Lipid profile comparison of tomato extract and atorvastatin supplementation in atherosclerosis rats

R S Iswari1*, M Dafip2, A I Kartika3, I R Apriliana1, I N Chamidah1, M Abduh1

1 Department of Biology. Faculty of Mathematics and Natural Sciences. Universitas Negeri Semarang, Indonesia
2 Department of Biomedical Science, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia
3 Medical Laboratory Technology, Faculty of Nursing and Health Science, Universitas Muhammadiyah Semarang, Indonesia

*Corresponding author: iswari_retno@yahoo.co.id

Abstract. The use of synthetic drugs, such as atorvastatin and simvastatin, often cause health problems caused by side effects. Tomatoes are a food source that contains antioxidants such as vitamins A, C and E in high amounts and increase because of the processing. The nutritional content of tomatoes in preventing hypercholesterolemia arising from oxidative stress as a result of accumulation of LDL-cholesterol in the endothelium. Therefore, this study considers steamed tomatoes in overcoming the condition of hypercholesterolemia. The study design uses the Post Test Randomized Control Group Design. Before being given as many as 24 white rats that were made hypercholesterolemia, then divided into 4 groups, namely K1 (negative control group), K2 (atherosclerosis rat control group), K3 (atherosclerosis group called atorvastatin), K4 (atherosclerosis group supplemented with 16 mg / tail / day). ANOVA -LSD test results showed the differences shown (p <0.05) between the control group (K) and the consultation group (K3 and K4).

1. Introduction
The body needs cholesterol to compose and regenerate cells. The cholesterol biosynthesis is occurred in liver, then distribute to the whole body by low density lipoprotein (LDL)[1]. High LDL-cholesterol is trigger hypercholesterolemia that causing cholesterol accumulation in the endothelial blood vessels. The accumulation of cholesterol have consequences in production high radical so that trigger inflammation and atherosclerosis [2].

Atorvastatin is a synthesized statin drug that acts as an inhibitor of HMG-CoA reductase [3]. The atorvastatin reduces plasma cholesterol levels by cut off mevalonate pathway in endogenous cholesterol synthesis. The drug also plays role to reduce triglyceride levels, but with improper mechanism. The impact of long-term use of atorvastatin is muscle problems such as cramps, impaired function and shortening of muscle mass, and initiate kidney damage. Furthermore, atorvastatin has potentially in and suppress macrophage cells thereby increase the production of free radical [4].

Exogenous antioxidant is needed to eliminate oxidative stress that mainly produces from cholesterol oxidation. Tomato have been proven to the ability for antiaterogenik. with nutritional content includes 46.92 µg lycopene, 586.441 µg β-carotene, 22.98 mg/ 100 g vitamin C and 0.41 mg/ 100 g α-tocopherol [5].
Therefore, this research conducted as a case study to observe the ability of tomatoes to reduce lipid profile in hypercholesterolemia rats compared with atorvastatin.

2. Methods
This study was started after get permission from Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine, Universitas Gadjah Mada-Dr. Sardjito General Hospital, number KE/0877/07/2019. Tomato extraction was carried out in a Biochemistry Laboratory, Department of Biology, Universitas Negeri Semarang (UNNES), while the treatment was carried out at the Experimental Animal Laboratory, Center of Nutrition, UGM. This study was carried out for 3 months. A total of 24 white rats (Rattus norvegicus) Sprague Dawley strain, male, healthy, aged 2-3 months, weight 150-200 grams in the same place. Twenty-four rats were divided by random in to 4 groups, equally. The K1 or control group, K2 (control group with atherosclerosis), K3 (atherosclerosis group which was given atorvastatin, and K4 (atherosclerosis group supplemented by 16 mg of tomato extract/individual/day. The treatment was carried out during the study period.

Each group of rats was adapted by cholesterol induction feed was carried out through feed or diet, where the rats was given standard feed AIN-93M for control normal and diet hypercholesterolemia for group 2,3 and 4. Furthermore total cholesterol levels were checked for make sure hypercholesterolemia (cholesterol level > 54 mg/dl).

2.1. Tomato extract preparation
As much as 2 kg tomato were thinly cut and then streamed for 30 minutes over low heat. Streamed tomato in the oven with a 40-50 °C to dry. Dried streamed tomatoes are crushed and sieved with a number 100 sieve until obtained soft powder. Then, as much as 50 grams of soft powder was distilled using soxhlet with 300 ml petroleum solvent. Pulps from distilled was aerated until dry and not smell of petroleum ether. The pulp was extracted again using soxhletation method of 65°C and 350 ml of methanol as a solvent. Soxhletation is stopped until a clear filter solution is obtained. The extract obtained was mixed in a beaker glass until homogeneous and roasted at temperature of 40 – 50 °C to form a paste. The paste was dissolved into olive oil to be condensed on rats.

2.2. Measurement of LDL, HDL and Total Cholesterol Levels
The LDL levels were measured on the 15th day after streamed tomato extract was given. Before blood drawing, rats were fasted for 14 hours. Measurement of LDL-cholesterol, HDL and total cholesterol in the blood serum of white rats using the CHOD-PAP (Cholesterol Oxidase the Aminophenazone method)

2.3. Foam cell identification
The preparation was made using HE staining (paraffin block) and embedding method [6]. Abdominal aortic vessels are prepared as preparations. The preparations were read out at the UGM Anatomical Pathology Laboratory, each preparation was counted by the number of foam cells by looking at 20 fields of 400x magnification. Preparations (PA) of each treatment were analyzed descriptively. The results of the calculation of the total number of foam cells were tested using data normality Saphiro-Wilk (p> 0.05). Normally distributed data continued with parametric tests.

The results of measurements of LDL, HDL, total cholesterol and foam cell counts were tested using one-way ANOVA, if significant effect was followed by LSD analysis, with a 95% confidence level. After that, the correlation between the number of foam cells and the dose of steamed tomatoes given was tested using Pearson. The criteria for correlation levels are classified into five groups: 0.00-0.20 (no correlation), 0.21-0.40 (weak correlation), 0.41-0.60 (moderate correlation), 0.61-0.80 (strong correlation) and 0.81-1 (perfect correlation). Data analysis was performed with the Stastical Package for Social Science (SPSS) 23 software for windows.
3. Results and Discussion

3.1. Lipid Profile

Initial measurements showed that cholesterol levels before homogeneous and normal treatment (Table 1). Whereas the administration of tomato extract significantly reduced cholesterol and HDL. Giving tomato extract at a dose of 16 mg of tomato extract/individual day, influences in reducing levels of free cholesterol and LDL-cholesterol. Compared to K2 which had a total cholesterol content of 62.52 mg/dl, and LDL-cholesterol of 41.10 mg/dl, the treatment group (K1, K3 and K4) had lower cholesterol. On the other hand, the administration of steamed tomato extract was shown to significantly increase the amount of HDL reaching 29.20 mg/dl above K1, K2 and K3 (Table 2).

Table 1. Cholesterol level before treatment in all groups

| Groups | Cholesterol (mg/dl) |
|--------|---------------------|
| K1     | 50.50               |
| K2     | 51.20               |
| K3     | 54.13               |
| K4     | 55.20               |

Table 2. Blood cholesterol profile levels of hypercholesterolemia rats after administration of tomato and atorvastatin extracts.

| Groups | Cholesterol Total (mg/dl) | LDL (mg/dl) | HDL (mg/dl) |
|--------|---------------------------|-------------|-------------|
| K1 (n=6) | 55.65b                  | 37.10b      | 24.73b      |
| K2 (n=6) | 62.52a                  | 41.10a      | 22.63a      |
| K3 (n=6) | 52.20c                  | 32.90c      | 24.28c      |
| K4 (n=6) | 47.68d                  | 27.80d      | 29.20d      |

Note: The alphabetical sign behind the mean indicates differences in significance between groups.

3.2. Foam cell

Observation of abdominal aortic anatomical pathology preparations showed that the treatment group had a smaller number of foam cells. The appearance of foam cells tends to be abundant in mice with hypercholesterolemia abnormalities (Figure 1). The K1 group had the most number of foam cells compared to the K2, K3 and K4 groups (Table 3).

The one-way ANOVA test results obtained a significant value of p <0.032, this shows that the administration of tomato extract has a significant effect and is related to the number of foam cells in endothelial blood vessels. The average number of foam cells in K1 is 1.16 cells; K2 2.61 cells; K3: 1.25 and K4: 1.

Table 3. Foam cell calculation in all groups

| Groups | Foam cell (cell/ µm²) |
|--------|------------------------|
| K1 (n=6) | 1.16                  |
| K2 (n=6) | 2.61                  |
| K3 (n=6) | 1.25                  |
| K4 (n=6) | 1.15                  |
Figure 1. Histopathology of abdominal aorta with 400x magnification. The red circle marks the foam cell 
a) K1 control (normal mouse); b) K2 (rat atherosclerosis); c) K3 (rat atherosclerosis with atorvastatin 
treatment); and d) K4 (16 mg / head / day).

High cholesterol intake increases cholesterol distributor such as LDL and VLDL. When the cholesterol 
distributed and trapped in tunica of blood vessel. It triggers more cholesterol accumulation in the endothelial 
wall of blood vessels to form ox-LDL and accumulate into plaque [7]. The LDL-cholesterol oxidation 
process releases free radicals that damage cells, thereby stimulating the production of cytokines such as 
Intereukin-1 (IL-1), IL-6 and tumor necrosis factor. alpha (TNF–α) [8,9]. Furthermore, cytokines stimulate 
monocyte cells to migrate into the intima and differentiate into macrophage cells. Macrophage cells will 
phagocytose ox-LDL through the receptor scavenger (SR) and turn into foam cells that are sticky to the 
endothelium. Foam cells bond together to form fatty streaks, then form plaques that cause atherosclerosis. 
This condition is reflected in the K2 group (Figure 1). The results of observation of abdominal aortic 
preparations Group K2 showed the greatest number of foam cells compared to groups K1, K3 and K4 
(Table 3). The appearance of foam cells tends to be abundant in mice with hypercholesterolemia 
abnormalities

Giving steamed tomato extract has high levels of antioxidants in inhibiting free radicals. Some 
carotenoids such as β-carotene and lycopene as well as vitamins C and E have higher amounts and activity 
after steaming. Steamed tomatoes have lysopene 46.92 mg / 100 g and antioxidant activity is 38.35% higher 
than raw tomatoes or processed by other methods [5], [10]. The action of antioxidants in tomato extract 
inhibits the activity of LDL which is oxidized by free radicals (ROS). Vitamin E and carotene have radical 
scavenger activity which binds electrons to free radicals, so it is not reactive and does not damage cells. 
Vitamin E protects cell membranes from being destroyed by free radicals such as H2O2, -O, etc. Meanwhile, Vitamin C with carotenoids will stimulate immune cells to release cytokines and reduce inflammation.
Specifically lycopene in tomatoes has 3 main roles in, namely: 1) Adduct formation, the addition of lycopene conjugated double bonds to form lycopene-peroxyl radicals (ROO-lycopene *); 2) electron transfer, donating cations (licopene + *) and anions (licopene- *); 3) Adding hydrogen atoms (H +) to reduce free radicals (Lycopene * + ROOH) [11, 12]. Reducing the oxidation process is shown by decreasing levels of indigenous antioxidants such as superoxide dismutase. Furthermore, lycopene dampens the differentiation of macrophages into foam cells by reducing lipid synthesis and decreasing the activity of the scavenger-A (SR-A) receptor regulator. Decreased sensitivity of SR-A causes a decrease in internalization of ox-LDL into foam cells [13].

Lycopene plays an important role in reducing cholesterol synthesis, by inhibiting 3-hydroxy-3-methyl-glutaryl-coenzym A (HMG-CoA) reductase as similar as atorvastatin mechanism [14]. Inhibition of the enzyme HMG-CoA reductase will cause HMG-CoA unable to form mevalonate as a raw material for making cholesterol in the liver. Cholesterol levels in the liver that are low cause an increase in regulation of LDL receptor activity to supply cholesterol from other parts of the body into the liver.

The decreased cholesterol synthesis rate increase LDL receptors in hepatocytes, increasing the uptake of LDL, intermediate density lipoprotein (IDL) and very low-density lipoprotein (VLDL) from circulation [15]. It is also a result of the activity of sterol regulatory element binding protein 2 (IDL) SREBP-2 that raises as lycopene effect [16]. In addition, to create cholesterol homeostasis, the liver produces HDL to carry cholesterol from other parts of the body into the liver, especially from endothelial blood vessels. This can reduce cholesterol and foam cells that have formed. LDL that enters the liver is hydrolyzed by lysosomes, cholesterol can be re-esterified into cholesterol esters through acyl CoA: acyl transferase (ACAT) or converted to bile acids [1].

The inhibitory effect of the HMG-CoA enzyme results in a reduction in the amount of cholesterol supply to the extrahepatic tissue, such as the ovum / testis and adrenal as a hormone-making material. This will trigger extrahepatic tissue to draw LDL from the circulation. Excess cholesterol in extrahepatic tissue is returned to the liver via HDL to be used as bile and secreted to feces.

Lycopene also has a mechanism in reducing intracellular cholesterol levels, namely as a ligand for Liver X Receptor (LXR) - Retinoid X Receptor (RXR). LXR acts as a cholesterol sensor, participates in lipid regulation, and cholesterol metabolism [17]. LXR forms a heterodimer bond with RXR to become active. LXR - RXR heterodimer bonds regulate genes involved in synthesis, absorption, excretion for cholesterol homeostasis, and lipoprotein metabolism such as the ATP-Binding Cassette Transporter A1 (ABCA1) and Caveolin-1 (Cav-1) genes. The lowest total cholesterol level of K4 compared to K2 and K3. HDL levels in group IV were highest when compared to groups III and II. This shows that the greater the dose of tomato extract given, the better the effect on decreasing total cholesterol and increasing. Tomato diet helps eliminate triglycerides and total cholesterol in the body through feces, this can be shown from the decrease in the amount of triglycerides and total cholesterol in the group given steamed tomato intake.

4. Conclusion
The results of this study concluded that couscous tomato extract at a dose of 16 mg /ind / day was best for the role of antioxidants in the body of hypercholesterolemia rats compared to atorvastatin cholesterol-lowering drugs.

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