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Manipulating the biological character of red algae extract (*kappaphycus alvarezii*) administration on the total cholesterol levels of wistar strain male rats (*rattus norvegicus*) induced by a high-fat diet

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Abstract

Background/aim: Cholesterol is recycled in the body. The liver excretes cholesterol into biliary fluids, which is then stored in the gallbladder, which then excretes it in a non-esterified form (via bile) into the digestive tract. Typically, about 50% of the excreted cholesterol is reabsorbed by the small intestine back into the bloodstream Cholesterol is one of several biological problems that can result in death, especially for high-risk groups, such as older people and those with hypertension and hypercholesterolemia, and can lead to atherosclerosis and reduce work productivity as well. Red algae extract (*Kappaphycus alvarezii*) affects the total cholesterol level because the content of β-carotene reduces the formation and oxidation from low-density lipoprotein proteins (LDL cholesterol). This study aimed to determine the effect of red algae extract (*Kappa hycusalvarezii*) administration with a dose of 140 mg/200grBW on the total cholesterol levels of Wistar strain male rats (*Rattus norvegicus*) induced by a high-fat diet. **Materials and methods:** This research used 30 rats that met inclusion requirements, and they were divided randomly into three groups: the normal group (K_0), feed group (K_1), and *Kappaphycus alvarezii* group (K_2). An examination of cholesterol levels was done for each group on the 31^{st} day. The sample was analyzed using the CHOD-PAP (Cholesterol oxidase para aminophenazone) method. The data were analyzed using a one-way ANOVA test. **Results:** Statistics based on Mann-Whitney U tests for significance levels between the group that had not been given and the other group that had been given the extract of red algae (*Kappaphycus alvarezii*) with a dose of 140 mg/200grBW for rats that had been given a high-fat diet were less than 0.05 (Sig<0.05), resulting in 0.001. **Conclusions:** Administering the extract of red algae (*Kappaphycus alvarezii*) with a dose of 140 mg/200grBW affected the total cholesterol level of Wistar strain male rats (*Rattus norvegicus*) inducted with a high-fat diet.

Keywords: cholesterol, cholesterol oxidase para aminophenazone, red algae extract

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INTRODUCTION

Kappaphycus alvarezii seaweed is included in the group of red algae (*Rhodophyceae*) and is widely found in Indonesia, especially in East Java, Sulawesi (Middle, Southeast, South), Bali, NTT, Maluku, and Irian (Winarno 1996). This seaweed is known as one of the carrageenan producers and can reach 61.59% (Suryaningrum 1988). The type of carrageenan produced by *Kappaphycus alvarezii* is mainly kappa carrageenan (Asian 1988).

Seaweed that contains agar, alginate, and carrageenan components has a strong influence on reducing plasma cholesterol levels. These components can reduce blood cholesterol by 39%, while alginate has a high potential to reduce blood cholesterol through inhibition of cholesterol absorption in the intestine (Herpandi et al. 2006).

The fiber contained in seaweed binds cholesterol that accumulates in the small intestines as the amount of chylomicron remnant that transports cholesterol from the intestine to the liver decreases, and the number of lipoproteins and receptors decreases. This can help maintain homeostasis in the liver. Furthermore, seaweed contains a small number of total lipids but

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contains a lot of omega-3, especially eicosapentaenoic acid (EPA). This content can also reduce LDL cholesterol in plasma (Matanjun and Muhammad 2010).

This compound, which is also considered to play a role in lowering cholesterol when finding in seaweed, is a dietary fiber, especially soluble fiber, and is found in the highest amounts in *K. alvarezii*, such as polyphenols, β-carotene (mg), Vitamin C, a-tocopherol, and iodine. Thick soluble fiber inhibits the digestion and absorption of dietary fat, resulting in lower delivery of cholesterol to the liver by remnants of chylomicron, along with regulation of the increase in low-density lipoprotein receptors and the decreased secretion of lipoproteins to maintain cholesterol homeostasis in the liver (Lecumberri et al. 2008).

Some seaweed and dietary fibers (especially polionic ones) interact with food cholesterol, causing excretion, and then lowering blood cholesterol levels. It has been reported that dietary fibers, including seaweed polysaccharides, reduce serum lipid levels primarily by interfering with the absorption of cholesterol in the jejunum and by reabsorbing bile acids in the ileum, resulting in increased excretion of cholesterol and bile acids in feces (Sima et al. 2018).

Although kapaphycus contains β-carotene, some studies have shown no significant effect of β-carotene supplementation on total serum cholesterol, HDL cholesterol, or triglyceride levels (Redlich et al. 1999, Van Poppel et al. 1994). Investigation of dimethylsulphoniopropionate (DMSP) has recently revealed that this compound from marine algae could serve as an effective antioxidant, and these antioxidants play a role in lowering cholesterol (Renuga et al. 2018).

Kasim states that 5% *Kappaphycus alvarezii* seaweed was able to reduce blood lipid levels in hypercholesterolemic Wistar rats (*hyperlipidemic*) to normal on day 18, and if the concentration was increased, then the decrease was faster (Kasim 2004). Also, Hardoko reports that *Kappaphycus alvarezii* seaweed was also able to quickly reduce blood sugar levels in Wistar rats that were hyperglycemic diabetic (dependence diabetic mellitus), depending on the concentration given (Hardoko 2004).

Today, people's lifestyle of eating fast food has become a habit. This can be seen from the percentage of data obtained regarding the level of consumption of fast food of 67.6%, while the percentage of respondents who have reasons for choosing fast food is more practical at 73% and for a good reason at 27%. Fast food is usually high in fat and sugar but low in vitamins, fiber, minerals, and micronutrients and is one of the causes of hypercholesterolemia in the community (Sinaga 2016, Al-Anber and Al-Qaisi 2019).

Hypercholesterolemia triggers atherosclerosis, which is the beginning of coronary heart disease. A shift in a lifestyle where Indonesians are commonly consuming foods that are low in fiber is increasing the incidence of hypercholesterolemia. In Indonesia, the prevalence of hypercholesterolemia is tending to increase. Studies in East Jakarta in 1993 and Depok in 2001 revealed an increase in average total cholesterol from 173 mg/dl to 218 mg /dl and LDL cholesterol from 106 mg/dl to 133 mg/dl. The proportion of hypercholesterolemia in 2011 in Kebon Kalapa Village, Bogor, amounted to 80.5%, with a proportion of CHD of 27.7%, according to data from the Indonesian Litbangkes Agency (Sari et al. 2014).

Dietary fibers, especially those that are water soluble, are known to play a role in reducing plasma cholesterol levels. One source of soluble dietary fiber is seaweed. Several studies have shown that seaweed has a strong influence on reducing plasma cholesterol levels (Herpandi et al. 2006).

This research aims to determine the effect of red algae (*Kappaphycus alvarezii*) extraction on the total cholesterol levels of Wistar strain male rats (*Rattus norvegicus*) induced by a high-fat diet.

MATERIALS AND METHODS

This research used the posttest control group design method and was conducted at Widya Mandala Catholic University. The population in this study included Wistar strain male rats (*Rattus norvegicus*) that were treated at the Animal Laboratory of the Faculty of Pharmacy Widya Mandala Catholic University. An ethical feasibility test in this study was obtained from the Hang Tuah University Research Ethics Commission (No. 29 / HC / EC / KEPUHT / 2017).

Research Sample

Research design

The subjects in this study were Wistar strain male rats (*Rattus norvegicus*) obtained from animal husbandry DrhRachmadPriyadi that were treated at the Animal Laboratory of the Faculty of Pharmacy, Widya Mandala Catholic University. The rats' body weight was 150–200 grams on average, and they were 2–3 months old. Healthy male rats were selected by the researchers based on agile movement, bright eyes, smooth fur, and good appetite.

Three groups were used in this study using the federal formula, which obtained a sample size of eight in each group so that the total number of samples needed included 24 experimental animals.

The sample was divided into three groups, namely:

1. The negative control group (Group 1, K1): After adapting for seven days, this group of white rats was given a standard diet for 28 days.
2. The positive control group (Group 2, K2): After adapting for seven days, this group of white rats was given a high-fat diet of 39 ± 5 g/day/kg per rat for 28 days.
3. Treatment group (Group 3, K3): After adapting for seven days, this group of white rats was given a high-

fat diet for 28 days. On the 15th day, this group was also given red seaweed extract (*Kappaphycus alvarezii*), with a dose of 140 mg/200grBW for 14 days. Based on the research of Edrianyah (2013), an ethanol extract of 70% red seaweed (Gracilariaverrucosa) at a dose of 80mg/200grBW and 160mg/ 200grBW can significantly reduce total cholesterol. The researchers used a dose of 140mg / 200grBW.

High-fat diet composition

A high-fat diet is made up of a composition that has been shown to increase total cholesterol in the Octavia's study (Octavia et al. 2015, Ebrahimia et al. 2019). The composition of the high-fat diet in this study included 2 kg of goat fat, 4 kg of lard, 10 kg of pure 521 (standard feed), 2 kg of catfish pellets, 2 kg of flour, 20 chicken egg yolks, and 20 tablespoons of butter. The procedure for making the high-fat diet was to pulverize the goat fat and lard in a blender until smooth and then mix all the ingredients and pour in as much as 2 L of distilled water. Then, the mixture was allowed to stand for 3–5 hours. After the feed had sufficiently hardened, then the feed was ground with a meat grinder and cut to 1 cm in size.

Extraction of Red Seaweed (Kappaphy Cusalvarezii)

Dried red seaweed (*Kappaphy cusalvarezii*) was extracted with 70% ethanol through a maceration method into a thick extract. Dried red seaweed (*Kappaphy cusalvarezii*) was made into powder and put into a container and then macerated with 70% ethanol and was stirred repeatedly. After approximately 24 hours, the liquid extract was taken and filtered. Then, the pulp was macerated again with 70% ethanol. Next, the pulp was put into a cup and evaporated using a water bath at a temperature of 70–80ºC until it became a thick extract.

Sample Analysis

Sample analysis was conducted using blood from the heart of the mice. Previously, rats fasted for 10–12 hours and were only given distilled water. Then, blood was taken to check cholesterol levels using the CHOD-PAP (Cholesterol oxidase para aminophenazone) method (Kusumawati 2004, Islam et al. 2020).

Statistical analysis

The data were analyzed using SPSS (Statistical Product and Service Solution Program), which included descriptive statistics and a homogeneity test.

If the data normality test was normally distributed or, in the homogeneity test, the data were homogeneous, the data were analyzed using one-way ANOVA but with Kruskal Wallis.

RESULTS

This research was conducted at the Widya Mandala Christian University Surabaya Animal Laboratory for 28 days. The study used 24 Wistar male rats (*Rattus norvegicus)* that were divided into three groups: an experimental animal group given standard diets, an experimental animal group fed high-fat diets, and an experimental animal group fed high-fat diets and red seaweed extracts (*Kappaphycus alvarezii*).

Data from the measurement of cholesterol levels of the experimental animals that were given standard diets, the experimental group that was given a high-fat diet, and the experimental group that was given a high-fat diet and red seaweed extract (*Kappaphycus alvarezii*) can be seen in **Table 1**.

The average cholesterol level of the experimental animals given the standard diet, the experimental animals that were given a high-fat diet, and the experimental animals that were given a high-fat diet and red seaweed extract (*Kappaphycus alvarezii*) can be seen in **Fig. 1**.

Based on Figure, the average cholesterol level of the experimental group that was given a standard diet was 73.38 mg/dl; the experimental group that was given a high-fat diet was 80.38 mg/dl; and the experimental group that was given a high-fat diet and red seaweed extract (*Kappaphycus alvarezii*) was 56.50 mg/dl.

Table 1. Cholesterol levels of experimental animals in this research

No	K (-) Negative control group (mg/dl)	K (+) Positive control group (mg/dl)	KP Treatment group (mg/dl)
1	76	92	57
2	79	86	59
3	57	77	64
4	74	83	46
5	61	76	54
6	65	74	62
7	88	70	58
8	87	85	52

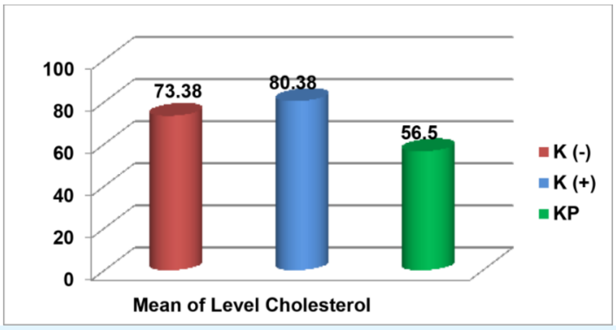

Fig. 1. Mean of Level Cholesterol of negative control group (K (-)), positive control group (K (+)), and treatment group (KP).

Table 2. Normality test results of cholesterol levels of the experimental group

Tests of Normality								
	Group	Kolmogorov-Smirnov[a]			Shapiro-Wilk			
		Statistic	df	Sig.	Statistic	df	Sig.
Cholesterol	Negative control	.147	8	.200*	.939	8	.599
	Positive control	.178	8	.200*	.968	8	.880
	Treatment group	.160	8	.200*	.968	8	.882

* This is a lower bound of the true significance.
a. Lilliefors Significance Correction

Table 3. Test of homogeneity of variances Cholesterol

Levene Statistic	df1	df2	Sig.
2.640	2	21	.095

Table 4. ANOVA test

	Sum of Squares	df	Mean Square	F	Sig.
Between groups	2410.083	2	1205.042	16.478	.000
Within groups	1535.750	21	73.131		
Total	3945.833	23			

This shows that the significance level for the experimental animals that were fed standard diets, the experimental animals that were given a high-fat diet, and experimental animals that were given a high-fat diet and red seaweed extract (*Kappaphycus alvarezii*) was p> 0.05. So it can be concluded that the data were normally distributed. The next step was to test the variance homogeneity.

The next requirement to fulfill the analysis requirements was to test the variance homogeneity of the data to test the similarity of the data variance of some experimental animal groups. The result of the homogeneity of the variance of the data using the Levene test is listed in **Table 3**.

The results of the analysis using the Levene test, as listed in **Table 3**, shows a significance of 0.095 (p> 0.05), and it can be concluded that the hypothesis is accepted because there was no difference in variance between the groups of data compared. In other words, the data variance was not homogeneous. For the next analysis, the parametric analysis was used with a one-way ANOVA test.

The one-way ANOVA test is a parametric statistical test that was used to compare whether there were differences in cholesterol levels between experimental animals fed standard diets, the experimental animal group fed high-fat diets, and the experimental animal group fed high-fat diets and red seaweed extracts (*Kappaphycus alvarezii*).

The results of the one-way ANOVA test analysis of differences in cholesterol levels between the experimental animal group fed standard diets, the experimental animal group fed high-fat diets, and the experimental animal group fed high-fat diets and red seaweed extracts (*Kappaphycus alvarezii*) can be seen in **Table 4**.

Based on the results of the one-way ANOVA test shown in **Table 4**, a significance value of p = 0.000 (p <0.05) was obtained, which means that H1 is accepted,

Table 5. The result of LSD analysis

Multiple Comparisons Dependent variable: cholesterol LSD						
(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Negative control	Positive control	-7.000	4.276	.117	-15.89	1.89
	Treatment group	16.875*	4.276	.001	7.98	25.77
Positive control	Negative control	7.000	4.276	.117	-1.89	15.89
	Treatment group	23.875*	4.276	.000	14.98	32.77
Treatment group	Negative control	-16.875*	4.276	.001	-25.77	-7.98
	Positive control	-23.875*	4.276	.000	-32.77	-14.98

*. The mean difference is significant at the 0.05 level.

so it can be concluded that there are differences in total cholesterol levels between the experimental animals given standard diets, the experimental animal group given a high-fat diet, and the experimental animal group that was given a high-fat diet and red seaweed extract (*Kappaphycus alvarezii*).

To determine which groups had significant differences, post hoc analysis was done. Post hoc analytic results with cholesterol level LSD tests between the experimental animal groups fed a standard diet, the experimental animal group fed a high-fat diet, and the experimental animal group fed high-fat diets and red seaweed extracts (*Kappaphycus alvarezii*) can be seen in **Table 5**.

Based on **Table 5**, there were significant differences between the experimental group given high-fat diets and the experimental group that was given a high-fat diet and red seaweed extract (*Kappaphycus alvarezii*), with p = 0.000 (p <0.05), and there were significant differences between the experimental group that was given a standard diet and the experimental group that was given a high-fat diet and red seaweed extract (*Kappaphycus alvarezii*), with p = 0.001 (p <0.05).

DISCUSSION

Based on the research data, the average cholesterol level in the experimental animal group that was given a high-fat diet (80.38 mg/dl) was higher than the average group of experimental animals that were given a standard diet (73.38 mg/dl). The provision of a high-fat diet for 28 days did not significantly increase cholesterol levels (p = 0.117). Increased cholesterol levels can occur because the administration of a high-fat diet causes high fat intake, so cholesterol levels increase.

The average cholesterol level in the experimental group that was given a high-fat diet and red seaweed extract (*Kappaphycus alvarezii*) (56.5 mg/dl) was lower than the average for the experimental group given a high-fat diet (80.38 mg/dl). However, in Ihedioha's research, it was found that 16-week-old male white rats (Rattusnorvegicus) had total cholesterol levels of 101.39+ 3.46, and mice aged 20 weeks had total cholesterol levels of 78.83 + 3.14, whereas, in this study, white rats were taken as samples that were 2–3 months old, or 8–12 weeks, so it was difficult to compare with the Ihedioha, Noel-Uneke, and Ihedioha (2013) studies. However, if rats in this study were ± 12 weeks old or ± 16 weeks, it could be said that the mean total cholesterol levels in the negative control group (K1), the positive control group (K2), and the treatment group (K3) were still below normal (Ihedioha et al. 2013).

In other research, the rats were given a high-fat diet without the treatment and had high cholesterol levels due to the administration of lard, which can increase blood cholesterol levels. According to a study that examined the intake of saturated fat, PUFA, and cholesterol to cholesterol levels, each saturated fat intake of 1% of the total energy a day is predicted to increase 2.7 mg/dl of plasma cholesterol levels (Hermanto et al. 2011).

The administration of red seaweed extract (*Kappaphycus alvarezii*) can significantly reduce cholesterol levels (p = 0.000). The decrease in cholesterol levels occurs because the extract of red seaweed (*Kappaphycus alvarezii*) has an essential fatty acid content, namely gamma-linolenic acid (GLA), that controls cholesterol synthesis in the liver, while its β-carotene content reduces the formation and oxidation of the protein low-density lipoprotein (LDL cholesterol). Whereas the levels of β-carotene found in seaweed have been examined, with the results showing that high concentrations of chlorophyll are produced when seaweed is planted in a deeper position under the sailor. This is probably due to the response to available blue-green light that can penetrate the seawater. As chlorophyll concentration increases, carotenoid content also increases. Increased carotenoid content is important for maintaining chlorophyll stability under excess light and high-temperature exposure (Anuonye et al. 2016).

Results of previous studies have shown that after 30 days of treatment, the administration of *E. spinosum* seaweed powder could maintain triglyceride levels and total cholesterol levels in rats. A seaweed dose effective in preventing a rise in total cholesterol and triglycerides is 20 mg / 200 g BW. Effective administration is four weeks of treatment. The red algae species *Eucheuma spinosum* can maintain total cholesterol and triglycerides in rats (Indriatmoko et al. 2015).

The extract of red seaweed (*Kappaphycus alvarezii*) contains triterpenoids (Edrianyah 2013) that can reduce cholesterol levels by inhibiting the enzyme HMG-CoA reductase, which is an enzyme in cholesterol synthesis. The compounds can provide inhibition to the pancreatic lipase enzyme, which plays a role in digesting triglycerides from food in the small intestine. The pancreatic lipase is responsible for the emulsification of lipids before intestinal absorption. Inhibition of pancreatic lipase inhibits fat absorption and reduces blood cholesterol and triglyceride levels. Triterpenoid compounds have a role in increasing the enzyme activity of lecithin cholesterol acylterase (LCAT). A plasma enzyme called LCAT converts free cholesterol into cholesterol esters, and then cholesterol ester is inserted into the nucleus of the lipoprotein particles to form HDL. HDL is circulated through the blood and includes more cholesterol from the blood and tissues back to the liver (Akusu et al. 2017, Mayore et al. 2018, Warditiani et al. 2015).

Based on the results of this study, it can be concluded that the administration of high-fat diets did not significantly increase cholesterol levels in the blood of the experimental animals, and the administration of red seaweed extract (*Kappaphycus alvarezii*) at a dose of 140 mg / 200grBW rat/day dissolved in aquadest solution for 14 days significantly decreased the cholesterol levels of the experimental animals.

CONCLUSION

The administration of red algae extracts (*Kappaphycus alvarezii*) at a dose of 140 mg / 200grBW rat/day dissolved in an aqua solution for 14 days significantly decreased cholesterol levels in the treatment group.

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