ANALYSIS OF MANGOSTEEN PEEL ETHANOL EXTRACT (GARCINIA MANGOSTANA L.) AGAINST THE EFFECTIVENESS OF ANTI-DYSLIPIDEMIA IN MALE RATS FED A HIGH-FAT DIET

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Abstract

Dyslipidemia is caused by the disruption of lipid metabolism due to the interaction of genetic and environmental factors. RISKEDAS data also showed that 15.9% of the population aged ≥ 15 years had a very high proportion of LDL (≥ 190 mg/dl), 22.9% had HDL levels less than 40 mg/dl, and 11.9% with very high triglyceride levels (≥ 500 mg/dl). Natural ingredients that have the potential to be an alternative are mangosteen peels. This experimental study uses a Pre-test and Post-test group-only control design approach that uses male Wistar rats as experimental animals, with as many as four male Wistar rats (Rattus norvegicus) in each treatment group—analyzed with One-Way Anova if the data were normally distributed with a follow-up test in the form of a Post Hoc Tukey HSD test to see noticeable differences between treatments. However, if the broadcast data is abnormal, the Kruskal-Wallis test is used as an alternative test. Total cholesterol in all rat treatment groups showed significant differences; this can be seen from the P value <0.05. Triglyceride levels in all treatment groups also showed substantial differences; this can be seen from P < 0.05 (P value = 0.018). The total cholesterol of rats before giving a high-fat diet in all treatment groups did not show significant differences (P value = 0.829). LDL levels also showed significant differences in all treatment groups; this can be seen from the value of P < 0.05. HDL levels also showed significant differences in all treatment groups; this can be seen from P < 0.05 (P value = 0.018). Mangosteen rind ethanol extract can significantly reduce total cholesterol, reduce triglyceride levels, reduce LDL levels, SGOT levels (Value = 0.007), and SGPT (P value < 0.05) compared to the control group. In contrast, mangosteen rind ethanol extract can significantly increase HDL levels compared to the control group (P value = 0.018).

Keywords: *mangosteen peel, dyslipidemia, ethanol, fat*

Background

Based on data from the World Health Organization (WHO) in 2008 estimates that the prevalence of dyslipidemia in various regions varies, namely by 30.3% in Southeast Asia, 36.7% in the Pacific West, where the majority is lower than in Europe 53.7% and 47.7% in America (1). RISKEDAS data also showed that 15.9% of the population aged ≥ 15 years had a very high proportion of LDL (≥ 190 mg/dl), 22.9% had HDL levels less than 40 mg/dl and 11.9% with very high triglyceride levels (≥ 500 mg/dl) (2). Dyslipidemia is caused by the disruption of lipid metabolism due to the interaction of genetic and environmental factors (3); (4). Some types of mixed dyslipidemia associated with forming atherogenic lipids can lead to premature cardiovascular disease. These include increased VLDL cholesterol manifested by increased TG and LDL and reduced HDL cholesterol (3,5).

In the management of dyslipidemia, several anti-dyslipidemia drugs are on the market, including statins (6), fibrates, niacin, ezetimibe, and bile acid binding resins (7). However, there have been reports of undesirable side effects (myopathy) from some 'super statins.' Thus, alternative treatments with possibly more minimal side effects are needed (8); (5)—one of the natural ingredients that has the potential to serve as such an alternative is mangosteen rind. Mangosteen peel is a shell that is discarded by consumers or can be called agricultural waste. This study aimed to analyze the antidislipidemia effectiveness of mangosteen peel extract..

Research Methods

This experimental study uses a Pre-test and Post-test group-only control design approach that uses male Wistar rats as experimental animals, with as many as four male Wistar rats (Rattus norvegicus) in each treatment group. This study was conducted in Maret 2023; the time taken was about 6 (six) weeks, with the first week for making ethanol extract of mangosteen fruit peel, the following week for the rat acclimatization process, and four weeks was the treatment of male Wistar rats. Surgical tools, laboratory glassware, aluminum foil, blender (Miyako), porcelain cup, desiccator, incubator, object-glass, cover glass, porcelain crutch, drying cabinet, microtube, light microscope, analytical balance sheet (Vibra AJ), oral sonde, electric oven (Stork), water bath (Yenaco), tube clamp, test tube rack, rotary evaporator, centrifugation, set of water content determination tools, UV spectrophotometer (Microlet 3000), injection syringe, furnace (Nabertherm), test tubes, animal scales (Presica). The ingredients used in this study were mangosteen fruit, methanol, Aquades, Na-CMC (Sodium-Carboxyl methylcellulose), simvastatin, husk, rat food pellets, phytochemical screening reagents, and ketamine.

The treatment experienced by each mouse in the group was as follows:

Table 1. Description of the Treatment of Each Group

No	Kelompok Uji	Perlakuan
1.	Normal	Test animals were not given specific treatment and were only given to eat and drink ad libitium.
2.	Control	Test animals were given 1 ml of 0.5% Na CMC suspension once a day for 14 days. Food and drinks are given ad libitum.
3.	Standard	Test animals were given oral suspension simvastatin 5 ml / kgBB

	(25 mg/kgBB)	once a day for 14 days. Food and drinks are given ad libitum.
4.	Mangosteen Peel Ethanol Extract - I (150 mg / kgBB)	The test animals were given mangosteen peel ethanol extract at a dose of 1.5 ml / kgBB once a day for 14 days. Food and drinks are given ad libitum.
5.	Mangosteen Peel Ethanol Extract - II (300 mg/kgBB)	The test animals were given mangosteen peel ethanol extract at a dose of 3 ml / kgBB once a day for 14 days. Food and drinks are given ad libitum.
6.	Mangosteen Peel Ethanol Extract - III (450 mg/kgBB)	The test animals were given mangosteen peel ethanol extract at a dose of 4.5 ml / kgBB once a day for 14 days. Food and drinks are given ad libitum.

Before the blood draw, the rats were fasted at least 8 hours before the blood draw. A blood draw is carried out by direct withdrawal from the heart of mice as much as 1 ml. Put in microtubes and allow to stand ± 20 minutes. Then the blood was centrifuged at a speed of 3000 rpm for 15 minutes to get rat blood serum. The determination of lipid profile is determined by the colorimetric method. Lipid profile examination is conducted at the Health Laboratory, North Sumatra Provincial Health Office. A blood draw is carried out by direct withdrawal from the heart of mice as much as 1 ml. Put in microtubes and allow to stand ± 20 minutes. Then the blood was centrifuged at a speed of 3000 rpm for 15 minutes to get rat blood serum. The determination of SGOT and SGPT levels is based on an enzymatic reaction using the Dyasis® kit reagent. The procedure for determining the activity of SGOT and SGPT catalysts is based on the work procedures of Dyasis®. SGOT and SGPT examinations are conducted at the Health Laboratory, North Sumatra Provincial Health Office.The study results were analyzed descriptively (Central tendency and Dispersion) from the research data in the form of lipid profiles (LDL, HDL, Total Cholesterol, and Triglycerides). In addition, lipid profiles were analyzed with One-Way Anova if the data were normally distributed with a follow-up test in the form of a Post Hoc Tukey HSD test to see noticeable differences between treatments. However, as an alternative test, if the distributed data is abnormal, the Kruskal-Wallis test is used.

Results And Discussion

The results of phytochemical screening on mangosteen peel ethanol extract samples can be seen in the following table. From table data 2, it can be seen that mangosteen peel ethanol extract contains several phytochemical compounds including Alkaloids, Saponins, Flavonoids, Tannins, as well as Steroids, and Terpenoids.

Table 2. Phytochemical Screening Results of Mangosteen Skin Ethanol Extract

Phytochemicals	Reagents	Result
Alkaloid	Bouchardart	+
	Mayer	+
	Dragondroff	-
	Wagner	+
Saponins	Aquadest + Alcohol 96%	-
Flavonoids	FeCl3 5%	+

Phytochemicals	**Reagents**	**Result**
	$Mg_{(s)} + HCl_{(p)}$	-
	NaOH 10%	-
	$H_2SO_{4\ (p)}$	-
Tannins	$FeCl_3$ 1%	+
Steroids and Terpenoids	Salkowsky	-
	Liberman Bouchard	+

Table 3. Results of Data Normality Test with Shapiro-Wilk Test on All Research Parameters

Parameters		**P-Value**	**Data Distribution**
Weight		0.391	Normal
Total Cholesterol Before Induction		< 0.05	Abnormal
Total Cholesterol After Induction		< 0.05	Abnormal
Lipid Profile After Treatment	Total Cholesterol	0.495	Normal
	Triglycerides	0.002	Abnormal
	HDL levels	< 0.05	Abnormal
	LDL levels	0.144	Normal
SGOT levels		< 0.05	Abnormal
SGPT Levels		0.067	Normal

From the data of the table 3, it can be seen that the data on body weight, total cholesterol and LDL levels from the lipid profile after treatment, and SGPT levels have a normal data distribution, while other parameters include: total Cholesterol before and after induction, triglyceride levels, HDL levels, and abnormally distributed SGOT levels. Based on the distribution of these data, data with normal data distributions are analyzed with parametric cynics while abnormal data is analyzed with non-parametric statistics.

Table 4. Comparison of Total Cholesterol Before and After High-Fat Diet Administration in All Treatment Groups

Treatment Groups	**Total Cholesterol (mg/dL)**	
	Sebelum Induksi	**After Induction**
Normal	118.50 (110-117)	117.00 (112-120)[b]
Standard	113.00 (100-119)	208.00 (208-211)[a]
Control	114.50 (110-110)	212.00 (212-218)[b]
Mangosteen Peel Ethanol Extract -I	114.50 (110-120)	209.50 (208-210)[b]
Mangosteen Peel Ethanol Extract -II	112.00 (100-120)	210.50 (207-210)[b]
Mangosteen Peel Ethanol Extract -III	118.00 (117-125)	208.50 (206-210)[b]
P-value	**0.829**	**0.018**

From the data table above, it can be seen that before being given a high-fat diet, the total cholesterol of rats before giving a high-fat diet in all treatment groups did not show significant differences (P value = 0.829).

This indicates that the entire cholesterol data of rats before being given a high-fat diet is uniform. However, the total cholesterol in all groups of rats after the high-fat diet showed a different distribution, where only the control, standard, mangosteen peel ethanol extract I, II, and III groups showed uniform total cholesterol.

Table 5. Comparison of Lipid Profiles across Rat Treatment Groups

Treatment Groups	Lipid Profile			
	Total Cholesterol *	**Triglycerides ****	**LDL***	**HDL****
Normal	117.00 (112-120)[b]	98.50 (97-100)a	54.00 ± 1.71a	64.00 (61-64)a
Standard	208.00 (208-211)[a]	105.50 (101-105)b	60.00 ±1.54b	62.70 (60-63)a
Control	212.00 (212-218)[b]	165.50 (162-179)c	107.00 ±4.22	29.50 (38-43)b
Mangosteen Peel Extract -I	209.50 (208-210)[b]	132.50 (132-135)d	83.75 ±2.62d	57.50 (56-59)b
Mangosteen Peel Extract -II	210.50 (207-210)[b]	120.50 (119-122)e	77.50 ± 1.29e	61.50 (61-63)a
Mangosteen Peel Extract -III	208.50 (206-210)[b]	112.00 (107-112)f	68.50 ±1.29f	61.00 (60-63)a
P-Value	**< 0.05**	**0.018**	**< 0.05**	**0.018**

*The data is displayed as Mean ± SD. The P value is obtained from the One Way ANOVA analysis; **Data is displayed as Median (Range). The P value is obtained from kruskal-wallis analysis; Different superscripts in the same column show significant differences.

From the data in the table above, it can be seen that all lipid profile data in the entire treatment group showed significant differences.

a) Total cholesterol in all rat treatment groups showed significant differences; this can be seen from the P value <0.05. The lowest mean total cholesterol was found in the standard group at 117.00 (112-120) mg/dL, followed by the standard group at 208.00 (208-211) mg/dL, mangosteen rind ethanol extract group I, II, III, and the group with the highest total cholesterol was the control group at 212.00 (212-218) mg/dL;

b) Triglyceride levels in all treatment groups also showed significant differences; this can be seen from the P value <0.05 (P value = 0.018). The trend of the lowest triglyceride levels was found in the standard group at 98.50 mg/dL, followed by the legal group at 105.50 mg/dL, mangosteen rind ethanol extract groups I, II, III, and the group with the highest triglyceride levels were the control group at 165.50 mg/dL.

c) LDL levels also showed significant differences in all treatment groups; this can be seen from the value of P < 0.05. The lowest average LDL level was found in the standard group at 54.00 ± 1.71 mg/dL, followed by the legal group at 60.00 ± 1.54 mg/dL, mangosteen fruit peel ethanol extract group I, II, III, and the group with the highest LDL level was the control group at 107.00 ± 4.22 mg/dL.

d) HDL levels also showed significant differences in all treatment groups; this can be seen from P < 0.05 (P value = 0.018). The highest HDL level trend was found in the standard group at 64.00 mg/dL, followed by the legal group at 61.50 mg/dL, mangosteen rind ethanol extract group I, II, and III, and the group with the lowest HDL level was the control group at 29.50 mg/dL.

Table 6. Comparison of SGOT and SGPT Levels in All Treatment Groups

Treatment Groups	SGOT levels (U/L)	SGPT Levels (U/L)
Normal	29.40 (22-35)[a]	40.15 ± 1.20[a]
Standard	109.00 (109-110)[b]	164.25 ± 1.29[b]

Control	168.50 (165-170)[c]	97.25 ± 1.50[c]
Mangosteen Peel Ethanol Extract -I	117.00 (118-120)[d]	100.75 ± 3.59[d]
Mangosteen Peel Ethanol Extract -II	118.50 (120-125)[e]	115.50 ± 4.51[e]
Mangosteen Peel Ethanol Extract -III	128.00 (120-128)[f]	142.50 ± 2.08[b]
P-Value	**0.007**	**< 0.05**

*The data is displayed as Mean ± SD. The P value is obtained from the One Way ANOVA analysis; **Data is displayed as Median (Range). The P value is obtained from kruskal-wallis analysis; Different superscripts in the same column show significant differences.

From the data table above, it can be seen that the SGOT and SGPT levels in all rat treatment groups show significant differences; this can be seen from the P value <0.05. The highest trend in SGOT levels was found in the control group, 168.50 U/L, and the lowest in the standard group, 29.40 U/L. Meanwhile, a similar picture was found in the SGPT level; the group with the highest SGPT level was found in the control group, which was 164.25 U/L, and the lowest was found in the standard group, which was 40.15 U/L.

Discussion

According to Ovale-Magallanes et al. (2017), mangosteen peel contains an organic compound, xanthones. Xanthone compounds are known as powerful anti-inflammatories and antioxidants and are considered analgesics (9). Research conducted by Pasaribu et al. (2012), ethanol extract of 96% mangosteen peel contains chemical compounds of the alkaloid group, flavonoids, glycosides, saponins, tannins, and steroids/triterpenoids (10). Antipyretic analgesics are compounds that can relieve pain and can reduce fever. Therefore, the content of flavonoids and alkaloids in mangosteen peel can have an analgesic effect. In addition, flavonoids can inhibit prostaglandins so that they have antipyretic effects (11).

This study showed that Ethanol extract of mangosteen peel showed significant improvement in lipid profile at the end of the study. Mangosteen peel ethanol extract at the highest dose showed the most optimal lipid profile improvement. This can be seen from the decrease in total cholesterol, triglyceride, and LDL levels and the increase in HDL levels of mangosteen peel ethanol groups II and III. However, this improvement in lipid profile in the mangosteen rind ethanol extract-III group did not exceed the modification shown in the standard group.

The anti-dyslipidemia effect of mangosteen rind ethanol extract may be related to the content of various phytochemicals in mangosteen fruit. Several studies have shown the potential of phytochemicals as anti-dyslipidemia (12). Polyphenol content can cause down-regulation of pro-inflammatory cell signal modulation such as nuclear factor-κB, activated protein-1, and mitogen-activated protein kinase by inhibiting the arachidonic acid cascade and eicosanoid derivatives (13). Another possible mechanism for the anti-dyslipidemia effect of polyphenolic compounds is the regulation of intestinal mycobiota. Polyphenolic compounds in the gut will interact with the gut microbiota to increase beneficial metabolite products such as short-chain free fatty acids (14).

In this study, SGOT and SGPT levels in the rats that received mangosteen peel ethanol extract were lower than the SGOT and SGPT levels of the control group. This suggests that mangosteen peel ethanol extract may protect liver tissue from NAFLD compared to the group that did not receive mangosteen peel

ethanol extract. However, the possibility of mild NAFLD in the rats that received mangosteen peel ethanol extract cannot be ruled out (13).

Conclusions And Suggestions

The conclusion that can be drawn from the results of this study is that mangosteen fruit peel ethanol extract can significantly reduce total cholesterol, reduce triglyceride levels, reduce LDL levels, reduce SGOT (Value = 0.007) and SGPT levels (P value <0.05) compared to the control group, while mangosteen fruit peel ethanol extract can significantly increase HDL levels compared to the control group (P value = 0.018).

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