Journal of
Fundamental and Applied Pharmaceutical Science
https://journal.umy.ac.id/index.php/jfap

Potential of Binahong Leaf Ethanol Extract (*Anredera cordifolia* (Ten.) Steenis) against Elevated HDL Levels (*Rattus corvegious*) Streptozonic-Induced Wistar Strain

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Abstract

Diabetes mellitus is frequently associated with decreased levels of high-density lipoprotein (HDL), which are prevalent cardiovascular disease risk factors. The use of synthetic drugs substantially increases the risk of atherosclerosis since they often have severe side effects, require high costs, and have poor prescription adherence. *Anredera cordifolia* (Ten.) Steenis, often known as the binahong plant, is a potentially effective complementary therapy for increasing HDL levels. Flavonoid compounds present in the ethanol extract of Binahong leaf have the potential to increase HDL levels. This work aims to evaluate the action of an ethanol extract from binahong leaves in raising HDL levels in rats of the Streptozotocin-induced Wistar strain (Rattus norvegicus). In this research, the Pretest-Posttest Control Group Design was used. The study involved the grouping of five Wistar strain rats (n=5) into five distinct groups: one received binahong leaf ethanol extract at concentrations of 25 mg/kg BW, 50 mg/kg BW, and 100 mg/kg BW, which served as the positive control group and received a negative control group supplemented with atorvastatin at 0.9 mg/kg BW. The CHOD-PAP method was applied to measure HDL levels. SPSS software was used to run the normality test, Wilcoxon test, one-way ANOVA test, and paired sample t-test. No statistically significant difference in HDL levels was found between treatment groups. The 50 mg/kg BW binahong leaf extract group had significantly higher HDL levels (p-value < 0.05). As a result, the ethanol extract obtained from binahong leaves presumably raises HDL levels in streptozotocin-induced Wistar rats.

Keywords: Binahong leaf ethanol extract; HDL; CHOD-PAP

Date of article
Received : 28 May 2024
Reviewed : 19 Jun 2024
Accepted : 31 Aug 2024

DOI
10.18196/jfaps.v5i1.22496

Type of article:
Research

INTRODUCTION

The Kemenkes RI data indicates a substantial rise in the prevalence of non-communicable diseases (NCDs).[1] The leading cause of mortality among non-communicable diseases (NCDs) is cardiovascular disease, with dyslipidemia being identified as one of the risk factors. In Indonesia, among those aged 15 years and older, Total cholesterol values exceed 200 mg/dL in roughly 28.8% of people, whereas 24.4% of those have an HDL score below 40 mg/dL, 27.9% have triglyceride levels above 150 mg/dL, and 72.8% have LDL counts above 100 mg/dL.[1] HDL is a lipoprotein that serves as a carrier

of endogenous cholesterol from tissues to the liver.[2] In individuals with diabetes mellitus, HDL levels are decreased, which is known to increase the likelihood of cardiovascular disease. Therefore, individuals who have been diagnosed with diabetes mellitus are more likely to be vulnerable to risk factors for cardiovascular issues.[3]

Long-term care of dyslipidemia is essential for controlling the lipid balance. In the context of managing dyslipidemia in individuals with diabetes, statin-class medications continue to be the primary course of action.[4] Nevertheless, the significant escalation in the risk of atherosclerosis is attributed to the inadequate adherence of patients to long-term statin medication.[5] Herbal medicine possesses the advantage of being relatively risk-free, in contrast to synthetic pharmaceuticals, which frequently induce severe adverse effects.

Anredera cordifolia (Ten.) Steenis, also famous as the binahong plant, is a promising solution for addressing dyslipidemia. The Binahong leaves contain alkaloids, flavonoids, saponins, phenols, triterpenoids/steroids, and tannins.[6] Flavonoid chemicals have been shown to lower total cholesterol, triglycerides, and LDL while increasing HDL levels.[7]

Previous research findings indicated that in individuals with diabetes mellitus, elevated blood glucose levels and lowered HDL levels were associated with insulin resistance, which in turn affected lipid metabolism.[8] As stated in reference,[9] insulin resistance triggers the activation of hormone-sensitive lipase (HSL), increasing the breakdown of triglycerides in the fat tissue of the body. This condition results in an excess of free fatty acids in the bloodstream. The liver utilizes these unbound fatty acids as both an energy source and a building block for the production of triglycerides. VLDL contains a high concentration of triglycerides because triglycerides formed from free fatty acids are incorporated into VLDL. The VLDL subsequently generates LDL and HDL, which are also abundant in triglycerides, via the activity of cholesterol ester transfer protein (CETP).[10] The hepatic lipase (HL) enzyme hydrolyzes the triglycerides present in LDL, resulting in the formation of tiny, dense LDL. This small, compact, low-density lipoprotein (LDL) is very susceptible to oxidation, making it highly likely to contribute to the development of atherosclerosis. The kidneys readily catabolized HDL, including a high amount of triglycerides, resulting in a drop in HDL levels. Thus, dyslipidemia frequently coexists with type 2 DM.[9]

It has been documented that STZ can induce diabetes mellitus, both type 1 and 2; therefore, the level of STZ is a significant factor in determining the type of diabetes.[11] STZ at moderate concentrations (40-55 mg/kg BB) induces type 2 DM ([12]). STZ induces type 2 diabetes mellitus by penetrating the pancreatic β cells through glucose transporter 2 (GLUT-2), which subsequently triggers a reduction in GLUT-2 expression. As a result of this condition, peripheral insulin receptor sensitivity is diminished, causing resistance to insulin and elevated blood glucose levels([13]). This study aims to evaluate the beneficial effects of an ethanol extract derived from binahong leaves in increasing HDL levels in Wistar rats (*Rattus norvegicus*) that have been stimulated with streptozotocin as well as to determine the most effective dosage.

METHOD

This research employs an experimental approach using the Pretest-Posttest Control Group Design. The study was carried out at the Laboratory of Pharmaceutical Technology at FKIK UMY and LPPT UGM.

Extraction

Four hundred fifty grams of binahong leaf powder sourced from Naturnonal Creatama Indonesia were soaked in 96% ethanol (1:5 w/v) for 6 days, followed by a further 2 days of soaking. Subsequently, the macerate was subjected to evaporation using a water bath maintained at a temperature of 500C, resulting in the formation of a dense extract. Four hundred fifty grams of binahong leaf powder acquired from Naturnonal Creatama Indonesia were soaked in 96% ethanol (1:5 w/v) for 6 days and then soaked again for 2 days. Subsequently, the macerate was subjected to evaporation using a water bath maintained at a temperature of 500C in order to yield a concentrated extract with a viscous consistency.

Phytochemical Screening

The phytochemical screening was conducted using the method described by Harborne (1987). It involved five tests to identify secondary metabolite chemicals, namely alkaloids, flavonoids, saponins, tannins, and steroids/triterpenoids.

Preparation of Test Animals

The subjects of the experiments consisted of 2-month-old male rats (*Rattus norvegicus*) from the Wistar strain, with a body mass ranging from 200 to 300 grams. Twenty-five rats were allocated into five groups using simple random sampling. The rats were acclimatized for a period of 5 days by being provided with a conventional diet in the form of AD II pellets and an adequate supply of mineral water.

STZ Induction

The STZ compound was dissolved in a citrate buffer with a pH of 4.5 and then well-mixed using a vortex. Intraperitoneal administration of a single dose of 40 mg/kg BW was used to induce STZ.

CMC-Na Suspension Manufacturing

500 mg of CMC-Na was diluted in hot distilled water as much as 10 ml, then stirred until a homogenous suspension formed, then diluted with 100 ml of distilled water in a measuring flask.

Treatment of Test Animals

The test animals were categorized into five categories: the negative control group was induced with 0.5% CMC-Na, the positive control group was injected with 0.9 mg/kg BW atorvastatin, and the sample group received binahong leaf extract at a range of doses from 25 mg/kg BW, 50 mg/kg BW, and 100 mg/kg BW.

On the day of acclimatization, an initial HDL level testing was performed. Following that, all rats were given a single dose of 40 mg/kg BW of STZ and allowed for three days to develop diabetes. Then, on the third day, HDL levels were measured, and the rats were treated for 14 days based on group assignment. HDL levels were measured again on the 17th day.

Blood Sampling

Blood samples were taken through the orbital sinus. Approximately 1.5 ml of dripping blood was taken and accommodated in a microtube that has been treated with EDTA. Plasma was obtained by centrifuging at 4000 rpm for 10 minutes.

HDL Level Check

Measurement of HDL levels was carried out using the CHOD-PAP enzymatic photometric test using the HDL reagent kit (DiaSys).

Data Analysis

The data obtained was analyzed using SPSS. Data normality test using Shapiro Wilk. The analytical method used was the one-way ANOVA test to compare HDL levels between groups, the Wilcoxon test, and the paired sample t-test to determine differences in levels before and after treatment.

RESULTS AND DISCUSSION

Plant Determination

Binahong leaf powder was identified at The Biological Laboratory of Ahmad Dahlan University with the determination number 136/Lab.Bio/B/IV/2022. The identification results showed that the plant used in this study was indeed binahong leaf powder.

Extraction

The extraction results obtained a viscous extract with a dark brownish-green color of 17.4 grams with a yield of 3.86%. The yield value in this study was higher than that of study,[14] which produced a yield value of 3.29%. Several factors, including the extraction method, type of solvent, length of extraction time, sample size, time, and storage conditions of the extract, can influence the yield of an extract.[15]

Phytochemical Screening

Alkaloid compounds, flavonoids, steroids, saponins, and tannins were found in binahong leaf extract, according to the findings of the phytochemical screening carried out in this study.

Data Analysis of HDL Levels

Measurement of HDL levels in this study was carried out 3 times. Initial HDL levels were measured on day 0 before the rats were treated. This aims to determine normal HDL levels as a reference value that will be compared with HDL levels after STZ-induced rats. The second measurement of HDL levels was carried out on the 3rd day after the mice were induced by STZ to ensure that there was a decrease in HDL levels in the mice. The third measurement of HDL levels on the 17th day after the rats were treated for 14 days aims to determine the final HDL levels after the treatment.

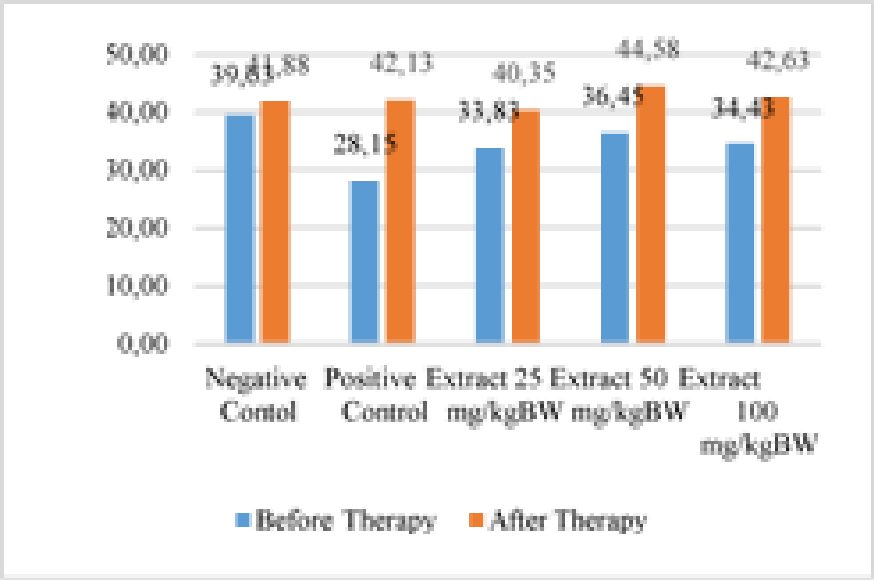

Figure 1. Average HDL Levels Beforeand After STZ Induction

According to the findings presented in Figure 1, the HDL levels in mice fell following the formation of STZ, and a statistically significant difference was seen ($p < 0.05$). This indicates that the STZ induction effectively lowered the HDL levels in mice. Consistent with the findings of the study,[16] HDL levels in rats decreased following STZ induction. Reduced levels of HDL are a characteristic feature of dyslipidemia in type 2 diabetes mellitus.[17]

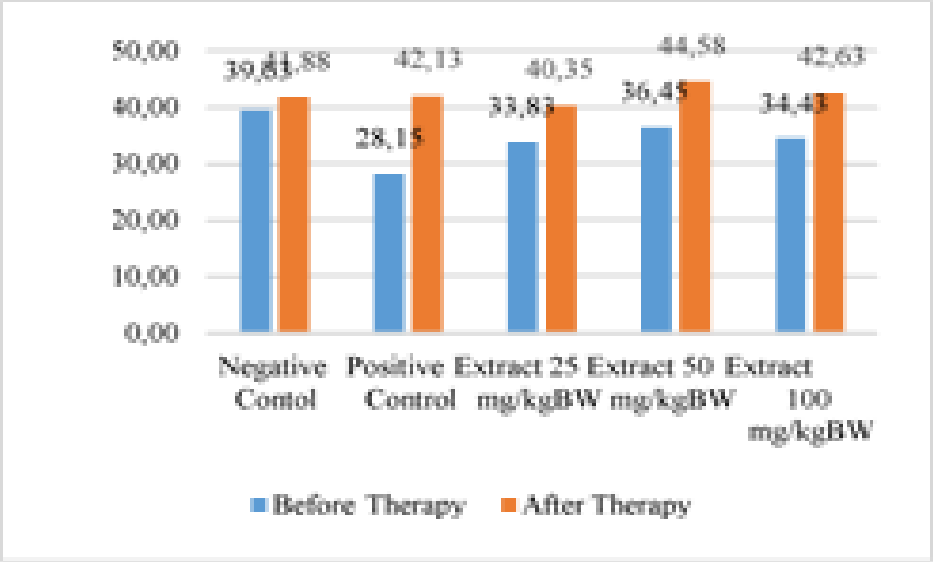

Figure 2. Average HDL Levels Before and After Therapy

According to Figure 2, there was a rise in HDL levels in all groups following a 14-day therapy. The normality test using Shapiro-Wilk exhibited a p-value greater than 0.05 as a parameter that data follows a normal distribution. Consequently, it is appropriate to proceed with the one-way ANOVA test, which resulted in a p-value above 0.05, meaning there was no significant difference in the increase of HDL levels across the treatment groups.

Table 1. Percentage of Increased HDL Levels

Group	Averag ebefore therapy (mg/dl)	Average after-therapy (mg/dl)	% Rate increases	P
Negative Control	39.63	41.88	6%	0.514
Positive Control	28.15	42.13	50%	0.020*
Extract 25 mg/kgBW	33.83	40.35	19%	0.303
Extract 50 mg/kgBW	36.45	44.58	22%	0.010*
Extract 100 mg/kgBW	34.43	42.63	24%	0.166

Note *: p-value <0.05 indicates a significant increase in HDL levels

The paired sample t-test results demonstrated a substantial rise in HDL levels in both the treatment group, which received a 50 mg/kg BW dose of the ethanol extract of binahong leaves, and the positive control group, which received atorvastatin medication. The elevation in

HDL levels in the extract is attributed to the presence of flavonoids, particularly quercetin, as the primary components in binahong leaves.

According to research,[18] the binahong leaf extract contains flavonoid compounds that inhibit the activity of HMG-CoA reductase, thereby impeding the synthesis of cholesterol in the liver. This phenomenon increases HDL and decreases triglyceride levels[9] as it indirectly inhibits VLDL synthesis. Furthermore, by increasing HDL antioxidant activity and the expression level of ATP-binding cassette (ABC) A1/G1, quercetin compounds can hasten reverse cholesterol transport (RCT).[9]

In this study, binahong leaf extract was administered at concentrations of 25, 50, and 100 mg/kg. The dosing variations were determined after a comprehensive review of multiple prior studies that have documented pharmacological activity in a range of diseases. Rats were administered binahong leaf extract at determined concentrations of elevated HDL levels, as shown in Table 1, and a significant result of HDL was observed in the treatment with 50 mg/kg BW dosage compared to the other dosages.

This is presumably because, at a dose of 25 mg/kgBW, it has not given an optimaleffect. In contrast, at a dose of 100 mg/kgBW, there is saturation, so increasing the dose does not provide a significant increase in effect. Some drugs, such as gabapentin, warfarin, and phenytoin, also show saturation or limited metabolism in humans. Pathological changes in the process of drug absorption, distribution, metabolism, and elimination cause this condition.[20] According to research,[9] efforts to increase HDL levels are classified as more difficult thanlowering LDL levels. However, the management of low HDL levels can be treated with non-pharmacological and pharmacological methods. Several factors affect HDL levels, including genetic factors, drugs and hormones, lifestyle, and comorbidities.[21]

Several rats in this study experienced injuries to their tails and legs. This is a manifestation of oxidative stress. Multiple research investigations indicate that patients with type 2 diabetes mellitus (DM) experience an elevated activity in reactive oxygen species (ROS), leading to oxidative stress.[22] Oxidative stress and inflammation in diabetes mellitus interfere with reverse cholesterol transport (RCT), thereby affecting HDL levels.[3]

CONCLUSION

Based on the findings of an in vivo study investigating the potential of an extract of ethanol derived from binahong leaves in animal models of type 2 diabetic dyslipidemia rats (*Rattus norvegicus*), it can be concluded that the ethanol extract has the capacity to elevate HDL levels in rats (*Rattus norvegicus*) Wistar strain induced by streptozotocin. The most effective dose of the ethanol extract was 50 mg/kgBW.

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