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Hematological and Biochemical Changes in Streptozotocin Induced Diabetic RatsTreated with Gliclazide Drug

Abdel-Fattah Amany[1], Risha Engy[1*], Abdalla Osama[2] and Hamed Mohamed[3]

ABSTRACT

Diabetes Type 2 is one of the most common diseases all over the world that menaces the economies of all countries, particularly for developing nations. The use of Sulphonylurea drugs especially gliclazide has been taken into consideration for treating the symptoms of diabetes. So, this study was conducted to investigate the effect of gliclazide on normal and diabetic rats via some hematological parameters and biochemical analysis. The experiment was performed on 80 male albino rats, distributed into 4 experimental groups , with 20 rats per groups. Streptozotocin (STZ) was injected at the dose of 55 mg/kg oncelly, intraperitoneal of 2nd group. The 1st group served as control, the 3rd group received 10mg dose of gliclazide and the 4th group received 55 mg dose of STZ oncelly with 10mg dose of gliclazide by gavages daily for 4weeks. Diabetic animals exhibited higher blood glucose levels with altered body weight . Liver transaminase enzymes, serum creatinine, urea, insulin and lipid profile levels were significantly increased ,while sreum total protine and albumin levels were significantly decreased in diabetic rats . Treatment with gliclazide ameliorated hyperglycemia, hyperlipidemia and kidney function. In addition, gliclazide minimized the histological alterations in the Pancrease, Liver and kidney of diabetic rats. In conclusion , this study strongly suggests that gliclazide exerts a protective role against diabetes-induced hepatic disease, renal injury and dyslipidemia , by ameliorating high liver enzymes, oxidative stress and hypercholesterolemia.

Key words: gliclazide; Streptozotocin; Diabetes:, hematological parameters; biochemical analysis; Liver enzymes.

INTRODUCTION

Diabetes mellitus is a public health hazzard caused by a relative or an absolute deficiency of insulin. Clinically, it is identified by symptomatic glucose intolerance and subsequently changes in lipid and protein metabolism (Cheng, 2018). It can classified into the following categories: Type1 diabetes IDDM (due to β-cell destruction, usually leading to absolute insulin deficiency), type 2 diabetes NIDDM (due to defect of insulin resistance), gestational diabetes mellitus and specific types of diabetes due to other causes, e.g., monogenic diabetes syndromes such as: neonatal diabetes and maturity-onset diabetes of the young [MODY], diseases of the exocrine pancreas (such as cystic fibrosis), and drug- -induced diabetes (such as in the treatment of HIV/AIDS or after organ transplantation). Diabetic complications include heart disease, stroke, retinopathy, neuropathy, hepatic problems, nephropathy and peripheral circulatory disorders (Chu et al., 2015; Lian et al., 2015; Tsai et al., 2017).

Sulfonylurea was first used as an oral Hypoglycemic agent that developed for treatment of the diabetes type 2. Drugs in the sulfonylurea class have several degrees of development and have different pharmacological properties. Gliclazide MR differs from other members of sulfonylurea. The sulfonylureas stimulate insulin secretion by binding to specific receptors on the pancreatic β cells leading to closure of ATP-sensitive K-channels (KATP) in the β cells and opening of voltage-gated calcium channels(Ashcroft and Gribble, 1999). Sulfonylurea monotherapy (gliclazide) is used for improving the blood glucose level, hyperlipidemia and remarkable improvements of the degenerative changes of the myocardium in STZ - induced diabetic rats (Colagiuri et al., 2018). Besides its anti-hyperglycaemic effect, gliclazide has antioxidant properties that related to the

* Corresponding author: engyrisha@yahoo.com
Clinical Pathology Department, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt [2] Clinical Pathology Department, Faculty of Vet. Medicine, Suez Canal University, Ismailia, Egypt
[3] Pathology Department, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt

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unique aminoazabicyclo-octane ring in its structure and protects against oxidative stress by regulating the antioxidant defence status of the pancreatic β- cells, increasing SOD and CAT activities(Jennings, 2000; Mikov et al., 2017).

Streptozotocin is a(2-deoxy-2-[3-methyl-3-nitrosourea] 1-D-glucopyranose) occurs in 2 anomeric forms, α and β, which can be separated by HPLC analysis. This drug is used for treating pancreatic islet cell tumors, some neuroendocrine tumors ,cytotoxicity for pancreatic β cells and stimulating diabetes within animals, this insulinopenia problem is called "Streptozotocin diabetes" that is brought on by the specific necrosis from the pancreatic beta cell (Islam and Code, 2017). It is used to induce both type I and type II Diabetes mellitus. Type I DM was induced by STZ administration at multiple low doses while type II DM was induced by STZ administration at single dosage. The present study involves the use of a single dose of STZ to achieve type II DM in rats (Ventura-Sobrevilla et al., 2011). Also, it caused diabetic kidney injury with hyperlipidemia and hperuricemia.

Therefore, the aim of the present study is to investigate the protective role of gliclazide against the streptozotocin induced diabetes in rats through some hematological and biochemical parameters.

MATERIALS AND METHODS

Experimental animal

In this study, eighty male albino rats of 1-2 month old (average body weight 170-220 gm) were obtained from Helwan farm of laboratory Animals (Ministry of Public Health) .The rats were put in their cages at least 1-2 weeks to allow them to naturalize prior starting of the experiment. During that period, rats had free access to water ad-libitum and standard diet (NRC, 1995)

Chemicals

Streptozotocin injection (2-Deoxy-2 [(methylnitrosoamino)-carbonyl] amino]-D-glucopyranose procured from Sigma - Aldrich co,UK.Gliclazide (Diamicron 60 mg) tables from Serdia Pharmaceuticals (India) Ltd.

Experimental design

Eighty male albino rats were randomly divided into four equal groups. The groups treated as follows. Group-1(control) had received physiological saline (control–ve), Group- 2 (STZ) administrated 55 mg/kg BW, single intraperitoneal injection of streptozotocin (control +ve), Group- 3 (GLIC) administrated (10mg/ kg BW) of gliclazide suspension orally once per day and Group- 4 (STZ+GLIC) administrated 55 mg/kg BWstreptozotocin I/P and gliclazide suspension (10mg/ kg BW) orally once per day. Rats were treated with their experimental dose daily for 4 weeks. During that period, the food and water intake was observed periodically along with alteration in body weight.

Blood samples

Bleeding from the medial canthus of the eye was described for collecting two separate blood samples on diabetic rats at the end of 4th week post treatment with drug.The first blood sample was collected in an EDTA coated eppendrof tubes that containing dipotassium salt of EDTA as anticoagulant (0.5mg/ml blood) and gently mix for hematological estimation. The second blood sample (without anticoagulant) were placed in an inclined position for 20 minutes at room temperature, then samples were stored on (refrigerator) and centrifuged for 10 minutes at 3000 rpm to completely separate the serum. Serum was carefully transferred to a 0.5 ml eppendrof tubes to be stored at-20°C until used for biochemical parameters.

Hematological parameters

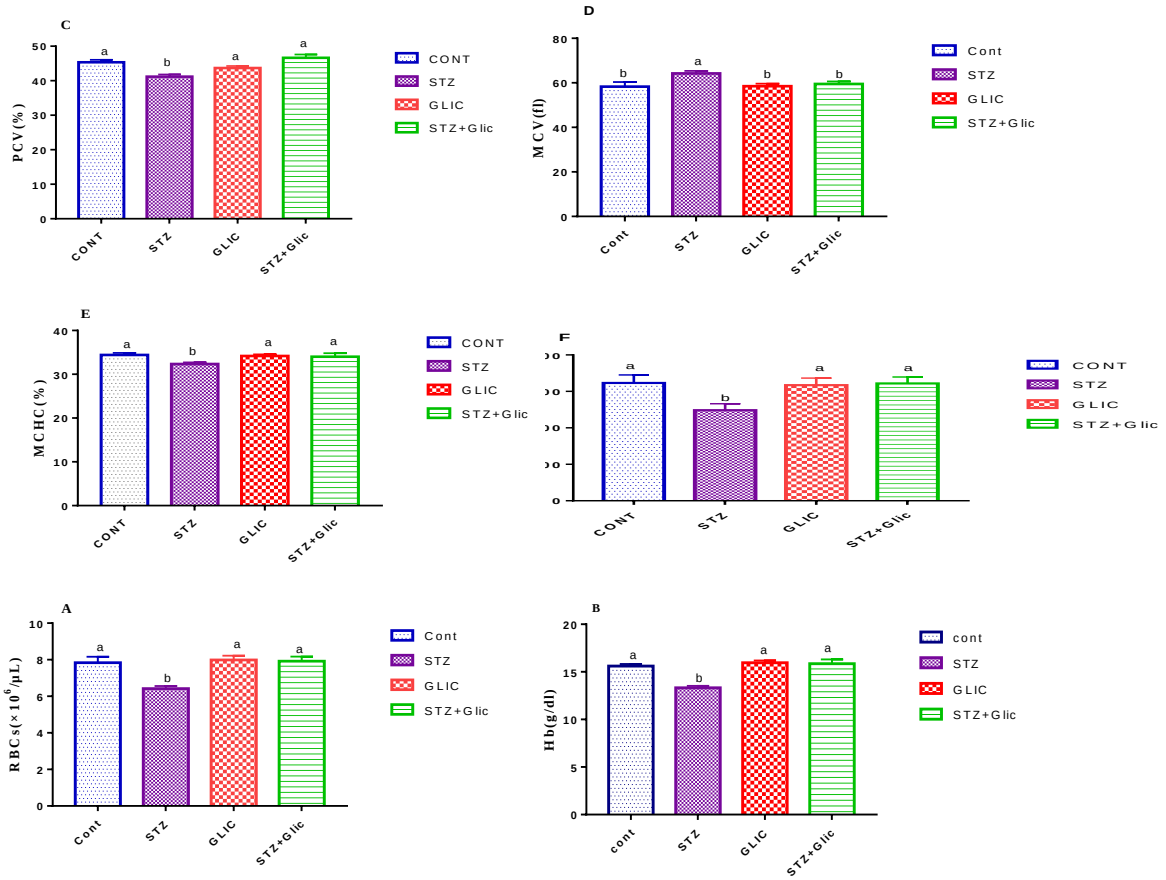
RBCs count, Hb concentration and PCV% were measured to calculate red cell indices, MCV (fl),MCHC (%) and MCH (pg) according to Feldman et al . (2000)

Total and differential leukocytic count

Leukocyte count was accomplished by manual hemocytometer method using

improved Neubauer counting chamber. Then, tow blood films were made manually from each blood sample and stained with giemsa stain for differential leukocytic count using light microscope at 100x magnification according to Feldman et al . (2000).

Fig. 1. The effects of gliclazide and streptozotocin on hematological parameters (RBCs(A), Hb(B), PCV(C), MCV(D), MCHC(E) and platelet count(F) resprctively in all rats. Control (normal saline), STZ (streptozotocin treated with 55mg/kg), Glic (gliclazide 10mg/kg), STZ+GLIC (streptozotocin treated with 55mg/kg and gliclazide 10mg/kg). Data was expressed as the mean ± SEM (n= 5 per group). Different letters indicates significant difference amang treatments ($p < 0.05$).

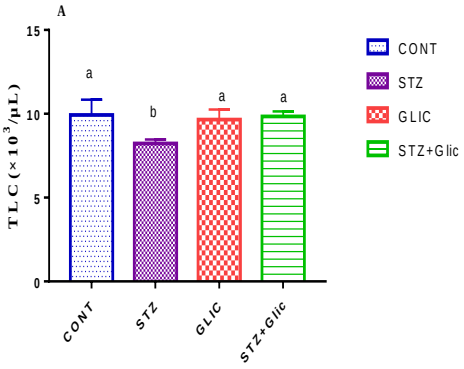


Selective biochemical parameters

All serum parameters were measured using cobas Integra 400 plus analyzer (Roche diagnostics, Germany). The colorimetric assay for determining ALT, AST and ALP activities in serum using Biolabo SA kits (Maizy,France) according to Reitman and Frankel (1957). While total protein was assayed calorimetrically using commercial kits (Stanbio, USA), according to Weichselbaum (1946) and Özkan (2007). Albumin was measured by using Diamond diagnostics kits according to Gendler (1984) and Tietz (1995). Globulin was measured by subtraction albumin from total protein and A/G ratio is determined by dividing the albumin concentration by the globulin concentration according to Kaneko et al., (1997).

The serum creatinine was determined according to quantitatively method described by Schwartz et al. (1987) using a kit supplied by Diamond, Egypt, Cairo. Urea was quantitatively determined according to enzymatic colorimetric method using a Diamond kits, as described byKaplan (1984). The cholesterol ,Triglycerides and HDL level were assayed

Fig. 2. The effects of gliclazide and streptozotocin on some leukocytic parameters TLC(A) in all rats. Control (normal saline),STZ (streptozotocin treated with 55mg/kg), Glic (gliclazide 10mg/kg), STZ+GLIC (streptozotocin treated with 55mg/kg and gliclazide 10mg/kg). Data was expressed as the mean ± SEM (n= 5 per group). Different letters indicate significant difference amang treatments ($p < 0.05$).



by using commercial geneis reagent kits according to Allain et al. (1974) and Siedel et al. (1983). The low density lipoprotein-cholesterol (LDL-C) was calculated according to the Friedewald equations described by Assmann et al. (1984). Blood

Fig. 3. The effects of gliclazide and streptozotocin on liver function markars (ALT (A), AST(B), ALP(C), Total protine(D), albumin(E) and globuline(F)) resprctively in all rats. . Control (normal saline),STZ (streptozotocin treated with 55mg/kg), Glic (gliclazide 10mg/kg), STZ+GLIC(streptozotocin treated with 55mg/kg and gliclazide 10mg/kg). Data was expressed as the mean ± SEM (n= 5 per group). Different letters indicate significant difference amang treatments ($p < 0.05$).

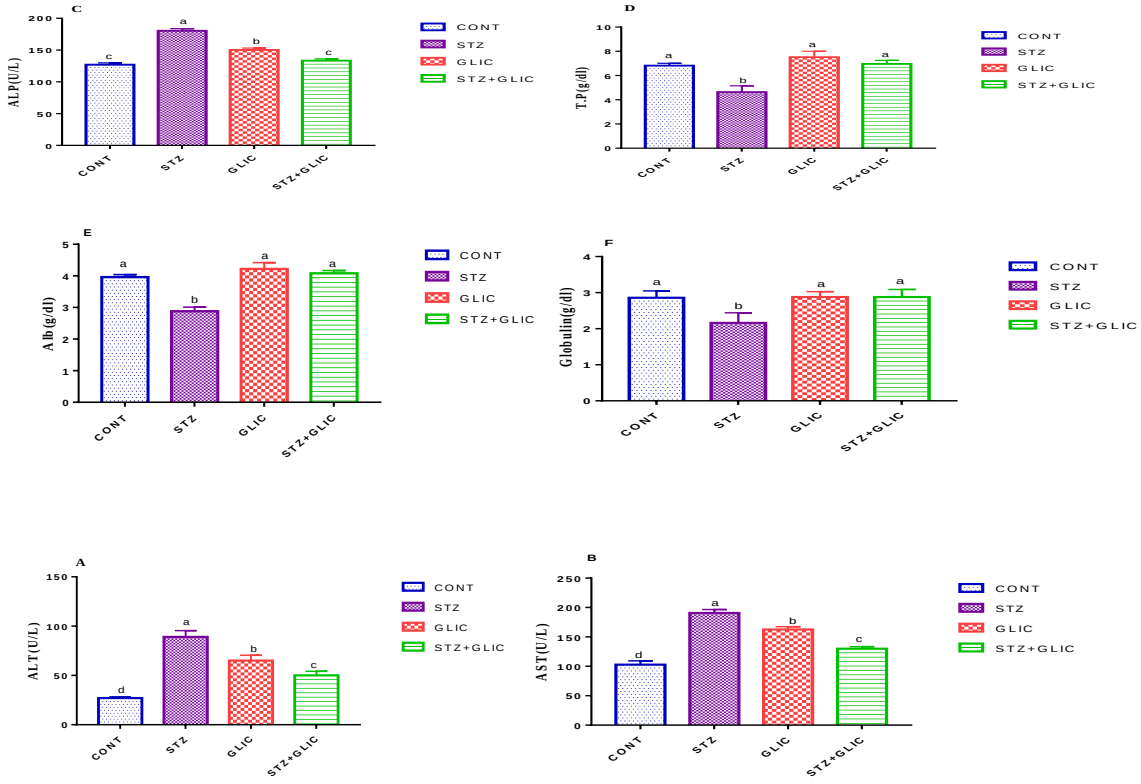
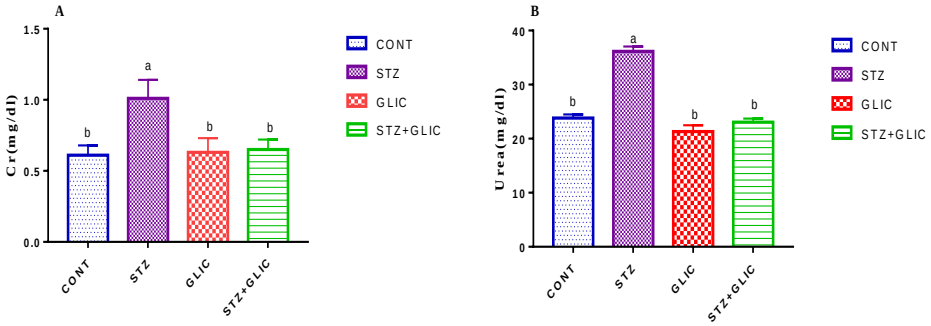
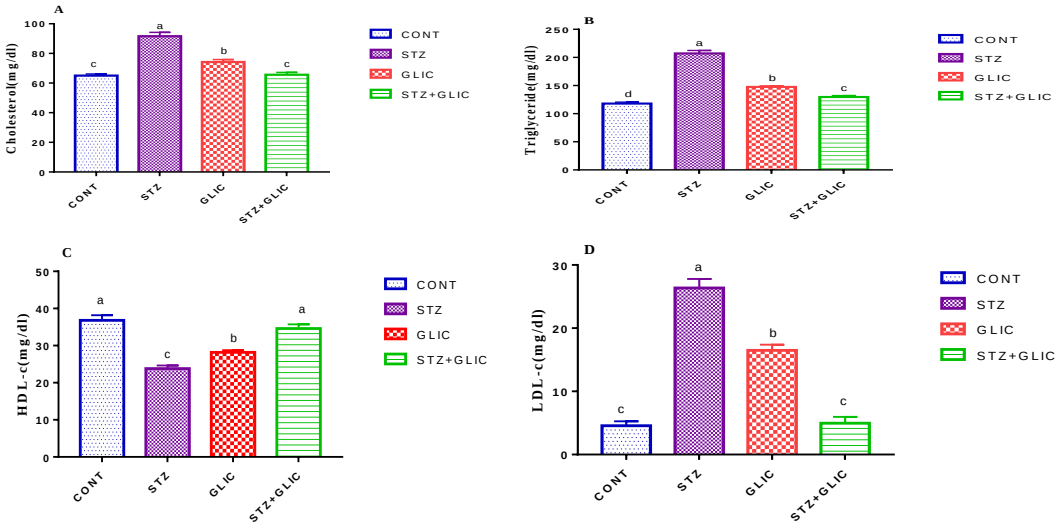


Fig. 4. The effects of gliclazide and streptozotocin on Kidney function markars (creatinine (A) and urea (B)) resprctively in all rats.Control (normal saline),STZ (streptozotocin treated with 55mg/kg) ,Glic (gliclazide 10mg/kg), STZ+GLIC (streptozotocin treated with 55mg/kg and gliclazide 10mg/kg). Data was expressed as the mean ± SEM (n= 5 per group). Different letters indicate significant difference amang treatments ($p < 0.05$).



glucose level was measured by using GOD-PAP method by enzymatic colorimetric test according to Besch et al. (1987). Enzyme-linked immunosorbent assay (ELISA) was used for the quantitative measurement of insulin using Pilkin kits purchased from United States (Chevenne et al., 1998).

Fig.5 The effects of gliclazide and streptozotocin on total lipid profile (cholesterol (A) ,triglyceride (B), HDL-c (C) and LDL-c (D)) resprctively in all rats. Control (normal saline),STZ (streptozotocin treated with 55mg/kg), Glic (gliclazide 10mg/kg), STZ +GLIC (streptozotocin treated with 55mg/kg and gliclazide 10mg/kg). Data was expressed as the mean ± SEM (n= 5 per group). Different letters indicate significant difference amang treatments ($p < 0.05$).



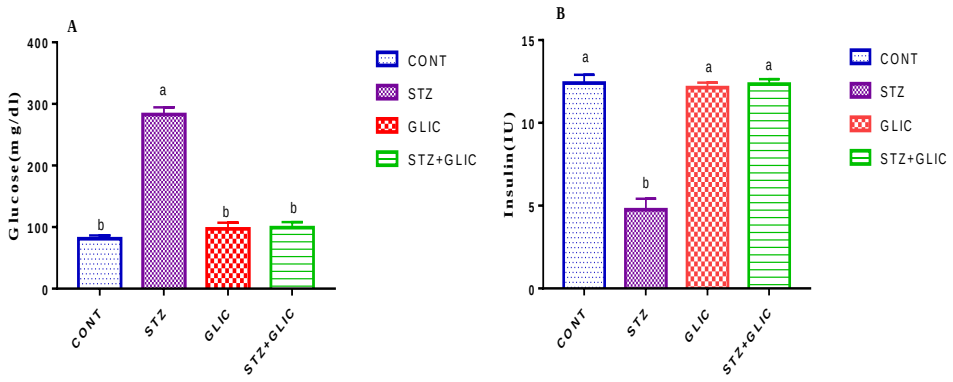
Statistical Analysis:

All our data were statistically analyzed by statistical software program (SPSS for windows, version20, USA). Data were expressed as means ± standard error of experimental study .ANOVA was used to know differences between means of all groups using Duncan multiple comparison tests. Difference results were considred a significant when $p< 0.05$ (Keuls, 1952).

RESULTS

Hematological Results

Fig. 6. The effects of gliclazide and streptozotocin on glucose (A) and insulin (B) levels in all rats. Control (normal saline),STZ (streptozotocin treated with 55mg/kg), Glic (gliclazide 10mg/kg), STZ+GLIC(streptozotocin treated with 55mg/kg and gliclazide 10mg/kg). Data was expressed as the mean ± SEM (n= 5 per group). Different letters indicate significant difference amang treatments ($p < 0.05$).



Erythrogram

The hematological results were showen in Fig. 1, (A-F). After 4 weeks, the streptozotocin treated group (Gp.2) emerged a significant decrease in RBCs count, Hb(g/dl) and PCV (%) compared to all experimental groups. On other hand, MCV(fl) was significantly increased in Gp.2 when compared to all investigatrd groups (Fig.1, D). While there were no significant change between all groups in MCH value (Fig.1, E). Furthermore,the MCHC value was significantly decreased in the STZ induced diabetic untreated group compared with other exposed groups (Fig.1,F). From the blood indices we found that diabetic untreated group suffer from macrocytic hypochromic anemia. Platelet count showed significant decrease in STZ treated group compared with all experimental groups (Fig.1).

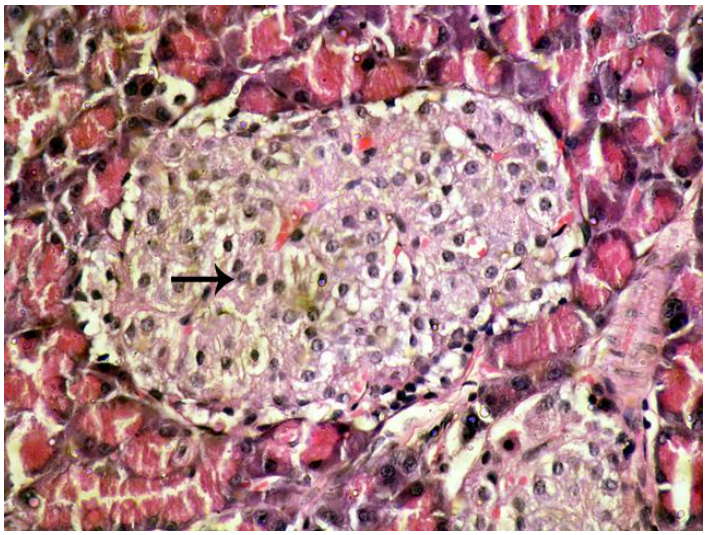


Photo (1): Pancreas of control group at the end of 4th wk is showing normal exocrine glands and normal endocrine gland having normal β cells (arrow). (HE, 400x).

Leukogram

Leukogram results were summarized in Fig. 2,(A), I/P administration of STZ (GP.2) significantly decreased WBCs count comparing with all experimental groups. At the same time, neutrophil (10^3/μL) and lymphocytic counts (10^3/μL) were decreased in STZ treated group (GP.2) but not sever enough to cause significant variation when compared with all investigated groups.

Biochemical Results

Liver function markers

Some biochemical results are displayed in Fig. 3, (A-F). ALT, AST and ALP levels were significantly increased in STZ treated group (GP.2) and GLIC (GP.3), being the highest in STZ treated group when compared with control and (GP.4). However;liver enzymes (ALT and AST) in STZ+GLIC (GP.4) were significantly increased compared to control group. Total protein, albumin and globulin levels were significantly decreased in STZ treated group (GP.2) compared with other investigated

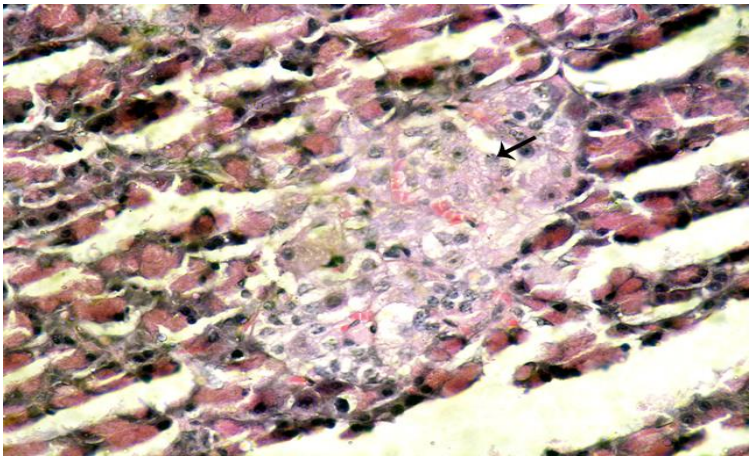


Photo (2): Pancreas of STZ group at the end of 4th wk is showing degenerative changes and necrosis of β cells (arrow). (HE, 400x).

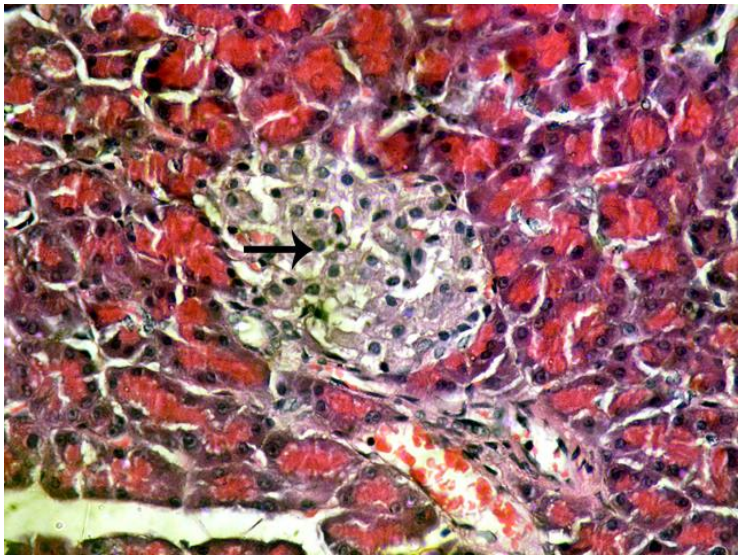


Photo (3): Pancreas of GLIC group at the end of 4th wk is showing normal exocrine glands and normal endocrine gland having normal β cells (arrow). (HE, 400x)

groups. Furthermore, the A/G ratio showed non significantly changes in whole experimental groups.

Kidney function markers

in GLIC and STZ+ GLIC treated groups as compared with control.

Lipid profile , glucose and insulin

As seen in Fig. 5,(A-D).Total cholesterol

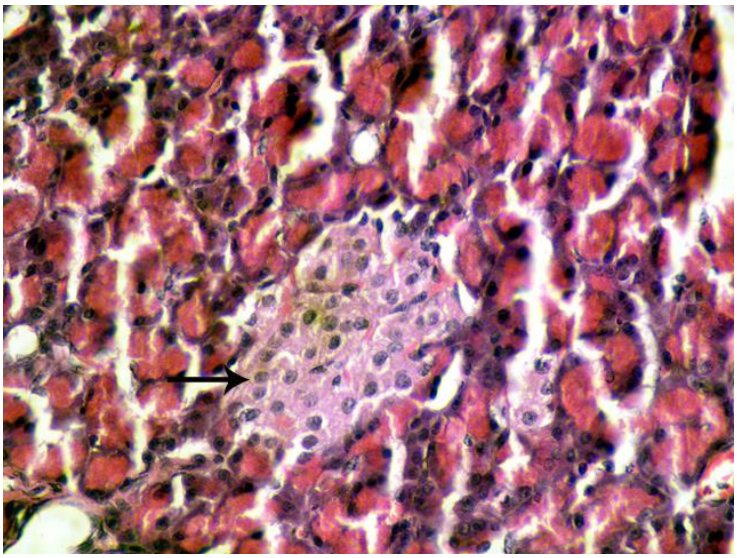


Photo (4): Pancreas of STZ+GLIC group at the end of 4th wk is showing normal exocrine glands and normal endocrine gland having normal β cells (arrow). (HE, 400x).

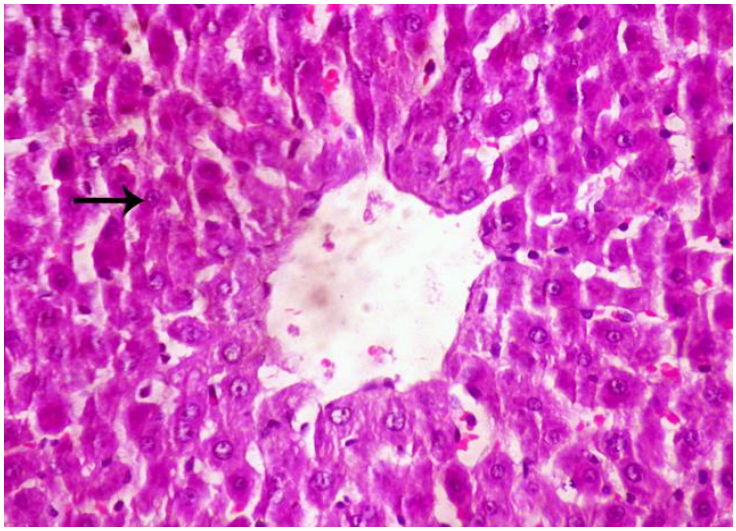


Photo (5): Liver of control group at the end of 4th wk Liver is showing normal hepatocytes and normal radial arrangements of hepatocytes around central vein (arrow). (HE, 400x).

As presented in Fig.4,(A-B).STZ treated rats (GP.2) showed a significant increase in both serum urea and creatinine levels when compared with all investigated groups. Moreover, no significant changes were showed in serum creatinine and urea levels

and triglyceride levels were significantly increased in STZ (GP.2) and GLIC (GP.3) groups as compared to both control and STZ+ GLIC groups. HDL-cholesterol level was significantly decreased in STZ (GP.2) and GLIC (GP.3) when compared to other experimental groups. No significant

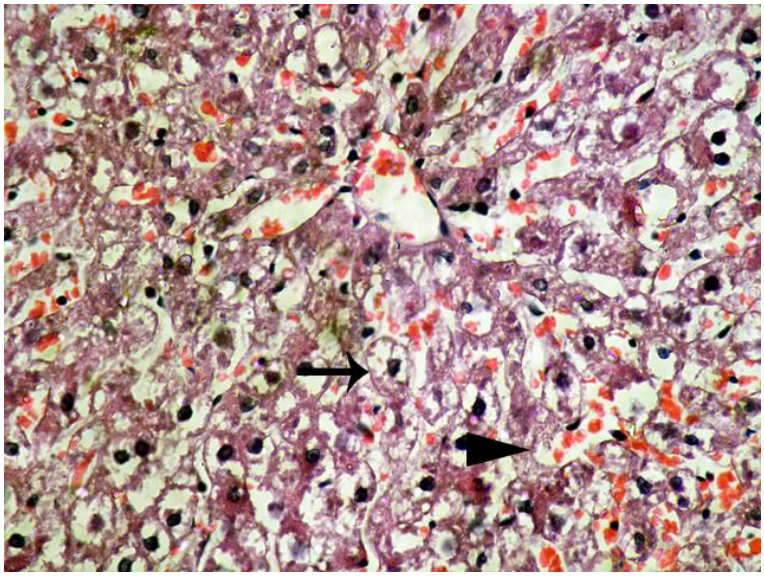


Photo (6): Liver of STZ group at the end of 4th wk is showing degenerative changes of hepatocytes (arrow) and dilation of hepatic sinusoids (arrow head) (HE, 400x).

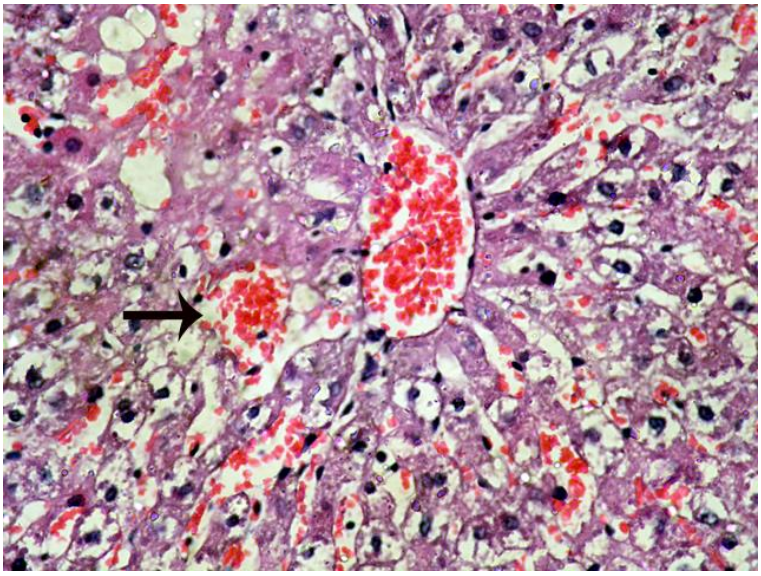


Photo (7): Liver of GLIC group at the end of 4th wk is showing necrosis of hepatocytes and severe congestion of hepatic sinusoids (arrow). (HE, 400x).

changes were observed between control and STZ+GLIC groups. Meanwhile the LDL-cholesterol level was significantly elevated in both GP.2 and GP.3, showing the highest significant elevate particulary in STZ group when compared with control and GP.4), but its level was insignificantly changed in STZ+GLIC group compared to control one.

As demonstrated in Fig.6,(A-B) at the end of 4th week, glucose level was significantly increased while insulin level was significantly decreased in STZ treated group compared to other investigated groups.

DISCUSSION

Diabetes is considered as a world wide health problem. STZ is a widely used

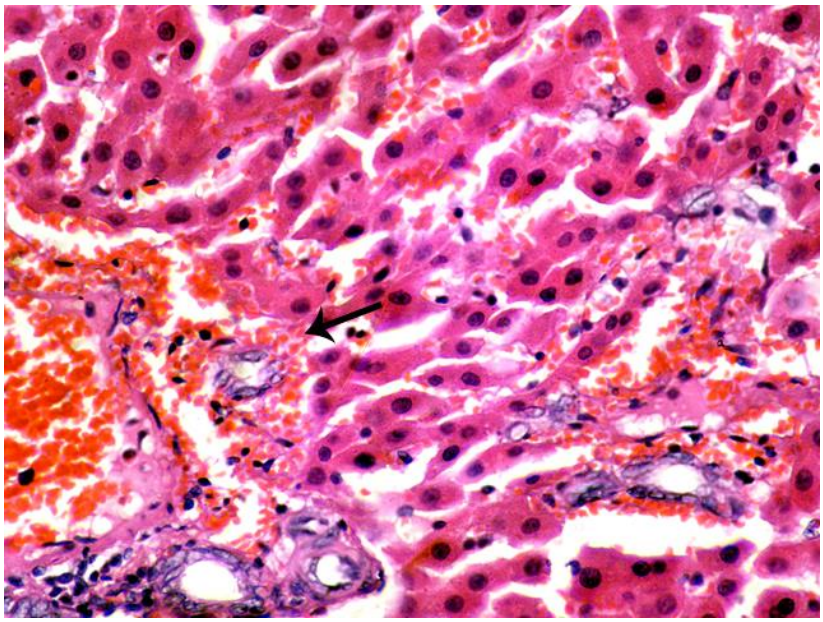


Photo (8): Liver of STZ+GLIC group at the end of 4th wk is showing hydropy of hepatocytes (arrow) with normal hepatic architecture. (HE, 400x).

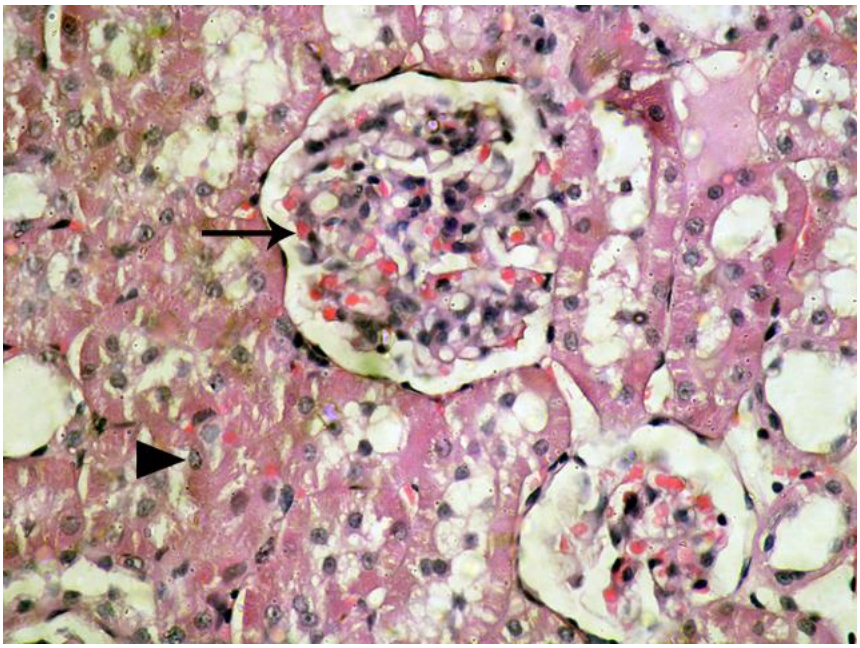


Photo (9): kidney of control group at the end of 4th wk is showing normal renal glomeruli (arrow) and normal renal tubules lines by normal renal tubular epithelium (arrow head).(HE, 400x).

inducer of Diabetic mellitus in experimental animals especially rats. It causes selective destruction of islet cells of Langerhans via generation of some types of oxygen free

radicals and alteration of endogenous scavengers of these reactive species (Nagy et ., 2012; Rouhi et al., 2017). Gliclazide is a second generation sulphonylurea and it

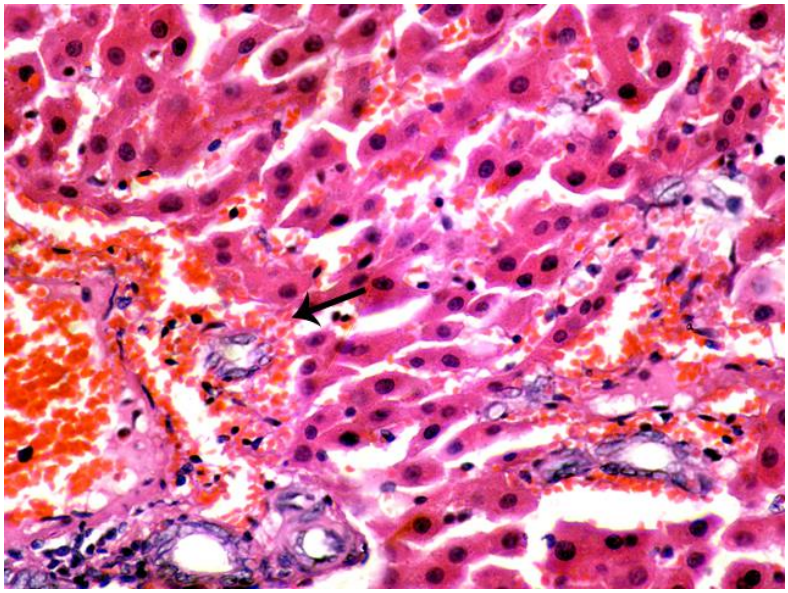


Photo (8): Liver of STZ+GLIC group at the end of 4th wk is showing hydropy of hepatocytes (arrow) with normal hepatic architecture. (HE, 400x).

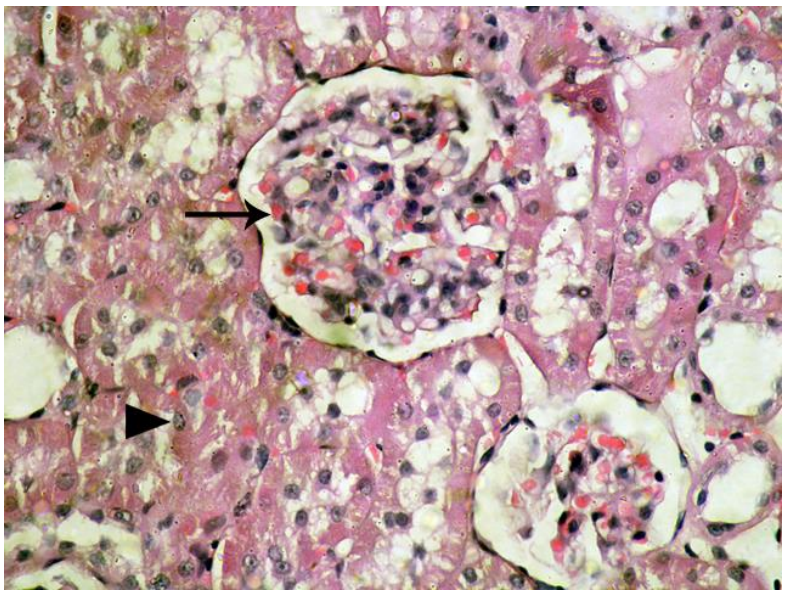


Photo (9): kidney of control group at the end of 4th wk is showing normal renal glomeruli (arrow) and normal renal tubules lines by normal renal tubular epithelium (arrow head).(HE, 400x).

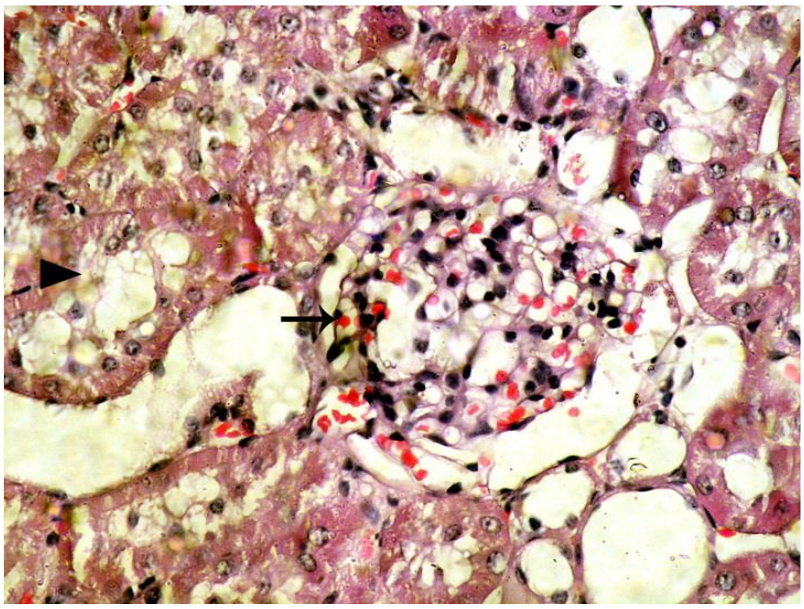


Photo (10): kidney of STZ group at the end of 4th wk is showing congestion of renal glomeruli (arrow) and degenerative changes in renal tubular epithelium lining renal tubules. (arrow head). (HE, 400x).

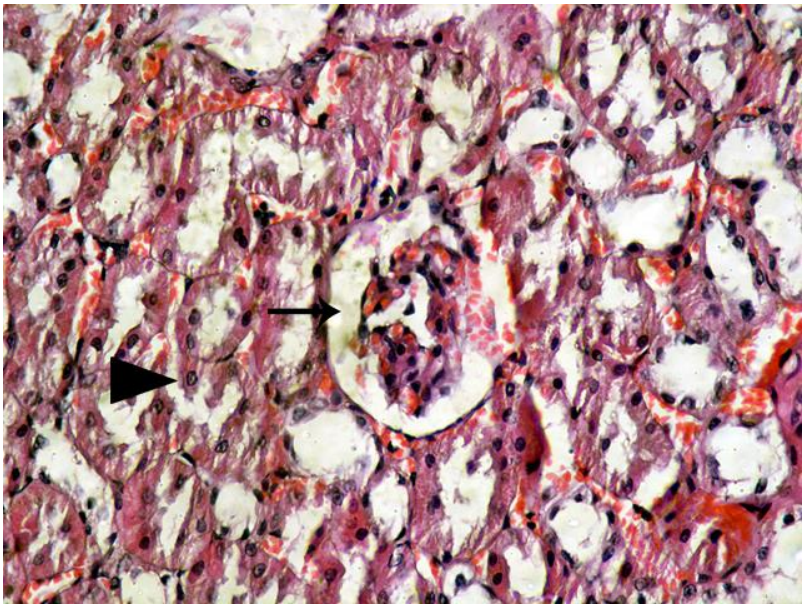


Photo (11): kidney of GLIC group at the end of 4th wk is showing normal renal glomeruli (arrow) and normal renal tubules lines by normal renal tubular epithelium (arrow head). (HE, 400x).

considered as an oral hypoglycaemic agent used in treatment of type II of Diabetic mellitus. It improves defective insulin secretion and decrease insulin resistance

(Sarkar et al., 2011). Our erythrogram results showed that, the intraperitoneal administration of STZ (55mg/kg BW) at the end of 4th week induce macroytic hypochromic anemia. which may be due to the increase in the non-enzymatic glycosylation of RBC membrane proteins that related to the increase of glucose level which leads to elevate the production of lipid peroxides causing hemolysis of RBCs. The lipid peroxidase considered as an oxidative stress marker which converts to hydroxyl radicals that causing hemolysis of RBCs (Sellamuthu et al., 2014). Our results also agree with (Nasirian et al., 2017) who approved a significant decrease in the hemoglobin and RBC count with STZ administration at the same dose. Furthermore, high levels of free radicals causes damage to cellular proteins, lipids membrane,nucleic acids, and cell death when comparing with the control and other treated groups (Rodriguez et al., 2006).

In our data, hematological parameters as RBC, Hb and PCV in diabetic rats treated with gliclazide (10mg/kg.BW) were significantly improved at the end of 4th week. This in the same line with Alper et al. (2006) and Oluwaseun et al.(2018) who reported that the diabetic rats treated with gliclazide at the same dose were significantly increased RBC, and Hb compared with untreated group.

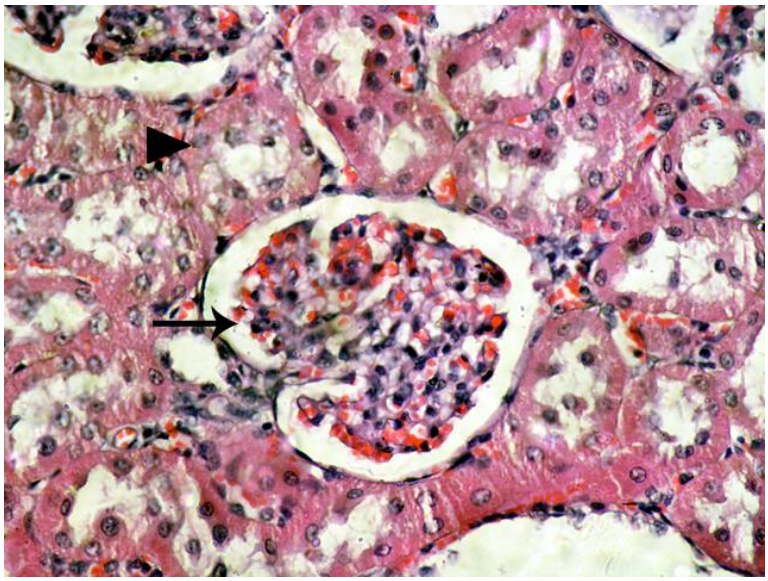


Photo (12): kidney of STZ+GLIC group at the end of 4th wk is showing normal renal glomeruli (arrow) and normal renal tubules lines by normal renal tubular epithelium (arrow head).(HE, 400x).

In the current study, the platelet count was significantly decreasd (thrombocytopenia) in induced diabetic untreated group compared with all investigated groups , this may be attributed to the shortened platelet survival which in the STZ diabetic rat is caused initially by a platelet defect. later, platelet factors become non-dominant at the end of 4th week. Also our result in accordance with Winocour et al. (1984) and Przygodzki et al. (2018) who observed that, I/P injection of STZ at single dose 200 mg/ kg BW induce thrombocytopenia , this decrease refered to lowered concentration of insulin in STZ diabetic rat which decline the activation of platelets.

Additionally platelet count insignificantly changed in Gp-3& Gp-4 groups comparing with control. This may be due to the ability

of gliclazide to reduce platelet adhesion, aggregation and hyperactivity and enhances fibrinolysis by acting on tissue plasminogen activator (Konya et al., 2010).

Leukogram in our work showed significant decrease in WBCs count in STZ treated rats which can be explained by the suppression of the immune system , and decrease of leukocyte production from the bone marrow leading poor defensive mechanisms against infection caused through STZ injection. These results agree with Sellamuthu et al. (2014) and Oyedemi et al. (2010) who mentioned that,I/P injection of STZ induce significant decrease in leukocytic count.

WBCs count in diabetic rats treated with gliclazide significantly reduced as compared to untreated group .This effect may be due to gliclazide helps in reduction of the high blood glucose level , prevent suppression of immune system especially WBCs and leukocytosis from the bone marrow which leading to improve defensive mechanisms against infection (Oluwaseun et al., 2018). In the same line, gliclazide at a dose 150 mg/ kg daily for 3 weeks significantly reduced leukocyte adhesion to endothelial cells in hyperglycemia and prevented retinal leukocytosis in STZ diabetic rats(Kinoshita et al., 2002).

Significantly in this study, the ALT, AST and ALP activities in streptozocin treated groups at 4th weeks showed increase from the negative control at the end of the experiment, which may refered to escape of these enzymes from liver cytosol into the blood stream as an effect of hepatic injury. It indicates the hepatocellular damage followed by cardiac tissue damage and is usually accompanied by a rise in AST activity and also due to insulin deficiency(Al-Logmani and Zari, 2009; Maiti et al., 2013; Sheweita et al., 2016). these results are similar to those found by Navarro et al. (1993); Babu et al. (2007) who agreed that liver enzymes were elevated significantly in STZ diabetic rat at one dose(60 mg/kg BW,I/P) for 45 days. Likely ,Intraperitoneal injection of STZ as a single dose 40 mg / kg BW for 30 days significantly increase liver enzymes activites (Sellamuthu et al., 2014). Our result confirmed histopathologicaly as the liver of STZ treated rats showed degenerative changes of hepatocytes and dilation of hepatic sinusoids.

In our study, there were a significant decrease in ALT, AST and ALP levels in diabetic group treated with gliclazide at 4th week when compared with diabetic untreated group. It is an indicator of repair in the liver damage and hepato protective nature, which improved and explained by our histopathological results which cleared hydropy of hepatocytes with normal hepatic architecture. In the same line, Vijayaraghavan et al. (2012) ; Prasath and Subramanian, (2013) and Al Mamun et al. (2017) mentioned that the treatment with gliclazide significantly reduced the elevated activity of liver enzymes that demonstrate amelioration in liver damage ,compared with diabetic rats.

Moreover, there were a significant increase in liver enzymes in gliclazide treated group (Gp-III) when compared with control group.This can be explained by the long duration of gliclazide administration or over doses cause asymptomatic elevation of hepatic enzymes (ALT, AST and alkaline phosphatase) that may be induce damage to liver cells or liver injury.

The present study revealed that, induction of diabetes by streptozotocin reduces the total protein, albumin and globulin levels till the end of 4th week. This may be reaveled to lowering protein synthesis because the adverse affect of STZ on hepatocyte by inducing oxidative damage to liver that including hepatic dysfunction, leakage of hepatic enzymes that responsible for protein synthesis and loss of albumin due to the damage of the proximal tubule leads to microalbuminuria in the early stage of streptozotocin-induced diabetes in rats,So we found that tubular reabsorption of albumin

was decreased in the proximal tubule of diabetic rats through decrease in endocytosis of albumin associated with decreased expression of megalin and also presence of proteinuria is due to an increased filtration of albumin at glomeruli in advanced diabetic nephropathy (Tojo et al., 2001). Our results in aggrement with Rao et al. (2005) and Al-Logmani and Zari (2009) who approved that , serum total protein and albumin levels was significantly decreased by the induction of streptozotocin.. This shows the STZ diabetic rats had defective in kidney functions (nephropathy) due to heavy loss of blood protein (albumin and globulin). In reverse, STZ diabetic rats at dose (35mg/kg/BW) for 12 weeks significantly increased level of albumin compared to control rats (Akileshwari et al., 2014).

In our data we found that, the levels of total protein, albumin and globulin were significantly improved in diabetic group treated with gliclazide (STZ+GLIC) when compared with control group. This can be explained by the immunostimulatory action of gliclazide which elevate globulin than albumin this activate to synthesis immunoglobulin from globulin especially IgG and the reduction of the high blood glucose level ,prevent further renal damages which can also help to improve the filtration ability of the kidney to prevent occurance of proteinuria(Onozato et al., 2004).
In our work showed that, I/P injection of streptoztocin caused a significant elevation of serum creatinine and urea that serve as clinical indicator of kidney function when compared with control group. That is reflected in diabetic nephropathyt that characterized by structural alterations like glomerulosclerosis, mesangial cell expansion, podocyte loss , renal fibrosis and changes in glomerular filtration rate. STZ diabetic rats show also notable urinary glucose excretion and elevated creatinine clearance resembling an early state of diabetic nephropathy, called diabetic hyperfiltration. This increase in glomerular filtration rate in diabetes might be observed early in the course of diabetic kidney disease, Which lead to irreversible damage of nephrons and development of progressive renal disease. Our results confirmed histopathologically as kidney showed congestion of renal glomeruli and degenerative changes in renal tubular epithelium lining renal tubules . In agree with our results Luippold et al. (2016) reported that, STZ administration in rats induce a significant increase in serum creatnine. Likely, STZ-induced diabetic rats significantly increased levels of serum urea lead to hyperuricemia , creatinine (SCr) and renal damages **(**Palsamy et al., 2010; Jiang et al., 2014; Kumar et al., 2014 and Qusti et al., 2016).

In the current study we found that, treatment of the diabetic rats with gliclazide suspension at the end 4th week was markedly ameliorated the kidney function markers and prevented hyperglycemia that induced structural alterations in the kidney at the end of 4th weeks, and this appeared in our result histopathologically where the kidney showed normal renal glomeruli and normal renal tubules with normal lining renal tubular epithelium. So, gilclazide suspension has renoprotective effects in diabetic rats (Onozatoet al. 2004). Also these results are similar to those found by Al Hroob et al. (2018) who inspected that the serum urea&creatinine levels reverted to their normal value after gliclazide administration in STZ diabetic rats.
Dyslipidemia is a risk factor for the development and progression of diabetic kidney disease. In the present work, diabetic rats with STZ (55mg/kgBW,I/P) showed a significant increase in serum triglycerides ,total cholesterol and LDL-cholesterol as compared to the control rats. while, HDLcholesterol was significantly decreased, increase the value of lipoproteins above normal, identifying dyslipidemia as a risk factor for the development of atherosclerosis. There is a correlation

between
dyslipidemia and hyperglycemia because LDL-cholesterol is easily glycated and is more susceptible to oxidation in the subendothelial layer .Glycated LDL-cholesterol crosses the endothelial layer and interacts with oxidizing agents, turning it into oxidized LDL, identified as an invasive agent that promotes recruitment of macrophage, resulting in foam cells and formation of atherosclerotic plaque (Ou et al., 2003; Putta and Kilari, 2014; Ayyasamy and Leelavinothan, 2016; Al Hroob et al., 2018).Our work agreed with Pushparaj et al. (2000) and Putta and Kilari (2014) who mentioned that, administration of STZ for 12 weeks at the same dose induced abnormal high level of lipid serum. It is reported that hypercholesterolemia and hypertriglyceridemia which may be caused due to insulin deficiency in diabetic state,and subsequently lipoprotein lipase enzyme inactivation resulting to hypertriglyceridemia (Pyorala et al., 1987).

The current study clarify thus gliclazide administration in (STZ+GLIC) treated group improved the bad effect of STZ on serum lipid profile at the end of 4th week. Our result in accordance with Al Mamun et al. (2017) who reported that, administration of gliclazide was significantly enhanced the hyperlipidemia action induced by STZ. This effect may attributed to inhibiting intestinal cholesterol absorption, decreasing hepatic cholesterol biosynthesis and increasing hepatic uptake of circulatin LDL-cholesterol and increasing excretion of biliary acids and cholesterol. These results indicate that gliclazide treatment has a benefical effect for protecting against
the risk of diabetic kidney by ameliorating the lipid profile (Oluwaseun et al., 2018). Also this is in agreement with published results by,Noda et al. (1997) who observed that gliclazide administration to diabetic rat ameliorated the elevated serum level of all lipid profile parameters.

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia associated with absolute or relative deficiency in insulin secretion from the beta cells of pancreas and/or desensitization of insulin receptors (Vijayaraghavan et al., 2012). In the present study, STZ-treated rats showed a significant increase in blood glucose levels at the end of 4th week. This associated with injection of STZ causes necrosis of beta cells followed by hypoinsulinemia and hyperglycemia which it was in agreement with previous studies recorded by Sellamuthu et al. (2014); Al-Jameel and El-Rahman, (2017) and Al Hroob et al. (2018) who observed that IP injection of STZ at single dose significantly elevated the serum glucose level causig T2DM.

Achievement of good glycemic control is essential to prevent or delay diabetes complications. So, diabetic rats treated with gliclazide (Gp.4) showed a significant reduction in blood glucose level which may be caused by an increase peripheral glucose utilization and decrease hepatic gluconeogenesis (Al Hroob et al., 2018). This also stated by Bhandari and Pillai, (2005) and Oluwaseun et al. (2018) who clarified that , the blood glucose level of diabetic rats treated with gliclazide at different doses was significantly reduced when comparing with pathogenic group.

In this study the diabetic untreated group with STZ showed that, insulin level decreased comparing with all investigated groups 4th week, which may referred to the fact that IP administration of STZ destroys some of the pancreatic β-cells, resulting in insulin deficiency and type 2 diabetes. It also causes necrosis of pancreatic β-cells through DNA alkylation lead to generation of nitric oxide and reactive oxygen species (Nain et al., 2012), which confirmed by our histopathological finding which showing degenerative changes and necrosis of β cells at the end of 4th week. Also we parallel with Hamza et al. (2018) who approved that, the intraperitoneal administration of STZ causes impaired in the β -cell function lead to reduced insulin production and secretion.

This defects correlated with degenerated in islet cells of Langerhans in T2DM rats.

Referring to our work, oral administration of gliclazide significanlly increased the lowered level of insulin in STZ diabetic groups. This may be associated with stimulating insulin secretion and increasing glucose uptake *in vivo*. Additionally, our data in agreement with pulido et al. (1997) and Oluwaseun et al. (2018) who observed that, diabetic rats treated with gliclazide showed an increase in insulin level which stimulating glucose uptake compared with untreated diabetic rats. Also these results confirmed microscopically as pancrease show normal exocrine glands and normal endocrine gland having normal β cells at 4th week.

CONCLUSION

In conclusion, gliclazide has strong hypoglycemic effects, these results suggest that gliclazide exhibits renoprotective effects in STZ-induced diabetic rats. it ameliorated hyperglycemia, elevated liver enzymes and hypercholesterolemia. Since the use of gliclazide preserved renal and hepatic function and prevented kidney and liver damage in diabetic rat

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