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Brief Report

Effects of ethanolic extracts of *Garcinia mangostana* fruit pericarp on oxidant-antioxidant status and foam cells in atherosclerotic rats

Dwi Laksono Adiputro[1], Husnul Khotimah[2], M. Aris Widodo[2], Rochmad Romdoni[3], Djanggan Sargowo[4]

[1]Department of Cardiology and Vascular Medicine, Ulin General Hospital, Faculty of Medicine, University of Lambung Mangkurat, Banjarmasin, South Kalimantan, Indonesia
[2]Department of Pharmacology, Faculty of Medicine, University of Brawijaya, Malang, East Java, Indonesia
[3]Department of Cardiology and Vascular Medicine, Dr. Soetomo General Hospital, Faculty of Medicine, University of Airlangga, Surabaya, East Java, Indonesia
[4]Department of Cardiology and Vascular Medicine, Saiful Anwar General Hospital, Faculty of Medicine, University of Brawijaya, Malang, East Java, Indonesia

Received February 20, 2013
Accepted March 17, 2013
Published Online March 29, 2013
DOI 10.5455/oams.170313.br.007

Corresponding Author
Dwi Laksono Adiputro
Department of Cardiology and Vascular Medicine, Ulin General Hospital,
Faculty of Medicine,
University of Lambung Mangkurat,
Jl. A. Yani Km 2 No. 43, Banjarmasin,
South Kalimantan, Indonesia.
d_adiputro@yahoo.com

Key Words
Catalase; Foam cells;
Glutathione peroxidase;
Hydrogen peroxide; Mangosteen

Abstract
The present study was aimed to determine whether ethanolic extract of mangosteen pericarp (EEMP) changed oxidant-antioxidant status and foam cell formation in atherosclerotic rats. Thirty two male Wistar rats were divided into five groups (n = 6 for each), including standard diet (control) group, hypercholesterolemic diet (HD) group, HD + EEMP at doses of 200, 400 and 800 mg/kg body weight. Analyses of serum H_2O_2 level, glutathione peroxidase (GPx) and catalase (CAT) activity were done using colorimetric methods. Foam cells counts were measured histologically with Oil Red O staining. One Way Analysis of Variance test was used to analyze the differences in all parameters. Hypercholesterolemic diet significantly increased H_2O_2 compared with the control group, but significantly decreased in EEMP-receiving group starting at dose of 200 mg/kg than that in HD group. There is no difference in GPx activity among all groups. Hypercholesterolemic diet decreased CAT activity compared with control group (P = 0.052), but was significantly increased in EEMP-receiving group at doses of 400 and 800 mg/kg. The levels of foam cells counts were significantly decreased in EEMP-receiving group at doses of 400 and 800 mg/kg than that in HD group. In conclusion, EEMP at dose of 400 mg/kg BW decreased oxidant levels due to modulation of catalase activity and also inhibited foam cells formation.

INTRODUCTION

Atherogenesis is a chronic inflammatory process that involves a complex interplay between circulating cellular and blood elements and the cells of the arterial wall. The "oxidation hypothesis" has been a central focus in the investigation of the pathogenesis of the atherosclerotic process [1, 2]. The earliest visible lesion of atherosclerosis is the fatty streak, an aggregation of cholesterol-loaded macrophages, the so-called foam cells, within the arterial wall [3].

Searching for new biologically active compounds, novel antioxidant agents derived from active phytochemicals, could improve the anti-atherosclerosis efficacy of standard drug treatment.

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 [3]. Various studies have revealed the potential effects of active

ingredients from the mangosteen rind, among others, as antioxidants [4]. Previous studies showed that α-mangostin from the pericarp of mangosteen has antioxidant properties and is a potent scavenger of peroxynitric anion, singlet oxygen, and superoxide radical [5-7].

The present study tried to analyze the effect of ethanolic extract of fruit mangosteen pericarp (EEMP) on oxidant and antioxidant indices in the atherosclerosis model. The hypothesis of this study was that EEMP can reduce levels of H_2O_2 and increase the activity of GPx and CAT. Morever, it was aimed to test whether EEMP can reduce levels of foam cells in atherosclerotic rats. These effects could contribute to a possible reduction in atheroslerotic lesions.

MATERIAL AND METHODS

Animals

Thirty male Wistar rats, weighing 110-145 g, purchased from Central Animal House of Bandung were housed in an air-conditioned room at 24 ± 2°C and 65-70% relative humidity with a 12 h light-dark cycle. The protocol used in this study was approved by the Research Ethics Committee for Animal Experimentation of the University of Brawijaya, Malang, Indonesia. Diets were made by following the American Institute of Nutrition's (AIN) recommendations. The animals were given water *ad libitum* during the experimental period. The composition of standard diet was 66% comfeed PAR-s, 33% wheat powder and water. The composition of high-cholesterol diet according to previous study [8] with modification, consisted of 53% comfeed PAR-s, 26.5% wheat powder, 0.1% sheep oil, 0.0013% cholic acid and 3.22% pig oil.

Collection of mangosteen fruit pericarp

Mangosteen fruits were obtained from mangosteen trees cultivated in forest area of Marabahan district, South Kalimantan. Every year, these fruits were harvested between October until September. Mangosteen fruit pericarps were collected from mature fruits, charcterized by dark purple of rinds and pleasant smell. The fruits which have been harvested have perfect rind structures and none of wound or scarring from plant infection. The fruits were rinsed in water, then the edible fleshes were collected. The fruit pericarps (exocarp and mesocarp) were collected in clean jar for subsequent processes [4].

Extraction and high performance liquid chromatography

In brief, the dried fruit pericarps (1 kg) were ground and then extracted with ethanol (10 liters) at room temperature. The extraction was repeated three times within 1 week, and the combined solution was concentrated. Ethanolic extract was obtained in three steps, including drying, extraction, and evaporation. Drying was done by cutting fruit pericarps in small pieces then overheated at 80°C or by sunlight exposure. Extraction was done by blending fruit pericarps to get 100 g powder. A hundred grams of powder were then added to 900 ml 70% ethanol in 1 liter erlenmeyer jar. To obtain maximal evaporation, they were mixed and then incubated at one night. Upper layers were collected and connected to evaporation apparatus, and the specimen was kept in freezer. High performance liquid chromatography (HPLC) was done using 4 standards, such as garcinone C (Chengdu), garcinone D (Chengdu), α-mangostin (Chengdu), and γ-mangostin (Chengdu).

Foam cells analysis

Foam cells analysis was done by aorta frozen section staining with Oil Red O. The quantification of foam cells was done by light microscope by three independent evaluators.

Glutathione peroxidase, catalase and H_2O_2 analysis

Commercial GPx and CAT analysis as well as H_2O_2 detection kits (Oxis Biotech international, Portland, OR, USA) were used to measure those enzyme activities in aorta, and H_2O_2 level in serum.

Statistical analysis

Data were presented as mean ± SD and differences between groups were analyzed using one way ANOVA with SPSS 15.0 statistical package. Post hoc test was used if the ANOVA was significant; $P < 0.05$ was considered statistically significant.

RESULTS

HPLC was performed to obtain several active compounds of ethanolic extracts of *Garcinia mangostana* fruit pericarp. We found xanthone compounds as to see in Table 1.

High cholesterol diet significantly increased H_2O_2 compared with control group ($P < 0.05$). The level of H_2O_2 was significantly decreased in EEMP-receiving group starting at dose of 200 mg/kg (body weight) than that in atherosclerotic group, although cannot reach levels at standard diet. Besides, there was no difference in GPx activities between all groups ($P > 0.05$). High cholesterol diet decreased CAT activity compared with control group ($P = 0.052$). The level of CAT activity was significantly increased in EEMP-receiving group at doses of 400 and 800 mg/kg to reach levels at standard diet group.

 DOI 10.5455/oams.170313.br.007

Table 1. Concentration of xanthone compounds in EEMP

Compounds	Percentage
Garcinone C	0.004
Garcinone D	0.002
α-mangostin	0.064
γ-mangostin	6.144

High cholesterol diet significantly increased foam cells counts compared with control group ($P < 0.05$). The levels of foam cells counts was significantly decreased in EEMP-receiving group at doses of 400 and 800 mg/kg than that in atherosclerotic group ($P < 0.05$), as seen in Fig.1.

DISCUSSION

We choose ethanolic extract in this study according to the results of previous study. The concentration of α-mangostin was highest in dichloromethane, ethanol, hexane and water, respectively [9]. Although the α-mangostin concentration was higher in dichloromethane compared to ethanol, dichloromethan was toxic so we choose ethanol. Ethanolic extract of garcinia fruit pericarp from Marabahan forest of South Kalimantan showed several xanthone compounds, including garcinone C (0.004%), garcinone D (0.002%), α-mangostin (0.064%), and γ-mangostin (6.144%). Previous reports showed that α-mangostin is a major antioxidant xanthone in mangosteen [10]. In the present study we found that γ-mangostin is the highest xanthone compound which is consistent with the study by Nakatani *et al* [11]. The concentration of active compounds is different compared with *Garcinia mangostana* species from another region as reported by other authors [10, 11]. The difference was determined by maturation of fruit, soil characteristics, and environmental factors.

The present study demonstrated that hypercholesterolemic diet significantly increased H_2O_2 level compared with the control group. Elevated H_2O_2 level was caused by the increasing activities of NADPH oxidase, xanthine oxidase and lipooxygenase [12]. Subsequently, superoxide radical will be dismutated spontaneously by superoxide dismutase to form H_2O_2. To maintain normal H_2O_2 level, other antioxidant defenses have been involved, including two main classes of enzymes. H_2O_2 is converted to water and O_2 by CAT or to water and an oxidized donor by peroxidases, such as the selenium-containing GPx [13].

Table 2. Foam cells and oxidant-antioxidant status in atherosclerotic rats receiving EEMP

	Standard diet (control)	Hypercholesterolemic diet	Hypercholesterolemic diet + EEMP		
			200 mg/kg	400 mg/kg	800 mg/kg
Foam cells (count)	3.4 ± 0.14	24.6 ± 2.19[a]	22 ± 2.12[a]	15.6 ± 2.5[abc]	12.6 ± 3.43[abcd]
H_2O_2 (µmol/l)	11.86 ± 0.51	31.78 ± 0.46[a]	27.65 ± 0.53[ab]	25.21 ± 0.7[abc]	21.42 ± 0.3[abcd]
GPx (nmol/mnt/ml)	0.44 ± 0.22	0.53 ± 0.08	0.48 ± 0.15	0.40 ± 0.13	0.47 ± 0.17
CAT (nmol/ml)	7.21 ± 1.81	4.26 ± 0.71	3.96 ± 0.74[a]	8.43 ± 4.62[b]	15.66 ± 3.01[abcd]

EEMP: ethanolic extract mangosteen pericarp; GPx: glutathione peroxidase; CAT: catalase; [a]$P < 0.05$ in comparison with standard diet group; [b]$P < 0.05$ in comparison with hypercholesterolemic; [c]$P < 0.05$ in comparison with hypercholesterolemic diet + EEMP of 200 mg/kg; [d]$P < 0.05$ in comparison with in hypercholesterolemic diet + EEMP of 400 mg/kg bw

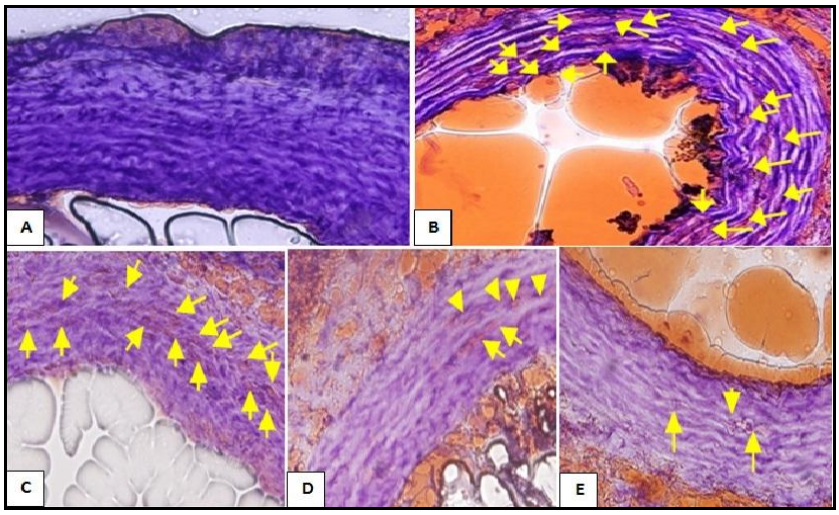

Figure 1.
Foam cells (yellow arrows) formation in atherosclerotic rats.
High cholesterol diet **(B)** significantly increased foam cells counts compared with control group **(A)**, but will significantly reduced n EEMP-receiving group at doses of 400 **(D)** and 800 mg/kg (body weight) **(E)**.

Administration of EEMP in atherosclerotic rats significantly decreased H_2O_2 level, starting at dose of 200 mg/kg than that of hyperchelosterolemic group, but cannot reach H_2O_2 level in standard diet group. In addition, administration of EEMP doesn't change an activity of GPx in aorta between groups. This finding indicated that H_2O_2 lowering effect doesn't come from an activity of aorta GPx. We suggested that H_2O_2 lowering effect is due to the decreasing activities of several enzymes which produce reactive oxygen species, such as NADPH oxidase, xanthine oxidase, and lipooxygenase. H_2O_2 lowering effect may also be contributed from a scavenging activity of active compounds in EEMP. We also found that EEMP is able to modulate CAT activity due to the scavenging activity of H_2O_2 or modulation of the Nrf-2 gene. This finding confirms a previous study which proved an activity of water and ethanolic extract of Garcinia mangostana as scavenger to H_2O_2 [14].

Finally, EEMP decreases foam cell counts significantly at doses of 400 and 800 mg/kg, although can't reach control levels. Decreasing foam cells counts in atherosclerotic rats receiving EEMP may be due to inhibition of H_2O_2 level and positive modulation to CAT. At the dose of 800 mg/kg we suggest that EEMP not only increases CAT activity but also increase its mRNA expression. This finding is consistent with a previous work, showing that over-expression of CAT could retard the development of atherosclerosis in the aorta of the ApoE(–/–) mice [15].

In conclusion, ethanolic extract of *Garcinia mangostana* pericarp at the dose of 400 mg/kg is able to decrease oxidant levels due to modulation of CAT activity in atherosclerotic rats. In addition, ethanolic extract of *Garcinia mangostana pericarp* also inhibits foam cells formation as lesion of atherosclerosis.

ACKNOWLEDGMENTS

The authors gratefully thank all technicians in Laboratory of Pharmacology (Mrs. Ferida & Mr. Moch Abuhari) and staff at Laboratory of Biomedical Science (Mr. Wibi Riawan), Faculty of Medicine, University of Lambung Brawijaya, Malang for their valuable techical assistances.

CONFLICTS OF INTEREST

None to declare

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 DOI 10.5455/oams.170313.br.007