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Decreasing Blood Cholesterol Levels in Rats Induced by Alginate of *Sargassum duplicatum* and *Turbinaria* sp. derived from Yogyakarta

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ABSTRACT— ***Dietary fiber has beneficial functional effects on health such as lowering blood cholesterol, improve digestive function, lowering the glycemic response and prevent degenerative diseases. Alginate is the main content of the cell walls of brown seaweed and are known to have the content of dietary fiber. However, until now there has never been a study to determine the effect of dietary fiber contained in the alginate mainly extracted from Sargassum and Turbinaria derived from Yogyakarta. This research aims to determine and perform testing of alginate extracted from brown seaweed Sargassum sp. and Turbinaria derived from the rocky beaches in Yogyakarta to decrease blood cholesterol levels. This study concluded that the alginate extracted from brown seaweed Sargassum sp. and Turbinaria sp. obtained from the rocky beach area Wonosari Yogyakarta have the ability to decrease blood cholesterol levels in hypercholesterolemic rats***

Keywords— alginate, dietary fiber, hypercholesterolemia, blood cholesterol levels

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1. INTRODUCTION

Alginate is the main content of the cell walls of brown seaweed or alginofit. Alginate in the market are mostly in the form of sodium alginate. Sodium alginate are soluble in water. One of the many functional foodstuffs belonging to Indonesia's seaweed that reach over 70% of the natural wealth of the sea. Brown seaweed is one of a class of seaweed in Indonesia and still not optimally utilized by the people of Indonesia (Handayani et al., 2004). Some type of brown seaweed are *Sargassum* sp. and *Turbinaria* sp. Brown seaweed are potential in Indonesia and many researchers have conducted the extraction of alginate, including those conducted by Mushollaeni (2004, 2007, 2010, 2011a, 2011b, 2012a, 2012b). The study has developed the technique of extraction of brown seaweed *Sargassum* sp. and *Turbinaria* sp. by the used of 5% HCl in the pre-extraction stage and 2,25% of Na_2CO_3 in extraction stage. However, no studies regarding the use of these two types of alginate in food products. Until today there has been no research on the health effects that occur from the consumption of food products containing both types of alginate. Whereas in some of the research that has been done on the type of alginate extracted from brown seaweed that does not exist in Indonesia, has showed that the consumption of diets containing alginate can lower blood cholesterol of hypercholesterolemia rats. Therefore, research is needed to identify and determine the best concentration in the use of both types of alginate in food products. The focus of this study is to determine the concentration of alginate of both types of brown seaweed which can lower blood cholesterol levels of rats with hypercholesterolemia conditions.

Alginate has an important role in the food industry. Use of alginate in the food industry related to the nature of the terrain, such as a thickener (Brownlee et al. 2005; Mancini et al. 2002; Gujral et al. 2001), to stabilize mixtures, dispersions and emulsions, as well as increasing the viscosity (Paraskevopoulou et al., 2006 ; Gomez-Diaz and Navaza 2004; Ferreira et al., 2005). Additionally, brown seaweed has the potential as a provider of dietary fiber (dietary fiber) naturally. Dietary fiber has the functional effects on health such as lowering blood cholesterol, improve digestive function and prevent degenerative diseases. Alginate contains higher food fiber other than agar-agar and carrageenan and also contains iodine 0,1-0,8% (Winarno, 1996). Given the important role of dietary fiber in an effort to solve the problem GAKI (Iodine Deficiency Disorders) and prevent the spread of degenerative diseases such as coronary heart disease, diabetes and hypercholesterolemia due to low dietary fiber consumption, it is necessary to optimally use of seaweed, namely through the availability and food-based approach. Therefore, brown seaweed *Sargassum* sp. and *Turbinaria* derived from the rocky beaches in Gunung Kidul in Yogyakarta and its potential has not been explored in abundance, it is very necessary research on their use in food products as well know the health effects if consumed. Expected long-term goals will have an impact on the types of alginates in the food industry in order to improve local potential areas, as well as possible in order to minimize dependence on imports alginate. This research aim to identify and test the alginate from seaweed species *Sargassum* sp. and *Turbinaria* derived from the rocky beaches in Gunung Kidul in Yogyakarta to decrease blood cholesterol levels.

2. MATERIALS AND METHODS

2.1. Materials

This research was conducted at the Laboratory of Process Engineering Tribhuwana Tunggadewi University and Physiology Laboratory Brawijaya University from January to June 2015. The materials used include raw materials, materials for alginate extraction and materials for analysis of rats blood. The raw materials used are brown seaweed Sargassum sp. and Turbinaria sp., which are obtained from the rocky beach area Wonosari Gunung Kidul in Yogyakarta. The chemicals that are used for the extraction of alginate include distilled water, $CaCl_2$ 1% and 74%, HCl 1%, 3% HCl, HCl 5%, 35% HCl, 0,5% KOH, 90% KOH, Na_2CO_3 2,25% , Na_2CO_3 10%, 95% Na_2CO_3, NaOCl 10%, NaOCl 12%, 95% isopropyl alcohol, and HNO_3. Materials for analysis of rats blood include distilled water, diethyl ether and chemicals for analysis of cholesterol. The tools used include alginate extraction tools, analyzers of blood cholesterol, and equipment for maintenance of rats. The main tools for the extraction and analysis of the quality of alginate include brand digital scales Mettler AE 160, pH meter brands Schott Gerate C6 832, VT-04 Viscotester Rion Co., LTD, and color reader CR-10 Minolta Japan. Equipment for analysis of cholesterol, HDL, LDL and TG of blood test rats includes syringe 10 ml, centrifuge blood to obtain blood serum, centrifuge tubes, tube cuvet and equipment biochemical and hematological blood analyzer, set of test animal maintenance equipment and surgical instruments. Equipment maintenance includes stables, chaff containers and equipment rations and drinking test animals.

2.2. Brown Seaweed Fractionations Procedure

Alginofit cleaned of dirt, cut ± 1 cm, washed and sun dried for 2-3 days until their water content ± 13.5%. Leaching I with $CaCl_2$ 1% (1:10 w/v) for 30 minutes and washed. Leaching II with HCl 5% (1:10 w/v) for 30 minutes at a temperature 30-40^{o}C and washed. Added 0.5% KOH solution (1:10 w/v) for 60 minutes at a temperature 50-60^{o}C and washed. Extracted with Na_2CO_3 2.25% (1:10 w/v) at 50-60^{o}C for 1 hour and filtered. Filtrate obtained, bleached in NaOCl 10% (1:10 w/v) was stirred and left for 5 hours. Precipitation with HCl 5% (1:10 w/v) to precipitate in the form of alginic acid, washed and filtered. Neutralized with 10% Na_2CO_3 at pH 6-7, stirred and filtered. Purification with isopropanol 95% (1:10 w/v) and dried at 50-60^{o}C for 17 hours, then milled to obtain powder of sodium alginate.

2.3. Experimental Design

This research is a descriptive experimental research with pre and post test control group design. Activity on this study aimed to determine the effect of alginate on the feed rats that have undergone hypercholesterolemia. Studied the influence is positive influences related to a decrease in blood cholesterol levels of rats with hypercholesterolemia. This study is used laboratory animals to test the effectiveness of the alginate. The population was Wistar rats. Samples were obtained by consecutive random sampling, with criteria Wistar rats were included in the inclusion criteria. Criteria of Wistar rats in this study are male with age 2 months, body weight 150-200 grams, healthy condition (active and not disabled). This study used 36 rats were divided into 6 groups: the negative control group, positive control, treatment with alginate from Sargassum 0.75% and 1%, treatment with alginate of Turbinaria 0.75% and 1%. Rats in hypercholesterolemic condition if the blood cholesterol is more than 130 mg / dl.

2.4. Determination of Biochemical Metabolic Parameters

In this research, adaptation of feed provided ad libitum for 1 week and continued with diet containing alginate accordance with the treatment. Weighing performed every 1 week. At the end of the experiment, rats dissected for examination of the blood cholesterol profile. Rats that had been anesthetized with diethyl ether, dissected for blood drawn through the heart as much as ± 8 ml syringe using a 10 ml syringe. Centrifuged to obtain blood serum and cholesterol analysis.

2.5. Statistical Analysis

The results of each group were compared after the data of all the samples collected. Data analysis was performed using SPSS 16.00 for windows. Hypothesis testing using parametric test One Way ANOVA. Defined true confidences of this test is 95%, and $p <0.05$ then obtained significant differences.

3. RESULT AND DISCUSSION

3.1. Pretreatment - Hypercholesterolemia Rats

The type of rats used in this study is Wistar rats and rats that have been bred specifically for clinical research in laboratory scale. Wistar rats in this study are 2 months old, weighing 150-200 grams and met the inclusion criteria. A total of 36 rats were divided into six groups: negative control, positive control and four treatments with the addition of alginate. Hypercholesterolemic rats condition is obtained by adding 1% of cholesterol in the standard feed and give propyl thio uracil (PTU) 0.01% in the sonde. The addition of the cholesterol in food is included in the method of exogenously induced hypercholesterolemia, while the PTU is a step in the induction of endogenous. This treatment was imposed after passing through a phase of adaptation and lasted for 50 days in all groups except the negative control group. At the end of this stage, the blood cholesterol levels of rats with the treatment of cholesterol and PTU is 130.85 to 140.02 mg / dl. This condition is a condition that is very high cholesterol levels because cholesterol negative control rats in this study were from 40.01 to 58.11 mg / dl. Based on the reference value of UB's Laboratory of Clinical Pathology (2015), normal cholesterol levels of white rats is 10-54 mg / dl.

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Herpandi et al. (2006) states that the condition of rats blood cholesterol levels ≥130 mg / dl is the condition of rats with hypercholesterolemia. This means that with the addition of cholesterol as much as 1% and PTU, has been able to increase blood cholesterol levels of rats. This treatment is in line with research conducted by Csont et al. (2002), Matos et al. (2005), Silva et al. (2000) and Herpandi et al. (2006) that in order to get the rats with hypercholesterolemia conditions required feed containing high cholesterol. In addition, the condition of hypercholesterolemia in rats can also be obtained with the addition of other ingredients containing high triglycerides and high cholesterol. Hardiningsih and Nurhidayat (2006), has been conducting research to create rat blood cholesterol levels be exceeded normal limits, causing hypercholesterolemia, by adding egg yolk, coconut oil and goat fat containing high cholesterol in the diet. PTU is a substance that serves to improve cholesterol levels by inhibiting synthesis conducted by thyroid hormone. PTU also known as antithyroid agents. PTU endogenously can damage the thyroid gland and can lead to increased levels of Low Density Lipoprotein (LDL) and blood cholesterol (Hartayo et al., 2008; Sutjiatmo et al., 2013).

3.2. Feed Consumption and Body Weight of Rats

The results showed that there was an increase in body weight of rats in all treatments and both negative and positive controls (Table 1). The increase in body weight of rats is related to the amount of feed intake. Feed given each day at each rats was 40 g and the mean residual feed was 5-10 grams per day. This indicates that the test rats can consume feed well and not too affected by the conditions of the treatment. Overall both control rats and rats with the treatment, are in good condition and there are no death cases. The behavior of all the rats in the control and treatment look normal, the body in a healthy condition, active and there are no signs of poisoning.

Table 1 : Results of measurement of body weight of rats in each group (g)

Treatments	*Pre test* (g)	*Post test* (g)	Increase in body weight (%)
0% K, 0% alginat	148,13	195,00	31,64% [a]
1% K, 0% alginat	152,34	206,00	35,22% [a]
1% K, 10% S	146,98	201,93	37,39% [a]
1% K, 15% S	147,73	198,00	34,03% [a]
1% K, 10% T	148,10	197,00	33,02% [a]
1% K, 15% T	149,62	198,07	32,38% [a]

Note: K (cholesterol), S (*Sargassum*), T (*Turbinaria*), the same letter show no significant difference ($p < 0.05$) and the repetitions is 5x

Results of analysis of variance showed no significant difference of the percentage increase in weight. Negative control group tended to have a lower body weight than the positive control group and the treatment group. This is possible because of the influence of induction with PTU. PTU is able to affect thyroid hormone action that would affect the increase in the concentration of LDL in the blood and can also result in weight gain. Moreover, the addition of 1%

cholesterol feed also affect weight gain (Abrams and Grundy, 1981; Hardiningsih and Nurhidayat, 2006; Sutjiatmo et al., 2013; Suzuki et al., 1979).

Weight gain in groups of rats with alginate 10% lower than the group with 15% alginate diet. This relates to the nature of alginate gel that can be automatically entered in the stomach so that if these conditions will result in the emergence of the full flavor of the stomach or cause a feeling of fullness for longer and slows gastric emptying. Physiological effect is due to the fiber content in the alginate (Hoad et al., 2004; Wolf et al. 2002). The condition results in rats consumption rate becomes lower. If the feed consumption is lower, then the weight will be low and weight gain will be slower.

The mean levels of total fiber alginate derived from *Sargassum* sp. and *Turbinaria* sp. in this study is 58.6% and 69.20%. Higher fiber composition in alginate of the type *Turbinaria* sp., Can lead to weight gain percentage rats receiving these types of alginate diet becomes lower than the diet with alginate from *Sargassum* sp. This is due to the high binding ability of fibers in water so as to inflate the stomach and lead to a longer satiety. Based on the observation time of surgery, the stomach of rats that consumed feed containing alginate of the type *Turbinaria* sp. looks bigger and filled with feed containing alginate which has been expanding. While on the stomach of rats in the control group negative and positive, tend to be empty or contains little food remains undigested.

3.3. Profile of Total Cholesterols

Efforts to improve blood cholesterol levels to achieve hypercholesterolemia rats done by two methods of induction is exogenously by adding 1% of cholesterol in food and endogenously by sonde 0.01% PTU. This rats were in hypercholesterolemic condition if the blood cholesterols were raised to 130 mg / dl. This induction method can raise blood cholesterol levels of rats to reach 130,85-140,02 mg/dl (Figure 1).

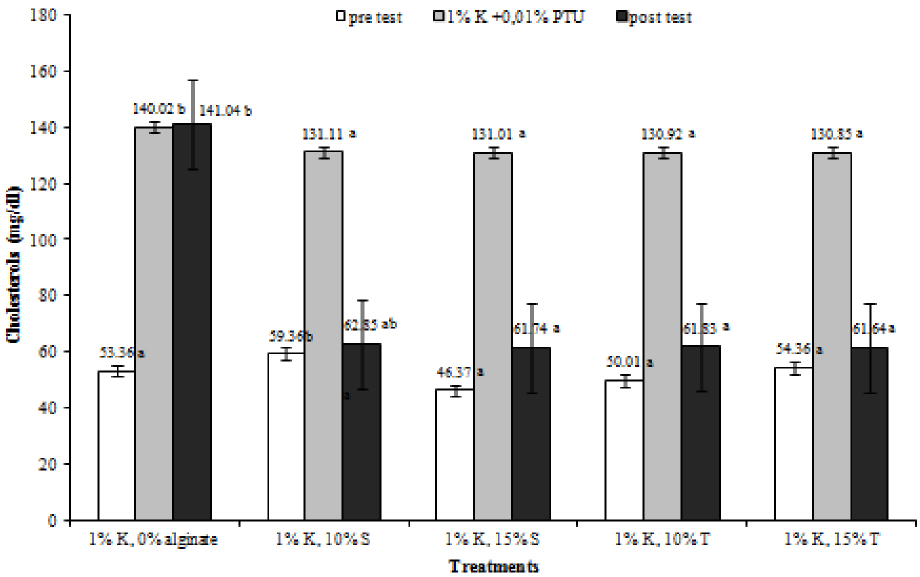

Figure 1: Pre-treatment levels of total cholesterol, after the addition of 1% cholesterol and 0.01% PTU and post treatment. Different letter on each value showed significant differences ($p < 0.05$)

The results showed a decrease in total blood cholesterol levels of the rats were subjected to treatment with alginate. Both types of alginate has demonstrated its ability to lower total blood cholesterol levels of rats up to 53%. Cholesterol-lowering effect is caused by the fibers contained in the alginate. Diet using alginate of the type *Turbinaria* sp. may result in a decrease in blood cholesterol levels of hypercholesterolemic rats better than Sargassum sp. This is due to the total fiber composition in alginate derived from *Turbinaria* sp. greater than *Sargassum* sp. Total fiber content in alginate *Sargassum* sp. and *Turbinaria* sp. in this study is 58.6% and 69.20%.

Behall (1997), Choudhary et al. (2005), and Moriceau et al. (2000) states that fiber in food can lower blood cholesterol levels. The mechanism is related to the ability of dietary fiber to form a gel in the stomach and increase the excretion of feces. Dietary fiber in the intestine can reduce the absorption of cholesterol in the hepatic circulation and immediately removing it through the feces (Samarghandian et al., 2011). Decreasing the amount of cholesterol that is absorbed is also caused by increased fat excretion and bile acids. Bile acids are the end products of metabolism of cholesterol. Bile acids formed from cholesterol in the liver. Dietary fiber can bind to bile acids and then the results will be excreted through feces. The high excretion of bile acids, indicates the higher the amount of cholesterol that has been converted into bile acids to emulsify fat. The end result of this mechanism is the low levels of total cholesterol and LDL (Anderson, 1994; Muchtadi et al., 1993).

4. CONCLUSIONS

This study concluded that the alginate extracted from brown seaweed *Sargassum* sp. and *Turbinaria* sp. obtained from the rocky beach area Wonosari Yogyakarta have the ability to decrease blood cholesterol levels in hypercholesterolemic rats.

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