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The effect of cacao beans extracts administration on SOD and ox-LDL concentration in oxidative stress conditioned rats

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ABSTRACT

Background: Psychosocial stress is one of the consequences of modern lifestyle changing and one of the potential cause of the oxidative imbalance. One of the most popular and effective methods to counter oxidative stress is by consuming antioxidant rich food or supplements. One of a potential source of natural antioxidant is cacao bean due to its rich flavonoids content such as *epicatechin*, *catechin*, and *procyanidin*. Thus, our research aimed to evaluate its antioxidant capacity, focusing on its effect on the level of SOD and ox-LDL in oxidative stress-conditioned-rat.

Methods: An experimental *Pre and Post Test Control Group Design* we conducted using 20 4-months old male Wistar rats which were divided into four groups and three groups (P1-P3) were treated with 70 mg, 140 mg, and 280 mg cacao bean extract while P0 act as a control. Plasma SOD level was examined in Food and Nutrient Study Center of Gadjah Mada University using the technique from Randox Laboratories while ox-LDL evaluation was conducted in Biochemistry Department Udayana University using ELISA with absorbance reading at 450 nm.

Results: SOD level begins to significantly increase at dosage 140 mg and continue to rise to 280 mg dose. Meanwhile, the ox-LDL level showed an interesting pattern. In P0, the level of ox-LDL at the end of the study showed a steep increase from 75.62 to 79.17 mg/dL. Meanwhile, all of the treatment group showed a significant decrease in the level of ox-LDL beginning at 70 mg dose and continuously decrease when the dose was doubled (140 mg). However, there were no differences in the level of ox-LDL between 140 mg dose of 280 mg. All the differences were statistically significant.

Conclusion: In conclusion, cacao bean extract is a potent antioxidant agent also has a great capacity to significantly lowering plasma ox-LDL level.

Keywords: Psychosocial stress, oxidative stress, cacao beans extract, SOD, ox-LDL
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INTRODUCTION

Lifestyle changes have induced many degenerative diseases partly due to increased oxidative stress originated either from food, environment, or metabolism.[1] Excessive oxidative stress would result in damage to many cellular components, producing secondary reactive substance such as malondialdehyde (MDA) and advanced glycation end-product (AGE).[2,3] An excessive amount of this substance has a deleterious effect toward the cell because of its capacity to induce apoptosis and tissue damage in various organs and, thus, initiate the development of many degenerative diseases such as cancer, type II diabetes mellitus, atherosclerosis, and etc.[1,3]

Psychosocial stress is one of the consequences of modern lifestyle changing and one of the potential cause of oxidative imbalance.[4] It consequences manifest mainly as increased level of corticosterone, catecholamine, and also had been proven to increase the level of MDA while decreasing the expression of SOD.[5] Those processes could result in increased level of Low-Density Lipoprotein (LDL), and it's oxidation process which is the underlying cause of atherosclerosis. It would also increase the blood pressure as the consequence of increased heart rate and contraction as well as increased sodium retention by the high level of corticosteroid hormone.[6] Corticosteroid hormone itself is a potent cause of insulin resistance which will lead to the development of T2DM.[3]

One of the most popular and effective methods to counter oxidative stress is by consuming antioxidant rich food or supplements. Antioxidants such as vitamin C, vitamin E, phenolic acid and the others are effective electron receptor and could effectively neutralize free radicals.[7] Many pieces of evidence had supported the effectiveness of such food that usually present in adequate amount in traditional diet.[8,9] For example, resveratrol within grape fruit has great antioxidant capacity and present in a high level in red wine which is why moderate consumption of wine per week is one of the best methods of cardiovascular disease preventions.[8] Anthocyanin containing fruit and purple sweet potato also has a significant antioxidant capacity and has been extensively studied albeit mainly as cholesterol lowering agent.[9] A Recent study also

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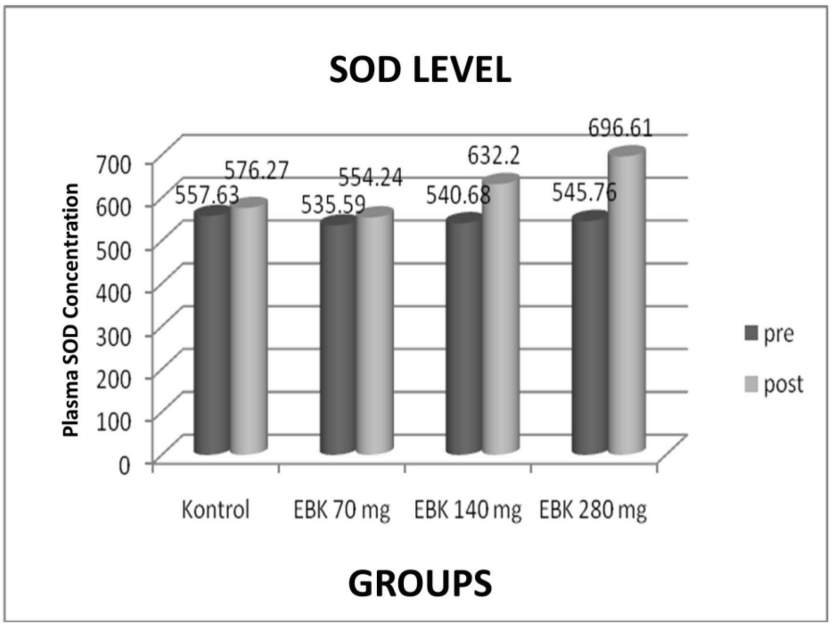

Figure 1 Comparison of the effect of Cacao Bean Powder to plasma SOD level between groups with different doses

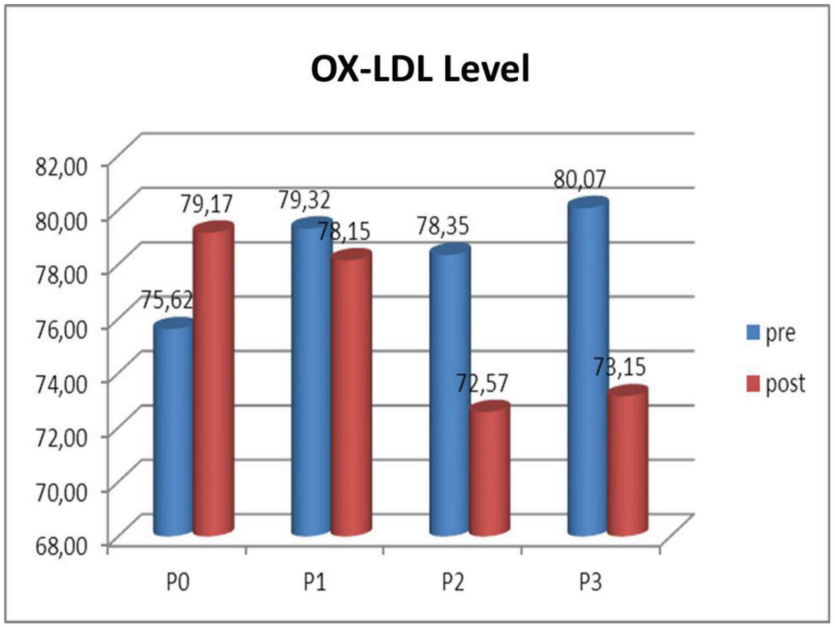

Figure 2 Comparison of different dosage of Cacao Bean Powder. P0 acted as control group. (P1: 70 mg; P2: 140 mg; P3: 280 mg)

pointed out flavonoid which presents in several fruits and vegetables as potential antioxidant.[9]

The natural antioxidant is regarded as the safest form of antioxidant compared to the synthetic ones. One of a potential source of natural antioxidant is cacao bean due to its rich flavonoids content such as *epicatechin*, *catechin*, and *procyanidin*.[10] Cacao is also widely planted and considerably cheap which is strengthening its position as a potent antioxidant source. However, there are still limited numbers of research evaluating the antioxidant properties of the cacao bean. Thus, our study aimed to evaluate its antioxidant capacity, focusing on its effect on the level of SOD and ox-LDL in oxidative stress-conditioned-rat.

METHODS

Study Design and Experimental Animals

An experimental *Pre and Post Test Control Group Design* we conducted using 20 4-months old male Wistar rats which body weight ranging from 180-200 grams. The animals were purchased from *Animal Laboratory Unit* of Pharmacology department Faculty of Medicine Udayana University. The animals were grouped into four groups (P0-P3) with five rats each. Each group was kept psychosocial stress under psychosocial stress for four weeks by putting them in 20 x 10 x 12 cm cage for 4 hours/day. We give only aquadest to P0 group, 70 mg cacao extract to P1, 140 mg of cacao extract to P2, and 280 mg of cacao extract to P3. The extracts were administered for two weeks. The blood was extracted from *medial canthus sinus orbitalis* for SOD and ox-LDL evaluation.

Cacao Bean Extract Preparation

The cacao extract was prepared by pulverizing 500 grams of peeled cacao bean into fine grains. Then, the product was macerated in 96% alcohol for 48 hours with a subsequent evaporating process using *vacuum evaporator*. The powder is then ready to be administered to the experimental animals.

SOD and ox-LDL Evaluation

Before drawing the blood sample, all rats were put under fasting condition for 10-12 hours. Then, the blood sample was obtained and collected in vacutainer. The blood then centrifuged at 3000 rpm for 15 minutes until the plasma was separated from the cellular component. The plasma was isolated and used to examine the SOD and ox-LDL. Plasma SOD level was analyzed in Food and Nutrient Study Center of Gadjah Mada University using the technique from Randox Laboratories while ox-LDL evaluation was conducted in Biochemistry Department Udayana University using ELISA with absorbance reading at 450 nm.

Statistical Analysis

All of the data were compiled and analyzed using one-way ANOVA using SPSS 16. A p-value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

The result of the experiment seemed promising since both the SOD level and ox-LDL level proved

showed positive results. Both results are shown in Figure 1 and Figure 2. SOD level begins to significantly increase at dosage 140 mg and continue to rise to 280 mg dose. Meanwhile, the ox-LDL level showed an interesting pattern. In P0, the level of ox-LDL at the end of the study showed a steep increase from 75.62 to 79.17 mg/dL. Meanwhile, all of the treatment group showed a significant decrease in the level of ox-LDL beginning at 70 mg dose and continuously decrease when the dose was doubled (140 mg) (Figure 2). However, there were no differences in the level of ox-LDL between 140 mg dose with 280 mg.

From the result, it clear that the effective dose of cacao extract that affected both SOD level and the ox-LDL level was 140 mg. At this level, it significantly increased the SOD level and also decreased ox-LDL concentration. Doubling the dose to 280 mg had little effect on both parameters. Meanwhile, though there was a difference in the concentration of both parameters at a lower dose, it was not statistically significant.

Lots study had evaluated the antioxidant effect of cacao bean extract or cocoa powder. 5% cocoa powder had been shown to increase antioxidant level and activity as well as attenuated inflammatory response in arthritis model-rat.[11] It is also observed that the increased antioxidant level was due to increased expression of SOD and Catalase especially mitochondrial SOD. Cocoa powder also found to increase SOD2 level in skeletal muscle of heart failure and T2DM patients. It also revealed that increased concentration of SOD2 was due to increase in expression rate of *SOD2* gene.[12]

The antioxidant properties of cocoa powder are due to its high level of several bioactive substances which mainly Flavanols.[10,13] Flavanols such as monomeric epicatechin and polymeric proanthocyanin are known to have strong anti-oxidant properties.[13] Flavanols from cocoa powder had been proven to stimulate nitric oxide synthetase, increase perfusion rate, maintaining arterial elasticity, lowering blood pressure, anti-coagulation, and anti-inflammation.[14] The anti-oxidant properties of cacao powder also proved to be higher compared to blueberry, cranberry, and pomegranate powder but its derived product, dark chocolate, did not have greater antioxidant capacity than pomegranate, although still higher than the other two.[14] These evidences strengthen the position of cacao bean as a potential anti-oxidative agent, not only because of its antioxidant potential but also its widespread plantation and market.

Comparing our study from the previous ones, we further confirmed the anti-oxidative potential of cacao bean powder. However, we also confirmed that the anti-oxidative property of cacao bean was also potent enough to lower plasma ox-LDL level. This was a significant finding since ox-LDL plays a central role in the pathogenesis of atherosclerosis, the main risk factors for MI and ischemic stroke. The result also gave a significant addition to the complex mechanism of cacao bean powder as potential therapeutic agent for atherosclerosis. However, further research is needed to translate this finding into the clinical one which will lead to its clinical application as atherosclerosis preventive agent.

CONCLUSION

In conclusion, cacao bean extract is a potent antioxidant agent mainly by increasing the level of plasma SOD level. It also has a great capacity to significantly lowering plasma ox-LDL level which made it a potential candidate as an anti-atherosclerotic agent.

REFERENCES

1. Kehrer JP, Klotz LO. Free radicals and related reactive species as mediators of tissue injury and disease: implications for Health. Crit Rev Toxicol. 2015;45(9):765-98
2. Ott C, Jacobs K, Haucke E, Navarrete Santos A, Grune T, Simm A. Role of advanced glycation end products in cellular signaling. Redox Biology. 2014;2:411-429.
3. Khansari N, Shakiba Y, Mahmoudi M. Chronic inflammation and oxidative stress as a major cause of age-related diseases and cancer. Recent Pat Inflamm Allergy Drug Discov. 2009 Jan;3(1):73-80
4. Colaianna M, Schiavone S, Zotti M, et al. Neuroendocrine Profile in a Rat Model of Psychosocial Stress: Relation to Oxidative Stress. Antioxidants & Redox Signaling. 2013;18(12):1385-1399.
5. Djordjevic J, Djordjevic A, Adzic M, Radojcic MB. Chronic social isolation compromises the activity of both glutathione peroxidase and catalase in the hippocampus of male Wistar rats. Cell Mol Neurobiol. 2010 Jul;30(5):693-700
6. Beaudry JL, Riddell MC. Effects of glucocorticoids and exercise on pancreatic β-cell function and diabetes development. Diabetes Metab Res Rev. 2012 Oct;28(7):560-73
7. Guerra-Araiza C, Álvarez-Mejía AL, Sánchez-Torres S, Farfan-García E, Mondragón-Lozano R, Pinto-Almazán R, Salgado-Ceballos H. Effect of natural exogenous antioxidants on aging and on neurodegenerative diseases. Free Radic Res. 2013 Jul;47(6-7):451-62
8. Willcox DC, Scapagnini G, Willcox BJ. Healthy aging diets other than the Mediterranean: A Focus on the Okinawan Diet. Mechanisms of ageing and development. 2014;136-137:148-162.
9. Pauwels EK. The protective effect of the Mediterranean diet: focus on cancer and cardiovascular risk. Med Princ Pract. 2011;20(2):103-11
10. Hu S, Kim BY, Baik MY. Physicochemical properties and antioxidant capacity of raw, roasted and puffed cacao beans. Food Chem. 2016 Mar 1;194:1089-94

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11. Ramos-Romero S, Pérez-Cano FJ, Ramiro-Puig E, Franch A, Castell M. Cocoa intake attenuates oxidative stress associated with rat adjuvant arthritis. Pharmacol Res. 2012 Sep;66(3):207-12
12. Ramirez-Sanchez I, Taub PR, Ciaraldi TP, et al. (−)-Epicatechin rich cocoa mediated modulation of oxidative stress regulators in skeletal muscle of heart failure and type 2 diabetes patients. International journal of cardiology. 2013;168(4):3982-3990.
13. Crozier SJ, Preston AG, Hurst JW, et al. Cacao seeds are a "Super Fruit": A comparative analysis of various fruit powders and products. Chemistry Central Journal. 2011;5:5.
14. Corti R, Flammer AJ, Hollenberg NK, Lüscher TF. Cocoa and cardiovascular health. Circulation. 2009 Mar 17; 119(10):1433-41.