

Effectivity of lemuru fish oil (*sardinella longiceps*) protection in periodontal tissue wistar rats induced by diabetes mellitus



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Rizka SA. Putri,¹ Dian W. Damaiyanti,^{1*} Hansen Kurniawan²

Abstract

Objective: To determine the effect of lemuru fish oil extract (*Sardinella longiceps*) on the number of fibroblasts in periodontal tissue of Wistar rats induced by diabetes mellitus using streptozotocin.

Material and Methods: Thirty-two Wistar rats male, were divided into 4 groups, group K0 (negative control), K1 (4ml/kgBB), K2 (8ml/kgBB), and K3 (4ml/kgBB). Rats were induced with nicotinamide and streptozotocin to create a state of diabetes. The experiment of giving lemuru fish oil extract was carried out for 21 days. The number of fibroblast cells in periodontal tissue with HE staining was performed.

The data were analyzed by One-Way ANOVA and Post-Hoc LSD test.

Results: The LSD test showed a significant difference between the group without treatment and the group using treatment lemuru fish oil. Group with Lemuru fish oil can increase the number of fibroblasts and the most effective dose is 8ml/kgBB.

Conclusion: Lemuru fish oil extract (*Sardinella longiceps*) can increase the number of fibroblasts in periodontal tissue of Wistar rats induced by diabetes mellitus.

Keywords: Diabetes mellitus, Fibroblast, Lemuru fish oil, Periodontitis
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¹Department of Oral Biology, Faculty of Dentistry, Hang Tuah University, Surabaya, Indonesia

²Department of Periodontics, Faculty of Dentistry, Hang Tuah University, Surabaya, Indonesia

Introduction

Diabetes mellitus (DM) is a chronic metabolic disease of carbohydrate, fat, and protein, characterized by hyperglycemia due to disorders of the pancreas where the body of people with diabetes mellitus cannot produce enough insulin.¹ Based on Indonesia's basic health research (Risksedas) in 2018, the prevalence of Diabetes Mellitus in Indonesia based on doctor's diagnosis in people aged ≥ 15 years increased from 1.5% in 2013 to 2.0% in 2018.²

Diabetes mellitus can cause several manifestations in the oral cavity, one of which is the emergence of periodontal disease.³ Periodontal disease can be related to diabetes mellitus because it results in an increase in glucose content in the biofilm layer and plaque on the tooth surface so that various kinds of adhering bacteria will reproduce properly. This is a factor that can worsen periodontal conditions resulting in gingivitis and periodontitis due to uncontrolled blood glucose levels.^{1,3}

Hyperglycemia in diabetes mellitus patients strongly induces protein glycation through Maillard non-enzymatic reactions resulting in advanced glycation end-products (AGEs) which bind to receptors for AGE (RAGE). AGEs accumulate in large amounts of periodontal tissue and bind to various cell receptors such as epithelial cells, fibroblasts, endothelial cells, and inflammatory cells in the periodontal tissues of DM patients. The

binding of AGEs with cells causes biological activity such as increased chemotaxis and monocyte cell activation, increased endothelial cell permeability, the release of inflammatory cytokines and growth factors by macrophages, increased procoagulant activity by endothelial cells and macrophages, and increased proliferation and synthesis of extracellular matrix fibroblasts and smooth muscle cells.^{4,5}

Fibroblasts are cells that are large in number and have an important role in periodontal connective tissue. In periodontal tissue, fibroblasts involved in the host immune response in periodontitis are human gingival fibroblasts (HGF) and periodontal ligament fibroblasts (PDF).⁶ Fibroblasts produce collagen fibers and differentiate into odontoblast cells and osteoblasts in the healing process. Fibroblasts also have an important role in gingival healing, namely by regulating the production of MMPs / TIMPs in periodontitis.^{7,8} Fibroblast cells have a function to maintain the integrity of the connective tissue structure and regulate connective tissue turnover by producing enzymes that can reduce MMP (matrix metalloproteinase).⁹

Glycemic control is the main key in the treatment of diabetes mellitus. Intensive treatment of hyperglycemia can reduce the risk of complications in the long term.¹⁰ Periodontal treatment by reducing the bacterial load and inflammatory response is also needed to improve glycemic control.⁵ Periodontal treatment can be performed mechanically with scaling and root planing. For

*Correspondence to: Dian W. Damaiyanti. Department of Oral Biology, Faculty of Dentistry, Hang Tuah University, Surabaya, Indonesia
damaiyanti@hangtuah.ac.id

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patients who can't respond to therapy mechanically properly, additional antibiotics are required during treatment. However, the use of antibiotics that are not following the dosage and time of use can cause resistance in the patient's body. With these considerations, several experts researched natural ingredients that can be an alternative treatment for periodontitis, one of which is by utilizing lemuru fish oil extract (*sardinella longiceps*).¹⁰

Lemuru fish oil comes from lemuru fish (*sardinella longiceps*) which is rich in unsaturated fatty acids (omega-3). Polyunsaturated fatty acid n-3 (PUFA), namely eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA) are the most dominant omega-3s found in fish oil.^{9,11} *Sardinella longiceps* contain 13.70% EPA and as much DHA as 8.91% which can function as an anti-inflammatory. The higher the levels of EPA and DHA contained in fish oil and which enter the connective tissue, the higher the inhibition of inflammatory progression will be.^{9,12}

Based on research conducted by Damaiyanti et al.¹³ it shown that giving lemuru fish oil extract at doses of 4ml/kg of weight, 8ml/kg of weight, and 16ml/kg of weight was proven to stimulate osteoblast cell proliferation and can be used as an alternative treatment to reduce the progression of periodontal tissue damage caused by diabetes mellitus.

Material and Methods

The type of research used was a true experimental laboratory with a randomized post-test control group.¹⁴ The sampling technique used was the simple random sampling method.¹⁴ The research sample was male *rattus norvegicus* strain wistar, 3-4 months old, had a heavyweight. 150-200 grams body, white and shiny hair, red and clear eyes, agile movements, and 16 feces are not flabby.

Wistar rats were acclimatized for 7 days before being given treatment. On day 8, Wistar rats induced nicotinamide 240 mg/kg of weight and streptozotocin 65 mg/kg of weight. After 7 days, the Wistar rats were examined. If Wistar rats experience an increase in fasting blood glucose of more than 126 mg/dL, the experimental animal is considered to have diabetes mellitus.^{13,15}

The sample was divided into 4 groups, namely K0, K1, K2, and K3. The K0 group was a negative control group that was given CMC-Na gel 2% without being given lemuru fish oil extract; K1 was the group that was given lemuru fish oil extract at a dose of 4ml/kg of weight; K2 is a group given lemuru fish oil extract at a dose of 8ml/kg of weight; K3 is

a group given lemuru fish oil extract at a dose of 16ml/kg of weight. The experimental treatment was given orally using a gastric swab for 21 consecutive days.¹³

On day 36 Wistar rats, each group was sacrificed by injecting ketamine 80 mg/kg of weight and xylazine 20 mg/kg of weight intraperitoneally. Then mandibular jaw decapitation was carried out for decalcification with EDTA 14%, fixation with 10% buffered formalin solution, cutting the mandibular tissue of Wistar rats by including the surrounding normal tissue in alcohol-xytol, after that made paraffin blocks and cutting tissue as thick as 5 millimicrons in microtomes. Then, HE stained the tissue with HE and examined the HPA by calculating the number of fibroblast cells in the mandibular periodontal ligament of the first molars using an Olympus CX-21 microscope with the addition of the optilab program. The microscope magnification of the preparations was carried out 400 times and viewed in 5 different fields of view then averaged. The data obtained were then performed statistical analysis using the One-Way ANOVA parametric statistical test then continued with the post-hoc LSD test with a significance level of 95% ($p = 0.05$) using the SPSS program version 20.¹³

Results

The data obtained from the research results were analyzed to obtain an overview of the distribution and a summary of the data to clarify the presentation of the results.

Table 1 shows the average number of fibroblast cells in the periodontal tissue of wistar rats in the K2 group and the smallest mean number of fibroblasts in the periodontal tissue of Wistar rats is in the K0 group.

Table 1. Results of the mean and standard deviation of the number of fibroblasts in the periodontal tissue of wistar rats

Group	Mean ± Standard Deviation
K0	165.5 ± 8.103
K1	523.25 ± 23,386
K2	543.00 ± 24,779
K3	500.5 ± 11,474

Table 2. Table of LSD Post-Hoc test results

Group	K0	K1	K2	K3
K0		0.000*	0.000*	0.000*
K1			0.155	0.106
K2				0.007*

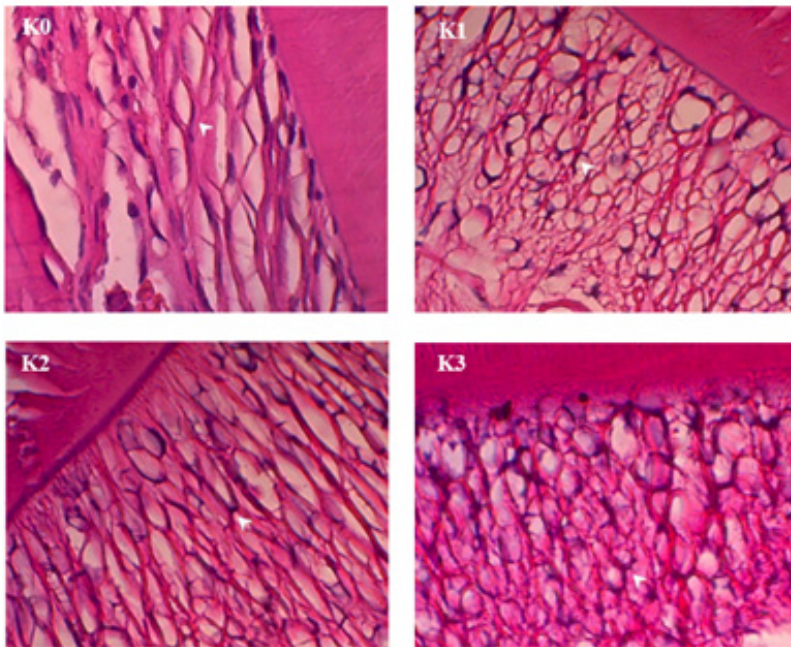


Figure 1. Histology of fibroblasts in periodontal tissue with 400x magnification (arrows show fibroblasts)

The normality test used the Shapiro-Wilk test because the number of research samples was ≤ 50 . The results of the Shapiro-Wilk test have a significance value of $p > 0.05$, so the number of fibroblast cells in the periodontal ligament of Wistar rats is normally distributed. The homogeneity test was carried out with the Levene test to determine the homogeneity of the data variations in each group. Based on the results of the Levene test periodontal fibroblasts in Wistar rats has a homogeneity variant of $p > 0.05$, namely $p = 0.217$. It can be concluded that the research data has a homogeneous variant.

The One-Way ANOVA test results show a significance value of $p = 0.000$ ($p < 0.05$) which indicates that there are significant differences between each group. Based on these results, the test can be continued using the Post-Hoc LSD test to determine significant differences in the number of fibroblasts in the periodontal tissue of Wistar rats between each group.

Based on Table 2, the results of the Post-Hoc LSD test showed that the groups that had significant differences ($p < 0.05$) were K0 with K1, K0 with K2, K0 with K3, and K2 with K3.

Discussion

The results of the post hoc LSD test in table 5 show that there is a significant difference between the K0 and K1, K2, and K3 groups. This proves that the administration of lemuru fish oil extract

therapy is effective in increasing the number of fibroblast cells because of the content of C20-22 n-3 polyunsaturated fatty acids (PUFA) which is widely known as omega-3 in lemuru fish oil.^{9,11,16} The types of PUFA that are predominantly contained in lemuru fish oil are eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA). EPA and DHA can help activate macrophages to secrete anti-inflammatory cytokines such as IL-10.^{17,18} These anti-inflammatory cytokines have a role in inducing fibroblasts to proliferate, migrate, and form extracellular matrices including synthesizing collagen. This can help reduce the production of pro-inflammatory cytokines in the healing process. So that the higher the levels of EPA and DHA contained in lemuru fish oil and that enter the connective tissue, the inhibition of the progression of inflammation will be higher.^{9,12,17}

The results also showed that the K0 group had the lowest mean number of fibroblast cells compared to the K1, K2, and K3 groups. This is because the K0 group was not given lemuru fish oil extract therapy so that the inflammatory process in that group increased and the fibroblast cell proliferation phase could not run optimally. Whereas in the K1, K2, and K3 groups, lemuru fish oil extract will help increase the proliferation phase of fibroblast cells which will release growth factors (PDGF, FGF, and TGF- β) to produce an extracellular matrix such as collagen. The more activated growth factor signals will direct the fibroblast cells to migrate to the injured area increasing the number of fibroblast cells in that area.^{19,20} Fibroblasts help reduce the inflammatory process to maintain the integrity of the connective tissue structure and regulate connective tissue turnover by producing enzymes that can decrease MMP (matrix metalloproteinase). So those fibroblasts have an important role in the healing process by suppressing the production of MMPs / TIMPs in the periodontal connective tissue and the inflammatory process can decrease.^{7,8,9,12}

Group K1 with K2 and K3 showed no significant differences between groups. It can be concluded that the administration of doses of 4 ml/kg of weight, 8 ml/kg of weight, and 16 ml/kg of weight has almost the same results and effects in increasing the number of fibroblast cells. This could have an impact on increasing the number of excess fibroblast cells, causing fibrosis, namely the development of excessive deposition of extracellular matrix components, including collagen. Fibrosis is the result of chronic inflammation induced by various stimuli such as infection, autoimmune reactions, allergic responses, chemical insults, radiation, and tissue injury.²¹

There was a significant difference between the K2 and K3 groups. This shows the effect of the dose of lemuru fish oil 8ml/kg of weight with 16ml/kg of weight in increasing the number of fibroblast cells optimally because lemuru fish oil has an anti-inflammatory role which will inhibit the work of monocytes and macrophages so that it can reduce the production of pro-inflammatory cytokines such as IL-1, IL-6, PGE2, and TNF- α . However, giving lemuru fish oil at a dose of 16 ml/kg of weight is not recommended because it can allow fibrosis.^{22,23} In addition, lemuru fish oil at a dose of 16 ml/kg of weight has a high level of viscosity, because oil is one of the most difficult lipids to dissolve in water. However, the condition is that a drug that is given in any way must have good solubility in water to produce maximum therapeutic effects. Giving lemuru fish oil at a dose of 16 ml/kg of weight has a high viscosity level so that it does not dissolve easily in water. This causes the absorption process to be incomplete so that the therapeutic response received by the body will not run optimally. Therefore, it is more advisable to give a small dose but it can give a bigger effect.^{23,24}

Based on the results of this study, it can be concluded that there is an effect of giving lemuru fish oil extract (*sardinella longiceps*) on the number of periodontal fibroblasts of wistar rats induced by diabetes mellitus. The dose of lemuru fish oil of 8ml/kg of weight in the K2 group was the most effective in helping to increase the number of fibroblast cells in the periodontal tissue of Wistar rats because the dose of 8ml/kg of weight given in the study could have a similar effect to the 16ml/kg of weight dose. Meanwhile, the dose of 4ml/kg of weight was not chosen as the most effective dose due to the possibility of obstacles that arise during the healing process in the inflammatory area because the dose given is too small so that the number of fibroblast cells in that group is small.

Conclusion

There is an effect of giving lemuru fish oil at a dose of 4 ml/kg of weight, 8 ml/kg of weight, and 16 ml/kg of weight in increasing the number of fibroblasts in periodontal tissue of Wistar rats induced by diabetes mellitus. Lemuru fish oil at a dose of 8ml/kg of weight is the most effective dose in increasing the number of fibroblast cells because it can create a similar effect to that produced by higher doses.

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Conflict of Interest

The authors report no conflict of interest.

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