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Protection Effect of Gray Mangrove Extract to Oxidative Stress after Mixed Periodontopathogen Bacteria Infection-Animal Models

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ABSTRACT
Periodontitis is caused by periodontopathogenic bacterial infection. There is a relationship between Reactive Oxygen Species (ROS) and the pathogenesis of periodontitis. The imbalance between ROS and antioxidant trigger an oxidative stress response which causes periodontal destruction. Leaves of gray mangrove have been reported to possess antimicrobial, antioxidant, and anticandidal activities. The aim of this study is to investigate the protective effect of the gray mangrove leaf extract on the lipid peroxidation level and catalase activities in saliva periodontitis Wistar rats. The experiment was post-test only control group design. Thirty-five male Wistar rats divided randomly into five groups. 1st group (G1) was a negative control without treatment, 2nd group (G2) was a positive control that induced by mixed periodontopathogenic bacteria, and 3rd,4th,5th group (G3, G4, G5) induced by mixed periodontopathogenic bacteria and treated with gray mangrove leaf extract at dose 0.25 g/kg, 0.5 g/kg, and 1 g/kg daily, respectively. After treatment, the rats were sacrificed. Salivary malondialdehyde level (mg/mL) was measured by the thiobarbituric acid (TBA) method. Catalase activity (U/mL) was measured by optical density method. Data were analyzed one way ANOVA and Least Significant Difference (LSD) test ($p<0,05$). The gray mangrove leaf extract demonstrated the capacity to significantly decrease the salivary malondialdehyde level ($p \leq 0.05$) and significantly increase salivary catalase activity ($p \leq 0.05$) in periodontitis rats. The gray mangrove leaf extract has a protective effect for oxidative stress which is characterized by a decreasing malondialdehyde level and an increasing catalase activity in salivary rats induced by mixed periodontopathogen bacteria.

Keywords: Gray Mangrove leaf extract, Periodontitis, Saliva, Malondyaldehyde, Catalase.

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INTRODUCTION

Periodontal disease is still a problem in the world and is an inflammatory disease in periodontal tissue consisting of oral hard tissue and soft tissue such as gingiva, periodontal ligament, alveolar bone, and cementum.[1,2] The cause of periodontitis is anaerobic gram-negative bacteria, among others Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Bacteroides forsythus, Treponema denticola, T.socranskii, and P. Intermedia.[3]

Bacterial colonization will occur such as cytokines such as IL-1α and β, IL-6, IL-8 and TNF-α, and PMN in inflammation. PMN outlays will produce free radicals such as superoxide anions, hydroxyl radicals, nitrous oxides and hydrogen peroxide that cause damage to the gingiva, periodontal ligaments and alveolar bone. This also creates an imbalance between oxidative antioxidants, which can increase damage to lipid membranes, proteins, carbohydrates, deoxyribonucleic acid (DNA).[4-6]

Lipid peroxidation is a reinforcing reaction formed by free radicals (hydroxyl radicals) with Poly Unsaturated Fatty Acid Poly (PUFA) on cell membranes that will produce toxic compounds. Among these toxic compounds, the main ones formed are malondialdehyde (MDA).[7,8] Thus, lipid peroxidation can be performed directly with an MDA level. When MDA levels are high in plasma, then it can be ascertained that the cells undergo oxidative stress. Some components of an enzyme that acts as a marker of antioxidant activity are catalase, superoxide dismutase (SOD), glutathione peroxide.[2] Catalase functions to catalyze 2 molecules of hydrogen peroxide solution into 2 molecules H_2O and O_2. Hydrogen peroxide is one form of the antioxidant compound resulting in periodontitis pathomechanism.[2,9,10] The principle of periodontal therapy is by reducing supra and subgingival plaque and calculus by appropriate action and maintaining oral hygiene.[3] Clearance of plaque and bacteria only by mechanical technique alone is not showing maximum results in the long term cause cannot eliminate the primary etiology so that the bacteria will be recolonized. Antibiotics are used as periodontal therapy support manually because antibiotics will kill the subgingival pathogenic bacteria that still exist after mechanical treatment.[3,11] Unfortunately, inadequate use of antibiotics and prolonged use of antibiotics contribute substantially to the increase in antibiotic resistance. Bacterial resistance to antibiotics has become a problem in Indonesian and world hospitals.[8,12]

Antibiotics consist of natural antibiotics and synthesis. Antibiotic synthesis has a bad effect if used carelessly. While natural antibiotics are generally derived from secondary metabolites resulting from the extract of a particular plant, which has medicinal properties.[13] The high level of flora biodiversity in Indonesia, many of which are utilized as medicinal plants. One of them is Avicennia marina. sp. In Indonesia there are many species of gray mangrove (Avicenna marina sp) which is a type of mangrove that is tolerant to the wide salinity range of other mangrove species. Gray mangrove leaves have a potential antioxidant component.[14] Hence, our study aims to prove the protective effect of the gray mangrove leaf extract on MDA level and catalase activity in salivary rats induced by mixed periodontopathogen bacteria.

MATERIALS AND METHODS

Animal preparation

Ethical clearance for this research was approved by the University of Hang Tuah Dentistry Ethics Committee No.142/KEPK/XII/2018. This study used 35 male rats aged 6 months (equivalent to 18 years of human) with a weight of 200-300 grams in acclimatization for 7 days. Then on the 7th day, the rat were divided and marked into 5 groups, namely groups 1st, 2 nd, 3 rd, 4th, and 5th. Each group consisting of 7 rats was placed in 1 cage. Each rat in each group was fed standard and drank the same amount during the experimental process. Food is given by being placed in small containers and given 3 times daily (every morning, afternoon, and evening). While the beverage is given in a 300 ml bottle containing water that is equipped with a small pipe and given ad libitum.[15]

Periodontitis animal model preparation

On day 8th, all groups of rats were given amoxicillin for 4 days and continued to be fed and drunk. The daily dose of amoxicillin for a rat weighing 300 grams was 81 mg amoxicillin solution added to 1.8 ml of *Natrium-Carboxyl Methyl Cellulose* (Na-CMC). Subsequently on day 12th, groups 1st (without any treatment as control group), 2nd, 3rd, 4th, and 5th induced a mixed periodontopathogen bacteria. Inoculation was done by bending 2 ml of $1x10^9$ cells/ml of live bacteria in PBS into 3 places, ie into the stomach using a spherical syringe, along the edge of the upper and lower left, right and left molar gingiva, and the last one through the anal into the colorectal area with a syringe spuit.[16] Giving done 3 times in 4 days. During that time, all groups were kept fed standard and drank an equal amount.[2,17] On day 12th, groups 3rd, 4th, and 5th were also simultaneously given the gray mangrove extract for 25 days at varying doses, ie G1 was given the gray mangrove extract with a dosage of 0.25 g/kg BW, G2 was given the gray mangrove extract with a dosage of 0.5 g/kg BW, and G3 was given the gray mangrove extract at 1 g/kg BW. Finally, on the 37th day, all groups of rats were anesthesia, saliva was collected, and rats were sacrificed. The salivary MDA level and catalase activity were measured by optical density as an indicator using spectrophotometry.

Statistical analysis

The data were analyzed using *Statistical Package for the Social Sciences* SPSS version 17.0 in one-way ANOVA and LSD test with a significant value of 95% (p<0,05).

RESULTS

Table 1: Mean value of salivary MDA level (nmol/mL) and catalase activity (U/mL) in each experimental group

Animal Groups	Mean ± Standart Deviation	
	MDA levels	Catalase activity
1st (G1) group (without any treatment as control group)	22.86 ± 0.99	15.26 ± 0.40
2nd (G2) group (mixed periodontopathogen bacteria)	63.28 ± 0.90	5.90 ± 0.39
3rd (G3) group (mixed periodontopathogen bacterial+gray mangrove extract 0,25 g/kg BW)	45.81 ± 0.78	8.95 ± 0.34
4th (G4) group (mixed periodontopathogen bacterial+gray mangrove extract 0,5 g/kg BW)	42.04 ± 0.64	10.63 ± 0.44
5th (G5) group (mixed periodontopathogen bacterial+gray mangrove extract 1 g/kg BW)	37.46 ± 0.43	11.09 ± 0.48

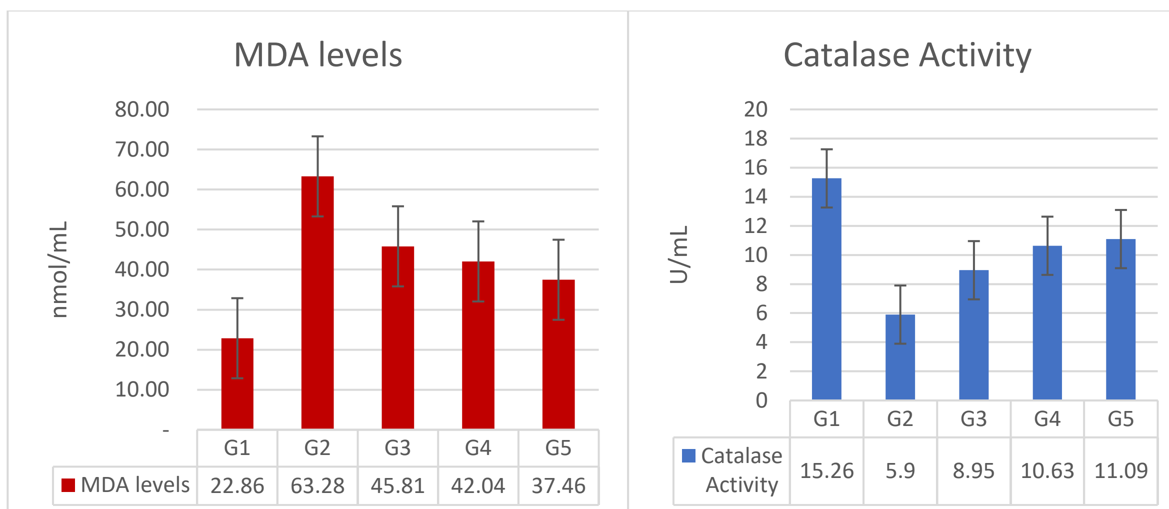

Figure 1: Mean value of salivary MDA level (nmol/mL) and catalase activity (U/mL) in each experimental groups.

Table 2: ANOVA and LSD test of *salivary MDA level* in each experimental groups

Groups	G1	G2	G3	G4	G5
1st (G1) group (without any treatment as control group)		0.000*	0.000*	0.000*	0.000*
2nd (G2) group (mixed periodontopathogen bacteria)			0.013*	0.002*	0.000*
3rd (G3) group (mixed periodontopathogen bacterial+gray mangrove extract 0,25 g/kg BW)				0.439	0.044*
4th (G4) group (mixed periodontopathogen bacterial+gray mangrove extract 0,5 g/kg BW)					0.199
5th (G5) group (mixed periodontopathogen bacterial+gray mangrove extract 1 g/kg BW)					

Note: * = p<0.05

Table 3: ANOVA and LSD test of salivary catalase activity in each experimental group

Groups	G1	G2	G3	G4	G5
1st (G1) group (without any treatment as control group)		0.000*	0.000*	0.000*	0.000*
2nd (G2) group (mixed periodontopathogen bacteria)			0.027*	0.008*	0.000*
3rd (G3) group (mixed periodontopathogen bacterial+gray mangrove extract 0,25 g/kg BW)				0.378	0.044*
4th (G4) group (mixed periodontopathogen bacterial+gray mangrove extract 0,5 g/kg BW)					0.234
5th (G5) group (mixed periodontopathogen bacterial+gray mangrove extract 1 g/kg BW)					

Note: * = p<0.05

Based on the LSD test, it was found that there was an increase in salivary MDA levels and decrease salivary catalase activity in the G2 group compared with the G1 group which showed significant difference (p <0.05). It is known that the highest value of salivary MDA levels and the lowest value of salivary catalase activity in the G2 group which are induced mixed periodontopathogen bacteria for 3 times in 4 days. While the lowest value of salivary MDA level and highest salivary catalase activity in the G1 group of rats which was negative control group without any treatment. The experimental periodontitis caused significant (p<0.05) reduction of the salivary catalase activity as well as an increase in salivary MDA levels (Table 1 and Figure 1) when compared to the control group without any treatment. This suggests that mixed periodontopathogen bacteria induction in the G2 group may increase salivary MDA levels and decrease salivary catalase activity. It is suggesting that oxidative stress is observed in periodontitis.

Furthermore, there was a decrease in salivary MDA levels and increase salivary activity showing significant differences (p<0.05) in G3, G4, and G5 group when compared with the G2 group. Administration of the gray mangrove extract with dose 0.25g/kg BW; 0.5g/kg BW; 1g/kg BB orally for 25 days increased salivary catalase activity and reduced salivary MDA levels compared with the G2 group.

DISCUSSION

This study, an increase in the salivary MDA levels and decrease the salivary catalase activity can be an indicator of increased free radical and oxidant activity, which can be ascertained that cells experience oxidative stress and can cause serious cell damage if they are massive or prolonged.[2,18.]

In this study showed the condition of periodontitis in the group with the induction of bacteria (G2), where there was a significant increase of salivary MDA levels and decrease salivary catalase activity compared with the control group. The primary etiological agent of periodontitis is a polymicrobial complex, predominantly Gram-negative anaerobic or facultative bacteria within the subgingival biofilm.[19] These bacterial species initiate the production of pro- inflammatory cytokines such as IL-1, IL-6, IL-8, TNF-α., and increase the number and activity of polymorphonucleocytes (PMN). PMNs produce ROS superoxide via the respiratory burst mechanism as part of the defense response to infection. High levels or activities of ROS cannot be balanced by the antioxidant defense system, which leads to oxidative stress and tissue damage.[20] ROS can directly cause tissue damage, involving lipid peroxidation, DNA damage, protein damage, and oxidation of important enzymes including catalase.[2,18,20]

Periodontitis begins with a bacterial attack. When an organism is exposed to a bacterial attack, the bacteria secrete LPS and DNA where it will trigger an immune response between bacterial pathogens and the host. This immune response will cause increase activated protein-1 (AP-1) and nuclear-kB factor (NF-kB), and prostaglandin production.[7,21] This will stimulate osteoclast activity resulting in bone resorption and will lead to periodontal tissue damage or periodontitis. AP-1 and NF-kB will increase the concentrations of metalloproteinases (MMPs) that eventually result in tissue damage, and also lead to the release of proinflammatory cytokines such as IL-1, IL-6, IL-8, TNF-α and thus activate fibroblasts and hyperresponsive polymorphonuclear (PMN) that will accelerate the production of reactive oxygen species (ROS).[2,3,22] Excessive ROS production can cause damage by various mechanisms

such as, through the process of lipid peroxidation, DNA damage, destruction of proteins, and secrete proinflammatory cytokines from monocytes and macrophages. If the levels of ROS continue to increase and not balanced with the levels of antioxidants in the body that cause oxidative stress. In periodontitis, persistent oxidative stress results in periodontal tissue destruction.[2,4,6,8]

In the early stages of lipid peroxidation, ROS targets are the double carbon bonds of PUFA unsaturated fatty acids. This double carbon bond will weaken the hydrogen carbon bonds that facilitate the release of hydrogen by free radicals. Finally, free radicals release hydrogen atoms and free-radical lipids (lipid-free radicals), which results in oxidation producing peroxyl radicals. Furthermore, the peroxyl radical may react with other PUFAs, releasing electrons and producing other lipid hydroperoxides and lipid-free radicals. This process can occur continuously in a chain reaction. These hydroperoxide lipids are unstable and their fragmentation produces products such as malondialdehyde, 4-hydroxynonenal, and others.[6,7,13]

In the event of oxidative stress, the body performs a homeostatic mechanism by producing endogenous antioxidants. However, how fast and how many antioxidants are produced depends on a variety of factors, so the body needs to be helped with the intake of exogenous antioxidant compounds. Some exogenous components that have antioxidant substances, among others, vitamin B, C and flavonoid compounds, saponins, tannins.[14,23] Gray Mangrove sp leaf extract is known to have high vitamin C levels of 15.32 mg and vitamin B levels of 2.64 mg, which acts as an antioxidant. Based on previous research, it is known vitamin C has a role in periodontitis although its role is not known with certainty. Although low vitamin C intake does not cause periodontitis, it is known that additional vitamin C is needed during tissue regeneration.[24] Vitamin C deficiency is associated with collagen synthesis damage that causes tissue dysfunction such as wound healing disorders and capillary rupture due to weak connective tissue capillary walls. Collagen regeneration to maintain dental tissue is essential in periodontal health, as it can be said that low vitamin C concentrations are a risk factor for periodontal disease. [23,25] Vitamin C is the most important antioxidant in extracellular fluid, Vitamin C can efficiently prevent superoxide, hydrogen peroxide, hypochlorite, hydroxyl radicals, peroxyl radicals, and exogenous radicals. Vitamin C is also effective in inhibiting lipid peroxidation by peroxyl radicals, preventing membrane peroxidation, and preventing cell damage due to oxygen radicals. In addition to vitamin C, vitamin B also has an antioxidant effect.[26,27] Based on previous research, it is known that vitamin B supplementation can affect the healing process in periodontal tissue.[28]

Gray mangrove leaves are also known to have flavonoid compounds, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, isocatechins, in which they act as antioxidants[29] and anti- inflammatory as saponin.[14,30] The antioxidant activity of the active compound is due to the presence of a phenolic hydroxy group in its molecular structure, which inhibits the action of enzymes involved in superoxide anion production reactions such as xanthine oxidase and protein kinase.[27] Besides, this active compound also inhibits cyclooxygenase, lipooxygenase, microsomal monooxygenase, glutathione-S-transferase, mitochondrial succinoxydase, NADH oxidase. The mechanism of flavonoids in inhibiting inflammation is by inhibiting capillary permeability and inhibiting arachidonic acid metabolism as well as the secretion of lysosomal enzymes from neutrophil and endothelial cells. While the anti-inflammatory mechanism of saponin is by inhibiting the formation of exudate and inhibiting the increase in vascular permeability. In addition to flavonoids, tannin also has antioxidant and anti-inflammatory activity, flavonoids required for efficient radical scavenging, inhibit the enzymes responsible for superoxide.[31]

Various components of antioxidants in gray mangrove leaf extract gives an illustration that the giving of gray mangrove leaf extract at a dosage of 0.25g/kg BW; 0.5g/kg BW; 1g/kg BB orally for 25 days in this study has been able to provide a therapeutic effect on oxidative stress in rats induced mixed periodontopathogen bacteria.

CONCLUSION

The gray mangrove leaf extract has a protective effect for oxidative stress which is characterized by a decreasing malondialdehyde level and an increasing catalase activity in salivary rats induced by mixed periodontopathogen bacteria.

CONFLICT OF INTEREST

All authors declared no conflict of interest.

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