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Original Research Article

Anti-diabetic Effect of Ethyl acetate Extract of *Spondias mombin* (Linn) Stem Bark in Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels due to insulin deficiency or resistance. *Spondias mombin* (Linn), commonly known as hog plum, is a tropical plant traditionally used for various medicinal purposes. This research aimed to assess the impact of ethylacetate extract from the stem bark of *Spondias mombin* (ESM) on blood glucose and various biochemical parameters in rats with diabetes induced by streptozotocin (STZ). STZ-induced diabetic rats were categorized into six groups, each comprising five rats (n = 5). The first group served as the healthy control; the second group included healthy rats given 100 mg/kg of ESM, while groups 3 to 6 contained STZ-induced rats treated with 0.6 mL/kg of water, 50 mg/kg of metformin, 50 mg/kg of ESM, and 100 mg/kg of ESM respectively over a 14-day period. Weekly blood glucose levels were measured using test strips and an Accu-Chek glucometer. Biochemical assessments, including lipid profile, liver function, and kidney function tests, were conducted following standard procedures. Histological examination of the rats' pancreas was performed after the experimental duration. The daily oral administration of ESM at doses of 50 and 100 mg/kg body weight in STZ-induced diabetic rats revealed a restoration of glucostasis, along with improvements in kidney, liver, and lipid dysfunction associated with diabetes. The extract positively influenced the pathological alterations in the pancreas caused by diabetes induction. *S. mombin* stem bark exhibits a glucose-lowering effect and ameliorates the pathological complications linked to diabetes.

Keywords: Diabetic, Streptozotocin, Biochemical parameters, Histopathology.

Introduction

Diabetes mellitus (DM) stands as one of the significant urgent health challenges facing the world in the 21st century. As reported in the 2021 Diabetes Atlas by the International Diabetes Federation, 6.7 million individuals lost their lives due to complications related to diabetes in 2021, translating to one death every five seconds. About 537 million people worldwide have diabetes with an estimation of 1 in 10 individuals (20-79 years old) exhibiting diabetes and related complications. It is estimated that by 2030, this figure is expected to rise up to 643 million, and 783 million in 2045.[1] Diabetes, lifelong (chronic) disease, is a group of metabolic disorders characterized by high levels of glucose in blood (hyperglycemia). It results from an insulin deficit, insulin resistance, or both. To regulate blood sugar levels, the pancreas β -cells release insulin.[2] Diabetes is usually a co-morbidity in a number of complicated diseases and conditions including obesity, inflammatory/immune disorders, renal disorders, cardiovascular and cerebrovascular diseases, and inflammation.[3] The complications associated with diabetes are evidently observed in both types of DM. Elevated blood sugar levels lead to long-term effects due to issues with insulin processing and irregularities in the metabolism of carbohydrates, fats, and proteins.[4,5]

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Conditions such as high blood pressure, eye disorders, advanced kidney disease, nerve damage, circulatory issues, and imbalances in electrolytes, weakened immune response, erectile dysfunction, and complications during pregnancy are all examples of complications associated with diabetes.[5]

Clinically, managing diabetes and diabetes complications without any side effects is still a problem. The pharmacokinetic characteristics, secondary failure rates, high cost, and concomitant unwanted effects of these medications limit their utilization.[6] Hence, it is therefore necessary to search for less expensive alternative medications. Due to their accessibility and affordability, a sizable segment of the rural population still relies heavily on herbs as medicine to treat disease.[7] There are more than 400 plant species that exhibit anti-diabetic action, and herbal medications are a significant component of traditional medicine. *Spondias mombin* (Linn) is a member of the Anacardiacae family.[8] It is undoubtedly native to the Caribbean, tropical America, and more recently, found in Nigeria and other West African nations. Its bark is greyish-brown, thick, rough, deeply grooved, and has blunt, spine-like projections.[8] This plant is frequently seen and obtained nearby in South/West Nigeria and is known locally as Iyeye (Yoruba) and Uvuru (Igbo).

Traditional medicine has shown that the entire *Spondias mombin* tree is medicinally and pharmacologically valuable. Its common medicinal and therapeutic uses include; antibacterial,[9] anti-viral,[10] wound-healing[11] as well as an abortificient.[12] Additionally, studies have indicated that it is utilized to treat a number of systemic and topical disorders, including inflammation of the mouth and throat, prostatitis, and herpes labialis.[13] The leaves of *S. mombin* possess antimicrobial, antibacterial, antiviral, antifungal, anti-edematogenic, hypoglycemic, and antioxidant effects, according to diverse studies.[14-16]

The anti-diabetic activity of crude methanol extract of *Spondias mombin* leaves in STZ-induced rats[17] and alloxan induced rats[18] has been reported. Omoboyowa et al.,[19] reported the anti-diabetic efficacy

of solvent fractions of *S. mombin* in *Drosophila melanogaster* with ethylacetate fraction reported to be most potent among the solvent extracts, therefore this study aims to investigate the anti-diabetic efficacy of the ethylacetate fraction of *S. mombin* stem bark in vertebrates with possible effects on the biochemical parameters.

Materials and Methods

Reagents and Chemicals
All chemicals utilized in this research were of analytical quality, and STZ was procured from Sigma-Aldrich (USA). The assay kits for assessing kidney and liver function, as well as lipid profiles, were sourced from Agappe Diagnostic (Switzerland), and the reagents for histological analysis and stains were also obtained from Sigma-Aldrich (USA).

Animals
Female Wistar albino rats, weighing between 150–200 g, used in this research were acquired from the animal facilities at the University of Ibadan, Nigeria. The animals were kept under standard environmental conditions and were provided with standard rat pellets (Ladokun feeds, Ibadan, Nigeria) and water ad libitum. They were acclimated to laboratory conditions for 14 days prior to the start of the experimental procedures. The care of the rats was conducted in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals. Ethical approval for the use of animals was secured from the Directorate of Research at Adekunle Ajasin University, Akungba-Akoko (AAUA/06/23/3641).

Plant collection and identification
Stem bark of *Spondias mombin* (Linn) was de-barked using clean knife at medicinal plant garden, Adekunle Ajasin University, Akungba-Akoko (AAUA), Nigeria on 12th August, 2023. Identification and authentication of the whole plant was carried out by Dr. Obembe of Department of Plant Science and Biotechnology, AAUA, and a voucher specimen number (PSBH-222) was assigned and submitted to the University Herbarium.

Extraction and Ethylacetate-Partitioned Fractionation of Crude Ethanol Extract
The stem barks were dried in the air at standard room temperature and humidity for three weeks, and then processed into powder with a mechanical blender. In summary, 115 g of the powdered sample was immersed in 1000 mL of ethanol. The resulting ethanol extract was concentrated using a rotary evaporator and kept for later use. The ethanol extract of *S. mombin* (45 g) was dissolved in 500 mL of distilled water and extracted sequentially with n-hexane to eliminate non-polar compounds (defat). After separation, the aqueous phase was subjected to extraction with 500 mL of ethyl acetate for 72 hours. The ethyl acetate fraction, once separated, was concentrated using a rotary evaporator and allowed to dry, resulting in the ethyl acetate fraction (ESM) used for the study.

Induction of Diabetes mellitus
Diabetes mellitus was induced in fasted Wistar albino rats through an intraperitoneal injection of streptozotocin (50 mg/kg) in a citrate buffer with a pH of 4.5 (Sigma Chemicals, USA). Four days post-injection, rats that had fasting blood glucose levels exceeding 200 mg/dL were classified as diabetic and included in this study.[20] Out of the 30 rats treated with STZ, 22 were confirmed to be diabetic with fasting blood glucose levels above 200 mg/dL. For the experimental study, 20 of these 22 diabetic rats were utilized. Both the diabetic rats and the control group received treatment with the ethylacetate fraction over a period of 14 days.

Anti-diabetic study
Following the successful induction of diabetes mellitus, both diabetic and normal rats were randomly assigned to six (6) groups, each consisting of five (5) rats (n = 5). Group 1 received 0.2 mL/kg of distilled water as the normal control; Group 2 consisted of normal rats treated with 100 mg/kg of ESM; Group 3 included STZ-induced rats administered 0.2 mL/kg of distilled water; Group 4 comprised STZ-induced rats treated with 50 mg/kg of metformin (standard drug); Groups 5 and 6 received daily doses of 50 mg/kg and 100 mg/kg of ESM fraction, respectively, following STZ induction. All treatments were administered orally via gavage for 14 days. Blood glucose levels were monitored using an Accu-Chek glucometer on days 1, 7, and 14 of treatment. On the 14th day, all rats were fasted overnight, after which blood samples were collected through ocular puncture for biochemical analyses, and the animals were euthanized. Pancreatic tissues were excised, with a small portion fixed in 10% formaldehyde for histological examination. The initial and final weights of the rats were measured using a Tree MRB-S 1201 digital weighing balance (USA).

Biochemical parameters
Serum urea levels were determined using standard procedures,[21] serum uric acid (Fossati et al., 1980), serum creatinine level,[22] serum aspartate and alanine transaminase (AST and ALT) assays,[23] total and direct bilirubin,[24] serum total cholesterol,[25] serum triglyceride [26] and serum HDL-cholesterol and LDL-cholesterol concentration[27] according to Agappe assay kits (Switzerland)

Pancreas Histology
The pancreas were entirely preserved in 10% formalin for 24 hours; tissues were embedded in paraffin; and sections were sliced at a thickness of 5 µm, stained using hematoxylin and eosin, and mounted in Canada balsam for observation under × 100 and × 400 magnifications.[28] Image-J was utilized for calibrating the scale bar and for quantifying the scoring, which was assigned on a scale of 10.

Statistical Analysis
The data collected were analyzed using R programming version 3 for macOS. All values were expressed as the mean ± S.E.M. of the measured variables. Statistical significance was evaluated using analysis of variance (ANOVA), followed by a post-hoc Tukey multiple range test for multiple comparisons. A P-value of 0.05 or less was considered statistically significant.

Results and Discussion

Streptozotocin is widely recognized as a standard model for inducing diabetes mellitus due to its toxic effects on pancreatic beta-cells, leading to cellular destruction, reduced insulin levels, and hyperglycemia.[29] Streptozotocin-induced hyperglycemia in experimental animals serves as a valuable tool for evaluating the efficacy of various hypoglycemic agents and has been extensively utilized in research.[30] The compound induces diabetes by selectively targeting and destroying pancreatic beta-cells through DNA alkylation.[31] However, administering non-toxic free radical scavengers and antioxidants may help protect pancreatic islets from streptozotocin's cytotoxic effects.[32] Consequently, this model provides an excellent foundation for investigating beta-cell protection and screening potential diabetes treatments.

Effects of ethylacetate fraction of S. mombin on glucose concentration and weight change
In *in vivo* studies, animal body weights are typically used as an index to assess overall health conditions.[33] STZ-induced diabetic rats in this study had significant ($P < 0.05$) lower body weight compared with normal control rats. However, when compared to STZ-induced rats treated with ESM and metformin the result showed no significant change in body weight (Fig. 1b and table 1). This finding is consistent with previous reports that STZ-induced rats treated with natural or synthetic compounds showed no or minimal changes in body weight.[34,35] Although, increase in body weight in STZ-induced rats administered natural supplement has also been reported.[36,37]
The effect of ethylacetate fraction of *S. mombin* stem bark on weekly change in blood glucose levels (BGLs) of normal and diabetic treated animals is presented in figure 1A. The result revealed significant ($p < 0.05$) decrease in glucose concentration of STZ induced rats treated with 50 mg/kg and 100 mg/kg of ethylacetate fraction of *S. mombin* (ESM) compared with the STZ induced rats**.** The diabetic animals treated with

50 mg/kg and 100 mg/kg ESM had a significantly ($P < 0.05$) higher BGLs at day 14 compared to the control.

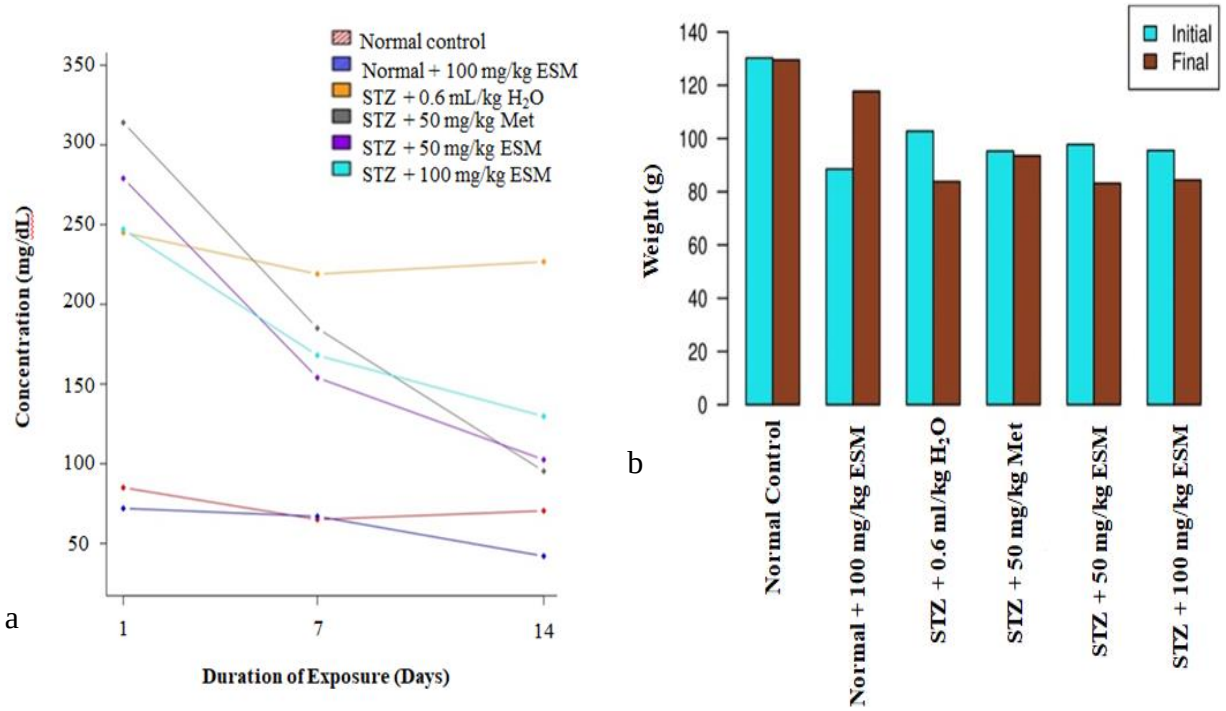

Figure 1a-b: Effect of ethylacetate extract of *S. mombin* on (a) Glucose concentration and (b) weight change of STZ-induced diabetic rats

Table 1: Relative initial and final body weight of animals and the corresponding weight gain/loss after induction of diabetes and respective treatments

Groups	Initial Body weight	Final Body weight	% change
Normal Control	130.25±18.91	129.47± 13.92 [a]	- 0.60
Normal +100 mg/Kg ESM	88.50±8.50	117.73± 6.44 [b]	+ 33
STZ+0.2 mL H2O	102.75±1.89	83.8175±2.66[c]	+ 18.43
STZ+50 mg/Kg MET	95.25±3.50	93.5075±4.11 [c]	- 1.83
STZ+50 mg/Kg ESM	97.75±2.50	83.1675±7.75[c]	+ 14.92
STZ+100 mg/Kg ESM	95.50±3.87	84.3950±2.71[c]	- 11.63

The pancreatic cells normally control the activity of the beta-cells of the Islets of Langerhans by secreting insulin, which helps to keep blood glucose levels within a specific range.[38] As a result of the destroyed beta-cells of the Islets of Langerhans in the pancreas,[39] the elevated blood glucose (hyperglycemia) observed in this study following the administration of STZ may be attributed to a reduction in the release of insulin. On the other hand, the reduced blood sugar levels and the ameliorative effects of metformin and ESM extract imply that the extract may have enhanced the pancreas's functional activity to release insulin. The uptake of glucose from the blood for energy production may have been aided by the extract's promotion of an increase in the pancreas' secretion of insulin in response to the STZ-induced hyperglycemia, which would have restored the animal's glycemic status.[40] ESM may therefore have an action similar to that of insulin or perhaps stimulate beta-cells to secrete more insulin. Similar to the report by Mondal et al.[41], the results showed that the extract can normalize hyperglycemia brought on by STZ. According to reports of Eluehike and Onoagbe,[42] *Spondias mombin* possess various phyto-constituents that might be responsible for the observed hypoglycemic activity.

Effects of ethylacetate fraction of S. mombin on liver function

Serum ALT and AST activities rise as a result of cellular leakage caused by hepatic membrane disruption.[49] In this study, the serum AST, ALT, Direct Bilirubin and Total Bilirubin of STZ-induced rats treated with ESM is presented in figure 2a-d. The results revealed a significant ($P < 0.05$) increase in AST activity in STZ-induced diabetic rats compared to control. Significant ($P < 0.05$) decrease was observed in AST activity of STZ-induced rats treated with 50 mg/kg metformin compared to STZ induced control (figure 2a). Significant ($P < 0.05$) decrease was observed in the ALT activity of STZ-induced rats treated with 50 mg/kg and 100 mg/kg ESM compared to STZ-induced control rats as shown in figure 2b. The direct Bilirubin concentration of STZ-induced rats treated with ESM showed non-significant ($P > 0.05$) increase compared

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with STZ-induced control rats (figure 2c). Treatment of STZ-induced rats with 100 mg/kg of ESM significantly ($P < 0.05$) reduces total bilirubin level compared to control rats as shown in figure 2d.

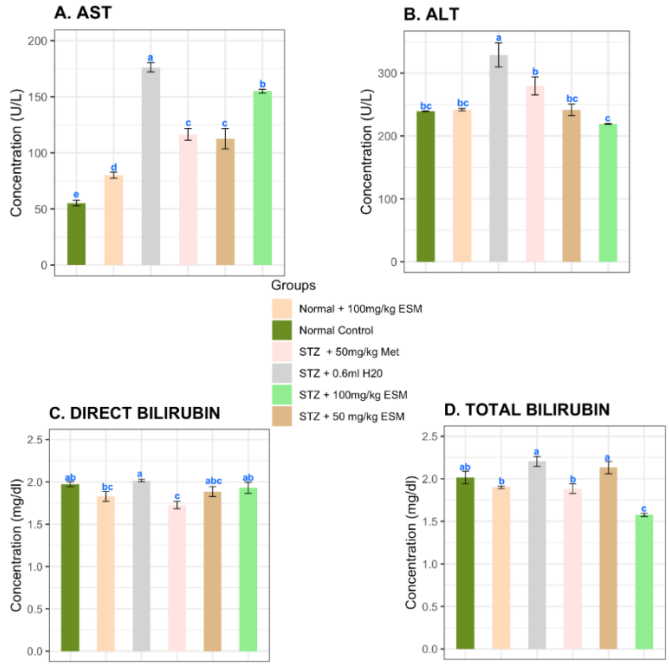

Figure 2 (a-d): Serum AST, ALT, Direct Bilirubin and Total Bilirubin of diabetic rats after ESM treatment. Bars with different alphabet showed significant difference at $P < 0.05$.

These findings suggest that ESM extract is effective in preventing diabetic-related hepatic injury. Similar results were reported by Ali et al.[50] that observed N-acetylcysteine (NAC) reduced ALT activity in serum and had hepato-protective impact on STZ-induced diabetic rats. Additionally, NAC therapy increased the activity of this enzyme in the mercury chloride-induced liver toxicity model.[51]

Effects on Lipid profile

Lipid abnormalities associated with diabetes, commonly referred to as "diabetic dyslipidemia," are characterized by elevated total cholesterol (T-Chol) and triglycerides (Tg), reduced high-density lipoprotein cholesterol (HDL-C), and an increased presence of small, dense LDL particles. Low-density lipoprotein cholesterol (LDL-C) levels may be either moderately elevated or within the normal range.[52] The most frequently observed lipid disorders in diabetes—hypertriglyceridemia and hypercholesterolemia—contribute significantly to coronary artery disease.[53]

The findings of this study indicate a significant ($P < 0.05$) reduction in total cholesterol and triacylglycerol concentrations in control rats, both with and without ESM administration, compared to STZ-induced control rats. STZ-induced rats treated with 50 mg/kg of metformin, as well as 50 and 100 mg/kg of ESM, exhibited a significant ($P < 0.05$) reduction in total cholesterol and triacylglycerol levels compared to STZ-induced control rats, as illustrated in Figures 3a and 3b. Additionally, treatment with 100 mg/kg of ESM significantly ($P < 0.05$) lowered LDL levels in STZ-induced rats compared to those receiving STZ alone (Figure 3d). Furthermore, HDL levels significantly ($P < 0.05$) increased in STZ-induced rats treated with metformin and ESM compared to STZ-induced control rats (Figure 3c).

This effect may be attributed to the inhibition of fatty acid synthesis. Under normal metabolic conditions, insulin activates lipoprotein lipase, an enzyme responsible for hydrolyzing triglycerides. However, insulin deficiency leads to the inactivation of these enzymes, resulting in hypertriglyceridemia.[54]

Effect on serum Urea, Uric Acid and Creatinine

In the excretion of waste materials, the kidneys are crucial. It has been extensively reported that complications of diabetes mellitus include elevations in the levels of metabolites such as albumin, urea, creatinine, and uric acid.[43,44] The considerable increase in blood levels of creatinine, uric acid, and urea after STZ administration shows that the glomerulus is unable to filter these metabolites, leading to a high concentration of these waste products in the serum. When compared with the STZ-induced rats, ESM and metformin treatment ameliorated the nephropathy by lowering serum urea, creatinine, and uric acid levels (Figure 4). The role of urea in the kidney's maximum water conservation involves increased urea reabsorption and, as a result, a tendency to increase serum urea.[45]

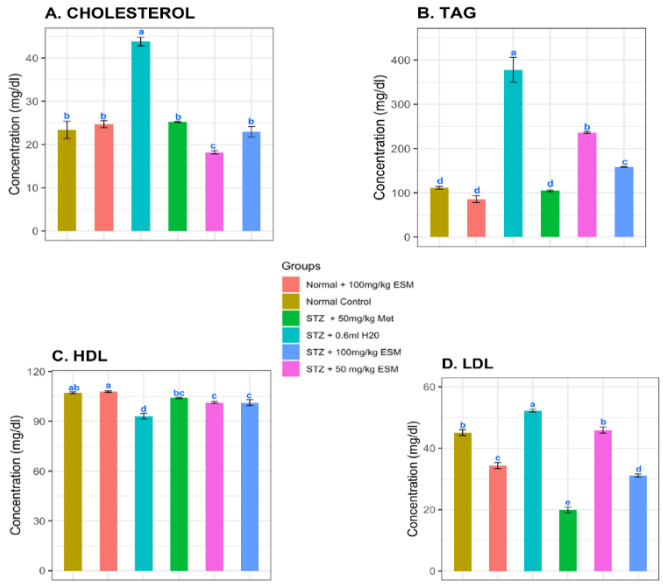

Figure 3 (a-d): Serum Cholesterol, TAG, HDL, and LDL of diabetic rats after diabetes after ESM treatment. Bars with different alphabet showed significant difference at $P < 0.05$.

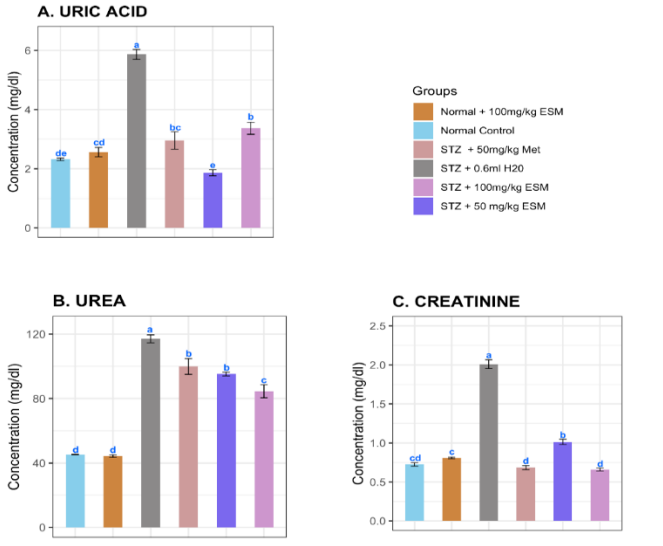

Figure 4 (a-c): Serum Urea, Uric Acid and Creatinine level of diabetic rats after ESM treatment. **A.** Bars with different alphabet showed significant difference at $P < 0.05$.

According to Han et al.[46] an increase in urine creatinine may indicate poor muscle health or inflammation, and it is also thought to be the

result of STZ-induced kidney disease. In the current study, the increase in urea level due to STZ induction could also indicate decreased protein intake and increased breakdown of proteins in muscles and other tissues into amino acids due to increased protease activity.[47] High uric acid levels in the body as a result of STZ-induced damage can cause uric acid crystals to form, resulting in gout.[48]

Histopathology of diabetic rats after ESM treatment

The interlobular duct, pancreatic acini cells, islet and its cellular components, vascular stroma and intercalated ducts are all visible across the various groups. Yellow arrows indicate mild, red arrows indicate a severe: observable signs of fibrosis, hemorrhage, presence of inflammatory red cells, degraded islet cells and poor outline connective tissue and collagen fibers. Control rats, normal rats giving 100 mg/kg of ESM and STZ-induced rats treated with 100 mg/kg of ESM Showed clear duct, intact islet and acinar cells, no observable hemorrhage or fibrosis. STZ-induced control rats and STZ-induced rats treated with 50 mg/kg of ESM showed a severe congested pancreatic duct, degenerated islet and acinar cells, infiltrated pancreatic parenchyma and severe hemorrhagic/fibrotic presentation and congested duct. STZ-induced rats treated with metformin showed mild pancreatic duct congestion/islet cell degeneration with no observable significantloss in acinar cells (Figure 5).

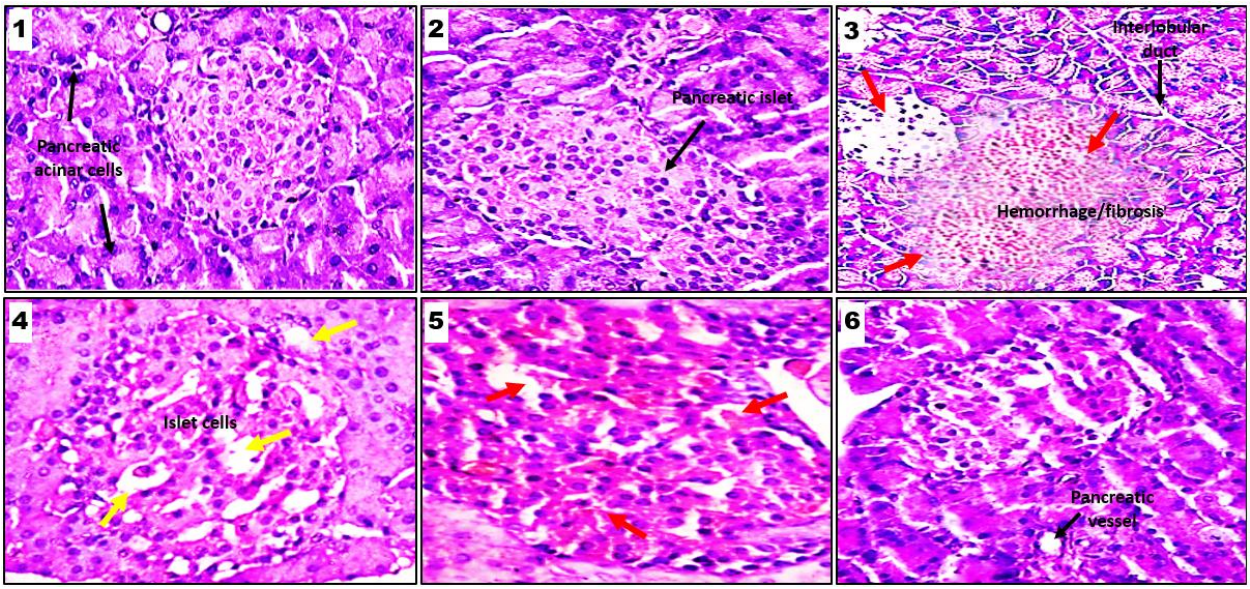

Figure 5: MGx200 views of pancreas micromorphological section demonstrated by H&E staining

Group 1: Normal rats + 0.2 mL of distilled water
Group 2: normal rats + 100 mg/kg b.w. of ESM
Group 3: STZ-induced rats + 0.2 mL of distilled water
Group 4: STZ-induced rats + 50 mg/kg b.w of metformin (standard drug)
Groups 5: STZ-induced rats + 50 mg/kg b.w of ESM
Groups 6: STZ-induced rats + 100 mg/kg b.w of ESM

Conclusion

The result of this study showed that ESM restored glucose homeostasis in STZ-induced rats and ameliorates diabetic complications such as hepatic damage, hyperlipidemia and nephropathy. ESM has favorable effect to restore histopathological changes of the pancreas in STZ induced rats. The anti-diabetic effect of the plant might be due to stimulation of glucose uptake by peripheral tissues, inhibition of endogenous glucose production or activation of gluconeogenesis in the liver and muscle. Further study to isolate and characterize the anti-diabetes compounds from this plant is hereby suggested.

Conflicts of Interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that anyliability for claims related to the content of this article shall be borne by them.

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