The Effect of 96% Ethanol Extract of Binahong Leaf on Hyperglycemia White Male Rats Using Total Cholesterol and Triglyceride Parameters

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Abstract. Hyperlipidemia is an increase in lipid levels such as cholesterol and triglycerides. Binahong leaves contain compounds that possess anti-hyperlipidemic properties. This study aims to determine the effect of 96% ethanol extract of binahong leaves on reducing cholesterol and triglyceride levels in hyperglycemic and hyperlipidemic rats. The testing animals were divided into 6 groups consisting of normal group, negative group (high fat and sucrose diets), positive group (atorvastatin), dose I group (12.5 mg / kg), dose II (25 mg / kg), and dose III (50 mg / kg). All groups, except the normal group, were induced with high fat and sucrose diets. The results obtained showed that the binahong leaves extract was effective in reducing cholesterol and triglyceride levels. The dose II group was comparable to the positive control in reducing cholesterol and triglyceride levels with the percentage reduction of 48.97% and 47.17%, respectively.

1. Introduction

An instantaneous lifestyle has a negative impact on the increasing prevalence of degenerative diseases, including coronary heart disease (CHD) or cardiovascular disease, diabetes mellitus, hypertension, and cancer. The latest data on the prevalence of diabetes has increased quite significantly over the last five years. In 2013, the prevalence rate of diabetes in adults in Indonesia reached 6.9% and it reached 8.5% in 2018 (Kementerian Kesehatan RI 2018). According to data published by the World Health Organization (WHO) in 2011, 25% of the world's population has high cholesterol levels.

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia associated with abnormalities in carbohydrate, fat and protein metabolism caused by decreased insulin secretion or decreased insulin sensitivity or both which causes chronic complications of microvascular, macrovascular and neuropathy (Dipiro 2015). Increased levels of fatty acids cause the pancreas to fail to provide sufficient insulin to cope with the increased demand for insulin. This often results in the difficulty in removing fatty acids, and a lack of inhibition of glucose release in the liver which can lead to hyperglycemia and hyperlipidemia (Tomkins et al. 2018).

Hyperlipidemia is an increase in total cholesterol, low density lipoprotein (LDL), triglycerides, and a decrease in high density lipoprotein (HDL) or a combination of abnormalities (Wells et al. 2012). In the long term, these metabolic disorders contribute to the development of various complications such as cardiovascular disease (CVD), retinopathy, nephropathy, neuropathy and a high risk of cancer (Alledredge et al. 2013).
Binahong plant (*Anredera cordifolia* (Ten) Steenis) is a traditional medicinal plant for cardiovascular disease such as the treatment of heart swelling and stroke and empirically as an antihyperlipidemic (Laela 2009). Binahong (*Anredera cordifolia* (Ten) Steenis) or in Chinese known as *Dheng San Chi* is a medicinal plant originated from South America. Binahong (*Anredera cordifolia* (Ten) Steenis) contains phenols, flavonoids, saponins, triterpenoids, steroids and alkaloids, in addition to having antioxidant activity (Setiaji 2009). The active compounds of flavonoids have many benefits for the body, one of which is that flavonoids can be used to decrease cholesterol. Flavonoids can help decrease cholesterol deposits in the walls of coronary blood vessels. The decrease of cholesterol in the blood vessels would not lead to other diseases caused by cholesterol, such as hypertension, stroke and heart disease (Nalole 2009).

Previous research have shown that the application of binahong leaf extract (*Anredera cordifolia* (Ten) Steenis) can reduce blood cholesterol levels of male white rats induced by foods high in fat and propylthiouracil (Fauziah et al. 2014). Binahong leaf methanol extract at a dose of 50 mg / kgBB for 14 days can reduce blood sugar levels by 75.64% in mice (Sukandar et al. 2011). 70% ethanol extract of binahong leaves at a dose of 50 mg / kgBB for 21 days can reduce blood total cholesterol and LDL levels by 55.29% and 81.31% (Dwintha et al. 2015).

2. Methodology

A. Tools

Macerator, centrifuge, Shanghai EYELA vacuum rotary evaporator, oven, microtube, water bath, vortex mixer, clinical photometer microlab 300 Elitechgroup Europe.

B. Materials

Binahong leaves (*Anredera cordifolia* (Ten) Steenis) obtained from the Bogor Institute for Spices and Medicinal Plants (BALITRO). The materials used for this study were atorvastatin, 96% ethanol, aquadest, Mayer reagent, Dragendorff reagent, Bouchardate reagent, ketamine, anhydrous acetic acid, ether, HCl, FeCl₃, Mg metal, NaOH, Na-CMC,H₂SO₄, gelatin, reagents cholesterol kit (Human) and Triglyceride reagent kit (Human).

C. Research Methods

Making binahong leaf powder

Binahong leaves were first cleaned, then washed with running water and weighed. Afterwards, they were dried with good air circulation and not being exposed to direct sunlight covered with a black flannel cloth, because drying with a temperature that is too high due to direct sunlight may damage active components. Simplicia was mashed until it formed a simplicia powder and afterwards it was sieved and weighed.

96% ethanol extract of binahong leaf

Binahong leaf extraction was carried out by maceration because extraction in this way is a simple method of extraction which does not have the potential to damage the active plant substances. Binahong leaf extraction was made by inserting dry simplicia powder into a macerator and adding 96% ethanol solvent. They were then soaked for 6 hours while occasionally being stirred so that the active substance contained in the simplicia was homogeneous. They were then left out for 18 hours protected from light while being stirred to flatten all parts of the simplicia powder so that it was soaked with 96% ethanol. Maserate was separated by filtration and the extraction process was repeated three times with the same type and amount of solvent. The maserate obtained was concentrated by means of a vacuum rotary evaporator until a thick extract was obtained.
Phytochemical screening/filtering
Phytochemical screening tests were carried out to determine the presence or the absence of alkaloids, flavonoids, phenols, saponins, tannins and triterpenoids/steroids in binahong leaves. The results of each test can be seen in Table 1.

Binahong leaf extract dosage
From the previous research, 70% ethanol extract of binahong leaves at a dose of 50 mg / kgBB for 21 days in male white rats can reduce blood total cholesterol and LDL levels by 55.29% and 81.31% respectively (Dwintha et al. 2015). In this study, three variations of the dose were used, namely a dose of 12.5 mg / kg, a dose of 25 mg / kg, a dose of 50 mg / kg.

Atorvastatin dosage
Atorvastatin was used as a comparison agent. The usual dose of atorvastatin used was 10-80 mg / day (Lacy et al. 2007). The dose given orally to humans was 40 mg / day. The dose for mice must be converted to 4.11 mg / KgBW.

Manufacturing high fat feed
High fat feed was made with a composition of 20% beef fat, 10% butter, 20% sucrose and 50% standard feed. Feed was made by heating butter, then melting it until the butter became oil. The beef fat was also heated until it melted. The sucrose was also heated with water until it dissolved. Standard feed was crushed until smooth then mixed and stirred until homogeneous. Afterwards, it was formed into pellets (Tatto 2017). High-fat feeding was carried out for 4 weeks.

Treatment of test animals
All rats were acclimatized on day 1-7 and groups II to VI were hyperlipidemic with high-fat diet on day 8-35. On the 36th day, blood was drawn to determine the initial levels of the mice. Then, on days 37-64, they were given treatment according to the division of each group. The final blood was drawn after being given the treatment on the 64th day and on the 65th day the final levels were measured.

Collection and examination of blood serum from test animals
Before taking the blood, rats were anesthetized using ketamine until they were unconscious. After anesthetizing the rats, blood was drawn in the corner of the mouse's eyes with a capillary tube. The capillary tube was turned afterwards. 2 mL of blood were collected, stored in a microtube and then centrifuged at 4000 rpm for 15 minutes in order to obtain serum. The blood was stored in a refrigerator, then the sample was ready to be analyzed (Vogel 2008).

Measurement of Total Cholesterol and LDL levels
a. Total cholesterol
10 µL of serum was taken, then mixed with 1000 µL of enzyme reagent (cholesterol reagent kit). Afterwards, it was vortexed and incubated for 5 minutes at 37°C or 10 minutes at 20-25°C. Levels were read with a clinical photometer.

b. Triglycerides
10 µL of serum was taken using a micropipette, then mixed with 1000 µL of triglyceride reagent kit. The solution was homogenized using vortex and incubated for 10 minutes at 25°C. Levels were observed with a clinical spectrophotometer.

Data analysis
Data on the reduction of cholesterol and triglyceride levels were analyzed using one-way analysis of variance (one way ANOVA). If the results of the one-way analysis test (one way ANOVA) were significant, the Tukey HSD test was carried out (Priyatno 2011).
3. Result and Discussion

Binahong plants (*Anredera cordifolia (Ten) Steenis*) contain phenols, flavonoids, saponins, triterpenoids, steroids and alkaloids, in addition to having antioxidant activity (Setiaji 2009). The active compounds of flavonoids have many benefits for the body, one of which is that flavonoids can be used to lower cholesterol. Flavonoids can remove cholesterol deposits in the walls of coronary blood vessels. The erosion of cholesterol in the blood vessels would not lead to other diseases caused by cholesterol, such as hypertension, stroke and heart disease (Nalole 2009).

Binahong leaf powder was extracted using the maceration method. The maceration method was chosen to minimize metabolite degradation (Hanani 2015). The maceration method is suitable for materials which are thermolabile. The extraction process was carried out using 96% ethanol as a solvent. 96% ethanol contains 4% water. Ethanol was easier to penetrate the cellular membrane, making it able to extract intracellular material from the plants used (Tiwari et al. 2011).

The high cholesterol-inducing agents used were 20% beef fat, 10% butter, 20% sucrose and 50% standard feed mixed homogeneously, formed in pellets. The composition of the standard feed, namely 13% moisture content, 19-21% protein, 5% fat, 5% fiber, 7% ash, 0.9% calcium, 0.6% phosphorus, ME 3000-3100 Kcal / kg (HI-PRO-VITE). Butter is rich in trans fatty acids (TFA) which cause the disruption of the activity of carnitine palmyoyltransferase-1 and 3-hydroxy-acyl-CoA dehydrogenase in the liver and an increase in liver triglycerols. This is due to the reduced oxidation of trans fatty acids in the liver, resulting in accumulation of lipids in the liver, heart and organs. (Mozaffarian et al. 2006). A diet high in sucrose for 3-5 weeks could increase blood glucose levels and hyperinsulinemia and also decrease insulin sensitivity in mice (Chicco et al. 2003). Diets high in sucrose markedly alter insulin-mediated glucose metabolism which can result in insulin resistance in mice (Pagliassoti et al. 2007).

Giving high cholesterol feed to mice aimed to increase the content of free fatty acids in plasma cells which resulted in a decrease in insulin sensitivity in peripheral tissues (Tatto et al. 2017). The free fatty acids in the cells were broken down to form acetyl Co-A, which in turn provided the energy needed by the cells. The energy produced has two important products, namely citrate ion and ATP, which have a strong inhibitory effect on the enzyme phosphofructokinase, a speed-limiting enzyme that increases glucose consumption in cells. Therefore, the use of glucose as an energy source has almost stopped and causes intense suppression of glucose consumption by cells, resulting in an increase in glucose levels (Guyton 1994).

The rats used in the study had hyperlipidemia with an average increase in total cholesterol levels of 226.8 mg / dL and triglyceride levels of 213.4 mg / dL. High-cholesterol feed induction caused mild dysfunction of pancreatic beta cells without completely interfering with insulin secretion (Srinivasan et al. 2005).

Statin class drugs are the first choice drugs to treat hyperlipidemia in diabetes patients (Chaudhury and Aggarwal 2018). The choice of a comparator drug atorvastatin was due to a greater reduction in total cholesterol when compared to other statins (except rosuvastatin). This is because atorvastatin has an active metabolite with activity equivalent to the parent compound which provides a longer duration of inhibition of the HMG-CoA reductase enzyme (Poli 2007) Blood draws were taken through the orbital sinus because it was easier and minimized the possibility of blood lysis when taking blood (Fatimah et al. 2018). Total cholesterol and LDL levels were measured using a clinical spectrophotometer.

Based on the results shown in Figure 1, 96% ethanol extract of binahong leaf doses I, II, and III had a decrease in total cholesterol levels. The result of decreasing total cholesterol levels in rats after giving various doses of 96%ethanol extract binahong leaves and comparative drug atorvastatin for 28 days was that the dose group III (50 mg / kgBB) had a better reduction in cholesterol levels than the dose I (12.5 mg / kgBB) and II groups (25 mg / kgBB).

Based on the results shown in Figure 2, 96% ethanol extract of binahong leaves doses I, II, and III had a decrease in triglyceride levels. The results of decreasing triglyceride levels in rats after giving various doses of 96% ethanol extract binahong leaves and comparative drug atorvastatin for 28 days, dose group II (25 mg / kgBB) had a better reduction in cholesterol levels compared to dose I (12.5 mg / kgBB) and III groups (50 mg / kgBB).
The final data on the examination of total cholesterol and triglycerides were statistically tested using data on the reduction of total cholesterol and triglyceride levels. The ANOVA test results for a one-way reduction in total cholesterol and triglycerides obtained sig = 0.001 (<0.05) and sig = 0.001 (<0.05), causing a significant difference between the treatment groups.

Flavonoids can erode cholesterol deposits in the walls of coronary blood vessels. The erosion of cholesterol in the blood vessels would not lead to other diseases caused by cholesterol, such as hypertension, stroke and heart diseases (Anggraini et al, 2018). The alkaloid compounds contained could also inhibit the activity of the pancreatic lipase enzyme. The reduced activity of this enzyme could also reduce triglyceride deposits that enter from the small intestine because these enzymes convert triglycerides into two monoglycerides and two free fatty acids, causing them to enter the blood vessels (Artha et al., 2017).

4. Conclusion
The application of 96% ethanol extract of Binahong leaf (*Anredera cordifolia* (Ten) Steenis) dose I (12.5 mg / kg), dose II (25 mg / kgBB) and dose III (50 mg / kgBB) had the effect of reducing total cholesterol levels and Hyperglycemia and hyperlipidemia in male white rats (*Rattus norvegicus*), which were comparable to atorvastatin positive controls. The highest percentage reduction in total cholesterol and triglycerides was found in the 96% ethanol extract of Binahong leaf (*Anredera cordifolia* (Ten) Steenis) dose II (25 mg / kgBW) with a percentage reduction of 48.97% and 47.17%, respectively.

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Appendices

Table 1. Results of Phytochemical Screening of Binahong Leaf Extract

| No. | Filtering | Result |
|-----|-----------|--------|
| 1.  | Alkaloids | +      |
| 2.  | Flavonoids| +      |
| 3.  | Phenol    | +      |
| 4.  | Saponins  | +      |
| 5.  | Tannins   | -      |
| 6.  | Steroids  | +      |
| 7.  | Terpenoids| -      |

Figure 1. Graph of Total Cholesterol Levels

Figure 2. Graph of Triglyceride Level