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Histological assessment of the possible protective role of glimepiride against progression of experimentally induced diabetic nephropathy in rats

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Abstract

Introduction: little is known about the potential effects induced by sulphonylureas independently of their anti-hyperglycemic action. The present study examined the effect of glimepiride treatment on the progression of renal histological changes in diabetic rats to determine whether therapeutic intervention with these agents would prevent the onset and progression of renal complications or not. **Material and methods**: forty-eight adult male albino rats were equally divided into four groups; control, normal rats receiving glimepiride, streptozotocin induced diabetic non-treated rats and glimepiride-treated diabetic rats. Blood glucose level measurement and histological evaluation of renal tissue elements using H&E, Masson trichrome, and PAS reaction techniques at four and eight weeks after treatment were performed. Stained sections were subjected to some morphometric measurements namely, glomerular tuft area (GTA), mesangial matrix index (MMI) and area per cent of connective tissue (CT). Statistical analysis for significance of obtained data was performed using analysis of variance and student-T test. **Results**: Glimepiride did not cause any histological renal impairment when used solely. Induction of diabetes had a significant negative impact on renal structure. In addition to significant elevation of blood glucose levels, increased kidney and kidney to body weight ratio was estimated. A variety of histological changes affecting the glomerular and tubular elements of renal tissue were detected and were more intense in the eighth week of the experiment. A significant increase in GTA, MMI and area per cent of C.T. were also found in diabetic rats. All the tested parameters showed a significant improvement in the glimepiride-treated group. **Conclusion**: glimepiride could attenuate most of the histological changes produced in case of experimentally induced diabetic nephropathy in spite of persistent hyperglycemia. **Recommendation**: glimepiride could be used in Type I and type II diabetics to protect or slow down the progression of diabetic nephropathy.

Key Words: Glimepiride, Diabetic nephropathy, Kidney, Protection**.**

INTRODUCTION

Streptozotocin is well known for its selective pancreatic islet beta-cell cytotoxicity and has been extensively used to induce severe and irreversible hyperglycemia in the experimental animals **(Mitra *et al.,* 1996)**. It impairs glucose oxidation, decreases insulin biosynthesis and secretion **(Mir *et al*., 2008).** The microvascular and macrovascular complications in diabetes are the major causes of morbidity and mortality in diabetic subjects. Therefore, the search for more effective and safer hypoglycemic agents has continued to be an interesting area of search **(Krishna *et al.,* 2004).**

The progression of renal complications associated with long-term poorly controlled diabetes mellitus occurs in human over a time course of ten to twenty five years. The histoarchitectural changes associated with the disease progression are well described in literature and include glomerular hypertrophy, thickening of glomerular basement membrane, mesangeal expansion and interstitial fibrosis **(Dalla Vestra *et al.*,**

2001). However, it has become evident that prolonged tight glycemic control slows down the progression of diabetic nephropathy **(McCarthy *et al.*, 2000).**

Sulphonylurea group has represented the backbone of non-insulin dependent diabetes mellitus therapy for more than 30 years. The insulinotrophic effect of sulphonylurea is augmented by glucose and they apparently increase beta cell sensitivity to glucose and non-glucose stimuli **(Pfeifer *et al.*, 1980)**. Glimepiride has been developed for glycemic control in diabetic patients and represents the third generation sulphonylurea. It effectively inhibits the development of oxidative stress in diabetes by possessing a potent extrapancreatic effect on glucose metabolism and may directly stimulate glucose transport activity through phospholipid signaling pathway **(Krauss *et al.*, 2003).**

The present study examined the effect of glimepiride treatment on the progression of renal histological changes in diabetic rats to determine whether therapeutic intervention with this drug would prevent the onset and progression of renal complications or not.

MATERIAL AND METHODS

Test drugs:

1. Streptozotocin (STZ):
   N-methylonitrosocarbamiloD-glucosamine (Sigma chemical company, St.Louis, USA) freshly prepared in citrate buffer (pH4.5).
2. Glimepiride (Amaryl, Avertis Pharma) 4mg tablets freshly dissolved in distilled water.

Animals:

Forty-eight adult male albino rats weighing 150-200 gm were used for this study. They were kept under normal laboratory conditions and given free access of food and water. They were acclimatized for one week before starting the experiment.

Experimental induction of diabetes:

Diabetes was induced by an intraperitonial injection of a single dose of streptozotocin (45mg/Kg body weight) after 18 hrs fasting **(Luno *et al.*, 1998).** Streptozotocin (STZ) powder was dissolved in 0.1mol/L freshly prepared citrate buffer (pH 4.5) 10 min before use. This drug has been shown to induce a diabetic state in 72 hrs as documented by examining tail blood samples using a glucometer (Smart-DC, Germany). Animals were classed diabetics when glycemia exceeded 11mmol/L (1mmol=18mg glucose) following **Park and Bendayan (1994).** Animals with blood glucose levels above 200mg/dl were selected for the study.

Experimental design:

Group I: control rats (n=12) were given distilled water orally at the same time where other groups were given the drug.

Group II: contained the rats (n=12) that were given glimepiride (0.36mg/Kg/day) through gastric gavage. This dose was equipotent with human therapeutic dose after adjustment using the equation provided by **Paget and Barnes (1964).**

Group III: diabetic rats (n=12) served as non-treated diabetic rats that received distilled water orally at the same time where other groups were given the drug

Group IV: diabetic rats (n=12) that were treated with glimepiride with the same dose used in group II.

Five rats were randomly selected from each group after 4 weeks then 8 weeks of treatment and blood glucose was measured. Thereafter, rats were weighed, sacrificed and the wet weight of the right kidney was recorded then renal tissue was processed for histological examination.

(P.S. At the end of the experiment, two untreated diabetic rats died within the eight week observation period).

Histological study:

The right kidneys of sacrificed rats were dissected and washed with saline then divided into two halves parallel to the major axis. Specimens were fixed in 10% buffered formol saline, routinely processed for 5µm paraffin sections and stained with hematoxylin and eosin, Masson trichrome (MT) and Periodic Acid-Schiff (PAS) reaction **(Drury and Wallington, 1967).**

Morphometric study:

Morphometric measurements of digitalized images from stained sections were performed by "Leica QWin 500" image analyzer computer system (England). Quantitative morphometric study included:

A. Detection of glomerular tuft area (GTA) in PAS stained slides. Ten randomly selected glomeruli per animal were examined under high magnification (X400). GTA was measured by manually tracing the glomerular tuft and results were expressed as means ±SD in micrometers squared **(Nakagawa *et al.*, 2003)**.
B. Assessment of mesangeal expansion (mesangeal matrix index= MMI) by determining the ratio of mesangial matrix area to glomerular tuft area **(McCarthy *et al.*, 2000)**. Mesangial matrix area was defined as the PAS positive area within the tuft area **(Yamashita *et al.*, 2002)**.
C. Detection of the area *per cent* occupied by the blue-stained connective tissue within the glomeruli and surrounding the tubules in Masson trichrome stained samples.

Statistical analysis:

Data were presented as means ± standard deviation. Comparison of data among different groups was carried out by one way analysis of variance (ANOVA). Results were considered significant when probability (p) was ≤ 0.05, highly significant when (p) ≤0.01 and very highly significant when p ≤0.001 **(Mould, 1989)**.

RESULTS

Effect on body weight and relative kidney to body weight (KW/BW)

Body weights of rats from group I and group II steadily increased over the time of the study with no significant increase in the body weight, kidney weight or KW/BW ratio in group II compared to group I. On the other hand, diabetic rats (group III and group IV) weighed significantly less than their respective control group at all time points considered throughout the study. Rats of group IV didn't show significant change in BW after the period of four weeks compared to group III, while at the eighth week a significant increase in BW became evident.

Till the fourth week of the experiment, there was no significant difference between kidney wt. of rats from all groups. However, a significant increase in kidney weight in group III was clear compared to group I at eight weeks. At the same time, KW of rats in group IV showed a significant decrease compared to group III and reached values very close to that of control group.

There was a significant increase in KW/BW ratio in group III compared to the control group all through the study. However, a significant decrease in this ratio was found in group IV in comparison to III, but it was still significantly higher than control group **(Table 1).**

Blood glucose level:

Glimepiride had no significant effect on blood glucose level in non-diabetic rats. However, animals of group IV showed a reduction of blood glucose level compared to group III which was statistically non significant **(Table 2)**.

Histological results

Group I: which included control rats demonstrated the normal histological architecture of renal tissue. Masson trichrome staining showed minimal amounts of collagen fibers confined to the Bowman's capsule, around the tubules and basal laminae of glomerular capillaries. PAS positive material was observed in the basement membrane of renal tubules as well as the brush border of the proximal convoluted tubules and basal laminae of glomerular capillaries **(plate 1).**

Group II: included rats receiving glimepiride which maintained really normal pattern all over the experimental period**.** The amount and distribution of collagen fibers and PAS positive material were also indistinguishable from normal **(plate 2).**

Group III: included diabetic non-treated rats and demonstrated a variety of histological changes in the glomeruli and tubules of all animals.

- After four weeks, glomerular capillary loops were frequently dilated and congested. The renal cortex showed multiple congested capillaries and interstitial mononuclear infiltrating cells. Masson trichrome staining revealed increased collagen deposition. It was evident in the intraglomerular capillaries, perivascular and peritubular regions mainly distal convoluted. The glomerular mesangium was mostly expanded as evident by increased

intraglomerular PAS positive material. Some tubules showed strong PAS reaction in their basal laminae while others revealed weak reaction in their brush borders **(plate 3).**

- After eight weeks, the glomerular changes were more prominent in the form of glomerular hypertrophy and hypercellularity with obliteration of Bowman's space. Confined areas of renal cortex showed variable grades of glomerular and tubular degeneration with some apparently normal ones in between. It varied from vacuolization of epithelial cells lining both renal convoluted tubules up to complete destruction of these cells. The degenerated tubules were surrounded by minimal mononuclear cellular infiltration**.** Masson trichrome stain showed increased intraglomerular connective tissue with focal collagen deposition. Intense PAS positive intraglomerular material was observed that caused collapse of capillary lumen in some glomeruli. Some PCT exhibited weak reaction in their brush border with focal interstitial strong reaction **(plate 4).**

Group IV: included the glimepiride treated diabetic rats and showed that the majority of the glomerular and tubular elements of the renal tissue were almost normal throughout the experiment. There was a detectable abnormal connective tissue proliferation in the renal cortex with patchy interstitial fibrosis around collecting tubules on examining Masson trichrome stained sections. PAS reaction was as normal as that of control group **(plate 5).**

Morphometric results and statistical analysis:

Quantitative morphometric studies showed that the mean gomerular tuft area (GTA) and mesangial matrix index (MMI) increased in diabetic rats (group III) in all the time intervals of the study. This increase was statistically significant compared to all groups of this experiment. The treated diabetic group (IV) exhibited an increase in GTA and MMI after four weeks interval. This increase was not as remarkable as that in group III. However, after eight weeks both parameters showed a significant decline that was very close to the control value.

The mean area *per cen*t of C.T. collagen in non treated diabetic rats revealed a statistically significant increase in comparison to group I and group II. On the other hand, the values of the treated diabetic group showed non-significant difference from the control values **(Table 3)**.

A positive correlation could be established between the effect of glimepiride in decrease of MMI in treated diabetic group and its protective effect against development of nephropathy.

DISCUSSION

Little is known about the potential effects induced by sulphonylureas independently of their anti-hyperglycemic action. The present study examined the effect of glimepiride treatment on the progression of renal histological changes in diabetic rats to determine whether therapeutic intervention with these agents would prevent the onset and progression of renal complications or not.

In the present study, blood glucose was significantly higher in streptozotocin-induced diabetes than in controls. Streptozotocin-induced diabetes is used as a model of type I diabetes, which is associated with low plasma insulin and hyperglycemia. STZ initiates the breaking of pancreatic beta cells and hence activates the repair process which consumes nicotinamide adenine dinucleotide (NAD) known to be indispensible for pro-insulin synthesis. The net result is apoptosis of beta cells with overt diabetes and nearly complete absence of insulin **(Mitra *et al.*, 1996)**.

Although STZ treatment is widely accepted model for type I diabetes, one of its drawbacks is its potential nephrotoxicity. **Song *et al.* (2003)** investigated this issue but didn't observe any clear STZ-dependent nephrotoxic effects. In a similar study, **Danda *et al.* (2005)** ruled out this possibility even after 14 weeks following administration of a higher dose of 55mg/Kg. Both studies attributed nephrotoxicity strictly to development of diabetes mellitus.

It was evident from the results of the present work that administration of glimepiride in

STZ-treated rats decreased blood glucose level; however this reduction was statistically insignificant. This could be attributed to relatively low dosage of STZ which was not enough to destroy all pancreatic beta cells. Moreover, glimepiride improves peripheral glucose uptake and decreases endogenous glucose production independent of its insulin secretagogue action. Furthermore, decreased glucose level could result from other nonrelated mechanisms like increased sensitivity of peripheral tissues to released insulin, insulin-like extrapancreatic effects, intensification of transmembrane transport of glucose and an increase in glycolysis activity as postulated by **Zueva *et al.* (2003)**.

The results of the present work revealed that diabetic rats had a significant reduction in body weight compared to control group which was in agreement with **Mooradian (1996).** Glimepiride treatment of diabetic rats resulted in improving glycemic control without significantly increasing the weight compared to control, but a significant increase in comparison to nontreated diabetic rats was estimated. This was in accordance with results of **Gottschalk *et al.* (2007)** but contradicted with **Radermecker and scheen (2006)** who detected a further reduction in body weight in glimepiride treated diabetic rats.

In this experiment, a significant increase in kidney weight and kidney weight relative to body weight was evident in diabetic rats. This might reflect the reduction in total body weight by simple dehydration of diabetic animals in addition to renal cellular hypertrophy, intraglomerular hyperplasia and increased extracellular matrix synthesis **(Kloke *et al.*, 1998)**. Glimepiride treatment produced a significant reduction in both parameters although the values were still higher than those of the control. These data could be explained by the antioxidant effect of glimepiride **(Krauss *et al.*, 2003).**

Light microscopic examination of diabetic kidneys showed glomerulopathy characterized by thickening of the glomerular basement membrane and mesangial matrix expansion. These findings were in agreement with **Paola (2002)** who added that these changes will lead to progressive reduction in the filtration surface of the glomeruli. **Laurie *et al.* (1989)** explained that mesangial expansion occurs not only as a result of mesangial cell proliferation as mentioned earlier, but also due to increased deposition of extracellular matrix composed mainly of type IV collagen, laminin and fibronectin.

It is known that diabetic patients may develop variable degrees of glomerular sclerosis and tubulo-interstitial fibrosis (**Brown *et al.*, 1982**). However, structural glomerular changes such as glomerular hypertrophy may be difficult to assess unless careful measurements are made. Glomerular hypertrophy was quantified as the glomerular tuft cross sectional area GTA by **Nakagawa *et al.* (2003)**. Glomerular sclerosis was defined as increment in intraglomerular PAS positive material associated with collapse of capillary lumens and amorphous hyaline material deposition with or without adhesions to the Bowman's capsule **(Yamashita *et al.*, 2002)**. Regarding the sequence of events concerning elements of diabetic nephropathy, it was believed that glomerular hypertrophy occurs first and is followed later by mesangial expansion in diabetic rats **(Osterby, 1986)**. However, the results of the present work showed that mesangial expansion occurred as early as four weeks duration and hence it may not be categorized as a late stage change.

Hyperglycemia of diabetes mellitus is thought to cause both non-enzymatic and oxidative glycosylation of tissue proteins including glomerular wall. So the collagen of the capillary wall form chemically irreversible advanced glycosylation end products (AGE) which accumulate in the vessel walls and are thought to be the cause of microvascular affection in diabetics (**Locatelli *et al.*, 2002**). Also oxidative glycosylation produces oxidants that possess reactivity similar to free radicals and damage proteins and is responsible for alterations in the glomerular basement membrane (**Ambrosioni, 1987**).

The present study revealed that glimepiride treatment improved the histological changes in diabetic rats. The degree of glomerular hypertrophy and mesangial expansion for the diabetic treated animals was less than that seen in diabetic rats. These results were

consistent with that of **Biederman *et al.* (2005**). On the other hand, former data of **Cortes *et al.* (1998)** who used tolazamide – an old member of sulphonylurea group- revealed that it markedly enhanced extracellular matrix synthesis and accumulation in mesangial cells probably by stimulating glutathione expression and glucose transport.

There are many possible mechanisms for renal protection observed with glimepiride. Firstly, **Krauss *et al.* (2003)** demonstrated that glimepiride administration caused a decrease of peroxides and malondialdehyde levels and an increase in the activity of superoxide dismutase and glutathione peroxidase following streptozotocin administration. Ultimately, they suggested that its renoprotective action could be attributed to its protective effect against the development of oxidative stress in diabetics as it acts as free radical scavenger. A second mechanism was introduced by **Asano *et al.* (1999)** via the restoration of normal mesangial contractility. Diminished mesangial contractility is responsible for the glomerular hyperfunction which is considered as a significant contributor to the development of glomerulsclerosis. However, functional activation of sulphonyle urea receptor 2 (SUR2) on mesangial cells by sulfonylurea induces elevation of intracellular Ca^{2+} resulting in limitation of the glomerular filtering surface area and hence hyperfiltration in already hypertrophied glomeruli. A third suggested mechanism for the renoprotective effect of glimepiride could be by acting as an exogenous competitive inhibitor of α-endosulfine, the endogenous ligand of a unique (SUR) that displaces it from its receptors. **Heron *et al.* (1998)** proved that both α-endosulfine and glimepiride compete for the same receptors and found that α-endosulfine inhibits glibenclamide-invoked currents in patch clamping experiments on insulinoma cells. More recently, **Yee *et al.* (2004)** found that α-endosulfine could act as a regulator of mesangial cell signal transduction, glucose uptake and glomerular filtration.

The increased fibrous tissue formation demonstrated in kidneys of diabetic rats in the present study could be explained by enhanced expression of collagen I mRNA and increased collagen I protein accumulation rate secondary to increased glucose uptake by mesangial cells **(Giannico *et al.*, 2007)**. While glimepiride stimulates glucose transport, it exerts a concomitant suppression of PAI-1transcriptional activity and/or enhanced PAI-1 mRNA degradation **(Derosa *et al.*, 2004)**. So, it is believed that a high turnover and increased matrix breakdown are involved.

While collagen I gene expression may be stimulated by increased glucose availability, there is a simultaneous regression of plasminogen activator inhibitor-1 (PAI-1) expression indicative of enhanced catabolic rate, fully counterbalancing the increased synthetic activity. The main candidate mediator effect is an elevated c-AMP level. Principally, the down regulation of steady-state PAI-1 mRNA content by agents that stimulate cAMP formation is well-documented major regulatory mechanism **(Heaton *et al.*, 2003)**. Furthermore, sulphonylureas are known to activate adenyl cyclase and inhibit the activity of phosphodiesterases, thus leading to an increase in cellular cAMP levels **(Skillman and Feldman, 1981)**. PAI-1 expression plays an important role in the pathogenesis of diabetic glomerulosclerosis **(Eddy, 2002)**. PAI-1 expression is up regulated by hyperglycemia in mesangial cells in tissue culture and has been demonstrated to be a pathogenic contributor to the development of nephropathy in experimental insulin-deficient diabetes **(Nicholas *et al.*, 2005).**

CONCLUSION

It can be concluded that glimepiride could attenuate most of the histological changes produced in case of experimentally induced diabetic nephropathy. Interestingly, these findings were detected in spite of persistent hyperglycemia. This suggested that elevated blood glucose level was not the mediators through which glimepiride exerted its prophylactic effect in diabetic nephropathy. Alternatively, it could be suggested that it acted directly on SUR2B in the kidney.

Ultimately, a potential new therapeutic field is opened in management of diabetes mellitus but it needs human trials to validate its protective role for diabetic patients.

RECOMMENDATION

Based on the obtained results in this study, it can be recommended that glimepiride can be used in Type I and type II diabetics to protect or slow down the progression of diabetic nephropathy.

Table (1): Effect of glimepiride on body weight (BW), kidney weight (KW), and relative kidney weight to body weight (KW/BW) in all studied groups.

Parameters	**BW (gm)**		**KW (mg)**		**KW/BW (mg/gm)**	
Duration in weeks	4w	8w	4w	8w	4w	8w
group I (Control)	308.3±2.3	335±2.7	1.1±0.04	1.18±0.004	3.5±0.04	3.5±0.05
group II (Glimepiride)	311.3±3.7	337±3.7	1.1±0.008	1.18±0.016	3.5±0.05	3.5±0.04
group III (Diabetic)	263.6±11.96*	259.5±3.8*	1.15±0.03	1.28±0.01*	4.4±0.08*	4.7±0.04*
group IV (Glimepiride-treated diabetic)	281.5±7.14*	291.6±8*#	1.13±0.03	1.15±0.01*#	3.7±0.04*#	3.9±0.07*#

Values are represented as mean ± SD (n=5).
[Significance was considered P<0.05]
*=Significant change compared to control.
=Significant change between glimepiride treated diabetics and diabetic untreated group.

Table (2): Effect of glimepiride on blood glucose (mmol/L) in all studied groups.

Parameter	**Blood glucose level (mmol/L)**	
Duration in weeks	4w	8w
group I (Control)	6.9±0.16	6.9±0.06
group II (Glimepiride)	6.9±0.24	6.98±0.23
group III (Diabetic)	15.6±4.2*	18.5±3*
group IV (Glimepiride-treated diabetic)	12.7*±2.5	16.1*±3.8

Values are represented as mean ± SD (n=5).
[Significance was considered P<0.05]
*=Significant change compared to control.

Table (3): Morphomertic comparison of the mean glomerular tuft area (GTA), mesangial matrix index (MMI) and mean area % Of C.T. in all studied groups.

Parameter / Groups	**GTA (μm²)**		**MMI**		**Mean Area % of C.T.**	
	4 w	8 w	4 w	8 w	4 w	8 w
group I (Control)	3828±79	3799±75	0.64±0.3	0.77±0.2	28.62±13.9	29.69±11.4
group II (Glimepiride)	3794±83	3812±63	0.66±0.2	0.75±0.1	29.03±12.3	29.83±9.4
group III (Diabetic)	5238±15*	6836±63*	0.08±0.7*	1.05±0.08*	36.0±11.2*	41.851±13.7*
group IV (Glimepiride-treated diabetic)	4129±37	4790±85#	0.72±0.1	0.87±0.2#	28.32±9.4	28.50±15.9#

Values are represented as mean ± SD (n=5).
[Significance was considered P<0.05]
*=Significant change compared to control group.
#=Significant change between glimepiride treated diabetics and diabetic untreated group.

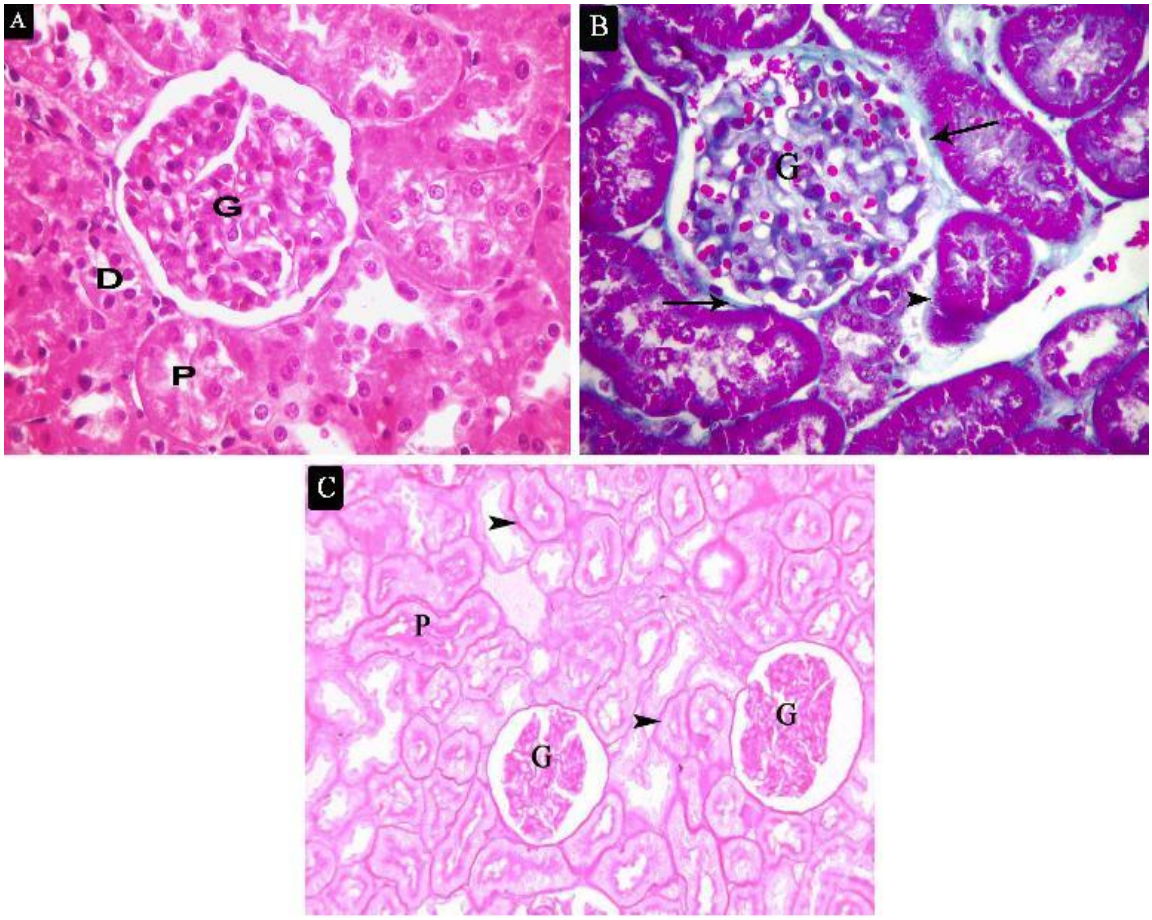

Plate 1: photomicrographs of sections in the right kidney of a control rat (group I) showing:

A. normal renal architecture: glomerulus (**G**), proximal (**P**) and distal (**D**) convoluted tubules (H&E X400).

B. normal distribution of connective tissue in the glomerulus (**G**), Bowman's capsule → and surrounding the tubules ► (M.T. X400).

C. strong PAS reaction in the glomeruli (**G**), brush border of the proximal convoluted tubules (**P**), basal laminae ► of tubules (PAS X200).

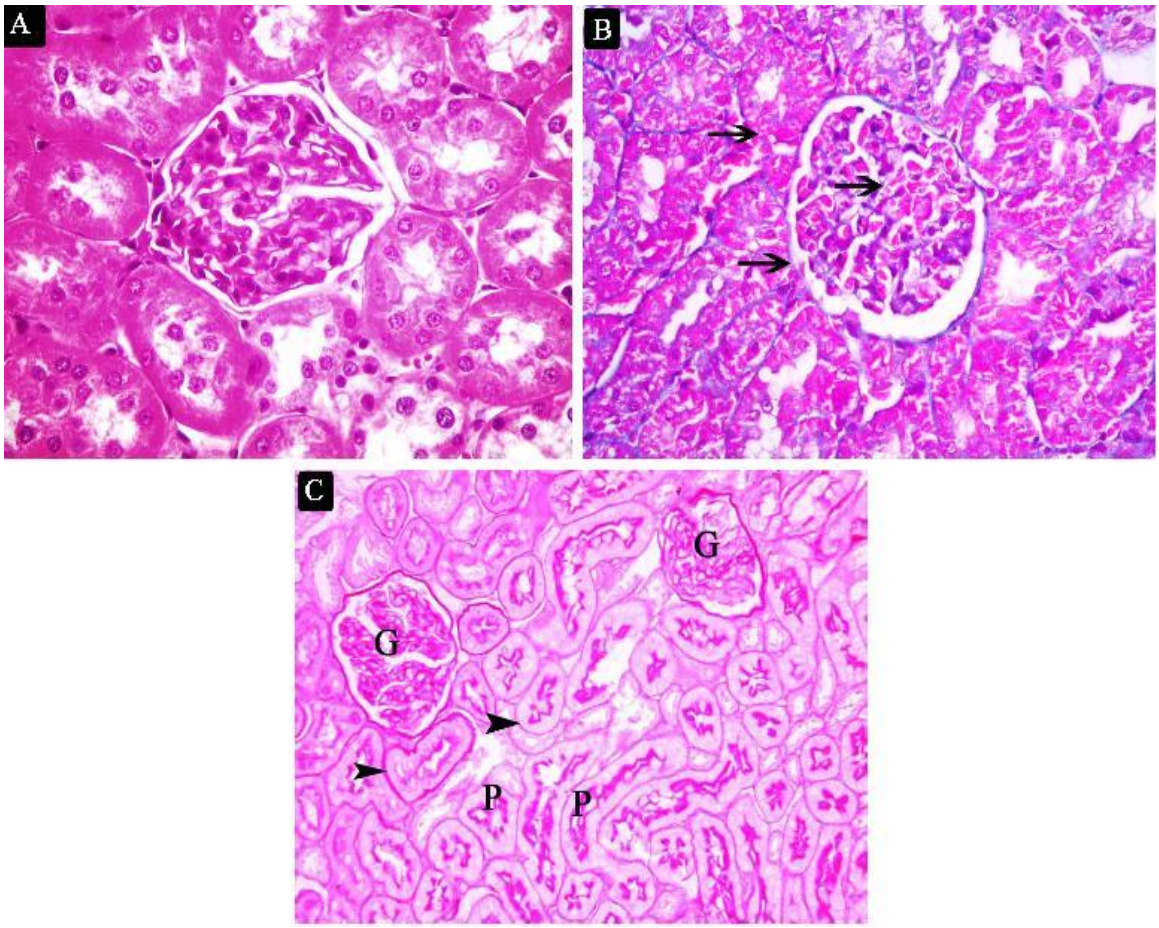

Plate 2: photomicrographs of sections in the right kidney of a glimepiride treated rat (group II) showing:

A. normal pattern of renal architecture (H&E X400).

B. normal distribution of connective tissue ⟶ (M.T. X400).

C. strong PAS reaction in the glomeruli (G), brush border of the proximal convoluted tubules (P), basal laminae of tubules ► (PAS X200).

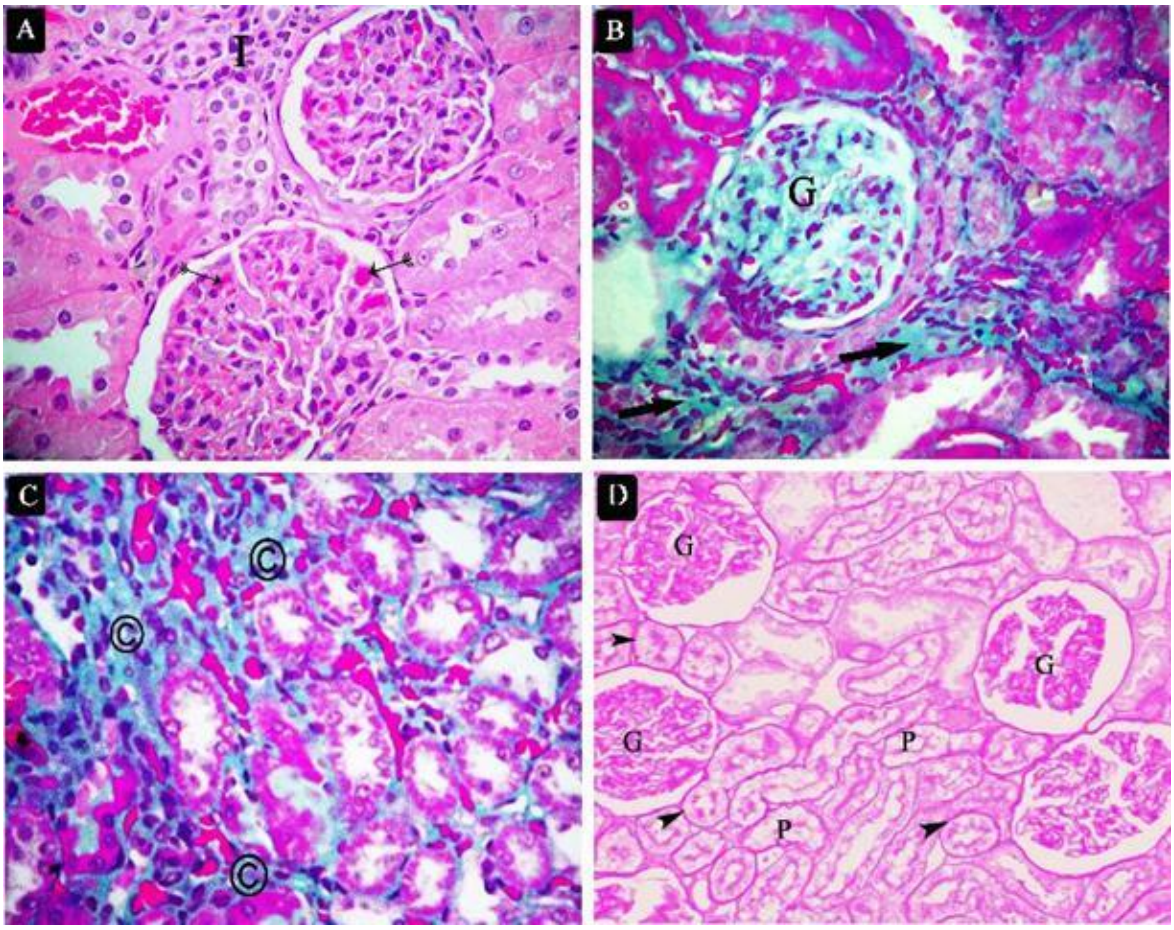

Plate 3: photomicrographs of sections in the in the right kidney of a diabetic rat (group III) after four weeks demonstrating:

A. dilated and congested glomerular capillary loops ⟶. Interstitial mononuclear infiltrating cells are seen (**I**) and some disturbed and vaculated cells of proximal and distal convoluted tubules (H&E X400).

B. intraglomerular (**G**) and interstitial collagen deposition ⟶ (M.T. X400).

C. increased peritubular collagen deposition © (M.T. X400).

D. expanded glomerular mesangium as evident by increased intraglomerular PAS positive material. Some tubules shows strong PAS reaction in their basal laminae (➤) while others reveal weak reaction in their brush borders (**P**) (PAS X200).

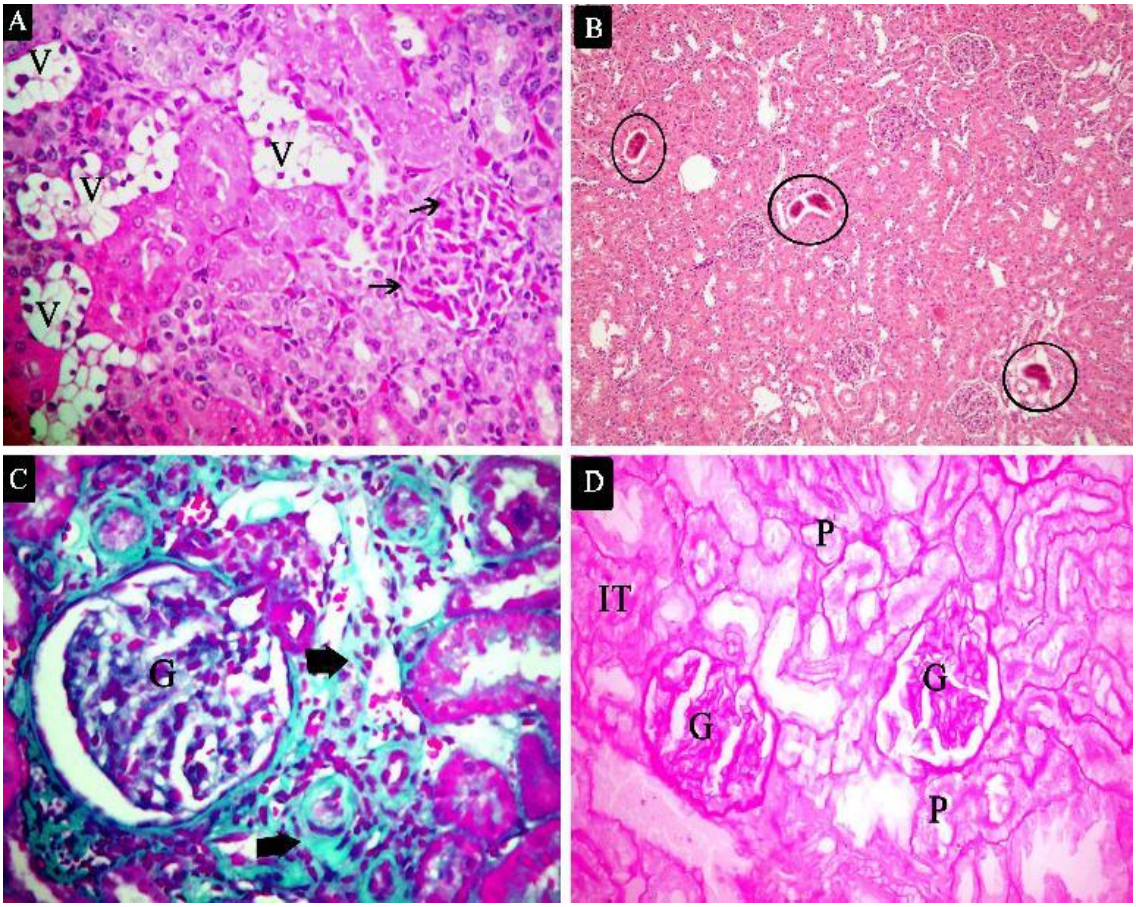

Plate 4: photomicrographs of sections in the in the right kidney of a diabetic rat (group III) after eight weeks showing:

A. glomerular obliteration of Bowman's space → and vacuolization of tubular epithelium are shown (**V**) (H&E X200).

B. destruction of some tubules ○ (H&E X100).

C. increased intraglomerular connective tissue (**G**) with focal collagen deposition in the interstitium → (M.T. X400).

D. intense PAS positive intraglomerular material (**G**) and some PCT exhibiting weak reaction in their brush border **(P)** with focal interstitial strong reaction (**IT**)(PAS X200).

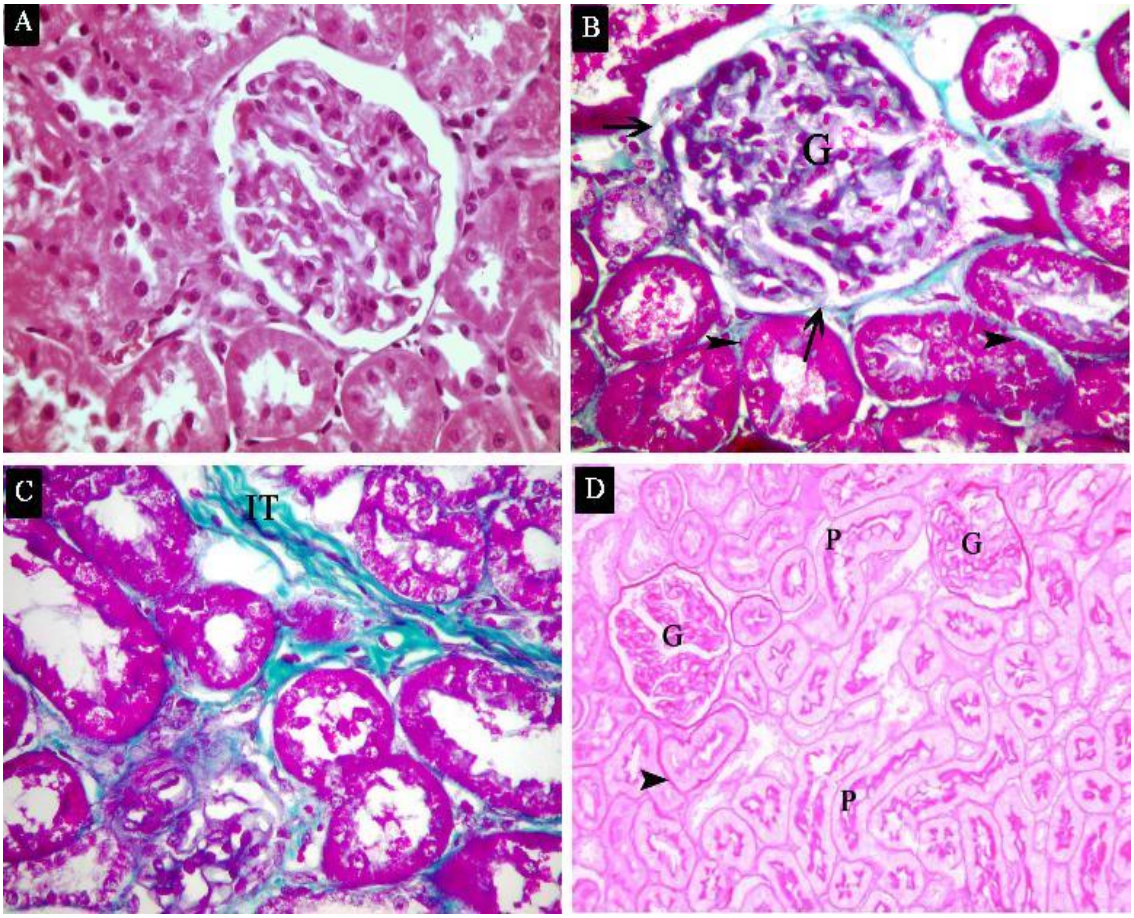

Plate 5: photomicrographs of sections in the right kidney of a glimepiride-treated diabetic rat (group IV) after eight weeks showing:

A. normal renal architecture (H&E X400).

B. normal distribution of connective tissue in the glomerulus **(G)**, Bowman's capsule ⟶ and surrounding the tubules ▸ (M.T. X400).

C. patchy interstitial fibrosis (**IT**)(M.T. X400).

D. strong PAS reaction in the glomeruli (**G**), brush border of the proximal convoluted tubules (**P**), basal laminae of tubules ▸ (PAS X200).

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تقييم هستولوجي للدور الوقائي المحتمل لعقار الجلميبريد في حالات الإعتلال الكلوي السكري المستحدث تجريبيا في ذكور الجرذان البيضاء

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لم يتم معرفة الكثير عن الدور الذي يمكن أن تلعبه مجموعة عقاقير السلفونيليوريا بغض النظر عن كونها مضادات لإرتفاع نسبة السكر في الدم و لذلك صممت الدراسة الحالية لفحص التأثير المحتمل لعقار الجليميبريد على تطور التغيرات الهستولوجية للكلى في ذكور جرذان التجارب البيضاء بالسكري المستحدث تجريبيا لتحديد ما اذا كان لهذا العقار دور وقائي في منع ظهور أو تقدم المضاعفات الكلوية لمرضى السكر.

و لهذا الغرض تم استخدام ثمانية وأربعين من ذكور الجرذان البيضاء البالغة و تم تقسيمهم الى أربع مجموعات: مجموعة ضابطة (I)، مجموعة تم اعطاؤها عقار الجليميبريد بمفرده (II)، مجموعة تم استحداث مرض السكر بها بإستخدام الستربتوزوتوسن و تركت بدون علاج (III)، و أخيرا مجموعة رابعة تم استحداث مرض السكر بها و عولجت بعقار الجليميبريد (IV) و بعد ذلك تم اجراء بعض القياسات الكيميائية الحيوية لتقييم أداء الكلى الوظيفي و كذلك فحص نسيجي للكلى عن طريق صباغتها بالهيماتوكسيلين و الإيوسين، و ماسون ثلاثي الألوان و حمض البيريوديك شيف بعد مرور أربع ثم ثمانية أسابيع من بداية التجربة و تلى ذلك إجراء بعض القياسات الشكلية على هذه العينات منها قياس مساحة الكبيبات الكلوية (GTA) و معامل مسراق الكبيبة (MMI) و النسبة المئوية لمساحة ألياف الكولاجين ثم تم تحليل النتائج لمعرفة دلالتها الاحصائية.

و قد أظهرت النتائج أن عقار الجليميبريد لم يتسبب في احداث أي تغيرات نسيجية في الكلى عند استعماله منفردا بينما كان لإحداث مرض السكر تجريبيا أثرا سلبيا ذو دلالة احصائية على تركيب الكلى النسيجي نتج عنه زيادة وزن الكلى بالنسبة لوزن الجسم ، كما تم رصد تغيرات عديدة في تركيب الكبيبات الكلوية و الأنابيب المتصلة بها في صورة زيادة في مساحة الكبيبات و إصابة بعضها بالتصلب كما لوحظ درجات متعددة من تضرر الأنابيب المختلفة وزيادة كمية ألياف الكولاجين المحيطة بها و كانت أكثر حدة في الأسبوع الثامن منها في الأسبوع الرابع و كان لعلاج هذه الجرذان بعقار الجليميبريد أثرا كبيرا في تحسن جميع المعايير المستخدمة في المجموعة الأخيرة من هذه الدراسة.

و على ضوء كل ما سبق تم استنتاج أن إستخدام عقار الجليميبريد يؤدي الى الحد من الإضطرابات النسيجية التي تم ملاحظتها في حالة إحداث السكر تجريبيا على الرغم من ارتفاع مستوى السكر في الدم و عليه فيوصى بإستخدام هذا العقار في علاج مرضى السكر من النوعين الأول و الثاني للحماية و تقليل فرصة الإصابة بالاعتلال الكلوي المصاحب لمرض السكر.