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Effect of Metformin and Glimepiride on Liver and Kidney Functions in Alloxan-Induced Diabetic Rats

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ABSTRACT: Metformin and glimepiride have been marked as potential hypoglycemic agents for the treatment of type 2 diabetes. The purpose of this study was to investigate the effect of daily oral administration of metformin (500 mg/kg) or glimepiride (0.5 mg/kg) alone or in combination for 28 days on some physiological parameters and histological alterations in liver and kidney of the diabetic rats. Diabetes was induced by a single intraperitoneal dose of alloxan (150 mg/kg) Data showed that metformin and glimepiride alone or in combination induced a significant decrease in serum glucose levels in the diabetic rats. Treating the diabetic rats with glimepiride increased the AST activity significantly. Meanwhile, administration of glimepiride or metformin had no significant effect on the activity of ALT. On the other hand, the combination of both drugs exhibited a significant reduction in the activities of AST and ALT as compared to that in the diabetic control rats. Glimepiride treatment revealed that total protein and globulin levels were increased, while metformin or the combined drug resulted in a significant decrease in albumin level which almost reached the normal value and an increase in the globulin than that of the diabetic rats. Creatinine level improved significantly in all the treated groups in comparison to that of the diabetic group and reached the normal values. On the other hand, no sign of improvement in the levels of urea was observed in the rats treated with metformin or the combined drug, while treatment with glimepiride decreased urea levels significantly. Histological examination of liver in the diabetic rats showed extensive necrosis of hepatocytes, cytoplasmic vacuolation, distended sinusoids with massive congestion. In addition, the kidney glomeruli increased in size and the renal tubules degenerated. The treatment with metformin or glimepiride ameliorated the hepatic injury, at the same time; the combined drug caused severe destruction in the liver cells. The treated rats also exhibited shrinking in the glomerular capillaries with widening of Bowman's space, mesangial matrix expansion, necrosis and vacuolation of renal tubules. In conclusion, the combination of biochemical and histological biomarkers provides useful and sensitive tools in the investigation of chronic effects induced by diabetes and anti-diabetic drugs.
Key words: Diabetes, Alloxan, Glimepiride, Metformin, Rats, Histopathological studies, Liver and Kidney.

INTRODUCTION

Diabetes is a hereditary, chronic metabolic disease characterized by hyperglycaemia and eventual glycosuria.(1) The prevalence of diabetes in Egypt is high and suggests that it is a major emerging clinical and public health problem.(2) Type 2 diabetes mellitus is an increasingly prevalent condition worldwide. The

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complications of this disease are known to significantly increase the morbidity and mortality of those affected, resulting in substantial direct and indirect costs.[3]

Much effort has been devoted to the development of optimal therapeutic regimens for the management of type 2 diabetes. Various pharmacologic agents are available for the management of type 2 diabetes, including first-and second generation sulfonylureas (glimepiride, gliclazide, glipizide, glyburide and glibenclamide), biguanides (metformin), α-glucosidase inhibitors (acarbose and miglitol), thiazolidinediones (pioglitazone and rosiglitazone), meglitinide analogues (repaglinide), amino acid D-phenylalanine derivatives (nateglinide), and insulin. Sulfonylureas work primarily by stimulating pancreatic insulin secretion, which in turn reduce hepatic glucose output and increase peripheral glucose disposal.[4]

Glimepiride is effective, well-tolerated, and well-established drug widely used in the management of Type 2 diabetes. The unique properties of glimepiride may provide advantages over other currently available insulin secretagogues.[5] Therapy with glimepiride improves the relative insulin secretory deficit found in type 2 diabetes mellitus (T2DM), has antihyperglycemic efficacy equal to other secretagogues with reduced potential for hypoglycemia and may have additional actions contributing to glycemic control in type 2 diabetes mellitus.[6] Glimepiride may cause prolonged hypoglycemia in patients with renal dysfunction, so the drug should be used cautiously, and the dose of this drug must be reduced in patients with a reduced glomerular filtration rate.[7]

Clinical studies have shown glimepiride to be safe and effective in reducing fasting and postprandial glucose levels, with dosages of 1-8 mg/day.[8] Also, oral administration of glimepiride (0.1 mg / kg body weight/day) for four weeks brought back transaminases activities to near the

control values.[9]

Metformin (N,N-dimethyl biguanide) is one of the oral drugs used for more than 40 years to treat patients with type 2 diabetes mellitus without causing over hypoglycaemia.[10] It has recently been recommended that metformin therapy be initiated at the time of diagnosis of type 2 diabetes, in conjunction with lifestyle modification.[11] Pharmacological studies have indicated that metformin acts by improving peripheral sensitivity to insulin inhibiting gastrointestinal absorption of glucose.[12] and decreasing hepatic glucose production.[13] Renal function must be assessed prior to and periodically during metformin therapy, particularly in the elderly in whom it is recommended that creatinine clearance be assessed in order to detect accurately any significant degree of renal dysfunction.[14]

Administration of metformin (100 mg/kg/day) for one month to the diabetic male albino rats showed a significant decrease in blood glucose level.[15] A case of serious hepatotoxicity possibly associated with metformin use at a dose of 500 mg/day for 3 weeks in an elderly patient with poorly controlled type 2 diabetes mellitus was reported.[11] The laboratory analysis showed elevation of AST and ALT, alkaline phosphatase, and total bilirubin concentrations. In this case, metformin appeared to cause a mixed-type (hepatocellular and cholestatic) hepatic damage.

Concerning the effect of hypoglycemic agent on protein levels, it was reported that administration of metformin (100 mg/kg/day) for one month caused no significant effect on serum protein levels. The serum protein levels were also insignificantly changed after the administration of gliclazide (7.2 mg/kg/ day) for 4 weeks as compared to that of the normal and diabetic control rats.[15]

Many reports have been recorded to study the effects of some oral anti-diabetic drugs on blood urea and creatinine as a

significant marker in diabetic renal dysfunction. Impairment of kidney functions is a prominent feature of diabetes. Elevated levels of urea and decreased concentrations of uric acid and creatinine were shown in diabetes.[16] It was stated that oral administration of metformin (500 mg/kg) for 21 days significantly reduces plasma urea and glucose levels in male diabetic rats as compared with that in the diabetic control group.[17] Also, serum creatinine exhibited significant decreases in streptozotocin-induced diabetic rats as compared to the control.[18]

Many studies to date suggest that combinations of different classes of oral antidiabetic drugs are more effective than the maximum doses of a single drug. In fact, many authors (19 - 22) now suggest the use of combination therapy early – if not initially – in the disease process. Combination therapy, in addition to being more effective than monotherapy, will often permit the utilization of submaximal doses of the agents used. Therefore, patients will derive the benefits of all drugs used, while minimizing the likelihood of intolerable side effects from any one class.[23] Several combination tablets are now available which help to defray the cost of diabetes management, and may improve adherence to prescribed therapy. Metformin and glimepiride seem to be particularly well suited for use in combination because of their different mechanisms of action. These drugs appear to have complementary effects in improving glycemic control, as well as beneficial effects on lipids and body weight.[24]

Several histological studies have been carried out in order to determine the histological changes in liver. In the liver of diabetic control rats there were a portal inflammation, focal necrosis, microvesicular flattening, apparent granular degeneration and hydropic swelling of hepatocytes.[25] Studies have also shown that hepatobiliary disorders, such as inflammation, necrosis or fibrosis of non-alcoholic fatty liver disease, cirrhosis, hepatocellular carcinoma, hepatitis C, acute

liver failure, and cholelithiasis could follow diabetes.[26]

Many authors reported histological abnormalities in kidney of rats that were injected with alloxan or streptozotocin (STZ). In glomeruli of diabetic animals, mesangial matrix expansion and compressed capillaries were observed.[27] Diabetic nephropathy is characterized histopathologically by thickening of the glomerular basement membrane and expansion of the mesangial area.[28] They also reported that periodic acid schiff (PAS) stained section of kidney showed no extracellular matrix (ECM) expansion in control rats, but a clear expansion of mesangial area was noted in diabetic rats at 24 weeks. Glomerular size was significantly greater in diabetic rats than in control rats at 12 and 24 weeks. The extent of mesangial expansion correlates well with the progression of glomerulosclerosis and renal dysfunction.[29] Also, the kidney section of STZ-diabetic control rats showed marked microscopic changes like tubular multifocal clarification and vacuolation compared to kidney of non-diabetic control rats.[30]

The aim of the present work is to study the effect of the oral anti-diabetic drugs represented by glimepiride and metformin individually and in combination on some physiological serum parameters (glucose, AST, ALT, total protein, albumin, globulin, urea and creatinine levels) and histological alterations in liver and kidney of the diabetic rats.

MATERIAL AND METHODS

Experimental animals

Male albino rats with body weight of 120-160 g. were fed with a standard diet of bread, tap water, milk and wheat grains ad libitum. After randomization into groups, rats were acclimatized for a period of 14 days in the new environment before initiation of the experiment. Rats were housed in clean cages at room temperature.

Chemicals

Alloxan monohydrate was used as an agent to produce hyperglycemia. Glimepiride (Amaryl)

and Metformin (Glucophage) as potential hypoglycemic agents for the treatment of diabetic rats.

Induction of diabetes

Alloxan was dissolved in sterile normal saline and injected immediately within few minutes to avoid degradation. Fasted rats were injected with alloxan in a dose of 150 mg/kg body weight intraperitoneally.[31] On the 14th day after alloxan administration, fasting blood samples were collected from the eye (venous pool) under ether anaesthesia for blood glucose estimation.[32]

The survived animals were considered diabetic when their blood glucose level was higher than 11 mmol/L.[33] Only uniformly diabetic rats were included in the study.

Experimental design

Rats were divided into five groups, 10 rats were used in each group as follows:

Group 1: Normal control rats were injected with normal sterile saline solution (the solvent of alloxan) then after 14 days they received 1 ml sterile saline solution / day for 28 days using an intragastric tube.

Group 2: Diabetic control rats received only 1 ml normal sterile saline solution / day for 28 days using an intragastric tube.

Group 3: Diabetic rats received glimepiride (0.5 mg/kg body weight) in 1 ml of sterile normal saline solution / day for 28 days using an intragastric tube.[34]

Group 4: Diabetic rats received metformin (500 mg/kg body weight) in 1 ml of sterile normal saline solution / day for 28 days using an intragastric tube.[17]

Group 5: Diabetic rats received a combined drug [glimepiride (0.5 mg/kg body weight) and metformin (500 mg/kg body weight)] in 1 ml of normal sterile saline solution / day for 28 days using an intragastric tube.

Blood sampling and Biochemical parameters measurements in serum

At the end of the 28th day, the animals were fasted overnight. Blood samples were collected from the abdominal vein after ether anaethesia .The blood was allowed to clot at room temperature; serum was separated by

centrifugation at 3000 xg for 15 minutes. Serum samples were used for biochemical determinations.

- Glucose was estimated by enzymatic colorimetric method [35].
- AST and ALT levels were determined according to Schmidt and Schmidt [36].
- Total protein level was estimated according to Biuret reaction [37]
- Albumin was estimated by Bromocersol Green (BCG) colorimetric method [38].
- Globulin was determined according to Tietz [39].
- Urea was estimated according to the urease-modified Berthelot reaction [40].
- Creatinine was estimated by kinetic method [41].

Microscopical techniques

Kidney and liver were removed promptly and fixed in Bouin's fluid for 24 hours. After fixation, the organs were then dehydrated through ascending grades of ethyl alcohol until they reached the absolute alcohol (1 hour) then they were transferred to xylol (3 changes, 5 minutes each). The organs were then placed in a mixture of melted wax and xylol (1:1) for about 10 minutes and then transferred to a paraffin wax (56^{o}C) for about 2 hours and then embedded. Sections were cut (3 micra thick) and stained with haematoxylin and eosin; (H and E).[42] for microscopic examination .

Statistical analysis:

Data are expressed as mean of six individual samples ± SD. The results were computed statistically (SPSS software package, Version 15) using one–way analysis of variance.[43] Values of $p < 0.05$ were considered statistically significant.

RESULTS

Biochemical parameters

Effect of the used antidiabetic drugs on the levels of:

1- Glucose:

Data of the present study show that the administration of glimepiride, metformin and the combination of them for 28 days decreased the levels of serum glucose

significantly as compared to that of the diabetic control group (Fig.1). On the other hand, the values were still more than that of the normal control rats in case of administration of both metformin and the combined drugs. Also, the present study shows that glimepiride was more effective than either metformin or the combined drug in the treatment of the diabetic rats. The mean values were 77.20±7.66, 162.20±75.28 and 124.20±42.79 mg/dl for treatment with glimepiride, metformin and the combined drug, respectively while the normal value of serum glucose level was 87.00±29.06 mg/dl.

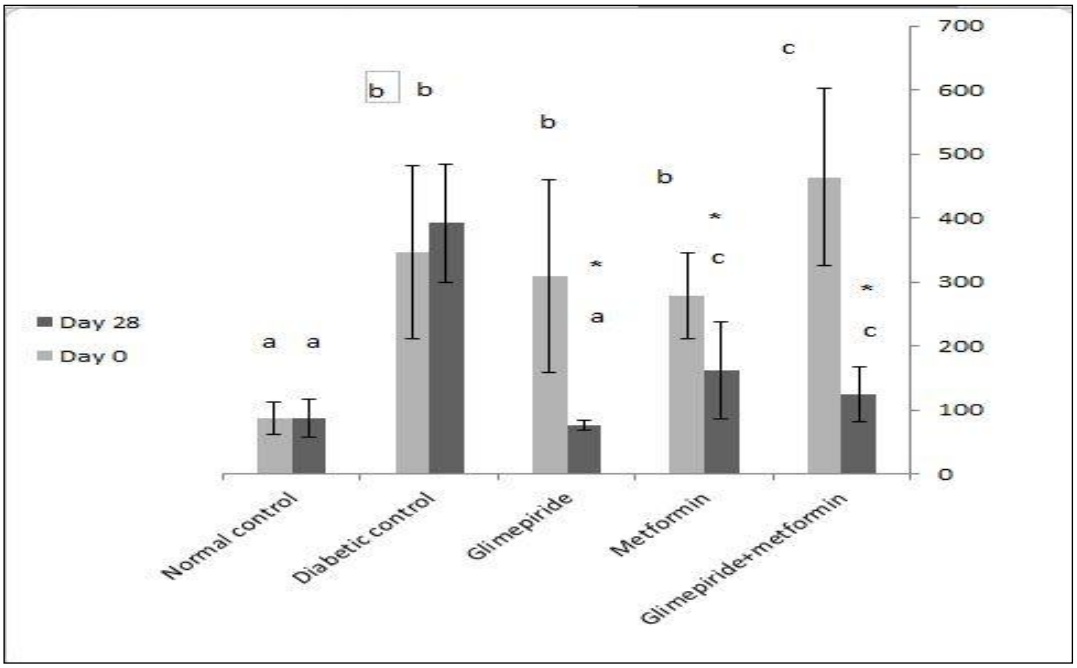

Fig. (1): Effect of the used antidiabetic drugs (glimepiride, metformin and the combined drugs) on the levels of glucose (mg/dl) in serum of the diabetic rats 28 days after treatments.

- Different letters differ significantly ($p<0.05$), while similar letters differ insignificantly.
- Letters indicate the significance between groups at a same day.
- Asterisk indicates the significance between the same group at day 0 and day 28.

2-AST and ALT:

Table (1) shows that the administration of alloxan to rats of diabetic control caused a significant increase in the activity of both serum AST and ALT as compared to that of the normal control group.

At the same time the obtained data showed that treatment with glimepiride induced a significant increase in serum AST levels in comparison to that of the diabetic control group. Contrary to glimepiride group, administration of the combined drug significantly decreases the levels of AST.

Table1: Mean ±S.D. of serum AST and ALT (U/L), total protein, albumin and globulin levels (gm/dl) 28 days after treatment of the diabetic rats.

Treatment	AST	ALT	Total protein	Albumin	Globulin
Normal control	66.8^{a}± 10.92	37.6^{a} ±3.36	5.28^{a}± 0.30	2.43^{a}± 0.10	2.85^{a}± 0.37
Diabetic control	115.8^{b}±32.71	72.6^{b} ±13.18	5.22^{a} ± 0.30	4.54^{b}± 0.21	0.68^{b}±0.16
Glimepiride	168.4^{c} ±9.45	84.6^{b}±9.45	6.08^{b}± 0.33	4.64^{b}± 0.94	1.44^{c} ± 0.74
Metformin	123.8^{b} ±36.81	77.4^{b} ±7.99	5.54^{a}± 0.50	3.57^{c} ±1.54	1.97^{c} ±1.16
Glimepiride+metformin	89.4^{a} ±4.67	56.2^{c} ±7.53	5.32^{a} ±0.38	2.13^{a}± 0.08	3.19^{d} ±0.39

Tabulated values are the average of six individual samples.
Different superscripts (a,b,c,d) differ significantly ($p<0.05$), while similar superscripts differ insignificantly.
Letters indicate the significance between treatments at the same day.

On the other hand, treatment with metformin exert no significant effect on the serum AST levels in comparison to the diabetic control group, while the value still significantly increased than that of the normal control rats. The mean values were 66.80±10.92, 115.8±32.71, 168.4±9.45, 123.8±36.81 and 89.4±4.67 (U/L) for the normal control, diabetic control, glimepiride, metformin and the combined drug, respectively.

Serum ALT levels of diabetic rats increased, as compared to that in the control group. While results of the present work did not show any significant alterations in serum ALT levels for glimepiride as well as for the metformin group in comparison to the diabetic control group. At the same time, an apparent

significant decrease in serum ALT levels was observed after administration of the combined drug in comparison to the diabetic control group but the value is still significantly higher than that of the normal control rats. The mean values were 37.6±3.36, 72.6±13.18, 84.6±9.45, 77.4±7.99 and 56.2±7.53 (U/L) for the normal control, diabetic control, glimepiride, metformin and the combined drug, respectively.

3 -Total protein, albumin, and globulin:

Table (1) shows that serum total protein levels remained unaffected in the diabetic rats as compared to that of the control rats. Also, administration of metformin or the combined drug caused no significant effect in serum protein levels as compared to diabetic control rats. On the other hand, administration of glimepiride induced a significant increase in serum protein level as compared to that of the diabetic control rats. The mean values were 5.28±0.30, 5.22±0.30, 6.08±0.33, 5.54±0.50 and 5.32±0.38 (gm/dl) for the normal control, diabetic control, glimepiride, metformin and the combined drug, respectively.

Data also show that there is a significant elevation in serum albumin level in the diabetic control rats when compared with the normal rats. While, no significant change was observed in serum albumin levels after treatment with glimepiride when compared to that of the diabetic control group. Moreover, a significant decrease in the mean values of serum albumin was observed after administration of metformin for 28 days as compared to the diabetic control group, but the value is still higher than that of the normal control group, while administration of the combined drug tend to bring levels significantly towards the normal value as shown in table (1).

Data of the current study indicate that there is a sudden significant decrease in mean serum globulin levels of diabetic control rats when compared with that in the normal control rats. Data also reveal that there is an obvious significant increase in serum globulin levels after treatment with

glimepiride, metformin and the combined drug in comparison to that in the diabetic control group. It is clear also that serum globulin levels in the combined drug treatment are found to be significantly higher in comparison to that of glimepiride and metformin groups and even in comparison to that of the control group. The mean values were 2.85±0.37, 0.68±0.16, 1.44±0.74, 1.97±1.16 and 3.19±0.39 (gm/dl) for normal control, diabetic control, glimepiride, metformin and the combined drug, respectively (Table 1).

4 – Urea and creatinine:

Fig. (2) reveals that the mean levels of serum urea increased significantly in the diabetic rats as compared to that of the normal rats. Concerning the treatment with glimepiride, data show that there is a significant decrease in the levels of serum urea as compared to that of the diabetic control group. While the treatment with the combined drug significantly increased the levels of serum urea as compared to that of the diabetic control group. Data also show that there is no significant change in the levels of urea in serum of rats after administration of metformin to the diabetic rats. The mean values were 33.2±3.83, 44.6±8.65, 38.4±4.51, 41.01±5.15 and 56.4±5.22 (mg/dl) for the control, diabetic control, glimepiride, metformin and the combined drug, respectively.

The data obtained indicate that mean serum creatinine levels of the diabetic rats are significantly increased when compared with that of the control rats. In contrast, single or combined administration of glimepiride, and / or metformin significantly decreased serum creatinine levels as compared to that of the diabetic rats and brought the levels toward the normal values. The mean values were 0.39±0.07, 0.73±0.22, 0.43±0.23, 0.59±0.17 and 0.52±0.05 (mg/dl) for the control, diabetic control, glimepiride, metformin and the combined drug, respectively (Fig.3).

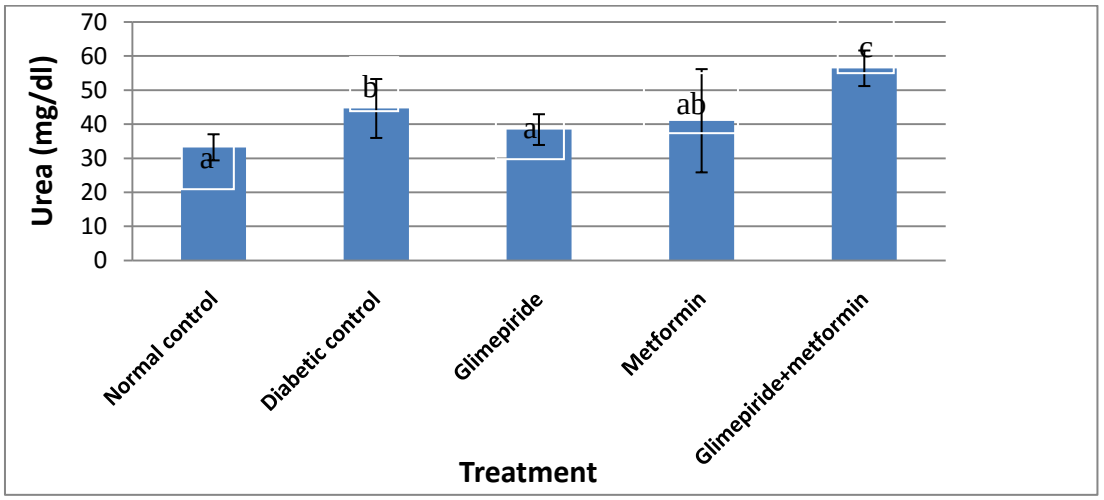

Fig. (2): Effect of the used antidiabetic drugs (glimepiride, metformin and the combined drugs) on serum urea levels (mg/dl) 28 days after treatment of diabetic rats.

- Different letters differ significantly ($p<0.05$), while similar letters differ insignificantly.
- Letters indicate the significance between groups at the same day.

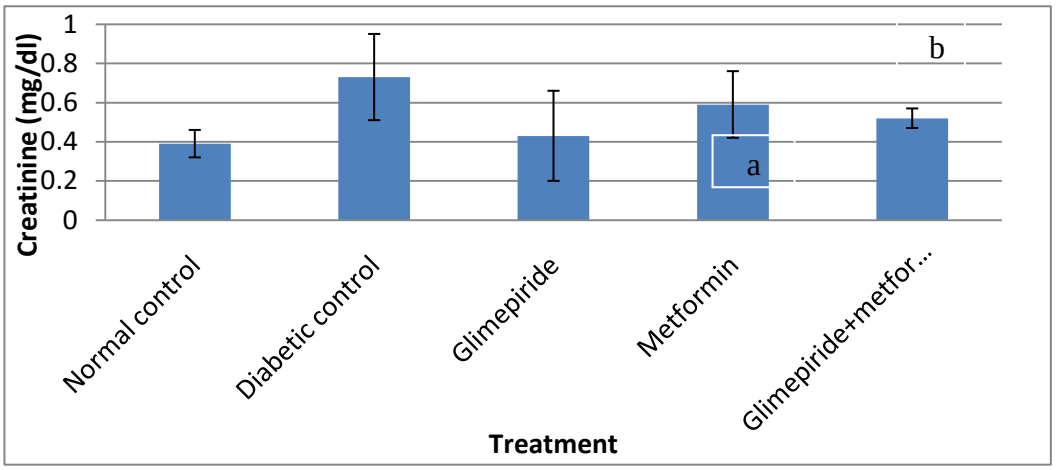

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Fig. (3): Effect of the used antidiabetic drugs (glimepiride, metformin and the combined drugs) on serum creatinine levels (mg/dl) 28 days after treatment of diabetic rats.

Different letters differ significantly ($p<0.05$), while similar letters differ insignificantly.

- Letters indicate the significance between groups at the same day.

Histopathological findings:

Liver

Rat liver of the control group generally exhibited a normal mural architecture. Hepatocytes showed a homogenous cytoplasm and a large spherical nucleus containing one or more nuclei and variable amounts of dispersed and peripheral heterochromatin. Hepatocytes are located among blood capillaries called sinusoids (s) forming cord like structure known as hepatic cell cords. The lumens of sinusoids contain mainly erythrocytes and white blood cells. Kupffer cells (k) are found to rest on the luminal surface of the sinusoids endothelium. In addition, blood vessels (BV) contain erythrocyte in normal shape as shown in fig. (4).

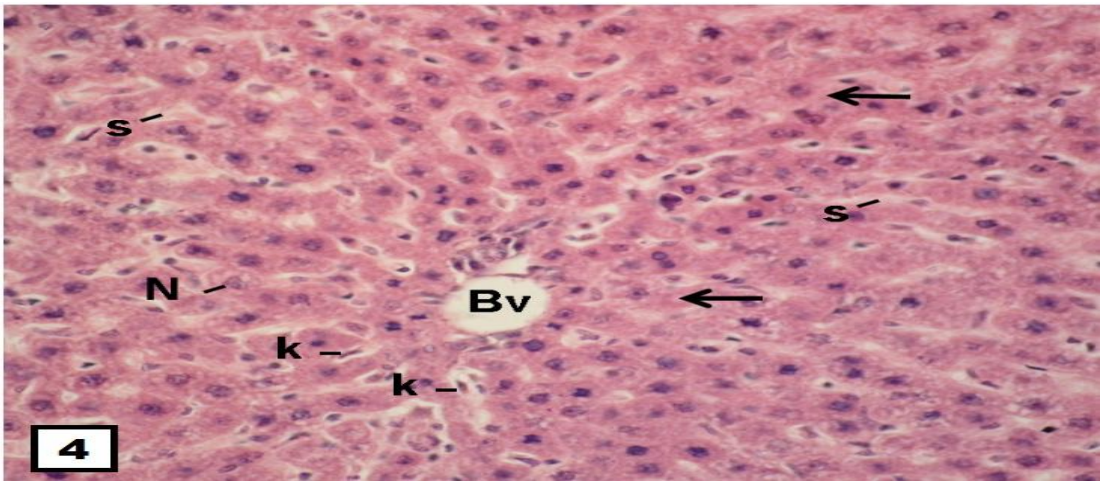

Fig.4: Light micrograph of transverse section in the liver of the albino rat, control group. Hepatocyte (arrows), S: Sinusoids, BV: Blood vessel, N: Spherical nucleus, K: Kupffer cells. HE Stain. X 400.

Light microscopic study of the liver of albino rat treated with alloxan 150 mg/kg body weight (fig.5) shows several changes. The liver cells are degenerated (double arrows). Vacuolation increased and some cells are completely filled with large vacuoles

presenting balloon like appearance (arrow heads). In addition, there is disorganization of hepatocytes with lysis of cytoplasm. Furthermore, there is dilation and congestion of blood sinusoids (s). Moreover, there are numerous hypertrophied kupffer cells (k) in sinusoids.

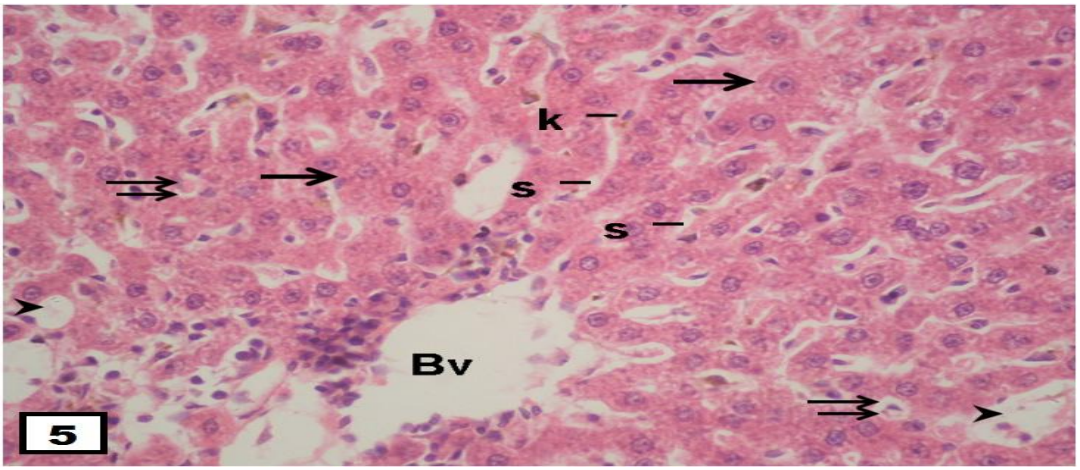

Fig.5: Light micrograph of transverse section in the liver of the albino rat, diabetic control group. Arrows point to swelling of hepatocytes, degenerated hepatocytes (double arrows), large vacuole (arrow heads), S: dilation and congestion of blood sinusoids, hypertrophied kupffer cells (k). HE Stain. X 400.

In glimepiride treated diabetic rats (fig 6), there is an apparent decrease in hepatocyte degeneration and vacuolation. Hepatocytes show some pyknosis of nuclei (Pyn) and they are still swollen. In addition, there is extensive dilation of sinusoids (S). The liver shows moderate disorganization of the hepatic cords.

The liver tissues of the albino rats treated with metformin (500mg/kg) body weight (fig.7) exhibited an apparent decrease in hepatocyte degeneration, vacuolation and pyknosis of nuclei as compared to diabetic control group. Furthermore, the liver cords arrangement appears normal.

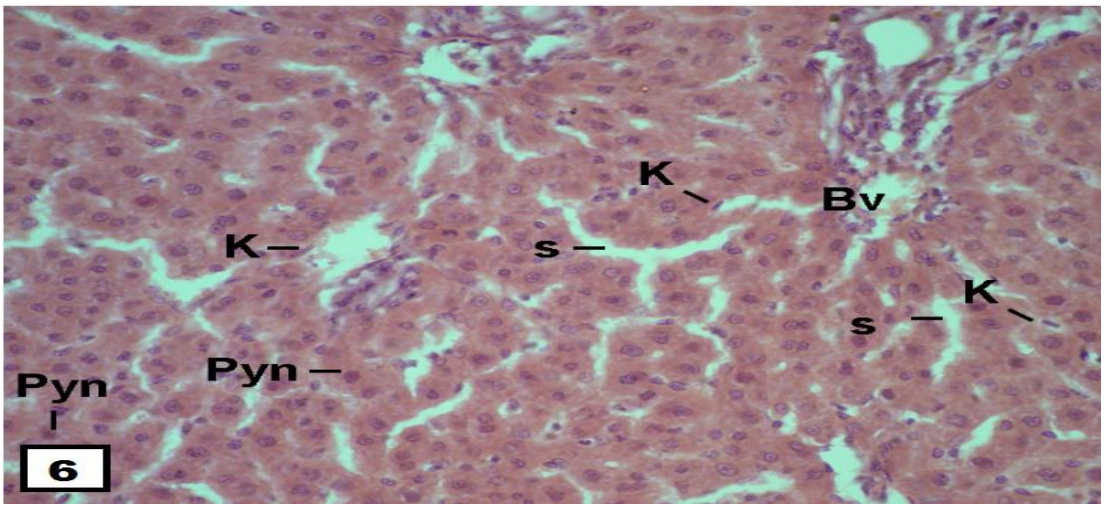

Fig.6: Light micrograph of transverse section in the liver of the diabetic albino rat after treatment with glimepiride (0.5 mg/kg. b.wt.) S: point to dilatation of blood sinusoids, pycnotic nuclei (Pyn), numerous kupffer cells (k). HE stain. X 400.

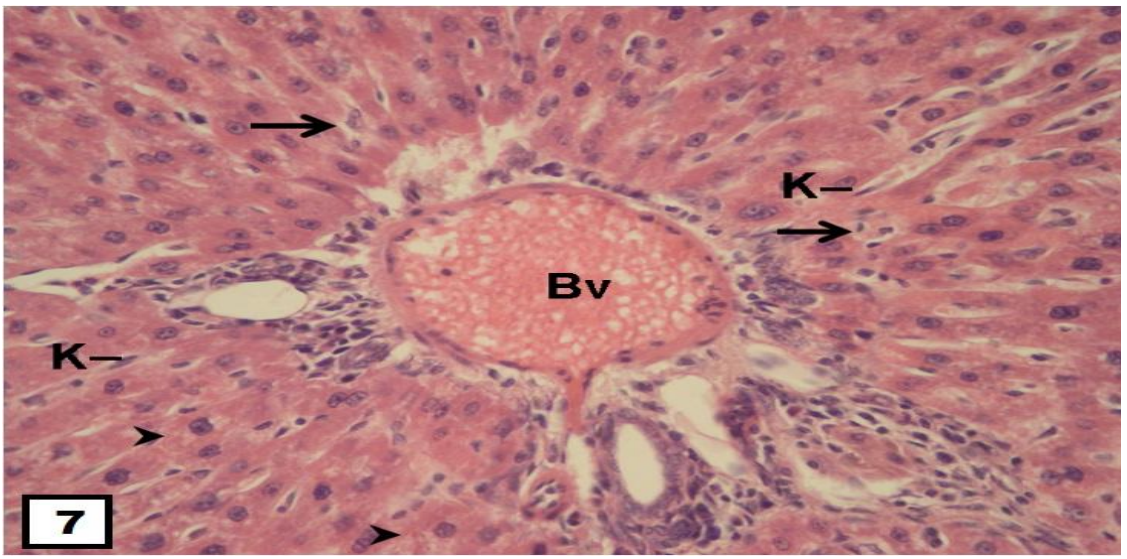

Fig.7: Light micrograph of transverse section in the liver of the diabetic albino rat after treatment with metformin (500 mg/kg. b.wt.) Arrows point to degenerated hepatocytes, swelling of hepatocytes (arrow heads), numerous kupffer cells (k). HE stain. X 400

Histological analyses demonstrate wide range of alteration in liver of diabetic albino rat treated with the combined drug for 28 days (figs. 8 and 9). The

hepatocytes are highly vacuolated and hypertrophied (arrows). Cytoplasmic vacuolation is the most obvious pathological alteration. The degenerative hepatocytes having lysed cytoplasm and deformed nuclei. Furthermore, the liver cord arrangement is highly disrupted and the hepatocytes could be distinguished only by the degenerated nuclei surrounded by small masses of cytoplasm (N). Sinusoids in most cases are distend with massive congestion (S).

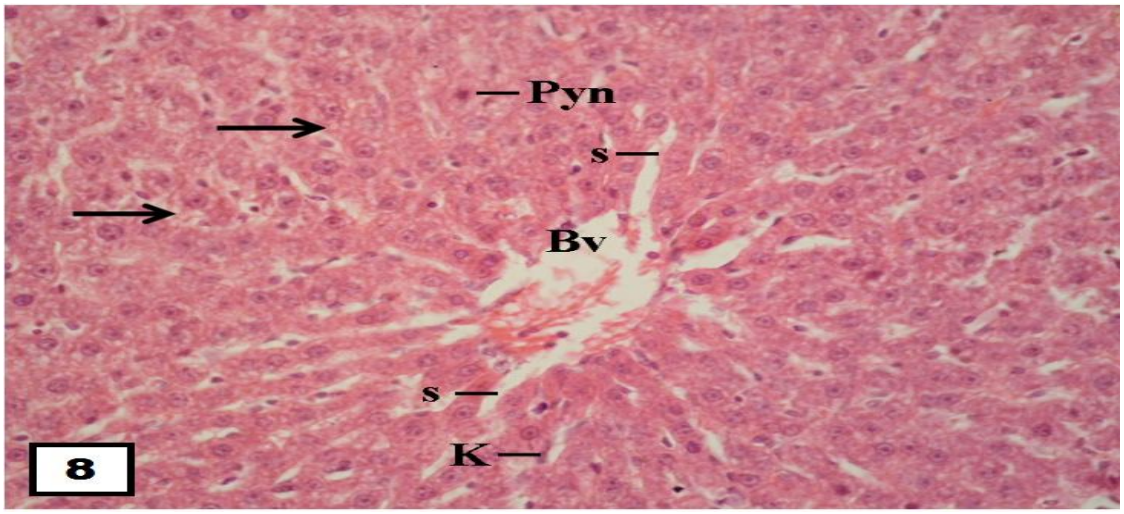

Fig.8: Light micrograph of transverse section in the liver of the diabetic albino rat treated with the combined drug (glimepiride 0.5 mg/kg. b.wt.) and (metformin 500 mg/kg. b.wt.) . Arrows point to hepatocyte swelling and vacuolation, dilation of blood sinusoids (S), kupffer cells (K), pycnotic nuclei (Pyn). HE stain. X 400.

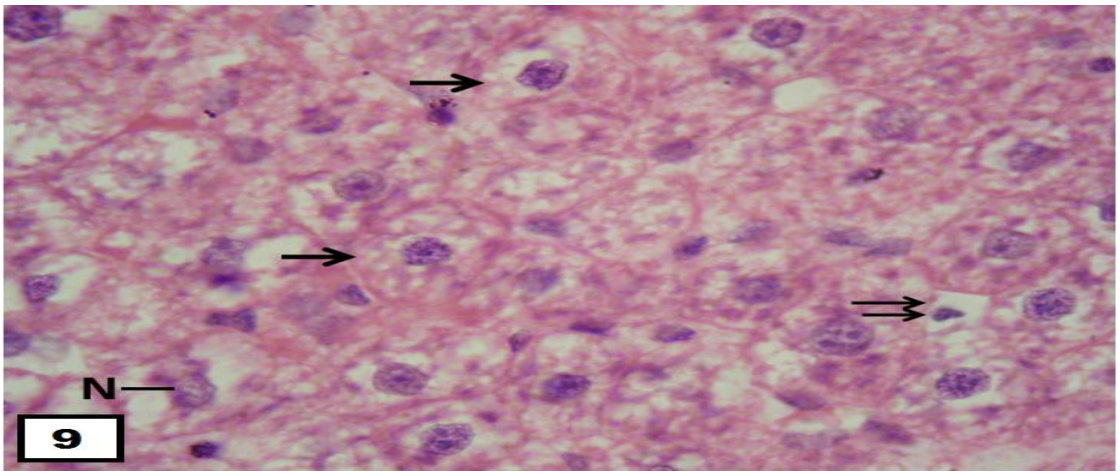

Fig.9: Light micrograph of transverse section in the liver of the diabetic rat treated with the combined drug (glimepiride 0.5 mg/kg. b.wt.) and (metformin 500 mg/kg. b.wt.). Arrows point to hepatocyte swelling and vacuolation, necrotic hepatocyte (double arrows), and deformed nuclei (N). HE stain. X 1000.

Kidney

The normal kidney (fig. 10) has a number of nephrons, the nephrons is made up of renal tubules (double arrow heads) and renal corpuscles. The renal corpuscles consist of a cluster of capillaries (glomerulus: G) surrounded by the Bowman's capsule (Bc). The microscopic picture of the glomerulus showed capillary space covered by endothelial cells on the inner side, which in their lumen contained nucleated blood cells. The space between capillaries is filled with mesangial cells. On the inner side of the Bowman's capsule, epithelial cells are found. The convoluted renal tubules are covered by tall columnar cells with a weak eosinophilic cytoplasm and apical microvilli or brush border towards the lumen.

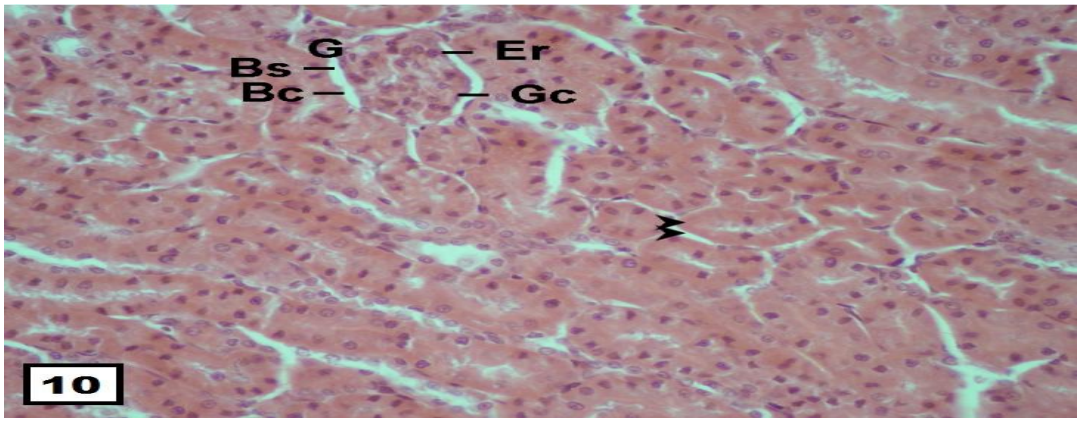

Fig.10: Light micrograph of transverse section in kidney of the albino rat, control group. G: Glomerulus, Gc: Glomerular Capillaries, Er: Erythrocytes, Bc: Bowman's capsule, Bs: Bowman's space, renal tubules (double arrow heads). HE stain. X 400.

Kidney sections of alloxan-diabetic control rats (fig. 11) show glomerular alterations. The glomerular size is significantly great (G) in diabetic rats resulting in a reduction in Bowman's space to the glomerulus (double arrows). Moreover, mesangial matrix expansion is noticed (arrow heads). Furthermore, degeneration of renal tubules (double arrow heads) including intracytoplasmic vacuoles in the epithelial cells of these tubules is frequently observed.

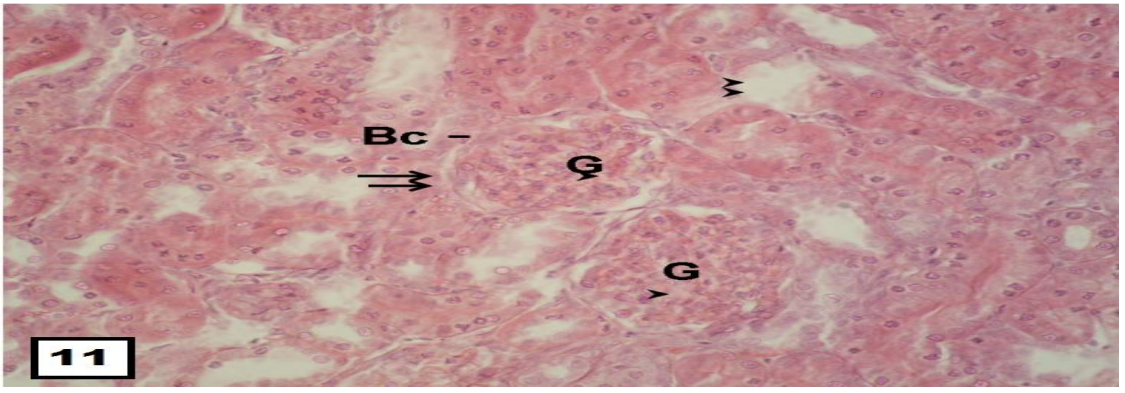

Fig.11: Light micrograph of transverse section in kidney of the diabetic albino rat. G: Glomerulus, arrow heads point to mesangial area expansion, double arrows point to adhesion of the glomerulus to Bowman's capsule (Bc) , renal tubule degeneration (double arrow heads). HE stain. X 400.

After 28 days of glimepiride treatment (0.5 mg/kg body weight), degenerative changes of renal corpuscles are noticed (figs. 12 and 13). It is presented by atrophy of glomerular capillaries (Gc) causing Bowman's space dilatation (Bs). Moreover, the glomerular network shows expansion of mesangial tissue (arrow heads) with some adhesion of Bowman's capsule to the glomerulus (double arrows). The degeneration of renal tubules is still observed in the kidney including complete lysis of cytoplasm and vacuolation (double arrow heads).

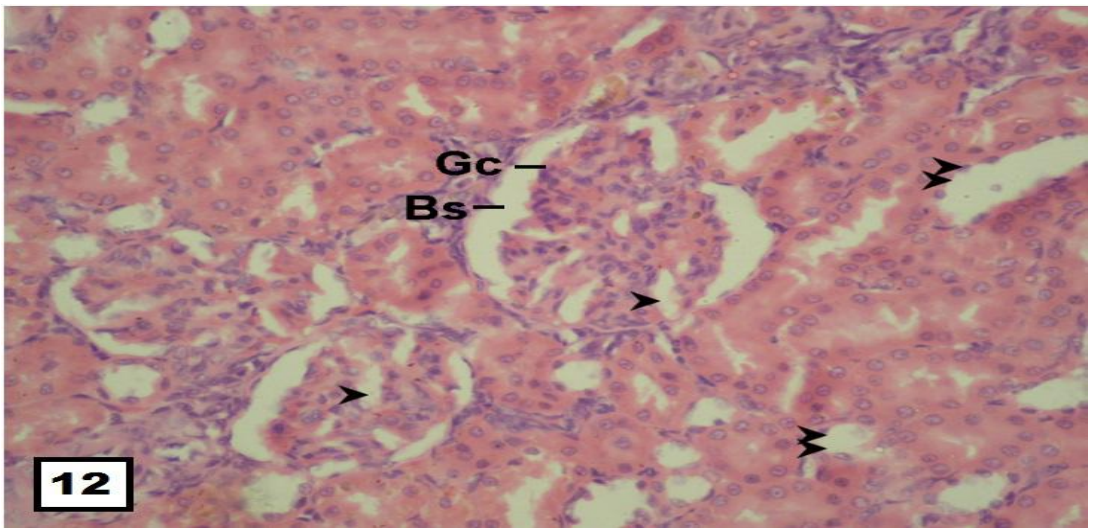

Fig.12: Light micrograph of transverse section in the kidney of the diabetic albino rat after treatment with glimepiride (0.5 mg/kg. b.wt.). The atrophy of glomerular capillaries (Gc) with bowman's space dilatation (Bs), mesangial area expansion (arrow heads), double arrow heads indicate degeneration of renal tubules. HE stain. X 400.

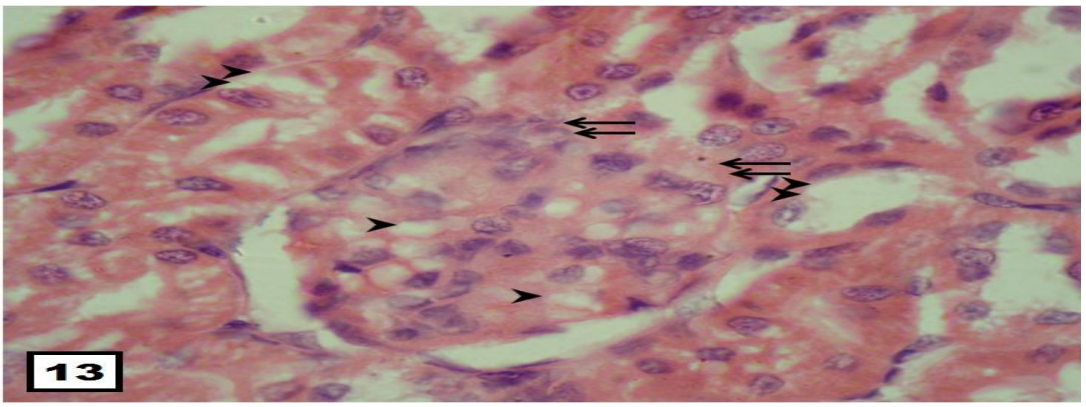

Fig.13: Light micrograph of transverse section in kidney of the diabetic albino rat after treatment with glimepiride (0.5 mg/kg. b.wt.). Arrow heads point to expansion of mesangial area, double arrows point to adhesion of the glomerulus to Bowman's capsule, degeneration of renal tubules (double arrow heads). HE stain. X 1000.

The intensity of the damage is still severe in diabetic rats treated with metformin (fig.14). The glomerular shows frequent shrinkage (arrow) and destruction increase of mesangial tissue (arrow heads) and widening of the Bowman's space consequently Bowman's space is enlarged (arrow). In addition, severe damage of the renal tubules displaying multifocal clarification and vacuolation (double arrow heads).

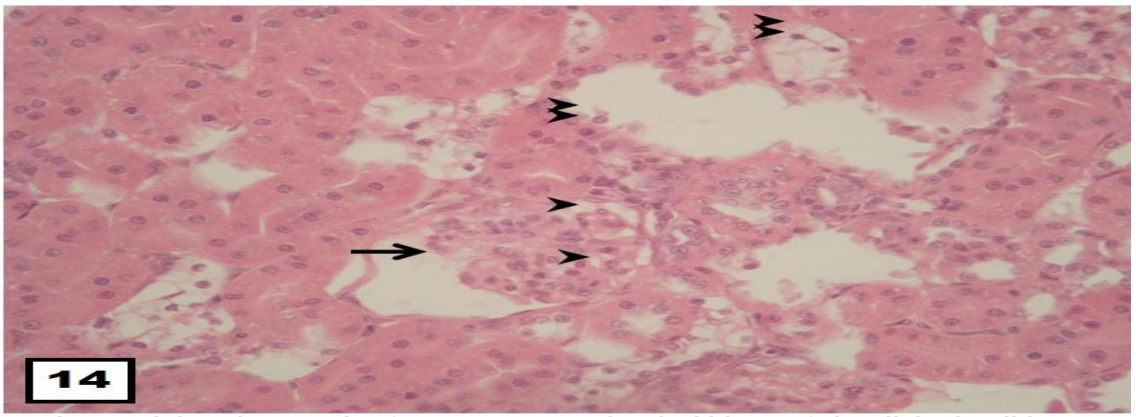

Fig.14: Light micrograph of transverse section in kidney of the diabetic albino rat after treatment with metformin (500 mg/kg. b.wt.). Arrow heads point to mesangial matrix expansion, arrow points to shrinkage of the glomerular capillaries and increased space with in the Bowman's space, double arrow heads point to complete degeneration of renal tubules. HE stain. X 400.

Treatment with the combined drug for 28 days shows glomerular alterations (fig. 15). The glomeruli of this group shows mesangial matrix expansion (arrow head), and compressed capillaries are observed (arrow). Furthermore, degeneration and necrosis of renal tubules are evident. Some tubules appear empty (double arrow heads); others show separation of the epithelial cells from its membrane causing a wide space in between the renal tubules (double arrow heads).

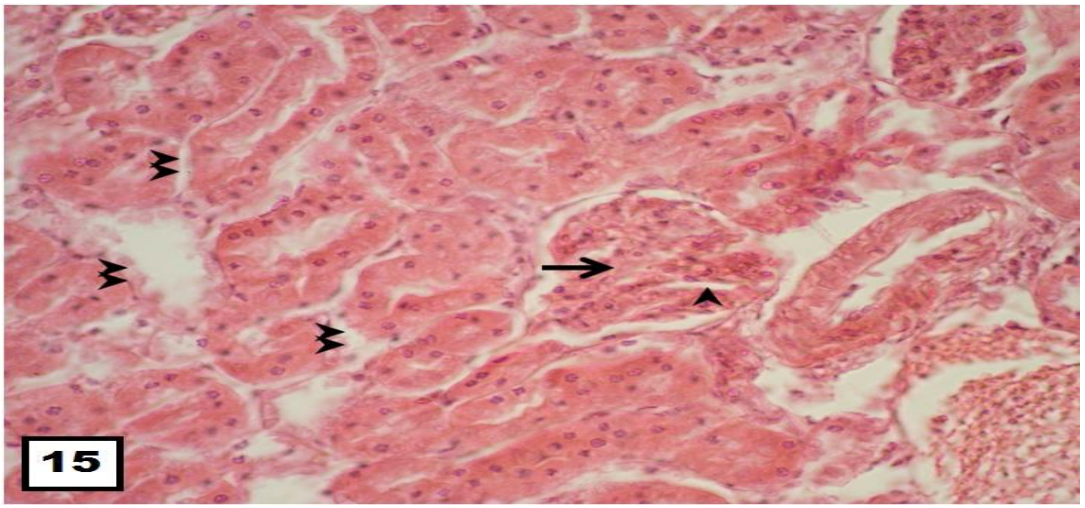

Fig.15: Light micrograph of transverse section in kidney of the diabetic albino rat after treatment with the combined drugs (glimepiride 0.5 mg/kg. b.wt. and metformin 500 mg/kg. b.wt.). Arrow head points to mesangial area expansion, arrow points to dilation of glomerular capillaries, degeneration and sparation of renal tubules from its basement membrane (double arrow heads). HE stain. X 400.

DISCUSSION

Administration of alloxan to rats, as expected, resulted in hyperglycemia, through the destruction of β- cells of the islets of Langerhans. The mechanism of alloxan action was fully described elsewhere.[44] The present data demonstrated that the administration of glimepiride, metformin and the combined drug for 28 days to diabetic rats induced a significant decrease in serum glucose levels. These findings run

paralleled with that obtained previously by many authors (17,34,45,).The antidiabetic effect of glimepiride may be due to that sulfonylurea drugs act on pancreatic β - cells, in which membrane ATP-sensitive K^+ channels are inhibited, to promote the release of insulin and thereby reduce the blood glucose level.(46) Also, it is well established that metformin reduces fasting blood glucose concentration by reducing rates of hepatic glucose production(47), its effect on the relative contribution of hepatic glycogenolysis.(48) and gluconeogenesis ;(49). Moreover, the antihyperglycemic effect of metformin could be linked to more than one mechanism. These mechanisms include the: a - improving peripheral sensitivity to insulin, b - inhibiting gastrointestinal absorption of glucose (12) and c - decreasing hepatic glucose production.(13) In addition, glimepiride and metformin seem to be particularly well suited for use in combination because of their different mechanism of action (50)

Enzymes directly associated with the conversion of amino acids to keto acids are ALT and AST .In our study, increases in the activities of serum AST and ALT in the diabetic as well as antidiabetic treated rats relative to their normal levels induce hepatic dysfunction and show severe hepatotoxicity. Our results are in agreement with those previously reported by Nathan et al.(11) It has been concluded that higher ALT is a risk factor for type 2 diabetes and indicates a potential role of increased hepatic gluconeogenesis and / or inflammation in the pathogenesis of type 2 diabetes.(51) The increase of the activities of AST and ALT in serum may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream which give an indication on the hepatotoxic effect of alloxan.(52) Histological examination of diabetic liver in the present study revealed dilatation and congestion of blood sinusoids between the hepatic cells with numerous hypertrophied kupffer cells.

Similarly, sinusoidal dilatation with increase in kupffer cells between the hepatic cells was shown.[53] sinusoidal fibrosis in diabetic patients was also observed.[54] In the present study, hepatocytes were swollen with marked cytoplasmic vacuolation and nuclei of many cells revealed clear signs of necrosis. Same results were also reported using streptozotocin (STZ) – induced diabetic rats.[53] In agreement with the present work, severe destruction of hepatic cells after streptozotocin injection in rats as focal necrosis, vacuolation, granular degeneration and swelling of hepatocytes were noted.[25] Also, cellular damage of liver cells and increase in serum aminotransferase that proves the damage were reported in alloxan-treated mice.[55]

The present results demonstrate that normoglycaemia with glimepiride or metformin treatment could ameliorate histological hepatic lesions, metformin is more potent than glimepiride. The improvement of liver histology associated with glimepiride or metformin in the present investigation could be attributed to its antidiabetic action resulting in alleviation of altered metabolic status in animals. Similarly, remarkable improvement in the histological alterations of liver was noticed in STZ-diabetic rats after treating with metformin (25mg/kg) for 28 days.[56]

Data show also that treatment of the diabetic rats with the combined drug caused a significant reduction in the activity of AST and ALT enzymes in serum as compared to that in the diabetic rats, but the readings didn't reach to the normal value. This result may be due to that metformin has been proven effective in both monotherapy and in combination with sulfonylureas.[12] As insulin sensitizer, metformin acts predominantly on the liver, where it suppresses glucose release.[57] Metformin has also been shown to inhibit intestinal absorption of glucose, while glimepiride stimulates insulin secretion.[58]

In addition, the present study also demonstrates the severe destruction in liver of diabetic rats treated with the combined drug (glimepirid and metformin) although the glucose and liver enzymes decreased significantly.

It was also evident from the results of the present work that only glimepiride group increases the total protein levels in alloxan induced diabetic rats. At the same, time serum albumin levels were found to be increased in both diabetic control rats and rats treated with either glimepiride or metformin. This increase may be ascribed to the renal damage. patients with early renal insufficiency and early renal damage had a significantly higher concentration of serum albumin.[59] The presence of abnormal amount of protein in the urine reflects systemic disease that result in an inability of the kidneys to normally reabsorb the proteins through the renal tubules.[60] In the present work, the presence of different amounts of serum globulin in the treated groups could be due to the challenge response towards the increase or decrease noticed in the concentration of albumin in these groups.

Diabetic hyperglycemia induces elevation of the plasma levels of urea and creatinine which are considered as significant markers of renal dysfunction.[61] The results of the current work show a significant increase in the levels of serum urea and creatinine in the diabetic group. After the treatment of alloxan diabetic rats with glimepiride, metformin or the combined drug, the level of creatinine was decreased significantly in the serum as compared with that of the diabetic group. Also, the results of the present work show that only with glimepiride treatment, the elevation of serum urea levels caused by diabetes is reduced. These findings may be due to that the main effect of the sulfonylureas in enhancement of insulin secretion and improvement of metabolism by both pancreatic and extrapancreatic

mechanisms.[18] On the other hand, treatment with metformin or the combined drug didn't bring urea levels to the normal values.

Histologically, the kidney sections of alloxan diabetic control rats of the present study show marked increase in the glomerular size, mesangial matrix expansion, necrosis and vacuolation of renal tubules. The glomerular enlargement is not due to the increase in the size of the mesangium or in the width of the basement membrane, but to enlargement of the intercapillary volume. Same results associated with increase in filtration surface were reported.[62] Studying normoglycaemia with glimepiride, metformin and the combination of both in the present investigation couldn't ameliorate the glomerular and tubular lesions that characterize diabetic nephropathy. Moreover, there is atrophy in the glomerular capillaries with widening of the Bowman's space as well as increase of serum albumin in the groups treated with glimepiride and metformin separately when compared with the normal group. This increase in the albumin level means that there is inability of the kidneys to reabsorb the produced protein through renal tubules as normally happened. This also proofs the inability of protein with intermediate molecular weight to enter the Bowman space (60). In the group treated with the combined drug, increased serum urea level indicates kidney failure. However, with prolonged diabetes, diabetic nephropathy will develop and characterized by proteinuria, a loss of renal function and a rapid progression to end-stage renal failure[63].

In conclusion, the combination of biochemical and histological biomarkers provides useful and sensitive tools in the investigation of chronic effects induced by diabetes and anti-diabetic drugs. Accordingly, frequent biochemical and laboratory analysis is important to check

occurrence of complications during the course of treatment.

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