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Original Article

ANTIOXIDANT AND ANTI-DYSLIPIDEMIC ACTIVITY OF BROWN SEAWEED (*SARGASSUM POLYCYSTUM*) EXTRACT IN RATS FEED WITH HIGH-FAT CONTENT

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ABSTRACT

Objective: This research aimed to obtain the antioxidant and antidyslipidemic activity of brown seaweed (*S. polycystum*) extract *in vivo*.

Methods: Two tests, such as antioxidant and anti-dyslipidemia, were conducted on the sample animals. Also, the number of Wistar rats was divided into 2 sections which consisted of 6 treatment groups, respectively. The test animals received high-fat feed for 35 d, after which there administered brown seaweed extract for 14 d. An antioxidant test consisting of 6 treatment groups, such as normal, negative, positive control (vitamin E), doses of 50, 100, and 200 mg/kg BW, was conducted in the first group. Meanwhile, the anti-dyslipidemia test consisting of 6 treatment groups, including normal, negative, positive (simvastatin), doses of 50, 100, and 200 mg/kg BW, was carried out in the second group. Hematological and statistical analysis was measured and performed using a 300 micro lab photometer as well as ANOVA, respectively.

Results: The antioxidant test results showed that superoxide dismutase (SOD) activity obtained a percentage increase of 63.41%, 75.01%, and 177.11%, while the dyslipidemia test results showed that after the administration of brown seaweed (*S. polycystum*) extract at a dose of 200 mg/kg BW, there was a significant difference between the negative controls, with p less than α (0.05), such as total cholesterol (0.000) and triglycerides (0.000). The percentage decrease in total cholesterol levels in the dose group was 31.98%, 41.47%, and 49.45%, with triglyceride levels of 30.65%, 37.78%, and 47.96%, respectively.

Conclusion: Considering these results, it was concluded that brown seaweed extract has an antioxidant activity on SOD parameters as well as an antidyslipidemic activity on total cholesterol and triglyceride parameters. Therefore, the most effective dose in improving the levels of total cholesterol and triglycerides is 200 mg/kg BW.

Keywords: Brown seaweed (*Sargassum polycystum*), Antioxidant, SOD, High-fat feed, Dyslipidemia, Cholesterol

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INTRODUCTION

Degenerative diseases have recently increased due to morbidity and mortality in developed countries. Furthermore, it is caused by high-fat feed that raises cholesterol levels, resulting in cardiovascular disorders such as atherosclerosis [1]. The high-fat feed also causes Low-Density Lipoprotein (LDL), which is easily oxidized, produces reactive oxygen species that leads to oxidative stress, and triggers an increase in the lipid peroxidation process. The body produces endogenous antioxidant compounds such as the enzyme superoxide dismutase (SOD). However, this cannot control the oxidation in the body without causing oxidative stress, which requires more significant amounts of antioxidants. This is performed by providing an external intake of exogenous antioxidants from natural and synthetic sources into the body [2].

Dyslipidemia is a lipid metabolism disorder due to genetic and environmental factors, which is indicated in the parameters of total cholesterol, LDL cholesterol, and triglycerides [3]. Plasma lipid levels increase due to high carbohydrate consumption, stored as fat. Therefore, the consumption of high-fat foods and a high-carbohydrate diet affects energy requirements and the formation of triglycerides and plasma cholesterol, as well as reduces HDL cholesterol in the body [4].

The decrease in plasma lipids helps in the treatment of dyslipidemia. Meanwhile, brown seaweed can reduce lipid levels in the blood. According to Akbarzadeh *et al.* (2018), brown seaweed (*Sargassum oligocystum*) lowers triglyceride levels in Wistar rats [5]. Therefore, an antidyslipidemic activity test is conducted on the total cholesterol and triglyceride parameters of brown seaweed extract in rats fed with a high-fat content feed. Furthermore, brown seaweed also acts as a source of antioxidants. This plant has flavonoids, steroids, triterpenoids, fucoidans, and components that act as a source of antioxidants, as stated by Ganapathi and Lutfiyana [6].

This research aims to obtain the antioxidant and antidyslipidemic activity of brown seaweed (*S. polycystum*) extract *in vivo*. During this research, brown seaweed was developed into natural medicines with antioxidant and antidyslipidemic activity.

MATERIALS AND METHODS

Materials

The materials used in this research included brown seaweed, vitamin E, high-fat feed consisting of animal fat, vitamins, cellulose, cholesterol, sucrose, cornflour, and casein, 20% EDTA, TEP, Aqudest, 20% TCA, 0.67% TBA, Carbonate buffer solution pH 10.2, Epinephrine solution 0.01 M, Chloroform–Ethanol 96% with a ratio of 3:5, H_2O_2 0.059 M, and Phosphate buffer 0.05 M pH 7. Furthermore, test animals used were male and female Wistar rats.

Tools

The tools used in this research were rat cages with food and water, rat scales, analytical scale, syringe, oral sonde, Eppendorf tube, measuring cup, beaker, stirring rod, stove, pestle mortar, micropipette, capillary tube, test tube, centrifugation devices, UV-Vis spectrophotometers, refrigerators, and pH meters.

Plant determination

The brown seaweed (*Sargassum polycistum*) was determined by Research Center for Oceonagraphy Indonesian Institute of Sciences, Jakarta, Indonesia with No. B-2716/IPK.2/IF/X/2016.

Preparation of test animals

Male Wistar rats were acclimatized for 7 d. During adaptation, the rats were fed and provided with water (ad libitum). After adaptation, the test animals were divided into 2 sections consisting

of 6 groups, respectively. The test animals other than the normal control group were administered high-fat feed for 35 d and then received brown seaweed extract for 14 d. Two tests were carried out, including antioxidant and anti-dyslipidemia tests [7].

Ethical approval

The pharmacodynamic test protocol on animals was ethically approved by the Health Research Ethics Committee of the Jakarta Veterans National Development University with No: 231/VI/2021/KEPK.

Animal treatment

A total of 24 healthy rats were divided into 6 groups, including normal (rats fed with a standard feed), a negative control (rats fed with a high-fat feed), test group I (brown seaweed extract at a dose of 50 mg/kg BW), test group II (brown seaweed extract at a dose of 100 mg/kg BW), test group III (brown seaweed extract at a dose of 200 mg/kg BW), and positive control (vitamin E), which consisted of 4 rats, respectively.

Blood sample

Blood samples were taken on day 0, day 14, day 35 after providing the high-fat feed. Blood was taken through the eyes of rats using±3 ml capillary tubes, it was collected in a test tube containing 20% EDTA, after which it was left for a while, then centrifuged at 3000 rpm for 10 min. A clear portion of the blood (plasma) was used to measure malondialdehyde levels.

Analysis of superoxide dismutase (SOD) activity

SOD activity was examined on red blood cells according to the modified Misra and Fridovich method with modification [8]. A total of 250 L red blood cell hemolysate was added to 400 L 96% (3:5) of the chloroform-ethanol mixture. The mixture was mixed for 1 min, then centrifuged at 3000 rpm for 10 min. The clear light-yellow filtrate was collected, after which a 50 L distilled water, 2775 L 0.0518 M carbonate buffer pH 10.2, and 125 L 0.01 M epinephrine solution were added. Afterward, it was mixed homogeneously and placed in a cuvette. Furthermore, the absorption measurements were performed after 1, 2, 3, and 4 min at a wavelength of 480 nm and 30 °C. The same method was also used for distilled (empty) water, with absorption readings taken after 1, 2, 3, and 4 min.

Antidyslipidemic activity test

The number of male Wistar rats was divided into 6 treatment groups, including normal, negative (high-fat feed), positive (simvastatin), and test groups with doses of 50, 100, and 200 mg/kg BW. After being treated with high-fat feed and sample administration, dyslipidemic rats were treated for 14 d. The lipid levels were observed on days 0, 7, 14 after being given a high-fat diet and 7, 14 d after being treated. In addition, the total cholesterol and triglyceride levels were analyzed using the "CHOD PAP" and "GPO PAP" methods, respectively [9].

***In vivo* toxicity test**

In stage I, 40 male and female DDY rats weighing 20-35 g were acclimatized (adapted) for approximately 7 d, then grouped randomly. As a result, each sex's bodyweight was evenly spread among four groups. Finally, in stage II, a total of 50 male and female DDY rats weighing 20-35 g were acclimatized to experimental animals for roughly 7 d before being randomly divided into 5 groups for each sex [10].

Stage 1 was divided into 4 groups consisting of 5 experimental animals, administered doses of 10, 50, 250, and 1250 mg/Kg BW. Therefore, the probe dose was increased by using a more significant concentration of the preparation when no death was recorded after 24 h.

Stage 2 was divided into 5 groups, each consisting of 5 experimental animals, which received doses: 1000; 2000; 3000; 4000, and 5000 mg/kg BW. First, observations were made after 24 h, counting the number of experimental animals that died in each group and sex. Afterward, the LD_{50} value was calculated.

RESULTS

Plant determination result

The brown seaweed was identified by Research Center for Oceonagraphy Indonesian Institute of Sciences, Jakarta, Indonesia as macroalgae *Sargassum polycystum* with this classification:

Divisio: Ochraphyta

Class: Phaeophyceae

Ordo: Fucales

Famili: Sargassaceae

Genus: Sargassum

Species: *Sargassum polycystum*

Measurement of superoxide dismutase (SOD) levels

The results of the antioxidant activity test in sample animals were used to determine the levels of superoxide dismutase (SOD), as shown in fig. 1. The average SOD activity in the negative control group was 20.84 U/ml, which was lower than the average SOD activity in the normal, positive, and treatment group. Meanwhile, the average result of SOD activity in the 50 mg/kg BW treatment group was 29.17 U/ml, with an increase of 63.41%, which was lower than the positive control group of 108.3 U/ml. The average result of SOD activity in the treatment group at a dose of 100 mg/kg BW 50 U/ml with an increase of 75.01% percent was lower than the positive control group of 108.3 U/ml. The dose of 200 mg/kg BW of brown seaweed extract with an increase of 177.11% increases the activity of SOD in the body as well as the positive control group.

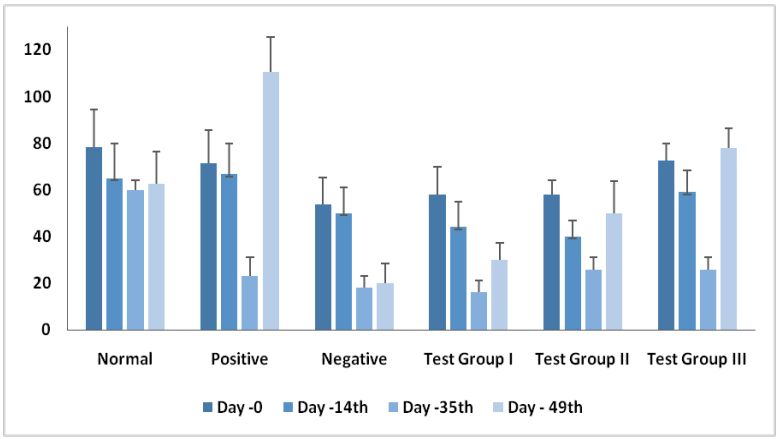

Fig. 1: The mean value of SOD before and after administration of brown seaweed extract, Data was given in mean+SD, n=5

Measurement of total cholesterol levels

The results of antidyslipidemic testing activity in experimental animals were measured for cholesterol levels, the results of which are shown in fig. 2.

Measurement of triglyceride levels

The results of testing for antidyslipidemic activity in experimental animals were measured for triglyceride levels, the results of which can be seen in fig. 3.

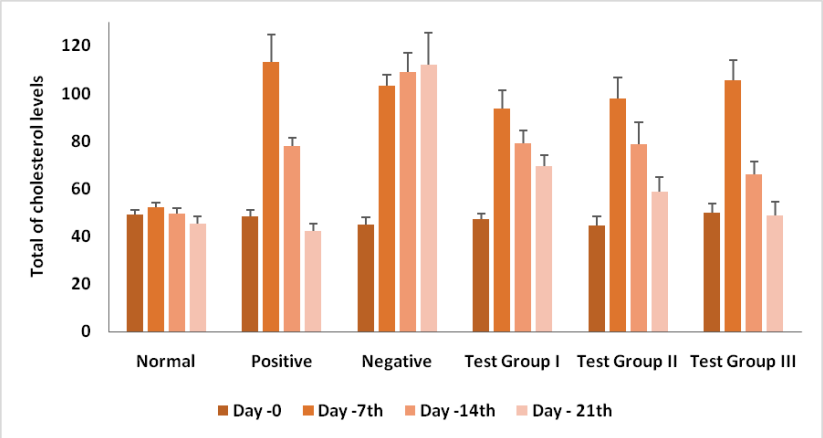

Fig. 2: Total cholesterol concentration, data was given in mean+SD, n=4

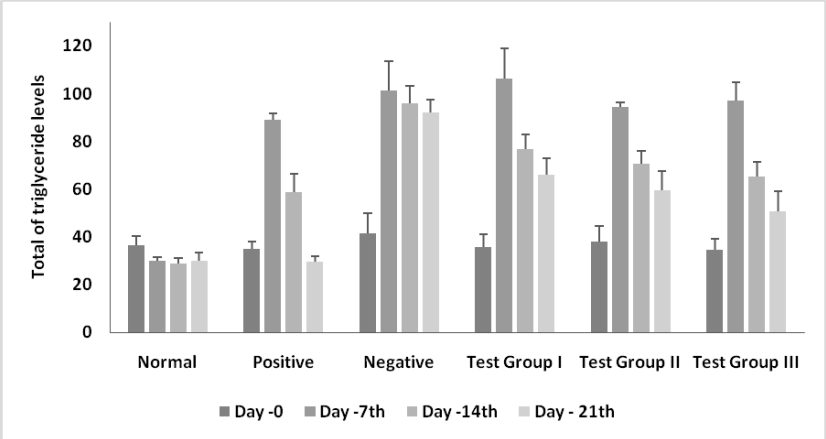

Fig. 3: Triglyceride concentration, data was given in mean+SD, n=4

***In vivo* toxicity test**

The 24h observations of stages I and II showed no death. Based on observations, the LD_{50} results of the preparation were 0.64 ml/20g BW or 320 mg/20g BW (16 g/Kg BW). LD_{50} 70% ethanol extract of brown seaweed>15g/kg BW. Therefore, this showed that brown seaweed's 70% ethanolic extract was practically non-toxic.

DISCUSSION

Antioxidant activity test of brown seaweed extract against SOD activity in rats

The measurement result of SOD activity for each treatment group are presented in fig. 2. The average SOD activity in the negative control group was 20.84 U/ml lower than the average SOD activity in the normal, positive, and treatment groups. Superoxide dismutase is an antioxidant produced by the body that neutralizes free radicals and protects cells from damage. Furthermore, it catalyzes the transformation of superoxide to hydrogen peroxide (H_2O_2) [11]. These results are supported by Yang RL's research, which stated that human subjects with high fat feeds experience oxidative stress conditions characterized by an increase in free radicals and a decrease in the status of antioxidant enzyme capacity [12]. The average increase in SOD activity in the treatment group was 50 mg/kg BW of 29.17 U/ml, which was lower than the positive control group of 108.3 U/ml. Therefore, the brown seaweed extract was unable to boost SOD activity and vitamin E, which was utilized as a positive control.

The average results of SOD activity from the treatment group at a dose of 100 mg/kg BW 50 U/ml showed an increase of 75.01% percent, which is lower than the positive control group 108.3 U/ml. Therefore, the brown seaweed extract did not increase the SOD activity and vitamin E, which was a positive control.

The dose of 200 mg/kg BW of brown seaweed extract with a percentage rise of 177.11% increases the activity of SOD in the body as well as the positive control group. These results showed that a high dose of administered seaweed extract leads to an increase in SOD activity. Furthermore, Widiyantoro *et al.* (2010). Stated that the administration of vitamin E as an exogenous antioxidant enhances the maintenance of SOD activity compared to the group that was not given vitamin E [13]. Also, another research discovered that flavonoids help maintain SOD activity in the body; hence, the antioxidant status is maintained [14].

Total cholesterol in antidyslipidemic activity testing of brown seaweed extract in rats

High-fat feeding for 14 d showed an increase in blood cholesterol levels. Oral administration for 14 d was conducted, and the lipid

levels in the administration of brown seaweed extract decreased, as shown in fig. 4. This was caused by the pharmacological activity of fucoxanthin, which happens to be a phenolic compound found in brown seaweed. Fucoxanthin is a pigment from the carotenoid group found in brown seaweed [15]. Furthermore, it affects the hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) and Acyl-CoA cholesterol acyltransferase (ACAT), which is one of the actions of synthetic dyslipidemia drugs, as well as a sterol-binding factor-binding transcription factor (SREBP-1): SREBP-1a. The activity is controlled by the sterol levels in cells, which regulates the genes associated with lipid and cholesterol production [16]. The most significant percentage decrease in total cholesterol levels at the III dose of 200 mg/kg BW was 49.45%. Statistical analysis showed a significant difference in doses I, II, and III with negative control ($p>0.05$). Therefore, the decrease in lipid levels at dose III had no significant difference in the positive group, while at doses I and II, there was a significant difference in the positive control group, implying that the effect of lowering total cholesterol at dose III is the same as that of the positive control.

Triglyceride levels in antidyslipidemic activity testing of brown seaweed extract in rats

The observation of triglyceride levels was performed 5 and 3 times after administering high-fat feed and 2 times after administering an extract of brown seaweed (*Sargassum polycystum*). The decrease in triglyceride levels is shown in fig. 5. The presence of a secondary component, such as fucoxanthin, which considerably reduces plasmatic and hepatic triglyceride concentrations [16], causes a reduction in triglyceride levels discovered in brown seaweed (*Sargassum polycystin*). The highest percentage decrease in triglyceride levels at the dose III of 200 mg/kg BW was 47.85%. Triglyceride levels at doses I, II, and III were significantly different ($p>0.05$) from the negative and positive controls, implying that brown seaweed extract reduces the plasma triglyceride levels, but this has not been able to match the positive control.

***In vivo* toxicity test**

This aims to determine the toxicity of brown seaweed extract in both sexes using 5 male and female rats each. Furthermore, the female rat was first subjected to an acclimatization process for 7 d before receiving treatment to adapt to their new environment and avoid false-positive results.

The 5 male and female rats, each weighing between 20-25 grams, were given a suspension at a dose of 0.64 ml/20g BW which was derived from the stock suspension extract solution in 0.5% CMC Na with a concentration of 500 mg/ml. Subsequently, from 24 h monitoring, no one died.

Based on the above observations, the LD50 results of the preparation are 0.64 ml/20g BW or 320 mg/20g BW (16 g/kg BW). Hence, the LD_{50} of 70% brown seaweed ethanol extract is>15g/kg BW, implying that brown seaweed's 70% ethanolic extract was practically non-toxic [7].

CONCLUSION

The extract of brown seaweed (*Sargassum polycystum*) has an antioxidant activity on SOD parameters and antidyslipidemic activity on total cholesterol and triglyceride parameters. Therefore, a 200 mg/kg BW dose is the most effective in improving total cholesterol and triglyceride levels.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

The author has no conflicts of interest to declare.

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