

Analysis of APO-B Serum Level in Balb/C Mice Hypercholesterolemic Against Temulawak Extract (*Curcuma xanthoriza* Roxb)

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Abstract

APO-B Serum levels is the most predictive value for the incidence of atherosclerosis and coronary heart disease. *Curcuma xanthorrhiza* Roxb contains curcumin, which can be used as an antioxidant, anti-inflammatory and antihypercholesterol. The mechanism of curcumin contained in ginger to reduce cholesterol is due to its function as a cholagoga or bile stimulant. This study aims to determine the effect of temulawak extract on the levels of APO-B Serum in hypercholesterolemia mice. This research were a true experimental study with a post-test only control group design carried out in February 2018. The extraction As much as 25 mice were divided into 5 groups where are group consisted of 5 mice. Positive control group (K+) were treated with high cholesterol feed and water, negative control group (K-) were given standard feed and water, treatment group 1 (P1) were given high cholesterol food and 25mg/kg BW of curcuma extract for 14 days, treatment group 2 (P2) were treated with foods high in cholesterol and 50mg/kg BW of curcuma extract for 14 days and treatment group 3 (P3) treated with high cholesterol and ginger extract 75mg/kg BW for 14 days. Examination of APO-B levels were measured using the spectrophotometric method. Data were analyzed using One-Way Anova. The results showed that the average of APO-B level at (K+) was 209.7 ± 1.02 mg / dL, at (K-) 115.3 ± 1.04 mg / dL, at (P1) 180.4 ± 1.07 mg / dL, at (P2) 147.6 ± 1.12 mg / dL, at (P3) 119.1 ± 1.10 mg / dL. Based on the results of statistical test it was found that there was a significant decrease in APO-B levels with $p\text{-value}=0.001$ at $\alpha 0.05$ ($p<\alpha$).

Keywords

Temulawak extract, apo-B serum levels, hypercolesterolemic.

INTRODUCTION

Coronary Heart Disease is still one of the diseases feared by most people because the

mortality rate of coronary heart disease is still high, in both developed countries and developing countries. According to the data from the World Health Organization (WHO),

in 2012 around 17.5 million people, deaths in the world were caused by heart and blood vessel disease. It represented 31% of the causes of death worldwide. One of the laboratory markers that can be assessed the risk of cardiovascular disease is by checking cholesterol levels.

Laboratory tests that can be carried out to include blood total cholesterol levels, levels of low density lipoprotein (LDL), high density lipoprotein (HDL), and Apolipoprotein B (APO-B). Apolipoprotein B is an amphipatic protein and the only protein known to require lipids for its secretions(1). In humans there are two APO-B isoforms, namely APO B-100 containing 4536 amino acids and APO B-48 containing 2152 amino acids. Both types of APO-B have the function of binding to LDL receptors and also play a role in the cholesterol (2).

The current dyslipidemia drug is nicotinic acid, Fibric acid, HMG CoA Reductase Inhibitor (statin), and bile acid binder. The use of statins in maximum doses such as atorvastatin (80 mg/day) has been shown to reduce LDL to 58% in hypercholesterolemic patients. The use of niacin at a dose of 3 g/day up to 4.5 gr/day can reduce LDL up to 25%. However, the use of statins and niacin can distrub side effects, such as hepatotoxic, myopathic and teratogenic effects on growing cells. Peptic gastritis or ulcer is a common reason for not

using niacin. Hepatysis occurs in up to 3% in individuals treated with niacin (3). Development of new drugs is needed which is safer, cheaper and more effective in improving lipid profiles.

One of the natural ingredients that is widely used in society empirically is temulawak. *Curcuma xanthorrhiza* is a native plant that is growing in Indonesia and its resources are very abundant in Madura, especially in Bangkalan. Bangkalan is a district that is well known for its richness of plants that can function as an alternative treatment. The part of this plant that is often used is the rhizome. This plant can grow well and adapt in the open or under the shade of trees to a shade level of 40%. Several studies have shown that ginger can be used as a drug, antimicrobial, antibacterial and antioxidant (4).

The chemical content of the temulawak could be distinguished from the starch fraction, which is the largest fraction, in the form of yellowish-white powder; the curcuminoid fraction, these oil yellow to reddish-giving component in the ginger rhizome and essential oil fraction, which is a component consisting of monoterpenes and sesquiterpenes derived compounds. Curcumin is a fraction of curcuminoids, which have a broad-spectrum biological activity. Curcumin in temulawak can work as an antioxidant, anti-inflammatory, and antihypercholesterol. The mechanism of

curcumin in ginger to reduce cholesterol is due to its function as a cholagoga or bile stimulant. The activity of the ginger rhizome is characterized by increasing production and secretion of bile; by increasing excretion of bile it will reduce high cholesterol levels (4). Based on the background described above, the researcher intends to conduct a research on the effect of temulawak extract on APO-B levels in Hypercholesterolemic mice

MATERIALS AND METHODS

This type of research is true experimental, using quantitative analysis methods. The population of this study was male mice (*Mus musculus*) of balb/c strain with a body weight of 20–30 grams, 2–3 months of age obtained from the Faculty of Veterinary Medicine, Airlangga University, Surabaya. The research was conducted at January 2018. Samples from this study were mice, male strain balb/c taken from the population randomly as many as 42 tails then grouped into 3 groups.

Material

Curcuma rhizome, quail egg yolk, 96% ethanol, 0.5% Na CMC (*Natrium Carboxymethyle Cellulose*), standard feed, drinking water, APO-B reagent kits from Human Companies.

Tool

Blades, blenders, analytic balance, waterbath shakers, Buchner, rotary evaporator, glassware, 30x20 cm mouse

cages, sonde, 1 mL syringe, centrifuge, bottles, surgical instruments, and photometers, sample cups, micropipets, Tubes.

Procedure

Method of making ethanol extract of curcuma rhizome

Extraction was carried out by maceration method, by means of 75 grams of dried ginger powder put into maserator. Then, as much as 96% ethanol solvent 400 mL were added and shaken for 1 hour to achieve a homogeneous condition in the waterbath shaker at a speed of 120 rpm for 1 hour. The solution was macerated for 24 hours at room temperature.

After 24 hours, the solution was filtered or separated using a Buchner filter. The residue from the filtering process was winded and remaserated again for 24 hours. The maceration was repeated up to 3 times. Then, the mixed solution filtering results 1 to 3 with the rotary vacuum evaporator at 50 until a concentrated extract with a concentration of 100% was obtained. Then, extract made a dose of 25 mg/kg BB, 50 mg/kg BB and 75 mg/kg BB using Na CMC 0.5% solvent.

Animal Preparation

A total of 25 mice were weighed and recorded their weight, they are divided into 5 treatment groups. Positive control group (K+) were treated with high cholesterol and drinking water, negative control group (K-) were treated with standard feed and drinking

water, treatment group 1 (P1) were given high cholesterol food and 25mg/kg BW of curcuma extract for 14 days, treatment group 2 (P2) were given high cholesterol food and 50 mg/kg BB of curcuma extract for 14 days and treatment group 3 (P3) were given high cholesterol and ginger extract 75 mg/kg BB for 14 days. Each group was placed in a cage measuring 30x20 cm cage. Each cage contained 5 mice and was adapted to be given a standard CP 511 feed and drinking water in ad libitum for 7 days as an acclimatization period. After 7 days of acclimatization, the mice were weighed again and the object of the study were mice weighing approximately 25 grams, mice that fit the criteria in groups K(+), P1, P2, and P3 were given quail egg yolks using a sonde to the stomach 1 time/day for 7 days. On the 8th day, one mouse was taken from each groups to be checked its total cholesterol levels.

Mice were fasted for 10 hours before being examined for total cholesterol levels. the blood of mice was taken using a 1mL syringe through the heart, then a total cholesterol level was examined using a POCT cholesterol device. Mice were included in the category where are hypercholesterolemia (cholesterol level above 82 mg/dL) from the examination of total cholesterol levels, then given the following treatment:

a. Positive control (K+) group was given high cholesterol and drinking water in ad

libitum.

- b. The negative control group (K-) was only given standard feed and drinking water in ad libitum
- c. The first treatment group (P1) was given temulawak extract using a sonde to the stomach at a dose of 25 mg/kg BB for 14 days, giving standard feed and drinking water in ad libitum.
- d. The second treatment group (P2) was given temulawak extract using a sonde to the stomach at a dose of 50 mg/kg BB for 14 days, standard feed and drinking water in ad libitum.
- e. The third treatment group (P3) was given temulawak extract using a sonde to the stomach at a dose of 75 mg/kg BB for 14 days, standard feed and drinking water in ad libitum.

The treatment for giving curcumin extract is given for 14 days. Mice were fasted on the 15th day for 10 hours but the mice were still given a drink. Mice were taken in 5 treatment groups or a total of 25 mice in the three treatment groups to be examined for APO-B levels. Mice anesthetized using chlorophome. After losing consciousness, mice are dissected using a scalpel and take the blood. blood was taken using 1 mL syringe through the heart as much as 1 mL from each mouse. Mice that have been taken for blood were not used anymore because they have been sacrificed (mice destroyed).

Examination of APO-B Levels

The blood that has been taken from mice then centrifuged to take the serum, then the serum was inserted into *Prestige 24 i*.

Analysis Techniques

Analysis of data used in this study was to compare differences in APO-B Serum levels in the three treatment groups using SPSS. If the data is normally distributed then it will use the Parametric statistical test One Way ANOVA test, but if the data is not normally distributed, it using the Kruskal-Wallis Non-Parametric statistical test.

RESULTS

The—results of the average, total cholesterol level in mice after giving a sonde of quail egg yolk for 7 days was 128 ± 1.02

Lmg/dl, where the total cholesterol level was normal in mice ranging from 26–82 mg/dL. Based on the average, it can be seen that mice have been hypercholesteloremic. After being given treatment in accordance with the treatment group for 14 days, the average examination results of APO-B levels were 209.7 ± 1.02 mg/dL in the Positive control group (K+); 115.3 ± 1.04 mg/dL in the negative control group (K-); 180.4 ± 1.07 mg/dL in treatment group 1 (P1); 147.6 ± 1.12 mg/dL in treatment group 2 (P2) and 119.1 ± 1.10 mg/dL in treatment group 3 (P3). The lowest average was found in the treatment group 3 given temulawak extract at a dose of 75 mg/kg BB.

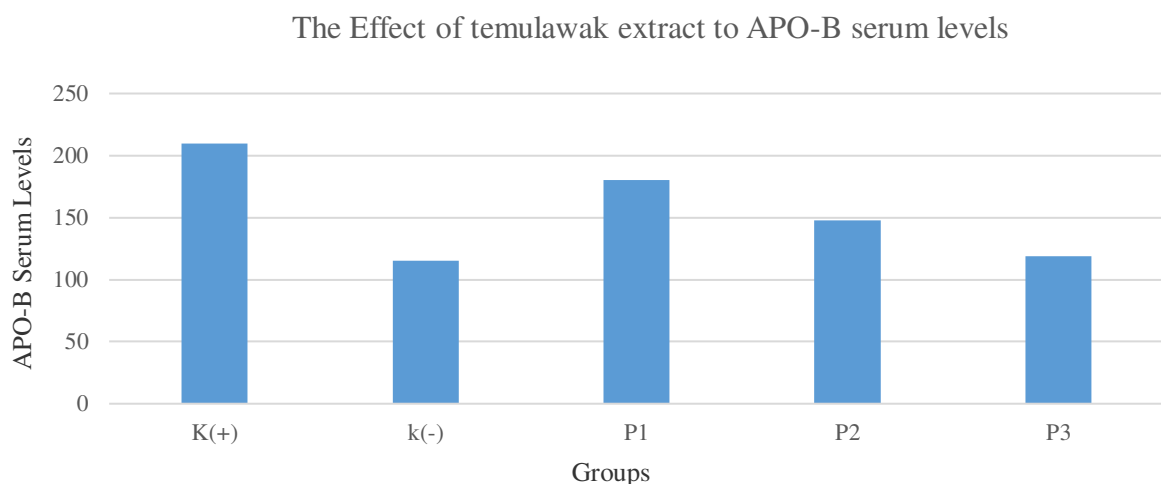


Fig 1. The effect of temulawak extract to APO-B serum levels

DISCUSSION

Effect of High Fat Feeding on Total Cholesterol Levels

In the positive control group (K+) was 128 ± 1.02 mg/dL. The results of this study are

in line with the research conducted by Santiago (5) which proved that each mouse given a high-fat feed diet had dyslipidemia or increased levels of lipid profiles.

High fat feed is given for 2 weeks, this

means that the mice remain in a state of dyslipidemia. The results of this study showed a higher lipid profile in the positive control group (K⁺) compared to the treatment group. This shows a high-fat diet has an positive effect on lipid profiles.

Fats contained in food will be broken down into cholesterol, triglycerides, phospholipids and free fatty acids when digested in the intestine. These four fat elements will be absorbed from the intestine and enter into the blood. the increase in absorption of fat will cause the condition of hypercholesterolemia. The higher the level of fat in the food, the higher the level of lipid profiles total cholesterol and Apo B in the body (6).

Effect of Temulawak Extract on APO-B Levels

The results of Apo B levels in the positive control group (K⁺) tended to be higher than in the treatment group (P1, P2 and P3), there were significant differences in Apo B levels in each groups, where the higher dose was given, the value Apo B levels will decrease. The Temulawak extract dose given is 25 mg/kg BB, 50 mg/kg BB and 75 mg/kg BB respectively. This is in line with the statement that the content of flavonoids in ginger in the form of hesperidin can inhibit the secretion of Apo B-100 by the liver, thus affecting the level of Apo B in the blood. Hesperidin is the main flavonoid in temulwak has been shown to be hypocholesterolemic. Hesperidin works

through a mechanism of inhibition of the enzyme activity HMGCoA reductase and ACAT and inhibits the secretion of apolipoprotein B by the liver (7).

Curcumin also contains an active ingredient, namely curcumin, curcumin plays a role in stimulating cholesterol-7 α -hydroxylase hepatic enzyme activity. This enzyme contained in liver cells catalyzes the change in cholesterol into bile salts. The increase activity of this enzyme shows an increase in cholesterol catabolism. The reaction of 7 α -hydroxylase in cholesterol biosynthesis is the first step that must be present in bile acid biosynthesis. As a result of stimulation of this enzyme by curcumin, the change in hepatic cholesterol into bile salts increases, resulting in reduced cholesterol levels in the liver. In order to meet the cholesterol needs, the number of LDL receptors in the liver will be increased, so that there is an increase in LDL uptake. The decreased cholesterol levels, LDL and increased levels of HDL plasma cholesterol (7).

In a previous study conducted by (4), the study concluded that ginger extract at a dose of 100 mg/kg BB and 400 mg/kg BB could reduce total cholesterol levels by >20% in rats given high fat feed, where total cholesterol consisted of LDL, HDL and triglycerides.

CONCLUSIONS

Based on the results of research that has been conducted on the effect temulawak extract on APO-B levels in mice, it can be concluded that there was a significant difference in the decrease in APO-B levels : in the positive control group (K+) tended to be higher than in the treatment group (P1, P2 and P3), there were significant differences in Apo B levels in each groups, where the higher dose was given, the value Apo B levels will decrease. Where is the lowest decrease occurred in the treated group 3 with an extract dose of 75 mg/kg.

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CONFLICT OF INTEREST

There are no conflicts of interest.

REFERENCES

1. Setiawan HG, Kaligis AHM, Assa YA. Description of serum apolipoprotein B (APO-B) levels in lacto-ovo vegetarians. *Ebiomedik*. 2017;5(1):2–5.
2. Cesar TB, Aptekmann NP, Araujo MP, Vinagre CC, Maranhão RC. Orange juice decreases low-density lipoprotein cholesterol in hypercholesterolemic subjects and improves lipid transfer to high-density lipoprotein in normal and hypercholesterolemic subjects. *Nutr Res* [Internet]. Elsevier Inc.; 2010;30(10):689–94. Available from: <http://dx.doi.org/10.1016/j.nutres.2010.09.006>.
3. Sargowo D. Lipid , Dyslipidemia and CV Impact : The urgency to Treat. 2012;0–18.
4. Anggraini S. Pengaruh Ekstrak Etanol Rimpang Temulawak (*Curcuma xanthorrhiza* Roxb.) Terhadap Kadar Kolesterol Total Pada Tikus Putih Hiperlipidemia. Universitas Muhammadiyah Surakarta. 2012;9.
5. Santiago VAJ, Jayachitra J, Shenbagam M. & Nalini N. d-limonene attenuates bloodpressure and improves the lipid and antioxidant status in high fat diet and L-name treated rats.*J. Pham*. 11,pp.752-758.2010.
6. Hermawati, Manalu W, Suprayogi A, Astuti DA. Dietary fiber supplements in the diet to improve the blood lipid parameters of hypercholesterolemia mice. *Makara Seri Kesehatan*. 2013;17(1):1-9.
7. Murfian AA, Aznam N. The effect of giving instant ginger on the total cholesterol level of male white rats Wistar hypercholesterolemia strain. *Jurnal Kimia Dasar*. 2017;6(4):159-466.