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The Effect of Lovastatin Produced from Aspergillus flavus UICC 360 on Decreasing Cholesterol Level Blood (Rattus novergicus L.) Sprague Dawley strain

Dadang Kusmana*, Yanti Raflianti*, Wibowo Mangunwardoyo**

Department of Biology, Faculty of Mathematic and Sciences, University of Indonesia, Depok, 16424*
Al-Izhar Senior High School. Jl. Fatmawati Kav. 49. South Jakarta. 12450 Indonesia**

***Abstract*-** Lovastatin is a competitive potential inhibitors of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase activity, which plays a role in cholesterol biosynthesis. In this study, rats were given extract lovastatin produced from the fermentation of *Aspergillus flavus* UICC 360 with various of doses (0.1, 0.2 and 0.4 mg/day) on the rats and examined the effect on serum total cholesterol, triglyceride, HDL and LDL in the blood of rats for 14 days treatment. The lovastatin dose was given at 0,1 mg/day not only decreased the total cholesterol, triglyseride and LDL, but also increased HDL eventhough was not sigificant, and the dose lovastatin given at 0.2 mg/day caused lowering the triglyceride (61.76%) at level ($p < 0.01$) compared with negative control (21.28%), also the dose lovastatin given of 0.4 mg/day was able to decrease LDL levels (90%) at level ($p<0,01$) compared to the negative control (59.74%).

***Index Terms*-** HDL-cholesterol, LDL- cholesterol, lovastatin, *Rattus novergicus*, total cholesterol, trygliceride

I. INTRODUCTION

Cholesterol is a lipid constituent compound beside triglycerides, phospholipids, cholesteryl-esters, and free fatty acids. Cholesterol is synthesized in the liver, intestine, adrenal cortex, and reproductive tissue such as ovary, testis, and placenta. Cholesterol plays several important roles, as the main component of cell membranes, as precursors of bile acid synthesis and as precursors in the synthesis of various steroid hormones including aldosterone, estrogen, and testosterone (1). A total cholesterol level in blood plasma of healthy people is normally at range 150 – 200 mg/dL. When total cholesterol level in blood plasma is above the normal threshold, it called hypercholesterolemia. According to Krieger (1998) (2) hypercholesterolemia is a risk factor for cardiovascular disease such as atherosclerosis, in the other hand, it can also cause of cardiovascular disease. Atherosclerosis can cause blood flow to the heart or the brain is blocked, allowing the occurrence of stroke of heart attack (3).

Lovastatin is the result of secondary metabolites produced from the fermentation process of various types of molds. Research on various types of mold in class Basidiomycetes and Deutomycetes showed that lovastatin can be produced such as *Monascus ruber*, *Penicillium brevicompactum*, dan *Aspergillus terreus* (4) (5) (6) studied the fermentation of rice (red yeast rice) from *Monascus purpureus* type of mold can produce lovastatin. It tested against *Spague Dawley rat strain*, thus decrease cholesterol level up to 20.78% with a provision for 24 days by 20 g/day/rat. Zulfiana (2003) (7) reported that from the extract of *Monascus purpureus* Went strain TISTR 3090 which is extracted from Tempe flour can reduce total cholesterol up to 39.55% after being administered for 3 days at a dose of 10 mg/kg body weight. The dry biomass and Tempe extract contain ergosterol from *Rhizopus spp*, can reduce cholesterol and triglyceride levels during 3 days treatment in white rat *Rattus norvegicus* strain wistar (8).

Previous study reported amount of 40 species of *Aspergillus spp*. from University of Indonesia Culture Collection (UICC) were screened and assayed. The result revealed that the *Aspergillus flavus* UICC 370 produced the highest lovastatin, this was the first report that those fungus was able to produce the lovastatin . This study aims to determine the effect of lavostatin produced by *Aspergillus flavus* UICC 360 in lowering total cholesterol triglycerides, HDL, and LDL level in the blood of rats.

II. MATERIALS AND METHODS

A. Animal experiment

The animals used were 25 white rats (*Rattus novergicus* strain *Sprague Dawley*), male, aged 2 months with a weight of ± 200 g.

B. Chemicals

Reagent Cholesterol Complete Test enzymatic-chlorometry method CHOD-POD Code HB006, 2 x 125 ml (Cypress Diagnostics 2008), Reagent Kit Triglyceride complete GPO enzymatic Cat. No. 063-0249A 3 x 50 mL (ST. Reagentsia), Reagent Kit cholesterol HDL Cholesterol complete (phosphotungstic precipitation Code HB007, 3 x 10 mL) (Crypess Diagnostics 2008) and technical ether.

C. Animal Test Preparation

A total of 25 experimental rats adapted in a cage for 14 days to adjust to the new environments. Rats were fed pellets and water ad *libitum* (without limit), the pellets were given in the container and placed in a cage, whereas the drinks was given in a bottle inserted through the wire cover. Cage was given pedestal of wood shavings to absorb dirt, and it changed every day. Cages were cleaned daily by soaking in disinfectant for 5 minutes then washed up with soap and rinsed with water until clean. The cages which were containing rats were placed on shelves in a room measuring 6 x 8 meters. The room was illuminated by 36 Watt fluorescent lamp for 12 hours every day with a room temperature

about 28 – 30^{o}C and relative humidity (Rh) between 70 – 80 % (10).

D. Treatment

The experimental design used in this study was Completely Randomized Design (CRD). All the rats were increased its cholesterol levels, by providing 2.5 ml of coconut oil in 14 days (7). Test materials used were three different doses of lovastatin extract namely, 0.1, 0.2, and 0.4 mg/day. The chemicals emulsified with CMC 1% (b/v) as much as 2.5 ml administered orally using *Gavage* needle, once in a day during 14 days (11). Analysis of total cholesterol, triglycerides, HDL, and LDL cholesterol blood of the rats performed in three stages: before, and after administration of coconut oil to raise cholesterol level, as well as post-treated during 14 days.

E. Preparation of test preparation materials

Fermentation was done in 30 units of 250 ml Erlenmeyer containing 100 mL medium at pH 6.5 Czapex Dox Broth (CDB) consist of 0.5 g KCl; 3 g $NaNO_3$; 1g K_2HPO_4; 0,5 g $MgSO_4$. $7H_2O$;0.01 g $FeSO_4.7H_2O$ and 30 g sucrosa in 1 L aquadest . A total of 2 mL of inoculums (3.5-4x10^7 cfu mL) *Aspergillus flavus* UICC 360 was added to the Erlenmeyer contains Czapek Dox Broth (CDB), then it incubated at a temperature of 26-28^{o}C, in a shaker incubator at 100 rpm for 48 hours. After fermentation process, the culture was filtered using filter paper (Whatman no. 1). The filtrate was added Sulfuric Acid 2 N to reach pH 3, whereas the mycelium was homogenized. The filtrate and mycelium were regrouped, and then extracted in three times using a separator funnel. The extraction was using ethyl-acetate in the same amount. After it is dried with evaporator, it would be obtained the lovastatin β-open-hydroxy-acid.

F. Determination of the dose of test preparation materials

According to recommendations of *National Cholesterol Education Program* (NCEP), dose of the use of lovastatin for humans is 10-80 mg daily. Based on these recommendations and for use in rats, after being converted multiplied by 0.018 (12) then the treatment was given with a half dose (0.1 mg/day), normal dose (0.2 mg / day) and two times the normal dose (0.4 mg/day).

G. Preparation of solution test substance

Stock solution made by dissolving 4 mg of test substance with 25 ml of 1% CMC. For the 0.4 mg dose, it is taken directly from a stock solution of 12.5 ml (for 5 rats), for a dose of 0.2 mg, it is taken as much as 6.25 ml of stock solution and then added with 6.25 ml CMC1% while the dose of 0,1 mg made by taking as many as 3.125 ml of stock solution are then added with 9, 375 ml of 1% CMC. Test material created new each time will provide the treatment.

H. Analysis of total cholesterol, triglycerides, HDL and LDL cholesterol in the blood of rats

The analysis used the serum which is taken from the veins at the tip of the rats (8). Analysis of total cholesterol used an *enzymatic-colorimetric test* cholesterol (Chod-POD), the analysis of triglycerides used an enzymatic Tryglyceride method (GPO-enzymatic), HDL cholesterol analysis used a HDL-cholesterol Phosphotungstic precipitation and analysis of LDL cholesterol used the Friedwald formula Cypress Diagnostic .

I. Data analysis

Data from the examination of total cholesterol, triglycerides, HDL, and LDL from rats' blood were analyzed using various analysis (Anova test). An advanced test using T test also conducted to detect the differences between treatment groups to determine the Smallest Real Difference (SRD) (13).

III. RESULTS AND DISCUSSION

A. Data analysis of total cholesterol, triglycerides, HDL, and LDLbefore and after administration of coconut oil.

The total cholesterol level increased (24.8%) to 77.44 mg/dL from the initial 62.04 mg/dL. Triglycerides level also increased (65.3%) of 48 ml/dL from the initial 79.36 ml/ dL, HDL cholesterol level decreased (2.96%) from 33.8 ml/dL from the initial to 32.8 ml/ dL, whereas LDL cholesterol level increased (30.26%) to 28.16 mg/ dL from the initial 19.64 mg/ dL (Figure 1). The statistical results of T test (paired samples test) analysis showed a correlation before and after the issuance of coconut oil. The elevation of cholesterol, triglycerides, LDL, level and the demotion of HDL levels after the issuance of different coconut oil different significantly ($p<0.01$) than before administration of coconut oil. Coconut oil which was administered to the rats containing 82.2 g saturated fatty acids, whereas saturated fatty acid content of 9.9 g of oleic acid and linoleic acid at 3.2 g per 100 ml (14) was a cause of increase of total cholesterol, triglycerides, and LDL level in the blood plasma of rats that were tested.

The increase was due to coconut oil and saturated fatty acid receptor can suppress the formation of *Low Density Lipoprotein* (LDL), thus the LDL cholesterol that was circulating in the blood increased (15) . Triglyceride was the major component of chylomicrons, and it Is also related with another lipoprotein metabolism such as *High Density Lipoprotein* (HDL). According to Fungwe et al (1993) (16) elevation of triglycerides in the liver and blood are the result of the reduction of fatty acid oxidation and additional triglycerides synthesized in order to facilitate cholesteryl-ester transportation in LDL. HDL cholesterol was decreased due to the absorption of fatty acids in the intestine, and it resulted triasilgliserol synthesis, subsequently it joined by chylomicrons from the HDL, thereby decreasing levels of HDL.

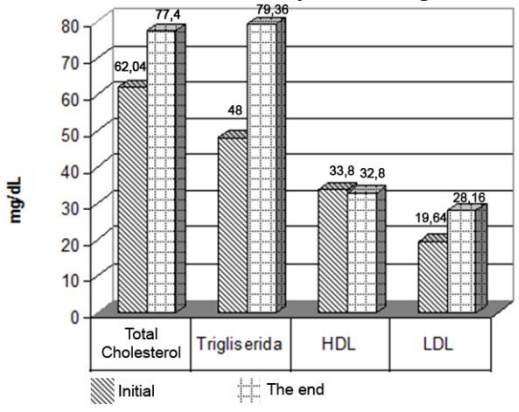

Fig. 1.Comparison of the average total cholesterol, triglycerides, HDL cholesterol and LDL cholesterol in the blood of rats before and after the issuance of coconut oil.

B. Analysis of total cholesterol in the blood of tested animals following administration of lovatatin fermentation extracts of Aspergillus flavus UICC 360.

Administration of Lovastation fermentation extract from *Aspergillus flavus* UICC 360 as test substance, decrease the total cholesterol in the blood of rats. The statistical analysis using Anova test resulted that total cholesterol level was not shown the difference significantly ($p > 0.05$). The average reduction of total cholesterol levels in the rats' blood in group A (dose 0.1 mg/day) is 37.19%, group B (dose 0.2 mg/ day) is 40,68% group C (dose 0.4 mg/day) is 41,78% and it closest to the total cholesterol of group E (as positive control/ group control) 42.95%. Group D (as negative control) also showed reduction of 32.99% (Figure 2). The reduction of the negative control happened because body has an auto regulation mechanism or regulation in order to maintain cholesterol levels remained normal. Cholesterol is mostly synthesized in the liver (80%), when the amount of cholesterol which is entering the liver through excessive food and will suppress intestinal cholesterol synthesis (17).

Lovastatin is an essential compound to overcome the hypercholesterolemia disease because of its activity could inhibit the hydcoxymetilglutaril-coenzyme A (HMG-CoA) reductase, which serves as a catalyst in the biosynthesis of cholesterol. HMG-CoA reductase is the main enzyme that will convert HMG-CoA to mevalonat, at the time when lovastatin in β-hydroxy-acid is at higher concentrations than HMG-CoA, thus the HMG-CoA reductase would prefer to bind with mevalonat so that the formation of mevalonat would not present, and as the consequence of that the formation of cholesterol would be inhibited.

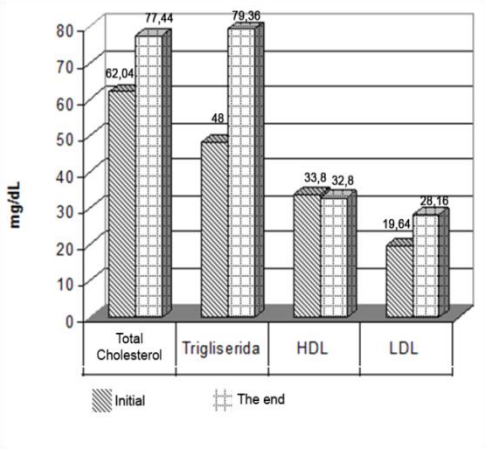

Fig. 2 Comparison of the average total cholesterol, triglycerides, HDL cholesterol and LDL cholesterol in the blood of rats, before and after the issuance of coconut oil.

Figure 3 showed comparison of the average total cholesterol before and after treatment A (0.1 mg /day), B (0.2 mg/dayC (0.4 mg / day), D (negative control) and E (the positive control). The decreasing of the total cholesterol after administration of the extract lovastatin produced by *Aspergillus flavus* UICC 360.. Tanzawa et al. (1982) (11) reported that administration of statin in rats in amount of 10 mg/kg body weight which is given orally will reduce the total cholesterol level 22.4%, compared to the control that the cholesterol levels increased with WR-1339.

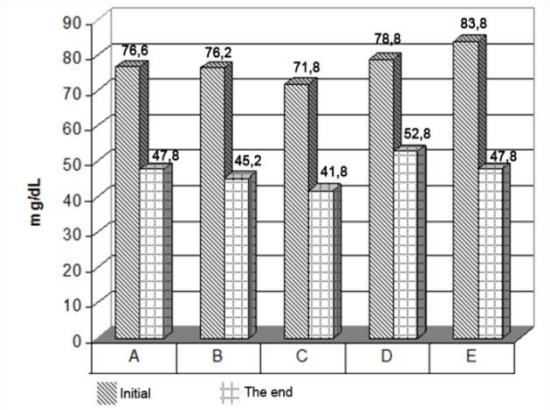

Figure 3. Comparison of the average total cholesterol before and after treatment A (0.1 mg /day), B (0.2 mg/dayC (0.4 mg / day), D (negative control) and E (the positive control)

C. Analysis of triglycerides levels in the blood of tested animals following administration of lovastatin fermentation extracts of Aspergillus flavus UICC 360.

Triglyceride levels decreased in all treatment groups. The decrease of triglyceride levels in the treated group A (dose 0.1 mg/day) is 61.76% higher than triglyceride levels in the treated group E (positive control), namely 54.40 %. Treatments group A (dose 0.1 mg/day) and B (dose of 0.2 mg/day) also showed reduced levels of triglycerides while still under the positive control group.

The result of statistical analysis of LSD test showed that the decrease of triglycerides levels was the most different ($p<0.01$) was between group D (negative control) with group A (dose 0.1 mg/day) and group C (0.4 mg dose/day) but when seen from the mean difference in the level of significance ($\alpha = 0.05$) were the most significantly different between group D (negative control) and group A (dose 0.1 mg/ day). The results of statistical analysis with LSD test can be concluded that administration of lovastatin fermentation extracts of *Aspergillus flavus* UICC 370 at a dose of A (0.1 mg/day) can lower triglyceride levels by as much as 61.76% (Figure 4).

Triglyceride is a form of fatty acids stored in adipose tissue. Triglyceride is used by the body as an energy source and the components of the cell. Triglyceride comes from fats in the diet and the results of carbohydrate and protein synthesis in the liver. Although required by the body, which is too high triglyceride levels in the blood may be a risk factor for heart disease. In general, normal levels of triglycerides is below 150 mg/dL(18). β-oxidation or esterification process can limit the amount of triglycerides.

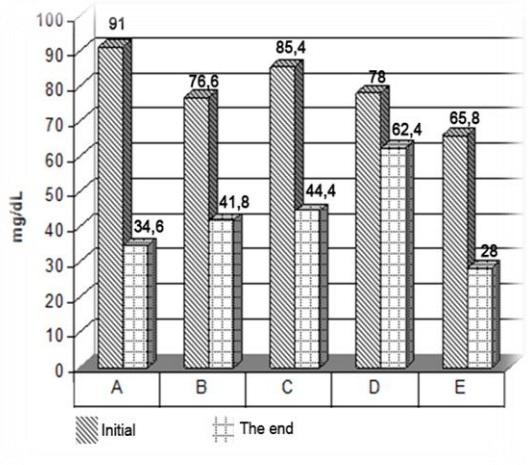

Fig. 4 Comparison of mean triglycerides before and after treatment A (0.1 mg/day), B (0.2 mg /day), C (0.4mg/day), D (negative control) and E (positive control)

Treatment with lovastatin can lower triglycerides, free cholesterol, and VLDL. In rats treated with lovastatin total cholesterol (6.4 mg/dL) and free cholesterol (8.6 mg/ dl) was slightly lower than rats given placebo (each 5.1 mg/dL and 6.2 mg/dL) lovastatin treatment group). While triglycerides for the group treated with lovastatin (32.2 mg/dL) and placebo (25.7 mg/dL) (19).

D. Analysis of High Density Lipoprotein (HDL) levels in blood of tested animals following administration of lovastatin fermentation extracts of Aspergillus flavus UICC 360.

The results of the analysis of HDL cholesterol levels after administration of extract of lovastatin in the treatment group A (dose 0.1 mg/day) was the average levels of HDL cholesterol increased by 0.4 mg/dL (1.1%), treatment group B (dose 0 , 2 mg/day) at 1 mg/dL (3%) and treatment C (dose of 0.4 mg/day) at 3 mg/dL (9.2%). The group C was the most widely increase HDL cholesterol of the three tested (dose of 0.4 mg / day), it has higher amount than average increase in HDL cholesterol levels by treatment group E (positive control) increased by 1.6 mg/dL (4.7%). The results of the analysis showed administration of lovastatin extract shown to increase levels of HDL cholesterol, but based on the results of statistical analysis by ANOVA showed that differences in the treatment of elevated levels of HDL cholesterol with lovastatin fermentation extract of *Aspergillus flavus* UICC 360 does not show significant differences (p> 0, 05). It may be associated with a less homogeneous population of rats.

HDL cholesterol is synthesized in the liver and small intestine. HDL cholesterol which synthesized in the liver consists of apoprotein and free cholesterol (20) HDL cholesterol can prevent atherosclerotic disease because it serves as a carrier of excess cholesterol from cells to the liver. The excess will be converted into bile acids and excreted. At the human body, the level of HDL cholesterol is inversely proportional to the atherosclerotic disease (1) (Figure 5).

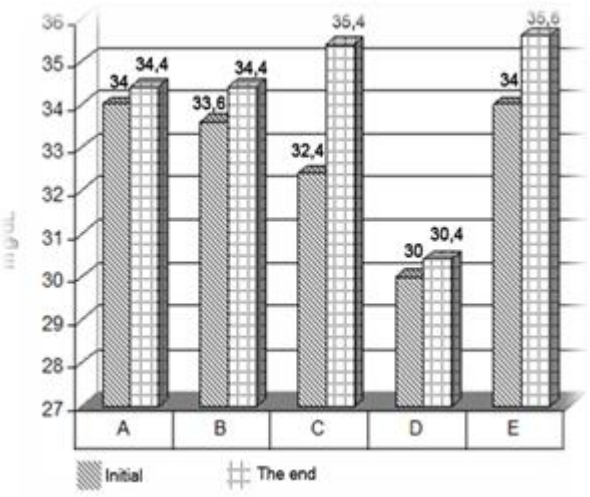

Fig. 5 Comparison of the average HDL cholesterol before and a after treatment A (0.1 mg /day), B (0.2 mg / day), C (0.4 mg day, D (negative control) and E (the positive control)

E. Analysis of low density lipoprotein cholesterol (LDL) level in blood of tested animals following administration of lovastatin fermentation extracts of Aspergillus flavus UICC 360.

The results of the analysis of LDL cholesterol levels after administration of extract of lovastatin in the treatment group A (dose 0.1 mg/day) was the smallest decline in average LDL cholesterol level as much 63.20%. Whereas, the greatest average decline of LDL levels happened at group C (dose of 0.4 mg/dL). (Figure 6) It was also greater than the average decrease in LDL cholesterol in the positive control group of 90%. Based on the results of statistical analysis, the treatment differences in reduction of LDL cholesterol levels with lovastatin fermentation extract of *Aspergillus flavus* UICC 360 showed significant differences (p> 0.05).

Ganong (1995) (21) reported that the presence of thyroid hormone can lower blood cholesterol levels by increasing the formation of low density lipoprotein (LDL) receptor. Administration of lovastatin as a controlling enzyme in cholesterol biosynthesis can stimulate expression of LDL receptors resulting indecreased levels of LDL cholesterol in the blood plasma (2) Expression of LDL receptor plays an important part in setting and maintaining cholesterol levels within the cell (15).

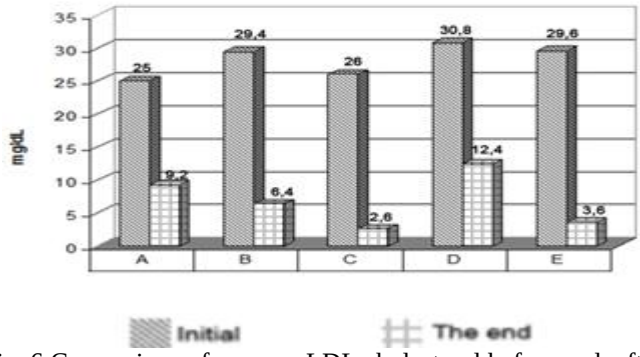

Fig. 6 Comparison of average LDL cholesterol before and after treatment A (0.1 mg/ day), B (0.2 mg/ day), C (0.4 mg/day, D (negative control) and E (positive control)

IV. CONCLUSION

The results of this study showed that lovastatin extracts of *Aspergillus flavus* UICC 360 that was administered to white rats (*Rattus novergicus* strain *Sprague Dawley* rats) at doses of 0.1% mg / day was not only decreased the total cholesterol, triglyseride and LDL, but also increased HDL eventhough was not sigificant, and the dose lovastatin given at 0.2 mg/day caused lowering the triglyceride (61.76%) at level ($p < 0.01$) compared with negative control (21.28%), also the dose lovastatin given of 0.4 mg/day was able to decrease LDL levels (90%) at level ($p<0,01$) compared to the negative control (59.74%).

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AUTHORS

First Author – Dr Dadang Kusmana MSi. Department of Biology, Faculty of Mathematic and Sciences, University of Indonesia, Depok, 16424. kusmana_dadang@yahoo.com

Second Author –Yanti Rafliyanti MSi, Al-Izhar Senior High School. Jl. Fatmawati Kav. 49. South Jakarta. 12450 Indonesia. yantirafliyanti@yahoo.com

Third Author – Dr. Wibowo Mangunwardoyo MSc**.** Department of Biology, Faculty of Mathematic and Sciences, University of Indonesia, Depok. 16424 w_mangunwardoyo@yahoo.com or. wibowo.mangun@ui.ac.id

Correspondence Authors – email: kusmana_dadang@yahoo.com and wibowo.mangun@ui.ac.id Telp : 62-21-7270163