

SOURSOP LEAVES FOR REDUCING BLOOD CHOLESTEROL LEVEL IN WHITE MALE RATS (*RATTUS NOVERGICUS*).

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ABSTRACT: Background: The increase of blood cholesterol level is one of the leading factors of atherosclerosis which may leads to heart disease. Patients' conformity of reducing blood cholesterol drugs is low, due to its price. Natural medicine is chosen as an alternative. It has been acknowledged over some generations that soursop leaves can be used as a medicine to reduce blood cholesterol rate.

Objective: To determine the effectiveness of soursop leaves to reduce blood cholesterol rate on white male rat. Simvastatin is used as the comparison.

Method: Experimental test is conducted in this study. The research subjects are divided into six groups, each of them consists of five rats. The first group is given aqua dest only, the second group is given simvastatin only, the third group is given simvastatin and soursop leaves extract, the fourth group is treated with 96% ethanol extract from 5% soursop leaves extract, the fifth group is treated with 96% ethanol extract from 15% soursop leaves extract, and the sixth group is treated with 96% ethanol extract from 25% soursop leaves extract.

Results: The data shows the difference in total plasma cholesterol rate in each group after 2 weeks of treatment. The most significant decrease in cholesterol levels occurred in the experimental group II for 47.58 mg/dl, given 96% ethanol extract with a dose concentration of 5%. Then followed by experimental group IV for 51 mg/dl after the administration of 96% ethanol extract dose 25% concentration, then decrease in total cholesterol levels in the experimental group III for 55.6 mg/dl given 96% ethanol extract with dose concentration of 15%. Besides the positive control group, the group treated with simvastatin experienced highest decrease in total plasma cholesterol levels for 39.42 mg/dl compare with other experimental groups. The increase in total plasma cholesterol levels occurred in CTF (after induction of yolk) is 234.6 mg/dl.

Conclusion: According to the experiment, it can be concluded that ethanol extract 96% of soursop (*Annona muricata L.*) leaves with the concentration 5%, 15%, 25% can decrease the total plasma cholesterol rates in rat (*Rattus norvegicus L.*)

Keywords: Soursop leaves, male white rat, cholesterol, ethanol extract

INTRODUCTION

Indonesian people have been acknowledging and utilizing herbal medicine as one of the curative method to control their health problems. Based on the data by Harborne (1987)[17], there are 1,040 species of plants in Indonesia used as herbal medicine which are mostly grown in tropic forest.

Cholesterol is sterol class of fats or lipids produced by the body. In certain rate, the cholesterol is needed by the body for the formation of essential components. The normal rate is 160-200 mg/dL [1]. Contrast with its function in normal rate, higher the cholesterol rate in blood, higher the risk of atherosclerosis. Atherosclerosis caused the thickening and hardening of blood vessels by cholesterol. Atherosclerosis is the first cause of heart disease. Decreasing the rate of blood cholesterol is one of the therapeutic treatment to prevent heart disease. It can be done by hypolipidemia drugs [32]. However, hypolipidemia is expensive, and not so many people are able to purchase it. Searching hypolipidemia in the nature is an interesting investigation [33]. According to the previous studies, soy (*Glycine max M.*), celery

(*Apiumgraveolens M.*), and field milk thistle (*Sonchus gagak L.*) is the herbal pharmacy which has already proven to lower the cholesterol rate. Plants are a source of chemical compounds, some of them are potential for the basic ingredients of medicine [7]. Soursop (*Annona muricata L.*) is a tropical plant. Its flower, fruit, root, and bark can be processed as herbal medicine. Radi, J., [29]. Empirically, soursop leaf is used by the people to decrease cholesterol rate, 3-5 dosage per day of soursop leaves is in boiled 3 glass of water until 1 glass of water left. The previous study has not been sufficient to prove the feasibility of soursop leaf as anti-cholesterol medicine. The current study is conducted to strengthen the previous results [27].

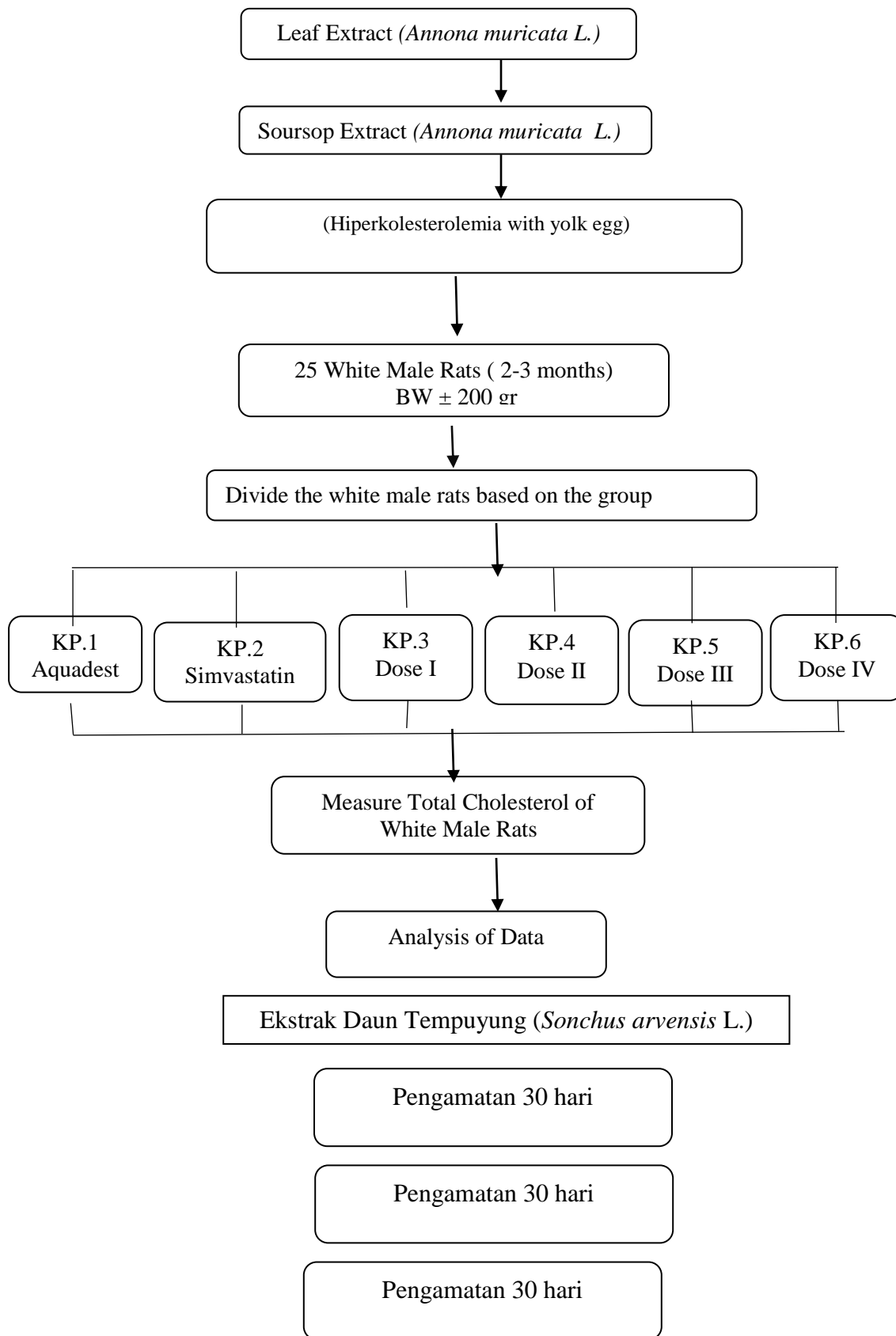
METHODOLOGY

Research design and subject

In this study, there are six groups of male rats, each group consists of five male rats with the following details

- Group 1: given aquadest
- Group 2: given simvastatin
- Group 3: given soursop leaf extract and simvastatin
- Group 4: given ethanol extract 96% from soursop leaf extract 5%
- Group 5: given ethanol extract 96% from soursop leaf extract 15%
- Group 6: given ethanol extract 96% from soursop leaf extract 25% [12].

Before the treatment, all research subjects were injected egg yolk to increase blood cholesterol.

Methodology of Research

March-April

Table 1. Rate of blood cholesterol (mg/dl) of rate male after injected with egg yolk Rate of blood cholesterol to rat plasma (hypercholesterolemia)

No	KKN	KKP	KE I	KE II (5%)	KE III (15%)	KE IV (25%)
1	73.3	62.7	60	59	51.9	60.3
2	44.5	46.5	42.5	46.5	40.5	52.1
3	38.4	47.0	22.8	47.0	42.0	43.5
4	44.0	37.9	35.4	47.0	41	45.5
5	43.0	49.1	36.4	38.4	47.0	53.6
Total Cholesterol	243.2	243.2	197.1	237.9	222.4	255
Average	48.64	48.64	39.42	47.58	55.6	51

Description:

- KN = aqua dest
- KP = simvastatin
- KE I = soursop leaf extract and simvasta
- KE II = ethanol extract 96% from soursop leaf extract 5%
- KE III = ethanol extract 96% from soursop leaf extract 15%
- KE IV = ethanol extract 96% from soursop leaf extract 25%



Figure 1. The average of plasma cholesterol increase (mg/dl) after injected with egg yolk (Proses hypercholesterolemia)

Table 2. Rate of blood cholesterol (mg/dl) of rate male after injected with egg yolk, simvastatin, and ethanol extract 96% from soursop (*Annona Muricata*) leaves

No	KKN	KKP	KE I	KE II (5%)	KE III (15%)	KE IV (25%)
1	124	253	158	110	120	101
2	126	268	130	121	111	89
3	128	222	122	144	96	91
4	122	210	112	144	105	111
5	119	220	74	131	122	119
Total Cholesterol	519	1173	596	650	554	511
Average	103.8	234.6	119.2	130	110.8	102.2

Description:

- KN = aqua dest
- KP = simvastatin
- KE I = soursop leaf extract and simvastatin
- KE II = ethanol extract 96% from soursop leaf extract 5%
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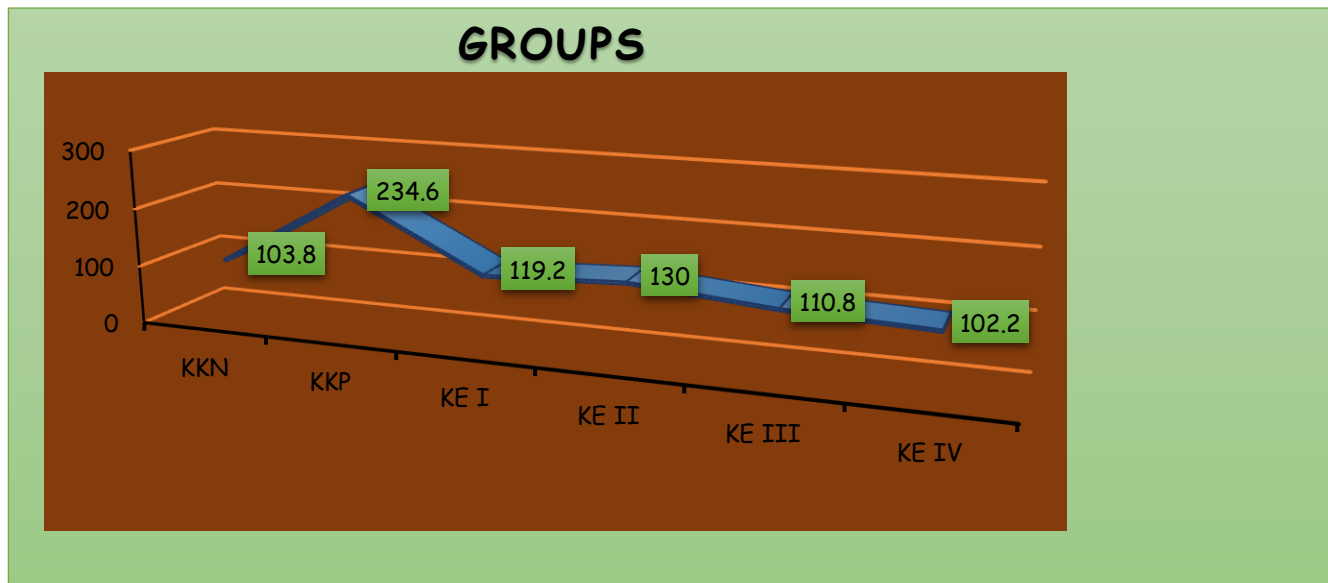


Figure 2. The graph of decrease in plasma cholesterol average (mg/dl) after injected with egg yolk, simvastatin, and ethanol extract 96% from soursop (*Annona Muricata*) leaves
Analysis data used SPSS 22.0 for Test of Normality, homogeneity and One way Anova Test.[8].

RESULT AND ANALYSIS

Animal used as the research subjects are healthy white rats line SD (*Rattus norvegicus L.*) with the body weight of 200-250 g and aged 2-3 months delivered from Husbandry Department of East Java Province Government. There are forty-five rats used in this study, and they were given adaptation time for two weeks' prior to the research [21].

In this research, soursop (*Annona muricata L.*) leaves are processed into extract to allow more active compounds to be extracted and increase the ability of blood cholesterol reducing ability. The active compounds extracted includes: flavonoid, tannin, and alkaloid [35].

There are many drug/drugs and vitamin combinations that can be used to cure high rate of blood cholesterol and fat. One of those is statin as the most common drug used to decrease cholesterol rate. To control the use of anti-cholesterol drug statin, simvastatin is commonly used. Simvastatin is chosen as it has strong feasibility in reducing blood cholesterol and it is safe for long-term consumption even though there are also some side effects of its long-term usage [6]. The determination of total plasma cholesterol rate in this study using centrifugal (Heraeus) which automatically describe the total cholesterol by enzymatic colorimetry method with Cholesterol FS reactor [4].

Principally, cholesterol ester changes into cholesterol and acid in solution with water. Then with cholesterol oxidation, it changes into 4-cholesten-3-one and hydrogen peroxide. Then with the help of peroxidase, it will form 4-p-benzikinoantipirin (pink-purple) which can be measured in wave length of 546 nm [16].

Based on the evaluation of measurement by Center Laboratory of Biology Research, Indonesian Institute of Sciences Cibinong, the raw oil material used in this study is soursop (*Annona muricata L.*) leaves from genus *magnolia le Annonaceae*. The investigation results shows that ethanol

extract soursop leaves, 96% contains alkaloid and tannin. In the previous study, reported that it contained acetogenin *Annonaceous* chemical compound which is a toxic compound which act to obstruct and halt the growth of cancer cell [37]. The result of yield assessment is 18.22%. The objective of yield assessment is to determine the comparison value of the extract obtained from the theory. So, the extract ratio produced by soursop leaves powder is 18.22%. The measurement of drying reducing of ethanol extract 96% soursop leaves resulted a percentage. The result shows that the value of drying reducing of soursop leaves still fulfill the standard of extract viscosity drying for 10-30%. In this study, there are three control groups: normal control (standard diet), negative control food standard and fat (egg yolk) and positive control (standard diet, egg yolk, and simvastatin). The grouping is conducted to observe the extend of fat induction (egg yolk) raise the total plasma cholesterol compare to normal condition, also to investigate the extend of 96% ethanol extract soursop(*Annonamuricata. L.*) leaves reduces the total cholesterol plasma in the rats with hypercholesterolemia compare to simvastatin drug [13]. The data shows the difference in total plasma cholesterol rate in each group after 2 weeks of treatment. The most significant decrease in cholesterol levels occurred in the experimental group II is 47.58 mg / dl were given 96% ethanol extract with a dose concentration of 5% and a decrease in plasma total cholesterol levels in the experimental group IV at 51 mg/dl were administered the extract with 96% ethanol dose concentration of 25% and a decrease in plasma total cholesterol levels in the experimental group III, 55.6 mg/dl were given 96% ethanol extract with dose concentration of 15%. Besides the positive control group were given simvastatin decreased the most in total plasma cholesterol levels be 39.42 mg/dl compare with other experimental groups.

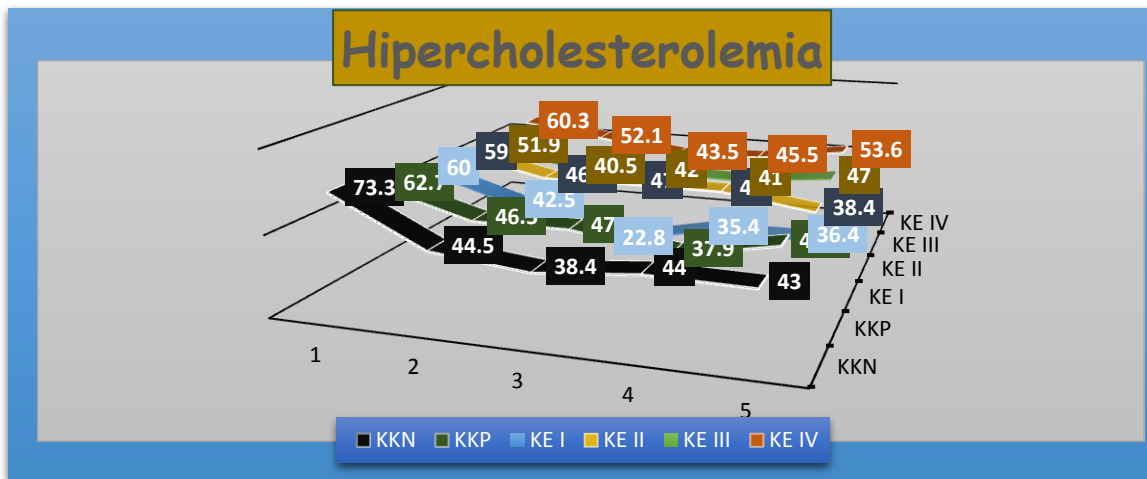


Figure 3: The average of plasma cholesterol increase (mg/dl) after injected with egg yolk (Procces hypercholesterolemia) The increase in total plasma cholesterol levels occurred in CTF (after induction of yolk) to 234.6 mg/dl.

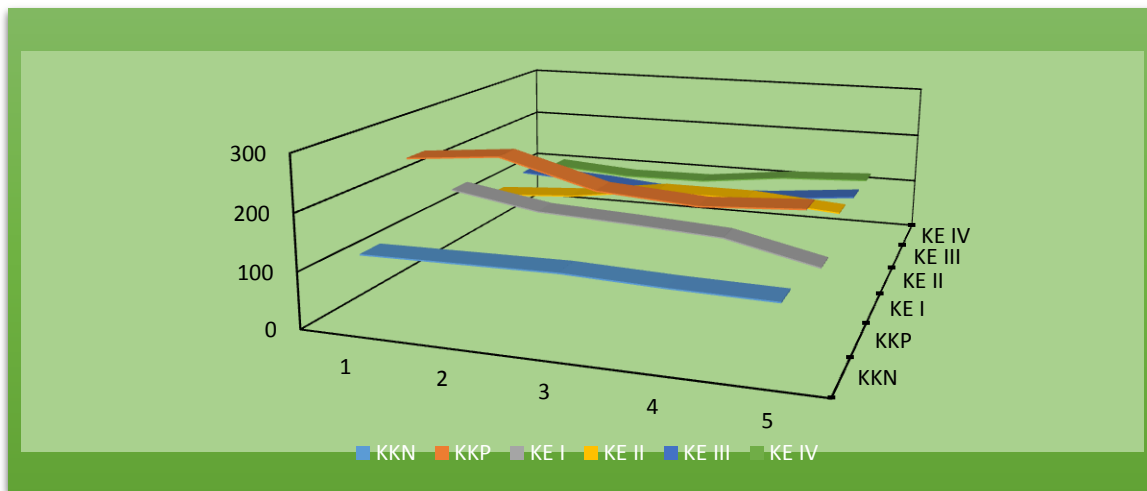


Figure 4: The average of plasma cholesterol decrease (mg/dl) after giving soursop leaf extract and simvastatin.

The data is tested using normality test with Kolmogorov-Smirnov method and homogeneity test with Levene method. It shows normal and homogenous distribution, then continued to parametric method.

The research hypothesis is statistically shown by one-way ANOVA (Analysis of Variance). ANOVA test is conducted to discover the difference in test solution potential to the effect group anti-cholesterol, then followed by several comparison tests using Least Significant Different (LSD) or Bonferroni. LSD test is conducted to determine the potential difference among the effects of anti-cholesterol in each treatment group. The test shows that there is a significant difference among KN, KP, KE I, II, KE, KE III, and KE IV. Statistical test performed in real level of $\alpha = 0.05$ with level of confidence 95% [20].

LSD test results showed that the positive control, dose 1 (KE II), dose 2 (KE III) and dose 3 (KE IV) had a significant difference ($\text{sig} < 0.05$) with a negative control. In the third dose group (KE IV) showed no significant difference ($\text{sig} > 0.05$) with positive control. The second dose group showed no significant difference ($\text{sig} > 0.05$) with the third dose but there was a significant difference with the positive control ($\text{sig} < 0.05$).

CONCLUSION

According to the experiment, it can be concluded that ethanol extract 96% of soursop (*Annona muricata L.*) leaves with the concentration 5%, 15%, 25% can decrease the total plasma cholesterol rates in rat (*Rattus norvegicus L.*) with hypercholesterolemia by egg yolk induction. The most significant decrease among the experimental groups is the use of ethanol extract 96% of soursop (*Annona muricata L.*) leaves with the concentration 5%, which showing the relation potential of ethanol extract 96% of soursop (*Annona muricata L.*) leaves with the ability to decrease blood cholesterol.

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