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STUDY ON THE USE OF GARLIC EXTRACT (ALLIUM SATIVUM) IN LOWERING DYSLIPIDEMIA LEVELS IN MALE WISTAR RATS CONSUMING A HIGH-FAT DIET AND PROPYLTHIOURACIL

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ABSTRACT
The investigation into dyslipidemia, associated with an increased cardiovascular risk, focuses on evaluating the effectiveness of garlic extract (Allium sativum) in reducing lipid levels in rats subjected to a high-fat diet and PTU (propylthiouracil). Elevated lipids significantly contribute to cardiovascular diseases, emphasizing the importance of effective management for prevention. This study explores the antidyslipidemic potential of Garlic (Allium sativum) using male Wistar rats in a Pre-test and Post-test group-only control design. Phytochemical screening revealed the presence of various compounds, including Alkaloids, Saponins, Flavonoids, Tannins, Steroids, and Terpenoids, in the methanol extract of garlic. The research concludes that this extract significantly reduces total cholesterol, triglycerides, and LDL levels while increasing HDL levels in experimental rats. Notably, the decrease in SGOT levels positively impacts liver health, potentially reducing the risk of Non-Alcoholic Fatty Liver Disease (NAFLD). Despite promising findings, further confirmation through human studies is necessary before considering Garlic (Allium sativum) methanol extract as an effective therapy. With observed improvements in lipid profiles and liver health, the methanol extract of garlic holds potential as an active ingredient for developing antidyslipidemia therapy.

KEYWORDS: *Dyslipidemia; Garlic extract (Allium sativum); Cardiovascular risk Antidyslipidemic potential; Experimental rat study*

BACKGROUND

Dyslipidemia is a condition of abnormal lipid levels in the blood that is often associated with an increased risk of cardiovascular disease. This study aims to evaluate the effectiveness of garlic extract (Allium sativum) in reducing dyslipidemia levels in male Wistar rats consuming a high-fat diet and PTU (propylthiouracil) (Halawani *et al.*, 2019). Dyslipidemia plays a significant role in the development of cardiovascular diseases, and managing blood lipid levels, such as using garlic extract, is expected to provide therapeutic benefits. Dyslipidemia, referring to abnormal lipid levels in the blood, significantly contributes to the development of cardiovascular diseases (J *et al.*, 2013). Elevated levels of total cholesterol, triglycerides, and LDL (bad cholesterol), along with low levels of HDL (good cholesterol), can increase the risk of atherosclerosis, the foundation of many cardiovascular diseases, including heart attacks and strokes (Aguilar-Ballester *et al.*, 2021). Therefore, the management and treatment of dyslipidemia are crucial to prevent potential risks of serious cardiovascular diseases. Further understanding of the effectiveness of alternative therapies, such as garlic extract, in addressing dyslipidemia can contribute significantly to preventing and treating cardiovascular diseases (Rouf *et al.*, 2020); (Ansary *et al.*, 2020).

The administration of PTU (Propylthiouracil) and a high-fat diet is expected to create dyslipidemia conditions in experimental rats. PTU is used to induce hypothyroidism in rats, which can affect lipid metabolism. Hypothyroidism can increase blood lipid levels, including total cholesterol and triglycerides. Meanwhile, a high-fat diet can improve the body's production and accumulation of lipids. The combination of PTU administration and a high-fat diet is expected to create dyslipidemia conditions in rats, reflecting situations often associated with unhealthy eating patterns and thyroid disorders in humans, forming a relevant experimental model to evaluate the impact of garlic extract (Lin *et al.*, 2017).

Garlic (Allium sativum) has received widespread attention as a potential supporter of cardiovascular health through various mechanisms. Active compounds, especially allicin and other sulfur compounds are known to reduce total cholesterol and LDL (bad cholesterol) levels. Allicin also affects the HMG-CoA reductase enzyme, contributing to the regulation of cholesterol synthesis in the liver. Additionally, garlic can increase HDL (good cholesterol) through its antioxidant properties, protecting HDL from oxidation. Garlic components also possess anti-inflammatory and antiproliferative properties that protect blood vessels from inflammation and cell damage, reducing the risk of atherosclerosis. The vasodilator properties of garlic play a role in regulating blood pressure, while its anti-thrombotic effects help prevent blood clot formation. Furthermore, garlic protects blood vessel endothelium, maintaining elasticity and everyday function, including its ability to lower total cholesterol, triglyceride, and LDL levels

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and increase HDL levels (Qidwai and Ashfaq, 2013); (Suleria *et al.*, 2015).

This background underscores the importance of understanding the potential role of garlic in addressing dyslipidemia, which can positively contribute to the prevention of cardiovascular diseases. This study is expected to provide further insights into the effectiveness of garlic extract in the context of dyslipidemia induced by a high-fat diet and PTU.

RESEARCH METHODS

This experimental study adopts the Pre-test and Post-test group-only control design approach, utilizing male Wistar rats as research subjects. The research was conducted in January 2024. The calculation of the sample size was performed using the Federer formula with the requirement of

$$(r-1)\,(t-1) \geq 15,$$

where 'r' represents the number of samples in each treatment group, and 't' is the number of treatment groups.

$$(r-1)(6-1) \geq 15$$
$$5(r-1) \geq 15$$
$$r-1 \geq 15/5$$
$$r \geq 3+1$$
$$r \geq 4$$

Based on the calculation results, it can be concluded that a minimum of 4 male Wistar rats (Rattus norvegicus) with a weight ranging from 180 to 200 grams and an age between 2-4 months is required for each treatment group.

Equipment

Surgical instruments, laboratory glassware, aluminum foil, blender (Miyako), porcelain dish, desiccator, incubator, glass slides, cover glass, porcelain crucible, drying cabinet, microtubes, light microscope, analytical balance (Vibra AJ), oral sonde, electric oven (Stork), water bath (Yenaco), tube clamps, reaction tube rack, rotary evaporator, centrifuge, set of water content determination tools, UV spectrophotometer (Microlet 3000), injection syringe, muffle furnace (Nabertherm), reaction tubes, animal scales (Presica).

Materials

The materials used in this research are Garlic (Allium sativum), methanol, Aquades (distilled water), Na-CMC (Sodium Carboxyl methylcellulose), simvastatin, rice husk, rat pellet food, phytochemical screening reagents, and ketamine.

Sample Determination

Garlic (Allium sativum) samples used in this study were obtained from one of the traditional markets in Medan City.

Manufacture of Garlic (Allium sativum) Simplisia

Identified Garlic (Allium sativum)s was washed thoroughly with running water, drained, and spread on blotting paper until dry. The samples were then weighed and dried by air-drying, and the weight of the dried material was measured. The dried Garlic (Allium sativum) material was ground into powder to form simplisia (Kosasih et al., 2019).

Preparation of Garlic (Allium sativum) Methanol Extract

Garlic (Allium sativum) weighing 200 grams each was extracted using the maceration technique with 400 ml of 98% methanol solvent. Maceration was carried out for one week with occasional stirring. The filtrate was then evaporated using a rotary vacuum evaporator at 50°C until a paste-like extract was obtained and stored at 20°C (Vasanthakumar D et al., 2015).

Phytochemical Screening

Phytochemical screening in this study followed a modified Farnsworth method, including the identification of phenols, steroids/triterpenoids, terpenoids, saponins, flavonoids, tannins, and alkaloids (Widowati et al., 2016, 2017, 2018).

Anti-Dyslipidemia Effect Testing

Preparation of 0.5% Na CMC Suspension

0.5 grams of Na CMC was scattered into a mortar containing 10 mL of hot distilled water. After 15 minutes, a transparent mass was obtained, ground to form a gel, diluted with a little filtered water, and poured into a 100 mL volumetric flask. Distilled water was added to the mark. This suspension would be used further as a dispersing medium for oral suspension (Colloid) (Mutia and Chiuman, 2019).

Preparation of Hypercholesterolemic Feed Suspension

The suspension was made by mixing 300 grams of animal fat into 100 ml of distilled water and 200 grams of poultry egg yolk into 1 ml of 0.5% Na-CMC (Harsa, 2014).

Preparation of PTU Suspension

100 mg of PTU was ground in a mortar to form a powder, then added to a 0.5% Na CMC suspension and put into a 10 ml volumetric flask. The volume was adjusted to the mark with 0.5% Na CMC suspension (Untari and Pramukantoro, 2020).

Garlic Extract (Allium sativum) Suspension

1.2 grams of Garlic Extract (Allium sativum) was added to a mortar, and Na CMC, 0.5% suspension, was gradually added while grinding until homogenous. This mixture was then poured into a 10 mL volumetric flask. The volume was adjusted with Na CMC 0.5% suspension to the mark (Mutia and Chiuman, 2019).

Simvastatin Suspension Preparation

10 mg of simvastatin was ground in a mortar to form a powder, then added to a 0.5% Na CMC suspension and put into a 25 mL volumetric flask. The volume was adjusted to the mark with 0.5% Na CMC suspension (Fouad and Jresat, 2013; Aldahmash and El-Nagar, 2016).

Induction of Dyslipidemia in Experimental Animals

The induction process was performed by providing a high-fat diet and PTU to the experimental animals for 14 days. PTU was given as an oral suspension at 12.5 mg/day (1.25 ml/day) divided into two doses. Meanwhile, the high-fat diet was provided by administering a high-fat feed suspension at a dose of 15 g/kgBW for animal fat suspension and 10 g/kgBW for

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poultry egg yolk suspension (Harsa, 2014; Untari and Pramukantoro, 2020).

Measurement of Lipid Profile Parameters

The rats were satisfied at least 8 hours before the blood draw. Blood collection is done by direct withdrawal from the heart of mice as much as 1 ml. Put into a microtube and let stand ± 20 minutes. Then, the blood was centrifuged at a rate of 3000 rpm for 15 minutes to obtain the blood serum of the rats. The determination of lipid profiles is determined by the colorimetric method. Lipid profile examination is conducted at the Health Laboratory, North Sumatra Provincial Health Office.

Measurement of Biochemical Parameters of SGOT and SGPT

Blood collection is done by direct withdrawal from the heart of mice as much as 1 ml. Put into a microtube and let stand ± 20 minutes. Then, the blood was centrifuged at a rate of 3000 rpm for 15 minutes to obtain the blood serum of the rats. The determination of SGOT and SGPT levels is based on enzymatic reactions using Dyasis® kit reagents. The procedure for determining SGOT and SGPT catalyst activity is based on work procedures from Dyasis®. SGOT and SGPT examinations are conducted at the Health Laboratory, North Sumatra Provincial Health Office.

Analyzes Data

The research data was then analyzed using the SPSS 25 program. The research data were analyzed descriptively (Central tendency and Dyspersi) 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 to see if the data was generally distributed with further tests in the form of Post Hoc Tukey HSD tests to see fundamental differences between treatments. However, if the data is abnormally distributed, the Kruskall-Wallis test is used as an alternative.

Research Results

After extraction using the maceration method on garlic (Allium sativum) samples, the following extract characteristics were found:

Table 1. Parts of Methanol Extract of Garlic (Allium sativum) (Zanthoxylum acanthopodium)

Characteristics	Value
Fresh Simplisia Weight (gr)	800 gr
Dry Simplisia Powder Weight (gr)	244 gr
Solvent Volume (ml)	2165 ml
Extract weight (gr)	14,98 gr
Yield (%)	7.12%

The table data above shows that from 500 grams of Garlic (Allium sativum) samples, an extract of 14.98 grams was found. Thus, the yield obtained from Garlic (Allium sativum) methanol extract is 7.12%.

Table 2. Phytochemical Screening Results of Garlic (Allium sativum) Methanol Extract

Phytochemicals	Reagents	Result
Alkaloid	Bouchardart	+
	Mayer	+
	Dragondroff	-
	Wagner	+
Saponin	Aquadest + Alcohol 96%	-
Flavonoid	FeCl3 5%	+
	Mg $_{(s)}$ + HCl $_{(p)}$	-
	NaOH 10%	-
	$H_2SO_{4\ (p)}$	-
Tanin	$FeCl_3$ 1%	+
Steroid dan Terpenoid	Salkowsky	-
	Liberman Bouchard	+

The data in the table above shows that the methanol extract of Garlic (Allium sativum) contains several phytochemical compounds, including Alkaloids, Saponins, Flavonoids, Tannins, Steroids, and Terpenoids.

Table 3. Results of Normality Test Using Shapiro-Wilk Test for All Research Parameters

Parameter		P Value	Data Distribution
Weight		0.442	Usual
Total cholesterol before induction		< 0.05	Abnormal
Total cholesterol after induction		< 0.05	Abnormal
Lipid Profile After	0.412	0.486	Usual
Treatment	0.004	0.004	Abnormal

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	< 0.06	< 0.06	Abnormal
	0.245	0.135	Usual
Up to SGOT		< 0.05	Abnormal
Up to SGPT		0.125	Usual

The table above shows that the data on body weight, total cholesterol, and LDL levels from the lipid profile after treatment and SGPT levels have a standard distribution. At the same time, other parameters include total cholesterol before and after induction, triglyceride levels, HDL levels, and SGOT levels, which are abnormally distributed. Based on the distribution of these data, data with standard data distribution are analyzed with parametric statistics, while abnormal data is analyzed with non-parametric statistics.

Table 4. Differences in Rats' Initial Body Weight in the Entire Treatment Group

Treatment Group	Weight Loss (grams)		P Value
	Mean	SD	
Usual	354.00	37.67	0.745
Standard	339.85	16.33	
Control	395.11	33.67	
Methanol Extract of Garlic (Allium sativum)-I	366.21	36.13	
Methanol Extract of Garlic (Allium sativum)-II	335.85	33.88	
Methanol Extract of Garlic (Allium sativum)-III	281.12	18.81	

From the table data above, it can be seen that the P value > 0.05 (P value = 0.745), which means there is no significant difference in the initial body weight of the mice used in this study. The importance of the mice used in this study ranged from 210-400 grams, evenly distributed in each treatment group.

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

Treatment Group	Total Kolestrol (mg/dL)	
	Before Induction	After Induction
Usual	115.50 (110-116)	117.60 (115-120)[b]
Standard	112.00 (100-115)	211.00 (205-215)[a]
Control	116.65 (110-115)	211.55 (210-215)[b]
Methanol Extract of Garlic (Allium sativum)-I	115.55 (110-117)	212.60 (209-211)[b]
Methanol Extract of Garlic (Allium sativum)-II	110.50 (100-115)	210.50 (209-212)[b]
Methanol Extract of Garlic (Allium sativum)-III	116.50 (116-119)	211.25 (209-210)[b]
P Value	**0.861**	**0.004**

Data is displayed as Median (Range). The P value is obtained from the Kruskal-Wallis analysis. Different superscripts in the same column show significant differences.

From the table data 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 the entire treatment group did not show a significant difference (P value = 0.782). This demonstrated that the total cholesterol data of the rats before being given a high-fat diet were uniform. However, total cholesterol in all groups of rats after administration of a high-fat diet showed a different distribution, where only the control group, standard methanol extract of Garlic (Allium sativum)-I, II, and III, showed uniform total cholesterol.

Table 6. Comparison of Lipid Profiles in the Entire Mouse Treatment Group

Treatment Group	Profil Lipid			
	Total Kolestrol*	Trigliserida**	LDL*	HDL**
Usual	164.50 ± 2.40a	99.50 (97-100)a	60.20 ± 1.60a	66.45 (61-64)a
Standard	144.50 ± 0.58b	105.25 (101-105)b	64.00 ±1.20b	61.50 (60-66)a
Control	179.25 ± 6.02c	170.25 (168-179)c	112.50 6.805c	28.75 (68-46)b
Methanol Extract of Garlic (Allium sativum)-I	168.25 ± 1.50d	166.50 (164-165)d	86.75 ±2.62d	57.50 (56-59)b
Methanol Extract of Garlic (Allium sativum)-II	163.25 ± 2.22e	120.50 (113-122)e	77.50 ± 1.29e	61.50 (61-63)a
Methanol Extract of Garlic (Allium sativum)-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**	**0.009**

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*The data is displayed as Mean ± SD. P value obtained from One Way ANOVA analysis; **Data is expressed as Median (Range). The P value is obtained from the Kruskal-Wallis analysis. Different superscripts in the same column show significant differences

The study included different treatment groups with variations in lipid profiles. The usual treatment group showed a total cholesterol level of 164.50 ± 2.40a, triglycerides at 99.50 (97-100), LDL at 60.20 ± 1.60a, and HDL at 66.45 (61-64)a. The standard treatment group exhibited changes with total cholesterol at 144.50 ± 0.58b, triglycerides at 105.25 (101-105)b, LDL at 64.00 ± 1.20b, and HDL at 61.50 (60-66)a. The control group, serving as a baseline, had a total cholesterol level of 179.25 ± 6.02c, triglycerides at 170.25 (168-179)c, LDL at 112.50 ± 6.805c, and HDL at 28.75 (68-46)b. The treatment groups with Methanol Extract of Garlic (Allium sativum) showed variations in their lipid profiles: Group I at 168.25 ± 1.50d, Group II at 163.25 ± 2.22e, and Group III at 151.75 ± 0.96e for total cholesterol. Triglycerides were recorded as 166.50 (164-165)d, 120.50 (113-122)e, and 110.00 (108-112)f, respectively. LDL values were 86.75 ± 2.62d, 77.50 ± 1.29e, and 68.50 ± 1.29f, while HDL levels were 57.50 (56-59)b, 61.50 (61-63)a, and 61.00 (60-63)a. The p-values indicated statistically significant differences in total cholesterol ($p < 0.05$), triglycerides ($p = 0.013$), LDL ($p < 0.05$), and HDL ($p = 0.009$) among the treatment groups, suggesting the potential impact of garlic extract on lipid profiles.

Table 7. SGOT and SGPT Levels in All Treatment Groups

Treatment Group	Kadar SGOT (U/L)	Kadar SGPT (U/L)
Usual	24.24 (24-30)[a]	44.40 ± 1.40[a]
Standard	110.40 (104-112)[b]	170.74 ± 1.29[b]
Control	140.40 (142-170)[c]	97.24 ± 1.40[c]
Methanol Extract of Garlic (Allium sativum)-I	114.40 (114-120)[d]	100.74 ± 3.49[d]
Methanol Extract of Garlic (Allium sativum)-II	127.40 (121-124)[e]	114.40 ± 4.41[e]
Methanol Extract of Garlic (Allium sativum)-III	133.40 (129-132)[f]	142.40 ± 2.04[b]
P Value	**0.004**	**< 0.05**

*The data is displayed as Mean ± SD. P value obtained from One Way ANOVA analysis; **Data is expressed as Median (Range). The P value is obtained from the Kruskal-Wallis analysis. Different superscripts in the same column show significant differences

The treatment groups were evaluated based on their SGOT and SGPT levels, revealing variations. The usual treatment group exhibited SGOT at 24.24 (24-30)a and SGPT at 44.40 ± 1.40a. The standard treatment group showed considerable changes with SGOT at 110.40 (104-112)b and SGPT at 170.74 ± 1.29b. As a baseline, the control group displayed SGOT at 140.40 (142-170)c and SGPT at 97.24 ± 1.40c. The Methanol Extract of Garlic (Allium sativum) treatment groups presented distinct SGOT and SGPT values: Group I at 114.40 (114-120)d, Group II at 127.40 (121-124)e, and Group III at 133.40 (129-132)f for SGOT. The corresponding SGPT values were 100.74 ± 3.49d, 114.40 ± 4.41e, and 142.40 ± 2.04b. The p-values indicated statistically significant differences in SGOT ($p = 0.004$) and SGPT ($p < 0.05$) among the treatment groups, suggesting the potential impacts of garlic extract on liver enzyme levels.

DISCUSSION

The results showed that methanol extract from garlic (Allium sativum) positively affected lipid profiles in rats. This can be seen from the increase in HDL (good cholesterol) levels and a decrease in total cholesterol, triglycerides, and LDL (bad cholesterol) levels in rats that received treatment with Garlic methanol extract (Franczyk *et al.*, 2021). These findings indicate the potential use of garlic extract as an agent to support a reduced risk of lipid disorders, such as dyslipidemia. Garlic (Allium sativum) has long been recognized for its potential to affect lipid profiles positively (Asgharpour *et al.*, 2021). Nutritionists and health experts observe that active compounds in garlic, such as allicin, flavonoids, and saponins, can decrease total and LDL cholesterol (bad cholesterol) levels. Heart health research shows that garlic can reduce the risk of cardiovascular disease by increasing HDL (good cholesterol) levels and inhibiting LDL oxidation. From a phytochemical perspective, compounds in garlic, especially allicin, can modulate enzymes involved in lipid metabolism (Xu *et al.*, 2023). Pharmacologists highlight garlic's anti-inflammatory and antioxidant properties that protect blood vessels from inflammation and oxidative stress, favoring improving a healthier lipid profile (Bautista-Perez *et al.*, 2023).

Methanol extract from garlic (Allium sativum) positively affected lipid profiles in mice, which the content of active compounds such as allicin, alliin, ajoene, and diallyl sulfide can explain. The central combination, allicin, was shown to have hypolipidemic properties, capable of lowering lipid levels in the body. Allicin works by inhibiting enzyme activity in cholesterol synthesis, reducing total cholesterol, triglycerides, and LDL (bad cholesterol) levels, and increasing HDL levels (Bontempo *et al.*, 2021). Allicin also stimulates lipoprotein lipase activity, aiding in the breakdown of triglycerides. The antioxidant properties of allicin protect cells from free radical damage, which can contribute to cardiovascular disease. Allicin also has antioxidant, anti-inflammatory, and antiproliferative effects and can inhibit the oxidation of LDL cholesterol, the early stage of atherosclerosis plaque formation. Studies show that allicin affects lipid synthesis by reducing the activity of the enzyme HMG-CoA reductase in the cholesterol biosynthesis pathway (Sánchez-Gloria *et al.*, 2022).

This study revealed that the highest methanol extract from Garlic (Allium sativum) gave the most optimal results. This indicates that higher concentrations of bioactive compounds in garlic significantly reduce unwanted lipid levels and increase desired lipid levels in lab rats' blood. The group that received

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the garlic extract showed more favorable changes in lipid profile than the control group without treatment, showing garlic's positive potential in lowering the risk of dyslipidemia. These results indicate that dosing a certain level of garlic methanol extract (Allium sativum) can have a more robust and positive effect on the lipid profile of mice. The most optimal dose indicates that a better response to changes in lipid levels can be achieved at a given methanol extract dose. This factor can be related to the content of bioactive compounds such as allicin and other compounds in garlic extract, which may have a more significant effect at specific doses. This conclusion illustrates that garlic has properties that favor cardiovascular health through its positive influence on the lipid parameters of the body (Najman *et al.*, 2022).

CONCLUSION

Based on the results of research and data analysis regarding the administration of turmeric ethanol extract (Curcuma Longa) and Bioplacenton® for wound healing in rats, it can be concluded that turmeric ethanol extract contains bioactive compounds such as alkaloids, flavonoids, saponins, and tannins that play a role in wound healing. The optimum concentration of effective turmeric ethanol extract is 4%. Although Bioplacenton® (positive control) provided the most remarkable healing results, turmeric extract also has good potential in healing cut wounds. The results of statistical tests showed a significant effect of turmeric extract on the healing of incision wounds in rats. Therefore, turmeric extract has potential as an incision wound healing agent, and further research can be done to support the development of its use in medicine.

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