

# THE EFFECT OF BLACK GARLIC (*ALLIUM SATIVUM* (L)) ON REDUCING TOTAL CHOLESTEROL LEVELS IN WHITE MALE RATS

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## ABSTRACT

Garlic (*Allium sativum*) has been used as a medicinal for hyperlipidemia. Various processing developments are used to reduce the smell of garlic. Black garlic is made with a high temperature and humidity heating process. The black garlic has therapeutic benefits of lowering cholesterol levels. The chemical content in black garlic are allicin, S-allyl cysteine (SAC), flavonoid compounds, and polyphenol compounds. The purpose of this study is to use spice plants as a medicine that is effective in reducing total cholesterol levels. This research is an in vivo study which is experimental with a pre and post test controlled group design. Samples divided into three groups with varying doses, group I named with a dose of 0.126 g/kg, group II with 0.252 g/kg, and group III with 0.504 g/kg. Control positive group use simvastatin with 0.252 g/kg single dose. The test results that have been carried out the seventh day indicate in total cholesterol levels in the three test groups. The average reduction in total cholesterol levels in all groups was 10.3 mg/dL. The best results were in group II with a decrease in the mean total cholesterol levels of 13 mg/dL. Based on the results of the study, it can be concluded that blackened garlic has antihyperlipidemic activity by reduced total cholesterol levels in white rats with the best dose of 0.252 g / g BW and is more effective than Simvastatin drug.

**Keywords:** hyperlipidemia, black garlic, total cholesterol, rats.

## 1. INTRODUCTION

Black garlic is fresh garlic (*Allium sativum* L.) that has been fermented for a period of time at controlled high temperature (60-90°C) under controlled high humidity (80-90%) (Yuan, 2016). When compared to fresh garlic, black garlic does not give off a strong flavor due to reduced levels of allicin. The increase in bioactivity of black garlic compared to fresh garlic was caused by changes in physicochemical properties. Studies on the basic findings of black garlic, such as its production, bioactivity, and application, have been carried out (Kimura, 2016). Previous research has suggested that using garlic (*Allium sativum* (L)) can reduce cholesterol levels (Rinjani, 2012). However, there has been a development in the use of garlic (*Allium sativum* L) by heating and controlling the humidity called black garlic (Choi, 2014). Based on this, research is needed to determine the effect of blackened garlic on total cholesterol levels of white rats. This is the finding of blackened garlic (*Allium sativum* (L)) which can act as an antihyperlipidemic. This information can provide an overview of the potential of blackened garlic (*Allium sativum* (L)) as an antihyperlipidemic.

## 2. MATERIALS AND METHODS

The tools used in this study were glass tools, porcelain plates, Autocheck tools, analytical scales, 1 mL spoid and sonde, animal rearing cages. Then , the materials used in this study



were black garlic, cholesterol strips, simvastatin 10 mg, aquadest, 90% ethanol, and 0.5% Na<sub>2</sub>C<sub>2</sub>O<sub>4</sub>. Dragendroff, Wagner, and Mayer reactions, magnesium bands, HCl, FeCl<sub>3</sub>, anhydrous acetic acid and concentrated H<sub>2</sub>SO<sub>4</sub> for screening phytochemicals of black garlic.

#### Simplicia Standardization

Simplicia standardization was carried out including the determination of water content, total ash content, water-soluble and ethanol-soluble extract content, and drying losses. (Depkes RI, 2000)

#### Phytochemical Screening

Performed through chemical reactions against alkaloids, steroids and terpenoids, and phenolics (Jones and Kinghorn, 2006). As well as flavonoids and saponins (Depkes RI, 1995).

#### Anticholesterol In Test Animal

This research was conducted at the Research and Development Laboratory of FARMASKA TROPIS, Faculty of Pharmacy, University of Mulawarman Samarinda. This type of research is the post test only control group design. Samples in this study were male Wistar strain rats aged 8 weeks with body weight between 180 mg - 200 mg with healthy conditions. Rats were adapted for 1 week with cage care and given standard feed. After 1 week the rats were randomized into 5 groups, each group consisting of 3 rats. The group consisted of a negative control group (K1), a positive control group (K2), a dose group 1 (D1) 0.126 g / day, a dose 2 (D2) 0.252 g / day, and a dose group 3 (D3) 0.504 g / day. Before the experimental animal treatment, high-fat feed was induced with the composition of flour, green beans, chicken fat, chicken egg yolk, duck egg yolk, and butter. Provision of high-fat feed for 3 months. In addition to high-fat feed, experimental animals were also induced with 0.01% propylthiouracil (PTU) and used cooking oil. Blackened garlic was given orally. Data collection in the form of blood samples to check cholesterol levels was carried out on the day 7, 14, and 28 via the coccygeal vein or caudal vein.

### 3. RESULT AND DISCUSSION

#### Results of Simplicia Standardization

Standardization is the process of guaranteeing the final product (drug) so that it has a certain parameter value that is constant and is determined in advance. To guarantee the quality of medicinal plant simplicia, it is necessary to establish quality standards. (Table 1).

Based on the results of the simplicia standardization, it was found that blackened garlic does not belong to the category of simplicia in general, as the definition of simplicia according to the Indonesian Ministry of Health (1995) is a natural ingredient used for medicine and has not undergone any process changes, and unless otherwise stated, it is generally in the form of ingredients that are has dried.

Tabel 1. Result Standardization Of Black Garlic

Parameters	Result	Standards FH
Dry shrinkage	30%	<10%
Water content	21,34%	<10%
Total Ash	65%	<2,9%
Total water-soluble extract	26%	>11,5%
Total ethanol soluble extract	11%	>11,4%

Standardization that has been carried out does not meet the requirements of the Indonesian Herbal Pharmacopoeia simplicia. Because the simplicia used is wet and cannot be dried. Black garlic simplicia has a jelly-like texture with a high water content. In making black garlicks, it involves a moisture process of up to 90% (Kimura, 2016). The purpose of determining the levels of dissolved compounds in certain solvents aims to provide an initial picture of the amount of compounds that can be extracted (Depkes RI, 2000). The results showed that black garlic contained compounds that were more soluble in water, namely 26%, while the ethanol soluble compound was 11%. Meanwhile, the determination of the total ash content aims to determine whether or not a treatment is good and provide an overview of the internal and external mineral content contained in simplicia (Depkes RI, 2000). The ash content of black garlic is thought to be 65% because the material used has a chewy texture like jelly.

#### Results of Phytochemical Screening

Phytochemical screening is an important test in an effort to reveal the potential of a medicinal plant resource. This screening is carried out to provide an overview of the class of compounds contained in blackened garlic.

Based on the results of phytochemical screening, it was found that blackened garlic contained flavonoids and polyphenols. The results of the blackened garlic phytochemical screening can be seen in Figure 1.

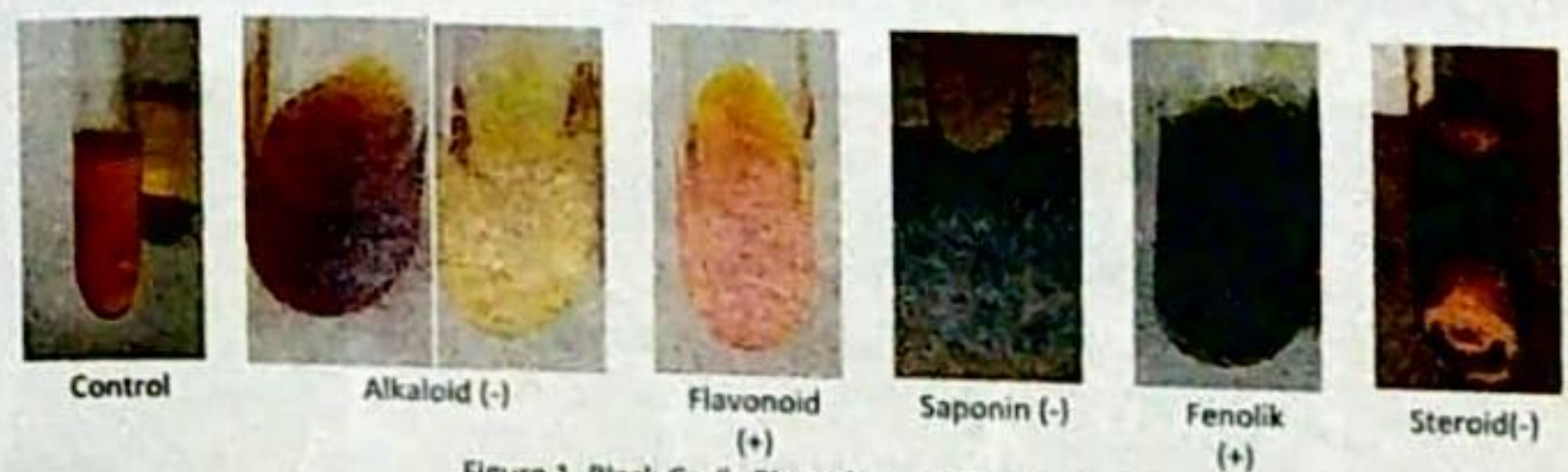


Figure 1. Black Garlic Phytochemical Screening Test

Phytochemical screening tests showed negative results for alkaloids, steroids and terpenoids, and saponins. However, flavonoids and phenolic compounds showed positive results. According to Choi, black garlic has components of chemical compounds, namely polyphenols and flavonoids (Choi, 2014).

#### Total Cholesterol Level Measurement Results

All groups of experimental animals were acclimatized for 1 week with standard feeding and drinking. The purpose of giving acclimatization or adaptation time is so that the test animals are accustomed to the conditions of the cage and so that they can observe the test animals that are suitable for the test treatment, namely active mice, with clean white fur and weighing 180-200 grams.

Then the rats were induced with high fat feed for 8 weeks to increase total cholesterol levels in all treatment groups. All test animals were grouped by randomization to eliminate the researcher intervention. The test animals were given a screen to regulate the amount of food given 20g per day.

The hyperlipid condition in the test animals was evidenced by the significant level of increasing total cholesterol levels in the blood of rats. After being induced, cholesterol levels



were measured using cholesterol strips and a constant cholesterol level was obtained. The test was continued by testing the effectiveness of black garlic in reducing cholesterol levels and stopping high-fat feeding. Based on the results of the study, the average level of total cholesterol for 7 days of treatment in each treatment showed varying results (Figure 1).

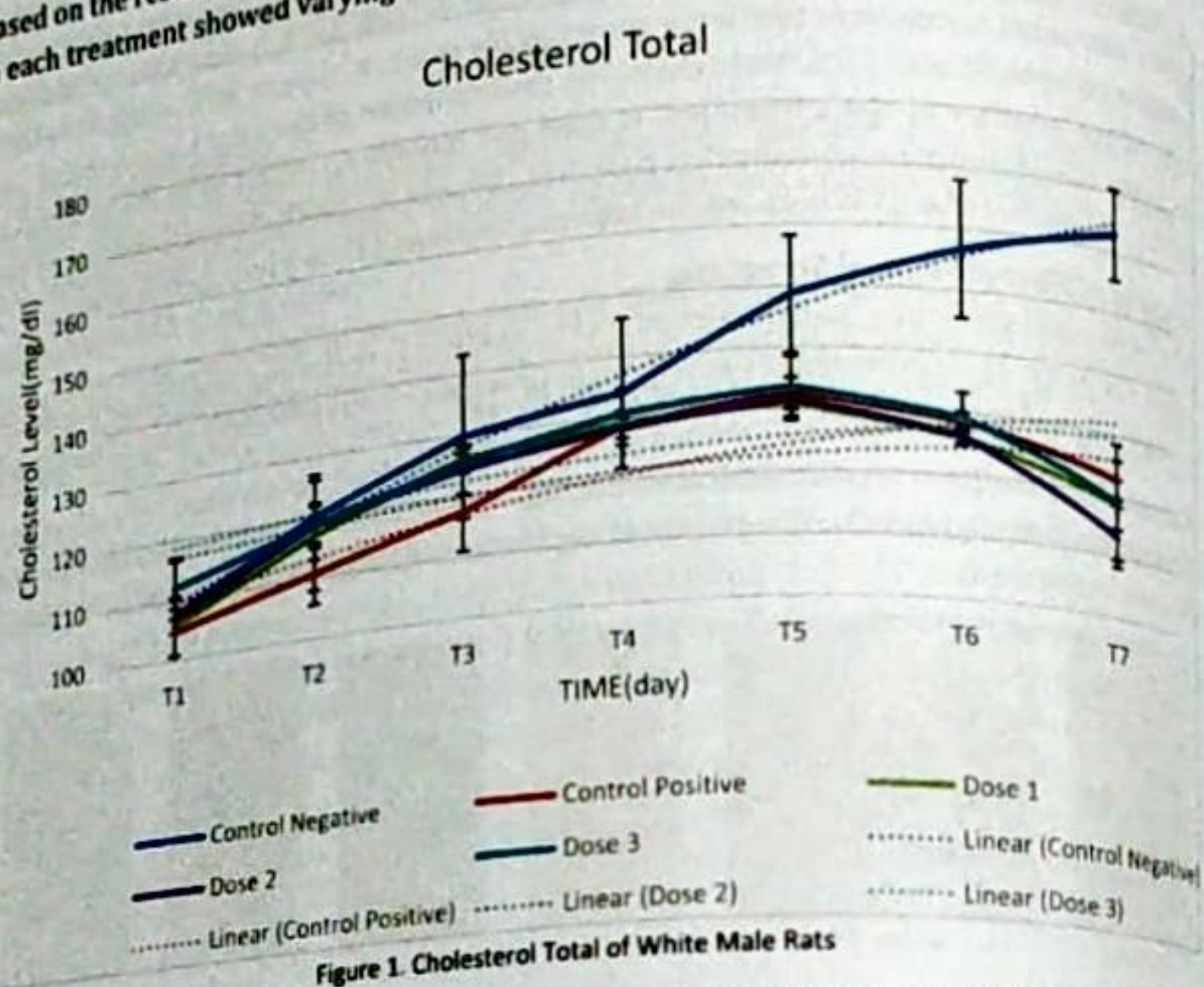


Figure 1. Cholesterol Total of White Male Rats

In this study, the data obtained from the measurement of blood serum total cholesterol levels of white rats induced by high-fat feed which had been given black garlic at a dose I 0.126 g / g BW, dose II 0.252 g / g BW, dose III 0.504 g / g BW, and 10 mg / g BW for simvastatin. In groups I and II, namely the negative control group and the positive control group (simvastatin), total cholesterol levels were not too different, the mean cholesterol levels for group I and group II were 163 mg / dl and 122.67 mg / dl. The mean level of total cholesterol in group I was included in levels above normal total cholesterol in rats because the rats in group I were not given treatment in the form of drug administration, resulting in an increase in total cholesterol levels. In group II total cholesterol levels also exceeded the normal limit of cholesterol levels in rats, this was due to the onset of the drug simvastatin 4-6 weeks (Adyana, 2000). Simvastatin as a HMG Co-A reductase enzyme prevent the formation of Acetyl CoA into mevalonate and prevent cholesterol formed. Then the dose groups I (0.126 g / g BW), II (0.252 g / g), and III (0.504 g / g) showed an average level of total cholesterol, respectively 118.67 mg / dl, 113.33 mg / dl, and 119 mg / dl. In addition, Figure 1. shows that the increase in the dose of blackened garlic did not show a significant decrease. At dose II, there was a more significant decrease than the positive control, dose I, and dose III.

Time (day)	Control Negative	Control Positive	Dose I	Dose II	Dose III
T1	107.33±2.8	104.67±4.0	105.67±4.9	107±9.6	112±5.1
T2	120±7	110.67±5.1	117±9.1	118.67±3.7	118.67±3.2
T3	129.67±13.4	117.33±6.4	126±1.7	124.33±4.0	125.33±1.5
T4	134±12.16	128.67±0.5	129±1	128.33±2.8	130.67±4.0
T5	148.67±9.5	132.67±2.5	131±1	131.67±3.5	133.67±4.9
T6	156.67±11.2	128.67±1.5	126±1	126.33±1.5	129.33±4.1
T7	163±7.5	122.67±3.2	118.67±4.1	113.33±5.0*	119±9.5

\*best result from group dose II in activity anti-hyperlipidemia by reducing total cholesterol

Based on the results of Figure 1.2, it can be seen that there is fat in the digestive organs at the positive dose, dose I, dose II, and dose III. Fat accumulation occurs due to the high-fat feed intake given. Diets containing high fat will be metabolized by the body into fatty acids. When too much fatty acids have accumulated, the body will store them into triglycerides or fat in the body.

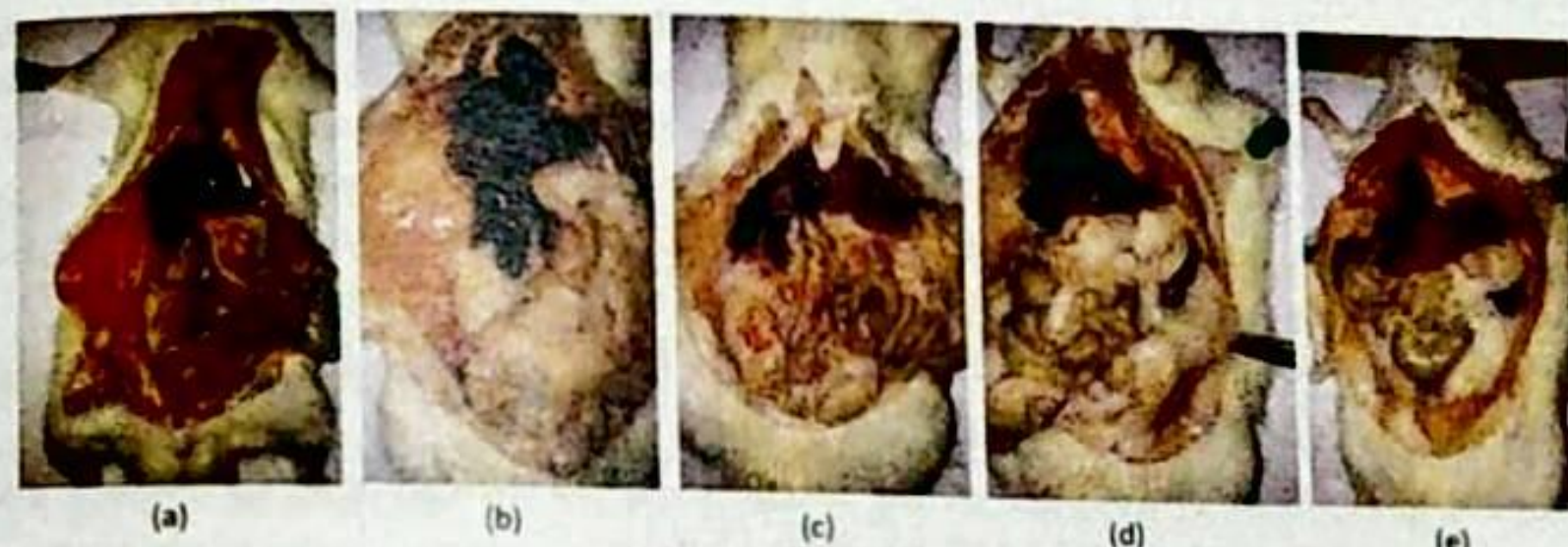


Figure 2. Organ *Rattus norvegicus* (a) dosesnegative (b) dosespositive (c) doses I (d) doses II (e) doses III

The Shapiro-Wilk normality test showed that the data were normally distributed ( $p > 0.05$ ). Anova test on total cholesterol levels showed that there were no significant differences between treatment groups ( $p > 0.05$ ).

#### 4. CONCLUSION

Based on the results of the study, it can be concluded that blackened garlic has antihyperlipidemic activity by reducing total cholesterol levels in white rats with the best dose of 0.252 g / g BW and is more effective than Simvastatin drug.