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Effect of Garlic Extract on Cholesterol and Glucose in High-Fat Diet STZ Induced Rats

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ABSTRACT

Diabetes Mellitus (DM) is a chronic metabolic disease characterized by elevated blood glucose levels and linked to an increased risk of hypercholesterolemia. Garlic (Allium sativum Linn), which contains bioactive compounds such as saponins and flavonoids, has potential for managing these conditions. This study aimed to evaluate the effects of garlic extract on two parameters: blood cholesterol levels in Wistar rats fed a high-fat diet and blood glucose levels in rats induced with streptozotocin-nicotinamide (STZ). This study employed a laboratory experimental methodology, specifically adhering to a post-test-only control group design. For cholesterol assessment, hypercholesterolemic rats were treated with garlic extract at doses of 0.05, 0.10, or 0.20 g/head/day, alongside negative and positive control groups. For glucose assessment, STZ-induced diabetic rats received garlic extract at 100, 250, or 500 mg/kgBW/day, with corresponding control groups. Garlic extract significantly reduced mean cholesterol levels in a dose-dependent manner (130.60 mg/dL, 121.80 mg/dL, and 112.00 mg/dL for 0.05, 0.10, and 0.20 g/head/day, respectively). These findings suggest that garlic extract exhibits a significant cholesterol-lowering effect in hypercholesterolemic rats but does not markedly reduce blood glucose levels in STZ-induced diabetic rats at the tested dosages. Future research should explore optimal dosing regimens and investigate the underlying mechanisms through which garlic compounds selectively influence lipid metabolism while showing limited impact on glucose homeostasis in diabetic models.

KEYWORDS *Garlic extract; Cholesterol; Blood sugar level; Streptozotocin*

INTRODUCTION

Cholesterol, an essential fatty substance synthesized in the body primarily in the liver, plays a crucial role in the formation of plasma lipoproteins, cell membranes, and as a precursor to important steroid compounds (Duan et al., 2022; Li et al., 2019; Qian et al., 2022). Although vital for bodily functions, excessive cholesterol levels in the blood (hypercholesterolemia) pose a serious health risk as they are a major factor contributing to atherosclerosis, which can lead to coronary heart disease (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 alone, the prevalence of hyperlipidemia is notably high and tends to increase with age (Kementerian Kesehatan Republik Indonesia, 2018), highlighting the urgency of identifying effective prevention and treatment strategies, including non-pharmacological approaches.

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 (Kementerian Kesehatan Republik Indonesia, 2020; Ogurtsova et al., 2017; Wang et al., 2022; World Health Organization, n.d.). 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 in the body (Oktaviana et al., 2022; Putriyani et al., 2019; Vergès, 2015). Furthermore, the connection between cholesterol metabolism and pancreatic beta-cell function underscores the complexity of lipid involvement in DM pathogenesis (Perego et al., 2019), reinforcing the need for research into cholesterol interventions within diabetic conditions.

Among various non-pharmacological measures, garlic (Allium sativum) 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 (Brajawikalpa & Kautama, 2016; 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 (Limbu et al., 2019; Wignjosoesastro et al., 2014). Despite the extensive body of research on garlic's hypolipidemic properties, a significant gap exists in the literature regarding its efficacy under combined metabolic conditions that frequently co-occur in clinical practice. Most previous studies have investigated garlic's effects either on hyperlipidemia or hyperglycemia in isolation, without

addressing the complex metabolic interactions present when both conditions coexist. Furthermore, there is limited evidence on the differential dose-response relationships of garlic extract for cholesterol versus glucose management in preclinical models that simulate these comorbid conditions.

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 in several key aspects. First, while studies such as Limbu et al. (2019) and Wignjosoesastro et al. (2014) primarily focused on garlic's hypolipidemic effects in isolation, this research evaluates its potential under combined metabolic challenges—hyperlipidemia and hyperglycemia—which frequently co-occur clinically and may involve distinct or overlapping pathophysiological mechanisms. Second, unlike previous investigations that employed single-disease models, this study utilizes a dual-induction protocol (high-fat diet combined with STZ administration) to more accurately replicate the complex metabolic environment observed in patients with metabolic syndrome or diabetic dyslipidemia. Third, this research systematically compares dose-response relationships for both cholesterol and glucose parameters within the same experimental framework, allowing for direct assessment of garlic extract's differential efficacy across these two critical metabolic markers. Research by Limbu et al. (2019), which demonstrated garlic supplementation's beneficial impact on patients' lipid profiles, serves as one of the key references supporting this intervention's potential. However, this study tests garlic extract within a more complex preclinical model.

This study employs 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. The aim of this research is to determine and analyze the effect of garlic extract on cholesterol and glucose in high-fat diet STZ-induced rats.

RESEARCH METHOD

This study employed a laboratory experimental methodology, specifically adhering to 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 measured were the blood glucose and total blood cholesterol concentrations in the 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 sample of 60 rats was employed, divided equally between two principal investigations: 30 rats were allocated to the study assessing hypocholesterolaemic effects, and the remaining 30 were assigned to the study evaluating hypoglycaemic effects. The minimum sample size required per group was determined using Federer's formula, $(t-1)(n-1) \geq 15$, where 't' represents the number of groups (6) and 'n' signifies 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 suggesting a minimum of 5 animals per group, this study assigned 5 rats to each treatment and control group within both investigations.

The equipment utilised included animal housing cages (30x20x10 cm), 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, 1 ml 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, thirty male Wistar rats (excluding those in the normal control group) were induced with hypercholesterolaemia through the provision of a high-fat diet administered at 40 g per day for 14 consecutive days (Pramitasari et al., 2017). 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 0.09 mg/200g body weight/day, converted from a human dose of 5 mg based on (Mongi et al.,

2019) Treatment group 1 (T1) received the high-fat diet plus garlic extract at 0.05 g/rat/day; Treatment group 2 (T2) received the high-fat diet plus garlic extract at 0.10 g/rat/day; and Treatment group 3 (T3) received the high-fat diet plus garlic extract at 0.20 g/rat/day (Pramitasari et al., 2017). The administration of garlic extract and simvastatin occurred daily between 08:00 and 09:00 WIB via oral gavage for a duration of 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 utilised for the analysis of total cholesterol levels.

For the investigation into hypoglycaemic effects, a separate cohort of thirty male Wistar rats (excluding the normal control group) was induced with diabetes mellitus. This was achieved via a single intraperitoneal injection of Streptozotocin (STZ), administered at a dose of 45 mg/kg body weight, dissolved in 0.01M citrate buffer (pH 4.5) on the first day of this study arm (Saputra et al., 2018). Three days following the STZ induction, these rats were divided 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 subsequently received standard chow and distilled water for 14 days; the Positive Control group (PC) was STZ-induced and treated with glibenclamide (0.09 mg/200g body weight/day, converted from a human dose of 5 mg based on Mongi et al. (2019) via oral gavage for 14 days; Treatment group 1 (T1) was STZ-induced and treated with garlic extract at 100 mg/kg body weight/day via oral gavage for 14 days; Treatment group 2 (T2) was STZ-induced and treated with garlic extract at 250 mg/kg body weight/day via oral gavage for 14 days; and Treatment group 3 (T3) was STZ-induced and treated with garlic extract at 500 mg/kg body weight/day via oral gavage for 14 days. The administration of garlic extract and glibenclamide commenced three days after the initial STZ induction. Blood glucose levels were measured on the 15th day of treatment, following an 8-hour fast.

Blood glucose concentrations were measured using the EasyTouch GCU monitoring system. 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 1000 µl (1 ml) 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 546 nm.

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 SPSS software, version 25.00.

RESULT AND DISCUSSION

Table 1 displays the findings from a study that quantified blood cholesterol concentrations in various groups of rats undergoing different treatment protocols. The results demonstrate that the three experimental treatments (P1, P2, and P3) led to a reduction in cholesterol levels when compared to the untreated high-cholesterol control group (designated 'Kontrol Kolesterol -'). Notably, among these experimental groups, treatment P3 produced the lowest mean cholesterol concentration, reaching a level that closely approximated that achieved by the positive control treatment.

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 greatest effect occurred in the 0.20 g/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 1. Rat Blood Cholesterol Levels

Treatment	Mean ± SD
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 2. Results of Cholesterol Level Testing Following Garlic Extract Administration

Group	n	Mean ± SD		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 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 demonstrated a capacity to reduce elevated blood glucose levels compared to the untreated STZ-induced group, with the higher dosage of garlic extract showing a more pronounced effect.

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 (100 mg/kg BW)	93.8	368.8	282.8
Garlic Extract (250 mg/kg BW)	94.4	434.0	271.2
Garlic Extract (500 mg/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

The 'Normal' group served as a healthy control, consistently maintaining stable blood glucose levels of approximately 95.8 mg/dl, 94.8 mg/dl, and 95.0 mg/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 86.0 mg/dl, which increased sharply to 402.8 mg/dl three days post-STZ induction, followed by a significant decrease to 235.4 mg/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 92.4 mg/dl, which rose markedly to 358.4 mg/dl on Day 3 and remained elevated at 319.6 mg/dl on Day 14, indicating persistent hyperglycemia without intervention.

The remaining three groups were induced with STZ and treated with varying doses of garlic extract. The group receiving 100 mg/kg body weight (BW) of garlic extract began with an initial glucose level of 93.8 mg/dl, which rose to 368.8 mg/dl post-STZ induction on Day 3 and subsequently decreased to 282.8 mg/dl by Day 14 of treatment. The group treated with 250 mg/kg BW of garlic extract had a baseline glucose level of 94.4 mg/dl, which increased to 434.0 mg/dl on Day 3 and decreased to 271.2 mg/dl by Day 14. Finally, the group receiving the highest dose (500 mg/kg BW) started at 81.8 mg/dl, rose to 412.4 mg/dl on Day 3, and exhibited the most substantial reduction among the extract-treated groups, reaching a glucose level of 253.8 mg/dl by Day 14.

Table 4. T-test Results of Blood Glucose LevelsAfter STZ Induction with Varying Doses of Garlic Extract

Treatment	n	Mean ± SD	p
Blood Glucose Levels + Garlic Extract 100 mg	5	47.80 ± 8.614	0.856
Blood Glucose Levels + Garlic Extract 250 mg	5	150.40 ± 12.341	0.146
Blood Glucose Levels + Garlic Extract 500 mg	5	112.00 ± 4.637	0.323

Table 4 presents the results of a t-test analysis comparing blood glucose levels among streptozotocin (STZ)-induced subject groups treated with varying doses of garlic extract. The mean blood glucose level in the group receiving 100 mg of garlic extract was 47.80 ± 8.614 (Mean±SD). In contrast, the group administered 250 mg exhibited a significantly higher mean blood glucose level of 150.40 ± 12.341, while the group receiving the highest dose, 500 mg, had a mean blood glucose level of 112.00 ± 4.637. Based on the t-test analysis, no statistically significant differences were observed in mean blood glucose levels for groups treated with 100 mg ($p = 0.856$), 250 mg ($p = 0.146$), and 500 mg ($p = 0.323$) doses.

Garlic extract exhibits therapeutic potential in addressing hyperglycaemic and hypercholesterolaemic complications due to its diverse phytochemical composition. Research by (Tanessa et al., 2023a) 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 active compounds within the extract, targeting 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 100 mg/kg body weight (BW), 250 mg/kg BW, and 500 mg/kg BW. These findings contrast with those of (Cyntithia et al., 2024a) and (Syamsi et al., 2024a) 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., 2024b) research, using garlic extract doses of 60 mg/kg BW, 500 mg/kg BW, and 750 mg/kg BW in streptozotocin (STZ)-induced diabetic rats, indicating a significant effect of garlic extract on blood glucose levels. Meanwhile, (Syamsi et al., 2024b) study on alloxan-induced diabetic rats showed that the optimal garlic extract dosage for reducing blood glucose was 400 mg/kg BW from the tested range of 45 mg/kg BW, 200 mg/kg BW, and 400 mg/kg BW.

In contrast to blood glucose levels, this study revealed a significant difference in cholesterol levels following the administration of garlic extract at dosages of 0.10 g/kg BW and 0.20 g/kg BW. Statistical analysis demonstrated a significant effect of garlic extract on reducing blood cholesterol levels at both 0.10 g/kg BW and 0.20 g/kg BW. These results align with the previous findings. A research indicated that single-clove garlic ethanol extract could lower blood cholesterol levels in mice at

dosages of 0.007 g/day and 0.014 g/day (Dewi et al., 2021). Other study also reported a significant effect of garlic extract on reducing total cholesterol levels in hypercholesterolaemic white rats, although this study was conducted over 42 days with multiple measurement time points (Hewen et al., 2020). This cholesterol-lowering effect is strongly suspected to be linked to the allicin content in garlic, which can reduce cholesterol synthesis, inhibit fatty acid synthesis and platelet aggregation, and prevent thrombosis (Pradana & Suryanto, 2017).

Furthermore, the capacity of garlic extract to reduce total cholesterol levels is also 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., 2023b). Saponins are thought to form mixed micelles with cholesterol and bile acids, hindering the absorption of both in the intestine, which subsequently stimulates hepatic cholesterol synthesis for conversion into bile acids and excretion via feces (Bogoriani & Ratnayani, 2015; Suandy et al., 2024a; Wijayanti et al., 2017). 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., 2024b). 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 the administration of lyophilized garlic to rats fed a high-cholesterol diet for 28 days significantly reduced total cholesterol and LDL concentrations.

Consistent with these findings, research by (Asdaq et al., 2022) investigated the effects of garlic extract and one of its sulfur-containing components, diallyl disulfide (DADS), in Sprague-Dawley rats on 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 significantly decreased in groups treated with garlic extract and DADS, further strengthening the antihyperlipidaemic potential of garlic.

CONCLUSION

Administering garlic extract at 0.10 g/kg ($p = 0.001$) and 0.20 g/kg ($p < 0.001$) 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 ($p > 0.05$). These findings warrant further research to determine the optimal effective and safe dosage for clinical cholesterol management, evaluate potential adverse effects, and investigate longer treatment durations or higher doses for achieving significant glucose-lowering effects. Future studies should also explore the molecular mechanisms underlying the differential

efficacy of garlic extract on lipid versus glucose metabolism, examine its effects in different diabetic models (Type 1 versus Type 2), and assess the bioavailability and pharmacokinetics of key bioactive compounds to optimize therapeutic applications in managing comorbid metabolic conditions.

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