

Cacao beans extract (*Theobroma cacao* L.) improve lipid profile but had no effect on blood NOx concentration in dyslipidemia white male rat (*Rattus norvegicus*)

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## ABSTRACT

**Background:** Dyslipidemia is a disorder of lipoprotein metabolism, including overproduction of total cholesterol, Low-Density Lipoprotein (LDL), triglyceride and deficiency High-Density Lipoprotein (HDL), which cause decreased level of antioxidant enzyme and lead to oxidative stress, marked with increased of lipid peroxidation. Oxidative stress decreases blood nitrate and nitrite (NOx) which are the metabolites of nitric oxide (NO). Cacao beans extract rich in flavanols antioxidants such as catechin, epicatechin, and procyanidin. **Objective:** The aim of this experiment is to evaluate the effects of cacao beans extract (*Theobroma cacao* L) to blood profile lipid and NOx of dyslipidemia white rat (*Rattus norvegicus*). **Methods:** This experiment was a pretest-posttest control group design. Rats divided into four groups P0 control group only given high cholesterol food, P1 given 70 mg, P2 given 140 mg and P3 given 280 mg of cacao beans extract. **Results:** The administration of cacao beans extract significantly decreased (p=0,000) total cholesterol, triglyceride and LDL in group P1, P2 and P3 and significantly (p=0,000) increased HDL, although not significantly increased NOx in P1, P2, and P3 (p=0,486). **Conclusion:** Cacao beans could inhibit oxidative stress showed significantly decreased total cholesterol, triglyceride, LDL and significantly increased HDL but not able increased blood NOx concentration in dyslipidemia white rat.

**Keywords:** Psychosocial stress, oxidative stress, cacao beans extract, NOx

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## INTRODUCTION

The incidence of cardiovascular diseases which often lead to death is increasing even in developing countries such as Indonesia. Cardiovascular disease not only occur in elderly but the incidence in adult and the young adult population also increased. It supported by the data of the Household Health Survey, 2004, that as many as 2.2% of Indonesia's population aged 15 years or older suffer from cardiovascular disease.<sup>1</sup>

Coronary heart disease (CHD) is a cardiovascular disease that causes most deaths, caused by interruption of blood flow to the heart muscle due to the narrowing of coronary arteries and atherosclerosis due to dyslipidemia.<sup>2</sup> Dyslipidemia is a disorder of lipid metabolism characterized by elevated levels of total cholesterol, triglycerides, low-density lipoprotein (LDL) and decreased levels of high Density Lipoprotein (HDL), causing a decrease in antioxidant enzymes and increased lipid peroxidation, which

have roles in the process of atherosclerosis.<sup>3</sup> Study in mice showed an induction prooxidant and increased lipid peroxidation in mice that received a diet high in cholesterol.<sup>4</sup>

Dyslipidemia is a condition that includes the increase in total cholesterol, LDL, triglycerides, and or decline of HDL levels. In rats normal levels of rat's total cholesterol is 10-54 mg / dL<sup>5</sup>, normal levels of rat's triglycerides is 26-145 mg / dL.<sup>6</sup> Increase in cholesterol achieved within two weeks, said dyslipidemia in case of weight gain > 20% or cholesterol total serum > 240 mg / dL.<sup>7</sup>

Some studies have been conducted to evaluate the effects of various antioxidants on oxidative stress and lipid profiles. Vitamin E supplementation significantly lowered lipid peroxidation and increased total antioxidant status in plasma.<sup>8</sup> Supplementation of vegetable juice had also been proven to lower triglyceride levels, the ratio of HDL / LDL blood and compounds malondialdehyde (MDA), as well as increased the activity of serum antioxidant enzymes such as glutathione peroxidase and superoxide dismutase.<sup>9</sup> Epidemiological research indicates foods high in beta carotene and vitamin E lowers the risk of atherosclerosis by preventing the oxidation of LDL. Beta-carotene and zeaxanthin also

prevent lipid peroxidation in liposomes.<sup>10</sup> Also, increased oxidative stress is proportional to a decrease in antioxidant activity and the accumulation Reactive Oxygen Species (ROS) which cause endothelial dysfunction.<sup>11,12</sup>

Nitric oxide is an endothelium-derived relaxing factor (EDRF) for the relaxation of the vascular smooth muscle, causing vasodilation of blood vessels and increases the blood flow.<sup>13</sup> NO in the network formed by L-arginine which substrate for enzyme endothelial nitric oxide synthase (eNOS) with NADPH as a cofactor, oxygen (O<sub>2</sub>) and tetrahydrobiopterin (BH<sub>4</sub>) to produce L-citrulline as well as nitrate and nitrite as intermediate.<sup>14,15</sup> NO metabolites that not used were oxidized into nitrite. Then, if the demand increase, nitrite within the tissue will reduce back to NO, a process catalyzed by the enzyme xanthine oxidase (XO). Because of their stability, the total serum levels of nitrite and nitrate (NOx) used as an indicator of body NO synthesis rate.<sup>15</sup>

The cacao bean (*Theobroma cacao* L.) is one of the several sources of natural antioxidants that widely cultivated in Indonesia. Cacao bean or chocolate is food that rich in flavonoid antioxidants. The flavonoid contained in the cacao bean are a monomer flavan-3-ol (flavanols) which include epicatechin and catechin as well as procyanidin oligomers such as flavanols. Consumption of foods rich in flavonoids has been shown to have health benefits for the heart and blood vessels.<sup>16</sup> The content of flavanols in cacao beans is varied quantitatively depending on the source and the fermentation process, but it also quantitatively different due to differences in the climate where the cacao plants grow.<sup>17</sup>

The benefits of cocoa beans have widely studied, but efficacy in addressing the state of oxidative stress caused by dyslipidemia still needs to be done further research, especially cocoa beans originated from areas of Bali. Therefore, this study aimed to determine the effect of cocoa bean (*Theobroma cacao* L.) extract on the lipid profile and blood NOx level of dyslipidemic male rats.

## METHODS

This research is a purely experimental design with pre and posttest control group design. The study was three months which lasted from June 2012 to August 2012. The study takes places in Department of Pharmacology and Biochemistry Medical Faculty Udayana University. The population in this study is a white male Wistar rats aged 4-5 months obtained from the Laboratory Animal Unit (ALU) Department of Pharmacology Medical Faculty Udayana University. The number of the sample determined by the formula Pocock,

each group of 5 mice with a body weight of 180-200 grams.

Independent variable is the level of blood lipid profile and blood NOx; control variables were sex, health, weight, food, age, and environment. All mice were given high-cholesterol food for 21 days, then performed a pre-test. Then dyslipidemia rats were randomly divided into four groups simple and still be given high-cholesterol foods, the control group P0, P1 treatment group given 70 mg extract of cocoa beans per cow per day, P2 and P3 given 140 mg given 280 mg for 14 days. After 14 days, blood sampling is done through orbital sinus medial canthus for inspection lipid profile and blood NOx examination. Foods high in cholesterol is a special mixture obtained from Department of Pharmacology Medical Faculty Udayana University.

The Extract of Cocoa seed extracted from the dried cocoa beans that have been peeled skin seeds. Blend until smooth cocoa beans, cocoa powder 500 grams of macerated using 96% ethanol for 48 hours, then evaporated using an evaporator to extract cocoa beans produced and ready to be used for research. This research has gained airworthiness Research Ethics Committee of Ethics of the Medical Faculty Udayana University / Sanglah Hospital.

The data obtained in this study were analyzed by One-Way ANOVA to determine differences between groups, with the degree of significance was set at  $p < 0.05$

## RESULT

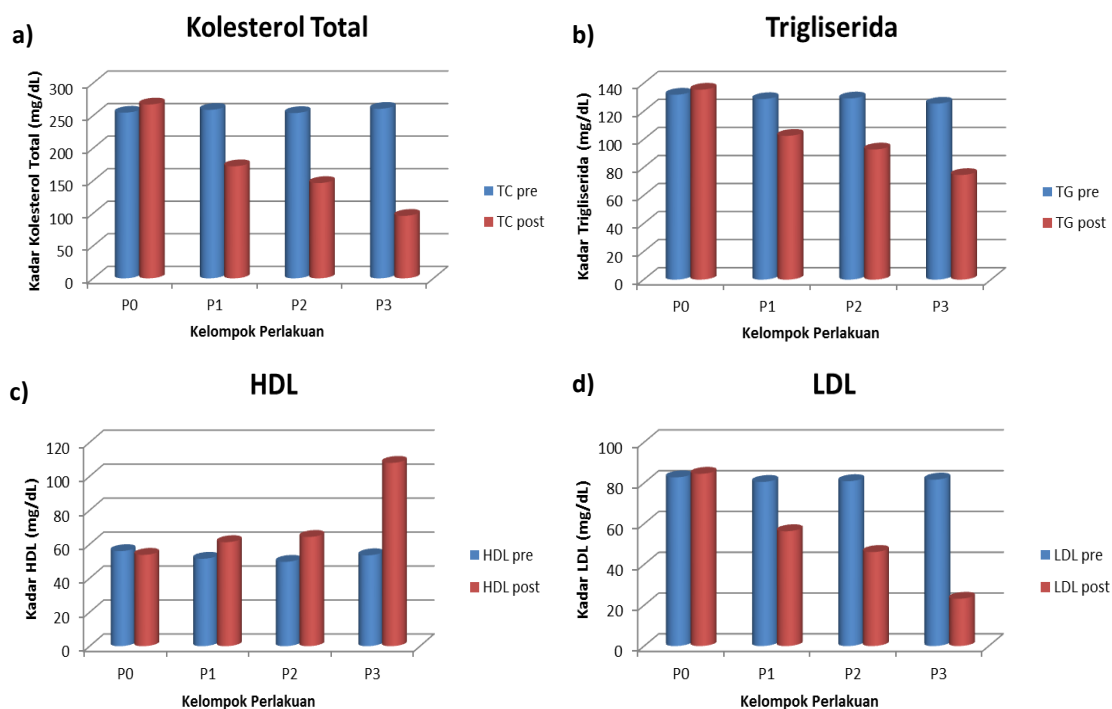
Results of research include examining lipid profile, and blood NOx levels can be seen in **Figures 1 and 2**.

It can be seen that the results of the lipid profile pre-test in the form of total cholesterol (a), triglycerides (b), HDL (c) and LDL (d) between the control group and the treatment group. The extract of cocoa beans P1, P2 and P3 was no statistical difference ( $P = 0.612$  for total cholesterol;  $P = 0.086$  for the triglycerides;  $P = 0.097$  for the HDL and  $P = 0.618$  for LDL). In the control group, namely the group gave high-cholesterol foods for five weeks without extract of cocoa beans on total cholesterol, triglycerides, HDL and LDL did not change, while the treatment group P1, P2 and P3 levels of total cholesterol, triglycerides and LDL decreased and HDL level increased. From the picture above can be seen that the results of the lipid profile after treatment showed no significant differences ( $P = 0.000$ ) (**Figure 1.**)

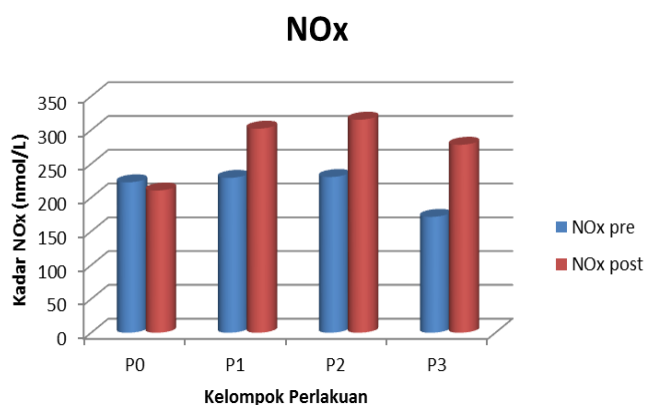
**Figure 2** presented the results of pre-test form NOx levels between the control group and the treatment group of cocoa seed extract P1, P2 and P3 there was no statistical difference ( $P = 0.577$ ).

From the picture above can be seen that the results of the NOx after treatment showed a significant difference ( $P = 0.486$ ). In the control group, namely, the group gave high-cholesterol foods for five

weeks without cocoa seed extract on NOx levels unchanged, while the treatment group P1, P2 and P3 NOx levels have increased but not statistically significant.



**Gambar 1.** Total Cholesterol (a), Triglicerida (b), HDL (c) and LDL (d) levels before and after granting cocoa bean extract within three dose variations.



**Gambar 2.** NOx levels before and after cocoa seed extract within three dose variations

## DISCUSSION

Results of this study showed that the extract of cocoa beans has a hypolipidemic effect but cannot increase the levels of NOx. Test results lipid profile in this study demonstrated the ability of cocoa seed extracts improves lipid profiles within normal limits. Total cholesterol in the control group after being given high-cholesterol food for five weeks remain high from 254.39 mg/dl to 267.03 mg/dl. While in the treatment group experienced a decrease mainly in the P3 group (260.39 mg/dl to

95.99 mg/dl). Decrease in total cholesterol in treatment group P1, P2 and P3 were statistically significant ( $P = 0.000$ ), the same thing happens to the variables of triglycerides and LDL. For HDL works in reverse, namely an increase in HDL levels in the treatment group P1, P2 and P3 were significantly ( $P = 0.000$ ), while the control group remained unchanged. Flavonoid content is quite high in grain kakao16 able to lower total cholesterol, triglycerides, LDL and increase HDL. The results are consistent with the provision of the

cocoa powder in mice hypercholesterolemia that show decreased levels of total cholesterol, triglycerides, LDL and MDA with increased HDL.<sup>18</sup>

Antioxidant flavonols contained in the cocoa bean extract works in the cell membrane by capturing the free radicals formed in the cell membrane. Mechanism of action of flavanols in the cell membrane to neutralize free radicals in unsaturated fatty acids (peroxyl polyunsaturated fatty acids) or PUFA-OO • membrane phospholipid cell and turn it into hydroperoxy polyunsaturated fatty acid (PUFA-OOH) that is no longer free radicals, thus lipid oxidation decrease.<sup>19</sup> These results are also by other studies such as the provision of black soybean seed extracts that contain anthocyanins can improve lipid profile because it can lower total cholesterol and triglycerides were significantly and increased HDL.<sup>20</sup> Purple sweet potato leaf aqueous extract showed hypolipidemic function in mice given high-cholesterol foods characterized by a decrease in total cholesterol, triglycerides, LDL and increased levels of HDL.<sup>21</sup>

Found an increase in plasma NOx levels were not statistically significantly different before and after the intervention in each group. The results of this study are not consistent with studies conducted by Murphy et al. namely providing cocoa powder containing 897 mg of flavanols after 6 hours has the same effect with the provision of cocoa powder containing 234 mg of flavanols for four weeks. They both inhibit The activation and platelet function so as to prevent the occurrence of atherosclerosis plaque. It made possible by the NO is oxidized to nitrate and nitrit.<sup>22</sup>

## CONCLUSION

Cocoa seed extract can prevent the occurrence of dyslipidemia is characterized by a decrease in total cholesterol, triglycerides, and LDL with increased levels of HDL, but has not been effective to increase NOx levels to prevent the occurrence of endothelial dysfunction.

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