# Effects of Golden Sea Cucumber (Stichopus Hermanii) Ethanol Extracts on Cholesterol Levels of Hypercholesterolemic Rats

*by* Farmindo Hartono

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# Effects of Golden Sea Cucumber (Stichopus Hermanii) Ethanol Extracts on Cholesterol Levels of Hypercholesterolemic Rats

**Farmindo Hartono[1], Indri Safitri Mukono[2], Maftuchah Rochmanti[3]**

*[1]Student of Faculty of Medicine, Airlangga University, [2]Professor of Medical Biochemistry Department, Faculty of Medicine, Airlangga University, [3]Doctorate of Pharmacology and Therapy Department, Faculty of Medicine, Airlangga University, Jl. Mayjen. Prof. Dr. Moestopo 47, Surabaya, 60132*

## Abstract

**Background:** Hypercholesterolemia is a condition characterized by an increase in cholesterol level in blood higher than normal limits. Hypercholesterolemia is a risk factor for cardiovascular disease with high mortality. Golden sea cucumbers are known to have antioxidant content. Objectives: This study aims to prove the effect of ethanol extract of golden sea cucumbers (*Stichopus hermanii*) on cholesterol levels in the blood of hypercholesterolemic rats.

**Method:** This study used a randomized post-test only control group method by randomizing 36 Wistar rats. Rats were divided into 4 groups namely KN, P1, P2, and P3 groups induced with high-fat feed. Ethanol extracts of golden sea cucumbers were given at different doses in the P1 group (4.25 mg/kgBW), P2 (8.5 mg/kgBW), and P3 (17 mg/kgBW) for 14 days. The blood cholesterol level was measured and compared with the KN group given the placebo.

**Result:** In the P1 and P3 groups, LDL levels were higher than in the KN group while in the P2 group were lower. In the measurement of HDL levels, obtained lower HDL levels in all groups of administration compared to the KN group. Total cholesterol and HDL level of all group were lower compared to the KN group.

**Conclusion:** When comparing LDL levels, there were no significant differences between the consenting group and the KN group. When comparing HDL levels, significant differences were seen in the P2 and P3 groups compared to the KN group. In the total cholesterol levels, a significant difference was only seen in the P2 group compared to the KN group.

***Keywords:*** *HDL, Hypercholesterolemia, Total Cholesterol, LDL, Stichopus hermanii.*

## Introduction

Hypercholesterolemia is a condition when LDL cholesterol (low-density lipoprotein) and total cholesterol in the blood increases above normal limits and HDL (high-density lipoprotein) cholesterol in the blood decreases below normal limits[1]. Hypercholesterolemia can occur due to weight, age, lack of exercise, stress, metabolic disorders, genetic disorders, and diets high in cholesterol and saturated fatty acids[2]. Total cholesterol and LDL cholesterol are harmful to health because they can cause atherosclerosis[3] in arteries, especially in the heart, brain, kidneys, eyes[4] so that they become one of the risk factors for cardiovascular disease such as coronary heart disease and stroke (coronary heart disease)[5].

Indonesia is a country with the biggest sea cucumber potential in the world[6]. Golden sea cucumber (*Stichopus*

**Corresponding Author:**
**Indri Safitri Mukono**
Professor of Medical Biochemistry Department, Faculty of Medicine, Airlangga University
e-mail: indrisafitri@yahoo.com

*hermanii*) is one of the species of sea cucumber that is often traded[6]. The use of golden sea cucumbers (*Stichopus hermanii*) is not only a food ingredient but can also be used in the health sector for treatment[7]. Golden sea cucumbers (*Stichopus hermanii*) contain glycoprotein, collagen, glycosaminoglycan, hyaluronic acid, chondroitin sulfate, dermatan sulfate, heparin, heparin sulfate, mucopolysaccharide, proteoglycans, docosahexaenoic acid (EPA-DHA), flavonoids, saponin and so on so that golden sea cucumbers can accelerate the process of wound healing, have antibacterial, antifungal and antioxidant activity[8–12].

Antioxidant activity of golden sea cucumbers can be seen in research (Revianti *et al*. 2016)[13] which states the administration of ethanol extracts of golden sea cucumbers has been shown to inhibit increased lipid peroxidase, decreased catalase activity, and increased horn (corneum) layer thickness. Research to observe the antioxidant activity of golden sea cucumbers against LDL, HDL and total cholesterol in the blood is important to be carried out as an alternative consideration for the treatment of hypercholesterolemia.

## Method

This research is a type of experimental research (true experimental study) with a randomized post-test only control group method.

**Extraction of golden sea cucumber:** The extraction process begins by freezing and drying the golden sea cucumbers that have been prepared using a freeze-drying tool. Then the golden sea cucumbers are ground into powder using a common blender. 200 grams of powder then dissolved using 96% ethanol for 1 day. After 1 day the solution is filtered using filter paper. The filter results are evaporated using an evaporator. Obtained golden sea cucumber extract which is then stored in a sterile state in the refrigerator at 4ºC.

**Adaptation period:** The experimental animals used were 36 male Wistar rats which would be divided into 4 groups the negative control group (KN) and 3 treatment groups (P1, P2, P3). Each group consisted of 9 male Wistar rats. The adaptation process was carried out for 7 days and during the process of adaptation all mice were treated the same, that is, given a standard rat feed.

**Induction period of high-fat feed:** During the induction period, all groups of rats will be given a high-fat feed with a composition of 40% chicken feed, 40% duck egg yolk, and 20% pork fat for 2 months or 60 days obtained from the Faculty of Veterinary Medicine Airlangga University to be induced to become hypercholesterolemia. On the 60th day, a random sample of blood was collected in 1 rat and LDL, HDL and total cholesterol levels were measured at the Regional Health Laboratory to confirm the state of hypercholesterolemia in mice.

**Giving golden sea cucumber extract:** The negative control group was not given golden sea cucumber extract and replaced with placebo in the form of aquadest. Group P1 was given golden sea cucumber extract at a dose of 4.25 mg/kgBW per day for 2 weeks. P2 group was given golden sea cucumber extract at a dose of 8.5 mg/kgBW per day for 2 weeks. P3 group was given golden sea cucumber extract at a dose of 17 mg/kgBW per day for 2 weeks. The entire group of ratscontinued to be given high-fat feed during the treatment period to avoid the possibility of cholesterol returning to normal due to the standard rat feed.

**Collection of blood samples:** Blood samples are collected at the end of the 2nd week. Rat blood was obtained by taking blood using the cardiac puncture method. Blood sampling is done according to the procedure that has been studied by (Beeton, Garcia and Chandy 2007). After the blood sample is obtained the rat will be put into the freezer and then it will be taken as rat waste.

**Measurement of LDL and HDL levels:** Measurement of LDL and HDL levels from rat serum will use the precipitation (direct) method by using an analyzer and conducted at the Regional Health Laboratory.

**Data Analysis:** The data obtained were tested for normality using *Shapiro Wilk* or *Kolmogorov Smirnov* (n <50) and obtained normal distribution data which was then analyzed by parametric statistics using ANOVA. Data analysis using SPSS 23 program and p value <0.05 was declared significantly.

## Result

The results of this study were carried out by comparing LDL, HDL, and total cholesterol of Wistar rats induced using high-fat feed in all groups of Wistar rats can be seen in Table 1. Table 1 shows the average and standard deviation of LDL, HDL and total cholesterol levels in KN group (induced high fat feed and given a

placebo containing aquadest), P1 group (induced high fat feed and given a golden cucumber extract sonde with a dose of 4.25mg/kgBW per day), P2 group (induced high fat feed and given sonde sea cucumber extract golden with a dose of 8.5mg/kgBW per day), and group P3 (induced high-fat feed and given a sonde extract of golden sea cucumbers with a dose of 17mg/kgBW per day).

Overall data analysis showed significant results between all groups at HDL levels (p = 0.019), but not significant between all groups at LDL levels (p = 0.366) and total cholesterol levels (p = 0.182).

## Discussion

Hypercholesterolemia, especially LDL cholesterol accompanied by an increase in free radicals in the blood will cause the LDL oxidation process which will eventually lead to atherosclerosis[3] which manifests clinically in coronary heart disease and stroke[14]. The content of flavonoids has antioxidant activity which has the potential to reduce LDL and total cholesterol and increase HDL. The mechanism is to prevent LDL oxidation thereby increasing the expression of LDL receptors so that LDL receptors will absorb LDL in the blood into cells as a whole. LDL will then be hydrolyzed by lysosomes and cholesterol will enter the cells thereby reducind cholesterol in the blood. The entry of cholesterol in the blood also inhibits the synthesis of enzymes that play a role in synthesizing cholesterolthereby reducing cholesterol synthesis[15]. In addition it is to increase the activity of lecithin cholesterol acyltranferase (LCAT). The LCAT activity and its activator will change discoid HDL to HDL3. HDL3 will then receive cholesterol from the tissue. Cholesterol is then esterified so that there is an enlargement in HDL3 and turns into HDL2. HDL2 will then carry cholesterol and cholesterol esters to the liver for extraction. An increase in LCAT activity will cause an increase in HDL resulting in an increase in the excretion of cholesterol by the liver through bile[16,17].

In this study, it can be seen that total cholesterol levels, P2 group have lower total cholesterol levels compared to total cholesterol levels in the KN group and close to normal, which is 54.88 mg/dL (normal <54 mg/dL)[18], but statistical test comparison of LDL levels and total cholesterol for each treatment group was not significant. This can be caused by giving extracts that are not long enough. Most studies on hypercholesterolemia provide treatment for 14 days[14,19,20] but research by Kasim, Kurniawati and Nurhidayat[21] which also has a hypercholesterolemia theme showed a decrease in total cholesterol. rats drastically on day 21 were given Angkak powder at a dose of 0.5g/day while on day 14 cholesterol levels were still high. It also found lower HDL levels in all treatment groups (P1, P2, and P3) compared to the KN group. This is thought to be due to the activity of the enzymes Hepatic Lipase (HL) and Endothelial Lipase (EL). HL is a lipolytic enzyme that is synthesized by hepatocytes and has TG lipase activity and phospholipase A1 activity. This enzyme has an important role in mediating HDL metabolism. HL has greater HDL activity than VLDL or chylomicrons and converts larger HDL particles into smaller, pre-HDL, and lipid-free or fat-free HDL remnants. The magnitude of the HL effect on HDL is highly dependent on the composition of HDL. HL postheparin activity is inversely correlated with low HDL-c levels in humans. Excessive HL expression in mice and rabbits results in a marked decrease in HDL-c and a reduction in HDL size[22,23].EL is also an enzyme that plays an important role in modulating HDL metabolism. EL is synthesized by endothelial cells, functions on the surface of vascular endothelium, and mainly has phospholipase A1 activity. EL hydrolyzes HDL more efficiently than other lipoprotein fractions. Adenoviral vectors that mediate over expression in EL in mice resulted in significant decreases in HDL-c and apoA-I levels. In addition, inhibition of EL antibody activity in rats significantly increases plasma HDL-c, phospholipid, and apoA-I levels and results in greater HDL[22].

**Table 1. Mean and Standard Deviation of LDL, HDL, and Total cholesterol level of each group**

| Characteristic | Mean ± Standard Deviation | | | | p |
|---|---|---|---|---|---|
| | KN | P1 | P2 | P3 | |
| LDL (mg/dL) | 14.14 ± 5.64 | 14.38 ± 3.89 | 13.13 ± 3.60 | 18.00 ± 8.44 | 0.365 |
| HDL (mg/dL) | 26.29 ± 2.22 | 25.50 ± 2.39 | 22.13 ± 3.40 | 23.00 ± 2.83 | 0.019 |
| Total cholesterol (mg/dL) | 70.14 ± 10.87 | 62.75 ± 10.12 | 54.88 ± 12.22 | 64.00 ± 17.47 | 0.182 |

## Conclusion

Hypercholesterolemia rats given ethanol extract of golden sea cucumbers (*Stichopus hermanii*) at a dose of 4.25 mg/kgBW and 17 mg/kgBW had higher LDL levels compared to hypercholesterolemia rats that were not given golden sea cucumber ethanol extract. Hypercholesterolemia rats given ethanol extract of golden sea cucumbers (*Stichopushermanii*) at a dose of 8.5 mg/kgBW had lower LDL levels, but the ratio of each group was not significant.

Hypercholesterolemia rats given ethanol extract of golden sea cucumbers (*Stichopus hermanii*) at a dose of 4.25 mg/kgBW, 8.5 mg/kgBW and 17 mg/kgBW had lower HDL levels compared to hypercholesterolemia rats without golden sea cucumber ethanol extract.

Hypercholesterolemia rats given golden sea cucumber ethanol extract (*Stichopus hermanii*) at a dose of 4.25 mg/kgBW, 8.5 mg/kgBW and 17 mg/kgBW had lower total cholesterol levels compared to hypercholesterolemia rats that were not given golden sea cucumber extract, but the ratio of each insignificant group.

**Conflict of Interest:** There was no conflict of interest in this study.

**Ethical Clearance:** The Ethical Clearance is taken from the health research ethics committee at the Faculty of Medicine at Airlangga University, Surabaya,Indonesia.

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