Research Article

In vitro and *in vivo* Antidiabetic Effect of the Aqueous Extract of Garlic (*Allium sativum* L.) Compared to Glibenclamide on Biochemical Parameters in Alloxan-induced Diabetic Mice.

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Received: 05-02-2023; **Revised:** 24-04-2023; **Accepted:** 02-05-2023; **Published on:** 15-05-2023.

ABSTRACT

Diabetes mellitus (DM) is the most common chronic disease. Scientific research has turned to traditional herbal remedies as a potential adjunct therapy to maintain better glycemic control with minimal side effects. Garlic has been known since ancient times as an essential regulator either in diet or in medicine. Today the use of garlic is growing and widespread all over the world. This study is carried out to evaluate *in vivo* and *in vitro* antidiabetic effects of garlic extract compared to glibenclamide on biochemical parameters in alloxan induced diabetic mice. In-vitro antidiabetic effect of the A. sativum aqueous extract was conducted using α-amylase assay. Meanwhile *in-vivo* antidiabetic activity was conducted using alloxan induced diabetic mice with an intraperitoneal injection of 160 mg/kg body weight. The diabetic mice were divided into five groups, two of which were given garlic extract orally (200 mg/kg and 400 mg/kg) and a group composed of diabetic mice were given the standard drug, glibenclamide, orally at a dose of 2.5 mg/kg. The control mice (normal and diabetic) were fed normal saline once a day for 28 days. The preventive effect of garlic extract was proved to have the same result as the standard drug, glibenclamide when given at the dose indicated above. This result was shown when experiments dealt with blood glucose, glycosylated hemoglobin levels, total cholesterol, triglycerides, total lipids, alanine aminotransferase (ALAT), and aspartate aminotransferase (ASAT), with significant increases in plasma insulin. In addition, the plant extract exhibited a considerable inhibitory effect on α-amylase activity with IC_{50} value of 680.54± 0.58µg/ml. The present study shows that garlic extract possesses significant, potent anti-diabetic activity in vitro and in vivo, and that this activity is dose related. The garlic extract also recovers metabolic alterations and preserves insulin secretion capacity.

Keywords: *Allium sativum* L., antihyperglycemic activity, α-amylase, glibenclamide, biochemical parameters.

QUICK RESPONSE CODE →

DOI: 10.47583/ijpsrr.2023.v80i01.015

DOI link: http://dx.doi.org/10.47583/ijpsrr.2023.v80i01.015

INTRODUCTION

Diabetes mellitus is a metabolic disturbance that progressively affects the functioning of various body systems, and it is characterized by hyperglycemia[i] caused by an inherited and/or acquired deficiency in the production of insulin by the pancreas, or by the ineffectiveness of the insulin produced[ii]. This insulin deficiency leads to an increase in the concentration of glucose in the blood and these damages many systems in the body, especially blood vessels and nerves. Hyperglycemia caused by a decrease in insulin production is called type 1 diabetes and hyperglycemia caused by insufficient use of insulin is called type 2 diabetes. Of these two types, type 2 diabetes is a major problem today and accounts for almost 95% of the total diabetic patients[iii] and is expected to increase to 439 million by 2030[iv].

Treatment of type 2 diabetes is complicated by several factors: typically, insulin resistance, hyperinsulinemia, impaired insulin secretion, and reduced insulin-mediated glucose uptake and utilization. This type is treated with metformin, glibenclamide, α-glucosidase and α-amylase inhibitors[v].

Since ancient times, plants have been an exemplary source of medicine. Ayurveda and other Indian books mention the use of plants in the treatment of various human ailments. India has about 45.000 species of plants and several thousand of them have been claimed to have medicinal properties[vi]. Commonly practiced treatments for diabetes include oral antidiabetic medications, insulin injections, and management through diet and exercise[vii].

In addition to the drugs currently available for the treatment of diabetes, traditional herbal medicines are also used worldwide for the treatment of diabetes. Inhibition of α-amylase enzymes elaborate in the digestion of carbohydrates can significantly decrease the postprandial increase of blood glucose after a mixed carbohydrate diet. Therefore, this can be a central strategy in the management of postprandial blood glucose level in type 2 diabetic patients and marginal patients[viii]. It can be concluded that natural α-amylase inhibitors from plant

sources offer a good strategy for the control of postprandial hyperglycaemia.

Studies have been conducted on oral antihyperglycemic agents from plants used in traditional medicine, and many plants have been found to have good activity[ix]. The World Health Organization (WHO) has also recommended the evaluation of the efficacy of plants in conditions where we lack modern and safe medicines[x]. For this reason, scientific research has been directed towards natural anti-diabetic products that produce minimal or no side effects[xi].

Among the plants, Garlic (*Allium sativum* L., Liliaceae) is a common spicy flavoring agent used since ancient times. Garlic has been cultivated for its characteristic flavor and medicinal properties. Although garlic has been used for centuries, and even today it is part of popular culture in many cultures, until recently its therapeutic and pharmacological properties have not been scientifically substantiated. In the last decade, some of the protective effects of garlic have been well established by epidemiological studies and animal experiments. are studying commercially available garlic preparations in the form of garlic oil, garlic powder, and pills that are widely used for certain therapeutic purposes, including lowering blood pressure and improving lipid profile[xii]. Garlic has been widely attributed to reducing risk factors for cardiovascular disease and cancer[xiii], stimulating immune function[xiv], protecting against liver disease[xv], and having an antioxidant effect[xvi]. In addition, garlic contains at least 33 sulfur compounds, several enzymes, 17 amino acids and minerals such as selenium[xvii]. It contains a higher concentration of sulfur compounds than any other Allium species. Sulfur compounds are responsible for both the pungent smell of garlic and many of its medicinal effects[xviii].

The objectives of the present study are threefold; to examine the influence of oral administration of garlic extract compared to the drug glibenclamide on biochemical parameters; to probe the activities of certain enzymes, alanine aminotransferase (ALAT), and aspartate aminotransferase (ASAT) in plasma; and to scrutinize the level of α-amylase inhibitory effects of this plant extract.

MATERIALS AND METHODS

Preparation of garlic extract

Fresh garlic (*Allium sativum* Linn) bulbs were harvest from Meknes Agouray Morocco in June 2018, peeled, washed, and chopped into small pieces.

The garlic juice was prepared by adding 100 g of garlic with 250 ml of distilled water and crushed in a mixing machine. The resultant slurry was squeezed and filtered through a fine cloth and the filtrate was quickly frozen at -10 °C until used[xix].

Animals

Male albino (Wistar mice) used in the research project were procured from Pasteur Institute, Casablanca, Morocco. The animals were separated into 5 groups (five mice per group) and housed in clean cages with temperature 22-24 °C, 12 hours light and 12 hours dark cycle, and relative air humidity 40–60%. Mice had free access to food and water. All protocols were carried out in accordance with the International Standards and Ethical Guidelines on Animal Welfare.

IN-VIVO ANTIDIABETIC ACTIVITY

Induction of diabetes

After one week of adaptation period with a nutritionally complete diet, mice were fasted overnight and injected intraperitoneally with a freshly prepared alloxan monohydrate solution at a dose of 160 mg/kg body weight[xx]. The diagnosis of diabetes was based on hyperglycemia (blood glucose levels above 200 mg/dl) on the 3rd day after the alloxan injection.

Experimental protocol

In this experiment, thirty-five male mice were used. The mice were randomly divided into five groups of 5 mice each. Garlic extract and a standard drug, glibenclamide, were fed by gastric gavage every day at a fixed time (10:00 AM) for four consecutive weeks as follows:

***Group N-C*:** Normal Control mice were administrated 1 ml of normal saline.

***Group DT-200*:** Diabetic Treated mice were administrated garlic extract (200 mg/kg body weight).

Group DT-400: Diabetic Treated mice were administrated garlic extract (400 mg/kg body weight).

***Group D-C*:** Diabetic Control mice were administrated 1 ml of normal saline.

Group DT-Glb: Diabetic Treated mice were administrated a standard drug, glibenclamide (2.5 mg/kg body weight).

The experimental period for each group of mice was 4 weeks. Body weights of animals by balance (accuracy: 0.01 g) per week were measured and at the end of the experiment, all animals were sacrificed.

Blood samples

At the end of the experimental period, mice were fasted for 12 h with free access to drinking water, and then sacrificed by the retro-orbital plexus puncture method using capillary tubes. Blood samples were centrifuged at 3000 rpm for 10 min at 4 °C, and the resulting plasma was stored until analysis.

Determination of biochemical parameters

Serum glucose, triglycerides, HbA1c, cholesterol, aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) levels were determined using an Abbott vitros 250 model auto-analyzer according to manufacturer instructions.

IN VITRO ANTIDIABETIC ACTIVITY

Amylase inhibitory effect

The different concentrations of aqueous extract of *allium sativum* were reacted with α-amylase enzyme and starch solution to study the inhibition potential of α-amylase according to the protocol described previously[xxi] with small modifications.

A mixture of 300µL of sample and 300µL of 0.02 M sodium phosphate buffer (pH=6.9) containing the enzyme α-amylase (240U/mL) was incubated at 37°C for 20 min. Then, 300 µL of 1% starch solution in 0.02 M sodium phosphate buffer was added to the reaction mixture.

The reaction mixture was incubated at 37 °C for 20 minutes. Then 1 mL of dinitrosalicylic acid (DNS) was added, and the reaction mixture was incubated at 37 °C for 20 minutes. Afterwards, the reaction mixture was incubated in a boiling water bath for 15 minutes, diluted by adding 2 mL of distilled water and the absorbance was measured at 560 nm in the spectrophotometer. Acarbose was used as a positive control.

The results were expressed as percent inhibition and calculated using the following equation:

$$\text{Inhibition } (\%) = \frac{(A_c - A_{cb}) - (A_s - A_{sb})}{A_c - A_{cb}} \times 100$$

Where A_c is the absorbance of the control (enzyme and buffer); A_{cb} is the absorbance of the control, blank (buffer without enzyme);

A_s is the absorbance of the sample (enzyme and inhibitor); and A_{sb} is the absorbance of the sample blank (inhibitor without enzyme). IC_{50} values (concentration of inhibitor required to inhibit 50% of the enzyme activity) were evaluated.

Glucose Tolerance Test (OGTT)

The Glucose Tolerance Test (OGTT) investigates the effect of different doses of extract of garlic on postprandial blood glucose levels. This extract and the standard hypoglycemic drug (glibenclamide) are suspended in a 0.9% NaCl saline solution and then administered orally to normal mice forty-five minutes before gastric gavage of a 5g/kg BW glucose solution.

Group 1 received glucose solution, the second group received a single dose of glibenclamide (20 mg/kg) before forty-five minutes of glucose administration, and the third and fourth groups received single doses of 200 mg/kg and 400 mg/kg of garlic extract respectively forty-five minutes before glucose administration. Blood glucose levels were monitored for 30, 60, 90 and 120 minutes after administration of the glucose solution.

Statistical analysis

Data were expressed as means±SEM (n=5) and compared by using GRAPHPAD Prism software (version 8) by one-way ANOVA followed by Student's t-test to compare means between the different treated groups. Differences were considered statistically significant at $p \leq 0.05$ in all tests.

RESULTS

IN VIVO ANTIDIABETIC ACTIVITY

Effect of administration of garlic extract on serum glucose level.

In the present study, after four weeks of treatment, a very significant (p<0.001) progressive decrease in serum blood glucose was detected in the group receiving 200 mg/kg, 400 mg/kg body weight of garlic extract (Fig.1).

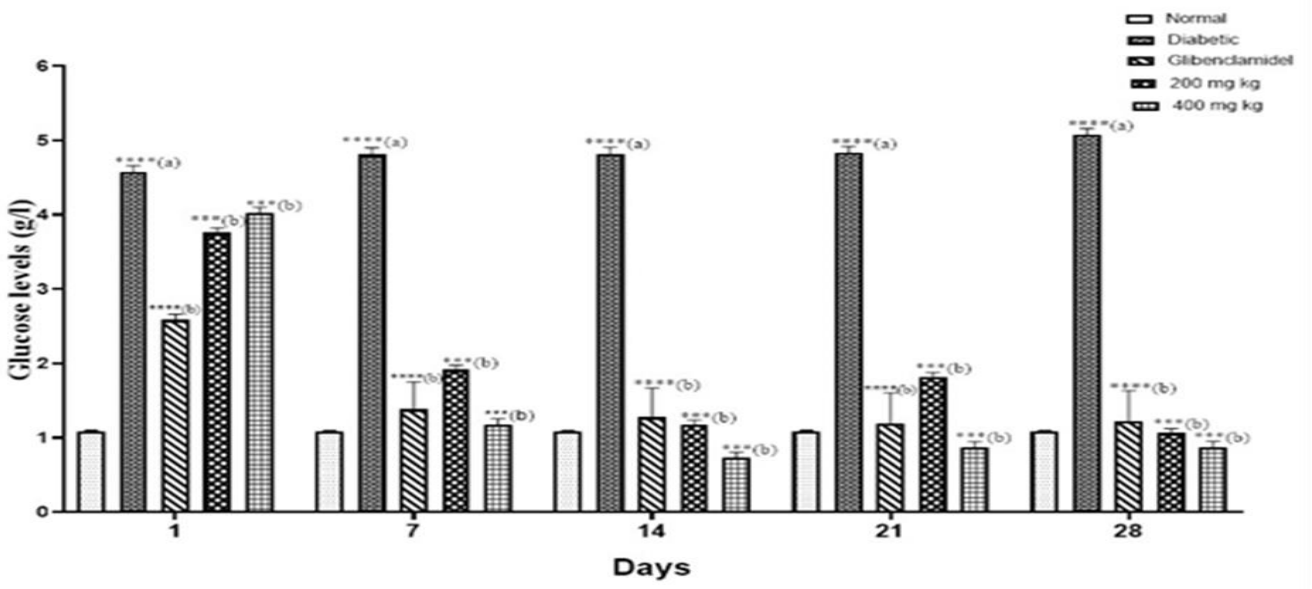

Figure 1: Effect of oral administration of garlic extract on serum glucose concentration for normal, diabetic, Glibenclamid treated and garlic extract treated male mice. Each column represents mean ± S.E.M. for five mice. One-way ANOVA followed by Student's t-test. ** p≤0.01, *** p≤ 0.001, **** p ≤ 0.0001 (a): compared to (N-C) (b): compared to (D-C).

Effect of administration of garlic extract on body weight gain, blood glycosylated hemoglobin and plasma insulin levels

Induction of diabetes by alloxane after four weeks of treatment in diabetic mice was associated with significant (p<0.05) decrease in body weight, increase in HbA1c and sharp decrease in insulin concentration compared to normal groups (Table 1).

Table 1. Effect of administration of garlic extract on body weight gain, blood glycosylated hemoglobin and plasma insulin levels. Each column represents mean ± S.E.M. for five mice. One-way ANOVA followed by Student's t-test.

Groups	Body weight gain (g)	HbA1c (٪)	Plasma insulin (µU/ml)
N-C	33.78± 5.18	2.24 ± 0.03	14.39 ± 3.60
D-C	26.89 $^{\#\#}$ ± 8.84	3.71 $^{\#\#\#\#}$ ± 0.06	4.64 $^{\#\#\#\#}$± 0.09
DT-200	34.74 ** ± 7.37	2.51 ****± 0.04	10.22 ****± 0.05
DT-400	35.07 **± 7.60	2.12 ****± 0.05	12.62 ****± 1.31
DT-Glb	35.44 ** ± 9.79	2.14 ****± 0.05	14.09 ****± 3.69

** p≤0.01, *** p≤ 0.001, **** p≤ 0.0001 (*): compared to (N-C), ($^{\#}$): compared to (D-C).

Diabetic mice treated orally with 200 and 400 mg/kg of garlic extract maintained a good phenotype with an increase in body weight, a decrease in HbA1c and a significant increase in plasma insulin, with a slight dose-dependent improvement.The results of 400 mg/kg BW treatment are similar to those obtained with glibenclamide treatment.

Glucose Tolerance Test (OGTT)

In Fig .2, the obtained results showed that after 30 minutes of glucose administration (5 g/kg), a strong increase in blood glucose levels in normal mice was observed, and then blood glucose levels drop up to 120 minutes.

In mice treated with garlic extract at a dose of 200 mg/kg and 400 mg/kg, the blood glucose level was slightly elevated after 30 minutes of glucose administration. From 60 min, the blood glucose level of mice treated with either 200 mg/kg or 400 mg/kg dose decreased gradually until 120 min.

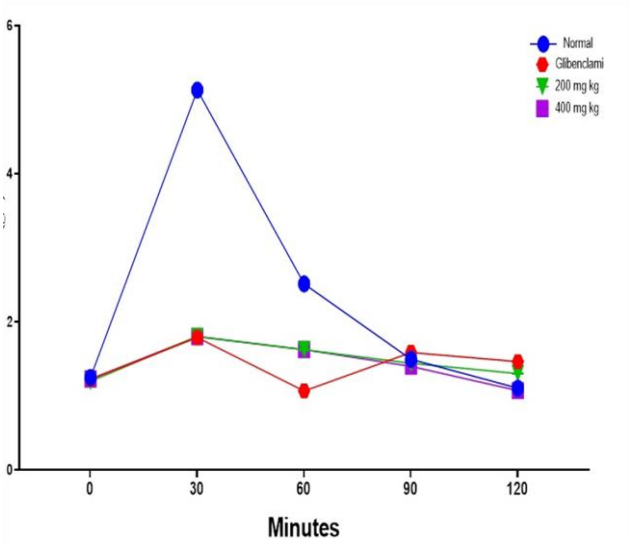

Figure 1: The evolution of blood glucose levels in mice as a function of the dose of garlic extract compared to glibenclamid.

For mice treated with Glibenclamide, the blood glucose level increased slightly after 30 minutes of glucose administration, and it starts to decrease from 60 minutes to reach its minimum value after 120 minutes. The results obtained (Fig. 2), show that garlic extract at the doses of 200 mg/kg and 400 mg/kg have a considerable effect on the reduction of postprandial glycaemia during the first 120 minutes following the administration of glucose dose (5g/kg).

Effect of administration of treatment by garlic extract on serum biochemical parameters

The biochemical parameters such as triglycerides and cholesterol in experimental groups of mice show a significant increase in the levels of triglycerides and cholesterol in alloxan-induced diabetic mice (D-C) when compared with normal control mice (N-C).

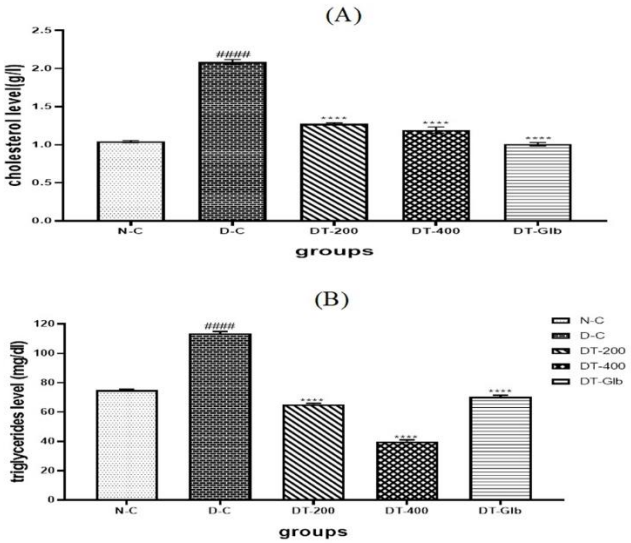

Figure 2: Effect of oral administration of garlic juice on serum cholesterol (A), and triglycerides (B) concentrations. Each column represents mean ± S.E.M. for five mice. N-C: Normal Control D-C: Diabetic Control DT-200: Diabetic Treated mice with garlic extrac extract at 200 mg/kg, DT-400: Diabetic treated mice with garlic extract at 400 mg/kg.

DT-Glb Diabetic Treated mice with glibenclamide at 2.5 mg/kg, Results are expressed as mean±SEM (n=5). One-way ANOVA followed by Student's t-test. * p≤0.05, ** p≤0. 01, *** p≤ 0.001, **** p≤ 0.0001 (#): compared to (N-C) (*): compared to (D-C).

Figure. 3 shows that administration of Glibenclamide at a dose of 2.5 mg/kg and *Allium sativum* extract to diabetic mice for 28 days resulted in the restoration of biochemical parameters levels towards near normalcy in a dose-dependent fashion.

Effect of garlic treatment on pathophysiological enzymes activities after four weeks of treatment in experimental groups

The effect of administration of aqueous garlic extract on serum ALT and AST activities are presented in Fig. 4. In the D-C group the serum ALT and AST activities were significantly (p<0.05) increased compared to the N-C group. In the DT-Glb, DT-200 and DT-400 groups serum ALT activity was significantly reduced (p<0.05) compared to the D-C group, while in the N-C groups it showed a significant increase (p<0.05) compared to the N-C groups. Similarly, in the DT-400 and DT-Glb groups, serum ALT activity was significantly decreased (p < 0.05) compared to the DT-200 groups.

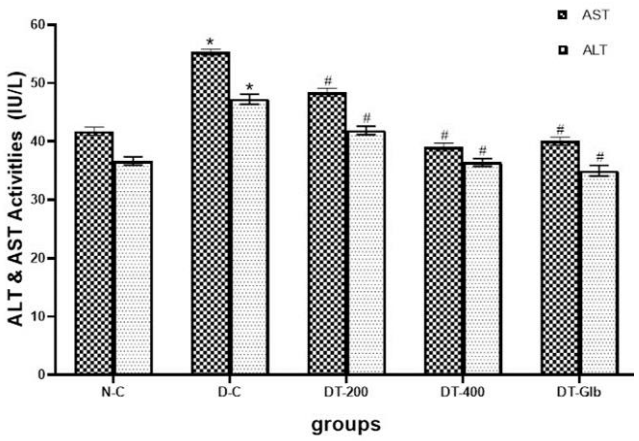

Figure 3: Effect of oral administration of garlic juice on the serum activities of ALT and AST. Each column represents mean ± S.E.M. for five mice. *Significant difference (p<0.05) with N groups in both charts. Significant difference (p<0.05) with D groups in both charts. Significant difference (p<0.05) with D groups in both charts. (N-C: Normal Control; D-C: Diabetic Control; DT-200: Diabetic Treated mice with garlic extract at 200 mg/kg; DT-400: Diabetic Treated mice with garlic extract at 400 mg/kg; DT-Glb Diabetic Treated mice with glibenclamide at 2.5 mg/kg).

In the DT-200, DT-400 and DT-Glb groups, serum AST activity was significantly reduced (p<0.05) compared to the D-C groups. Serum AST activity in the DT-400 groups was not significantly different from that in the DT-Glb groups, but in compared to the DT-200 groups, it showed a significant decrease (p<0.05). In addition, the DT-200 groups showed a significant increase (p<0.05) compared to the N-C groups.

In vitro antidiabetic activity

The α-amylase inhibitory potency of the extract and acarbose molecule are presented in Table 2.

Table 2. The effect of *A. sativum* aqueous extract on α-amylase enzyme.

Studied agents	Aqueous extract of *A. sativum*	Acarbose
IC_{50} µg/mL of α-amylase	680.54± 0.58	243.28± 3.49

The inhibitory effect of the extract on α-amylase was highly effective with IC50 of 680.54±0.58 µg/mL, and significantly more potent than the acarbose molecule (p<0.05) with IC50 of 243.28± 3.49 for the enzyme α-amylase.

DISCUSSION

Aside from its general use as a condiment, garlic (*Allium sativum*) is known for its pharmacological and nutritional properties[xxii]. Garlic has long been believed to possess a hypoglycemic effect[xxiii].

Alloxan has been widely used for inducing type I diabetes in a variety of animals by affecting degeneration and necrosis of pancreatic β-cells[xxiv]. The present results showed that alloxan-induction results in a decrease in body weight of diabetic mice, which is possible due to catabolism of fats and protein. Daily oral administration of *Allium sativum* extract to diabetic mice for 28 days at doses of 200 and 400 mg/kg significantly improves body weight in diabetic mice.

The present data showed that the garlic extract significantly decreased serum glucose in treated diabetic mice in a dose-dependent manner as compared with diabetic control mice. Moreover, the experimental effects of the extract on weight damage compared favorably with Glibenclamide.

The potency of glucose-lowering effects of garlic was supported experimentally in a number of studies. The first report considering the beneficial effects of allicin, a biologically active sulfoxide from garlic in diabetic mice was published as far back as by[xxv]. Further studies have demonstrated that sulfur-containing amino acids from garlic possess a direct hypoglycemic action, potentiate the effects of insulin on the body, and increase the hepatic glycogen synthesis in diabetic mice and rabbits[xxvi xxvii xxviii] One more mechanism of hypoglycemic is to sparing insulin from –SH inactivation by reacting with endogenous thiol. Inactivation of insulin by sulfhydryl group is a common phenomenon. Garlic can effectively combine with compounds like cysteine glutathione, and serum albumins and enhance serum insulin[xxix].

S-allyl cysteine sulfoxide (SAC) composed of garlic is a precursor of garlic oil that has a direct stimulating effect on insulin secretion from the pancreas which has been demonstrated[xxx xxxi]. It is also possible that garlic juice directly stimulates insulin secretion, as improving effects of garlic juice on fasting blood glucose were found in the present study.

High blood glucose levels react non-enzymatically with hemoglobin to form glycosylated hemoglobin (HbA1C). Therefore, the rate of glycosylation is proportional to the blood glucose concentration[xxxii]. The estimation of glycosylated hemoglobin is a well-accepted biochemical parameter useful in the diagnosis and management of the disease. Increased glycated hemoglobin levels are associated with loss of cell function β and have been implicated in the complications of diabetes mellitus[xxxiii]. Results showed that oral administration of *Allium sativum* tends to decrease glycosylated hemoglobin levels by improving blood glucose homeostasis.

Diabetes mellitus is considered one of the most powerful factors significantly increasing the risk of cardiovascular disease of atherosclerotic origin[xxxiv]. Increased serum lipid levels in diabetes are a risk factor for coronary heart disease. Furthermore, lipids play an essential role in the pathogenesis of diabetes mellitus.

In our study, increased triglycerides and cholesterol levels were observed in diabetic mice. In addition, the lipid content of cell membranes appears to be disrupted by diabetes as evidenced by increases in non-enzymatic glycation, lipid peroxidation and the cholesterol/phospholipid ratio[xxxv]. The high level of non-esterified fatty acids in plasma has been proposed as a major cause of insulin resistance and may reduce insulin secretion in diabetes[xxxvi]. Therefore, the improved in vivo hypoglycemic response to insulin deficiency in diabetic mice treated with garlic juice may be explained by the decrease in blood lipid concentrations.

In normal cases, insulin activates lipoprotein lipase, hydrolyses triglycerides[xxxvii] and increases the uptake of fatty acids into the adipose tissue. In insulin deficiency, lipolysis is not inhibited, and we have increased lipolysis, which ultimately leads to hyperlipidaemia. In insulin-deficient diabetes, the serum concentration of free fatty acids is elevated due to the influx of free fatty acids from fat deposits, where the balance of the free fatty acid-lipolysis cycle of triglycerides is shifted in favour of lipolysis.

Oral administration of garlic extracts in diabetic mice significantly reduced serum triglycerides and cholesterol compared to a standard anti-diabetic drug (Glibenclamide) administered at a dose of 2.5 mg/kg. The results are consistent with other work that has shown that administration of fresh garlic improved the lipid profile, including reduction in serum cholesterol levels[xxxviii]. With respect to the cholesterol-lowering property of garlic, it has been suggested that some garlic constituents may act as inhibitors of certain enzymes such as hydroxy methyl glutaryl-CoA reductase, which is involved in cholesterol synthesis[xxxix]. Consistent with this idea, in vivo treatment of garlic extract has been shown to reduce lipid peroxidation products[xl].

Among the objectives of this study is to examine the effect of garlic extract administration on the serum activities of aminotransferases (AST, ALT) which are used in the evaluation of hepatic disorders. An increase in the activity of these enzymes reflects active hepatic damage/inflammatory hepatocellular disorders[xli]. According to these results, the increase in serum AST, ALT, activities may be mainly due to the leakage of these enzymes from the hepatic cytosol into the bloodstream[xlii], which gives an indication of the hepatotoxic effect of alloxan.

In addition, the increased catabolism of proteins accompanying gluconeogenesis and urea formation observed in the diabetic state could be responsible for the elevation of these tissue transaminases. The increase in alanine transaminase activity is due to hepatocellular damage and is generally accompanied by an increase in aspartate transaminase[xliii] [xliv] [xlv] demonstrated that the administration of several plant extracts in the context of diabetes could restore changes in the activities of serum enzymes such as transaminases: AST and ALT. In the present study, daily oral administration of glibenclamide at a dose of 2.5 mg/kg and an Allium *sativum* extract to diabetic mice for 28 days at doses of 200 and 400 mg/kg resulted in a reduction in ALT and AST activity in plasma compared to the mean values of the diabetic group and may therefore alleviate liver damage caused by alloxan-induced diabetes. These results are consistent with those obtained by[xlvi] in rats.[xlvii] also demonstrated liver protection by S-alkyl cysteines and alliin on hepatocytes in vitro. Therefore, garlic can also be used for liver protection[xlviii].

The ability of aqueous extract of *A. sativum* to inhibit α-amylase was tested to screen for hypoglycaemic effects. This enzyme suggested that the compounds responsible for the anti-diabetic activity of garlic are extractable in water, which supports the traditional use of an aqueous extract of *A. sativum* in the treatment of diabetes.

CONCLUSIONS

In conclusion, the results of the present study showed that the aqueous extract of A. sativum significantly lowered blood glucose levels in diabetic mice. This extract was able to adjust some serum biochemical parameters, especially the levels of cholesterol, triglycerides, ASAT and ALAT, which were decreased. The studied extract also showed a promising inhibitory effect on α-amylase. Therefore, this study suggests that the aqueous extract of A. sativum could be considered as an alternative agent for the treatment of DM. Furthermore, garlic should be considered as a beneficial candidate for future experimental studies on diabetes mellitus.

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Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.