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(Research Article)

Anti-Dyslipidemia effectiveness of andaliman fruit extract (*Zanthoxylum acanthopodium*) in Wistar rats given a high fat diet and PTU (Propylthiouracil)

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Abstract

The andaliman fruit contains many phytochemicals, such as phenols, saponins, flavonoids, triterpenoids, and alkaloids, and has various pharmacological effects such as: anti-inflammatory, antioxidant, antidiabetic, and antibacterial. This study aimed to explore the antidislipidemia effectiveness of andaliman fruit extract. This experimental study used the Pre-test and Post-test group-only control design approach using Wistar male rats as experimental animals. The sample size in this study was calculated using the Federer formula; the calculation showed that at least four male Wistar rats (Rattus norvegicus) were needed in each treatment group. First, the research data were analyzed descriptively (Central tendency and Dispersion) of lipid profile data (LDL, HDL, Total cholesterol, and Triglycerides), color, texture, and weight. Then the research data in the form of lipid profiles were analyzed with One-Way Anova if the data were normally distributed with further tests in the form of Post Hoc Tukey HSD tests to see the fundamental differences between treatments. However, if the data were not normally distributed, the Kruskall-Wallis test was used as an alternative. The results showed that the total cholesterol of rats before being given a high-fat diet in all treatment groups did not show significant differences (P value = 0.782). This indicates that all rats' cholesterol data were uniform before being given the high-fat diet. However, after a high-fat diet, total cholesterol in all groups of rats showed different distributions. Only the control, standard, andaliman fruit methanol extract-I, II, and III groups had uniform total cholesterol. The methanol extract of andaliman fruit could significantly reduce total cholesterol, triglyceride, LDL, and SGOT levels compared to the control group. In contrast, the methanol extract of andaliman fruit could dramatically increase HDL levels compared to the control group.

Keywords: Anti-Dyslipidemia; Andaliman Fruit; Methanol

1. Introduction

The disruption of lipid metabolism causes dyslipidemia due to the interaction of genetic and environmental factors. 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. Several anti-dyslipidemia drugs are on the market in managing dyslipidemia, including statins, fibrates, niacin, ezetimibe, and bile acid-binding resins. However, there have been reports of unwanted side effects (myopathy) from some 'super statins.' In addition, fibrate-class drugs mainly used to treat hypertriglyceridemia and low HDL cholesterol require high doses to show significant efficacy. Thus, alternative treatments with possibly more minimal side effects are needed. The andaliman fruit contains many phytochemicals, such as phenols, saponins, flavonoids, triterpenoids, and alkaloids (1). Therefore, andaliman fruit has various pharmacological effects such as: anti-inflammatory, antioxidant, antidiabetic, and antibacterial (2–5)

Based on the background description above, it can be seen that the andaliman fruit has various phytochemicals and health benefits. However, no studies still explore the anti-dislipidemia benefits of andaliman fruit. Therefore, researchers are interested in exploring the antidislipidemia effectiveness of andaliman fruit extract.

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2. Research Methods

This experimental study uses a Pre-test and Post-test group-only control design approach using male Wistar rats as experimental animals. The sample size in this study was calculated using the Federer formula; these calculations show that at least four male Wistar rats (Rattus norvegicus) are needed in each treatment group. Wistar rats used as experimental animals in this study are male with body weights of 180-200 grams and rat ages between 2-4 months. The materials used in this study are andaliman fruit, methanol, distilled water, Na-CMC (sodium-carboxyl methylcellulose), simvastatin, husk, rat food pellets, phytochemical screening reagents, and ketamine in the study of phytochemical tests using a modified Farnsworth method consisting of identification of phenols, steroids/triterpenoids, terpenoids, saponins, flavonoids, tannins and alkaloids. The induction process was carried out by giving experimental animals a high-fat diet and PTU for 14 days. PTU was given as an oral suspension at 12.5 mg/day (1.25 ml/day) divided into two doses. At the same time, the high-fat diet was shown by giving a high-fat feed suspension at 15 gr/kgBB for fat animal suspension and 10 gr/kgBB for poultry egg yolk suspension. The research data were analyzed descriptively (Central tendency and Dispersion) from the data in lipid profiles (LDL, HDL, Total cholesterol, and Triglycerides), color, texture, and weight. Then the research data in the form of lipid profiles were analyzed with One-Way Anova if the data were normally distributed with further tests in the form of Post Hoc Tukey HSD tests to see fundamental differences between treatments. However, if the data is not normally distributed, the Kruskall-Wallis test is used as an alternative test.

3. Results and discussion

Table 1 Characteristics of Methanol Extract of Andaliman Fruit (Zanthoxylum acanthopodium)

Characteristics	Value
Fresh Simplisia Weight (gr)	500 gr
Dry Simplisia Powder Weight (gr)	212 gr
Solvent Volume (ml)	2120 ml
Weight of Extract (gr)	15,47 gr
Yield (%)	7.28%

Table 2 Phytochemical Screening Results of Methanol Extract of Andaliman Fruit

Phytochemistry	Reagents	Results
Alkaloids	Bouchardart	+
	Mayer	+
	Dragondroff	-
	Wagner	+
Saponins	Aquadest + Alcohol 96%	-
Flavonoids	FeCl3 5%	+
	Mg $_{(s)}$ + HCl $_{(p)}$	-
	NaOH 10%	-
	H_2SO_4 $_{(p)}$	-
Tannins	$FeCl_3$ 1%	+
Steroids and Terpenoids	Salkowsky	-
	Liberman Bouchard	+

From the data in Table 1, it can be seen that from 500 grams of andaliman fruit samples, an extract of 15.47 grams was obtained. Thus, the yield obtained from the methanol extract of andaliman fruit is 7.28%.

From the data in Table 2, it can be seen that the methanol extract of andaliman fruit contains several phytochemical compounds including Alkaloids, Saponins, Flavonoids, Tannins, and Steroids and Terpenoids.

All parameters evaluated in this study including body weight, total cholesterol, lipid profile, SGOT, and SGPT levels were analyzed for normality using the Shapiro-Wilk test. The results of the normality analysis can be seen in the table below. The data in Table 3 shows that body weight, total cholesterol and LDL levels from the lipid profile after treatment, and SGPT levels have normal data distribution. In contrast, other parameters, including total cholesterol before and after induction, triglyceride levels, HDL levels, and SGOT levels, are generally not distributed. Therefore, based on the distribution, data with standard distribution were analyzed with parametric statistics, while non-normal data were analyzed with non-parametric statistics.

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

Parameters		**Nilai P**	**Distribusi Data**
Weight Loss		0.395	Normal
Total cholesterol before induction		< 0.05	Not Normal
Total cholesterol after induction		< 0.05	Not Normal
Lipid profile after treatment	Total cholesterol	Normal	Normal
	Triglycerides	Not Normal	Not Normal
	HDL levels	Not normal	Not Normal
	LDL Level	Normal	Normal
SGOT levels		< 0.05	Not Normal
SGPT Levels		0.056	Normal

Table 4 Initial Weight Gain of Rats in All Treatment Groups

Treatment Group	**Body Weight (grams)**		**P-value**
	Mean	**SD**	
Normal	244.00	37.67	0.972
Standard	239.56	16.23	
Control	248.86	23.67	
Andaliman Fruit Methanol Extract-I	247.50	26.12	
Andaliman Fruit Methanol Extract-II	238.45	23.44	
Andaliman Fruit Methanol Extract-III	241.12	14.81	

From the data in Table 4, it can be seen that the P value> 0.05 (P value = 0.972) means that there is no significant difference in the initial body weight of the rats used in this study. The range of body weight of rats used in this study ranged from 210-300 grams which were evenly distributed in each treatment group. To evaluate the anti-dyslipidemia effect of andaliman fruit, a high-fat diet was administered to the control, standard, methanol extract of andaliman fruit-I, II, and III groups. Before and after the administration of the high-fat diet, total cholesterol in all rats was measured, and non-parametric statistics analyzed all entire cholesterol data. The results of the analysis can be seen in the following table. 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 = 0.782). This indicates that the entire cholesterol data of rats before being given a high-fat diet is uniform. However, the total cholesterol in all group rats after the high-fat diet showed a different distribution. Only the control, standard, andaliman fruit methanol extract-I, II, and III groups offered uniform total cholesterol.

Table 5 Comparison of Total Cholesterol Before and After Administration of High-Fat Diet in All Treatment Groups

Treatment Group	**Total cholesterol (mg/dL)**	
	Before Induction After Induction	**Before Induction After Induction**
Normal	115.80 (110-116)	117.60 (114-120)[b]
Standard	112.00 (100-115)	211.00 (204-218)[a]
Control	116.64 (110-118)	211.45 (210-215)[b]
Andaliman Fruit Methanol Extract-I	115.45 (110-117)	212.60 (209-211)[b]
Andaliman Fruit Methanol Extract-II	110.50 (100-115)	210.50 (209-212)[b]
Andaliman Fruit Methanol Extract-III	116.50 (116-119)	211.24 (209-210)[b]
p-value	0.782	0.012

Table 6 Comparison of Lipid Profile in All Rat Treatment Groups

Treatment Group	**Profil Lipid**			
	Total cholesterol *	**Triglycerides ****	**LDL***	**HDL****
Normal	134.50 ± 2.40a	99.50 (97-100)a	60.20 ± 1.60a	63.45 (61-64)a
Standard	144.50 ± 0.58b	105.25 (101-105)b	64.00 ±1.20b	61.50 (60-63)a
Control	179.25 ± 6.02c	170.25 (168-179)c	112.50 3.805c	28.75 (38-43)b
AFM-I Extract	168.25 ± 1.50d	133.50 (134-135)d	83.75 ±2.62d	57.50 (56-59)b
AFM-II Extract	163.25 ± 2.22e	120.50 (113-122)e	77.50 ± 1.29e	61.50 (61-63)a
AFM Extract-III	151.75 ± 0.96e	110.00 (108-112)f	68.50 ±1.29f	61.00 (60-63)a
P Value	< 0.05	0.013	< 0.05	0.014

* Data are shown as Mean ± SD. P values obtained from One Way ANOVA analysis; **Data are shown as Median (Range). P values obtained from Kruskal-Wallis analysis; Different superscript in the same column indicates significant difference.

From the data table above, it can be seen that all lipid profile data in all treatment groups show 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 normal group which was 134.50 ± 2.40 mg/dL, followed by the standard group at 144.50 ± 0.58 mg/dL, andaliman fruit methanol extract group I, II, III, and the group with the highest total cholesterol was the control group at 179.25 ± 6.02 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 = 0.013). The trend of the lowest triglyceride levels was found in the normal group at 99.50 mg/dL, followed by the standard group at 105.25 mg/dL, the andaliman fruit methanol extract group I, II, III, and the group with the highest triglyceride levels was the control group at 170.25 mg/dL.
c. LDL levels also showed significant differences in all treatment groups, this can be seen from the P value <0.05. The lowest average LDL level was found in the normal group at 60.20 ± 1.60 mg/dL, followed by the standard group at 64.00 ± 1.20 mg/dL, andaliman fruit methanol extract group I, II, III, and the group with the highest LDL level was the control group at 112.50 ± 3.80 mg/dL.

HDL levels also showed significant differences in all treatment groups, this can be seen from the P-value <0.05 (P value = 0.014). The trend of the highest HDL levels was found in the normal group which was 63.45 mg/dL, followed by the standard group at 61.50 mg/dL, the andaliman fruit methanol extract group I, II, III, and the group with the lowest HDL levels was the control group at 28.75 mg/dL. Another parameter that was also assessed in all groups of rats at the end of the study was liver function, namely: SGOT and SGPT levels. Comparison of SGOT and SGPT levels in all rat treatment groups can be seen in the table below.

Table 7 Comparison of SGOT and SGPT Levels in All Treatment Groups

Treatment Group	Levels SGOT (U/L)	Levels SGPT (U/L)
Normal	26.25 (26-30)[a]	45.50 ± 1.50[a]
Standard	110.50 (108-112)[b]	170.75 ± 1.29[b]
Control	160.50 (162-170)[c]	97.25 ± 1.50[c]
AFM Extract-I	116.50 (118-120)[d]	100.75 ± 3.59[d]
AFM Extract-II	127.50 (121-124)[e]	115.50 ± 4.51[e]
AFM Extract-III	133.50 (129-132)[f]	142.50 ± 2.08[b]
P-Value	0.022	< 0.05

*Data are shown as Mean ± SD. P values obtained from One Way ANOVA analysis; **Data are shown as Median (Range). P values obtained from Kruskal-Wallis analysis; Different superscript in the same column indicates significant difference.

From the data in Table 7, it can be seen that the levels of SGOT and SGPT in all rat treatment groups show significant differences, this can be seen from the P value <0.05. The highest trend of SGOT levels was found in the control group, which was 160.50 U/L and the lowest in the normal group, which was 26.25 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 170.75 U/L and the lowest was found in the normal group which was 45.50 U/L.

The results of this study showed that methanol extract of andaliman fruit showed significant improvement in lipid profile at the end of the study. The highest dose of andaliman extract 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 from the Andaliman Fruit Methanol Extract-II and III groups. However, this improvement in lipid profile in the Andaliman Fruit Methanol Extract-III rat group did not exceed the improvement shown in the standard group.

The anti-dyslipidemia effect of methanol extract of andaliman fruit may be related to the content of various phytochemicals in andaliman fruit. Several studies have shown the potential of phytochemicals as anti-dyslipidemia. For example, Polyphenol content can cause down-regulation of pro-inflammatory cell signals modulation such as nuclear factor-κB, activated protein-1, and mitogen-activated protein kinase through inhibition of the arachidonic acid cascade and eicosanoids derivatives. 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, and gut microbiota such as Akkermansia municiphilia sp. restore inflammatory conditions in the gut, improve gut permeability and insulin sensitivity. Furthermore, these improvements to the gut microbiota protect the gut-liver axis, thereby reducing lipid profiles in the body (6,7)

Other studies that discuss the anti-dyslipidemia effect of andaliman fruit are still limited. However, Ahmad et al. (2021) reported that nanoparticles from andaliman fruit have anti-inflammatory effects that induce blood vessel repair in arteriosclerosis. The results of his research show results that align with this study's results. The anti-inflammatory development and improvement of atherosclerosis are related to the advancement of lipid profile because the oxidation level causes the formation of atherosclerosis by lipid oxidants in blood vessels (8)

In addition, the methanol extract of andaliman fruit also significantly reduced SGOT and SGPT levels compared to the control group. This decrease in SGOT and SGPT levels is associated with improving Non-Alcoholic Fatty Liver Disease (NAFLD). Several studies have shown that NAFLD is a risk factor for the formation of arteriosclerosis. This is because NAFLD causes dysfunction of the vascular endothelium. Thong and Quynh (2021) reported that both SGOT and SGPT correlate with NAFLD, but the use of SGOT and SGPT separately may show errors in confirming mild NAFLD. In severe NAFLD cases, SGOT will increase slightly; in milder cases, SGOT levels can be found in average amounts. Therefore, using SGOT and SGPT unilaterally may allow errors in confirming mild NAFLD (9).

In this study, the SGOT and SGPT in the rats that received methanol extract from andaliman fruit were lower than the levels of SGOT and SGPT in the control group. This suggests that the methanol extract of andaliman fruit can protect liver tissue from NAFLD compared to the group that did not receive the methanol extract of andaliman fruit. However, the possibility of mild NAFLD in the rats that received the andaliman fruit extract cannot be ruled out.

4. Conclusion

The conclusion that can be drawn from the results of this study is that methanol extract from andaliman fruit can significantly reduce total cholesterol, triglyceride levels, LDL levels, and SGOT levels compared to the control group. In contrast, methanol extract from andaliman fruit can dramatically increase HDL levels compared to the control group.

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