was a positive control group, and the other groups were induced by mixed periodontopathogen bacteria and treated with Avicennia marina sp leaf extract on various concentration. Group-1 was negative control group without any treatment, group-2 was a positive control group induced by mixed periodontopathogen bacteria, and the other groups-3, 4, and 5 were induced by mixed periodontopathogen bacteria and treated with Avicennia marina leaf extract on serial dose: K3 (0,25 gr/kg/day), K4 (0,5 gr/kg/day), K5 (1 gr/kg/day). These animals were kept in position for 35 days to promote microbial dental plaque accumulation and inflammation.28,29 After 35 days the ligature was removed. The animals were weighed before treatment administration at the beginning of the experiment, and tretment dose was improved according to our previous studies.28 Avicennia marina sp leaf extract on various concentration were given for 15 consecutive days after mix periodontopathogen bacteria induction. At the end of the experimental period, the animals were sacrificed under anesthesia and then the surrounding gingiva were removed for catalase level analyses, according to the delivered standardizes procedure.29

Biochemical analysis for catalase activity measurement. For biochemical gingival catalase analysis, all collected samples were obtained from gingivo-mucosal tissues. Then the samples were immediately stored in a deep freeze (at approximately–80 °C) for subsequent laboratory analysis. The homogenate was then centrifuged at 10,000 rpm for 15 min, and the supernatant used for the determination of catalase activity according to the method developed by Bradley et al. And was estimated by colorimetric measurement at 460 nmon an ELISA
plate reader. The amount of enzyme necessary to produce a change in absorbance per 1 s was defined as 1 unit of catalase activity.

**Statistical analysis.** Gingival catalase level (mg/ml) of each group was measured. All of datas were analyzed by one way ANOVA and LSD multiple comparison test at 5% significance level. For statistical analysis, differences between the groups were tested by analysis of variance using SPSS software, version 17.0 (SPSS Inc.).

**RESULT**

There were a significant differences in the gingival catalase activity which can be seen in distribution table 1.

**Table 1. Average effect of Avicennia marina sp extract on catalase activities in Wistar rats induced gingival mix periodontopathogen bacteria(nmol/min/g)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>Std.Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.6570</td>
<td>0.01703</td>
</tr>
<tr>
<td>2</td>
<td>2.9810</td>
<td>0.01449</td>
</tr>
<tr>
<td>3</td>
<td>2.6590</td>
<td>0.01595</td>
</tr>
<tr>
<td>4</td>
<td>2.2560</td>
<td>0.01350</td>
</tr>
<tr>
<td>5</td>
<td>1.9590</td>
<td>0.01663</td>
</tr>
</tbody>
</table>

**Fig 1. Graphic of Avarage gingival catalase activity(nmol/min/g)**
Table 2. ANOVA and LSD test effect of Avicennia marina sp extract on catalase activities in Wistar rats induced gingival mix periodontopathogen bacteria

<table>
<thead>
<tr>
<th>GROUP</th>
<th>GROUP</th>
<th>Mean Difference (I-J)</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP-1</td>
<td>GROUP-2</td>
<td>-1.3240</td>
<td>.000</td>
</tr>
<tr>
<td>GROUP-3</td>
<td>GROUP-4</td>
<td>-1.0020</td>
<td>.000</td>
</tr>
<tr>
<td>GROUP-4</td>
<td>GROUP-5</td>
<td>-0.3020</td>
<td>.000</td>
</tr>
<tr>
<td>GROUP-2</td>
<td>GROUP-3</td>
<td>0.3220</td>
<td>.000</td>
</tr>
<tr>
<td>GROUP-4</td>
<td>GROUP-5</td>
<td>0.7250</td>
<td>.000</td>
</tr>
<tr>
<td>GROUP-3</td>
<td>GROUP-4</td>
<td>1.0220</td>
<td>.000</td>
</tr>
<tr>
<td>GROUP-4</td>
<td>GROUP-5</td>
<td>0.9700</td>
<td>.000</td>
</tr>
</tbody>
</table>

This study showed that gingival catalase level was significantly higher in group-2 than group-1. Gingival catalase level in treatment group was significantly lower than control positive group.

DISCUSSION

The inability to examine initiation and progression of periodontal disease and to assess certain therapies in humans has led to a great interest in the use of animal models in periodontal research. Over the past two decades, various investigations have been implemented to understand the pathogenesis of periodontitis. Although only mechanical and surgical treatment modalities were used for many years, much advancement has been made thanks to detailed research on both causative microorganisms and the host-mediated response.31,32

Periodontitis can be seen in the control positive group with the induction of mix periodontopathogen bacteria. Periodontitis occurs due to increased reactive oxygen species due to the inflammatory process. At the time of the occurrence of inflammation, gingival cells will a first tissue defense against the presence of pathogenic bacteria, lipopolysaccharide (LPS), lipoteichoic acid (LTA), peptidoglycan, and on the network that has been damaged. On the pathogenesis of periodontitis, this is largely mediated by complement complex causes the formation of membrane-attact so that lysis bacterial cells with vasoactive and chemotactic component which is the mechanism of phagocytosis. Macrophages stimulated by bacteria or by the interaction of complement, so the release of interleukin-1 (IL-1), tumor necrosis factor , and the synthesis and the release of neutrophils chemotactic factor (IL-8). This resulted in
migration of neutrophils that occurs opsonization process, then lysis by ROS, such as the system H₂O-myeloperoxidase or lipid peroxidation-O₂. However, ROS are unstable oxygen compounds, that in this study which comes from the induction mix periodontopathogen bacteria that causes the increase of reactive oxygen compounds. Catalase is an endogenous antioxidant. Antioxidants are chemical compounds that have the capability to provide hydrogen radical electron. As a result, these compounds can alter radically the nature of any change nonradical and radical oxidation by antioxidants. Antioxidants consist of endogenous antioxidant produced by the body itself and exogenous antioxidants derived from food. If there is an increasing number of free radicals in the body (can be caused by inflammation, food, drugs, radiation, etc.) then the body will do the defense that is by increasing the activity of endogenous antioxidants such as superoksid dismutase, catalase, and glutathione, but in the event of exposure Excessive oxidant antioxidant body will not be able to handle it, so that the body requires external supply of antioxidants (flavonoids, vitamin A, vitamin C, vitamin E, selenium, zinc, and L-cysteine).  

Today, new periodontal treatment strategies are focused more on learning about the role of the host response and host-modulatory agent. It is emphasized that the adjunctive use of hostmodulatory agents can help to increase therapeutic responses, can be efficacious in slowing the progression of disease, and can make the responses more presumably in the susceptible host. For this reason, numerous host-modulatory agents such as anti-inflammatory and anti-oxidant agents were investigated to cope with the breakdown of the soft and hard periodontal tissues.

Recently, evidence has emerged that reduced total antioxidant capacity and enhanced oxidative damage within the oral cavity lead to the progression of periodontal tissue destruction. Moreover, periodontitis patients show increasing levels of LPO and ROS, which cause a state of oxidative stress. Reactive oxygen species are significant signalling molecules in several cellular processes. When there is excessive production, these molecules act as a toxic substance that leads to cellular damage (proteins, lipids, and DNA). There is a defence mechanism against ROS provided by enzymatic and non-enzymatic antioxidants to prevent their deleterious actions. In healthy people equilibrium is present between the production of ROS and tissue concentration of antioxidants.

Antioxidants have been extensively used as food additives and may playa crucial role in the treatment of many degenerative and chronic diseases such as periodontitis. Considering
increased oxidative stress and reduced total antioxidant capacity in periodontal disease, researchers are using antioxidant agents to treat or alleviate this disease.\textsuperscript{33,34}

\textit{Avicennia marina sp} leaf extract treatment on serial dose 0.25 gr/kg/day; 0.5 gr/kg/day and 1 gr/kg/day caused increasing gingival catalase activity significantly from the positive control group. It showed that \textit{Avicennia marina sp} leaf extract has protective effects against free radicals, although this increase has not been able to restore the initial state (negative control group).

Periodontal tissue damage induced by the mix of bacteria periodontopathogen compounded by the existence of a series of mechanisms involving ROS compounds. During phagocytosis, macrophages and neutrophils as effector cells also produce toxic oxygen combined fagosom and lysosomes become fagolisosom responsibility to help kill and swallow microorganisms. Most of those involved in the process of phagocytosis is hydrogen peroxide (H2O2), superoxide anions (O2\textsuperscript{-}), and nitrogen oxide (NO), is directly toxic to the bacteria. Everything is generated through the oxidation of NADPH and other enzymes in a process called respiratory burst, due to increased consumption of oxygen in the inflammation. Macrophage activity is very efficient in destroying pathogens, this activity in vivo is usually in conjunction with local tissue damage caused by the release of antimicrobial mediators as free radicals. Have gingival epithelial cells of the immune system, cell membranes, the GCF (gingival creviculer fluid). One form of the antioxidant defense system were found (exogenous antioxidants) contained in the GCF to counteract the effects of oxidants and free radicals, but the number was limited. In the event of exposure or excessive production of oxidants, it will be seen an increase of antioxidant activity as a process of defense. In addition, because the numbers are limited, it is necessary to the existence of additional endogenous antioxidants.\textsuperscript{33,34}

In summary, the results of this study reveal that \textit{Avicennia marina sp} leaf extract contains compounds that are antioxidants. \textit{Avicennia marina sp} leaf extract can decrease rat gingival catalase level. The components that contain antioxidants are very instrumental in securing the stability between the consumption of oxidants and free radicals and antioxidants in the body's production so as to provide protective and therapeutic effects on disease periodontitis because it proved to reduce the activity of catalase. Then it can be concluded that alternative therapies of periodontitis can be done by giving exogenous intake of antioxidants.\textsuperscript{35,36,37}
ACKNOWLEDGEMENTS

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REFERENCES


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Committee

Moderator

Table Clinic Lecturer

Speaker

Table Clinic Participant

Seminar Participant