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The Effect of Biji Mete (*Scomberomorus commerson*) in the Feed to the Decline in Blood Cholesterol Level Hypercholesterolemic Male Mice (*Mus musculus*)

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Abstract

Cashew nut *(Anacardium occidentale)* is one type of seed that very useful because contains omega-3. This research purpose for study about the influence of Cashew Nut in feed to against lowering total blood cholesterol levels of male mice (*Mus musculus*) that hypercholesterolemia. This research was conducted use 5 treatments, there are negative control, positif control, treatment 1 (K1) with ratio standard feed and cashew nut (2:1), treatment 2 (K2) with ratio standard feed and cashew nut (1:1) and treatment 3 (K3) with ratio standard feed and cashew nut (1:2). The parameters that measured are total cholesterol blood of mice with CHOD-PAP method. The principle that used in the method is determination of total cholesterol in the blood after enzymatic hydrolisis and oxidation. The results of the research shown that cashew nut give influence to the total blood cholesterol level of mice, it contains in the final result, there are (-) = 86 mg/dL, (+) = 174 mg/dL, K1 (2:1) = 124 mg/dL, K2 (1:1) = 94 mg/dL dan K3 (1:2) = 75 mg/dL.

Keywords : *Total Cholesterol levels, Cashew Nut, Hypercholesterolemia, Omega-3, Mice*

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INTRODUCTION

Cholesterol for the body to function as a stabilizing component of cell membranes, as a precursor of bile salts and as components of some steroid hormones. It is also a pre substance for the formation of vitamin D, a precursor of cholesterol is converted into ubiquinone, dolichol and in the skin is converted into cholecalciferol, which is a form of vitamin D. Cholesterol circulates in the blood are not in free form, but rather in the form of lipoprotein (Iswari, RS and Manalu, W . , 2011)

Cholesterol circulates in the blood is packaged in the form of lipoproteins such as chylomicrons, VLDL, LDL and HDL. Increased cholesterol in the blood is associated with plaque formation arteriosclerosis that can clog blood vessels, causing heart attacks and strokes. Although high levels of HDL cholesterol atherogenic, high levels of HDL cholesterol is protective because HDL particles act issued cholesterol to tissues and return it to the liver (Iswari, RS and Manalu, W. , 2011)

Cholesterol elevated levels in the blood is associated with the formation of atherosclerotic plaques that can clog blood vessels, causing heart attacks and strokes (Champe , P. , 2010) .

Atherosclerosis is a disease of the formation of plaque on the walls of arteries, causing narrowing of the vessel lumen and vessel elasticity decrease. The formation of plaque on the walls of blood vessels causing impaired blood flow. Plaque is formed composed of smooth muscle cells, connective tissue, fat and dirt that accumulate in the arterial wall intimate. Naturally, these events occur in humans and animals, only the reaction speed will be faster if the diet contains plenty of saturated fatty acids and a source of energy derived from fat (Iswari , RS and Manalu , W. , 2011) .

Atherosclerotic disease can be caused by improper nutrition, particularly by fat, cholesterol and triglycerides in the blood. Increased blood cholesterol is a major factor likelihood of atherosclerosis, while the influence of triglycerides is still uncertain. Reduced levels of HDL (High Density Lipoprotein) can increase the likelihood of atherosclerosis (Winarto, 2004).

Unsaturated fatty acids ω - 3 is believed to be closely linked to health, which can control blood cholesterol levels, reduce the risk of degenerative diseases and to improve the intelligence of children because DHA is a major fatty acid constituent of brain phospholipids. In cardiovascular disease, omega-3 fatty acids have shown a beneficial impact on the cardiovascular system. The risk of disease diabetes, obesity, asthma, and others proved to be reduced by increasing the intake of omega - 3 (Andarwulan , 2011).

Cashew fruit is actually a real (true fruit) or cashew nut shaped like kidney and stick to the artificial fruit. The content of unsaturated fatty acids in cashew include monounsaturated fatty acids MUFA (monounsaturated fatty acids) in the form of omega - 9 and PUFA (Polyunsaturated Fatty Acids) in the form of omega - 6 and omega - 3

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unsaturated fatty acid content is exactly what effect against cholesterol (Rismundar , 1981).

Based on research conducted by Akinhanmi, TF, Atasie, VN, and Akintokun, PO (2008) showed that the cashew (Anacardium occidentale) containing omega - 3 and can be used as a source of omega - 3.

With omega - 3 found in cashew and based on previous research, this study was conducted to determine the effect of cashew kernels in the feed to lower total blood cholesterol levels of male mice (Mus musculus) were hypercholesterolemia by using the CHOD -PAP method.

MATERIALS AND METHODS

Object Research.

Seeds Cashes (Anacardium occidentale). Experimental mice that male mice (Mus musculus), aged 1.5 - 2 moon by weight ± 15.82 grams.

Study Design

The experiment was conducted in April 2014 to May 2014, in the Research Laboratory of the Faculty of Mathematics and Natural Sciences and Laboratory Mulawarman Samarinda Pharmacies M. Yamin Samarinda.

Research using cashew (Anacardium occidentale) that has been mashed, then put in a standard feed mice with several different treatments. Feeding was done 2 times a day are at 9:00 and 17:00 pm. Then the drinks given Prophil Thio Uracyl (PTU) (except for the negative control) Feeding and drinking is done every day for 40 days. In this study used five male mice (Mus musculus). The mice were divided into 5 treatments as follows:

Specification:

(-) : Negative control (-) , fed a standard 12 g / day

(+) : A positive control (+) , fed a standard 12 g / day and given PTU 0.01 %

K1 : 1 treatment, were fed a standard 8 g / day and cashew 4 g / day (2: 1 ratio between the standard feed and cashew) , and 0.01 % PTU

K2 : treatment 2, were fed a standard 6 g / day and cashew 6 g / day (ratio of 1 : 1 between the standard feed and cashew) , and 0.01 % PTU

K3 : 3 treatment, fed a standard 4 g / day and cashew 8 g / day (ratio 1:2 between the standard feed and cashew), and 0.01 % PTU

Originally adapted mice for 10 days with standard feed to get used to the new environment, then after 10 days, blood was taken mice as total blood cholesterol levels early (Day 0), in which the blood sample is taken through the veins with cut off the tail, then the blood in a centrifuge at a speed of 4000 rpm for 5 minutes to get the blood serum. As much as 0.01 mL of blood serum analyzed. On the next day is day 1 up to day 10 added 0.01 % PTU in mice drink bottled drinking 60 mL (except negative control) in order to raise total blood cholesterol levels in mice. Then measured levels of total blood cholesterol. Then on day 11 and day 40 samples added cashew (Anacardium occidentale) in accordance with the standard feed each treatment to lower blood total cholesterol in mice. Every 10 days the mice were taken for analysis of blood serum total

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blood cholesterol by using a photometer 5010 with a wavelength of 546 nm and prior to blood collection, mice were fasted for 12 hours beforehand.

Sample Analysis.

Mice experiments put into the capillary tube, and then cut the tail to collect blood from veins. Blood inserted into the tube lid. Blood obtained was then centrifuged at a speed of 4000 rpm for 5 min to obtain serum and then analyzed the total cholesterol. Analyzed with CHOD - PAP method. The principle used is the determination of total cholesterol in the blood after enzymatic hydrolysis and oxidation.

The principle of measurement of total cholesterol by enzymatic colorimetric based on the following reaction:

$$\text{Kolesterol ester} + H_2O \xrightarrow{\text{Chol. esterase}} \text{Kolesterol} + \text{Asam Lemak}$$

$$\text{Kolesterol} + 1/2\ O_2 + H_2O \xrightarrow{\text{Chol. oksidase}} \text{Kolesterol} + H_2O_2$$

$$2H_2O_2 + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroksidase}} \text{Quinoneimine} + 4\ H_2O$$

Analysis Procedure:

1. Taken 0.01 mL of blood serum was added 1 mL of reagent CHOD - PAP
2. Mixed for 1 minute
3. Incubated at 37ºC for 10 minutes
4. Read the absorbance at 500 nm wavelength with a blank , color spectrum will be stable for 2 hours with Photometer 5010
5. Cholesterol levels calculated by the formula calculation :

   Levels of total cholesterol (mg/dL) =

$$\frac{\text{Absorbance of sample}}{\text{Absorbance Standard}} \text{X } 200 \text{ mg/dL}$$

RESULTS

Serum Total Cholesterol.

Mice with K3 treatment had higher levels of total blood cholesterol the most low (75 mg/dL). Then followed by treatment K2 (94 mg/dL) and K1 (124 mg/dL). Then for mice treated with (-) had higher levels of total cholesterol 86 mg/dL, whereas the (+) levels of total cholesterol reaches 174 mg / dL.

No	Sampel Code	Day ke-0	Day ke-10	Day ke-20	Day ke-30	Day ke-40
1	(-)	80	86	98	102	86
2	(+)	85	112	136	154	174
3	K1	86	136	138	142	124
4	K2	88	115	118	127	94
5	K3	85	140	139	99	75

Table 1. Level Mice Cholesterol (*Mus Musculus*)

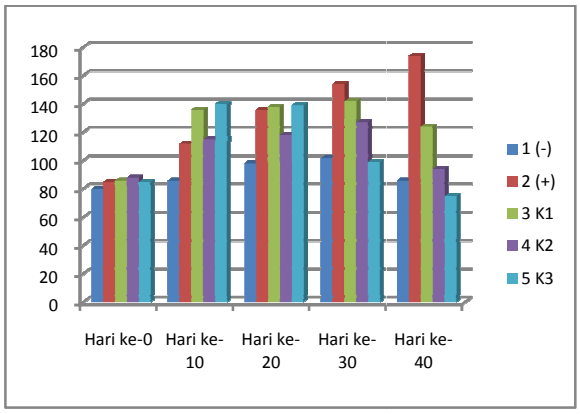

Chart 1. Total Level Cholesterol Mice

DISCUSSION

The results of the study total blood cholesterol levels of male mice (Mus musculus) are shown in Table 1. Mice that had

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been moved to a new environment, adapted to the standard feeding and drinking plain water for 10 days. After 10 days the mice were taken blood and blood serum analyzed by using a photometer 5010. The results can be seen in Chart 2.

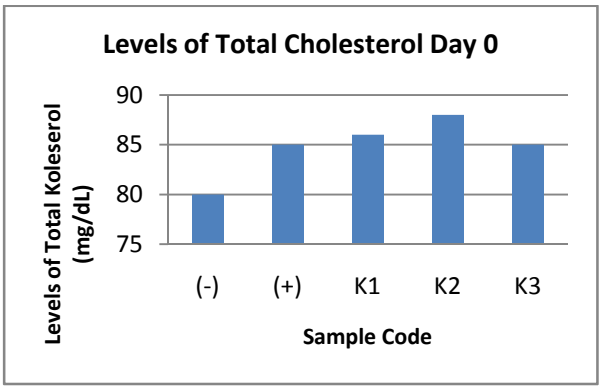

Figure 2. Levels of Total Cholesterol Day 10

2. It can be seen from the graph of mice (-) : 80 mg / dL; mice (+): 85 mg / dL; K1 mice: 86 mg / dL; K2 mice: 88 mg / dL and mice K3 : 85 mg / dL . Based on Hernawati (2013), explained that the range of total blood cholesterol levels in normal mice is 26-82 mg / dL. Can be seen from the above that there are mice that have high levels of cholesterol in the top of the range of total blood cholesterol levels to normal mice. This may imply that the mice are likely to experience interference with the metabolism system, but is not very significant because it can be said mice suffered little disruption due to not adapt to the new environment.

On day 1, the mice drinks were added 0.01 % PTU (except for negative treatment), in which the beverage filling was done 2 days. In mice feed was given the usual standard feed. The treatment is done during the first day until day 10 and then analyzed the levels of total cholesterol to - 2 mice. So that the results obtained from the graph 3.

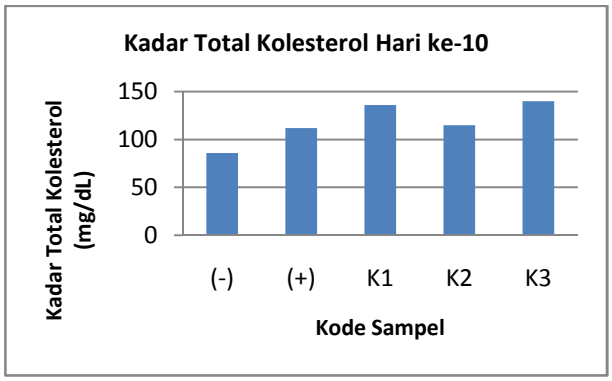

Chart 3. Levels of Total Cholesterol Day 10

Based on the data above it can be seen that the mice (+) increased levels of total cholesterol in mice that K1 of 86 mg/dL to 136 mg/dL, in mice K2 ie an increase of 88 mg/dL to 115 mg/dL and in mice K3 the increase occurred from 85 mg/dL to 140 mg/dL. From the results obtained it can be said that PTU were added to the beverage mice have managed to raise total blood cholesterol levels of male mice (Mus musculus). PTU is an antithyroid substances that can damage the thyroid gland to inhibit the formation of thyroid hormones. Thyroid hormones can lower blood cholesterol levels by increasing the formation of LDL in the liver, resulting in increased spending cholesterol from the circulation. Thyroid hormone deficiency results in decreased

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cholesterol catabolism, resulting in increased blood cholesterol (Ganong, 1995).

Then on day 11 of treatment has been paid to mice in the form of additional feed cashew nuts in the food (except for the treatment of negative and positive). In mice with positive treatment or positive control, was not given the addition of cashew nuts in the food, this is a positive treatment caused of this will serve as a comparison with the treatment in mice given cashew nut, so it can be seen whether there are differences in levels of total cholesterol. On the drink for each treatment is still given PTU 0.01 % except in the negative treatment. It aims to maintain the condition hypercholesterolemic in mice in order to see whether the addition of cashew kernels in the feed can affect the levels of total cholesterol mice.

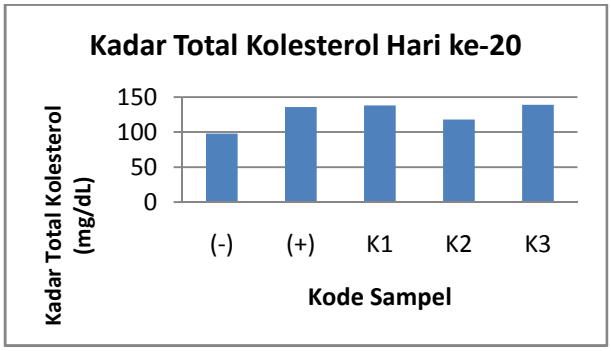

Chart 4. Levels of Total Cholesterol Day 20

The results obtained on day 20 it can be seen that the levels of total cholesterol was increased except at the 3rd treatment that K3 mice which had declined, although only slightly, namely from 140 mg/dL to 139 mg/dL. This suggests that in mice treated K3 with 1:2 can be said that cashew took effect on levels of total blood cholesterol mice. Then in the treatment of K2 and K3 are still increased, indicating that cashew seed still not affect the levels of total cholesterol mice that still increased. As well as in mice (+) an increase to 136 mg/dL, this is because the mice untreated adding cashew nut so that no reduction occurred.

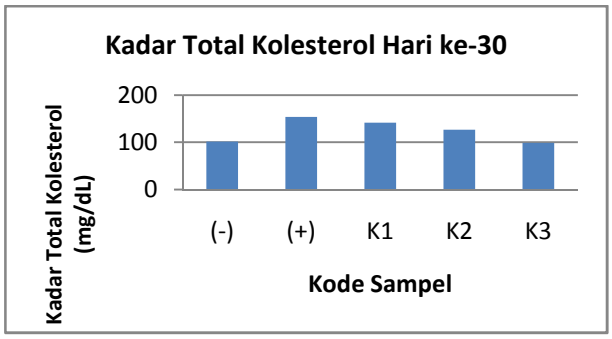

Chart 5. Levels of Total Cholesterol Day 30

On the results of the analysis of the levels of total blood cholesterol 30th day can be seen that the treatment given to each mouse still showed the same results as in the analysis of day 20, whereas levels of total cholesterol

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mice was increased except at the 3rd treatment is K3 are decreased. In mice K3 can be seen that significant reductions ranging from 139 mg/dL to 99 mg/dL, so it can be said on the addition of cashew kernels has the most considerable effect on the levels of total cholesterol mice and can eliminate the effect hypercholesterolemic are maintained with the addition of PTU 0.01%.

In the analysis results of 40 days total cholesterol mice had decreased total cholesterol approaching normal mice is 26-82 mg/dL. Chart 6 can be seen below.

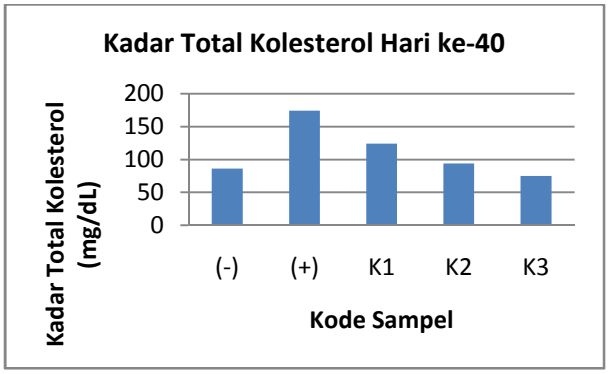

Chart 6. Levels of Total Cholesterol Day 40

From the chart 6 it can be seen in mice (+) an increase to 174 mg/dL because not given additional treatment cashew. While the decrease in total cholesterol occurred in mice given the addition of cashew nuts, it indicates that in mice treated with the addition of cashew kernels have a significant impact in lowering total blood cholesterol hypercholesterolemic mice. K1 mice decreased from 142 mg/dL to 124 mg/dL. In mice K2 decreased from 127 mg/dL to 94 mg/dL, whereas in mice K3 reduction of 99 mg/dL to 75 mg/dL. Decreased cholesterol levels in mice given cashew nut caused by the mechanism of production of lipoprotein transport in the liver that are secreted into the blood (Elisabeth, 1992).

Unsaturated fatty acids, especially omega - 3 can inhibit the synthesis of VLDL and LDL production was consequently reduced. High levels of secreted VLDL and LDL that can lead to deposition of cholesterol in the blood, because VLDL and LDL are transport proteins that carry triglycerides, cholesterol and phospholipids from the liver to the rest of the network. While HDL transports cholesterol to actually be further broken down in the liver into bile acids and excreted through the body excretion. In other words, the omega - 3 effect on the increase in cholesterol levels (Elisabeth, 1992).

CONCLUTION

From this study it can be concluded that the addition of cashew kernels in the standard feed hypercholesterolemic mice can

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affect the levels of total cholesterol mice, in which the optimum condition is achieved at a ratio (1:2) between the feed and cashew.

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