Hypocholesterolemic and Hypoglycemic Effects of Soursop Fruit (*Annona muricata*) Ethanolic Extract in High Fat Diet and Alloxan Induced Wistar Rats

Evi Sovia[1](✉), Nurbaiti Nazarudin[2], Ilma Fiddiyanti[3], Dinyar Supriyadi[4], Wini Fatimah Junaidi[5], Hidayah Dwijayanti[5], Helmi Makarim[5], and Aprilia Grace Sweetasari[6]

[1] Pharmacology Departement, Faculty of Medicine, Universitas Jenderal Achmad Yani, Cimahi, Indonesia
evi.sovia@lecture.unjani.ac.id

[2] Ear Nose and Throat Disease Department, Faculty of Medicine, Universitas Jenderal Achmad Yani, Cimahi, Indonesia

[3] Radiology Department, Faculty of Medicine, Universitas Jenderal Achmad Yani, Cimahi, Indonesia

[4] Clinical Pathology Department, Faculty of Medicine, Universitas Jenderal Achmad Yani, Cimahi, Indonesia

[5] Faculty of Medicine, Universitas Jenderal Achmad Yani, Cimahi, Indonesia

[6] Neurology Department, Faculty of Medicine, Universitas Jenderal Achmad Yani, Cimahi, Indonesia

Abstract. Annona muricata (soursop) is a plant belonging to the Annonaceae family. It's a medicinal herb that's been used for centuries as a natural cure for a number of diseases. Several investigations in animals showed that the bark and leaves of the plant possessed antihypertensive, vasodilator, anti-spasmodic, and cardio depressive. However, research on soursop fruit is still limited. This study aims to investigate the effect of soursop fruit ethanolic extract on blood glucose, total and Low-Density Lipoprotein (LDL) cholesterol levels in high fat diet and alloxan-induced rats (Rattus novergicus). The study was done experimentally with pre and posttest control group design using 30 rats divided into 6 groups consisted of normal group with standard diet, and five groups induced by high fat diet and alloxan then given 0.9 mg/kg of simvastatin, 0.5 mg/kg glibenclamide, 250 and 500 mg/kg of soursop fruit ethanolic extract (SFEE), and one diabetic hyperglycmia group as positive control without treatment. Blood glucose, total and LDL cholesterol levels measurement was conducted after 21 days of treatment. Data was analyzed using dependent t-test. Results shows significant reduction in blood glucose, total and LDL cholesterol level within treatment groups ($p < 0.05$). Furthermore, 500 mg/kg SFEE has roughly the same effectiveness as 0.9 mg/kg of simvastatin in reducing the total cholesterol level ($p > 0.05$), but not for LDL cholesterol. Meanwhile, the effectiveness of 250 and 500 mg/kg SFEE in lowering blood glucose levels is the same as glibenclamide ($p > 0.05$). In conclusion, SFEE shows hypoglycemic and hypocholesterolemic effect on both dosages.

Keywords: Annona muricata · Hypoglicemic · Hypocholesterolemic

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1 Introduction

Diabetes is a serious, chronic disease that occurs when the pancreas unsuccessful to produce enough insulin or when the body fails to use the insulin that is produced effectively. Diabetes is a major public health issue, and it is one of four priority noncommunicable diseases (NCDs) that world leaders have identified for action. Diabetes has been steadily increasing in both the number of cases and the prevalence over the last few decades [1]. Dyslipidemia is the most common risk factor for cadiovascular diseases (CVD) in people with Types 1 and 2 diabetes. It is critical to identify and treat lipid abnormalities [2]. Oral hypoglycemic agents, such as biguanides and sulphonylureas, are available alongside insulin for the treatment of diabetes mellitus, however they have side effects. Herbal remedies are gaining popularity due to their effectiveness, lack of side effects in clinical trials, and low cost [3].

Soursop *(Annona muricata* L.) is a species of Annonaceae that has been extensively researched in recent decades due to its therapeutic potential the Annonaceae family's medicinal uses were first reported many years ago, and this species has since gained popularity due to its bioactivity and traditional uses [4]. The major pharmacological activities of soursop include cytotoxicity, antileishmanial activity, anti-viral activity, anticarcinogenic and genotoxic effects, wound healing, and antimicrobial activity [5].

Despite claims that all morphological parts of the plant are useful in traditional medicine, no scientific studies have been conducted to establish the hypocholesterolemic and hypoglycemic effects of the soursop fruit. Hence, the current study was designed to investigate the hypocholesterolemic and hypoglycemic properties of soursop fruit alcoholic extract in high fat diet and alloxan-induced diabetic hyperlipidemic rats.

2 Materials and Methods

2.1 Plant Material

The research material was two kg of soursop fruit obtained from the Citatah Karst Area plantation (Cipatat, West Bandung), which was extracted with 96% ethanol as a solvent to produce 190 g of concentrated paste at the Laboratory of the School of Biological Sciences and Technology ITB Bandung.

2.2 Preparation of Soursop Fruit Ethanol Extract

Soursop fruit ethanol extract was prepared by weighing two kilograms of soursop fruit flesh, then soaked in 5 L of 96% ethanol solution for 72 h at room temperature, stirring occasionally. The solution was then filtered and the filtrate was concentrated using a rotary evaporator to obtain a concentrated residue [6]. The concentrated residue was dried, weighed and dissolved with 1% Na-CMC during the period of use. Administration to rats was carried out orally through an oral probe.

2.3 Animal Experiment

Experimental animals were male wistar rats aged 6–10 weeks, weighing 100–250 g, and in good health obtained from Biofarma.

2.4 Experimental Animal Treatment

Ethical approval is obtained from the research ethic commission team of Universitas Jenderal Achmad Yani No. 011/UH1.10/2020. A total of 30 rats that passed the selection were randomly divided into six groups. Each group was given the induction-intervention treatment except for the normal group. The induction period consisted of administering a high-fat diet (HFD) orally for 14 days followed by injection of alloxan 125 mg/kg in saline solution intraperitoneally. The intervention period was carried out with therapy based on the treatment group for 21 days [7]. The test groups were divided into six groups, that are: 1) normal gruop that given standard diet and saline solution injection followed by 1% Na-CMC, 2) diabetic hyperlipidema group that induced by high fat diet and alloxan followed by 1% Na-CMC, 3) and 4) were standard groups that that induced by a high-fat diet and alloxan followed by glibenclamide 0.5 mg/kg in 1% Na-CMC or simvastatin 0.9 mg/kg in 1% Na-CMC, 5) group that induced by a high fat diet and alloxan followed by followed by soursop fruit ethanol extract (SFEE) 250 mg/kg in 1% Na-CMC, and 6) group that induced by high fat diet and alloxan followed by SFEE 500 mg/kg in 1% Na-CMC. Blood glucose, total cholesterol and LDL cholesterol levels of rats were measured before and after the treatment period by taking blood samples through the tail vein.

2.5 Measurement of Glucose, Total Cholesterol and LDL Cholesterol in Rat Blood

In general, the measurement of blood glucose and cholesterol levels could be done through laboratory analysis and electrode-based biosensors. Examination of cholesterol levels using a spectrophotometer produces precise measurement numbers, but requires a long time, high cost, and invasive venous blood sampling. Meanwhile, electrode based biosensors requires a small volume of blood and provides a measurement value that is relatively the same as the spectrophotometric method [8].

2.6 Statistic Analysis

The data obtained will be analyzed with a paired dependent t-test to see the level of change in blood glucose, LDL and total cholesterol levels. The data were expressed as means $\pm$ SD. Values of $p < 0.05$ were taken to imply statistical significance and $p < 0.01$ to imply statistical very significance.

3 Results

Soursop fruit extract given to experimental animals can reduce blood glucose levels of rats. The paired dependent t-test was carried out to compare differences in blood glucose levels before and after treatment, which are listed in Table 1.

In the control group, blood glucose levels before treatment (90 ± 6.2 mg/dl) were not significantly different from after treatment (112.6 ± 33.4 mg/dl) with p value $= 0.264$ (> 0.05). There was a significant decrease ($p < 0.05$) in blood glucose levels between

Table 1. Effect of SFEE on blood glucose level in high fat diet and alloxan induced rats

Groups	Blood glucose levels (mg/dl)		P value
	Before treatment	After treatment	
Normal	90 ± 6,2	112,6 ± 33,4	0,264
Diabetic hyperglicemia	336,4 ± 47,55	319,6 ± 13,09	0,225
Glibenclamide 0.5 mg/kg	287,4 ± 65,4	81,2 ± 13,27	0,003*
SFEE 250 mg/kg	280,2 ± 63,89	96,2 ± 14,21	0,004*
SFEE 500 mg/kg	288,2 ± 15,46	88 ± 5,05	0,000*

SFEE: Soursop fruit ethanolic extract: *p < 0.05

Table 2. Effect of SFEE on total cholesterol level in high fat diet and alloxan induced rats

Groups	Total cholesterol level (mg/dl)		P value
	Before treatment	After treatment	
Normal	114,80 ± 0,83	114,67 ± 0,58	0,62
Diabetic hyperglycemia	118,20 ± 1,09	120,67 ± 1,53	0,09
Simvastatin 0.9 mg/kg	118,60 ± 1,51	68,00 ± 3,67	0,000*
SFEE 250 mg/kg	118,20 ± 1,09	113,60 ± 1,14	0,001*
SFEE 500 mg/kg	118,80 ± 1,64	107,80 ± 1,64	0,001*

SFEE: Soursop fruit ethanolic extract: *p < 0.05

after treatment (96.2 ± 14.2 mg/dl) compared to before treatment (280.2 ± 63.89 mg/dl) in the group given SFEE at a dose of 250 mg/kg. Likewise, blood glucose levels in the group given SFEE at a dose of 500 mg/kg decreased significantly between before treatment (288.2 ± 15.46 mg/dl) and after treatment (88 ± 5.05 mg/dl). The experimental animal group that was given glibenclamide at a dose of 0.5 mg/kg experienced a significant decrease in blood glucose levels ($p < 0.05$) from 287.4 ± 65.4 mg/dl to 81.2 ± 13.27 mg/dl.

Table 2 shows that the highest mean total cholesterol level after treatment was found in the hyperlipidemic diabetes group, which was 120.67 ± 1.53 mg/dL and the lowest was in the group given simvastatin (68 ± 3.67 mg/dL).

The results of the paired dependent t-test showed a significant difference between total cholesterol levels before and after treatment in the group given simvastatin and SFEE at doses of 250 and 500 mg/kg ($p < 0.05$). This proves that the administration of soursop fruit ethanol extract at a dose of 250 and 500 g/kg and simvastatin 0.9 mg/kg for 21 days can reduce total cholesterol levels.

The results of the measurement of LDL cholesterol levels are presented in Table 3 which shows LDL cholesterol levels before and after treatment with SFEE for 21 days. Table 3 shows the highest LDL cholesterol levels after treatment in the diabetic hyperglycemia group, which is 127,80 ± 1,92 mg/dl and the lowest in the simvastatin 0.9 mg/kg

Table 3. Effect of SFEE on LDL cholesterol level in high fat diet and alloxan induced rats

Groups	LDL cholesterol level (mg/dl)		P value
	Before treatment	After treatment	
Normal	105,00 ± 1,10	104,60 ± 1,81	0,198
Diabetic hyperglycemia	126,60 ± 1,14	127,80 ± 1,92	0,109
Simvastatin 0.9 mg/kg	126,00 ± 1,58	89,20 ± 3,76	0,000*
SFEE 250 mg/kg	126,60 ± 1,14	121,20 ± 3,11	0,010*
SFEE 500 mg/kg	126,80 ± 1,3	108,40 ± 5,94	0,001*

SFEE: Soursop fruit ethanolic extract: *$p < 0.05$

group, which is 89,20 ± 3,76 mg/dl. The results of the analysis using the paired dependent t-test resulted in a significant decrease in LDL cholesterol levels in the simvastatin, SSFE 250 and 500 mg/kg groups ($p < 0.05$). This proves that the administration of simvastatin 0.9 mg/kg and SFEE doses of 250 mg and 500 mg/kg for 21 days can reduce LDL cholesterol levels.

4 Discussion

The results of this study support previous studies showing that soursop fruit extract can reduce blood glucose levels [9]. Soursop fruit contains antioxidants such as flavonoids, tannins, saponins, beta-carotene, and alkaloids that work to increase regeneration and inhibit pancreatic beta cell degeneration so that it can maximize the action of the pancreas in insulin secretion. The insulin produced will be captured by insulin receptors on cell membranes, especially the liver, muscle, and adipose tissue. Through the role of cAMP-dependent protein kinase-C by increasing the signal transduction strength between insulin and its receptors so that target organs are more sensitive to insulin [10, 11].

Soursop fruit can also inhibit glycogenolysis and activate glucokinase so that hepatic glucose phosphorylation increases, causing a decrease in peripheral blood glucose levels. Flavonoids, tannins, saponins, beta-carotene, and alkaloids contained in soursop fruit act as alpha-amylase and alpha-glucosidase inhibitors which cause the absorption of glucose in the intestine to be inhibited [5, 12].

Tannins and saponins play an active role in reducing glucose secretion in intestinal cells by inhibiting the enzyme DPP-4 which will increase the level of GLP-1 which is synthesized by L-cells in the small intestine, so that less glucose is absorbed from the small intestine into the blood vessels. GLP-1 will also maintain a feeling of fullness longer after eating by inhibiting gastric emptying [13].

Tannins and flavonoids have the same mechanism of action as glibenclamide in increasing insulin secretion through the role of blocking potassium channels that are sensitive to ATP-sensitive K channels on the beta-pancreatic cell membrane, then membrane depolarization occurs which causes Ca++ channels to open so that Ca++ ions can

enter beta cells. And causes an increase in intracellular calcium which will stimulate beta cell granules to secrete insulin [5].

The results of this study are in accordance with previous research which states the ability of soursop fruit to reduce cholesterol levels and suppress cell damage that can occur due to high blood cholesterol. According to previous research, it was stated that the condition of hypercholesterolemia can be characterized by oxidative stress. Changes in the integrity of membrane-bound organelles in body cells due to high cholesterol can trigger the release of reactive oxygen molecules from the mitochondrial membrane and the activation of membrane-bound enzymes such as NADPH reductase which produces reactive oxygen that causes lipid peroxidation [14, 15].

Soursop fruit has a high content of antioxidants such as tannins, alkaloids, flavonoids, phenolics, and ascorbic acid so that it can neutralize radical substances formed in body cells [16, 17]. These results are consistent with previous studies which stated that soursop fruit contains flavonoids which function to suppress cholesterol synthesis in the liver by the HMG-CoA reductase enzyme by competitively binding to its cofactor site, similar to patent cholesterol drugs of the statin class such as simvastatin [18, 19].

5 Conclusion

The experiment results of the present study showed that soursop fruit ethanolic extract has hypocholesterolemic and hypoglycemic effects. The extract contains some phytochemical compounds that may contribute to its activities. This study supported the traditional usage of the extract as antidiabetic and antihyperlipidemic.

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