Effect of carrageenan fiber of seaweed (Eucheuma cottonii) and tuna fish (Katsuwonus pelamis) in the diet on the plasma lipid of rats (Rattus norvegicus) Strain Sprague Dawley

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Abstract

This research was aimed to determine the best carrageenan seaweed (Eucheuma cottonii) to tuna precook oil ratio in the diet given to rat (Sprague dawley) on the lipid profile of their blood plasma. The experiment was arranged in a completely randomized design and replicated four times. Those treatments were diet composition namely 0% carrageenan and 0% fish oil (M_0K_0), 5% carrageenan and 5% fish oil (M_1K_1), 10% carrageenan and 10% fish oil (M_2K_2), 15% carrageenan and 15% fish oil (M_3K_3), 10% carrageenan (M_0K_2) 10% fish oil (M_2K_0). Diet with high cholesterol level was feed to 24 mice for 21 days, until the rat has reached hypercholesterolemia condition. The average cholesterol level in the rat with hypercholesterolemia condition was 312.75 mg/dL, or 137% higher than that of before treatment. Afterwards, in the next 21 days, the diet with various karagenan and fish oil content was given. The decrease of cholesterol level in the rat blood plasma upon treatment was then monitored. Results showed that carrageenan and or fish oil in the diet has improved the lipid level in the rat blood plasma, M_2K_2, M_1K_1, M_3K_3, M_0K_2, M_2K_0 were 62.25%, 85.96%, 63.15%; 47.81%, 34.08%, 62.36%, 44.32%, 62.50%, 49.49%; 38.69%, 50.58%, 50.44%; 37.34%, 47.44%, 50.48%; and 1.7%, 8.7%, 4.25% respectively. The synergistic effects were showed between carrageenan and fish oil mixture. The mixture gave the best profile rat lipid plasma at 10% ratio.

Keywords: carrageenan, tuna fish oil, plasma lipid

Introduction

Coronary heart disease (CDH) is one of serious health problems that responsible for 70% and 23.39% death in USA and Indonesia, respectively (Sargowo, 2001). The disease is caused by atherosclerosis, fat and blood cells accumulation in the inner part of coronary which causes plaque. Atherosclerosis is related to cholesterol level of low density lipoprotein (LDL) and high density lipoprotein (HDL). Therefore, the disease can be decreased by monitoring the LDL and HDL content in blood.

Coronary heart disease can be prevented by consuming marine fish and dietary rich in fiber, such as fish oil and seaweed. Oil from tuna precook is rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which are able to lower cholesterol level in blood. Carrageenan seaweed, on the other hand, has been used as dietary fiber from sea origin for the same purpose. The combination of both constituents can be applied to lower cholesterol level in blood.

Materials and Methods

Materials used in this research were seaweed (Eucheuma cottonii) and tuna fish oil (Katsuwonus pelamis); rats (Rattus norvegicus) Strain Sprague Dawley Sprague-Dawley, ± 4 weeks of age and the weight gap was not more than 10 g.

Feed materials

Feed materials for experimental rats consisted of seaweed carrageenan, tuna fish oil, , maize oil, casein, egg embryo as source of cholesterols, mineral and vitamin mixture, and dextrose.

Chemicals

Ethanol, ether, Chloroform, glacial asetic acid, aquaest, H_2SO_4, Chloride,acid oxide-mercury, natrium hidroxida, Potassium sulphate, hexana, aceton, and buffer phosphate reagen kit for analysis of total cholesterol, HDL dan triglyceride (Boehringer).

Methods

Production of carrageenan

To produce carrageenan from seaweed (Eucheuma cottonii) extraction method used by methanol (Winarno, 1990). Chronology of carrageenan production process is as follows: 1kg of seaweed were washed to remove sand, salt, and objects other foreign. Seaweed and then soaked for 24 hours then boiled in a pressure cooker equipment at the temperature of 120°C for 15 minutes. Then the seaweed is cooked at the temperature of 90-100°C for 3 hours is intended to solve the cell wall of seaweed, and then continue with the screening. Separation of carrageenan done with the container through the addition of metanol or metanol (1:15 v / v) intended to get good quality with carrageenan. Sediment and
filtered and carrageenan results methanol dried container with the 100°C temperature in the oven for 3 days, then after a dry mashed and filtered, then weighed the weight.

**Analysis of Fatty Acid Composition of Tuna Fish Oil**

In the test tube's 5mg fish oil with added 1mL toluene, then added 2mL acid chloride solution of 5% in methanol, incubation temperature at 50 °C for 12 hours. Next added 5mL Sodium chloride solution of 5%, and extracted 2 times each with 5mL heksana. Extracting heksana plus potassium bicarbonate solution with 2% to neutral and then dried with Sodium sulphate anhydrate (Method AOCS, 1990). Extracting hexane evaporated with vacuum evaporator until the volume reached 0.6 mL. Methyl ester fatty acid (FAME) analyzed by the method gas chromatography -spekrofotometri mass with the type of equipment: KG-MS (GC-17A Ver.3 Shimadzu GCMS QP-5050 class-5000; Shimadzu Release 2.2. Sample size: 0.2 μL; column: DM 5MS J & W Scientific 122-5532; size column: 30m x 0.25 mm (id) x 0.25 μm; detector Type: MS; Integrator: HP Deskjet 810c printer; helium gas carrier. Operational mode: programmed temperature, the temperature field: 60-320 °C, increased 15 °C / min, temperature Injector; 300 ° C; Bath temperature 320 ° C

**Adapting period**

Twenty-four rats tails adapted for one week and given a standard diet. Standard diet contains carbohydrates, fat, protein, vitamins and minerals with the composition as follows: water content of 11-13%, 19-21% of protein, fat 4,5-7%, fiber 3-4%, 4-6% ash, 0,7-0,9% calcium, phosphorus 0,6-0,75%, with the energy content of 2950-3100 Kal / kg next mouse blood taken through the plasma tail, and analyzed the data to obtain initial lipid profile.

**Pretreatment (hypercholesterolemic diet)**

Rats divided into six groups based on the weight of the next randomly assigned diet and the standard yellow duck eggs as a source of cholesterol (5mL/ekor/hari) for 21 days to increase the rate of blood plasma cholesterol to the conditions hiperkolesterolemik (total cholesterol, 300mg/dL). At the end of this blood is taken through the tail, and plasma lipid profile (total cholesterol, HDL, LDL, and Triglyceride) analyzed.

**Treatment of carrageenan diet and fish oil**

After the conditions reached hiperkolesterolemik (total cholesterol + 300 mg / dl) of each group was given a diet with the rate of fish oil and carrageenan, which vary. The compositions of each diet for each group are listed in Table 1.

<table>
<thead>
<tr>
<th>Diet (g)</th>
<th>M0K0</th>
<th>M1K1</th>
<th>M2K2</th>
<th>M3K3</th>
<th>M0K2</th>
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<tr>
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</table>

Modified from AIN-93 (Suprijana, 1997)
Analysis lipid profile
Total Cholesterol Test

The rate of cholesterol measured by the method Rajani sat down-PAP (cholesterol Oxidase-p-aminophenase) with the principles of the enzymatic test colorimetric.

Blood serum taken as much as 0.01 ml and mixed with 1 mL reagent (commercial kit) and then inserted into the test tube and mixed until a homogeneous. After a homogeneous mixture and then incubated the temperature 37ºC for 5 minutes. Then read on absorbance at 546 nm.

The calculation of the rate of total cholesterol was done by using the formula:
\[ \text{The rate of cholesterol (mg / dl)} = \left(\frac{\text{absorbance sample}}{\text{absorbance standard}}\right) \times 200 \text{mg/dL} \]

Test Hing Density Lipoprotein / HDL (Boehringer Kits) (Rajani sat down Method-PAP)

Before testing the rate of HDL, conducted sample preparation is 200 µL of blood serum is mixed with 500 µL reagent precipitation then incubated for 10 minutes at room temperature. Following the centrifuge at 4,000 rpm for 10 minutes so that the resulting supernatant ready for analysis. Stages of the HDL analyzes rate with the method CHOPD-PAP is taken 100 µL supernatant and mixed by 1000 µL reagent solution. After mixed incubated the temperature 37 º C for 5 minutes. Then read on absorbance at 546 nm. The calculation of the rate of HDL made with the formula:
\[ \text{The rate of HDL (mg / dl)} = \left(\frac{\text{absorbance sample}}{\text{absorbance standard}}\right) \times 200 \text{mg/dL} \]

Low Density Lipoprotein (Barraas, 1994)

LDL rate is calculated directly using the formula:
\[ \text{The rate of LDL} = \text{Kindergarten - (HDL + MH / 5)}; \]

with the assumption of MH / 5 is VLDL

Triglyceride Test / MH (GPO-P AP Method)

Triglyceride test plasma determined using the method calorimetric enzymatic combination Test Kit (Boehringer-mechanism)

Taken in mL 0.01 blood serum, and then mixed with 1 mL reagent (kit). After that incubated the temperature 37 ºC for 5 minutes, then read on absorbance at 546 nm. Triglyceride rate calculation is done by using the formula:
\[ \text{Triglyceride rate (mg / dl)} = \left(\frac{\text{absorbance sample}}{\text{absorbance standard}}\right) \times 200 \text{mg/dL} \]

Results and Discussion

Total Cholesterol

The results of analysis of the rate of total serum cholesterol showed that rat-free fiber groups of carrageenan and fish oil has a higher total cholesterol compared with a group of mice received the addition of fiber carrageenan and fish oil. In Figure 1 it can be seen that the rate of total serum cholesterol groups: M2K2, M1K1, M3K3, M2K0, M0K2, M0K0 succession is 109.75; 164.25; 168; 179.5; 180.25; and 355 mg / dl.

The descriptive seen that the rate of plasma cholesterol rats that received the addition of fish oil and 5% carrageenan (M1K1), 10% (M2K2), 15% (M3K3), and 10% carrageenan (M0K0), fish oil 10% (M2K0) experienced a decrease of 47.81% 62.25% 44.32% 38.69% 37.34% if compared with the control (fiber-free carrageenan and fish oil (M0K0) 1.73%.
Duncan test further showed that the addition of carrageenan treatment, the addition of fish oil, and adding a mixture of fish oil and carrageenan showed no real different, but very different when compared with the real treatment that does not use carrageenan and fish oil in a decrease in total cholesterol.

Decrease the effects caused by suspected in the hamper the ability of carrageenan absorption cholesterol in the intestine and increase excretion bile acids. Absorption retardation is associated with the ability to increase carrageenan viskositas Lumen intestine and disrupting the establishment of misel, and increase excretion bile acids, as carrageenan that can dissolve binding bile acids (sequestration of how acids). As bile acids made from cholesterol in the liver. With the increase excretion bile acids, cholesterol is absorbed from the blood and bile acids into metabolism. (Wolever et al, 1997). While the fatty acid Omega-3 influence lipid in the blood plasma, which impedes the formation of fat cells protrin and Triglyceride in VLDL, so that the lower level and VLDL cholesterol in the blood, and reduce the formation of amino acids by the liver cells. (Bruckner, 1986)

According to Muchtadi et al (1993) point cholestrol excretion is in the best conversion into bile acids (200-300 mg / day); point is the synthesis of other steroid hormones (40 mg / day); sweat, hair, and skin (50 mg / day) and through the urine (1 mg / day).

Other mechanisms decrease cholesterol caused by the increased production proponiat acid metabolism as a result of fiber by intestinal microbes. Acid propionat this will push the activity of enzyme β-hidroksi-β-metil glutaril-COA reduktase (HMG-COA reduktase) so hampered cholesterol biosynthesis (Harianto, 1996).

**HDL Cholesterol**

HDL serum analysis results showed that rat-free fish oil group and fiber carrageenan, which is lower compared with other groups of rats. In Figure 2, that the rate of HDL serum groups M2K2, M3K3, M2K0, M0K2, M1K1, M0K0 succession is 63.15; 59.1; 49.5; 48.57; 47.3 and 28.12 mg / dl.

Treatment of carrageenan and fish oil have had a significant effect on the increase in HDL. Duncan test results further showed 110.13% increase in HDL (M2K2), 86.28% (M3K3), 75.52% (M2K0), 37.02% (M0K2), 34.09% (M1K1), and 5, 04% (M0K0), in the blood.

HDL is the only carrier that works for the transfer of excess cholesterol from the network peripheral to the heart and is useful in lowering the risk aterosklorosis. Conversely sent by the LDL cholesterol from the liver to the network peripherals and dumped there, so LDL is because aterogenik cause coronary vessel in the management or aterosklorosis. With terjadinnya decrease LDL, HDL, the more will be needed to meet the shortage of cholesterol in the liver to form bile acids. Such conditions will stimulate synthetic HDL in the liver so that the rate of HDL in the blood will increase. Result of the high-fiber, bile acids are lost in the intestine to the outside so absorbed with Feces can serve and return to the heart (point enterohepatik) decreased. According to Kahl's (1999) each 1-point increase in HDL can reduce the risk of suffering from coronary disease was 2-3%.

Effect of fiber food HDL is likely decrease the amount of HDL due to a decrease in total cholesterol, but will decrease this imbangi with the increase in the amount of HDL due to the effects of fiber hipotriglyceride. Anonymous (2001) in cardiovascular medicine hypertriglyceride states can lower HDL, it may happen that the vice hipotriglyceride increase HDL Anonymous (2001)

To increase HDL blood through improvements in diet is not easy. So far the effects of fiber, which can increase HDL is not reported. Diet can increase HDL, among others, reported by Kahl's (1999) 6 capsule that consumption of fish oil (Omega 3) per day for 6 weeks HDL increase of 10%. Another thing that can increase HDL is the hormones estrogen and exercises...
**LDL Cholesterol**

The results of analysis of serum LDL mice showed that the groups free of carrageenan and fish oil are higher than with other groups of rats. In Figure 6, the rate of mouse serum LDL groups M2K2, M3K3, M1K1, M2K0, M0K2, and M0K0 is 29.2; 84.1; 97.25; 104.3; 106.72 and 259.42 mg / dl.

The rate of plasma LDL rats that received additional treatment M0K0, M1K1, M2K2, M3K3, M0K2, M2K0 and M0K0 experienced a decrease of 8.74% 57.18% 85.96% 62.50% 50.58%; and 47.44% compared with a group of free carrageenan and fish oil.

Duncan test results further showed that the addition of carrageenan treatment, the addition of fish oil, did not differ significantly, but very real, if different than the treatment with a mixture of fish oil and carrageenan, a group that does not use carrageenan and fish oil in a decrease in total cholesterol.

Some research shows that water-soluble fiber foods can directly affect metabolism and LDL. McCall, et al (1992) found that the fiber psyllium lower LDL synthesis. Fernandes et al (1994) stated that the pectin in the guinea pig cause LDL particles that formed small, increase the LDL renovation, and to the receptor LDL hepatic more binding LDL.

Fatty acid Omega-3 can improve Feces excretion can serve on the steroid, the composition of fatty acid found in the lipoprotein, resulting fluiditas lipoprotein will be increased and will affect the activity of lipolytic enzyme, change speeds and synthetic katabolisme VLDL (Bruckner, 1986)

**Triglyceride**

Triglyceride analysis of the rate of rat plasma showed almost the same pattern with total cholesterol (Figure 4). Duncan test further showed that carrageenan and fish oil can significantly reduce the rate of Triglyceride. Hipotriglyceride effects those are most evident in the treatment of carrageenan and fish oil.

![Fig 3. Rat Blood Plasma LDL, at the beginning and the end of Experiment](image)

![Fig 4. Rat Blood Plasma Triglyceride, at the beginning and the end of Experiment](image)
In a descriptive decrease in the rate of Triglyceride by the treatment of carrageenan and fish oil are M2K2, M1K1, M2K0, M0K2, M3K3, and M0K0 respectively 63.15% 62.36% 50.48% 50.44% 49.49% And 4.25% increase in triglyceride.

As we have stated previously that the treatment of carrageenan and fish oil can improve excretion bile acids that work to help absorption of fat / triglyceride. When excretion bile acid increased, the absorption of fat also will be disrupted, consequently, can reduce the rate of Triglyceride plasma.

The possibility of carrageenan and fish oil products can bind digestion of fat (fatty acid and glycerol) can also hinder the absorption and lead to a decrease in blood Triglyceride the more significant.

Conclusions

Carrageenan and tuna fish oil in dietary could improve lipid profile of rat blood plasma. There was synergistic effect between carrageenan and tuna fish oil in dietary on lipid profile of rat. The best combination effect of seaweed carrageenan and tuna fish oil on lipid profile was achieved by giving 10% tuna fish oil and 10% carrageenan in dietary feed.

References


Sargowo. 2001. Role of triglyceride rate and lipoprotein as risk factor of coronary heart disease (preliminary research). Live sciences Journal, 13 (2) : 121 – 128