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Research Article

Anredera cordifolia Leaves Extract as Antihyperlipidemia and Endothelial Fat Content Reducer in Male Wistar Rat

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ABSTRACT

The objective of this research was to evaluate antihyperlipidemia and endothelial fat content reducer of ethanol leaves extract of *Anredera cordifolia* **(**binahong**)** in male Wistar rat. The rats were given by high fat feed and pure cholesterol (on week 0, 3rd, 7th and 11th). The doses of binahong extract which was used in this study were 50 mg/kg body weight (bw), 100 mg/kg bw, 200 mg/kg bw. The result showed that blood cholesterol in statin group was decreased 75.85%, binahong leaves extract of 50, 100, 200 mg/kg bw decreased 55.29%, 63.45%, 67.70%, respectively, and 46.52% decreased in negative control group. Triglycerides was decreased 61.64% in statin group 41.08%, 47.59% and 50.66% in binahong leaves extract of 50, 100, 200 mg/kg bw respectively and 20.17% in negative control group, while LDL level decreased 97.14% in statin group, 81.31%, 89.01%, 95.33% in 50, 100, 200 mg/kg bw binahong extract, respectively and 69.69% in negative control group. On the other hand, it showed that no significantly increase in HDL level in all of extracts groups which were 2.93% 3.56% 3.66%, respectively in binahong leaves extract of 50, 100, 200 mg/kg bw, while HDL level in the statin group was increased 9.86%, and 1.63% in the negative control group. Binahong leaves extract with doses of 50 mg/kg bw, 100 mg/kg bw and 200 mg/kg bw, significantly reduced total cholesterol level, triglycerides, LDL level compared to negative control group ($p < 0.05$) and had no influence in HDL level. Binahong leaves extract with doses of 50,100 and 200 mg /kg bw could reduce fat deposits in the endothelial cell of blood vessels.

Keywords: *Anredera cordifolia*, anthyperlipidemia, endothelial fat reducer

INTRODUCTION

Cardiovascular diseases are diseases with high prevalence of causing death. The death that caused by heart disease can be prevented by reducing the risk factors, such as hypertension, hypercholesterolemia, diet, lack of exercise, smoking, and obesity. One of heart diseases is coronary heart that can be developed to cardiac ischemia. Ischemia can cause angina pectoris and myocardial infarction which then can lead to heart attacks and death[1]. One of plant that has many benefits in treating the disease is binahong (*Anredera cordifolia* (Ten.) Steenis)[2]. Organs of this plants that can be used were leaves, stems, roots and flowers, but leaves the most often used for health or as herbal medicine. Binahong has the latin name *Anredera cordifolia* (Tenore) Steen. This plant is actually derived from China under the name Dheng san chi[3]. Binahong is a creeper and is perennial (old age), the length can reach 5 m. Soft stems, cylindrical. Single leaf heart shape, 5-10 cm long, 3-7 cm wide, can be eaten, flower-shaped compound bunches, long-stemmed, appearing axillary, shaped root rhizomes, fleshy soft[3]. Atherosclerosis is a disease marked by thickening and hardening of arteries. The beginning of the formation of atherosclerosis is the thickening of fat in the endothelial cells. The complication related to atherosclerosis can cause death in various ways, such as arteriosclerosis which occurs in coronary blood vessels that are responsible for the occurrence of coronary heart disease. The same thing could happen in the arteries which influence blood supply to the brain, it can cause stroke[4]. The objective of this research was to evaluate antihyperlipidemia and endothelial fat content reducer of ethanol leaves extract of *Anredera cordifolia* **(**binahong**)** in male Wistar rat.

MATERIAL AND METHODS

Materials

Amyl alcohol, ammonium, aquadest, hydrochloric acid, sulfuric acid, sodium CMC, leaves of binahong (*Anredera cordifolia*), ethanol, ethyl acetate, gelatin, chloroform, pure cholesterol, vanillin liquid in sulfuric acid, sodium oxide, propylthiouracil, statin, bismuth nitrate potassium iodide, mercuric chloride, anhydride acetic, reagent kit for cholesterol, triglyceride, HDL and LDL.

Plant preparation

Leaves of *Anredera cordifolia* was collected from Lembang - Bandung, Indonesia, were thoroughly washed with tap water, wet sortation, cut, dried and grinded into powder. The plant was identified in Herbarium Bandungense, School of Life Science and Technology, Bandung Institute of Technology, Indonesia. Crude drug

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Table 1: Phytochemical screening of crude drug, extract and fractions of binahong leaves

	Crude drug	Ethanol extract	n-Hexane fraction	Ethyl acetate fraction	Water fraction
Saponin	+	+	+	+	+
Quinone	-	-	-	-	-
Flavonoid	+	+	+	+	+
Tannin	-	-	-	-	-
Alkaloid	+	+	+	+	+
Steroid/Triterpenoid	+	+	+	+	+

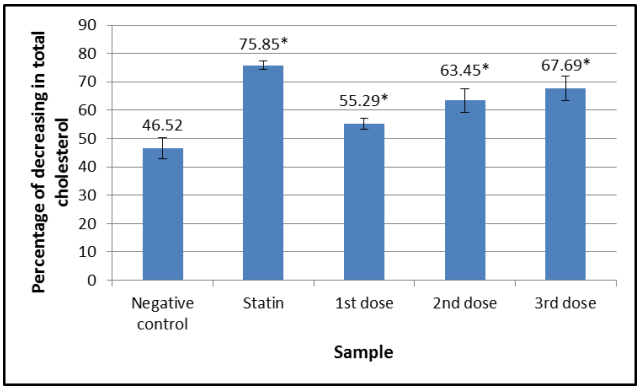

Figure 1. Percentage of decreasing in total cholesterol, * = significantly different to negative control ($p<0.05$)

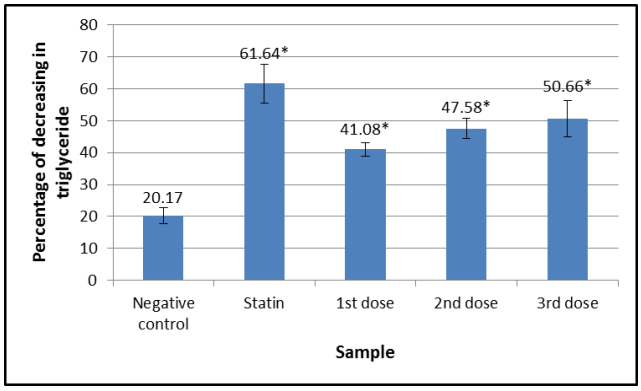

Figure 2. Percentage of decreasing in triglyceride, * = significantly different to negative control ($p<0.05$)

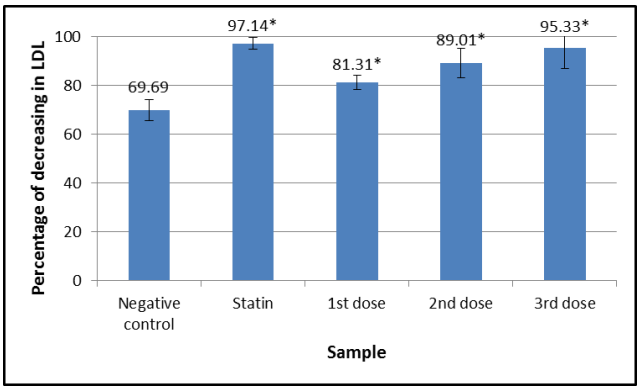

Figure 3. Percentage of decreasing in LDL, * = significantly different to negative control ($p<0.05$)

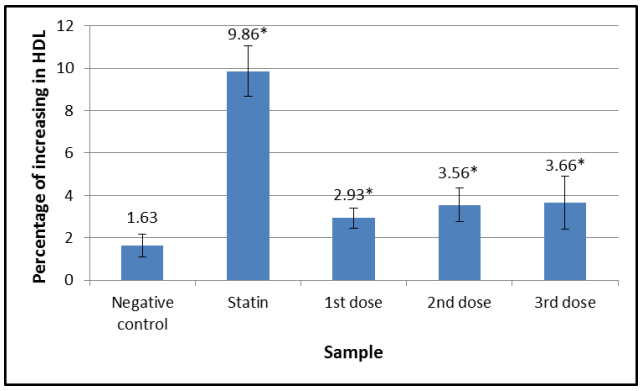

Figure 4. Percentage of increasing in HDL, * = significantly different to negative control ($p<0.05$)

was extracted by reflux using ethanol 70 %, and then fractionated was conducted by liquid-liquid extraction using n-hexane and ethyl acetate, so there were 3 fractions: n-hexane, ethyl acetate and water fractions.

Experimental Animals

Experiments were performed with the approval by the Animal Research Ethics Committee of ITB (No. 02/KEPHP-ITB/11-2014). Male white Wistar rats, weighted 200-250 g and aged approximately 3 months.

Phytochemical screening

Phytochemical screening of crude drug, ethanolic extract, n-hexane, ethyl acetate and water fraction of binahong (*Anredera cordifolia*) leaves were performed to determine the presence of saponin, quinone, flavonoid, tannin, alkaloid, steroid/triterpenoid group[5].

Animal Models for Endothelial Fat Content

Animals was induced by high fat feed which contained 1% cholesterol, 5% duck egg yolk, fat goat 10%, 1% coconut oil and 83 % standard feed, for 12 weeks, which on week 0, 3rd, 7th and 11th, the rats were given pure cholesterol orally. After that, they were being prepared for 21 days administration of test extract. Then the animals were divided into 5 groups, which were statin group (simvastatin 3.6 mg/kg bw), ethanol leaves extracts of binahong with doses of 50 mg/kg bw (the first dose), 100 mg/kg bw (the second dose), 200 mg/kg bw (the third dose) and negative control group (vehicle).

RESULTS

This research was started by phytochemical screening of crude drug, extract and fractions of binahong leaves. The result of phytochemical screening could be seen in Table 1. The percentage of decreasing in total cholesterol could

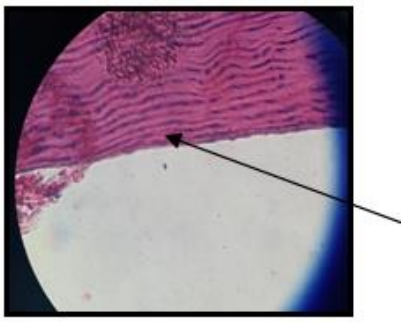

Figure 6. Endothelial cells in normal rat

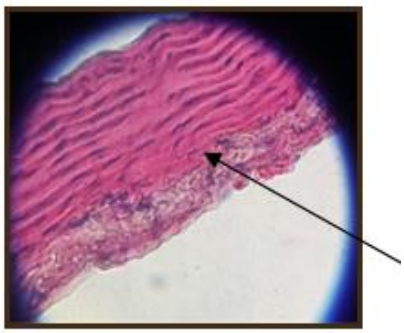

Figure 7. Fatty layer of endothelial cells after 12 weeks induction

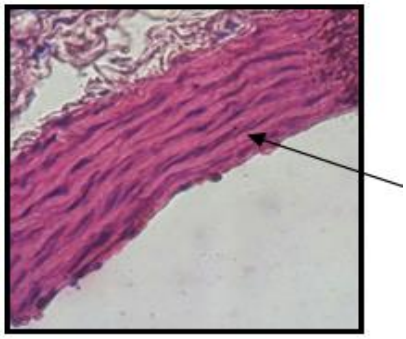

Figure 8. Fatty layer of endothelial cells after treating by statin for 3 weeks

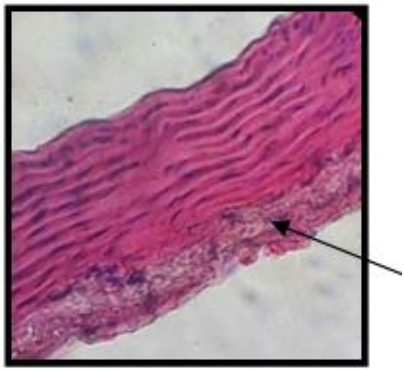

Figure 9. Fatty layer of endothelial cells after treating by the first dose for 3 weeks

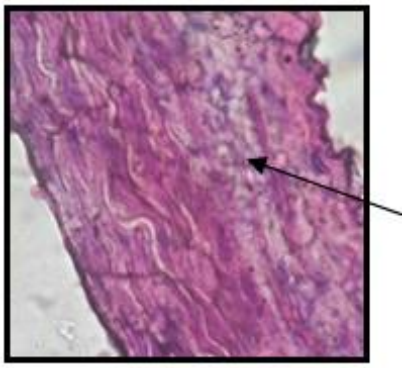

Figure 10. Fatty layer of endothelial cells after treating by the second dose for 3 weeks

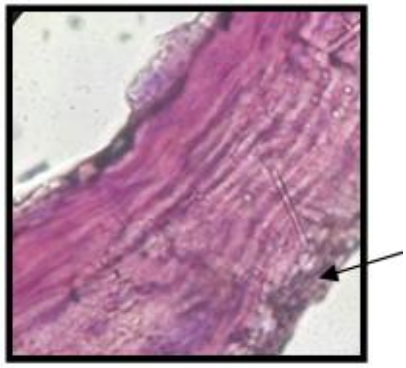

Figure 11. Fatty layer of endothelial cells after treating by the third dose for 3 weeks

be seen in Fig 1. The third dose gave the highest percentage of decreasing in total cholesterol compared to the first dose and the second dose. The percentage of decreasing in total triglyceride could be seen in Fig 2. The percentage of decreasing in total triglyceride of the third dose were the highest compared to the first dose and the second dose. The percentage of decreasing in total LDL could be seen in Fig 3. The percentage of decreasing in total LDL of the first dose were the lowest compared to the second dose and the third dose. The percentage of increasing in total HDL could be seen in Fig 4. The third dose showed the highest increasing in total HDL compared to the first dose and the second dose. The percentage of decreasing in fatty layer could be seen in Fig 5. The third dose had the highest percentage of decreasing in fatty layer and the lowest was given by the first dose. The layer of endothelial cells in normal rat could be seen in Fig 6. The layer of endothelial cells for 12 weeks after giving high fat feed and pure cholesterol orally could be seen in Fig 7. The layer of endothelial cells after treating by statin for 3 weeks could be seen in Fig 8. The layer of endothelial cells after administration by the first dose (50 mg/kg bw) could be seen in Fig 9. The layer of endothelial cells after treating by the second dose (100 mg/ kg bw) could be seen Fig 10. The layer of endothelial cells after treating by the third dose (200 mg/kg bw) for 3 weeks could be seen Fig 11. The layer of endothelial cells after treating by vehicle for 3 weeks in negative control group could be seen in Fig 12.

DISCUSSION

In the previous studies revealed that binahong (*Anredera* cordifolia) leaves had many activities such as antifungal[6], antibacterial[7], improve kidney failure in rats[8], hematoma[9], antihyperuricemic[10], and anti-inflammatory[11-13]. Based on the acute and subchronic toxicity study of binahong ethanol extract showed that the extract was safe[14]. Previous research by Urmila[15], exposed that methanol leaves extract of *Basella alba* which was the same family with binahong (*Anredera cordifolia*) had effect on hyperlipidemia. The selection of plant such as binahong (*Anredera cordifolia*) as object of this research was not only because of increasing in prevalency of the need of cardiovascular medicine, but also giving integrated research regarding mechanism in reducing of the risk of atherosclerosis. In the previous research showed that binahong leaves (*Anredera cordifolia)* could be used to manage the risk factors of hypertension and hypercholesterolemia which contributed to the coronary heart diseases[16]. This research aimed to study the effect of binahong leaves extract in hypercholesterolemia and reduction of fat accumulation on endothelial cells of male Wistar rats 200-250 g. On the week 0, 3rd, 7th, 11th, the rats were induced by pure cholesterol orally for 7 days. The induction of cholesterol is to accelerate increasing in cholesterol level and optimize the cholesterol level. The optimum cholesterol level would damage completely and cause accumulation of fat on endothelial cells. Then, the extract sample was given for 3 weeks. This method was modified from previous research[17]. Based on Figure 1 and

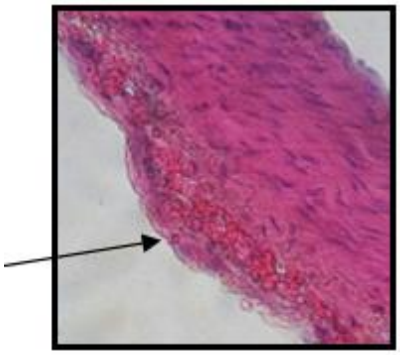

Figure 12. Fatty layer of endothelial cells in negative control for 3 weeks

statistically analysis we could see that all of groups could reduce the total cholesterol and there were significantly different with the negative control group ($p<0.05$). Extract 200 mg/kg bw had no significant different with statin, while extract 50 mg/kg bw showed significant difference with statin ($p<0.05$). The result demonstrated that ethanol leaves extract of binahong was very efficient in reducing of total cholesterol level. In negative control group, the total cholesterol level also decreased 57.1%, its might be due to its high fat feed was replaced by normal feed. Based on this data it can be concluded that by diet only, the total cholesterol level would decrease slowly by itself. It was similar with the previous research which found that 96% ethanol leaves extract of binahong (*Anredera cordifolia*)[16] and methanol leaves exctract of *Basella alba*[15] gave effect on reducing in cholesterol blood level. The addition of cholesterol in diet is related with increasing in concentration of total cholesterol in plasma and LDL plasma[18]. Shaul[19] exposed that LDL would increase endothelial NO synthase (eNOS) which could increase nitric oxide (NO) and then damaged endothelial cell. As well as the total cholesterol, all of groups also showed significant reduction in triglycerides compared to negative control group ($p<0.05$) (Figure 2). Extract 50 mg/kg bw, 100 mg/kg bw and 200 mg/kg bw showed the similar level with statin group in reduction of triglycerides, and had no significant difference with statin. The result reported that ethanol leaves extract of binahong (*Anredera cordifolia*) with doses of 50 mg/kg bw until 200 mg/kg bw was really efficient in reducing

the level of triglycerides. The negative control also gave the reduction in triglycerides level due to the replacement of high fat feed with the normal feed. It was similar with the previous research which reported that methanol leaves exctract of *Basella alba*[15] and 96% ethanol leaves extract of binahong (*Anredera cordifolia*)[16] had reducing in triglyceride level. The significant reduction of LDL was also showed by all of groups compared to the negative control ($p<0.05$) (Figure 3). All of groups which were extract 50 mg/kg bw, 100 mg/kg bw and 200 mg/kg bw and statin group also showed different level of reduction in LDL, and there were significant differences among the four groups. Nevertheless, ethanol leaves extract of binahong (*Anredera cordifolia*) 50 mg/kg bw, 100 mg/kg bw and 200 mg/kg bw still have the potential to reduce the level of LDL. The negative control also showed reduction of LDL level 29.06% which might be caused by the same factor as before. This result was similar with Urmila's study which exposed that methanol leaves exctract of *Basella alba*[15] could reduce LDL level. Based on the above data, it was showed that all of groups reduce LDL level so that the risk of fat accumulation on endothelial cell would also decrease, because of only LDL that could bring cholesterol to the tissue that can cause lipid accumulation which lead to fatty layer and then develop to be atheroma which has a role in the occurrence of cerebral or cardiac stroke. Based on statistically analysis, ability to decrease LDL of extract 50 mg/kg bw, 100 mg/kg bw and 200 mg/kg bw showed significant difference compared to statin group ($p<0.05$). Extract 200 mg/kg bw could decrease LDL greater than extract 50 mg/kg bw, 100 mg/kg bw and significantly different to negative control ($p<0.05$). Based on Figure 4 and statistically analysis, it could be seen that all of groups had significant increase in HDL compared to negative control group ($p<0.05$). Statin group could increase HDL level 9.86%, while the other groups only slightly increased the HDL level, which were 2.93%, 3.56 %, 3.66 % for extract 50, 100 and 200 mg/kg bw, respectively. It was similar with Urmila's research which reported that increasing in HDL level also given by methanol leaves exctract of *Basella alba* [15].

Based on Figure 7, it could be concluded that the model of the damage of endothelial cells by lipid was really formed and also clarified that there was lipid accumulation in week 12th. The significant increase of LDL level would be accompanied by the reduction of HDL level and the risk of LDL accumulation in blood vessels would be increased, as shown in microscopic observation. Cholesterol reduction also occurred in negative control group, might be due to Wistar rats were normal which was treated by high fat feed, so its body may be stress while providing compensation in decreasing in cholesterol, but not quite able to provide large reduction. The result showed significant difference in increasing in HDL between statin group and the negative control ($p<0.05$), while the extract groups gave no significant difference with the negative control. Based on this data it can be assumed that the ethanol leaves extract of binahong did not decrease cholesterol by inhibiting HMG-CoA enzyme reductase likes statin.

Inhibition of HMG-CoA reductase would decrease cholesterol synthesis in the liver which was usually occurs as compensation and then would increase HDL in circulation or tissues, so it could fulfill the needs of liver cholesterol to form bile acids from cholesterol or formation of cell components. The same reason also lead to decrease in LDL and VLDL as a result increasing in formation and metabolism of HDL. However, the other mechanism might be occured. All of groups showed the significant decrease in fatty layer compared to negative control group ($p<0.05$). Statin group could decrease fatty layer 68.3%, and the other groups (50, 100, 200 mg/kg bw) only slightly decreased the fatty layer level, which were 39.5%, 54.6% and 55.9%, respectively. Based on microscopic observation in reducing in thickness of fatty

layer on endothelial cells, it could be seen that reduction of plaque was linear with decreasing in LDL. The absence of lipid deposits in the first dose, second dose, third dose and statin group revealed that there was combination of inhibition of fatty layer formation or other alternatives which could decrease the plaque. Fatty layer could be formed through induction by giving high fat feed. Ethanol leaves extract of binahong (*Anredera cordifolia*) 50 mg/kg bw, 100 mg/kg bw and 200 mg/kg bw had antihypercholesterolemic activity which were significantly different compared to negative control and could reduce the total cholesterol level, triglycerides, LDL and prevent the forming of fatty layer on endothelial cells.

CONCLUSION

Binahong leaves extract with doses of 50 mg/kg bw, 100 mg/kg bw and 200 mg/kg bw reduced total cholesterol level, triglycerides, LDL level significantly different compared to negative control group ($p< 0.05$), and had no influence in the HDL level.

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