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INTERNATIONAL JOURNAL OF CANCER AND BIOMEDICAL RESEARCH

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Metformin treatment improves the hepato-renal dysfunctions induced in Type-II diabetes in male rats

Mona M. Elwan, Eman E. El-Nahass, Mohamed A. Basyouny, Ehab O. Elshennawy, and Sabry A. El-Naggar

Zoology Department, Faculty of Science, Tanta University, Tanta, Egypt

■■■ ABSTRACT

Background: Metformin is one of the alternative drugs to treat Diabetic Mellitus type 2 (T2DM). One of the chronic symptoms of T2DM is the dysfunctionality of some vital organs. **Aim:** This study evaluated the role of metformin treatment to improve the liver and kidney functions of T2DM rats. **Material and Methods:** Forty rats were divided into four groups (n=10) as follows: group 1 (Gp1) was orally administered 200 µl of distilled water. Gp2 was administered metformin (200 mg/Kg) daily for two months. Gp3 and 4 were fed on a high-fat diet for 5 weeks, then the rats were injected with streptozotocin (STZ) (40 mg/Kg) once through intraperitoneal (i.p.). injection Gp4 was injected with STZ as in Gp3 then administered with metformin as in Gp2. All rats were sacrificed after 2 months to analyze the hematological, biochemical, and histopathological changes. **Results:** Metformin administration decreased the total body weight of both healthy and T2DM rats and improved the hepato-renal dysfunctions in T2DM rats as evidenced by decreasing the serum levels of ALT, AST, urea, and creatinine. Metformin also decreased the level of malondialdehyde ,activities of superoxide dismutase, and catalase, while increased the activity of glutathione. On the other hand, metformin increased the levels pof C-peptide and decreased glucose levels. Furthermore, histopathological investigations showed an improvement in the liver and kidney tissues upon treatment of T2DM rats with metformin. **Conclusion:** Long-term treatment of T2DM rats with metformin can improve hepato-renal dysfunctions.

Keywords: Hepato-renal dysfunction; histopathology; Metformin, Type 2 Diabetes

■■■ ARTICLE INFO

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease worldwide (Ahmed, 2002; Patlak, 2002). T2DM is known as a non-insulin-dependent type with a long-term metabolic disorder (Kumar et al., 2020). T2DM is caused by a combination of environmental, genetic, and behavioral risk factors (Chen et al., 2011). More than 90% of diabetic adults who are diagnosed with T2DM show progressive microvascular and macrovascular complications. Therefore, there is a need to gradually intensify therapy to achieve and maintain glycemic control (Brown et al., 2003; Schwartz and Katz, 2016; Rena et al., 2017). Insulin insensitivity is the main characteristic for T2DM patients, which may lead to decreased insulin production, and eventual pancreatic β-cell failure which in turn

decreases glucose transport into the liver, muscles, and fat cells (Fujioka, 2007; Cerf, 2013).

Metformin (Met) is well-known as an anti-hyperglycemic agent for T2DM management, which was originated naturally from glargine found in Galega officinalis (Bailey and Day, 2004). Met used as glucose-lowering agents through inhibiting gluconeogenesis in hepatocytes and decreases glucose absorption from the intestine (Viollet et al., 2012). Met is a unique anti-diabetic drug because it does not only cause severe hypoglycemia but is also able to improve protein synthesis. Met inhibits hepatic glucose synthesis, improves glucose uptake, increases insulin sensitivity, and reduces glucose absorption from the gastrointestinal tract (Brown et al., 2008). Met

activates adenosine monophosphate (AMP)-activated protein kinase and inhibits mitochondrial complex I, oxidative phosphorylation, and ATP production (Rena et al., 2017). Treatment with Met in elderly diabetic individuals with renal impairment could activate 5'-adenosine monophosphate protein kinase (AMPK), subsequently, switching off ATP-consuming anabolic mechanisms (Collier et al., 2006; Rena et al., 2017).

In addition to its use as an anti-diabetic drug, Met was recently used as in other clinical settings, including obesity, cancer, antioxidant, hepato-reno-protection (Scheen et al., 2015; Chukwunonso et al., 2016; Mobasher, 2021). Besides the previous uses of Met, it can be also be used as a cardio-protective agent, augment the immune system, and increase the efficiency of probiotics (Nasri et al., 2013). Recently, Met has been used in pre-clinical trials in patients with COVID-19 (Samuel et al., 2021).

Based on the above listed biological effects of Met, this study aimed to evaluate the role of long-term treatment with Met on the functionality of liver and kidney in T2DM induced in experimental rats.

MATERIALS AND METHODS
Chemicals

Streptozotocin (STZ) and Metformin (Met) were purchased from Sigma-Aldrich (France). Met was diluted by phosphate buffer saline (PBS), and the concentration was adjusted to 200 mg/kg b. w.t. in 200 µl for oral administration. Cholesterol was purchased from Sigma-Aldrich (St Quentin Fallavier, France). All biochemical kits including aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine, superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) kits were purchased from Biodiagnostic Company, Egypt.

Animals

Forty male Sprague Dawley rats (150 ± 5 g) were purchased from Helwan university. Rats were housed for 1 week at the Animal Facility, Faculty of Science, Tanta University, Egypt one week before experimentation. The experimental protocol was approved by the institutional Ethics Committee at faculty of Science, Tanta University, Egypt (No. IACUC-SCI-TU-00375). Rats were given drinking tap water and normal experimental pelleted animal food *ad libitum*. Normal diet composed of protein 21%, fats 3.2%, carbohydrates 68.2%, and fibers 3.44%. High fat diet (HFD) consisted of 68% powdered normal diet, 30% melted animal abdominal fats until become homogenous in dough-like consistency and 2% extra pure cholesterol (El-Wakf et al., 2015).

Induction of T2DM in rats: (Furman, 2021)
Experimental protocol

The rats were divided into four groups (*n* = 10/each). Group 1 (Gp1): served as a control rats that were administered orally with 200 ml distilled water. Gp2: Rats were administered with Met (200 mg/Kg), daily for 60 consecutive days. Gp3: Rats that were fed on high-fat diet (HFD) and then treated via intraperitoneal (i.p.) injection of with a single dose of STZ (40 mg/Kg). Gp4: HFD-rats were injected i.p. with STZ as in Gp3 and then administered with Met as in Gp2. At the end of the experiment, all rats were sacrificed after 2 months under ethyl proper anaesthesia and gross examinations were performed macroscopically on all groups during the sacrifice.

Determination of the percentage of the change in total body weight

Rats were weighed at the beginning and at the end of the experiment. The percentage of the change in the total body weight (% T.B.W.) was determined as (final b.wt − initial b.wt / initial b.wt) × 100.

Hematological and biochemical analysis

The total count of red blood cells (RBCs), hemoglobin content (Hb), hematocrit (Hct%), blood platelets and the total count of white blood cells (W.B.Cs) were determined by using an auto-hematology analyzer (BC-3200, Mindray, China). ALT and AST were determined as described by Reitman and Frankel, (1957). Serum cholesterol, triglycerides, and HDL were determined using a quantitative kit based on the previously described methods (Burstein et al., 1970; Allain et al., 1974; Fossati and Prencipe, 1982). Low-density lipoprotein cholesterol (LDL) was calculated according to Friedewald et al. (1972) as follows: LDL=total

cholesterol–HDL–VLDL. Malondialdehyde (MDA) was assessed based on the methods of Li and Chow (1994). Superoxide dismutase (SOD) activity was assessed as described by Paoletti and Mocali (1990). Catalase activity was measured following the methodology of Aebi (1984). According to Paglia and Valentine (1967), the level of reduced glutathione (GSH) was determined.

Histopathological investigations

Tissue specimens of the livers and kidneys were fixed in 10% formalin. Tissues were processed in different grades of alcohol and xylene. Sections (5μm) were prepared from paraffin blocks using a microtome (mark, company), stained with Haematoxylin and eosin H&E, and observed under a light microscope (Optica light microscope, B- 350) to examine gross cellular damage according to Bancroft and Layton (2012).

Statistical analysis

All data are presented as mean (M) ± standard deviation (SD). One–way analysis of variance (ANOVA) was applied to determine the significant differences among treatment groups. If there a significant difference between means, Tukey post hoc comparisons among different groups were performed. For all statistical tests P values ≤ 0.05 was considered to be statistically significant. Data and statistical analysis were performed using Excel 365 (Microsoft Corporation, USA), and Minitab version 19).

RESULTS

Treatment with Met decreased the body weight change in T2DM rats

The body weight of the control group increased from 175 g to 195 g from wk-5 to wk-14. A slight decrease in the final body weight was noticed in the group of rats injected with STZ (40 mg/Kg). Treatment of T2DM rats with Met led to a significant decrease in the body weight when compared to T2DM rats (Figure 1). Naive Healthy rats treated with Met for two months did not show change the relative liver and kidney weight. In T2DM rats administered with Met or not, the relative liver and kidney weight did not show change a significant change when

compared to the control group (data not shown).

Effect of Met on the hematological parameters in T2DM rats

The results showed that the Hb level, total RBCs counts and Hct % value did not show a significant change among all groups under the study. However, the white blood cells (WBCs) and platlets counts dercreased significantly in T2DM-rats and T2DM-rats treated with Met (Table

1).

Effect of Met on the liver and kidney functions in T2DM rats

As compared to the untreated T2DM rats, the levels of liver transaminases (ALT and AST) in the group of rats that were administrated Met alone did not show significant changes. However, the levels of these enzymes were increased in T2DM rats when compared to the control rats. Treatment of T2DM rats with Met led to significant decreases in the activities of ALT and AST enzymes when compared with T2DM rats alone. The levels of urea and creatinine did not show significant changes in rats that were administrated with Met when compared to the control rats (Table 2). In T2DM rats, however, the levels of urea and creatinine were increased. Administration of Met into T2DM rats decreased both urea and creatinine levels when compared to T2DM rats (Table 2).

Effect of Met on the lipids profile in T2DM rats

The results showed that the levels of cholesterol, triglycerides, LDL, HDL, and VLDL in the group of control rats administered with Met for two months did not show significant changes when compared their levels in the control group. However, the levels of these parameters wereincreased in the T2DM rats when compared to control rats. Administration of Met into T2DM rats decreased the levels of cholesterol, and LDL but increased the triglycerides level as compared to untreated T2DM rats. The level of HDL was increased in T2DM/Met groups when compared to the control group (Table 3).
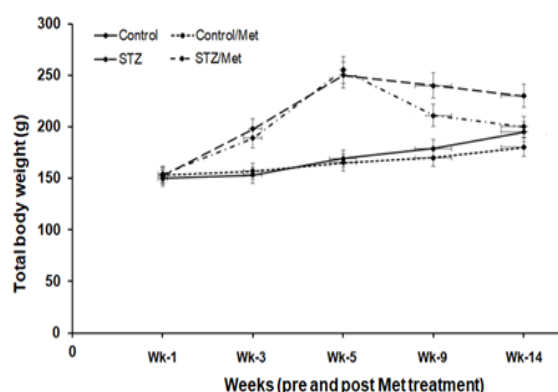
Figure 1. Kinetic of body weight change of both naïve and T2DM rats post treatment with Met for two months .

Effect of Met treatment on the oxidative stress biomarkers in the liver tissues of T2DM rats

The results showed that the activities of SOD, CAT, GSH and MDA in the liver tissues of control rats administered with Met did not show significant changes when compared to the control group. In T2DM rats, the activities of SOD, CAT, and GSH in T2DM rats were significantly decreased; while the activity of MDA was significantly increased. T2DM rats administered with Met showed improvement in the antioxidant status of their liver tissues as indicated by the increase in the activities of SOD, CAT, and the decrease in levels of MDA (Table 4).

Effect of Met treatment on C-peptide and glucose levels in T2DM rats

The C-peptide level was increased in rats administered with Met alone; however, its levels was significantly decreased in T2DM rats. Administration of T2DM rats with Met induced increases in the level of C-peptide (Figure 2). In contrast, the levels of glucose in diabetic rats administrated with Met were increased when compared to control rats and rats administrated with Met alone (Figure 2).

Histopathological investigations of liver tissues

Examination of the liver sections of the control group (Gp1) showed normal architecture of hepatocytes radiation from the central vein. The liver strands were alternating with narrow blood sinusoids lined by endothelial cells and distinct phagocytic Kupffer cells (Figure 3A). Liver sections of Met-treated mice showed normal central vein. Normal hepatic lobulation, binucleated hepatocytes and regular blood sinusoids with distinct phagocytic Kupffer cells were distinct (Figure 3B). The liver sections of T2DM rats (G3) exhibited marked disorganization of hepatic architecture, congested central veins, cellular infiltration, some hepatocytes showed eosinophilia, and others are degenerated with darkly stained nuclei (pyknotic). Also, irregular and dilated blood sinusoids were noticed (Figure 3C). While treatment the mice with T2DM/Met (G4) showed normal hepatic architecture, some hepatocytes showed megakaryocytic nuclei with faint stained ones (karyolitic), and slight apparent irregular and dilated blood sinusoids with distinct Kupffer cells were seen (Figure 3D).

Histopathological investigations of kidney tissues in T2DM after Met treatment

Normal histological structure of the kidney tissue of the control group (G1) exhibited normal renal parenchyma that appeared as the cortex region, normal glomeruli with normal bowman's corpuscle, and normal renal tubules (Figure 4A). Kidney sections of the Met treated group (G2) exhibited normal architecture with no visible lesions; some renal tubules were intermixed with each other's (Figure 4B). kidney sections of T2DM rats treated group of mice (G3) showed disorganized and destructed glomeruli with distorted Bowman´s capsule with widening space, most of the renal tubules were disorganized, dilated, destructed, and degeneration of the epithelial cells lining was exhibited with hyaline casts (Figure 4C). While kidney sections of T2DM/Met rats treated group (G4) exhibited normal-like structure of the glomeruli with regular Bowman´s space (Figure 4D).

DISCUSSION

Patients with T2DM are more vulnerable to several complications such as cardiovascular disease, obesity, retinopathy, and neuropathy (Chen et al., 2011). Met was used in malaria, influenza, and adult-onset diabetes treatments and to manage hyperglycemia in T2DM. Chemically, Met is a hydrophilic base with rapid passive diffusion through cell membranes (Graham et al., 2011).
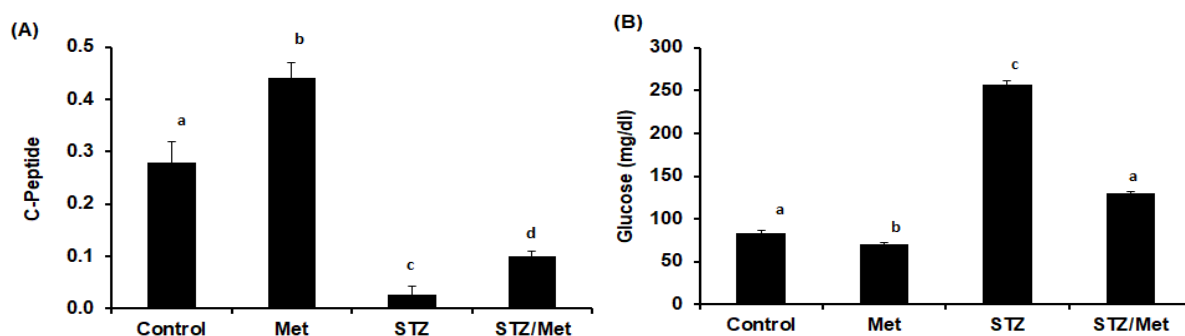
Figure 2. Shows the level of C-peptide (A) and glucose levels (B) in the different group under study.

Table 1. The hematological parameters in different groups of rats treated with Metformin.

Groups	Hb (g/dL)	RBCs (x10^6 /µL)	Hct (%)	WBCs (x10^3 /µL)	Plat. (x10^3 /µL)
Control	11.5 ± 0.35	8.7 ±0.41	38.6 ± 3.3	12.8 ± 0.5[a]	881.6 ± 30.4[a]
Met alone	11.3 ± 0.5	8.7 ± 0.1	41.9 ± 2.5	12 ± 0.8[a]	740 ± 38.2[b]
STZ	12.3 ± 0.45	8.5 ± 0.25	44 ± 2.57	9.5 ± 0.36[b]	730 ± 33.6[b]
STZ/Met	12 ± 0.35	8.3 ± 0.26	45 ± 3.93	6.5 ± 0.56[c]	727 ± 30.5[b]
P-value	0.07 n.s.	0.305 n.s.	0.138 n.s.	< 0.001	< 0.001

Means that do not share a letter are significantly different (Tukey's test, p < 0.05). Met: Metformin, STZ: streptozotocin, STZ/Met, streptozotocin/Metformin.

Table 2. Alanine transaminases (ALT), aspartate transaminase (AST), urea and creatinine levels in the sera of different groups under study.

Group	ALT (U/L)	AST (U/L)	Urea (mg/dl)	Creatinine (mg/dl)
Control	49.3±4.16[c]	130.0±2.65[b]	22.00±2.0[c]	0.62±0.10[b]
Met	47.3±8.39[c]	128.0±2.00[b]	25.00±2.0[c]	0.72±0.03[a,b]
STZ	97.7±5.9[a]	183.0±2.65[a]	40.00±2.[a]	0.90±0.10[a]
STZ/Met	80.3±3.06[b]	131.3±3.06[b]	32.67±2.08[b]	0.67±0.12[a,b]
P-value	< 0.001	< 0.001	< 0.001	0.03 n.s.

Means that do not share a letter are significantly different (Tukey's test, p < 0.05). Met: Metformin, STZ: streptozotocin, STZ/Met, streptozotocin/Metformin.

Table 3. Cholesterol, triglycerides (TG), high density lipoprotein (HDL-C), low density lipoprotein (LDL-C) and very low density lipoprotein (VLDL) levels in the different groups under the study.

Group	Cholesterol (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL (mg/dl)
Control	43.3±1.5[c]	53.00±2.65[b]	38.3±1.15[a,b]	10.00±1.0[c]	14.00±1.0[b]
Met	41.0±1.0[c]	51.00±1.00[b]	36.3±1.5[b]	7.00±1.0[c]	11.00±1.0[c]
STZ	76.3±3.1[a]	60.67±2.08[b]	36.0±2.7[b]	20.67±1.5[a]	12.33±0.6[b,c]
STZ/Met	53.7±1.5[b]	79.33±7.02[a]	43.0±2.7[a]	16.54±1.9[b]	17.00±1.0[a]
P-value	< 0.001	< 0.001	0.013	< 0.001	< 0.001

Means that do not share a letter are significantly different (Tukey's test, p < 0.05). Met: Metformin, STZ: streptozotocin, STZ/Met, streptozotocin/Metformin.

Table 4. Malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) levels in the liver tissues of the different groups under study.

| Group	MDA (nmol/g)	SOD (U/g)	CAT (U/g)	GSH (mg/g)
Control	34.75±1.71[c]	41.25±2.22[a]	84.00±4.08[a]	12.50±1.29[a]
Met	39.50±1.29[c]	41.50±1.29[a]	78.50±1.29[a]	11.00±0.82[a,b]
STZ	79.50±2.65[a]	26.25±2.22[c]	53.25±2.75[c]	5.35±0.51[c]
STZ/Met	50.50±1.29[b]	36.00±2.16[b]	70.50±1.29[b]	9.75±1.32[b]
P-value	< 0.001	< 0.001	< 0.001	< 0.001

Means that do not share a letter are significantly different (Tukey's test, p < 0.05). Met: Metformin, STZ: streptozotocin, STZ/Met, streptozotocin/Metformin.
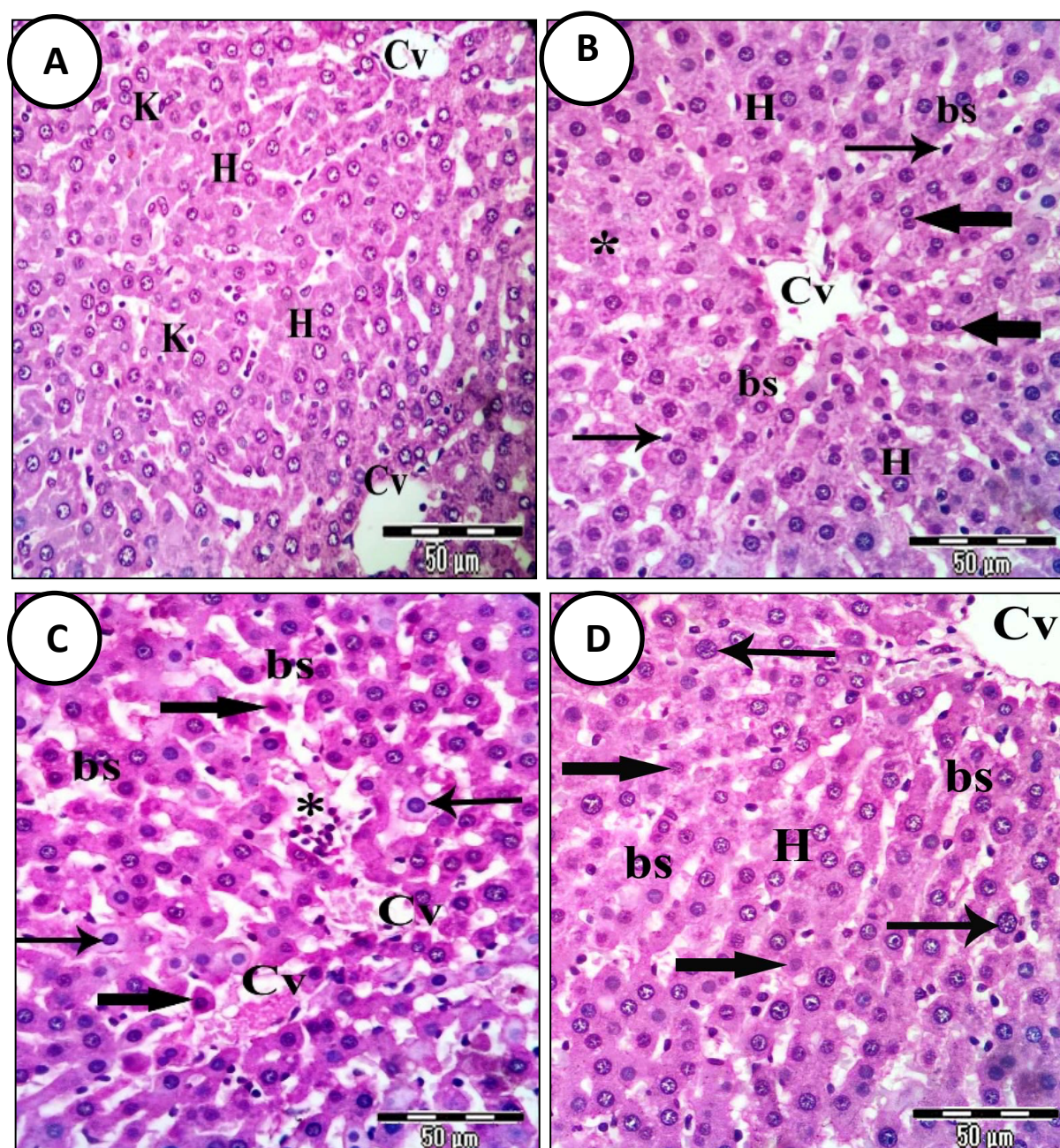
Figure 3. Photomicrographs of the liver sections stained with Haematoxylin & Eosin. (A) The liver section of the control group of mice (G1) exhibits normal hepatic structure, central vein (Cv), radial hepatic strands (H), and regular blood sinusoids (bs) lined with normal kupffer cells (K). (B) The liver section of Met-treated group showing the central vein (Cv), some of hepatocytes (H) are binucleated (thick arrows), and irregular blood sinusoids with distinct kupffer cells (arrows) as well as necrotic area (*). (C) The liver section of STZ treated mice (G3) exhibits marked disorganization of hepatic architecture, congested central veins (Cvs), cellular infiltration (*), some hepatocytes show eosinophilia (thick arrows), others are degenerated with pyknotic nuclei (thin arrows). Also, irregular, and dilated blood sinusoids (bs). (D) The liver section of STZ/Met-treated group (G4) showing normal-like structure of hepatic architecture, normal strands of hepatocytes (H), some hepatocytes with megakaryocytic nuclei (arrows), others with karyolitic ones (thick arrows), slightly irregular and dilated blood sinusoids (bs) with kupffer cells (x 400).
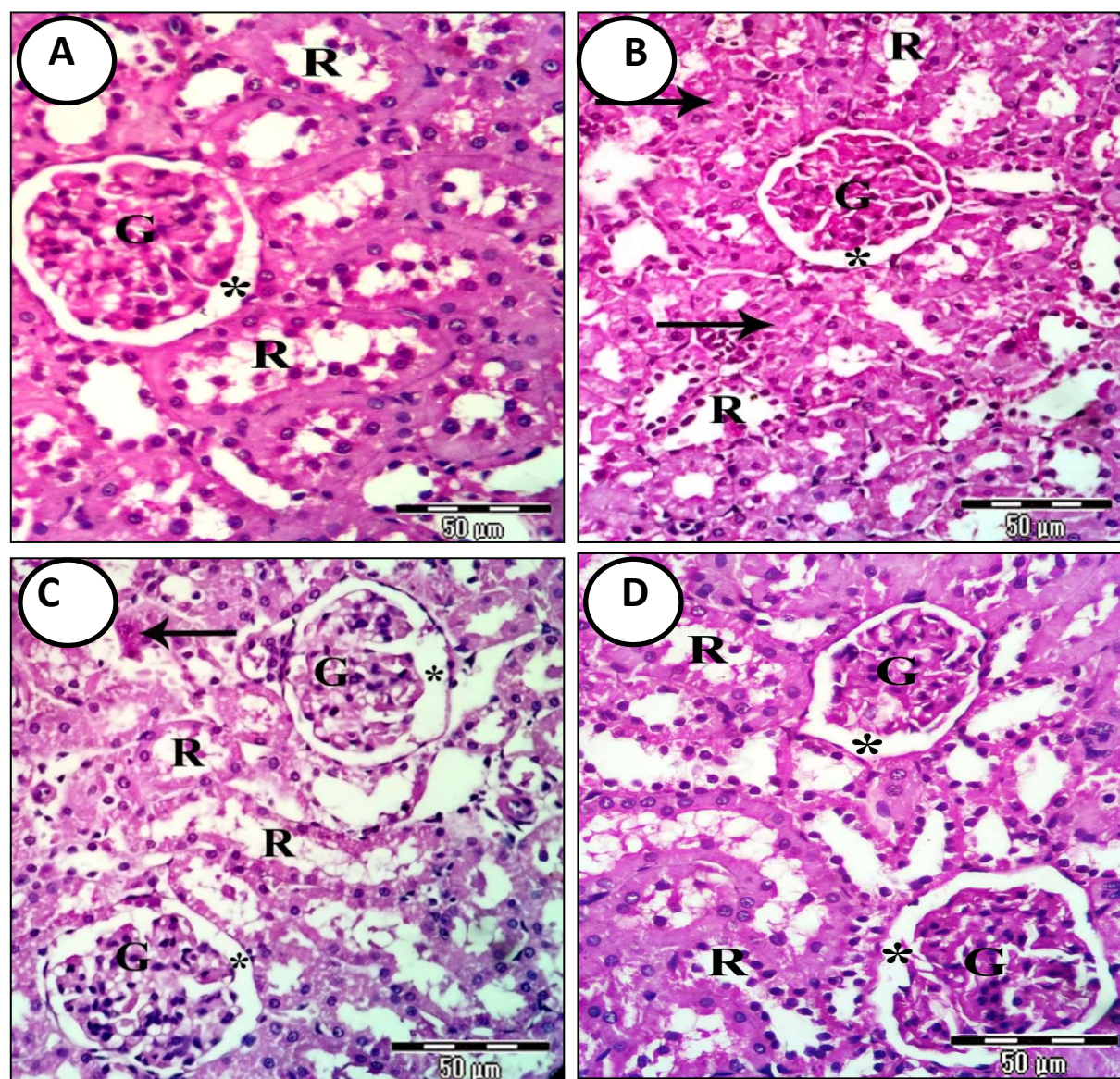
Figure 4. Photomicrographs of kidney sections stained with H&E. (A) Kidney section of the control group of mice (G1) reveals normal glomeruli (G) with Bowman´s space (*) and regular renal tubules (R). (B) Kidney section of the Met-treated group (G2) showing intact glomeruli (G), regular Bowman´s space (*), some renal tubules were intermixed with each other (arrows). (C) The kidney section of STZ-treated group (G3) exhibits disorganized and destructed glomeruli (G), widening of Bowman´s space (*), dilation of the renal tubules (R), mostly tubules lost their characteristic appearances, degeneration of the lining epithelial cells and existence of hyaline casts. (D) Kidney section of STZ/Met treated group (G4) showing normal-like structure of the glomeruli (G), a slight widening of the Bowman´s space (*), and more or less normal renal tubules (R), (x 400).

Met is commonly used for the treatment of T2DM patients and prediabetic patients (Ortega et al., 2014). Compared to insulin and chlorpropamide therapy, Met have shown better control of diabetes complications and mortality (Rafieian-Kopaei and Baradaran, 2013). Upon Met treatment, the incidence of T2DM decreased by 34% (Nasri e al., 2013). It has been reported that Met is also able to reduce the serum glucose levels in T2DM patients (Konopka et al., 2019). It also decreased the capability of hepatocytes to produce glucose via activation of AMPK. Furthermore, in muscle tissues, Met stimulates glucose transport and insulin signaling (Seo-Mayer et al., 2011). A clinical study also

supported the capability of Met to reduce the incidence of angina, myocardial infarction, peripheral vascular disease, stroke, and sudden death in T2DM patients (Kim et al., 2015). Our data showed that the Hb level, total RBCs counts, and Hct% value didn´t show a significant change among all groups under the study. However, the WBCs and platelets counts decreased significantly in T2DM-rats and T2DM-rats treated with Met. This study clearly showed that the long-term treatment of T2-DM with Met did not cause any significant changes in the hematological parameters. These finding was in agreement with previous studies by (Muhammad et al. 2012; Charity et al. 2019; Fagbohun et al. 2020.

This study investigated the efficacy of Met treatment on the liver and kidney functions in diabetic rats. The data showed that ALT and AST levels were increased significantly in the T2DM group when compared to the control. Treatment of T2DM rats with Met led to a significant decrease in ALT and AST enzymes when compared with T2DM-rats alone. The levels of urea and creatinine were increased in T2DM rats. Administration of Met in T2DM rats decreased both urea and creatinine levels when compared to T2DM rats alone. These findings agreed with previous studies by (Khadre et al. 2011; Albasher et al. 2020). In previous studies Met administered for short terms. However, few studies explained the effects of Met treatment in long period. Therefore this study extended to find the possile toxic effect of Met on the hepato-renal biochemical and histological parameters.

Cholesterol, LDL, non-HDL (VLDL+LDL), and triglycerides levels were increased in the diabetic rats. Met administration decreased the levels of cholesterol, and LDL but increased the triglycerides level as compared to T2DM rats only. The level of HDL was increased in T2DM/Met groups when compared to the control group, these lipids profiling were compatible with previous studies that investigated lipids levels upon treating diabetic rats with Met (Anurag and Anuradha, 2002; Mishra et al., 2019).

Furthermore, the results showed that SOD, CAT, GSH, and MDA in liver tissues of control rats administered with Met did not show a significant changes when compared to the control group. The activities of SOD, CAT, and GSH in STZ-induced in T2DM rats were significantly decreased; however, the MDA activity was significantly increased in the diabetic group. STZ-induced rats administered with Met showed improvement in the antioxidant status of their liver tissues evidenced by the increase in the SOD, CAT activities, and decrease in MDA activities. These findings were in line with previous studies that reported the oxidants/antioxidants status were increased in diabetic rats treated with Met (Vilela et al., 2016 and Mobasher et al., 2020).

The present study indicated that diabetic rats showed hematological, biochemical, and histological alterations in the liver and kidney tissues as compared to the control and Met treated groups. The prominent histopathological alterations due to T2DM induction are represented by marked disorganization of hepatic architecture and congestion of blood vessels. Some hepatocytes showed hyper-eosinophilia, others showed degeneration with pyknotic nuclei, disorganization of the glomeruli, destruction, and dilation of the renal tubules. While TD2M/Met group showed improvement in the hepatic and renal tissues as denoted by normal hepatic strands, some hepatocytes showed mega karyolitic nuclei, others showed karyolitic ones, others are dilated with pyknotic nuclei. The same findings were reported by Al-amri et al. (2020) who found that diabetes experimentally induced in rats caused severe congestion in the glomeruli, as well as swelling in the tubule epithelium.

However, rats post-treated with Met revealed enhancement in the pathological changes in the kidney of rats evidenced by the typical structure of the kidney. But these results unparalleled to that attained by Adaramoye et al. (2012) who recorded that Met hydrochloride and glibenclamide had a toxic effect on some organs of male rats. We believed that short-term treatment with a therapeutic dose of met might not show the obvious toxic effect. While the long-term treatment could show toxic effects if present.

CONCLUSION

Treatment of diabetic rats (T2DM) with Met for long period improves hepato-renal dysfunctions as evidenced by restoration of some hematological, biochemical, and histopathological alterations.

CONFLICT OF INTEREST

All authors declare no conflicts of interest.

FUNDING

No fund was received for this work.

AUTHOR CONTRIBUTION

All authors conceived, designed the study, and contributed to manuscript revisions

DATA AVAILABILITY

All data generated or analyzed during this study are included in this published article. More detailed data is available from corresponding author on reasonable request.

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