



## ARTICLE

### EFFECTS OF ETHANOL EXTRACT OF CINNAMON BARK (*Cinnamomum burmanii*) ON THE LIPID PROFILE AND MALONDIALDEHYDE OF DYSLIPIDEMIC RATS

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#### ABSTRACT

Dyslipidemia is a risk factor for atherosclerosis. Dyslipidemia conditions and increased malondialdehyde (MDAs) can cause oxidative stress. Cinnamon contains antioxidant compounds that have a hypolipidemic effect. This study aimed to determine the effect of cinnamon bark ethanol extract on triglycerides, total cholesterol, LDL, HDL, and MDA in dyslipidemic rats. In this study, rats were divided into five groups, a negative control group: which received standard feed, a positive control group: which received a high-fat diet and PTU, the groups that received a high-fat diet, PTU, cinnamon bark ethanol extract (EECB) 125 mg/Kg BW, 250 mg/Kg BW, 500 mg/Kg BW. The results of the analysis showed a decrease in triglyceride levels in all groups given EECB ( $p < 0.05$ ). Total and LDL cholesterol levels decreased in the 125 mg/Kg BW EECB group, and malondialdehyde decreased in the 500 mg/Kg BW EECB group ( $p < 0.05$ ). HDL levels did not increase. The ethanol extract of cinnamon bark was able to improve lipid profiles because it contains cinnamaldehyde and quercetin that can inhibit HMG CoA reductase activity, as well as flavonoids, tannins, and cinnamate can reduce triglyceride and MDA levels.

**Keywords:** cinnamon, dyslipidemia, lipid profile, malondialdehyde,

#### АБСТРАКТ

Дислипидемия является фактором риска развития атеросклероза. Состояние дислипидемии и повышенный уровень малондиальдегида (МДА) могут вызывать окислительный стресс. Корица содержит антиоксидантные соединения, обладающие гиполипидемическим действием. Целью данного исследования было определить влияние этанолового экстракта коры корицы на триглицериды, общий холестерин, ЛПНП, ЛПВП и МДА у крыс с дислипидемией. В этом исследовании крысы были разделены на пять групп, группа отрицательного контроля: получавшая стандартный корм, группа положительного контроля: получавшая высокожировую диету и РТУ, группы, получавшие высокожировую диету, РТУ, этаноловый экстракт коры корицы (ЕЕСВ) 125 мг/кг БВ, 250 мг/кг БВ, 500 мг/кг БВ. Результаты анализа показали снижение уровня триглицеридов во всех группах, получавших ЕЕСВ ( $p < 0,05$ ). Уровни общего холестерина и холестерина ЛПНП снизились в группе 125 мг/кг БВ ЭЭКБ, а малондиальдегида - в группе 500 мг/кг БВ ЭЭКБ ( $p < 0,05$ ). Уровень ЛПВП не увеличился. Этанольный экстракт коры корицы способен улучшать липидный профиль, поскольку содержит циннамальдегид и кверцетин, которые могут ингибировать активность НМГ СоА редуктазы, а также флавоноиды, танины и циннамат, способные снижать уровень триглицеридов и МДА.

**Ключевые слова:** корица, дислипидемия, липидный профиль, малондиальдегид.

## INTRODUCTION

Dyslipidemia is still a health problem throughout the world and is a risk factor for atherosclerosis cardiovascular disease. Dyslipidemia is a condition characterized by increased levels of triglycerides (TG), decreased levels of high-density lipoprotein (HDL), and increased low-density lipoprotein (LDL).<sup>1,2,3</sup>

Increased levels of LDL cholesterol are the most dominant cause of fat deposition on the artery walls, causing atherosclerosis.<sup>3</sup> In physiological conditions there is a state of balance between free radicals and antioxidants in the body. When there is a disruption in the balance between free radicals and antioxidants, a condition called oxidative stress occurs. LDL-cholesterol is easily oxidized by free radicals such as reactive oxygen species (ROS). The results of lipid peroxidation can trigger the formation of malondialdehyde (MDA). Increased plasma MDA can cause oxidative stress in atherosclerosis. Malondialdehyde is used as a biomarker of oxidative stress.<sup>4</sup> In addition to LDL cholesterol, triglycerides are also atherogenic because they play a role in causing the risk of atherosclerosis.<sup>5</sup>

The target in the management of dyslipidemia is to improve the lifestyle by engaging in physical activity, adjusting dietary patterns, and improving plasma lipid profiles.<sup>2</sup> Improvement of plasma lipid profiles can reduce LDL cholesterol, and triglyceride levels, and increase HDL cholesterol levels.<sup>1</sup> Natural ingredients have been used for a long time by the people of Indonesia to prevent or treat disease. Antioxidants in natural products have been widely studied to fight the effects of free radicals as well as being hypolipidemic because they can lower cholesterol and triglycerides. One of the natural ingredients known to be beneficial for health that has antioxidant effects and can reduce plasma lipid levels is cinnamon.<sup>6,7</sup> In general, the part of the cinnamon plant that is used as herbal medicine is the bark of the stem. The cinnamon bark contains cinnamaldehyde,

cinnamate, cinnamic acid, essential oils, eugenol, safrole, cinnamaldehyde, cinnamyl acetate, quercetin, tannins, polyphenols, and flavonoids.<sup>6,8</sup> The main compounds in the ethanol extract of cinnamon bark are cinnamaldehyde and eugenol. They can suppress lipid peroxidation so they can prevent lipid peroxidation and reduce MDA levels.<sup>9</sup> Cinnamaldehyde, quercetin, and polyphenols in cinnamon provide antihyperlipidemic effects. Cinnamaldehyde lowers plasma cholesterol levels by inhibiting HMG-CoA reductase activity.<sup>7,10,11</sup> Various antioxidant compounds in cinnamon bark dissolve in ethanol extract preparations which have a strong effect as antioxidants.<sup>12</sup> In this study, giving a high-fat diet and PTU can disrupt fat metabolism resulting in dyslipidemia. Giving EECB doses of 125 mg/Kg BW, 250 mg/KgBW, and 500 mg/Kg BW can improve plasma lipid profiles and reduce MDA levels in dyslipidemia.<sup>13</sup>

This study aimed to determine the effect of ethanol extract of cinnamon bark at doses of 125 mg/Kg BW, 250 mg/Kg BW, and 500 mg/Kg BW on triglycerides, total cholesterol, LDL cholesterol, HDL, and MDA levels in dyslipidemic rats.

## MATERIAL AND METHODS

This research is an experimental laboratory research with a post-test design. This research has received permission from the Health Research Ethics Commission, Faculty of Medicine, Universitas Padjadjaran No 1186/UN6.C.10/PN/2018.

### Tools and Materials

The tools used in this study were rat cages, rat feed, and drinking places, 18G of feeding tubes, scales, gloves, measuring cups, and scalpels. The tools and consumables used to check total cholesterol, cholesterol, and MDA levels include micro-hematocrit capillary tubes, centrifuge tubes, digital balances, cuvettes, micropipettes, test tubes, water baths, spectrophotometer, tissue, and gloves.

The materials used were cinnamon extract at a dose of 125 mg/KgBW/day, 250

mg/KgBW/day, and 500 mg/KgBW/day, standard pellets for rat feed, high-fat diet feed, water for rats to drink, Propyl Thio Uracyl (PTU) 0.01%, and MDA test kit butylated hydroxytoluene (BHT) solution, Sodium Dodecyl Sulphate (SDS) solution, ethylenediaminetetraacetic acid disodium salt dehydrate (Na<sub>2</sub>EDTA) solution, acetic acid, thiobarbituric acid (TBA) solution, 0, 8% 1000 µL and CHOD-PAP reagent, and 10 µl standard reagent, triglyceride test reagent, LDL test reagent.

### Animal Models

In this study, the inclusion criteria for the experimental animals were male white rats of the Wistar strain, body weight 200-250 grams, 2-3 months old, and healthy. Experimental animals were kept by following the rules and procedures in the Experimental Animal Laboratory under the coordination of the Study Center of the Faculty of Medicine, Universitas Jenderal Achmad Yani Cimahi Indonesia. The experimental animal laboratory consists of two animal maintenance rooms, one service room with room temperature ranges from 26–28 °C, exhaust fans, lamps, and sunlight from closed windows. The rat cages used were 30 x 50 x 15 cm in size with 5 rats per cage. Standard feed was given 10% of body weight or around 20-25 grams/head/day as well as drinking water ad libitum. Animal maintenance was carried out according to Standard Operational Procedures that apply in the Experimental Animal Laboratory.

Rats used in this study were acclimatized for 7 days in animal cages at the Experimental Animal Laboratory, Faculty of Medicine Universitas Jenderal Achmad Yani Cimahi Indonesia. After acclimatization, rats were weighed to meet the inclusion criteria. Rats were randomly divided into 5 groups, where the number of rats in each group was 5. Hyperlipidemia in rats was induced by administering a high-fat diet and propylthiouracil (PTU).

The positive control group (PC) was fed with standard pellets for 28 days. The

Negative Control Group (NC) was fed with standard pellets; given a high-fat diet and PTU orally for 28 days. Dosage group 1 (D1) was fed with standard pellet, high-fat diet, PTU for 14 days, then on days 15-28 was given ethanol extract of cinnamon bark at a dose of 125 mg/kgBW orally. Dosage group 2 (D2) was given standard feed, high-fat diet, and PTU for 14 days then on days 15-28 was given ethanol extract of cinnamon bark at a dose of 250 mg/kgBW orally. Dosage group 3 (D3) was fed with a standard pellet, given a high-fat diet, PTU for 14 days, and on days 15-28 was given ethanol extract of cinnamon bark at a dose of 500 mg/kg BW orally. On the 29th day, blood was taken from the rat's orbital vein to check levels of triglycerides, total cholesterol, LDL cholesterol, HDL cholesterol, and plasma MDA.

### Production of Ethanol Extract of Cinnamon Bark

Simplicia powder is the initial process of making extracts. The production of bark cinnamon powder was done by drying it with a tool that didn't damage the chemical content in it until the powder with a certain degree of fineness can be obtained. Extract from simplicia dry powder then macerated using 96% ethanol solvent. Nine hundred grams of Simplicia dry powder were put into the macerator, soaked for the first 6 hours while occasionally stirred, then let stand for 18 hours. Next maserate then separated by centrifugation, decantation, or filtration. The screening process was repeated at least once with the same type of solvent and the volume of the solvent was half the volume of the solvent in the first screening. All collected fibers were then steamed with a vacuum evaporator, low-pressure evaporator, or can use "rotavapor" until the thick extract was obtained.

### Cholesterol Measurements

The total cholesterol was measured using the colorimetric enzymatic method (CHOD/PAP) because it was easy, and the results were precise and thorough. The blank

tube consists of 1.000 ul of reagent blank solution, the standard tube consists of 1.000 ul of reagent solution and 10 ul of standard solution, and the sample tube consists of 1.000 ul of reagent and 10 ul of sample plasma. The tubes were then mixed until homogeneous, incubated at 37°C for 10 minutes, and read the absorption of standard and sample against the blank using a spectrophotometer at a wavelength of 492-546 nm. The color formed was stable for 30 minutes.

#### Triglyceride (TG) Measurements

The triacylglyceride level was measured using the enzymatic method because it was easy, and the results were precise and more thorough. The blank tube consists of 1.0 ml of reagent solution, the standard tube consists of 1 ml of reagent solution and 10 ul of standard solution, and the sample tube consists of 1 ml of reagent and 10 ul of sample plasma. The tubes were then mixed until homogeneous, left at room temperature for 20 minutes or at 37°C for 10 minutes, and read the absorption of standard and sample against the blank using a spectrophotometer at a wavelength of 520-546 nm.

#### MDA Measurements

The material for MDA measurements was plasma. SDS, BHT, acetic acid EDTA, and TBA were added to the plasma and then incubated at 100°C in a water bath for 10 minutes and closed each of the tubes. Each tube was immersed in an ice water bath, then centrifuged at 3000 rpm for 10 minutes. The supernatant was taken from each tube and then read the absorbance using a spectrophotometer at a wavelength of 532 nm.

#### Data Analysis

The data were analyzed statistically to assess the average levels of total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, and MDA for each group. Before the different tests were carried out, all data were tested for normality and homogeneity.

Furthermore, normally distributed and homogeneous data were analyzed using the one-way ANOVA test and the Tukey post hoc follow-up test.

## RESULTS

Dyslipidemia in experimental animals was induced by giving a high-fat diet which resulted in increased triglyceride and cholesterol levels. Propylthiouracil (PTU) administration was aimed to increase blood cholesterol concentrations by increasing endogenous cholesterol biosynthesis so the rat's cholesterol levels can increase.

Lipid profile levels of rats induced by high fat, PTU, and ethanol extract were shown in Table 1. The highest mean triglyceride levels were seen in the positive control group at a dose of 149.07 mg/dL, and the lowest was in the group that received ethanol extract of cinnamon bark at a dose of 125 mg/dL. The data from the triglyceride measurement results were normally distributed but not homogeneous so the differences between the treatment groups were assessed by the Kruskal-Wallis test. There was a significant difference between each group with  $p < 0.05$ . The Mann-Whitney post hoc test was carried out and the results showed that triglycerides decreased significantly in all groups that received EECB compared to the positive control group.

The highest mean total cholesterol level was in the positive control group and the lowest was in the group receiving EECB at 125 mg/Kg BW. The data were normally distributed and homogeneous, then the different test was carried out using the one-way ANOVA test. There was a significant difference between each group with  $p < 0.05$ . Tukey's post hoc test showed that an EECB dose of 125 mg/Kg BW could significantly reduce total cholesterol compared to the positive control group.

The highest mean LDL cholesterol level was in the positive control group. The data were normally distributed and homogeneous, then the different test was carried out using the one-way ANOVA test. There was a

significant difference between each group with  $p < 0.05$ . Follow-up tests using post-Hoc Tukey showed that there was a significant decrease in LDL cholesterol levels in the EECB group at a dose of 125 mg/Kg BW compared to the positive control group.

The results of HDL cholesterol measurement showed that the highest mean HDL level was in the group that received EECB at a dose of 500 mg/Kg BW and the lowest was in the group that received EECB at a dose of 125 mg/Kg BW. The results of the ANOVA test

showed no significant difference in HDL levels between all groups with  $p < 0.05$ .

From the results of this study, the highest mean levels of MDA were seen in the positive control group and the lowest mean levels of MDA were seen in the group that received EECB at a dose of 500 mg/Kg BW. The results of the ANOVA test showed that there was a significant difference in MDA levels between groups. Follow-up test with post-Hoc Tukey showed MDA levels decreased in the 500 mg/Kg BW EECB group compared to the positive control group.

**Table 1 Plasma Lipid and MDA Profiles of Rats Induced by a High-Fat Diet**

Group	TG	Total Cholesterol	LDL	HDL	MDA
NC	79.21 ± 8.40	78,86 ± 3,89	65.58 ± 4.10	13,29 ± 0,95	0.1302 ± 0.013
PC	149.07 ± 21.98	93.51 ± 2,64	81.00 ± 7.24	12,51 ± 7,37	0.2446 ± 0.029
D1	77.61 ± 8.76	76,28 ± 1,55	67.45 ± 3.64	8,83 ± 4,74	0.2120 ± 0.024
D2	85.52 ± 3.52	81,56 ± 11,48	68.41 ± 10.38	13,15 ± 1,56	0.1898 ± 0.021
D3	88.57 ± 8,67	85,95 ± 7,33	71.46 ± 6.89	14,49 ± 1,54	0.1534 ± 0.010
<i>p-value</i>	<i>0.005</i>	<i>0.004</i>	<i>0.017</i>	<i>0,201</i>	<i>0.000</i>

Remarks: NC Negative Control, PC Positive Control. D1 EECB dose of 125 mg/Kg BW, D2 EECB dose of 250 mg/Kg BW, D3 EECB dose of 500 mg/Kg BW

## DISCUSSION

The decrease in triglyceride levels by EECB is due to the presence of antioxidants. Antioxidants that play a role in lowering triglyceride levels are polyphenols. The polyphenol is Methylhydroxy Calcone Polymer (MHCP). MHCP on lipid metabolism influences adipose cells by inactivating Hormone Sensitive Lipase (HSL) and increasing lipoprotein lipase (LPL) synthesis.<sup>3</sup> HSL inactivation will inhibit the hydrolysis of TG deposits in adipose cells into FFA so that the amount of free fatty acid (FFA) in circulation will decrease. If FFA in the blood decreases, then the amount of FFA that will enter the liver will decrease, so the process of esterification of FFA to TG will decrease. The result is a decrease in the secretion of Very Low-Density Lipoprotein (VLDL) by the liver into the blood circulation. Increased LPL enzyme synthesis will increase the hydrolysis of chylomicrons to become remnant chylomicrons and increase the hydrolysis of

VLDL to Intermediate Density Lipoprotein (IDL). The result is a decrease in the number of VLDL and chylomicrons in the blood so that the concentration of TG in the blood also decreases. This is because TG is the main lipid component that composes chylomicrons and VLDL. In addition, the flavonoid and tannin compounds contained in cinnamon sticks provide a hypolipidemic effect. This effect is known because flavonoids and tannins can inhibit the absorption of fat in the intestine, this can cause triglyceride and cholesterol levels to decrease in plasma.<sup>3,14</sup>

Cholesterol in the body comes from two sources, namely half by synthesis by the body itself and the other half comes from fat consumed from the daily diet. HMG CoA reductase is an enzyme that plays an important role in the synthesis of cholesterol in the body. Inhibition of HMG CoA reductase activity can lead to lower plasma cholesterol levels. Antioxidant compounds that influence reducing LDL levels in the blood in the

cinnamon extract are flavonoids and Cinnamaldehyde. Flavonoid compounds in the form of quercetin will inhibit the HMG-CoA reductase enzyme in the liver, thereby reducing cholesterol synthesis.<sup>7,10</sup> The reduced synthesis of cholesterol in the liver has an impact on the formation of VLDL in the liver which will decrease. The decreased formation of VLDL causes the synthesis of LDL cholesterol in plasma to decrease because cholesterol is the most important element in the LDL cholesterol fraction. In addition, the role of flavonoids and other antioxidants in cinnamon sticks is to prevent the oxidation process of low-density lipoprotein (LDL) by acting as radical-scavenging antioxidants and lipophilic antioxidants, which are able to scavenge free radicals before free radicals attack cell membranes which are composed of lipids. LDL cholesterol is a compound that is susceptible to oxidation, if LDL is oxidized by free radicals, foam cells will form which can lead to the formation of atherosclerotic plaques and a high risk of developing coronary heart disease.<sup>4</sup>

Oxidized LDL can trigger the formation of other free radical molecules in the form of malondialdehyde. Thus, an increase in LDL cholesterol levels can cause an increase in plasma MDA levels. In the body, various antioxidant enzymes can fight free radicals, but if the number of free radicals exceeds the ability of these antioxidants, conditions of oxidative stress can occur.<sup>15,16</sup> An increase in MDA compounds is a biomarker of oxidative stress that occurs in the body.<sup>16</sup> Antioxidants found in cinnamon include cinnamaldehyde, quercetin, flavonoids, tannins, and cinnamates. The antioxidants in the ethanol extract of cinnamon bark work against free radicals resulting from oxidation of plasma lipids, so that EECB given to dyslipidemic rats can reduce MDA levels.<sup>11,12</sup> Ethanol extract of cinnamon bark has a high content of polyphenols and flavonoids which act as secondary antioxidants which work as radical-scavenging antioxidants and lipophilic antioxidants, which are able to scavenge free radicals before free radicals attack cell

components such as free lipids and prevent chain reactions from occurring so that no damage occurs. further such as oxidative stress.<sup>7</sup> The antioxidant effect of EECB is expected to reduce plasma lipid oxidation and the risk of developing atherosclerosis.<sup>10</sup> Antioxidants in the EECB in this study have been shown to reduce plasma lipid levels and MDA.

## CONCLUSION

The effect of ethanol extract of cinnamon bark (*Cinnamomum burmanii*) on lipid profiles, namely triglycerides, total cholesterol, LDL cholesterol, HDL cholesterol, and plasma MDA has been proven in this study. Giving EECB to dyslipidemic rats showed that the group that received EECB doses of 125 mg/Kg BW, 250 mg/Kg BW, and 500 mg/Kg BW ( $p < 0.05$ ) can decrease TG levels. Total cholesterol and LDL cholesterol decreased significantly in the group that received an EECB dose of 125 mg/KgBW. Meanwhile, MDA levels decreased significantly in the group that received EECB at a dose of 500 mg/Kg BW. HDL levels did not differ between treatment groups.

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