A Study of the Protective Role Versus the Curative Role of Pentoxifylline on Experimentally Induced Diabetic Nephropathy in a Rat Model

Original Article

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ABSTRACT

Introduction: Chronic diseases have become major health issues encountering people globally and on top of the list comes "Diabetes Mellitus (DM)". One of its serious complications is "Diabetic Nephropathy (DN)".

Aim of the Work: Demonstration of the possible effect of pentoxifylline (PTX) on induced diabetic nephropathy in rats as a cure or prophylactic therapy.

Materials and Methods: Seventy adult male albino rats were randomly distributed into five groups; group I (control group), group II (PTX group), group III (diabetic nephropathy group), group IV (diabetic rats received pentoxifylline as a prophylactic treatment one week after induction of diabetes) and group V (diabetic rats received pentoxifylline as a curative treatment starting after sixth week of induction of diabetes). Induction of diabetes was done by intraperitoneal injection of a single dose of streptozotocin (50 mg/kg). A single dose of PTX (200 mg/kg) was given daily by gastric intubation. At the end of experiment (after 8 weeks), renal specimens were collected and processed for paraffin blocks and stained with H&E and immunohistochemical stains and examined by light microscope. Other samples were prepared to obtain semithin sections stained with toluidine blue to be examined by light microscope and ultrathin sections to be examined by transmission electron microscope (TEM).

Results: In diabetic nephropathy, there were shrunken glomeruli. The epithelial cells of renal tubules showed vacuolations, degenerative changes and complete shedding. There was anti-NF-κb strong positive reaction of renal tubules. In TEM examination, there were thickening of the glomerular filtration barrier, fusion of foot processes of podocytes, damage of renal tubules. Histopathological examination of the kidney showed improvement in the prophylactic and the curative groups. There was a significant decrease in anti-NF-κb positive reaction in groups II, IV and V.

Conclusion: Pentoxifylline had both prophylactic and curative effects against diabetic nephropathy.

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Key Words: Diabetic nephropathy; NF-κb; pentoxifylline; TEM.

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INTRODUCTION

One of the most common vascular complication of diabetes is Nephropathy which affects microvascular circulation of the kidneys causing diabetic nephropathy (DN) which affects up to thirty percent of patient with insulin dependent diabetes type-l[1]. The increased risk of mortality in cases of DN occurs as a result of progressive increased damage of the glomerular filtration process[2].

A viscous circle of hyperlipidemia, hyperglycemia and hypertension and renin angiotensin system activation collectively involved in pathogenesis of DN[3]. An inflammatory process develops in which specific pro-inflammatory cytokines are involved in diabetes complication on blood[4].

Diabetic nephropathy may be diffuse or nodular (Kimmelstiel-Wilson lesion)[5]. The changes within the glomerulus include thickening of the basement membrane, increase in the number of mesangial cells and increase in mesangial matrix. This matrix invades the glomerular capillaries and produces deposits called Kimmelstiel-Wilson nodules. The mesangial cells and matrix can progressively expand and consume the entire glomerulus, shutting off filtration[6].

Pentoxifylline (PTX) is a synthetic dimethyl xanthine derivative which is structurally related to caffeine and theophylline[7]. It has been in use for a long time in treatment of intermittent claudication to improve vascularity and relieve the condition[8].

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Many clinical trials focused on the role of PTX in modifying the nephropathy effects of diabetes[9]. It has been hypothesized that it works against inflammation, fibrosis and it has anti-proliferative activity. Literature shows that it has benefits in nephropathy and alleges that PTX reduces TNF-alpha and proteinurea but there is still debate on its net effect and its overall efficiency and effectiveness[10].

Our work aims to demonstrate the possible prophylactic and curative effects of pentoxifylline on diabetic nephropathy in male rat model.

MATERIAL AND METHODS

Animals

Seventy male adult albino rats were obtained from the animal house of Vaccines and Sera Company (VACSERA), Egypt. They ranged from 200 to 250 grams. Rats were kept in Clinical Pharmacology Department, faculty of medicine, Ain Shams University. They were housed in medium sized cages, one rat per cage and left 1 week for acclimatization and standard diet with water were given and kept under regular dark and light cycles. Blood Glucose level was measured and insured all rats were free of diabetes.

Ethical considerations

The Experiment done under the guidelines approved by the Committee of Animal Research Ethics, Ain Shams University, Faculty of Medicine.

Drugs

Streptozotocin (STZ) and pentoxifylline (PTX) were obtained from Sigma–Aldrich Co.

Animal groups

The animals were randomly assigned to 5 groups.

Group I (thirty rats): served as control group. It was further subdivided into three equal subgroups:

- **Group Ia:** serving as negative control. It received no drug.
- **Group Ib:** serving as control for group II. It received 200 ml/kg distilled water using gastric intubation daily.
- **Group Ic:** serving as control for group III. It was injected intra-peritoneal with a single dose of 1 ml/kg of sterile citrate buffer solution.

Group II (PTX treated group): they received PTX in a single daily dose throughout the duration of experiment (8 weeks).

Group III (Diabetic nephropathy group): animals were injected with STZ and left with no treatment for seven weeks.

Group IV (Prophylactic group): rats were confirmed to be diabetic and received PTX as a prophylactic treatment starting from one week after induction of diabetes and lasting for further 7 weeks.

Group V (Curative group): diabetic rats received a single daily dose of PTX as a curative treatment after 6th week of induction of diabetes and continued for another 2 weeks (till the end of the experiment).

Experimental Design

Only single dosage of 50 mg/kg of STZ in 0.1 citrate buffer was introduced through intraperitoneal injection in every rat. The rats were infused with glucose for one day to avoid hypoglycemia. The blood glucose was estimated using a test strip glucometer (Accu-chek, Roche Germany) using one drop of tail blood obtained by tail puncture after 3 days of administering STZ. Rats with a blood sugar level of 270 mg/ml or greater were accepted as diabetic[11,12].

Pentoxifylline was dissolved in distilled water (1 gm/L) and given orally in a single daily dose of 200 mg/kg body weight[13] by gastric intubation.

At the end of experimental period, the body weight of rats was measured and blood samples were collected from their medial epicanthus. Animals were anaesthetized using urethane with a dose of 1.2gm/kg[14], anterior abdominal wall was incised and the kidney was carefully dissected out and obtained.

Histopathological studies

1. Specimens were subjected to formalin fixation. After fixation, ascending grades of ethanol were used to dehydrate tissues, cleared in xylol and embedded in paraffin blocks. Serial sections of 5 micrometer thickness were cut and stained with Hematoxylin and Eosin (H&E) for routine histological examination[15].

Some sections from all specimens were picked upon positive slides for immunohistochemical staining for nuclear factor kappa (NF-κb).

The tissue sections were deparaffinized and antigen retrieval was done. Sections were immersed in hydrogen peroxidase for ten minutes in order to block the peroxidase enzyme, followed by incubation of sections with rabbit polyclonal anti-NFkb/p65 (Rel A) Ab-1 antibody (Cat. No. RB-1638-R7, Ready to use, Lab Vision) that followed also by washing. Sections were then covered with 4–5 drops of Ultra Vision biotinylated goat anti-polyvalent secondary antibody; incubated at room temperature for 10 min. The technique used in staining was a standard avidin-biotin complex staining procedure. Then slides were counter stained with hematoxylin. Positive immunoreaction for nuclear factor kappa appeared in cytoplasm[16].

Transmission electron microscope (TEM)[17] was used to examine ultrathin sections in Al-Azhar University. 2.5% gluteraldehyde and phosphate buffer was used for fixation at four°C for two hours. Three successive washes for 10 minutes each, were done in a phosphate-puffer-solution (PBS). Then there were post fixed in one percent osmium tetroxide. Ascending concentrations of ethanol; 50, 70, 90 and 100% were used to dehydrate specimens. Acetone was added then specimens were put inside the capsules

which were completely filled with Araldite 502 resin and polymerized at sixty degrees for one day.

LEICA Ultracut (UCT) ultramicrotome was used to cut capsules into 1 um sections (semi-thin) then stained with 1% toluidine blue for approximately 30 seconds. The semi-thin sections slides stained with toluidine blue stain were photographed using microscope of the Olympus® 268 Nm with an Olympus® camera attached through a 1/2 X picture adaptor and a 100 X oil objective in the Anatomy Department, Faculty of Medicine, Ain Shams University.

Ultramicrotome was used to prepare ultrathin sections (80-90 nm) to be stained by double stain- technique of 2% uranyl acetate for ten minutes, followed by ten minutes immersion in Reynold's solution of lead citrate. Then they were washed and left to dry to be kept in grid box till examination by Transmission electron microscopy.

Image Analysis

The result images of anti-NF kappa immunostained sections were analyzed on Intel® Core I3® based computer using Video Test Morphology® software (Russia) with a specific built-in routine for measuring the density of the stain. Five slides were prepared from each group, 5 random fields from each slide were subjected to analysis.

Statistical Analysis

The mean values and standard deviation (SD) were calculated using the computer program SPSS (Statistical package for social science) version 17.0. Analysis of variance (one way ANOVA) was done followed by Post Hoc test to compare groups. Significance of data was determined by the probability value (*P. value*). The difference was non-significant at $P > 0.05$, significant ≤ 0.05 and highly significant ≤ 0.001.

RESULTS

Histopathological Results

Heamatoxylin and Eosin Stain(Hx&E)

Examination of Hx&E stained sections of the rat kidney of control group (both subgroups) showed that the renal cortex contained renal corpuscles, proximal convoluted tubules and distal convoluted tubules. The parietal layer of the Bowman's capsule was formed of flat epithelial cells surrounding Bowman's space. The glomerulus of the renal corpuscle was formed of a tuft of blood capillaries (Figure 1A). Group II (PTX group) showed the same histological picture as control group apart from congested capillaries in the glomerulus (Figure 1B). In group III, the parietal layer of Bowman's capsule was apparently thick in certain area and in other areas was disrupted and discontinued, the Bowman's space was wide and irregular and the renal glomerulus was shrunken (Figure 1C). Group IV showed reguler distinct Bowman's space and renal glomerulus was formed of tuft of blood capillaries similar to the control group (Figure 2A). However, there was apparently thickened parietal layer of Bowman's capsule in some areas (Figure 4C). Group V showed apparent thin parietal layer of Bowman's capsule and some congested capillaries (Figure 2B).

Examination of Hx&E stained sections of the rat kidney of control group showed that the proximal convoluted tubules had narrow lumen and were composed of cuboidal cells with eosinophilic cytoplasm and basal vesicular nuclei. The distal convoluted tubules had wide lumen and were formed of cuboidal epithelial cells with apical vesicular nuclei (Figure 3A). Group II (PTX group) showed the same histological picture as control group with extravasation of blood cells in intertubular space (Figures 3B, C). In group III, the proximal convoluted tubules were irregular with destroyed apical brush border and markedly degenerated lining epithelial cells. There were loss of their cell lining, vacuolated cytoplasm, rarified cytoplasm and darkly stained nuclei (Figure 4A). The distal convoluted tubules were destroyed with complete detachment of the lining cells from their basement membrane into the lumen in some tubules (Figure 4B). Group IV showed that the lining cells of proximal convoluted tubules were cuboidal with esinophilic cytoplasm and vesicular basal nuclei and the lining cells of distal convoluted tubules were cuboidal with apical vesicular nuclei similar to the control group (Figure 2A). However, cystic dilatation of some tubules and degenerated cells of the epithelial lining of some distal convoluted tubules were noticed (Figure 4C). In group V, most of proximal convoluted tubules were with narrow lumen and their lining cells showed intact epithelium with basal vesicular nuclei. Some of the epithelial cells lining distal convoluted tubules were lost while others had wide lumen and their lining cells had apical vesicular nuclei (Figure 4D).

Anti-NF kappa immunohistochemical Stain

Examination of renal sections of control group stained with anti-NF kappa immunohistochemical stain revealed the renal cortex of rat kidney of control group with weak cytoplasmic positive reaction of the cells of the renal glomerulus (Figure 5A). Group II showed the glomerulus with weak reaction of the cytoplasm of its cells similar to the control group (Figure 5B). Group III showed weak positive reaction of the cytoplasm of cells of the glomerulus (Figure 5C). Group IV showed weak cytoplasmic reaction of the cells of the glomerulus (Figure 6A). Group V showed weak cytoplasmic reaction of the cells of renal glomerulus (Figure 6B).

The renal tubules showed weak positive reaction of cytoplasm of the epithelial cells of proximal and distal convoluted tubules of control group and group II (Figure 7A,B). In group III, there was a strong positive reaction of the cytoplasm of the lining cells of renal tubules (Figure 7C). Group IV showed faint cytoplasmic reaction of proximal and distal convoluted tubules (Figure 8A). Group V showed weak reaction of proximal and distal convoluted tubules. Apparently PCT had increased reaction than DCT (Figure 8B).

Toluidine Blue Stain

Examination of semithin sections of rat kidney of the control group stained with toluidine blue revealed that the Bowman's capsule's (BC) parietal layer was made up of basic squamous epithelium. Podocytes had large irregular open face nuclei. Mesangial cells with small dark nuclei were seen between blood capillaries of the glomerulus (Figure 9A). Group II showed that BC parietal layer was made up of basic squamous epithelium. The mesangial cells appeared with dark nuclei between the congested blood capillaries of the glomerulus (Figure 9B). Group III showed focal thickening of BC parietal layer, wide irregular Bowman's space and congested blood capillaries of the glomerulus (Figure 9C). Group IV showed that the nuclei of the cells of BC parietal layer bulged in some areas. Podocyte appeared on the periphery of the glomerulous sending its branches. The mesangial cells had dark stained nuclei. There were some congested capillaries (Figure 10A). Group V showed thin BC parietal layer, the podocytes with open face nuclei on the periphery of the renal corpuscle and the mesangial cell with dark nuclei. The glomerular capillaries were congested (Figure 10B).

Proximal convoluted tubules (PCT) of the control group appeared with narrow lumen and were composed of cuboidal epithelial cells with luminal brush border and rounded basal vesicular nucleus (Figure 11A). Group II showed PCT with narrow lumen and their lining cells with apical brush border, vesicular nucleus and basal infoldings (Figure 11B). Group III showed shedding of the cells of PCT into the lumen, cytoplasmic vacuolation and extravasated blood in the intertubular space (Figure 11C). In group IV, most of the lining cells of the PCT had minimal amount of vacuolations in their cytoplasm with vesicular rounded basal nuclei. There was congested interstitial capillaries filled with red blood cells (RBCs) around the tubules (Figure 12A). Group V showed PCT with narrow lumen and their lining cells appeared with vesicular rounded basal nuclei (Figure 12B). Some proximal convoluted tubules showed hydropic degeneration (Figure 14B).

The distal convoluted tubules (DCT) of the control group were lightly stained with wide lumen and formed of cuboidal epithelial cells with apical vesicular nucleus (Figure 13A). Group II showed the DCT with wide lumen and its lining cells are cuboidal with apical vesicular nuclei similar to the control group (Figure 13B). In group III, the cells of DCT showed swollen cytoplasm with vesicles. There was irregular membrane of the nuclei of the lining cells of DCT (Figure 13C). Group IV showed the DCT with wide lumen. Most of their lining cells had vesicular apical nuclei. There were expelled cells into the lumen and some cells with ballooning of the cytoplasm. There were congested interstitial blood capillaries filled with RBCs around the tubules (Figure 14A). Group V showed DCT with wide lumen and apical vesicular nuclei of their lining cells (Figure 14B).

Transmission Electron Microscope Examination

Examination of ultrathin sections of rat kidney of the control group showed the major processes and foot processes of podocyte in the subpodocytic space. The smooth regular glomerular basement membrane surrounded the fenestrated capillary endothelium (Figure 15A). Group II showed podocyte major processes and foot processes resting on thin glomerular basement membrane of a congested blood capillary. There were filtration slits between foot processes similar to the control group (Figure 15B). In group III there were ballooning and fusion of foot processes of podocyte with thick glomerular basement membrane. A wide area of degeneration of the mesangial matrix was noticed (Figure 15C). In group IV, the glomerulous was with thin glomerular basement membrane. However, foot processes were fused together in some areas (Figure 16A). Group V showed thin glomerular basement membrane (Figure 16B). Podocyte was with vacuolated cytoplasm and some foot processes were fused (Figure 16C).

The apical border of the epithelial cells of PCT of the control group showed numerous microvilli. Their cytoplasm contained mitochondria and basal euchromatic nuclei with regular membrane and there were basal infoldings of the cell basement membrane (Figure 17A). Group II showed PCT lining cell with numerous mitochondria, dense bodies and euchromatic nucleus in the cytoplasm similar to the control group (Figure 17B). Group III showed the lining cells of PCT with cellular debris in the lumen, destroyed apical microvilli, marked vacuolation and degenerated nuclei (Figure 17C). Group IV showed PCT with apical microvilli, few dense bodies in the cytoplasm and basal infolding. There were extravasated red blood cells around the tubule (Figure 18A). Group V showed cellular debris expelled from ruptured cells of PCT into the lumen. PCT cells appeared with preserved apical microvilli, some vacuolations, numerous mitochondria and dense bodies in the cytoplasm with preserved basal infolding (Figure 18B).

The cell lining of DCT of control group was cuboidal with few microvilli projecting into the lumen, numerous long densely packed mitochondria and apically situated rounded nuclei with regular membrane and prominent nucleolus (Figure 19A). Group II showed the epithelial cell lining of DCT with few apical microvilli, numerous mitochondria, membrane bounded vesicles, euchromatic nucleus and basal infoldings similar to the control group (Figure 19B). In group III, there were loss of apical microvilli and marked cytoplasmic vacuolation. The cytoplasm showed rounded membranous vesicular bodies containing degenerated cytoplasmic organelles. The nucleus showed dispersed chromatin and the nuclear membrane is not clearly demarcated from the surrounding cytoplasm. Decreased basal infolding of the basement membrane of the cell lining of DCT was noticed (Figure 19 C). Group IV showed distal convoluted tubules with few short apical microvilli , elongated mitochondria, apical euchromatic nucleus and extensive basal infolding. There was a dilated blood capillary (Figure 20A). Group

V showed the lumen of DCT with cellular debris expelled from a ruptured cell. The lining cell of distal convoluted tubule had few apical microvilli, numerous mitochondria, dense bodies and vesicular nucleus with basal infolding similar to the control group (Figure 20B).

Results of Measuring the Blood Glucose Level

The PTX group (119.0±11.59) showed non-significant decrease to control group (126.9±23.22).

The diabetic group showed a high significant increase in the mean blood glucose level (518.8±54.08) as compared to the control group (126.9±23.22) and PTX group (119.0±11.59).

The preventive group (486.8±164.31) and the curative group (532.5±70.83) showed a high significant increase in the mean blood glucose level as compared to the control group (126.9±23.22) and PTX group (119.0±11.59) and showed a non-significant difference as compared to the diabetic nephropathy group (518.8±54.08) (Bar Chart 1).

Results of Measuring the Body Weight

The PTX group (293.60±39.49) showed non significance to control group (253.9±45.43).

The diabetic group (178.0±21.75) showed a high significant decrease in the mean body weight (518.8±54.08) as compared to the control group (253.9±45.43) and PTX group (293.60±39.49).

The preventive group (166.80±62.50) and the curative group (157.40±38.94) showed a high significant decrease in the mean body weight as compared to the control group (253.9±45.43) and PTX group (293.60±39.49) , and showed a non-significant difference as compared to the diabetic nephropathy group (178.0±21.75) (Bar Chart 2).

Results of Measuring the Density of Positivity in NF kappa immunostained sections

The PTX group (69.28±5.43) showed non-significant increase in density of positivity compared to control group (67.58±4.88).

The sections stained with NF kappa immunostain of DN rats (88.72±5.59) showed highly significant increase in density of positivity compared to control group (67.58±4.88).

The DN group (88.72±5.59) showed significant increase in density of positivity compared to the PTX group (69.28±5.43).

The preventive group (70.30±5.79) and curative group (72.40±4.24) respectively showed non significance compared to control group (67.58±4.88).

The preventive group (70.30±5.79) and curative group (72.40±4.24) respectively showed highly significant decrease in density of positivity compared to DN group (88.72±5.59) as shown in (Bar Chart 3).

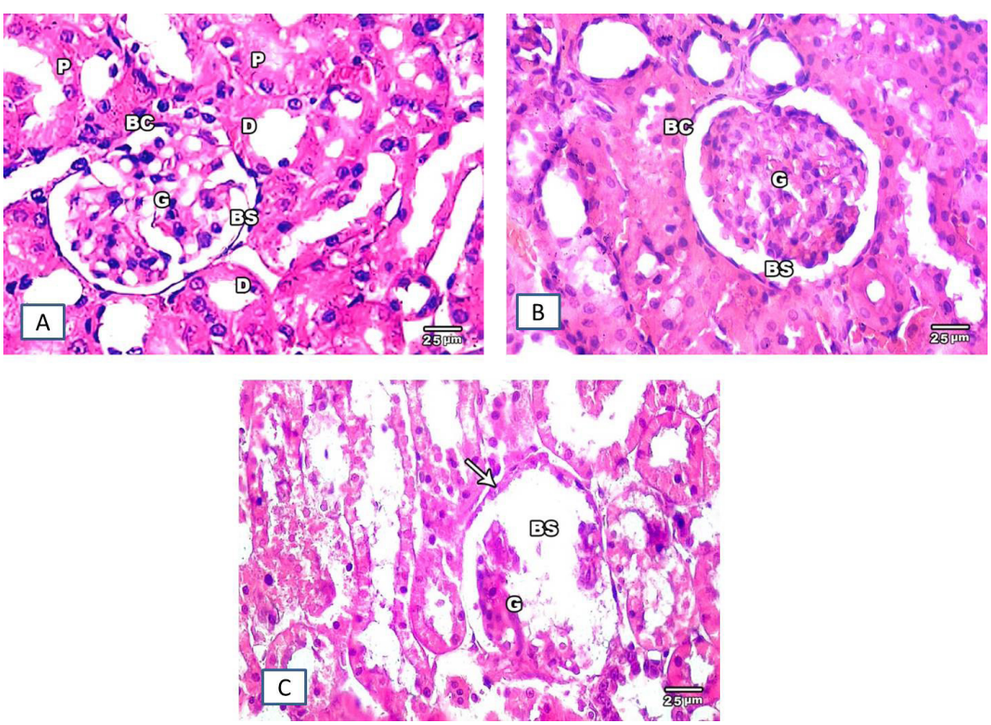


Fig. 1{A-C}: A photomicrograph of a section of rat kidney A. control group: showing the renal cortex with renal corpuscles, proximal convoluted tubules (P) and distal convoluted tubules (D). The parietal layer of the Bowman's capsule (BC) is formed of flat epithelial cells surrounding Bowman's space (BS). The glomerulus (G) of the renal corpuscle is formed of a tuft of blood capillaries. B. group II: showing flat epithelial cells of the parietal layer of Bowman's capsule (BC), regular Bowman's space (BS) and congested capillaries in the glomerulus (G). C. group III: showing that the parietal layer of Bowman's capsule is thick in certain area and in other areas is disrupted and discontinued (arrow), wide irregular Bowman's space (BS) and shrunken renal glomerulus (G). (Hx&E x400)

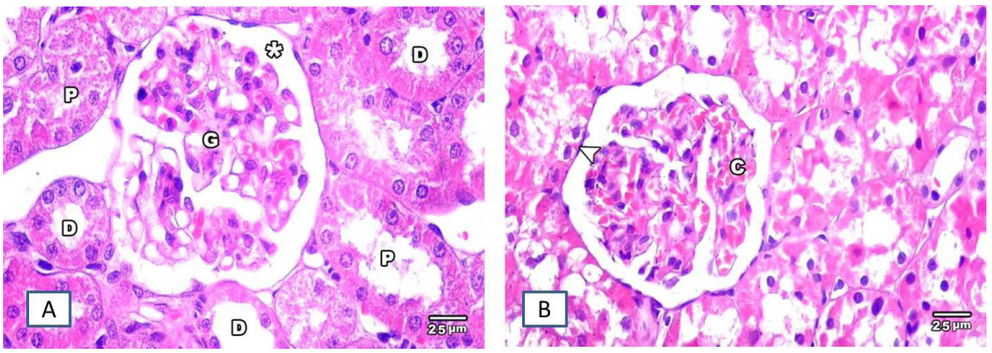


Fig. 2 {A-B}: A photomicrograph of a section of rat kidney A.group IV: showing the reguler distinct Bowman's space (star) and renal glomerulus (G) formed of tuft of blood capillaries. The lining cells of proximal convoluted tubules (P) are cuboidal with esinophilic cytoplasm and vesicular basal nuclei. The lining cells of distal convoluted tubules (D) are cuboidal with apical vesicular nuclei. B. group V: showing apparent thin parietal layer of Bowman's capsule (arrow head). There are some congested capillaries (C). (Hx&E x400)

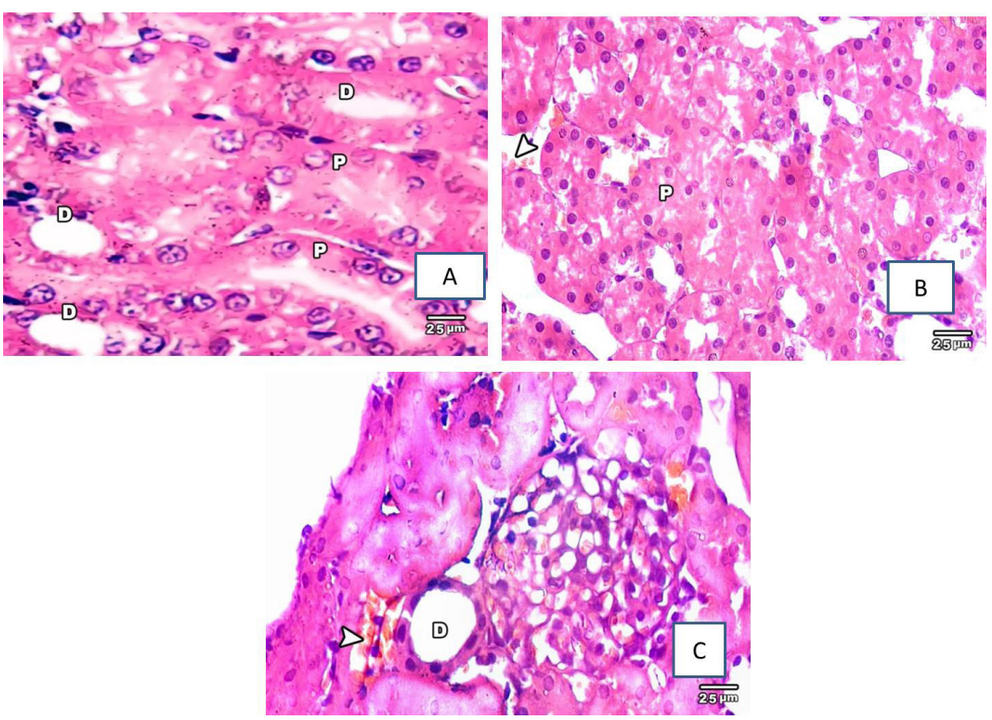


Fig. 3{A-C}: A photomicrograph of a section of rat kidney showing renal tubules. A. control group: showing the proximal convoluted tubules (P) have narrow lumen and are composed of cuboidal cells with eosinophilic cytoplasm and basal vesicular nuclei. The distal convoluted tubules (D) have wide lumen and are formed of cuboidal epithelial cells with apical vesicular nuclei. B&C. group II: similar to control group with extravasation of blood cells in intertubular space (arrow head). (Hx&E x400)

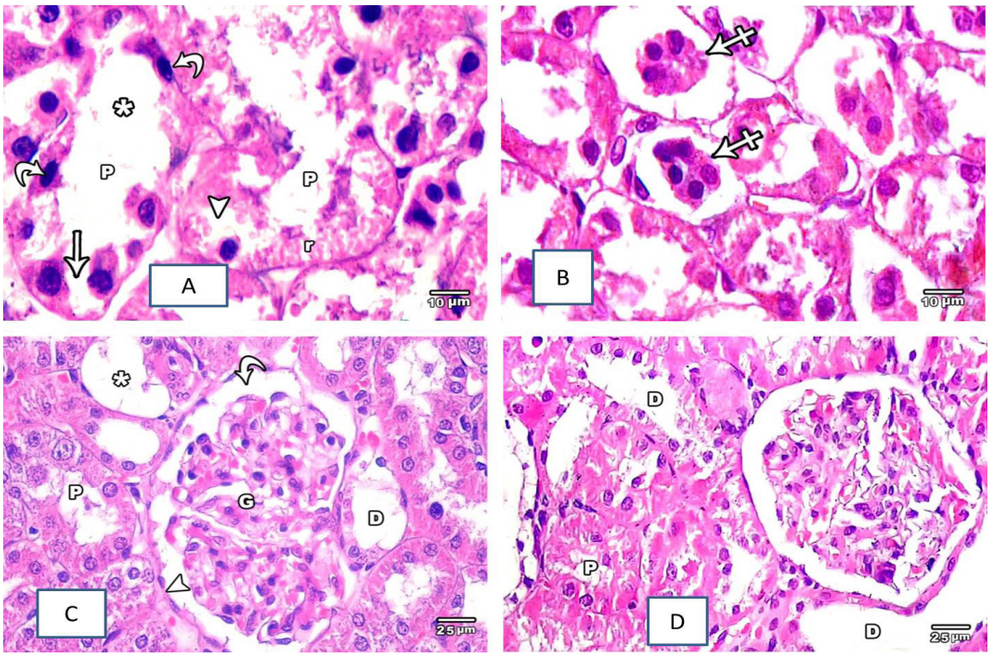


Fig. 4{A-D}: A photomicrograph of a section of rat kidney showing renal tubules. A. group III: showing irregular proximal convoluted tubules (P) with destroyed apical brush border and markedly degenerated lining epithelial cells. Notice loss of their cell lining (star), loss of cells (arrow), vacuolated cytoplasm (arrow head), rarified cytoplasm (r) and darkly stained nuclei (curved arrow). B. group III: showing destroyed distal convoluted tubules (D) with complete detachment of the lining cells from their basement membrane into the lumen in some tubules (crossed arrow). C. group IV: showing apparently thickened parietal layer of Bowman's capsule (arrow head) in some areas. There is reguler distinct Bowman's space (curved arrow). The renal glomerulus (G) is formed of a tuft of blood capillaries. Most of renal tubules have vesicular nuclei apart from cystic dilatation (star) of some tubules and the degenerated cells of the epithelial lining of some distal convoluted tubules (D). D. group V: showing most of proximal convoluted tubules (P) are with narrow lumen and their lining cells show intact epithelium with basal vesicular nuclei. Some of the epithelial cells lining distal convoluted tubules (D) are lost while others have wide lumen and their lining cells have apical vesicular nuclei. (Hx&E A&B x1000, C&D x400)

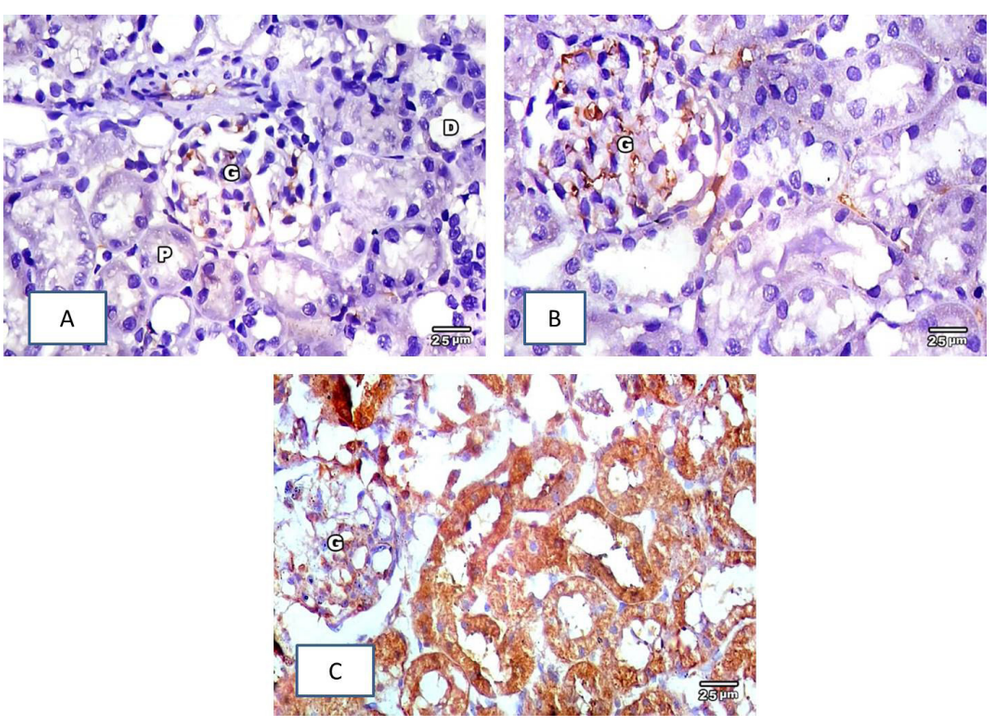


Fig. 5{A-C}: A photomicrograph of a section of the renal cortex of rat kidney stained with anti-NF kappa immune-histochemical stain A. control group: showing weak cytoplasmic positive reaction of the cells of the renal glomerulus (G). P for proximal convoluted tubules and D for distal convoluted tubules. B. group II: showing weak reaction of the cytoplasm of the cells of the glomerulus (G) similar to control group. C. group III: showing weak positive reaction of the cytoplasm of cells of the glomerulus (G). (anti-NF kappa immune-histochemical stain x400)

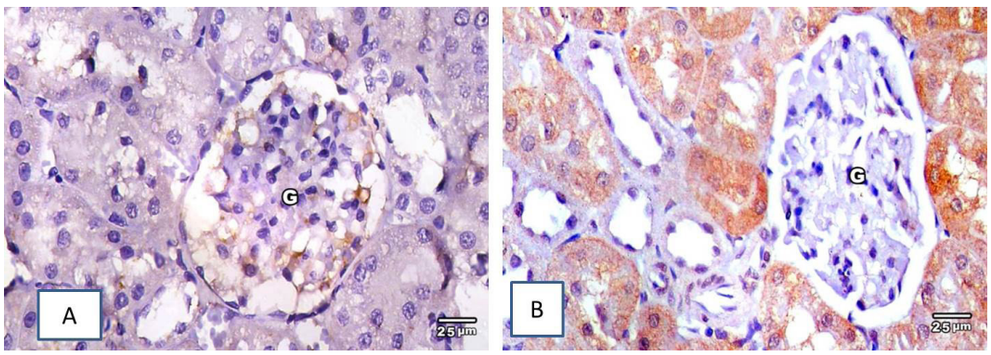


Fig. 6 {A-B}: A photomicrograph of a section of the renal cortex of rat kidney stained with anti-NF kappa immune-histochemical stain. A. group IV: showing weak cytoplasmic reaction of the cells of the glomerulus (G). B. group V: showing weak cytoplasmic reaction of the cells of renal glomerulus (G). (anti-NF kappa immune-histochemical stain x400)

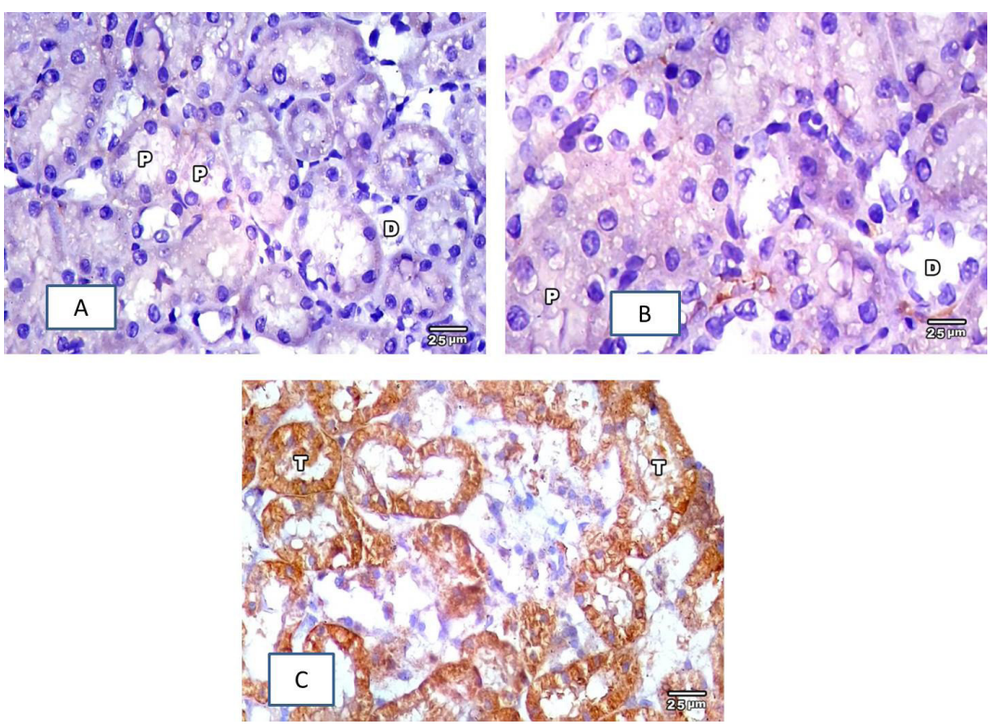


Fig. 7{A-C}: A photomicrograph of a section of the renal cortex of rat kidney stained with anti-NF kappa immune-histochemical stain showing A: control group with weak positive reaction of cytoplasm of the epithelial cells of proximal (P) and distal (D) convoluted tubules. B. group II with weak cytoplasmic reaction of the lining epithelial cells of renal tubules (proximal convoluted tubules (P) and distal convoluted tubules (D)) similar to control group. C: group III showing strong positive reaction of the cytoplasm of the lining cells of renal tubules. (anti-NF kappa immune-histochemical stain x400)

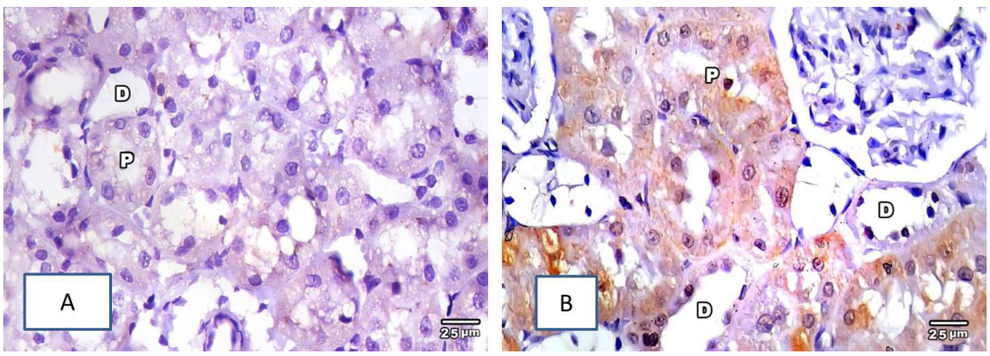


Fig. 8{A-B}: A photomicrograph of a section of the renal cortex of rat kidney stained with anti-NF kappa immune-histochemical stain showing A. group IV: showing faint cytoplasmic reaction of proximal (P) and distal (D) convoluted tubules. B. group V: showing weak reaction of proximal (P) and distal (D) convoluted tubules. Apparently PCT (P) have increased reaction than DCT (D). (anti-NF kappa immune-histochemical stain x400)

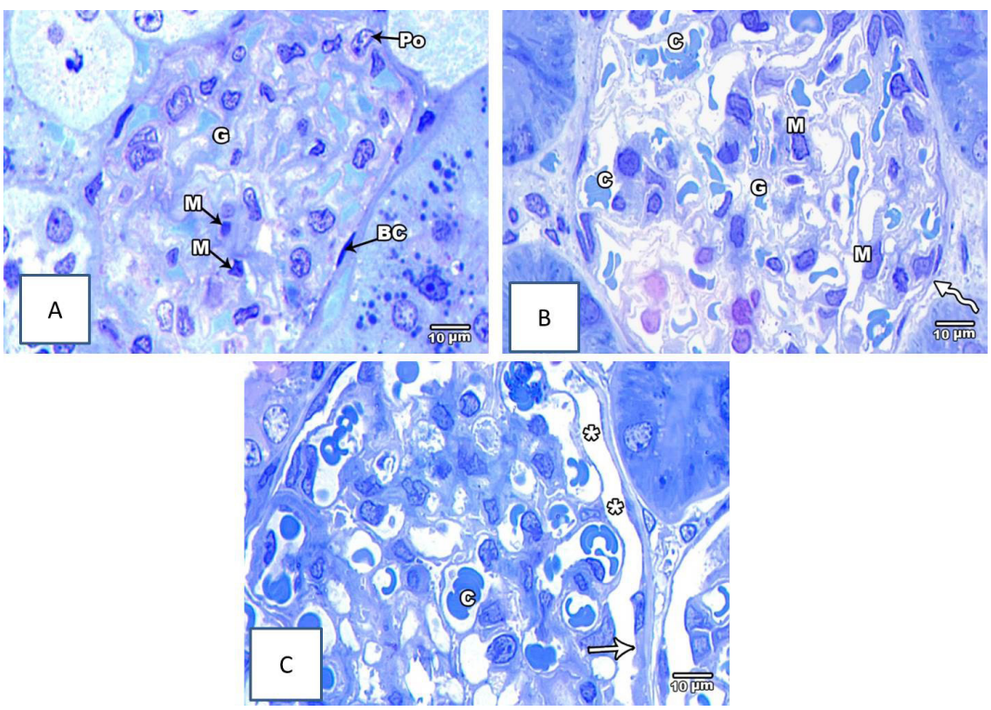


Fig. 9{A-C}: A photomicrograph of a semithin section of the renal cortex of rat kidney showing the renal corpuscle. A. control group: the parietal layer of the Bowman's capsule (BC) is formed of simple squamous epithelium. Podocytes (Po) have large irregular open face nuclei. Mesangial cells (M) with small dark nuclei are seen between blood capillaries of the glomerulus (G). B. group II: showing the parietal layer of Bowman's capsule (wavy arrow) formed of simple squamous epithelium. Notice the mesangial cells (M) with dark nuclei between the congested blood capillaries (C) of the glomerulus (G). C. group III: showing focal thickening of parietal layer of Bowman's capsule (arrow), wide irregular Bowman's space (star) and congested blood capillaries (C) of the glomerulus. (Toluidine blue stain x1000)

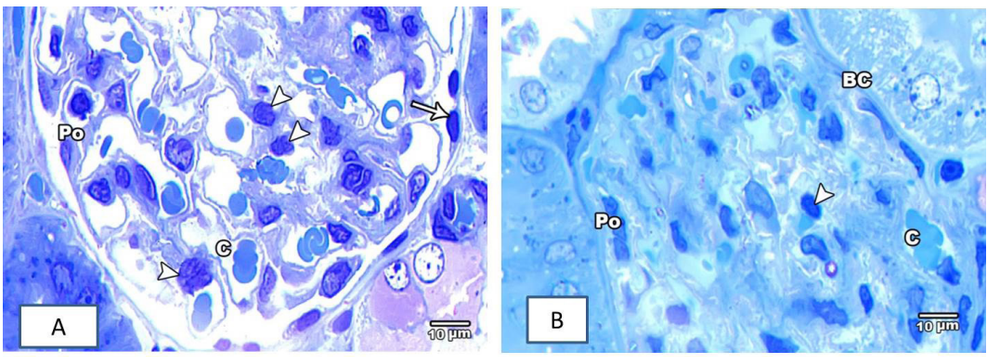


Fig. 10 {A-B}: A photomicrograph of a semithin section of the renal cortex of rat kidney showing the renal corpuscle. A. group IV: showing the nuclei of the cells parietal layer of Bowman's capsule bulge in some areas (arrow). Podocyte (Po) appear on the periphery of the glomerulous sending its branches. The mesangial cells (arrow head) have dark stained nuclei. There are some congested capillaries (C). B. group V: showing thin parietal layer of Bowman's capsule, podocytes (Po) with open face nuclei on the periphery of the renal corpuscle and mesangial cell (M) with dark nuclei. There is congestion in the glomerular blood capillaries (C). (Toluidine blue stain x1000)

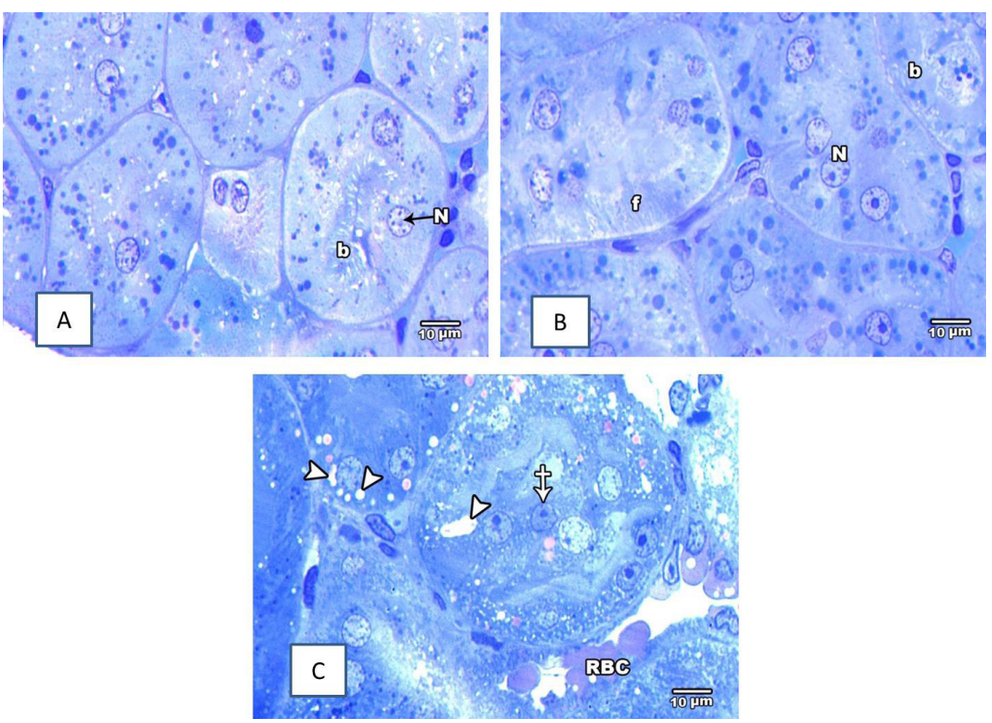


Fig. 11 {A-C}: A photomicrograph of a semithin section the renal cortex of rat kidney. A. control group: showing proximal convoluted tubules with narrow lumen and are composed of cuboidal epithelial cells with luminal brush border (b) and rounded basal vesicular nucleus (N). B. group II: showing proximal convoluted tubules with narrow lumen and their lining cells with apical brush border (b), vesicular nucleus (N) and basal infoldings (f). C. group III: showing shedding of the cells of proximal convoluted tubules into the lumen (crossed arrow), cytoplasmic vacuolation (arrow head) and extravasated blood (RBC) in the intertubular space. (Toluidine blue stain x1000)

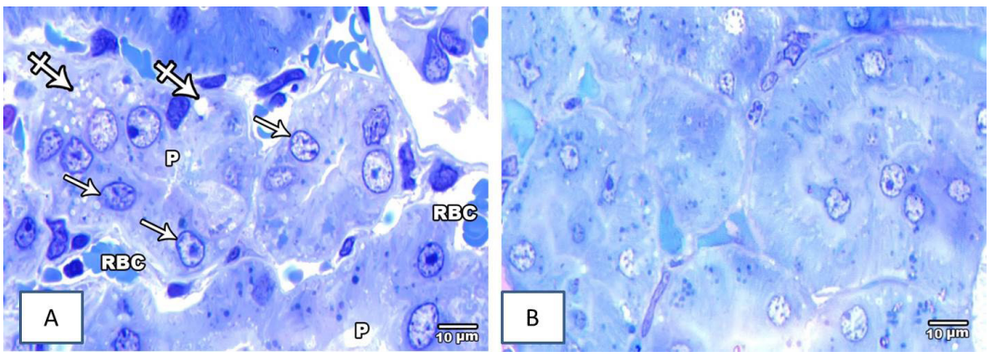


Fig. 12 {A-B}: A photomicrograph of a semithin section the renal cortex of rat kidney. A. group IV: showing proximal convoluted tubules (P) with most of their lining cells having cytoplasm with minimal amount of vacuolations (crossed arrow) and vesicular rounded basal nuclei (arrow). There are congested interstitial capillaries filled with RBCs (RBC) around the tubules. B. group V: showing proximal convoluted tubules with narrow lumen and their lining cells appear with vesicular rounded basal nuclei. (Toluidine blue stain x1000)

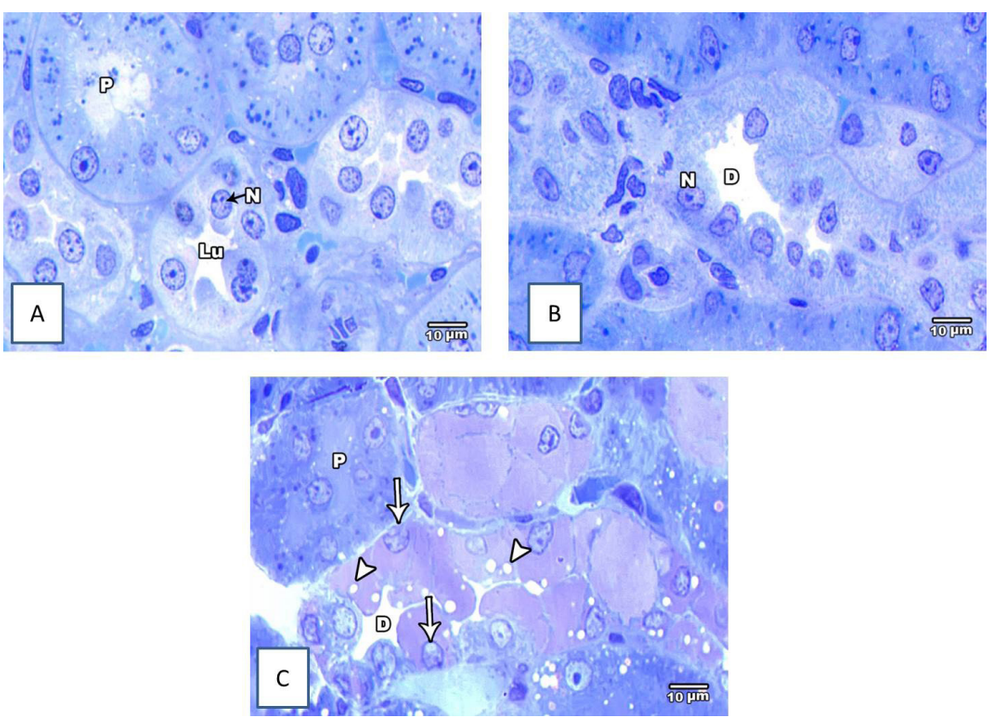


Fig. 13{A-C}: A photomicrograph of a semithin section of the renal cortex of rat kidney. A. control group: showing the lightly stained distal convoluted tubules with wide lumen (Lu) and formed of cuboidal epithelial cells with apical vesicular nucleus (N). B. group II: showing the distal convoluted tubule (D) with wide lumen and its lining cells are cuboidal with apical vesicular nuclei (N). C. group III: showing the cells of distal convoluted tubules (D) with swollen cytoplasm with vesicles (arrow head). Notice the irregular membrane of the nuclei of the lining cells (arrow) of DCT. P for proximal convoluted tubules. (Toluidine blue stain x1000)

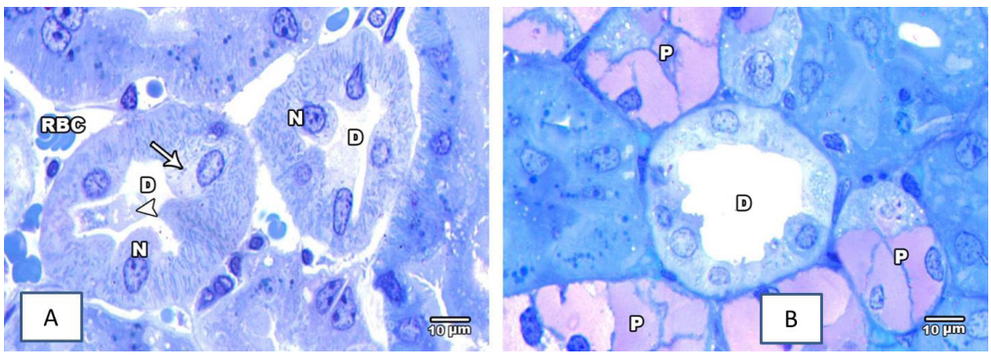


Fig. 14{A-B}: A photomicrograph of a semithin section of the renal cortex of rat kidney. A. group IV: showing the distal convoluted tubules (D) with wide lumen. Most of their lining cells have vesicular apical nuclei (N). There are expelled cells into the lumen (arrow head) and some cells with ballooning of the cytoplasm (arrow). Notice the congested interstitial blood capillaries filled with RBCs (RBC) around the tubules. B. group V: showing distal convoluted tubule (D) with wide lumen and apical vesicular nuclei of their lining cells. Proximal convoluted tubules (P) show hydropic degeneration. (Toluidine blue stain x1000)

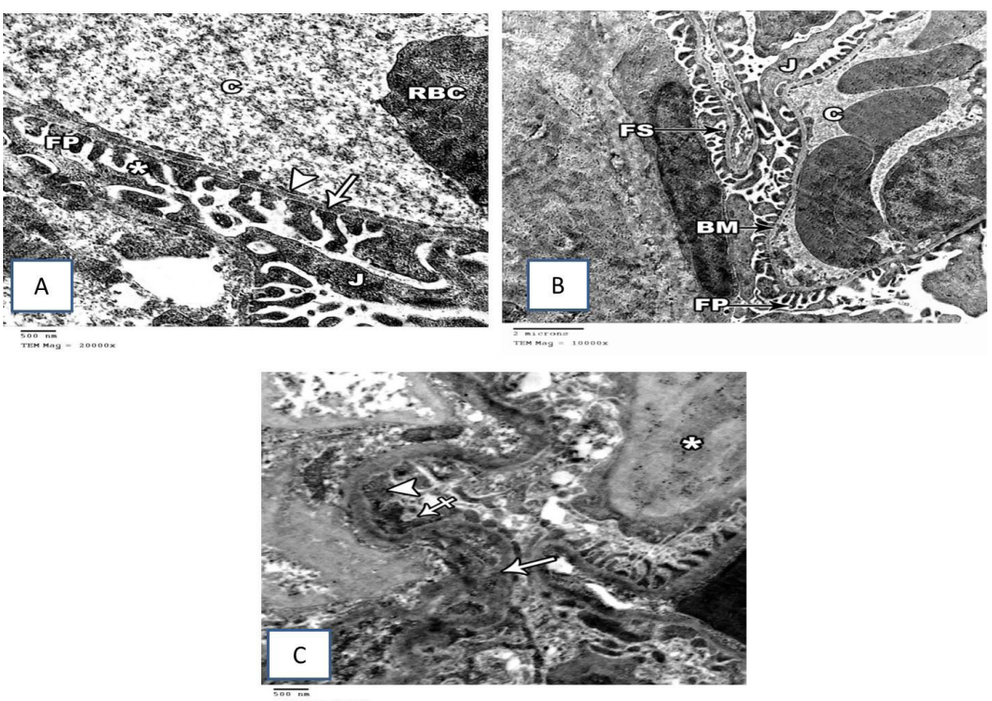


Fig. 15 {A-C}: A transmission electron micrograph of renal cortex A: control group showing the major processes (J) and foot processes (FP) of podocyte in the subpodocytic space (star). The smooth regular glomerular basement membrane (arrow) surrounds the fenestrated capillary endothelium (head arrow). Notice the glomerular capillary (C) with red blood cell (RBC) inside it. B: group II showing podocyte major processes (J) and foot processes (FP) resting on thin glomerular basement membrane (BM) of a congested blood capillary (C). Notice filtration slits (FS) between foot processes. C: group III showing ballooning (arrow head) and fusion (crossed arrow) of foot processes of podocyte with thick glomerular basement membrane (arrow). Notice the wide area of degeneration of the mesangial matrix (star). (Uranyl acetate & lead citrate)

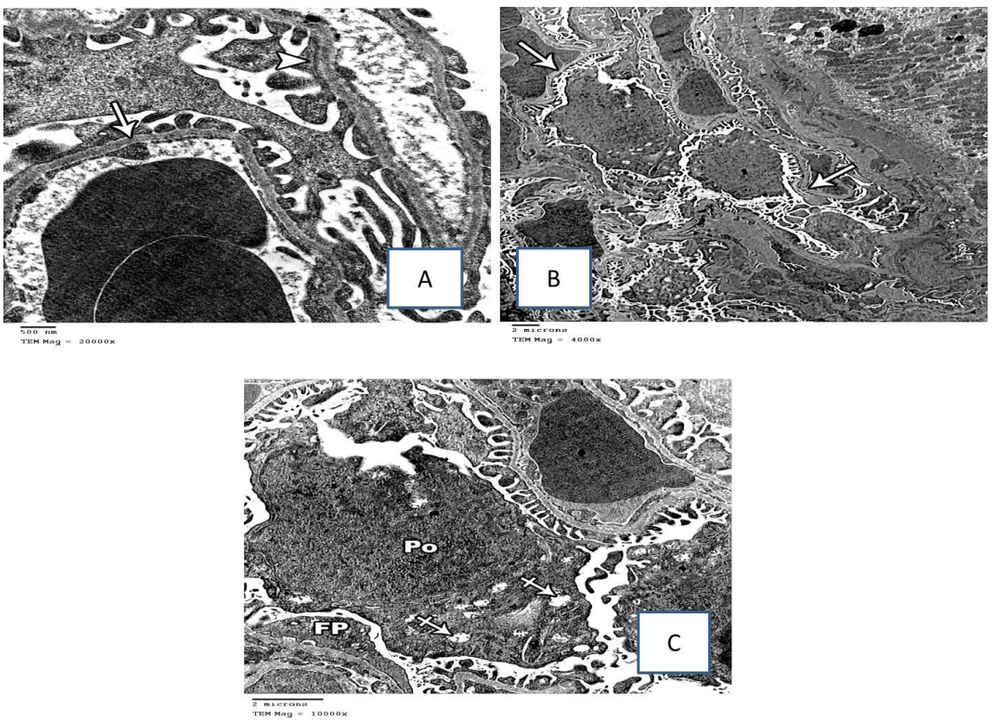


Fig. 16{A-C}: A transmission electron micrograph of renal cortex. A. group IV: showing thin glomerular basement membrane (arrow). Foot processes (FP) are fused together in some areas. B. group V: showing thin glomerular basement membrane (arrow). C. group V: showing podocyte (Po) with vacuolated cytoplasm (crossed arrow). Some foot processes (FP) are fused. (Uranyl acetate & lead citrate)

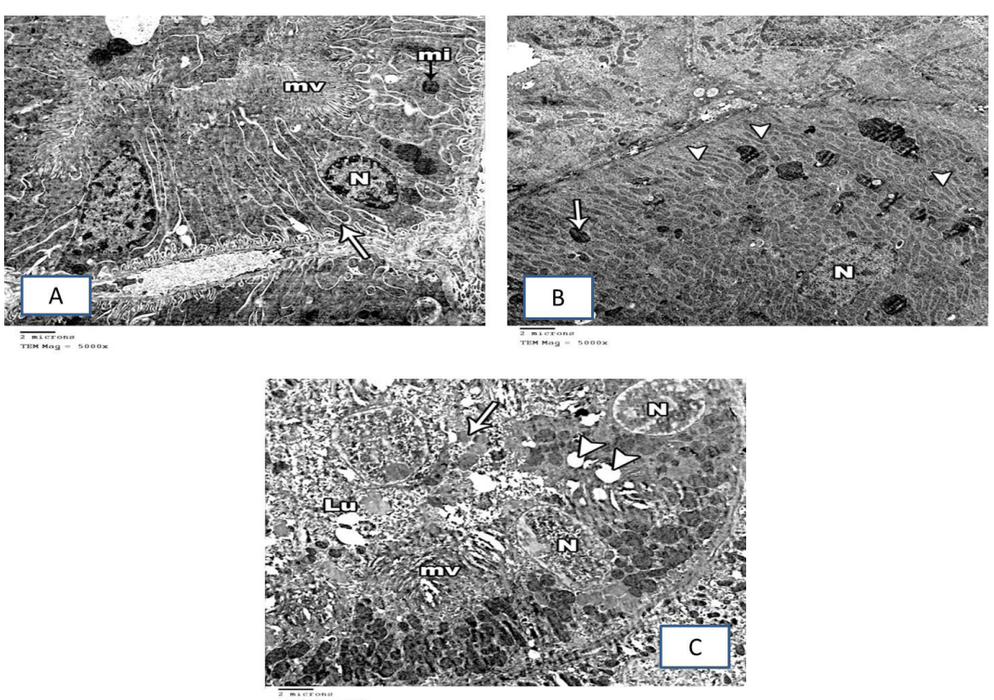


Fig. 17{A-C}: A transmission electron micrograph of renal cortex. A: control group showing the epithelial cells of PCT with their apical border showing numerous microvilli (mv). Notice the mitochondria (mi), basal euchromatic nuclei (N) with regular membrane and basal infolding (arrow) of the cell basement membrane. B:group II showing a PCT lining cell with numerous mitochondria (arrow head), dense bodies (arrow) and euchromatic nucleus (N) in the cytoplasm. C:group III showing the lining cells of proximal convoluted tubule with cellular debris (arrow) in the lumen (Lu), destroyed apical microvilli (mv), marked vacuolation (arrow head) and degenerated nuclei (N). (Uranyl acetate & lead citrate)

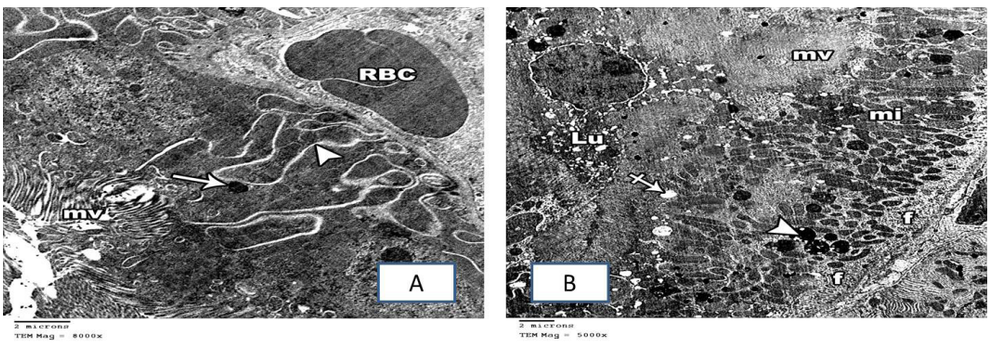


Fig. 18 {A-B}: A transmission electron micrograph of renal cortex. A. group IV: showing proximal convoluted tubule with apical microvilli (mv), few dense bodies (arrow) in the cytoplasm and basal infolding (arrow head). There are extravasated red blood cells (RBC) around the tubule. B. group V: showing cellular depris expelled from ruptured cells of PCT into the lumen (Lu). Proximal convoluted tubule cells appear with preserved apical microvilli (mv), some vacuolations (crossed arrow), numerous mitochondria (mi) and dense bodies (arrow head) in the cytoplasm with preserved basal infolding (f). (Uranyl acetate & lead citrate)

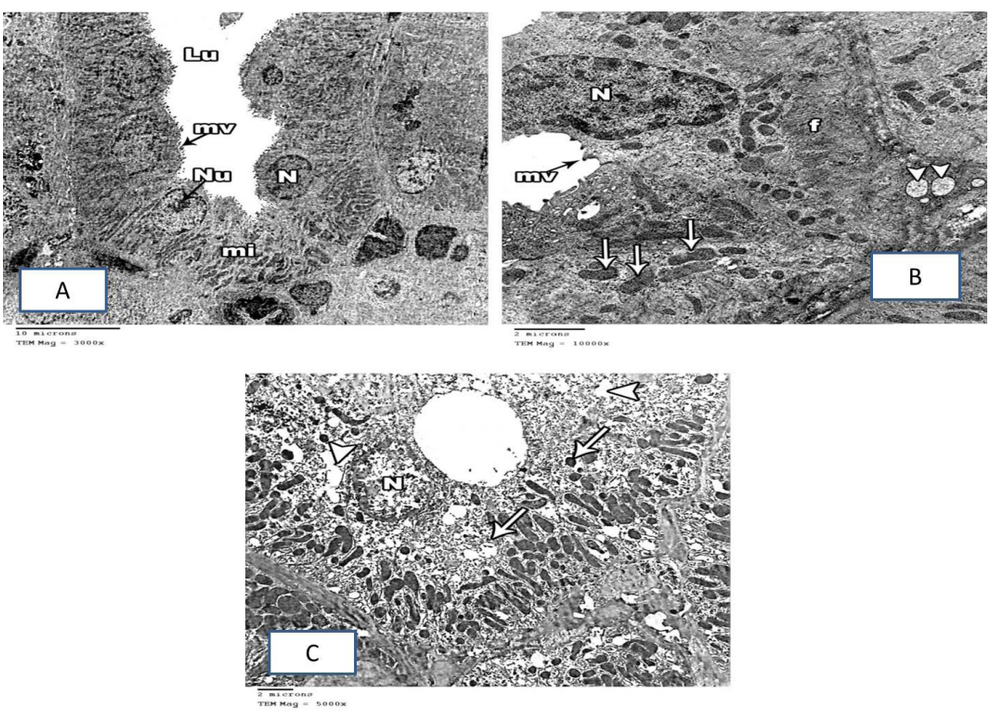


Fig. 19{A-C}: A transmission electron micrograph of renal cortex. A: control group showing cuboidal cell lining DCT with few microvilli (mv) projecting into the lumen (Lu), numerous long densely packed mitochondria (mi) and apically situated rounded nuclei (N) with regular membrane and prominent nucleolus (Nu). B:group II showing the epithelial cell lining of DCT with few apical microvilli (mv), numerous mitochondria(arrow), membrane bounded vesicles (arrow head), euchromatic nucleus (N) and basal infoldings (f). C: group III showing loss of apical microvilli, marked cytoplasmic vacuolation (arrow head). The cytoplasm shows rounded membranous vesicular bodies containing degenerated cytoplasmic organelles (arrow). The nucleus (N) shows dispersed chromatin and the nuclear membrane is not clearly demarcated from the surrounding cytoplasm. Notice the decreased basal infolding of the basement membrane of the cell lining of DCT. (Uranyl acetate & lead citrate)

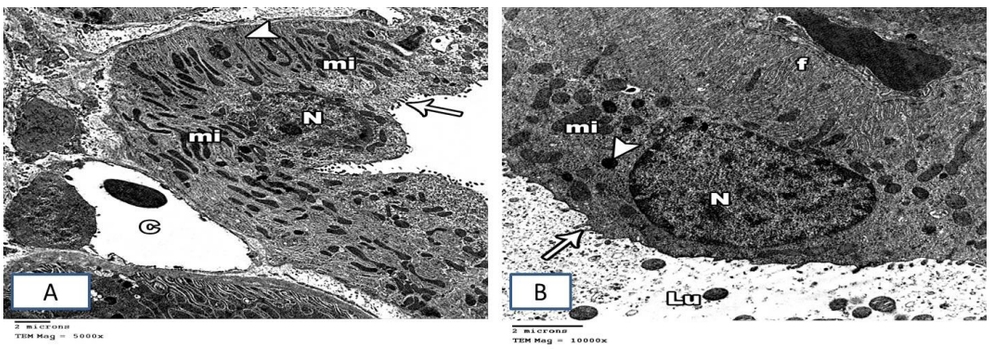
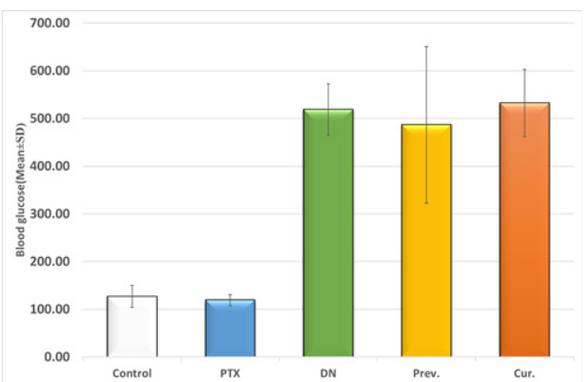
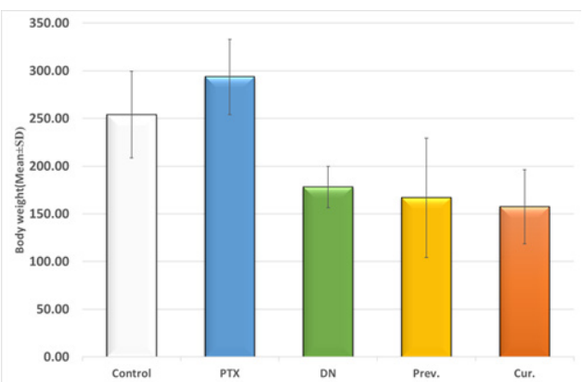


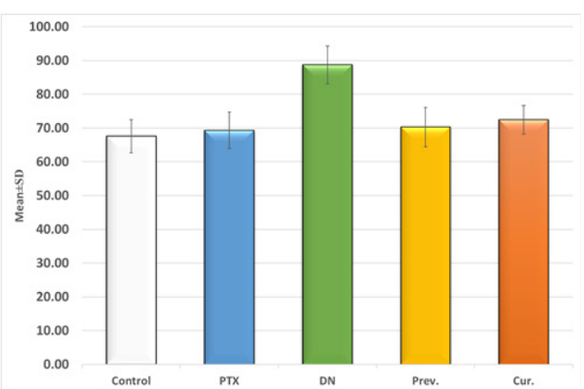
Fig. 20 {A-B}: A transmission electron micrograph of renal cortex. A. group IV: showing distal convoluted tubules with few short apical microvilli (arrow), elongated mitochondria (mi), apical euchromatic nucleus (N) and extensive basal infolding (arrow head). There is a dilated blood capillary (C). B. group V: showing the lumen (Lu) of DCT with cellular depris expelled from a ruptured cell. The lining cell of distal convoluted tubule has few apical microvilli (arrow), numerous mitochondria (mi), dense bodies (arrow head) and vesicular nucleus (N) with basal infolding (f). (Uranyl acetate & lead citrate)



Bar chart 1: The Mean±SD of blood glucose in all groups



Bar chart 2: The Mean±SD of Body weight (last week) in all groups



Bar chart 3: Comparison of density of positivity for anti-NF kappa immunostain between different groups (mean±SD)

DISCUSSION

Diabetic nephropathy (DN) in type 1 diabetic patients is the largest cause of chronic kidney disease in the working age group[18]. There are currently no viable methods to prevent its onset and progression. The literature indicates that pentoxifylline (PTX) can enhance renal hemodynamics, decrease urinary protein excretion, and relieve or postpone renal failure in DN patients[19].

The aim of this study is to investigate the possible prophylactic and the curative effects of pentoxifylline against the diabetic nephropathy and to assess the role of inflammation in the pathogenesis of diabetic nephropathy.

In the current work, adult male albino rats were chosen as an animal model to avoid the effect of female hormones which may play a role in decreasing the risk of developing kidney failure relative to males[20]. In the current study, successful diabetic rat model was performed using STZ-induced method of induction of type I diabetes mellitus. STZ-induced method of induction of type I diabetes mellitus is the most commonly used[21]. STZ, which causes pancreatic -cell damage and absolute insulin shortage is frequently used in rats to induce experimental type-1 diabetes[22]. The microscopic changes in the kidney in group III (diabetic nephropathy group) were more obvious in the renal cortex[23], so the present study focused on the histopathological changes of the renal cortex.

In the present study, histological examination of the renal sections of diabetic nephropathy group revealed marked histo-pathological changes. The renal corpuscle appeared destructed with thickened parietal layer of Bowman's capsule, wide irregular Bowman's space and shrunken glomeruli. There was interstitial heamorrhage. There were apparent increased number of mesangial cells and increased mesangial matrix. These findings are in agreement with Kitada *et al*. who reported that when comparing with control rats, diabetic rats had higher levels of mesangial matrix proteins, mesangial matrix percentage, and type IV collagen accumulation. There was no significant mesangial matrix buildup or nodular lesions in the glomeruli or tubular fibrosis[24].

In TEM examination of the present study, there were ballooning and fusion of foot processes of podocyte. In DN, loss of glomerular cells has been observed[25]. It could be mediated by increased advanced glycation end-product (AGE)[26], RAS activation[27] and oxygen free radicals[28]. Moreover, hyperglycemia causes separation of podocytes from GBM. Interestingly, podocytes are found in diabetic patients' urine and it positively correlates to severity of nephropathy[29]. As a result, Hyperglycemia causes the NADPH oxidase to produce reactive oxygen species (ROS), which begins podocyte death[28].

Thick glomerular basement membrane was found in the current investigation. The loss of negatively charged proteins in GBM results in increased protein loss. Protein glycation in the basement membrane reduces renal barrier efficacy and increases leakage. As a compensation, basement membrane thickening may occur[30]. Phospholipid peroxidation and plasma protein deposition on GBM cause enhanced glomerular permeability and BM thickness[20].

In the present study, the lining cells of the PCT demonstrated destruction of apical microvilli, marked vacuolation, rarified cytoplasm with darkly stained nuclei. The DCT in diabetic nephropathy group showed complete detachment of the lining cells from their basement membrane into the lumen in some tubules. The cells of distal convoluted tubules showed swollen cytoplasm (hydropic degeneration) with numerous small rounded vesicles scattered in the cytoplasm. Their nuclei were squeezed.

The finding of the current study is in accordance with Donder *et al*. who mentioned that there were histopathologic changes in DN including thickening of basement membrane, as well as tubular vacuolation and mesangium proliferation[31] and in agreement with Schlöndorff and Banas who stated that there were also thickening of the glomerular basement membrane, expansion of podocyte slit membranes, and mesangial hypertrophy in DN[32].

Tubule cell vacuolization may be a form of adaptive mechanism under a demanding condition (Diabetes) and subsequent cell disruption. It is linked to glycogen deposition and lipid vacuoles formation[30]. Diabetic patients had substantial dilatation of the distal tubules, as well as separation and tubular epithelial degradation[33].

Several growth factors, including (IGF-1), (PDGF), (VEGF) and (EGF), contribute to early tubular system proliferation in diabetes[34]. Hyperglycemia and the endogenous (RAS) promote the production of VEGF[35]. DN happens usually due to multiple contributors, including genetics, hyperglycemia, polyol pathway activation, RAS activation, oxygen free radicals production, activation of the protein kinase C pathway, increased advanced glycation end-product (AGE), and glomerular hyperfiltration[36].

Inflammation process sheds light on the hypothesis that diabetic kidney disease (DKD) is caused not only by uncontrolled hemodynamics and hyperglycemia, but also by a persistently active innate immune system and a low-grade inflammatory state in diabetic patients[37]. The transcription factor nuclear factor Kappa (NF-κB) regulates gene expression and controls the expression of several genes involved in inflammation, immunity, apoptosis, and chemoattractant protein-1[38]. It is found in the human kidney's glomeruli, interstitium, and tubules. Hyperglycemia is known to enhance NF-B expression[39]. Proteinuria and interstitial cell infiltration are linked to NF-B activation in DKD[38]. Proteinuria is known to further activate NF-B and contribute to chronic proteinuria in a cyclic way[40].

Inflammatory cytokines are considered to enhance vascular endothelial cell permeability, contribute to glomerular hypercellularity and GBM thickening, promote

endothelial cell death, and can be directly harmful to renal cells at the cellular level[41]. In DN, the cellular hypertrophy is another aspect of histological alteration. Biological effects of the transforming growth factor beta (TGF-b) caused by hyperglycemia in kidney cells can lead to cellular hypertrophy and promotion of extracellular matrix formation[42].

All cell types of the kidney including endothelial cells, tubulointerstitial cells, podocytes and mesangial cells can be destroyed in diabetic individuals. Any damage or dysfunction of one cell type, on the other hand, spreads to the rest of the cells[43]. Progressive thickness of GBM and tubules, as well as the increasing buildup of extracellular matrix components, are caused by an increase in expression and deposition of collagen IV, laminin, and fibronectin and mononuclear cell infiltration[42,44]. Tubulointerstitial fibrosis and tubular atrophy develop when tubulointerstitial abnormalities progress[30].

Active oxygen can increase plasminogen activator inhibitor-1 expression in the mesangium while decreasing the expression of plasminogen activators, inhibiting the capacity of glomerular mesangial cells to deconstruct extracellular matrix and worsening extracellular matrix aggregation. As a result, it leads to the glomerular and renal tubular cell damage[19]. There is increasing data that suggests that the metabolic and hemodynamic abnormalities seen in DN are caused by increased generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS)[45].

In the present study, histological examination of the renal sections of diabetic nephropathy group stained with anti-NF kappa immunostain revealed strong positive reaction of the cytoplasm of the lining cells of renal tubules for NF kappa. However, a weak positive reaction of the cytoplasm of cells in the glomerulus was observed. This might be because of that NF-κB is mainly localized in tubular epithelial cells, interstitial cells, and, to a lesser extent, in the glomeruli[46].

Clinical and experimental investigations have revealed that hyperglycemia causes the buildup of advanced glycation end-products (AGE) in diabetes patients' tissues, which bind to the cellular receptor RAGE. The AGE/RAGE interaction sets off a signalling cascade that includes an increase in the nuclear transcription factor (NF-B). As a result, there is an increase in oxygen free radicals accumulation[47].

Inflammation, especially pro-inflammatory cytokines is important factors in the development of microvascular diabetes problems. Neuropathy, for example, occurs as a result of microvascular, enzymatic and metabolic alterations[4]. Inflammatory cytokines including IL-1, IL-6, and TNF- have a role in nephropathy development[41]. In addition, changes induced by chronic hyperglycemia lead to dysregulation of pro-inflammatory cytokines[4]. TNF is cytotoxic to glomerular, mesangial and epithelial cells and may induce significant renal damage[48].

In the present study, light microscopic examination of group IV showed normal glomerulus except there was apparently thickened parietal layer of Bowman's capsule in some areas. However, there were some congested capillaries. The congested capillaries may be due to that PTX improves the effectiveness of microcirculation, decreases platelet aggregation and lowers plasma viscosity[49]. It inhibits the phosphodiesterase and can cause vasodilatation of blood vessels[50].

In the present study, improvement of renal tubules occurred. The distal convoluted tubules showed more improvement than the proximal convoluted tubules apparently in contrast to that the histological changes were markedly seen in PCT while less effect were seen in distal ones[23].

In group IV of the present study, proximal convoluted tubules showed that most of cells had vesicular nuclei with minimal amount of vacuolations. Distal convoluted tubules showed the most of the lining cells with vesicular nuclei. This is in agreement with Seifi *et al*. who reported that aside from its function in reducing nephropathy, PTX may have a number of renal protecting benefits by lowering malondialdehyde levels while concurrently replenishing intracellular glutathione and reducing oxidative damage to the kidneys[51].

In TEM examination of group IV of the present study, there was normal glomerulus with thin basement membrane. There were some congested blood capillaries and the surrounding parietal layer of Bowman's capsule was thin. Podocyte foot processes were fused together in some areas. Proximal convoluted tubules showed preserved apical microvilli, dense bodies in the cytoplasm, normal basal infolding similar to control group but there were dilated blood capillary and extravasated red blood cells around the tubule.

The finding of the current study is in accordance with Davila-Esqueda *et al*. who found that in diabetic rats treated with pentoxifylline, decreased GBM thickening, improvement of podocyte flattening, fenestration in the endothelial cell layer and decreased albuminuria were observed[52].

Distal convoluted tubules of group IV of the present study showed extensive basal infolding and elongated mitochondria. There were few short apical microvilli and vesicular nucleus with prominent nucleolus similar to control group but there was a dilated blood capillary or extravasated red blood cells between tubules. In sections stained with anti-NF kappa immune-histochemical stain of group IV of the present study, there was weak cytoplasmic reaction of the cells of the glomerulus similar to control group. Proximal and distal convoluted tubules showed decreased cytoplasmic reaction as compared to group III.

These findings are in agreement with Gallardo *et al*. who said that in individuals with chronic renal failure, inflammation is a mortality risk factor. Preventing

extracellular fluid volume expansion and using medicines like PTX may help to decrease inflammatory and oxidating process[53]. The current study's findings support the notion that pentoxifylline has the ability to suppress the inflammatory cytokines formation in diabetics since the early onset of the disease[54,55].

In the present study, in group V (the curative group) the diabetic rats were treated with PTX after 6 weeks of induction of diabetes mellitus for confirmation of development of diabetic nephropathy as An *et al*. reported that at the end of the 3rd week, 24-h urinary protein excretion was significantly increased in the DN group[19]. In light microscopic examination of group V of the present study, there was normal histological picture of the glomerulus. However, there was congestion in blood capillaries. Some cells lining the PCT exhibited hydropic degeneration and irregular nuclear membrane. DCT were normal with vesicular nuclei except some nuclei had irregular nuclear membrane and there were some extravasated red blood cells.

In TEM examination of group V of the present study, there was thin glomerular basement membrane similar to control group. Podocyte showed vacuolated cytoplasm and fused foot processes in some sections. PCT showed cellular debris in their lumen. PCT cells appeared with preserved apical microvilli, numerous mitochondria and dense bodies in their cytoplasm with preserved basal infolding similar to the control group. There were some vacuolations in the cytoplasm. Improvement of DCT was observed in the current study as there were few apical microvilli, numerous mitochondria, dense bodies, vesicular nucleus and preserved basal infolding, but the lumen contained cellular debris expelled from ruptured cell.

This is in agreement with An *et al*.[19] who stated that PTX was able to alleviate renal tissue damages of DN after significant reversion of the pathological changes of kidneys in DN rats.

Pentoxifylline (PTX) is a methyl xanthine phosphodiesterase inhibitor that has a role in antioxidation, anti-inflammation and immunity regulation[56]. Furthermore, several studies have demonstrated the therapeutic benefits of PTX in diabetic and non-diabetic renal disease. It has been observed that PTX can protect against drug induced nephrotoxicity[57-59]. Furthermore, PTX has been shown to reduce renal hypertrophy, renal disease progression, and urinary albumin excretion in animal models with DN and remnant kidney[60]. Moreover, some studies have shown that PTX decreases glomerular and tubulo-interstitial injury in diabetics[61]. In another research, PTX improved microalbuminuria and proteinuria in diabetic and non-diabetic renal disease patients[62]. PTX restored the normal histology as well as the quantity of apoptotic cells[33].

In sections stained with anti-NF kappa immune-histochemical stain of group V of the current study, there was negative cytoplasmic reaction of renal glomerulus. Proximal and distal convoluted tubules showed decreased reaction than group III. Apparently PCT showed increased reaction than DCT. These results are in agreement with Coimbra *et al*. who stated that PTX inhibits activation and down-regulates the activated NF-κb. Also, it lowers the plasma level of pro-inflammatory cytokines as TNF-α, IL1, IL6[63].

When PTX introduced to rats for 8 weeks, insulin resistance decreased, TN alpha elevation was ameliorated, leukocyte infiltration as well as endothelial pyknosis in DN[64]. This was concurrent to another research in which there was a decrease in hypoxia-inducible factor 1-alpha (HIF-1α) and VEGF expression in the kidney of rodents with diabetes[65].

Pentoxifylline helps the kidneys by maintaining GFR. Concurrent reductions in inflammatory markers such as TNF-alpha, IL-6, and high-sensitivity C-reactive protein indicate that inflammatory damage to the kidneys has been mitigated[66]. It was also found to inhibit the renal inflammatory reaction, and prevent proteinuria significantly in the STZ diabetic rats[67]. Pentoxifylline is an effective treatment for diabetic nephropathy, and its anti-oxidative action may contribute, at least in part, to its efficacy[19].

In the present study, measuring the mean blood glucose level showed high significant increase in the diabetic group as compared to the control group. The group IV and the group V showed a high significant increase in the mean blood glucose level as compared to the control group and group II and showed a non-significant difference as compared to the diabetic group. This goes with other previous studies who reported that the PTX had no change or improvement in the blood glucose level in the diabetic patients[68]. PTX had no sensible effect on rats' blood glucose level[19].

These results are against Garcia *et al*. who noticed decreases in glycemia ranged from 32 to 67% after PTX treatments and stated that the pentoxifylline hypoglycemic effects are similar to those of glibenclamide[69].

Measuring the mean body weight in the present study showed a significant decrease in the group III as compared to the control group. This is in agreement with Zafar and Naqv who stated that STZ induced diabetes showed significant reduction of body weight, ill looking and polydepsia in diabetic rats[70]. Ewenighi *et al*. demonstrated that the weight loss in diabetic rats might be due to increased glycogenolysis, lipolysis and gluconeogenesis which lead to loss of tissue protein and muscle wasting[71].

In the current study, the group IV and the group V showed a high significant decrease in the mean body weight as compared to the control group and group II and showed a non-significant difference as compared to the group III. These results are with Feyli *et al*. who stated that the diabetic rats received PTX showed non-significant difference in body weight when compared with control[72].

CONCLUSION

Pentoxifylline was found to have a great effect on preventing and improving the histological changes of diabetic nephropathy and is recommended to be used as an adjuvant therapy in diabetes mellitus.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Rahimi, R., Nikfar, S., Larijani, B. and Abdollahi, M. (2005): A review on the role of antioxidants in the management of diabetes and its complications. Biomedicine & Pharmacotherapy, 59(7), 365-373.
2. Mora-Fernández, C., Domínguez-Pimentel, V., de Fuentes, M. M., Górriz, J. L., Martínez-Castelao, A. and Navarro-González, J. F. (2014): Diabetic kidney disease: from physiology to therapeutics. The Journal of physiology, 592(18), 3997-4012.
3. Usuelli, V., Nasr, M. B., D'Addio, F., Tezza, S., Abdi, R. and Fiorina, P. (2015): The rise, fall, and resurgence of immunotherapy in type 1 diabetes. Pharmacological research, 98, 31-38.
4. Navarro-Gonzalez, J. F., & Mora-Fernandez, C. (2008): The role of inflammatory cytokines in diabetic nephropathy. Journal of the American Society of Nephrology, 19(3), 433-442.
5. Weidner, N., Cote, R. J., Suster, S. and Weiss, L. M. (2009): Modern Surgical Pathology E-Book. Elsevier Health Sciences.
6. Schlöndorff and Banas 2009 Schlöndorff D. and Banas B (2009): The mesangial cell revisited: no cell is an island. Journal of the American Society of Nephrology: JASN; 20 (6): 1179–87.
7. Zhang, M., Xu, Y. J., Saini, H. K., Turan, B., Liu, P. P., and Dhalla, N. S. (2005): Pentoxifylline attenuates cardiac dysfunction and reduces TNF-α level in ischemic-reperfused heart. American Journal of Physiology-Heart and Circulatory Physiology, 289(2), H832-H839.
8. Salhiyyah, K., Broderick, C., Forster, R. and Abdel-Hadi, M. (2020): Pentoxifylline for intermittent claudication. Cochrane Database of Systematic Reviews, (10).
9. Yavari, V., Ostovan, M. A., Kojuri, J., Afshariani, R., Hamidian, A., Roozbeh, J., & Pakfetrat, M. (2014): The preventive effect of pentoxifylline on contrast-induced nephropathy: a randomized clinical trial. International urology and nephrology, 46(1), 41-46.
10. Shahidi, S., Hoseinbalam, M., Iraj, B., & Akbari, M. (2015): Effect of pentoxifylline on microalbuminuria in diabetic patients: a randomized controlled trial. International journal of nephrology, 2015.
11. Hayashi, K., Kojima, R. and Ito, M. (2006): Strain differences in the diabetogenic activity of streptozotocin in mice. Biological & pharmaceutical bulletin. Jun; 29(6): 1110–9.
12. Akbarzadeh A, Norouzian D, Mehrabi MR, *et al*. (2007): Induction of diabetes by streptozotocin in rats. Indian J. Clin. Biochem.; 22:60–64.
13. Sharifi, Z. N., Movassaghi, S., Mohamadzadeh, F., Asl, S. S., Pourheydar, B. and Mehdizadeh, M. (2015): Reduction in ischemic brain injury following the administration of pentoxifylline after transient global ischemia/reperfusion in a rat model. Medical journal of the Islamic Republic of Iran, 29, 193.
14. El-Lithy, G. M., El-Bakly, W. M., Matboli, M., Abd-Alkhalek, H. A., Masoud, S. I., and Hamza, M. (2016): Prophylactic L-arginine and ibuprofen delay the development of tactile allodynia and suppress spinal miR-155 in a rat model of diabetic neuropathy. Translational Research, 177, 85-97.
15. Druury RAB and Wallington EA (1980): Carleton's histology technique.5th edition, oxford university press, 241-242.
16. Li, W., Tan, D., Zenali, M. J., & Brown, R. E. (2010): Constitutive activation of nuclear factor-kappa B (NF-kB) signaling pathway in fibrolamellar hepatocellular carcinoma. International journal of clinical and experimental pathology, 3(3), 238.
17. Woods, A.E. and Stirling, J. W. (2008): Electron microscopy. In: Bancroft J.D. and Gamble M. (Eds.), Theory and Practice of Histological Techniques. 6th ed., Churchill Livingstone Elsevier, Edinburgh. P: 601-636.
18. Eboh, C., & Chowdhury, T. A. (2015): Management of diabetic renal disease. Annals of translational medicine, 3(11).
19. An, Z. M., Dong, X. G., Guo, Y., Zhou, J. L., & Qin, T. (2015): Effects and clinical significance of pentoxifylline on the oxidative stress of rats with diabetic nephropathy. Journal of Huazhong University of Science and Technology [Medical Sciences], 35(3), 356-361.
20. Seppi, T., Prajczer, S., Dörler, M. M., Eiter, O., Hekl, D., Nevinny-Stickel, M., ... & Lechner, J. (2016): Sex differences in renal proximal tubular cell homeostasis. Journal of the American Society of Nephrology, 27(10), 3051-3062.
21. Islam, M. (2013): Animal models of diabetic neuropathy: progress since 1960s. Journal of diabetes research, 2013.
22. Wu, J. and Yan, L. J. (2015): Streptozotocin-induced type 1 diabetes in rodents as a model for studying mitochondrial mechanisms of diabetic β cell glucotoxicity. Diabetes, metabolic syndrome and obesity: targets and therapy, 8, 181.

23. El-Mahdy N. A., El-Masry T. A., El-Desouky K. I. and Ghanem S. K. (2016): The Renal and Cardio Protective Effects of Aliskiren and Pentoxifylline Alone and in Combination on Streptozotocin Induced Diabetic Rats. British Journal of Pharmaceutical Research; 12,1.
24. Kitada, M., Ogura, Y. and Koya, D. (2016): Rodent models of diabetic nephropathy: their utility and limitations. International journal of nephrology and renovascular disease, 9, 279.
25. Kalluri, R. (2006): Proteinuria with and without renal glomerular podocyte effacement. Journal of the American Society of Nephrology, 17(9), 2383-2389.
26. Chuang, P. Y., Yu, Q., Fang, W., Uribarri, J. and He, J. C. (2007): Advanced glycation endproducts induce podocyte apoptosis by activation of the FOXO4 transcription factor. Kidney international, 72(8), 965-976.
27. Jia, J., Ding, G., Zhu, J., Chen, C., Liang, W., Franki, N. and Singhal, P. C. (2008): Angiotensin II infusion induces nephrin expression changes and podocyte apoptosis. American journal of nephrology, 28(3), 500-507.
28. Susztak, K., Raff, A. C., Schiffer, M. and Böttinger, E. P. (2006): Glucose-induced reactive oxygen species cause apoptosis of podocytes and podocyte depletion at the onset of diabetic nephropathy. Diabetes, 55(1), 225-233.
29. Nakamura, T., Ushiyama, C., Suzuki, S., Hara, M., Shimada, N., Ebihara, I. and Koide, H. (2000): Urinary excretion of podocytes in patients with diabetic nephropathy. Nephrology Dialysis Transplantation, 15(9), 1379-1383.
30. Pourghasem, M., Shafi, H. and Babazadeh, Z. (2015): Histological changes of kidney in diabetic nephropathy. Caspian journal of internal medicine, 6(3), 120.
31. Donder E., Dogan M.M., Kuloglu T., Dabak Ö.D., Kocaman N. and Ozkan Y. (2013): The investigation of the effects of enalapril and losartan on ghrelin immunoreactivity in kidney of streptozotocin-induced diabetic rats. Fırat Tıp Dergisi. ; 18:1–6.
32. Schlöndorff D. and Banas B (2009): "The mesangial cell revisited: no cell is an island". Journal of the American Society of Nephrology: JASN. 20 (6): 1179–87.
33. Sönmez, M. F. and Dündar, M. (2016): Ameliorative effects of pentoxifylline on NOS induced by diabetes in rat kidney. Renal Failure, 38(4), 605-613.
34. Vallon, V. and Thomson, S. C. (2012): Renal function in diabetic disease models: the tubular system in the pathophysiology of the diabetic kidney. Annual review of physiology, 74, 351-375.
35. Feliers, D., and Kasinath, B. S. (2010): Mechanism of VEGF expression by high glucose in proximal tubule epithelial cells. Molecular and cellular endocrinology, 314(1), 136-142.
36. Yung, S., Chau, M. K., Zhang, Q., Zhang, C. Z. and Chan, T. M. (2013): Sulodexide decreases albuminuria and regulates matrix protein accumulation in C57BL/6 mice with streptozotocin-induced type I diabetic nephropathy. PLoS One, 8(1), e54501.
37. Garcia F. A., Pinto S. F., Cavalcante A. F., Lucetti L. T., Menezes S. M., Felipe C. F., Alves A. P., Brito G. A., Cerqueira G. S. and Viana G. S. (2014): Pentoxifylline decreases glycemia levels and TNF-alpha, iNOS and COX-2 expressions in diabetic rat pancreas. Springerplus, 5; 3: 283.
38. Sanz, A. B., Sanchez-Niño, M. D., Ramos, A. M., Moreno, J. A., Santamaria, B., Ruiz-Ortega, M., ... & Ortiz, A. (2010): NF-κB in renal inflammation. Journal of the American Society of Nephrology, 21(8), 1254-1262.
39. Sakai, N., Wada, T., Furuichi, K., Iwata, Y., Yoshimoto, K., Kitagawa, K., ... & Kobayashi, K. I. (2002): p38 MAPK phosphorylation and NF-κ B activation in human crescentic glomerulonephritis. Nephrology Dialysis Transplantation, 17(6), 998-1004.
40. Mezzano, S., Aros, C., Droguett, A., Burgos, M. E., Ardiles, L., Flores, C., ... & Egido, J. (2004): NF-κB activation and overexpression of regulated genes in human diabetic nephropathy. Nephrology Dialysis Transplantation, 19(10), 2505-2512.
41. Navarro-Gonzalez, J. F., & Mora-Fernandez, C. (2008): The role of inflammatory cytokines in diabetic nephropathy. Journal of the American Society of Nephrology, 19(3), 433-442.
42. Ziyadeh, F. N., Hoffman, B. B., Han, D. C., Iglesias-De la Cruz, M. C., Hong, S. W., Isono, M., ... & Sharma, K. (2000): Long-term prevention of renal insufficiency, excess matrix gene expression, and glomerular mesangial matrix expansion by treatment with monoclonal antitransforming growth factor-β antibody in db/db diabetic mice. Proceedings of the National Academy of Sciences, 97(14), 8015-8020.
43. Maezawa, Y., Takemoto, M. and Yokote, K. (2015): Cell biology of diabetic nephropathy: Roles of endothelial cells, tubulointerstitial cells and podocytes. Journal of diabetes investigation, 6 (1), 3-15.
44. Najafian, B., Alpers, C. E. and Fogo, A. B. (2011): Pathology of human diabetic nephropathy. In Diabetes and the Kidney, 170, 36-47.
45. Pollock, J. S. and Pollock, D. M. (2011): Endothelin, nitric oxide, and reactive oxygen species in diabetic kidney disease. In Endothelin in Renal Physiology and Disease, 172, 149-159.

46. Gómez-Garre, D., Largo, R., Tejera, N., Fortes, J., Manzarbeitia, F., and Egido, J. (2001): Activation of NF-κB in tubular epithelial cells of rats with intense proteinuria: role of angiotensin II and endothelin-1. Hypertension, 37(4), 1171-1178.
47. Schmidt, A. M., Yan, S. D., Wautier, J. L. and Stern, D. (1999): Activation of receptor for advanced glycation end products: a mechanism for chronic vascular dysfunction in diabetic vasculopathy and atherosclerosis. Circulation research, 84(5), 489-497.
48. Ortiz, A., Bustos, C., Alonso, J., ... & Egido, J. (1995): Tumer necrosis factor as a mediator of glomerular damage. Journal of Nephrology, 8(1), 27-34.
49. Zhang, M., Xu, Y. J., Mengi, S. A., Arneja, A. S., & Dhalla, N. S. (2004): Therapeutic potentials of pentoxifylline for treatment of cardiovascular diseases. Experimental & Clinical Cardiology, 9(2), 103.
50. Kabbesh, N., Gogny, M., Chatagnon, G., Noireaud, J., Thorin, C., Desfontis, J. C., & Mallem, M. Y. (2012): Vasodilatory effect of pentoxifylline in isolated equine digital veins. The Veterinary Journal, 192(3), 368-373.
51. Seifi, B., Kadkhodaee, M., Delavari, F., Mikaeili, S., Shams, S. and Ostad, S. N. (2012): Pretreatment with pentoxifylline and N-acetylcysteine in liver ischemia reperfusion-induced renal injury. Renal Failure, 34(5), 610-615.
52. Davila-Esqueda, M. E., Vertiz-Hernandez, A. A., and Martinez-Morales, F. (2005): Comparative analysis of the renoprotective effects of pentoxifylline and vitamin E on streptozotocin-induced diabetes mellitus. Renal failure, 27(1), 115-122.
53. Gallardo, J. M., de Carmen Prado-Uribe, M., Amato, D. and Paniagua, R. (2007): Inflammation and oxidative stress markers by pentoxifylline treatment in rats with chronic renal failure and high sodium intake. Archives of medical research, 38(1), 34-38.
54. Cooper, A., Mikhail, A., Lethbridge, M. W., Kemeny, D. M. and Macdougall, I. C. (2004): Pentoxifylline improves hemoglobin levels in patients with erythropoietin-resistant anemia in renal failure. Journal of the American Society of Nephrology, 15(7), 1877-1882.
55. Navarro, J. F., Mora, C., Muros, M., & García, J. (2005): Additive antiproteinuric effect of pentoxifylline in patients with type 2 diabetes under angiotensin II receptor blockade: a short-term, randomized, controlled trial. Journal of the American Society of Nephrology, 16(7), 2119-2126.
56. Navarro-González, J. F., Mora-Fernández, C., de Fuentes, M. M., Chahin, J., Méndez, M. L., Gallego, E., ... & García, P. (2015): Effect of pentoxifylline on renal function and urinary albumin excretion in patients with diabetic kidney disease: the PREDIAN trial. Journal of the American Society of Nephrology, 26(1), 220-229.
57. Nasiri-Toosi, Z., Dashti-Khavidaki, S., Khalili, H., & Lessan-Pezeshki, M. (2013): A review of the potential protective effects of pentoxifylline against drug-induced nephrotoxicity. European journal of clinical pharmacology, 69 (5), 1057-1073.
58. Kim, Y. K., Choi, T. R., Kwon, C. H., Kim, J. H., Woo, J. S. and Jung, J. S. (2003): Beneficial effect of pentoxifylline on cisplatin-induced acute renal failure in rabbits. Renal failure, 25(6), 909-922.
59. Akpolat, T., Akpolat, I., Öztürk, H., Sarıkaya, Ş., Coşar, A. M., Bedir, A., and Kandemir, B. (2000): Effect of vitamin E and pentoxifylline on glycerol-induced acute renal failure. Nephron, 84(3), 243-247.
60. Navarro, J. F., Milena, F. J., Mora, C., León, C., & García, J. (2006): Renal pro-inflammatory cytokine gene expression in diabetic nephropathy: effect of angiotensin-converting enzyme inhibition and pentoxifylline administration. American journal of nephrology, 26(6), 562-570.
61. Navarro-González, J. F., Muros, M., Mora-Fernández, C., Herrera, H., Meneses, B., & García, J. (2011). Pentoxifylline for renoprotection in diabetic nephropathy: the PREDIAN study. Rationale and basal results. Journal of diabetes and its complications, 25(5), 314-319.
62. Badri, S., Dashti-Khavidaki, S., Lessan-Pezeshki, M., and Abdollahi, M. (2011): A review of the potential benefits of pentoxifylline in diabetic and non-diabetic proteinuria. Journal of Pharmacy & Pharmaceutical Sciences, 14(1), 128-137.
63. Coimbra R, Melbostad H, Loomis, W., *et al*. (2006): LPS-Induced Acute Lung Injury is Attenuated by Phosphodiesterase Inhibition: Effects on Proinflammatory Mediators, Metalloproteinases, NF-κB, and ICAM-1 Expression. The Journal of Trauma: Injury, Infection, and Critical Care ;60 : 115-125.
64. El-Bassossy, H. M., El-Moselhy, M. A., and Mahmoud, M. F. (2011): Pentoxifylline alleviates vascular impairment in insulin resistance via TNF-α inhibition. Naunyn-Schmiedeberg's archives of pharmacology, 384(3), 277.
65. Sun, H. K., Lee, Y. M., Han, K. H., Kim, H. S., Ahn, S. H. and Han, S. Y. (2012): Phosphodiesterase inhibitor improves renal tubulointerstitial hypoxia of the diabetic rat kidney. The Korean journal of internal medicine, 27(2), 163.
66. Renke, M., Tylicki, L., Rutkowski, P., Knap, N., Zietkiewicz, M., Neuwelt, A., ... & Rutkowski, B. (2010): Effect of pentoxifylline on proteinuria, markers of tubular injury and oxidative stress in non-diabetic patients with chronic kidney disease-placebo controlled, randomized, cross-over study. Acta Biochimica Polonica, 57(1).

67. Han, K. H., Han, S. Y., Kim, H. S., Kang, Y. S., and Cha, D. R. (2010): Prolonged administration enhances the renoprotective effect of pentoxifylline via anti-inflammatory activity in streptozotocin-induced diabetic nephropathy. Inflammation, 33(3), 137-143.

68. Campbell R. K. (1993): Clinical update on pentoxifylline therapy for diabetes-induced peripheral vascular disease. Annuals of pharmacotherapy; 27: 1099-1105.

69. Garcia, F. A., Rebouças, J. F., Balbino, T. Q., da Silva, T. G., de Carvalho-Júnior, C. H. R., Cerqueira, G. S., ... & de Barros Viana, G. S. (2015): Pentoxifylline reduces the inflammatory process in diabetic rats: relationship with decreases of pro-inflammatory cytokines and inducible nitric oxide synthase. Journal of inflammation, 12(1), 1-10.

70. Zafar, M., & Naqvi, S. N. U. H. (2010): Effects of STZ-Induced diabetes on the relative weights of kidney, liver and pancreas in albino rats: a comparative study. International Journal of Morphology, 28(1).

71. Ewenighi C, Dimkpa U, Onoh L, *et al*. (2015): Estimation of blood glucose level and body weight in allaxon induced diabetic rat treated with aqueous extract of garcinia kola seed. Ulutas Meical Journal.; 1: 26-30.

72. Feyli S.A., Ghanbari A. and Keshtmand Z. (2017): Therapeutic effect of pentoxifylline on reproductive parameters n diabetic male mice. Andrologia; 49:1-7.

الملخص العربى

دراسة الدور الوقائي مقارنة بالدور العلاجي لمادة البنتوكسيفيلين على اعتلال الكلى السكري المستحدث في الفئران

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المقدمة: يعتبراعتلال الكلي السكري في مرضى السكري من النوع الأول هو أكبر سبب لمرض الكلى المزمن في الفئة العمرية العاملة.

الهدف من البحث: الهدف من الدراسة الحالية هو إظهار الضرر الناتج من اعتلال الكلي السكري وتقييم الدور الوقائي والعلاجي المحتمل للبنتوكسيفيلين في اعتلال الكلي السكري لدي الفئران البيضاء الذكور البالغة.

المادة و طرق البحث : تم استخدام سبعين من ذكور الفئران البيضاء البالغة ثم تم تقسيمهم عشوائيا إلى خمس مجموعات على النحو التالي:

المجموعة الأولى (المجموعة الضابطة) : تكونت من ثلاثين فأرا.

المجموعة الثانية: (المجموعة غير المصابة بداء السكري) (عشرة فئران) : تلقت البنتوكسيفيلين كجرعة يومية واحدة ٢٠٠ مجم / كجم مذابة فى ماء الشرب طوال مدة التجربة.

المجموعة الثالثة: (مجموعة اعتلال الكلي السكري) (عشرة فئران): تم حقنها بالاستربتوزوتوسن بجرعة٥٠ مجم / كجم عن طريق الحقن داخل الغشاء البريتوني كجرعة وحيدة لكل فأرمن أجل إصابته بداء السكرى وبعد التأكد من حدوث ارتفاع في مستوي السكر (بوصوله لمعدل ٢٧٠ مجرام / مل) تم ملاحظة تاثيره على الكلي.

المجموعة الرابعة(عشرة فئران): تلقت الفئران المصابة بمرض السكري جرعة يومية واحدة من البنتوكسيفيلين كعلاج وقائي يبدأ من الأسبوع الاول بعد تحريض مرض السكري ويستمر لمدة ٧ أسابيع أخرى.

المجموعة الخامسة(عشرة فئران) : تلقت الفئران المصابة بمرض السكري جرعة يومية واحدة من البنتوكسيفيلين كعلاج بعد الأسبوع السادس من تحريض مرض السكري لمدة أسبوعين آخرين.

في نهاية الفترة التجريبية تم قياس وزن الجسم لكل مجموعة وأخذ عينات الدم منها لقياس مستوى السكر في الدم ثم تم أخذ عينات الكلى وتم تمريرنصفها و معالجتها لوضعها فى قالب من البارافين لعمل صبغة الهيماتوكسيلين والأيوسين والصبغة المناعية ضد العامل النووي كابا لاظهار الالتهابات ثم تم فحص الشرائح بواسطة المجهر الضوئي. النصف الآخر من العينات تم اعدادها وصبغها بصبغه التولويدين الازرق وكذلك لفحصها تحت الميكروسكوب الالكتروني النافذ. تم تحليل الصور الناتجة من المقاطع المصبوغة مناعيا ضد العامل النووي كابا باستخدام برنامج الكمبيوتر

(SPSS) (الحزمة الإحصائية للعلوم الاجتماعية) الإصدار ١٧,٠.

النتائج: كشف الفحص النسيجي بالمجهر الضوئي لمجموعة اعتلال الكلي السكري عن تغيرات مرضية نسجية ملحوظة في شكل كبيبات كلوية مدمرة مع اتساع فراغ بومان وزيادة سمك الطبقة الجدارية لمحفظة بومان. كما تضررت الأنيبيبات الكلوية الملتوية القريبة والبعيدة.

وفي فحص الميكروسكوب الالكتروني لوحظ زيادة سمك الغشاء القاعدي الكبيبي وزيادة سمك الطبقة الجدارية لكبسولة بومان. كما وجد التصاق في الافرع القدمية لخلية البودوسيت. هناك اندماج عمليات القدم للخلية مع الغشاء القاعدي الكبيبي السميك. أظهرت الخلايا المبطنة للأنابيب الملتوية القريبة والبعيدة فجوات كثيرة و ميتوكوندريا متحللة وفقدان النتوءات القاعدية. كما وجد ترسب قطرات دهنية في الخلايا المبطنة للانابيب الملتوية القريبة.

قد أظهرت النتائج تحسنا ملحوظا بعد العلاج الوقائي والعلاجي باستخدام البنتوكسيفيلين. كما أظهرت نتائج قياس كثافة الإيجابية في الشرائح المصبوغة مناعيا انخفاضًا ذو دلالة احصائية في كثافة الإيجابية مقارنة بمجموعة اعتلال الكلي السكري مما يؤكد دور البنتوكسيفيلين كمضاد للالتهابات.

الاستنتاج: قد تم استنتاج ان للبنتوكسيفيلين القدرة علي تحسّين التغيرات النسيجية المرضية لاعتلال الكلية السكري إما كعلاج وقائي أو علاجي ولذلك يوصى باستخدامه كعلاج مساعد في مرض السكري.