Role of Glimepiride in Ameliorating Histological Changes in kidney of Experimentally Induced Diabetes Mellitus in Rats

Review Article

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ABSTRACT

Introduction: Several complications are accompanied with diabetes mellitus. The most common is diabetic nephropathy. The most common 3rd generation of sulfonylurea is glimepiride, which has other effects on glucose metabolism in addition to its hypoglycemic action.

Aim of the Work: Goal of this work is to study role of Glimepiride in ameliorating histological Changes in kidney of experimentally induced diabetes in rats.

Material and Methods: Forty male albino rats were divided randomly into two main groups. group I was control group and the experimental was group II, which subdivided to subgroup IIA, ten rats given glimepiride by mouth for eight consecutive weeks, subgroup IIB, ten rats injected with streptozotocin via an intraperitoneal injection, and subgroup IIIC, ten rats injected with streptozotocin via an intraperitoneal injection and then given glimepiride by mouth for 8 consecutive weeks. The kidneys were removed from all groups and processed for histological study.

Results: Cortex of the kidney in diabetic subgroup IIB revealed glomeruli dilated and congested. The proximal and distal tubules revealed destruction and degeneration of their epithelial cell lining; profound perivascular, glomerular, and peritubular collagen connective tissue fibers precipitations. EM study showed histological glomerular changes includes irregular thickening of the glomerular basement membrane (GBM) and the foot processes of the podocytes are extensively effaced. In subgroup IIC, the glomeruli and the tubules were apparently as that of the control group and the distribution of the glomerular and interstitial connective tissue was apparently normal. EM study revealed the GBM, podocytes foot processes were apparently like control group.

Conclusion: Glimepiride could ameliorate the progression of diabetic nephropathy after experimentally induced diabetes mellitus.

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Key Words: Diabetes mellitus, glimepiride, nephropathy, streptozotocin.

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INTRODUCTION

Diabetes mellitus is a chronic deteriorating disease as the main sign is hyperglycemia outcoming from resistance to insulin action or insufficient insulin secretion, or both, that is accompanied with many complications[1,2]. The chronic elevation of blood glucose level of diabetes is accompanied with long- dated dysfunction, damage, and eventually failure of different body organs, mainly kidneys, eyes, heart, and nerves[3].

Diabetic affection of the kidney is the more common life-threatening sequel of diabetic disease. It is usually due to metabolic derangements of glucose homeostasis, such as elevated blood and tissue levels of glycosylated proteins and changes in hemodynamic circulation inside the renal tissue[4].

Studies on diabetic patients have demonstrated that hyperglycemia resulted in glycosylation of proteins and liberations of more reactive oxygen species (ROS). Under these conditions, destruction can occur to cellular biomolecules such as lipid, protein, carbohydrate, and DNA[5].

Streptozotocin (STZ) is an antineoplastic drug commonly used in the management of different neoplastic diseases. It has high toxicity to the beta-cells of pancreas which resulted in its inflation and ultimately degeneration of the beta cells[6]. It deteriorates the oxidative process of glucose and diminishes insulin synthesis and proper secretions[7]. The diabetogenic possessions of STZ occur through uptake of STZ in beta cells by glucose transporter (Glut2)[8], increased oxidative stress due to nitric oxide (NO) liberations and reactive oxygen species (ROS) manufactures[9]. Experimental diabetes in laboratory animals is so important in understanding the pathogenesis and management of the disease, and a convenient dose of STZ is commonly used for induction of experimental diabetes[10].

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Sulphonylurea was the backbone for management of type II diabetes mellitus for many decades[11]. 3rd generation sulfonylurea used commonly for management of diabetes mellitus type II, Glimepiride (Amaryl)is most commonly using of these drugs[12].

The goal of this work is to study role of Glimepiride in ameliorating histological Changes in kidney of experimentally induced diabetes mellitus in albino rats.

MATERIAL AND METHODS

Drug and Chemicals

Glimepiride (Amaryl)

Glimepiride 2 mg tablets obtained from (Sanofi-Aventis company, Cairo Egypt). prepared freshly on distilled water and given by mouth through intragastric tube at a dose of 0.36 mg/kg/day[13].

Streptozotocin (STZ)

STZ obtained from (Cornell-lab-chemical company, Cairo). prepared freshly in citrate buffer (PH 4.5). STZ injected at a dose of 65 mg /kg to male rat via single intraperitoneal injection 18 hours postprandial[14].

STZ induced diabetic condition within 72h as proved through examining tail blood samples from rats by using a glucometer.

Animals and Experimental Design

40 adult male rats weighing 180-200 grams were included in this work. In the animal house of Faculty of Medicine, Tanta University, the animals were kept in a specific clean, pathogen-free environment. The rats were maintained in clean properly ventilated cages with steel wire tops at room temperature and free access to water and food ad libitum. The experiment steps done according to the rules and regulations laid down by the research ethical committee on animal's experimentation of Tanta Faculty of Medicine (approval code 1180/04/12).

The rats were divided as follows:

Group I: (Control group) (10rats): divided into

- Subgroup IA: (Negative control) (5 rats): Rats kept without any treatment along the whole experimental study.
- Subgroup IB : (STZ vehicle control) (5 rats): Rats injected with 0.1 ml of citrate buffer by intraperitoneal injection.

Group II (Experimental group): This group is formed of thirty adult male rats and divided into:

- Subgroup IIA: (glimepiride treated) (10 rats): Rats received glimepiride by intragastric gavage for 8 consecutive weeks.
- Subgroup IIB: (STZ treated) (10 rats): Rats received Streptozotocin (STZ) a single intraperitoneal injection.
- Subgroup IIC: (STZ& Glimepiride treated) (10 rats):

Rats received Streptozotocin (STZ) a single intraperitoneal injection, after induction of hyperglycemia within 72 hours, then given glimepiride by intragastric gavage for 8 consecutive weeks.

Rats with random blood glucose level over 200 mg/dl to be considered diabetic. Blood glucose level was measured by glucometer.

5 rats chosen randomly and victimized from every group; four weeks from the beginning of the work, then other 5 rats were victimized after 8 weeks from the beginning of the experiment.

The whole rats were victimized beneath ether -induced anesthesia.

The abdomen of rat opened through midline incision, both kidneys were removed, splitting into parts, fixed in the proper fixation, and treated for light and EM examination.

At the end, victimized rats were safely gathered in a special pack in related safety and health precaution rules.

Histological study

Examination by light microscope

Specimens fixed promptly in 10% formal saline solution, dehydrated in ascending concentrations of ethyl alcohol, cleared in xylene, impregnated and paraffinized. Then, sections of 5µm thickness were cut and mounted on slides. Then, sections were stained with hematoxylin & eosin (H&E) and Mallory`s trichrome stains[15].

Electron microscopy (E.M) study

The renal specimens were cut and fixed using 4% phosphate buffered glutaraldehyde (0.1 mol/L, pH 7.4), then post-fixed using 1% phosphate-buffered osmium tetroxide. After that, specimens were dehydrated in ascending grades of alcohol then embedded at the apex of inverted polythene beam capsule filled with liquid resin.

Ultrathin sections (70- 80nm) were cut using ultramicrotome. Ultrathin sections were double stained with uranyl acetate and lead citrate to be studied and photographed by Transmission EM[16]. JEOL-JEM-100 transmission EM was utilized for examination the grids at Electron Microscopy lab., Faculty of Medicine, Tanta University, Egypt.

RESULTS

Examination by light microscope

Light microscopic study of sections of kidney of control rats (subgroup IA&IB): stained with H&E- showed normal histological architecture of the renal cortex with the glomeruli formed by a tuft of capillaries and surrounded by the Bowman's space, the proximal convoluted tubule is composed of a simple cuboidal type of epithelium with eosinophilic, granular appearing cytoplasm and a brush

border. Distal convoluted tubules may be differentiated from proximal convoluted tubules by the absence of a brush border and a larger more clearly defined wide lumen (Figure 1 A1, A2). Mallory`s trichrome sections displayed the minimal amount of collagen connective tissue fibers around both tubules and glomeruli (Figure 2 A).

Renal cortex of rats treated with glimepiride (Subgroup IIA)

Light microscopical study of H&E- stained kidney sections, the histological architecture of the renal cortex was preserved all over the whole study, apparently as that of the control subgroup (IA&IB) (Figure 1 B1,B2). Mallory`s trichrome sections displayed the minimal amount of collagen connective tissue fibers around both tubules and glomeruli as that of the control subgroup (IA&IB) (Figure 2B).

Renal cortex of diabetic rats (Subgroup IIB)

After 4 weeks: studying renal cortex of rats revealed that the marked congestion and dilatation of capillaries of the glomeruli. Widing of capsular space in some glomeruli. Destruction and degeneration of the epithelial lining of both proximal and distal tubules, also peritubular infiltration and intraluminal debris were evident (Figure 1 C1,C2). Mallory`s trichrome sections displayed moderate amount of amount of collagen connective tissue fibers around both tubules and glomeruli (Figure 2C).

After 8 weeks: there was manifest deterioration in the histological architecture of the renal cortex in form of more glomerular and tubular degeneration. The epithelial cells lining both proximal and distal tubules showed vacuolization of the cytoplasm, other cells showed complete destruction (Figure 1 D1,D2). Mallory`s trichrome sections displayed excessive amount of collagen connective tissue fibers around both tubules and glomeruli (Figure 2D).

Renal cortex of diabetic rats treated with glimepiride (Subgroup IIC)

After 4 weeks: marked similarity of most of the glomeruli and the tubules were evident nearly like to control group (Figure 1 E1,E2). Mallory`s trichrome sections displayed minimal amount of collagen connective tissue fibers around both tubules and glomeruli (Figure 2E).

After 8 weeks: The most of glomeruli and the tubules were apparently like control group (Figure 1 F1,F2). Mallory`s trichrome sections displayed minimal amount of collagen connective tissue fibers around both tubules and glomeruli was apparently like the control group (Figure 2F).

Electron microscopic examination

Renal cortex of control albino rat (subgroupIA&IB)

Ultrastructural examination of the cortex of kidney of control rats displayed the visceral layer of the renal corpuscle was in contact with the capillary endothelium and was made of podocytes from which several major processes arise. Each major process gave rise to many secondary minor processes (pedicles) that were resting on a well-developed basal lamina called the glomerular basement membrane (GBM) which appeared homogenous and formed of outer and inner low electron dense layers (lamina rara) in-betweens middle high dense one (lamina densa) was evident (Figure 3A).

Renal cortex of the control rat treated with glimepiride (subgroup II A)

It showed similar findings to control group along whole length of the study (Figure 3A1).

Renal cortex of the diabetic albino rat (subgroup IIB)

After 4 weeks: electron microscopic study renal cortex of rats revealed glomerular changes in the form of irregular thickening of the (GBM), elongated, slender, disturbed foot processes of the podocytes. The foot processes became pleomorphic (Figure 4D).

After 8 weeks: The (GBM) became thicker with obvious fusion of podocytes pedicles with the basement membrane (effacement of podocytes pedicles). Focal electron dense bodies within the cytoplasm of the podocytes were evident (Figure 4D1).

Renal cortex of the diabetic rat treated with glimepiride (subgroup IIC)

After4weeks: GBM showed mild thickening. Podocytes with foot processes were seen nearly like control group (Figure 5G).

After 8 weeks: revealed that GBM and podocytes foot processes were apparently as like to control group (Figure 5G1).

Proximal convoluted tubules

Proximal convoluted tubules of the control rat (subgroup IA&IB)

The cells of the proximal convoluted tubules (PCT) showed multiple microvilli forming their distinctive brush border. Every cell showed a rounded basal nucleus and long rod like abundant mitochondria coordinated in-between basal infoldings (Figure 3B).

Proximal convoluted tubules of the control rat treated with glimepiride (subgroup IIA)

Cells of these tubules showed similar findings to those of the control group along the whole length of study (Figure 3B1).

Proximal convoluted tubules of the diabetic rat (subgroup IIB)

After 4 weeks: study of cells of PCT showed degenerative changes including irregular indented nucleus, The mitochondria showed disturbed cristae with loss of

their pattern of arrangement in between basal infoldings (Figure 4E).

After 8 weeks: There were extensive degenerative changes in the shape and pattern of the mitochondria. Degenerated areas of cytoplasm could be seen (Figure 4E1).

Proximal convoluted tubules of the diabetic rat treated with glimepiride (subgroup IIB)

After 4 weeks: cells of the PCT showed that nucleus, mitochondrial distribution, and apical microvilli were similar to control group (Figure 5H).

After 8 weeks: cells of the PCT were apparently like control group (Figure 5H1).

Distal convoluted tubules

Distal convoluted tubules (DCT) of the control rat (subgroup IA&IB)

Examination of the cells of this control group showed rounded nucleus. Mitochondria were less than that of the proximal tubules. The cells showed few microvilli, so it lacks brush border (Figure 3C).

Distal convoluted tubules of the control rat treated with glimepiride (subgroupIIA)

The cells of these tubules were apparently similar to control group (Figure 3C1).

Distal convoluted tubules of the diabetic rat (subgroupIIB)

After 4 weeks: Nucleus was irregular (indented) with peripheral condensed chromatin. The mitochondria showed disturbed cristae (Figure 4F).

After 8 weeks: Nucleus was marked irregularity (indented) with peripheral condensed chromatin. The mitochondria showed more disturbed cristae (Figure F1).

Distal convoluted tubules of the diabetic rat treated with glimepiride (subgroupIIC)

After 4 weeks: The nucleus showed mild indentation. The mitochondria and the apical microvilli were nearly like a control group (Figure 5K).

After 8 weeks: Nucleus, the mitochondria and apical microvilli were apparently as control group (Figure 5K1).

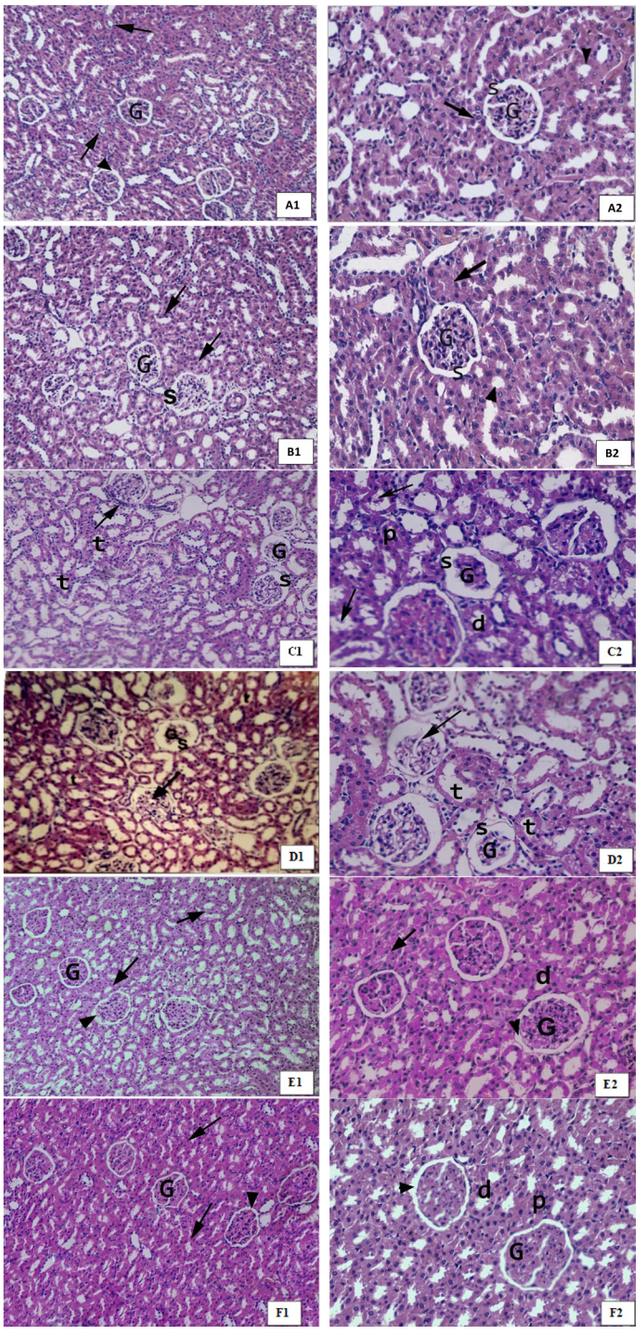

Fig. 1: Representative photomicrographs of hematoxylin and eosin (H&E) stained renal sections from different experimental groups: (A1): showed normal renal cortex, in the (control subgroupIA&IB) contains renal corpuscles formed of the glomeruli (G) with Bowman`s space (arrow`s head), and convoluted tubules (arrows). (A2): showed normal renal cortex, in the (control subgroupIA&IB) contains renal corpuscles formed of the glomeruli (G) with its tuft and Bowman`s space (S) proximal (arrow) and distal (arrow`s head) convoluted tubules. (B1): a section in (subgroupIIA) showed apparently the same as the control. (B2): a section in (subgroupIIA) showed apparently the same as the control. (C1): showed a section in (subgroupIIB) after 4 weeks showed shrunken glomeruli (G), widening of the Bowman`s space (S) and tubular degeneration (t). Periglomerular and peritubular infiltrating cells with collagen connective tissue fibers are seen (arrow). (C2): showed a section in (subgroupIIB) after 4 weeks showed shrunken glomerulus (G), widening of Bowman`s space(S) with tubular degeneration, both proximal (p) and distal (d) tubules. Intraluminal debris are seen (arrows). (D1): showed a section (subgroupIIB) after 8 weeks showed shrunken glomeruli (G), widening of the Bowman`s space (S) and tubular degeneration (t). Dilated glomerular capillaries are seen (arrow). (D2): showed a section (subgroupIIB) after 8 weeks with dilated congested glomerular capillaries (arrow), vacuolization of the epithelium lining of the convoluted tubules(t). Shrunken glomerulus (G) and wide Bowman`s space (S) are seen. (E1) showed a section in (subgroupIIC) after 4 weeks with the glomerulus (G) with its Bowman`s space (arrow`s head) and most of the tubules (arrows) are nearly like the control group. (E2) showed a section in (subgroupIIC) after 4 weeks Bowman`s space (arrow`s head), most of the proximal (arrow) and distal tubules (d) are nearly like the control group. (F1) a section a section in (subgroupIIC) after 8 weeks showed the glomeruli (G), Bowman`s space (arrow`s head) and the tubules (arrows) are apparently as that of the control group. (F2) a section (subgroupIIC) after 8 weeks showed the glomerulus (G), Bowman`s space (arrow`s head) and the tubular epithelium of both proximal (p) and distal(d) tubules are apparently as the control group. (H&E stain; A1, B1, C1, D1, E1, and F1 X 200; A2, B2, C2, D2, E2, and F2, X 400).

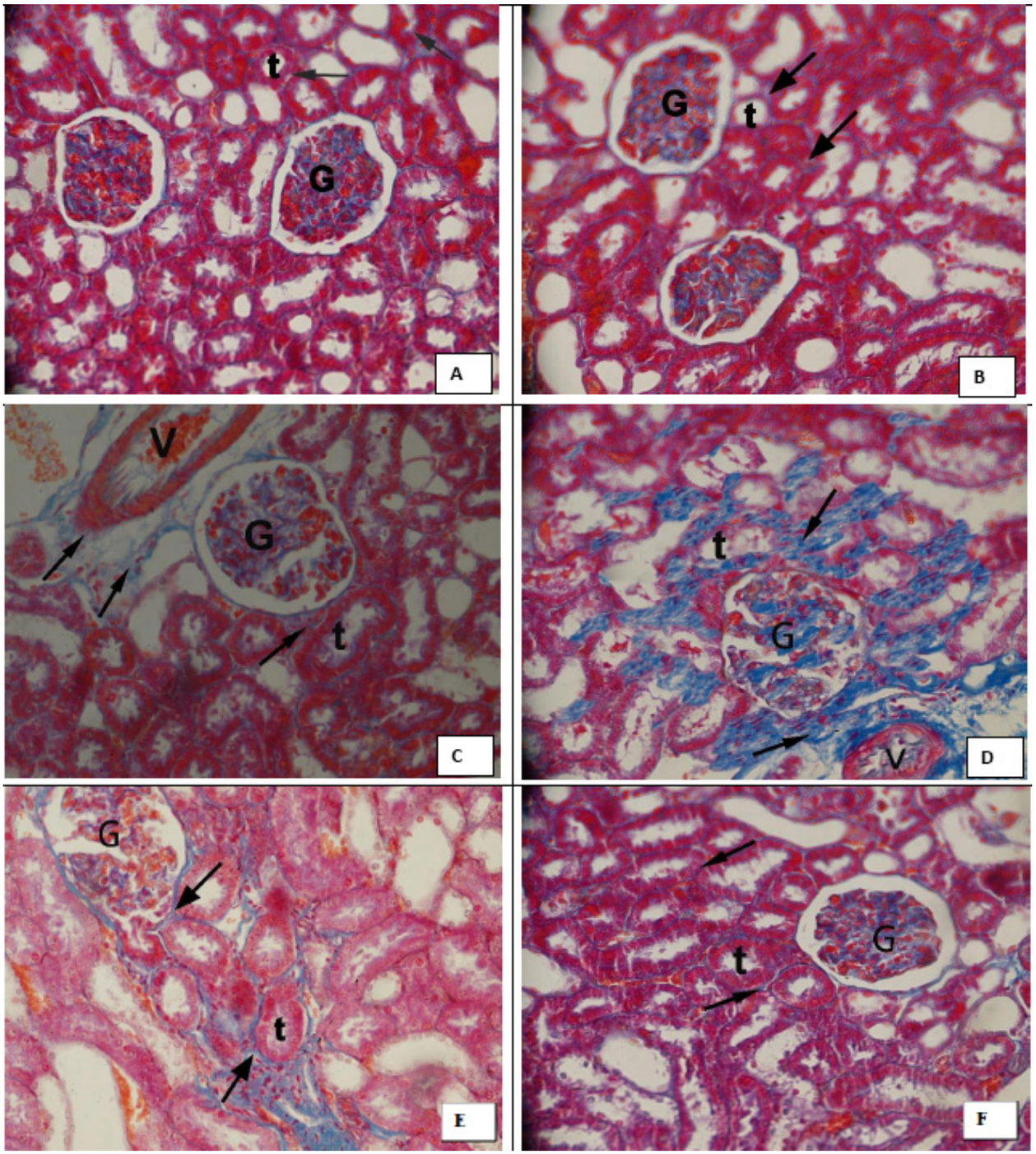

Fig. 2: Representative photomicrographs of Mallory`s Trichrome stained renal sections from different experimental groups: (A): a section in subgroup(IA&IB) showed restrictive distribution of collagen connective tissue fibers (arrows) between renal tubules and the intraglomerular(G).(B): a section in (subgroupIIA) showed apparently the same collagen connective tissue distribution as the control.(C): a section in subgroup(IIB) after 4 weeks showed increase peritubular, perivascular (v) and intraglomerular (G) collagen connective tissue deposition (arrows).(D): a section in (subgroup IIB) after 8 weeks showed extensive peritubular, perivascular (V) and intraglomerular (G) collagen connective tissue deposition (arrows).(E): a section (subgroupIIC) 4 weeks showed mild deposition of collagen connective tissue fibers (arrows) in the peritubular (t) and intraglomerular regions (G). (F): a section (subgroupIIC) after 8 weeks showed the restrictive distribution of the peritubular (t) and interglomerular (G) collagen connective tissue fibers (arrows) is apparently as that of the control group. (Mallory`s Trichrome stain; A, B, C, D, and F X 400).

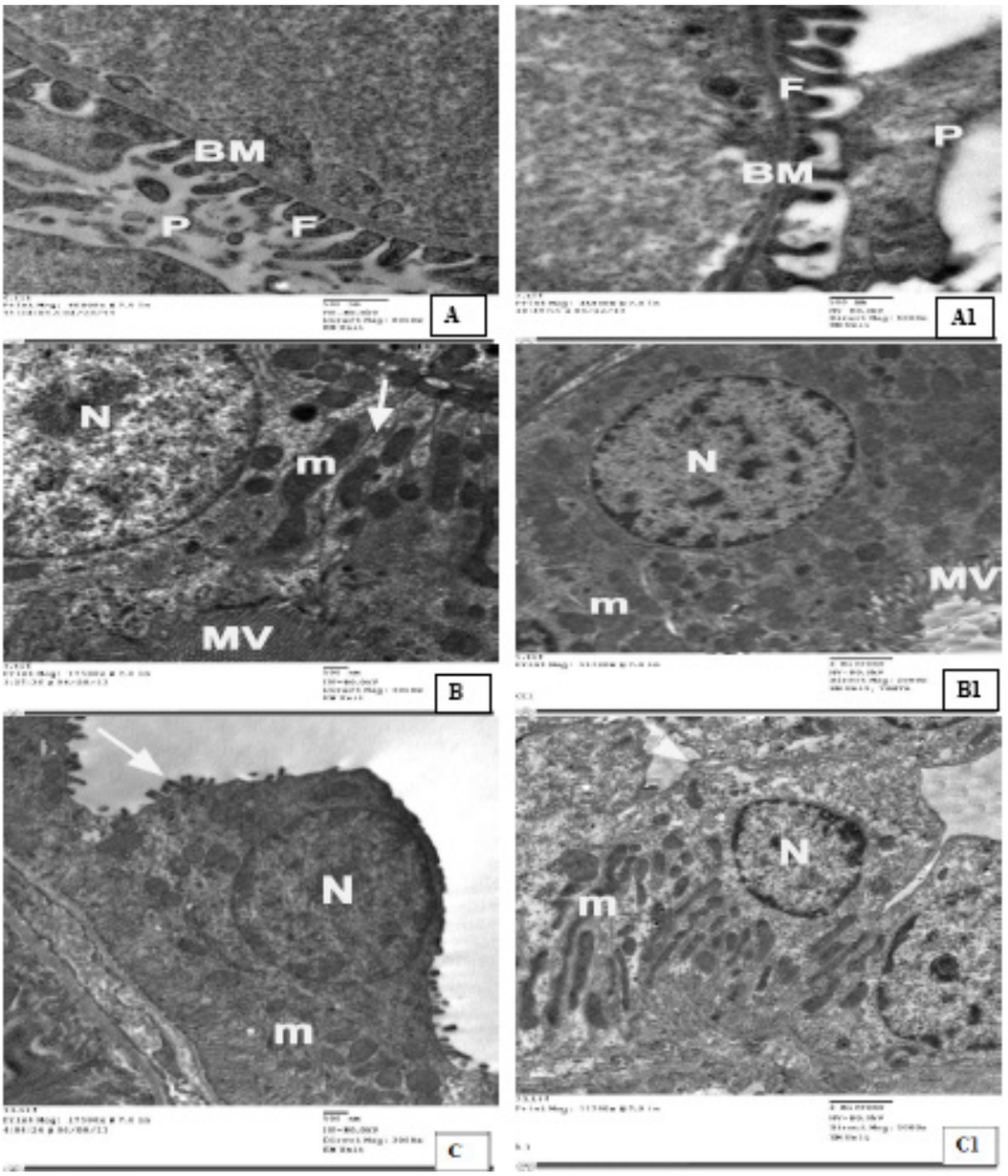

Fig. 3: Representative photomicrographs of renal ultrathin sections from different experimental groups: (TEM) (A): An ultra-thin section from the kidney of adult male albino rat showed: In subgroup IA&IB, showing podocyte (p) with arrangement of their foot processes (F) resting on glomerular basement membrane (BM). (Fig. 3A). (A1): Subgroup IIA presents similar histological findings as IA&IB subgroup. (Fig.3 A1). (B):In subgroup IA&IB, a proximal convoluted tubule of an adult control albino rat showed the nucleus (N), basolateral folding of plasma membrane (arrow) separated by columns of cytoplasm containing elongated mitochondria(m) in-between with apical microvilli (MV) (Fig 3B), and (B1):in Subgroup IIA presents similar histological findings as Subgroup IA&IB showed rounded nucleus (N), mitochondria(m) apical microvilli (MV) (Fig.3 B1). (C): In Subgroup IA&IB, a distal convoluted tubule of an adult control albino rat showed rounded nucleus (N), mitochondria (m) and few microvilli (arrow) (Fig.3 C) and (C1):in Subgroup IIA presents similar histological findings as IA&IB subgroup (Fig.3 C1). (TEM: A and A X8000; B X3000; B1 X2000; C and C1X3000)

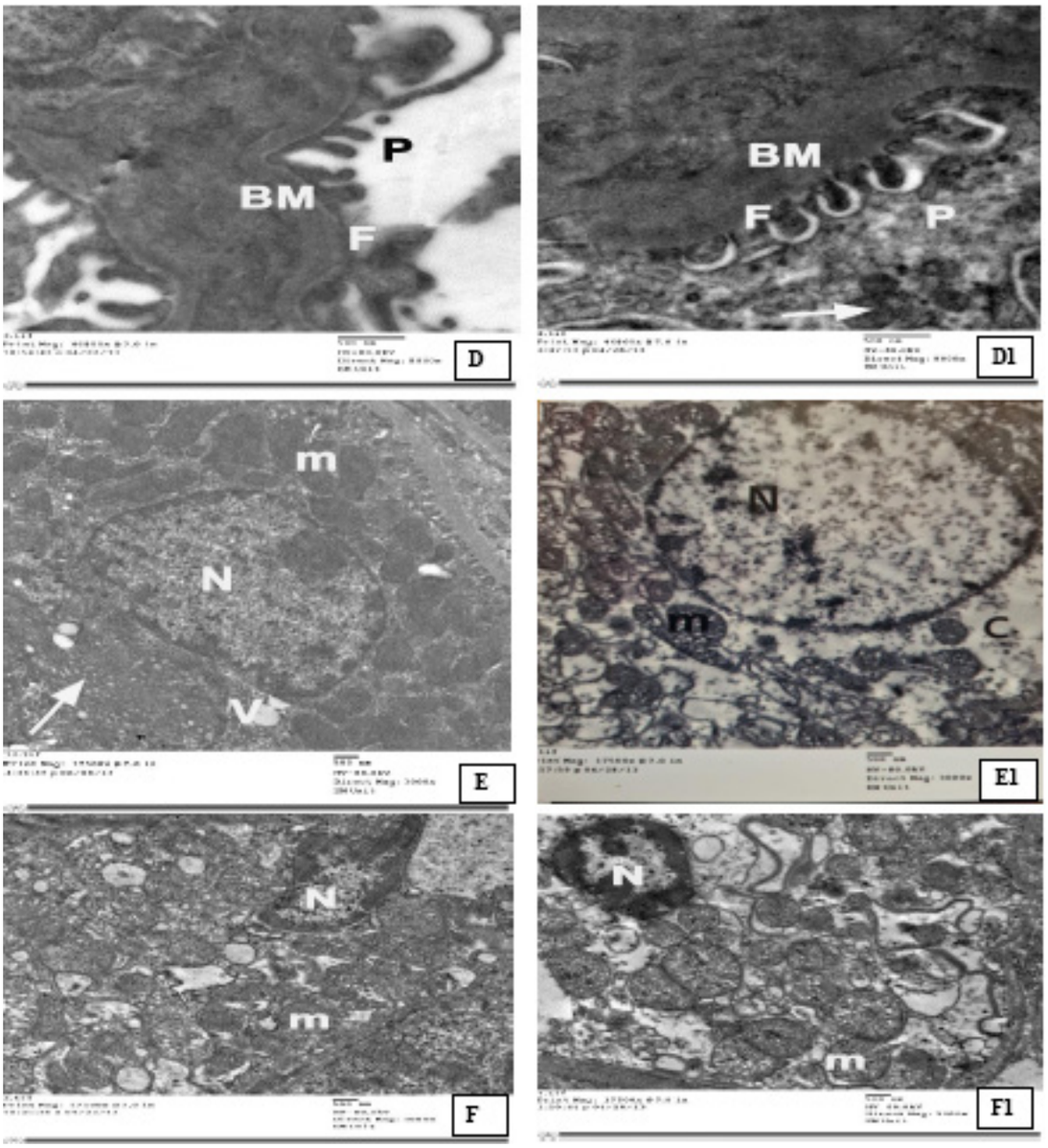

Fig. 4: Representative photomicrographs of renal ultrathin sections from experimental Subgroup IIB (TEM): In subgroup IIB, after 4 weeks thickening of glomerular basement membrane (BM), effacement of podocytes foot processes (F) (Fig.4D). In subgroup IIB, after 8 weeks showed more thickening of glomerular basement membrane (BM), fused podocytes foot processes (F). intracytoplasmic deposits (arrow)are seen in the cytoplasm of the podocytes (p) (Fig.4D1). In subgroup IIB a PCT of an adult diabetic albino rat after 4 weeks showing (irregular)indented nucleus (N), intracytoplasmic vacuoles (v). Apical microvilli (arrow) are seen (Fig.4E). Also, a PCT of an adult diabetic albino rat after 8 weeks showed extensive degenerative changes in the shape and pattern of mitochondria (m). degenerated areas of cytoplasm (c) are seen (Fig.4E1). In subgroup IIB a DCT after 4 weeks showed irregular indented nucleus (N) with peripheral condensed chromatin, vacuolated mitochondria (m) with disturbed cristae are seen (Fig4.F). In subgroup IIB a DCT after 8 weeks showed a distal convoluted tubule with indented nucleus (N) with peripheral condensed chromatin, vacuolated mitochondria (m) with disturbed cristae are seen (Fig4.F1). (TEM: D and D1X8000; E, E1, F, and F1 X3000)

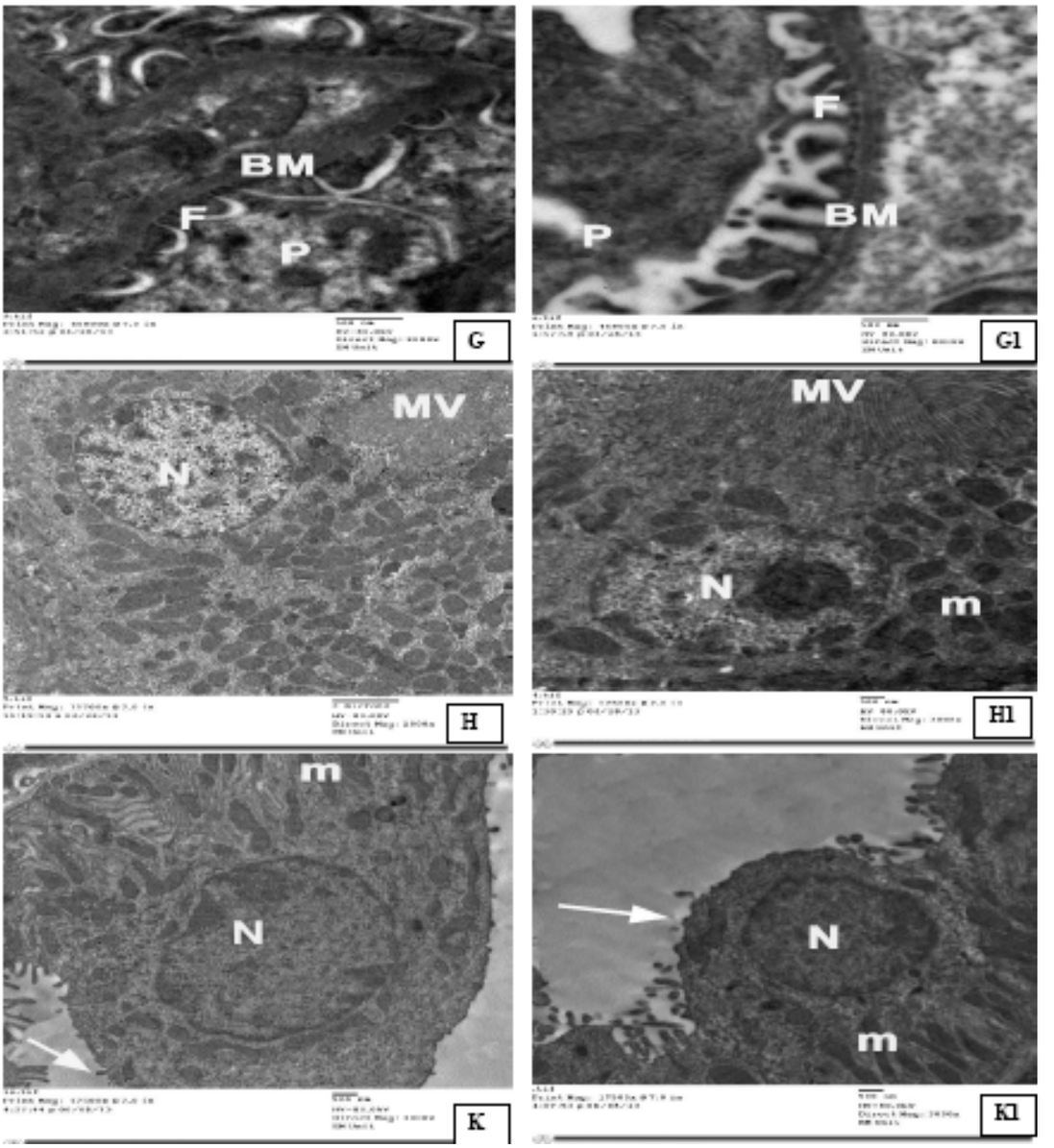

Fig. 5: Representative photomicrographs of renal ultrathin sections from experimental Subgroup IIC (TEM): In subgroup IIC, after 4 weeks showed mild thickening of GBM, podocytes (P) foot processes (F) are nearly like the control (Fig. 5G). and after 8 weeks showed the thickness of the glomerular basement membrane (B.M), podocytes with foot processes (F) are apparently as the control (Fig.5G1). In subgroup IIC, after 4 weeks a PCT with the nucleus (N), mitochondria (m) and apical microvilli are nearly like the control (Fig.5H), and after 8 weeks showed rounded nucleus (N) with prominent nucleolus. Mitochondria (m) and apical microvilli are seen apparently as the control. (Fig.5H1). In subgroup IIC, after 4 weeks showed a DCT mild indented nucleus (N). Mitochondria (m), the apical microvilli (arrow) are seen nearly like the control (Fig.5K), after 8 weeks showed the nucleus (N), mitochondria (m) and the apical microvilli are apparently as the control. (Fig.5K1). (TEM: G and G1 X8000; H X 2000, H1, K, and K1 X3000)

DISCUSSION

The goal of current work was to demonstrate the efficacy of glimepiride therapy on kidney of experimentally induced diabetic rat, to know that treatment by glimepiride could inhibit the onset and deterioration of renal nephropathy.

The glucose lowering effect of glimepiride pertains to its ability to upgrade the insulin secretions and promote its actions[17]. Also, it prevents the evolution of oxidative stress in diabetes through a strong extra pancreatic effect on metabolism of glucose and may directly promote transport of glucose activity through the phospholipid coding pathway[18,19]. It may extend anti-inflammatory effects by induction of nitric oxide liberations or through selective inhibition of the cyclooxygenase pathway[20].

Groop[11] stated that there was more debate about the mechanism of action of sulfonylurea and which they lowered blood glucose level through affection on other extrapancreatic structures in addition to excitation of insulin release.

In the present study, control rats treated with glimepiride (subgroup IIA) there were no changes in histological architecture in kidney throughout the whole study and the renal tissue was apparently like a control one. This eliminated any deleterious impact of glimepiride on kidney.

In relation to kidney of diabetic rats (subgroup IIB) revealed harmful histopathological changes in the kidney after four weeks and more harmful lesions after eight weeks. The glomerular capillaries were congested and dilated. The glomeruli were shrunken. Variable degrees of renal tubular changes were present. Some tubules showed vacuolization of their lining epithelium, Others showed complete destruction of their lining cells. These results agreed with Hagras *et al*.[13] who used STZ for induction of diabetes. Hyperglycemia of diabetes mellitus caused oxidative stress which produced DNA damage[21].

Moreover, Dobashi *et al*.[22], Horie *et al*.[23], and (Schnackenberg & Wilcox)[24] reported that oxidative stress of diabetes mellitus had affected whole structures of the cortex of kidney, i.e., glomeruli, tubules, interstitial tissue, and blood vessels. Hyperglycemia is correlated with clear deterioration of antioxidative defense, especially glutathione (GSH) and ascorbate (AA) depletion[25,26].

This could explain the damage in the glomeruli and the renal tubular cells secondary to hyperglycemia.

In this study, transmission electron microscopic study of diabetic kidney showed glomerular affection in form of thickened GBM, with fusion of podocytes pedicles with the GBM. These findings agreed with Paola[27] who mentioned that these findings would markedly decrease the glomerular filtration area.

Laurie *et al*.[28] reported that mesangial extension occurred in diabetic kidney due to mesangial cell proliferation and increased precipitation of extracellular matrix.

In this study, the changes in the glomerular basement membrane could be explained according to Locatelli *et al*.[29] who reported that high blood glucose level of diabetes cause both non-enzymatic and oxidative glycosylation of tissue proteins involving glomerular basement membrane.

This coincided with Ambrosioni *et al*.[30] who stated that oxidative glycosylation produced many oxidants that reacted like free radicals which destroyed proteins and caused histopathological changes in basement membrane of glomeruli.

The kidney convoluted tubules of this diabetic group showed mitochondrial changes in the form of disturbed cristae. This could be elucidated in relating Ong *et al*.[31] who reported that diabetes mellitus is a chronically inflammatory disease accompanied with liberation of inflammatory cytokines and chemokine genes.

Hyperglycemia produced oxidative stress then creation of reactive oxygen species (ROS), that are recognized to have an important factor in the pathogenesis of diabetic affection of the kidney. Ceriello[32] and Lee *et al*.[33], They added that chronic hyperglycemia significantly decreases the level of glutathione (GSH) which is potent natural antioxidant.

This was reported by Swaminathan and shah[34] who stated that (ROS) caused alteration in the expression of gene for the normal endogenous antioxidants.

Experimental studies had stated that diabetic rats were commonly associated with increased levels of mRNA encoding tumor necrosis factor α (TNF α) within the glomerular and proximal tubule cells[35].

Moreover, Gomez-Cambronero *et al*.[36] said that (TNF-α) was recognized to cause mitochondrial damage. They added that mitochondrial damage was closely associated with glutathione depletion. This may explain the mitochondrial change found by electron microscopy in this work.

In the present work, as regards to Mallory`s trichrome stain, the increased amount fibrous tissue elucidated in the kidneys of the diabetic rats could be explained according to Eddy[37]. and Nicholas *et al*.[38] who stated that plasminogen activator inhibitor-1(PAI-1) expression was increased by hyperglycemia in cells of mesangium and had been explained to be a pathogenic factor to the evolution of nephropathy and glomerulosclerosis in experimental induced diabetes.

Moreover, Sakai *et al*.[39], Yamagishi *et al*.[40] and Ritz[41] had reported that advanced glycated end- product (AGE) that were accumulated secondary to hyperglycemia suppressed de novo protein synthesis and stimulated transforming growth factor (TGF-β) mRNA expression in epithelial cells of proximal tubules throughout over generation of intracellular reactive oxygen species. TGF-β had been elucidated to be an important mediator of the

process of fibrosis and had been recorded to be increased in the kidneys of diabetes in both animals and human[42].

As related to the kidney of diabetic rats treated with glimepiride, (subgroup IIC), Renal tissue of rats showed that improvement of most of the glomeruli and tubules which appeared nearly like to control after four weeks and more improvement of most of the glomeruli and tubules which apparently as the control after eight weeks of this study, but there are few tubules still affected.

This could be demonstrated by several mechanisms. Krauss *et al*.[43] suggested that glimepiride protected the kidney as it inhibits the evolution of oxidative stress of diabetes and had antioxidant effects.

Asano *et al*.[44] stated that glimepiride restored the normal mesangial contractility which was reduced in diabetics and caused glomerular hyperfunction that helped the development of glomerulosclerosis. A third suggested mechanism for renal protection by glimepiride was that it acted as a competitive inhibitor for alpha endosulphin[45].

This demonstration was supported by Yee *et al*.[46] who found that alpha endosulphin regulated signal transformation of mesangial cells, uptake of the glucose and filtration process of glomeruli.

CONCLUSION

We can conclude that glimepiride could ameliorate the progression of diabetic nephropathy after experimentally induced diabetes.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

دور الجليميبريد في تحسين التغيرات النسيجية في كلية الجرذان المستحثة تجريبيا بداء السكري

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مقدمه: داء السكري هو مرض مزمن متدهورحيث ان العلامة الرئيسية هي ارتفاع السكر في الدم الناتج عن مقاومة عمل الأنسولين أو عدم كفاية إفراز الأنسولين، أو كليهما، والذي يصاحبه العديد من المضاعفات . يصاحب الارتفاع المزمن في مستوى الجلوكوز في الدم بسبب داء السكري خلل وظيفي طويل الأمد وتلف وفشل في نهاية المطاف في أعضاء الجسم المختلفة، وخاصة الكلى والعين والقلب والأعصاب. يعد الاعتلال السكري للكلى هو الأكثر شيوعًا لداء السكري والتي تهدد الحياة. عادة ما يكون ذلك بسبب الاضطرابات الأيضية في توازن الجلوكوز، مثل ارتفاع مستويات الدم والأنسجة من البروتينات الغليكوزيلية والتغيرات في الدورة الدموية داخل أنسجة الكلى.تعتبر السلفونيل يوريا هو العمود الفقري لعلاج داء السكري من النوع الثاني منذ عده عقود . يستخدم الجيل الثالث من السلفونيل يوريا بشكل شائع لعلاج داء السكري من النوع الثاني، ويستخدم جليميبريد (أماريل) بشكل شائع لهذه الأدوية.

الهدف من البحث : تهدف هذه الدراسة الي القاء الضوء علي دور الجليميبريد في تحسين التغيرات النسيجية في كلية الجرذان المستحثة تجريبيا بداء السكري

مواد وطرق البحث : تمت هذه الدراسة علي اربعين من ذكور الجرذان البيضاء البالغة والتي يتراوح وزنها بين ١٧٠-٢٠٠ جرام وقسمت إلي ٤ مجموعات كما يلي: مجموعة (I): ضمت عشرة جرذان وهي المجموعة الضابطة. مجموعة (IIA) تكونت من عشرة جرذان اخذوا الجليمبرايد(الاماريل)بالفم لمدة ثمانية اسابيع.مجموعه(IIB) تكونت من عشرة جرذان تم استحداث سكر لهم بواسطة ستريبتوزوتوسين.مجموعه (IIC) تكونت من عشرة جرذان تم استحداث سكر لهم لهم بواسطة ستريبتوزوتوسين وأخذت الجليمبرايد(الاماريل)بالفم لمدة ثمانية اسابيع.وقد تم اختيار خمسة جرذان عشوائيا من كل مجموعة بعد اربعة ثم ثمانية اسابيع من بدء التجربة.و تم استخراج الكلى من كل مجموعه وتم تجهيزه للفحص بواسطه المجهرين الضوئى والالكتروني النافذ.

النتائج : اظهرت النتائج لفحص النسيج الكلوي الخاص بالجرذان المصابه بداء السكري في المجموعه الثانيه (IIB) وجود احتقان في الأنيبيبات الكلوية واتساع في فراغ محفظة بومان كما اوضحت الأنيبيبات الملتفة تنكسا وتهتكا للجدار المبطن لها. و باستخدام صبغة الملوري ثلاثي الالوان كان هناك ترسبا في الياف الكولاجين في الأنيبيبات الكلوية و حول الأنيبيبات الملتفة. وأوضح الميكروسكوب الالكتروني عدم انتظام في سمك الغشاء القاعدي الكبيبى و التصاق الزوائد القدمية مع هذا الغشاء.كما اظهرت الأنيبيبات الملتفة فقدان الترتيب المعتاد من الميتوكوندريا مع وجود اماكن ذات كثافة قليلة في السيتوبلازم.و في المجموعه (IIC) كانت الغالبية العظمى من الأنيبيبات الكلوية وا الأنيبيبات الملتفة مماثلة تقريبا للمجموعة الضابطة. و باستخدام صبغة الملوري ثلاثي الالوان كان ترسب الياف الكولاجين في الأنيبيبات الكلوية وكذلك حول الأنيبيبات الملتفة اقل من المجموعة السابقة .واوضح الميكروسكوب الالكتروني سمك الغشاء القاعدي الكبيبى سمكه طبيعيا تقريبا وكذلك الزوائد القدمية وخلايا الأنيبيبات الملتفة القريبة والبعيدة تقريبا مماثلة للمجموعة الضابطة.

خلاصة البحث: و يمكن استنتاج أن استحدام الجليمبرايد من الممكن ان يحسن التغيرات النسيجية في كلية الجرذان المستحثة تجريبيا بداء السكري