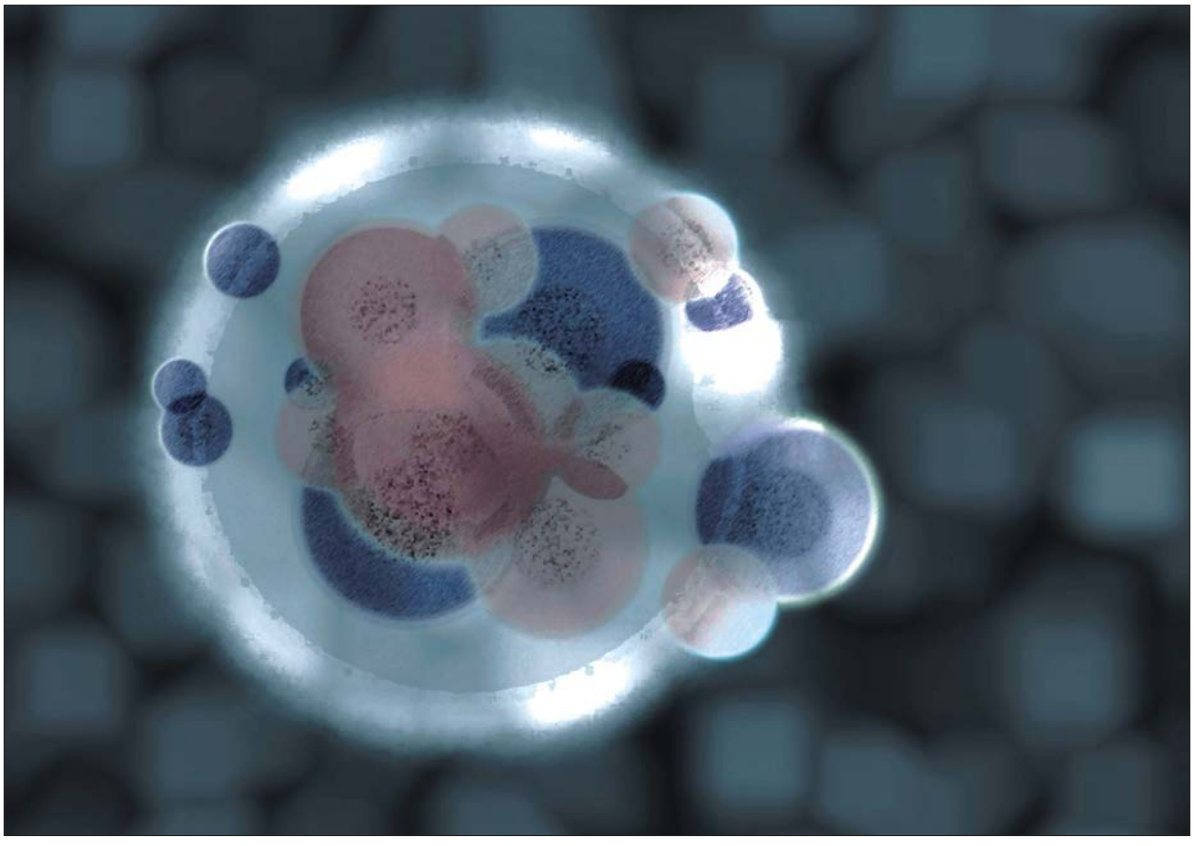

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Effects of Garlic (*Allium sativum*) on Blood Sugar and Nephropathy in Diabetic Rats

[1]S.M.A. Shariatzadeh, [1]M. Soleimani Mehranjani, [1]M. Mahmoodi, [1]M.H. Abnosi,
[1]H.R. Momeni, [2]A.R. Dezfulian and [1]M. Noori
[1]Department of Biology, Arak University, P.O. Box 879, Arak, Iran
[2]Department of Histology and Embryology,
Ahvaz Gondey Shapoor Medical Science University, Iran

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 singe 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; Obineche *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; Patumraj *et al*., 2000). Garlic, containing compounds such as S-allyl cysteine sulphoxide (allicin) which has antioxidant effects and increases the amount of catalase and glutathione peroxidase and also SACS (S-allyl cysteine solfuxide) which stimulates insulin secretion in isolated β-cells in normal rats (Augusti and Sheela, 1996; Liu *et al*., 2005; Thomson *et al*., 2007).

Corresponding Author: S.M.A. Shariatzadeh, Department of Biology, Faculty of Sciences, Arak University, Arak, Iran
Tel: 08612771446 Fax: 08612770050

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 glucosemeter 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 which 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:

$$V = \frac{\sum_{i=1}^{n} P \times a(p) \times t}{M^2}$$

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

$$V_{Vglomeruli} = \frac{\sum_{i=1}^{n} P(Glomeruli) \times a(p)_{Glomeruli} \times M^2_{Cortex}}{\sum_{i=1}^{n} P(Cortex) \times a(p)_{Cortex} \times M^2_{Glomeruli}}$$

$$V_{Total\ glomeruli} = V_{Vglomeruli} \times V_{Cortex}$$

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^2 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 in 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

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

Groups of rats	Mean first blood sugar (mg dL^{-1})	Mean final blood sugar (mg dL^{-1})	p-value
Control	81.5±3.3	80.5±2.6	0.600
Control+extract	82.2±4.5	83.2±4.8	0.600
Diabetic	238.3±12.1	294.0±16.1	0.001
Diabetic+extract	234.3±14.7	161.8±64.0	0.010

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 inducement of diabetes and treatment with aqueous-ethanolic extract of garlic

Groups of rats	Mean first b.wt. (g)	Mean final b.wt. (g)	p-value
Control	202.0±10	278.0±13.0	0.001
Control+extract	199.0±8.9	261.0±12.5	0.001
Diabetic	212.6±8.4	222.6±13.8	0.100
Diabetic+extract	205.0±8.6	206.8±21.9	0.800

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

Table 3: Comparing the mean kidney weight (g), the mean total volume of kidney (mm^3), volume of medulla (mm^3), cortex (mm^3) and the mean total volume of glomeruli (mm^3) in four different groups of rats, four weeks after experimental inducement of diabetes and treatment with aqueous-ethanolic garlic extract

Groups of rats	Mean kidney weight	Mean total volume of kidney (mm^3)	Mean volume of medulla (mm^3)	Mean volume of cortex (mm^3)	Mean total volume of glomeruli (mm^3)
Control	0.91±0.08^a	313.6±24.8^a	36.9±2.6^a	276.7±24.5^a	3.9±0.4^a
Control+extract	0.75±0.11^a	229.0±48.4^c	29.7±7.6^a	199.3±41.2^c	3.2±0.6^c
Diabetic	1.23±0.09^b	380.8±28.7^b	67.9±7.2^b	312.9±25.9^b	4.5±0.4^b
Diabetic+extract	1.19±0.16^b	395.3±38.5^b	71.9±10.7^b	323.4±29.5^b	4.1±0.3^a

Values are Mean±SD (ANOVA, Tukey test, $p>0.05$), Means with the same letter(s) do not show a significant difference (ANOVA, Tukey test, $p>0.05$)

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 complication 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 duo to its free SH group (Mathew and Augusti, 1973). On other hand, antioxadiative property of S-allyl cystein 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 organosulfor can

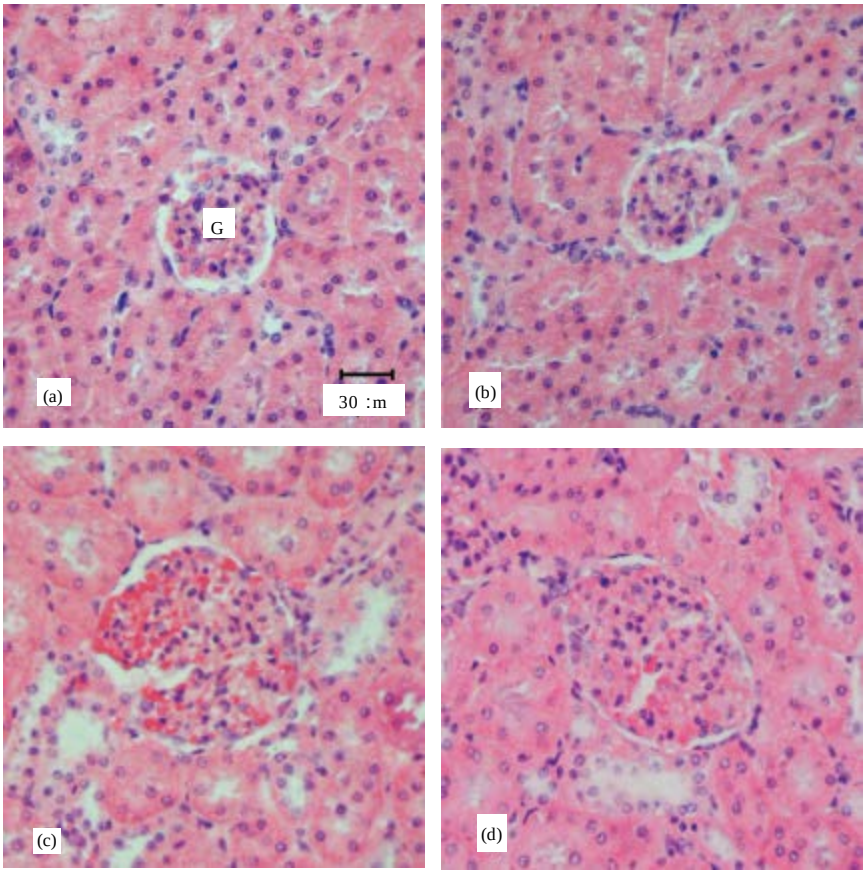

Fig. 1: H and E stained light micrograph of rat kidney tissue showing glomerular size in different groups of rats four weeks after experimental inducement of diabetes mellitus and treatment with aqueous-ethanolic extract of *Allium sativum* (garlic) (50 mg/kg/day), (G = glomerulus). (a) Control group, (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 organosolfur 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 conformed by the other investigations (Bilous, 2001; Bulut *et al*., 2001), this may be due to hyperplasia and hypertrophy of tubular and mesengial cells of the kidney (Bulut *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 Meki, 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 anti-oxidant along with releasing free radicals (Anwar and Meki, 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 (Bulut *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 (Baluchnejadmojarad *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

Ahmad, M.S. and N. Ahmed, 2006. Antiglycation properties of aged garlic extract: Possible role in prevention of diabetic complications. J. Nutr., 136: 796S-799S.

Ahmed, N., 2005. Advanced glycation endproducts-role in pathology of diabetic complications. Diabetes Res. Clin. Pract., 67: 3-21.

Al-Qattan, K., M. Thomson and M. Ali, 2008. Garlic (*Allium sativum*) and ginger (*Zingiber officinale*) attenuate structural nephropathy progression in streptozotocin-induced diabetic rats. Eur. J. Clin. Nutr. Metabol., 3: e62-e71.

Anwar, M.M. and A.R. Meki, 2003. Oxidative stress in streptozotocin-induced diabetic rats: Effects of garlic oil and melatonin. Comp. Biochem. Physiol. A Mol. Integr. Physiol., 135: 539-547.

Augusti, K.T. and C.G. Sheela, 1996. Antiperoxide effect of S-allyl cysteine sulfoxide, an insulin secretagogue, in diabetic rats. Cellular Mol. Life Sci., 52: 115-119.

Baluchnejadmojarad, T., M. Roghani, H. Homayounfar and M. Hosseini, 2003. Beneficial effect of aqueous garlic extract on the vascular reactivity of streptozotocin-diabetic rats. J. Ethnopharmacol., 185: 139-144.

Banerjee, S.K. and S.K. Maulik, 2002. Effect of garlic on cardiovascular disorders: A review. Nutr. J., 1: 4-4.

Bilous, R., 2001. Renal Structural Damage in IDDM and NIDDM Functional Relationships, Diabetic Nephropathy. 1st Edn., Renal Structural Damage in IDDM and NIDDM Functional Relationships, Diabetic Nephropathy. UK, ISBN: 9780470846445.

Bor, M.V., E.S. Elamail and N. Altan, 2000. Serum antiotensin converting enzyme activity in streptozotocin induced rats. Turk. J. Med. Sci., 30: 311-313.

Bulut, H.E., B. Ongarlioglu, C. Kaloglu, O. Ozdemir and S. Ayan, 2001. Effects of experimental diabetes and insulin treatment on rabbit renal morphology, A quantitative and qualitative study. Turk. J. Med. Sci., 31: 209-216.

El-Demerdash, F.M., M.I. Yousef and N.I. El-Naga, 2005. Biochemical study on the hypoglycemic effects of onion and garlic in alloxon-induced diabetes rats. Food Chem. Toxicol., 43: 57-63.

Engerman, R.L. and T.S. Kern, 1989. Hyperglycemia and development of glomerular pathology: Diabetes compared with galactosemia. Kidney Int., 36: 41-45.

Grover, J.K., S. Yadav and V. Vats, 2002. Medicinal plants of India with antidiabetic potential. J. Ethropharmacol., 81: 81-100.

Heidari, Z., H.R.M. Sagheb, H. Rafighdoost, A.A. Moein, M.H.N. Mugahi and B. Minaii, 2003. A stereological study of diabetic kidney following administration of difference dose of streptozotocin. DARU, 11: 1-6.

Jamison, J.R., 2003. Garlic (*Allium Sativum*): Clinical Guide to Nutrition and Dietary Supplements in Disease Management. 1st Edn., Churchill Livingstone, London, pp: 541-546.

Kiernan, J.A., 1999. Histological and Histochemical Methods: Theody and Practice. 3rd Edn., A Hodder Arnold Publication, USA, ISBN-10: 0750649364.

Lauer, M.E., V.C. Hascall and A. Wang, 2007. Heparan sulfate analysis from diabetic rat glomeruli. J. Biol. Chem., 282: 843-852.

Lee, M.J., D. Feliers, M.M. Mariappan, K. Sataranatarajan and L. Mahimainathan *et al*., 2007. A role for AMP-activated protein kinase in diabetes-induced renal hypertrophy. Am. J. Physiol. Renal. Physiol., 292: F617-F627.

Liu, C.T., H. Hse, C.K. Lii, P.S. Chen and L.Y. Sheen, 2005. Effects of garlic oil and diallyl trisulfide on glycemic control in diabetic rats. Eur. J. Pharmacol., 516: 165-173.

Mason, R.M. and N.A. Wahab, 2003. Extracellular matrix metabolism in diabetic nephropathy. J. Am. Soc. Nephrol., 14: 1358-1373.

Mathew, P.T. and K.T. Augusti, 1973. Studies on the effect of allicin (diallyl disulphide-oxide) on alloxan diabetes. I. Hypoglycaemic action and enhancement of serum insulin effect and glycogen synthesis. Indian J. Biochem. Biophys., 10: 209-212.

Nishi, S., M. Ueno, S. Hisaki, N. Iino and S. Iguchi *et al*., 2000. Ultrastructural characteristics of diabetic nephropathy. Med. Electron. Microscopy, 33: 65-73.

Obineche, E.N., E. Mensah-Brown, S.I. Chandranath, I. Ahmed, O. Naseer and A. Adem, 2001. Morphological changes in the rat kidney following long-term diabetes. Arch. Physiol. Biochem., 109: 241-245.

Osterby, R., 1992. Glomerular structural changes in type 1 (insulin-dependent) diabetes mellitus: Causes, consequences and prevention. Diabetologia, 35: 803-812.

Patumraj, S., S. Tewit, S. Amatyakul, A. Jariyapongskul, S. Maneesri, V. Kasantikul and D. Shepro, 2000. Comparative effects of garlic and aspirin on diabetic cardiovascular complications. Drug Deliv., 7: 91-96.

Pendsey, S., 2002. Practical Management of Diabetes. 1st Edn., Jaypee Brothers, New Dehli, pp: 113-153.

Rasch, R. and J. Dorup, 1997. Quantitative morphology of the rat kidney during diabetes mellitus and insulin treatment. Diabetologia, 40: 802-809.

Sheela, C.G. and K.T. Augusti, 1992. Antidiabetic effects of S-allyl cysteine sulphoxide isolated from garlic *Allium sativum* Linn. Indian J. Exp. Biol., 30: 523-526.

Thomson, M., Z.M. Al-Amin, K.K. Al-Qattan, L.H. Shaban and M. Ali, 2007. Anti-diabetic and hypolipidaemic properties of garlic (*Allium sativum*) in stereptozotocin-induced diabetic rats. Int. J. Diabetes Metabol., 15: 108-115.

Vestra, M.D. and D. Fioretto, 2003. Diabetic nephropathy: Renal structural studies in type 1 and type 2 diabetic patients. Int. Congress Series, 1253: 163-169.