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Hypolipidemic and Hypoglycemic Potentials of Garlic Extract in High-Fat Diet and Streptozotocin-Induced Wistar Rats

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ABSTRACT

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by elevated blood glucose levels and often associated with hypercholesterolemia. Garlic (*Allium sativum Linn.*), rich in bioactive compounds such as allicin, saponins, and flavonoids, has been widely reported to possess lipid- and glucose-lowering properties. This study aimed to evaluate the hypolipidemic and hypoglycemic effects of garlic extract in Wistar rats subjected to a high-fat diet and streptozotocin (STZ) induction. A laboratory experimental design with a post-test-only control group was employed. For cholesterol assessment, hypercholesterolemic rats were administered garlic extract at doses of 0.05, 0.10, and 0.20g/head/day, while diabetic rats induced with STZ received doses of 100, 250, and 500 mg/kg body weight per day, with corresponding control groups. Garlic extract significantly reduced mean cholesterol levels in a dose-dependent manner (130.60mg/dL, 121.80mg/dL, and 112.00mg/dL for 0.05, 0.10, and 0.20g/head/day, respectively). In contrast, mean blood glucose levels showed a marginal decrease with increasing doses (282.80 mg/dL, 271.20mg/dL, and 269.27mg/dL at 100, 250, and 500mg/kgBW/day, respectively). However, no statistically significant differences were observed among groups ($p=0.706$, one-way ANOVA). These findings suggest that garlic extract exhibits a potent cholesterol-lowering effect in hypercholesterolemic rats but has a limited impact on blood glucose reduction under the tested conditions. Further studies are warranted to determine the optimal dosage and underlying mechanisms of action.

INTRODUCTION

Cholesterol, an essential fatty substance synthesized primarily in the liver, plays a crucial role in the formation of plasma lipoproteins and cell membranes, and serves as a precursor to essential steroid compounds (Duan et al., 2022; Kheirmandparizi et al., 2021; Qian et al., 2022). Although vital for bodily functions, excessive cholesterol levels in the blood (hypercholesterolemia) pose a serious health risk because they are a significant factor in atherosclerosis, which can lead to coronary heart disease (Gadidala et al., 2023; Ibrahim et al., 2023). High cholesterol levels represent a significant global health concern, with the World Health Organization (WHO) projecting a substantial increase in the number of affected individuals in the coming years (Siregar, 2022). In Indonesia, the prevalence of hyperlipidemia is notably high and tends to increase with age, highlighting the urgency of identifying effective prevention and treatment strategies, including non-pharmacological approaches (Kementerian Kesehatan Republik Indonesia, 2021).

The importance of managing cholesterol levels becomes even more relevant in the context of other metabolic diseases such as Diabetes Mellitus (DM). DM is characterized by chronic hyperglycemia resulting from impaired insulin secretion or action and also has a high and rising prevalence in Indonesia (Indrahadi et al., 2021; Wang et al., 2022; World Health Organization, 2021). There is a strong correlation between DM and dyslipidemia; individuals with DM are at greater risk of elevated total cholesterol levels. This is partly due to disrupted lipid metabolism, influenced by insulin resistance or insulin deficiency, which affects cholesterol synthesis and absorption (Oktaviana et al., 2022). Furthermore, the connection between cholesterol metabolism and pancreatic beta-cell function underscores the complexity of lipid involvement in DM pathogenesis, reinforcing the need for research into cholesterol interventions within diabetic conditions (Galli et al., 2023).

Among various non-pharmacological measures, garlic (*Allium sativum Linn.*) has long been recognized for its potential health benefits, particularly in managing lipid levels. Garlic contains bioactive compounds such as allicin, which is believed to inhibit HMG-CoA reductase, an enzyme crucial to cholesterol synthesis (Najman et al., 2021). Numerous studies have demonstrated garlic's positive effects on reducing serum cholesterol, triglycerides, and LDL levels while increasing HDL levels in both experimental animals and humans (Kodariah & Mendrofa, 2022; Vezza et al., 2024). The novelty of this study lies in exploring the effects of garlic extract specifically in an animal model experiencing comorbid conditions: hyperlipidemia induced by a high-fat diet alongside hyperglycemia (DM-like conditions) induced by Streptozotocin (STZ). This study differs from previous research that primarily focused on garlic's hypolipidemic effects or on hyperlipidemia models alone by evaluating its potential under combined metabolic challenges—hyperlipidemia and hyperglycemia—which frequently co-occur clinically.

This study will employ laboratory experimental methods using Wistar rats. A disease model will be developed by administering a high-fat diet to induce hyperlipidemia and injecting Streptozotocin (STZ) to create hyperglycemic conditions. This research aims to determine and analyze the effect of administering a garlic ethanol extract on reducing total cholesterol levels in male Wistar rats subjected to a high-fat diet and STZ-induced hyperglycemia.

METHOD

This study employed a laboratory experimental methodology, specifically a post-test-only control-group design. The primary objectives were twofold: firstly, to evaluate the effect of administering garlic extract on reducing blood cholesterol levels in Wistar rats subjected to a high-fat diet, and secondly, to assess its impact on lowering blood glucose levels in Wistar rats induced with Streptozotocin (STZ). This design incorporated both control and treatment groups, the latter receiving the garlic extract, followed by meticulous measurement and rigorous analysis of the pertinent biological parameters. Confounding variables were carefully controlled throughout the experiment to enhance the evidence supporting the treatment's influence. The independent variable in this study was the dosage of garlic extract, while the dependent variables were blood glucose and total blood cholesterol concentrations in Wistar rats. The research was conducted at the Pharmacy Laboratory, Universitas Sumatera Utara, Medan, spanning the period from November 2024 to January 2025.

The experimental animals utilised were male Wistar strain rats, sourced from the USU Pharmacy Laboratory. Inclusion criteria stipulated that rats should be approximately 3 months of age, possess a body weight ranging between 180 and 200 grams, be in a healthy condition, and exhibit normal activity levels. Any rats that became ill or died during the course of the treatment period were excluded from the study analysis. A total of 60 rats were employed, divided equally between two principal investigations: 30 were allocated to the study assessing hypocholesterolaemic effects, and the remaining 30 to the study evaluating hypoglycaemic effects. The minimum sample size per group was determined using Federer's formula: $(t-1)(n-1) \geq 15$, where 't' represents the number of groups (6) and 'n' represents the number of subjects per group. The calculation yielded a minimum requirement of $n \geq 4$ subjects per group. Considering this calculation and adhering to WHO recommendations that suggest a minimum of 5 animals per group, this study assigned 5 rats to each treatment and control group in both investigations.

The equipment utilised included animal housing cages (30x20x10cm), an oven, a blender, a Mettler Toledo analytical balance, 1 cc tuberculin injection syringes, an EasyTouch GCU device with corresponding test strips for blood glucose and cholesterol measurement, measuring cylinders, glass funnels, stirring rods, volumetric flasks, beaker glasses, 1ml gastric tubes (sondes), lancets, tube racks, scissors, knives, filter cloth, and protective gloves. Key materials comprised the garlic extract, standard rat chow, components for the high-fat diet (including 1%

Cholesterol, 5% duck egg yolk, 10% goat fat, 1% coconut oil, BR-1 feed, cholic acid, and wheat flour), Streptozotocin (STZ), 0.01M citrate buffer (pH 4.5), simvastatin, glibenclamide, chloroform for euthanasia, EDTA as an anticoagulant, 70% alcohol for disinfection, and distilled water.

For the investigation into hypocholesterolaemic effects, 30 male Wistar rats (excluding those in the normal control group) were induced to develop hypercholesterolaemia by feeding a high-fat diet at 40 g per day for 14 consecutive days. Following this induction period, the rats were allocated into six groups, each containing five animals (n=5): the Normal Control group (NC) received standard chow; the Negative Control group (NeC) received the high-fat diet only; the Positive Control group (PC) received the high-fat diet supplemented with simvastatin (at a dose of 0.09mg/200g body weight/day, converted from a human dose of 5 mg. Treatment group 1 (T1) received the high-fat diet plus garlic extract at 0.05g/rat/day; Treatment group 2 (T2) received the high-fat diet plus garlic extract at 0.10g/rat/day; and Treatment group 3 (T3) received the high-fat diet plus garlic extract at 0.20g/rat/day. Garlic extract and simvastatin were administered daily between 08:00 and 09:00 WIB via oral gavage for 14 days (from day 15 to day 28 of the experimental period). On the 29th day, subsequent to an 8-hour fasting period, the rats were euthanised using chloroform inhalation. Blood samples were collected via intracardiac puncture, transferred into EDTA-containing tubes, and centrifuged at 4000 rpm for 10 minutes. The resulting plasma supernatant was used to analyse total cholesterol levels.

To investigate the hypoglycaemic effect of garlic extract, thirty male Wistar rats were used in this experiment. Diabetes mellitus was induced in all rats, except the normal control group, by a single intraperitoneal injection of streptozotocin (STZ) at 45 mg/kg body weight, dissolved in 0.01 M citrate buffer (pH 4.5), on the first day of the study. Three days after STZ induction, the animals were randomly assigned into six groups (n=5): the Normal Control group (NC) received standard chow and distilled water without STZ induction for 14 days; the Negative Control group (NeC) was STZ-induced and received standard chow and distilled water for 14 days; the Positive Control group (PC) was STZ-induced and treated with glibenclamide (0.09mg/200 g body weight/day, converted from the human dose of 5 mg via oral gavage for 14 days; Treatment group 1 (T1) was STZ-induced and treated with garlic extract at 100mg/kg body weight/day; Treatment group 2 (T2) received 250mg/kg body weight/day; and Treatment group 3 (T3) received 500mg/kg body weight/day via oral gavage for 14 days. The administration of garlic extract and glibenclamide began three days after STZ induction. Blood glucose levels were measured on the 15th day of treatment after an 8-hour fast.

Blood glucose concentrations were measured using the EasyTouch GCU monitoring system. Although this device is primarily calibrated for human blood, it has been widely used in rodent studies. No specific correction factor was applied; however, all measurements were conducted under identical conditions across groups, ensuring reliable relative comparisons. Capillary blood samples were procured from the tip of the tail, after cleansing the area with 70% alcohol and making a minor incision. A droplet of blood was applied directly onto the glucose test strip inserted into the device, and the resultant glucose level was displayed on the monitor. Plasma total cholesterol levels were quantified using the enzymatic colorimetric CHOD-PAP (Cholesterol Oxidase Para-Aminophenazone) method. Specifically, 10 microlitres (μl) of plasma were combined with 1000μl (1ml) of the CHOD-PAP reagent. This mixture was incubated at room temperature for 20 minutes, after which the absorbance was measured using a spectrophotometer set to a wavelength of 546nm.

The data obtained from these experiments were subjected to statistical analysis. Specifically, a one-way analysis of variance (ANOVA) was conducted, and significant differences between groups were further examined using a post-hoc Least Significant Difference (LSD) test. All statistical procedures were executed using statistic software. Ethical approval for this study was granted by the Health Research Ethics Committee at Universitas Prima Indonesia (No. 085/KEPK/UNPRI/IV/2024).

RESULTS

Table 1. Rat blood cholesterol levels

Treatment	Mean ± SD (mg/dL)
Normal cholesterol	47.80 ± 8.614
Cholesterol control -	150.40 ± 12.341
Cholesterol control +	93.00 ± 7.778
Cholesterol P1	130.60 ± 5.128
Cholesterol P2	121.80 ± 3.194
Cholesterol P3	112.00 ± 4.637

Table 1 presents the findings from a study that quantified blood cholesterol levels across groups of rats undergoing different treatment protocols. The results demonstrate that the three experimental treatments (P1, P2, and P3) reduced cholesterol levels compared with the untreated high-cholesterol control group (designated 'Cholesterol Control -'). Notably, among these experimental groups, treatment P3 produced the lowest mean cholesterol concentration, approaching that of the positive control.

Table 2. Results of cholesterol level testing following garlic extract administration

Group	n	Mean ± SD (mg/dL)		p
		Before	After	
Hypercholesterolaemia + garlic extract 0.5 g	5	150,40 ± 12,341	130,60 ± 5,128	0,011
Hypercholesterolaemia + garlic extract 0.10 g	5	150,40 ± 12,341	121,80 ± 3.194	0,001
Hypercholesterolaemia + garlic extract 0.20 g	5	150,40 ± 12,341	112,0 ± 4,637	0,000

Table 2 presents data from an investigation into the effect of varying doses of garlic extract on cholesterol levels in Wistar rats. Rats administered a daily dose of 0.50 g garlic extract exhibited a reduction in mean cholesterol levels to 130.60±5.128 ($p=0.011$). The 0.10 g/day group showed a more pronounced reduction to 121.80±3.194 ($p=0.001$). The most significant effect occurred in the 0.20g/day group, with cholesterol levels declining to 112.0±4.637 ($p<0.001$). These results indicate a dose-dependent relationship, with higher doses correlating to stronger cholesterol reduction and greater statistical significance.

Table 3. Blood glucose level measurements (mg/dl)

Group	Blood glucose Level (mg/dL)		
	Day 0*	Day 3**	Day 14***
Normal	95.8	94.8	95.0
Glibenclamide (Positive control)	86.0	402.8	235.4
STZ-Induced (Negative control)	92.4	358.4	319.6
Garlic extract (100mg/kg BW)	93.8	368.8	282.8
Garlic extract (250mg/kg BW)	94.4	434.0	271.2
Garlic extract (500mg/kg BW)	81.8	412.4	253.8

* Day 0: Baseline blood glucose levels prior to induction
** Day 3: Blood glucose levels 3 days post-STZ induction
*** Day 14: Blood glucose levels 14 days post-treatment

Table 3 shows the successful induction of hyperglycemia in rats by streptozotocin (STZ) on day 3, as evidenced in all groups except the normal control. By day 14, both the standard drug Glibenclamide and garlic extract reduced elevated blood glucose levels compared with the untreated STZ-induced group, with the higher garlic extract dosage showing a more pronounced effect.

The 'Normal' group served as a healthy control, consistently maintaining stable blood glucose levels of approximately 95.8mg/dl, 94.8mg/dl, and 95.0mg/dl on days 0, 3, and 14, respectively. The 'Glibenclamide' group acted as a positive control; these rats were induced with STZ and subsequently treated with glibenclamide, a well-established anti-diabetic drug. Their initial glucose level was 86.0mg/dl, which increased sharply to 402.8 mg/dl three days post-STZ induction, followed by a significant decrease to 235.4mg/dl by Day 14 after treatment. In contrast, the 'STZ Induction' group served as a negative control. These rats were rendered diabetic with

STZ but received no treatment. Their baseline glucose level was 92.4mg/dl, which rose markedly to 358.4mg/dl on day 3 and remained elevated at 319.6mg/dl on day 14, indicating persistent hyperglycemia without intervention.

Table 4. T-test results of blood glucose levels after 14 days of garlic extract administration in STZ-Induced wistar rats

Treatment	n	Mean ± SD (mg/dL)	p
STZ + Garlic Extract 100 mg/kg BW	5	282.80 ± 25.41	0.856
STZ + Garlic Extract 250 mg/kg BW	5	271.20 ± 32.65	0.146
STZ + Garlic Extract 500 mg/kg BW	5	253.80 ± 29.70	0.323

Table 4 presents the results of a t-test comparing blood glucose levels in STZ-induced rats after 14 days of garlic extract administration. The mean values were 282.80mg/dL, 271.20 mg/dL, and 253.80mg/dL for doses of 100, 250, and 500mg/kg BW, respectively. Although a decreasing trend was observed with higher doses, the differences were not statistically significant ($p>0.05$).

DISCUSSION

Garlic extract exhibits therapeutic potential in addressing hyperglycaemic and hypercholesterolaemic complications due to its diverse phytochemical composition. Research by Tanessa et al. (2023) demonstrated that garlic extract effectively reduced blood glucose and cholesterol levels in Wistar rats. This efficacy is hypothesized to arise from the synergistic action of the extract's active compounds, which target receptors via antagonistic mechanisms, thereby producing a more pronounced therapeutic effect. However, the results of this study indicated no statistically significant difference in blood glucose reduction between groups of rats administered garlic extract at dosages of 100mg/kg body weight (BW), 250mg/kg BW, and 500mg/kg BW. These findings contrast with those of Cyntithia et al. (2024) and Syamsi et al. (2024), who reported significant reductions in blood glucose levels following garlic extract administration. This discrepancy may be attributable to the lower extract dosages employed in the present study. Cyntithia et al. (2024) reported that garlic extract doses of 60mg/kg BW, 500mg/kg BW, and 750mg/kg BW in streptozotocin (STZ)-induced diabetic rats significantly reduced blood glucose levels. Meanwhile, a study by Syamsi et al. (2024) on alloxan-induced diabetic rats showed that the optimal garlic extract dosage for reducing blood glucose was 400mg/kg BW from a tested range of 45mg/kg BW, 200mg/kg BW, and 400mg/kg BW.

In contrast to blood glucose levels, this study revealed a significant difference in cholesterol levels following administration of garlic extract at 0.10g/kg BW and 0.20g/kg BW. Statistical analysis demonstrated a significant effect of garlic extract on reducing blood cholesterol levels at both 0.10g/kg BW and 0.20g/kg BW. These results align with the previous findings. Research indicated that single-clove garlic ethanol extract could lower blood cholesterol levels in mice at dosages of 0.007g/day and 0.014g/day (Dewi et al., 2021). Another study also reported a significant effect of garlic extract on reducing total cholesterol levels in hypercholesterolaemic white rats (Elsherbiny & Fawzy, 2021). This cholesterol-lowering effect is strongly suspected to be linked to garlic's allicin content, which can reduce cholesterol synthesis, inhibit fatty acid synthesis and platelet aggregation, and prevent thrombosis (Jain et al., 2025).

Furthermore, the garlic extract's capacity to reduce total cholesterol levels is associated with its saponin, alkaloid, and flavonoid content. Saponins can form insoluble complexes with cholesterol, thereby inhibiting its intestinal absorption, while flavonoids can neutralize free radicals that protect the pancreas (Tanessa et al., 2023). However, this pancreatic protection is likely preventive rather than corrective, as the present study showed no significant decrease in blood glucose levels. The treatment duration (14 days) may have been insufficient for β-cell regeneration or restoration of insulin-secretion capacity, resulting in a limited hypoglycemic effect despite possible cellular protection.

Saponins are thought to form mixed micelles with cholesterol and bile acids, thereby hindering the intestinal absorption of both, which subsequently stimulates hepatic cholesterol synthesis for conversion into bile acids and excretion via feces (Cao et al., 2024; Oakenfull & Sidhu, 2023; Suandy et al., 2024). Alkaloids in garlic also contribute to cholesterol reduction by inhibiting pancreatic lipase activity, thus increasing fecal fat excretion and reducing hepatic fat uptake for cholesterol conversion (Suandy et al., 2024). It should be noted, however, that this study did not include fecal lipid measurements; therefore, the explanation regarding lipid excretion is based on previously reported mechanisms rather than direct experimental data.

The combination of saponins, flavonoids, and alkaloids in garlic extract has the potential to lower cholesterol levels in diabetic animal models. Research by Najman et al. (2021) also supports this, demonstrating that administering lyophilized garlic to rats fed a high-cholesterol diet for 28 days significantly reduced total cholesterol and LDL concentrations.

Consistent with these findings, Asdaq et al. (2022) investigated the effects of garlic extract and one of its sulfur-containing components, diallyl disulfide (DADS), in Sprague-Dawley rats fed a high-fat diet. The results showed that both garlic extract and DADS significantly lowered triglyceride (TG), total cholesterol (TC), and LDL levels in hyperlipidaemic rats. The atherogenic index also decreased significantly in the garlic extract- and DADS-treated groups, further strengthening the antihyperlipidaemic potential of garlic.

CONCLUSION

Administering garlic extract at 0.10g/kg and 0.20 g/kg body weight significantly reduced blood cholesterol levels in Wistar rats. In contrast, paired t-test results showed no significant effect on blood glucose levels across all experimental groups. These findings warrant further research to determine the optimal, effective, and safe dosage for clinical cholesterol management and evaluate potential adverse effects.

AUTHOR'S DECLARATION

Authors' contributions and responsibilities

VV: Writing original draft, visualization, conceptualization; **EG**: Writing original draft (supporting), review and editing; LC: Supervision (lead), validation, visualization, review, and editing.

Availability of data and materials

All data are available from the authors.

Competing interests

The authors declare no competing interests.

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