

Extract of mangosteen increases high density lipoprotein levels in rats fed high lipid

Dwi Laksono Adiputro*, M. Aris Widodo**, Rochmad Romdoni***, and Djanggan Sargowo****

ABSTRACT

*Department of Cardiology and Vascular Medicine
Faculty of Medicine
Lambung Mangkurat
University/Ulin General
Hospital, Banjarmasin

** Department of
Pharmacology
Faculty of Medicine
Brawijaya University, Malang
*** Department of Cardiology and Vascular Medicine
Faculty of Medicine
Airlangga University/
Dr. Soetomo District General
Hospital, Surabaya
**** Department of
Cardiology and Vascular
Medicine
Faculty of Medicine
Brawijaya University/
Saiful Anwar General Hospital,
Malang

Correspondence :
dr. Dwi Laksono Adiputro
Department of Cardiology and
Vascular Medicine
Faculty of Medicine
Lambung Mangkurat
University/
Ulin General Hospital
Jl. A. Yani Km 2 No. 43
Banjarmasin, South Kalimantan
E-mail:
d_adiputro@yahoo.com

BACKGROUND

In cardiovascular medicine, *Garcinia mangostana* has been used as an antioxidant to inhibit oxidation of low density lipoproteins and as an antiobesity agent. The effect of *Garcinia mangostana* on hyperlipidemia is unknown. The aim of this study was to evaluate the effect of an ethanolic extract of *Garcinia mangostana* pericarp on lipid profile in rats fed a high lipid diet.

METHODS

A total of 40 rats were divided into five groups control, high lipid diet, and high lipid diet + ethanolic extract of *Garcinia mangostana* pericarp at dosages of 200, 400, and 800 mg/kg body weight. The control group received a standard diet for 60 days. The high lipid diet group received standard diet plus egg yolk, goat fat, cholic acid, and pig fat for 60 days with or without ethanolic extract of *Garcinia mangostana* pericarp by the oral route. After 60 days, rats were anesthetized with ether for collection of blood by cardiac puncture. Analysis of blood lipid profile comprised colorimetric determination of cholesterol, triglyceride, low density lipoprotein (LDL), and high density lipoprotein (HDL).

RESULTS

From the results of one-way ANOVA it was concluded that there were significant between-group differences in cholesterol, trygliceride, LDL, and HDL levels ($p=0.000$). Ethanolic extract of *Garcinia mangostana* pericarp significantly decreased cholesterol, trygliceride, and LDL levels, starting at 400 mg/kg body weight ($p=0.000$). Ethanolic extract of *Garcinia mangostana* pericarp significantly increased HDL level starting at 200 mg/kg body weight ($p=0.000$).

CONCLUSION

Ethanolic extract of *Garcinia mangostana* pericarp has a beneficial effect on lipid profile in rats on a high lipid diet.

Key words: Mangosteen, lipid profile, high lipid diet, rats

Ekstrak Kulit Buah Manggis meningkatkan kadar high density lipoprotein pada tikus diet tinggi lemak

ABSTRAK

LATAR BELAKANG

*Di bidang kardiovaskuler, buah manggis bersifat antioksidan untuk menghambat oksidasi low density lipoprotein (LDL) dan antiobesitas. Efek buah manggis terhadap hiperlipidemia belum diketahui. Tujuan penelitian ini adalah untuk mengevaluasi efek ekstrak etanol dari kulit buah manggis (*Garcinia mangostana*) terhadap profil lipid pada tikus yang diberikan diet tinggi lemak.*

METODE

Empat puluh ekor tikus terbagi menjadi lima kelompok meliputi kontrol, kelompok diet tinggi lemak, dan kelompok diet tinggi lemak + ekstrak etanol kulit buah manggis dosis 200; 400; dan 800 mg/kgBB. Kelompok kontrol mendapat diet standar selama 60 hari. Kelompok diet tinggi lemak mendapat diet standar ditambah kuning telur, minyak kambing, asam kolat, dan minyak babi selama 60 hari dengan atau tanpa ekstrak etanol kulit buah manggis per oral. Setelah 60 hari, tikus dianestesi dengan eter dan dilakukan pungsi darah dari jantung. Analisis profil lipid yang meliputi kadar kolesterol, trigliserida, LDL, dan high density lipoprotein (HDL) dilakukan dengan metode kolorimetrik.

HASIL

Uji ANOVA menyimpulkan bahwa terdapat perbedaan bermakna kadar kolesterol, trigliserida, LDL, dan HDL pada berbagai kelompok perlakuan ($p=0,000$). Ekstrak etanol kulit buah manggis menurunkan kadar kolesterol, trigliserida, LDL secara bermakna dimulai pada dosis 400 mg/kgBB ($p=0,000$). Ekstrak etanol kulit buah manggis meningkatkan kadar HDL secara bermakna dimulai pada dosis 200 mg/kgBB ($p=0,000$).

KESIMPULAN

Disimpulkan bahwa ekstrak etanol kulit buah manggis mempunyai efek menguntungkan terhadap profil lipid pada tikus yang mendapat diet tinggi lemak.

Kata kunci: *Manggis, kulit buah, profil lipid, diet tinggi lemak, tikus*

INTRODUCTION

The mangosteen tree has long been cultivated in tropical countries. The tree presumably originated from Southeast Asia or Indonesia, and may also be found in the Malay Peninsula, Myanmar, Thailand, Kambodia, and Vietnam. The mangosteen pericarp is 0.6-1 cm thick and contains a violet pigment.⁽¹⁾ Communities of the abovementioned countries have utilized the pericarp or the fruit flesh in traditional medicine against abdominal pain, diarrhea, dysentery, wound infections, suppuration, and chronic ulcers.⁽²⁾

A variety of studies have revealed the potential of the active principles of mangosteen, among others as antioxidant, antibacterial, antifungal, antimalarial, anti-inflammatory, cytotoxic, and inhibitor of the human immunodeficiency virus (HIV), aromatase inhibitor, and inducer of quinone reductase.⁽³⁾ In cardiovascular medicine, the mangosteen can potentially inhibit the oxidation of low density lipoprotein (LDL). In obese individuals, the mangosteen is capable of reducing C-reactive protein level.⁽⁴⁾ In addition, the active substances in mangosteens can also decrease oxidative stress due to cardiac reperfusion.⁽⁵⁾

On the basis of the concept of natural intelligence, the potential of active substances of plant origin becomes more limited if the pharmacological actions are only displayed by one active substance. The substances isolated from mangosteen pericarps contains many xanthenes, among others α -mangostin, β -mangostin, γ -mangostin, gartanin, 8-deoxygartanin, and mangostanol.^(6,7) α -mangostin is the principal active substance and has been frequently used in traditional medicine throughout the world as an anti-inflammatory,⁽⁸⁾ antibacterial,⁽⁹⁾ and anticancer agent.⁽¹⁰⁾

To date it has been demonstrated that mangosteen pericarps contain more than 85 active substances. A study by Nakatani et al.⁽¹²⁾ stated that ethanolic extracts of mangosteen pericarp contains 7.7% α -mangostin and 39% β -mangostin. A study by Tewtrakul et al.⁽¹³⁾ found higher IC_{50} values for nitric oxide (NO) inhibition and prostaglandin- E_2 (PGE_2) release in isolates of α -mangostin and β -mangostin in comparison with ethanolic extract of mangosteen pericarp.

However, none of the abovementioned studies have investigated the effect of mangosteen pericarp on the lipid profile of rats on a high lipid diet. Therefore the purpose of the present study was to analyze the role of an ethanolic extract of mangosteen pericarp on the lipid profile of rats on a high lipid diet. The hypothesis of this study was that ethanolic extracts of mangosteen pericarp is capable of reducing total cholesterol, triglyceride, and LDL levels in rats receiving a high lipid diet. In addition, it was expected that ethanolic extracts of mangosteen pericarp may increase high density lipoprotein (HDL) levels in rats on a high lipid diet.

METHODS

Study design

The present study was an in vivo experimental study using animal models, conducted at the Pharmacological and the

Biomedical Laboratories of the Faculty of Medicine, Brawijaya University from March 2010 to June 2010.

Experimental animals

The experimental animals were albino rats (*Rattus norvegicus*) obtained from the Pharmacological Laboratory of the Faculty of Medicine, Brawijaya University. The rats used were male rats aged 6-8 weeks and weighing 100-150 grams. The rats were kept in open ventilated cages and were acclimatized for 2 weeks before being subjected to the intervention. The rats were kept in a single caging system, where each cage contained one rat. The cages were plastic boxes measuring 45 cm x 35.5 cm x 14.5 cm, with wire covers. The cages had beddings of rice hulls that were changed daily. The animal feed given daily was feed for adult rats composed of confed PAR-S 66%, wheat flour 33%, and water. This was the composition of the feed for the standard diet. The feed given to each rat was \pm 40 gram per day and renewed daily. For the high lipid diet, the composition of the feed was comfeed PAR-S 53%, wheat flour 26.5%, goat fat 0.1%, cholic acid 0.0013% and pig fat 3.22%.

The experimental animals were divided into one group of rats receiving the standard diet, one group receiving the high lipid diet, and three groups receiving high lipid diet + ethanolic extract of mangosteen pericarp at dosages of 200, 400, and 800 mg/kgBW, respectively. The intervention was conducted for 60 days. Each intervention group consisted of 8 rats, making up a total of 40 rats.

Preparation of ethanolic extract of mangosteen pericarp

The preparation of the ethanolic extracts of mangosteen pericarp was performed in three stages, comprising the processes of drying, extraction, and evaporation. Drying was done by washing the mangosteen pericarps obtained from forests at Marabahan, South Kalimantan, and subsequently comminuting the cleansed

material. The pieces of pericarp were then either heated in an oven at 80°C or placed to dry in the sun. Extraction was performed by blenderizing the material and weighing off a portion of 100 grams. The 100 gram portion of the blenderized pericarp material was put into an Erlenmeyer flask of 1 liter capacity, then 900 mL of 70% ethanol was added. The soaked mixture was shaken well, then left to settle overnight. Evaporation was done by decanting the upper layers of the ethanol-pericarp mixture into an evaporating flask. The latter was placed in an evaporator, the water bath was filled completely, the device was installed and connected to an electricity source (220 V). The ethanolic pericarp extract dripping into the receiver flask was collected and transferred to a plastic bottle for storage in a freezer.

Collection of blood samples

Blood samples were obtained from the heart of each rat after the intervention period of 60 days. The blood collecting procedure was started by putting the rat into a glass container into which ether-saturated cotton wads had been placed for inhalation anesthesia. The anesthetized rat was sectioned by opening the abdomen up to the chest. After exposing the heart, the cardiac apex was punctured and the blood slowly withdrawn using a 5 mL syringe until the maximum amount of blood was obtained. The blood sample was subsequently transferred into an Eppendorf tube without added EDTA. The tube was then code-labelled and transported to the Clinical Pathology Laboratory of the Faculty of Medicine, Brawijaya University, for analysis of serum lipid

profile, comprising total cholesterol, triglyceride, LDL, and HDL levels. The analysis was performed according to the procedural steps outlined in the kit.

Data analysis

To determine differences in cholesterol, triglyceride, LDL, and HDL levels, ANOVA and post hoc tests were done. The level of significance was set at $p < 0.05$. The statistical analysis was performed with SPSS version 13.

Ethical clearance

This study was approved for ethical clearance by the Committee on Research Ethics, Faculty of Medicine, Brawijaya University, Malang.

RESULTS

After administration of the intervention for 60 days, the highest increase in body weight was found in the group receiving a high lipid diet and ethanolic extract of mangosteen pericarp at a dosage of 800 mg/kg BW, the increase being 1.84 gram/day. Mean feed consumption did not differ in the five groups ($p > 0.05$), making them comparable (Table 1).

Rats receiving the high lipid diet for 60 days had significantly increased total cholesterol, triglyceride, and LDL levels, as compared with rats receiving the standard diet ($p = 0.000$). In addition, rats on the high lipid diet also had a significant reduction in HDL levels, as compared to rats on the standard diet ($p = 0.000$).

Administration of ethanolic extract of mangosteen pericarp at dosages of 400 mg/

Table 1. Increase in body weight and feed consumption of the various groups

	Standard diet	High lipid diet	High lipid diet + ethanolic extract of mangosteen pericarp		
			200 mg/kg BW	400 mg/kg BW	800 mg/kg BW
Increase in body weight (g/d)	1.54	1.58	1.16	1.37	1.84
Feed consumption (g/d)	19.13	14.33	13.16	18.30	18.70

Values are mean

Table 2. Total cholesterol, triglyceride, LDL, and HDL levels in various group

	Standard diet	High lipid diet	High lipid diet + ethanolic extract of mango steen pericarp		
			200 mg/kgBB	400 mg/kgBB	800 mg/kg BB
Total cholesterol (mg/dL)	105.80 ± 10.06	193.00 ± 36.72 ^a	167.40 ± 23.42 ^a	136.25 ± 10.81 ^{abc}	101.6 ± 5.13 ^{bd}
Triglyceride (mg/dL)	96.80 ± 8.32	133.20 ± 8.32 ^a	124.00 ± 18.21 ^a	97.00 ± 7.44 ^{bc}	91.8 ± 7.89 ^{bc}
LDL (mg/dL)	52.20 ± 10.94	144.80 ± 35.50 ^a	116.20 ± 24.97 ^{ab}	87.75 ± 10.14 ^{abc}	48.6 ± 8.73 ^{bcd}
HDL (mg/dL)	34.20 ± 3.83	21.60 ± 2.30 ^a	26.00 ± 0.71 ^{ab}	29.25 ± 1.26 ^{ab}	34.4 ± 5.13 ^{bcd}

Values represent mean ± SD; LDL= low density lipoprotein; HDL= high density lipoprotein; mg/kgBW= milligram/kilogram body weight; mg/dL: milligram/deciliter; ^aThere was a significant difference with the standard diet (p<0.05); ^bThere was a significant difference with high lipid diet without ethanolic extract of mangosteen pericarp (p<0.05); ^cThere was a significant difference with high lipid diet + extract at 200 mg/kgBW (p<0.05); ^dThere was a significant difference with high lipid diet + extract at 400 mg/kgBW (p<0.05)

kgBW and 800 mg/kgBW, respectively, to the high lipid diet group, resulted in decreased cholesterol levels. Administration of ethanolic extract of mangosteen pericarp at the dosage of 800 mg/kgBW to the high lipid diet group was capable of reducing cholesterol levels down to the levels in the standard diet group. Similar results were obtained for triglyceride levels. For LDL levels, administration of ethanolic extract of mangosteen pericarp was capable of reducing LDL levels, starting with the dosage of 200 mg/kgBW and restoring the LDL levels to standard diet levels at a dosage of 800 mg/kgBW. Similarly, administration of ethanolic extract of mangosteen pericarp significantly increased HDL levels, starting at a dosage of 200 mg/kgBW and approaching standard diet HDL levels at a dosage of 800 mg/kgBW (Table 2).

DISCUSSION

The present study found increases in cholesterol, triglyceride, and LDL levels, with a reduction in HDL level in the rats receiving the high lipid diet. This was caused by the intake of saturated fatty acids contained in goat fat, pork fat, egg yolk, and cholic acid. These results were consistent with those of previous studies on high lipid diets in rats.^(14,15) Consumption of long-chain saturated fatty acids (C>10) led to increases in cholesterol and triglyceride levels. These

increases caused increases in LDL levels, necessary for transporting the cholesterol to peripheral tissues for oxidation or to adipose tissues for storage.⁽¹⁶⁾ A higher proportion of lipids to proteins in the lipoproteins is associated with a lower density of the lipoproteins. Thus the higher the LDL levels, the lower the HDL levels. Furthermore, increases in triglyceride levels lead to increases in chylomicron and very low density lipoprotein (VLDL) levels, as transporters of triglycerides. LDL is the last stage of VLDL catabolism, therefore raised VLDL levels also increase LDL levels. Increased cholesterol levels result in downregulation of native LDL receptors, thus inducing LDL uptake by receptor scavengers, ultimately leading to foam cell formation.⁽¹⁷⁾

Administration of ethanolic extract of mangosteen pericarp reduced blood cholesterol and triglyceride levels at dosages of 400 mg/kgBW and 800 mg/kgBW. The mechanism of the reduction in blood cholesterol levels is due to inhibition of cholesterol formation. In cholesterol formation there is a stage of squalene synthesis before becoming cholesterol. At this stage there is the combination of two molecules of farnesyl pyrophosphate and elimination of pyrophosphate radicals. The combination of two molecules of farnesyl pyrophosphate is marked by the combination of two farnesyl pyrophosphate radicals. This combination is

inhibited by the antioxidant properties of the ethanolic extract of mangosteen pericarp, thus inhibiting squalene formation. The antioxidant properties of ethanolic extract of mangosteen pericarp has been proven by several studies. The study of Chonmawanang et al.⁽¹⁸⁾ demonstrated the potential of mangosteen pericarp as inhibitor of superoxide radicals through the mechanism of transfer of electrons or hydrogen atoms.


As to the mechanism of increased cholesterol katabolism, cholesterol is converted into bile salts. The process of conversion of cholesterol into bile salts requires the availability of oxygen, NADPH, and cytochrome p450. The study of Foti et al.⁽¹⁹⁾ demonstrated that the active substances in mangosteens are capable of inhibiting cytochrome p450. Therefore, the mechanism of cholesterol reduction by mangosteen pericarp in the present study was caused by inhibition of cholesterol synthesis.

The reduction in triglyceride levels in the present study was caused by increased VLDL and chylomicron katabolism. VLDL is a triglyceride transporter originating in the liver, while chylomicrons are triglyceride transporters from the intestines. VLDL and chylomicron katabolism is effected by lipoprotein lipases that hydrolyze triglycerides into free fatty acids and glycerol.

Administration of ethanolic extract of mangosteen pericarp increased HDL levels, starting at a dosage of 200 mg/kgBW. The increase in HDL in the present study is due to increased synthesis and secretion by the liver and intestines. The main function of HDL is as a storage site of apolipoproteins C and E, which are needed in the katabolism of chylomicrons and VLDL, where apolipoprotein C is a cofactor of lipoprotein lipase and apolipoprotein E is a ligand for LDL receptors.⁽²⁰⁾

The clinical implication of these study results are that the potential in rats of mangosteen pericarp extract may be applied to human subjects. A limitation of this study was that the supporting parameters associated with lipid metabolism were not studied in detail.

CONCLUSION

Ethanolic extract of mangosteen pericarp has beneficial effects on the lipid profile of rats receiving a high lipid diet, in the form of reduced total cholesterol, triglyceride, and LDL levels and increased HDL levels. The benefits of mangosteen supplementation in patients with hypercholesterolemia need to be further investigated. 

REFERENCES

1. Akao Y, Nakagawa Y, Linuma M, Nozawa Y. Anti-cancer effect of xanthenes from pericarps of mangosteen. *Int J Mol Sci* 2008;9:355-70.
2. Cui J, Hu W, Cai Z, Liu Y, Li S, Tao W, et al. New medicinal properties of mangostins: analgesic activity and pharmacological characterization of active ingredients from the fruit hull of *Garcinia mangostana* L. *Pharmacol Biochem Behav* 2010; 95:166-72.
3. Han AR, Kim JA, Lantvit DD, Kardono LBS, Riswan S, Chai H, et al. Cytotoxic xanthone constituents of the stem bark of *Garcinia mangostana* (mangosteen). *J Nat Prod* 2009;72: 2028-31.
4. Udani JK, Singh BB, Barret ML, Singh VJ. Evaluation of mangosteen juice blend on biomarkers of inflammation in obese subjects: a pilot, dose finding study. *Nutr J* 2009;8:48.
5. Buelna-Chontal M, Correa F, Hernandez-Resendiz S, Zazueta C, Pedraza-Chaverri J. Protective effect of α -mangostin on cardiac reperfusion damage by attenuation on oxidative stress. *J Med Food* 2011;14:1370-74.
6. Suksamrarn S, Suwannapoch N, Ratananukul P, Aroonlerk N, Suksamrarn A. Xanthenes from the green fruit hulls of *Garcinia mangostana*. *J Nat Prod* 2002;6:761-3.
7. Nilar LJ, Harrison LJ. Xanthenes from the heartwood of *Garcinia mangostana*. *Phytochemistry* 2002;60:541-8.
8. Moongkarndi P, Kosem N, Kaslungka S, Luanratana O, Pongpan N, Neungton N. Antiproliferation, antioxidation and induction of apoptosis by *Garcinia mangostana* (mangosteen) on SKBR3 human breast cancer cell line. *J Ethnopharmacol* 2004;90:161-6.
9. Suksamrarn S, Suwannapoch N, Phakhodee W, Thanuheranlert J, Ratananukul P, Chimnoi N, et al. Antimycobacterial activity of prenylated

- xanthones from the fruits of *Garcinia mangostana*. *Chem Pharm Bull* 2003;51:857-9.
10. Moongkarndi P, Kosem N, Luanratana O, Jongsomboonkusol S, Pongpan N. Antiproliferative activity of Thai medicinal plant extracts on human breast adenocarcinoma cell line. *Fitoterapia* 2004;75:375-7.
 11. Chin Y, Kinghorn AD. Structural characterization, biological effects, and synthetic studies on xanthones from mangosteen (*Garcinia mangostana*), a popular botanical dietary supplement. *Mini Rev Org Chem* 2009;5:355-64.
 12. Nakatani K, Atsumi M, Arakawa T, Oosawa K, Shimura S, Nakahata N, et al. Inhibitions of histamine release and prostaglandin E₂ synthesis by mangosteen, a Thai medicinal plant. *Biol Pharm Bull* 2002;25:1137-41.
 13. Tewtrakul S, Wattanapiromsakul C, Mahabusarakam W. Effects of compound from *Garcinia mangostana* on inflammatory mediators in RAW264.7 macrophage cells. *J Ethnopharmacol* 2009;121:379-82.
 14. Lee YJ, Choi DH, Cho GH, Kim JS, Kang DG, Lee HS. *Arctium lappa* ameliorates endothelial dysfunction in rats fed with fat/cholesterol diets. *BMC Compl Alternative Med* 2012;12:116 doi: 10.1186/1472-6882-12-116.
 15. Silva MAA, Vechetti-Junior IJ, do Nascimento AF, Furtado KS, Azevedo L, Ribeiro DA, et al. Effects of swim training on liver carcinogenesis in male Wistar rats fed a low-fat or high-fat diet. *Appl Physiol Nutr Metab* 2012;37: 1101-9.
 16. Edwar Z, Suyuthie H, Yerizel E, Sulastri D. Pengaruh pemanasan terhadap kejenuhan asam lemak minyak goreng sawit dan minyak goreng jagung. *J Indonesian Med Assoc* 2011;61:248-52.
 17. McLaren JE, Michael DR, Ashlin TG, Ramji DP. Cytokines, macrophage lipid metabolism and foam cells: Implications for cardiovascular disease therapy. *Prog Lipid Res* 2011;doi:10.1016/j.plipres. 2011.04.002.
 18. Chomnawang MT, Surassmo S, Nukoolkarn VS, Gritsanapan W. Effect of *Garcinia mangostana* on inflammation caused by propionibacterium acnes. *Fitoterapia* 2007;78:401-8.
 19. Foti RS, Pearson JT, Rock DA, Wahlstrom JL, Wienkers LC. In vitro inhibition of multiple cytochrome P450 isoforms by xanthone derivatives from mangosteen extract. *Drug Metab Disp* 2009; 37:1848-55.
 20. Ansell BJ, Watson KE, Fogelman AM, Navab M, Fonarow GC. High-density lipoprotein function: recent advances. *J Am Coll Cardiol* 2005; 46:1792-8.