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The hepatoprotective effect of cacao beans whole extracts (*Theobroma cacao* L.) in oxidative stress mice

Ida Ayu Dewi Wiryanthini[1*], I Wayan Gede Sutadarma[1], Ni Wayan Sucindra Dewi[2], I Wayan Surudarma[1]

ABSTRACT

Background: Increased production of reactive oxygen species (ROS), causing accumulation of oxidative damage. Carbontetraclorida (CCl_4) is known as a toxic material and causing hepatocellular damage initiated by oxidative stress, which increased blood serum *glutamic pyruvic transaminase* (SGPT) concentration. Cacao beans (*Theobroma cacao* L.) extracts have antioxidant properties from *flavonols,* consisting of *catechin, epicatechin,* and *procyanidin*. This study aims to investigate the effect of hepatoprotective of cacao beans (*Theobroma cacao* L.) extracts in oxidative stress mice induced by CCL_4.

Methods: The study was conducted at the Department of Biochemistry and Department of Pharmacology and Therapy, Faculty of Medicine Udayana University using the *Pretest-Postest Control Group Design*. About 20 male mice were divided into 4 groups, as control or placebo group (P0), intervention group by 15 mg extracts of cacao beans (P1), 30 mg (P2), and 60 mg (P3) for 7 days. On the eight-day induced by 0,55 mg/gram mice bodyweight of CCl_4. All groups were examined for blood SGPT concentration before and after the intervention. Data were analyzed using SPSS version 20 for Windows.

Results: The SGPT level prior to the administration of cacao bean extract and CCl4 was 18.56±0.19 µ/L in P0 group, followed by 18.41±0.28 µ/L (P1), 18.36±0.21 µ/L (P2), and 18.16±0.47 µ/L (P3), but statistically not significant (p=0.260). However, after CCl4 administration, the SGPT levels were 38.63±0.37 µ/L in P0 but significant decrease in the P1 (28.14±0.34 µ/L), P2 (20.72±0.35 µ/L), and P3 (18.68±0.25 µ/L) following cacao beans whole extracts administration (p=0.000).

Conclusion: This study concluded that cacao beans extracts administration could inhibit the oxidative stress and protect hepatocellular damage induced by CCl_4 by decreasing the blood SGPT concentration.

Keywords: CCl_4, Oxidative Stress, Cacao Beans, Flavanols, SGPT

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[1]Department of Biochemistry, Faculty of Medicine, Universitas Udayana, Bali, Indonesia
[2]Department of Pharmacology and Therapy, Faculty of Medicine, Universitas Udayana, Bali, Indonesia

*Corresponding to:
Ida Ayu Dewi Wiryanthini;
Department of Biochemistry, Faculty of Medicine, Universitas Udayana, Bali, Indonesia;
wiryanthini@unud.ac.id

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INTRODUCTION

The liver has a significant role in human survival.[1] Its important roles include being responsible for the metabolism and detoxification of xenobiotics or other hazardous chemicals.[1] In essence, metabolism is closely related to oxidation-reduction reactions involving oxygen or electron exchange.[2] Free radicals are part of metabolic products that are volatile and very reactive.[3] The use of drugs, hazardous chemicals, poor nutritional status, and environmental pollution can increase free radicals' production in the body and encourage oxidative stress.[4] In this condition, the liver cells become vulnerable to free radical attack and at risk of damage and death. Given the liver's critical role for survival, the adverse effects of oxidative stress need to be anticipated so that liver damage can be avoided. Theoretically, oxidative stress can be prevented by providing antioxidant intake from outside the body so that the balance between prooxidants and antioxidants can be maintained.[5]

Free radicals are chemical compounds with one or more unpaired electrons in the outer portion of their orbit so that they tend to attract electrons from other compounds causing free radicals to be very reactive.[6] Oxidant is a compound that can attract electrons.[6] By definition, oxidants and free radicals are often considered to be the same because they both have similar properties, that are both have the ability to attract electrons.[6] But free radicals have high reactivity and not all oxidants are free radicals as well as all free radicals are oxidants. A study by Harman D said that vegetables and fruits are natural sources of antioxidants that can be used to prevent oxidative stress.[7] Vitamin E, Beta Carotene, or vitamin C are natural antioxidants that effectively neutralize free radicals.[7] Besides the three compounds mentioned above, other natural compounds, such as flavonoids, carotenoids,

and alkyl sulfide compounds, have antioxidant properties.[8]

The use of antioxidants has been widely known to prevent and counteract the formation of free radicals. In general, antioxidants can be divided into two groups, namely enzymatic and non-enzymatic antioxidants.[3] Enzymatic antioxidants, which are also called preventive antioxidants consist of superoxide dismutase, catalase, and glutathione peroxidase.[3] Non-enzymatic antioxidants, also called chain breaker antioxidants, are divided into fat-soluble antioxidants such as tocopherol and carotenoid flavonoids, quinones, and bilirubin, and water-soluble antioxidants such as ascorbic acid, uric acid, metal-binding proteins, and heme-binding proteins.[3]

A condition where the amount of antioxidants is lower than free radicals is called oxidative stress.[4] Oxidative stress causes damage to nucleic acids, fats, and proteins that affect cell health conditions and their viability or induce various cellular responses by forming secondary reactive compounds and cell death due to necrosis or apoptosis.[9] At present, the use of natural antioxidants is considered safer than synthetic antioxidants, because they are obtained from plant extracts. Cocoa or cacao beans (*Theobroma cacao* L.) or are one source of natural antioxidants that are easily cultivated in Indonesia and have a relatively affordable price.[10] Previous studies have shown that cacao beans that have been processed into cacao powder contain flavanols. The types of flavanols found in cacao powder are epicatechin, catechin, and procyanidin.[10,11]

In previous studies, it was found that oxidative stress can be induced by administering a solution of Carbontetrachlorida (CTL_4) orally at a dose of 0.55 mg / g BW in male mice aged 2,5-3 months with a bodyweight of 25-30 grams.[12,13] Free radicals that are formed will oxidize lipids from the liver cell membrane so that they are damaged and break easily.[14] Oxidative stress caused by membrane lipid peroxidations was associated with the hepatocellular injury.[14] Rupture of liver cells will be accompanied by the release of Serum Glutamic-Pyruvate Transaminase (SGPT) into the blood and the magnitude of elevated SGPT Serum levels is directly proportional to the degree of liver cell damage.[15] This event is particular and can be used as an indicator to show how much the degree of liver cell damage is happening. Cacao bean extract can be said to have the efficacy of hepatoprotector if the increase in SGPT of mice that have been conditioned by drinking extracts is smaller than the increase in SGPT of control mice animals.[16]

Based on those mentioned above, this study could be used as supporting data to determine the hepatoprotective effect of cacao beans whole extracts (*Theobroma cacao* L.) in oxidative stress mice.

METHODS

An experimental study through the pre- and post-test control group design approach was conducted among 20 male-mice (*Mus musculus*) in this study. They were aged 2,5-3 months with bodyweight between 25-30 grams obtained from the Animal Laboratory Unit Department of Pharmacology and Therapy of the Faculty of Medicine, Udayana University. They were divided into 4 groups and each group consisted of 5 mice. Group P0 (control) was given aquades, group P1 (cacao bean extract 15 mg), group P2 (cacao bean extract 30 mg), and group P3 (cacao bean extract 60 mg) given for 7 days. On the 8th day, all mice were given CCl4 at a dose of 0.55 mg/gram bodyweight. Blood is drawn from all mice through the medial canthus sinus orbitalis to examine SGPT levels through the ELISA method and presented in µ/L units.

Cacao bean is extracted by blending 500 grams of dried cacao beans until smooth and then put into a beaker and macerated for 24 hours in 96% alcohol immersion. After that, it is filtered and the filtrate is collected in a dark-colored bottle. Evaporation is carried out with a vacuum evaporator at 700°C and the residue is weighed for use in experiments. Of the 500 grams of cacao beans, 25 grams of cacao bean extract are obtained. All data obtained in this study were analyzed with the One-Way Anova test by SPSS version 20 for Windows.

RESULTS

Table 1 and Figure 1 show SGPT levels among groups before and after the administration of cacao beans. The SGPT level prior to the administration of cacao bean extract and CCl4 was 18.56±0.19 µ/L in P0 group, followed by 18.41±0.28 µ/L (P1), 18.36±0.21 µ/L (P2), and 18.16±0.47 µ/L (P3), but statistically not a significant difference (p=0.260) (Table 1 and Figure 1). However, after CCl4 administration, the SGPT levels were 38.63±0.37 µ/L in P0 but significant decrease in the P1 (28.14±0.34 µ/L), P2 (20.72±0.35 µ/L), and P3 (18.68±0.25 µ/L) following cacao beans whole extracts administration (p=0.000) (Table 1 and Figure 1).

Figure 1 below shows that the administration of cacao bean extract starting at a dose of 15 mg can reduce blood SGPT levels compared to controls. The results of this study indicate that the amount of Cacao bean extract is effective for lowering blood SGPT levels starting at a dose of 15

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Table 1. The SGPT blood levels analysis before and after cacao beans whole extracts administration by One-Way ANOVA.

Variables	P0	P1	P2	P3	p
SGPT (mean±SD)(μ/L)					
Pre-Test	18.56±0.19	18.41±0.28	18.36±0.21	18.16±0.47	0.260
Post-Test	38.63±0.37	28.14±0.34	20.72±0.35	18.68±0.25	0.000*

*Statistically significant if p-value less than 0.05; P0=control group was given aquades; P1= cacao bean extract 15 mg for 7 days; P2= cacao bean extract 30 mg for 7 days; P3= cacao bean extract 60 mg for 7 days

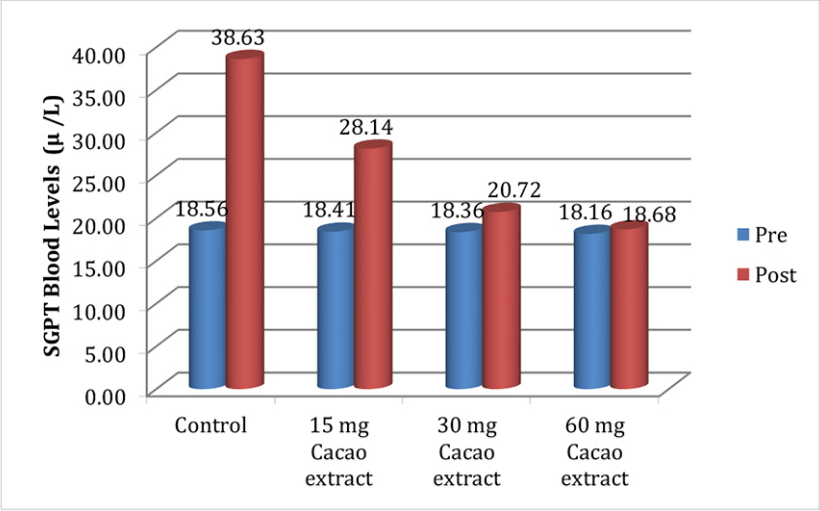

Figure 1. Graph of Decreased SGPT Levels After Giving Cacao Beans Extract

mg, so increasing the dose will increase the effect of reducing the SGPT levels of the blood of mice experiencing oxidative stress after being induced with CCl4 (Figure 1).

DISCUSSION

The liver has a pivotal role in human survival due to responsible for the metabolism and detoxification of other hazardous chemicals. The liver comprises hepatocytes, biliary epithelial cells (cholangiocytes), stellate cells, Kupffer cells, and liver sinusoidal endothelial cells.[17] Each of these cell types possesses a cooperatively unique function in regulating the liver at different levels.[18] However, hepatocytes have been known as a primary epithelial cell population of the liver and make up the most liver volume to perform many liver functions.[19] The oxidative stress, which could impair the hepatocyte functions, also influences the liver's hepatoprotective properties by free radicals by secreting SGPT on the bloodstream as the initial marker.[20]

The results of this study are in accordance with the study of guava fruit extract, buni, and carrots can reduce SGPT levels in mice that have been induced CCl_4.[21] The inhibitory mechanism of decreasing SGPT levels due to flavanols (epicatechin, catechin, and procyanidin) found in cacao bean extract works by capturing free radical scavenger against hydroxyl radicals, superoxide anions, peroxyl radicals, and alkoxyl as well as chelating agents.[10] Inhibited oxidative stress can prevent liver cell damage that is marked by decreased levels or release of cytoplasmic components, namely SGPT.[22]

Based on the results of this study, it can be concluded that the administration of cacao bean extract can overcome oxidative stress and prevent liver damage by lowering the SGPT levels of the blood of mice that have been carved with carbon tetrachloride (CCl4). A dose of 60 mg of cacao bean extract is more effective in reducing SGPT levels so that it is more effective in preventing liver cell damage. A previous study found that decreasing SGPT levels which indicate for preventing liver cell damage was related to the phenol-rich cocoa extract through regulating the Bax gene responsible for cell apoptosis.[16,23] In addition, previous studies also found that the polyphenolic compounds of *Theobroma cacao* L had an antioxidant properties which could against the free radicals particularly in liver cell damage by flavonoid compounds.[24,25]

CONFLICT OF INTEREST

The authors declare that there is no competing interest regarding the manuscript.

ETHICS CONSIDERATION

Ethics approval has been obtained by the Ethics Committee, Faculty of Medicine, Universitas Udayana, prior to the study being conducted.

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None

AUTHOR CONTRIBUTIONS

All of the authors equally contribute to the study from the conceptual framework, data gathering, and data analysis until reporting the study results through publication.

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