SEAWEED EXTRACTS IMPROVE LIPID PROFILE OF WISTAR RAT

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ABSTRACT

Hypercholesterolemia or hyperlipidemia has been established as an important risk factor of cardiovascular disease. Patients with hypercholesterolemia usually require a prolonged treatment; and the newer and more potent generation of antilipid agents are costly.

In Bali there are several types of seaweed that are generally consumed by the local people and known by the local names of *Bulung Boni* (*Caulerpa* spp.) and *Bulung Sangu* (*Gracilaria* spp.). Preliminary studies on the effect of *Bulung Boni* and *Bulung Sangu* extracts appeared to improve lipid profile, but the available data are still very limited both in extent and depth, and further investigations were considered relevant and needed.

This experimental study used completely blind randomized design, using a total of 24 Wistar rats divided into six sample groups of equal size, all fed with a diet high in cholesterol content. The six sample groups were respectively designated as negative control group, positive control group, and four treated sample groups, respectively fed orally with a dose of 20 mg and 60 mg extracts of *Bulung Boni* per 100g of body weight per day, and 20 mg and 60 mg extracts of *Bulung Sangu* per 100g body weight per day. Each treatment was repeated four times.

Our study showed that rats fed with high-cholesterol diet and treated with oral *Bulung Boni* or *Bulung Sangu* extract at a dose of 20 mg and 60 mg/100 g bw/ day were associated with statistically significantly increased plasma HDL levels ($p < 0.05$), and statistically significant decreased plasma LDL and total cholesterol levels ($p < 0.05$) as compared with those of rats fed with high cholesterol diet without treatment with *Bulung Boni* or *Bulung Sangu* extracts.

From our data it could be implied that *Bulung Boni* and *Bulung Sangu* extracts improve lipid profile in the Wiatar rat by significantly increasing plasma HDL level, and lowering LDL and total cholesterol levels.

Key words: Seaweed extracts, *Caulerpa spp*, *Gracilaria* spp, HDL cholesterol, LDL cholesterol, total cholesterol, and anti-lipid agent

INTRODUCTION

In some countries such as Japan, Korea, China, Vietnam, Indonesia, Peru, Scandinavia, Scotland, and Philippines, seaweed has been used as a source of food, medicine, and raw material for various types of industries. Seaweed has potential nutrient content such as carotenoids, vitamins, fatty acids, carbohydrates, minerals, and other essential substances. Carotenoids have important biological functions as an antioxidant, and immunostimulatory which can prevent the disease, anti-inflammatory, anti-stress, anti-aging, and protect the skin from the harmful effects of ultraviolet radiation [1,2]. Carotenoids are antioxidant that are potential in protecting against membrane lipid peroxidation [3]. Carotenoids are derived from natural sources more safe than synthetic carotenoids [4].

Antioxidants can protect the body from free radicals attack and reduce its negative impact. Free radicals are necessary for the survival of several physiological processes in the body, especially for electrons transport, but the excessive free radical can harm the body because it can damage macromolecules such as protein in cells, and DNA (deoxyribo nucleic acid). Macromolecular damage can improve cell death [5].

In addition besides effects of oxidants, cholesterol also affects the development of degenerative diseases. The development of people live style that consume more fatty foods, especially of saturated fatty acid intake tend to cholesterol to be higher than the level of need. Intake of foods with high cholesterol content can increase cholesterol levels in the blood. This condition called hypercholesterolemia. One of the major atherosclerosis risk factors are dyslipidemia, and the prevalence of dyslipidemia in Indonesia has increased [6].

Anticipating the effect of hypercholesterolemia in cardiovascular disease, has developed several hypolipidemic drugs such as niacin, gemfibrozil, and the class of statins. The treatment of patients with hypercholesterolemia require a long period, and high costs, the study should be developed to obtain more effective drugs with a cheaper price, and less side effects. Natural ingredients from the sea, such as seaweeds need to be explored because of its carotenoids content that has function as an antioxidant and lowering blood cholesterol levels.

In Bali there are several types of seaweed that has long been used as a source of food by people. Types of seaweeds local name are *Bulung Boni* (*Caulerpa* spp.) and *Bulung Sangu* (*Gracilaria* spp). However studies on the effect of *Bulung Boni* and *Bulung Sangu* to improve lipid profile are very limited, therefore it is still very relevant for further study.The results of study can provide information to the public about *Bulung Boni* and *Bulung Sangu* benefits in improving of lipid profile in cases of dyslipidemia.

MATERIAL AND METHODS

Material in this study consist of *Bulung Boni* (*Caulerpa* spp.), *Bulung Sangu* (*Gracilaria* spp.). White rats used were male rats, wistar strain, 2.5 up to 3 month of age, 200 g to 225 g of weight, profile thio Uracil (PTU), standard diet, foods high in cholesterol is made in the Laboratory of Pharmacology Faculty of Medicine Udayana University with a mixture of 1% dried pig brain, 5% egg yolk cooked, 10% lard, 1% coconut oil, and 83% standard diet with drinking water containing 0.01% PTU [7], and cholesterol kit.

This experimental study used completely blind randomized design, using a total of 24 Wistar rats divided into six sample groups of equal size, all fed with a diet high in cholesterol content. The six sample groups were respectively designated as negative control group (KN), positive control group (KP), and four treated sample groups, respectively fed orally with a dose of 20 mg and 60 mg extracts of *Bulung Boni* per 100g of body weight per day (BB20 and BB60), and 20 mg and 60 mg extracts of *Bulung Sangu* per 100g body weight per day (BS20 and BS60). Each treatment was repeated four times.

Preparation of seaweed extracts

Dried seaweed were grinded until smooth and become powder. Seaweed powder is mixtured with 96% ethanol solution with a ratio of 3:1, then stirred with a magnetic stirrer for one hour at room temperature. Furthermore, filtered with Whatman paper No. 42 in order to obtain a filtrate, then concentrated by rotary evaporator.

Seaweed extracts treated in rats

In one cage was placed as many as four rats that had previously adapted for one week in the laboratory. Standard diet, cholesterol diet, and beverages rats administered daily *ad libitum*. Seaweed extract was administered orally by zonde with dose 20 mg and 60 mg/100 g body weight rat/day according to treatment.

Blood sample

After 30 days treatment**,** rats was fasted for 18 hours. Bloods sample was taken through the sinus orbitalis as much as 2 cc. Measurement of cholesterol levels using enzymatic CHOD PAP Test colorimeter method. Blood samples of rats as much as 1 cc put into test tube, and plasma from blood was separated with centrifuge for 20 minutes at 1500 rpm. 10 µl of plasma was mixed with 1000 µl of reagent. Sample tubes incubated for 10 minutes at temperature of 20-25^{0}C. Samples inserted into the spectrophotometer with a wavelength of 500 nm [9].

Data Analysis

Analysis of HDL, LDL,and total cholesterol performed with test of homogeneity of variance using Levene's Test at 5% significance level. If the variance is not homogeneous, the data must be transformation. To determine the effect of treatment, the data were analyzed with analysis of variance at 5% significance level. If the F test showed a significant difference , the treatment was tested furthermore by BNJ at 5% significance level.

RESULTS

Levels of high density lipoprotein (HDL)

Levene's test showed that the treatment with *Bulung Boni* and *Bulung Sangu* extracts on observations of HDL cholesterol, LDL, and total cholesterol, have a homogeneous variance ($p > 0.05$) (Table 1).

Table 1. *Levene's* Test for Homogeneity of Variances

Variable	MS effect	MS error	F	P
HDL	2,0870	1,0409	2,0049	0,1266
LDL	149,0894	77,1862	1,9315	0,1387
Total Cholesterol	118,7493	63,5314	1,8691	0,1500

The highest plasma HDL level found in negative control 78.25 ± 1.72 mg / dl, then BB60 62.34 ± 1.19 mg / dl, BS60 61.53 ± 3.84 mg / dl, 58.44 ± BS20 1.19 mg / dl, BB20 55.68 ± 1.44 mg / dl, and the lowest in the positive control with plasma HDL level 45.29 ± 1.11 mg / dl (Figure 1).

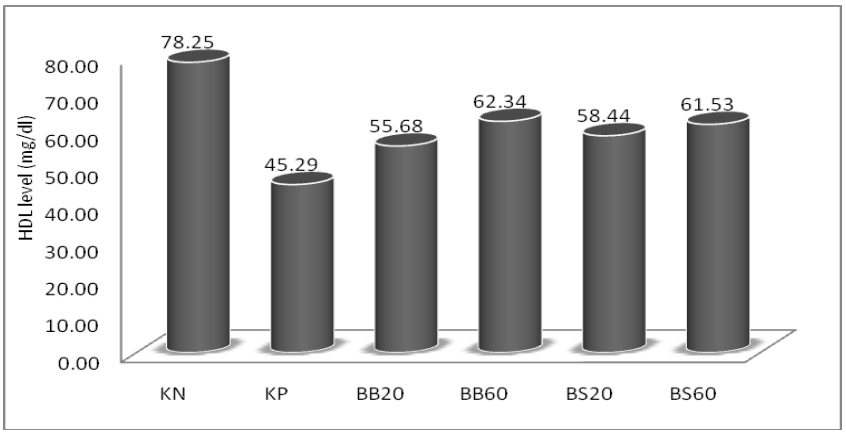

Figure 1. .Plasma HDL Level Wistar Rat in Negative Control (KN), Positive Control (KP), BB20, BB60, BS20, and BS60

Analysis of variance of plasma HDL level Wistar rats treated high-cholesterol diet with *Bulung Boni* and *Bulung Sangu* extracts showed significant differences ($p < 0.05$) in various treatments. To determine the effect of each treatment on plasma HDL performed with multiple comparation test. Plasma HDL level in positive control (KP) was significantly lower compared with KN, BB20, BS20, BB60, and BS60. Plasma HDL level in BB20 was significantly higher compared with positive controls, but did not differ significantly with BS20. Plasma HDL level in BB60 was significantly higher compared with the KP, BB20, and BS20, but non significantly different with BS60. Plasma HDL level in KN was significantly higher compared with KP, BS20, BB20, BS60, and BB60.

Levels of low density lipoprotein (LDL)

The lowest plasma LDL level showed in negative control was 21.26 ± 3.29 mg/dl, then BB60 48.00 ± 10.97 mg/dl, BS60 53.86 ± 20.89 mg/dl, 113.18 ± BB20 18.91 mg/dl, BS20 117.33 ± 26.41 mg/dl, and positive controls with the highest average level of plasma LDL 233.38 ± 19.86 mg/dl (Figure 2)

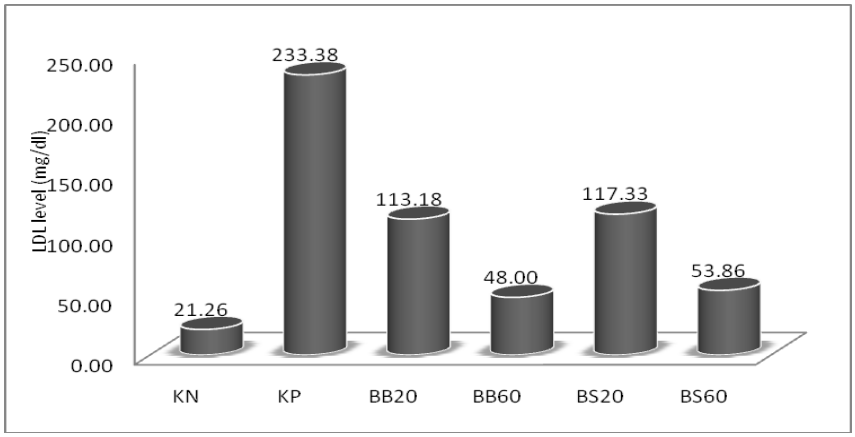

Figure 2. Plasma LDL Level Wistar Rat in Negative Control (KN), Positive Control (KP), BB20, BB60, BS20, and BS60

Analysis of variance of plasma LDL level Wistar rats treated high-cholesterol diet with *Bulung Boni* and *Bulung Sangu* extract showed significant ($p < 0.05$) in various treatments. To determine the effect of each treatment on plasma LDL level performed with multiple comparation test. Plasma LDL level in KP was significantly higher compared with KN, BB60, BS60, BB20, and BS20. Plasma LDL level in BB20, significantly higher compared with BB60, BS60, and KN, but did not differ significantly with BS20. Plasma LDL level in BB60 significantly lower compared with the KP, BS20, BB20 but non significantly different with BS60. Plasma LDL level in negative control were significantly lower compared with BB60, BS60, BB20, BS20, and KP.

Levels of total cholesterol

The highest plasma total cholesterol level found in positive control 311.80 ± 18.47 mg/dl, then BS20 196.07 ± 25.86 mg/dl, BB20 17.42 ± 190.07 mg/dl, BS60 132.96 ± 18.11 mg/dl, BB60 128 ± 10.79 mg/dl, and the lowest in the negative control with total cholesterol 114.98 ± 4.59 mg/dl (Figure 3).

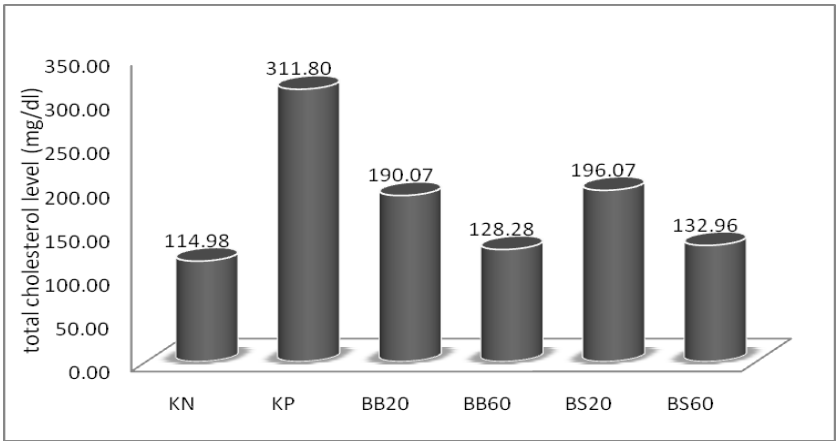

Figure 3. Plasma Total Cholesterol Level Wistar Rat in Negative Control (KN), Positive Control (KP), BB20, BB60, BS20, and BS60.

Analysis of variance of total cholesterol Wistar rats treated high-cholesterol diet with *Bulung Boni* and *Bulung Sangu* extract showed significant ($p < 0.05$) in various treatments. To determine the effect of each treatment on plasma total cholesterol level performed with multiple comparation test. Plasma total cholesterol level in positive control (KP) was significantly higher compared with negative control (KN), BB20, BS20, BB60, and BS60. Total cholesterol level in BS20 were significantly higher compared with KN, BB60, BS60, but non significantly different with BB20. Total cholesterol level in BB60 significantly lower compared with positive control (KP), BS20, and BB20, but non significantly different with BS60, and KN.

DISCUSSIONS

Levels of high density lipoprotein (HDL)

High-cholesterol diet with *Bulung Boni* and *Bulung Sangu* extracts dose of 20 mg/ 100 g bw rat/day resulted in plasma HDL level were significantly higher than treated only high-cholesterol diet (positive control). *Bulung Boni* and *Bulung Sangu* extracts with a higher dose is 60 mg/100 g rat bw/day can increase plasma HDL level that were significantly higher

compared with dose of 20 mg/100 g bw rat/day. *Bulung Boni* and *Bulung Sangu* extracts dose of 60 mg/100 g bw rat/day can increase plasma HDL level above 60 mg/dl, approximately 62.34 ± 1.19 mg/dl in BB60, whereas in BS60 61.53 ± 3.84 mg /dl. This plasma HDL level were high. Plasma HDL level of less than 40 mg /dl are low, whereas above 60 mg/dl is high[10]. HDL cholesterol level above 60 mg/dl can be considered as a protective effect against heart disease [11].

Bulung Boni extract can increase plasma HDL level in rats, therefore significantly higher than fed only high cholesterol diet, but did not differ significantly with *Bulung Sangu* extract. This is due to in *Bulung Sangu* and *Bulung Boni* there are several types of the same carotenoid such as beta carotene, chlorophyll b, antheraxanthin, astaxanthin free, and neoxanthin, therfore the ability to increase plasma HDL level did not differ significantly. Any increase in plasma HDL cholesterol level at 1 mg/dl can reduce the risk of coronary heart disease in men by 2% and 3% in women [12]. High level of HDL in the blood will accelerate the process of transporting cholesterol to the liver, therefore reducing the possibility of accumulation of cholesterol in the blood vessels [13]. Dietary carotenoids may act as a hypocholesterolemic agent, and inhibiting the enzyme HMG-CoA reductase is an important role in cholesterol synthesis [14].

Levels of low density lipoprotein (LDL)

In rats fed high-cholesterol diet with *Bulung Boni* and *Bulung Sangu* extract with a dose of 20 mg/100 g bw rat/day had plasma LDL level are significantly lower than without *Bulung Boni or Bulung Sangu* extract. In BS20 117, 33 ± 26.41 mg/dl, and BB20 113.18 ± 18.91 mg/dl. *Bulung Boni* and *Bulung Sangu* extract with higher dose, that was 60 mg/100 g bw rat /day can reduce plasma LDL level that were significantly lower compared with dose of extract 20 mg/ 100 g bw rat /day. Plasma LDL level in BB60 48.00 ± 10.97 mg/dl, while in BS60 plasma LDL level higher at 53.86 ± 20.89 mg /dl, but did not differ significantly with BB60. This shows that *Bulung Boni* and *Bulung Sangu* extracts with a dose of 60 mg/100 g bw rat /day can reduce plasma LDL level therefore achieve to optimal LDL level.

LDL level less than 100 mg /dl is optimal LDL level, 100-129 mg/dl is near optimal level of LDL, 130-159 mg/dl is the highest normal range, 160-189 mg/dl is high LDL category, more than 190 mg/dl, very high category [10].

Levels of total cholesterol

In Wistar rats fed only high-cholesterol diet without *Bulung Boni* and *Bulung Sangu* extracts (positive control) resulted in total cholesterol level was significantly higher compared with the treatment BS20, BB20, BS60, BB60, and KN, approximately 311.80 ± 18.47 mg/dl, it is called hypercholesterolemia, because of total cholesterol levels in the blood more than 240 mg/dl.

Increased cholesterol levels can be caused by the diet contains too much cholesterol and fat, therefore the body is unable to control it, the excretion of cholesterol into the colon trough bile acid is very limited, and the production of cholesterol in the liver more than the body needed[15].

High-cholesterol diet with *Bulung Boni* or *Bulung Sangu* extract with a dose of 60 mg/100 g bw rat/day resulted in total cholesterol level were significantly lower compared with dose of 20 mg/100 g bw rat/day, but did not differ significantly with negative control (KN). This shows that *Bulung Boni* and *Bulung Sangu* extracts with a dose of 60 mg/100 g bw rat /day can lower total cholesterol level near to total cholesterol in rats treated only standard diet (negative control). *Bulung Boni* and *Bulung Sangu* extracts can lower cholesterol level below 200 mg /dl, this is due to carotenoid content in *Bulung Boni* and *Bulung Sangu* function as hypocholesterolemic.

Carotenoid has a very important biological functions as an antioxidant, immune system, prevent degenerative diseases, anti-inflammatory, anti-stress, inhibit lipid peroxidation, and lower blood cholesterol level [16]

CONCLUSSION

Bulung Boni and *Bulung Sangu* extracts improve lipid profile by increasing plasma HDL level, lowering LDL and total cholesterol levels Wistar rat fed high cholesterol diet significantly.

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