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Cinnamomum burmannii Bl. Bark Ameliorate Lipid Profile and Platelet Aggregation in Dyslipidemia Mice through Antioxidant Activity

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Abstract

BACKGROUND: *Cinnamomum burmannii* Bl. has a higher coumarin, flavonoids, saponins, and alkaloids.

AIM: We investigated the antioxidant, anti-platelet aggregation, and anti-dyslipidemia activity of cinnamon bark extract (CBE) in dyslipidemia mice.

METHODS: Mice were divided randomly into six groups (n = 5) that consist of normal control, negative control, positive control (atorvastatin), and test groups of CBE at doses 300, 400, and 500 mg/kg BW. All groups except normal control were given dyslipidemic-induced feed for 14 days. After that, the induction of dyslipidemia was stopped, then continued with suspension of atorvastatin (positive control) and the test group was given CBE for 7 days. Then, it was measured malondialdehyde (MDA), superoxide dismutase (SOD), bleeding time, coagulation time, total cholesterol, triglyceride, low density lipoprotein (LDL), and high-density lipoprotein (HDL).

RESULTS: The CBE has antioxidant activity by decreased MDA concentrations and increased SOD activity in dose group 300; 400; and 500 mg/kg BW compared to negative control. The anti-platelet aggregation of CBE showed that the effects of prolong bleeding time and coagulation time and improve the decreased plasma absorbance after the addition of ADP. There was a decrease in total cholesterol for the three dose groups, respectively, 20.14%, 24.42%, and 35.76%. Triglyceride levels decreased by 4.09%, 8.74%, and 12.5%. LDL levels decreased by 38.17%, 53.8%, and 67.96%. HDL levels increased by 27.29%, 67.8%, and 72.64%.

CONCLUSION: CBE has antioxidant, anti-platelet aggregation and anti-dyslipidemia activity, and potential to prevent cardiovascular disease.

Introduction

A lot of people currently are not attention to the diet they have, starting from eating foods that tend to contain high calories, as well as lack of exercise, causing the accumulation of fat in the body. Excess fat in the body can cause dyslipidemia. Dyslipidemia is defined as a change in lipid metabolism in which the concentration of lipids or lipoproteins in the blood vessels becomes abnormal and has an impact on the levels of lipoproteins in the blood circulation, as well as on the concentrations of their components [1]. The abnormal concentration of lipids in the blood vessels is characterized by an increase in total cholesterol, triglycerides, low density lipoprotein (LDL) cholesterol, and a decrease in high-density lipoprotein (HDL) cholesterol [2]. Data in Indonesia show that there are 21.2% of the Indonesian population aged 15 years with abnormal cholesterol levels (based on NCEP ATP III, with cholesterol levels ≥200 mg/dL) where there are more women than men and more urban than rural, 12.4% had very high LDL proportions (≥190 mg/dL), 24.3% had HDL levels <40 mg/dL, and 11.9% had very high triglyceride levels (≥500 mg/dL) [3]. Abnormal fat levels in the blood circulation in the long-term can increase the risk of atherosclerosis and cardiovascular disease (CVD). CVD is caused by impaired function of the heart and blood vessels, such as coronary heart disease (CHD), heart failure or heart failure, hypertension, and stroke [4].

CVD was the leading cause of death in Asia in 2019, causing 10.8 million deaths, which were approximately 35% of the total deaths in Asia. From 1990 to 2019, the number of CVD deaths in Asia increased from 5.6 million to 10.8 million, the proportion of CVD deaths in total deaths increased from 23% to 35%, and crude CVD mortality rates increased continuously in both men and women. Whereas the growing crude CVD mortality rates indicate the increasing burden of CVD in Asian populations, the fall in age-standardized CVD mortality implies that the trend in the crude mortality rate was driven mainly by demographic changes. The impact of demographic changes and increasing

CVD epidemics in Asia is likely the consequence of complex effects from changes in socioeconomic, living environments, lifestyles, prevalence of CVD risk factors, and capacities to prevent and treat CVD. Most CVD deaths (87%) were due to coronary heart disease (CHD) (47%) or stroke (40%) [5].

The main etiology of CHD is the formation of plaque on the walls of blood vessels, also known as atherosclerosis. Atherosclerosis involves free radicals, inflammatory mediators, platelets, and lipids in the process. When LDL accumulates in the tunica intima, LDL activates the endothelium to produce leukocyte adhesion molecules and chemokines that function to increase monocyte and T cell aggregation. Monocytes then turn into macrophages and release cytokines, proteases, and vasoactive molecules. T cells recognize local antigens in the lesion and release pro-inflammatory cytokines and plaque formation [6]. In conjunction with the atherosclerotic process, reactive oxygen species (ROS) and NO are involved in lipid peroxidation, a process that converts polyunsaturated fatty acids into lipid radicals. Lipid peroxidation induces oxidative stress which can cause DNA damage, disruption of cell membranes, and the development of various diseases. The lipid radicals formed will then react with oxygen to form lipid peroxy radicals. These lipids peroxy radicals will then react with other polyunsaturated fatty acids and so on to form a stable molecule. The heart and blood vessels are organs that are very sensitive to oxidative stress (a condition where there is an imbalance between the number of free radicals and antioxidants), where levels of free radicals or ROS are in excess, while antioxidants are insufficient to trap free radicals. Levels of oxidative stress high concentrations can cause damage to DNA, proteins, and endothelial cell membranes in the heart and blood vessels [6], [7]. The formation of platelet plugs (thrombosis and embolism) also contributes to the atherosclerosis process and increases the risk factors for CVD [8]. Platelets or platelets are one of the important factors in the process of blood clotting [9]. To reduce the prevalence of CVD, the formation of blood clots (thrombus) in blood vessels can be medically prevented with antiplatelet agents. Antiplatelet (antiplatelet) drugs are used to reduce platelet aggregation and inhibit thrombosis, thereby reducing the risk of atherosclerosis [10]. So far, CVD therapy uses a combination of various chemical drugs that are consumed for a long time, such as aspirin (as an antiplatelet), ACE inhibitors (inhibiting the renin-angiotensin-aldosterone system), – blockers (lowering heart rate and contractility of the heart so that cardiac output decreases), and the lipid-lowering drug class (atorvastatin). The chemical drugs used have significant side effects for the wearer, for example, the consumption of the HMG-CoA reductase class of drugs, in the long-term can cause side effects such as rhabdomyolysis and myopathy [11]. Statins are currently used for the treatment of dyslipidemia, but they cause side effects commonly relate to skeletal muscles and include muscle cramping, soreness, weakness, and fatigue and myopathy [12].

Atherosclerosis is a CVD that can be prevented with ingredients that have activities to reduce oxidative stress, platelet aggregation, and improve dyslipidemia conditions. One of the natural sources that are safe for consumption in the long-term is cinnamon (*Cinnamomum burmannii* L.) Cinnamon has been used traditionally by the wider community as an anti-fungal, anti-coagulant, anti-bacterial, analgesic, antipyretic, anti-inflammatory, and anti-viral, anti-cancer, antihypertensive, and anti-hyperglycemic [13]. Cinnamon bark is rich in phenols and flavonoids, including cinnamyl alcohol, coumarin, cinnamic acid, cinnamaldehyde, anthocyanins, and essential oils with a glucose constituent, protein, crude fat, pectin, etc [14].

Cinnamaldehyde has ability to scavenge free radicals (radical scavenger). Cinnamic acid (Cinnamic acid) can prevent the formation of free radicals, remove radicals before damage occurs, repair oxidative damage, and remove damaged molecules in cells [15]. Coumarins and their derivatives have many biological activities, including stimulating the formation of skin pigment, affecting enzyme activity, blood anticoagulation, anti-microbial activity, and exhibiting activity to inhibit carcinogenic effects [16]. Coumarins have a phenol group and a 1,2-pyrone group, where compounds with these groups can donate electrons to neutralize oxygen-nucleated free radicals and reduce the enthalpy of dissociation of the O-H group [13]. Coumarins are Vitamin K antagonists that have pharmacological activity as anticoagulants, coumarins inhibit the synthesis of prothrombin and prevent the formation of blood clotting factor preparations by inhibiting hepatic synthesis of four coagulation proteins that depend on vitamin K (factor II, VII, IX, and X) [17]. *C.burmannii*, possess anti-inflammatory activity showed by significantly decrease in production of pro-inflammatory mediators NO and PGE2 level, also pro-inflammatory cytokines IL-6, IL-1 β, and TNF-α level in activated RAW246.7 macrophages, to prevent chronic disease related to inflammation such as ateroskeloris [18].

Several cinnamon species with different species such as *C. cassia*, *C. zeylanicum*, and *C. tamala* have been shown to have antihyperlipidemic activity. Based on the previous research, it is known that cinnamon contains coumarins and is proven to be used as an anti-obesity and hypolipidemic because it has the effect of lowering cholesterol and lipid levels in the blood in mice and rats fed a high-fat diet [19], [20]. According to the other research, coumarin which has been isolated from Ionidium suffruticosum can reduce LDL levels, triglycerides, phospholipids, and significantly increase HDL levels in rats fed a high-fat diet. The use of coumarin isolated from Ionidium suffruticosum can prevent atherosclerosis and CHD [21]. Research for *C. burmanni* has not been widely carried out. *C. burmannii* is a cinnamon originating from Indonesia and has a higher coumarin content than other cinnamons [22], [23]. Therefore,

this study was conducted to reduce cases of CHD through a very specific study, namely, the antioxidant, anti-platelet aggregation and anti-dyslipidemia activity of *Cinnamomum burmannii* Bl. in Dyslipidemia Mice.

Materials and Methods

Materials

Cinnamomum burmannii Bl. purchased from Aromatic Medicine Research Institute (BALITRO, Indonesia), ethanol purchased from Bratachem (Indonesia), Atorvastatin (PT. Kalbe Farma, Indonesia), adenosine diphosphate (ADP) (Sigma Aldrich, Germany), trichloroacetic acid (TCA) (Sigma Aldrich, Germany), thiobarbituric acid (TBA) (Sigma Aldrich, Germany), 1,1,3,3-tetraethoxypropane (TEP) (Sigma Aldrich, Germany), Epinephrine (Sigma Aldrich, Germany), Cholesterol, Triglyceride, and HDL kit purchased from Biolabo (France)

Extraction procedure Cinnamomum burmannii Bl. bark extract (CBE)

Cinnamomum burmannii Bl. was determined by the Indonesian Institute of Sciences, Center for Plant Conservation in the Botanical Gardens, Bogor, Indonesia. Making cinnamon bark extract (CBE) by maceration method by weighing 2000 g of simplicia powder macerated with 70% ethanol assisted by stirring for 6 h. After that it was filtered, the macerated pulp was then macerated again using 70% ethanol solvent 5 times. All the obtained macerates were collected and concentrated using a rotary evaporator at a temperature of 45–55°C to obtain a thick ethanol extract. The extract obtained from the rotary evaporator was then heated over a water bath for several days and dried so that all the ethanol residue evaporated and until a thick extract was obtained. The thick ethanol extract was weighed carefully and then calculated the value of drug extract ratio (native), which is the equivalent amount of simplicia which produces 1 g of extract and yield.

Animals and in vivo experimental design

The research was conducted at the Pharmacology Laboratory and Biochemistry Laboratory, Faculty of Pharmacy, Pancasila University, Srengseng Sawah, South Jakarta, Indonesia. This study was an experimental study to examine the antioxidant, anti-platelet aggregation, and anti-dyslipidemia effects of the 70% ethanol extract of cinnamon bark (*Cinnamomum burmannii* Bl.) CBE in dyslipidemia mice. The research was conducted after obtaining ethical approval with the number 475/UN2.F1/ETIK/PPM.00.02/2021 from The Ethic Committee of The Faculty of Medicine, University of Indonesia, Indonesia. All animals were treated and housed in a temperature of 23°C ± 2°C and humidity of 55 ± 15% and were submitted to a 12-h light/dark cycle and allowed free access to standard laboratory chow and tap water.

Antioxidant, anti-platelet aggregation, and anti-dyslipidemia effects were tested on dyslipidemia mice. A total of 30 mice that will be used in the study were acclimatized for a week for environmental adjustment, control of health and body weight, and uniformity of food. Experimental animals were divided into six groups, with each group consisting of five mice. Normal control group, negative control group, positive control group (atorvastatin), and dose group of 300 g/kgBW; 400 g/kgBW; and 500 g/kgBW. All groups, except normal controls, were fed a dyslipidemia-inducing diet (80% egg yolk, 15% sucrose solution, and 5% animal fat). The administration was given once a day for 14 days so that the mice experienced dyslipidemia. After 14 days, the induction was stopped and the test was continued with atorvastatin as a positive control and CBE at three different doses, while normal and negative controls were only given standard feed and drinking water, for 7 days. Determination of bleeding time, coagulation time, decreased plasma absorption after the addition of ADP, measurement of MDA levels, superoxide dismutase (SOD) activity, measurement of total cholesterol, triglyceride, LDL, and HDL cholesterol levels were carried out on days 0, 14, and 21.

Antioxidant assays

MDA levels were measured using the TBARs (Wills) method. A total of 200 L of plasma was added with 1.0 mL of 20% TCA and 2 mL of 0.67% TBA. The solution was homogenized and heated on a water bath for 10 min, then cooled. After cooling, the solution was centrifuged at 3000 rpm for 10 min, then the filtrate was taken. The pink colored filtrate was measured with a UV-VIS spectrophotometer at a wavelength of 532 nm. Concentrations are determined using the standard curve using 1,1,3,3-TEP.

Measurement of SOD activity was carried out using the adrenochrome assay method. 100 L of filtrate into another test tube, added 2800 L of sodium carbonate buffer pH = 10.2 and 100 L of 0.01 M epinephrine (Sigma) solution into a test tube, the absorbance was measured after 1, 2, 3, and 4 min at a wavelength of 480 nm [24].

$$\text{SOD activity measurement formula: \% detention} = \frac{A - B}{A} \times 100$$

$$\text{SOD activity (unit/mL)} = \frac{\text{\% detention x 1 unit / 10 } \propto L}{50\ \%} \times 100$$

A = Average difference in absorbance/min without sample (Blangko); B = Average difference in

absorbance/min sample; 50% = Units of SOD activity defined as the amount of SOD required to cause 50% inhibition of epinephrine oxidation (SOD_{50}) [24].

Anti-platelet aggregation test

Determination of bleeding time

The mice were put into a holder, the tails of the mice were gently cleaned with 70% alcohol, then the tails of the mice were injured at distance of 2 cm from the base of the tails of the injured mice with a maximum wound depth of 2 mm, the blood that came out was absorbed on filter paper. The time interval between the onset of the first drop of blood until the blood stops flowing is the bleeding time [25].

Coagulation time

Blood samples were obtained from the orbital sinus vein, which was taken with a capillary tube, the blood that came out was absorbed by a capillary tube. The capillary tube containing blood is broken for 0.5 cm, every 15 s intervals until the formation of fibrin threads is observed in the broken part, the time required for the formation of fibrin threads is the coagulation time [25].

Decreased plasma absorbance

Blood samples from the orbital sinus veins in the eye, the blood that comes out is accommodated with an Eppendorf tube previously added with 3.18% sodium citrate then centrifuged at 1500 rpm for 15 min, blood plasma is obtained then blood plasma is taken as much as 250 μL then added NaCl 0.9% as much as 3 mL and then the plasma absorption was measured using a spectrophotometer with a wavelength of 600 nm. Plasma uptake was remeasured after administration of 30 μL ADP 5 M as a platelet aggregation inducer and incubation at 37°C for 20 min. Measurement of plasma uptake is calculated by calculating the percentage difference in plasma absorption before and after administration of the platelet aggregation inducer ADP, the calculation method is as follows [26]:

$$\frac{A-B}{A}\times 100\% = \%\ \text{Decreased plasma absorption after ADP induction}$$

A: Decreased plasma absorption after ADP induction; B: Plasma Absorbance after ADP induction

Anti-dyslipidemia effect test

Determination of total cholesterol and triglyceride levels

Blank tubes, standard tubes, and sample tubes consisting of aqua distillate and cholesterol or triglyceride reagents of 1000 μL were prepared. Then, 10 μL of aqua distillate was put into a blank tube, 10 μL of cholesterol standard was put into a standard tube and 10 μL of mice blood plasma was put in a sample tube then mixed, incubated for 10 min at room temperature. The absorption was read at a wavelength of 500 nm.

Determination of HDL cholesterol levels

Prepared standard tubes and sample tubes containing 150 μL of sample and standard each. Then, 15 μL of the precipitation kit reagent was put into a standard tube and the sample tube was then mixed homogeneously and incubated for 10 min at room temperature, then centrifuged for 15 min at 4000 RPM. HDL cholesterol levels were determined by the enzymatic colorimetric method using a cholesterol kit.

Measurement of LDL levels

LDL levels can be determined indirectly using the Friedewald formula:

LDL cholesterol = Total cholesterol – (triglycerides + HDL cholesterol) 5

Data analysis

Data were analyzed with SPSS 22.0. Data on MDA levels, SOD activity, bleeding time, coagulation time, and decreased plasma absorption after the addition of ADP, total cholesterol, triglyceride, LDL, and HDL levels obtained from each treatment group (mean ± SD) tested for normality (Shapiro–Wilk test) and homogeneity (Levene's test). If the data are normally distributed, then a one-way ANOVA test is performed. If the data are not normally distributed, the Kruskal–Wallis's test will be performed. If the results show a significant difference, then proceed with the *post hoc* LSD analysis test for normal and homogeneous data; Mann–Whitney *post hoc* test for abnormal or inhomogeneous data. Differences between means were regarded significant at p value < 0.05

Results

The plant determination in this study showed that the plant used was a species of *Cinnamomum burmannii* Bl. From the maceration of 2000 g of cinnamon bark simplicia powder with 70% ethanol as a solvent, the extract weight was 291.78 g with a yield calculation of 14.59%.

Antioxidant activity of CBE in dyslipidemia mice

The MDA level and SOD activity in each experimental animal group is shown in Figure 1a and b. Oxidative stress increased in mice that received a high-fat diet (dyslipidemia), which was indicated by increased MDA levels and decreased activity of SOD in the day-14 at group test.

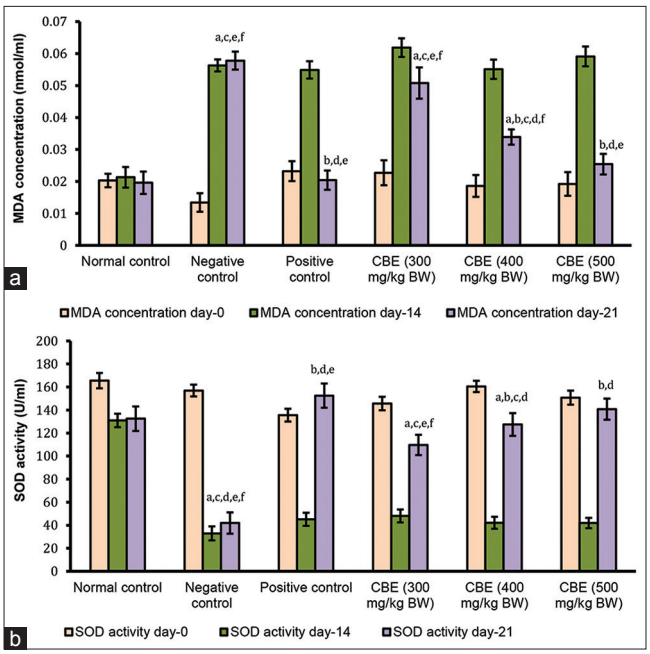

Figure 1: Antioxidant activity of CBE at day-0, day-14, and day-21 in dyslipidemia mice. (A). Decrease the MDA concentration (nmol/mL). (B). Increase the SOD activity (U/ml). Data in mean±SD. (a) p < 0.05 versus normal group at day-21, (b) p < 0.05 versus negative group at day-21, (c) p < 0.05 versus positive control at day-21, (d) nilai p < 0.05 versus CBE (300 mg/kg BW) at day-21, (e) nilai p < 0.05 vs CBE (400 mg/kg BW) at day-21, (f) nilai p < 0.05 versus CBE (500 mg/kg BW) at day-21

Figure 1a showed that the MDA levels in the negative control group on day-21 increased in the highest average MDA levels compared to the other groups. The group that received atorvastatin as a positive control and CBE had antioxidant effects and significantly reduced MDA levels. Statistical results showed that MDA levels in the normal group had no significant difference with the positive group (atorvastatin) and the CBE group at a dose of 500 mg/kgBW ($p < 0.05$).

Figure 1b showed that the SOD activity in the negative control group is the lowest compared to other test groups. The results of the measurement of SOD activity in the positive control group (atorvastatin) showed an increase in the activity of the SOD enzyme. The CBE group at a dose of 500 mg/kg BW gave a higher average SOD enzyme activity than the other dose groups ($p < 0.05$).

Anti-platelet aggregation activity of CBE in dyslipidemia mice

To evaluate the anti-platelet aggregation activity of CBE in dyslipidemia mice, this can be done by measuring bleeding time, coagulation time, and inhibition of platelet aggregation triggered after addition of ADP to blood plasma. In this study, the results of determining the bleeding time and coagulation time are shown in Table 1 and the inhibition of platelet aggregation induced by ADP is shown in Figure 2.

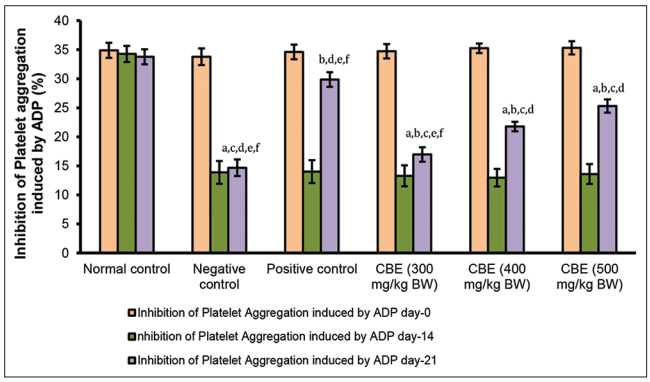

Figure 2: Increase the inhibition of platelet aggregation induced by ADP day-0, day-14, and day-21 in dyslipidemia mice (%). Data in mean ± SD. (a) p < 0.05 versus normal group at day-21, (b) p < 0.05 versus negative group at day-21, (c) p < 0.05 versus positive control at day-21, (d) nilai p < 0.05 versus CBE (300 mg/kg BW) at day-21, (e) nilai p < 0.05 versus CBE (400 mg/kg BW) at day-21, and (f) nilai p < 0.05 versus CBE (500 mg/kg BW) at day-21

On day 0, 14, and 21, the average bleeding time under normal conditions (normal control) was 53.89–54.11 s. On the 14th day, the test group that received high-fat diet induction had bleeding times ranging from 35.65 to 37.40 s. This proves that the dyslipidemia mice model has a significantly different bleeding time with normal controls. At the end of the test on day 21, the bleeding time of the test group that received atorvastatin and the three test doses ranged from 93.98 to 243.83 s, while the negative group was 38.4 s. Based on the results of statistical analysis, there was a significant difference in bleeding time on day 21 for all groups ($p < 0.05$). There was an increase in bleeding time in the positive control and CBE groups at doses of 300, 40, and 500 mg/kg BW compared to the negative group as well as the positive control ($p < 0.05$).

The coagulation time in the normal control group was 48.85 ± 1.39 s. On day 21, the average coagulation time in the group receiving atorvastatin and the test material ranged from 72.81 to 151.44 s. Based on the results of statistical analysis, there was a significant difference in the coagulation time on day 21 for all groups ($p < 0.05$). There was an increase in coagulation time in the positive control and the three test doses compared to the negative control. The positive control group had a significant difference with the CBE group at doses of 300, 400, and 500 mg/kg BW. This shows that the CBE group can increase the coagulation time but not significantly when compared to the positive control ($p < 0.05$).

The average inhibition of platelet aggregation induced by ADP in the normal group was 33.79%. The average inhibition of platelet aggregation in the

Table 1: Effect of CBE on bleeding time and coagulation time in dyslipidemia mice

Groups	Bleeding time (s)			Coagulation time (s)		
	Day-0	Day-14	Day-21	Day-0	Day-14	Day-21
Normal control	53.11 ± 1.02	53.86 ± 1.30	54.11 ± 1.72	50.10 ± 1.61	50.86 ± 1.35	48.85 ± 1.39
Negative control	53.38 ± 1.45	36.40 ± 1.43	38.40 ± 1.96	51.38 ± 1.45	33.40 ± 1.83	38.40 ± 1.96
Positive control	53.40 ± 1.93	36.73 ± 1.27	243.83 ± 2.88	50.46 ± 1.85	34.13 ± 1.42	151.44 ± 2.98
CBE (300 mg/kg BW)	53.07 ± 2.41	36.01 ± 1.24	93.98 ± 2.44	51.26 ± 1.66	34.01 ± 2.38	72.81 ± 2.81
CBE (400 mg/kg BW)	53.35 ± 1.38	35.65 ± 0.82	152.17 ± 2.88	51.28 ± 2.17	33.65 ± 1.18	92.93 ± 3.20
CBE (500 mg/kg BW)	53.32 ± 2.03	37.40 ± 1.14	204.51 ± 2.73	50.49 ± 0.71	34.40 ± 1.83	120.98 ± 2.72

CBE: Cinnamon bark extract.

positive control group and the three test groups ranged from 16.96% to 29.86%. Based on the results of statistical analysis, there was a significant difference in the inhibition of platelet aggregation on day 21 of all groups ($p < 0.05$). There was an increase in inhibition of platelet aggregation in the positive control and the three doses of CBE compared to the negative control which had inhibition of platelet aggregation by 14.67%. The positive control group had a significant difference with the CBE group at doses of 300, 400, and 500 mg/kg BW. This shows that CBE can increase the inhibition of platelet aggregation, but it is not significant when compared to positive control ($p < 0.05$).

Anti-dyslipidemia activity of CBE in dyslipidemia mice

To evaluate the anti-dyslipidemia activity of *Cinnamomum burmannii* Bl. can be done by measuring total cholesterol (TC), triglyceride (TG), LDL, and HDL level. In this study, the results of determining the anti-dyslipidemia are shown in Figure 3a-d.

In this study, the measurement of total cholesterol levels on day 0 ranged from 53.5 to 61.96 mg/dL; triglyceride levels between 39.52 and 41.8 mg/dL, LDL levels between 11.74 and 18.82 mg/dL and HDL levels between 30.48 and 39.56 mg/dL. In the group of mice fed high-fat diet to mice for 14 days, there was a significant increase in cholesterol total, triglyceride, LDL levels, and decrease in HDL. The profile showed that the mice had dyslipidemia after being fed a high-fat diet for 14 days. Administration of atorvastatin and CBE can reduce blood lipid profile in dyslipidemia mice. On the 21st day, cholesterol total at the dose group of 300, 400, and 500 mg/kg BW was 79.14 mg/dL; 72.56 mg/dL and 68.84 mg/dL, respectively. The cholesterol total results of these dose variations were significantly different when compared to the negative control average of 104.28 mg/dL, so that the administration of CBE with three different doses in dyslipidemia mice could significantly reduce total cholesterol levels in mice significant ($p < 0.05$). The triglyceride level in the dose of 300, 400, and 500 mg/kg BW was 58.16 mg/dL; 54.7 mg/dL; and 53.18 mg/dL, respectively. Triglyceride levels from these dose variations were significantly different when compared with the negative control of 63.06 mg/dL, so that the administration of CBE with three different doses in dyslipidemia mice could significantly reduce triglyceride levels in mice ($p < 0.05$). In measuring LDL

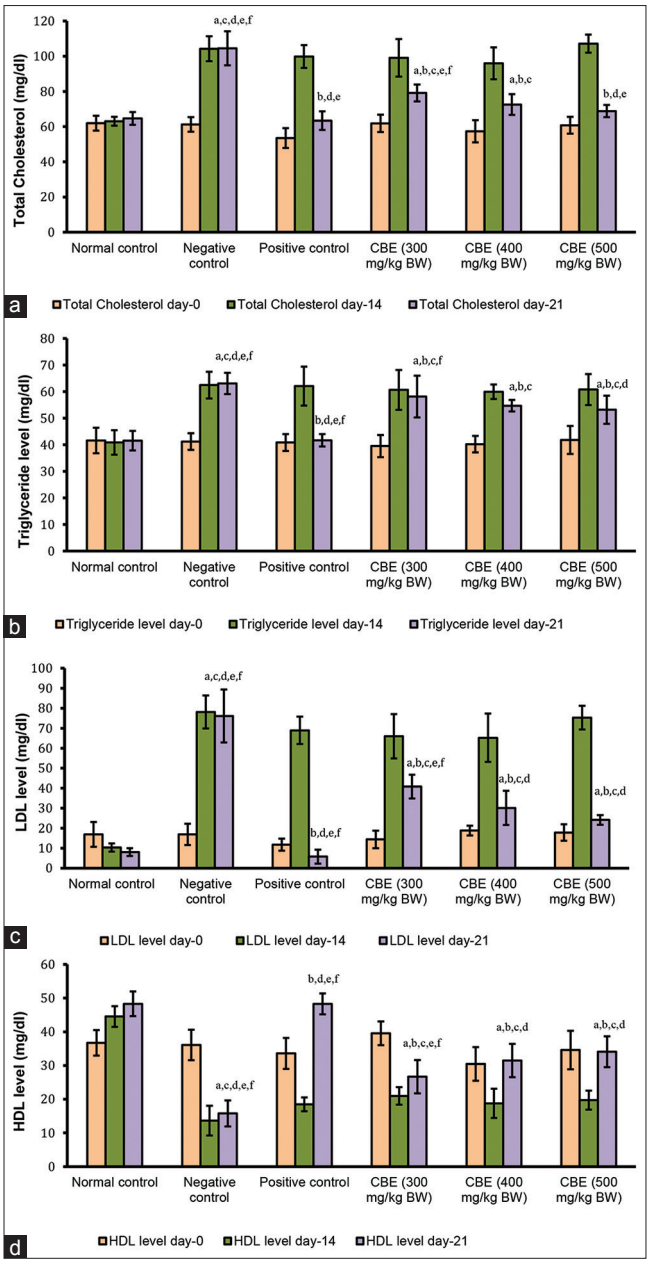

Figure 3: Anti-dyslipidemia of Cinnamomum burmannii Bl. at day-0, day-14, and day-21 in dyslipidemia mice. (A-C). Decrease the Total cholesterol level, trygliserida level, and LDL level (mg/dL). (D). Increase the HDL level (mg/dL). Data in mean±SD. (a) $p < 0.05$ versus normal group at day-21, (b) $p < 0.05$ versus negative group at day-21, (c) $p < 0.05$ versus positive control at day-21, (d) nilai $p < 0.05$ versus CBE (300 mg/kg BW) at day-21, (e) nilai $p < 0.05$ versus CBE (400 mg/kg BW) at day-21, and (f) nilai $p < 0.05$ vs CBE (500 mg/kg BW) at day-21

levels, dose of 300, 400, and 500 mg/kg BW group were 40.82 mg/dL; 30.14, and 24.12 mg/dL, respectively. The LDL levels from these dose variations were significantly

different when compared with the negative control LDL levels of 76.14 mg/dL, so that the administration of CBE with three variations of doses in dyslipidemia mice could reduce. LDL mice significantly ($p < 0.05$). HDL levels in the dose group of 300, 400, and 500 mg/kg BW were 26.68 mg/dL; 31.48 mg/dL; and 34.08 mg/dL. The HDL levels from these dose variations were significantly different when compared to the negative control average of 15.78 mg/dL, so that the administration of ECB with three different doses in dyslipidemia mice can increase HDL mice significantly. The percentage changes in the blood lipid profile in the dyslipidemia model animal (day-14) and after administration of atorvastatin and CBE (day-21) are shown in Table 2.

Discussion

Cinnamon has been used for many years to taste the foods, but also possesses great medicinal values. Several studies have shown that cinnamon could be effective and safe in treatment of serious illnesses including CVD. In this study, cinnamon bark simplicia (*Cinnamomum burmannii* Bl.) was extracted using a cold method, namely, maceration. The cold method was chosen because it is feared that there are compounds that can be damaged in heating. In addition, the maceration method also uses simple tools. The extraction used 70% ethanol because there is water content that can increase the polarity of the ethanol. The increase in polarity makes ethanol easier to penetrate cellular membranes to extract compounds from plants [27]. In addition, according to the previous studies, coumarins can be extracted well in polar solvents such as ethanol, methanol, and especially water [28] and cinnamaldehyde can be extracted well in ethanol solvent [29].

In this research can be seen that the dose of 500 mg/kgBW has the lowest MDA level, so it can be interpreted that this dose has the best ability to reduce MDA levels of the three doses. This indicates that the higher the dose of CBE given, the lower the MDA levels in mice. This can occur due to the presence of coumarin compounds, cinnamaldehyde and cinnamic acid in CBE [13], [15], [16], [17]. This finding is linear with other studies which prove that ethanol extract of *Cinnamomum zeylanicum* and *Cinnamomum cassia* increased the levels of SOD, glutathione peroxidase (GPx), and GSH in the liver of streptozotocin-induced rat [30]. Another study also reported that *Cinnamomum burmannii* (Nees and T. Nees) Blume and *Eleutherine palmifolia* (L.) Merr. extract combination can reduce MDA level and increase activity of SOD in hyperlipidemic heart of mice that experience oxidative stress [31]. Quercetin and kaempferol from *C. burmannii* also act as powerful natural antioxidants [32]. Hydrogen donation is the main mechanism of phenolics as antioxidants. The lower strength of the O–H bond present in phenolics corresponds to a higher scavenging activity. Quercetin and kaempferol identified in *Cinnamomum* species have a C2-C3 double bond and a C-3 hydroxyl group, while the most active hydroxyl groups are those attached to the C4 and C3 positions [33].

In this study, the measurement of SOD activity in the negative control group was the lowest compared to the other test groups. This can happen because the mice in the negative control group that only received high-fat diets experienced oxidative stress. The free radicals formed in the mice in the negative control group were neutralized by the natural SOD enzyme, resulting in a decrease the activity of the enzymatic antioxidant (endogenous antioxidant). The SOD activity in the positive control group (atorvastatin) showed an increase in activity. This is because the administration of atorvastatin can act as a secondary antioxidant that works by donating electrons to free radicals. Due to the addition of exogenous antioxidants, endogenous antioxidants are not used too much. Therefore, in positive group, the SOD activity is high [24].

This study also assessed the anti-platelet aggregation activity of *Cinnamomum burmannii* Bl. to help reduce the risk of CVD. Among traditional medicine, cinnamon extract has anticoagulant and antiaggregation properties for the platelets. Bleeding time was observed to see the effect of the test material on the formation of a temporary hemostatic plug, namely, the hemostasis process of the platelet phase. The time from the onset of injury to the formation of a temporary hemostatic plug in the injured area is called the bleeding time or mass. Based on the previous research, the normal range of bleeding time in mice is 45.17–62.67 s [34]. In another study, it was also reported that the normal range of bleeding time in mice ranged from 52.47 to 54.94 s [25]. In this study, the bleeding time in the normal control group was 48.85 ± 1.39 s. This is quite in accordance with the results obtained in the previous studies. The increase in bleeding time, especially in the positive control

Table 2: Percentage of changes in lipid profile at dyslipidemia mice (day-14) and after treatment (day-21)

Groups	TC		TG		LDL		HDL	
Increase TC Day-14 (%)	Increase TG Day-14 (%)	Decrease TC Day-21 (%)	Increase TG Day-14 (%)	Decrease TG Day-21 (%)	Increase LDL Day-14 (%)	Decrease LDL Day-21 (%)	Decrease HDL Day-14 (%)	Increase HDL Day-21 (%)
Normal control	1.78	2.57	1.78	1.66	38.86	21.90	21.23	8.44
Negative control	70.17	0.23	51.55	0.99	361.82	2.56	62.22	15.69
Positive control (atorvastatin)	86.65	36.51	52,.01	32.86	487.39	91.20	45.00	161.26
CBE (300 mg/kg BW)	60.25	20.14	53.44	4.09	359.11	38.17	47.02	27.29
CBE (400 mg/kg BW)	67.42	24.42	48.96	8.74	246.65	53.80	38.45	67.80
CBE (500 mg/kg BW)	76.31	35.76	45.41	12.50	322.45	67.96	42.95	72.64

HDL: High-density lipoprotein, LDL: Low-density lipoprotein, CBE: Cinnamon bark extract, TC: Total cholesterol, TG: Triglyseride.

group, was caused by the mechanism of action of the test material which causes irreversible inhibition of the P2Y12 receptor on platelets so that the ADP response to platelet aggregation is reduced [35].

The time of coagulation or blood clotting aims to see the effect of the test material on the secondary hemostatic plug, namely, the hemostasis process in the coagulation phase where fibrinogen will turn into insoluble fibrin and close the surface of the platelet plug [36]. In this study, the coagulation time in the normal control group was 48.85 ± 1.39 s. This is in accordance with the previous research, the normal range of coagulation time in mice is 43.18–49.38 s [34]. In the test of coagulation time, it was seen that there was an increased the coagulation time in the positive control of atorvastatin. This is because the loss of tissue factor procoagulant activity is associated with loss of plasma P-selectine [35], [37].

Platelets in plasma will form aggregations when induced by ADP. The plasma before the addition of ADP showed turbidity of plasma containing unaggregated platelets and was measured on a spectrophotometer resulting in initial plasma uptake. After the addition of ADP, plasma absorption will decrease because the platelets in the plasma begin to form platelet aggregates and then settle so that plasma turbidity decreases. The administration of compounds that have ability to inhibit platelet aggregation causes these inhibitory compounds to inhibit the work of ADP so that little aggregation is formed [38]. In this study, the inhibition of platelet aggregation induced by ADP in the normal group was 33.79%. Based on the previous research, the normal range for decreasing plasma absorption is 34.29% [25]. There is a closeness in the value of decreasing plasma uptake in the two studies. In this test, it was seen that there was a decrease in plasma absorption, especially in the positive control group and CBE at a dose of 500 mg/kg BW. The increase in the decrease of plasma absorption is due to the mechanism of action of atorvastatin which causes irreversible inhibition of the P2Y12 receptor on platelets so that the ADP response to platelet aggregation is reduced [35], [37]. In the CBE group at doses of 300, 400, and 500 mg/kgBW there was an increase in bleeding time, an increase in coagulation time, and an increase in a decrease in plasma absorption. This indicates the presence of antiplatelet activity in CBE which is thought to be derived from coumarin, volatile oil and flavonoid content. Coumarins are Vitamin K antagonists that have pharmacological activity as anticoagulants, coumarins inhibit prothrombin synthesis and prevent the formation of blood clotting factor preparations by inhibiting hepatic synthesis of 4 coagulation proteins that depend on vitamin K (factors II, VII, IX, and X). Flavonoids can inhibit platelet aggregation by inhibiting the cyclooxygenase metabolic pathway, essential oils can increase the antiplatelet effect by inhibiting platelet aggregation by induction of ADP (adenosine-5-diphosphate) [17]. Other studies mention other Cinnamomum species, namely, C. tamala which contains Cinnamaldehyde, eugenol, -linalool, cinnamyl acetate, cinnamic acid, coumarin useful for curing and treating various diseases such as reducing the oxidative damage and useful as anti-coagulant agent, CVDs and used to induce blood circulation [39]. The study of cinnamon components showed that eugenol, cinnamaldehyde, 2-hydroxycinnamaldehyde, 2 methoxycinnamaldehyde have the most antiaggregatory effect. This research showed that some of the cinnamon ingredients have mild anticoagulant effects and inhibit platelet aggregation [40]. The other review also shown that eugenol and cinnamaldehyde can inhibit platelet aggregation induced by various agonists (especially arachidonic acid) by inhibiting thromboxane A2 (TXA2). Cinnamaldehyde also can inhibit *in vitro* platelet aggregation induced by collagen, thrombin, arachidonic acid, and ADP. Cinnamaldehyde represses the release of arachidonic acid (AA) from platelet membrane phospholipids and then decreases thromboxane A2 production. Cinnamaldehyde markedly prolonged the bleeding and coagulation times in mice. Antithrombotic effects and prolong tail bleeding time of mice from cinnamaldehyde were also demonstrated *in vivo*. Cinnamaldehyde may be prohibition of thrombosis formation *in vivo* due to inhibition of platelet aggregation and the mechanism may be associated with interaction of platelet and smooth muscle cell through platelet derived TXA2, a potent agonist for platelet activation and clot formation [41], [42].

Furthermore, it would be suggested that cinnamon can reduce other risk factors associated with CVD, such as hyperlipidemia. In this regard, it was shown, increased HDL cholesterol levels and decreased the concentration of triglyceride and LDL cholesterol levels in administration of cinnamon to hypercholesterolemia rats [43]. In the present study, administration of ECB with three different doses in dyslipidemia mice could significantly improve the lipid profile of mice. The results of this study were linear with the results of other studies which also showed C. cassia reduced lipid and lipoprotein profiles. Cinnamon consumption of 4 g/kgBW for 30 days reduced total cholesterol, TGs, and LDL [43]. In another study, The administration of 225 mg/kg *E. palmifolia and* 75 mg/kg *C. burmannii* for 120 days significantly neutralization of oxidative stress, and normalization of lipid and lipoprotein levels, in hyperlipidemic mice [31].

Several studies have supported the antihyperlipidemic mechanisms *C. burmannii* extract. Quercetin from *C. burmannii* can inhibit cholesterol synthesis by inhibiting the activity of HMG-CoA reductase [44]. Moreover, tannic acid and gallic acid have a high affinity for cholesterol [45]. *C. burmannii* extract can act as an antihyperlipidemic agent due to the high amount of cinnamaldehyde in the bark. The level of cinnamaldehyde in cinnamon bark is about 65–80% and eugenol which is about 5–10% in the bark [31].

This hypothesis is supported by previous research which found that the addition of 2% cinnamaldehyde to the diet for 5 weeks significantly reduced the BW of obesity-induced mice. This finding also showed a reduction of total cholesterol and triglyceride resulting from cinnamaldehyde compound [46].

Hyperlipidemia is associated with increased platelet activity following various agonists and procoagulant state. It seems that cholesterol plays a critical role in activating of platelets [47]. Therefore, platelets of hypercholesterolemic patients with high levels of LDL exhibit high aggregability and enhanced activity [48]. Platelets are activated by native LDL (nLDL) through apoE Receptor and Oxidized LDL through CD36 and scavenger receptor-A (SRA) [49]. Absorption of cholesterol by the platelets is associated with increased sensitivity to epinephrine and ADP. On the other hand, hypercholesterolemia is associated with high mean platelet volume (MPV) and low platelet count and platelets are more susceptible to activation [50]. The cinnamon water extract also reducing the expression of CD11b, CD36, and SRA and then inhibits differentiation of monocytes into macrophages [51] so indirectly reduces foam cells formation and ultimately limits the formation of atherosclerotic plaque. Cinnamon decreases triglyceride, LDL cholesterol, and total cholesterol and increases HDL cholesterol in serum. Since LDL and Ox-LDL are high affinity ligands for CD36 which lead to platelet activation, a reduction in LDL levels by cinnamon, decreases platelet activation. In addition to cinnamon extract, different cinnamon compounds have inhibitory effects on platelet function and lipid levels. Since lipid lowering effect of cinnamon can ultimately lead to decreased platelet activation, it seems that, both mechanisms associated with a reduction of risk for CVD.

Conclusions

The CBE has antioxidant activity by decreasing the concentration of MDA and increasing the activity of SOD, anti-platelet activity by prolong bleeding time and coagulation time, and the higher dosage of extract of cinnamon bark will give greater ability in lowering total cholesterol, triglycerides, LDL levels, and increasing HDL levels. Therefore, *Cinnamomum burmannii* Bl. has the potential to prevent CVD.

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