THE ANALYSIS of TOTAL CHOLESTEROL LEVELS IN MICE (*Mus musculus*) MALES WHO WERE GIVEN EXTRACTS of METHANOL LEAF *CEMBA* (*Acacia pennata*)

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Abstract: This study was conducted to determine the activity of the leaf extract *Cemba* (*Acacia pennata*) antihyperkolesterolemia against total cholesterol levels in mice (*Mus musculus*) male ICR strain. *Cemba* leaves extracted by using 96% methanol by maceration. This study is a completely randomized design (CRD) that consist of five treatments and five repetition i.e normal group, hiperkolesterol group, the group of the giving of the EDC(Ap) with doses of 100, 250, 500 mg/Kg. The extract was dissolved using Carboxy Methyl Cellulose (CMC) 0.5% and given orally in mice which had previously been given a high cholesterol feed. Data were analyzed using Analysis of Variance (ANOVA) using SPSS Program is 2.0 and followed by Tuckey test (α 0.05). The results showed the methanol extract of leaves cemba with the dose of 500 mg / kg hads the highest effectiveness in lowering total cholesterol levels in mice which have hypercholesterolemia with a 47.88% percentage drop. These results are significantly different from hypercholesterolemia group with -7.20 percentage. The granting of leaves extracts *cemba* (Acacia pennata) are able to decrease the total cholesterol levels of mice (*Mus musculus*) male hipercholesterolemia.

Keywords: Anti-hypercholesterolemia, Total Blood Cholesterol, Methanol Extract of *Cemba* Leaf, Male Mice (*Mus musculus*) ICR strain.

INTRODUCTION

The progress of the times today makes most of the community is experiencing a lifestyle change that is affected by urbanization and modernization as well as the lack of sports activities, including eating patterns. In terms of diet, the public tended to choose things that are fast and instant regardless of the side effects thereof, so that it can lead to the appearance of various diseases, such as cancer, diabetes mellitus, hypertension, stroke, atherosclerosis, cataracts, and coronary heart disease (PJK). One of the causes of the onset of the degenerative diseases is because excess cholesterol in the body resulting in a State called hiperkolesterolemia.

Hiperkolesterolemia especially low-density lipoprotein (LDL) cholesterol which is accompanied by an increase in free radicals in the blood, it will cause the occurrence of oxidation of LDL that ultimately lead to atherosclerosis (Sanchez *et.al*,2003; Prior, 2003; Micallef *et.al*., 2007). Anticipating the danger of hiperkolesterolemia against cardiovascular disease has developed several drugs such as Gemfibrozil, Niacin hipolipidemik, and the Statins (Jones *et.al*., 1998).

Considering the treatment of patients hiperkolesterolemia take a long time, and require high costs as well as the presence of side effects which cannot be avoided, then the research need to be developed to get the drug more effectively with cheaper prices, and reduce the effects of beside

caused. Natural materials such as plants need to be explored because dimanfaatakan has not been optimally. As one of the efforts to optimize plant utilization of Indonesia, then the research needs to be done on plants (Singh, 2011). The plants contain compounds of secondary metabolites in the form of flavonoids and phenolic are useful as free radical-catcher (Cos *et.al*, 2001).

Several studies report that the content of tannin in vegetables or plants can play a role in preventing or lowering the risk of heart disease korener. In addition, the fragrance of pandan leaves contain alkaloids, saponins and tannins, polyphenols, flavanoida which is able to inhibit the activity of the enzyme HMG-Coa work reductase and inhibit the absorption of cholesterol in the digestive tract (Amelia, 2014).

Cemba (*Acacia pennata*) by the people of Enrekang Regency, South Sulawesi utilized leaves to add to the flavour of the meat processed on the cuisine which is a special food called cemba cuisine.

Based on explanation above, need to be examined on the merits of the methanol extracts of the plant as a medicinal herb cemba antihiperkolesterolemia. In this study are expected by administering cemba leaf extract can lower cholesterol levels in male mice. As for the purpose of peneltian is to know how the granting of cemba leaf extract against total cholesterol levels decrease mice males.

RESEARCH METHODS

This research is conducted on wants a December 2015 – March 2016, research conducted in the laboratory of biology and chemical engineering laboratory, UNM.

Animal Test

Animal testing is used in the form of mice strain ICR male, healthy, and normal activities observe in the laboratory biological science faculty UNM. Mice age 2 months as many as 25 of the tail is divided into 5 groups each treatment consists of 5 mice tails in one enclosure. The mice body weight range 20 – 30 grams are divided into 5 groups of treatment.

Experimental Design

Mice are adapted for 2 weeks with a given commercial feed in the form of a standard feed flour ADII and in drinking water ad libitum feed given before treatment (in the form of cholesterol feeding powdered egg yolk) so that the way of life and food become uniform. On the third week, the mice are classified according the Group respectively. The Group of mice are determined based on the weight of the body is divided into 5 groups of treatment. As for the moderate groups as follows:

1. The group I (normal group) is a group of male mice are only given standard feed and aquades during the trial period.
2. Group II (hiperkolesterol group) is a group of male mice given standard feed for 2 weeks (the period of adaptation). Then 2 the next week given the feed of cholesterol. After 2 weeks given the Na-CMC 0.5% as much as 0.2 mL/day for 2 weeks.
3. Group III, i.e. the group of male mice given standard feed for 2 weeks. Then 2 the next week given the feed of cholesterol. After 2 weeks given the leaf extract 100 mg/kgBB doses cemba as much as 0.2 mL/day for 2 weeks.
4. Group IV, i.e. the group of male mice given standard feed for 2 weeks. Then 2 the next week given the feed of cholesterol. After 2 weeks given the leaf extract 250 mg/kgBB doses cemba as much as 0.2 mL/day for 2 weeks.
5. Group V, i.e. the group of male mice given standard feed for 2 weeks. Then 2 the next week given the feed of cholesterol. After 2 weeks given the leaf extract 500 mg/kgBB doses cemba as much as 0.2 mL/day for 2 weeks.

Tools and Materials

Tools

The tools used in this research is an oven, the total cholesterol level gauges (Necso), glass (Pyrex) 250 ml and 1000 ml flask, Erlenmeyer flask (Pyrex), analytical balance, balance Ohaus, a measuring cup (Pyrex), autoclave, autoclave, scissors, wire test, rang funnels, funnel, buhner, blender, spoit, oven, syringe, rotary vacum evaporator (Hanshin), vacum and animal maintenance test enclosure.

Materials

Cemba leaves, methanol 96%, ICR strain mice males aged 2 months weight 20-30 g, egg yolk, feed mice AD II, alcohol 70%, , wathmen 41 filter paper, strip cholesterol (Necso), cotton and tissue.

Sterilization Tools

For sterilization is used the oven. It is aimed so that the tools used are free of microorganisms.

Work Procedures

Making Leaf Powder Cemba (Acacia pennata)

Cemba leaves as many as 2000 g washed, dried until dry, once dry, blended into a powder. Cemba leaves used are old leaves that are dark green.

Sample Extraction

Cemba leaves powder as much as 210 gram soaked with 1500 ml 96% methanol for 24 hours at room temperature, submersion is repeated three times. The results of the marinade or maserat filtered using filter paper and then concentrate with rotary vacuum evaporator until retrieved a thick extract (Kristiani dkk, 2013; Suratiningsih dkk, 2013: Kusuma dkk, 2014).

Manufacture of Feed High Cholesterol

Feeding cholesterol used in this research was made from a mixture of egg yolk of chickens ras and feed standard ADII by comparison 1:9. Yolk Chicken breeds are boiled for 30 minutes or until cooked, then yellow eggs that had been cooked on a baking sheet until crushed into powder. The egg yolk and then in the oven at a temperature of 40 ° C – 45 ° C for 24 hours. Standard feed ADII weighed and blended then mixed with egg yolk and flour, stirring until well blended. Feeding cholesterol that has been so then wrapped with clear plastic so that it is not contaminated.

The Process of Granting Cemba Leaf Extract to Mice Males

The male mice are held and the clipped part of the nape with the fingers. The male mice are conditioned as comfortable as possible in order not to experience stress. Fill the syringe with the leaf extract as much 0.2 mL cemba then given orally to animal testing mice males.

The Process of Blood Sampling On Mice Male

The tail of the male mice are in alcohol over 70% with the use of cotton. The male mice are no tail and cut about 1 mm from the tip of the tail with a sterile scissors. Blood in the strip in the capacity of cholesterol by as much as 15 µ L. Cholesterol levels tested using gauges cholesterol

multi check (Necso). The tail of the male mice that had alcohol in order for blood is not flowing continuously and given antibiotics.

Measurement Period Blood Cholesterol Levels

Every 14 days conducted measurements of cholesterol levels. Measuring cholesterol levels done after a period of adaptation as the initial cholesterol levels and the granting of preferential treatment (after mice were given cemba leaf extract) as well as after the given feed cholesterol.

Data Analysis

The data obtained were analyzed using analysis of variants (FR F)/ANOVA on ranks confidence α: 0.10 with SPSS program. Then proceed with the further test Tuckey which also use SPSS statistical program.

RESULTS AND DISCUSSION

Table 1. The average male mice are cholesterol levels before and after treatment

No	Treatment	Average cholesterol level (mg/dL) mice male (*Mus musculus*)			Percentage decrease cholesterol level (mg/dL) mice
		Stage I	Stage II	Stage III	
1	Normal	99,4[a]	117,4[a]	110[a]	6,05 %
2	hipercholesterol	97,2[a]	158[b]	166,8[b]	-7,20 %
3	EDC(Ap) 100 mg/kg BB	107,2[a]	147[b]	121,8[a]	20,63 %
4	EDC(Ap) 250 mg/kg BB	108,4[a]	158[b]	119,2[a]	31,75 %
5	EDC(Ap) 500 mg/kg BB	104,8[a]	163,2[b]	103,6[a]	48,77 %

Description: the same Letter in a column indicates "different is not real". The letters that differ in one column shows "real different". A different letter between one column with another column showing "real different" EDC (Ap) (leaf extract Cemba (a. pennata)

The results of the analysis using ANOVA (Analysis of Variance) followed by α 0.05 TUCKEY test. Table 1 shows the average levels of total cholesterol (mg/dL) male mice at the phase I all treatment showed a different result is not real and is still in the normal range is 80-130 mg/dL. Average levels of total cholesterol (mg/dL) mice males the most high on treatment group EDC(Ap) 100 mg/kgBB of 108.2 mg/dL and the average levels of total cholesterol (mg/dL) are the lowest in the group of hiperkolesterol treatment of 97.2 mg/dL.

Average levels of total cholesterol (mg/dL) mice males on the second stage shows different results between group normal with the Group hiperkolesterol and the group provided cemba leaf extract. The levels of total cholesterol (mg/dL) to the most high in the group the EDC(Ap) 500 mg/kgBB mice males of 163.2 mg/dL and the average levels of total cholesterol (mg/dL) at least a normal group of 117.4 mg/dL.

Average levels of total cholesterol (mg/dL) mice males on the third stage shows different results between group normal with the group hiperkolesterol. However, it is no different with the given group cemba leaf extract. Average levels of total cholesterol (mg/dl) mice males in the group hiperkolesterol showed different results with normal group and the group provided cemba

leaf extract. The levels of total cholesterol (mg/dL) to the most high in the group hiperkolesterol of 166.8 mg/dL and average levels of total cholesterol (mg/dL) are the lowest in the group given cemba leaf extract dose 500 mg/KgBB (of 103.6 mg/dL. Data on the average levels of total cholesterol (mg/dL) mice at each treatment can be seen in table.

On the group hiperkolesterol is not happening total cholesterol levels decrease on mice male but on the contrary an increase in total cholesterol levels of 7.20% mg/dL. The normal visible presence of total cholesterol levels decrease in male mice of 6.05%. Similarly, with the group the EDC(Ap) a dose of 100 mg/kg, 250 mg/kg and 500 mg/kg mice males showed a decrease in total cholesterol levels in a row the following 20,63 31,75%,%, and% 48,77.

Hiperkolesterol induction by feeding cholesterol can increase the levels of total cholesterol mice males. This is because all mice induced by feeding cholesterol increase total cholesterol levels exceed the normal cholesterol levels 130 mg/dL. One of the causes of the rise in cholesterol levels in the blood are the consumption of foods containing cholesterol or saturated fat. Sources of cholesterol comes from animal products such as meat, spleen, brain, kidney, egg yolks and shrimp. Yolk chicken race contains cholesterol 9.09 mg/g (Kartika, 2012). Ramadan (2011) stated that the feeding standards and high cholesterol can increase total cholesterol levels of 85 to 115 mg/dL mg/dl and trigliserid levels of 87 mg/dl being 127 mg/dl.

Cholesterol diet will go into the path of exogenous and mempungaruhi serum cholesterol. Cholesterol levels in the blood are very dependent on the path of biosisntesis, an enzyme that very role i.e. HMG-CoA reductaseCholesterol diet will go into the path of exogenous and mempungaruhi serum cholesterol. Cholesterol levels in the blood are very dependent on the path of biosisntesis, an enzyme that very role i.e. HMG-CoA reductase In the network there is a continuous reesterifikasi and lipolysis. However, if speed is reesterifikasi unbalanced with a speed of lipolysis then free fatty acids will be released into the blood plasma (Ramadan, 2011).

Own hiperkolesterolemia mechanism starting from the intake of saturated fat and cholesterol that comes from the feed rich in cholesterol fat will be digested in the intestine resulting in free fatty acids, triglycerides, phospholipids and cholesterol. Furthermore, these compounds will be changed to kilomikron after being absorbed by the intestine. There is the rest of the solution of kilomikron in the form of free cholesterol along with the apoprotein form VLDL (Very Law Density Lipoporotein). Furthermore the enzyme lipoprotein lipase endothelial cells will transform into VLDL IDL (Intermediate Density Lipoproteins) which persist for 2-6 hours before turning into LDL (Law Density Lipoproteins) (Soucha, 2006).

The normal average levels of total cholesterol in phase III of 110 mg/dl. Different case with the group hiperkolesterol with an average total cholesterol level at phase III of 166.8 mg/dl, the cholesterol levels exceed normal cholesterol. While the average total cholesterol level at phase III group of EDC (Ap) a dose of 100 mg/kg, 250 mg/kg and 500 mg/kg murine males showed a decrease in total cholesterol levels, with an average total cholesterol levels in a row 121.8 mg/dL, 119.2 mg/dL, and 103mg/dL. The existence of a significant difference between the group of EDC(Ap) a dose of 100 mg/kg, 250 mg/kg, 500 mg/kg with the group hiperkolesterol, as well as the absence of any real difference with normal group show that cemba leaf extract can lower total cholesterol levels of murine hiperkolesterolemia.

This signifies that granting cemba leaf extract with various doses (100 mg/kgBB, 250 mg/kgBB and 500 mg/kgBB mice male can lower total cholesterol levels of male mice hiperkolesterolemia. The most effective dose to lower total cholesterol levels of male mice i.e., 500 mg/kg with a decrease of 48.77 59.6% or mg/dL. Cemba leaf extract capability in lowering cholesterol levels total male mice are allegedly due to secondary metabolite compounds the flavanoid, saponins and tannins contained in leaf extract cemba that serve as antioxidants. Ramli (2008) states that, plant phytochemicals compounds have Mimosaceae with the polyphenol mainly flavanoid and tannins which are often found at each plant. Similar plants of the genus Acacia namely Acacia catechu, extracts of the leaves have antioxidant abilities that do not vary markedly with the ability of synthetic antioxidant BHT.

Flavonoid antioxidant potential deterrent is the formation of free radicals. This compound is able to prevent adhesions and damage to blood cells, HDL. Based on previous research (Rofida, 2015), flavonoids can affect LDL cholesterol metabolic processes by increasing the ability of LDL to be bound to receptors LDL receptor bound to termetabolisme will be the cholesterol esters in

the network. HDL cholesterol ester-binding will be present on the network and then eskresi into the intestine. In addition, the flavonoids also have lower LDL oxidase activity. Flavonoids may reduce LDL concentration of lipids, reduces oxidative stress by inhibiting the macrophage cell and activate the oksigenase cellular antioxidant. Thus, it is a natural antioxidant flavonoids that are able to protect against the concentration of lipids and lipoproteins in the arteries. With a decrease in LDL oxidase then formation of foam cells will lower the risk of occurrence of inhibited so that atherosclerosis.

Compounds, the polyphenols and Saponins are also known to lower cholesterol levels. According to Umarudin et al. (2012) tannin is the polyphenols compounds that act as antioxidants. Polyphenols are reportedly able to decrease the total cholesterol levels and inhibit the formation of atherosclerosis through effects antioxidant against LDL oxidation and can increase the production of nitric oxide (NO). Nitric oxide is a vasodilator endogenous capability as antiaterosklerosis. Some of the traditional herbal tanin derivative known to be effective in the inhibition of HMG-Coa reductase that may become agents of hipolipidemia.

A number of studies have shown that saponins lower serum cholesterol levels in a variety of animals including humans (Avci, 2006). There are several mechanisms of the effects of saponins in the reduction of cholesterol. The mechanism is the formation of insoluble complexes with β-Hydroxysteroid so it can lower the cholesterol intestinal absorption and increasing excretion of sterols in the stool. Saponins can also increase the adsorption of bile acids into fibers by means of the formation of the complex, so the formation of large misel weights can prevent the reabsorption of bile acids and cause the loss of bile acids which are offset by an increase in the conversion of cholesterol into bile acids in the liver (Fauzana, 2015).

The results of this research show that the methanol extract of leaves of cemba quite effectively lowers total cholesterol levels in male mice are experiencing hiperkolesterolemia making it potentially as a medicinal herb to lower total cholesterol levels.

CONCLUSION

Based on the research that has been done, it can be concluded the grant cemba leaf extract was able to decrease the total cholesterol level male mice are hiperkolesterolemia.

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