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Research Article

Evaluation of Hepatic Histopathological Changes in Empagliflozin-treated Male Rats with Alloxan-Induced type 2 Diabetes

Amal Mahmood Alwan[1], Raya Zaid Ali[1], Abthal Noori Mahdi[1], Aya Azam Jamal[2], Dorees Taher Hasan[2], Wurood Khalid Mohammed[1]

[1]Departement of Biology, College of Education for Pure Science, University of Diyala, Diyala, Iraq
[2]College of Education for Pure Science, University of Diyala, Diyala, Iraq

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Abstract

Empagliflozin is a selective sodium-glucose cotransporter-2 (SGLT2) inhibitor widely used in the management of type 2 diabetes (T2DM). In addition to its glucose-lowering activity, empagliflozin possess anti-inflammatory and metabolic regulatory properties. Diabetes is a chronic metabolic disorder characterized by persistent hyperglycemia and is frequently associated with structural and functional alterations in multiple organs, including the liver. This study evaluated the histopathological effects of empagliflozin on hepatic tissue in an alloxan-induced diabetic rat model. Fifteen adult male rats were randomly assigned into three groups (n = 5 per group): a healthy control group, an untreated diabetic group, and a diabetic group treated with empagliflozin (5 mg/kg/day) for 30 days. Diabetes was induced using alloxan monohydrate, and liver tissue was subsequently collected for histopathological examination using hematoxylin and eosin staining. Microscopic examination revealed severe hepatic alterations in untreated diabetic rats, including hepatocellular necrosis, central vein congestion, sinusoidal dilatation, hemorrhage, nuclear degeneration, and disruption of normal hepatic architecture. In the empagliflozin-treated group, partial preservation of liver structure was observed; however, several pathological features persisted, including inflammatory cell infiltration, sinusoidal hemorrhage, hepatocellular hypertrophy, nuclear pyknosis, and early hepatic steatosis. Although empagliflozin treatment appeared to attenuate some histopathological abnormalities, complete restoration of normal liver structure was not achieved within the experimental period. The findings suggest that the observed hepatic injury was primarily associated with alloxan-induced toxicity and diabetes-related pathologic processes. Under the conditions of this study, empagliflozin exhibited limited hepatoprotective activity despite modest preservation of hepatic architecture. Further studies involving larger sample sizes, extended treatment durations, and molecular investigations are required to clarify the potential role of empagliflozin in preventing or ameliorating diabetes-associated liver injury.

Corresponding Author: Raya Zaid Ali, Department of Biology, College of Education for Pure Science, University of Diyala Diyala Governorate, Iraq. ***Email***: raya.zaid@uodiyala.edu.iq

1. INTRODUCTION

Diabetes is one of the most important public health problems and can be considered a silent killer because of the delay in its detection during the initial stages [1]. This disease highlights metabolic disorders characterized by increased glucose levels. The World Health Organization (WHO) diagnosed individuals with diabetes with urination, dry mouth, weakness, blurred vision, decreased wound healing rate, tingling in the hand and foot, pain, or numbness and stated that diabetes is a widespread phenomenon with a global impact [2]. Diabetic nephropathy (DN), a prominent consequence of diabetes, can cause end-stage renal disease or cardiorenal death [3]-[4]. Diabetes has been classified into four types, with type 2 diabetes (T2DM) being the most common, accounting for approximately 95 percent of all types of diabetes [5]-[6]. Potential mechanisms of metabolic stress-induced cell death in type 2 diabetes include endoplasmic reticulum (ER) stress, oxidative stress [7], and ceramide production (Sokolowska et al., 2019), culminating in ER pathway activation. Endogenous apoptotic cells or mitochondria [8]-[9]. Empagliflozin, a selective sodium-glucose cotransporter 2 (SGLT2) inhibitor, is a relatively new class of antidiabetic medications with anti-inflammatory and metabolic regulation properties, in addition to lowering blood glucose levels [10].

It prevents transporters in the kidneys from reabsorbing glucose and has been shown to significantly reduce the risk of heart disorders and death from cardiovascular disease in people with and without diabetes, making these medications a good choice for people with CHF. It has beneficial properties, especially in the prevention of HF, which are independent of its hypoglycemic effects [11]. Sodium-glucose cotransporter-2 inhibitors were a new class of diabetes drugs. SGLT inhibition leads to sodium and glucose reabsorption in the proximal convoluted tubule of the kidney [12]. Empagliflozin inhibits sGLT $4, increases urinary glucose excretion, and reduces renal glucose reabsorption. The diuretic and natriuretic properties of empagliflozin cause intravascular spasms by reducing salt and iodine volume. In addition, empagliflozin is associated with weight loss and lower blood pressure without an increase in heart rate [13]. This study aimed to examine the histological effects of empagliflozin on the liver in an alloxan-induced diabetic rat model to determine its safety beyond glycemic control and to clarify its possible involvement in modifying diabetes-associated hepatic changes.

2. METHOD

2.1. Induction of diabetes

Five male rats were isolated for the control group (without any treatment), and the remaining 10 rats were injected subcutaneously with alloxan, which is of Indian origin, at a full dose of 150 mg/kg of rat weight, where 1 gm was dissolved. Alloxan in 10 mL of the physiologically undefined solution. The animals were starved for 24 h before 10 mL of physiologically defined saline. After injection, they were provided with food and allowed to drink a glucose solution containing percentage 5 to prevent hypoglycemia. After 72 h, the blood glucose level was checked using a German-made diabetes device to confirm the presence of diabetes.

2.2. Distribution division and of the animals

In this study, 15 white male rats were housed inside cages at the Department of Life Sciences, College of Education for Pure Sciences, University of Diyala. They were 3 - 4 months old, and their body weight ranged between 167 and 200g. Subsequently, was divided into three groups. Throughout the experiment, a physiologically undefined solution was administered to the control group. The second group was divided into two groups that were injected with alloxan to induce diabetes. The first secondary group had induced diabetes, whereas the second secondary group had diabetes and was treated with the drug empagliflozin at a concentration of 5 mg/kg of animal weight, which was continued for 30 days, and the amount of the drug that was dosed to the rat used was calculated. In this study, according to the following equation:

$$X/D = WRat/1000 \ [1]$$

AS X: the amount of drug that must be injected into experimental animals, measured in mg / kg of body weight.
D: The specified drug dose is [5] mg / kg of body weight
W: Rat weight used in the experiment.
At the end of the experiment, all animals were dissected, the organ under study was removed, and tissue sections were prepared. A 10% formalin solution was used to preserve the samples for 24 h, after which the water tap was opened to wash them, and then it was transferred to a concentration of 70% alcohol to preserve the samples. All the steps required to prepare the slides were performed. The slides were stained with hematoxylin–eosin and examined and photographed using an optical microscope equipped with a digital camera.

3. RESULTS

The results of the current study showed that inducing diabetes in male rats for 30 days led to a group of changes in the liver compared with the liver of males in the control group. These changes include the appearance of severe necrosis of hepatic cells, congestion of the central vein, bleeding within the sinusoids and their expansion, and death of most liver nuclei. Hepatocytes burst and necrotize the central vein wall, and active transport vesicles appear, as shown in Fig 1.

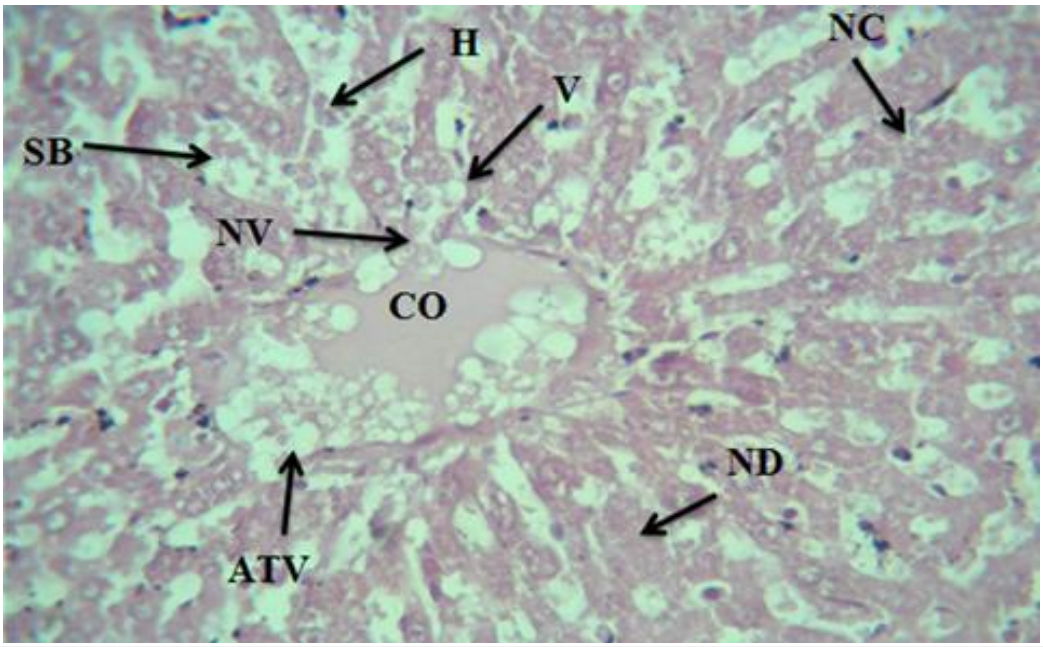

Figure 1: Cross-section of the liver tissue of male rats treated for 30 days with alloxan-induced diabetes. It shows the occurrence of congestion of the central vein CO, severe necrosis and degeneration of hepatocytes (NC), necrosis of the wall of the central vein (NV), death of the nuclei of most hepatocytes (ND), dilatation of the sinusoids (SB), and hemorrhage. H, ATV active transport vesicles (H&E 40).

The current results also showed that the histological changes in the liver in the experimental group with induced diabetes were disorganized hepatic cells and sinusoids in the form of ropes, but they were irregular, death of some nuclei in the hepatic cells, with the appearance of fine granules scattered in the hepatic cells; and a decrease in the number of Kupffer cells, as shown in Figure 2.

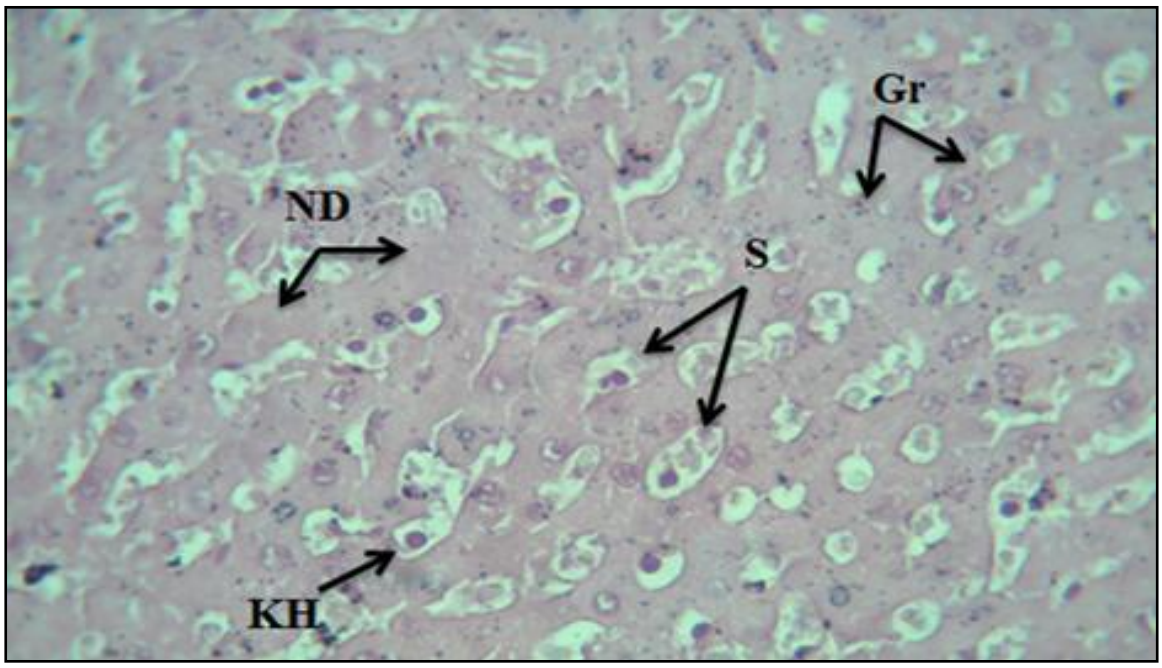

Figure 2: Irregularity of the blood sinusoids in the liver tissue of male rats with induced diabetes, appearance of fine granules in the hepatic cells (GR), death of most (ND) cell nuclei, and enlargement of Kupffer cells (KH). (H&E 40X).

The results of the current study also showed that in the liver of male white rats with diabetes induced by alloxan during the experiment period of 30 days, there were histopathological changes in the liver that included congestion of the central vein, necrosis of its wall, the occurrence of necrosis, rupture, intrasinusoidal hemorrhage, and death of nuclei in some hepatic cells, as shown in Figure 3.

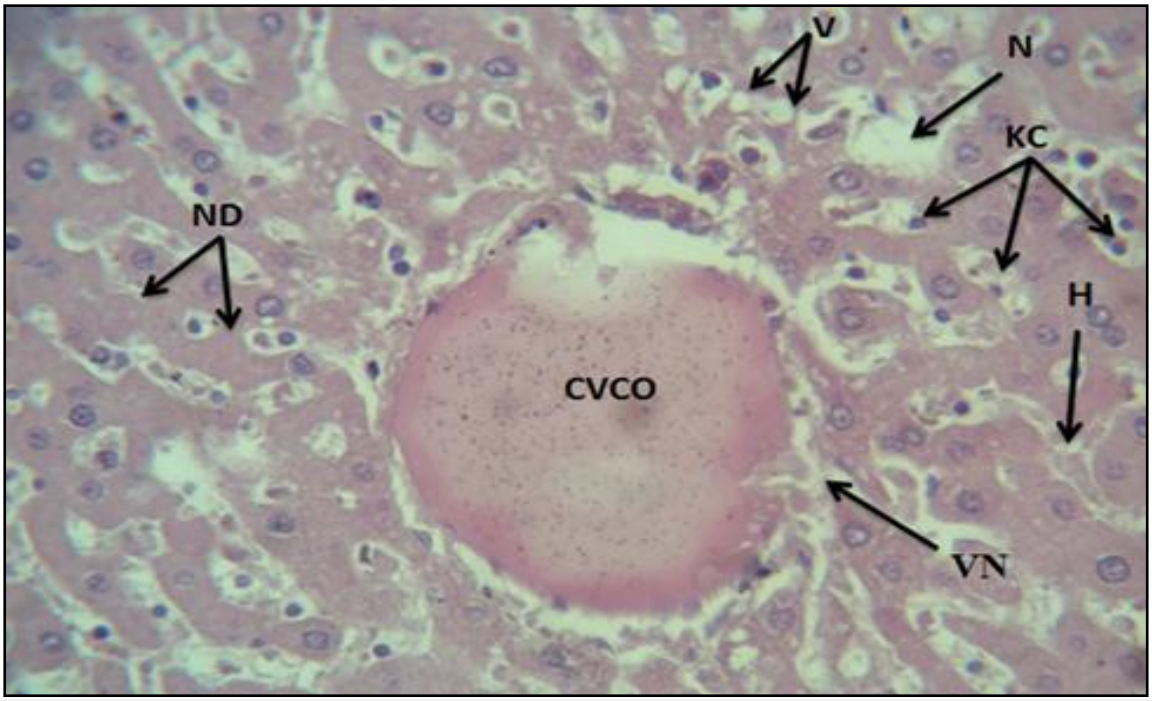

Figure 3: A cross-section of liver tissue in male diabetic rats treated with 5 mg / kg empagliflozin. It shows severe central vein congestion (CVCO), hemorrhage (H), Kupffer cells (KC), vascular changes (V), necrosis (N), nuclear death (ND), and vessel necrosis (VN). Color (H&E, 40)

We also observed that liver tissue in the diabetic group treated with empagliflozin exhibited histopathological damage, characterized by infiltration and accumulation of inflammatory cells around the central vein, hemorrhage, dilation of the hepatic sinusoids, nuclear pyknosis and necrosis, hepatocellular enlargement, and the presence of tissue ruptures, as shown in Figure 4.

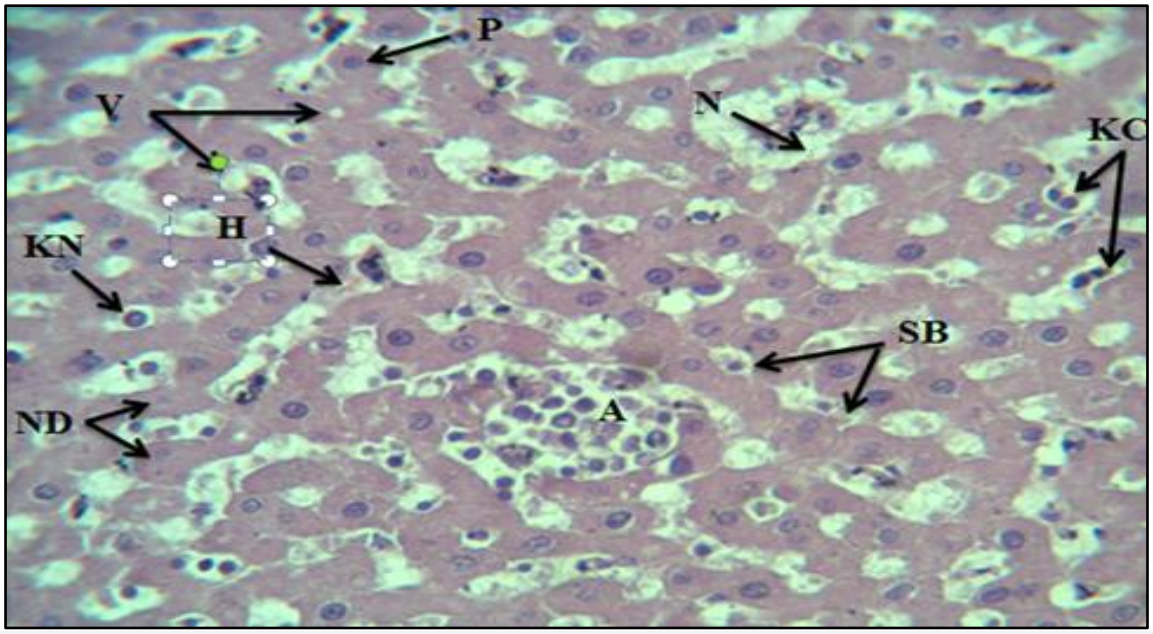

Figure 4: Liver sections from diabetic rats treated with empagliflozin for 30 days. Histopathological examination revealed infiltration and aggregation of inflammatory cells (A), hemorrhage within the hepatic sinusoids (H), tissue rupture (V), necrosis (N), nuclear degeneration/death (ND), irregularity of the hepatic sinusoidal cords (SB), nuclear pyknosis (P), a reduced number and enlargement of Karl Wilhelm von Kupffer cells (KC), and Kupffer cell necrosis (KN). Hematoxylin and eosin (H&E) staining, 40 × magnification.

The results of the study also showed that liver tissue from diabetic male rats treated with empagliflozin exhibited lipid accumulation, which indicates of hepatic steatosis (fatty liver). Histopathological examination revealed dilated hepatic sinusoids containing lipid deposits, infiltration and enlargement of Kupffer cells, more Kupffer cells, hypertrophy of some hepatocytes, and nuclear pyknosis in some cells. These findings are illustrated in Fig. 5.

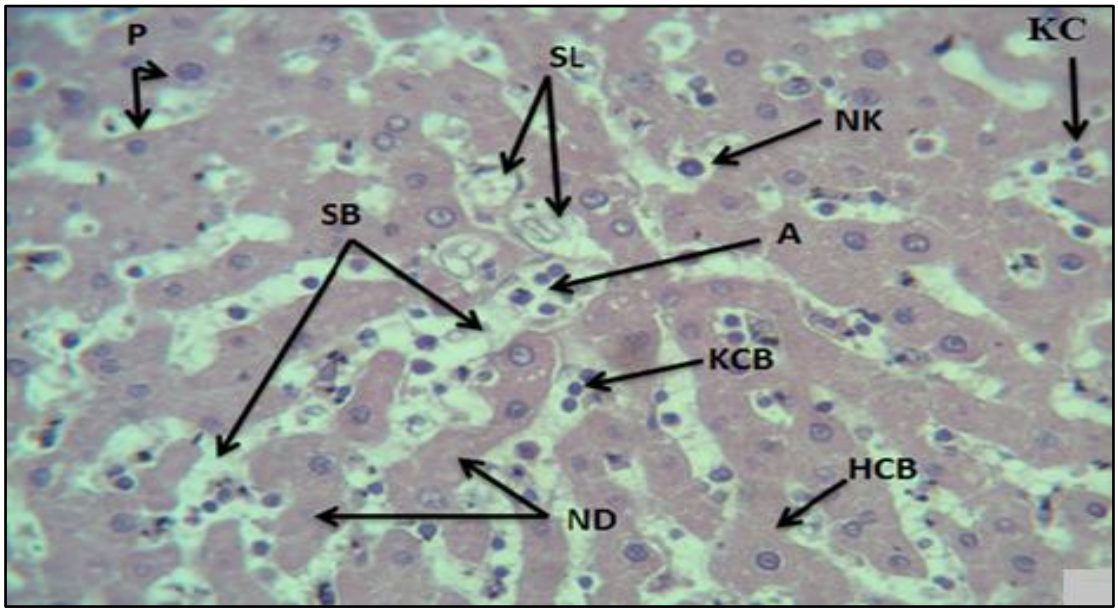

Figure 5: Liver section from diabetic male rats treated with empagliflozin for 30 days. Histopathological examination revealed the onset of hepatic steatosis with lipid accumulation within the hepatic sinusoids (SL), dilation of the sinusoids (SB), Kupffer cell infiltration (A), Kupffer cell hyperplasia and enlargement (KCB), nuclear necrosis (NK), nuclear pyknosis (P), nuclear degeneration/death in some cells (ND), and hypertrophy of some hepatocytes (HCB). Hematoxylin and eosin (H&E) staining, 40 × magnification.

4. DISCUSSION

In agreement with previous studies [14], [15], the present study demonstrated that alloxan-induced diabetes caused significant histopathological alterations in liver tissue, including hepatocellular necrosis, sinusoidal dilatation, hemorrhage, congestion of the central vein, and disruption of the normal hepatic cord architecture. These pathological changes are primarily attributed to chronic hyperglycemia, which enhances oxidative stress through excessive production of reactive oxygen species (ROS), leading to cellular injury, lipid peroxidation, and ultimately hepatocyte death [7], [16]. In addition, diabetes-associated metabolic stress promotes endoplasmic reticulum stress and mitochondrial dysfunction, thereby aggravating hepatocellular degeneration and loss of nuclear integrity [8], [9]. Although empagliflozin has well-documented antioxidant and anti-inflammatory properties, particularly in renal and cardiovascular tissues [11], [17], the findings of the

current study revealed only limited histological improvement in liver tissue following treatment. Residual pathological features, including inflammatory cell infiltration, sinusoidal hemorrhage, nuclear pyknosis, and hepatocellular hypertrophy, were still observed, indicating that empagliflozin was unable to completely reverse the hepatic damage induced by diabetes under the conditions of the present experiment.

The limited hepatic response observed in this study may be attributed to several factors. First, alloxan exerts direct cytotoxic effects and induce oxidative damage in tissues beyond pancreatic β-cells, thereby contributing to persistent hepatic injury even after antidiabetic treatment [18]. Second, the treatment duration of 30 days may not have been sufficient to achieve complete hepatic recovery. Furthermore, the administered dose of empagliflozin (5 mg/kg) may not have provided the optimal therapeutic effect required for effective hepatoprotection [19]. The liver's central role in drug metabolism, particularly through cytochrome p450 enzymes, may also generate reactive metabolites that contribute to cellular stress and potentially reduce the overall efficacy of the treatment. The development of fatty changes in the liver (hepatic steatosis) observed in the empagliflozin-treated group is more likely related to diabetes-associated disturbances in lipid metabolism rather than a direct effect of the drug itself. Insulin resistance, a hallmark of DM, promotes increased mobilization of free fatty acids from adipose tissue, enhanced hepatic fatty acid uptake, and triglyceride accumulation within hepatocytes, ultimately leading to hepatic steatosis [20]. These metabolic alterations may persist despite partial glycemic control, contributing to the histopathological abnormalities observed in the liver tissue of treated animals.

Furthermore, the infiltration of inflammatory cells observed in the liver tissue suggests the activation of immune-mediated pathways, which may be triggered by drug metabolites capable of forming antigenic complexes that provoke immune responses against hepatocytes [21], [22]. Persistent inflammation can also worsen tissue injury by promoting the release of proinflammatory cytokines and enhancing oxidative stress within the hepatic microenvironment. Collectively, these findings highlight the multifactorial pathogenesis of diabetes-induced liver injury, involving oxidative stress, metabolic dysregulation, and inflammatory processes.
Although empagliflozin provides well-established systemic benefits in the management of diabetes mellitus, including glycemic control and protection against cardiovascular and renal complications, its hepatoprotective effects appeared limited under the conditions of this study. The persistence of histopathological abnormalities following treatment shows that empagliflozin alone may not be sufficient to fully reverse diabetes-associated hepatic damage. Therefore, longer treatment durations, optimization of dosage regimens, and the use of combination therapeutic approaches may be necessary to achieve more effective protection and recovery of liver tissue in diabetic conditions.

5. CONCLUSION

The findings of the present study demonstrate that alloxan-induced diabetes mellitus causes significant histopathological damage to liver tissue in male rats, characterized by hepatocellular degeneration, necrosis, sinusoidal alterations, inflammatory cell infiltration, and disruption of normal hepatic architecture. Although treatment with empagliflozin resulted in modest structural improvements, it did not completely reverse the diabetes-induced hepatic lesions within the 30-day treatment period. The occurrence of fatty changes in the liver tissue of empagliflozin-treated rats is more likely associated with diabetes-related metabolic disturbances, particularly alterations in lipid metabolism, rather than a direct effect of the drug itself. These findings shows that the hepatoprotective effects of empagliflozin may be limited under the experimental conditions employed in this study. Further investigations are warranted to determine the optimal dosage and treatment duration of empagliflozin and to clarify its precise role in protecting against diabetes-associated liver injury. Future studies should also explore combination therapeutic approaches and evaluate the underlying molecular mechanisms involved in hepatic recovery and metabolic regulation.

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REFERENCES

[1] R. M. M. Khan, Z. J. Y. Chua, J. C. Tan, Y. Yang, Z. Liao, and Y. Zhao, "From pre-diabetes to diabetes: Diagnosis, treatments and translational research," Medicina, vol. 55, no. 9, p. 546, 2019, doi: 10.3390/medicina55090546.
[2] World Health Organization and International Diabetes Federation, Classification of Diabetes Mellitus. Geneva, Switzerland: World Health Organization, 2019.
[3] C. Faselis, A. Katsimardou, K. Imprialos, P. Deligkaris, M. Kallistratos, and K. Dimitriadis, "Microvascular complications of type 2 diabetes mellitus," Current Vascular Pharmacology, vol. 18, no. 2, pp. 117–124, 2020, doi: 10.2174/1570161117666190502103733.
[4] D. Moriconi et al., "Glomerular hyperfiltration predicts kidney function decline and mortality in type 1 and type 2 diabetes: A 21-year longitudinal study," Diabetes Care, vol. 46, no. 4, pp. 845–853, 2023, doi: 10.2337/dc22-2003.
[5] M. I. Harris, Diabetes in America. Bethesda, MD, USA: National Institutes of Health, 2013.
[6] American Diabetes Association, "Classification and diagnosis of diabetes: Standards of medical care in diabetes—2021," Diabetes Care, vol. 44, no. 1, pp. S15–S33, 2021, doi: 10.2337/dc21-S002.
[7] A. M. Ali et al., "Antidiabetic potency, antioxidant effects, and mode of actions of Citrus reticulata fruit peel hydroethanolic extract, hesperidin, and quercetin in nicotinamide/streptozotocin-induced Wistar diabetic rats," Oxidative Medicine and Cellular Longevity, vol. 2020, Art. no. 1730492, 2020, doi: 10.1155/2020/1730492.

[8] E. N. Gurzov and D. L. Eizirik, "Bcl-2 proteins in diabetes: Mitochondrial pathways of β-cell death and dysfunction," Trends in Cell Biology, vol. 21, no. 7, pp. 424–431, 2011, doi: 10.1016/j.tcb.2011.03.001.
[9] D. A. Cunha et al., "Death protein 5 and p53-upregulated modulator of apoptosis mediate endoplasmic reticulum stress–mitochondrial dialog triggering β-cell apoptosis," Diabetes, vol. 61, no. 11, pp. 2763–2775, 2012, doi: 10.2337/db12-0123.
[10] S. Steiner, "Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes," Zeitschrift für Gefäßmedizin, vol. 13, no. 1, pp. 17–18, 2016, doi: 10.1056/NEJMoa1504720.
[11] V. Quagliariello et al., "The SGLT-2 inhibitor empagliflozin improves myocardial strain, reduces cardiac fibrosis and pro-inflammatory cytokines in non-diabetic mice treated with doxorubicin," Cardiovascular Diabetology, vol. 20, Art. no. 150, 2021, doi: 10.1186/s12933-021-01346-y.
[12] R. Saad et al., "The molecular effects of SGLT2i empagliflozin on the autophagy pathway in diabetes mellitus type 2 and its complications," Journal of Diabetes Research, vol. 2022, Art. no. 8337823, pp. 1–11, 2022, doi: 10.1155/2022/8337823.
[13] J. Cai et al., "AMPK: The key to ischemia-reperfusion injury," Journal of Cellular Physiology, vol. 237, no. 11, pp. 4079–4096, 2022, doi: 10.1002/jcp.30875.
[14] K. Houguig, S. Rkha, M. Rayadi, and N. Ouzennou, "Factors influencing therapeutic observance in diabetic subjects in the province of Essaouira (Morocco): A cross-sectional study," Ethiopian Journal of Health Sciences, vol. 32, no. 4, pp. 747–754, 2022, doi: 10.4314/ejhs.v32i4.11.
[15] A. Elksnis, M. Martinell, O. Eriksson, and D. Espes, "Heterogeneity of metabolic defects in type 2 diabetes and its relation to reactive oxygen species and alterations in beta-cell mass," Frontiers in Physiology, vol. 10, Art. no. 107, 2019, doi: 10.3389/fphys.2019.00107.
[16] E. Sokolowska and A. Blachnio-Zabielska, "The role of ceramides in insulin resistance," Frontiers in Endocrinology, vol. 10, Art. no. 577, 2019, doi: 10.3389/fendo.2019.00577.
[17] Z. A. Jigheh et al., "Empagliflozin alleviates renal inflammation and oxidative stress in streptozotocin-induced diabetic rats partly by repressing HMGB1-TLR4 receptor axis," Iranian Journal of Basic Medical Sciences, vol. 22, no. 4, pp. 384–391, 2019, doi: 10.22038/ijbms.2019.31788.7651.
[18] I. A. Okafor, U. S. Nnamah, and J. Nnaka, "Liver function analyses and spleen histology assessment following the co-administration of cisplatin and methanolic extract of Portulaca oleracea in Wistar rats," Natural Product Sciences, vol. 26, no. 3, pp. 252–258, 2020, doi: 10.20307/nps.2020.26.3.252.
[19] E. Ferrari and M. Rasponi, "Liver–heart on chip models for drug safety," APL Bioengineering, vol. 5, no. 3, Art. no. 031504, 2021, doi: 10.1063/5.0048986.
[20] K. Reyes-Gordillo, R. Shah, and P. Muriel, "Oxidative stress and inflammation in hepatic diseases: Current and future therapy," Oxidative Medicine and Cellular Longevity, vol. 2017, Art. no. 3140673, 2017, doi: 10.1155/2017/3140673.
[21] N. Sandhu and V. Navarro, "Drug-induced liver injury in GI practice," Hepatology Communications, vol. 4, no. 5, pp. 631–645, 2020, doi: 10.1002/hep4.1503.
[22] C. Ronco and L. Di Lullo, "Cardiorenal syndrome," Heart Failure Clinics, vol. 10, no. 2, pp. 251–280, 2014.

BIOGRAPHIES OF AUTHORS

Amal Mahmood Alwan is a lecturer in the Department of Biology, College of Education for Pure Sciences, University of Diyala, Iraq. She received their Ph.D. in Biological Sciences with a specialization in Animal Histology. Their research interests include animal histology, tissue pathology, experimental biology, and related areas of biology sciences. She has authored and coauthored several scientific publications in national and international journals. She can be contacted at amal.m.alwan@uodiyala.edu.iq. Google Scholar:

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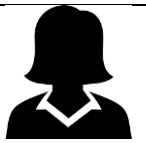

Raya Zaid Ali is a lecturer in the Department of Biology, College of Education for Pure Sciences, University of Diyala, Iraq. She holds a Ph.D. in Biology Science with a specialization in Immunogenetics from the University of Diyala. Her research interests include genetics, immunology, and physiology. She has published several scientific papers in their field. She can be contacted at raya.zaid@uodiyala.edu.iq

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Abthal Noori Mahdi is an assistant lecturer at Department of Biology, College of Education for Pure Sciences, University of Diyala, Iraq. She specializes in animal histology within the field of biology sciences. Their academic interests include histology, cell biology, and laboratory sciences. She contributes to teaching and scientific activities in her department. She can be contacted at abthalnoori3@gmail.com

Scopus

Aya Azam Jamal works at the College of Education for Pure Science, University of Diyala, Iraq, in the field of biology and laboratory sciences. She contributes to administrative and scientific activities within the college. Their professional interests include biology, laboratory sciences, and scientific research. She can be contacted at ayaazam08@gmail.com

Scopus

Doris Taher Hassan works at the College of Education for Pure Science, University of Diyala, Iraq, in the field of biology and laboratory sciences. She contributes to administrative and scientific activities within the college. Their professional interests include biology, laboratory sciences, and scientific research. She can be contacted at lwy88941@gmail.com

Scopus

Wurood Khalid Mohammed works at the College of Education for Pure Science, University of Diyala, Iraq, in the field of biological and laboratory sciences. She contributes to administrative and scientific activities within the college. Their professional interests include biology, laboratory sciences, and scientific research. She can be contacted at Wroodkhaled89@gmail.com

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