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Biochemical and Ultrastructure Changes in the Kidney of Streptozotocin-Induced Diabetic Rat

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Abstract: Diabetes mellitus alters the cellular production of eicosanoids in a number of tissues, including the kidney and these agents have in true been implicated in the pathogenesis of diabetic nephropathy. The aim of this work was to study the effect of streptozotocin-induced diabetes on a kidney by light and electron microscopy. Twenty four adult male white albino rats were collected and classified into 2 main groups. The first (treated) group by a single Streptozotocin (STZ) injection (60 mg/kg) intra-peritoneal (i.p). The second group was considered control. The blood samples were collected for Fasting Blood Sugar (FBS), cholesterol, creatinine and triglycerides The survival rate in diabetic rats was 66.6% in contrast to 100% in controls. There was a significant weight loss ($p = 0.01$) in diabetic rats while a highly significant weight gain ($p = 0.002$) was recorded in control ones. The kidney weight in diabetic rats showed an extremely significant increase ($p = <0.004$). The rats injected with STZ have shown severe hyperglycaemia ($p<0.0001$). Serum cholesterol showed a highly significant rise in both diabetic ($p = 0.007$) and control rats ($p = 0.001$). Both creatinine values ($p<0.0001$) and serum triglycerides ($p = 0.0004$) were significantly higher in diabetic rats than in control rats. The kidney of induced diabetic rats showed a Histopathological and ultrastructure changes in both kidney. In conclusion, STZ-induced diabetes mellitus had deleterious effects in both structure and function of the rat kidney. Intensive management of diabetes mellitus in human is recommended to delay or prevent such diabetic nephropathy.

Key words: Streptozotocin, blood sugar, cholesterol, creatinine, triglycerides, histological, ultrastructure changes

INTRODUCTION

Different metabolic disorders including hypertension, hypercholesterolemia, obesity, together with diabetes prevalence and an increasing aging population result in significant global increase in chronic and end-stage kidney disease (White *et al*., 2005). Diabetes mellitus alters the cellular production of eicosanoids in a number of tissues, including the kidney and these agents have in turn been implicated in the pathogenesis of diabetic nephropathy (De Rubertis and Craven, 1993). Diabetes mellitus accelerates the usual age-related renal functional and morphological deterioration like hypertrophic changes which have received much more attention because of their possible role in development of the ultimate structural destruction (Medeiros *et al*., 2006). Renal hypertrophy and hyperfilteration are inherent features in the course of experimental and human diabetes mellitus (Thora *et al*., 1997; Seyer-Hansen, 1983; Dnyanmote *et al*., 2006). A variety of clinical and experimental studies have been conducted in order to characterized several metabolic, functional and structural changes in alloxan and Streptozotocin (STZ) diabetic rats including diabetic renal changes (Thora *et al*., 1997; Oskov *et al*., 1965; Rasch, 1979). There are several techniques including chemical destruction, surgical removal of the beta cell mass or even pancreatectomy for induction of experimental diabetes (Islas-Andrade *et al*., 2000; Frankel *et al*., 1984). The long-term cortical renal alterations caused by diabetes (thickening of the peripheral basement membranes and expansion of mesangial regions) illustrate the potential renal damage by diabetes (Osterby *et al*., 1984). The three-dimensional ultrastructure of the Glomerular Basement Membrane (GBM) and Mesangial Matrix (MM) in STZ-induced diabetic rats and in one case of a human diabetic nephropathy were examined by Tanaka *et al*. (1996) using the quick-freezing and deep-etching method. They illustrated that in the diabetic rats, the GBM inner layer was diffusely enlarged and the meshwork structures in the GBM middle layer and the MM were markedly irregular due to the rupturing of the fine fibrils. These irregularities and enlargements of the mesh pores in the diabetic rats develop during the experimental period and were significantly different from those in the control rats. Another authors (Ina *et al*., 2002) who dealt with electron microscopical study of the kidney in the diabetic rats showed that lysosomes were conspicuous in the podocytes of diabetic rats and the podocytes had an increased capacity for endocytosis in the early stage of diabetic nephropathy without increased urinary protein excretion.

The aim of this work was to study the effect of STZ-induced diabetes on the rat kidney through light and

electron microscope study in order to prevent such parenchymatous changes at the early stage of diabetic nephropathy.

MATERIALS AND METHODS

Twenty four male adult white albino rats were used in this study. They have been collected from the animal house in Faculty of Medicine, Umm Alqura University where this study has been carried out. The rats were classified into two main groups, 12 animals as treated group and the other 12 animals as control. All animals were weighted before starting the study and blood samples were collected through their tails for measurement of Fasting Blood Sugar (FBS), cholesterol, creatinine and triglycerides. They were fed with a standard diet and tap water *ad libitum*. The animals were maintained in cages under 12-h cycle of light and dark and the room temperature was kept at 20-25°C.

Experimental type 1 diabetes was induced in the first (treated) group by a single streptozotocin (sigma Aldrich Co., St. Louis, USA) injection (60 mg/kg body weight intra-peritoneal, dissolved in 50 mmol/l sodium citrate buffer, pH 4.5) those rats were fasted overnight before streptozotocin administration. The second group was considered as a control and received sodium citrate buffer alone. In the treated group, before the streptozotocin injection and 24 h later, blood glucose was measured using a glucometer Accu-Chek Adantage (Roche, Sao Paulo, SP, Brazil) to confirm the development of diabetes mellitus. Only animals showing sever hyperglycaemia (FBS levels greater than 250mg/dl where the normal FBS is 70-110 mg/dl) and diabetic signs such as weight loss and polydipsia were considered to have developed diabetes mellitus.

All diabetic and control animals were followed up through the total period of study (8 weeks) with frequent evaluations of blood sugar in the induce-diabetic group. Four diabetic animals expired most likely due to sever hyperglycaemia, therefore four control animals had been excluded from the study. Eight weeks after induction of diabetes, all surviving rats (8 treated and 8 control) were weight again and killed with an overdose of pentobarbital (60 mg i.p). Blood samples were collected from the inferior vena cava to evaluate for FBS, creatinine, cholesterol and triglycerides. Following laparotomy the right kidneys were dissected and excised from all animals and weight. Each kidney was divided longitudinally into two parts: one part was immersed in 10% formaldehyde saline. After fixation, the tissues were embedded in paraffin and five micron-thick tissue sections were cut from the paraffin blocks, all of the kidney sections were stained using haematoxylin and eosin (H & E) to be studied by light microscopy. The other part of the kidney was immersed into glutaraldehyde (volume fraction 2.5%). After the process of chemical fixation, chemical dehydration, drying and conductivity enhancement, all samples were observed and photographed using transmission electron microscope (Leica Stereoscan 260, England).

All data in this study were recorded as mean ± Standard Error of Mean (SEM). Student's t test was used and both t and probability (p) values were estimated. The lower and upper limits of 95% Confidence Interval (CI) as well as the 95% CI of the differences were also estimated mainly in the diabetic animals. The results were considered significant when the two-tailed p value was less than 0.05 (Budneck, 1987).

RESULTS

General characteristics of experimental animals: The control rats were freely moving in their individual cages through the study, while the induced-diabetic rats appeared to be lethargic and displayed restricted movements, in spite of absence of marked motor disorders in any of the studied rats.

Survival rate: It has been found that in treated (diabetic) group four rats out of twelve have expired while no animals in the control group died indicating the survival rate in the diabetic rats was 66.6% and in the control rats was 100%.

Body weight: The recorded results of body weight of both diabetic and control rats showed that the initial body weight of diabetic rats ranged 195-238 gm with a mean of 216.8±5.4 gm and the lower limit of 95% Confidence Interval (CI) was 204.1 and upper limit was 229.6. The final weight after 8 weeks of induction of diabetes ranged 172 -220 gm with a mean of 196.2±5.9 gm and the lower limit of 95% CI was 182 and upper limit was 210. The mean difference between initial and final body weight of diabetic rats was -20.6 gm and 95% confidence interval of the difference was -37.8 to -3.4 and t value was 2.57 and p was 0.02 indicating a significant loss of weight in diabetic animals (Table 1). The initial body weight of control rats ranged 195-230 gm with a mean of 208.8±4.1 gm and the lower limit of 95% CI was 199 and upper limit was 218.6. The final weight after 8 weeks ranged 215-245 gm with a mean of 230±3.9 gm and the lower limit of CI was 220.6 and upper limit was 239.3. The mean difference between initial and final body weight of control rats was 21.12 and 95% confidence interval of the difference was 8.8 to 33.4. The t value was 3.6 and p was 0.002 i.e. highly significant weight gain during the period of study (Table 1).

Morphology of the kidney: In the diabetic rats there was a marked increase in kidney size as noticed by naked eyes compared to control ones. The recorded kidney weight of both diabetic and control rats showed that the

Table 1: The initial and final body weight of both diabetic and control rats (Final: after 8 week of induction of diabetes)

	Weight of diabetic rats (gm)		Weight of control rats (gm)	
No	Initial	Final	Initial	Final
1	225	205	220	235
2	195	180	210	245
3	232	210	195	215
4	210	187	230	243
5	218	208	199	218
6	238	220	200	222
7	198	172	205	228
8	219	188	212	234
Mean±SEM	216.8±5.4	196.2±5.9	208.8±4.1	230±3.9
	t = 2.57; p = 0.02		t = 3.6; p = 0.002	

Table 2: Kidney weight of both diabetic (after 8 week of induction of diabetes) and control rats

No	Kidney weight in diabetic rats (gm)	Kidney weight in control rats (gm)
1	1.65	1.50
2	1.55	1.38
3	1.48	1.46
4	1.62	1.30
5	1.44	1.34
6	1.35	1.20
7	1.60	1.25
8	1.50	1.40
Mean±SEM	1.52±0.03	1.35±0.03
	t = 3.34; p = 0.004	

kidney weight after 8 weeks of induction of diabetes ranged 1.35-1.65 gm with a mean of 1.52±0.03 gm and the lower limit of 95% Confidence Interval (CI) was 1.43 and upper limit was 1.6. The kidney weight of control rats after 8 weeks of starting the study ranged 1.2-1.5 gm with a mean of 1.35±0.03 gm and the lower limit of 95% CI was 1.26 and upper limit was 1.43. The mean difference between kidney weight of control and diabetic rats was 0.17 gm and 95% confidence interval of the difference was 0.06 to 0.27 and t value was 3.34 and p was 0.004 indicating highly significant increase in kidney weight of diabetic rats in contrast to the control ones (Table 2).

Blood sugar: The recorded findings of biochemistry of induced-diabetic rats showed that the initial FBS values ranged 78-108 mg/dl with a mean of 96.1±3.5 mg/dl and the lower limit of 95% CI was 87.7 and upper limit was 104.5. The final FBS values after 8 weeks of induction of diabetes ranged 255-402 mg/dl with a mean of 312.1±16.5 mg/dl and the lower limit of 95% CI was 273 and upper limit was 351.2. All animals showed marked hyperglycaemia (normal FBS: 70-110 mg/dl). The mean difference between initial and final FBS of the diabetic rats was 216 gm and 95% CI of the difference was 179.7 to 252.2 and t value was 12.7 and p was < 0.0001 i.e. extremely significant increase in blood sugar after injection of streptozotocin (Table 3).

The blood sugar values of the control rats showed that the initial FBS ranged 88-110 mg/dl with a mean of 98.7±2.8 mg/dl and the final FBS levels after 8 weeks of starting the study ranged 76-110 mg/dl with a mean of 95.1±4.3 mg/dl. In the control rats the t value was 0.6 and p was 0.1 i.e. non-significant changes in blood sugar (Table 4).

Serum cholesterol: The initial values of cholesterol in the induced- diabetic rats ranged 138-264 mg/dl with a mean of 183.8±14.4 mg/dl and the lower limit of 95% CI was 149.7 and upper limit was 218. The final values after 8 weeks of induction of diabetes ranged 184-342 mg/dl with a mean of 258.8±18.8 mg/dl and the lower limit of 95% CI was 214.3 and upper limit was 303.4. Three animals showed an increase in cholesterol above normal levels (N: 150-250 mg/dl). The mean difference between the initial and final cholesterol levels of the diabetic rats was 75 mg/dl while the 95% CI of the difference was 24.08 to 125.9 and t value was 3.15 and p was 0.007 i.e. highly significant increase in blood cholesterol in diabetic rats in contrast to the controls (Table 3). The initial values of blood cholesterol in the control rats ranged 128-215 mg/dl with a mean of 178.7±11.4 mg/dl and final values after 8 weeks were 198-298 mg/dl with a mean of 241.3±10.2 mg/dl. Only one rat showed hypercholesterolemia. In the control rats the t value was 4.14 and p was 0.001 i.e. highly significant increase in cholesterol levels without marked true hypercholesterolemia (Table 4).

Serum creatinine: The initial values of creatinine in the diabetic rats ranged 0.3-1.4 mg/dl with a mean of 0.8±0.1 mg/dl and the lower limit of 95% CI was 0.5 and upper limit was 1.2, while the final values after 8 weeks induction of diabetes ranged 1.7-3.4 mg/dl with a mean of 2.4±0.2 mg/dl and the lower limit of CI was 1.9 and upper limit was 2.9. All animals showed high creatinine above normal ranges (N: 0.7-1.4 mg/dl). The mean difference between initial and final creatinine levels of diabetic rats was 1.5 mg/dl and 95% CI of the difference was 1 to 2.1 and t value was 6.2 while p was < 0.0001

Table 3: The initial and final biochemical findings in diabetic rats (FBS: Fasting Blood Sugar)

	FBS (mg/dl)		Cholesterol (mg/dl)		Creatinine (mg/dl)		Triglyceride (mg/dl)	
No	Initial	Final	Initial	Final	Initial	Final	Initial	Final
1	98	268	165	238	0.6	3.0	134	188
2	105	320	174	184	1.2	2.1	82	158
3	88	350	264	310	0.9	2.8	154	220
4	100	295	164	215	0.4	1.9	112	205
5	108	310	222	342	1.1	2.5	145	198
6	78	297	189	236	1.4	3.4	99	234
7	90	402	155	300	0.3	1.7	121	142
8	102	255	138	246	1.2	2.2	148	267
Mean±SEM	96.1±3.5	312±16.5	183.8±14.4	258.8±18.8	0.8±0.1	2.4±0.2	124.3±9	201.5±14.2
	t = 12.7; p<0.0001		t = 3.15; p = 0.007		t = 6.2; p<0.0001		t = 4.5; p = 0.0004	

Table 4: The initial and final biochemical findings in control rats (FBS: Fasting Blood Sugar)

	FBS (mg/dl)		Cholesterol (mg/dl)		Creatinine (mg/dl)		Triglyceride (mg/dl)	
No	Initial	Final	Initial	Final	Initial	Final	Initial	Final
1	97	105	165	246	1.1	1.3	145	205
2	103	110	189	234	0.8	0.9	134	120
3	110	98	210	298	0.4	1.2	98	150
4	90	86	168	232	1.3	0.8	78	138
5	88	104	210	248	0.9	1.6	110	88
6	92	76	145	198	1.4	1.8	122	144
7	14	100	128	250	1	0.8	88	96
8	90	82	215	228	0.6	1.1	160	186
Mean±SEM	98.7±2.8	95.1±4.3	178.7±11.4	241.3±10.2	0.9±0.1	1.1±0.1	116.8±10.1	140.8±14.3
	t = 0.6; p = 0.1		t = 4.14; p = 0.001		t = 1.4; p = 0.1		t = 1.3; p = 0.1	

i.e. extremely significant impairment of kidney function (Table 3) The initial creatinine levels in the control rats ranged 0.4-1.4 mg/dl with a mean of 0.9±0.1 mg/dl and the final values after 8 weeks were 0.8-1.8 mg/dl with a mean of 1.1±0.1 mg/dl. Only two rats showed high creatinine above normal. In the control rats the t value was 1.4 and p was 0.1 i.e. non-significant changes in the kidney function (Table 4).

Serum triglycerides: The initial values of triglycerides in the diabetic rats ranged 82-154 mg/dl with a mean of 124.3±9 mg/dl and the lower limit of 95% CI was 103 and upper limit was 145.7. The final triglyceride levels after 8 weeks of induction of diabetes ranged 142-267 mg/dl with a mean of 201.5±14.2 mg/dl and the lower limit of 95% CI was 167.9 and upper limit was 235.1. Six animals showed high triglycerides above normal (N: 40-165 mg/dl) The mean difference between initial and final triglyceride levels in the diabetic rats was 77.1 mg/dl and 95% CI of the difference was 40.9 to 113.2 and t value was 4.5 and p was 0.0004 i.e. extremely significant increase in diabetics more than control rats (Table 3) The initial values of triglycerides in the control rats ranged 78-160 mg/dl with a mean of 116.8±10.1 mg/dl and the final levels after 8 weeks ranged 88-205 mg/dl with a mean of 140.8±14.3 mg/dl. Two animals showed an increase in triglyceride levels above normal. In the control rats the t value was 1.3 and p was 0.1 i.e. non-significant changes (Table 4).

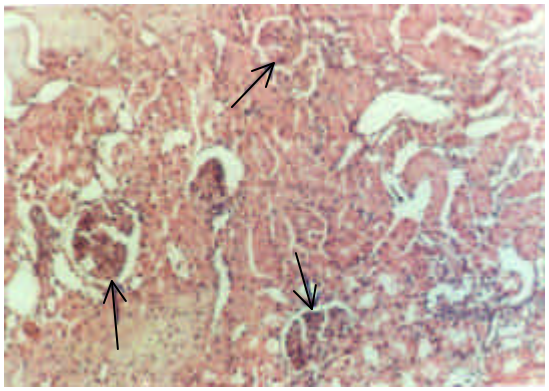

Fig. 1: Photomicrograph (medium power view) in the kidney of STZ-induced diabetic rat showing loss of arrangement of convoluted tubules and four renal corpuscles (arrows) (H&E x200)

Histological findings: The kidney of STZ-induced diabetic rats showed a loss of normal arrangement of convoluted tubules and slightly little number (4 renal corpuscles,) per medium power view (Fig. 1) in contrast to the well-arranged convoluted tubules and 5 renal corpuscles in the same power view of control rats (Fig. 2). The Bowman's capsule of STZ- diabetic rats became thick and fibrosed with loss of separation between it and the glomerular tuft with fibrosed and cavitated glomerular capillary loops (Fig. 3).

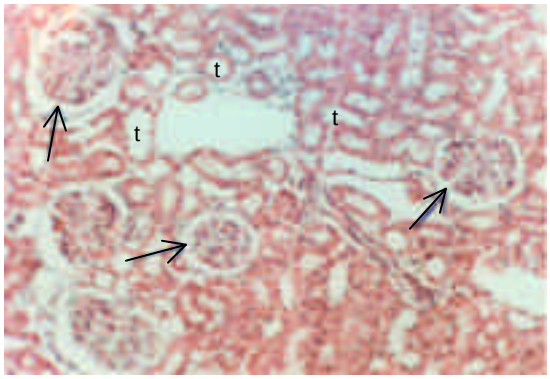

Fig. 2: Photomicrograph (medium power view) in the renal cortex of a control rat showing well-arranged transverse and oblique sections of renal convoluted tubules (t) and about five renal corpuscles (arrows). (H&E x200)

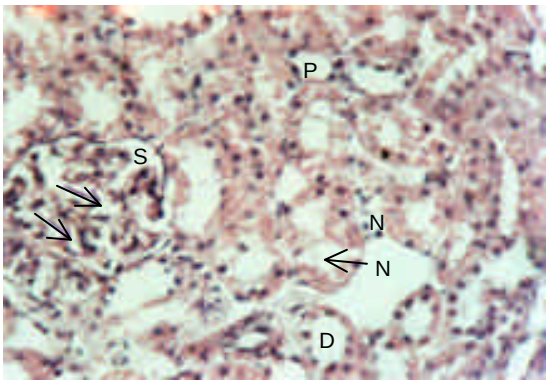

Fig. 3: Photomicrograph in the kidney of STZ-Diabetic rat showing narrowing of Bowman's capsule space (S) and glomerular capillary loops surrounded by vaculated strips (arrows). Proximal (P) and distal (D) convoluted tubules show irregular outline, pale cells with vaculated cytoplasm and pyknotic nuclei (N) (H&E x400)

Some cortical fields in STZ-diabetic rats showed glomeruli with different extensions of inflammatory infiltrates (Fig. 4) in which the small intra-renal arteries showed onion ring image. Those findings were absent in the control group (Fig. 5).

The proximal and distal convoluted tubules showed irregular outlines, presence of pyknotic cells with pale and vacuolated cytoplasm (Fig. 3) in contrast to the control non diabetic rats (Fig. 6). No significant changes in the medulla were seen between diabetic and controlled rats apart from some irregularities in the collecting tubules (Fig. 7).

Ultrastructure findings: In the induced-diabetic rats the renal glomeruli showed thick wrinkled glomerular basement membrane with loss of its trilaminar

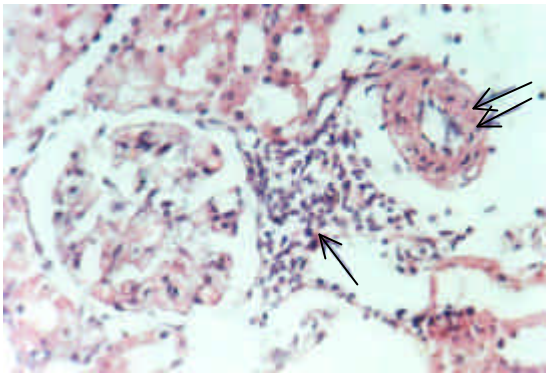

Fig. 4: Photomicrograph in the kidney of STZ-Diabetic rat showing glomeruli with different extensions of inflammatory infiltrates (single arrow). The small intra-renal arteries show onion ring image (double arrows) (H&E x400)

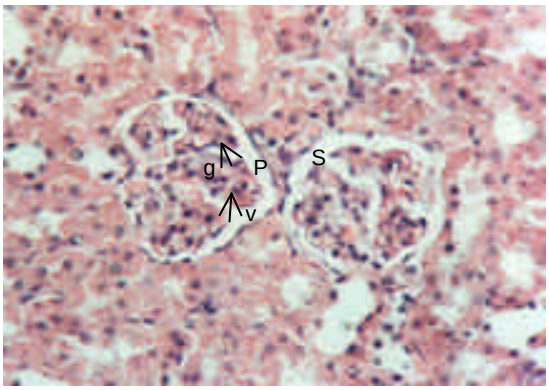

Fig. 5: Photomicrograph in the kidney of a control rat showing renal corpuscle with a glomerulus (g) surrounded by visceral layer (v) and a parietal layer (p) of Bowman's capsule and separated by a space (S)(H&E x400)

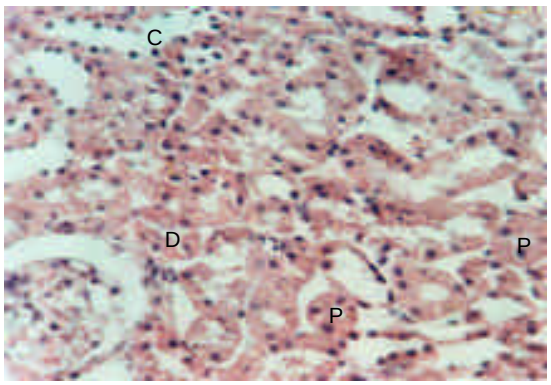

Fig. 6: Photomicrograph in the kidney of a control rat showing proximal convoluted tubules (P), distal convoluted tubules (D) and collecting tubules (H&E x400)

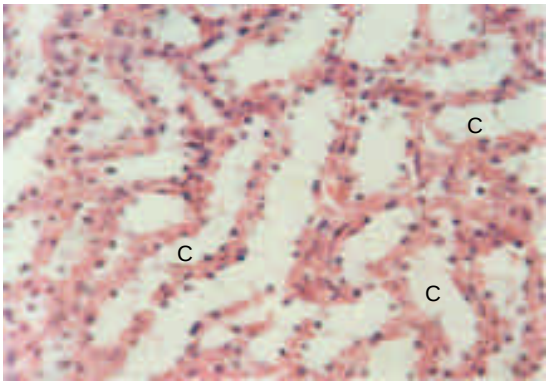

Fig. 7: Photomicrograph in the kidney of STZ-Diabetic rat showing irregular outlines of collecting tubules © (H&E x400)

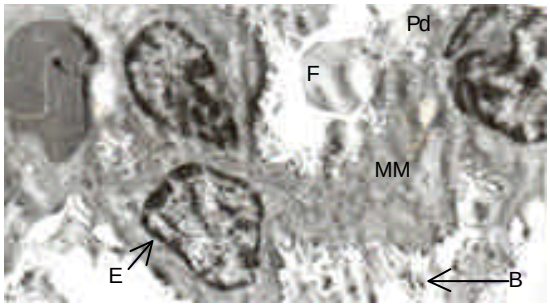

Fig. 8: Electron micrograph in the renal cortex of STZ-Diabetic rat showing renal glomerulus with wrinkled glomerular basement membrane (B), diffuse mesangial matrix (MM) and thickened glomerular endothelium (E) with disintegrated cristae. Fused crowded and irregular podocytes (pd) with broad foot processes (F) are shown (x9250)

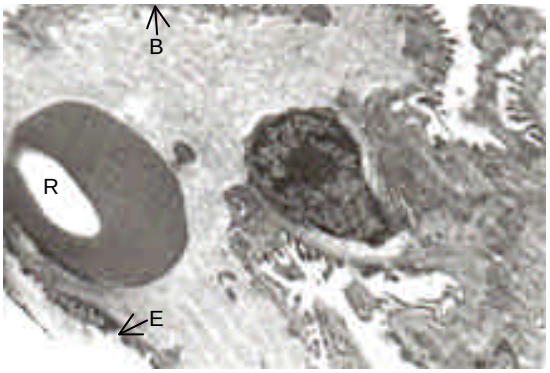

Fig. 9: Electron micrograph in the renal cortex of a control rat showing glomerular blood capillaries (b) lined with fenestrated endothelium (E) with nuclei pulging into capillary lumen. Trilaminar glomerular basement membrane (B) and red blood cells (R) within the capillary lumen are demonstrated (x11500)

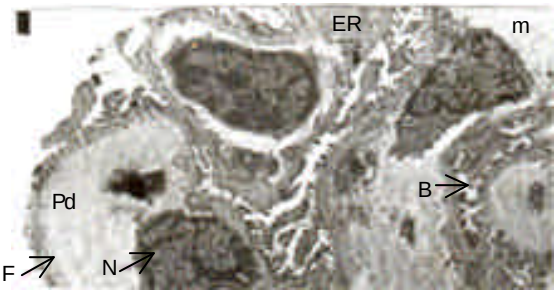

Fig. 10: Electron micrograph in the renal cortex of a control rat showing the primary processes of podocytes (pd) and their foot processes (F) resting on glomerular basement membrane (B). Mesangial cells (m), podocytes with large euchromatic nucleus (N) and cytoplasm containing rough Endoplasmic Reticulum (ER) are also shown (x9000)

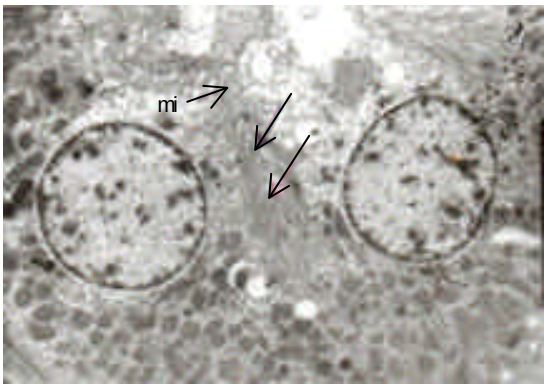

Fig. 11: Electron micrograph in the renal cortex of a control rat showing proximal convoluted tubules with multiple microvilli (mi) forming brush border (arrows) (x6750)

appearance. The glomeruli also showed thickened and fibrosed glomerular endothelium with disintegrated cristae. Diffuse mesangial matrix with irregular apoptotic mesangial cells containing irregular heterochromatic nuclei were also shown. Primary and secondary podocytes appeared broad as a result of their fusion and wide spaces between the cells were noticed (Fig. 8) in contrast to the control group (Fig. 9 and 10).

Proximal tubules in control rats showed multiple microvilli forming the brush border (Fig. 11) while in the induced-diabetic rats they were not intact with loss of their brush borders (Fig. 12). The renal cortex of STZ-diabetic rats showed irregular convoluted tubules with heterochromatic nuclei, loss of basal infoldings, scattered rounded mitochondria with less electron dens bodies and vesicular (oedematous and vaculated) cytoplasm, with thick basement membrane (Fig. 13) in which the cells became distorted with multiple endocytic vacuoles in a pale cytoplasm. In a high power view of

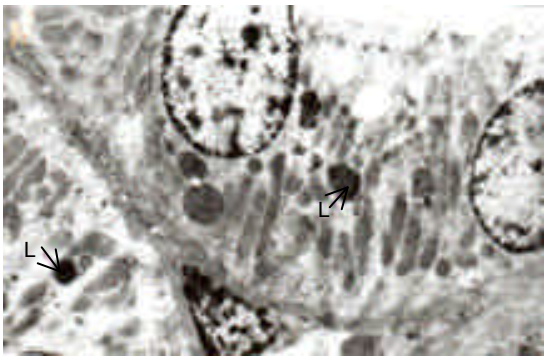

Fig. 12: Electron micrograph in the renal cortex of STZ-diabetic rat showing affected proximal convoluted tubules with lysosomes (L) and loss of their brush border (x9250)

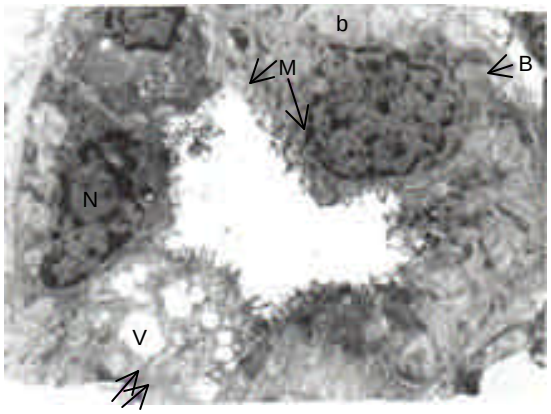

Fig. 13: Electron micrograph in the renal cortex of STZ-diabetic rat showing proximal convoluted tubules with vacuoles (V), irregular heterochromatic nuclei (N), scattered rounded mitochondria (M), less electron dens bodies (arrows) and thick basement membrane (B) with loss or disarranged basal infoldings (b) (x9250)

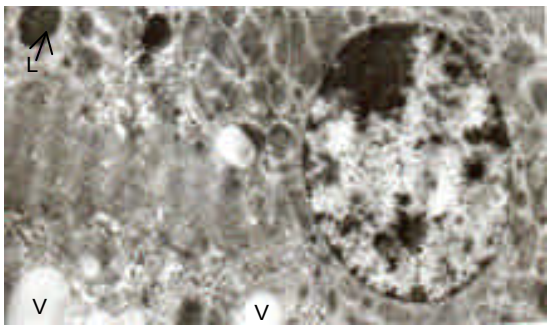

Fig. 14: Electron micrograph (high power view) in the renal cortex of STZ-diabetic rat showing convoluted tubule with lysosomes (L), vesicular cytoplasm and vaculation of renal tubule (V) (x13500)

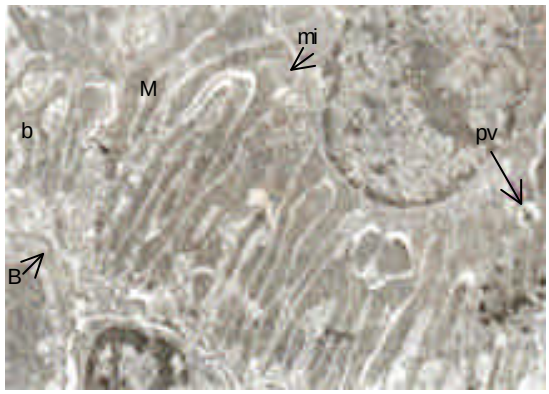

Fig. 15: Electron micrograph in the renal cortex of a control rat showing a proximal convoluted tubules with Trilaminar basement membrane (B), apical microvilli (mi), pinocytic vesicles (pv) and elongated mitochondria (M) enclosed within the short basal infoldings (b) (x12500)

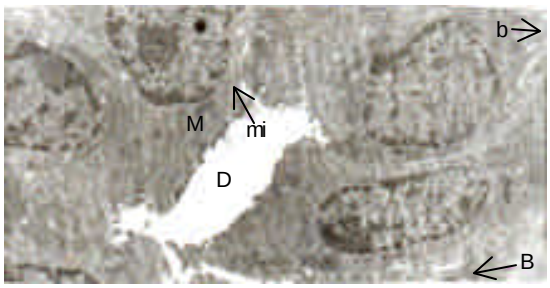

Fig. 16: Electron micrograph in the renal cortex of a control rat showing the epithelial lining of the distal convoluted tubule cells (D) resting on a basement membrane (B). These cells show apical few short microvilli (mi) and basal infoldings (b) with longitudinal mitochondria (M) in between (x11500)

diabetic kidney the lysosomes, endocytic vacuoles and rounded mitochondria were clearly seen (Fig. 14) in contrast to the proximal convoluted tubules in the control group (Fig. 15).

The distal convoluted tubules of induced-diabetic group showed a marked disruption, thickening of the basement membrane, loss of podocytic foot processes, disruption of tubular basal infoldings with multiple lysosomes and fibrosed tubules (Fig. 13 and 14) in contrast to the distal convoluted tubules in control rats (Fig. 16).

DISCUSSION

The present study demonstrated a survival rate of 100% in control rats and 66.6% in STZ-induced diabetic rats which approximately coincided with Saito *et al*. (2006) who reported 100% survival rate in control group and 69.2 % in the diabetic rats.

Permanent endothelial dysfunction in women with diabetes was significantly improved by oestrogen (Lee *et al*., 2001). Obineche *et al*. (2001) reported that vascular endothelial cells in female diabetic rats were less injured than in male diabetic rats mainly in 4-week-model. However, in 10-week-model the same damage occurred in both male and female diabetic rats. To avoid such effect of oestrogen, male rats were used in this study. The significant loss of weight of diabetic rats recorded in this study didn't agree with Saito *et al*. (2006) who recorded no weight gain in the diabetic rats through their period of study inspite of a significant difference between the final body weight of control and diabetic rats. However we agreed with Besse *et al*. (1993) who recorded a significant weight loss in diabetic rats. The diabetes caused by STZ administration increases fat mobilization in skeletal muscle (Stearns *et al*., 1979) inducing significant weight loss. This study showed a significant increase in kidney weight in diabetic rats in contrast to the control ones which agreed with Medeiros *et al*. (2006) who reported that diabetes accelerates the usual age-related renal functional and morphological deterioration like hypertrophic changes which have received much more attention because of their possible role in development of the ultimate structural destruction. The normal range of rat creatinine is extensive, reflecting the variability due to strain, age and sex differences (Aguila and Mandarim-de-Lacerda, 2003). A decreased creatinine excretion can be expected as a result of competition for creatinine secretion with ketoacids (in diabetic ketoacidosis) leading to an increased serum creatinine which coincided with our results which showed a significant rise in serum creatinine in diabetic rats in contrast to the control animals. Renal dysfuncin in diabetic rats is characterized by a significant decrease in creatinine clearance and consequently raised serum creatinine (Murali *et al*., 2003) which was observed in the present study.

The hallmark of diabetic lipid abnormalities is raised triglyceride levels and low HDL-C plasma concentrations while total cholesterol and LDL-C don't differ from those in non-diabetics (Carlson *et al*., 2001) which coincided with the present study which showed high triglyceride in six diabetic rats and high cholesterol in only three diabetic ones.

In type 2 diabetes there is a 2-4 fold excess risk of coronary heart disease. The excess risk is related to dyslipidaemia than to hyperglycaemia which in turn more closely like to the occurrence of microvascular complications such as retinopathy and nephropathy (Kamel *et al*., 1961). For this reason, we have studied both cholesterol and triglycerides in addition to the blood sugar.

The number of glomeruli per kidney was greater in treated diabetic, Spontaneously Hypertensive Rats (SHR) which represent our control rats than in non-treated diabetic (SHR) which represent our diabetic rats (Medeiros *et al*., 2006). Those findings more or less coincided with our results which showed slightly reduced number of glomeruli (renal corpuscles) in diabetic rats in contrast to the control animals. The previous findings have also been supported by other reference (Gross *et al*., 2005) who reported that the rat offsprings of either hyperglycaemic and diabetic mothers have fewer nephrons. The induced-diabetic kidney cells in this study became distorted with multiple endocytic vacuoles in a pale cytoplasm, in addition, primary and secondary podocytes appeared broad as a result of their fusion with apparent wide spaces between the cells.

However lysosomes, endocytic vacuoles and rounded mitochondria were clearly seen. Those findings are agreed with Ina *et al*. (2002) who reported that in conventional electron microscopy, lysosomes were conspicuous in the podocytes of diabetic rats. Such podocytes had an increased capacity for endocytosis in the early stage of diabetic nephropathy.

Our study demonstrated the renal glomeruli with wrinkled glomerular basement membrane, diffuse mesangial area, apoptotic mesangial cells and thickened glomerular endothelium with disintegrated cristae. However Tanaka *et al*. (1996) illustrated that in the diabetic rats, the GBM inner layer was diffusely enlarged and the meshwork structures in the GBM middle layer and the mesangial matrix were markedly irregular due to the rupturing of fine fibrils. In this study, the Bowman's capsule of diabetic rats as well as basement membrane became thick and fibrosed with loss of separation between the capsule and the glomerular tuft and so less primary and secondary podocytes appeared. Such cortical renal alterations coincided with those caused by diabetes (thickening of the peripheral basement membrane and expansion of the mesangial regions) as reported by Osterby *et al*. (1984). Apoptosis is associated with loss of glomerular cells in rats with long - term STZ- induced diabetes mellitus and to a considerably lower degree in controls of the same age and strain (Pesce *et al*., 2002). However our findings showed a reduced number of glomeruli which also have had diffuse mesangial matrix with irregular apoptotic mesangial cells containing irregular heterochromatic nuclei.

In summary, STZ-induced diabetes had different deleterious effects on both structure and function of the kidney. In order to delay or prevent such diabetic nephropathy, intensive management of diabetes in human is recommended.

Abbreviations: FBS: Fasting blood sugar, gm: gram, STZ: Streptozotocin.

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