Kurdish Studies
May 2024
Volume: 12, No: 4, pp. 1030-1036
ISSN: 2051-4883 (Print) | ISSN 2051-4891 (Online)
www.KurdishStudies.net

DOI: 10.53555/ks.v12i4.3101

Insulin Secretion, Transaminase Activities And Alterations In Liver Histology In Experimental Type II Diabetic Albino (Wistar) Male Rats Following Treatment With *Mangifera Indica*

Zubia Begum[1*], Rehana Perveen[2], Hina Abrar[3], Noor Jahan[4], Dr. Rana Asif Hussain[5], Dr. Muhammad Zuhair Yusuf[6]

[1*,2]Department of Pharmacology, Institute of Baqai Pharmaceutical Science (BIPS), Baqai Medical University, Karachi, Pakistan
[3,4]Department of Pharmacology, Dow College of Pharmacy, Dow University of Health Sciences, Karachi, Pakistan
[5]Department of Pharmacognosy, Institute of Baqai Pharmaceutical Science (BIPS), Baqai Medical University, Karachi, Pakistan
[6]Department of Biological and Biomedical Sciences, Aga Khan University, Karachi, Pakistan

***Corresponding Author:** Zubia Begum
*Pharm-D, M.Phil., Ph.D. Scholar (Pharmacology), Department of Pharmacology, Institute of Baqai Pharmaceutical Science (BIPS), Baqai Medical University (BMU), Karachi, Pakistan Email: drzubia04@gmail.com

Abstract
Male albino (Wistar) rats were used to study the ameliorative effects of *Mangifera indica* (*M. indica*) ethanolic extract at doses of 200 and 400 mg/kg respectively, glibenclamide (05 mg/kg) and sitagliptin (10 mg/kg) were used as standard drugs in streptozotocin (45 mg/kg) induced diabetic rats (positive control). The aim of the research study was to assess the biochemical changes in type II diabetic rats model treated with different doses of ethanolic leaves extract of *M. indica.* Biochemical parameters were included serum insulin concentration, serum transaminase levels, bilirubin levels and evaluate the histopathological examination of the liver. The results of the investigation revealed that the significant ($p<0.001$) increment of the serum insulin level in 200 and 400 mg/kg ethanolic extract of *M. indica* (treated groups). In streptozotocin diabetic rats, the activities of liver enzymes in serum were increased and after 28 days of treatment with 200 and 400 mg/kg ethanolic extract of *M. indica,* transaminase levels were effectively reduced. Histopathological study revealed hepatic histological changes which were more noticeable in diabetic inducer group. *M. indica* leaves extract have positive effective results on biochemical parameters in diabetic type II rats. The biochemical protective effects of *M. indica* were associated with improved histological hepatocellular integrity and architecture.

Keywords: Animal model, Streptozotocin, Type II diabetes, *Mangifera indica*, Insulin assay, Liver enzymes assay

INTRODUCTION

The metabolism of carbs, fats, and proteins is disturbed by diabetes, a complex and multivarious group of disorders [1] marked by elevated fasting and postprandial blood glucose levels. Two primary types of diabetes; type 1 and type 2. Type 1 diabetes occurs by deficiency of insulin release due to autoimmune destruction of the beta cells of the pancreas. While, type 2 diabetes is caused by resistance to insulin action and secretion [2].

For the medical community, managing diabetes without any side effects is still an obstacle. Several medications, including sulfonylurea, thiazolidines [3] and biguanides [4] are currently available to treat diabetes and reduce blood glucose level. Many scientists working in the field of diabetes research have recently examined the therapeutic potential of numerous plant extracts [5].

Mangiferin, phenolic acids, benzophenones, and other antioxidants such flavonoids, ascorbic acid, carotenoids, and tocopherols are just a few of the phytochemicals that have been linked to the health benefits of mango plant leaves. The biological effects of *M. indica* extracts, such as their anti-cancer, anti-diabetic, anti-oxidant, anti-microbial, anti-obesity, lipid-lowering, and anti-diarrheal properties, have been investigated [6]. The aim of the research work was to evaluate the ability of *M. indica* leaves extract to reduce the biochemical activities in diabetes type II induced rats and also investigate the histopathological examination of the liver.

MATERIALS AND METHODS

Plant material collection

Mangifera indica (MI) leaves were collected from cultivated trees at the University of Karachi's Department of Botany. Plant leaves wer identified as voucher specimens for the herbarium by a plant taxonomist; G H. NO. 95717.

Reagents and instruments

Ethanol (Analytical grade 99.99%), Streptozotocin (BioShop Canada, Lot No. 5K40517), Standard drugs (glibenclamide and sitagliptin), Ethanolic extract of *M. indica* (200 and 400 mg/kg), ELISA kit (Crystal Chem Cat # 90060), AST kit (Cat. No:

5.17520.0001), ALT kit (Cat. No: 5.17530.0001), ALP kit (Cat. No: 5.17640.0001), GGT kit (Cat. No: 5.17526.0001) Bilirubin Direct (Cat. No: 5.17646.0001), Bilirubin Total (Cat. No: 5.17645.0001).
The following instruments were used in the research work; Rotary evaporator (EYELA N-1000, Tokyo Rikakikai Co., Tokyo, Japan), Glucometer (Exactive vital), Eppendorf centrifuge machine (5810 R), Automatic selectra ProM chemical analyzer (ELITech Group - Clinical Systems).

Extraction of *Mangifera indica* leaves
M. indica leaves were collected and washed to remove dust particles and subsequently air-dried in a shaded area. Following the drying process, the leaves were grated, ground, and subjected to ethanol extraction. The ethanol was then removed from the plant extract using a rotary evaporator, resulting in the formation of a dense, dark greenish gummy residue [7,8].

Selection of animals
Male albino (Wistar) rats, with a weight range of 180-200±5 grams, were obtained from the animal house of the Baqai Institute of Pharmaceutical Sciences (BIPS), Baqai Medical University in Karachi, Pakistan. The rats were provided with a standard diet and had unrestricted access to water. They were housed in a controlled environment with a 12-hour light-dark cycle, maintaining a temperature of approximately 25±2°C and a relative humidity of 55–65% [9].

Ethical consideration
The research was authorized under reference # BMU-EC/02-2021 by the Board of Advanced Studies and Research (BASR) at Baqai Institute of Pharmaceutical Sciences (BIPS), Baqai Medical University, Karachi, Pakistan.

Induction of diabetes in albino male rats
Streptozotocin (STZ) was freshly dissolved in 0.1 M citrate buffer (pH = 4.5) at a dose of 45 mg/kg and administered intraperitoneally within 15 minutes of dissolution. The assessment of fasting glucose concentration on the 3-5 days after STZ treatment was used to confirm diabetes. Albino rats with glucose levels more than 200 mg/dl were diagnosed with Type II diabetes and ready for the experiment.

Experimental design
The rats were divided into six groups, each consisting of 12 albino male rats. Over a period of 28 days, extract and standard drugs were administered orally according to the following schedule: Group A (control group) received distilled water; Group B (diabetic inducer group) was given a single dose of streptozotocin at 45 mg/kg via the intraperitoneal route [10]. Group C and D (streptozotocin-induced Type II diabetic rats) were treated with standard drugs; glibenclamide (5 mg/kg) [11] and sitagliptin (10 mg/kg) [12], respectively. Group E and F (streptozotocin-induced Type II diabetic rats) received the ethanolic leaves extract of *M indica* at doses of 200 and 400 mg/kg, respectively.

Biochemical examination
After completion of the 28-day treatment period, rats were anesthetized using ketamine at a dose of 87.5 mg/kg (Aledani et al., 2020). Blood samples were then collected and allowed to coagulate at room temperature, followed by centrifugation at 3000 rpm for 15 minutes (Iheanacho et al., 2017). The clear, non-hemolyzed supernatant serum was promptly separated and divided into two portions, one for insulin assay and the other for enzymes assay.

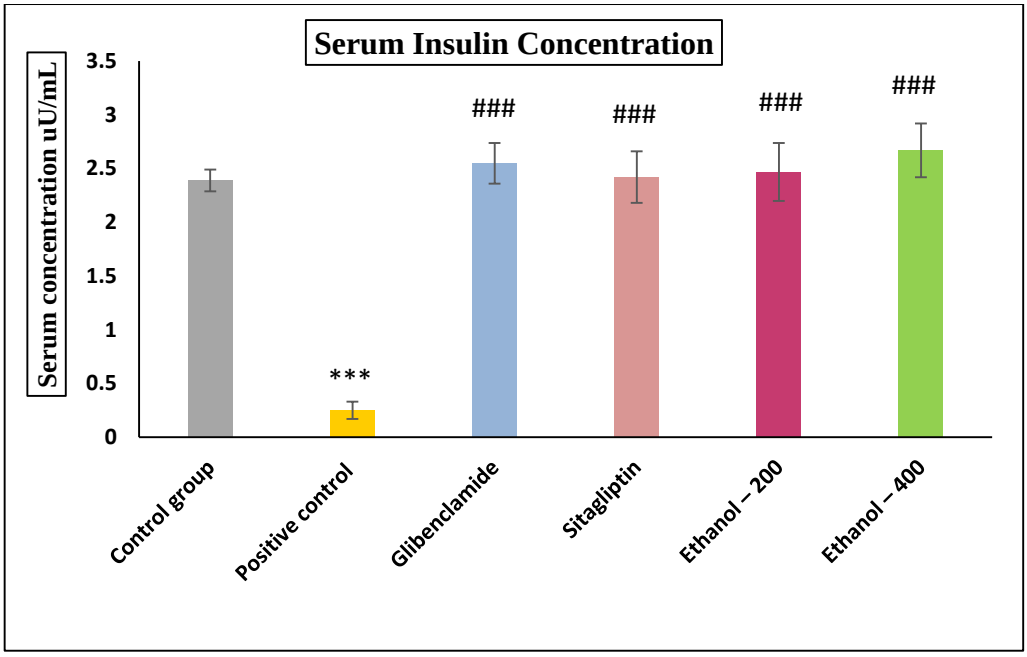
Insulin assay: The serum insulin level was measured by ELISA kit. **Enzyme assay:** aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma glutamyl transferase (GGT) activities were determined by IFCC method. Alkaline phosphatase (ALP) activity was determined according to the recommendation of the German clinical chemistry association. Total bilirubin (TB), and direct bilirubin (DB) were determined by Malloy-Evelyn modified method.

Preparation of liver tissue for histopathological examination
The animal tissues were rinsed with saline and immersed in 10% normal buffer formalin for subsequent histopathological assessment. After 24 hours, the tissues underwent standard protocol embedding in paraffin wax. Sections of five micrometers in thickness were cut from these blocks, placed on poly-1-lysine coated glass slides, and stained with hematoxylin and eosin using standard procedures and histopathological changes in all groups were then examined under a light microscope [18,19].

Statistical Analysis
The data were subjected to analysis using the one-way ANOVA test in SPSS version 21. The results were presented as Mean±SEM. Statistical significance was determined at $p<0.05$.

RESULTS

Effect of ethanolic leaves extract of *Mangifera indica* on serum insulin concentration
Comparing the control group with diabetic inducer group, the serum insulin level decreased highly significantly ($p<0.001$). After the treatment with ethanolic leaves extract of *M. indica*, serum insulin level increased highly significantly ($p<0.001$) as compared with diabetic inducer group (Graph 1). The ethanolic extract of *M. indica* (treated groups: 200 and 400 mg/kg) showed comparable elevation in serum insulin level as control group and standard groups (glibenclamide and sitagliptin).

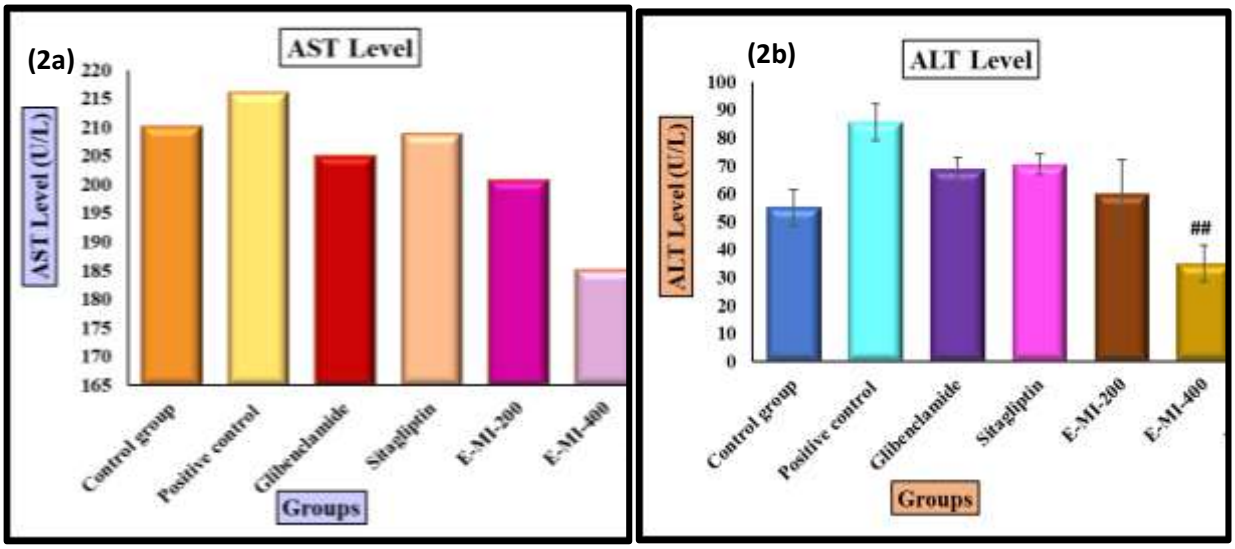


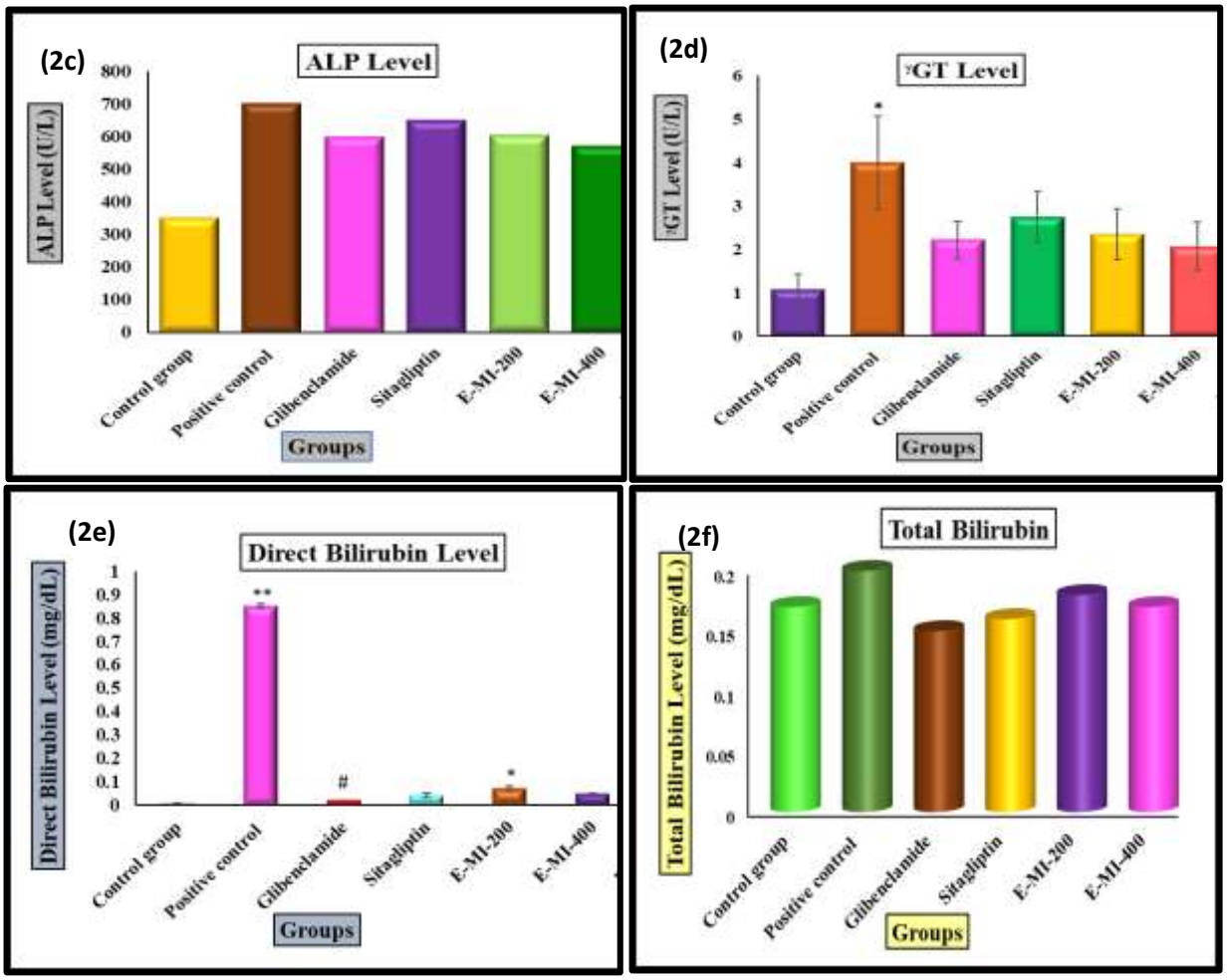
Graph 1. Effect of ethanolic leaves extract of *Mangifera indica* on serum insulin level: Comparison of serum insulin level between control, positive control, standard group (glibenclamide and sitagliptin) and treated groups (ethanolic leaves extract of *M. indica*).

Values shown as Mean±SEM
Level of significance p<0.001*** = All groups compared with control group
Level of significance p<0.001### = All groups compared with positive control group

Effect of ethanolic leaves extract of *Mangifera indica* on liver function profile

Graph 2, shows the effect of oral administration of ethanolic extract of *M. indica* leaves at doses of 200 mg/kg and 400 mg/kg on serum AST, ALT, ALP, GGT and bilirubin activities in all experimental groups. As shown in graph 2a, the diabetic inducer group showed the highest AST activity, which was non-significantly higher than treated groups, and standard groups. The AST levels in the treated groups did not exhibit any significant alterations compared to the control group. As indicated in the graph 2b, there was an observed increase in ALT activity following streptozotocin administration; however, this increase did not reach statistical significance when compared to the control group. Notably, ALT activity demonstrated significant ($p<0.01$) decrease following the administration of dose 400 mg/kg of the *M. indica* extract as compared with the positive control group. The diabetic inducer group exhibited the increased ALP level and GGT ($p<0.05$) level as compared with the control group. However, administration of *M. indica* leaves extract to diabetic rats showed reduction but this reduction non-significant to the control group (graph 2c, 2d). Bilirubin (direct and total) was reduced in the treated groups of both doses; this reduction was statistically insignificant as compared with the positive control group (grap h e, f) however, near to the standard groups, which confirm and reflec t its effectiveness in improving liver functions without negative effects.

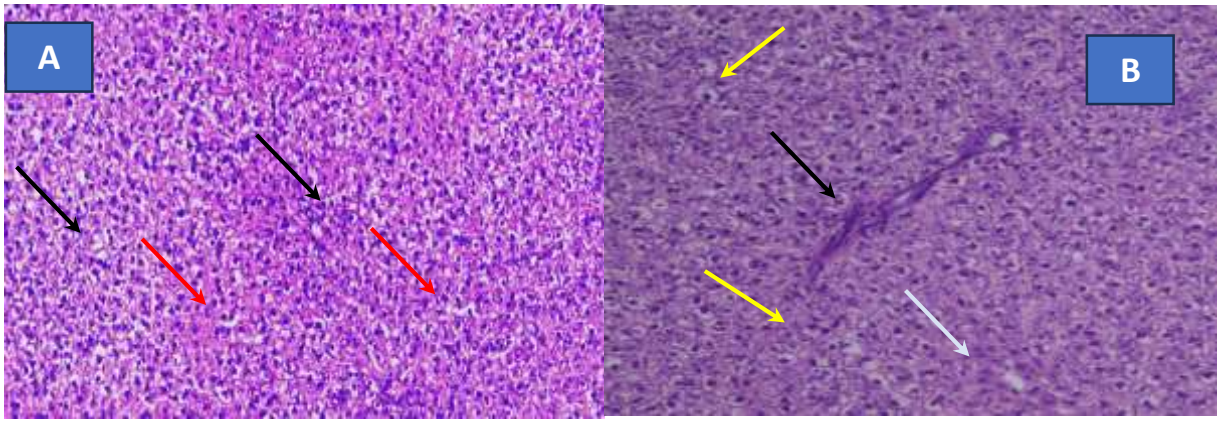




Graph 2. Effect of ethanolic extract of *M. indica* (200 mg/kg and 400 mg/kg) on liver enzymes in albino rats: (a) Aspartate aminotransferase (AST), (b) Alanine aminotransferase (ALT), (c) Alkaline phosphatase (ALP), (d) Gamma glutamyl transferase (GGT), (e) Direct bilirubin (DB), and (f) Total bilirubin (TB).
Level of significance $p<0.05$*, $p<0.01$** = All groups compared with control group
Level of significance $p<0.05^{\#}$, $p<0.01^{\#\#}$ = All groups compared with positive control group
E-MI = Ethanolic extract of *M. indica*

Histopathology evaluation

Slide A displays a photomicrograph of a liver section from the control group, revealing a normal lobular pattern with intact hepatocytes. In slide B, the liver section from the diabetic positive control group shows degeneration of hepatocytes, vacuolization of cytoplasm, swelling, and congested central vein. Slides C and D illustrate liver sections from rats treated with the standard drugs glibenclamide and sitagliptin, respectively, showcasing restored hepatocyte morphology, a preserved centrilobular vein, and minimal sinusoidal disruption. Slide E representing rats treated with *M. indica* at a dose of 200 mg/kg, displays improvement in hepatocyte distortion. Slide F depicting rats treated with *M. indica* at a dose of 400 mg/kg, exhibits significant recovery of hepatocyte distortion and sinusoidal integrity.



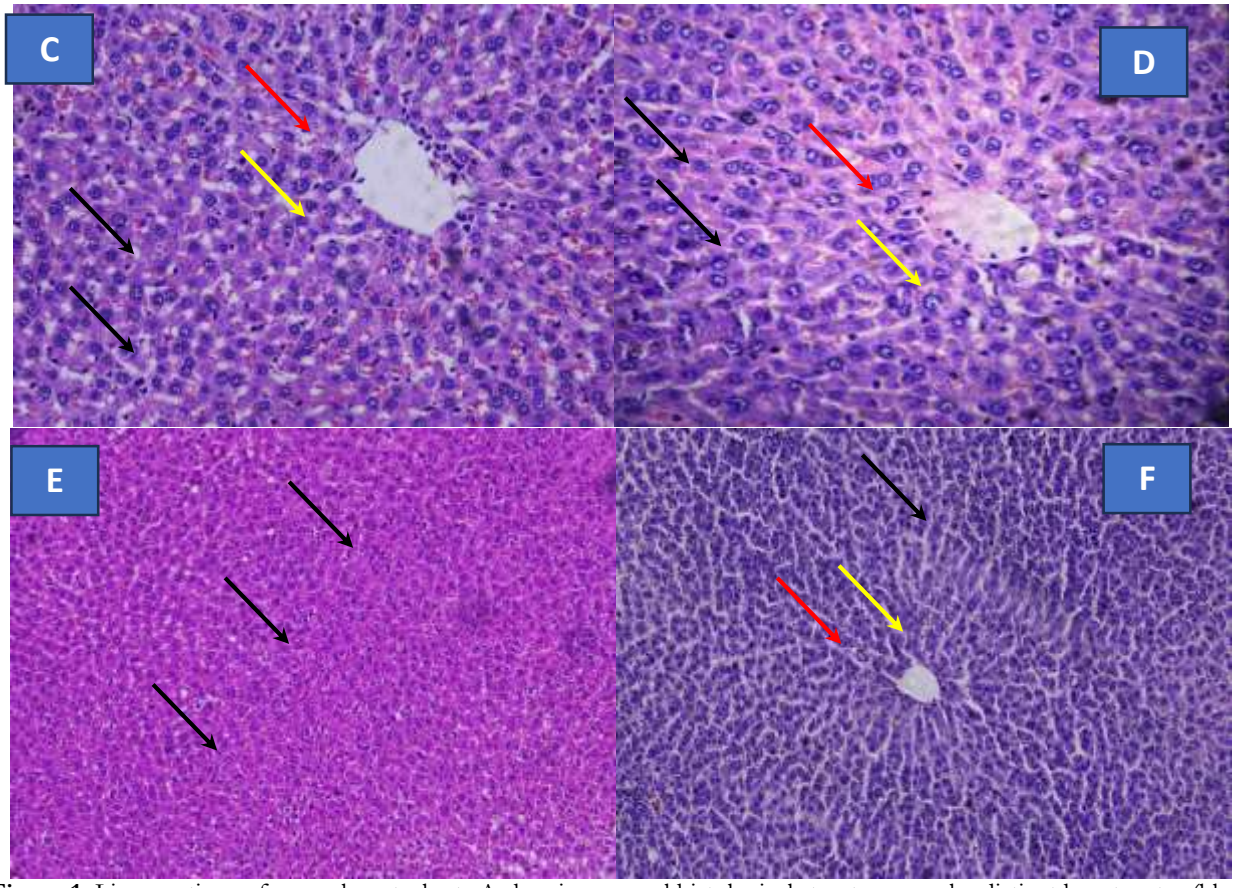


Figure 1. Liver sections of normal control rats A showing normal histological structure, regular distinct hepatocytes (black arrow) with sinusoidal spaces (red arrow). B. Streptozotocin induced diabetes (positive control rats) showing (yellow arrow) degeneration of hepatocytes, vacuolization of cytoplasm, congested central vein (black arrow) and portal vein (sky-blue arrow). C and D standard groups glibenclamide and sitagliptin showing restoration of liver architecture, hepatocytes (black arrow), sinusoid (yellow arrow) and normal central vein (red arrow). E. Diabetic rats treated with *M. indica* extract (200 mg/kg) showing with less pathological changes and regenerating hepatocytes (black arrow). F. Diabetic rats treated with *M. indica* extract (400 mg/kg) illustrating improved histological structure of hepatocyte (black arrow), central vein (red arrow) and sinusoid (yellow arrow). (A, B C, D, E and F, magnification X-10 and 40).

DISCUSSION

Diabetes mellitus is a metabolic disorder characterized by the loss of glucose homeostasis and the absence of insulin secretion [20]. Certain herbal extracts have been proven to effectively treat diabetes and prevent its long-term complications without any adverse effects. *M. indica* is known for its anti-diabetic, antioxidant, anti-viral, and anti-inflammatory properties [21]. This study assessed the hepatoprotective properties of *M. indica* extract in a STZ-diabetic rats model. Changes in different biochemical parameters, including serum insulin concentration, liver enzymes and histopathological slides were determined in the diabetic rats treated with *M. indica* extract, and compared with positive control group, control group, and standard drugs (glibenclamide and sitagliptin). The treatment of *M. indica* extract showed significant hypoglycemic effects after 28 days, consistent with previous reports of hypoglycemic effects from various parts of the *M. indica* extract [22, 23, 24]. In research study, STZ-induced diabetic rats showed significant ($p<0.001$) reduction in insulin concentration as compared with control group, these results correlate well with the observation of previous study, STZ's diabetogenic effect, which results in the destruction of β-cells and a decrease in insulin containing secretory granules, may be responsible for this [25, 26]. After 28 days of treatment with *M. indica* extract showed significant ($p<0.001$) elevation in insulin concentration as compared with the positive control group. ALT, AST, and ALP are specific markers used to assess hepato-cellular damage leading to liver cell necrosis [27]. In research study, ALT, AST, ALP, GGT and bilirubin enzyme activities were elevated in diabetic rats which is consistent with the findings of previous studies [28,29,30]. The activity of transaminases and bilirubin in the diabetic rats was decreased after treatment with ethanolic leaves extract of *M. indica* in dose dependent manner. This decrease suggests that the *M. indica* may have a hepatoprotective effect. The number of hepatic inflammatory reactions to diabetes mellitus have been documented [31]. The liver sections of STZ diabetic rats showed significant histopathological changes, including central vein congestion, hepatocyte degeneration, inflammation, and cytoplasmic vacuolization. The study results align with previous research indicating similar histopathological changes following diabetes induction using STZ injection [32, 33]. *M. indica* ethanolic extract and standard drugs (glibenclamide and sitagliptin) treatment showed protective effects against hepatic changes associated with diabetes in diabetic rats, resulting in less pathological changes and improved liver architecture as compared with diabetic inducer group. Consequently, *M. indica* extract exhibits hepatoprotective effects and this might be due to the presence of phytochemical constituents in the leaves part of the plant.

Conclusion

The study findings suggested that *M. indica* leaves ethanolic extract had antidiabetic effects in type II diabetic rats and may be a promising nutritional therapy for the treatment of type II diabetes. *M. indica* leaves extract is regarded as a safe medication because it did not have any negative effects on the liver as compared with diabetic inducer rats.

Conflict of interest: The authors declare no conflict of interest

REFERENCES

1. Xu, Y.; He, Z.; King, G.L. 2005. Introduction of hyperglycemia and dyslipidemia in the pathogenesis of diabetic vascular complications. *Curr. Diabetes Rep.*, *5*, 91–97.
2. Eizirik, D.L., Pasquali, L. and Cnop, M., 2020. Pancreatic β-cells in type 1 and type 2 diabetes mellitus: different pathways to failure. *Nature Reviews Endocrinology*, *16*(7), 349-362.
3. Derosa, G. and Maffioli, P., 2011. Thiazolidinediones plus metformin association on body weight in patients with type 2 diabetes. *Diabetes Research and Clinical Practice*, *91*(3), 265-270.
4. Cicero, A.F., Tartagni, E. and Ertek, S., 2012. Metformin and its clinical use: new insights for an old drug in clinical practice. *Archives of medical science: AMS*, *8*(5), 907.
5. Lee J, Noh S, Lim S, Kim B. 2021. Plant Extracts for Type 2 Diabetes: From Traditional Medicine to Modern Drug Discovery. *Antioxidants* (Basel), 10(1):81.
6. Kumar, M., Saurabh, V., Tomar, M., Hasan, M., Changan, S., Sasi, M., Maheshwari, C., Prajapati, U., Singh, S., Prajapat, R.K. and Dhumal, S., 2021. Mango (*Mangifera indica L.*) leaves: Nutritional composition, phytochemical profile, and health-promoting bioactivities. *Antioxidants*, *10*(2), 299.
7. Azmir, J., Zaidul, I.S.M., Rahman, M.M., Sharif, K.M., Mohamed, A., Sahena, F., Jahurul, M.H.A., Ghafoor, K., Norulaini, N.A.N. and Omar, A.K.M., 2013. Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of food engineering*, *117*(4), 426-436.
8. Doughari, J.H., El-Mahmood, A.M. and Tyoyina, I., 2008. Antimicrobial activity of leaf extracts of Senna obtusifolia (L). *African Journal of Pharmacy and Pharmacology*, *2*(1), 7-13.
9. Thirumalai, S. and Sinha, K.K., 2011. Product recalls in the medical device industry: An empirical exploration of the sources and financial consequences. *Management Science*, *57*(2), 376-392.
10. Punithavathi, V.R., Anuthama, R. and Prince, P.S.M., 2008. Combined treatment with naringin and vitamin C ameliorates streptozotocin-induced diabetes in male Wistar rats. *Journal of Applied Toxicology: An International Journal*, *28*(6), 806-813.
11. Trivedi, N.A., Mazumdar, B., Bhatt, J.D. and Hemavathi, K.G., 2004. Effect of shilajit on blood glucose and lipid profile in alloxan-induced diabetic rats. *Indian journal of pharmacology*, *36*(6), 373.
12. Mega, C., Teixeira de Lemos, E., Vala, H., Fernandes, R., Oliveira, J., Mascarenhas-Melo, F., Teixeira, F. and Reis, F., 2011. Diabetic nephropathy amelioration by a low-dose sitagliptin in an animal model of type 2 diabetes (Zucker diabetic fatty rat). *Journal of Diabetes Research*, *2011*.
13. Iheanacho, S., Ogueji, E., Yaji, A., Dada, O., Mbah, C., Ifejimalu, A. and Ibrahim, B.U., 2017. Effects of herbal plants (Zingiber officinale and Hibiscus sabdariffa) as dietary additives on serum biochemistry and some metabolites in Clarias gariepinus (Burchell, 1822). *J. Coast. Life Med*, *5*(12), 516-520.
14. Bellinger, L.L. and Mendel, V.E., 1975. Hormone and glucose responses to serial cardiac puncture in rats. *Proceedings of the Society for Experimental Biology and Medicine*, *148*(1), 5-8.
15. Maurya AK, Tripathi S, Ahmed Z, Sahu RK. 2012. Antidiabetic and antihyperlipidemic effect of Euphorbia hirta in streptoztocin induced diabetic rats. *Der Pharmacia lettre*, 4:703-707.
16. Gandhi GR, Sasi Kumar P. 2012. Antidiabetic effect of merremia emarginata burm. F. in streptozotocin induced diabetic rats. *Asian Pac. J. Trop Biomed.* 2:281-286.
17. Bancroft JD, Steven A. 1990. Theory and practice of Histological technique 3rd edi, Churchill living stone, New York, USA. 281-282.
18. Cardiff, R.D., Miller, C.H. and Munn, R.J., 2014. Manual hematoxylin and eosin staining of mouse tissue sections. *Cold Spring Harbor Protocols*, 2014(6), 073411.
19. Fischer, A.H., Jacobson, K.A., Rose, J. and Zeller, R., 2008. Hematoxylin and eosin staining of tissue and cell sections. *Cold spring harbor protocols*, *2008*(5), 4986.
20. Hameed, I., Masoodi, S.R., Mir, S.A., Nabi, M., Ghazanfar, K. and Ganai, B.A., 2015. Type 2 diabetes mellitus: from a metabolic disorder to an inflammatory condition. *World journal of diabetes*, *6*(4), 598.
21. Samanta, S., Chanda, R., Ganguli, S., Reddy, A.G. and Banerjee, J., 2019. Anti-diabetic activity of mango (*Mangifera indica*): a review. *MOJ Bioequiv Availab*, *6*(2), 23-26.
22. Mistry, J., Biswas, M., Sarkar, S. and Ghosh, S., 2023. Antidiabetic activity of mango peel extract and mangiferin in alloxan-induced diabetic rats. *Future Journal of Pharmaceutical Sciences*, *9*(1), 1-13.
23. Gondi, M. and Prasada Rao, U.J.S., 2015. Ethanol extract of mango (*Mangifera indica L.*) peel inhibits α-amylase and α-glucosidase activities, and ameliorates diabetes related biochemical parameters in streptozotocin (STZ)-induced diabetic rats. *Journal of food science and technology*. *52*, 7883-7893.
24. Kulkarni, V.M. and Rathod, V.K., 2018. Exploring the potential of *Mangifera indica* leaves extract versus mangiferin for therapeutic application. *Agriculture and Natural Resources*, *52*(2), 155-161.
25. Rawi, S.M., Mourad, I.M. and Sayed, D.A., 2011. Biochemical changes in experimental diabetes before and after treatment with mangifera indica and psidium guava extracts. *Int J Pharm Bio Sci*, *2*(2), 29-41.

26. Akhani, S.P., Vishwakarma, S.L. and Goyal, R.K., 2004. Anti-diabetic activity of Zingiber officinale in streptozotocin-induced type I diabetic rats. *Journal of pharmacy and Pharmacology*, *56*(1), 101-105.
27. Muruganandan, S., Srinivasan, K., Gupta, S., Gupta, P.K. and Lal, J., 2005. Effect of mangiferin on hyperglycemia and atherogenicity in streptozotocin diabetic rats. *Journal of ethnopharmacology*, *97*(3), 497-501.
28. Alanazi, A. Z., Alqahtani, F., Mothana, R. A. A., Mohany, M., Abuohashish, H. M., Ahmed, M. M. and Al-Rejaie, S. S. 2020. Protective Role of Loranthus regularis against Liver Dysfunction, Inflammation, and Oxidative Stress in Streptozotocin Diabetic Rat Model. *Evidence-Based Complementary and Alternative Medicine*. 2020, 8.
29. Idris, A. S., Mekky, K. F. H., Abdalla, B. E. E. and Ali, K. A. 2011. Liver function tests in type 2 Sudanese diabetic patients. *International Journal of Nutrition and Metabolism*, 3:17–21.
30. Nwanjo, H. U. 2007. Studies on the effect of aqueous extract of Phyllanthus niruri on plasma glucose level and some hepatospecific markers in diabetic Wistar rats. *International Journal of Laboratory Medicine*, 2:1–18.
31. Lee, H and Lim, Y. 2019. "Gamma-tocopherol ameliorates hyperglycemia- induced hepatic inflammation associated with NLRP3 inflammasome in alloxan-induced diabetic mice," *Nutrition Research and Practice*, vol. 13, no. 5, 377–383.
32. Ahmed, D., Kumar, V., Verma, A., Shukla, G.S. and Sharma, M., 2015. Antidiabetic, antioxidant, antihyperlipidemic effect of extract of Euryale ferox salisb. with enhanced histopathology of pancreas, liver and kidney in streptozotocin induced diabetic rats. *Springerplus*, *4*(1), 1-17.
33. Abolfathi, A.A., Mohajeri, D., Rezaie, A. and Nazeri, M., 2012. Protective effects of green tea extract against hepatic tissue injury in streptozotocin-induced diabetic rats. *Evidence-based complementary and alternative medicine*, *2012*.