Effects of Garlic (*Allium sativum*) on Blood Sugar and Nephropathy in Diabetic Rats


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**Abstract:** The aim of this study was to investigate the short term effect of aqueous-ethanolic extract of garlic in preventing and curing nephropathy following induced diabetes in rats using streptozotocin (STZ), with the help of stereological methods. Thirty two male wistar rats were randomly divided into control, control+extract, diabetic and diabetic+extract groups (n = 8). Diabetes was induced by intraperitoneal injection of 60 mg kg⁻¹ of streptozotocin (STZ). Treatment with aqueous-ethanolic extract of garlic (50 mg kg⁻¹ day⁻¹) was followed for four weeks, then the left kidneys were excised and stereological studies were carried out. The results revealed that blood sugar of the diabetic rats was reduced significantly following treatment with garlic extract. The final body weight of both diabetic and diabetic+extract groups didn’t increase significantly, but in control and control+extract groups the body weight increased significantly (p<0.001). A significant increase in the kidney weight of the diabetic and diabetic+extract rats was found (p<0.001) compared to the control ones. The volume of cortex, medulla and total volume of kidney increased significantly in the diabetic and diabetic+extract groups compared to the control ones. The total glomerular volume increased significantly in diabetic rats compared to the other groups (p<0.05). In conclusion the aqueous-ethanolic extract of garlic influences the volume of total glomeruli but has no effect on the volume of other kidney components.

**Key words:** Kidney structure, stereology, diabetes, nephropathy, garlic

### INTRODUCTION

Diabetes is the most common endocrine disorder and important metabolic disturbance which high concentration of plasma blood sugar is a definitive sign of it (Ahmad and Ahmed, 2006; Pendsey, 2002). Insulin-Dependent Diabetes Mellitus (IDDM) is a disease displaying a wide range of complicating factors including nephropathy (Ahmad and Ahmed, 2006; Al-Qattan et al., 2008; Lauer et al., 2007). Kidney morphological changes as a result of diabetes occur in glomeruli, tubules and interstitial tissue and accumulation of extra cellular matrix proteins in the mesangium, glomerular basement membrane and tubulointerstitium is also seen (Ahmed, 2005; Mason and Wahab, 2003; Nishi et al., 2000; Vestra and Fioretto, 2003). Renal hypertrophy occurs rapidly, with significant increase in kidney weight being evident within days of onset of type 1 diabetes (Lee et al., 2007; Ohineche et al., 2001). Despite the fact that insulin is the most important medicine in curing diabetes, investigators have been trying to use medicinal plants as an additional treatment beside insulin for a long time (El-Demerdash et al., 2005; Sheela and Augusti, 1992). Garlic is one of the most popular herbs used worldwide to reduce various risk factors associated with cardiovascular diseases (El-Demerdash et al., 2005; Thomson et al., 2007). Actually, garlic contains a variety of effective compounds that exhibit anticoagulant (anti-thrombotic), antioxidant, antibiotic, hypocholesterolemic, hypoglycemic, as well as hypotensive activities (Banerjee and Maulik, 2002; Thomson et al., 2007). Most of the studies showed that garlic can reduce blood glucose levels in diabetic mice, rats and rabbits and increase plasma insulin in diabetic rats (Augusti and Sheela, 1996; Banerjee and Maulik, 2002; Grover et al., 2002; Jamison, 2003; Patmoraj et al., 2000). Garlic, containing compounds such as S-allyl cysteine sulfoxide (allicin) which has antioxidant effects and increases the amount of catalase and glutathione peroxidase and also SACS (S-allyl cysteine sulfoxide) which stimulates insulin secretion in isolated β-cells in normal rats (Augusti and Sheela, 1996; Liu et al., 2005; Thomson et al., 2007).
Therefore, since garlic plays an important role in reducing oxidative stress and also blood sugar in diabetes (Augusti and Sheela, 1996; Banerjee and Maulik, 2002; Thomson et al., 2007), it is suggested as a substitute to anti-diabetic chemical agents. So, the aim of this study was to investigate the short term effect of aqueous-ethanolic extract of garlic in preventing and curing nephropathy caused by streptozotocin (STZ) induced diabetes in rats using stereological methods.

MATERIALS AND METHODS

Male Wistar rats (n = 32), with mean body weight of 204±20 g, were kept in the animal house of Arak University while the temperature was maintained 22±2°C with 12 h light: 12 h dark cycle and free access to diet during the experiment. The animals were randomly divided into four groups of control, control+extract, diabetic and diabetic+extract (n = 8). To induce diabetes, streptozotocin (STZ) was injected intraperitoneally (60 mg kg⁻¹ b wt.) and rat with a blood sugar >200 mg dL⁻¹ was considered as diabetic rat. The blood sugar of rats was measured by glucometer at the beginning of the experiment to confirm the normal blood sugar and also 10 days later to check the rise in blood sugar in the diabetic rats. After the establishment of diabetes, both extract treated groups were treated with garlic extract (50 mg kg⁻¹ day⁻¹) orally for four weeks. The extract was prepared in laboratory using percolation technique with 85% ethanol as solvent. The blood sugar was also measured at the end of the experiment, then the rats were anesthetized by ether and their left kidneys were taken out, de-capsulated, weighed and fixed in Bouin fixative solution. The fixed kidneys were embedded in 7% agar, cut into 1 mm thick slices and paraffin blocks were made, then from 4 µm thick sections were prepared and stained using Hematoxylin and Eosin (H and E) method (Kiernan, 1999).

Stereological study: Sections were analyzed stereologically with a light microprojector. Images of the sections were projected on working table and a fine grid of points was superimposed. Point counting method using Cavalieri principle was carried out to estimate the volume of kidney, medulla and cortex, according to the following formula:

\[ V = \sum_{i=1}^{n} P \times a(p) \times t / M^t \]

where as, the total volume of glomeruli was obtained using the following equation:

\[ V_{\text{glomeruli}} = \frac{\sum_{i=1}^{n} P(\text{Glomeruli}) \times a(p)_{\text{glomeruli}} \times M^t_{\text{glomeruli}}}{\sum_{i=1}^{n} P(\text{Cortex}) \times a(p)_{\text{cortex}} \times M^t_{\text{glomeruli}}} \]

\[ V_{\text{glomeruli}} = V_{\text{glomeruli}} \times V_{\text{cortex}} \]

which V stands for absolute volume, \( \sum_{i=1}^{n} P \) for total number of points, a(p) for area of point (grid), t for section thickness and M for square of profile magnification.

Statistical analysis: The paired sample t-test was used to compare the mean blood sugar and the mean body weight of the rats. One way ANOVA and Tukey test was carried out to find the differences between the mean values gained stereologically. p-value less than 0.05 was considered significant.

RESULTS

Blood sugar: The final blood sugar reduced significantly in diabetic+extract group compared to the first blood sugar of the rats (p<0.01) and also compared to the final blood sugar in the diabetic group (45% reduction and p<0.001), while the extract had no effect on the blood sugar in the control group (Table 1).

Body and kidney weight: There was a significant increase in the mean body weight of the control and control+extract groups compared to the first body weight of the rats (p<0.001), where both diabetic and diabetic+extract groups such difference was not seen (p>0.05) (Table 2). Regarding control rats compared to the control+extract group no significant difference was found in the mean kidney weight (p>0.05). The same finding was achieved when comparing diabetic and diabetic+extract groups.

Table 1: Comparing the first and final blood sugar (mg dL⁻¹) in four different groups of rats. Four weeks after experimental induction of diabetes and treatment with aqueous-ethanolic extract of garlic

<table>
<thead>
<tr>
<th>Groups of rats</th>
<th>Mean first blood sugar (mg dL⁻¹)</th>
<th>Mean final blood sugar (mg dL⁻¹)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>81.5±3.3</td>
<td>80.5±2.6</td>
<td>0.600</td>
</tr>
<tr>
<td>Control+extract</td>
<td>82.3±4.5</td>
<td>82.3±4.8</td>
<td>0.600</td>
</tr>
<tr>
<td>Diabetic</td>
<td>238.3±12.1</td>
<td>294.0±16.1</td>
<td>0.001</td>
</tr>
<tr>
<td>Diabetic+extract</td>
<td>234.3±14.7</td>
<td>161.8±64.0</td>
<td>0.031</td>
</tr>
</tbody>
</table>

Values are Mean±SD (paired sample t-test)

Table 2: Comparing the first and final body weight (g) in four different groups of rats. Four weeks after experimental induction of diabetes and treatment with aqueous-ethanolic extract of garlic

<table>
<thead>
<tr>
<th>Groups of rats</th>
<th>Mean first b. wt. (g)</th>
<th>Mean final b. wt. (g)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>202.0±10.9</td>
<td>278.0±13.0</td>
<td>0.001</td>
</tr>
<tr>
<td>Control+extract</td>
<td>150.0±8.9</td>
<td>261.0±12.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Diabetic</td>
<td>212.6±8.4</td>
<td>222.6±13.8</td>
<td>0.100</td>
</tr>
<tr>
<td>Diabetic+extract</td>
<td>205.0±8.6</td>
<td>206.8±21.9</td>
<td>0.800</td>
</tr>
</tbody>
</table>

Values are Mean±SD (paired sample t-test)
groups (p>0.05) (Table 3). However as Table 3 shows a significant increase in mean kidney weight was seen in diabetic groups compared to the control rats (p<0.001) (Table 3).

**Volume of kidney, cortex and medulla:** Comparing the mean total volume of kidney in diabetic rats and diabetic+extract ones no significant difference was found (p>0.05), but when the mean volume of kidney in diabetic group was compared to the control group (p<0.005) and the control+extract group, a significant difference was found (p<0.001). Also the mean volume of kidney in diabetic+extract group compared to the control and control+extract groups had increased significantly (p<0.001). In addition a significant increase in kidney volume was also found when both control and control+extract groups were compared (p<0.001) (Table 3).

The mean volume of cortex showed no significant difference in the diabetic+extract rats compared to the diabetic ones (p>0.05). Meanwhile comparing control and control+extract groups, a significant difference was seen (p<0.001). When comparing the mean cortex volume in diabetic rats with the control group (p<0.02) and control+extract group (p<0.001) a significant difference was observed (Table 3).

The mean volume of medulla in control and control+extract was not significantly different (p>0.05). The same result was found comparing diabetic and diabetic+extract groups (p>0.05). While both diabetic groups showed a significant increase when compared to the control ones (p<0.001) (Table 3).

**Volume of glomeruli:** The mean total volume of the glomeruli in diabetic group showed a significant increase when compared with control ones (p<0.05). Comparing the total volume of the glomeruli in diabetic+extract rats to the diabetic ones a significant reduction was found (p<0.05), while showing no significant difference to control group (p>0.05), but a significant increase was found when compared to the control+extract group (p<0.001) (Table 3).

**Histological findings:** Structure of the interstitial tissue, tubules and the glomeruli appeared normal in the control and control+extract groups and bowmen’s space was also recognizable. However, glomeruli seemed slightly smaller in the control+extract group compared to the control ones (Fig. 1a, b). In both diabetic groups glomerular hypertrophy was seen while histopathological changes were not found in the tubules and interstitial tissue (Fig. 1c, d).

**DISCUSSION**

Nowadays, the importance of diabetes study and methods of its treatment is considerable. As we know one of the important complications of diabetes is nephropathy which results in histopathological changes in kidney and body weight loss due to lack of insulin and change in the metabolism of food material in the body. Many synthetic medicines have been developed for the treatment of diabetes, however these medicines have side effects and limitations in terms of efficacy (Thomson et al., 2007). Therefore in the recent years, special attention has been paid to use medicinal plants in treatment of diabetes and selecting a substitute for insulin (El-Demerdash et al., 2005).

The results of this investigation showed that aqueous-ethanolic extract of garlic caused a highly significant reduction in blood sugar level in streptozotocin induced diabetes. Earlier studies on the hypoglycemic activity of garlic have showed variable results (Augusti and Sheela, 1996; Sheela and Augusti, 1992; Thomson et al., 2007).

It has been reported that the garlic may affect the insulin secretion from β-cells, release of bound insulin or increase of insulin sensitivity (Thomson et al., 2007). As mentioned above the allicin of garlic is the responsible component for enhancing serum insulin activity due to its free SH group (Mathew and Augusti, 1973). On other hand, antiradioxidative property of S-allyl cysteine sulfoxide (allicin) might be another reason of garlic beneficial effect on diabetes (Augusti and Sheela, 1996).

Therefore, treatment with garlic extract which contain compounds such as S-allyl cysteine and organosulfur can
Fig. 1: H and E stained light micrograph of rat kidney tissue showing glomerular size in different groups of rats four weeks after experimental induction of diabetes mellitus and treatment with aqueous-ethanolic extract of *Allium sativum* (garlic) (50 mg/kg/day), (G = glomerulus). (a) Control group, (b) Control+extract group, (c) Diabetic group and (d) Diabetic+extract group. As the figures show the glomeruli size was the same in the control and control+extract groups. The same was seen comparing diabetic and diabetic+extract groups, while being bigger than the control ones.

Gradually normalize oxidative stress and causes an increase in serum insulin levels in diabetic rats (Augusti and Sheela, 1996), delaying the side effects of diabetes.

In this study, inducing diabetes, prevented growth and increase in body weight in diabetic rats compared to the weight gain found in the control rats. Treatment of rats with aqueous-ethanolic extract of garlic did not compensate the reduction of body weight, although in some studies, treatment with garlic extract has been effective and caused a significant increase in the body weight of diabetic rats (Baluchnejadmojarad et al., 2003; Grover et al., 2002). This difference in results may be due to the kind of extraction, the dose recommended and how long it is used. In this study, although the amount of dose consumed had been effective (50 mg/kg/day) it hadn’t increased the body weight in diabetic rats, meanwhile some studies have shown that using active compounds of garlic such as S-allyl cysteine and organosulfur can lead to a weight gain in alloxan induced diabetes in rats (Grover et al., 2002). This may be related to the applied specific compound of garlic and the different agent to induced diabetes.

According to earlier studies, during diabetes mellitus, the blood sugar increases and results in lack of sugar in the cells; forcing, the cells to use amino acids and fatty acids as a source of energy which eventually leads to the reduction of proteins and fats in the body which causes body weight loss. On the other hand, lack of insulin reduces rRNA and mRNA synthesis which is another factor in reduction of the body proteins (Engerman and Kern, 1989).
In the present study, a significant increase in the kidney weight was found which is confirmed by the other investigations (Bilous, 2001; Bulut et al., 2001), this may be due to hyperplasia and hypertrophy of tubular and mesangial cells of the kidney (Bilut et al., 2001; Obineche et al., 2001; Rasch and Dorup, 1997). Although garlic can effectively normalize the oxidative stress in streptozotocin induced diabetic rats (Anwar and Mek, 2003; Augusti and Sheela, 1996; Banerjee and Maulik, 2002; El-Demerdash et al., 2005). In this study using aqueous-ethanolic extract of garlic didn’t prevent kidney weight gain in diabetic rats, this may be due to the amount of used streptozotocin (60 mg kg⁻¹), since earlier studies have shown that streptozotocin can cause a reduction in the amount of antioxidant along with releasing free radicals (Anwar and Mek, 2003; El-Demerdash et al., 2005).

Corresponding to the earlier studies, a significant increase in the total volume of the kidney (Bor et al., 2000; Bulut et al., 2001; Heidari et al., 2003), cortex (Bilut et al., 2001; Heidari et al., 2003), medulla (Heidari et al., 2003; Obineche et al., 2001) and glomeruli (Bilous, 2001; Bulut et al., 2001; Rasch and Dorup, 1997; Vestra and Fioretto, 2003) was found in the diabetic rats compared to the control ones as a result of hypertrophy and hyperplasia in the tubules, interstitial tissue (Bilous, 2001; Bor et al., 2000; Bulut et al., 2001), elements in the cortex (Bilous, 2001; Obineche et al., 2001), medulla (Obineche et al., 2001) and glomeruli (Bilous, 2001; Bulut et al., 2001; Heidari et al., 2003) in the diabetic rats. Hypertrophy in glomeruli can also be due to gradual clogging of some of the glomeruli which puts an overload on the remaining glomeruli (Osterby, 1992). In addition through this hypertrophy an increase in the thickness and length of the glomerular capillaries occur (Bilous, 2001).

Treating the diabetic rats with aqueous-ethanolic extract of garlic caused a significant reduction in the total volume of glomeruli and prevented glomerular hypertrophy, this suggests that garlic extract with it’s anti-oxidant and hypoglycemic property (Augusti and Sheela, 1996; Banerjee and Maulik, 2002; Thomson et al., 2007) can only have an effect on glomerular hypertrophy but no influence on the volume of the cortex and the medulla.

In conclusion, although application of high dose of garlic is toxic (Baluchnejadmohamad et al., 2003), but it is also reported that the high dose of garlic has no further hypoglycemic effect (Al-Qattan et al., 2008). Therefore, considering the useful and effective garlic compounds on reducing blood sugar and delaying nephropathy, it’s necessary to study the role of garlic in long term on recovering some of the diabetes side-effects in the kidney.

REFERENCES


