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Glutathione peroxidase-lowering effects of the mangosteen pericarp extract on CCl_4-induced hepatic injury in rats

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Abstract

This study aimed to investigate whether an ethanolic extract of *Garcinia mangostana* pericarp is able to modulate the glutathione peroxidase and glutathione system in the liver of CCl4-induced hepatic injury. Thirty one male Sprague Dawley rats were divided into five groups, including control (untreated) group; CCl_4 group (at dose 0.55 mg/kg body weight dissolved in coconut oil, at day eight); EEGM group (at doses 900; 1080; and 1296 mg/kg body weight/day for eight days then received CCl_4 at dose 0.55 mg/kg body weight dissolved in coconut oil). Glutathione peroxidase and GSH/GSSH ratio were assayed by colorimetric method. The level of GPx was insignificantly lower in the CCl_4 group compared to the untreated control group ($P > 0.05$). Out of the 900 mg/kg BW, 1080 mg/kg BW, and 1296 mg/kg BW doses of *Garcinia mangostana* extract, only the two highest doses significantly decreased in GPx level compared to the CCl_4 group or untreated control group ($P < 0.05$). The level of the GSH/GSSG ratio in the liver homogenate were not significantly different between groups ($P > 0.05$). Mangosteen pericarp extract decrease the activity of glutathione peroxidase liver on CCl_4-induced hepatic injury of rats.

Keywords : Antioxidant, hepatotoxicity, liver injury, mangosteen, pericarp

Introduction

The model of hepatotoxicity can be achieved by carbontetrachloride (CCl_4) exposure. This toxic substance will biotransformed in the liver to produce several reactive metabolites such as trichloromethane (CCl3) and trichloromethyl peroxy (CCl3 O•O•). This radical will induces lipid peroxidation of membrane, then impairs its function and finally produces liver injury [1, 2]. CCl_4 causes severe liver damage with necrotic and apoptotic hepatocellular injury. CCl_4 was metabolized into trichloromethyl radical (CCl_3•) by the cytochrome p450, then reacts with oxygen to form trichloromethylperoxy radical (CCl_3OO•), resulting in protein and DNA damage and lipid peroxidation [3]. Therefore, diminishing free radical directly and retarding the propagation of the oxidative chain reaction are practicable strategies for preventing CCl_4-induced hepatotoxicity. In addition CCl_4-induced hepatic injury is a widely used experimental model in the screening of hepatoprotective drugs [4].

Supplementation with exogenous antioxidants and phytochemicals from plant sources help to reduce the lipid peroxidation, oxidative damage and liver injury. The mangosteen fruit is a rich source of phenolic compounds. Various phenolic compounds in mangosteen include xanthone, tannins, and anthocyanins. Of these phenolic compounds, only xanthone is most frequently investigated [5]. Previous studies showed that α-mangostin from the pericarp of mangosteen has antioxidant properties and potent scavenger of peroxynitric anion, singlet oxygen, and superoxide radical [6-8]. Therefore, this study aimed to investigate whether an ethanolic extract of *Garcinia mangostana* (EEGM) pericarp is able to modulate the glutathione peroxidase and glutathione system in the liver of CCl4-induced hepatic injury.

Material and Methods

Animals

Thirty one male Sprague Dawley rats, weighing 180–201 g, purchased from BALITVET of Bogor was housed in an air-conditioned room at 24 ± 2◦C and 65–70% relative humidity with a 12 h light-dark cycle. These animals were divided into five groups, including control (untreated) group; CCl_4 group (at dose 0.55 mg/kg body weight in coconut oil, at day eight); EEGM group (at doses 900; 1080; and 1296 mg/kg body weight/day for eight days then received CCl_4 at dose 0.55 mg/kg body weight in coconut oil). The protocol used in this study was approved by the

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Ethic Committee for Animal Experimentation of the Indonesian University. Diets were made by following American Institute of Nutrition (AIN) recommendations. The animals were given water *ad libitum* during the experimental period. The composition of standard diet is 66% comfeed PAR-s, 33% wheat powder, and water.

Ethanolic extract of mangosteen fruit pericarp

Mangosteen fruit pericarp was obtained from BALLITRO, Cimanggu, Bogor. Simplicia in dry conditions was pulverized, using a grinder, then soaked in 50% ethanol. Before the pot is inserted solvent, 50% ethanol solution was made by entering the distilled water and 50% ethanol. After so solvent, the solvent is then used as desired, then put in a pot that already contains crude drug material. The solution was stirred for approximately 3 hours for homogeneous and its soluble active substances deposited during the last 24 hours. After it was filtered, the filtrate was put in a closed glass container and stored. In the waste was added by 2 L of 50% ethanol solution, then shaken again for ± 1 hour and followed by filtration The second filtrate was combined with the first filtrate, and then inserted into the evaporator flask to be evaporating with a rotary evaporator (Buchi). Evaporation was performed at a temperature of 45^{o}C, 45 rounds per minute to obtain the ethanol extract.

Liver tissue homogenization

Rat liver tissue was removed from the -80° storage and allowed to stand at room temperature. All the liver in each group was weighed and the weight recorded. One hundred mg of liver tissue was inserted into the microtube. The liver was crushed by positron homogenizer for 2-3 minutes until homogenized, then added 1 mL of PBS (phosphate buffer saline) 0.05 M pH 7.2 then centrifuged at 4000 RPM, at a temperature of 4°C for 15 minutes. The supernatant then separated from the pellet was inserted into another clean tube, then added with PBS again to a volume of 1 mL. The samples were stored in a refrigerator (-80ºC) until ready to use.

Glutathione peroxidase analysis

A commercial glutathione peroxidase (RANDOX-RANSEL® Glutathione peroxidase, Crumlin, County Antrim, United Kingdom) detection kit were used to measure liver glutathione peroxidase level.

GSH/GGSG ratio analysis

A commercial GSH/GSSG ratio (Microplate Assay for GSH/GSSG (Reduced/Oxidized Glutathione) catalog number GT 40 (Oxford Biomedical Research, Oxford, MI, USA) detection kit was used to measure the liver GSH/GGSG ratio.

Ethics

This research has been approved by the research ethics committee of Faculty of Medicine, University of Indonesia, Jakarta, Indonesia.

Statistical analysis

Data were presented as mean ± SD and differences between groups were analyzed using 1-way ANOVA with SPSS 15.0 statistical package. The post Hoc test was used if the ANOVA was significant. $p < 0.05$ was considered statistically significant.

Results

Table 1 presents the GPx levels in the liver from each experimental group. The level of GPx was insignificantly lower in the CCl_4 group compared to the untreated control group ($P > 0.05$). Out of the 900 mg/kg BW, 1080 mg/kg BW, and 1296 mg/kg BW doses of *Garcinia mangostana* extract, only the two highest doses significantly decreased in GPx level compared to the CCl_4 group or untreated control group ($P < 0.05$). There was no significant difference between the effects of these two highest doses. Besides, the first dose of the *Garcinia mangostana* extract significantly reduced the GPx level than that CCl_4 group ($P < 0.05$).

Table 1. The levels of liver glutathione peroxidase induced by CCl_4

			CCl_4 + EEGM (mg/kg BW)		
Level	Control	CCl_4	900	1080	1296
GPx (U/mg protein)	58.19 ± 15.18	49.92 ± 21.17	37.50 ± 12.96[a]	25.16 ± 8.15[ab]	36.08 ± 20.23[ab]

Note: values are presented as mean ± SD; [a]p<0.05; in comparison with the control group; [b]p<0.05; in comparison with CCl_4 group; CCl_4: carbon tetrachloride; EEGM: ethanolic extract of the *Garcinia mangostana* pericarp; mg/kg BW: milligram/kilogram body weight; U/mg protein: units/milligram protein.

Table 2 presents the GSH/GSSG ratio in the liver homogenate from each experimental group. The level of the GSH/GSSG ratio in the liver homogenate was not significantly different between groups ($P > 0.05$).

Table 2. The levels of GSH/GSSG ratio liver induced by CCl_4

			CCl_4 + EEGM (mg/kg BW)		
Ratio	Control	CCl_4	900	1080	1296
GSH/GSSG	54.55 ± 10.69	58.64 ± 31.81	60.66 ± 26.39	54.50 ± 30.84	70.78 ± 37.55

Note: values are presented as mean ± SD; [a]p<0.05; in comparison with the control group; [b]p<0.05; in comparison with CCl_4 group; CCl_4: carbon tetrachloride; EEGM: ethanolic extract of the *Garcinia mangostana* pericarp; mg/kg BW: milligram/kilogram body weight.

Discussion

In this study, liver damage was performed by induction with CCl_4. Increased oxidative stress is a feature of the liver damage induced by CCl_4 which involving Kupffer cells and the activity of neutrophils [9, 10]. Increased lipid peroxidation in the endoplasmic reticulum membrane occurs as a result of tricholoromethyl radical of CCl_4 [11, 12]. In detail, CCl_4 will accumulate in hepatocytes, when activated by the oxidase will trigger the homolytic fission by termination of it's C-Cl bond. This activation takes place in the liver endoplasmic reticulum through the electron transport system of nicotinamide adenine dinucleotide phosphate towards oxygen. This reaction will lead to the formation of reactive metabolites, including trichlorometyl radical (CCl3•) and peroxy trichlorometyl radicals (CCl3OO•) [13]. Furthermore, both these radicals can form covalent bonds with the multiple molecular structure, especially lipid in the endoplasmic reticulum membrane to form of lipid radicals. Lipid radical will reacts with molecular oxygen to form peroxy radicals, which would be involved in the initiation of lipid peroxidation [14]. Lipid peroxide products will disrupt membrane at cellular and subcellular levels. These events will trigger hepatocytes damage, then release of intracellular enzymes, such as GOT, GPT and ALP into the blood [15].

In this study, we evaluated the changes in CCl_4-induced GPx activity and found that the induction of CCl_4 decrease in the activity of GPx although not found significant differences ($P > 0.05$). This insignificant result may be caused by the role of catalase as a second line of defense against H_2O_2 increase, so does not decrease the activity of GPx or affinity CCl4-induced free radicals is greater toward catalase. This finding was confirmed by insignificant results of the GSH / GSSG ratio in this study.

Treatment with EEGM at a dose of 900 mg / kg before the induction of CCl4 turns the GPx activity was significantly lower than the control group ($P < 0.05$). Similarly, EGGM at doses of 1080 and 1296 mg / kg prior to induction of CCl4 also triggered a significant decrease in GPx activity compared with the control group and the CCl4 group. This finding indicates that the combination EGGM and CCl4 increasing the formation of free radicals that will trigger the catalysis by GPx. α-mangostin work as free radical scavenger through two mechanisms, namely the transfer of a hydrogen atom or a radical adduct formation. Both of these mechanisms will produce the mangostin radical [16]. These mechanisms indicates that the radical formation EEGM act as prooxidant. Fluctuating phenomenon by doses EEGM 1080 and 1296 mg/kg body weight may be due to the termination reaction (neutralization) between two molecules of free radicals. The reaction between the radical CCl3 or CCl3OO may result radical EEGM. On the other side, the radical EEGM can react with the radical CCl3 or CCl3OO to yield a neutral product. Previous *in vitro* study showed that γ-mangostin induced the intracellular peroxide [17]. Another study found that α-mangostin significantly decreased the levels of GSH, and increased GPx activity [18].

Conclusion

In conclusion, we suggested that treatment with the mangosteen pericarp extract on CCl_4-induced hepatic injury decrease the glutathione peroxidase liver of rats.

Declaration of interest

The author(s) declare(s) that there is no conflict of interests regarding the publication of this article.

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