Various tomato extract dose effect on the lipid profile of hypercholesterolemia rats

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Abstract. The influence of tomato lycopene on hypercholesterolemia has been studied many times. Post Test Randomized Control Group Design is used as the research design. A total of 24 white rats were made hypercholesterolemia before treatment, then they were divided into 4 groups randomly, i.e. K1 (control group), K2, K3, and K4 (Treatment groups 1, 2 and 3). Treatment groups were administered with tomato extract. K2 (given 6 mg / head / day), K3 (12 mg / head / day), K4 (24 mg / head / day). The result of ANOVA test showed significant difference (p <0.05) among control group (K) and treatment group (K2, K3 and K4). The results of LSD testing showed a significant difference in the group (p <0.05), on triglyceride levels, total cholesterol and LDL-cholesterol. So it can be concluded that tomato extract can decline the status of lipid profile in terms of triglycerides, total cholesterol and LDL-cholesterol.

1. Introduction

The more rampant patterns and modern lifestyle such as excess junk food consumption, have threatened cardiovascular health of Indonesia society. Junk food contains a lot of sodium, saturated fat and cholesterol that have an impact on the imbalance conditions of the blood flow. The high level of salt consumption and high lipids without the balancing consumption of high-fiber can cause high blood pressure and hypercholesterolemia. High blood pressure and hypercholesterolemia increase the risk of hypercholesterolemic atherosclerosis and cause coronary heart disease.

Preventive efforts are needed to prevent the occurrence of atherosclerosis. Consumption of natural ingredients that are relatively safe and easy to obtain, for example tomato plant, is recommended. Tomato plants are vegetable plants that can potentially be a medicinal herb, easy to find and the fruit of this plant has been proven to help decreasing cholesterol levels [1]. This potential use of the tomatoes is caused by the fact that tomatoes contain Lycopene. Lycopene is a red carotenoids that is dissolved in fat. Lycopene can be synthesized by plants and some microorganisms, but cannot be synthesized by animals and humans. Lycopene is composed of carbon chains 40 open (acyclic) with 13 double bonds (11 conjugated and 2 non conjugated). Has the form of the trans-isomer in vivo and sis.
In natural conditions, lycopene of tomatoes can undergo isomerization of cis-transe induced by light, heat energy and chemical reactions, and also, the pH of the stomach. The cis form is easier to absorb and has better bioavailability. In addition, tomatoes are rich in vitamin C and some antioxidants such as vitamin E and lycopene. Tomato is a rich source of lycopene, β-carotene, folate, potassium, vitamin C, flavonoids and vitamin E which serve - individually or in a group - to protect lipoproteins and vascular cells from oxidation [2].

Based on the previous research, there is an increase in the antioxidant status of the male Wistar strain rats fed with tomato extract in the dose of 1.08 mg/head/day [3]. It has also been proven that the tomato extract dose of 20-40 mg/kg was able to prevent the formation of plaque on artery coronary [4]. Some of the previous researches have suggested the need to do more research to find out the influence of the consuming tomato extract against cholesterol profile of hypercholesterolemia rats. The objective is to explain the role of the tomato extract as anti-hypercholesterolemia that is able to decrease the potential of atherosclerosis in hypercholesterolemia mice. Furthermore, the objective is also to prove the role of lycopene in tomato to cholesterol profile by observing the decrease in three indicators, namely, triglycerides, total cholesterol and LDL-cholesterol.

2. Methods

This research is a laboratory experimental research that utilizes The Post Test only Control Group Design [5]. This research’s objects are 20 male white rats from Wistar strain by the age 2 months which weights are around 200-210 grams. First, those rats’ blood cholesterol level was raised by giving them a standard feed of pellet and 2 ml pork oil. Those are given directly by gastric gavage method for 30 days [6]. Every 100 mg of pork oil contains 28.4 g saturated fatty acids and 95 g cholesterol [7]. After 1 month, the cholesterol level for each group of mice was measured to ensure that they have suffered hypercholesterolemia. Rats are said to be hypercholesterolemia if the total cholesterol levels > 54 mg/dl [8].

Then, those twenty white rats are divided randomly into four groups. The control group (K) is fed with standard feed and drinking water ad libitum for 21 days without any extracts of tomato. Treatment group 1 (P1) is fed with standard feed + 8 mg/rats/day of tomato extract. Group treatment 2 (P2) is fed with standard feed + 16 mg/rats/day of tomato extract. Group treatment 3 (P3) is fed with standard feed + 32 mg/rats/day of tomato extract. The tomato extract was given per oral with gavage method, for as long as 21 days. At day 22, rats are not fed for 12 hours, and then their blood samples are taken through the retroorbital plexus. The blood samples then are measured for the levels of triglycerides, total cholesterol, and LDL-cholesterol. Measurement of triglycerides, total cholesterol, and LDL-cholesterol is conducted using a kit’s reagent of Cholesterol and triglycerides, LDL-cholesterol from the Dyasis according to the manufacturer instructions.

Tomato extract was made by preparing 2 kg of tomatoes, cut them into thin pieces then steamed for 30 minutes with low heat. Steamed tomatoes then put in the oven and dried at a 40-50 °C and then when they were dry, they would be pounded until became fine powder and sifted with the number 100 sieve. Then we took 50 g of that fine powder and placed it into tubes containing filter paper. Next, we prepared a round bottom flask filled with 300 ml petroleum ether as solvent and then commenced the distillation process using the soxhlation tool. The resulting dregs was then aerated until dry and free from petroleum ether odor. The dregs then was dissolved with 350 ml solvent methanol with the soxhlation method at 65 °c temperature. Soxhlation would be stopped when the filter solution is is clear. The resulting extract was into a beaker glass and then spread into petri dishes and put in the oven with temperature of 40-50 °C until it became pasta. The pasta was then dissolved with olive oil and then given to mice.

The data is tested using one way analysis of variance for the levels of triglycerides, total cholesterol, and LDL-cholesterol contained in the blood serum of white rats (Rattus norvegicus). After that an LSD (Least Significance Difference) test is used.
3. Results and discussion
Measurement of the mice’s cholesterol after consumption of high cholesterol diet (pellets plus pork oil for 30 days) indicates the hypercholesterolemia condition. The average total cholesterol is presented in Table 1.

| Group | Average cholesterol levels (mg/dl) |
|-------|-----------------------------------|
| K     | 78.6                              |
| P1    | 76.8                              |
| P2    | 76.61                             |
| P3    | 73.03                             |

Table 1 shows that the average total cholesterol levels in blood serum of white rats from the four groups is in hypercholesterolemia condition, because all of the cholesterol level is more than 54 mg/dl.

After consumption of steamed tomato extract, the lipid profile is significantly improved. We can see that the treatment groups (P1, P2 and P3) have lower lipid profile compared to the control group. The results are presented in Table 2.

| Group | Triglycerides (mg/dl) | Total cholesterol (mg/dl) | LDL (mg/dl) |
|-------|-----------------------|---------------------------|-------------|
| K     | 48.48                 | 64.20a                    | 43.30a      |
| P1    | 37.8b                 | 54.65b                    | 34.90b      |
| P2    | 31.64c                | 49.68c                    | 29.10c      |
| P3    | 22.44d                | 41.52d                    | 22.80d      |

Table 2 shows that giving steamed tomatoes as much as 32 mg/head/day has the most effect on the levels of triglycerides, total cholesterol and LDL-cholesterol. Compared to the control group (K), all treatment groups (P1, P2 and P3) have better lipid profile.

Consumption of a high cholesterol diet can lead to the occurrence of hypercholesterolemia which is characterized by increased levels of total cholesterol. High intake of saturated fat and cholesterol cause increased cholesterol concentration. Any intake of saturated fat as much as 1% of the total daily energy may increase 2.7 mg/dl of cholesterol levels [9].

Saturated fatty acids increases the cholesterol levels through several mechanisms, i.e. suppressing the LDL receptor activity, inhibits the synthesis of cholesterol at the heart, increase the transfer of free cholesterol and decrease the affinity of LDL against receptor [10]. Cellular cholesterol inhibits the activity of HMG CoA reductase enzyme by gene transcription through suppression effect of sterol regulating element (SRE 1) [11]. LDL receptor gene also contains a nucleotide sequence SRE 1, which led to a decrease in LDL receptor synthesis because of suppressing cholesterol presence [12]. Increasing levels of LDL results in suppression of HDL and inability to dispose excess cholesterol from the blood, thus further decrease of HDL.

Lycopene’s role in cholesterol metabolism is in inhibition of the enzyme HMG CoA reductase’s work, an enzyme that is involved in cholesterol synthesis in the liver; that could bring about the hypocholesterolemia. This is also in accordance with research suggesting that lycopene on tomatoes can inhibit cholesterol synthesis by inhibiting the activity of HMG-CoA reductase enzyme, increasing LDL cholesterol degradation by macrophages, and increasing LDL receptors control on LDL level so it will be reduced [13]. Lycopene’s mechanism in inhibiting HMG-CoA reductase enzyme is similar to the mechanism of statin [14]. Lycopene competed with HMG CoA reductase’s substrate on the enzyme’s active side. Lycopene changes enzyme conformation when binding to the enzyme’s active side, this causes change in the structure of HMG-CoA reductase and substrates cannot stick [15].
Decreased activity of the enzyme 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA reductase) cause reduction in cholesterol synthesis in the liver. Low intracellular cholesterol levels will trigger the liver increase the regulation of LDL receptor activity in supplying cholesterol. An increase in LDL receptor regulation is a result of increase in sterol regulatory element binding protein 2 (SREBP-2) process, a transcription activator that plays an important role in the maintenance of homeostasis of cholesterol [16]. LDL entering the heart undergoes hydrolysis by Lysosomes, cholesterol can experience re-esterification and change into ester via acyl transferase (ACAT) or converted into bile acids [17, 18]. Consumption of lycopene result in decreasing ACAT activity and inhibit the accumulation of ester cholesterol will result in decreased accumulation of cholesterol and inhibits ester [19].

4. Conclusion

Based on the results of this study, it is proven that consumption of tomato extract can decrease the levels of triglycerides, total cholesterol, and LDL cholesterol.

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