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Therapeutic Benefits of Garlic against Alloxan-Induced Diabetic in Rats

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Abstract
Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. The present study was carried out to investigate the effects of garlic (Allium sativum Linn) juice on biochemical parameters, enzyme activities and lipid peroxidation in alloxan-induced diabetic rats. Alloxan was administered as a single dose (150mg/kgBW) to induce diabetes. A dose of 1ml of garlic juice/100g body weight (equivalent to 0.4 g/100g BW) was orally administered daily to alloxan-diabetic rats for four weeks. The levels of glucose, urea, creatinine and bilirubin were significantly ($p < 0.05$) increased in plasma of alloxan-diabetic rats compared to the control group. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and alkaline and acid phosphatases (AlP, AcP) activities were significantly ($p < 0.05$) increased in plasma and testes of alloxan-diabetic rats, while these activities were decreased in liver compared with the control group. Brain LDH was significantly ($p < 0.05$) increased. The concentration of thiobarbituric acid reactive substances and the activity of glutathione S-transferase in plasma, liver, testes, brain, and kidney were increased in alloxan diabetic rats. Treatment of the diabetic rats with repeated doses of garlic juice could restore the changes of the above parameters to their normal levels. The current results showed that garlic juice exerted antioxidant and antihyperglycemic effects and consequently may alleviate liver and renal damage caused by alloxan-induced diabetes.
Keywords: *Rats; Alloxan; Garlic; Biochemical parameters; Enzymes; Lipid peroxidation.*

1. Introduction

Diabetes mellitus is an endocrine disorder that is characterized by hyperglycemia [1]. Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger. If left untreated, diabetes can cause many complications. Acute complications can include diabetic ketoacidosis, nonketotic hyperosmolar coma, or death. Serious long-term complications include heart disease, stroke, chronic kidney failure, foot ulcers, and damage to the eyes [2]. A number of investigations, of oral antihyperglycemic agents from plants used in traditional medicine, have been conducted and many of the plants were found with good activity [3]. The World Health Organization (WHO) has also recommended the evaluation of the plants' effectiveness in conditions where we lack safe, modern drugs [4]. This has leaded an increasing demand of research on natural antidiabetic products which produces minimal or no side effects. 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 nowadays is part of popular in many cultures, but until recently there has been little scientific support of its therapeutics and pharmacological properties. In the past decade, some protective effects of garlic have been well established by epidemiological studies and animal experiments. Elkayam et al (2003) investigate the commercially available garlic preparations in the form of garlic oil, garlic powder and pills are widely used for certain therapeutic purposes, including lowering blood pressure and improving lipid profile [5]. Garlic has been largely attributed to the reduction of risk factors for cardiovascular diseases and cancer [6], stimulation of immune function [7], hepatoprot-ection [8] and antioxidant effect [9]. In addition, garlic contains at least 33 sulfur compounds, several enzymes, 17 amino acids, and minerals such as selenium [10]. It contains a higher concentration of sulfur compounds than any other Allium species. The sulfur compounds are responsible both for garlic's pungent odor and many of its medicinal effects. In the 1973s, Jain et al and Jain and Vyas showed that the ingestion of garlic juice resulted in better utilization of glucose [11,12]. Augusti and Sheela, and Sheela and Augusti, consistently showed that S-allyl cysteine sulfuxide (alliin), a sulfur containing amino acid in garlic has a potential to reduce diabetic condition in rat almost to the same extent as did glibenclamide and insulin [13,14]. Therefore, the purpose of the current study was to examine the influence of oral administration of garlic on the levels of free radicals, biochemical parameters, and the activities of some enzymes in plasma and different tissues of alloxan-induced diabetic rats.

2. Materials and methods

2.1.Preparation of garlic juice

Fresh garlic (*Allium sativum* Linn) bulbs were purchased from the local market in New Domiatta, Egypt., peeled, washed, and chopped into small pieces. About 250 ml of distilled water per 100g of garlic were added 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 °Cuntil used. Alloxan (hydrate) LR, $C_4H_2N_2O_4$. H_2O, was purchased from Sigma-Aldrich Chemical (St. Louis, MO, USA) by Gamma trade Company (Cairo). Alloxan was dissolved in saline solution (0.9% sodium chloride, pH7). The dose of alloxan used was 150mg/kg BW as a single dose [1].

2.2. Animals and treatments

Twenty-four adult male albino rats (100–160 g) were obtained from the Animal House of the Faculty of Medicine (Domiatta), University of Al-Azhar, Egypt. The animals were housed in standard cages under 12-hour light/dark cycle maintained on a standard feed and water ad libitum. Rats were fed pellets consisted of 30% berseem hay, 25% yellow corn, 26.2% wheat bran, 14% soybean meal, 3% molasses, 1% CaCl2, 0.4% NaCl, 0.3% mixture of minerals and vitamins (0.01g/kg diet of vitamin E), and 0.1% methionine. The chemical analysis of the pellets [15] showed that they contained 17.5% crude protein, 14.0% crude fiber, 2.7%crude fat and 2200 Kcal./kg diet. After one weeks of acclimation, animals were divided into two groups. The first group (8 rats) was used as control and received double distilled water as vehicle. The second group (16 rats) was injected subcutaneously (s.c.) with a single dose of alloxan (150mg/kg BW), and divided into two subgroups (8 rats per each) after stabilization of diabetes forone week (animals having fasting blood glucose concentration ≥200 mg/dL (11.1 mmol/L) were considered diabetic and used for the investigation). The first subgroup was kept as diabetic. The second subgroup received 1ml garlic juice/100g BW/day by gavage for four weeks. Prior to administration of alloxan, the animals were fasted for 12h with free access drinking water. At the end of the experimental period, animals were sacrificed. Serum was obtained for further biochemical analysis.

Enzyme Assessments

At the end of the experimental period, rats were fasted for 12h, and then sacrificed by cervical decapitation and fasting blood samples were collected from the sacrificed animals in tubes with heparin. Plasma samples were obtained by centrifugation at 860 g for 20 min and stored at –20^{o}C till measurements. Also, liver, testes, kidney, and brain were immediately removed and washed using chilled saline solution. Tissues were minced and homogenized (10% w/v), separately, in ice cold 1.15% KCl–0.01M sodium, potassium phosphate buffer (pH 7.4) in a Potter–Elvehjem type homogenizer. The homogenate was centrifuged at 10,000g for 20min at 4^{o}C, and the resultant supernatant was used for different enzyme assays. Plasma, liver and testes alanine aminotransferase (ALT; EC 2.6.1.2) and aspartate aminotransferase (AST; EC 2.6.1.1) activities were assayed by the method of Reitman and Frankel (1957) [16]. Plasma, brain, liver and testes lactate dehydrogenase (LDH, EC1.1.1.27) activity was determined by the method of Cabaud and Wroblewski (1958) [17]. Alkaline phosphatase (AlP; EC 3.1.3.1) activity was measured at 405nm by the formation of paranitrophenol from para nitrophenyl phosphate as a substrate [18]. Acid phosphatase (AcP; EC 3.1.3.2) activity was measured using the method of Moss (1984) [19]. Plasma, liver, brain, testes and kidney glutathione S-transferase (GST; EC 2.5.1.18) activity was determined according to Habig et al. (1974) [20], using para nitrobenzyl chloride as a substrate. Thiobarbituric acid-reactive substances (TBARS) were measured in plasma, liver, brain, testes and kidney by using the method of Tappel and Zalkin (1959) [21]. Protein concentration in liver, testes, brain and kidney supernatants was assayed by the method of Lowry et al. (1951) [22] using bovine serum albumin as a standard.

Biochemical Assays

Stored plasma samples were analyzed for glucose level by using the method of Trinder (1969) [23]. Plasma urea, creatinine and total bilirubin concentrations were determined by the methods of Patton and Crouch (1977); Henry et al. (1974) and Pearlman and Lee (1974) [24, 25,26] respectively.

Statistical analysis

Data were analyzed as a completely randomized design using the General Linear Model procedure. Means were statistically compared using least significant difference (LSD) test at 0.05 significant level [27,28].

3. Results

The effects of oral administration of garlic juice on plasma glucose, urea, creatinine and total bilirubin are summarized in Table 1. The experimentally induced-diabetes increased ($p < 0.05$) the level of plasma glucose by 199% of control level (Table 1). However, treatment of alloxan-diabetic rats with the juice of garlic reduced their plasma glucose levels by 68% compared with the diabetic group.

Table 1: Plasma bilirubin, creatinine, urea and glucose levels in control, diabetic, and diabetic treated male rats with garlic (G) (Means ± SE)

Parameters (mg/dl)	Control	Diabetic	Diabetic + G
Bilirubin	0.83 ± 0.070^{b}	1.24 ± 0.083^{a}	0.96 ± 0.098^{b}
Creatinine	0.75 ± 0.018^{b}	0.93 ± 0.14^{a}	0.68± 0.023^{ab}
Urea	32± 2.50^{b}	49 ± 2.67^{a}	41± 1.58^{ab}
Glucose	94± 5.64^{b}	289± 7.20^{a}	90± 4.86^{b}

Values are the means of eight rats.
^{ab}Within rows, between control and treated animals, means with different superscript letters differ significantly ($P < 0.05$).

In alloxan-diabetic rats the activities of plasma AST, ALT, LDH, AlP and AcP were significantly ($p < 0.05$) increased by 49, 60, 37, 51 and 58%, respectively, relative to their normal levels (Table 2).

Table 2: Assay of plasma enzyme activities and thiobarbituric acid-reactive substances (TBARS) in control, diabetic, and diabetic treated male rats with garlic (G) (Means ± SE)

Parameter	Control	Diabetic	Diabetic + G
AST (U/dl)	41± 1.02[c]	65± 2.34[a]	50± 0.94[b]
ALT (U/dl)	50± 2.07[c]	84± 4.37[a]	66± 2.78[b]
LDH (U/l)	1174 ± 92[b]	1499± 54[a]	1317± 43[a]
AlP (U/l)	46± 3.03[c]	75± 3.40[a]	64± 2.67[ab]
AcP (U/l)	12.3 ± 0.78[c]	19.8 ± 0.68[a]	14.1 ± 0.28[b]
GST (lmol/h)	0.64 ± 0.015[a]	0.62 ± 0.009[a]	0.72 ± 0.011[a]
TBARS (nmol/ml)	0.68± 0.06[b]	0.86± 0.05[a]	0.79± 0.06[a]

Values are the means of eight rats.
[abc]Within rows, between control and treated animals, means with different superscript letters differ significantly (P < 0.05).

In contrast, the activities of AST, ALT, LDH, AlP and AcP were significantly ($p < 0.05$) decreased in the liver tissue of alloxan-diabetic rats (Table 3) by 47%, 38%, 41%, 35% and 36%, respectively and increased in testes by 38%, 32%, 35%, 31% and 33%, respectively compared to the control values (Table 4).

Table 3: Assay of liver enzyme activities and thiobarbituric acid-reactive substances (TBARS) in control, diabetic, and diabetic treated male rats with garlic (G) (Means ± SE)

Parameter	Control	Diabetic	Diabetic + G
AST*	117 ± 3.35[a]	77 ± 3.24[c]	97± 2.36[b]
ALT*	113 ± 5.74[a]	79± 3.38[c]	103± 7.33[ab]
LDH**	2236 ± 176[a]	1316 ± 61[c]	1725 ± 150[b]
AlP*	348 ± 23.0[a]	222± 7.6[c]	285 ± 18.1[bc]
AcP*	17.1 ± 1.20[a]	12.3 ± 0.71[c]	13.3 ± 0.35[bc]
GST***	0.90 ± 0.052[c]	1.55 ± 0.059[a]	1.27 ± 0.008[b]
TBARS****	25.4 ± 1.14[b]	31.6 ± 1.10[a]	27.3 ± 1.07[b]

Values are the means of eight rats.
[abc]Within rows, between control and treated animals, means with different superscript letters differ significantly (P < 0.05).
* IU/mg: international unit, the amount of the enzyme that under defined assay conditions will catalyze 1 mole of substrate per minute, per mg protein.
** IU/g: international unit, the amount of the enzyme that under defined assay conditions will catalyze 1 mole of substrate per minute, per gram tissue.
*** GST specific activity: lmol/h/mg protein.
**** TBARS is expressed as nmol/g tissue.

Also, brain LDH activity was significantly ($p < 0.05$) increased by 58% in alloxan-diabetic rats (Table 5). The present study showed that the levels of free radicals were significantly ($p < 0.05$) increased in plasma, liver, testes, brain and kidney by 28%, 16%, 22%, 38% and 22%, respectively in alloxan-diabetic rats as compared to control values (Tables 2–5).

Table 4: Assay of testes enzyme activities and thiobarbituric acid-reactive substances (TBARS) in control, diabetic, and diabetic treated male rats with garlic (G) (Means ± SE)

Parameter	Control	Diabetic	Diabetic + G
AST*	95± 5.52[c]	139 ± 6.38[a]	122 ± 3.00[b]
ALT*	84± 5.95[b]	109± 3.76[a]	93± 5.16[ab]
LDH**	1090 ± 82[b]	1420 ± 67[a]	1345 ± 77[a]
AlP*	496 ± 19[b]	645 ± 22[a]	552 ± 34[ab]
AcP*	13.1 ± 0.44[b]	15.7 ± 1.01[a]	14.2 ± 0.51[ab]
GST***	0.88 ± 0.01[b]	1.17 ± 0.01[a]	1.03 ± 0.04[a]
TBARS****	17.5 ± 0.39[c]	21.1 ± 0.76[a]	18.7 ± 0.74[ab]

Values are the means of eight rats.
abcWithin rows, between control and treated animals, means with different superscript letters differ significantly (P < 0.05).
* IU/mg: international unit, the amount of the enzyme that under defined assay conditions will catalyze 1 mole of substrate per minute, per mg protein.
** IU/g: international unit, the amount of the enzyme that under defined assay conditions will catalyze 1 mole of substrate per minute, per gram tissue.
*** GST specific activity: lmol/h/mg protein.
**** TBARS is expressed as nmol/g tissue.

While, after treatment of alloxan-diabetic rats with garlic, the level of free radicals was significantly ($p < 0.05$) decreased in plasma and tissues as compared with the mean value of diabetic group (Tables 2–5).

Table 5: Assay of brain and kidney enzyme activities and thiobarbituric acid-reactive substances (TBARS) in control, diabetic, and diabetic treated male rats with garlic (G) (Means ± SE)

Organ	Parameter	Control	Diabetic	Diabetic + G
Kidney	GST**	0.85 ± 0.027[b]	1.18 ± 0.047[a]	1.08± 0.068[a]
	TBARS***	23.7 ± 0.55[b]	26.6 ± 0.86[a]	26.7 ± 0.49[a]
Brain	LDH*	1298± 54[c]	1992 ± 84[a]	1749 ± 59[b]
	GST**	0.52 ± 0.001[a]	0.52 ± 0.004[a]	0.53 ± 0.006[a]
	TBARS***	26.7 ± 0.80[c]	37.4 ± 1.04[a]	28.5 ± 1.44[b]

Values are the means of eight rats.
[abc]Within rows, between control and treated animals, means with different superscript letters differ significantly (P < 0.05).
* IU/g: international unit, the amount of the enzyme that under defined assay conditions will catalyze 1 mole of substrate per minute, per gram tissue.
** GST specific activity: lmol/h/mg protein.
*** TBARS is expressed as nmol/g tissue.

Finally, the activity of GST was significantly ($p < 0.05$) increased in liver, testes and kidney of both diabetic and garlic-treated diabetic rats compared with the control values (Tables 3–5).

Discussion

Aside from its general use as a condiment, garlic (Allium sativum) is known for its pharmacological and nutritional properties. Garlic has long been believed to possess a hypoglycemic effect [29]. The present data indicated that the garlic juice significantly decreased serum glucose in treated diabetic rats in a dose-dependent fashion as compared with diabetic control rats (table 1). The hypoglycemic potency of garlic has been attributed to the sulphur compounds [di propenyl (2- disulphide) and 2-propenyl propyl disulphide respectively][30]. The mechanism of hypoglycemic action probably involves direct or indirect stimulation of insulin secretion[31]. Further, Augusti suggested that these disulphide compounds have the effect of sparing insulin from–SH inactivation by reacting with endogenous thiol-containing molecules such as cysteine, glutathione, and serum albumins [32]. Also, plasma urea, creatinine and total bilirubin (Table 1) are consistent with the finding of Augusti and Sheela (1996) and Campos et al. (2003) in rats, Kumar and Reddy (1999) in mice and Jain and Vyas (1975) in rabbits [33,34, 35, 36]. Orekhov and Grunwald (1997) found that garlic indirectly affects atherosclerosis by reduction of hyperlipidemia, hypertension, and probably diabetes mellitus and prevents thrombus formation [37]. Augusti and Sheela (1996) reported that garlic acts as an insulin secretagogue in diabetic rats. Another proposed mechanism is due to spare insulin from sulfhydryl group. Inactivation of insulin by sulfhydryl group is a common phenomenon. Garlic can effectively combine with compounds like cysteine and enhance serum insulin [38]. Jain and Vyas (1975) proposed that garlic can act as an antidiabetic agent by increasing either the pancreatic secretion of insulin from the beta cells or its release from bound insulin [36].

The diabetic hyperglycemia induces elevation of plasma levels of urea and creatinine which are considered as significant markers of renal dysfunction [39]. The results in Table 1 showed significant ($p < 0.05$) increase in the level of plasma urea and creatinine in the diabetic groups by 40% and 68% of control level, respectively. These results indicated that diabetes could be lead to renal dysfunction. While, after treatment of alloxan-diabetic rats with garlic, the level of urea was significantly ($p < 0.05$) decreased in plasma by 14% compared to the mean value of diabetic group (Table 1). Similarly, the elevation of creatinine level caused by diabetes was declined after administration of garlic by 26% ($p < 0.05$) compared with the diabetic group (Table 1). These results are in agreement with other previous studies on root extract of panax ginseng [40] and herbal Derris reticulata [41].

The increase in the activities of plasma AST, ALT, LDH, AlP and AcP (Table 2) indicated that diabetes may be induced hepatic dysfunction. Supporting our finding it has been found by Douaouya et al,(2016)that liver was necrotized in diabetic patients [1]. Therefore, the increment of the activities of AST, ALT, LDH, AlP and AcP in plasma may be mainly due to the leakage of these enzymes from the liver cytosol into the Blood stream, which gives an indication on the hepatotoxic effect of alloxan [42]. On the other hand, treatment of the diabetic rats with garlic caused reduction in the activity of these enzymes in plasma compared to the mean values of diabetic group (Table 2). These results are in agreement with those obtained by Ohaeri (2001) in rats [43]. The reduction in liver enzyme activities (Table 3) is mainly due to leakage of these enzymes into the blood stream as a result of alloxan toxicity which leads to the liver damage. However, treatment of alloxan diabetic groups with garlic for 28 consecutive days could restore the activities of the above enzymes to their normal levels. A possible explanation for the differential effects of garlic on the activities of AST, ALT, LDH, AlP and AcP in plasma and liver is that these treatments may inhibit the liver damage induced by alloxan. Furthermore, the improvement of the liver damage by oral administration of garlic juice could be confirmed through studying their effect on the level of plasma bilirubin. The results in Table 1 showed that the experimentally induced diabetes increased ($p < 0.05$) the level of plasma bilirubin by 55% of control. However, garlic intake produced significant ($p < 0.05$) decrease in plasma bilirubin of alloxan-diabetic rats by 25% compared to the diabetic rats. Rana et al. (1996) reported that the increase in plasma bilirubin (hyper-bilirubenimia) may be resulted from the decrease of liver uptake, conjugation or increase bilirubin production from hemolysis. Also, the elevation in plasma bilirubin indicates liver damage as confirmed by the changes in the activities of plasma (Table 2) and liver (Table 3) enzymes [44].

Like many chronic diseases, diabetes is widely believed to increase oxidative stress. In diabetes an increase in oxidative stress arises due to compromise in natural antioxidant mechanisms and an increase in oxygen free radical production [45]. The induction in the levels of free radicals in alloxan diabetic rats, and the decrease in these levels after treatment of alloxan-diabetic rats with garlic (Tables 2–5) are in agreement with those obtained by Douaouya et al, (2016) [1]. Also, Rajani et al. (2008) reported that garlic was effective in preventing or ameliorating oxidative stress [46]. Maintenance of free radical levels in garlic-treated diabetic animals might be due to the presence S-allyl cysteine sulfoxide in garlic [33].

Glutathione S-transferases (GSTs) are a family of enzymes that catalyze the addition of the tripeptide glutathione to endogenous and xenobiotic substrates which have electrophilic functional groups. They play an important role in the detoxification and metabolism of many xenobiotic and endobiotic compounds [47]. So far, few studies have been directed towards the influence diabetes millitus and hypoglycemic garlic on the activity of GST [48]. The increment in the activity of GST (Tables 3–5) is in consistent with the induction in the generation of free radicals (Tables 2–5). Increased GST activity might be one of the defense mechanism in these animals to detoxify or neutralize the toxic metabolites, e.g. ketone bodies, generated in liver by the diabetes. Chandra et al.(2004) suggested that garlic oil may effectively normalize the impaired antioxidants status in streptozotocin induced-diabetes. The effects of this antioxidant may be useful in delaying the complicated effects of diabetes as retinopathy, nephropathy and neuropathy due to imbalance between free radicals and antioxidant systems [49]. From the above results, it could be concluded that garlic is able to normalize the blood glucose levels. In addition, this plant juice could ameliorate the impaired renal function, inhibit liver damage and induced free radicals associated with alloxan diabetes.

Conclusion

The present results showed that local spices of Allium sativum exerted antioxidant and antihyperglycemic effects and consequently may alleviate and protect pancreas damage caused by alloxan-induced diabetes. Further, it is concluded that the plant must be considered as an excellent candidate for future studies on diabetes mellitus. In addition, comprehensive pharmacological investigations, including chronic experimental studies, should be carried out.

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