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The Prophylactic Effect of *Eucheuma spinosum* on Total Blood Cholesterol Levels in *Rattus norvegicus* Dexamethasone-Induced

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Abstract

Hyperlipidemic is one of the main risk factors for heart disease caused by the formation of atherosclerosis. *Eucheuma spinosum* contains a natural antioxidant flavonoid, which has an antiatherogenic effect that can inhibit the formation of cholesterol in the liver so that it can prevent the development of atherosclerosis. This study aims to determine the effect of prophylactic administration of *Eucheuma spinosum* on the total cholesterol levels of *Rattus novergicus* induced by dexamethasone. This study was an experimental design with a post-test-only control group design. This study would use 24 white male Rattus norvegicus divided into three groups; a negative control group (K-) without treatment, a positive control group (K+) induced dexamethasone 8 mg/Kg BW on day 14 until day 20, a treatment group (KP) given *Eucheuma spinosum* extract 200 mg/Kg BW on day 8 until day 20 as Prophylactic and on day 14 until day 20 induced dexamethasone 8 mg/Kg BW. After completing the treatment period, all groups were terminated and taken total calculation of cholesterol levels through the aorta of rats. Data was analyzed statistically computerized. The results of the study of total cholesterol levels that used Kruskal-Wallis test showed differences between groups ($p<0.001$). There was a decrease in total cholesterol levels in the treatment group ($p<0.024$) compared with the positive control group. The administration of *Eucheuma spinosum* extract as a prophylactic dose of 200 mg/Kg BW for 14 days could reduce the total cholesterol level of Rattus norvegicus induced by dexamethasone compared to those not given *Eucheuma spinosum* extract.

Keywords: dexamethasone, *Eucheuma spinosum*, flavonoid, total cholesterol

Original Research Article

INTRODUCTION

Hyperlipidemia is a term that refers to a disease in which the condition of the lipids in the body increases significantly beyond normal limits; this event can be caused by genetic or non-genetic factors (Stewart et al., 2020). The World Health Organization (WHO) notes that as many as 2.6 million deaths worldwide are caused yearly by hyperlipidemia. Based on the record issued by the WHO, it is also known that Europe is the region with the highest prevalence of hyperlipidemia, at 54%. In Indonesia, based on Riskesdas records from 2014, the prevalence of hyperlipidemia was 39.8% (Santosa et al.,

2018). Since childhood, hyperlipidemia has been a major risk factor for heart disease caused by atherosclerosis formation (Stewart et al., 2020).

Cholesterol is a sterol class of lipid material most human cells synthesize, especially in the liver. It is an integral structural component of cell membranes, serving as a fundamental component of vitamin D, steroid hormones, and bile acids (Narwal et al., 2019). Cholesterol is transported in the plasma primarily as low-density lipoprotein (LDL). The main transfer route from the tissues to the liver is through high-density lipoprotein (HDL) (Ridayani et al., 2018). Normally, cholesterol levels are below 200 mg/dL in healthy individuals. A cholesterol level between 200 and 239 mg/dL is considered a high threshold, and 240 mg/dL or more is regarded as a biomarker for cardiovascular disease, including heart attack, stroke, type II diabetes, peripheral arterial disease, and high blood pressure (Narwal et al., 2019).

High cholesterol levels (> 5.2 mmol/L) are the most severe predisposing factors that can cause plaque formation in blood vessels called atherosclerosis. Atherosclerosis is an inflammatory disease indicated by the discovery of lipid deposits in the form of cholesterol and cholesterol esters in the artery walls. Atherosclerosis is known to be the biggest trigger for heart disease and can cause death. There is a consistent relationship between LDL levels and coronary heart disease, so that the ratio of LDL and HDL cholesterol ratio can be used as a biomarker (Stewart et al., 2020).

Dexamethasone is a synthetic steroid drug with immunosuppressive, anti-inflammatory, and antiallergic properties (Sinner, 2019). Dexamethasone, a glucocorticoid member, affects the distribution of fat in the body; consequently, the number of lipids will increase. This causes a hyperlipidemic condition with an abnormal increase in blood lipid levels (Dolatabadi, 2015). Lipids in the blood circulate as lipoproteins, which consist of cholesterol, triglycerides, phospholipids, and proteins (Jovandaric & Milenkovic, 2020).

Algae are ancient photosynthetic organisms, ranging from prokaryotic cyanobacteria to eukaryotic microalgae. Algae are generally classified based on their color, shape, and life cycle (Lee & Ryu, 2021). The fundamental pigment component of algae consists of red *(Rhodophyta)*, green *(Chlorophyta),* and brown (*Phaeophyta).* Algae has been ingested by humans for a long time, predominantly in Asian nations (Diharmi et al., 2019). Algae are known to have many bioactive compounds, such as antioxidants and sulfated polysaccharides. It has been studied that they are very beneficial for health (Widyaswari et al., 2021). Indonesia has 555 species of algae, about 55 of which are used as food. Among the 55 species, *Eucheuma spinosum* is known to be the most widely cultivated. Even so, using *Eucheuma spinosum* in the medical field is not optimal (Widyartini et al., 2023). *Eucheuma spinosum* is the most important source of many bioactive metabolites compared to other algae (Podungge et al., 2021).

Eucheuma spinosum contains bioactive compounds that can be used as anti-oxidant, anti-bacterial, anti-tumor, anti-hypercholesterolemic, anti-inflammation, and amylase inhibition activity such as flavonoids, alkaloids, tannins, saponins, and their derivatives (Inayah and Masruri, 2021). Among those biological functions, the most noteworthy attribute of algae is its exceptional antioxidant activity. This is significant as antioxidants play a role in neutralizing free radicals, thereby enhancing immunity and reducing the risk of disease within the human body (Ha *et al.*, 2022).

An antioxidant is a type of chemical compound that provides an electron to an unpaired and reactive free radical, thereby diminishing the oxidative impact of the radical. Many natural compounds derived from herbs possess the ability to function as external antioxidants and have been demonstrated through clinical research to be successful in their antioxidant role (Sukweenadhi *et al.*, 2020). Antioxidants play a crucial role in safeguarding cells from oxidative stress and upholding the equilibrium of harmful oxygen molecules. Various types of free radical species exist, such as superoxide anions (O2 -), hydroxyl radicals (OH•), hydrogen peroxide (H2O2), hypochlorite anions (ClO-), and singlet oxygen (Sharifi-Rad *et al.*, 2020).

Antioxidants are categorized into two groups based on how they work: enzymatic and nonenzymatic. Enzymatic antioxidants follow a multistep process, converting harmful oxidative

byproducts into hydrogen peroxide (H2O2) and eventually into water, utilizing cofactors like copper, zinc, manganese, and iron. Several enzymatic antioxidants, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), shield the human body from the harmful effects of reactive oxygen species (ROS) (Moussa, Judeh and Ahmed, 2019). An enzyme called tyrosinase is pivotal in regulating melanin synthesis. On the other hand, nonenzymatic antioxidants like Vitamin C and E, polyphenol, flavonoids, and carotenoids disrupt the chain reaction off free radicals (Damongilala *et al.*, 2023).

Flavonoid compounds, one of the antioxidants, are known to lower cholesterol in the body for people with hyperlipidemia. The mechanism of action of flavonoids lowers cholesterol in the body, namely by inhibiting the action of the HMG-CoA reductase enzyme, which helps the formation of cholesterol in the blood. By inhibiting this mechanism in the liver, total cholesterol levels in the blood will decrease, reducing the prevalence of other diseases such as heart disease, high blood pressure, and stroke (Anggraini & Nabillah, 2018).

MATERIALS AND METHODS

Research design

This research design was true experimental with a post-test-only control group in the biochemistry laboratory at Hang Tuah Faculty of Medicine, Surabaya. The sample used in this study was 24 male *Rattus norvegicus*, which was divided into three groups.

Material

The tool used in this study was experimental animals' place to eat and drink, experimental animal cages measure 40 cm x 30 cm x 15 cm, analytical scales, Torsion balance scales (for measuring rat body weight), husk, sonde, cotton, Eppendorf tube, syringe, sample container, water bath, vacuum rotary evaporator, porcelain cup, a set of glass tools, set of beaker glass, measuring cup, pipette and stirring rod, glass funnel, filter paper, centrifuge tube, centrifuge (to separate blood serum from experimental animals), spectrophotometer, surgical equipment.

The material used in this study was Dexamethasone, *Eucheuma spinosum* extract, aquadest, Alcohol 95%, Ketamine, and CMC-Na 1%.

Making *Eucheuma spinosum* Extract

The following are the steps needed to extract a sample of *Eucheuma spinosum* (Podungge et al., 2017): wash fresh *Eucheuma spinosum* seaweed and drain. *Eucheuma spinosum* is cut into small pieces and weighed, then stored in a jar. Then, at a ratio of 1:1 (w/v), add to each pot of ethanol solvent.

Soak the extract in an ethanol solution at room temperature for 3x24 hours. Filter the soaked results using Whatman filter paper no. 42. Filtrate and residue will be formed due to this filtering. At 40 °C, evaporate the filtrate to form a thick extract. The evaporation technique uses a vacuum rotary evaporator.

Experimental animal treatment stage

First, divide the 24 *Rattus norvegicus* into three groups that will be treated differently. Then, for seven days, adapt the experimental animals to their new surroundings. During this adjustment period, the experimental animals continued receiving standard feed in the form of pellets and water (Handajani, 2021). The negative control group would be filled with eight *Rattus norvegicus* without treatment. From the 14th to the 20th day, eight *Rattus norvegicus* were induced dexamethasone intraperitoneally at a dose of 8 mg/kg BW in the positive control group. The third group was a treatment group consisting of eight *Rattus norvegicus*, which were given *Eucheuma spinosum* extract 200 mg/Kg BW on day 8 until day 20 as Prophylactic and on day 14 until day 20 induced dexamethasone 8 mg/Kg BW intraperitoneally.

At the end of the experiment, the rats fasted for 12 hours and drank only filtered water before taking samples. Sampling would be done through the heart of the rat. Retrieval steps will be carried out in the following order (Putra, 2016):

First, inject 0.3 mL of ketamine (20–40 mg/kg) intramuscularly, and wait for the rat to become weak and lose consciousness before grabbing it (Krissanti et al., 2023). The rat is placed on its back, facing upward, and then pricked with a needle on the soles of the front and back legs so that the position does not change. Touch the rat's chest, which is between the two front legs of the rat, to estimate the position of the rat's heart. Then, perform surgery on the rat's thorax until a heart is found using a scalpel or surgical scissors. Next, puncture the syringe with a diameter of 2.5 cc in the rat's heart perpendicularly and take 3 ml of rat blood. Put the blood sample taken into the sample tube and centrifuge at 3000 rpm for 10 minutes. Take serum from a sample tube that has been mixed up using a pipette. Put the serum into a new tube to determine the total blood cholesterol level (Putra, 2016).

Determination of cholesterol levels

Determination of total cholesterol levels in the blood would be done using the colorimetric enzymatic method, namely by using enzymes to see the occurrence of color changes. This color change would be measured by using a spectrophotometer with a 450–550 nm wavelength. By using the colorimetric enzymatic method, it will be necessary to use a tool, namely the Cobas Integra 400 Chemistry Autoanalyzer (Kuswari et al., 2021)

The reagent that will be used to make a diagnosis is Cobas Integra 400. With this reagent, total cholesterol concentrations in serum and plasma can be determined quantitatively. This reagent is an In Vitro Diagnostic reagent.

Data Analysis

Data analysis would be carried out descriptively. First, a normality test would be carried out by using the Shapiro-Wilks test. The second step that needed to be done was to do an ANOVA test to see if the data was normally distributed. With the ANOVA test, the three groups would be compared by using the 5% significance level. If there was untypical data distribution in the Shapiro-Wilks test, the Kruskal-Wallis test was used to compare the data, with a significance level of 5%. Later, the data to be analyzed came from the results of trials in the negative control, positive control, and treatment group. Each would be compared and resulted in three data analysis groups.

RESULT

Total Cholesterol Level

Total cholesterol levels of the experimental group without treatment, the group of experimental animals induced by dexamethasone, and the group of experimental animals that were given *Eucheuma spinosum* Prophylactic, induced dexamethasone intraperitoneally can be seen in Table 1.

Table 1. Total cholesterol level in each group

No.	Negative Control (mg/dl)	Positive Control (mg/dl)	Treatment (mg/dl)
1	73	111	62
2	68	144	51
3	62	83	72
4	63	118	78
5	51	95	105
6	51	79	105
7	42	73	75
8	54	144	73

Note:
Negative control : the group that was not given treatment

Positive control : dexamethasone-induced group
Treatment : group that was given prophylactic *Eucheuma spinosum extract* from day-8 until day-20, induced dexamethasone-induced from day-14 until day-20

Table 2. Descriptive statistical analysis of variable cholesterol total degree

Group	Mean (mg/dl)	Standard deviation
Negative control	58	10.25392
Positive control	105.875	28.09391
Treatment	77.625	18.94305

Note:
Negative control : the group that was not given treatment
Positive control : dexamethasone-induced group
Treatment : group that was given prophylactic *Eucheuma spinosum* extract from day 8 until day 20 induced dexamethasone from day 14 until day 20

Table 2 explains that there is an increase in total cholesterol between the experimental group of animals without treatment and the experimental group of animals induced by dexamethasone. Furthermore, if the mean total cholesterol of the experimental group induced by dexamethasone is compared with the group of experimental animals given *Eucheuma spinosum* Prophylactic and induced by dexamethasone, it shows a decrease. More details can be seen in Figure 1.

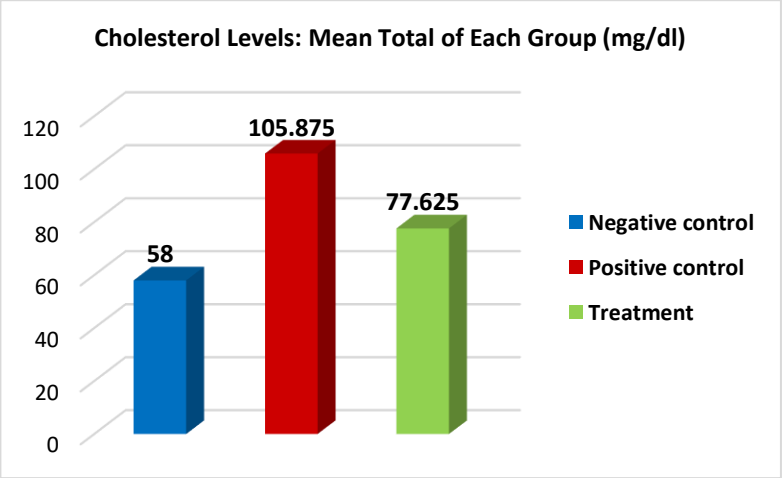

Figure 1. Cholesterol levels: mean total of each group

Note:
Negative control : the group that was not given treatment
Positive control : dexamethasone-induced group
Treatment : group that was given prophylactic *Eucheuma spinosum* extract from day 8 until day-20 induced dexamethasone from day 14 until day-20

Normality and Homogeneity Test

The data was then tested for normality and homogeneity. According to the normality test, the significance value of the experimental group without treatment was 0.879 ($p > \alpha$), 0.295 ($p > \alpha$) for the group of experimental animals induced by dexamethasone, and 0.265 ($p > \alpha$) for the group of experimental animals given *Eucheuma spinosum* Prophylactic and induced by dexamethasone. Because these three groups have $p > \alpha$, we can conclude that the data in these three sets are normally distributed.

After carrying out the normality test, before processing statistical data, a homogeneity test was done first to determine if the data was normally distributed. The homogeneity test used in this experiment was the Levene test. The Levene test showed a significance value of 0.030 ($p < \alpha$), indicating the variances of the experimental group without treatment, the group of experimental animals induced by dexamethasone, and the group of experimental animals given *Eucheuma spinosum* Prophylactic and induced by dexamethasone were not homogeneous. Because the data was normally

distributed and the variance was not homogeneous, the next step was to perform a non-parametric statistical test, namely the Kruskal-Wallis test.

Kruskal-Wallis Test Result, a Non-parametric Statistical Test

Based on the Kruskal-Wallis test, a significance value of p = 0.001 (p < α) was obtained, which meant that there was a difference in mean total cholesterol levels between the experimental animal groups without treatment, the experimental animal group induced by dexamethasone, and the experimental animal group was given *Eucheuma spinosum* as prophylactic and induced dexamethasone. Furthermore, a post hoc analysis test must be carried out using the Mann-Whitney U test to find out which group had a significant difference in average cholesterol.

Mann-Whitney U post hoc test results

Table 3 shows a difference in average cholesterol levels between the negative control group and the positive control group because the significance value between the two is 0.001 (p < α). Furthermore, a significance value of p = 0.027 (p < α) is obtained, indicating that the mean total cholesterol level differs significantly between the negative control and the treatment groups. Finally, the significance between the positive control group and the treatment group is p = 0.024 (p < α), indicating that the average total cholesterol level differs significantly.

Table 3. Mann-Whitney U post-hoc test result

	Negative control	Positive control	Treatment
Negative control	-	.001	.027
Positive control	-	-	.024
Treatment	-	-	-

Note:
Negative control : the group that was not given treatment
Positive control : dexamethasone-induced group
Treatment : group that was given prophylactic *Eucheuma spinosum* extract from day 8 until day 20 induced dexamethasone from day 14 until day-20

DISCUSSION

Based on research data, the mean total cholesterol level of the dexamethasone-induced experimental group (105.875 mg/dl) is higher than the mean total cholesterol level of the experimental group without treatment (58 mg/dl). This shows that administering dexamethasone by intraperitoneal injection at a dose of 8 mg/Kg BW for 6 days starting on the 14th day can significantly increase total cholesterol levels (p = 0.001). The elevation of cholesterol levels induced by dexamethasone in this experiment confirms a statement from research conducted in 2015 by the Department of Biology, Payame Noor University, I.R. of Iran. A. Arab Dolatabadi stated in his journal that intraperitoneal injection of dexamethasone with a dose of 1mg/kgBB increases the cholesterol levels of rats (Dolatabadi, 2015).

An increase in total cholesterol levels in the blood can occur due to dexamethasone's mechanism of action by stimulating lipolysis and the functioning of the fatty lipase enzyme. Increasing these two activities can cause an increase in the amount of free fatty acids in the blood, which will disrupt the balance (Koorneef et al., 2022). Fatty acids will undergo a process known as -oxidation, converting them to acetyl-CoA. If the blood level of acetyl-CoA is too high, it will be transported to the liver, where it will be the most essential raw material in forming cholesterol (Ojha & Symonds, 2017). The HMG-CoA synthase enzyme will catalyze the condensation of several acetyl-CoA molecules in the liver to form HMG-CoA. HMG-CoA would be reduced to mevalonate with the help of the HMG-CoA reductase enzyme. New cholesterol will be formed when the liver can produce mevalonate (Zeka et

al., 2017). This increase in cholesterol synthesis activity will lead to a condition of excess cholesterol levels in the blood called hypercholesterolemia (Rodwell & Murray, 2018).

The administration of corticoid treatment is recognized for its ability to elevate the production of VLDL by the liver. Additionally, corticoids might encourage the intestine to create VLDL as well. The diminished activity of liver lipoprotein lipase could potentially account for the elevated levels of VLDL-TG, thereby contributing to a disrupted lipid metabolism that results in hyperlipidemia (Nkono *et al.*, 2022).

In 2013, a study published in the *Universitas Gajah Mada* journal stated that treating of *Eucheuma spinosum* powder with 10 mg/200grBB and 20 mg/200grBB can significantly reduce LDL cholesterol levels after four weeks of treatment. Based on this knowledge, the administration of *Eucheuma spinosum* as a prophylactic can effectively lower cholesterol levels (Dinna & Mustofa, 2013). In this study, the group that was given *Eucheuma spinosum* prophylactic and induced by dexamethasone showed an average total cholesterol level of 77.625 mg/dl, which was lower than the mean total cholesterol level in the experimental animal group that was induced only by dexamethasone (105.875 mg/dl). The results of statistical tests indicated a significant decrease in cholesterol levels ($p = 0.024$).

Prophylactic administration of *Eucheuma spinosum* extract lowered total cholesterol levels due to its antioxidant content. Flavonoids are antioxidants found in *Eucheuma spinosum*. Flavonoids are a class of the large family of polyphenols, and they have a major role in regulating metabolism in the body. Flavonoids can regulate lipid and carbohydrate metabolism, reduce hyperglycemia and dyslipidemia, improve metabolism in adipose tissue, and reduce oxidative stress (Ajebli & Eddouks, 2019). Flavonoids in seaweed *(Eucheuma spinosum)* will play a role of inhibiting HMG-CoA reductase in the liver. In carrying out their role of inhibiting the activity of the HMG-CoA reductase enzyme, flavonoids work as competitive inhibitors that will form bonds with the cofactor reductase site (Zeka et al., 2017). As a result, the HMG-CoA reductase enzyme cannot work and cannot convert HMG-CoA to mevalonate, a cholesterol-forming component. Based on this theory, the mechanism for reducing cholesterol occurs possibly through the role of flavonoids, which inhibit the HMG-CoA Reductase enzyme so that it cannot synthesize cholesterol (Zeka et al., 2017).

CONCLUSION

Based on the results of this study, it can be concluded that intraperitoneal injection of dexamethasone at a dose of 8 mg/Kg BW, which was carried out for 6 days starting from the 14th day until the 20th day, significantly increased the total cholesterol levels of the experimental animals, and that administration of *Eucheuma spinosum* extract as a Prophylactic at a dose of 200 mg/Kg BW, given from day 8 to day 20, resulted in a significant difference in the total blood cholesterol levels of experimental animals given the Prophylactic and those induced by dexamethasone alone.

CONFLICT OF INTEREST

All authors declare that there is no conflict of interest in this study

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None

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