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Anti-diabetic and hypolipidaemic properties of garlic (*Allium sativum*) in streptozotocin-induced diabetic rats

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Abstract
In this study the hypoglycaemic, hypocholesterolaemic and hypotriglyceridaemic effects of garlic were studied in streptozotocin (STZ)-induced diabetic rats. Compared to normal (non-diabetic) rats, STZ-induced diabetic rats had approximately 200% higher serum glucose, 50% higher serum cholesterol and 30% higher serum triglyceride levels as well as 86% higher urinary protein levels. Daily treatment of STZ-induced diabetic rats with an extract of raw garlic (500mg/kg intraperitoneally) for seven weeks significantly lowered serum glucose, cholesterol and triglyceride levels. Compared to control diabetic rats, garlic-treated rats had 57% less serum glucose, 40% lower serum cholesterol levels and 35% lower triglyceride. In addition, urinary protein levels in garlic-treated diabetic animals were 50% lower compared to the diabetic controls. In contrast, the increased urine output and water intake of diabetic rats were not affected by garlic treatment. These results indicate that raw garlic possesses a beneficial potential in reversing proteinuria in addition to reducing blood sugar, cholesterol and triglycerides in diabetic rats. Therefore, garlic could be of great value in managing the effects and complications of diabetes in affected individuals.

Key words: *Diabetes, Garlic, hypoglycaemic activity, hypolipidaemic activity, proteinuria*

Introduction

Garlic (A*llium sativum*) is one of the most popular herbs used worldwide to reduce various risk factors associated with cardiovascular diseases. Garlic, a member of the *Liliaceae* family, is a common food for flavour and spice and it is one of the herbs most commonly used in modern folkloric medicine. Garlic was an important medicine to the ancient Egyptians as listed in the medical text *Codex Ebers* (ca. 1550 BC) especially for the working class involved in heavy labour because it was an effective remedy for many aliments such as heart problems, headache, bites, worms and tumours.

Garlic is stated to possess many therapeutic benefits. Garlic's strong odour is largely due to sulphur-containing compounds (e.g. S-allylcysteine sulphoxide), which are believed to account for most of its medicinal properties.[1] Actually, garlic contains a variety of effective compounds that exhibit anticoagulant (anti-thrombotic),[2,3,4,5,6] antioxidant,[7,8] antibiotic,[9,10,11] hypocholesterolaemic,[12] hypoglycaemic,[1] as well as hypotensive activities.[12-13]

As mentioned above, although a large number of sulphur-thiosulphinates are present in sufficient quantities at normal consumption levels (3-5 g per day). Allicin has been shown to be important in many health effects of garlic.[14] However, the anti-cancer effect of garlic might be shared between allicin and other unidentified compounds.[15] Garlic contains about 1% alliin, which is converted enzymatically by allicinase to allicin, and other sulphur-containing compounds.[16]

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Garlic has been found to be effective in lowering serum glucose levels in STZ-induced as well as alloxan-induced diabetic rats and mice. Most of the studies showed that garlic can reduce blood glucose levels in diabetic mice, rats and rabbits.[14] Augusti and Sheela consistently showed that S-allyl cysteine sulphoxide, (allicin), a sulphur-containing amino acid in garlic (200 mg/kg body weight), had a potential to reduce the diabetic condition in rats almost to the same extent as did glibenclamide and insulin.[17-18] Aged garlic extract was also effective in preventing adrenal hypertrophy, hyperglycaemia and elevation of corticosterone in mice made hyperglycaemic by immobilization stress.[19] In addition, Liu and co-workers reported that both garlic oil and diallyl trisulphide improved glycaemic control in STZ-induced diabetic rats.[20] Ingestion of garlic juice resulted in better utilization of glucose in glucose tolerance tests performed in rabbits, while allicin at a dose of 250 mg/kg was 60% as effective as tolbutamide in alloxan-induced diabetic rabbits.[21]

In contrast, garlic powder intake (6.25% by weight in diet) for 12 days reduced hyperphagia and polydipsia, but did not alter either hyperglycaemia or hypoinsulinaemia in STZ-induced diabetic mice.[22] Similarly, Baluchnejadmojarad and Rohgani found no hypoglycaemic effect of an aqueous extract of garlic in rats with STZ-induced diabetes although they did observe a significant effect of garlic on vascular reactivity.[23-24] Liu and co-workers have speculated that these inconsistent results are at least partly due to the use of different preparations or derivatives of garlic in the different studies.[20] Staba and coworkers have established that the chemicals present in a garlic product are largely dependent on the processing conditions, such as temperature, duration of preparation, and extraction solvents used.[25]

In humans, the hypoglycaemic effect of garlic is not well documented. Most reports have shown a significant effect of garlic on blood glucose of normal healthy individuals but not in diabetic patients. Thus the role of garlic in diabetes treatment/prevention in humans is yet to be confirmed.[13]

The aim of the present study was to investigate the efficacy of an aqueous extract of raw garlic in controlling serum glucose, cholesterol, triglyceride and urine protein levels in STZ-induced diabetic rats treated daily intraperitoneally (IP) for a period of 7 weeks. Since there have been variable reports about the use of different preparations of garlic, as discussed above, an aqueous extract of raw garlic was used in the present study. Use of this preparation is also consistent with our previous work with garlic.[4-5, 26, 27,28]

Materials and Methods

Extract preparation

Aqueous garlic extract was prepared from locally available garlic bulbs. The garlic bulbs were peeled on crushed ice. Then 50 g of the peeled garlic was cut into small pieces and homogenized in 70 ml of cold, sterile 0.9% NaCl in the presence of some crushed ice. The homogenization was carried out in a blender at high speed using 30 second bursts for a total of 10 minutes. The homogenized mixture was filtered 3 times through cheesecloth, the filtrate was centrifuged at 2000 RCF for 10 minutes and the clear supernatant was diluted to 100 ml with normal saline. The concentration of this garlic preparation was considered to be 500 mg/ml on the basis of the weight of the starting material (50 g/100 ml). The aqueous extract of garlic was stored in small aliquots at -20°C until use. The stability of the preparation during storage has been previously established in platelet aggregation studies (unpublished observations).

Treatment of Diabetic Rats

Male Sprague-Dawley rats weighing 250-280 g (parents purchased from Laboratory Animals Inc., England) and maintained on a normal diet and filtered tap water *ad libitum* were used in the experiment. For baseline data, blood was drawn from all animals by cardiac puncture under ether anaesthesia and allowed to clot. Immediately, the clotted blood was centrifuged at 3500 RPM for 30 minutes. The serum was separated and stored at –80°C for later analysis.

The animals were randomly divided into a healthy group (8 rats) and a streptozotocin (STZ)-treated group (initially 20 rats). The STZ-treated rats were injected IP with 60 mg streptozotocin/kg body weight in a volume of 0.5 ml saline following an overnight fast.[29] After a period of three days, blood was drawn from the fasting STZ-treated animals and serum was prepared and stored for later analysis as described above. Serum glucose levels were determined immediately and the STZ-treated rats determined to be diabetic due to a high serum glucose level (>350 mg/dl) were randomly divided into two groups containing 8 animals each: Group 1, the control diabetic group, was injected IP daily with saline for the treatment period, and Group 2, the garlic-treated group, was injected IP daily with 500 mg/kg of the garlic extract. After periods of two, five and seven weeks, blood was drawn from the fasting rats by cardiac puncture, and serum was prepared and stored for later analysis as described above.

Rats were weighed before the start of the experiment and then weekly during the experimental period. Animals were monitored for general health during the treatment period. Twenty-four hour water intake was measured daily. Twenty-four hour urine output was measured in each treatment group before STZ administration and at two, five and seven weeks by housing the animals in metabolic cages and collecting the urine in a calibrated 250 ml attached container. The collected urine was centrifuged at 3000 RPM for 10 min to remove any particulate material. Urine samples were then stored at -80°C for protein determination.

At the end of the experiment, the rats were sacrificed under sodium pentobarbitone anaesthesia according to the guidelines for euthanasia in the *Guide for the Care and Use of Laboratory Animals*.[30]

Assays

Serum glucose, cholesterol and triglyceride were determined spectrophotometrically using kits supplied by CARO Co., Germany. Urinary protein was determined by the Coomassie Blue dye binding method of Bradford.[31]

Statistical Analysis

The data are expressed as mean ± SEM. Readings within a group were compared using the one-way ANOVA analysis and readings between groups were compared using the independent sample test. Statistical analysis was performed using SPSS (Version 14). A level of $p < 0.05$ was considered to be significant.

Results

Figure 1 shows changes in the serum glucose levels in STZ-induced diabetic rats in response to 500 mg/kg garlic extract administration. It is clear from the data that the serum glucose levels of the control diabetic animals continued to increase during the 7 weeks of the experiment compared to the post-STZ injection level. In contrast, the garlic-treated diabetic rats showed significantly reduced serum glucose levels during the treatment period when compared to the control diabetic rats. At weeks 2, 5 and 7 of garlic extract treatment, the serum glucose levels of the garlic-treated diabetic rats were reduced by 29%, 68% and 57%, respectively in comparison to control diabetic rats.

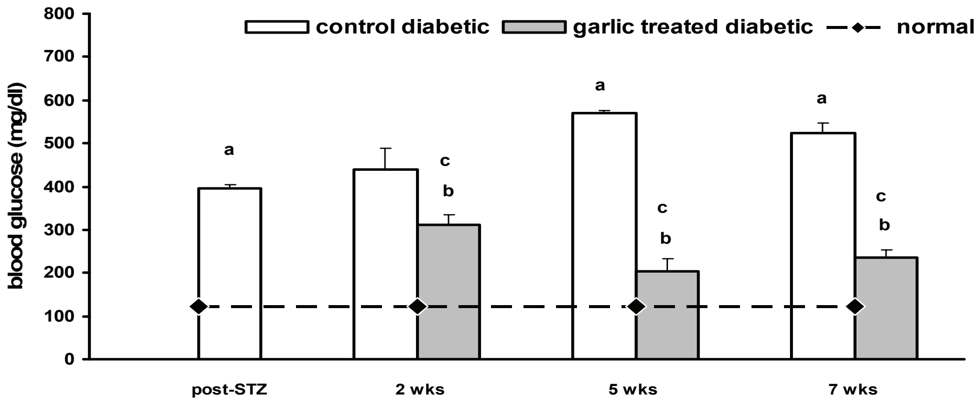

Figure 1: Serum glucose levels in STZ-induced diabetic rats treated with aqueous extract of garlic. Glucose levels were measured in serum of normal rats, STZ-induced diabetic rats (control diabetic) and garlic-treated STZ-induced diabetic rats (garlic-treated diabetic). Normal glucose levels were averaged over the experimental period and are depicted as a broken line. Analysis was done after STZ injection (post-STZ), and after 2, 5 and 7 weeks of treatment.
a: Significantly increased compared to normal ($p<0.05$), b: Significantly decreased compared to control diabetic ($p<0.05$).
c: Significantly decreased compared to post-STZ ($p<0.05$).

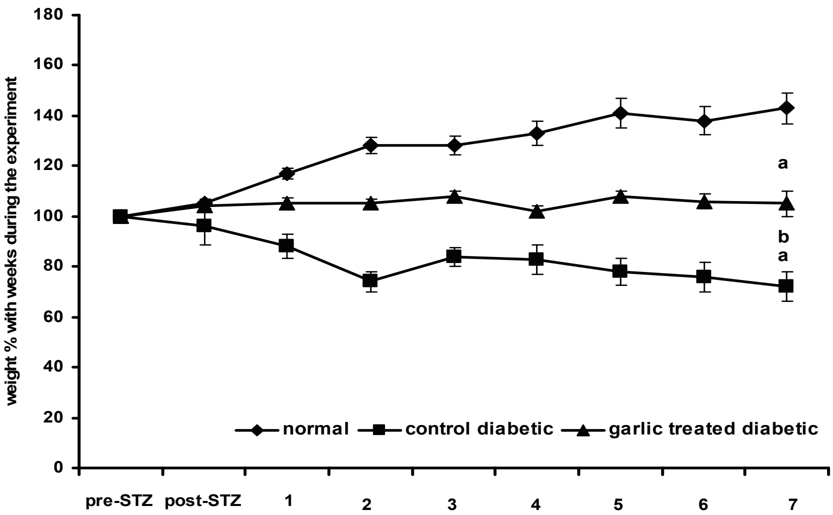

Figure 2: Weights of normal and STZ-induced diabetic rats over the garlic treatment period. Weights were measured in normal rats, STZ-induced diabetic rats (control diabetic) and garlic-treated STZ-induced diabetic rats (garlic treated diabetic). The animals were weighed before STZ injection (pre-STZ), one week after STZ-injection (post-STZ), and then weekly during the treatment period of 7 weeks. Weights are plotted as percentiles with the starting weights all standardized to 100%. a: Significantly decreased compared to normal at week 7 ($p<0.05$), b Significant difference between control diabetic and garlic treated diabetic rats ($p<0.05$).

However, garlic treatment did not reduce serum glucose of diabetic animals to normal levels.

Normal rats gained weight significantly throughout the experimental period, while both the control diabetic and garlic-treated diabetic animals had significantly lower body weights when compared to normal animals (Figure 2). However, garlic-treated diabetic rats maintained their initial weights during the 7-week treatment period although at the end of the experiment their body weights were significantly less than those of normal rats. In contrast, the control diabetic rats showed significant weight loss when compared to both the normal rats and the garlic-treated diabetic rats at the end of the 7-week experiment.

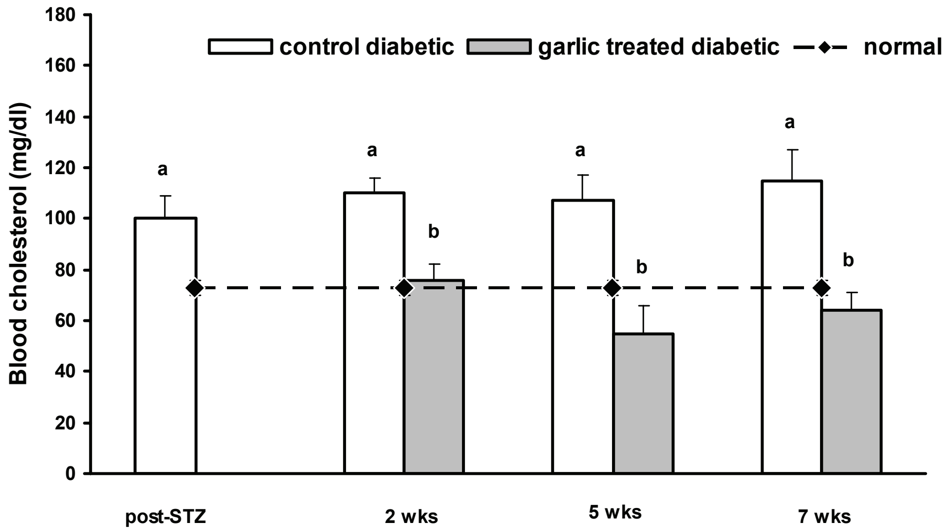

Figure 3: Serum cholesterol levels in STZ-induced diabetic rats treated with aqueous extract of garlic. Cholesterol levels were measured in serum of normal rats, STZ-induced diabetic rats (control diabetic) and garlic-treated STZ-induced diabetic rats (garlic treated diabetic). Normal cholesterol levels were averaged over the experimental period and are depicted as a broken line. Analysis was done after STZ injection (post STZ), and after 2, 5 and 7 weeks of treatment.
a Significantly increased compared to normal ($p < 0.05$), b Significantly decreased compared to diabetic control ($p < 0.05$).

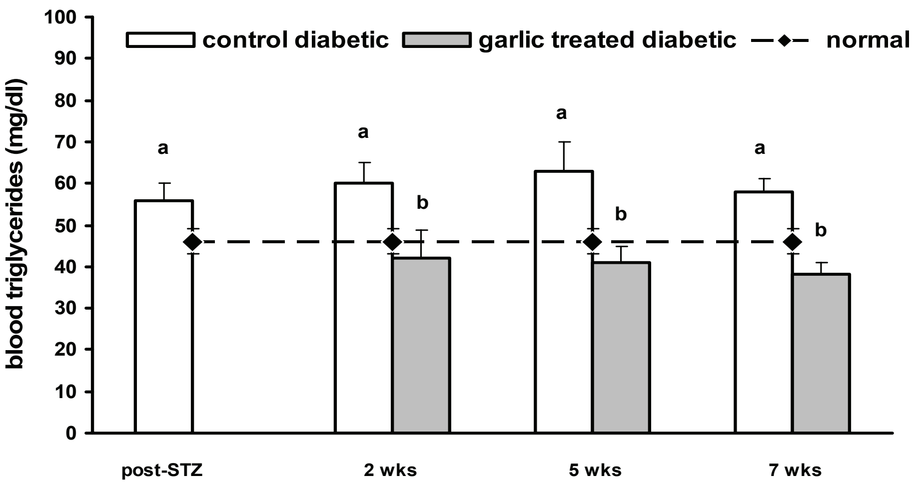

Figure 4: Serum triglyceride levels in STZ-induced diabetic rats treated with aqueous extract of garlic. Triglyceride levels were measured in serum of normal rats, STZ-induced diabetic rats (control diabetic) and garlic-treated STZ-induced diabetic rats (garlic treated diabetic). Normal triglyceride levels were averaged over the experimental period and are depicted as a broken line. Analysis was done after STZ injection (post STZ), and after 2, 5 and 7 weeks of treatment. a: Significantly increased compared to normal ($p < 0.05$), b: Significantly decreased compared to diabetic control ($p < 0.05$).

In Figure 3, one week after STZ-injection (post-STZ), the serum cholesterol levels of the diabetic rats were significantly higher than the normal levels. After treatment, the serum cholesterol levels of garlic-treated diabetic rats were significantly lower in comparison with the control diabetic rats. The serum cholesterol reduction elicited by garlic was sustained throughout the course of treatment with serum cholesterol levels below the normal level after 5 and 7 weeks of garlic treatment. Figure 4 shows a reduction in serum triglyceride levels to normal levels in garlic-treated diabetic rats through the 7 weeks of treatment. In contrast, both cholesterol and triglyceride levels remained elevated in the control diabetic animals throughout the experimental period.

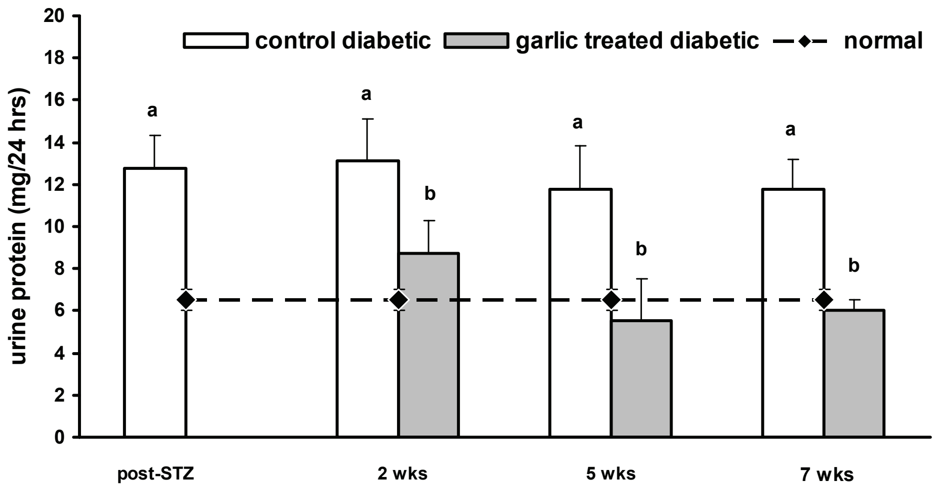

Figure 5: Urinary protein levels in STZ-induced diabetic rats treated with aqueous extract of garlic. Protein levels were measured in urine of normal rats, STZ-induced diabetic rats (control diabetic) and garlic-treated STZ-induced diabetic rats (garlic treated diabetic). Normal urinary protein levels were averaged over the experimental period and are depicted as a broken line. Analysis was done after STZ injection (post STZ), and after 2, 5 and 7 weeks of treatment.
a Significantly increased compared to normal ($p<0.05$). b Significantly decreased compared to control diabetic ($p<0.05$).

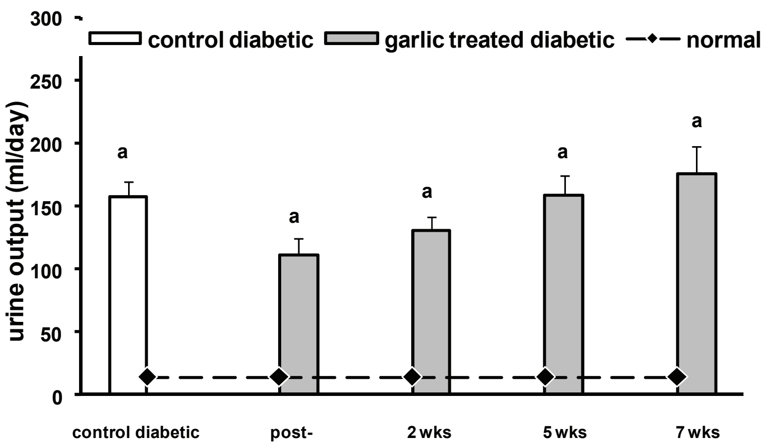

Figure 6: Urine output in STZ-induced diabetic rats treated with aqueous extract of garlic. Urine output was measured in rats after STZ-injection (post STZ) and after 2, 5 and 7 weeks of garlic treatment of STZ-induced diabetic rats. Normal urine output levels were averaged over the experimental period and are depicted as a broken line. Urine output levels in control diabetic rats were averaged over the experimental period and are presented as an open bar.
a Significantly increased compared to normal ($p<0.05$).

The urinary protein levels of garlic-treated diabetic rats were significantly lower than urinary protein levels in the control diabetic rats (Figure 5). One week after STZ-injection (post-STZ), the urinary protein level of the control diabetic rats was double that of the normal rats and remained elevated throughout the experimental period. In contrast, at weeks 2, 5 and 7 of garlic treatment, urinary protein levels in garlic-treated diabetic rats were lowered by 28%, 53% and 49%, respectively, with the urinary protein levels at 5 and 7 weeks of garlic treatment reaching the normal level. In the analysis of the data collected on urine output, it was found that the effect of garlic treatment was insignificant (Figure 6).

In fact, the urine output of the garlic-treated diabetic rats remained elevated during the 7 weeks of treatment. In contrast, the garlic-treated diabetic rats had decreased water intake after 2 weeks of treatment, after which

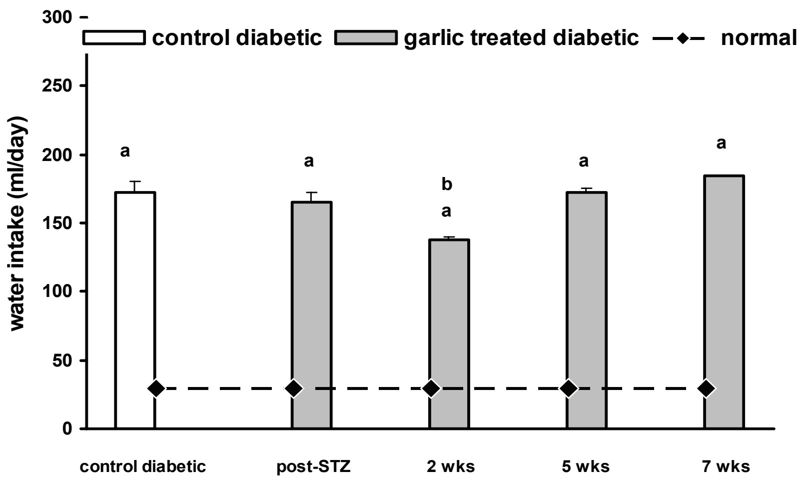

Figure 7: Water intake in STZ-induced diabetic rats treated with aqueous extract of garlic. Water intake was measured in rats after STZ-injection (post STZ) and after 2, 5 and 7 weeks of garlic treatment of STZ-induced diabetic rats. Normal water intake levels were averaged over the experimental period and are depicted as a broken line. Water intake levels in control diabetic rats were averaged over the experimental period and are presented as an open bar. a Significantly increased compared to normal ($p<0.05$).b Significantly decreased compared to control diabetic ($p<0.05$).

there was no significant reduction in water intake in the garlic-treated diabetic animals compared to the control diabetic rats (Figure 7).

Discussion

Diabetes mellitus is the most common endocrine disorder that affects more than 194 million people worldwide. If nothing is done to control this disease, the number will exceed 333 million by 2025 (6.3% of population). In 2003, Kuwait was among the five countries of the world with the highest diabetes prevalence in the adult population (12.8%).[32]

In addition to the primary effects of diabetes, diabetes is accompanied by increased risk factors such as hyperglycaemia, dyslipidaemia, hypertension, decreased fibrinolytic activity, increased platelet aggregation, and severe atherosclerosis.[33-34] Many synthetic drugs have been developed for the treatment of diabetes. However, these drugs have limits in terms of efficacy and side effects.[35] Therefore, there is much interest in discovering natural treatments without negative side effects that can reduce these risk factors in diabetic patients.

Garlic has been reported to possess a variety of medicinal properties including hypoglycaemic, hypocholesterolaemic and hypolipidaemic activities.[36] However, previous studies on the hypoglycaemic activity of garlic preparations have produced variable results.[7,17-24] Since we are interested in the beneficial effects of consumption of whole garlic, we chose to study the complete aqueous extract. In addition, in our previous studies, we have obtained consistent results using an aqueous extract of garlic in both rats and rabbits.[4,5,6,7,8,9,10,11,12, 26, 27,28]

In these experiments, the aqueous extract of garlic was given IP since, in previous experiments, both oral and IP administrations were found to be beneficial and IP administration required less handling of the animals.[6] The dosage was chosen to be 500 mg/kg since it is a safe amount of garlic to be given daily and does not cause toxicity.[14, 36] In addition, we have previously administered this dose (500 mg/kg) via the IP route with no detrimental effects in terms of toxicity and mode of administration.[26, 28]

The effects of the extracts of raw garlic were observed over a period of seven weeks. STZ-induced diabetic rats showed significant elevation of serum glucose, cholesterol and triglyceride levels. Our results confirmed that raw garlic has significant hypoglycaemic, hypocholesterolaemic and hypolipidaemic effects. Therefore, the present study reinforces the findings of previous papers that garlic had a significant effect in reducing blood glucose,[17,18,19,20,21, 37, 38] cholesterol[12] and triglyceride levels[8] in diabetic animal models.

We also observed that the weight loss that occurred in STZ-induced diabetic rats was attenuated by garlic treatment. In addition, at 7 weeks, the garlic-treated diabetic group had a lower mortality (one out of eight) compared to the control diabetic group (three out of eight). These changes may be a reflection of the improved health of the garlic-treated diabetic animals.

Proteinuria is a major predictor of glomerular injury and elevated rates of protein excretion are selective markers of progressive nephropathy.[39] STZ-induced diabetic rats are characterized by the development of proteinuria.[40] Furthermore, it has been shown that STZ has no long-term direct effects on the kidney, but STZ has secondary effects on the kidney as a result of the development of diabetes mellitus.[41] Our results showed that raw garlic may alleviate renal damage caused by STZ-induced diabetes, which was manifested by the significant lowering of urinary protein levels in the raw garlic-treated rats. Similar results were observed in alloxan-induced diabetic rats by El-Demerdash *et al*. who emphasized the alleviating effect of garlic on renal damage.[42] However, garlic treatment did not alleviate the characteristic polydypsia or polyuria observed in the STZ-induced diabetic rats. This is in agreement with the results of Liu *et al*. who also observed no change in water intake or urine output in STZ-induced diabetic rats treated with garlic oil or diallyl trisulphide.[20]

It is not clear how garlic actually works in alleviating hyperglycaemia. The hypoglycaemic action of garlic could possibly be due to an increase in pancreatic secretion of insulin from β-cells, release of bound insulin or enhancement of insulin sensitivity. It has been previously suggested that garlic (allicin) can enhance serum insulin by effectively combining with compounds like cysteine, which would spare insulin from SH group reactions which are a common cause of insulin inactivation.[21] Another mechanism proposed by Augusti and Sheela states that the antioxidant effect of S-allyl cysteine sulfoxide, an isolated product from garlic, may contribute to its beneficial effect in diabetes.[7] Jain and Vyas postulated that garlic may act as an anti-diabetic agent by increasing either the pancreatic secretion of insulin from the β-cells or release of bound insulin.[43] This explanation is supported by the results of Liu and coworkers who reported that treatment of STZ-induced diabetic rats with garlic oil or diallyl sulphide resulted in increased serum insulin levels.[20]

The results of this study strongly suggest that garlic may be very useful in the alleviation of diabetic complications as well as in the prevention of the development of atherosclerosis and nephropathy generally observed in diabetic patients. In the future, further work is needed to investigate the active ingredients in garlic. In addition, more parameters should be studied such as insulin enhancement, HbA_{1c} level, free radical production, etc., to elucidate the mechanism of action of the active constituents of garlic.

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