Oxidative Stress Biomarkers Modulation of Parotid Gland by Lemuru Fish Oil from Cigarette Smoke-Induced Rat

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Abstract

Periodontal disease worsened by cigarette, and local factors like calculus and ligation installment. Nicotine in a cigarette can cause inflammation and will impact an increase in free radicals. The increasing of free radical causes cellular damage and stimulated macrophages to produce pro-inflammatory cytokine. The increasing of free radical causes oxidative stress biomarkers imbalance such as an increase of malondialdehyde (MDA), and superoxide dismutase (SOD) level and decrease of catalase level. Lemuru (Sardinella longiceps) fish oil contains EPA and DHA act as an anti-inflammatory agent which can modulate oxidative stress biomarkers so oxidant and antioxidant levels will balance. The study aimed to know modulation oxidative stress biomarkers by lemuru fish oil on parotid gland Rattus novergicus induced with cigarette smoke and ligation. This study used 16 samples rats which inducted with smoke and ligation on lower incisive tooth of Wistar rats. Samples divided into two groups, P1 is non-therapy and P2 is therapy with lemuru fish oil until 10 days. Oxidative stress biomarkers MDA and catalase level analyze using spectrophotometry assay, MDA using the Thiobarbituric Acid method, while catalase using the hydrogen peroxide method. Superoxide dismutase analyzed using ELISA assay, with the Nitroblue Tetrazolium method. There is significant difference on level *MDA (p=0,000) and catalase (p=0,002) between-group (p<0,05). While in SOD there is no significant difference p=0,848 (p<0,05).* **Conclusion:** lemuru fish oil therapy can modulate oxidative stress biomarkers by decreasing the level of MDA and increase catalase level in parotid gland Rattus novergicus inducted with cigarette and ligation.

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Introduction

Smoking is a bad habit that has become a common thing in daily life and it is widespread in society. The age of smokers begins to spread in adolescence. The prevalence of smokers is highest for men aged 15-19 years. At that age, generally smoke 1 until 4 cigarettes a day and it is included in the group of light smokers [1,2]

The chemicals contained in cigarettes are toxic, consisting of nitrosamines and reactive oxygen which can form free radicals such as nitric oxide (NO), nitrite peroxide (NO2) in the gas phase and quinone (Q), semiquinone (HQ) and hydroquinone (HQ) HQ2) in the tar phase. The content of cigarettes plays a role in inflammation in the periodontal tissue, inhibits the healing process, inhibits attachment, damages cell membranes, decreases the immune system in saliva, and indirectly nicotine also produces free radicals or oxygen that is reactive Reactive Oxygen Species (ROS). Cigarrete smokers have a bigger chance to suffer from alveolar bone loss, increased pocket depth and tooth loss, compared to nonsmokers.[3,4]

The condition of the oral cavity due to the effect of cigarettes is compounded by the accumulation of plaque. The accumulation of plaque that increased will mineralize so the calculus is formed. Rough calculus surface facilitates retention of plaque bacteriak.[5,6] This condition will cause the gingiva become susceptible to inflammation and resulting inflammation of the gums known as gingivitis which can progress to periodontitis.[7,8,9]

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An experiment using silk ligature shows nicotine causes bone resorption.[10] Silk ligature size 3 is needed to trigger inflammation of periodontal tissues around mandibular anterior incisivus' subgingival. Periodontal inflammation is periodontal diseases caused by bacterially derived factors and antigens that stimulate a local inflammatory reaction and activation of the innate immune system. Ligature plays a role as local factor. It can induce inflammation of periodontal tissue and causes damage.[8,11]

Free radicals from smoke causes cellular damage such as lipid, protein, DNA, carbohydrate, neutrophil alteration, and stimulate macrophages to release proinflammation cytokines IL-1, IL-6, *tumor necrosis factor-α (*TNF-α), prostaglandin E2 (PGE2) which play a role on periodontal damage. Macrophages play a significant part in immunity and immune responses, they carry on phagocytosis of parasites and microbes, regulate lymphocyte activation and proliferation and they are essential in the activation process of T- and B-lymphocytes by antigens and allogenic cells.[3, 12] The proinflammatory cytokines regulate host responses to infection, immune responses, inflammation, and trauma which make the disease worse (proinflammatory).[12,13]

Malondialdehyde (MDA) is one of the most well-known secondary products of lipid peroxidation. Lipid peroxidation results can caused by an increase in oxidative stress such Reactive oxigen species (ROS). That's why MDA is used as a benchmark of oxidative stress levels of smokers.[14] Several study was found that exposure to clove cigarette smoke in Wistar rats for 30 days caused MDA levels to increase. When ROS is produced, the Thioredoxin (TRX) system is stimulated and transduces redox signals to change the activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx), to eliminate free radicals.[15] Smoking habbit affects on healing and this habbit needs to be stopped because it can disrupt the treatment. Supportive treatment is also needed to reduce free radicals' effects on the smokers[15,16,17]

Lemuru fish oil has anti-inflammatory, antibacterial and antioxidant effects, which can be used for alternative therapies in tissue damage due to increased oxidative stress. EPA and DHA act as anti-inflammatory, in accelerating healing.[18] The presence of omega 3 in lemuru fish oil (Sardinella longiceps) will be responded by the body by secreting anti-inflammatory cytokines, one of which is IL-10. IL-10 works to inhibit the process of formation of proinflammatory cytokines. If the inflammatory process is inhibited, then oxidants will also decrease. The content of vitamin A in lemuru fish oil also acts as an antioxidant. Increased antioxidants will reduce oxidants, so oxidative stress is also suppressed, it will affect the decrease in MDA levels, by increasing catalase and SOD activity. [14,15,19.]

Based on this, the researchers wanted to find out modulation oxidative biomarkers by Lemuru fish oil on parotid gland Wistar rats induced by cigarette smoke and ligation.

Materials and methods

Ethical clearance

All experiments were approved by the Faculty of dentistry Animal Care Committee komite etik penelitian kesehatan gigi (KEPKG) and performed following the guidelines of the Faculty of Dentistry, Universitas Hangtuah Council on Animal Care with number certificate 089/KEPK/VIII/2018.

Animal Experimental Design

The research conducted is a type of true experimental laboratory research with a posttest only control group design with modification of Kubota experiment.[10] The experimental animals used in this study were the Wistar strain Rattus novergicus.[20] The parameters seen in this study are MDA levels. Sixsteen rats were divided into two groups. Treatment group 1 (P1) and treatment group 2 (P2). Cigarette dose is given 1 cigarette per day for one rats which is a mild smoker condition. Non-filter cigarettes selected because the content of clove cigarettes has a greater nicotine content than the type of filter cigarettes.[11]

Group P1 is the treatment group, wherein the treatment of experimental animals was induced by cigarette smoke for 28 days. Day 28 of ligation was installed in experimental teeth around the anterior mandibular incisor, while cigarette smoke was induced for 7 days. The 35th day of ligation was released and local factors cleared. Induction of secondhand smoke is carried out on day 35 for 10 days, then sampling is carried out on the 45th day.

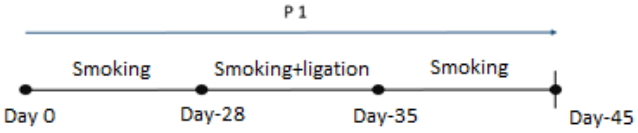

Figure 1. Group P1 Design Treatment

P2 group is the treatment group where the treatment of experimental animal was induced by cigarette smoke for 28 days. On the 28th day, ligation was placed on the teeth of experimental animals around the lower anterior incisor teeth while still inducing cigarette smoke for 7 days. The 35th day of ligation was removed and local factors cleared. Induction of cigarette smoke again carried out on the 35th day for 10 days. On the 35th day he was given lemuru (Sardinella longiceps) fish oil therapy for 10 days. On the 45th day, sampling was terminated.

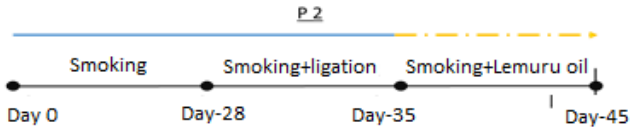

Figure 2. Group P2 Design Treatment

Sample Preparation

The parotid gland was separated and 1 mL of cold PBS was added. Then homogenized using a 5000 rpm homogenizer / sonicator for 5 minutes, then centrifuged 8000 rpm for 10 minutes. from the process obtained a supernatant and store at minus 20 for further testing.

Biochemical Analysis

MDA

Measurement of MDA levels in Wistar rats through a spectrophotometer based on reactions with Thiobarbbituric acid (TBA) using a commercial kit. Measurement MDA protocol was modified from Pratama (2019) experiment.[22] Then determine the curve used, namely y = 0.0972x-0.0240; RSQ = 0.9974. The absorbance sample is measured with a Spectrophotometer at the maximum wavelength of the TBA test (λ532 nm) and the absorbance value is then plotted on the standard MDA curve that has been made to calculate the sample concentration.[19]

SOD

Determine the SOD activity through an indirect method using nitroblue tetrazolium (NBT) (Sigma Aldrich, Singapore) according to the manufacturer's instructions. The IC_{50} (50% inhibition activity of SOD or SOD-like materials) can be determined by a colorimetric method. The absorbance was monitored at 450 nm. The percent of inhibition was normalized by the protein content and presented as SOD activity units/ml.

CATALASE

Catalase test, the supernatant is added with H2O2 60mM and let it sit for 5 minutes. The levels were read by a spectrophotometer with a wavelength (λ) of 240nm and then the absorbance value was obtained. The absorbance value is entered into the formula for calculating the enzyme activity of the catalase and the final result is the catalase enzyme activity value in units of U /mg.[17]

Results

The result was analyzed the description of distribution and summarization of data in order to clarify the presentation of results. Then hypothesis testing was used using analytic statistics with a significance value of 95% (p <0.05) using the SPSS version 23 program.

Data presented in table 1 shows general characteristics different result on bone height, between, ligation only groups and ligation with smoking induction group in produce periodontitis condition, This data was obtained from preliminary study to maintain right methods induced periodontitis by cigarette smoke. With these findings we can used the methods to futher investigation in salivary oxidative biomarkers induced with cigarrete smoke and ligation.

Group	N	Average Bone height (mm)	Standar Deviation
No Smoking	7	3,5414	0,07267
Smoking	7	2,7871*	0,1749

Table 1. The average and standard deviation of the anterior mandibular bone height in periodontitis produce with cigarrete smoking.
*show decrease of bone height.

Based on analysis there is significant difference on MDA level and Catalase Level between group significance value p <0.05 . Meanwhile result on SOD level showed there is no significant between group with significance value p > 0.05.

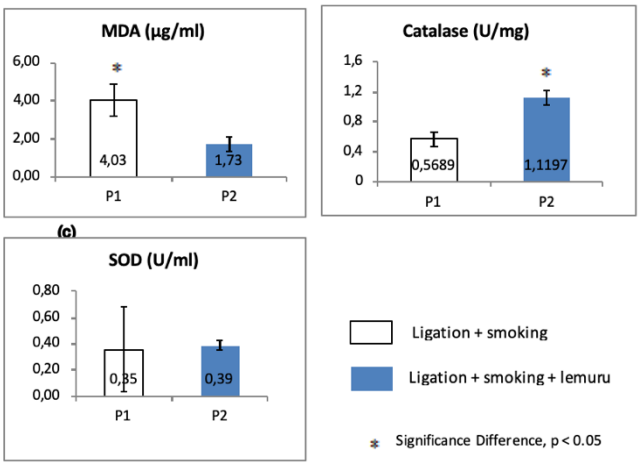

Figure 3. Effect of cigarette smoking on MDA, catalase and SOD levels. MDA level (µg/ml) (a) Catalase, U/mg (b) SOD, U/ml (c) levels were measured as described in materials and methods. Values are mean ± SD of each group. * Indicates a *P*<0.05 is statistically significant between groups.

Discussion

This research was conducted to determine the effectiveness of lemuru fish oil (Sardinella longiceps) in reducing Malondialdehyde (MDA) levels in the parotid gland of Wistar (Rattus novergicus strain wistar) rats induced by cigarette smoke and ligation. Ligation was carried out by binding silk size (silk ligature) size 3.0 on the subgingival area around the mandibular anterior incisor. The presence of cigarette smoke accompanied by ligation aims to induce inflammation in dental periodontal tissue.

Exposure to cigarette smoke can increase the production of excess free radicals and ultimately cause oxidative stress, which is characterized by increased levels of MDA, so that it will cause damage to cell death and indirect damage to the cells of the salivary gland acini.[19] Smoking, which is an important risk factor for periodontitis, induces oxidative stress in the body and causes an imbalance between reactive oxygen species (ROS) and antioxidants, such as superoxide dismutase (SOD).[6,11,15] The pathology associated with ROS is derived from their ability to modify cellular and extracellular macromolecules. Dismutation of the superoxide radical (O2•−), which is released from ROS, is catalyzed by SOD, which transforms it into hydrogen peroxide (H2O2). H2O2 is scavenged by catalase, peroxiredoxin-2 (Prx2), and glutathione, by transforms it into H2O and O2.[15]

This study shows that the higher average MDA level is in group of rats induced by cigarette smoke and ligation (4.04438 ± 0.689550). While the lower average MDA level was in group of rats induced by cigarette smoke and ligation with lemuru fish oil therapy (1.74038 ± 0.333328). Giving induction of cigarette smoke with a light dose of 1 non filter cigarette per day has been able to provide an effect of increasing levels of MDA in the parotid glands of wistar rats. Therefore, tissue breakdown associated with ROS has been measured with end products of lipid peroxidation like MDA.[14,21]

Catalase level higher in group of rats induced by cigarette smoke and ligation with lemuru fish oil therapy (1.119±0.1609) and lower on group of rats induced by cigarette smoke and ligation (0.5689±0.1262), results presented here reveal that soluble components of tobacco smoke produce a reversible inhibition of catalase. Group with lemuru fish oil therapy had ability to switch the balance toward a pro-oxidative state is to block the antioxidant defense. Meanwhile there is no difference in SOD level between group. This condition maybe because after ligation was removed the inflammation is decrease and level of oxidant was slightly lower and follow by SOD level. SOD is practically insensitive to soluble tobacco components, there are published studies indicating that GPx and SOD decrease their activity in the presence of tobacco smoke.[22] Data findins in this result, sugest that catalase is the most sensitive antioxidant enzyme to tobacco inhibitory effect than SOD. Cigarette smoking interferes with redox homeostasis in the body, alters antioxidants levels, and influences the periodontal disease activity.[15]

Lemuru fish oil which acts as an anti-inflammatory product, aims to inhibit the occurrence of the inflammatory process, so that free radical numbers will drop. The content of Vit A in lemuru fish oil also acts as an antioxidant which aims to inhibit the production of free radical excess. Oxidant activities and balanced antioxidants can reduce oxidative stress levels in the body, so that lipid peroxidation and MDA levels will also decrease. MDA is the result of the fatty acid peroxidation process which is not saturated by free radicals, but it is also used as a sign of oxidative stress.[14] When Oxidative stress arrised anti oxidant such catalase will increase to balance the oxidative reaction.[18,20] This research

shows that lemuru oil can induce body homeostatis to overcome the oxidants produced by tobacco cigarettes by modulating the body's oxidative and anti-oxidant activity, that can caused further damage such as periodontitis.

Conclusions

Lemuru fish oil therapy can modulate oxidative stress biomarkers by decreasing the level of MDA and increase catalase level in parotid gland Rattus novergicus inducted with cigarette and ligation.

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Declaration of Interest

The authors report no conflict of interest.

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