NEPHROPROTECTIVE ROLE OF LOSARTAN AND VITAMIN E AGAINST STREPTOZOTOCIN-INDUCED DIABETIC NEPHROPATHY IN RATS: Histological and Immunohistochemical study
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ABSTRACT

Introduction: Diabetes mellitus (DM), one of the major metabolic disorders, is characterized by high blood glucose levels due to the inability of body cells to utilize glucose properly. Diabetes is recognized for severe complications such as diabetic nephropathy, neuropathy, and retinopathy. The aim of the present study was to evaluate the protective role of the angiotensin receptor blocker; losartan potassium and the antioxidant; vitamin E against streptozotocin-induced diabetic nephropathy.

Material and Methods: forty male albino rats were divided into 5 groups each of 8. The 1st group acts as a healthy sham group and received citrate buffer. The 2nd group acts as an untreated diabetic group. The 3rd group acts as a diabetic group, treated with losartan potassium at dose 1mg/kg/day. The 4th group acts as a diabetic group received vitamin E at dose of 1gm/kg/day. The 5th group acts as a diabetic and received both losartan and vitamin E at the same doses used for 3rd and 4th groups respectively. Diabetes was induced by single intraperitoneal injection of streptozotocin (STZ) at 40mg/kg, and the treatment continued for 8 weeks. Serum glucose, triglycerides and total cholesterol as well as creatinine were measured at the end of the 8th week. Histochemical study, using H&E and PAS stains and immunohistochemical study of glomerular Cu,Zn-SOD were carried out.

Results: The rats of the diabetic non-treated group showed high serum glucose, triglycerides and cholesterol as well as creatinine levels and their kidney showed class II glomerulosclerosis with high score of the glomerular Cu,Zn SOD indicating oxidative renal damage. Treatments with losartan and/or vitamin E tend to normalize the studied parameters.

Conclusion: The pathogenesis of the diabetic nephropathy is mediated even partially through oxidative stress. Losartan potassium and vitamin E have a renoprotective effect against diabetic nephropathy. However, vitamin E had more protective effect than losartan and both had no additive renoprotective effects.

1. Introduction

Diabetes mellitus (DM) is a major global health problem and metabolic disorder, which characterized by high blood glucose levels due to the inability of body cells to utilize glucose properly. As the disease progress, individuals are at risk of developing
specific complications such as retinopathy, nephropathy, neuropathy and atherosclerosis (Gabir et al., 2000; Schlienger, 2013). The complications are major causes of morbidity and mortality in DM. It has been estimated that by the year 2030, there will be 8.6 million adults with DM in Egypt, making it the country with tenth largest population of diabetics in the world (Shaw et al., 2010). It was found that oxidative stress, which results from imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms, is involved in the pathogenesis of diabetic nephropathy (Bonnefont-Rousselot et al., 2000). Increased ROS production results in formation of cytokines and growth factors which participate in the pathogenesis of DM (Brown et al., 2005; Navarro-Gonzalez and Mora-Fernandez, 2008; Elmarakby et al., 2010; Wada and Makino, 2013).

In the diabetic kidney, superoxide anion (O₂•−) is generated through numerous pathways and factors, including mitochondrial electron-transport chain, NAD (P)H oxidase, uncoupled endothelial nitric oxide synthase (eNOS), angiotensin II, oxidized-LDL (low density lipoprotein), and advanced glycation end products (AGEs) (Brownlee, 2005; Forbes et al., 2008). Overproduction of superoxide results in the formation of secondary reactive oxygen species, activation of glycolytic damaging pathways, induction of proatherogenic genes, and reduction of anti-atherogenic enzymes such as eNOS and prostacyclin synthase, leading to diabetic renal injury (Forbes et al., 2008). Thus, superoxide excess is considered as a key component in the pathogenesis of diabetic nephropathy (DN).

The superoxide dismutase (SOD) family of antioxidant enzymes is a major defense system against the superoxide anion, converting superoxide into hydrogen peroxide (H₂O₂) and molecular oxygen (O₂) (Faraci and Didion, 2004). The hydrogen peroxide is subsequently detoxified to water (H₂O) by catalase or glutathione peroxidase. Three SOD isoforms have been identified in mammalian species; cytosolic CuZn-SOD (SOD1), mitochondrial Mn-SOD (SOD2), and extracellular CuZn-SOD (SOD3) (Faraci and Didion, 2004). There is substantial evidence that SOD activity in peripheral blood cells is reduced in the diabetic patients with DN as compared with those without diabetic
complication (Hodgkinson et al., 2003; Bahatia et al., 2003; Colak et al., 2005). In addition, recent studies have implicated SOD1 and SOD2 gene polymorphisms in human DN risk (Mollsten et al., 2007; Al-Kateb et al., 2008).

In patients with diabetes, Angiotensin II (AngII) is believed to play a central role in the progression of renal damage not only through hemodynamic effects but also nonhemodynamic effects, including stimulation of growth factors and cytokines and alterations in extracellular matrix metabolism (Leehey et al., 2000; Manley, 2000). Early animal research demonstrated that in diabetes, angiotensin-converting enzyme (ACE) inhibition reduced proteinuria and this finding has been demonstrated in humans both with and without diabetes mellitus (Beevers and Lip, 2001). Angiotensin receptor blockers (ARBs) provide an efficacious treatment option for the prevention of renal disease progression in patients with hypertension and/or diabetes (Schmieder et al., 2011). Losartan is an Angiotensin receptor blocker (ARB) and has a renoprotective role in hypertensive azotemic patients with type 2 diabetes (Weil et al., 2013). Losartan recruits microvasculature and increases glucose use in muscle. Increased muscle microvascular perfusion is associated with increased muscle delivery and action of insulin (Wang et al., 2013).

Several evidences suggest the potential benefits of the antioxidants in diabetics with cardiovascular complications. Oral supplementation of vitamin E was shown to prevent abnormalities of endothelium-dependent relaxation of the aorta and coronary arteries of STZ-diabetic rats (Keegan et al., 1995). Also, vitamin E supplementation in diabetes was reported to reduce oxidative stress and protect against free radical generation and so block many complications of diabetes (Baydas et al., 2002) that was suggested to be due to protection of the polyunsaturated fatty acids on the membrane from lipid peroxidation, and efficient scavenging of peroxy radicals (Vannucchi et al., 1999). Furthermore, dietary vitamin E was reported to reduce the increased concentration of lipid peroxides in the hepatic tissues of STZ-diabetic rats through decreasing their increased phospholipase A2 activity and phosphatidylethanolamine hydrolysis and, thereby, reduces the accumulation of superoxide radical and decreases the generation of oxidative
damage substances, increases membrane fluidity and lowers oxidative damage (Rhee et al., 2005).

This study was carried out in order to evaluate the prophylactic effect of vitamin E supplementations on diabetic nephropathy and the possible antioxidative and protective action of the angiotensin receptor blocker; losartan in STZ-induced diabetic rats.

2. Materials and Methods

2.1. Materials

Streptzotocin (Sigma- Aldrich Company, USA), presented in powder form, purity more than 99%, dissolved in freshly prepared sodium citrate buffer pH 4.5. Vitamin E available in the form of dl-alpha-tocopheryl acetate soft gelatin capsule (1000 mg) (Pharco, Egypt). Losartan potassium; available in the form of film coated tablets (50 mg) (Unipharma, Egypt). Glucose kits from Spinreact, Spain. Lipid profile kits: Tri glycerides and Total cholesterol kits from Bio Care Company, Egypt; and Creatinine kits from Diamond Company. Primary antibody for Cu,Zn-SOD and the detection kits from New Test Co., Egypt.

2.2. Animals:

This study was conducted on 40 adult male wistar albino rats 6-8 weeks old, weighing between 170 and 200 g. Animals were fed a standard diet and housed in the animal laboratory at the medical research center at Ain Shams faculty of medicine. They were placed at room temperature at 25 c with a 12:12-h light/dark cycle.

2.3. Experimental design:

The animals were randomly divided into 5 groups each consisted of 8 rats. The 1st group acts as a control group injected with citrate buffer only. the 2nd group acts as untreated diabetic group. The 3rd group: Diabetic group received losartan potassium at dose 1mg/kg/day for 8 weeks (Kedziora-Kornatowska, 1999). The 4th group: Diabetic group received vitamin E at a dose of 1 gm /kg/ day for 8 weeks (Je et al., 2001).The 5th: Diabetic group received both vitamin E at a dose of 1gm / kg/day and losertan potassium at dose 1 mg/kg/day orally for 8 weeks.
2.4 Induction and diagnosis of diabetes mellitus:

Diabetes was induced by an intraperitoneal (ip) injection of a single dose of STZ (40 mg/kg in freshly prepared citrate buffer pH 4.5). The animals were allowed to drink 5% glucose solution overnight to overcome drug induced hypoglycemia. Control rats were injected by the buffer alone (Gojo et al., 2007). Diabetes was verified 48-72 hours later by measuring blood glucose levels (after an overnight fasting) with the use of glucose oxidase reagent strips. Rats having blood glucose level of ≥ 300 mg/dl were considered to be diabetic.

2.5 Blood collection and sera preparation

At the end of experiment, blood was collected by direct heart puncture. The blood was left to clot. Serum was separated by centrifugation at 3000 rpm for 15 min and stored at –20°C for biochemical analysis of total triglycerides cholesterol, and creatinine.

2.6 Biochemical analysis

Serum glucose was measured according to Trinder, (1969), total Triglycerides and cholesterol were evaluated by enzymatic colorimetric assay according to Buccolo, (1973) and Natio and Kalpan, (1984) respectively. Creatinine was analyzed according to Murray, (1984).

2.7 Histopathological examination

Just after blood collection, the animals were sacrificed and the kidneys were immediately kept in 10% formalin and then processed to form paraffin blocks from which three sections were cut at 4-5µ to be stained with H&E and Periodic Acid Schiff (PAS) for detection of glomerulo-sclerosis and immunohistochemical staining for examination of superoxide dismutase (SOD).

**Scoring for glomerulosclerosis:**

The glomeruli demonstrating sclerosis were counted and each scored from 0 to 4 according to the percentage of glomeruli involved (0% = 0, 0–25% =1, 25–50%=2, >50%
=3, and global sclerosis = 4). Twenty five glomeruli were counted to get the final score, with a range from 1 to 100 for each case (Mauer et al., 1984).

The procedure of immunostaining was conducted according to the manufacture instructions and the method described by Chang et al., (2005):

The avidin-biotin-peroxidase complex staining method was employed for the immunohistochemical study. The tissue sections were mounted on +ve charged slides and deparaffinized in xylene and dehydrated in a decreasing alcohol gradient. Endogenous peroxidase activity was blocked with 0.3% H2O2 in methanol, followed by washing with standard PBS. The tissues were then blocked with 5% NSA using the Vectastatin ABC/DAB Elite Kit (Mouse IgG type). Excess serum was blotted. A primary antibody specific for human Cu-Zn SOD, ready to use (New Test Co., Egypt) was incubated overnight at 4C. The tissues were washed in PBS and incubated with biotinyl-conjugated secondary antibodies in an immunostaining kit for primary rabbit/mouse antibody. The immunohistochemical stain was performed using the avidin-peroxidase complex technique (ABC kit – Dako- USA). The ABC reaction was developed in the presence of Diamino Benzidine supplement with hydrogen peroxide (DAB). Lastly, sections were counterstained with Mayer's Hematoxylin. Sections were then studied with an optical photomicroscope (Olympus, Japan).

**Scoring for immunostaining:**

Glomerular staining was graded in each glomerulus semiquantitatively, and each was scored from “0 to 4+” according to the extent of staining (0=0–5%; 1=6–25%; 2=26–50%; 3=51–75% 4=>75%). Twenty five glomeruli were counted to get the final score, with the final score range from 1 to 100 for each case (Changet al., 2005).

**2.8 Statistical analysis**

The collected data of the present study were tabulated and analyzed using the student “t” test and paired "t" test according to Armistag, (1983) using computer with SPSS version 16 programme. Statistical significance was accepted at P value <0.05 or lower.
3. Results

The effects of STZ-diabetes induction as well as modulating effects of Losartan or/and vitamin E are shown in table 1. It was observed that there is a significant increase in serum glucose level as it was changed from 119.67±3.44 in control group to 601.83±9.3 in diabetic group (p < 0.001). Moreover, there is a significant increase in serum creatinine as it was changed from 0.483±0.036 in control group to 0.717±0.089 in diabetic group (p<0.001). Furthermore there is a significant increase in serum triglycerides as it was changed from 91.8±11.33 in control group to 423.5±9.09 in diabetic group (p< 0.001) and a significant increase in serum total cholesterol as it was changed from 54.88±0.69 in control group to 115.8±15.76 in diabetic group (p < 0.001).

Oral losartan treatment at dose of 1mg/kg significantly lowered the serum glucose level as it was changed from 601.83±9.3 in diabetic group to 308.0±232.54 in diabetic group received losartan (p <0.011). Moreover, there is a significant decrease in serum creatininne as it was changed from 0.717±0.089 in diabetic group to 0.495±0.076 in diabetic group received losartan (p <0.001). Furthermore, there is a significant decrease in serum triglycerides as it was changed from 423.5±9.09 in diabetic group to 180.02±169.42 in diabetic group received losartan (p< 0.006) and there was a significant decrease in serum total cholesterol as it was changed from 115.8±15.76 in diabetic group to 86.75±11.52 in diabetic group received losartan (p < 0.004).

Oral vitamin E treatment at dose of 1gm/kg significantly lowered the serum glucose level as it was changed from 601.83±9.3 in diabetic group to 219.83±90.15 in diabetic group received vitamin E. Moreover, there is a significant decrease in serum creatininne as it was changed from 0.717±0.089 in diabetic group to 0.468±0.141 in diabetic group received vitamin E.(p <0.004), Furthermore, there is a significant decrease in serum triglycerides as it was changed from 423.5±9.09 in diabetic group to 77.92±16.11in diabetic group received vitamin E.(p< 0.001) and there is a significant
decrease in serum total cholesterol as it was changed from 115.8±15.76 in diabetic group to 53.33±11.97 in diabetic group received vitamin E (p < 0.001).

Treatment of diabetic rats with both losartan at dose of 1mg/kg and vitamin E at dose of 1gm/kg significantly reduced serum glucose, creatinine, triglycerides and total cholesterol levels compared to the diabetic non-treated group. Moreover, it was noticed that treatment with both losartan and vitamin E had no additive effects compared to treatment with each of them alone.

Histopathological results (score of glomerulosclerosis using H&E and PAS) of STZ induced diabetic group and other groups studied are shown in table (2). There was a significant increase in the score of glomerulosclerosis in diabetic group (8.2±0.9) compared to other groups, however the administration of vit E alone or with Losartan had a significant lowering effect (p<0.001) on glomerulosclerosis (3.8±0.8 and 4.9±1.1 respectively) than administration of Losartan alone (6.2±1.3)

Immunohistochemical results of CuZnSOD examination in different groups studied are shown in table (3). Diabetic group showed significant increase (p<0.001) in the level of CuZnSOD (35.4±3.5) than other groups that received vit E alone (10.3±1.6); Losartan alone (10.9±1.2) or both vit E and Losartan (11.2±9) which are near each other and near the level of CuZnSOD in the control group (10±1.7).
4. Discussion

In diabetic patients with renal complications, there are significant changes such as increased lipid peroxidation, dyslipidemia and irregularities in the metabolism of proteins, carbohydrates and lipids. Increased lipid peroxidation is one of the main causes of diabetic complications (Alwakeel et al., 2009). There are two common mechanisms that lead to increased oxidative stress in diabetes; the first is the increased free radical production during elevated auto-oxidation of glucose, and the second is the hyperglycaemia-induced reduction in the levels of protective endogenous antioxidants such as vitamins A, E, C and glutathione and the decrease in the activities of antioxidant enzymes such as glutathione peroxidase (GSHpx) (Ceriello et al., 1997). Studies showing that treatment with antioxidants may prevent diabetes and hyperglycaemia-induced nephropathy suggest that oxidative stress is a major factor in the development of diabetic renal complications (Baburao Jain and Anand Jain, 2012).

In our study a single intraperitoneal injection of streptozotocin (STZ) at a dose of 40 mg/kg resulted in development of type1diabetes, the dose of STZ lies within the range to produces moderate diabetes, in which blood glucose levels are 4-5 times normal, by causing substantial but incomplete depletion of pancreatic insulin (Junod et al., 1969). STZ causes cellular toxicity and local immune responses in the β cells of the pancreas leading to its destruction (Bolzan and Bianchi, 2002).

The elevation of serum creatinin levels was reported by Kamper et al., (2010) who considered that the elevated creatinin in STZ diabetic rats is a significant markers of diabetic nephropathy.

A positive correlation between hyperglycaemia and the development of nephropathy in diabetic patients demonstrated by Ceriello et al., (2000) who reported that hyperglycemia and dyslipidemia in uncontrolled diabetes are important causal factors in the development and progression of diabetic kidney disease and that multiple biochemical mechanisms may explain the adverse effect of them on renal function. Oxidative stress results from hyperglycemia and dyslipidemia, which promote the
formation of advanced glycation end products (AGE) as well as protein kinase C (PKC) leading to increase the formation of different cytokines such as TGF-β, TNF-α, CTGF and interleukins which play a role in the pathogenesis of the diabetic nephropathy leading to a significant increase in serum urea and creatinin levels in diabetic rats. By examining the glomeruli of the diabetic rats using PAS stain there was thickening of the glomerular basement membrane and mesangial expansion indicating class (IIb) glomerulosclererosis, the average score of glomerulosclerosis in diabetic rats was significantly higher than that of control rats (p< 0.001), also there was a significant increase in the glomerular Cu, Zn-SOD protein in glomeruli as assessed by the immunostaining than control group (p < 0.001). In a study carried out by Ichihara et al., (2006), the glomerulosclerosis of diabetic rats was reported to be progressive on using PAS stain in STZ diabetic rats at 8 to 24 weeks after induction of diabetes , in contrast Kitching et al., (2000) stated that there was no significant difference in the glomerular histology between control and STZ diabetic rats on using PAS stain. Kitching et al., (2000)and Chang et al., (2005) found that the glomerular Cu, Zn- SOD is increased in STZ diabetic rats and type 2 diabetic patients. This increase could be explained by the fact that the SOD is an inducible antioxidant enzyme that induced for protecting the glomeruli against the enhanced lipid peroxidation status. Sechi et al., (1997) and Sharma et al., (2006) found an increasing in Cu, Zn- SOD mRNA levels in renal tissue in diabetic rats, so the SOD can be used as a marker of oxidative stress. The up regulation of SOD isoenzymes in STZ rats supports the hypothesis that there is increased oxidant stress in experimental DN and that (ROS) play an important role in tissue damage associated with diabetes.

Eight weeks after induction of diabetes, at the end of the experiment, diabetic rats showed significant increase in the fasting serum glucose levels, creatinin level, triglycerides and total cholesterol when compared with control rats.

Angiotensin II (Ang II) is a major mediator of progressive renal injury. Some observations suggest local renal tissue activation of the renin angiotensin aldosterone
system (RAAS) or increased intra-renal sensitivity to Ang II in DM (Lewis, 2002). Apart from the Ang II hemodynamic actions include the generation of reactive oxygen species (ROS) (Asaba et al., 2005).

By studying the effect of prophylactic treatment with angiotensin II receptor blocker (ARB), losartan potassium, there was a significant decrease in the serum creatinine, triglycerides and total cholesterol when compared with the diabetic rats received no treatment. Our results about the hypoglycemic effect of losartan was in agreement with (Chan et al., 2003) who stated that the treatment with ARB decreased the plasma glucose level in STZ diabetic rats due to partial inhibition of the sodium-glucose cotransporters (SGLTs) in the renal proximal tubular cells leading to decrease the glucose reabsorption in the renal tubules, in addition to enhancing the glucose utilization in peripheral tissues and reduction of hepatic gluconeogenesis via non-insulin mediated mechanisms. Tikellis et al., (2004) also reported that there was an increase in β-cell mass of the pancreas after losartan treatment and this could be due to increased proliferation rates of the residual β-cells that escaped the STZ toxic effect, β-cell differentiation from exocrine progenitors (neogenesis), a reduction in β-cell apoptotic rates, or the combined action of the all 3 mechanisms. The significant decrease in serum creatinine observed in our results is in agreement with Murali and Goyal, (2001) who stated that treatment with losartan (2 mg/kg) significantly prevented the raise in creatinine and that treatment with losartan significantly increased creatinine clearance in STZ-diabetic rats. Also, Remuzzi et al., (2004) reported that Losartan treatment uniformly decreased the risk of ESRD in patients with type 2 diabetes, this is may be explained by the fact that losartan affects the ROS levels indirectly via lowering the blood glucose levels, also the vasoactive Ang-II also has proinflammatory and profibrogenic actions and is implicated in the pathogenesis of diabetic nephropathy which will be blocked by losartan and that losartan treatment significantly reduces MDA levels and thus improves NO bioavailability for endothelial cells, thus improving endothelial function (Kamper et al., 2010). On examining the glomeruli of the diabetic rats received the losartan using PAS stain the average score of glomerulosclerosis was significantly lower than score diabetic rats received no treatment.
(P<0.01) indicating that the losartan had a renoprotective effect and prevent the progression of glomerulosclerosis in experimental diabetic nephropathy. These results are in agreement with (Lee et al., 2011) who showed that there was a decrease in the score of glomerulosclerosis in type 2 diabetic rats on using losartan for 25 weeks by reducing blood pressure and by decreasing inflammatory and sclerosing effects of Ang II, also the immunostaining score for Cu, Zn- SOD protein in glomeruli was decreased significantly than that diabetic rats (p<0.001) this could be explained by the decrease in the production of ROS in the kidney on using losartan.

In the present work, prophylactic treatment with vitamin E significantly lowered the serum glucose, creatinine, serum triglycerides, and total cholesterol levels when compared with the diabetic rats received no treatment. These results were in agreement with (Jain et al., 1996) who reported that vitamin E supplementation (100 IU/day) for 3 months can significantly lower glycated hemoglobin (GHb) in type 1 diabetic patients. The glomeruli of the diabetic rats received the vitamin E showed significant decrease in the glomerulosclerosis score than the diabetic rats received no treatment (p<0.001) indicating that the vitamin E had a renoprotective effect and prevent the progression of glomerulosclerosis in experimental diabetic nephropathy as discussed earlier. The immunostaining score for Cu, Zn- SOD protein in the glomeruli was significantly decreased than that diabetic rats received no medical treatment (p<0.001). This could be explained by the decrease in the production of ROS in the kidney on using vitamin E which is mediated through its protection of cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction. This would remove the free radical intermediates and prevent the oxidation reaction from continuing (Traber et al., 2011).

In the present study by using losartan potassium with the vitamin E at the selected doses and regimen together resulted in significant decrease in the serum glucose, creatinine, triglycerides and total cholesterol levels when compared with the diabetic rats.
received no treatment, but there no additive effects noticed compared with the groups treated with either losartan potassium or vitamin E alone.

There was not a significant difference between the effect of treatment with either losartan or vitamin E alone on the serum levels of glucose, creatinine or triglycerides. However there was a significant decrease (p<0.001) in serum total cholesterol level more with vit E treatment, indicating that the vitamin E has a better lowering effect on the total cholesterol than losartan. As regarding glomerulosclerosis score in both groups there was a significant decrease in the group received vitamin E when compared with the group received losartan ( p<0.001) indicating that the vitamin E has a better protective effect on the kidney than losartan which may be due to better lowering effect of the vitamin E on the cholesterol than losartan as the hypercholesterolemia is considered as a contributing factor to the progression of renal failure (Paczek et al., 1997). Vitamin E block many complications of diabetes by reducing oxidative stress and, hence, protects from oxidative damage and dyslipidemia (Baydas et al., 2002). Vitamin E has a potential to protect partially from hyperglycaemia-induced endothelial dysfunction as it protects against free radical generation and acts as an efficient scavenger of radicals (Dhein et al., 2003). There was no significant difference in the level of Cu,Zn –SOD immunostaining in both groups.

In the current study no significant difference was found in the levels of serum glucose, creatinine, triglycerides, total cholesterol levels in the two groups that treated with either vitamin E alone or both losartan and the vitamin E. Also non- significant decrease in the scores of glomerulosclerosis or immunostaining of the Cu,Zn –SOD was found, indicating that there was no additive effect of both drugs together.

There was no significant decrease in the serum glucose, creatinine, triglycerides levels in the groups treated by either Losartan alone or Losartan with vit E. However there was a significant decrease (p<0.001) in serum total cholesterol level in the group treated with both losartan and vit E, indicating that the treatment with vitamin E with
losartan has a better lowering effect on the total cholesterol than losartan alone. There was a significant decrease in score of glomerulosclerosis in the group received both vitamin E and losartan when compared with the group received losartan only (p<0.025) indicating that the both vitamin E and losartan together had a better protective effect on the kidney than losartan alone. These results may be due to the protective effect of vitamin E. however there was no significant difference in the score of Cu,Zn –SOD immunostaining.

5. Conclusion

We concluded that the oxidative stress play a role in the development of the diabetic nephropathy in experimental diabetic rats. The vitamin E administration had a renoprotective effect as it acts as an antioxidant, also the angiotensin II receptor blocker had a prophylactic effect on the kidney which may be mediated through its antioxidant effect, however it is being less than that of the vitamin E and the combination between the vitamin E and the angiotensin II receptor blocker had no additive effect on the kidney function.
References


Losartan on Diabetic Nephropathy in a Type 2 Diabetic Rat Model. Diabetes Metab J.; 35(2): 130–137.


### Tables and figures:

**Table 1:** Effect of Losartan or/and vitamin E on serum biochemical parameters in STZ-induced diabetic rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>54.88±0.69</td>
<td>91.8±11.33</td>
<td>0.483±0.036</td>
<td>119.67±3.44</td>
</tr>
<tr>
<td>Diabetic</td>
<td>115.8±15.76</td>
<td>423.5±9.09</td>
<td>0.717±0.089</td>
<td>601.83±9.3</td>
</tr>
<tr>
<td>Diabetic + Losartan</td>
<td>86.75±11.52</td>
<td>180.0±169.4</td>
<td>0.495±0.076</td>
<td>308.0±232.5</td>
</tr>
<tr>
<td>Diabetic + Vitamin E</td>
<td>53.33±11.97</td>
<td>77.92±16.11</td>
<td>0.468±0.141</td>
<td>219.83±90.15</td>
</tr>
<tr>
<td>Diabetic + Losartan + Vitamin E</td>
<td>53.1±8.55</td>
<td>94.93±33.99</td>
<td>0.471±0.044</td>
<td>215.67±167.76</td>
</tr>
</tbody>
</table>

STZ= streptozotocin  
D= Diabetic  
L= Losartan  
E= Vitamin E  
value; (Mean ± SD)
**Table 2:** Effect of Losartan or/and vitamin E on Hematoxylin and Eosin stain (H&E) and periodic acid Schiff (PAS)-stained renal sections in STZ-induced diabetic rats. (Score of glomerulosclerosis)

<table>
<thead>
<tr>
<th>Group</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.4 ± 0.5</td>
</tr>
<tr>
<td>Diabetic</td>
<td>8.2 ± 0.9</td>
</tr>
<tr>
<td>Diabetic + Losartan</td>
<td>6.2 ± 1.3</td>
</tr>
<tr>
<td>Diabetic + Vitamin E</td>
<td>3.8 ± 0.8</td>
</tr>
<tr>
<td>Diabetic + Losartan + Vitamin E</td>
<td>4.9 ± 1.1</td>
</tr>
</tbody>
</table>

There was a statistical significant difference in the score of glomerulosclerosis between the studied groups, being the largest in diabetic groups with the best improvement was observed with vit E administration (p<0.001).
Table 3: Effect of Losartan or/and vitamin E on immunostaining for detection of Cu,Zn SOD in the glomeruli in renal sections in STZ-induced diabetic rats. (Score of immunostaining)

<table>
<thead>
<tr>
<th>Group</th>
<th>score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 ± 1.7</td>
</tr>
<tr>
<td>Diabetic</td>
<td>35.4 ± 3.5</td>
</tr>
<tr>
<td>Diabetic + Losartan</td>
<td>10.9 ± 1.2</td>
</tr>
<tr>
<td>Diabetic + Vitamin E</td>
<td>10.3 ± 1.6</td>
</tr>
<tr>
<td>Diabetic + Losartan + Vitamin E</td>
<td>11.2 ± 0.9</td>
</tr>
</tbody>
</table>

There was a significant increased in the expression of CuZN SOD in the glomeruli of diabetic groups (p<0.001). However there was no significant difference between score of its expression in the other groups treated (P>0.05).
Figure (1): Light microscopy demonstration of glomeruli of wistar albino rats as shown by the periodic acid-Schiff (PAS)-stained sections show the development and progression of diabetic glomerulosclerosis in diabetic rats (the arrows pointing to glomerular basement membrane and mesangial cells) A) Control group. B) Untreated STZ diabetic rats. C) Diabetic rats received losartan. D) Diabetic rats received vitamin E. E) Diabetic rats received losartan and vitamin E. (PAS stain x200).

N.B: arrow with one head points to the mesangial expansion and arrow with two heads points to the basement membrane thickening.
Figure (2): Light microscopy of immunohistochemical demonstration of Cu,Zn-SOD in glomeruli of Wistar albino STZ diabetic rats showing significant changes (the arrows pointing to the Cu,Zn-SOD in the mesangial cells of the glomeruli).  
وظائف الكلى في فئران التجارب المحدث بها مرض السكرى من النوع الأول:

دور مضادات الأكسدة ومانع مستقبل الإنجيوتينسين II

يعتبر داء السكري مشكلة صحية كبيرة وقد ازداد بشكل كبير في جميع أنحاء العالم. هذا ويتميز داء السكري بمضاعفات تؤثر على عدة أجهزة، بما في ذلك الكلى، هذا وقد أصبح داء السكري في العديد من البلدان السبب الأكثر شيوعًا لمرحلة المرض الكلي. هذا وقد اعتبر مؤخراً ان الأكسدة التي تنتج عن احتلال التوازن بين انتاج أنواع الأكسجين التفاعليه والميات الدفاع مضاده للأكسدة، سبب ضائع في اعتلال الكلى السكري، نتيجة إنتاج أنواع الأكسجين التفاعليه التي ينتج عنها السيتوكيتات وعوامل النمو التي تشارك في التسبب في اعتلال الكليه السكري.

وقد أجريت هذه الدراسة لتوضيح دور أنواع الأكسجين التفاعليه في اعتلال الكليه السكري ودراسة الأثر الوقائي للفيتامينات (E) على اعتلال الكليه السكري في الحيوانات المصابه بداء السكري وتأثيرات المضادة للأكسدة المحتملة لمانع مستقبلات الأنجيوتينسين II.

أجريت هذه الدراسة على خمس مجموعات من ذكور فئران التجارب البيضاء البالغه اولى هذه المجموعات لم تتولن أي عقاقير، أما الثانية فقد حفظت داخل الغشاء البريدي بجرعه واحدة (40مجم/كم) من مادة الاستربينزوتوسين لاحثد النوع الأول من داء السكري، المجموعة الثالثة وقد تتولن أفرادها عقار اللوزراتن بجرعه مقدارها (1مجم/كم) يوميا لمدة 8 اسابيع والمجموعة الرابعة وقد تتولن أفرادها عقار الفيتامينات E بجرعه مقدارها (1جم/كم) يوميا لمدة 8 اسابيع. المجموعة الخامسة وقد تتولن أفرادها عقار اللوزراتن بجرعه مقدارها (1مجم/كم) وفاتامين E بجرعه (1جم/كم) يوميا لمدة 8 اسابيع.

وقد تم قياس مستوى السكر والدهون الثلاثيه والكوليسترول الكلي في الدم لتقييم العواقب الإيجابية لداء السكري كما تم تقييم وظيفة الكلى على طريق قياس مستوى الكرياتينين في الدم والفحص التشريحي المرضي للكل. والفحص الكيميائي المناعي للسوبر أكسيد ديمسيتروزت لتقييم الأكسدة داخل الكليه.
و يمكن تلخيص نتائج هذه الدراسة كالاتي: أدى حقن الوريد بجرعة مقدارها 40 مجم/كم من النوع الأول وتظلي هذا بظهور زيادة محلوز في مستوى السكر في الدم الذي كان محصبآ أيضاً بزيادة في مستوى الكرياتين، الدهون الثلاثية والكوليسترول الكلي في الدم. هذا وعند اجراء فحص لكبيبات الكلى في الفنارين المحدث بها داء السكرى وجد أنه هناك تصلب قد حدث بها من الدرجة الثانيه وكذلك عند اجراء الفحص المناعي للسوبر اكسيد ديسمونتاز وجود أن هناك زيادة داخل كبيبات الكلى و الذي يستخدم كدليل على زيادة الأكسدة التفاعليه في الكليه.

وعند دراسة التأثير الوقائي لدواء اللوزراتن بجرعة مقدارها (1مجم/كم) لمدة ثمانية أسابيع لدى هذا الي انخفاض محلوز في مستوى السكر و الكرياتين و الدهون الثلاثية والكوليسترول الكلي في الدم وكذلك إلى تراجع في تصلب كبيبات الكلى و نقص في مستوى السوبر اكسيد ديسمونتاز في كبيبات الكلى.

و بدراسة التأثير الوقائي لفيتامين ه بجرعة مقدارها (1مجم/كم) لمدة ثمانية أسابيع لدى هذا الي انخفاض محلوز في مستوى السكر و الكرياتين و الدهون الثلاثية والكوليسترول الكلي في الدم. وكذلك إلى تراجع أكثر في تصلب كبيبات الكلى و نقص في مستوى السوبر اكسيد ديسمونتاز في كبيبات الكلى.

ووجد أنه عند الجمع بين العقرين اللوزراتن (1مجم/كم) و الفيتامين ه (1 جم/كم) معاً لمدة 8 أسابيع ادى هذا إلى انخفاض محلوز في مستوى السكر و الكرياتين و الدهون الثلاثية والكوليسترول الكلي في الدم. وكذلك إلى تراجع في تصلب كبيبات الكلى و نقص في مستوى السوبر اكسيد ديسمونتاز في كبيبات الكلى.

وعند مقارنة تأثير التثبيبات الوقائي للوزراتن و فيتامين ه و العقرين معاً لم يحدث أي نقص محلوز في مستوى السكر أو الكرياتين أو الدهون الثلاثية ولكن يوجد هنا نقص محلوز في الكوليسترول الكلي عند استخدام فيتامين ه (ه) بمفرده أو عند استخدام فيتامين ه مع اللوزراتن مما يشير إلى أن فيتامين ه له تأثير أكبر على نقص مستوى الكوليسترول في الدم. وعند مقارنة تصلب الكبيبات في المجموعات الثلاثة وجد أن تأثير فيتامين ه (ه) بمفرده أو عند اعطائه مع اللوزراتن ادى إلى تراجع محلوز في تصلب كبيبات الكلى غير أنه لم يوجد أي فرق محلوز بين مستوى السوبر اكسيد ديسمونتاز في كبيبات الكلى للمجموعات الثلاثة.
ومن هذه النتائج نستخلص أن الاكسدة التفاعليه تلعب دورا في اعتلال الكليه السكري و أن هناك دور وقائي لاستخدام للوزراتن و فيتامين (E) على تطور اعتلال الكليه السكري ولكن يبدو أن دور فيتامين 
(5) أكثر فاعليه من الوزراتن وان استخدام العقاران معا لم يودي إلى احداث اي زيادة ايجابيه للنتائج.