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Research Report

The activity of *Stichopus hermanii* extract on triglyceride serum level in periodontitis

Rima Parwati Sari and **Syamsulina Revianti**
Department of Oral Biology
Faculty of Dentistry, University of Hang Tuah
Surabaya - Indonesia

ABSTRACT

***Background**: The level of triglyceride can be used as a parameter of hypercholesterolemia. Periodontitis can make the condition of hypercholesterolemia worse. Stichopus hermanii extract is a source of saturated fatty acid containing omega-3 which can decrease triglyceride blood level. **Purpose**: The aim of this research was to investigate the effect of Stichopus hermanii extract in triglyceride blood level of wistar rats which got periodontitis. **Methods**: The samples of this research were 30 rats divided into 5 groups, namely group K(–) as negative control group (without treatment), group K(+) as positive control group (induced with periodontopathogen mix), group P1 as treatment group1 (induced with periodontopathogen and Stichopus hermanii extract mix, 0.09 ml/kgW), Group P2 (induced with periodontopathogen and Stichopus hermanii extract mix, 0,18 ml/kgW), and group P3 (induced with periodontopathogen and Stichopus hermanii extract mix, 0,36 ml/kgW). Then, all of those rats were sacrificed and all serum was measured for their level of triglyceride. **Results**: All data was analyzed with ANOVA test showing a significant result. LSD test showed a significant different between group K(–) and group K(+), and between group K(+) and group P2 and P3. **Conclusion**: Stichopus hermanii extract can decrease the triglyceride blood level in wistar rats with periodontitis.*

***Key words**: Hypercholesterolemia, triglyceride, Stichopus hermanii extract, periodontitis, omega-3*

ABSTRAK

***Latar belakang**: Kadar trigliserida dalam darah dapat digunakan sebagai parameter hiperkolesterolemia. Periodontitis dapat memperburuk kondisi hiperkolesterolemia. Stichopus hermanii ekstrak mengandung asam lemak jenuh terutama omega-3 yang dapat berfungsi menurunkan kadar trigliserida dalam darah. **Tujuan**: Tujuan dari penelitian ini adalah untuk mengetahui pengaruh ekstrak Stichopus hermanii terhadap kadar trigliserida dalam darah tikus wistar yang mengalami periodontitis. **Metode**: Sampel penelitian ini adalah 30 ekor tikus wistar yang dibagi dalam 5 kelompok. Kelompok K(–) sebagai kelompok kontrol negatif (tanpa perlakuan), kelompok K(+) sebagai kelompok kontrol positif (tikus diinduksi periodontopatogen campuran), kelompok P1 sebagai kelompok perlakuan 1 (tikus diinduksi periodontopatogen campuran dan Stichopus hermanii ekstrak 0,09 ml/kgW), kelompok P2 (tikus diinduksi periodontopatogen campuran dan Stichopus hermanii ekstrak 0,18 ml/kgW) dan P3 (tikus diinduksi periodontopatogen campuran dan Stichopus hermanii ekstrak 0,36 ml/kgW). Kemudian semua tikus dikorbankan dan diukur kadar trigliserida serumnya. **Hasil**: Semua data dianalisis dengan uji ANOVA dan menunjukkan hasil yang signifikan, dan uji LSD menunjukkan terdapat perberbedaan yang bermakna antara kelompok K(–) dengan K(+) dan kelompok K(+) dengan P2 dan P3. **Kesimpulan**: Ekstrak Stichopus hermanii dapat menurunkan kadar trigliserida darah pada tikus wistar yang mengalami periodontitis.*

***Kata kunci**: Hiperkolesterolemia, trigliserida, ekstrak Stichopus hermanii, periodontitis, omega-3*

Correspondence: Rima Parwati Sari, c/o: Bagian biologi Oral, Fakultas Kedokteran Gigi Universitas Hang Tuah. Jl. Arif Rahman Hakim No. 150 Surabaya 60111, Indonesia. E-mail: rima.sari@yahoo.com

INTRODUCTION

Periodontal disease is recognized as an infection of periodontal tissues caused by bacteria related with the immune response of hosts which can lead to periodontal tissue destruction. Meanwhile, periodontitis is an inflammation disease of tissues supporting teeth caused by a specific organism that later can cause progressive destruction of periodontal ligament and alveolar bone accompanied by pocket depth formation, gingival recession or a combination of both.[1]

Periodontal disease, can also become a predisposing factor for cardiovascular disease due to several reasons, such as invasion of gram-negative bacteria, levels of pro inflammatory cytokines, immune system, fibrinogen, and the number of white blood cells.[2] This is supported by a research conducted by Kartika *et al.,*[3] stating that the induction of bacterial periodontopathogen can increase triglyceride serum level. Similarly, the high-cholesterol diet can also exacerbate inflammatory response of periodontitis caused by bacterial pathogens due to high cholesterol foods that can increase the amount of lipid deposition in various organs. As a result, the number of inflammatory molecules that circulate in the systemic tissues is increased.[4,5]

Triglycerides are the major fats in the body that is closely associated with cholesterol, which both have a relation that cannot be separated in metabolism process.[6] Triglycerides are even a form of three basic human fats. Unlike cholesterol stored in liver tissue or blood vessel walls, triglycerides are stored in fatty cells under the skin. High triglyceride levels will alter cholesterol metabolism of very low density lipoprotein (VLDL) into a form of large-VLDL (L-VLDL). This L-VLDL form then will become the low-density lipoprotein (LDL), which finally will aggravate bad cholesterol content in blood vessels.[7]

The mechanism of triglycerides formation into bad fat can be clearly seen in figure 1. The consumption of foods containing fat (cholesterol and triglycerides) then can trigger the releasing of gallbladder bile acids (produced by liver) to intestine. Bile acids are actually needed to form micelles or droplets of emulated fat. Considering that the

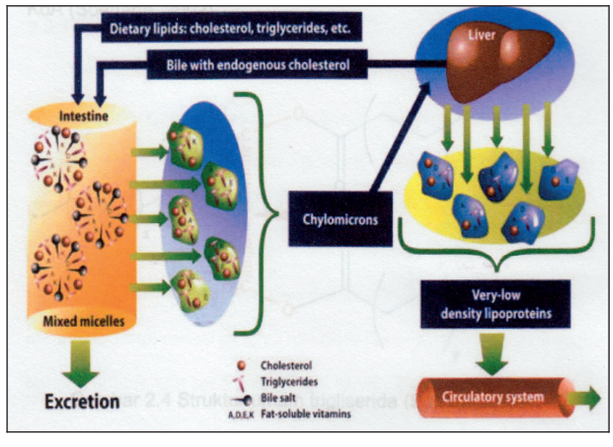

Figure 1. The changing mechanism of TG to VLDL.[8]

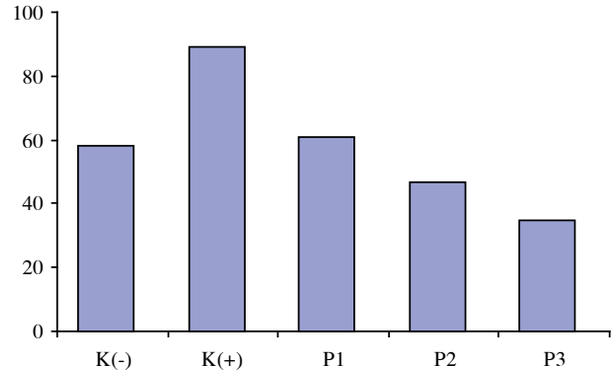

Figure 2. The mean of triglyceride serum levels.

most of the human body contains water, cholesterol and other fatty nutrition then should be in the form of emulated ones (an emulsifier is need), so they are easily absorbed in intestine. In intestine, mixed micelles (cholesterol, triglycerides, bile acids, proteins) are absorbed, while some are discarded. Mixed micelles absorbed (chylomicrons) are then secreted in liver into VLDL.

VLDL actually contains most of fat, cholesterol, and small amounts of protein. VLDL is known as bad cholesterol which then enters bloodstream and causes atherosclerosis, the narrowing or hardening of arteries. This condition causes heart disease and stroke. Thus, when atherosclerosis occurs in arteries leading to brain (carotid artery), it will trigger to stroke. Meanwhile, when it occurs in arteries leading to the heart (coronary arteries), it will trigger to heart attack.[8]

Foods that are low in fat and rich in omega-3 are considered to be an effort to cope with atherosclerosis. In early observations based on empirical facts, it is known that Eskimo community whose life is inseparable from fish consumption does not easily get heart diseases. The level of total cholesterol, triglycerides, and LDL cholesterol were lower in Eskimo population who has fish as their daily food than the population that has left the daily consumption of fish.[9] Therefore, it needs to pursue marine resources for the treatment of atherosclerotic disease.

Indonesia is a country that has the largest marine resources, with a variety of marine resources and its utilization. One of the marine resources rich in omega 3 is a sea cucumber or gamat. Gamat has many kinds, one of which is sea cucumber/gold gamat (Stichopus variegates/*Stichopus hermanii*). Stichopus hermanii contains 86% protein, 80% collagen, minerals, mucopolysaccharide, glicosaminoglycans (GAGs), natural antiseptics, chondroitin sulphate, omega-3, 6, and 9, amino acid.[10] Since it is rich in omega-3 EPA and DHA, it can lower cholesterol levels in rats infected with Porphyromonas gingivalis, and can also be useful as an additional treatment for periodontal diseases.[11]

Based on the description above, it is necessary to do a research focused on the effects of gold sea cucumber extract (*Stichopus hermanii*) on triglyceride serum levels in rats infected with periodontitis.

MATERIALS AND METHODS

This research is a laboratory experimental research with completely randomized design.[12] This research was conducted in Laboratory of Biochemistry, Faculty of Medicine, University of Airlangga, in Laboratory of Microbiology, University of Airlangga, and in Laboratory of Regional Public Health, Surabaya. The samples were 30 white male rat (*Rattus novergicus*), age 2–3 months with the weight of 200 grams. The samples were devided into 5 groups, 6 rats each before treated, the adaption process of the rats to the environtment was conducted for 1 week under monitoring.

Bacteria then were taken from the wobbly tooth of patients suffering from periodontitis by using sterilized paper points entered on its pocket. The revocation was actually conducted four times by using different sterilized paper points, and then incubated in incubator for 3 days. Afterwards, by using a micropipette, it was taken little for making preparations followed by coloring process in order to see what kind of bacterial colonies dominated. Then, it was known that bacteria dominating were gram (–) bacteria. Colonies of bacteria were then diluted equivalent to Mc Farland 0.5 in order to be ready to be given to control group, treatment group 1, treatment group 2, and treatment group 3.[13]

The procedures of this research began with the acclimatization of the research animals for 7 days in a laboratory. Before given treatment, white rats were fasted approximately 18 hours, but were still given water to drink. If there was rat which got pain, it would be excluded from this research. Those white rats were divided into five groups: first, negative control group, K(–), which was not induced with the mix of periodontopathogen bacteria and *Stichopus hermanii* extract; second, positive control group, K(+), which was induced with periodontopathogen bacteria, but not induced with *Stichopus hermanii* extract; third, treatment group 1, (P1), which was induced with periodontopathogen bacteria and *Stichopus hermanii* extract about 0.09 ml; treatment group 2, (P2), which was induced with periodontopathogen bacteria and *Stichopus hermanii extract* about 0.18 ml; and treatment group 3, (P3), which was induced with periodontopathogen bacteria and *Stichopus hermanii* extract about 0.36 ml.

Afterwards, the induction of periodontopathogen mix was conducted for 4 weeks, in which 5 days were for induction process, and 2 days were not for induction.[11] During induction process, a syringe was used to put the extract into the mouth of wistar rats which was then washed manually by the researcher.

The procedures of *Stichopus hermanii* extract induction was conducted on the same day, namely in groups P1, P2 and P3 for in 14 days. Furthermore, those rats in all the three groups were sacrificed, and their blood then was taken for the examination process of serum triglyceride levels.

Data obtained from the examining results of triglyceride serum levels in each group then were tabulated. Next, the statistical test, ANOVA test, was also conducted, and then the test was followed by Least Significant Difference (LSD) for significance test in each group.

RESULTS

The mean of serum triglyceride levels in each group can be seen in figure 2. The results of ANOVA test showed the differences of the mean of triglycerides serum levels. Meanwhile, the results of LSD test shows that there were significant differences (sig < 0.05) between K(–) group and K(+) group (p = 0.006), K(+) group and P1 group (p = 0.026), P2 group (p = 0.001) and P3 group (p = 0.000), P1 group and P3 group. There was no significant differences (sig > 0.05) between K(–) group and P1 group (p = 0.541), P2 group (p = 0.559) and P3 group (p = 0.103), P1 group and P2 group (p = 0.237), and P2 group and P3 group (p = 0.282) as can be seen in table 1.

DISCUSSION

Triglycerides are fats contained in blood which tends to rise due to alcohol consumption, weight gain, sugar and fat rich diet, and lack of exercise. The increasing of triglycerides triggers the risks of heart disease and stroke. From chemistry point of view, triglyceride is considered as a substance consisting of glycerol binding fatty acid chain. Thus, consuming foods containing fat will increase triglycerides in blood and cholesterol levels.[7]

Besides that, poor oral hygiene, such as periodontitis, can also increase triglycerides. This fact is also supported by some researches showing that the severity of periodontal disease is positively related to cholesterol plasma levels. It means that periodontitis is associated with the increasing of triglycerides.[3] Thus, this research also prove that the

Table 1. The results of LSD test

Dependent Variable	Treatment group	K(+)	P1	P2	P3
Levels of TG serum	K (–)	0.006*	0.541	0.559	0.103
	K (+)	-	0.026*	0.001*	0.000*
	P1	-	-	0.237	0.029*
	P2		-	-	0.282

Note: *Significant difference

induction of periodontopathogen mix can significantly elevate the levels of triglycerides serum.

Pathogenesis of these conditions are actually due to lipopolysaccharide (LPS) produced by periodontopathogen (on a negative- gram bacteria) that can inhibit the metabolism of fats resulting in the increasing of triglycerides later. Under the conditions of inflammation and periodontitis, body attempts to localize by producing cytokines, namely interleukin-1β (IL-1β) and tumor necrotizing factor-α (TNF-α) which have effect on fat metabolism by influencing the production of other cytokines and the use of amino acid and other ingredients included in the metabolism of fat. Thus, if IL-1β and TNF-α are increasing, hyperlipidaemia will potentially occur. It is because pro-inflammatory cytokines can increase intracellular cholesterol levels in some cell types, for instance, IL-1β can increase cholesterol accumulation in mesengial cells by regulating receptor of receiver via LDL receptor dysregulation. This leads to IL-1β and TNF-α that can accelerate the increasing of triglycerides serum.[14,15]

In addition, LPS derived from periodontopathogen can also trigger the decreasing of lipoprotein lipase activities during infection, while the function of lipoprotein lipase is breaking triglycerides. If triglycerides cannot be broken down, it then can be buried and can cause the increasing of cholesterol levels.[14] This possibility can occur since triglycerides are metabolized in liver from fatty acid derived from carbohydrate lipolysis, proteins, fats, and alcohol consumed daily which with APO-B lipoproteins can form VLDL excreted into blood circulation. VLDL then will be separated from triglycerides by lipase lipoprotein enzyme altering into intermediate density lipoprotein (IDL) and then into LDL that can very easily be oxidized and can also damage high-density lipoprotein (HDL) later, as a result, it finally will aggravate cholesterol content in blood vessel.[7]

Patients with periodontal disease also have high levels of C-reactive protein serum (CRP) and fibrinogen, called as acute-phase proteins (acute phase proteins), in which patients have more white blood cells than those in healthy individuals. CRP can only be synthesized by hepatocytes, and its making is controlled by IL-6, a cytokine that has proagglomeration and pro inflammatory activities often isolated from bacterimist patients. The levels of CRP serum can be increased 100 times, while the levels of fibrinogen can be increased 10 times in response to antimicrobial diseases. Fibrinogen is actually an essential component for agglomerating working in platelet function and considered as an ingredient for the formation of fibrin. Fibrinogen is also considered as an acute phase protein which can be increased as a result of systemic infections. Thus, the increasing level of fibrinogen in thrombosis can become a risk indicator of atherosclerosis.[16]

The induction of *Stichopus hermanii* containing omega-3 was proved to be effective in lowering triglyceride serum levels based on the results of LSD test which showed that there were no significant differences between K(+) group and all the other treatment groups induced with *Stichopus hermanii* ($p > 0.05$). Omega-3 actually consists of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), but gold sea cucumber (*Stichopus hermanii*) has high levels of EPA and DHA, 25.69% and 3.69%. The high level of EPA in *Stichopus hermanii* can indicate that the speed of repairing damaged tissue and the prevention of the prostaglandins formation causing inflammatory and cholesterol reduction in blood.[17] Meanwhile, DHA is considered as the major fatty acid in sperm, brain, and eye's retina. Thus, the high level of DHA intake can lower blood triglycerides causing heart disease.[18]

The mechanism of omega-3 fatty acids disease in lowering hyperlipidemia is by increasing the excretion of cholesterol in feces and suppressing the synthesis of triglycerides in liver so that it can alter the composition of fatty acids contained in lipoproteins. As a result, lipoprotein fluidity does not only become increasing, but also affects the activity of lipolytic enzymes as well as increases the speed of synthesis and catabolism of VLDL in plasma. On the other words, it can also be said that omega-3 can affect lipolysis of fatty tissue, so triglycerides are not formed by the reaction of free fatty acids and glycerol.[19] It can be concluded that golden sea cucumbers extract (*Stichopus hermanii* extract) decreasing of triglyceride serum levels in rats which got periodontitis.

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