

The Role of Sembung (*Blumea balsamifera*) Leaf Extract in Preventing Atherosclerosis in Hyperlipidemia Rat Models

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Abstract. This study aims to prove that administering BBLE as a natural antioxidant can prevent atherosclerosis by maintaining lipid profiles, antioxidant enzymes, and netrin-1 levels in hyperlipidemia in rat models. The research subjects were 20 adult male Wistar rats (*Rattus norvegicus*), which were divided into 2 groups using a randomized *pretest* and *posttest* control group design. Before treatment and after treatment for 3 months, lipid profiles, MDA, SOD, and netrin-1 were examined. The control group was only given high-cholesterol diets (HCD), while the treatment, apart from HCD, was also given BBLE 4mg/day. The data obtained was tested using paired *t-test* and group *t-test*. The results of the study showed that there was a significant decrease in netrin-1 in the control group ($p < 0.05$) after being given HCD for three months. In the treatment group, it also decreased but it was not significant ($p > 0.05$). Netrin-1 levels in the treatment group were higher than the control ($p < 0.05$). The lipid profile experienced a significant increase in HDL in the treatment group accompanied by a significant decrease in MDA and an increase in SOD ($p < 0.05$) when compared with the control group. This study concludes that administering BBLE at a dose of 4 mg/day to rats given HCD caused an increase in netrin-1 levels accompanied by improvements in lipid profiles and prevention of oxidative stress. The findings of this study reveal the novelty of BBLE in treating and maintaining blood vessel function in mice given HCD by increasing netrin-1 levels.

Keywords: *Blumea balsamifera*; Dyslipidemia; Herbal medicines; Herbal products; Oxidative stress.

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INTRODUCTION

Atherosclerosis is the most prevalent metabolic cardiovascular disease, which not only strikes underdeveloped nations but is also on the rise in wealthy countries, therefore lowering the global illness burden and generating a substantial economic effect (Kim et al., 2023). Atherosclerosis is characterized by a complicated inflammatory response and lipid accumulation beneath the artery endothelium. During the early stages of atherosclerosis, huge levels of plasma low-density lipoprotein (LDL) cholesterol collect beneath the damaged endothelium and then activate endothelial cells (ECs), encouraging monocyte adhesion and recruitment (Doran, 2022;

Engelen et al., 2022).

This condition is also triggered by hyperlipidemia, which causes increased levels of total cholesterol (TC), low-density lipoprotein (LDL) cholesterol (LDL-c), and triacylglycerol (TG) in the blood, as well as a decrease in blood concentrations of high-density lipoprotein (HDL) cholesterol (HDL-c) (El-Tantawy & Temraz, 2019). According to a 2018 Riskesdas assessment, 72.8% of Indonesians had hyperlipidemia, with LDL levels above 100 mg/dl and total cholesterol levels over 200 mg/dl (Rahmawaty et al., 2022). Malondialdehyde (MDA) is an essential indicator of lipid peroxidation and oxidative stress, whereas SOD and GSH-PX are two types of antioxidant enzymes that can prevent atherosclerosis (Batty et

al., 2022; Poprac et al., 2017). In addition to being significant as an inflammatory response in the development of atherosclerosis, the oxidized alteration of LDL induced by elevated levels of reactive oxygen species (ROS) is thought to be an early indication of atherosclerosis (Ley et al., 2011).

Functional and structural changes in the vascular endothelium are determined by various types of proteins in the endothelial environment (Krüger-Genge et al., 2019). Netrin-1 is a laminin-related extracellular protein that has a role in atherosclerosis. Netrin-1 prevents macrophage migration from arteries, causing atherosclerosis (Yimer et al., 2018). Netrin-1 prevents macrophage migration from arteries, causing atherosclerosis (van Gils et al., 2012). Netrin-1 also enhances nitric oxide (NO) production while decreasing NADPH oxidase-4 expression, increasing mitochondrial activity, and, ultimately, reducing infarct size (Bongo & Peng, 2014). Netrin-1 is a biomarker for atherosclerosis since it is lower in persons with coronary artery calcification compared to those lacking (Muñoz et al., 2017). However, its expression is decreased in atherosclerotic plaques (carotid, femoral, and aortic) (Oksala et al., 2013).

Statins are still the first medications prescribed in pharmacological methods to lower blood cholesterol levels (Tokgözoğlu & Zamorano, 2020). However, this drug's adverse effects include hepatotoxicity, muscular disease, acute renal failure, cataracts, and an increased risk of diabetes (Golomb & Evans, 2008). On the other hand, the high cost of traditional treatment has an impact on lower-income populations (Y.-C. Li & Huang, 2015). The presence of antioxidants from both within and outside the body influences endothelial function (Di Pietro et al., 2020; Widhiantara et al., 2018).

Antioxidants can be derived from sources outside the body, such as medicinal plants. According to reports, more than 19,871 medicinal plants are utilized as traditional ingredients in Indonesia, with 16,218 recognized and only around 9,600 species known to have medical characteristics. People utilize herbal therapeutic plants because of their cultural legacy, empirical safety and efficacy, low cost, and widespread availability. Sembung (*Blumea balsamifera*) is a medicinal plant that is commonly utilized by the inhabitants of Bali Island in Indonesia. This plant's leaves are boiled, and the resulting liquid is consumed warm.

Previous research has confirmed the

pharmacological effects of *B. balsamifera* leaf extract (BBLE) as an antidiabetic in vitro (Kusumawati et al., 2022), antibacterial and anticancer effects, and the ability to increase testosterone, luteinizing hormone (LH), and SOD levels in hypercholesterolemic rats model (Widhiantara et al., 2023). However, there has been no evidence of the pharmacological benefits of BBLE in hyperlipidemic rats, including improvements in blood profiles, antioxidant enzyme levels, and Netrin-1 levels. This research aims to determine the role of BBLE as an alternative anti-atherosclerosis drug by improving blood profiles, maintaining antioxidant enzyme levels, and increasing netrin-1 levels in the hypercholesterolemia rats model.

METHODS

Research Design

This is an experimental laboratory study with a pre-test and post-test control group structure. A total of 20 male Wistar rats were adapted for two weeks, with eligibility criteria (age four months, body weight 200-300 g, and in good health). After the adaption time was completed, blood was collected for a pre-test evaluation, followed by a group split into the control and treatment groups. The control group consisted of Wistar rats fed solely a high cholesterol diet (HCD), whereas the treatment group consisted of Wistar rats fed a high-fat diet as well as 4 mg sembung leaf extract/day/rat. The therapy lasts three months. After 3 months of treatment, blood samples were drawn to determine the research variables as a post-test result.

Ethics Permission

The Animal Ethics Committee, Faculty of Veterinary Medicine, Udayana University has permitted this research with an Animal Ethics Approval certificate No. B/99/UN14.2.9/PT.01.04/2023.

Preparation of *Blumea balsamifera* leaf extract (BBLE)

The fresh, medium-sized green leaves of sembung (*B. balsamifera*) are washed with running water to remove any extraneous organic matter and dirt. The sample is air dried to eliminate water, then diced and dried in an oven at 50°C for 24 hours, resulting in a dry sample or simplicia suitable for processing at the following stage. Simplicia is processed in a blender and sieved through a 20-mesh sieve until a powder is

formed. Sembung leaf powder weighing roughly 250 grams is wet with 70% ethanol solvent (Merck, Germany) at a powder-to-solvent ratio of 1:10, mixed until equally dispersed, and left for 24 hours (maceration). The macerate was separated by filtering with Whatman filter paper no. 42 (Sigma Aldrich, Germany). The collected maserate is evaporated using a vacuum rotary evaporator at 50°C, 80 rpm, and 80 kPa pressure to give a thick extract. The yield of the thick extract is calculated as the difference between the weight of the container containing the extract and the weight of an empty container. The *B. balsamifera* leaf extract used in this research is given the term BBLE (Widhiantara et al., 2021, 2023).

Rats models and treatments

The research began with a two-week acclimatization period. In this period, Wistar rats are fed a standard diet that includes protein (20% - 25%), fat (5%), carbs (45% - 50%), crude fiber (5%), ash (4%), vitamins, and minerals. After two weeks (passing the acclimation phase), blood samples were collected through the retro-orbital sinus to gather pre-test data, including a lipid profile, Netrin-1, malondialdehyde (MDA), and superoxide dismutase (SOD). The following phase was random allocation, which involved dividing

20 mice into two groups of ten each. A control group of 10 rats received HCD prepared from a mixture of lard oil (10%), duck egg yolk (5%), and regular meal. The treatment group (a total of ten rats) received HCD and BBLE at a daily dose of 4 mg/kg.BB. The therapy lasts three months, with HCD administered *ad libitum*. After three months of therapy, blood samples were collected to acquire post-test results (Rosiana & Widhiantara, 2020; Subawa et al., 2022).

Lipid Profiles

Determination of total cholesterol

Total cholesterol was determined using the FS Cholesterol Kit (DyaSys, USA) (Cat. No. 1 1300 99 10030). Briefly, it can be described as follows; 10 µl of Wistar rat serum and standards have been prepared. Next, 10 µl of distilled water is prepared as an empty reagent for control. 1,000 µl of the prepared mixture of reagents and standards was mixed, and incubated for 10 min at 20°C–25°C. The sample absorption value was read using UV-Vis spectrophotometry at a wavelength of 500 nm for 60 minutes and compared with the blank reagent. Calculation of total cholesterol levels using the following equation (Jawi et al., 2024):

$$\text{Cholesterol} \left(\frac{\text{mg}}{\text{dl}} \right) = \frac{A_{\text{sample}}}{A_{\text{Std}}} \text{Conc. Std} \cdot \frac{\text{Std}}{\text{Cal} \left(\frac{\text{mg}}{\text{dl}} \right)}$$

Triglycerides levels

TG FS (DyaSys, USA) (Cat. No. 1-5700-99-10-030), used in determining serum TG levels. Brief working procedure, 10 µl of Wistar rat serum and 1 µl of distilled water were prepared. At the same time, reagent standards were also prepared, 1,000 µl each. A mixture of distilled water and reagents was used as a blank. Each

blank and sample were mixed and incubated for 10 min at 20°C–25°C. The sample absorption value was read using UV-Vis spectrophotometry at a wavelength of 500 nm for 60 minutes and compared with the blank reagent. The calculation of total cholesterol levels uses the following equation (Jawi et al., 2024):

$$\text{TG} \left(\frac{\text{mg}}{\text{dl}} \right) = \frac{A_{\text{sample}}}{A_{\text{Std}}} \text{Conc. Std} / \text{Cal} \left(\frac{\text{mg}}{\text{dl}} \right)$$

High-density lipoprotein levels (HDL)

Determination of HDL levels was performed by using HDL Precipitant (DiaSys, USA) (Cat No. 1: 3540 99 90 885). The first test procedure was precipitation: namely, as much as 200 µl of sample or standard and 500 µl of precipitation reagent were mixed and incubated for 15 minutes at room temperature, then centrifuged for 20 minutes at 2,500 g. Within 2 hours after centrifugation, 0.1

ml of the clear supernatant was transferred to the reaction solution to determine HDL cholesterol levels. The determination of HDL cholesterol was carried out by preparing 100 µl of sample supernatant and 100 µl of standard, respectively. A total of 1,000 µl of cholesterol reagent was added to the standard and sample, mixed, and incubated for 10 minutes at room temperature. Then the absorbance value of the sample or

standard was measured using a UV-Vis spectrophotometer at a wavelength of 500 nm. The results were then compared with the reagent blank

value within 45 minutes. The calculations are carried out using the following equation (Jawi et al., 2024):

$$HDL - Choles \left(\frac{mg}{dl} \right) = \frac{\Delta \Delta sample}{\Delta A Std} Conc. Std \left(\frac{mg}{dl} \right)$$

The standard concentration is the total cholesterol concentration in a standard cholesterol solution.

Low-density lipoprotein levels (LDL)

Determination of LDL levels was performed by using the LDL Precipitant Kit (DyaSys, USA) (Cat No. 1: 4330 99 90 885). The first test procedure was through precipitation; namely, a 100 µl sample and 1,000 µl of precipitation reagent were prepared, mixed, and incubated for

15 minutes at room temperature, then centrifuged for 20 minutes at 2,500 g. Within 1 hour after centrifugation, 100 µl of the clear supernatant was transferred to the reaction solution to determine LDL cholesterol. The determination of LDL cholesterol was carried out similarly to HDL cholesterol. The results were compared with the reagent blank value within 45 minutes. The calculations are carried out using the following equation (Jawi et al., 2024).

$$LDL - Choles \left(\frac{mg}{dl} \right) = Total\ cholesterol - Cholesterol\ in\ supernatant$$

Oxidative stress assay

Malondialdehyde (MDA)

Rat Malondialdehyde ELISA Kit (Bioassay Tech Laboratory, China) MDA levels (Cat. No. E0156Ra) were used to perform the MDA assay. All of the reagents, samples, and standards were prepared, and then the samples and ELISA reagents were added to each well and incubated at 37°C for 1 hour. The ELISA plate was cleaned five times before adding substrate solutions A and B and incubating for 10 minutes at 37°C. When a stop solution was introduced, the color changed from blue to yellow. The optical density (OD value) of each well was assessed using a microplate reader set to 450 nm for 10 minutes.

Superoxide dismutase (SOD)

SOD levels were determined using the Rat Super Oxidase Dismutase ELISA Kit (Bioassay Tech Laboratory, China) (Cat. No. E0168Ra) was used to perform the SOD assay. All the reagents, samples, and standards were prepared following the manufacturer's procedure. Samples and ELISA reagent were added to each well and incubated for 1 hour at 37°C. The ELISA plate was washed five times, and substrate solutions A and B were added and incubated for 10 minutes at 37°C. A stop solution was added, and the color change was observed. The OD values for each well were read using a microplate reader set up at 450 nm for 10 minutes.

Netrin-1 levels

The Rat Netrin-1 ELISA Kit (MyBioSource, USA) (Cat No. MBS163009) was used in this study. Each well was filled with samples and ELISA reagent and incubated for 1 hour at 37°C. The ELISA plate was then rinsed five times before substrate solutions A and B were added. After 10 minutes at 37°C, the stop solution was added, and the color changed from blue to yellow. Each well OD value was determined using a microplate reader set to 450 nm for 10 minutes.

Data analysis

The provided data was analyzed using the SPSS 23.0 software program (IBM Corporation, Armonk, NY). The Kolmogorov–Smirnov and Levene tests were used to determine the normality and homogeneity of the data. The data were examined using a paired t-test (pre-and post-test) and an independent test between groups with a confidence interval of $p < 0.05$ to assess the significant difference between treatments. Figures and tables were used to describe the results of the observations.

RESULTS AND DISCUSSIONS

Netrin-1 levels

The control group's netrin-1 level before treatment was 134.08 ng/L. After being given high cholesterol feed for 3 months, the average netrin-

1 level decreased to 99.92 ng/L. This decrease was

statistically significant, but when compared to the treatment group given 4 mg of BBLE every day for three months, the netrin-1 level after treatment was higher than the control group, namely 107.782 (Table 1). Statistically this result is significantly different ($p < 0.05$) (Figure 1). Thus, giving 4 mg of BBLE/day for 3 months affects netrin-1 levels in the blood, even though high cholesterol feed is given.

Table 1. Netrin-1 levels before (pre-test) and after (post-test) treatment.

Groups	Netrin-1 levels \pm SD (ng/dl)		P-value
	Pre-test	Post-test	
Control	134.08 \pm 23.29	99.92 \pm 10.17	$p > 0.05^*$
BBLE	133.88 \pm 20.52	107.78 \pm 5.26	$p < 0.05$

Note: BBLE: *Blumea balsamifera* leaves extracts. Results are presented as mean \pm SD. P-value was tested by Independent t-test (pre-and post-test) and independent test between groups with a significant difference at $P < 0.05$.

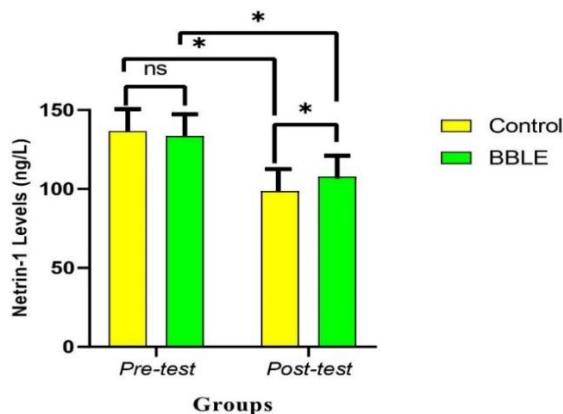


Figure 1. Netrin-1 levels of hypercholesterolemic Wistar rats. Based on the paired t-test (pre-and post-test) and an independent test between groups, the * sign indicates a significant difference ($P < 0.05$), whereas NS indicates no significant

difference ($p > 0.05$).

Netrin-1 is a protein secreted by endothelial cells that is important for maintaining endothelial function. Netrin-1 belongs to the family of secreted laminin proteins that function as chemoattractants or chemorepulsives in a variety of biological processes (Claro & Ferro, 2020). In inflammation, netrin-1 plays a role in inhibiting monocyte migration in inflamed tissues (Ziegon & Schlegel, 2021). This function is via the DCC/neogenic receptor to cause a chemoattractive effect and UNC5, to cause a chemorepulsive effect (Ziegon & Schlegel, 2021). If endothelial function is disrupted, such as an increase in LDL levels, as a result of the high-fat diet in this study, oxidative stress will occur, leading to a decrease in netrin-1 synthesis from the endothelium, resulting in a drop in netrin-1 in control rats.

The result of this research is by in vitro research on endothelial cells given TNF- α , thereby increasing inflammation and reducing netrin-1 (Passacuale et al., 2015). In the group of rats given BBLE, netrin-1 levels were higher, meaning that BBLE which contains flavonoids was able to reduce oxidative stress because its antioxidant properties were very strong (Mansour et al., 2023). As well as anti-inflammatory, so that endothelial function is better. The results of this study are also in accordance with in vitro endothelial research where aspirin was administered as an anti-inflammatory (Passacuale et al., 2015).

Oxidative stress

MDA and SOD levels

The effect of BBLE on oxidative stress in this study was proven by examining MDA and SOD levels. The MDA levels in the blood of rats after being given high-cholesterol feed were different from the MDA levels given high-cholesterol feed with BBLE for 3 months as presented in the following table. Both MDA and SOD after treatment were significantly different compared to the control. ($p < 0.05$) (Figure 2).

Table 3. Comparison of MDA and SOD levels in this study groups

Groups	MDA (mmol/mL)		SOD (U/mL)	
	Pre-test	Post-test	Pre-test	Post-test
Control	1.230	1.230	2.36	2.33
BBLE	1.190	0.670	2.23	4.40
p-Value	$p > 0.05$	$p < 0.05$	$p > 0.05$	$p < 0.05$

Note: BBLE: *Blumea balsamifera* leaves extract. Results are presented as mean \pm SD. P-value was tested by Independent t-test (pre-and post-test) and independent test between groups with a significant difference at $p < 0.05$.

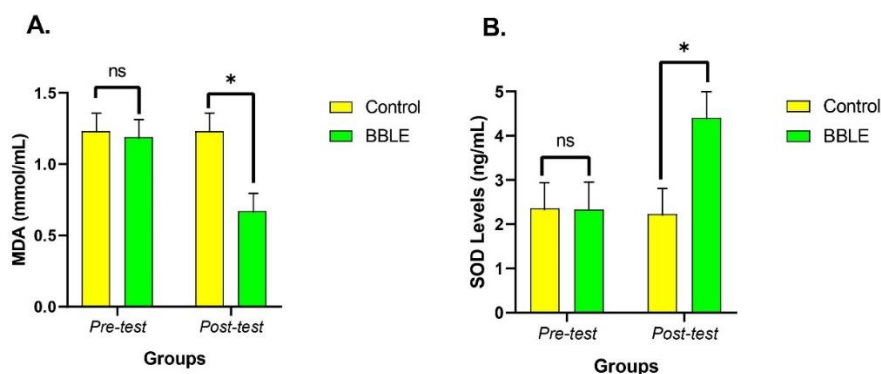


Figure 2. Results of measuring A) MDA and B) SOD levels in the control and BBLE groups. Based on the paired t-test (pre-test and post-test) and an independent test between groups, the * sign indicates a significant difference ($p < 0.05$), whereas NS indicates no significant difference ($p > 0.05$).

In this investigation, administering BBLE dramatically increased SOD levels while decreasing the oxidative stress marker, MDA, as compared to pre-test circumstances or the control group. Under normal settings, superoxide dismutase (SOD) protects the body by adjusting the redox state, and extracellular SOD (EC-SOD or SOD3) is the primary plasma antioxidant enzyme. These antioxidant enzymes play an important role in modifying cell redox state and are abundant in blood vessels, notably the arterial walls (Mohammedi et al., 2015). Previous study has shown that polyphenolic components in garlic extract (*Allium cepa*) can boost SOD activity in the blood and liver of hyperlipidemic mice while decreasing TC, TG, LCL-c, and MDA levels (Li et al., 2021). Polyherbal extracts were found to lower MDA and nitric oxide levels while increasing antioxidant enzyme and glutathione activity in mice fed a salt diet for 28 days (Olorunnisola et al., 2021). Apart from having an impact on the blood profile, HCD was also able to reduce liver function in model mice by reducing markers of liver function such as parenchymal degeneration, fatty degeneration, and necrosis. Apart from that, there was an increase in AST and ALT levels in the liver which acts as a biomarker

in hypercholesterolemia studies (Iswari et al., 2020).

Lipid profiles

Triglyceride (TG), HDL and LDL levels

As presented in Figure 3, there was a change in the blood lipid profile after feeding high cholesterol feed for 3 months. In the two groups of rats, there were significant differences in lipid profiles ($p < 0.05$). LDL in the control group was 46.37, while in the group given BBLE, LDL was lower, namely 30.69. Statistically this figure is significantly different ($p < 0.05$). HDL, which is good cholesterol, was found to be higher in the group of mice given high-cholesterol feed and BBLE than the control. In controls, the average HDL after being given high cholesterol feed was 59.80. In the treatment group, the average HDL was 75.86. Statistically this figure is significantly different ($p < 0.05$). Blood triglyceride levels also showed a significant difference between the control group and the BBLE group. The control group's triglyceride level after giving high cholesterol feed for 3 months was 151.34, while the treatment group's triglyceride level was 135.68 ($p < 0.05$).

Table 2. TG, HDL, and LDL levels before (pre-test) and after (post-test) treatment.

Groups	LDL \pm SD (mg/dL)		HDL \pm SD (mg/dL)		TG \pm SD (mg/dL)	
	Pre-test	Post-test	Pre-test	Post-test	Pre-test	Post-test
Control	43.81 \pm 5.96	45.87 \pm 3.33	63.70 \pm 4.96	59.45 \pm 1.80	153.41 \pm 8.18	150.17 \pm 6.13
BBLE	44.97 \pm 2.91	30.68 \pm 8.77	61.62 \pm 2.70	75.86 \pm 2.27	149.57 \pm 2.60	135.68 \pm 0.88
p-value	$p > 0.05$	$p < 0.05^*$	$p > 0.05$	$p < 0.05^*$	$p > 0.05$	$p < 0.05^*$

Note: BBLE: *Blumea balsamifera* Extract. Results are presented as mean \pm SD. P-value was tested by Independent t-test (pre-and post-test) and independent test between groups with a significant difference at $p < 0.05$.

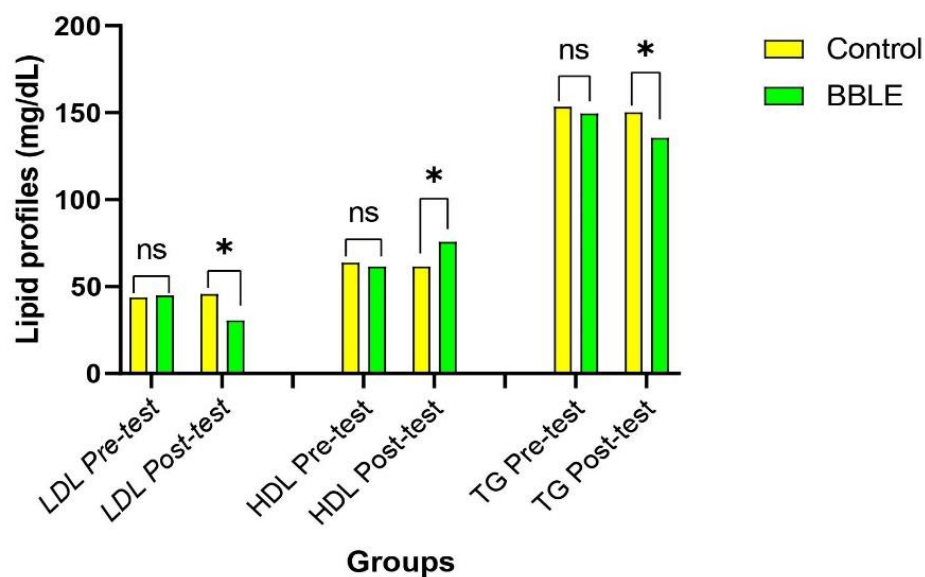


Figure 3. A) LDL, B) HDL, and C) Triglycerides (TGs) levels of hypercholesterolemic Wistar rats. Based on the paired t-test (pre-and post-test) and an independent test between groups, the * sign indicates a significant difference ($p < 0.05$), whereas NS indicates no significant difference ($p > 0.05$).

Apart from being an antioxidant, BBLE which contains flavonoids also causes a decrease in lipid absorption from the digestive tract, resulting in a decrease in LDL levels (Widhiantara et al., 2023). The results of this study are by previous studies that provide herbal medicine as a hypolipidemic (Haselgrübler et al., 2019; Laouani et al., 2024; Shaikh et al., 2022). The BBLE in this study caused higher levels of netrin-1, lower LDL, Tryglycerides, and higher HDL. The results of this study are in accordance with research conducted to measure differences in blood netrin levels in patients with coronary heart disease and dyslipidemia. In studies of patients suffering from coronary heart disease, it was proven that there were differences in netrin-1 levels in the blood between controls and patients with coronary heart disease (Inderjeet et al., 2022; Mutlu et al., 2020). Similar research shows that tomato juice supplementation is effective in improving the plasma lipid profile levels of hypercholesterolemia model albino mice by reducing total cholesterol, LDL-c, and triglycerides, as well as increasing HDL levels (Iswari, 2009). Lakum leaf extract at a dose of 40 mg/200 g BW was effective in reducing cholesterol and triglyceride levels and increasing HDL levels in mice induced by a high-fat diet (Sopandi et al., 2019).

Netrin-1 levels in coronary patients with dyslipidemia are lower than controls. This

research also proves that there is a decrease in netrin-1 levels in the blood of rats that experience increased cholesterol due to being fed high-cholesterol feed for 3 months, which is accompanied by an increase in LDL cholesterol which will cause oxidative stress and inflammation resulting in a decrease in netrin-1. Netrin-1 levels in the blood, which is netrin-1 produced by the endothelium, are greatly influenced by inflammation (Layne et al., 2017). Administration of BBLE in this study inhibited the inflammatory process because the diterpenoid content of BBLE is a strong anti-inflammatory, increasing netrin-1. The BBLE also contains flavonoids which have anti-inflammatory properties by inhibiting the JAK/STAT pathway, thereby reducing the expression of pro-inflammatory cytokines (Al-Khayri et al., 2022; Widhiantara et al., 2022; Yin et al., 2021). Decreased inflammation in the endothelium causes increased levels of netrin-1 in the blood (Jawi et al., 2024).

This study highlights the importance of new sources of bioactive compounds that can be used to improve and reduce the adverse effects of HCD. Administration of BBLE was able to reduce LDL and triglyceride levels but increased HDL levels in the hypercholesterolemia rats model. The antioxidant properties contained in BBLE improve the function of the endothelium of hypercholesterolemic mice by increasing netrin-1

levels while preventing cell damage from oxidative stress. The spotlight also goes to BBLE because it contains compounds such as quercetin which has antioxidant properties. Overall, the results of this research can contribute to the identification of medicinal plants that are empirically used by local communities in Indonesia to treat diseases. Future research should investigate the effects of BBLE in other animal models and human subjects to determine its effectiveness as a therapeutic intervention in metabolic disorders (Nguyen-Huu et al., 2024).

CONCLUSIONS

This study found that administering BBLE at a dosage of 4 mg/kg BW had anti-atherogenic effects. It has been shown to modulate lipid profiles, decrease oxidative stress by lowering MDA levels and boosting SOD levels, and boost Netrin-1 protein levels in mice fed a high-cholesterol diet for three months. BBLE supplementation can help prevent and cure disorders connected with hyperlipidemia, such as atherosclerosis. However, further study is needed to fully understand this process, such as in the modulation of HMGCR, LDL receptors, catalase, NO, and MPO levels in hyperlipidemia mice's livers or other clinical metabolic syndrome illnesses. Further research is also needed to understand the mechanisms underlying the anti-atherogenic effects of BBLE and its potential use in the treatment of hypercholesterolemia.

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