

Altered Kidney Morphology and Enzymes in Streptozotocin Induced Diabetic Rats

Morfología y Enzimas Renales Alteradas en Ratas con Diabetes Inducida por Estreptozotocin

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SUMMARY: We studied the effects of streptozotocin (STZ)-induced diabetes on kidney morphology, anatomy, architecture and on the activities of aminotransferases (AST and ALT), alkaline phosphatase (ALP) and pseudocholinesterase (PChE) in albino rats. The aim of the study was to investigate the association between diabetic kidney complications and kidney enzyme alterations. This study was performed in the Department of Anatomy and Institute of Pharmaceutical Sciences, Baqai Medical University, Karachi and Pathology department of College of Physicians & Surgeons (CPSP) Pakistan in 2007-08. Diabetes was induced by a single dose of STZ (45 mg/kg, b.w.) given intraperitoneally in sodium citrate buffer at pH 4.5. Eighty (80) albino rats were divided into five groups: control (A) and STZ treated (B, C, D, and E) which were sacrificed 2, 4, 6 and 8 weeks post treatment respectively. Histopathology of kidney showed lesions similar to human glomerulosclerosis, glomerular membrane thickening, arteriolar hyalinization and tubular necrosis. Increased levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and pseudocholinesterase (PChE) were observed in the kidney. It seems that the diabetic complications in the kidney are likely to be associated with alterations in enzyme levels.

KEY WORDS: Streptozotocin; Aminotransferases; Alkaline phosphatase; Pseudocholinesterase; STZ-diabetes; Rats.

INTRODUCTION

Streptozotocin (STZ) is a naturally occurring nitrosourea with molecular weight of 265 and empirical formula of C₁₄H₂₇N₅O₁₂ (Dorr & Fritz, 1980). It is widely used to induce insulin-dependent diabetes mellitus in experimental animals because of its toxic effects on islet beta cells (Punithavathi *et al.*, 2008; Fadillioglu *et al.*, 2008). The diabetogenic action of STZ is the direct result of irreversible damage to the pancreatic beta cells resulting in degranulation and loss of capacity to secrete insulin (Gu *et al.*, 1997). The effects of STZ on different organs have been extensively studied. STZ has various biological actions, including the production of acute and chronic cellular injury, carcinogenesis, teratogenesis and mutagenesis (Magee & Swann, 1969). STZ is a nitrosourea compound which generally shares similar fate of disposition with other nitrosoureas and is a drug of choice in islet cell carcinoma and malignant carcinoid tumors. It is diabetogenic, hepatotoxic, nephrotoxic and also causes gastric ulceration (Piyachaturawat *et al.*, 1988; 1990). STZ given

intravenously or intraperitoneally to laboratory mice in multiple sub-diabetogenic doses, induces pronounced pancreatic insulinitis with eventual destruction of insulin-secreting beta cells and diabetes mellitus. In an experimental study in rats, streptozotocin given intraperitoneally in a dose of 45 mg/kg body weight of animals, effectively produced hyperglycaemia (Punithavathi *et al.*; Fadillioglu *et al.*). In another study in rats, STZ injected in a dose of 65 mg/kg body weight effectively produced hyperglycemia and gastric mucosal ulcerations (Piyachaturawat *et al.*, 1988; 1990). The incidence and severity of lesions produced by STZ in pancreas, liver, kidney and GIT, progressively increased with time from one to six weeks post treatment (Piyachaturawat *et al.*). Studies have shown an association between specific diabetic complications and liver enzyme alterations (Arkila *et al.*, 2001) but only limited data is available on the possible association between diabetic nephropathy and kidney enzyme alterations (Ramesh *et al.*, 2007).

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MATERIAL AND METHOD

This study was performed in the Department of Anatomy and Institute of Pharmaceutical Sciences, Baqai Medical University, Karachi and Pathology department of College of Physicians & Surgeons (CPSP) Pakistan in 2007-08. STZ was obtained in powder form from Sigma Chemical Company, St.Louis, USA.

Immediately before use, STZ was dissolved in 10 mM sodium citrate buffer, pH 4-4.5, made isotonic by the addition of an appropriate volume of 0.25 M NaCl (Bennett & Pegg, 1981). After taking approval from the ethical committee, a total of 80 adult male albino rats (body weight 100-200 gm) of Jinnah Postgraduate Medical College (J.P.M.C) strain, originally obtained from Charles River Breeding Laboratories Brooklyn, Massachussets, USA were used in the study. All rats were housed in metabolic cages on a 12-h light/dark cycle at a temperature of 22-24°C (Petlevski *et al.*, 2006). The animals were divided into five groups; A, B, C, D, and E. There were 40 animals in group 'A' while groups B, C, D, and E each comprising of 10 animals. The group 'A' was control and groups B, C, D, and E were treated with STZ. After an overnight fast, the animals of groups B, C, D, and E were injected with a single dose of STZ (45 mg/kg, b.w.) intraperitoneally in sodium citrate buffer at pH 4.5 (Punithavathi *et al.*; Fadillioglu *et al.*; Ramesh, B. & Pugalendi, 2006). After the administration of STZ, the animals were given 1% sucrose solution to prevent hypoglycemia. Plasma glucose was estimated before the administration of STZ, after 24 hours and at the end of 2nd, 4th, 8th and 12th weeks by taking blood samples from the tail vein (Petlevski *et al.*; Thulesen *et al.*, 1997). Animals in group 'A' (control) were administered sodium citrate buffer pH 4.5 in a dose of 0.2 ml/100 grams body weight, intraperitoneally. After an overnight fast, the animals of group B, C, D and E were sacrificed at the end of 2nd, 4th, 8th and 12th weeks respectively while ten animals from the control group were sacrificed at the end of 2nd, 4th, 8th and 12th weeks respectively (Ren *et al.*, 2009). The kidneys were identified and observed for any gross appearance and color change with magnifying glass and tissues were preserved for histopathological studies and enzymatic estimation. For histopathology, the kidney tissues were immediately transferred to 10 % formal saline for paraffin embedding and staining with Hematoxylin & Eosin (H&E), Periodic Acid-Schiff (PAS) (Malatiali *et al.*, 2008) and Masson's trichrome and Gomori's methanamine silver nitrate (G.M.S.) stains. For enzyme estimation, kidney tissues were immediately excised, washed in ice-cold saline, blotted on tissue paper and immediately transferred to phosphate buffer (pH 7.4) for preservation. Kidney tissues were homogenized (100g L⁻¹) in cold (0.14 mol L⁻¹) KCl

using a Teflon homogenizer (Measuring & Scientific Equipment, UK). Homogenates were centrifuged in labofuge 15000 centrifuge at temperature 4°C and 5000 rpm for 20 minutes, placed in cold chambers. Supernatants were stored at -20°C until analysis (Petlevski *et al.*). AST and ALT activities were determined by the colorimetric method using Randox Diagnostic No. 146 kit. Activity of ALP was determined spectrophotometrically by AMP method using Roche diagnostic kit 396460. Whereas, PChE activity was determined by the colorimetric method using a commercial kit no. CE190.

In statistical analysis, data are shown as means ± S.E.M. The statistical significance of the changes in enzyme levels of control and STZ treated animals were evaluated by using the ONE-WAY ANOVA test of variance. The difference in enzyme levels was regarded as statistically significant if the F-value was greater (P value < 0.05) and was regarded as statistically non-significant if the F-value was lesser. Sigma Stat program for Windows, version 2.0 (Jandel Corporation, USA), was used for statistical analysis.

RESULTS

The gross examination showed no changes in the color on the external surface of the kidneys taken from animals in groups B, C, D and E.

GROUP-'B': Sections of kidneys from group 'B' showed no signs of pathology in the cortex and the medulla.

GROUP-'C': Some of the sections of kidneys from group 'C' showed mild infiltration of the lymphocytes in the interstitial spaces. Some of the glomeruli appeared to be distorted and slightly expanded. There were signs of tubular necrosis with loss of their brush border in some of the sections of proximal convoluted tubules as shown on Figures 5 and 7.

GROUP-'D': In about 1/3 of sections of group-'D', lymphocytic infiltration was pronounced and large aggregates of lymphocytes were seen in the interstitium. Variable number of proximal convoluted tubules showed signs of tubular necrosis. The epithelial lining cells were disrupted with pyknotic nuclei, vacuolated cytoplasm, broken membranes and loss of brush border. Variable number of glomeruli showed foci of hyalinized and eosinophilic deposits in the extracellular mesengium in the form of lobule causing mesengial expansion as shown in Figures 1, 4 and 6. Some of the arterioles at the vascular poles also showed hyaline change as shown in Figure 2. Glomerular membrane also appeared to be affected by hyaline change.

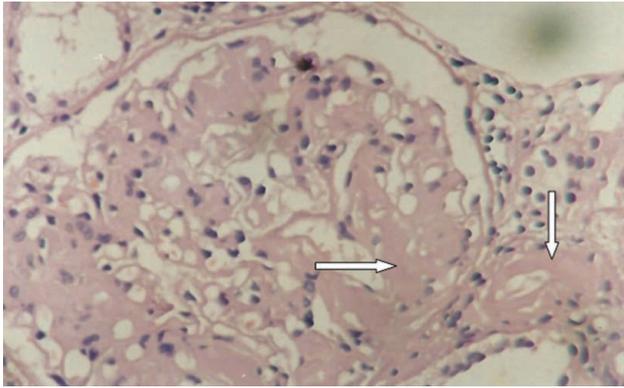


Fig. 1. Photomicrograph of 3 microns thick H & E stained paraffin section from the cortex of kidney in a streptozotocin treated rat (group-E) showing a glomerulus with severe nodules in diabetic glomerulosclerosis (right arrow) & a hyalinized arteriole (down arrow). x 400.

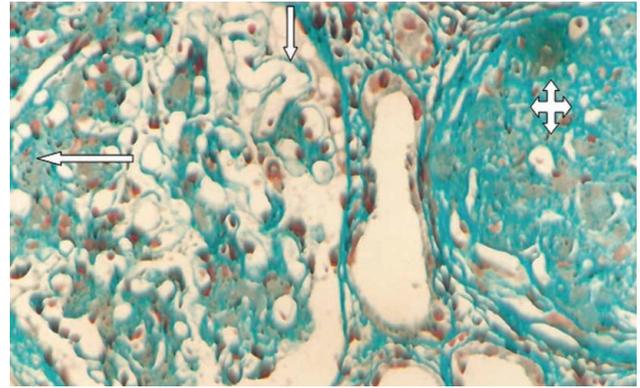


Fig. 4. Photomicrograph of 3 microns thick Mason's Trichrome stained paraffin section from the cortex of kidney in a streptozotocin treated rat (group-D) showing a glomerulus with diffuse glomerulosclerosis (quad arrow), increased mesangial matrix (left arrow) and thickening of capillary wall (down arrow). x 400.

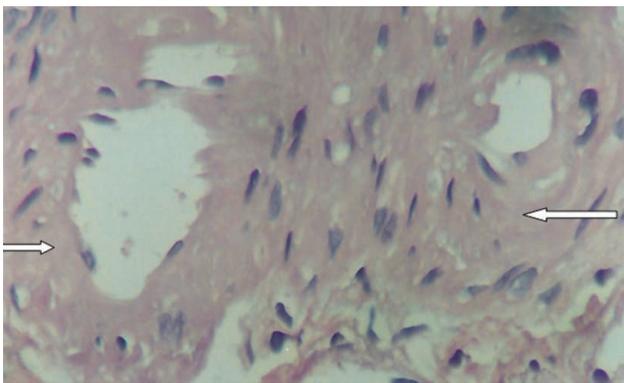


Fig. 2. Photomicrograph of 3 microns thick H & E stained paraffin section from the cortex of kidney in a streptozotocin treated rat (group-E) showing thickening (hyalinization) of blood vessels (arrows). x 400.

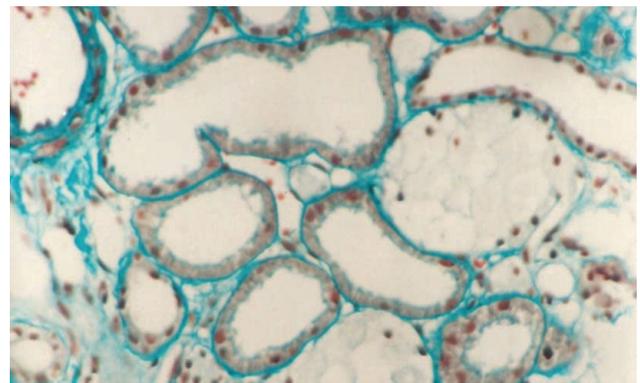


Fig. 5. Photomicrograph of 3 microns thick Mason's Trichrome stained paraffin section from the medulla of kidney in a streptozotocin treated rat (group-E) showing tubular atrophy and glycogen vacuolization of renal tubular epithelial cells. X 400.

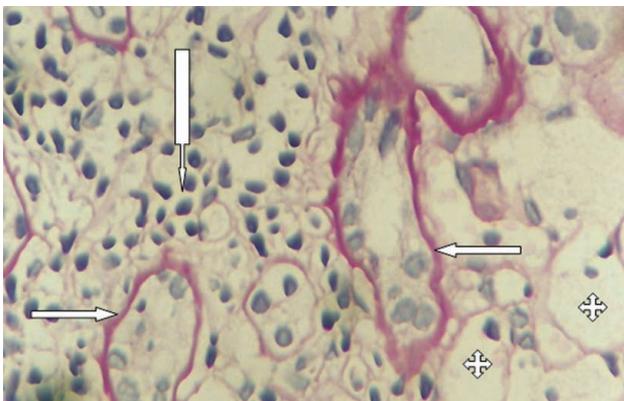


Fig. 3. Photomicrograph of 3 microns thick PAS stained paraffin section from the medulla of kidney in a streptozotocin treated rat (group-E) showing diffuse thickening of tubular basement membrane (arrows), lymphocytic infiltrate (down arrow) and tubular atrophy (quad arrows). x 400.

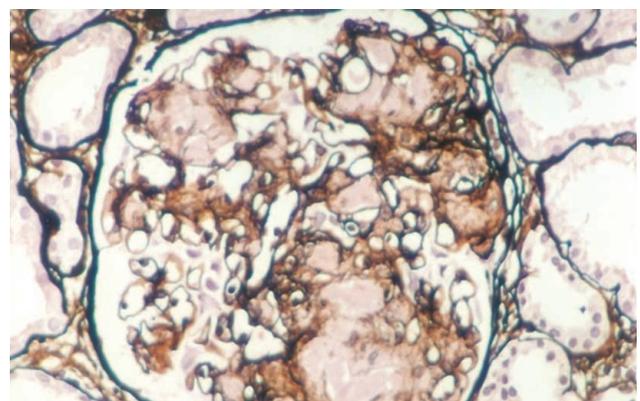


Fig. 6. Photomicrograph of 3 microns thick G.M.S. stained paraffin section from the cortex of kidney in a streptozotocin treated rat (group-E) showing a glomerulus with diabetic nodular glomerulosclerosis and thickening of the capillary wall. x 400.

GROUP-‘E’: Most of the kidney sections from group ‘E’ showed signs of advanced nephrotoxicity. In most of the glomeruli, there were deposits of hyaline material in the mesangium of the lobules of the glomerulus. In many of the glomeruli, the hyaline deposits were diffusely and evenly spread throughout the glomerulus. There was a diffuse infiltration of the glomerular tuft with eosinophilic material and also heavy focal deposition as shown in Figures 1, 4 and 6. The diffuse infiltrate appeared to be in the basement membranes of the capillaries, and the capillary bed had been obliterated in places. In place, this deposition caused complete hyalinization of many glomeruli as shown in figure 1. In many of the glomeruli, lesions characteristic of diabetic

glomerulosclerosis were present. It consisted of round, practically acellular, hyalinized nodule in the glomerular tuft. There was a considerable deposit of hyaline material thickening in Bowman’s capsule as shown in figure 6. Both afferent and efferent arterioles appeared as narrow ovals and also showed quite marked strongly eosinophilic hyaline thickening as shown in Figure 2. The nodules were scattered among some non-affected normal glomeruli which is a characteristic of diabetes mellitus. In most of the sections, areas of lymphocytic infiltrate were seen in the interstitium as shown in figure 3. Widespread tubular necrosis, thickening of tubular basement membrane, with sloughing of the epithelial cells was also present as shown in Figures 3, 5 and 7.

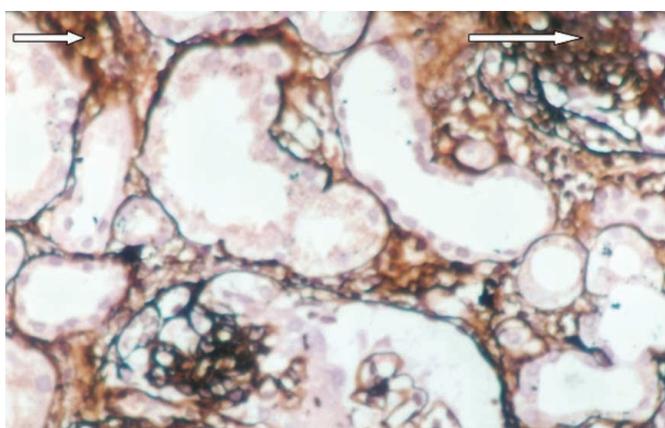


Fig. 7. Photomicrograph of 3 microns thick G.M.S. stained paraffin section from the medulla of kidney in a streptozotocin treated rat (group-E) showing tubular atrophy and interstitial fibrosis (right arrows). x 400.

Tables I and II show the enzyme activities in control and STZ-induced diabetes in rats. The mean values of the levels of AST, ALT, ALP and PChE were 12.2 ± 2.7 IU/L, 11.9 ± 1.72 IU/L, 54.5 ± 8.83 IU/L and 3848.5 ± 272.38 IU/L in group ‘A’ respectively. This study showed an increase in the levels of AST, ALT, ALP and PChE in the kidney in groups B, C, D, and E when compared with the control group (Table I). A significant increase ($P < 0.04$) in the AST levels, a significant increase ($P < 0.055$) in the ALT levels, a significant increase ($P < 0.001$) in the ALP levels and a significant increase ($P < 0.008$) in the PChE levels were observed when treated groups B, C, D, and E were compared with group A (Table II)

Table I. Effects of streptozotocin-induced diabetes on the activity of kidney enzymes in albino rats. Results are expressed as means + SEM for control and STZ-treated rats. Ten animals were used in each group.

Enzymes (IU/L)	Group-A (control)	Group-B (2 weeks)	Group-C (4 weeks)	Group-D (8 weeks)	Group-E (12 weeks)	P-Value
AST	12.2±2.7	138.2±30.95	102.2±17.86	123.0±19.59	129.2±25.5	P < 0.04
ALT	11.9±1.72	165.3±29.92	123.0±22.01	133.5±19.94	157.8±27.2	P < 0.055
ALP	54.5±8.83	467.6±83.01	483.7±80.37	502.9±60.57	530.0±65.44	P < 0.001
PChE	3848.5±272.38	5032.2±888.42	5632.6±543.43	5976.2±498.3	6665.4±646.67	P < 0.008

Table II. Statistical analysis of the difference in mean levels of AST, ALT, ALP and PchE in kidney between groups: A, B, C, D and E in albino rats. KEY: F = Treatment level i.e. Groups A - E.

S.#	Variables (Enzymes)	F-Values	P-Values	Degree of freedom (d.f.)	Conclusion
1.	AST	F = 4.16	P < 0.04	4.8	AST is significantly different
2.	ALT	F = 3.69	P < 0.055	4.8	ALT is significantly different
3.	ALP	F = 14.96	P < 0.001	4.8	ALP is significantly different
4.	PChE	F = 7.63	P < 0.008	4.8	PChE is significantly different

DISCUSSION

Studies have shown an association between specific diabetic complications and liver enzyme alterations (Arkkila *et al.*) but only limited data is available on the possible association between diabetic nephropathy and kidney enzyme alterations (Ramesh *et al.*, 2007). The present study was designed to observe the altered morphology of kidney and kidney enzyme levels in STZ-induced diabetes at various time intervals and to investigate a possible association between diabetic nephropathy and kidney enzymes alterations. Elevated activities of serum aminotransferases are a common sign of kidney damage and are observed more frequently among people with diabetes than in general population (Fadillioglu *et al.*; Sivajothi *et al.*, 2007; Al-Shamsi *et al.*, 2006; McAnuff-Harding *et al.*, 2006; Ohaeri, 2001).

Present observations on the kidney sections showed progressive damage which increased with the duration of time and the severity of hyperglycaemia. Severe hyperglycaemia induced by the streptozotocin caused the renal damage. Several researchers have described different kidney lesions with their possible mechanisms. Most kidney sections showed lesions similar to human glomerulosclerosis, glomerular membrane thickening, arteriolar hyalinization and widespread tubular necrosis. Progressive glomerulosclerosis associated with decreased kidney function, resulting in end stage renal failure is the major finding in diabetic nephropathy. There are multiple components of extracellular matrix and neither the exact component nor the factors responsible for its increase in diabetic nephropathy is known. O'Donnel *et al.* (1988) and Harvey *et al.* (1992) proposed that the glomerular damage in diabetic kidney was due to the increased production of Kallikrein and prostaglandin E2 which caused hyperfiltration and vasodilatation in diabetes. Diabetes mellitus (hyperglycaemia) caused increase in cellular production of eicosanoids from kidney tissues as investigated by DeRubertis & Craven (1993). These eicosanoids included vasodilatory prostaglandins (PGE 2 and PGI 2). There also occurred smaller concurrent increase in thromboxane (TX) A2 within one week after induction of diabetes. This increase in eicosanoids has been linked to the glucose-induced activation of the glomerular protein kinase-C signaling system that enhances phospholipase A2 activity and therefore release of membrane bound arachidonic acid for oxygenation. The eicosanoids thromboxane (TX) was involved in the pathogenesis of glomerular structural changes (basement membrane thickening and mesangial matrix expansion). Thromboxane and high glucose also caused direct stimulation of matrix protein production. Striker *et al.*

(1996) showed histological lesions closely resembling human diabetic glomerulosclerosis in mice. It was associated with an increase in type-IV collagen and laminin synthesis. Growth hormone might promote the susceptibility of this complication. Kelly *et al.* (1998) presented an ideal model of diabetic nephropathy. In the diabetic rats, the most florid lesion was seen after 12 weeks of streptozotocin treatment, with kidneys exhibiting vacuolated tubules, hyalinized arterioles, medullary fibrosis and necrosis and severe glomerulosclerosis. Gambaro *et al.* (1999) reported that untreated diabetic animals developed clear evidence of nephropathy, namely expansion of glomerular extracellular matrix, as expressed by glomerular basement membrane thickening and increased mesangial deposition of type-IV collagen. It might be due to increased mesangial proliferation. Song *et al.* (1999) attributed the increased mesangial matrix accumulation to decreased glomerular proteinase (collagenase and cathepsin B) activity. Oscika *et al.* (2000) proposed that the streptozotocin-induced diabetes caused an increase in glomerular PKC activity which played a central role in the development of diabetic nephropathy. Petersen *et al.* (2008) reported that progressive kidney fibrosis precedes end-stage renal failure in patients with diabetes mellitus. Elevated intra-renal transforming growth factor-beta (TGF-beta) is thought to underlie disease progression by promoting deposition of extracellular matrix and epithelial mesenchymal transition. Recently, Ohtomo *et al.* (2008) have proposed that in diabetic nephropathy, decreased activities of matrix metalloproteinase (MMP)-2, MMP-9 and plasmin contribute to mesangial matrix accumulation. Megsin, a novel member of the serine protease inhibitor superfamily, is predominantly expressed in mesangial cells and is up-regulated in diabetic nephropathy and its overexpression spontaneously induces progressive mesangial expansion by inhibiting plasmin and MMP activities.

In the present study, progressive widespread tubular necrosis with loss of brush border was observed in most kidney sections. These findings of present study are in agreement with the findings of Ramesh *et al.*, Kim *et al.* (2008) and Renno *et al.* (2008) who showed tubular epithelial changes, enlargement of lining cells of tubules and accumulation of glycogen in the kidney tubules. Glycogen accumulation in the renal tubules could be attributed to hyperglycaemia induced by streptozotocin-induced diabetes. Ren *et al.* reported marked enlargement of kidney weight/body weight (KW/BW), tubulointerstitial fibrosis and fibronectin in streptozotocin-induced diabetic rats from 8 to 16 weeks. Activin betaA, mainly located in tubular epithelial cells, was significantly higher in diabetic groups than that in the non-diabetic groups. High glucose may facilitate the synthesis of activin betaA, transforming growth factor (TGF)-beta, P-Smad2/3 and fibronectin. Activin A is involved in

tubulointerstitial fibrosis in diabetes by inducing the production of fibronectin through Smad signal pathway.

In the present study, we observed infiltration of lymphocytes in the interstitial spaces that increased with the increase in streptozotocin-induced hyperglycemia. These findings of present study are in agreement with the findings of Ramesh *et al.* Our study on the kidney sections showed early hypertrophy of glomeruli. Diabetic glomerular hypertrophy constitutes an early event in the progression of glomerular pathology which occurs in the absence of mesangial expansion. The relationship of glomerular hypertrophy to hyperglycaemia early in streptozotocin-induced diabetes could not be ascertained as proposed by Malatiali *et al.*

Our study showed that the aminotransferases (AST and ALT) levels were significantly increased in the kidney of STZ-treated animals as shown in Table-I. The increase in aminotransferases levels may be due to the cellular damage in the kidney caused by STZ-induced diabetes. Although ALT is also present in mitochondria and cytosol, the mitochondrial form is low in activity and is very unstable. The detailed mechanism by which enzymes are released from the cytosol and mitochondria is not completely known. Experimental studies have shown that subtle membrane changes are sufficient to allow passage of intracellular enzymes to the extracellular space. Cell damage increases permeability causing cytosolic isoenzymes to spill into the interstitium and from there into the peripheral blood (Garella, 1997). It had been shown by Rogers *et al.* (1986) that mitochondrial activity was decreased and cytoplasmic AST activity was increased 3-4 fold in STZ diabetic rats. Voss *et al.* (1988) proposed that STZ in hyperglycemic animals caused a time dependent rise in AST, ALT, and ALP levels. The increase in the levels of AST and ALT in diabetic rats after 1-3 weeks treatment was also reported by many other workers (Fadillioğlu *et al.*; Sivajothi *et al.*; Al-Shamsi *et al.*; McAnuff-Harding *et al.*; Ohaeri *et al.*). Okada *et al.* (1997) reported that AST activity was lower than the amount of enzyme in diabetic rat tissues. It is suggested that this may be due to the inactivation of cytosolic AST in the diabetic rat tissues by a glycation reaction, accompanied by impairment in glucose utilization in STZ induced diabetes.

In our study, the levels of ALP were significantly increased in the kidney of treated animals as shown in table I and II. The mechanism of release of membrane bound enzyme is less well understood. Alkaline phosphatase is a membrane bound glycoprotein enzyme. Leibovitch *et al.* (1991) observed increased levels of serum ALP in pathological conditions involving the kidneys. The increase in serum ALP might be derived from injury to the brush

border membrane of the renal tubular cells. Renal function impairment might also be responsible for the increased serum ALP. Nuyts *et al.* (1994) reported that human intestinal alkaline phosphatase (hALP), a specific marker of proximal tubular S3 segment, was elevated in the urine of microalbuminuric diabetic patient. This suggested that tubular alterations were present at an early stage of diabetic nephropathy especially at S3 segment. Increase in the levels of ALP in diabetic rats was also reported by Sivajothi *et al.*, Al-Shamsi *et al.* and McAnuff *et al.*

In the present study, the levels of PChE in the kidney of treated animals were found to be significantly increased as shown in the table I. Song *et al.* (1996) reported increased serum cholinesterase with increase triglyceride levels in diabetics. They pointed out a possible association between increased serum cholinesterase and vascular complications. Other researchers reported a significant increase in cholinesterase activity in STZ induced diabetes (Abbott *et al.*, 1993; Kutty *et al.*, 1994). Cholinesterase might have a role in altered lipoprotein metabolism in hypertriglyceridaemia associated with insulin deficiency in diabetes mellitus.

CONCLUSION

It may be concluded that the streptozotocin through its direct alkylating action can cause cellular necrosis and selective destruction of the beta cells producing hyperglycaemia at a dose of 45 mg/kg body weight. It may also be stated that streptozotocin by producing diabetes (hyperglycaemia) and hypoinsulinemia alters various metabolic and enzymatic functions of kidney resulting in various pathologic lesions. It may also be concluded that the diabetic complications in kidney are associated with alterations in enzyme levels.

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RESUMEN: Se estudiaron los efectos de la diabetes inducida por estreptozotocin (STZ) sobre la morfología, anatomía, arquitectura y sobre las actividades de aminotransferasas (ALT y AST), fosfatasa alcalina (ALP) y pseudocolinesterasa (PChE) en los riñones de ratas albinas. El objetivo del estudio fue investigar la asociación entre las complicaciones renales diabéticas y la alteración de las enzimas renales. Este estudio se realizó en el Departamento de Anatomía y el Instituto de Ciencias Farmacéuticas, Universidad de Medicina Baqai, Karachi y el departamento de Patolo-

gía de Colegio de Médicos y Cirujanos (CPSP) Pakistán entre el 2007-2008. La diabetes fue inducida por una dosis única de STZ (45 mg / kg de peso corporal) administrada por vía intraperitoneal en tampón de citrato de sodio a pH 4.5. Ochenta (80) ratas albinas fueron divididas en cinco grupos: control (A) y STZ tratados (B, C, D y E), que se sacrificaron a las 2, 4, 6 y 8 semanas después del tratamiento, respectivamente. La histopatología del riñón mostró lesiones similares a la glomerulosclerosis en humanos, engrosamiento de la membrana glomerular, hialinización arteriolar y necrosis tubular. Aumento de los niveles de aspartato aminotransferasa (AST), alanina aminotransferasa (ALT), fosfatasa alcalina (ALP) y pseudocolinesterasa (PChE) fueron observados en el riñón. Parece que las complicaciones de la diabetes en el riñón están directamente asociadas con alteraciones en los niveles de las enzimas.

PALABRAS CLAVE: Estreptozotocin; Aminotransferasas; Fosfatasa alcalina; Pseudocolinesterasa; STZ-diabetes; Ratas.

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