Effect of garlic extract (Allium sativum) administration of the total cholesterol level for white rats (Rattus norvegicus) with hypercholesterolemia

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Abstract. Hypercholesterolemia is one of the non-contagious diseases which is the main cause of death globally. Accumulation of excessive cholesterol or hypercholesterolemia will be atherosclerosis which causes strokes and cardiovascular diseases such as coronary heart disease. Garlic has an active agent allicin which an alkaloid compound that was contained in garlic, can be used to reduce cholesterol levels, in addition: vitamin C and niacin. Knowing that effect of garlic extract (Allium sativum) to decrease cholesterol total levels in white rats (Rattus norvegicus) which are deliberately hypercholesterol. Experimental research through Pre test-post test with control group design approaching in laboratory. Research sample was 25 white rats selected randomly and divided into 5 groups are normal control group and treatment group. Data analysis by One Way ANOVA showed that total cholesterol levels on the 32, 33, 37 and 42 days was significance value of p <0.05, its means the statistical test showed that given the garlic extract (Allium sativum) can reduce total cholesterol levels in white rats with hypercholesterolemia. There is the effect of garlic extract (Allium sativum) on decreasing cholesterol total levels in white rats (Rattus norvegicus) with hypercholesterolemia and a significant value (p <0.05).

1. Introduction
Hypercholesterolemia is one of the non-communicable diseases which is the main cause of death globally [1]. Excess cholesterol or what is called hypercholesterolemia will cause atherosclerosis which will cause strokes and cardiovascular diseases such as coronary heart disease [2,3,4,5]. Globally 2015 World Health Organization (WHO) data, this increase in cholesterol caused 2.6 million deaths (4.5% of all deaths) and 29.7 million Disability Adjusted Life Year (DALYs), or 20% of the total DALYS [6]. Data in Indonesia on residents aged> 15 years found abnormal total cholesterol of 35.9%, High-Density Lipoprotein (HDL) low at 22.9%, Low-Density Lipoprotein (LDL) not optimal with near optimal
borderline combined categories high 60.3% and very high 15.9%, abnormal triglycerides with high borderline category 13.0% and very high category 11.9% [7].

Garlic has an active agent allicin which is an alkaloid compound that is contained in garlic which can be used to reduce cholesterol levels, besides that in garlic there are also: vitamin C, niacin, germanium and sulfur-bounding compounds [2,6].

Based on the description of the data above, the researchers felt interested to find out more about the effect of giving garlic extract (Allium sativum) on the reduction of total cholesterol levels in Sprague dawley strain white rats that were hypercholesterol.

2. Method

Experimental laboratory study with pretest-posttest design with control group. The study sample was 25 randomly selected white rats divided into 5 groups, namely the control group and the treatment group. This research was conducted for 30 days. Research, maintenance and measurement of total cholesterol levels were carried out in the dry lab in the Faculty of Medicine, Universitas Nusa Cendana. The extraction process was carried out at the Bioscience Laboratory of the Universitas Nusa Cendana. The process of making garlic extract by maceration method. Giving hypercholesterol cholesterol feed consists of 30 grams of quail egg yolk PTU (Propiltiourasil) 0.1% and water up to 1000 mL. Making hypercholesterol feed is by using PTU 100 mg dissolved in 1000 mL of distilled water, quail egg yolk is dissolved with PTU solution that has been made. For 25 days this feed was given to mice with each volume of 0.5 cc per mouse by means of pressure [9] and followed by examination of cholesterol levels; after that, it was continued with the administration of the treatment in the form of simvastatin 1.8 mg / 200 gram per day, dose I garlic extract: 0.108 grams, dose II: 0.144 grams, dose III: 0.18 grams. Examination of total cholesterol levels was carried out on the 1st, 5th and 10th day after administration of the test material.

3. Result

3.1. Extraction

Garlic used in this study came from the Kapan area, South Timor Tengah Regency, East Nusa Tenggara Province. Garlic is peeled and then weighed 1000 grams. Next, the garlic is washed and dried in an oven at 400C for 2 days. Then the garlic is smoothed by blending 800 grams. Garlic is then soaked in 2 liters of 70% ethanol for 3x24 hours. The next process is evaporation carried out in the Bioscience Laboratory Undana on the 4th day using a Vacuum rotatory evaporator for 14 hours; so that we get 144,282 grams of thick garlic extract.

3.2. Body-weight measurement

In general, the weight gain of the test animals occurred on the 0th day until the 32nd day; whereas on the 37th day and 42nd day there was a decrease in body weight in the test animals. Weight gain occurred on day 0 to day 32 because the test animals were induced hypercholesterol feed in the form of a mixture of egg yolks and propylthiouracil drugs are given for 25 days where this condition caused a state of hypercholesterolemia. Feed consumption of hypercholesterol causes an increase in cholesterol in the blood, cholesterol is a type of fat. Fat stored in adipose tissue is not directly used but stored in the form of triglycerides; until it is needed to produce fat energy, it will be hydrolyzed to free fatty acids and glycerol [10].

This administration aims to reduce the condition of hyperthyroidism in the condition of normal mice to be a hypothyroid state so that there is an increase in cholesterol levels in the blood; its manifestations are slow metabolism and becoming fat [11].

The weight loss that occurred on the 37th day and 42nd day was suspected because the treatment of the test material in the form of simvastatin and garlic extract was given to reduce the state of hypercholesterol.
3.3. Cholesterol total level measurement

There was an increase in total cholesterol levels on the 32nd day in the positive control group, treatment group I, treatment group II and treatment group III. This is because on the 32nd day hypercholesterolemia feed has been induced for 25 days and then the total cholesterol level is checked; Whereas on the 33rd day, 37th day and 42nd day there was a decrease in total cholesterol levels in the positive control group, treatment group I, treatment group II and treatment group III because simvastatin and garlic extract were given treatment. Results of data analysis and discussion.

### Table 1. The results of the One Way Anova test analysis of all groups of test animals

| Cholesterol Level Total Day | Between Groups | Within Groups | Sig. | Nilai p |
|-----------------------------|----------------|--------------|------|---------|
| Day 0                       | Total          | Total        | .306 | >0.05   |
| Cholesterol Level Total Day 32 | Between Groups | Within Groups | .000 | <0.05   |
| Cholesterol Level Total Day 33 | Between Groups | Within Groups | .022 | <0.05   |
| Cholesterol Level Total Day 37 | Between Groups | Within Groups | .048 | <0.05   |
| Cholesterol Level Total Day 42 | Between Groups | Within Groups | .001 | <0.05   |

Annotation: p<0.05 : significant

Table 1 shows that total cholesterol levels on day 32, day 33, day 37 and day 42 have a significance value of p <0.05, which means that this statistical test shows that administration of garlic extract (*Allium sativum*) can reduce total cholesterol levels in white rats with hypercholesterolemia; So that H0 is rejected and H1 is accepted. A follow-up test from One Way Anova carried out a post hoc test to compare cholesterol change data between groups.

### Table 2. The results of the Post hoc test analysis of all groups of test animals

| Dependent Variable | Group of test animals | Group of test animals | Sig. | Annotation |
|--------------------|-----------------------|-----------------------|------|------------|
| Normal control group | Positive control group | Treatment group I | .000 | Significant |
| Treatment group I | Treatment group I | .001 | Significant |
| Treatment group II | Treatment group II | .000 | Significant |
| Treatment group III | Treatment group III | .001 | Significant |
| Normal control group | Treatment group I | .973 | |
| Positive control group | Treatment group II | .975 | |
| Treatment group I | Treatment group III | .998 | |
| Treatment group II | Normal control group | .998 | |
| Positive control group | Positive control group | .001 | Significant |
| Treatment group II | Normal control group | .000 | Significant |


| Treatment group | Cholesterol Level | Normal control group | Positive control group | Significant |
|-----------------|------------------|----------------------|------------------------|-------------|
| Treatment group I | 1.996 | 1.000 | 0.999 | Positive control group |
| Treatment group II | 1.997 | 0.997 | 0.999 | Normal control group |
| Treatment group III | 0.026 | 0.026 | 0.026 | Treatment group III |
| Normal control group | 1.999 | 0.999 | 0.999 | Treatment group II |
| Positive control group | 0.998 | 0.998 | 0.998 | Normal control group |
| Treatment group I | 0.025 | 0.025 | 0.025 | Treatment group I |
| Treatment group II | 0.053 | 0.053 | 0.053 | Treatment group II |
| Treatment group III | 0.129 | 0.129 | 0.129 | Normal control group |
| Normal control group | 1.000 | 1.000 | 1.000 | Treatment group I |
| Positive control group | 0.999 | 0.999 | 0.999 | Treatment group II |
| Treatment group I | 0.836 | 0.836 | 0.836 | Normal control group |
| Treatment group II | 0.349 | 0.349 | 0.349 | Treatment group III |
| Treatment group III | 1.000 | 1.000 | 1.000 | Normal control group |
| Normal control group | 0.998 | 0.998 | 0.998 | Treatment group I |
| Positive control group | 0.998 | 0.998 | 0.998 | Treatment group II |
| Treatment group I | 0.129 | 0.129 | 0.129 | Normal control group |
| Treatment group II | 0.086 | 0.086 | 0.086 | Treatment group III |
| Treatment group III | 0.401 | 0.401 | 0.401 | Normal control group |
| Normal control group | 0.522 | 0.522 | 0.522 | Treatment group I |
| Positive control group | 0.522 | 0.522 | 0.522 | Treatment group II |
| Treatment group I | 0.999 | 0.999 | 0.999 | Normal control group |
| Treatment group II | 0.538 | 0.538 | 0.538 | Treatment group III |
| Treatment group III | 0.401 | 0.401 | 0.401 | Normal control group |
| Normal control group | 0.025 | 0.025 | 0.025 | Positive control group |

**Notes:**
- The table shows the cholesterol levels for different treatment groups and control groups.
- Significance is indicated by the `Significant` column.
Table 2 shows the post hoc test to find out the strength of differences between treatment groups, data shows total cholesterol levels on day 32: normal control group towards positive control group, treatment group I, treatment group II, treatment group III, showing significant (significant) difference; positive control group, treatment group I, treatment group II, treatment group III for the normal control group showed significant differences due to the normal control group not given the test material treatment.

Data on 33rd day: treatment group I against treatment group III; Treatment group III on treatment group I showed a significant difference in strength; this was due to multilevel dosing between treatment group I and treatment group III. The 37th day data did not have the strength of differences between groups.

Data on 42 days: normal control group toward positive control group, treatment group I, treatment group II, treatment group III showed the strength of differences between groups; positive control group, treatment group I, treatment group II, treatment group III towards the normal control group showed there was a significant strength of difference between groups.

Table 3. Results of analysis of comparison of hypercholesterol levels before (day 32) and after administration of garlic extract (day 33, day 37 and day 42)

| Group of test animals | No. Variable | Measurement results of hypercholesterol level (mg/dl) |
|-----------------------|--------------|-----------------------------------------------------|
|                       |              | Day 32 | Day 33 | Day 37 | Day 42 |
| Treatment group I     | T3           | 277    | 204    | 172    | 144    |
|                       | T25          | 238    | 131    | 130    | 113    |
|                       | T13          | 241    | 140    | 131    | 138    |
|                       | T28          | 212    | 124    | 114    | 109    |
|                       | T24          | 238    | 136    | 134    | 104    |
|                       | p value      |        | 0.022  | 0.003  | 0.150  |
| Treatment group II    | T27          | 244    | 138    | 137    | 141    |
|                       | T9           | 258    | 231    | 152    | 130    |
|                       | T5           | 216    | 116    | 108    | 100    |
|                       | T10          | 262    | 157    | 141    | 139    |
|                       | T2           | 269    | 127    | 125    | 120    |
Table 3 shows the comparison of hypercholesterol levels before (day 32) and after administration of garlic extract (day 33, day 37 and day 42), among others: treatment group I hypercholesterolemia levels before administration of garlic extract (day 32nd) compared to after giving garlic extract (day 33 and day 37): has a value of \( p < 0.05 \), which means that there are significant differences in the level of hypercholesterolemia before and after administration of garlic extract; While the comparison (day 32) with (day 42): has a value of \( p > 0.05 \), which means that there are no significant differences. Treatment group II hypercholesterol levels before administration of garlic extract (day 32) and after administration of garlic extract on (33\textsuperscript{rd}, 37\textsuperscript{th} and 42\textsuperscript{nd}): have a value of \( p > 0.05 \) which means that there is no significant differences. Treatment group III hypercholesterol level before giving garlic extract (day 32) compared to after giving garlic extract at (33\textsuperscript{rd} day): has a value of \( p < 0.05 \) which means that there are significant differences in the level of hypercholesterolemia before and after administration garlic extract; While the comparison (day 32) with (days 37 and 42): has a value of \( p > 0.05 \), which means that there are no significant differences.

The normal control group was the control group that was not given test material treatment. This group was used by researchers to determine normal total cholesterol levels in research rats that were not treated with hypercholesterol cholesterol.

### Table 4. The measurement results of cholesterol total levels of test animals (mg/dl)

| Group of test animals | No. Variabel | Day 0 | Day 32 | Day 33 | Day 37 | Day 42 |
|-----------------------|--------------|-------|--------|--------|--------|--------|
| Normal control group  | T15          | 137   | 195    | 198    | 196    | 195    |
|                       | T22          | 119   | 153    | 156    | 154    | 154    |
|                       | T12          | 100   | 175    | 177    | 176    | 174    |
|                       | T16          | 152   | 171    | 173    | 170    | 170    |
|                       | T17          | 137   | 172    | 172    | 171    | 170    |
| Positive control group| T7           | 169   | 253    | 156    | 120    | 141    |
|                       | T23          | 155   | 236    | 144    | 141    | 139    |
|                       | T11          | 177   | 282    | 164    | 152    | 139    |
|                       | T20          | 167   | 245    | 144    | 104    | 108    |
|                       | T21          | 153   | 234    | 164    | 162    | 152    |
| Treatment group I     | T3           | 151   | 277    | 204    | 172    | 144    |
|                       | T25          | 115   | 238    | 131    | 130    | 113    |
|                       | T13          | 157   | 241    | 140    | 131    | 138    |
|                       | T28          | 165   | 212    | 124    | 114    | 109    |
|                       | T24          | 158   | 238    | 136    | 134    | 104    |
| Treatment group II    | T27          | 112   | 244    | 138    | 137    | 141    |
|                       | T9           | 137   | 258    | 231    | 152    | 130    |
The positive control group was the group given the treatment in the form of hipcholesterol cholesterol and given simvastatin drug test material. The dose of simvastatin given to mice is 1.8 mg / 200 g bb per day. In Table 4, the average total cholesterol level can be seen a decrease in total cholesterol levels from the state of hypercholesterolemia on the 33rd day, while on the 37th day and 42nd day, a decrease in the same number. Based on the statistical results on Table 2 post hoc tests on day 32: the positive control group towards the normal control group showed a significant difference in strength; 42nd day data: positive control group towards normal control group showed significant strength difference. There was a decrease in hypercholesterolemia levels through the statin drug mechanism inhibiting 3-hydroxy-3-methylglutaril coenzyme A reductase converting acetyl-CoA to mevalonic acid. This reaction is one step in the formation of cholesterol in the liver. Statins reduce LDL levels and increase HDL levels [12].

The decrease in total cholesterol levels by the drug simvastatin was in accordance with the research conducted by Nurmelis in 2015 about monitoring lipid-cholesterol profiles in normal mice and hypercholesterol cholesterol mice after the administration of cat whiskers (Orthosiphon stamunueus) extract in the positive control group given simvastatin drugs and research results showed a decrease in total cholesterol levels in the test group dose of 250,500,1000 mg/kg BB which was significantly different from normal controls in normal cholesterol mice (p <0.05) [13].

The treatment group I was the group given the garlic extract (Allium sativum) with a dose: 0.108 grams. In Table 4 the average total cholesterol level decreased the level of hypercholesterolemia on the 33rd day, 37th and 42nd day. Based on Table 2 the post hoc test on the 33rd day (one day after administration of garlic extract) the treatment group I to the treatment group III showed a significant difference in strength on the 42nd day (after 10 days of giving garlic extract) to the treatment group I to Normal controls show the power of significant differences. In Table 3 Comparison of the results of the analysis of hypercholesterol levels before administration of garlic extract (day 32) with after administration of garlic extract (day 33, day 37 and day 42) showed results including treatment group I day 32nd with 33rd day having significant value; 32nd day with 37th day showing significant results; 32nd day with 42nd day addressing results is not significant. The above data shows that giving garlic extract at a dose of 0.108 grams can reduce the level of hypercholesterolemia. This decrease occurs because garlic contains many alkaloid compounds, namely allicin. The mechanism for reducing blood cholesterol by allicin is thought to be able to bind to the SH-group which is a functional part of coenzyme-A so that there is direct inhibition of the enzyme 3-hydroxy-3-methylglutaril coenzyme (HMG-CoA) reductase by allicin.

The treatment group II was the group given the garlic extract (Allium sativum) with a dose of 0.144 grams. In Table 4 the average total cholesterol level in the treatment group II decreased the level of hypercholesterolemia. Based on Table 2 the post hoc test on day 42: treatment group II for the normal control group showed a significant difference in strength. In Table 3 Comparison of the results of the analysis of hypercholesterol levels before administration of garlic extract (day 32) with after administration of garlic extract (day 33, day 37 and day 42) showed results including treatment group II results statistics show before and after administration of garlic extract did not show a significant difference. There was no significant change in hypercholesterolemia levels before and after the administration of garlic extract at a dose of 0.144 grams, possibly not working effectively in inhibiting the HMG CoA reductase enzyme in the liver.

The treatment group III was the group given 0.18 grams of garlic extract. In Table 4 the average total cholesterol level in the treatment group III showed a decrease in the level of hypercholesterolemia. Based on Table 2 the post hoc test on the 32nd day: Treatment group III for the normal control group showed significant results; Data on the 33rd day: treatment group III on treatment group I showed a
significant difference in strength, this was due to multilevel dosing between treatment group I and treatment group III. Data on 42 days: treatment group III on the normal control group showed a significant difference in strength between groups. Table 3 results of comparison of hypercholesterolemia levels before administration of garlic extract (day 32) with after administration of garlic extract (day 33, day 37 and day 42) show significant results between 32nd day and 33rd day. The dose of extract given to this group is a dose that is increased 2 times the dose that should be given to humans, therefore the effect directly decreases hypercholesterolemia levels after one day of administration. This decrease occurs because Garlic contains many alkaloid compounds, namely allicin.

The mechanism for reducing blood cholesterol by allicin is thought to be able to bind to the SH-group which is a functional part of coenzyme-A so that there is direct inhibition of the enzyme 3-hydroxy-3-methylglutaral coenzyme (HMG-CoA) reductase by allicin. In addition, garlic is also contained in vitamin C (ascorbic acid) in protecting the heart as follows: prevents endothelial damage that normally initiates inflammatory responses and lipoprotein adhesions, destroys existing plaques by binding to lipoproteins and expelling them from the body, building collagen and increasing vessel elasticity blood [8]. There is also nicotinic acid (niacin) can reduce cholesterol levels by suppressing the activity of the lipoprotein lipase enzyme through inhibition of free fatty acid flow from adipose tissue, thereby reducing VLDL production in the liver and can inhibit fat mobilization so that total cholesterol production and LDL cholesterol can decrease [8]. The results of this study indicate that administration of garlic extract (Allium sativum) can reduce total cholesterol levels in Sprague Dawley rats with hypercholesterolemia; this study is in accordance with the results of research conducted by Pramitasari, Riana and Bahrudin regarding the effect of giving garlic extract (Allium sativum) on the improvement of lipid profile in wistar hypercholesterolemic Rattus norvegicus strains at various doses: 0.05 g / head / day, 0.1 g / head / day and 0.2 g / head / day obtained from garlic resulted in a significant decrease in total cholesterol (KT), LDL, TG, LDL / HDL ratio, and the ratio of KT / HDL at a dose of 0, 2 g / tail can reduce cholesterol almost to normal [3]. Brajawikalpa, Kautama with the title of the effect of giving garlic ethanol extract to total cholesterol, LDL and HDL levels in hypercholesterolemic cholesterol rats with a dose: 3.6 mg, 7.2 mg, 10.8 mg and 18 mg. Conclusion: Garlic ethanol extract has an influence on levels of total cholesterol, LDL and HDL; a dose of 3.6 mg / 200 g BB can reduce total cholesterol, LDL and increase HDL. The higher the garlic ethanol extract the greater the decrease in total cholesterol, LDL and increased HDL [8].

4. Conclusion

There is an effect of garlic extract (Allium sativum) on decreasing total cholesterol levels in white rats (Rattus norvegicus) with hypercholesterol with significant value (p <0.05). There are significant differences in hypercholesterolemia levels before being given garlic extract and after administration of garlic extract, among others: treatment group I before giving garlic extract (day 32) with after giving garlic extract (day 33) significant value 0.022 and before administration of garlic extract (32nd day) with after administration of garlic extract (42nd day) significant value 0.003; Treatment group III before giving garlic extract (day 32) with after giving garlic extract (day 33) significant value 0.007 which means smaller than p-value <0.05.

For further research, it is necessary to quantitatively test phytochemicals so that it can determine the amount of each group of compounds contained in garlic ethanol extract (Allium sativum) specifically which can reduce cholesterol levels. The measurements of total cholesterol levels can use an examination method that provides a higher level of accuracy such as laboratory testing of CHOD-PAP (cholesterol oxidase phenol amino phenzone).

5. References

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