The Cardioprotective Effect of Polysaccharide Sulphate Isolated from Brown Algae (*Sargassum polycystum)*

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Abstract. The incidence of atherosclerosis is characterized by an increase in the value of low-density lipoprotein (LDL) and a decrease in the value of high-density lipoprotein (HDL) as well as an increase in the total white blood cell count which can indicate the occurrence of atherosclerosis. This study used 18 rats which were divided into 6 groups of 3 each, namely a normal control group, a negative control group (CMC 0.5%), a positive control group (Simvastatin 20 mg/kg BW), and 3 groups given a sulfate polysaccharide isolate compound test material (dosage of 250, 50, and 10 mg/kg of body weight). The results showed that sulfated polysaccharide isolates had an effect in reducing white blood cells significantly between doses of 250 mg/kg BW and 50 mg/kg BW as well as reducing SGOT levels. Unfortunately it did not reduce the SGPT level. The results of the Mann-Whitney post hoc test showed that administration of sulfated polysaccharides at an optimal dose of 250 mg/kg BW reduced the number of foam cells in the atherosclerotic white rats' (*Rattus norvegicus*) aortas that were given a high-fat diet and had activity in reducing CKMB levels compared to other doses.

1 Introduction

Cardiovascular disease is a non-communicable disease which is the main cause of death in the world [1]. In 2019 more than 17 million premature deaths or under the age of 70 years were caused by non-communicable diseases, 38% of which were caused by cardiovascular disease [2]. As many as three quarters or around 80% of deaths caused by cardiovascular disease occur in countries with low to middle income, 40% of which are premature deaths [2-3]. In Indonesia, cardiovascular disease is ranked as the biggest cause of death with a

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percentage of 35% of 1.8 million deaths in 2016 [4]. Based on Basic Health Research data for 2018, the prevalence of heart disease with a doctor's diagnosis is 1.5% or 15 out of 1000 people [5].

Atherosclerosis is the most common cause of various types of cardiovascular disease such as claudication, myocardial infarction, heart failure, and stroke [6]. Atherosclerosis is a condition caused by a chronic inflammatory process on the inner walls of medium to large sized arteries including the aorta, coronary arteries, carotid arteries, and lower extremist arteries which causes plaque formation or what is called atheromas [7]. There are several risk factors that contribute to atherosclerosis, namely increased serum cholesterol and blood pressure, diabetes, obesity, smoking, family history and lifestyle [8]. Atherosclerosis is characterized by an increase in the low-density lipoprotein (LDL) value and a decrease in the high-density lipoprotein (HDL) value. [9]. In addition, an increase in the total white blood cell count can indicate the occurrence of atherosclerosis [10].

White blood cells have a role in the inflammatory process that occurs in atherosclerosis [11]. White blood cells are part of human blood cells that have a role in the immune system or help the body fight infections and various types of diseases [9]. Atherosclerosis is closely related to hypercholesterolemia characterized by the accumulation of lipids and fibrous elements in the large arteries (lipid abnormalities). However, the occurrence of inflammation and the role of white blood cells is also an important part of atherosclerosis [11].

Treatment of atherosclerosis focuses on several aspects, namely; blood pressure reduction, fat reduction and inhibition of blood clotting. Generally, statin and aspirin class drugs are used in the treatment of atherosclerosis, these two drugs also have anti-inflammatory activity in atherosclerosis. [12]. Although the use of statins is commonly used in lowering cholesterol and reducing morbidity and mortality in cardiovascular disease, statins have several side effects in the new onset of diabetes mellitus 2, neurological, neurocognitive and hepatotoxic effect [13]. In addition, aspirin, which has analgesic, anti-pyretic and anti-inflammatory effects, also has side effects including digestive and respiratory disorders and can increase platelet aggregation [14]. Due to the side effects of the statin and aspirin class drugs, researchers are looking for other therapies to treat inflammation in atherosclerosis with minimal side effects.

Algae including brown algae (Phaeophyceae) are the most abundant resources in the oceans which are rich sources of functional metabolites such as polysaccharides, proteins, peptides, lipids, amino acids, polyphenols and mineral salts [15]. Brown algae have different types, one of which is *Sargassum* sp. containing compounds such as polysaccharides, alginate and laminarin [16]. Polysaccharides isolated from algae have different characteristics such as molecular size, type of glycosidic bond, and type of ratio of the constituent monosaccharides related to differences in their biological activities. [17]. The main polysaccharide that has biological activity (is bioactive) in the brown algae species *Sargassum polycystum* is sulfated polysaccharide [18]. Sulfated polysaccharides have stronger pharmacological activity than ordinary polysaccharides such as acting as anticoagulants, antioxidants and anti-inflammatories [19-20]. Fucoidan is a type of sulfated polysaccharide that plays a role in atherosclerosis therapy including reducing total cholesterol, triglyceride, LDL levels, increasing HDL levels and in inflammatory processes [21]. [22] stated that treatment using polysaccharides from algae is the therapy of choice because it is more economical, abundantly available, and has low toxicity.

Therefore this study was conducted to determine the potency of sulfated polysaccharide isolates from brown algae (*Sargassum polycystum*) against white blood cells in atherosclerosis-model rats (*Rattus norvegicus*). The research used white rats (*Rattus norvegicus*) as an experimental animal model that was given a high-fat diet.

2 Material and methods

The materials used in the research were Carboxy Methyl Cellulose (CMC 0.5%), 96% ethanol, ether, sulfated polysaccharide isolate (Marianti A. Manggau Collection), duck egg yolk, butter (BlueBand®), rat feed, simvastatin tablets, and white rats (*Rattus norvegicus*).

2.1 Preparation of test animals

The test animals used in the study were male white rats (*Rattus norvegicus*) with a body weight of 200-300 g. A place for keeping test animals was prepared, namely cages, husks, places to eat and places to drink. The test animals were first acclimatized for 7 days in order to adapt to their new environment [23].

2.2 Sample preparation

Sulfated polysaccharides were extracted from brown algae (*Sargassum polycystum*) by maceration. 400 g samples of dry brown algae were put into an infusion pot, then 2 L of aquadest which had been mixed with 17 mL of 0.1 N HCl was added. The sample mixture was heated for 4 h (stirred once every 10 min). After 4 h, it was cooled and then filtered. $CaCl_2$ 2% with a ratio of 1 sample: 2 $CaCl_2$ 2% was added to the filtered result. A filtered yield of 1250 mL was obtained so that 2500 mL of $CaCl_2$ 2% was needed. 25 g of $CaCl_2$ were weighed out and then mixed. The solution was left to separate the precipitate and filtrate. The filtrate was removed and 96% ethanol was added to it with a ratio of 1:1. The precipitate was removed and evaporated.

2.3 Preparation of 0.5% CMC colloidal solution

5 g of CMC was put into a beaker, then hot distilled water with a temperature of 70°C was added little by little and stirred using a magnetic stirrer until a colloidal solution of CMC was formed, then distilled water was added to a volume of 1000 mL in a volumetric flask [24].

2.4 Preparation of sulfated polysaccharide suspension

Brown algal sulfated polysaccharidemade was prepared in the 0.5% CMC suspension with doses of 10 mg/kg BW, 50 mg/kg BW, and 250 mg/kg BW. The isolate that had been weighed for each dose was dispersed in 5 ml of 0.5% CMC which had been made previously and then crushed until homogeneous, and put into a 10 mL volumetric flask to which the colloidal CMC solution was then added to bring it up to the 10 mL mark.

2.5 Preparation of simvastatin suspension

A stock suspension of simvastatin was prepared by weighing 20 tablets of simvastatin at a dose of 20 mg each, calculating the average weight and then grinding until smooth. It was then weighed according to the conversion calculation results and suspended with 0.5% CMC little by little until it was homogeneous. Then, it was transferred to a volumetric flask and the volume was made up to the mark [25].

2.6 Modeling high-fat diet white rats

A total of 18 male white rats which had previously been acclimatized for 7 days were divided into 6 groups, namely 1 group as a normal control and the other 5 groups were fed a high-fat diet. The high-fat diet given consisted of butter and duck egg yolks. For 1 day, 400 g of butter was used. The butter was first heated on an electric stove with a maximum temperature of 120°C, while stirring occasionally. This continued until it was not thick but it was not allowed to melt. It was cooled for a while then used.

As for the duck egg yolks, they were first separated from the egg whites and then combined in a container. In 1 day, 10 eggs were used. The rats were each fed this high-fat diet orally according to the weight of each rat. Induction of the high-fat diet was given three times a day for 28 days [23].

2.7 Initial total cholesterol, HDL and triglyceride measurements

Initial measurement of total cholesterol, HDL, and triglyceride levels was carried out by taking blood sample from each group of test animals after being induced by the high-fat diet. 2 mLs of blood was taken through the lateral vein of the rats' tails using a sterile syringe and then accommodated in a vacutainer tube. Then the blood in the tube was allowed to clot for approximately 15 minutes and then centrifuged at 3000 rpm for 15 minutes to obtain serum. The resulting serum was immediately separated into an Eppendorf tube. The blood serums from the 6 groups, namely normal control, negative control, positive control, PST, PSS, and PSR which had been separated were put into the auto analyzer to measure total cholesterol, HDL, and triglyceride levels [26].

2.8 Analysis groups

In this study, 18 rats were used which were divided into 6 groups with each group consisting of 3 rats. The treatment used was the normal control group, namely no treatment (without administration of polysaccharide sulfate), a negative control group (0.5% CMC induction), a positive control group (simvastatin induction) and a group given brown algae sulfate polysaccharide (*Sargassum polycystum*) at differing dose levels. The treatment used consisted of:

- Group 1 : group without treatment, given standard feed.
- Group 2 : high-fat diet white rat model given 0.5% CMC colloidal solution (negative control).
- Group 3 : high fat diet white rat model given simvastatin suspension of 20 mg/kgBW (positive control).
- Group 4 : a high-fat diet white rat model given a sulfated polysaccharide suspension at a dose of 250 mg/kg BW (PST).
- Group 5 : a high-fat diet white rat model given sulfated polysaccharide suspension at a dose of 50 mg/kg BW (PSS).
- Group6 : a high-fat diet white rat model given sulfated polysaccharide suspension at a dose of 10 mg/kg BW (PSR).

Polysaccharide sulfate (sample), simvastatin (positive control) and CMC 0.5% (negative control) were administered orally for 14 days in a high-fat diet white rat model, then blood samples were taken again on day 14 after being given the sample, simvastatin, and CMC.

2.9 Measurement of total cholesterol, HDL and triglyceride

Measurement of total cholesterol, HDL and triglyceride levels after treatment was carried out by taking blood samples from each test animal group on the 14th day after the induction of sample (sulfated polysaccharide suspension), simvastatin and CMC. 2 mL of blood was taken through the lateral vein of the rats' tails using a sterile syringe and then accommodated in a vacutainer tube. Then the blood in the tube was allowed to clot for approximately 15 minutes and then centrifuged at 3000 rpm for 15 minutes to obtain serum. The resulting serum was immediately separated into an Eppendorf tube. The blood serums from the 6 groups, namely normal control, negative control, positive control, PST, PSS, and PSR which had been separated were put into the auto analyzer to measure total cholesterol, HDL and triglyceride levels [26].

2.10 Measurement of White Blood Cell (WBC)

Measurement of white blood cells including total white blood cells, neutrophils, lymphocytes, monocytes, eosinophils and basophils was carried out by taking fresh blood samples from rats through the lateral tail veins and orbital sinuses using a sterile syringe and capillary tube then collected in a vacutainer tube containing EDTA. Measurement of white blood cells was carried out at the Hasanuddin University Hospital laboratory using an autoanalyse tool or instrument (Hematology analyzer) [27].

2.11 Measurement of SGOT and SGPT levels

The levels of SGOT and SGPT was measured by taking blood from each group of test animals on the 14th day after treatment. Blood was taken through the lateral vein of the rats' tails using a 2 mL syringe and through the orbital sinus of the eye using a capillary tube and then collected in a red vacutainer tube. Then measurements of SGOT and SGPT levels were carried out at the Hasanuddin University Hospital Laboratory using a humalyzer.

2.12 Measurement of CKMB levels

Measurement of CKMB levels after treatment was carried out by taking blood from each group of test animals on the 14th day which was taken through the lateral vein of the rats' tails using a 2 mL syringe injection and then placed in a vacutainer tube. The blood sample was then centrifuged for 10 min at 4000 rpm. After centrifugation, the serum was put into an Eppendorf tube and stored at -20°C. CKMB levels in the serum were then measured using a humalyzer.

2.13 Animal test surgery

Euthanasia of the rats was done by lower neck dislocation with the help of the influence of inhalation of the anaesthetic diethyl ether then the lower abdomen of the rats was split open to the chest then the aortic organs were removed. The aortic organs were taken and cleaned with physiological NaCl then fixed in 10% neutral buffered formalin [28].

2.14 Preparation of aortic organ histopathology from the test animals

The aortic organ was fixed deeply in neutral buffered 10% formalin for 24 h. Then the aortic organ was inserted into the tissue cassette and dehydrated using 70%, 80%, 90% alcohol and

2x absolute alcohol. Each stage was carried out for 30 minutes. The clearing process was carried out using xylol and paraffin (ratio 1:3, 1:1, 3:1) for 30 min each. This was then followed by liquid paraffin infiltration for 2 h and embedding into the block. Next, the organs were sectioned transversely with a rotary microtome 3-5 µm thick. The slices were then placed on an object glass and dried at 35°C using a hot plate, then stained using Hematoxylin Eosin (HE). Once dry, they were covered using a cover glass [29].

2.15 Histopathological observation of aortic organs in test animals

Observation of aortic histology preparations was carried out using an electric binocular microscope to view atherosclerotic lesions and then the photos were collected. Observations were carried out by anatomical pathology specialists. Parameters were analysed by counting the number of foam cells in rat aorta preparations with HE staining.

2.16 Data Analysis

The data obtained from the measurement of total cholesterol, HDL, triglyceride, WBC, SGOT, SGPT and CKMB levels were collected from each treatment group and then analysed using statistical methods with the help of SPSS software. The results of data analysis are discussed and conclusions drawn.

3 Results and discussion

3.1 Total Cholesterol Levels

Examination of total cholesterol levels plays a very important role because it can be used as a reference by a doctor to make a diagnosis. Therefore, the analysis must be carried out carefully to minimize errors in the analysis process.

Statistical analysis of all groups showed that the treatment groups had a significantly different effect on the lipid profile. Based on the results, the normal control group and the negative control group had a significance value of 0.059 ($p > 0.05$), which means that there was no significant difference. Meanwhile, the comparison between the normal control group and the positive control group, PST 250 mg/kg BW, PSS 50 mg/kg BW and PSR 10 mg/kg BW obtained significance values ($p < 0.05$) respectively 0.001; 0.001; 0.013; 0.037. These results indicate that there were significant differences between the treatment groups. Data from the results of this statistical analysis in full can be seen in Table 1.

Table 1. Results of measuring average value of total cholesterol levels on day 28 (after induction of a high-fat diet) and day 42 (after treatment) (mean value ± SD, n = 3).

Test Group	Measurement (10^3/µL)	
	After induction on a high fat diet	After treatment
Normal control	37.67 ± 3.06	37.00 ± 3.00
Negative control	53.33 ± 7.57	56.67 ± 7.02
Positive control	64.33 ± 6.66	55.43 ± 8.69
PST 250 mg/kg BW	66.33 ± 4.04	56.50 ± 6.25
PSS 50 mg/kg BW	57.67 ± 3.78	54.33 ± 3.51
PSR 10 mg/kg BW	54.67 ± 8.14	50.67 ± 9.02

Description: PST (High dose sulfated polysaccharide)
PSS (Medium dose polysaccharide sulfate)
PSR (Low dose polysaccharide sulfate)

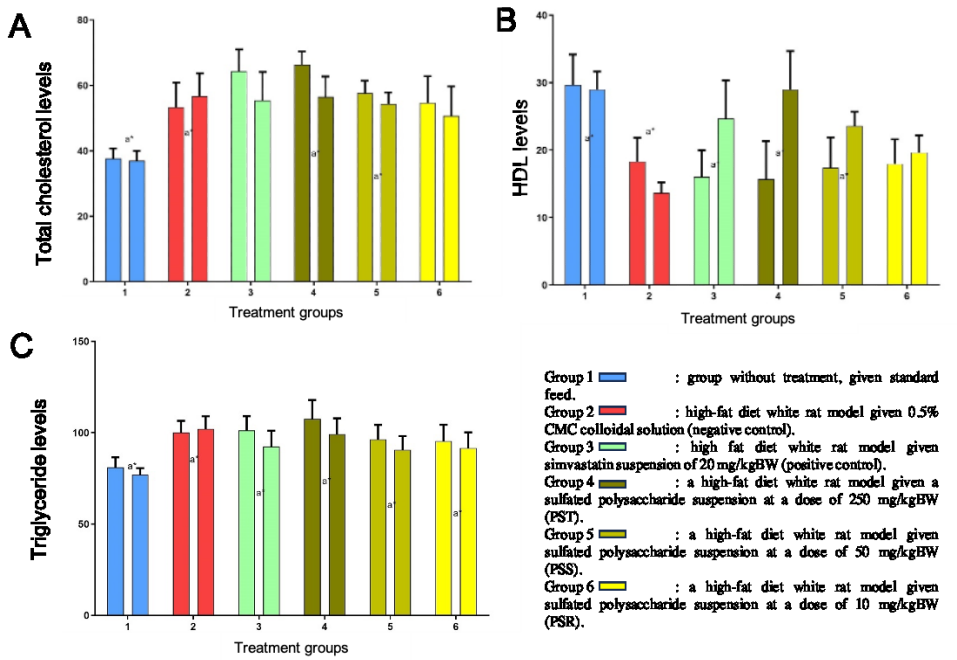


Fig. 1. Histogram of average total cholesterol levels (A), average HDL levels (B) and average triglyceride levels (C), all groups of animal test evaluated on day 28 (after induction of a high-fat diet) and day 42 (after treatment) (mean ± SD, n = 3).

Based on the results in Table 1, which was then depicted in the form of a histogram as in Figure 1A, the average value of total cholesterol levels obtained in rats with negative control on day 28 was 53.33 mg/dL and on day 42 was 56.67 mg/dL, higher than group 1 (normal control) which was given standard feed. This is because the negative group induced by a high-fat diet was only given CMC so it didn't reduce cholesterol levels and will continue to increase. As for the normal control, they were only given standard feed and were not given any treatment so that the total cholesterol level was not affected.

Meanwhile, the average total cholesterol level in group 4 (PST 250 mg/kg BW) at the examination on day 42 was 56.50 mg/dL, which was higher than group 5 (PSS 50 mg/kg BW) and group 6 (PSR 10 mg/dL) and group 3 (positive control) which was given simvastatin at a dose of 20 mg/kg BW. These results indicate that the treatment groups that were given simvastatin and sulfated polysaccharides had an effect in the form of a decrease in total cholesterol levels. This is because simvastatin is a statin class drug which is the first line of defence in reducing total cholesterol levels in the blood [30]. Regarding the previous research, it is showed that sulfated polysaccharides have a better effect than simvastatin in reducing total cholesterol levels [22, 31].

3.2 HDL levels

HDL contains cholesterol as its main core lipid and contributes about 20-30% of total cholesterol in the blood. High HDL levels actively protect the body from atherosclerotic cardiovascular disease. In addition to total cholesterol, HDL levels are also very important to measure and will be very helpful in making a diagnosis and identifying disorders/disease. The statistical analysis between the normal control group and the negative control group and

PSR 10 mg/kg BW showed significance values of 0.067 and 0.058 ($p > 0.05$), which means that there was no significant difference. Meanwhile, the comparison between the normal control group and the positive control group, PST 250 mg/kg BW and PSS 50 mg/kg BW obtained significance values ($p < 0.05$) respectively of 0.022; 0.019; 0.042 (Table 2).

Table 2. Results of measuring average HDL levels on day 28 (after induction of a high-fat diet) and day 42 (after treatment) (mean value ± SD, n = 3).

Test Group	Measurement (10^3/µL)	
	After induction on a high fat diet	After treatment
Normal control	29.67 ± 4.50	29.00 ± 2.64
Negative control	18.33 ± 3.51	13.67 ± 1.53
Positive control	16.00 ± 4.00	24.67 ± 5.69
PST 250 mg/kg BW	15.67 ± 5.68	29.00 ± 5.68
PSS 50 mg/kg BW	17.39 ± 4.51	23.61 ± 2.08
PSR 10 mg/kg body weight	18.00 ± 3.60	19.67 ± 2.52

Description: PST (High dose sulfated polysaccharide)
PSS (Medium dose polysaccharide sulfate)
PSR (Low dose polysaccharide sulfate)

Figure 1B shows the average HDL levels in normal control rats on day 28 which was 29.67 mg/dL and on day 42 which was 29.00 mg/dL, meaning that there was no significant decrease in HDL levels. In contrast the negative control group experienced a significant decrease in HDL levels where on day 28 the average HDL level was 18.33 mg/dL and on day 42 it was 13.67 mg/dL. This was because in the normal control group they were only given standard feed and this did not have a significant effect on HDL levels, while in the negative control group CMC administration made HDL levels decrease, inversely proportional to total cholesterol levels.

Meanwhile, the average HDL level in group 4 (PST 250 mg/kg BW) experienced an increase where on day 42 the average HDL level was 29.00 mg/dL, greater than group 5 (PSS 50 mg/kg BW) and group 6 (PSR 10 mg/kg BW) and group 3 (positive control) which were given simvastatin at a dose of 20 mg/kg BW. These results indicate that the treatment groups that were given sulfated polysaccharides or simvastatin experienced an increase in HDL levels. This is because simvastatin, in addition to lowering total cholesterol levels, can also increase HDL levels in the blood [30]. As for sulfated polysaccharides, besides having a better effect than simvastatin in reducing total cholesterol levels, sulfated polysaccharides are also able to increase HDL levels [22, 31].

3.3 Triglyceride levels

Triglycerides are one of the parameters of the lipid profile, present in plasma as part of lipoproteins. The main carrier of triglycerides in the post-absorptive (fasting) state is VLDL. The concentration of triglycerides in the blood is closely related to the risk of atherosclerosis and its complications. So that in addition to evaluating the total cholesterol and HDL levels, the measurement of triglyceride levels is equally important. The statistical analysis showed a significance value of 0.02 ($p \leq 0.05$), which means that the treatment group had a significantly different effect on triglyceride levels. The comparison between the normal control group and negative control, PSS 50 mg/kg BW and PSR 10 mg/kg BW obtained a significance value of 0.070, 0.185, and 0.237 respectively ($p > 0.05$), which showed that there was no significant difference. Meanwhile, a comparison between the normal control group and the positive control and PST 250 mg/kg BW obtained significance values ($p < 0.05$) of 0.014 and 0.033 respectively.

Table 3. Results of measuring average triglyceride levels on day 28 (after induction of a high-fat diet) and day 42 (after treatment) (mean value ± SD, n = 3)

Test Group	Measurement (10^3/µL)	
	After induction on a high fat diet	After treatment
Normal control	81.00 ± 5.56	77.00 ± 3.61
Negative control	100.00 ± 6.55	102.00 ± 7.00
Positive control	101.33 ± 7.76	92.33 ± 8.73
PST 250 mg/kg BW	107.66 ± 10.26	99.34 ± 8.50
PSS 50 mg/kg BW	96.32 ± 8.02	90.68 ± 7.51
PSR 10 mg/kg BW	95.33 ± 9.07	91.67 ± 8.50

Description: PST (High dose sulfated polysaccharide)
PSS (Medium dose polysaccharide sulfate)
PSR (Low dose polysaccharide sulfate)

Based on Table 3 which was then depicted in the form of a histogram as shown in Figure 1C, the average triglyceride levels in the normal control group rats decreased on day 42 with an average triglyceride level of 77.00 mg/dL, in contrast to the negative control group which experienced an increase in triglyceride levels with an average on day 42 of 102.00 mg/dL. This is because the normal control group was only given standard feed and this did not have a significant effect on triglyceride levels. As for the negative control group, it was because the administration of CMC would increase triglyceride levels, directly proportional to total cholesterol levels and inversely proportional to HDL levels.

However, the average triglyceride level in group 4 (PST 250 mg/kg BW) experienced the highest decrease where on day 42 the average triglyceride level was 99.34 mg/dL, greater than group 5 (PSS 50 mg/kg BW) and group 6 (PSR 10 mg/kg BW) and also greater than group 3 (positive control) who were given simvastatin at a dose of 20 mg/kg BW. These results indicate that the treatment groups that were given sulfated polysaccharides or simvastatin were able to decrease triglyceride levels. This is because in addition to increasing HDL levels and reducing total cholesterol levels in the blood, simvastatin can also reduce triglyceride levels [30]. The same held true for the sulfated polysaccharides [22, 31].

The evaluation of total cholesterol, HDL, and triglyceride levels as well as the results of statistical data analysis show that the administration of sulfated polysaccharides from brown algae (*Sargassum polycystum*) had a positive effect on the improvement of blood lipid profile in white rats (*Rattus norvegicus*) with a high-fat diet. Sulfated polysaccharides had an effect that was not significantly different between doses of 250 mg/kg, 50 mg/kg, and 10 mg/kg. However, from these three doses and from the three lipid profile examinations, it was found that the 250 mg/kg BW dose had the best effect compared to the other two dose variations and from the data obtained the 250 mg/kg BW dose polysaccharide sulfate treatment group also yielded a better effect compared to simvastatin 20 mg/kg BW. It can be said that, the higher the dose of sulfated polysaccharides, the greater the effect given in lowering total cholesterol and triglyceride levels and increasing HDL levels.

This result is also in line with the research conducted by [31], who reported that sulfated polysaccharide compounds were able to reduce total cholesterol, triglyceride levels, and increase HDL. Followed by the research conducted by [32], it was stated that the sulfated polysaccharide from *S. polycystum* has several biological activities, namely antioxidant activity, anti-TNF-α, and anti-cholesterol. A dose of 200 mg it could reduce total cholesterol, LDL, triglycerides and increase serum HDL levels of male white rats fed a high-fat diet.

3.4 White blood cell level

After 6 weeks of observation, fresh blood samples were taken from the rats via the lateral tail veins and orbital sinuses using a sterile syringe and capillary tube and then collected in a

vacutainer tube containing EDTA. Measurement of leukocyte cells using an autoanalyse instrument (Hematology analyzer). This procedure was conducted at the Pathology Laboratory of Hasanuddin University Hospital.

The results of the effect of sulfated polysaccharide isolates from brown algae on white blood cell levels in male white rats, shown in Table 4.

Table 4. Results of measurements of average white blood cell level on day 28 (after induction) and day 42 (after treatment) (mean value ± SD, n = 3)

Test Group	Measurement (10^3/µL)	
	After induction on a high fat diet	After treatment
Healthy control	12.75 ± 0.15	11.97 ± 0.88
Negative control	17.19 ± 0.18	16.27 ± 0.73
Positive control	18.50 ± 1.20	12.28 ± 1.72
PSS 250 mg/kg BW	16.85 ± 1.87	11.16 ± 1.92
PSS 50 mg/kg BW	16.68 ± 0.8	13.67 ± 0.23
PSS 10 mg/kg BW	16.17 ± 0.61	14.84 ± 0.62

Description: PSS (Polysaccharide Sulphate Isolate)

Table 4 shows that there was a significant difference in white blood cell levels in white rats between the healthy group and the other treatment groups on day 28 (after induction of a high-fat diet). The statistical analysis showed a significance value of 0.00 ($p < 0.05$) which means that in these data there is a significant difference in the value of white blood cell levels between treatment groups. Based on the results of the post-hoc test comparing the healthy group with the negative group, the positive group, PSS 250 mg/kg BW, PSS 50 mg/kg BW, and PSS 10 mg/kg BW there were significance values ($p < 0.05$) of 0.002, 0.00, 0.003, 0.005 and 0.013 respectively, indicating that there was a significant difference between the healthy groups compared to other groups, where there was an increase in white blood cells after induction of a high-fat diet in the negative group, the positive group, PSS 250 mg/kg BW, PSS 50 mg/kg BW, and PSS 10 mg/kg kg BW.

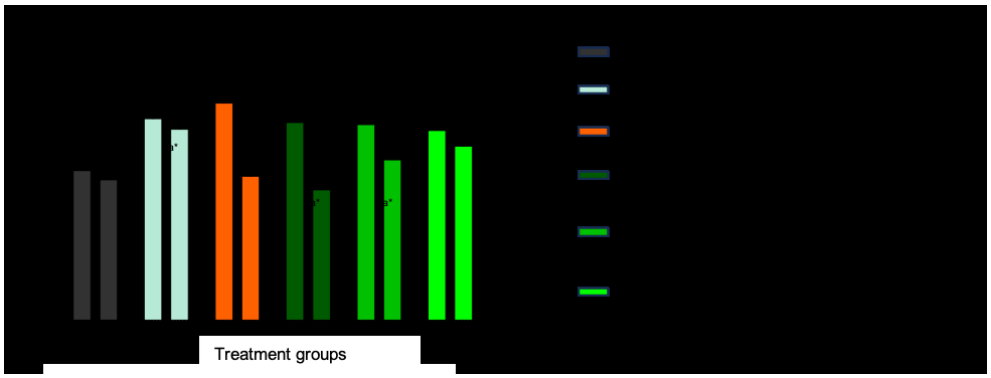


Fig. 2. Histogram of average white blood cell levels on day 28 (after induction) and day 42 (after treatment) in test animals (mean ± SD, n = 3).

Based on figure 2, the average value of white blood cell levels in the healthy control group (without treatment) can be seen by comparing the measurements on the 28th day (after induction) and the 42nd day (after treatment). After induction, the average value of white blood cell levels were 12.75 ± 0.15 and the average WBC level after treatment was 11.97 ± 0.88. Furthermore, the statistical test results obtained a significance value of 0.208 ($p > 0.05$)

which means there was no significant difference, because the healthy controls (blanks) were not given any treatment so it did not affect the white blood cell levels of the test animals.

Comparison of the test results in the treatment group 2 (negative control, CMC 0.5%) on the 28th day (after induction) and the 42nd day (after treatment) obtained the average value on the 28th day (after induction) of 17.19 ± 0.18 while the average after treatment was 16.27 ± 0.73 with a percentage decrease of 0.91 ± 0.55. Based on statistical testing, a significance value of 0.103 ($p > 0.05$) was obtained, which means that there was no significant difference, indicating that the administration of 0.5% CMC colloidal solution had no effect on white blood cell levels. From the results obtained, it appears that the CMC used as a negative control did not show any analgesic effect that occurred. This is because the negative control does not contain active substances that can reduce white blood cells.

Based on statistical testing in treatment group 3 (positive control, Simvastatin 20 mg/kg BW), by comparing measurements on the 28th day (after induction) and the 42nd day (after treatment) a significance value of 0.014 ($p < 0.05$) was obtained which means that there was a significant difference between the measurements after induction and after treatment. The average value obtained at the positive group after induction was 18.50 ± 1.20 with the final average of 12.28 ± 1.72. This is due to statin class drugs including simvastatin being the first line of defence in the therapy of dyslipidemia and primary and secondary prevention of atherosclerotic cardiovascular disease which works by inhibiting cholesterol biosynthesis by acting as a competitor inhibitor of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG- CoA) reductase [33].

Tests on treatment groups 4 and 5, namely the administration of sulfated polysaccharide isolates at doses of 250 mg/kg BW and 50 mg/kg BW obtained significance ($p < 0.05$) which means that there is a significant difference between the measurements after induction and after treatment. The average value of treatment group 4 obtained after induction was 16.85 ± 1.87 and the final average was 11.16 ± 1.92 while in the treatment group 5 the average valued obtained after induction was 16.68 ± 0.8 and the final average was 13.67 ± 0.23.

Sulphate polysaccharide isolates including fucoidan from brown algae have the potential to affect cardiovascular disease. Fucoidan can reduce LDL by inhibiting the formation of cholesterol in the synthesis of the enzyme HMG-CoA reductase and stimulating LDL receptors in the liver and other peripheral tissues. Foam cell formation may be reduced in the presence of fucoidan due to inhibition of adhesion molecules, which reduces migration of immune cells to the intima layer [34].

Based on statistical tests in the treatment group 6, using sulfated polysaccharide isolates of 10 mg/kg BW by comparing the measurements on the 28th day (after induction) and the 42nd day (after treatment) a significance value of 0.05 was obtained ($p > 0.05$) which means there was no significant difference between the measurements after induction and after treatment. However, based on the average value results obtained after induction of 16.17 ± 0.61 and after treatment of 14.84 ± 0.62, there was a decrease of 1.33 ± 0.53 in the average value.

This study showed that sulfated polysaccharide isolates had an effect that was not significantly different between doses of 250, 50, and 10 mg/kg BW, but the higher the dose, the greater the effect, and from this study it was found that sulfated polysaccharide isolates at a dose of 250 mg/kg BW has great potential in reducing white blood cells in the event of atherosclerosis as indicated by the percentage reduction in white blood cell levels which is larger than other doses and does not differ much from the positive control results, namely simvastatin 20 mg/kgBW. Therefore, it can be concluded that polysaccharide sulfate isolate can be developed as an alternative drug for the treatment of atheroschlerosis.

3.5 AST levels

AST levels were measured in white rats that were given a high-fat diet and also after being given treatment in the form of sulfated polysaccharide isolates from brown algae. The following table shows the average value of SGOT levels from the measurement results obtained (Table 5 and Figure 3).

Table 5. The results of measuring the average value of AST levels on day 28 (after induction) and day 42 (after treatment) (mean value ± SD, n = 3) and the value of % decrease

Test Group	Measurement		% Decrease (-)	% Increase (+)
	After Induction	After Treatment		
Normal Control	134.3± 31.37	116±42	18.3	-
Negative Control	58± 16.83	96.3±48	-	38.3
Positive Control (SST 20 mg/kg BW)	178.3±150.0	104±11	74.3	-
PSS 250 mg/kg BW	139.7±26.72	117±28	22.7	-
PSS 50 mg/kg BW	170.7± 33.24	95±22	75.7	-
PSS 10 mg/kg BW	80±13	61±17	19	-

Description: PSS (Polysaccharide Sulfate)
SST (Simvastatin)

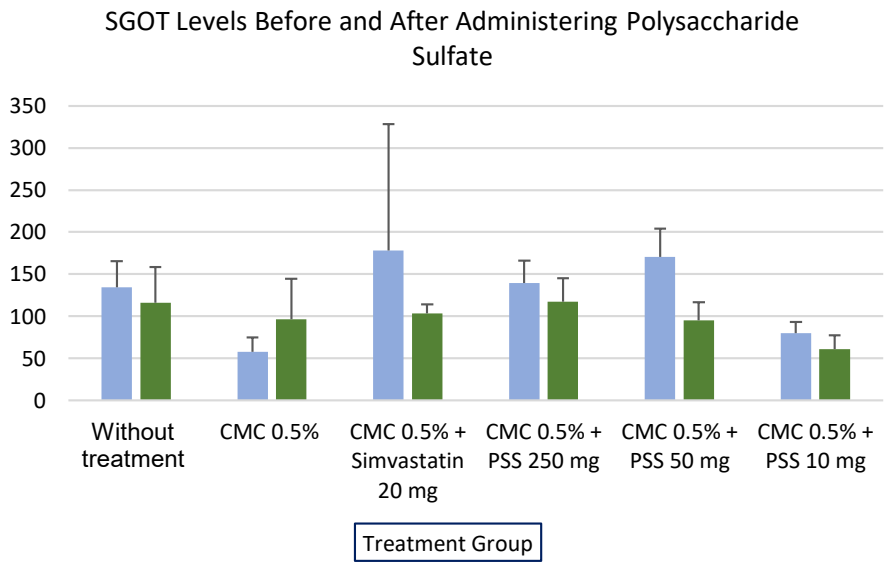


Fig. 3. Histogram of average AST levels on day 28 (after induction) and day 42 (after treatment) in test animals (mean ± SD, n=3). Left bar on 28th day (before), right bar after 42th day (after).

Based on the measurement results, the average value of SGOT levels in the normal (healthy) group after induction was 134.3 ± 31.37 and after treatment it was 116 ± 42 so that the percentage decrease was 18.3. The results of statistical data obtained a significance value of 0.704 ($p > 0.05$), which means there was no significant difference. The negative control group (0.5% CMC administration) after induction was 58 ± 16.83 and after treatment the SGOT level value was 96.3 ± 48 so that the percentage increase was +38.3. Meanwhile, based

on the results of statistical data, a significance value of 0.337 (p> 0.05) was obtained, which meant that there was no significant difference, if the administration of 0.5% CMC colloidal solution had no effect on AST levels. In the measurement results, the average value of AST levels in the positive control group (administration of simvastatin 20 mg/kg BW) after induction was 178.3 ± 150.0 and after treatment it was 104 ± 11, so that the percent reduction was 74.3. It was seen that there was a decrease in SGOT levels after treatment. Based on the results of statistical data, a significance value of 0.02 (p> 0.05) was obtained, indicating that the decrease was significantly different after induction and after treatment.

On groups that were given sulfated polysaccharides, with a dose of 250 mg, 50 mg, and 10 mg each, different statistical data were obtained, namely group 4 (sulfated polysaccharide 250 mg) obtained a significance value of 0.006 (p <0.05) which means there was a significant difference between the measurements after induction and after treatment. Group 5 (50 mg sulfated polysaccharide) obtained a significance value of 0.001 (p <0.05), which means that there was a significant difference between the measurements after induction and after treatment. Group 6 obtained a significance value (p> 0.05), which means that there was no significant difference between the measurements after induction and after treatment. The average value of group 4 after induction was 139.7± 26.72 and after treatment it was 117 ± 28 so that the percentage decrease was 22.7. Group 5 after induction had a value of 170.7 ± 33.24 and after treatment 95 ± 22 so that the percentage decrease was 75.7. Group 6 after induction had a value of 80 ± 13 and after treatment 61 ± 17 so that the percentage decrease was 19. So, based on the average value after induction and after treatment in groups 4 and 5, there was a decrease in AST levels. Whereas in group 6 there was no significant decrease in AST levels.

Based on the results of the percentage decrease in SGOT levels in each group when compared between groups, in group 4 (PSS 250 mg) and group 5 (PSS 50 mg) the percentage reduction in SGOT levels was significantly different from group 2 (negative control) but not significantly different from group 3 (positive control). This positive control group is a group induced by the drug simvastatin in which the statin drug class is a drug used to lower cholesterol, with its mechanism of action namely reducing cholesterol formation in the liver by competitively inhibiting the action of the HMG-CoA reductase enzyme.

3.6 SGPT Levels

SGPT levels were measured in white rats after having been given a high-fat diet and after being given treatment in the form of sulfated polysaccharide isolates from brown algae. The Table 6 and Figure 4 shows the average value of SGPT levels after the measurement.

Table 6. Results of measuring average value of SGPT levels on day 28 (after induction) and day 42 (after treatment) (mean value ± SD, n = 3) and the value of % reduction

Test Group	Measurement		% Decrease
	After Induction	After Treatment	
Normal Control	35±6	42.33±19.08	7.3
Negative Control	40,66± 18.93	36.66± 16.80	4
Positive Control (SST 20 mg/kg BB)	57±19.28	57± 4.35	0
PSS 250 mg/kg BW	66.66±14.46	59.66± 8.96	7
PSS 50 mg/kg BW	43.66± 12.85	36.66±2.30	7
PSS 10 mg/kg BW	59.66± 12.58	33±10.58	26.6

Description: PSS (Polysaccharide Sulfate)
SST (Simvastatin)

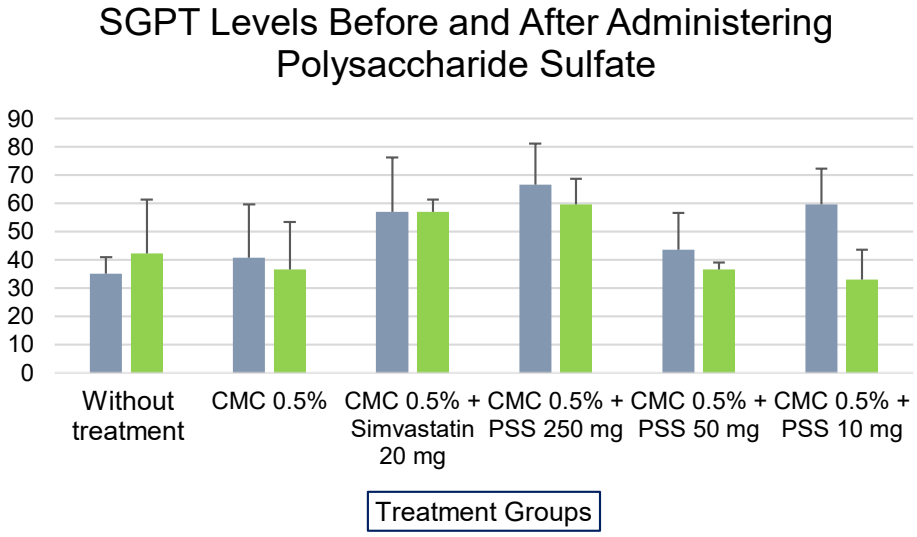


Fig. 4. Histogram of average SGPT levels on day 28 (after induction) and day 42 (after treatment) in test animals (mean ± SD, n=3). Left bar on 28th day (before), right bar after 42th day (after).

In the measurement results, the average value of SGPT levels in the normal control group after induction was 35 ± 6 and after treatment the value of SGPT levels was 42.33 ± 19.08 so that the percentage decrease is -7.3. And based on the results of statistical data a significance value of 0.660 ($p > 0.05$) was obtained which means that there was no significant difference in the normal control group during the treatment. In the negative control group (0.5% CMC administration) after induction it was 40.66 ± 18.93 and after treatment the SGPT level value was 36.66 ± 16.80 so that the percentage decrease was 4, while the statistical results obtained significance of 0.772 ($p > 0.05$) which means there was no significant difference. So, the administration of 0.5% CMC colloidal solution had no effect on SGPT levels. In the measurement results, the average value of SGPT levels in the positive control group (administration of simvastatin 20 mg/kg BW) after induction was 57 ± 19.28 and after treatment it was 57± 4.35, so that from these results there was no percentage decrease in SGPT levels from after induction to after treatment. Based on the results of the statistical data, a significance value of 1.000 ($p > 0.05$), was obtained indicating that the decrease was not significantly different.

The average value of SGPT levels in group 4 (administration of 250 mg sulfated polysaccharides) after induction was 66.66 ± 14.46 and after treatment it was 59.66 ± 8.96 so that the percentage reduction was 7. From the results of statistical data a significance value of 0.403 ($p > 0.05$) was obtained which means that there was no significant difference between the measurements after induction and after treatment. In group 5 (given polysaccharide sulfate 50 mg/kg) after induction it was 43.66± 12.85 and after treatment 36.66 ± 2.30 so that the percentage decrease was 7. From the results of statistical data a significance value of 0.489 ($p > 0.05$) was obtained. In group 6 (given polysaccharide sulfate 10 mg/kg) after induction the value was 59.66 ± 12.58 and after treatment 33 ± 10.58 and the percentage decrease was 26.6. From the results of statistical data a significance value of 0.113 ($p > 0.05$) was obtained. So, based on the statistical results, there was no significant difference between the measurements after induction and after treatment.

In this study, it was shown that sulfated polysaccharides had an effect at doses of 250 and 50 mg/kg BW in reducing SGOT levels in white rats that were given a high-fat diet and had atherosclerosis. But sulfated polysaccharides had no demonstrable effect on reducing SGPT

levels. Based on the literature, SGOT is an enzyme that is released during myocardial infarction. AST levels increase in patients with myocardial infarction due to damage to myocardial cells which can cause tissue death in the myocardium. Myocardial infarction is the main cause of cardiovascular death caused by atherosclerosis in which fat accumulates on the artery walls and causes narrowing and blockage of blood flow in the arteries. It can be concluded that sulfated polysaccharides can be developed for use as drugs in the treatment of myocardial infarction caused by atherosclerosis. Sulfated polysaccharides from brown algae (*Sargassum polycystum*) given to white rats with atherosclerosis had an effectivity in reducing white blood cell levels in these test animals.

3.7 Creatinine Kinase Myocardial Band (CKMB) levels

Measurement of Creatinine Kinase Myocardial Band (CKMB) levels is a specific and sensitive indicator of myocardial infarction (IM). CKMB levels can increase when there is plaque which causes the flow of blood and oxygen to the heart to decrease (Table 7).

Table 7 Results of measuring average value of CKMB levels on day 28 (after induction) and day 42 (after treatment) (mean value ± SD, n = 3)

Test Group	Measurement	
	After high fat induction	After treatment
Healthy control	220.667 ± 40.017	192.667 ± 33.292
Negative control	262.000 ± 98.077	216.333 ± 119.169
Positive control	270.667 ± 77.501	170.667 ± 63.042
PS 250 mg/kg BW	323.000 ± 64.861	114.333 ± 18.583
PS 50 mg/kg BW	218.333 ± 90.340	88.667 ± 17.010
PS 10 mg/kg BW	250.333 ± 50.213	173.667 ± 89.540

Description: PS (Polysaccharide sulfate)

Based on the results of statistical analysis using Tukey HSD Post Hoc Tests, between the average CKMB levels after treatment in the healthy control group (192.667 ± 33.392) compared to the average CKMB levels after treatment in the negative control group (216 ± 119.169 mg/dL) and the positive control group (170.667 ± 63.042), the results for sulfated polysaccharide fraction at a dose of 250 mg/kg BW (114.333 ± 18.583 mg/dL), polysaccharide sulfate fraction at a dose of 50 mg/kg BW (88.667 ± 17.010 mg/dL) and polysaccharide sulfate fraction at a dose of 10 mg/kg BW (173.667 ± 89.540 mg/dL) were not significantly different ($p>0.05$). This proved that there was no difference between the healthy control group and the negative control treatment group, the positive control group, the sulfated polysaccharide fraction group at a dose of 250 mg/kg BW, the sulfated polysaccharide fraction group at a dose of 50 mg/kg BW, and the sulfated polysaccharide fraction group at a dose of 10 mg/kg BW.

On the 42nd day measurement (after treatment) for all treatment groups of test animals, data were obtained in the form of CKMB levels in the test animals after induction in each group. The initial analysis was carried out using the Shapiro-Wilk normality test to see the normality of the data. The results of this test found that the data were normally distributed ($p \geq 0.05$). Because the results obtained were normally distributed, it could be followed with the ANOVA test. ANOVA statistical test analysis on the 42nd day measurement data (after treatment) for the CKMB level data obtained a significance value of 0.385 ($p> 0.05$), which means there was no significant difference.

Testing the healthy control group (without treatment) by comparing the measurements of the 28th day (after induction) and the 42nd day (after treatment) yielded an average value after induction of 220.667 ± 40.017 while the average value after treatment was 192.667 ± 33.292. Based on the statistical test results, a significance value ($p> 0.05$) was obtained,

which meant that there was no significant difference, because the healthy controls (blanks) were not given any treatment so that it did not affect the CKMB levels of the test animals (Figure 5).

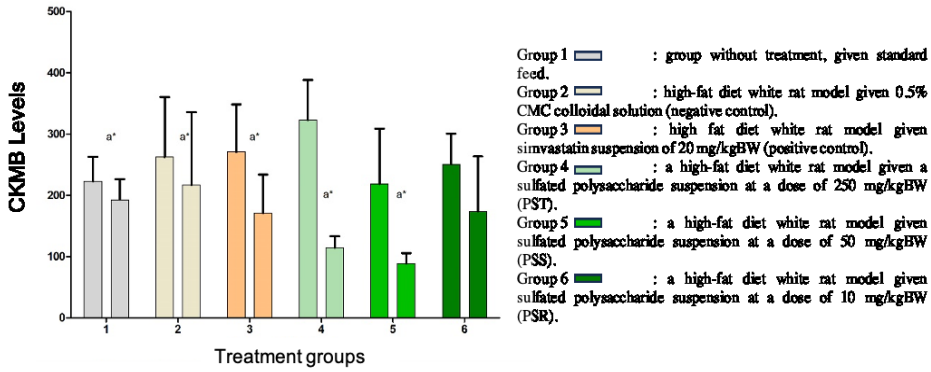


Fig. 5. Histogram of average CKMB levels on day 28 (after induction) and day 42 (after treatment) in test animals (mean ± SD, n = 3)

Testing of the negative control group (CMC 0.5%) by comparing the measurements of the 28th day (after induction) and the 42nd day (after treatment) yielded an average value after induction of 262.000 ± 98.077 and a final average value of 216.333 ± 119.169. Based on the statistical test results, there was no significant difference, so it can be said that the administration of 0.5% CMC had no effect on the CKMB levels obtained. This may mean that administration of 0.5% CMC colloidal solution for 14 days has no effect on CKMB levels and CMC only functions as a suspending agent and does not provide a pharmacological effect, so it does not affect the results of observations and there is no risk of bias.

In the statistical test analysis in the positive control group (simvastatin 20 mg/kg BW), by comparing the measurements on the 28th day (after induction) and the 42nd day (after treatment) a significance value was obtained ($p < 0.05$) which means there was a significant difference in the measurements after induction and after treatment. The average value at the positive group yielded an average after induction of 270.667 ± 77.501 and the final average was 170.667 ± 63.042. This is due to statins being a class of antihyperlipidemic drugs that can reduce total cholesterol levels by 20% and reduce the risk of vascular disease by 24%. Simvastatin which is used as a comparison also has an anti-cholesterol mechanism by competitively inhibiting the HMG-CoA reductase enzyme which has a function as a catalyst in the formation of cholesterol (Nuralifah, 2020).

Tests on the sulfated polysaccharide group at doses of 10 mg/kg BW and 50 mg/kg BW obtained significance values ($p > 0.05$) which means that there was no significant difference between blood sampling after induction and after treatment. The average value of the sulfated polysaccharide group at a dose of 10 mg/kg BW yielded an average value after induction of 250.333 ± 50.213 and the final average was 173.667 ± 89.540 while the average value of the sulfated polysaccharide group at a dose of 50 mg/kg BW yielded an average value after induction of 218.333 ± 90.340 and the final average was 88.667 ± 17.010.

Testing on the sulfated polysaccharide group at a dose of 250 mg/kgBW yielded the average value after induction of 323.000 ± 64.861 and after treatment it was 114.333 ± 18.583. The statistical test results obtained a significance value ($p < 0.05$) which means that there was a significant difference in the measurements on the 28th day (after induction) and the 42nd day (after treatment).

S. polycystum is one of the seaweeds that contains a lot of chemicals and has the potential to be utilized and developed. The chemical constituents of *S. polycystum* include proteins,

vitamin C, tannins, iodine, phenols, alginates and fucoidans which can be used as food and medicine. In research [35] one of the sulfated polysaccharides in the form of fucoidan can treat hypercholestromia by reducing LDL concentrations and increasing HDL. Fucoidan can also reduce lipid peroxidation associated with the release of enzymes in the heart and myocardial damage [35].

This study shows that polysaccharide sulfate has an effect that is not significantly different between doses of 10 mg/kg BW and 50 mg/kg BW, but is significantly different at a dose of 250 mg/kg BW, indicating that at this dose polysaccharide sulfate has the potential to significantly reduce CKMB levels, as seen from statistical tests, and did not differ greatly from the results of the positive control, namely simvastatin.

3.8 Histopathology analysis

This study was conducted with the aim of determining the effect of the administration of sulfated polysaccharides from brown algae (*Sargassum polycystum*) on the histopathological appearance of the aorta of white rats (*Rattus norvegicus*) model of atherosclerosis. Administration of sulfated polysaccharides from brown algae (*Sargassum polycystum*) was expected to cause aortic histological changes in rats fed a diet of duck egg yolk and butter, namely by reducing foam cells.

3.8.1 The Effect of a high-fat diet and sulfated polysaccharides on the number of foam cells

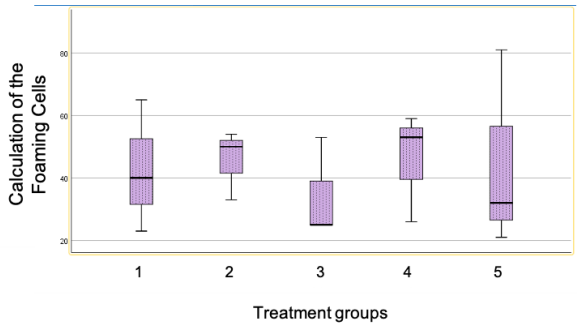
Based on the histopathological observation of the aortas using a microscope, data on the number of foam cells was obtained. Following are the results of calculating the number of foam cells in the atherosclerotic white rat (*Rattus norvegicus*) aortas.

Table 8. Average Number of Aortic Foam Cells in Atherosclerotic Model Rats After Administration of Polysaccharide Sulfate Suspension

Group name	Average Number of Foam Cells
Normal Control	42.66 ± 21.27
Negative Control	45.66 ± 11.15
Sulfated polysaccharide dose of 250 mg/kg BW	34.33 ± 16.16
Sulfated polysaccharide dose of 50 mg/kg BW	46.00 ± 17.57
Sulfated polysaccharide dose of 10 mg/kg BW	44.66 ± 31.94

Based on Table 8, it can be seen that the average number of foam cells in the normal control group is 42.66 ± 21.27. The negative control group was 45.66 ± 11.15. The sulfated polysaccharide group at a dose of 250 mg/kg BW was 34.33 ± 16.16, the sulfated polysaccharide group at a dose of 50 mg/kg BW was 46.00 ± 17.57 and the sulfated polysaccharide group at a dose of 10 mg/kg BW was 44.66 ± 31.94. The group with the highest average number of foam cells of sulfated polysaccharides at a dose of 50 mg/kg BW found at 46.00 ± 17.57, and the group with the lowest average number of foam cells was in the sulfated polysaccharide group at a dose of 250 mg/kg BW of 34 .33 ± 16.16.

Hypothesis testing was carried out using Kruskall-Wallis to determine differences within the entire group. The graph for calculating the number of foam cells in the atherosclerotic rat model for each group is shown in the histogram (Figure 6).



Group 1 : group without treatment, given standard feed.
Group 2 : high-fat diet white rat model given 0.5% CMC colloidal solution (negative control).
Group 3 : high fat diet white rat model given simvastatin suspension of 20 mg/kgBW (positive control).
Group 4 : a high-fat diet white rat model given a sulfated polysaccharide suspension at a dose of 250 mg/kgBW (PST).
Group 5 : a high-fat diet white rat model given sulfated polysaccharide suspension at a dose of 50 mg/kgBW (PSS).
Group 6 : a high-fat diet white rat model given sulfated polysaccharide suspension at a dose of 10 mg/kgBW (PSR).

Fig. 6. Histogram of the calculated value of the number of foaming cells in the atherosclerotic rat model

The results of the Kruskall-Wallis test obtained a significance value of 0.848 ($p>0.05$) which indicated that there was no significant difference in each treatment group.

3.8.2 Microscopic observation results of aorta of atherosclerotic model rats

Aortic histopathological profile data was determined by observation and determination carried out by an anatomical pathology specialist from Ibnu Sina Hospital. The histopathological images of the rat aortas are shown in the following figure.

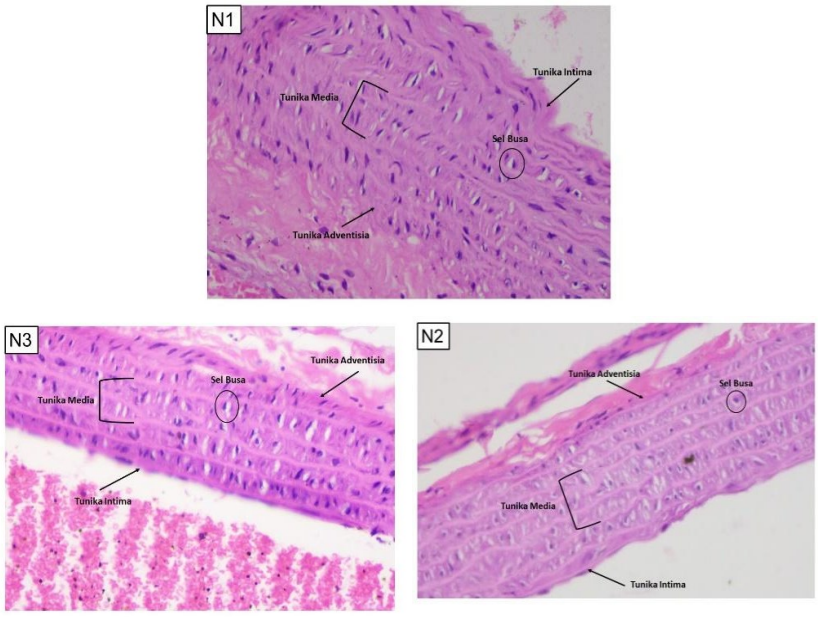


Fig. 7. Aortic histopathology of normal control group rats.

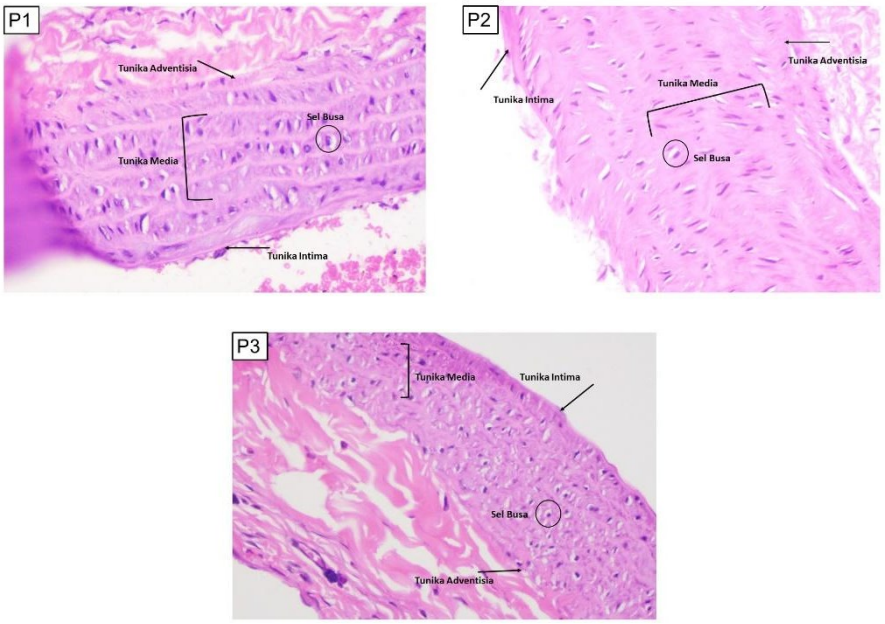


Fig. 8. Histopathological images of the aortas in the negative control group.

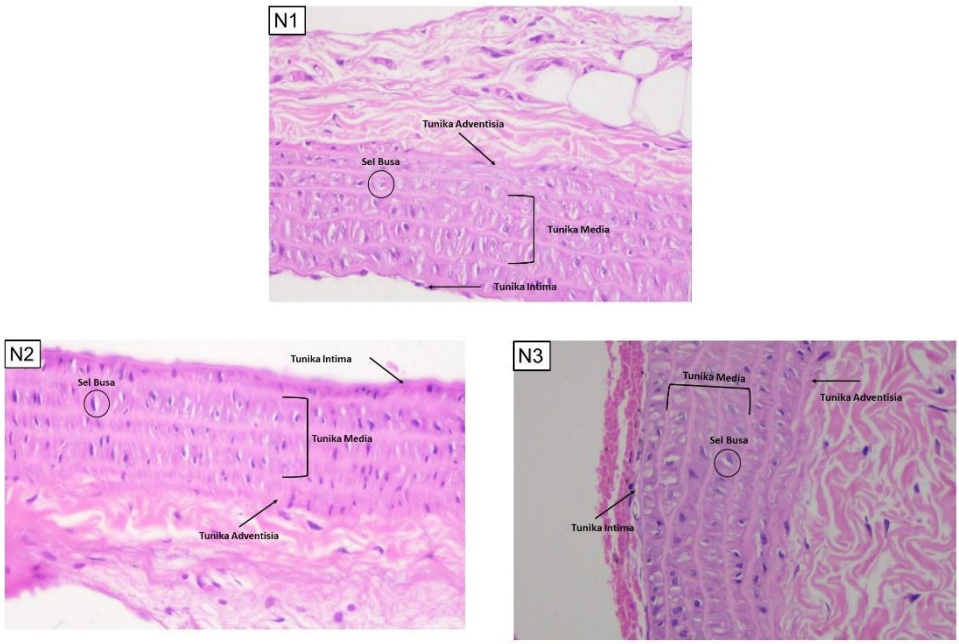


Fig. 9. Histopathological images of aortas of the sulfated polysaccharide group at a dose of 250 mg/kg BW.

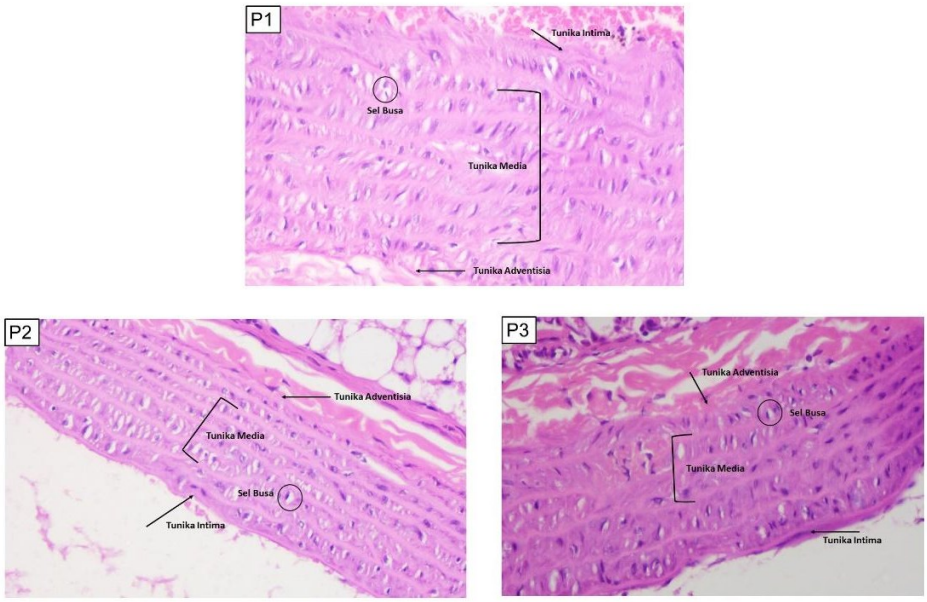


Fig. 10. Histopathological images of the aortas of the sulfated polysaccharide group at a dose of 50 mg/kg BW.

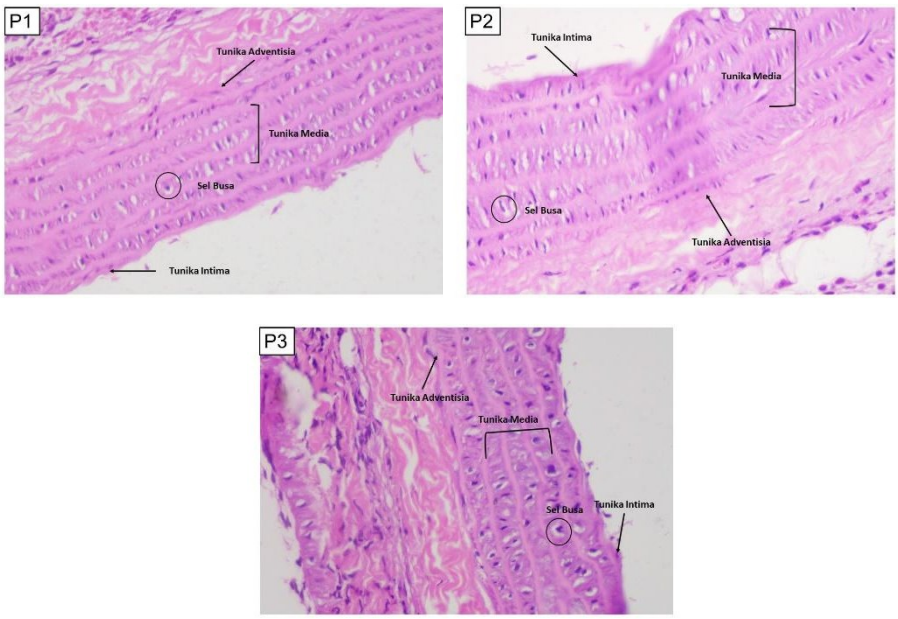


Fig. 11. Histopathological images of the aortas of the sulfated polysaccharide group at a dose of 10 mg/kg BW.

Figure 7 shows the histopathological images of the aortas of the white rat model of atherosclerosis in the normal control group which was given standard food and found the presence of foam cells and macrophages. N1 shows the tunica intima, media and adventitia which are not neatly arranged, while on N2 and N3 it shows the tunica intima, media and

adventitia which are neatly arranged. In Figure 8 the negative control group that was given a high-fat diet and 0.5% CMC shows histopathological images of the aortas with the presence of foam cells and fatty streaks, with neatly arranged tunica intima, tunica media and tunica adventitia. Figure 9, the polysaccharide sulfate treatment group at a dose of 250 mg/kg BW, shows aortic histopathological features with less accumulation of foam cells than the other groups. Macrophages are present within the intima tunica media and tunica adventitia which are neatly arranged in P1 and P3 while in P2 they are not neatly arranged. Figure 10, the polysaccharide sulfate treatment group at a dose of 50 mg/kg BW, shows that there was still a lot of accumulation of foam cells compared to the other groups, macrophages and tunica intima, tunica media and tunica adventitia which were neatly arranged in P1 and P3 while in P2 they were not neatly arranged. Figure 11, the polysaccharide sulfate treatment group at a dose of 10 mg/kg BW, shows accumulation of foam cells, the presence of macrophages and tunica intima, tunica media and tunica adventitia which were neatly arranged in P2 and P3 while in P1 they were not neatly arranged. Based on the images above, it appears that the fewest foam cells were the polysaccharide sulfate treatment group at a dose of 250 mg/kg BW.

The research was conducted by giving a high-fat diet in the form of duck egg yolks and margarine. According to research by [36-37] white rats that were given 2 g duck egg yolk per day for 28 days and 5 gram of margarine per day for 28 days experienced a resulting increase in total cholesterol and triglyceride levels and microscopically visible intima foam cells and aortic media [36-37]. Hyperlipidemia conditions can cause endothelial dysfunction so that LDL can more easily enter the walls of blood vessels and can increase the production of oxygen free radicals and can cause LDL to be oxidized to LDL-ox. Oxidized LDL can be captured by macrophages through scavenger receptors and when exposed to LDL oxidized by macrophages they become foam cells. Foam cells or fatty streaks are fatty streaks that microscopically appear as foam cells that occur in the subendothelium of blood vessels and are early markers of atherosclerotic plaque growth [38].

The results of this study indicate that in each treatment group there was a histological change in the aorta where inhibition of foam cell formation occurred which tended to increase with higher doses. However, there was no significant difference between the treatment groups ($p>0.05$). The changes in the aortas of the treated rats were that the rat aortas experienced a decrease in the number of foam cells after 14 days of polysaccharide sulphate suspension with 3 dose variations. Based on the literature, *S. polycystum* has strong antioxidant activity as evidenced by the DPPH method and has no side effects that need to be considered [39-40].

Administration of ethanol extract from *Sargassum* sp. has a protective effect against the formation of ROS. The formation of ROS is the initial process of atherosclerosis which can be prevented by giving antioxidants. Giving antioxidants with low concentrations can significantly delay or inhibit the oxidation process [41]. Figure 7 (N1, N2, N3) shows an accumulation of foam cells in the tunica intima and tunica media. The reason for the accumulation of foam cells in the aorta of rats that were given standard food is unclear, but it is thought that the foam cells were caused by exposure to anaesthetic in the form of ether, which was carried out when blood was drawn because in this study blood samples were also taken to see lipid levels in rats.

4 Conclusion

Based on the research results obtained, it can be concluded that administration of sulfated polysaccharides from brown algae (*Sargassum polycystum*) was able to have an effect on improving lipid profiles in white rats given a high-fat diet and a dose of 250 mg/kg BW, which had the best activity in reducing total cholesterol levels, increasing HDL levels and

reducing triglyceride levels. Moreover, doses of 250 mg/kgBW, 50 mg/kg BW and 10 mg/kg BW of sulfated polysaccharides from brown algae (*S. polycystum*) had activity in reducing white blood cell levels in rat models of atherosclerosis and are capable in reducing SGOT levels, but had no effect on reducing SGPT levels in white rats given a high-fat diet. At a dose of 250 mg/kg BW they had greater activity in reducing CKMB levels compared to other doses. In terms of histopathological features, the relationship between the administration of sulfated polysaccharides from brown algae (*S. polycystum*) to the number of aortic foam cells in atherosclerotic white rats leads to the conclusion that administration of sulfated polysaccharides from brown algae (*S. polycystum*) is able to inhibit the formation of foam cells in model white rat aortas with atherosclerosis but there was no significant difference.

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