

## Effect of Metformin Hydrochloride Administration and its Withdrawal on the Kidneys of Adult Male Albino Rats: Histological and Biochemical Studies

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### Abstract

**Background:** Metformin hydrochloride is a very potent anti-diabetic drug that has become the drug of choice for the treatment of type 2 diabetes.

**Aim of Study:** This work aims to demonstrate the effect of metformin hydrochloride administration and its withdrawal on the histological structure and function of the kidney of adult male albino rats.

**Material and Methods:** Sixty adult male albino rats were used in this work. They were divided equally into six groups; I- First control group (Group C1), II- Second control group (Group C2), III- Third control group (Group C3), IV- Treated group (Group T), V- First recovery group (Group R1) and VI- Second recovery group (Group R2). Each adult male albino rat of groups C1 and T was given 0.55ml of distilled water and 0.55ml of distilled water (contained 27.45mg metformin hydrochloride) respectively for four weeks. Each adult male albino rat of groups C2 and R1 was given 0.55ml of distilled water and 0.55ml of distilled water (contained 27.45mg metformin hydrochloride) respectively for four weeks, then, left for two weeks without treatment. Each adult male albino rat of groups C3 and R2 was given 0.55ml of distilled water and 0.55ml of distilled water (contained 27.45mg metformin hydrochloride) respectively for four weeks, then, left for four weeks without treatment. The treatments were given once/day orally. The specimens were collected at three time intervals; at the end of the 4th week for groups C1 & T, at the end of the 6th week for groups C2 & R1 and at the end of the 8th week for groups C3 & R2. The blood samples were collected for measuring urea and creatinine. The kidneys were used for light & electron microscopic examinations, and morphometric study.

**Results:** Light and electron microscopic examination and morphometric studies revealed that metformin hydrochloride induced various signs of degeneration, necrosis, inflammation and fibrosis. Biochemical study revealed that metformin hydrochloride induced deterioration in the kidney functions which were reflected by significant increase in the serum levels of urea and creatinine. On metformin hydrochloride

withdrawal, most of the histological and all the biochemical effects were recovered specially in the second recovery group where the recovery was directly proportionate with the duration of its withdrawal.

**Conclusion:** Metformin hydrochloride induced various deleterious changes in the histological structure and function of the kidney. These changes were improved on its withdrawal.

**Key Words:** *Animals – Rat – Metformin hydrochloride – Kidney – Creatinine – Oxidant – Antioxidant.*

### Introduction

**METFORMIN** hydrochloride is a type of biguanide, a class of oral antihyperglycemic drugs that have been utilized in the treatment of diabetes mellitus for many years [1]. It acts primarily at the liver by reducing glucose output and, secondarily, by augmenting glucose uptake in the peripheral tissues, chiefly muscle [2]. The metformin hydrochloride absorption occurs in the small intestine and decreases slightly with increasing doses. It is a highly ionized, water soluble substance. Its renal clearance is 4-fold greater than creatinine clearance [3]. Metformin hydrochloride is recommended as the first-line medication for type 2 DM because of its low cost, favorable adverse effect profile, and a possible beneficial effect on cardiovascular risk [4-7]. However, it is frequently avoided in patients with chronic kidney disease because of the concern of drug accumulation and lactic acidosis [8]. Although metformin-associated lactic acidosis is a very rare event, the mortality associated with it is close to 50%, as it is excreted through the kidney. The likelihood of metformin associated lactic acidosis is substantially higher in patients with kidney impairment [9,10]. Metformin is contraindicated in patients with diabetic ketoacidosis or diabetic precoma, renal dysfunction, and acute conditions which have the potential for altering

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renal function such as: Dehydration, severe infection and shock [11]. It has previously been shown in animal models that metformin hydrochloride exerts a nephrotoxic effect [12]. Moreover, given data by [13] showing a higher mortality risk with metformin use in advanced chronic kidney disease that is dose-dependent. Some studies, however, have shown the possible safety of metformin hydrochloride use in dialysis patients with type 2 diabetes mellitus [14-16]. Regulatory and professional society guidelines suggest that metformin hydrochloride may be an option in patients with mild to moderate chronic kidney disease [17]. From this view the effect of metformin hydrochloride on the kidneys is controversial. So, the current work aims to demonstrate the effect of metformin hydrochloride administration and its withdrawal on the histological structure and function of the kidney of adult male albino rats.

### Material and Methods

**Drug:** Metformin hydrochloride (Glucophage®) was available in tablet forms, manufactured by Minipharm Company for Pharmaceutical and Chemical Industries, 10th of Ramadan, Egypt under licence Merck Sante s.a.s. France subsidiary of Merck KGaA-Germany. The human therapeutic dose ranged from 500 to 2550mg/day [18]. In this work, the average therapeutic dose (1525mg) of metformin hydrochloride was used. The equivalent dose of adult rat was calculated according to the formula of Paget and Barnes [19] to be 27.45mg/rat. The Glucophage tablet which contained 1000mg was dissolved in 20ml of distilled water. So, the adult rat received 0.55ml of distilled water that contained 27.45mg of metformin hydrochloride. The distilled water alone or the distilled water that contained the drug was given orally by gastric tube as a single daily dose for the control and treated male albino rats respectively.

**Animals:** Sixty adult male albino rats of local strain were used in this work. The average weight of the adult male rats was about 150g. They were obtained and provided by veterinary care by the Animal House of Faculty of Medicine (Girls), Al-Azhar University during the year of 2020. The adult male albino rats were housed in stainless steel cages (40 X 27.5 X 19.5cm). Each cage contained 5 adult male albino rats. The rats were observed and maintained on balanced water and diet (standard diet pellets-EI-Nasr-Company, Abo-Zaabal-Egypt). The rats were divided equally into the following 6 groups:

I- *First control group (Group C1):* Each rat was given 0.55ml of distilled water.

II- *Second control group (Group C2):* Each rat was given 0.55ml of distilled water for 4 weeks. Then, the distilled water was stopped for 2 weeks.

III- *Third control group (Group C3):* Each rat was given 0.55ml of distilled water for 4 weeks. Then, the distilled water was stopped for 4 weeks.

IV- *Treated group (Group T):* Each rat was given 0.55ml of distilled water (contained 27.45 mg metformin hydrochloride) for 4 weeks.

V- *First recovery group (Group R1):* Each rat was given 0.55ml of distilled water (contained 27.45mg metformin hydrochloride) for 4 weeks. Then, the distilled water that contained the drug was stopped for 2 weeks.

VI- *Second recovery group (Group R2):* Each rat was given 0.55ml of distilled water (contained 27.45mg metformin hydrochloride) for 4 weeks. Then, the distilled water that contained the drug was stopped for 4 weeks.

*Collection of the specimens and preparation for examination:*

The specimens were collected at three time intervals; at four weeks (Groups C1 & T), six weeks (Groups C2 & R1) and eight weeks (Groups C3 & R2). The rats were anesthetized under isoflurane. Blood samples were collected from the retro-orbital sinus of each rat by a fine capillary glass tube. The collected blood was centrifuged at 3000rpm for 10 minutes. The clean supernatant serum was aspirated by automatic pipette into Eppendorf tubes and kept frozen at -20 until the time of biochemical study. Immediately after blood collection, rats were sacrificed, and the two kidneys of each rat were excised and used for histological study (one kidney was prepared for light microscopic examination and morphometric study, and the other one was prepared for electron microscopic examination).

I- *Histological study:*

1- *Light microscopic examination:* The kidneys were fixed by immersion in 10% formalin for three days [20]. The specimens were dehydrated in ascending grades of ethyl alcohol and cleared in benzene. The specimens were impregnated for three changes in paraffin and were finally embedded in paraffin wax. The paraffin blocks were cut into serial transverse sections at 5gm thick with a rotary microtome. Successive transverse paraffin sections were attached to an albumenized glass slides. The haematoxylin and eosin stain [21] was used to study the kidney architectures. The Masson's trichrome stain [21] was used to demonstrate the collagen

fibers. The images were taken by a microscope (Leica) DM750 connected to a digital camera in Anatomy Department, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt.

*2- Transmission electron microscopic examination:* The cortices of the kidneys were cut into small pieces. The specimens were immediately fixed in cold glutaraldehyde (5%) for 24 hours and washed in 0.1ml phosphate buffer (pH 7.2) for 20 minutes (3 changes), then post fixed with 1% osmium tetroxide for 1.5 hours. The specimens were washed again in phosphate buffer, dehydrated in ascending grads of alcohol and embedded in epoxy resin. The semi-thin sections (1 $\mu$ m thick) were cut on an LKB ultratome, stained with toluidine blue, and examined by light microscope to determine the area that subjected to ultrathin cutting. The ultrathin sections (60nm thick) were mounted on copper grids, and stained with uranyl acetate and lead citrate [21]. The ultrathin sections were examined using a transmission electron microscope (JEOL1010 EX II, Japan) at the Regional Mycology and Biotechnology Center, Al-Azhar University, Cairo, Egypt.

*3- Morphometric study:* The image analyser computer system Leica Qwin 500 (England) at the Regional Mycology and Biotechnology Center, Al-Azhar University, Cairo, Egypt was used to evaluate the width of the Bowman's spaces of the studied groups by using haematoxylin and eosin stained sections. The ten slides of ten rats of each group were taken. Then, 5 intact renal corpuscles were randomly selected in each slide, at power field X 100, to measure the widths of Bowman's spaces by measuring 4 widths for each Bowman's capsule. The surface area of collagen fibres of the studied groups was evaluated by using Masson's trichrome stained sections, and was measured in 5 fields in each section by using magnification X 100.

*II- Biochemical study:* The biochemical study to measure urea, creatinine was done at the Regional Mycology and Biotechnology Center, Al-Azhar University, Cairo, Egypt. Serum urea and creatinine were determined spectrophotometrically according to the methods of [22,23] respectively.

#### *Statistical analysis:*

Statistical analysis of the measured width of the Bowman's space, collagen fibers, urea and creatinine of the studied groups were carried out using the SPSS statistical package. The data were analyzed (expressed as means  $\pm$  SD) and statistical significance was determined by using one-way

ANOVA followed by a Tuckey post-Hoc test for multiple comparisons.  $p$ -value  $\leq 0.05$  were considered to be statistically significant [24].

## **Results**

### *I- Control groups (Groups; C1, C2 & C3):*

Light and electron microscopic examination of groups; C1, C2 and C3 showed insignificant differences, so their data were pooled together.

Light microscopic examination of the haematoxylin and eosin stained transverse sections of the kidneys of the control groups; C1, C2 & C3 showed that the renal cortices were consisted of renal corpuscles, proximal and distal convoluted tubules and interstitial tissues. The renal corpuscles consisted of renal glomeruli that were surrounded by Bowman's capsules. The Bowman's capsules consisted of parietal and visceral layers of epithelial cells that were separated by the Bowman's spaces. The visceral layer of epithelial cells (Podocytes) lined the glomerular side of the Bowman's space. The parietal layer of epithelial cells lined the Bowman's capsule on the opposite side. The glomeruli appeared as large cellular masses of basophilic nuclei and eosinophilic cytoplasm. The proximal convoluted tubules had narrow lumina. They were lined by single layers of cuboidal or columnar cells that had central rounded basophilic nuclei and extensive brush borders on their luminal surfaces. The distal convoluted tubules had wide lumina. They were lined by single layers of cuboidal cells that had central rounded basophilic nuclei. The interstitial tissues consisted of interstitial cells with basophilic nuclei and blood vessels Fig. (1A). The sections of the kidney that were stained with Masson's trichrome stain showed normal distribution of collagen fibers in the parietal layer of the Bowman's capsule, the renal glomeruli, the basement membranes of the renal tubules, the interstitial tissues and the wall of the blood vessels Fig. (2A).

Electron microscopic examination of the kidneys of the control groups; C1, C2 & C3 showed that the glomeruli consisted of network of capillaries covered by the podocytes and supported by mesangial cells which are modified smooth muscle cells. The glomerular capillary lumina contained red blood cells and lined by endothelial cells lied on thin glomerular basement membranes. The glomerular basement membrane lied between the endothelial cells, mesangial cells and the podocytes. The endothelial cells had euchromatic nuclei with small clumps of heterochromatin. Their cytoplasm formed thin sheets broken by numerous small circular fenestrations. The podocytes had euchro-

matic nuclei with small clumps of heterochromatin and primary foot processes that divided into numerous secondary foot processes. The secondary foot processes were separated by filtration slit gaps. Thin membranes called filtration slit membranes bridged the gaps between the secondary foot processes. The mesangial cells had euchromatic nuclei with large clumps of heterochromatin Figs. (3A, 4A). The proximal convoluted tubules had thin basement membranes. They were lined by cells that had brush borders that were composed of numerous closely packed microvilli. The proximal convoluted tubule cells had nearly rounded euchromatic nuclei with small clumps of heterochromatin. The nuclei had prominent eccentric nucleoli and well-defined nuclear envelopes with apparent nuclear pores. Their cytoplasm contained few lysosomes and numerous rounded or elongated mitochondria Figs. (5A, 6A). The distal convoluted tubules had thin basement membranes. They were lined by cells that had nearly rounded or oval euchromatic nuclei with small clumps of heterochromatin. The nuclei had prominent eccentric nucleoli and well-defined nuclear envelopes with apparent nuclear pores. Their cytoplasm contained rounded or elongated mitochondria Figs. (7A, 8A). The interstitial tissues consisted of interstitial cells that had euchromatic nuclei with small clumps of heterochromatin and few collagen fibers Figs. (5A, 6A, 7A, 8A).

Morphometric study revealed insignificant differences in the collagen fibers and width of the Bowman's spaces between the groups C 1, C2, C3 and R2 (Table 1) and Graph (1 C, D).

Biochemical study revealed insignificant differences in the levels of urea and creatinine between the groups C1, C2, C3 and R2 (Table 1) and Graph (1A, B).

## II- Treated group (Group T):

Light microscopic examination of the haematoxylin and eosin stained transverse sections of the kidneys of group T showed that numerous renal corpuscles had wide Bowman's spaces with disruption of their parietal epithelial cells. Some glomeruli were congested. Many proximal and distal convoluted tubules were hardly differentiated. Some tubular cells appeared exfoliated. Other cells had vacuolated cytoplasm, nuclei with fading of its basophilia and shrunken deeply stained nuclei. Some areas of the interstitial tissues were infiltrated by inflammatory cells and hemorrhage as compared to the control one Fig. (1A, B). The kidney sections that were stained with Masson's trichrome stain demonstrated apparent increase in the collagen

fibers distribution in the parietal layer of the Bowman's capsule, the renal glomeruli, the basement membranes of the renal tubules, the wall of the blood vessels and the interstitial tissues as compared with the control one Fig. (2A, B).

Electron microscopic examination of the kidneys of group T showed that the glomeruli consisted of network of capillaries covered by the podocytes. The glomerular capillaries lined by endothelial cells lied on thick and irregular glomerular basement membranes. Some endothelial cells had nuclei with condensed clumps of heterochromatin. Their cytoplasm formed thin sheets broken by numerous small circular fenestrations in some areas. However, the fenestration couldn't be detected in certain areas. Some podocytes had irregular nuclei with large clumps of heterochromatin. The majority of the secondary foot processes were fused, the filtration slit gaps appeared narrow and obliterated. The filtration slit membranes couldn't be identified as compared with the control one Figs. (3B, 4B) and Figs. (3A, 4A). Some proximal convoluted tubules had ill-defined and thick basement membranes. Some of their cells had shrunken nuclei with condensed clumps of heterochromatin. Other cells had euchromatic nuclei with small clumps of heterochromatin. Their cytoplasm contained lysosomes, irregular mitochondria, mitochondria with destructed cristae and degenerated mitochondria. The microvilli appeared short and disrupted in certain places as compared with the control one Figs. (5B, 6B) and Figs. (5A, 6A). Some distal convoluted tubules had irregular and thick basement membranes. Some of their cells had shrunken nuclei with condensed clumps of heterochromatin. Other cells had euchromatic nuclei with small clumps of heterochromatin. Their cytoplasm contained vacuoles and degenerated and irregular mitochondria as compared with the control one Figs. (7B, 8B) and Figs. (7A, 8A). The interstitial tissues consisted of interstitial cells and numerous collagen fibers. Some interstitial cells had nuclei with condensed clumps of heterochromatin as compared with the control one Figs. (5B, 7B, 8B) and Figs. (5A, 6A, 7A, 8A).

Morphometric study revealed significant increase in the collagen fibers and width of the Bowman's spaces of group T when compared with those of the groups; C1, C2, C3 and R2 (Table 1) and Graph (1 C, D).

Biochemical study revealed significant increase in the levels of urea and creatinine of group T when compared with those of groups; C1, C2, C3 and R2 (Table 1) and Graph (1A, B).

### III- First recovery group (Group R1):

Light microscopic examination of the haematoxylin and eosin stained transverse sections of the kidneys of group R1 showed that numerous renal corpuscles had wide Bowman's spaces. Some glomeruli were congested. Numerous proximal and distal convoluted tubules were hardly differentiated. Some tubular cells appeared exfoliated. Other cells had vacuolated cytoplasm, nuclei with fading of its basophilia and shrunken deeply stained nuclei. Some areas of the interstitial tissues were infiltrated by homogenous eosinophilic material as compared to the control one Fig. (1A, C). The kidney sections that were stained with Masson's trichrome stain demonstrated apparent increase in the collagen fibers distribution in the parietal layer of the Bowman's capsule, the renal glomeruli, the basement membranes of the renal tubules, the wall of the blood vessels and the interstitial tissues as compared with the control one Fig. (2A, C).

Electron microscopic examination of the kidneys of group R1 showed that the glomeruli consisted of network of capillaries covered by the podocytes. The glomerular capillaries lined by endothelial cells lied on thick and irregular glomerular basement membranes in certain areas. Some endothelial cells had nuclei with condensed clumps of heterochromatin. Some podocytes had irregular nuclei with large clumps of heterochromatin. The majority of the secondary foot processes were fused, the filtration slit gaps appeared narrow and obliterated. The filtration slit membranes couldn't be identified in certain areas as compared with the control one Figs. (3C, 4C) and Figs. (3A, 4A). Some proximal convoluted tubules had ill-defined and thick basement membranes. Some of their cells had shrunken nuclei with condensed clumps of heterochromatin. Other cells had euchromatic nuclei with small clumps of heterochromatin. Their cytoplasm contained lysosomes, irregular mitochondria, and degenerated mitochondria. The microvilli appeared short and disrupted in certain places as compared with the control one Figs. (5C, 6C) and Figs. (5A, 6A). Some distal convoluted tubules had irregular and thick basement membranes. Some of their cells had nuclei with condensed clumps of heterochromatin. Other cells had euchromatic nuclei with small clumps of heterochromatin. Their cytoplasm contained mitochondria with destructed cristae as compared with the control one Figs. (7C, 8C) and Figs. (7A, 8A). The interstitial tissues consisted of interstitial cells and numerous collagen fibers as compared with the control one Figs. (5C, 6C, 7C, 8C) and Figs. (5A, 6A, 7A, 8A).

Morphometric study revealed significant increase in the collagen fibers and width of the Bowman's spaces of group R1 when compared with those of the groups; C1, C2, C3 and R2. On the other hand, it revealed insignificant decrease in the collagen fibers and width of the Bowman's spaces of group R1 when compared with those of the group T (Table 1) and Graph (1C, D).

Biochemical study revealed significant increase in the levels of urea and creatinine of group R1 when compared with those of groups C1, C2, C3 and R2. However, it revealed insignificant decrease in the levels of urea and creatinine of group R1 when compared with those of group T (Table 1) and Graph (1A, B).

### IV- Second recovery group (Group R2):

Light microscopic examination of the haematoxylin and eosin stained transverse sections of the kidneys of group R2 showed an improvement in the renal corpuscles, renal tubules and interstitial tissues. However, few cells with fading of its basophilia and shrunken deeply stained nuclei were present as compared with groups; C1, C2, C3, T & R1 Fig. (1A-D). The kidney sections that were stained with Masson's trichrome stain demonstrated apparent decrease in the collagen fibers distribution in the parietal layer of the Bowman's capsule, the renal glomeruli, the basement membrane of the renal tubules, the wall of the blood vessels and the interstitial tissues than those of groups; T & R1 to be more or less similar to those of groups; C1, C2 & C3 Fig. (2A-D).

Electron microscopic examination of the kidneys of group R2 showed that the renal glomeruli appeared more improved than those of groups T & R1 and more or less similar to groups C1, C2 & C3. However, fusions of few secondary foot processes were present in certain areas Figs. (3D, 4D), Figs. (3C, 4C), Figs. (3B, 4B) and Figs. (3A, 4A). The proximal convoluted tubules appeared more improved than those of groups; T & R1 and more or less similar to groups C1, C2 & C3 Figs. (5D, 6D), Figs. (5C, 6C), Figs. (5B, 6B) and Figs. (5A, 6A). The distal convoluted tubules appeared more improved than those of groups T & R1 and more or less similar to groups C1, C2 & C3 Figs. (7D, 8D), Figs. (7C, 8C), Figs. (7B, 8B) and Figs. (7A, 8A). The interstitial tissues appeared more improved than those of groups; T & R1 and more or less similar to groups; C1, C2 & C3. However, few interstitial cells had euchromatic nuclei with large clumps of heterochromatin Figs. (5D, 7D, 8D), Figs. (5C, 6C, 7C, 8C), Figs. (5B, 7B, 8B) and Figs. (5A, 6A, 7A, 8A).

Morphometric study revealed significant decrease in the collagen fibers and width of the Bowman's spaces of group R2 when compared with those of groups T and R1. It also revealed insignificant difference in the collagen fibers and width of the Bowman's spaces between the groups R2, C1, C2 and C3 (Table 1) and Graph (1 C, D).

Biochemical study revealed significant decrease in the levels of urea & creatinine of group R2 when compared with those of groups T and R1. It also revealed insignificant difference in the levels of urea & creatinine between the groups R2, C1, C2 and C3 (Table 1) and Graph (1A, B).

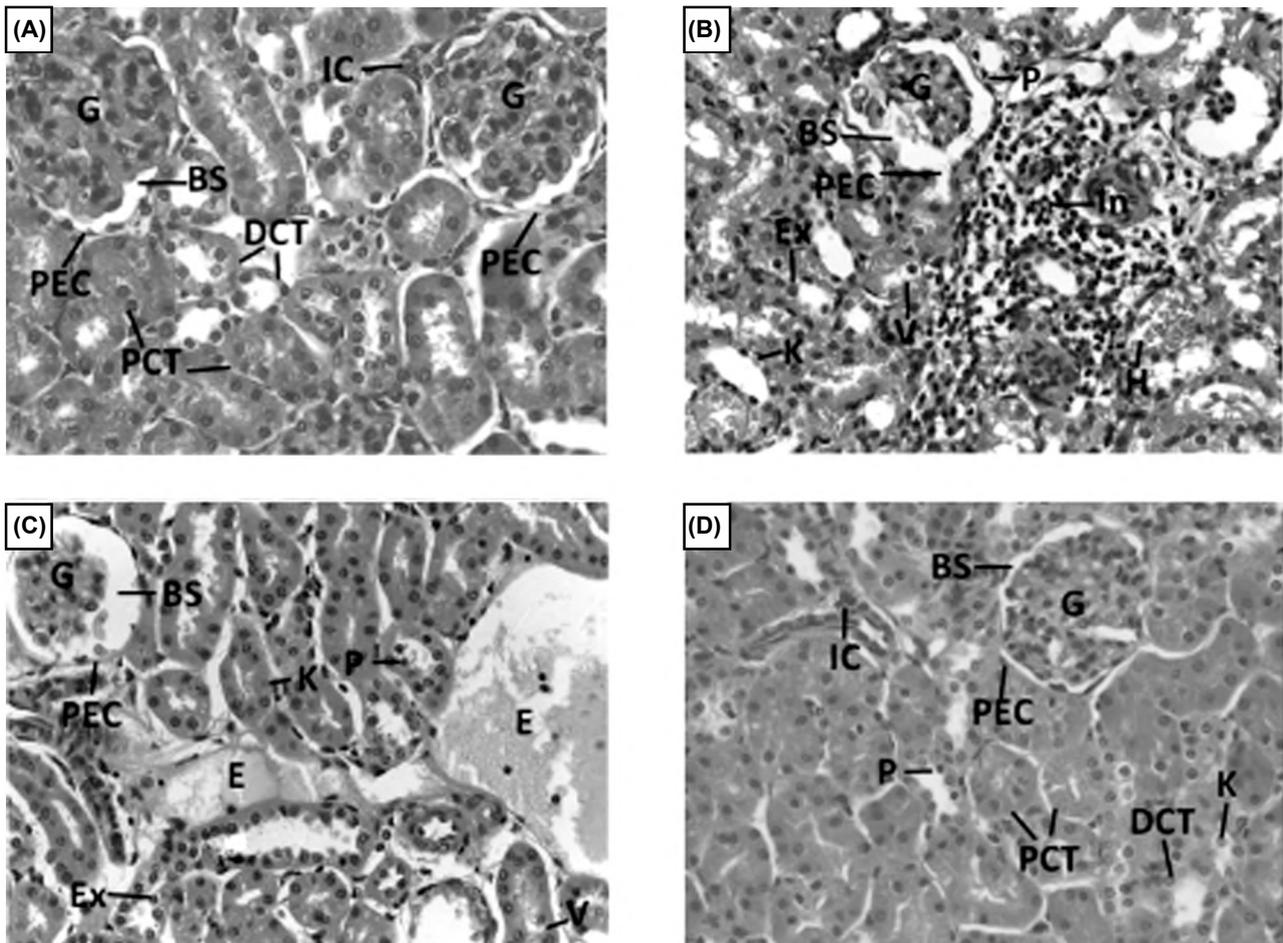


Fig. (1 A): A photomicrograph of a transverse section of the kidney of adult male albino rats of groups; C 1, C2 & C3 shows that the renal glomeruli (G) appear as large cellular masses of basophilic nuclei and eosinophilic cytoplasm. The Bowman's capsule consists of parietal layer of epithelial cells (PEC) which is separated from the visceral layer by the Bowman's Space (BS). The Proximal Convoluted Tubules (PCT) are lined by single layers of cuboidal or columnar cells that have extensive brush borders. The Distal Convoluted Tubules (DCT) are lined by single layers of cuboidal cells. The interstitial tissues consists of Interstitial Cells (IC). (H & E X400).

Fig. (1B): A photomicrograph of a transverse section of the kidney of adult male albino rats of group T shows that the Bowman's Space (BS) appears wide with disruption of its Parietal Epithelial Cells (PEC). The glomerulus is congested (G). Some tubular cells are exfoliated (Ex). Other cells have vacuolated cytoplasm (V), nuclei with fading of its basophilia (K) and shrunken deeply stained nuclei (P). The interstitial tissues contains inflammatory cells (In) and hemorrhage (H). (H & E X400).

Fig. (1 C): A photomicrograph of a transverse section of the kidney of adult male albino rats of group R1 shows that the Bowman's Space (BS) appears wide. The Parietal Epithelial Cells (PEC) appear normal. The glomerulus is congested (G). Some tubular cells are exfoliated (Ex). Other cells have vacuolated cytoplasm (V), nuclei with fading of its basophilia (K) and shrunken deeply stained nuclei (P). The interstitial tissues contains homogenous eosinophilic material (E) (H & E X400).

Fig. (1D): A photomicrograph of a transverse section of the kidney of adult male albino rats of group R2 shows that the parietal layer of epithelial cells (PEC) and the Bowman's Space (BS) appears normal. The glomeruli (G), the Proximal Convoluted Tubules (PCT), the Distal Convoluted Tubules (DCT) and the Interstitial Cells (IC) have normal appearance. Few nuclei with fading of its basophilia (K) and shrunken deeply stained nuclei (P) are seen. (H & E X400).

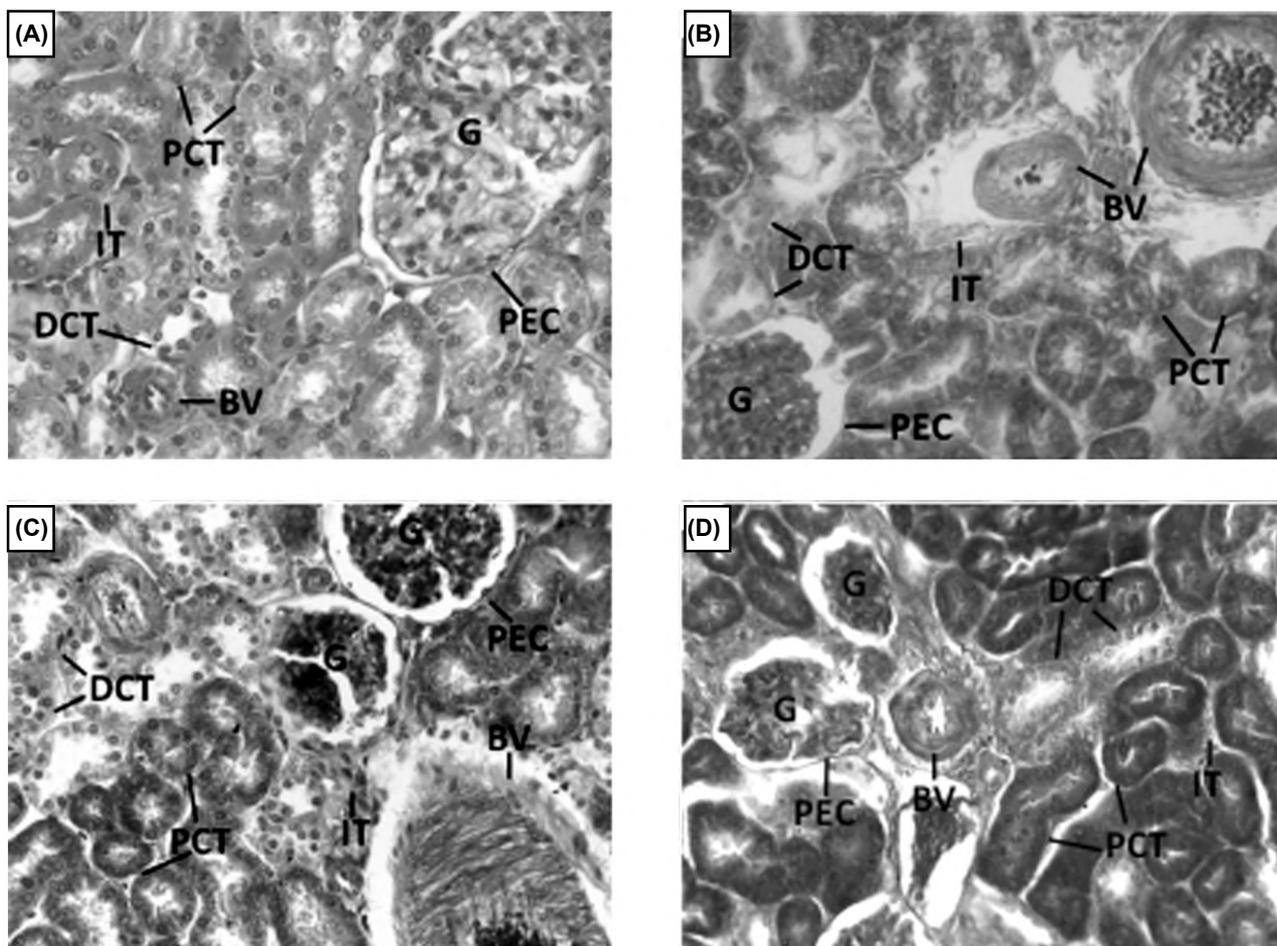


Fig. (2A): A photomicrograph of a transverse section of the kidney of adult male albino rats of groups; C1, C2 & C3 shows normal distribution of collagen fibers in the parietal epithelial layer of Bowman's capsule (PEC), the renal glomeruli (G), the basement membranes and brush borders of the Proximal Convoluted Tubules (PCT) and the basement membranes of the Distal Convoluted Tubules (DCT), the wall of the Blood Vessels (BV) and the Interstitial Tissues (IT). (Masson's trichrome X400).

Fig. (2B): A photomicrograph of a transverse section of the kidney of adult male albino rats of group T shows apparent increase of collagen fibers distribution in the parietal epithelial layer of Bowman's capsule (PEC), the renal glomeruli (G), the basement membranes and brush borders of the Proximal Convoluted Tubules (PCT) and the basement membranes of the Distal Convoluted Tubules (DCT), the wall of the Blood Vessels (BV) and the Interstitial Tissues (IT). (Masson's trichrome X400).

Fig. (2C): A photomicrograph of a transverse section of the kidney of adult male albino rats of group R1 shows apparent increase of collagen fibers distribution in the parietal epithelial layer of Bowman's capsule (PEC), the renal glomeruli (G), the basement membranes and brush borders of the Proximal Convoluted Tubules (PCT) and the basement membranes of the Distal Convoluted Tubules (DCT), the wall of the Blood Vessels (BV) and the Interstitial Tissues (IT). (Masson's trichrome X400).

Fig. (2D): A photomicrograph of a transverse section of the kidney of adult male albino rats of group R2 shows normal distribution of collagen fibers in the parietal epithelial layer of Bowman's capsule (PEC), the renal glomeruli (G), the basement membranes and brush borders of the Proximal Convoluted Tubules (PCT) and the basement membranes of the Distal Convoluted Tubules (DCT), the wall of the Blood Vessels (BV) and the Interstitial Tissues (IT). (Masson's trichrome X400).

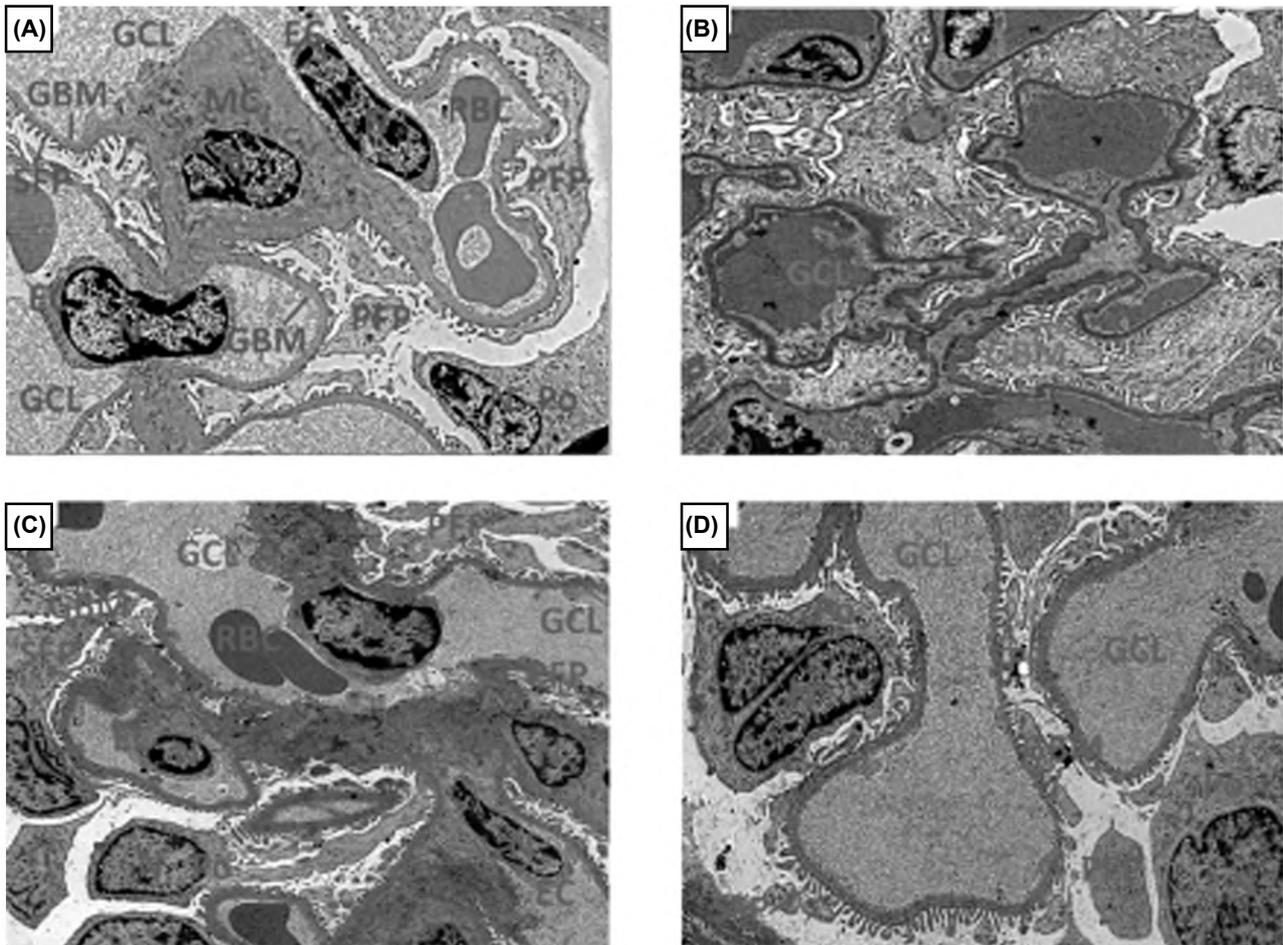


Fig. (3A): An electron micrograph of the kidney of adult male albino rats of groups; C 1, C2 & C3 shows that the Glomerular Capillary Lumina (GCL) contain Red Blood Cells (RBC) and is lined by Endothelial Cells (EC) that have euchromatic nuclei with small clumps of heterochromatin (N). The Glomerular Basement Membrane (GBM) appears thin. The Mesangial Cells (MC) have euchromatic nuclei with large clumps of heterochromatin (N). The podocytes (Po) have euchromatic nuclei with small clumps of heterochromatin (N), Primary Foot Processes (PFP) and Secondary Foot Processes (SFP). (TEM X5000).

Fig. (3B): An electron micrograph of the kidney of adult male albino rat of group T shows that the glomerular capillary lumina are lined by Endothelial Cells (EC) that have nuclei with condensed clumps of heterochromatin (N). The glomerular basement membranes appears irregular and thick (GBM). The podocytes (Po) have euchromatic nuclei with small clumps of heterochromatin (N), primary (PFP) and Secondary Foot Processes (SFP). (TEM X5000).

Fig. (3C): An electron micrograph of the kidney of adult male albino rat of group R1 shows that the Glomerular Capillary Lumina (GCL) contain RBCs (RBC) and are lined by Endothelial Cells (EC) that have euchromatic nuclei with condensed clumps of heterochromatin (N). The glomerular basement membranes appears thick and irregular in certain areas (GBM). The podocytes (Po) have euchromatic nuclei with small clumps of heterochromatin (N), primary (PFP) and Secondary Foot Processes (SFP). (TEM X5000).

Fig. (3D): An electron micrograph of the kidney of adult male albino rats of group R2 shows the Glomerular Capillary Lumina (GCL). The Glomerular Basement Membrane (GBM), the podocytes (Po) with their nuclei (N), the primary (PFP) and the Secondary Foot Processes (SFP) have normal appearance. (TEM X5000).

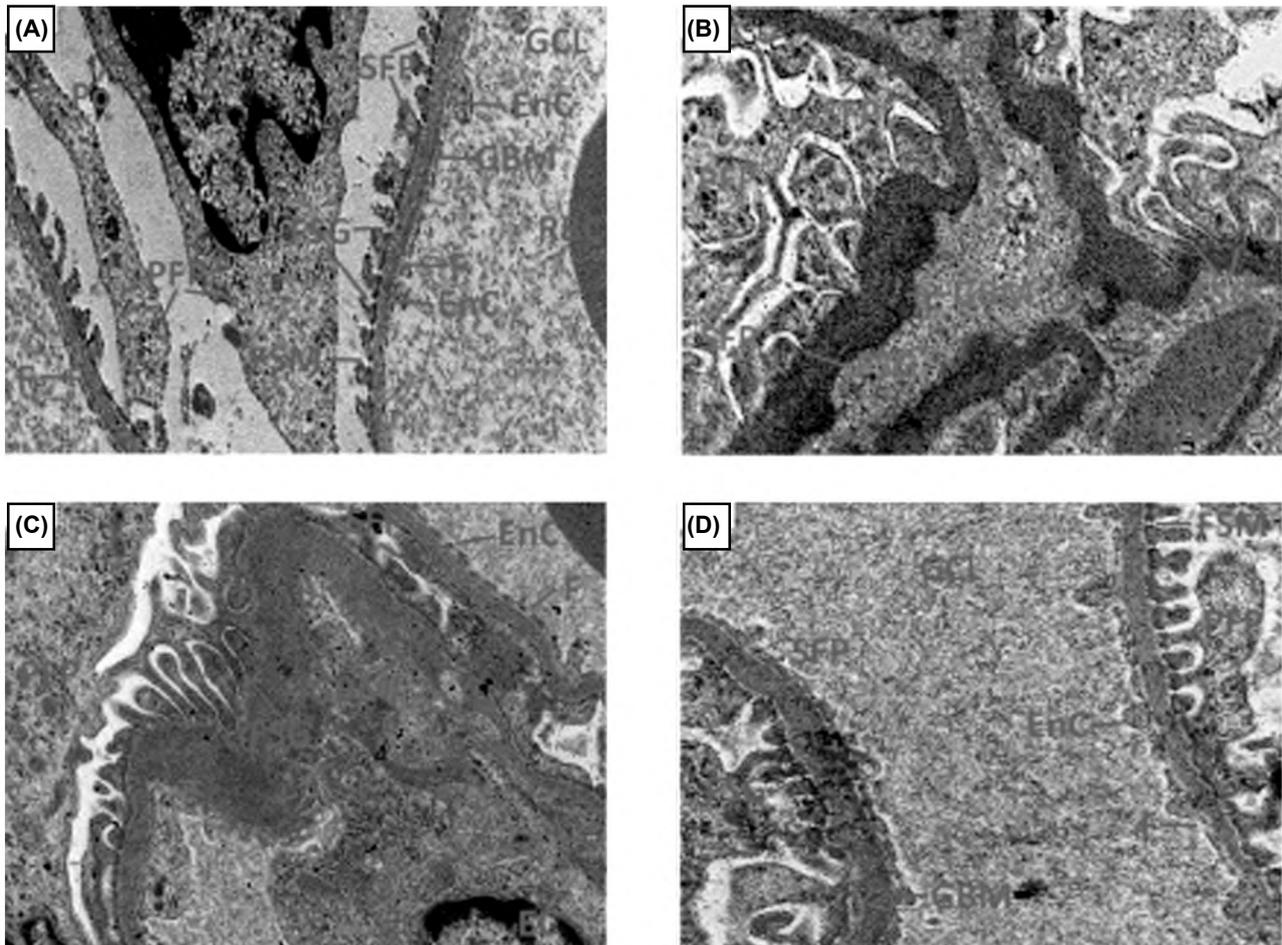


Fig. (4A): An electron micrograph of the kidney of adult male albino rats of groups; C 1, C2 & C3 shows that the Glomerular Capillary Lumen (GCL) contains Red Blood Cell (RBC) and is lined by endothelial cells that have cytoplasm (EnC) broken by numerous small fenestrations (F). The Glomerular Basement Membrane (GBM) appears thin. Part of a podocyte cell (PO) appears and has euchromatic nucleus (N), Primary Foot Process (PFP) and Secondary Foot Processes (SFP) which are separated by Filtration Slit Gaps (FSG) that are bridged by Filtration Slit Membranes (FSM). (TEM X30,000).

Fig. (4B): An electron micrograph of the kidney of adult male albino rats of group T shows that the glomerular capillary lumen contains Red Blood Cells (RBC). The Glomerular Basement Membrane (GBM) appears irregular and thick. The podocytes have Primary Foot Processes (PFP) and secondary foot processes which are fused (SFP). The filtration slit gaps appeared narrow and obliterated (FSG). (TEM X30,000).

Fig. (4C): An electron micrograph of the kidney of adult male albino rats of group R1 shows that the glomerular capillary lumen is lined by endothelial cells (EN) that have cytoplasm (EnC) broken by numerous small fenestrations (F) in some areas. The glomerular basement membrane appears irregular and thick in certain areas (GBM). The podocytes have Primary Foot Processes (PFP) and secondary foot processes which are fused (SFP). The filtration slit gaps appear narrow and obliterated (FSG). (TEM X30,000).

Fig. (4D): An electron micrograph of the kidney of adult male albino rats of group R2 shows that the Glomerular Capillary Lumen (GCL) is lined by endothelial cells that have cytoplasm (EnC) broken by numerous small fenestrations (F). The Glomerular Basement Membrane (GBM), the Primary Foot Processes (PFP), the Filtration Slit Gaps (FSG) and its overlying Filtration Slit Membranes (FSM) have normal appearance. Few secondary foot processes are fused in certain areas (SFP). (TEM X30,000).

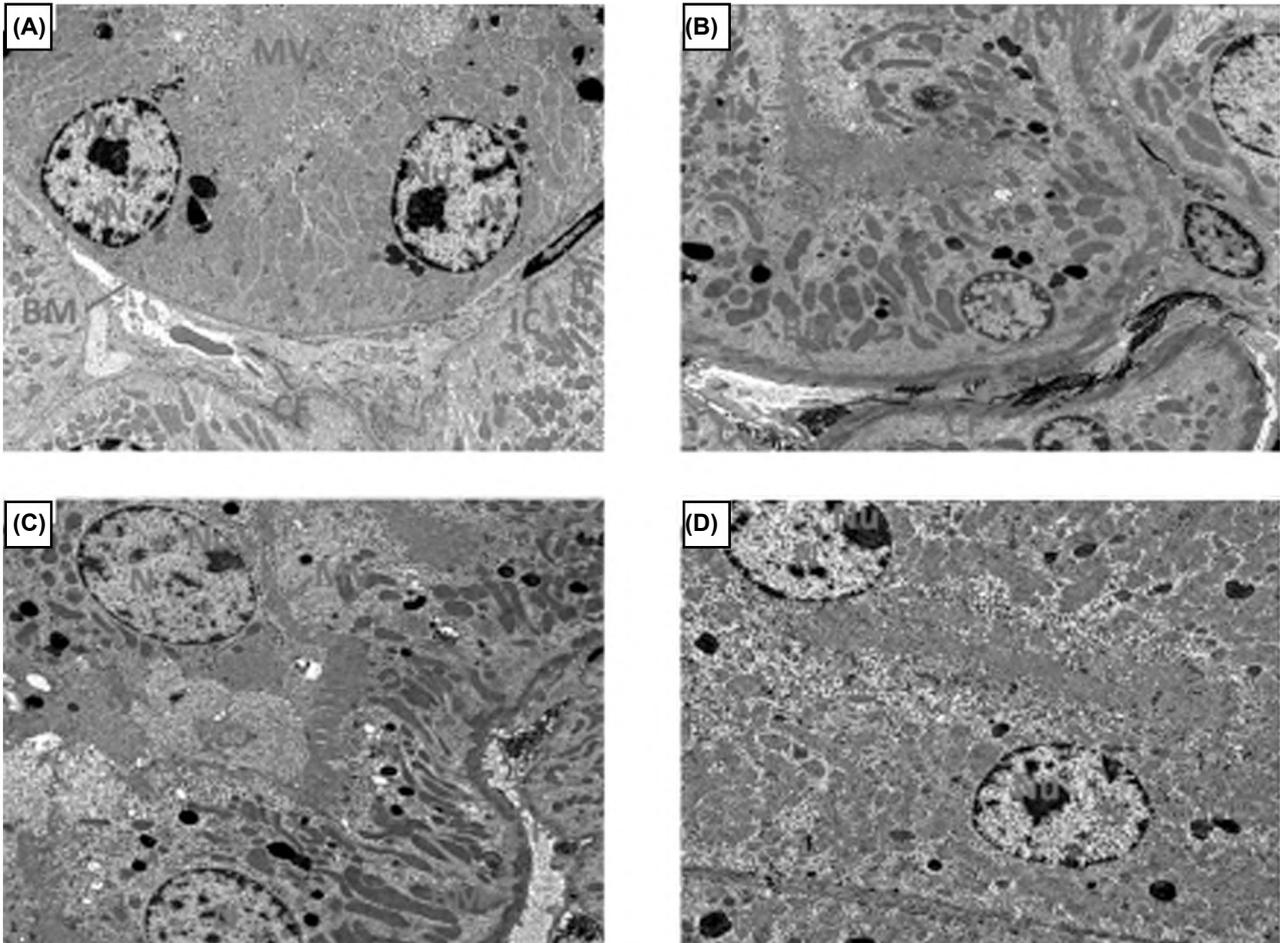


Fig. (5A): An electron micrograph of the kidney of adult male albino rats of groups; C1, C2 & C3 shows that the Proximal Convoluted Tubule (PCT) has thin Basement Membrane (BM). Their cells have numerous closely packed microvilli (MV) and nearly rounded euchromatic nuclei with small clumps of heterochromatin (N) and prominent nucleoli (Nu). The interstitial tissue consists of few Collagen Fibers (CF) and Interstitial Cell (IC) with euchromatic nucleus (N). (TEM X5000).

Fig. (5B): An electron micrograph of the kidney of adult male albino rats of group T shows that the Proximal Convoluted Tubule (PCT) has ill-defined and thick Basement Membrane (BM). The microvilli appear short and disrupted (MV). One cell has euchromatic nucleus with small clumps of heterochromatin (N), another cell has shrunken nucleus with condensed clumps of heterochromatin (P). The interstitial tissue consists of numerous Collagen Fibers (CF) and an Interstitial Cell (IC) with euchromatic nucleus (N). (TEM X5000).

Fig. (5C): An electron micrograph of the kidney of adult male albino rats of group R1 shows that the Proximal Convoluted Tubule (PCT) has ill-defined and thick Basement Membrane (BM). Their cells have euchromatic nuclei with small clumps of heterochromatin (N) and eccentric nucleoli (Nu). The microvilli appear short and disrupted (MV). The interstitial tissue consists of numerous Collagen Fibers (CF). (TEM X5000).

Fig. (5D): An electron micrograph of the kidney of adult male albino rats of group R2 shows that the Proximal Convoluted Tubule (PCT) has thin Basement Membrane (BM). Their cells have numerous closely packed microvilli (MV), and euchromatic nuclei with small clumps of heterochromatin (N) and prominent eccentric nucleoli (Nu). The interstitial tissue consists of few Collagen Fibers (CF). (TEM X5000).

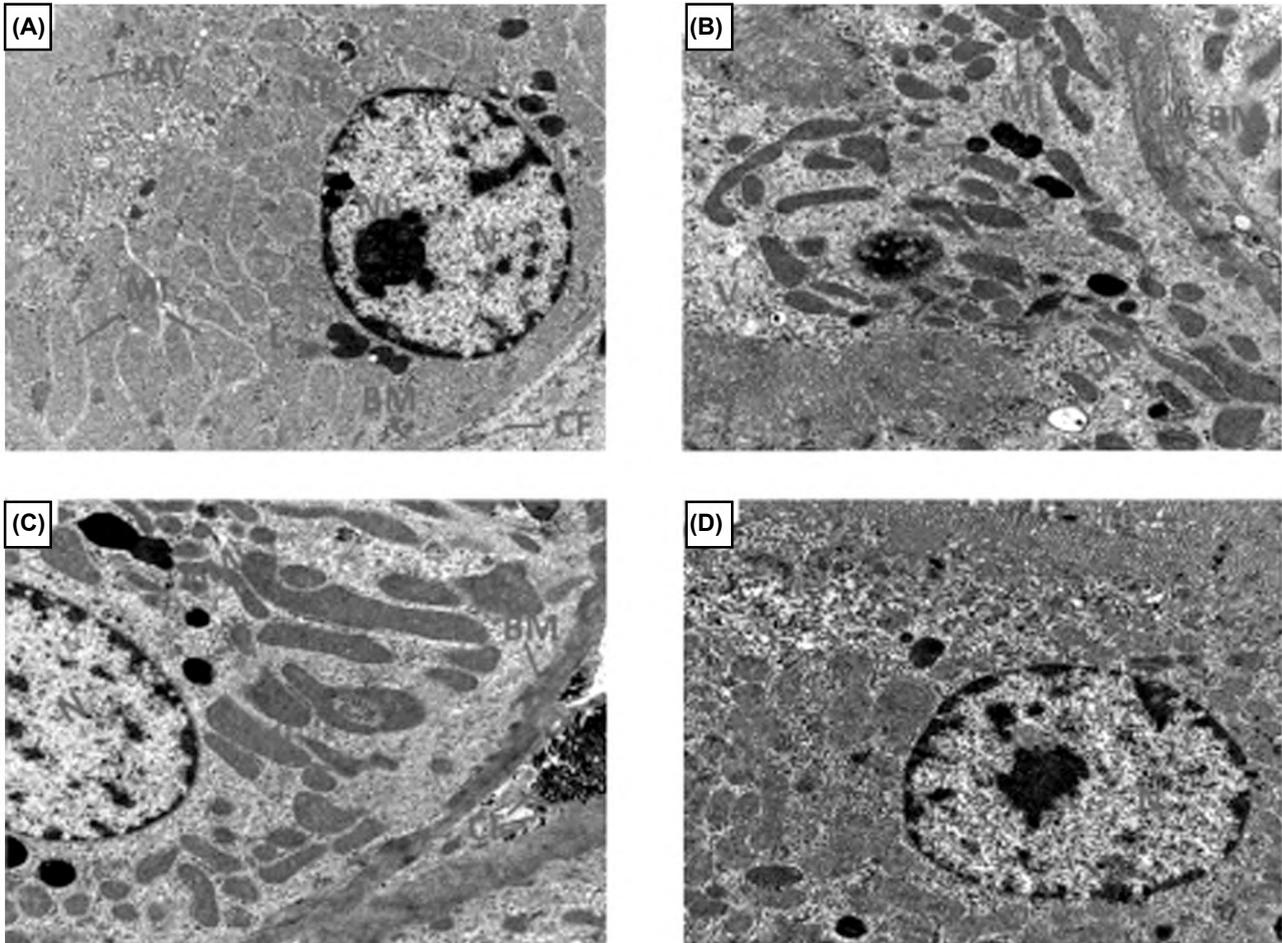


Fig. (6A): An electron micrograph of the kidney of adult male albino rats of groups; C1, C2 & C3 shows that the proximal-convoluted tubule has thin Basement Membrane (BM). Its cell has numerous closely packed microvilli (MV), nearly rounded euchromatic nucleus with small clumps of heterochromatin (N) and eccentric nucleolus (Nu). The nuclear envelope has apparent Nuclear Pores (NP). Its cytoplasm contains lysosomes (L) and numerous rounded or elongated mitochondria (Mi). The interstitial tissue consists of few Collagen Fibers (CF). (TEM X10000).

Fig. (6B): An electron micrograph of the kidney of adult male albino rats of group T shows that the proximal convoluted tubule has ill-defined and thick Basement Membrane (BM). Its cell has shrunken nucleus with condensed clumps of heterochromatin (P). The cytoplasm contains lysosomes (L), irregular mitochondria, mitochondria with destructed cristae (Mi) and Degenerated Mitochondria (DMi). The microvilli appear short and disrupted (MV) (TEM X10000).

Fig. (6C): An electron micrograph of the kidney of adult male albino rats of group R1 shows that the proximal convoluted tubule has ill-defined and thick Basement Membrane (BM). Its cell has euchromatic nucleus with small clumps of heterochromatin (N). The cytoplasm contains lysosomes (L), irregular mitochondria (Mi) and Degenerated Mitochondria (DMi). The interstitial tissue consists of numerous Collagen Fibers (CF). (TEM X10000).

Fig. (6D): An electron micrograph of the kidney of adult male albino rats of group R2 shows that the proximal convoluted tubule has closely packed microvilli (MV). Its cell has euchromatic nucleus with small clumps of heterochromatin (N), prominent eccentric nucleolus (Nu) and well-defined nuclear envelope with apparent Nuclear Pores (NP). The cytoplasm contains lysosomes (L) and numerous rounded or elongated mitochondria (Mi). (TEM X10000).

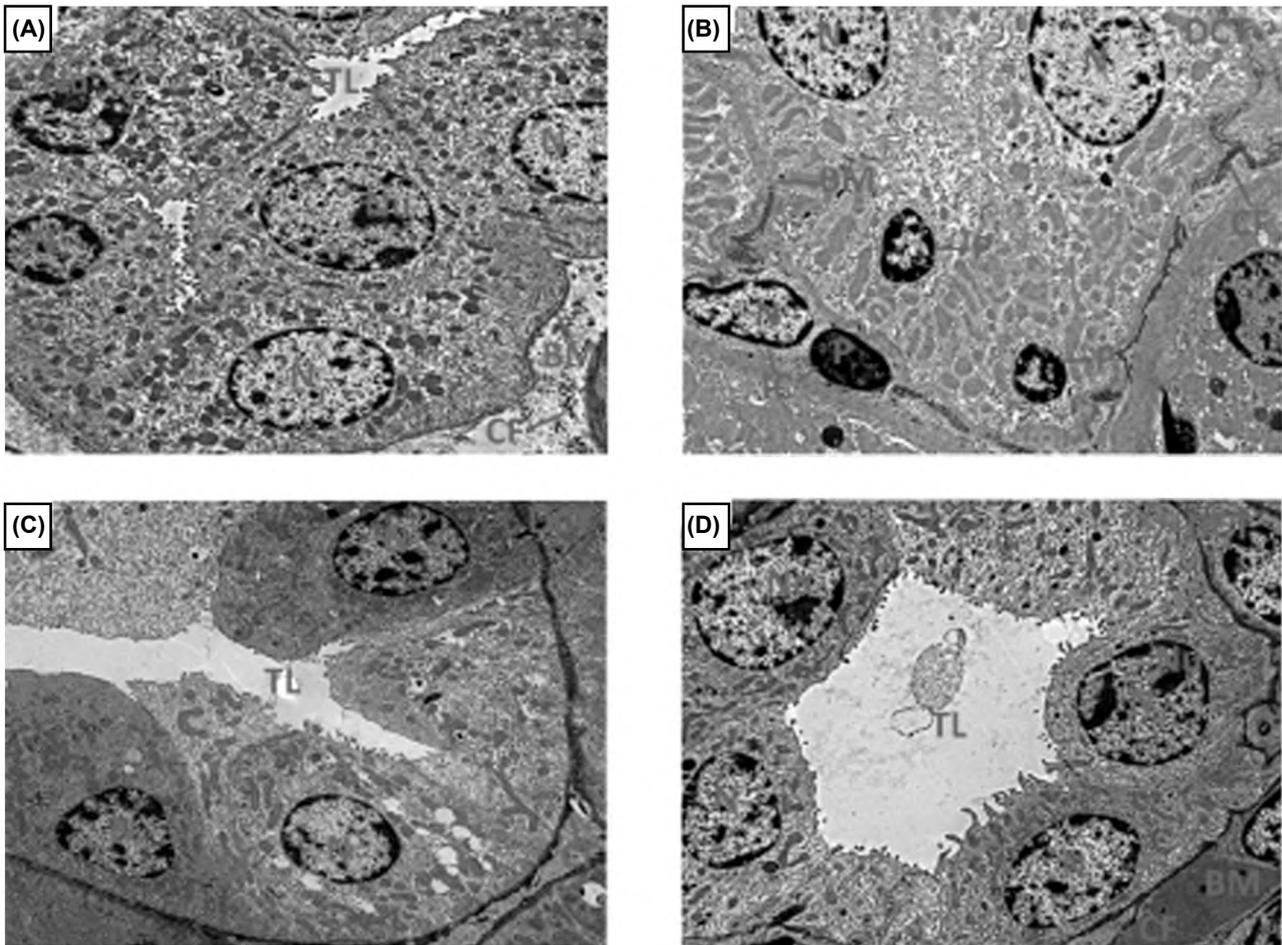


Fig. (7A): An electron micrograph of the kidney of adult male albino rats of groups; C1, C2 & C3 shows that the Distal Convoluted Tubule (DCT) has thin Basement Membrane (BM). Its cells have euchromatic nuclei with small clumps of heterochromatin (N) and prominent eccentric nucleoli (Nu). The Tubular Lumen (TL) appears. The interstitial tissue consists of few Collagen Fibers (CF). (TEM X5000).

Fig. (7B): An electron micrograph of the kidney of adult male albino rat of group T shows that the Distal Convoluted Tubule (DCT) has irregular Basement Membrane (BM). Two of its cells have shrunken nuclei with condensed clumps of heterochromatin (P). The other cells have euchromatic nuclei with small clumps of heterochromatin (N). The interstitial tissue consists of numerous Collagen Fibers (CF) and two Interstitial Cells (IC); one has euchromatic nucleus (N) and the other one has a nucleus with condensed clumps of heterochromatin (P). (TEM X5000).

Fig. (7C): An electron micrograph of the kidney of adult male albino rat of group R1 shows that the Distal Convoluted Tubule (DCT) has thin Basement Membrane (BM). Its cells have euchromatic nuclei with small clumps of heterochromatin (N). The Tubular Lumen (TL) appears. The interstitial tissue consists of numerous Collagen Fibers (CF). (TEM X5000).

Fig. (7D): An electron micrograph of the kidney of adult male albino rats of group R2 shows that the Distal Convoluted tubule (DCT) has thin Basement Membranes (BM). Their cells have euchromatic nuclei with small clumps of heterochromatin (N) and prominent eccentric nucleoli (Nu). The Tubular Lumen (TL) appears. The interstitial tissue consists of few Collagen Fibers (CF) (TEM X5000).

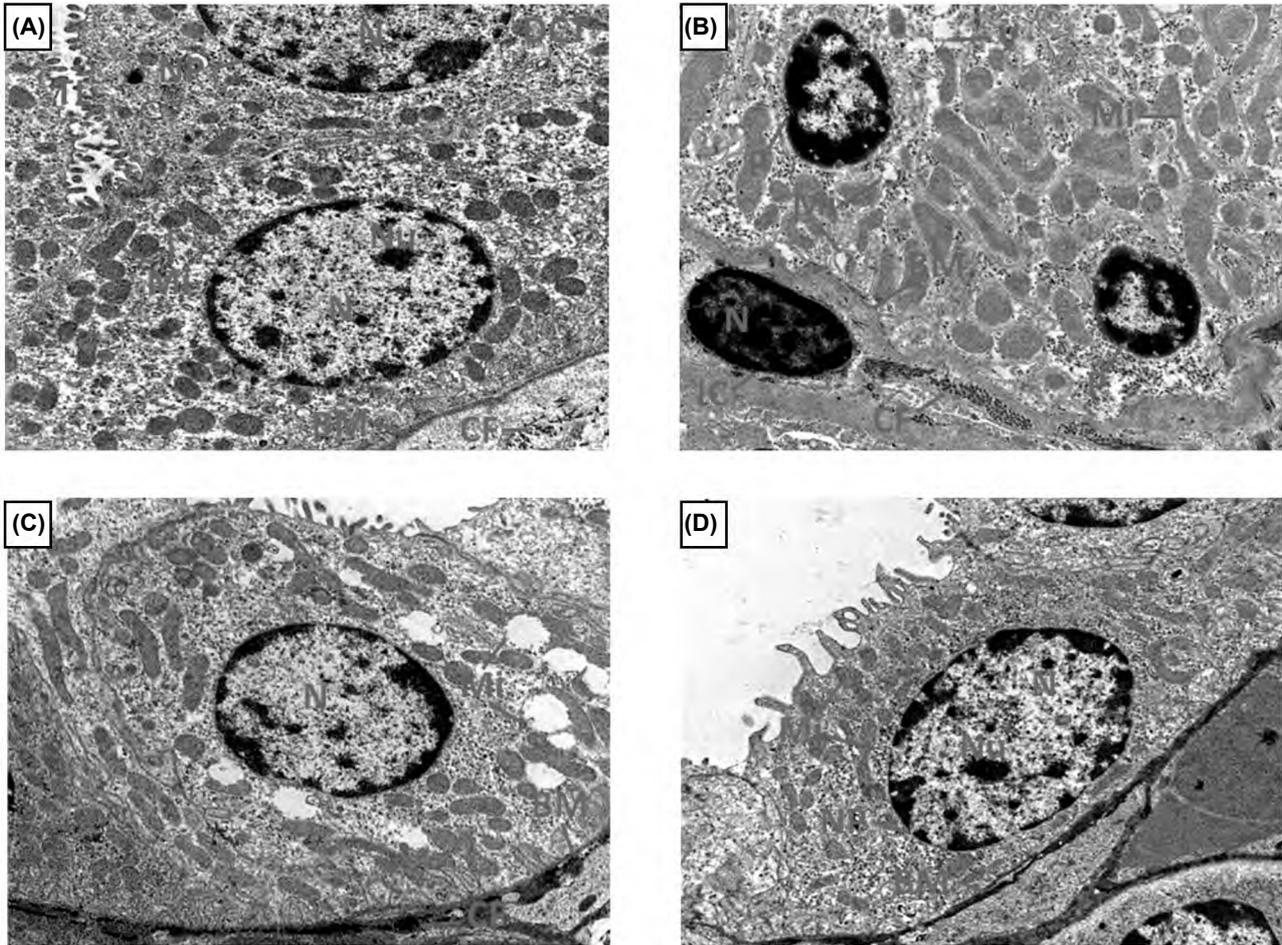


Fig. (8A): An electron micrograph of the kidney of adult male albino rats of groups; C1, C2 & C3 shows that the Distal Convoluted Tubule (DCT) has thin Basement Membrane (BM). Its cells have euchromatic nuclei with small clumps of heterochromatin (N). The nuclei have prominent eccentric nucleoli (Nu) and well-defined nuclear envelope with apparent Nuclear Pores (NP). Their cytoplasm contains rounded or elongated mitochondria (Mi). The Tubular Lumen (TL) appears. The interstitial tissue consists of few Collagen Fibers (CF). (TEM X10000).

Fig. (8B): An electron micrograph of the kidney of adult male albino rats of group T shows that the distal convoluted tubule has irregular and thick Basement Membrane (BM), its cells have shrunken nuclei with condensed clumps of heterochromatin (P), cytoplasmic vacuoles (V), mitochondria with destructed cristae and irregular mitochondria (Mi). The interstitial tissue consists of numerous Collagen Fibers (CF) and an Interstitial Cell (IC) that has nucleus with condensed clumps of heterochromatin (P). (TEM X10000).

Fig. (8C): An electron micrograph of the kidney of adult male albino rats of group R1 shows that the distal convoluted tubule has thin Basement Membrane (BM). Its cell has euchromatic nucleus with small clumps of heterochromatin (N). The cytoplasm contains numerous mitochondria with destructed cristae (Mi). The interstitial tissue consists of numerous Collagen Fibers (CF). (TEM X10000).

Fig. (8C): An electron micrograph of the kidney of adult male albino rats of group R2 shows that the distal convoluted tubule has thin Basement Membrane (BM). Its cell has euchromatic nucleus with small clumps of heterochromatin (N). The nucleus has prominent eccentric nucleolus (Nu) and well-defined nuclear envelopes with apparent Nuclear Pores (NP). Its cytoplasm contains rounded or elongated mitochondria (Mi) The Tubular Lumen (TL) appears. The interstitial tissue consists of few Collagen Fibers (CF). (TEM X10000).

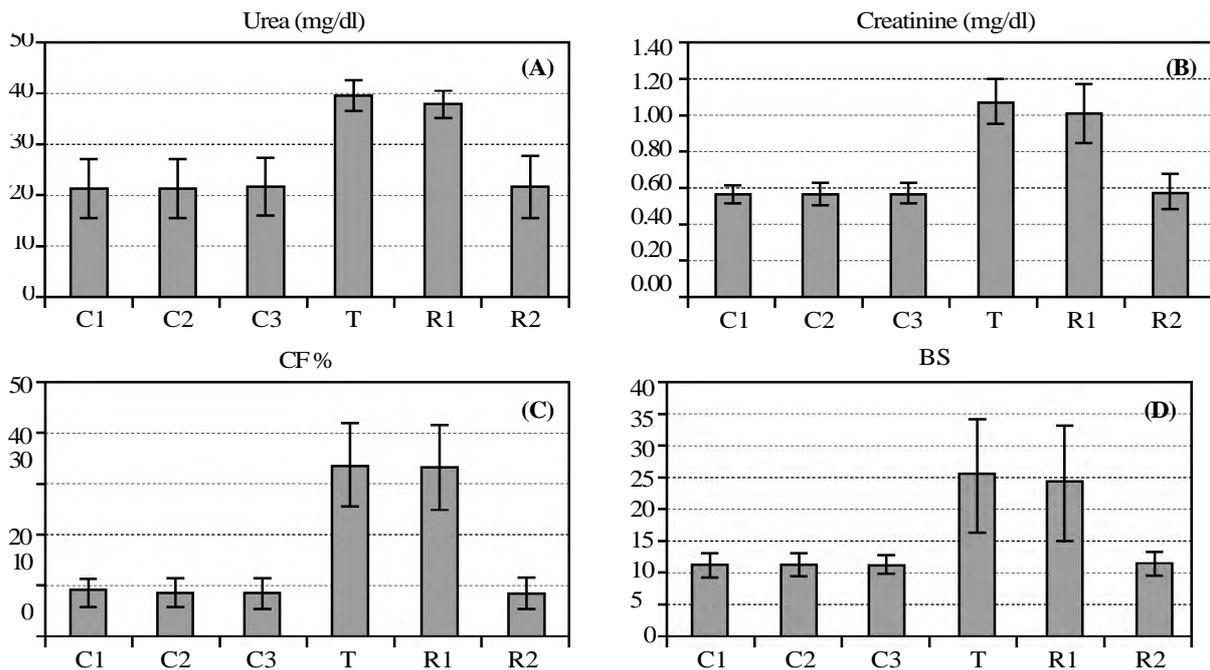
Table (1): Levels of urea and creatinine, percentage of collagen fibers and width of Bowman's space of studied groups.

| G  | Urea (mg/dl)                        |       | Creatinine (mg/dl)                 |           | CF%                                |             | BS                                 |             |
|----|-------------------------------------|-------|------------------------------------|-----------|------------------------------------|-------------|------------------------------------|-------------|
|    | Mean ±                              | Range | Mean ±                             | Range     | Mean ±                             | Range       | Mean ± SD                          | Range       |
|    | Test value* 32.665*<br>p-value 0.05 |       | Test value* 58.612<br>p-value 0.05 |           | Test value* 59.282<br>p-value 0.05 |             | Test value* 16.810<br>p-value 0.05 |             |
| C1 | 21.30±5.                            | 13-30 | 0.57±0.                            | 0.5-0.68  | 8.50±2.85                          | 4.24-12.32  | 11.11±1.74                         | 8.14-13.4   |
| C2 | 21.40±                              | 14-31 | 0.57±                              | 0.5-0.67  | 8.39±3.01                          | 4.21-12.22  | 11.20±1.81                         | 8.13-13.5   |
| C3 | 5.48 21.70                          | 13-30 | 0.06 0.57                          | 0.5-0.66  | 8.36±3.01                          | 4.11-12.18  | 11.08±1.49                         | 9.12-13.3   |
| T  | ±5.58 39.                           | 35-44 | ±0.06 1.                           | 0.87-1.25 | 33.84±8.                           | 19.25-45.34 | 25.19±8.95                         | 10.45-35.44 |
| R1 | 60±2.84                             | 33-42 | 07±0.12                            | 0.65-1.15 | 27 33.41±                          | 18.25-44.15 | 24.19±9.08                         | 9.55-35.44  |
| R2 | 37.90±2.                            | 13-29 | 1.00±0.                            | 0.4-0.72  | 8.25 8.36±                         | 4.14-12.13  | 11.29±1.86                         | 8.98-14.3   |

Post Hoc analysis using LSD test  
Parameters

| p-values  | Urea (mg/dl) | Creatinine (mg/dl) | CF%   | BS    |
|-----------|--------------|--------------------|-------|-------|
|           | C1 vs. C2    | 0.964              | 0.982 | 0.962 |
| C1 vs. C3 | 0.857        | 0.982              | 0.954 | 0.989 |
| C1 vs. T  | <0.05        | <0.05              | <0.05 | <0.05 |
| C1 vs. R1 | <0.05        | <0.05              | <0.05 | <0.05 |
| C1 vs. R2 | 0.821        | 0.876              | 0.952 | 0.943 |
| C2 vs. C3 | 0.892        | 0.964              | 0.992 | 0.962 |
| C2 vs. T  | <0.05        | <0.05              | <0.05 | <0.05 |
| C2 vs. R1 | <0.05        | <0.05              | <0.05 | <0.05 |
| C2 vs. R2 | 0.857        | 0.858              | 0.990 | 0.971 |
| C3 vs. T  | <0.05        | <0.05              | <0.05 | <0.05 |
| C3 vs. R1 | <0.05        | <0.05              | <0.05 | <0.05 |
| C3 vs. R2 | 0.964        | 0.893              | 0.998 | 0.933 |
| T vs. R1  | 0.444        | 0.112              | 0.860 | 0.680 |
| T vs. R2  | <0.05        | <0.05              | <0.05 | <0.05 |
| R1 vs. R2 | <0.05        | <0.05              | <0.05 | <0.05 |

\*: One Way ANOVA. CF: Collagen Fibers. C2: Second control group. R1: First recovery group.  
P: Parameters. BS: Bowman's Space. C3: Third control group. R2: Second recovery group.  
G: Groups. C1: First control group. T: Treated group.



Graph (1): Levels of urea (A) and Creatinine (B), percentage of collagen fibers (C) and width of Bowman's space (D) of studied groups.

CF: Collagen FIBERS. BS: Bowman's Space. C1: First control group. C2: Second control group. C3: Third control group. T: Treated group. R1: First recovery group. R2: Second recovery group.

## Discussion

In the current work, light and electron microscopic examination and morphometric study of the kidney of metformin hydrochloride treated adult male albino rats showed various signs of degeneration and necrosis in the form of significant widening of the Bowman's spaces, fusion of the secondary foot processes of the podocytes, narrowing and obliteration of the filtration slit gaps and ill-defined filtration slit membranes, exfoliation of the cells of the renal tubules, loss of integrity and distortion of brush borders of the proximal convoluted tubules, cytoplasmic vacuoles, presence of irregular and degenerated mitochondria and mitochondria with destructed cristae and nuclear changes in the form of karyolysis and pyknosis. Also various signs of inflammation and fibrosis were demonstrated in the form of significant increase in the collagen fibers, presence of interstitial edema, inflammatory cellular infiltration and thickening of the glomerular and tubular basement membranes. These findings coincide with [25] who observed distortion and necrosis in the liver and kidney tissues with marked necrosis and degeneration of seminiferous tubules as well as defoliation of spermatocytes, seminiferous tubule atrophy and the decrease in spermatogenic cells in metformin treated rats. Also, [26] reported that administration of  $\geq 900$ mg/kg/day of metformin hydrochloride resulted in increased incidence of necrosis and inflammation of the parotid salivary gland of male rats given 1200mg/kg/day.

In the current work, glomerular congestion and interstitial hemorrhage were present. These findings are in line with [2] who reported sporadic adverse reactions associated with metformin hydrochloride as vasculitis, allergic pneumonitis, cholestatic jaundice, and hemolytic anemia. Therapeutic doses of metformin in type 2 diabetic patients lower circulating levels of several coagulation factors such as plasminogen activator inhibitor, von Willibrand factor, tissue type plasminogen activator and factor VII. Also, [27] revealed that hemodialysis patients with diabetes mellitus, metformin users had a significantly higher risk of ischemic and hemorrhagic stroke than did nonusers.

In the present work, the effect of metformin hydrochloride on the histological structure of the kidney of adult male albino rat was confirmed by measuring serum urea and creatinine levels as indicators of kidney function. Significant increase in their levels was detected, demonstrating impairment in the kidney function. This finding coincides with [28] who reported that 1 of 22 (4.5%) patients

treated with metformin in general practice had serum creatinine levels exceeding predefined safety limits. Also, [29] reported that the patients with type 2 diabetes mellitus who received metformin hydrochloride therapy for at least 6 months had a greater decline in glomerular filtration rate. The continuation of metformin therapy was significantly associated with a decline in renal function.

In the present work, the possible cause of the deleterious effects of the metformin hydrochloride of the kidney can be explained by [30,31] who found that metformin caused oxidative stress since it increased the levels of Reactive Oxygen Species (ROS) and lowers the aconitase activity. The superoxide anion, the hydroxyl radical or hydrogen peroxide were highly reactive molecules that were collectively termed reactive oxygen species. ROS could chemically modify membranes, proteins or DNA and therefore high ROS levels could lead to a variety of pathologies. Also, [25,32] stated that metformin hydrochloride treatment to the male rats caused significant increase in testicular Lipid Peroxidation (LPO) as assessed by the accumulation of Malondialdehyde (MDA). The increased testicular LPO levels were accompanied by significant decrease in the activities of testicular antioxidants as Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione (GSH). Lipid peroxidation had been suggested as one of the molecular mechanisms involved in drug-induced tissue injuries. Moreover, [33] assumed that metformin could produce oxidative stress due to DNA fragmentation. High concentrations of metformin increased cumene hydroperoxide (CumOOH)-induced DNA damage. DNA damage can have biological consequences, such as transcription and/or replication inhibition, ultimately leading to cell-death.

In the current work, stoppage of metformin hydrochloride treatment led to reversibility of most deleterious effects on the histological structure of the kidney and biochemical parameters. The reversibility was directly proportionate with the duration of the stoppage. Where the deleterious effects on the histological structure of the kidney and biochemical parameters were markedly improved in group R2 (the drug stopped for 4 weeks) than those in group R1 (the drug stopped for 2 weeks). This finding coincides with [34] who said that a patient developed encephalopathy temporally related to metformin therapy that resolved after its withdrawal. Also, [35] reported that a 78-year-old male presented with a 10-day history of abdominal pain, vomiting, diarrhea, and jaundice after receiving metformin 850mg/day for 2 weeks. Laboratory analysis showed severe hepatocellular and chole-

static hepatic injury. Discontinuation of metformin treatment led to significant subjective improvement after 1 week, and all hepatic abnormalities resolved by 2 months.

#### Conclusion:

Metformin hydrochloride induced various deleterious changes in the histological structure and function of the kidney. These changes were improved on its withdrawal.

#### Recommendation:

Renal function of all patients who undergo metformin hydrochloride treatment should be monitored, and any detectable deterioration requires rapid withdrawal of the drug.

#### Acknowledgement:

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## تأثير إعطاء هيدروكلوريد الميتفورمين وإنسحابه على كلى ذكور الفئران البيضاء البالغة (دراسات نسيجية وكيميائية حيوية)

الخلفية: يعتبر هيدروكلوريد الميتفورمين دواء فعال جداً لمرض السكري والذي أصبح الدواء المفضل لعلاج مرض السكري من النوع ٢. الهدف من هذه الدراسة: يهدف هذا العمل إلى توضيح تأثير إعطاء هيدروكلوريد الميتفورمين وإنسحابه على التركيب النسيجي ووظيفة الكلى لذكور الفئران البيضاء البالغة.

المواد والطرق: تم استخدام ستين فأراً من الذكور البيضاء البالغة. تم تقسيمهم بالتساوي إلى ستة مجموعات:

- المجموعة الضابطة الأولى (المجموعة C1).
- المجموعة الضابطة الثانية (المجموعة C2).
- المجموعة الضابطة الثالثة (المجموعة C3).
- المجموعة المعالجة (المجموعة T).
- المجموعة الإنتعاش الأولى (المجموعة R1).
- المجموعة الإنتعاش الثانية (المجموعة R2).

تم إعطاء ذكور الفئران البيضاء من المجموعتين C1 و T 0.55 مل من الماء المقطر و0.55 مل من الماء المقطر (يحتوى على ٢٧.٤٥ مجم هيدروكلوريد الميتفورمين) على التوالي لمدة أربع أسابيع. وتم إعطاء ذكور الفئران البيضاء من المجموعتين C2 و R1 0.55 مل من الماء المقطر و0.55 مل من الماء المقطر (يحتوى على ٢٧.٤٥ مجم هيدروكلوريد الميتفورمين) على التوالي لمدة أربعة أسابيع. ثم تم إيقاف العلاج لمدة أسبوعين. كما تم إعطاء ذكور الفئران البيضاء من المجموعتين C3 و R2 0.55 مل من الماء المقطر و0.55 مل من الماء المقطر (يحتوى على ٢٧.٤٥ مجم هيدروكلوريد الميتفورمين) على التوالي لمدة أربعة أسابيع. ثم تم إيقاف العلاج لمدة أربعة أسابيع. وقد أعطيت العلاجات مرة واحدة فى اليوم عن طريق الفم. وتم جمع العينات على ثلاث فترات زمنية، فى نهاية الأسبوع الرابع للمجموعتين C1 و T، وفى نهاية الأسبوع السادس للمجموعتين C2 و R1 وفى نهاية الأسبوع الثامن للمجموعتين C3 و R2، وقد تم جمع عينات الدم لقياس اليوريا والكرياتينين. وتم استخدام الكلى للفحص المجهري الضوئى والإلكترونى والدراسة المورفومترية.

النتائج: أظهرت الفحص المجهري الضوئى والإلكترونى والدراسات المورفومترية أن هيدروكلوريد الميتفورمين سبب علامات مختلفة من الإنتكاس والإلتهاب والتليف. كما أظهرت الدراسة الكيميائية أن هيدروكلوريد الميتفورمين قد تسبب فى تدهور وظائف الكلى والذي إنعكس بزيادة معنوية فى مستويات اليورى أو الكرياتينين فى الدم. وقد أدى إنسحاب هيدروكلوريد الميتفورمين إلى تحسن معظم التأثيرات النسيجية وجميع التأثيرات الكيميائية الحيوية خاصة فى مجموعة الإنتعاش الثانية حيث كان التحسن متناسباً بشكل طردى مع مدة إنسحاب الدواء.

الإستنتاج: أدى إعطاء هيدروكلوريد الميتفورمين إلى تغيرات ضارة مختلفة فى البنية النسيجية ووظيفة الكلى. وقد تحسنت هذه التغيرات عند إنسحابه.