

Antidiabetic effect of garlic (*Allium sativum* L.) in normal and streptozotocin-induced diabetic rats

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Abstract

Objective: The antidiabetic effect of garlic ethanolic extract (*Allium sativum* L.) was investigated in normal and streptozotocin-induced diabetic rats.

Research methods and procedure: In the present study, oral administration of garlic extract (0.1, 0.25 and 0.5 g/kg body wt.) for 14 days on the level of serum glucose, total cholesterol, triglycerides, urea, uric acid, creatinine, aspartate amino transferase (AST) and alanine amino transferase (ALT) in normal and streptozotocin-induced diabetic rats were evaluated.

Results: Oral administrations of the garlic extract significantly decreased serum glucose, total cholesterol, triglycerides, urea, uric acid, creatinine, AST and ALT levels, while increased serum insulin in diabetic rats but not in normal rats ($p < 0.05$). A comparison was made between the action of garlic extract and glibenclamide (600 μ g/kg), the known antidiabetic drug. The antidiabetic effect of the extract was more effective than that observed with glibenclamide.

Conclusion: It is concluded that the plant must be considered as excellent candidate for future studies on diabetes mellitus.

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Keywords: *Allium sativum*; Garlic; Diabetes; Rat

Introduction

Present number of diabetics worldwide is 150 million and this is likely to increase to 300 million or more by the year 2025 (King et al., 1998). Reasons for this rise include increase in sedentary lifestyle, consumption of energy rich diet, obesity, higher life span, etc. (Yajnik, 2001). Regions with greatest potential are Asia and Africa, where diabetes mellitus (DM) rates could rise to 2–3-folds than the present rates (ADA, 1997). Many

herbal medicines have been recommended for the treatment of diabetes (Marles and Farnsworth, 1995; Alarcon-Aguilara et al., 1998). Plant drugs are frequently considered to be less toxic and more free from side effects than synthetic ones (Pari and Umamaheswari, 2000).

Garlic (*Allium sativum* L., Liliaceae) is a common spicy flavoring agent used since ancient times. Garlic has been cultivated in all over Iran for its characteristic flavor and medicinal properties (Zargari, 1997). 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

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decade, some protective effects of garlic have been well established by epidemiological studies and animal experiments. 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 (Elkayam et al., 2003). A clinically used glibenclamide (a sulphonylurea drug) is known to lower the serum glucose by stimulating β -cells to release insulin.

The purpose of this research was to experimentally assess the anti-diabetic effect of garlic alcoholic extract used in normal and streptozotocin-induced rats and to compare it with glibenclamide as a reference standard.

Materials and methods

Animals

Male Wistar rats weighing 200–250 g were housed in clean cages with temperature (22–24 °C), 12-h light/12-h dark cycle and relative air humidity 40–60%. Rats had continuous access to food and to tap water. Permission for the study was obtained from the country government.

Preparation of diabetic rat

The animals were injected with streptozotocin (70 mg/kg, i.p.). Five days after injection, the rats with fasting blood glucose higher than 180 mg/dl were used for the experiments. Eight rats were used in each experiment. Each animal was used once only in all of experiments. The food and water were removed from cages 12 h before testing.

Preparation of garlic extract

Fresh garlic bulbs (*A. sativum* L.) were purchased from a retail food store (Tehran, Iran) in June 2003, identified by department of botany of Teacher Training University (Voucher number: 45012, deposited in: Farabi Herbarium, director: Dr. F. Ghahremani nejad). Dried and ground bulbs (about 100 g) were submitted to extraction with 300 ml ethanol (80%) in a Soxhlet apparatus for 72 h. After extraction, the solvent was filtered and then evaporated by Rotavapor. The obtained garlic alcoholic extract was stored at –20 °C until being used.

Drug administration

Garlic extract was suspended in distilled water and administered orally through orogastric tube at doses of

0.1, 0.25 and 0.5 g/kg body wt. The volume of administered extract was 1 ml for each animal.

Experimental design

In the present experiment, 54 rats (30 diabetic, 24 normal rats) were used. The rats were divided into nine groups. Six rats were used in each group.

Group 1: Normal control rats were administered 1 ml of distilled water.

Groups 2–4: Normal rats were administered garlic alcoholic extract (0.1, 0.25 and 0.5 g/kg body wt.) daily using an intragastric tube for 14 days.

Group 5: Diabetic control rats were administered 1 ml of distilled water.

Groups 6–8: Diabetic rats were administered garlic alcoholic extract (0.1, 0.25 and 0.5 g/kg body wt.) daily using an intragastric tube for 14 days.

Group 9: Diabetic rats were administered glibenclamide orally (600 μ g/kg body wt.) in aqueous solution daily using an intragastric tube for 14 days.

Biochemical assays

After 14 days of treatments, blood samples were drawn from heart. Serum glucose, insulin, total cholesterol, triglycerides, urea, uric acid, creatinine, aspartate amino transferase (AST) and alanine amino transferase (ALT) levels were determined. Serum glucose was estimated by oxidase method (Barham and Trinder, 1972). The serum insulin was estimated by using the radioimmunoassay kit (diasorin, Italy), total cholesterol and triglyceride by the method of Rifai et al. (1999). Serum urea was assayed by the method of Tomas (1998a, b), while uric acid was measured by the method of Fossati et al. (1980). Serum creatinine was estimated by the method of Tomas (1998a, b). Serum AST and ALT were assayed by the method of Moss and Henderson (1999).

Statistical analysis

All the data were expressed as mean \pm S.E.M. Statistical analysis was carried out using one-way ANOVA followed by Tukey post hoc test. The criterion for statistical significance was $p < 0.05$.

Results

There was a significant elevation in serum glucose, total cholesterol, triglycerides, urea, uric acid, creatinine, AST and ALT while the serum insulin level significantly decreased in the diabetic rats.

Effect of garlic extract on serum glucose and insulin levels in normal and diabetic rats

Fig. 1 showed that the effect of the garlic extract on serum glucose and insulin in normal and diabetic rats. The results showed that serum glucose of diabetic rats increased while serum insulin decreased, when compared with normal rats. The administration of the garlic extract at doses of 0.25, 0.5 g/kg body wt., and glibenclamide tended to bring serum glucose ($F_{4,25} = 14.073$, $p < 0.001$) and insulin ($F_{4,25} = 13.784$, $p < 0.05$) significantly toward normal values, while normal rats did not exhibit any significant alterations in serum glucose and insulin levels duration of the experiment. The garlic extract was found to be more effective than glibenclamide. The administration of the garlic extract (0.1, 0.25 and 0.5 g/kg body wt.) did not change serum glucose ($F_{3,20} = 3.401$, $p > 0.05$) and insulin ($F_{3,20} = 1.439$, $p > 0.05$) levels in normal rats.

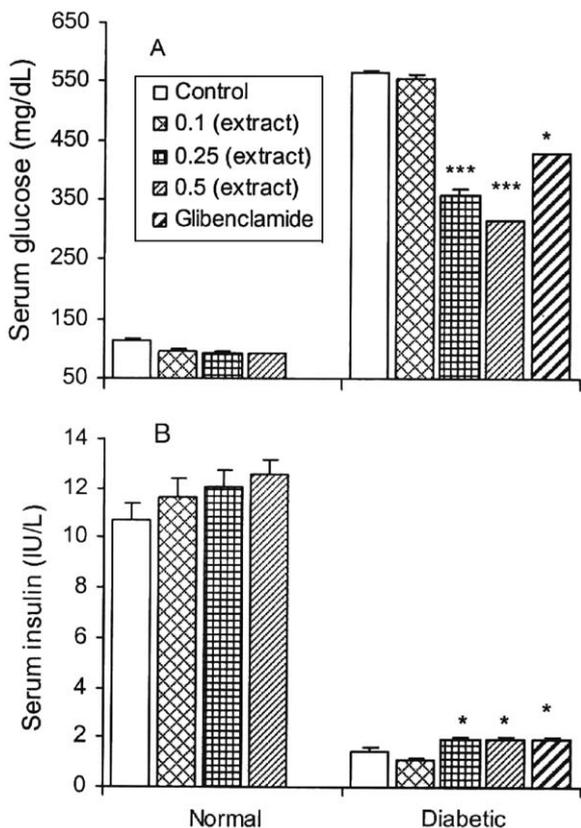


Fig. 1. Effect of oral administration of garlic alcoholic extract at doses of 0.1, 0.25 and 0.5 g/kg body wt. on serum glucose (A) and insulin (B) levels in normal and diabetic rats. Glibenclamide (600 μ g/kg) was treated only diabetic rats. Each column represents mean \pm S.E.M. for six rats. Control group administrated with water as a vehicle. * $p < 0.05$, *** $p < 0.001$ different from control diabetic group.

Effect of the garlic extract on serum triglycerides and total cholesterol levels in normal and diabetic rats

Fig. 2 showed that the effect of the garlic extract on serum triglycerides and total cholesterol in normal and diabetic rats. The results showed that the serum triglycerides and total cholesterol increased, when compared with normal rats. The administration of the garlic extract (0.1, 0.25, 0.5 g/kg body wt.) and glibenclamide significantly decreased serum triglycerides ($F_{4,25} = 20.578$, $p < 0.001$) and total cholesterol ($F_{4,25} = 11.677$, $p < 0.001$), when compared with control diabetic rats. The garlic extract at doses of 0.25 and 0.5 g/kg body wt. were found to be more effective than glibenclamide. The administration of the garlic extract (0.1, 0.25 and 0.5 g/kg body wt.) did not change serum triglycerides ($F_{3,20} = 0.366$, $p > 0.05$) and total cholesterol ($F_{3,20} = 4.893$, $p > 0.05$) levels in normal rats.

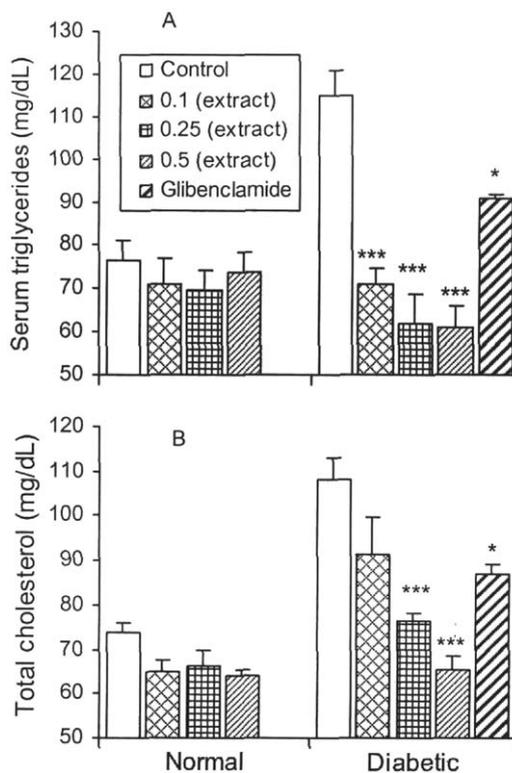


Fig. 2. Effect of oral administration of garlic alcoholic extract at doses of 0.1, 0.25 and 0.5 g/kg body wt. on serum triglycerides (A) and total cholesterol (B) levels in normal and diabetic rats. Glibenclamide (600 μ g/kg) was treated only diabetic rats. Each column represents mean \pm S.E.M. for six rats. Control group administrated with water as a vehicle. * $p < 0.05$, *** $p < 0.001$ different from control diabetic group.

Effect of the garlic extract on serum urea, uric acid and creatinine levels in normal and diabetic rats

Fig. 3 showed that the effect of the garlic extract on the serum urea, uric acid and creatinine in normal and diabetic rats. The results showed that serum urea, uric acid and creatinine increased, when compared with normal rats. The administration of the garlic extract (0.25, 0.5 g/kg body wt.) and glibenclamide significantly decreased serum urea ($F_{4,25} = 8.434$, $p < 0.001$), uric acid ($F_{4,25} = 3.198$, $p < 0.05$) and creatinine ($F_{4,25} = 4.412$, $p < 0.01$), when compared with control diabetic rats. The plant extract at a dose of 0.5 g/kg body wt. was found to be more effective than glibenclamide. The administration of the garlic extract (0.1, 0.25 and 0.5 g/kg body wt.) did not change serum

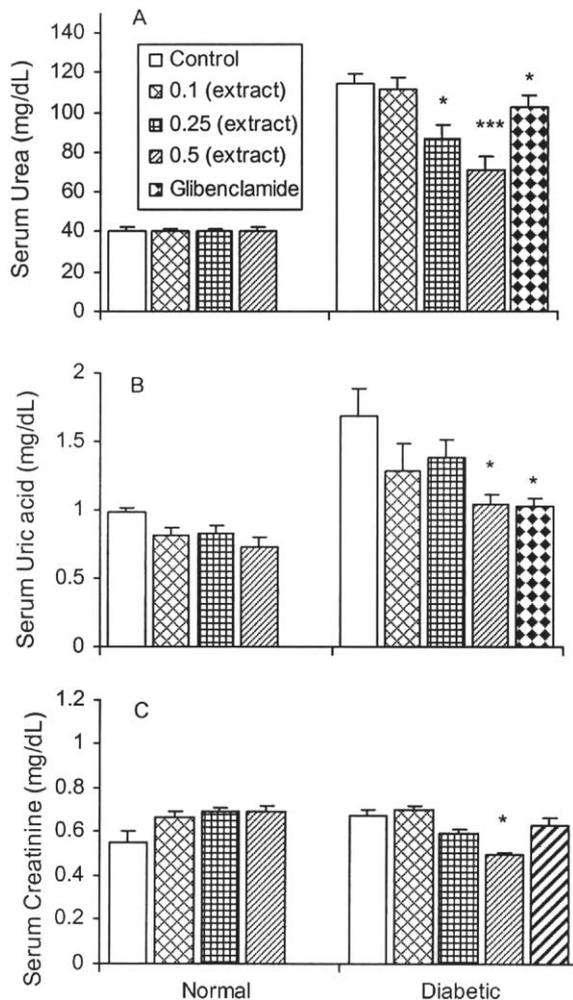


Fig. 3. Effect of oral administration of garlic alcoholic extract at doses of 0.1, 0.25 and 0.5 g/kg body wt. on serum urea (A), uric acid (B) and creatinine (C) levels in normal and diabetic rats. Glibenclamide (600 μ g/kg) was treated only diabetic rats. Each column represents mean \pm S.E.M. for six rats. Control group administrated with water as a vehicle. * $p < 0.05$, *** $p < 0.001$ different from control diabetic group.

urea ($F_{3,20} = 0.029$, $p > 0.05$), uric acid ($F_{3,20} = 2.69$, $p > 0.05$ levels) and creatinine ($F_{3,20} = 3.425$, $p > 0.05$) in normal rats.

Effect of the garlic extract on serum aspartate amino transferase (AST) and alanine amino transferase (ALT) levels in normal and diabetic rats

Fig. 4 showed that the effect of the garlic extract on the serum AST and ALT levels in normal and diabetic rats. The results showed that serum AST and ALT levels increased, when compared with normal rats. The administration of the garlic extract (0.5 g/kg body wt.) significantly decreased AST ($F_{4,25} = 2.813$, $p < 0.05$) and ALT ($F_{4,25} = 3.53$, $p < 0.05$) levels, when compared with control diabetic rats but not glibenclamide. The administration of the garlic extract (0.1, 0.25 and 0.5 g/kg body wt.) did not change AST ($F_{3,20} = 3.17$, $p > 0.05$) and ALT ($F_{3,20} = 1.574$, $p > 0.05$) levels in normal rats.

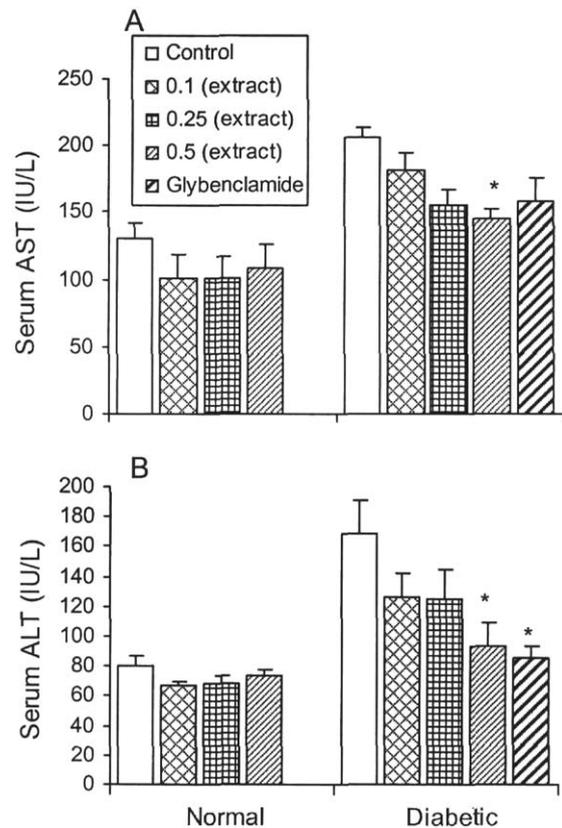


Fig. 4. Effect of oral administration of garlic alcoholic extract at doses of 0.1, 0.25 and 0.5 g/kg body wt. on serum AST (A) and ALT (B) levels in normal and diabetic rats. Glibenclamide (600 μ g/kg) was treated only diabetic rats. Each column represents mean \pm S.E.M. for six rats. Control group administrated with water as a vehicle. * $p < 0.05$ different from control diabetic group.

Discussion

Streptozotocin as an antibiotic and anticancer agent has been widely used for inducing type I diabetes in a variety of animals by affecting degeneration and necrosis of pancreatic β -cells (Merzouk et al., 2000).

The present data indicated that the garlic alcoholic extract significantly decreased serum glucose, triglycerides, cholesterol, urea, uric acid, AST and ALT, while increased serum insulin levels in treated diabetic rats as compared with control diabetic rats. In agreement with the present results, several studies have shown the hypoglycaemic effect of garlic, attributed mainly to allicin-type compounds (Chang and Johnson, 1980; Mathew and Augusti, 1973). The hypoglycaemic potency of garlic has been attributed to the sulphur compounds [di (2-propenyl) disulphide and 2-propenyl propyl disulphide, respectively]. The mechanism of hypoglycaemic action probably involves direct or indirect stimulation of insulin secretion (Carson, 1987). Further, it is also suggested that these disulphide compounds have the effect of sparing insulin from –SH inactivation by reacting with endogenous thiol-containing molecule such as cysteine, glutathione, and serum albumins (Augusti, 1996). The garlic extract might enhance glucose utilization because it significantly decreased the blood glucose level in glucose-loaded rats. It may be due to restoration of delayed insulin response or due to inhibition of intestinal absorption of glucose. This could be due to potentiation of the insulin effect of plasma by increasing the pancreatic secretion of insulin from existing β -cells or its release from bound insulin.

The administration of garlic extract significantly decreased serum triglycerides and cholesterol in diabetic rats. In contenance with the present data, other workers have reported that administration of fresh garlic or etheric garlic extracts was shown to improved lipid profile including reduction of serum cholesterol levels (Knipschild and Ter-Riet, 1989). Short-term experiments using primary hepatocyte cultures, which have proved useful as tools for screening the anticholesterogenic properties of garlic, also confirmed the cholesterol and lipid lowering effect of garlic (Yeh and Yeh, 1994). According to these investigators, the triacylglycerol lowering effect appears to be due to inhibition of fatty acid synthesis. Garlic decreased plasma triglycerides in lard-fed rats as well (Chi et al., 1982). With respect to the cholesterol lowering property of garlic, it has been suggested that some constituents of garlic may act as inhibitors for some enzymes such as hydroxy methyl glutaryl CoA reductase, which participates in cholesterol synthesis (Gebhardt, 1991; Gebhardt and Beck, 1996). Consistent with this idea, it has been shown that in vivo treatment of garlic extract reduces the lipid peroxidation products (Balasenthil et al., 2000).

Our data showed that uric acid levels were increased in diabetic rats. This may be due to metabolic disturbance in diabetes reflected in high activities of xanthine oxidase, lipid peroxidation and increased triglycerides and cholesterol (Madinov et al., 2000; Anwar and Meki, 2003). Moreover, protein glycation in diabetes may lead to muscle wasting and increased release of purine, the main source of uric acid as well as in activity of xanthine oxidase (Anwar and Meki, 2003). Our data showed that garlic extract decreased the serum urea and creatinine levels in diabetic rats. Elevation of the serum urea and creatinine, as significant markers, are related to renal dysfunction in diabetic hyperglycemia (Almadal and Vilstrup, 1988).

Serum enzymes including AST and ALT are used in the evaluation of hepatic disorders. An increase in these enzyme activities reflects active liver damage. Inflammatory hepatocellular disorders result in extremely elevated transaminase levels (Foreston et al., 1985; Hultcrantz et al., 1986).

In accordance with these findings, streptozotocin treatments has a significant role in the alteration of liver functions since the activity of AST and ALT were significantly higher than those of normal value. On the other hand, treatment of the diabetic rats with the garlic extract caused reduction in the activity of these enzymes in plasma compared to the mean values of the diabetic group and this is in agreement with that of Sheweita et al. (2001).

It is concluded that the plant must be considered as excellent candidate for future studies on DM. In addition, further comprehensive pharmacological investigations, including experimental chronic studies, should be carried out.

Acknowledgements

We would like to thank Deputy Research of the Science & Research Institute, Islamic Azad University for financial support of the project. We also thank Dr. Ghahramaninejad from the Department of Botany, Teacher Training University, for approving the authenticity of the plant used in the research.

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