



Biochemical Studies of *Averrhoa carambola* Ethanol Leaf and Fruit Extracts on Alloxan-induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The use of herbal medicines has always been an option to treat diseases such as diabetes cancer and other ailments. This study aimed to evaluate the effect of *Averrhoa carambola* leaf and its fruit ethanol extracts on biochemical parameters and histopathology features. Forty five albino rats were assigned into 9 groups of 5 rats each. Except for the normal control group, others were induced to be diabetic by a single intraperitoneal injection of alloxan. Group 1 (normal control) and Group 2 (diabetic untreated) received water. Group 3 received 2.5mg/kg glibenclamide, Groups 4-9 received graded doses (250mg/kg, 500mg/kg and 750mg/kg) of leaf and fruit extracts of *Averrhoa carambola*. Treatment lasted 14days. At the end of the treatment, blood samples and organ (pancreas) were collected from the animals for biochemical and histopathological evaluation.

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Treatment of alloxan-induced diabetic rats significantly ($p < 0.05$) reduced the glucose concentration across all treated groups. Following induction of diabetic mellitus, the serum activities of liver enzymes (Alanine amino transferase, Aspartate aminotransferase and Alkaline phosphatase), low-density lipoprotein cholesterol (LDL-C) and triglyceride were significantly ($p < 0.05$) elevated in diabetic untreated group with corresponding reduction in serum protein, albumin and high-density lipoprotein cholesterol (HDL-C) compared to the normal control and treated groups. However, the fruit extracts exhibited a high increase in urea and creatinine levels especially at dose of 750mg/kg body weight when compared with the leaf though not significantly higher than the diabetic untreated group. From histopathology studies, the groups treated with the leaf extract of *Averrhoa carambola* showed greater structural improvement and an increase in pancreatic islet cells. Therefore this study showed that although ethanol extracts of *Averrhoa carambola* leaf and fruit have antidiabetic properties, the leaf extract possesses more antidiabetic effect.

Keywords: Alloxan; *Averrhoa carambola*; diabetes mellitus; histopathology; lipid profile.

1. INTRODUCTION

Diabetes mellitus is a prevalent metabolic disorder characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1]. Alloxan-induced diabetic rat models are commonly used to study diabetes due to their ability to mimic the pathophysiological changes observed in humans, particularly in type 1 diabetes [2]. This model is instrumental in investigating potential therapeutic interventions, especially the effects of various plant extracts.

Diabetes significantly affects liver and kidney function [3]. The liver, which is the centre for glucose metabolism, experiences increased stress due to elevated blood sugar levels. This hyperglycemic environment leads to hepatic steatosis, insulin resistance, and the generation of reactive oxygen species (ROS), which can cause hepatocyte damage. Consequently, liver function enzymes, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), often rise, indicating liver injury [4].

Diabetes also causes a severe risk to renal health. Prolonged hyperglycemia can activate pathways that result in nephropathy, characterized by glomerular hyperfiltration and subsequent damage to renal structure [5]. Elevated blood sugar levels can lead to accumulation of advanced glycation end-products (AGEs) and induce inflammation, exacerbating kidney injury. This condition is often marked by increased proteinuria and elevated creatinine levels, reflecting compromised renal function [6].

Plant extracts have gained attention as potential therapeutic agents due to their rich

phytochemical composition, which may exert beneficial effects on metabolic health. These extracts often contain antioxidants, anti-inflammatory compounds, and bioactive substances that can modulate metabolic pathways [7]. For instance, they may reduce oxidative stress, enhance insulin sensitivity, and improve lipid metabolism, alleviating the adverse effects of diabetes on the liver and kidneys [8].

The plant *Averrhoa carambola* also known as star fruit is an aged plant belonging to oxalidaceae family with many medicinal purposes [9]. Traditionally, the juice from the fruit is used in the management of fever and stimulation of appetite [10]. A decoction of *carambola* leaves has been reported to be used in the treatment of diabetes [11]. Several studies have highlighted the anti-inflammatory [12], Analgesic [13], hypolipidemic and hypocholesterolemic [14] activities of *Averrhoa carambola*. These properties are as a result of important phytochemicals like saponin, alkaloid, and other various biological activities [15]. This study was aimed at evaluating the anti diabetic properties of *Averrhoa carambolale* leaf and fruit extracts on alloxan-induced rats.

2. MATERIALS AND METHODS

2.1 Sample Collection

The plant materials (Fresh leaves and matured ripped fruits) of *Averrhoa carambola* were collected from Umueme Obike in Ngor Okpala Local Government Area in Imo State, Nigeria. Botanical identification and authentication were performed by Dr. Hyginus C. Ogbuchi, Crop Science and Biotechnology, Faculty of Agriculture and Veterinary Medicine Imo State University where a voucher

(001/CSB/IMSU/2021) specimen was assigned at herbarium for reference.

2.2 Experimental Animals

Forty five (45) healthy male albino rats weighing 120 to 170g were used for the anti-diabetic study. All animals were obtained from the Faculty of Veterinary medicine, University of Nigeria Nsukka. They were acclimatized for seven days in the animal house, Department of Biochemistry Federal University of Technology Owerri Imo State, Nigeria and maintained in a clean environment of about 27°C twelve hours light and twelve hours of darkness with standard feed and allowed free access to clean water.

2.3 Extraction Procedure of *Averrhoa carambola* Leaf

The fresh leaves of *Averrhoa carambola* were thoroughly washed with clean tap water and dried for about two weeks under room temperature. The fresh leaves were ground into powder using a warring blender. 10grams of the leaves powder were suspended in 100ml of absolute ethanol and were allowed to stand for 48 hours. The suspension was then filtered with muslin cloth, the filtrate was again filtered with Whatman no1 filter paper. The resultant filtrate was evaporated in a water bath at 50°C and dried using a vacuum desiccator. The extract was transferred to an air tight bottle and stored in a refrigerator at 4°C prior to use.

2.4 Processing and Extraction of *Averrhoa carambola* Fruit

Fresh ripe fruits from *Averrhoa carambola* were properly washed with purified tap water, drained and later smashed with a manual blender. The smashed fruit materials (10g) were soaked in 100mls of ethanol for 48 hours at room temperature. After two days of occasional shaking, the whole material was filtered using a muslin cloth and the filtrate evaporated under reduced pressure using a water bath. The subsequent filtrate was dried in a vacuum desiccators after being evaporated in a water bath at 50°C. The extract was put into an airtight bottle and kept in a 4°C refrigerator until needed.

2.5 Induction of Diabetes

Diabetes was induced in the experimental rats by a single intra-peritoneal injection of 150mg/kg body weight of alloxan monohydrate (Sigma-

Aldrich, U.S.A) using distilled water as a vehicle following the method described by Carvalho [16]. Three days after alloxan administration, blood samples were collected from the tail vein of the rats, and their blood glucose levels were measured using an Acucheck glucometer and test strip to confirm diabetes. Rats that exhibited blood glucose level greater than 200mg/dl were considered diabetic and were selected for the experiment.

2.6 Experimental Design

Forty five (45) male albino rats of average weight 120-170g were classified into nine groups of five rats per group and subjected to treatments as follows:

- Group 1 – Normal control, received food and water
- Group 2 – Diabetic untreated group, received food and water
- Group 3- Diabetic control received glibenclamide (2.5mg/kg)
- Group 4 – Diabetic rats received 250mg/kg *Averrhoa carambola* leaf extract
- Group 5 – Diabetic rats received 500mg/kg *Averrhoa carambola* leaf extract
- Group 6 – Diabetic rats received 750mg/kg *Averrhoa carambola* leaf extract
- Group 7 – Diabetic rats received 250mg/kg *Averrhoa carambola* fruit extract
- Group 8 – Diabetic rats received 500mg/kg *Averrhoa carambola* fruit extract
- Group 9 – Diabetic rats received 750mg/kg *Averrhoa carambola* fruit extract

2.7 Blood Glucose Determination

This was determined based on the method outlined by Yakubu [17], Uhou [18]. A drop of blood was collected through the tail of all diabetic rats and dropped on the dextrose reagent pad and inserted into a micro processor digital blood glucometer and readings were recorded.

2.8 Animal Sacrifice and Blood Sample Collection

This was done as described by Stephen [19]. After (14 days) of treatment, the animals were made unconscious by placing them in a desiccator with 10% chloroform. Blood samples were collected through cardiac puncture with the help of syringe and needle and dispensed into lithium heparin bottle and was centrifuged for 10 minutes at 4000rpm. Serum was separated from

the plasma using Pasteur pipette for biochemical analysis.

The serum activities of liver marker enzymes (AST, ALP and ALT), and serum levels of albumin, Total protein, Triglycerides, Cholesterol, LDL, HDL, Urea and Creatinine were assayed using an auto-analyzer (Cobas C111 Chemistry Analyzer).

2.9 Histopathology Study

Microscopic tissue slides were processed using standard procedures described by Tedong [20]. Paraffin section of 4-5µm were stained with hematoxylin eosin

2.10 Statistical Analysis

Data obtained from the investigation were subjected to statistical analysis using the statistical package for Social Science (SPSS). One way analysis of variance (ANOVA) was used to assess the differences between groups. Duncan's multiple comparisons were used to differentiate between analytical

parameter means. Statistical significant level was considered at $P < 0.05$.

3. RESULTS

In the treated diabetic groups, a significant decrease in glucose level was observed (day 2-14) in comparison with the diabetic untreated group as shown in Fig. 1. However, maximum reduction in blood glucose level was observed in the group treated with 750mg/kg leaf extract of *Averrhoa carambola*.

The results of alanine transaminase (ALT) of the normal and test animals are presented in Fig. 2. The diabetic untreated rats showed a significant increase ($p < 0.05$) in ALT activity compared to normal control, standard drug as well as all the treated groups. In comparison with 2.5mg/kg of the standard drug (glibenclamide) with the normal control and test groups, 250mg/kg and different doses of ACFE had significant increases in ALT activity. No significant different was observed in the normal control and groups treated with 750mg/kg of leaf extract.

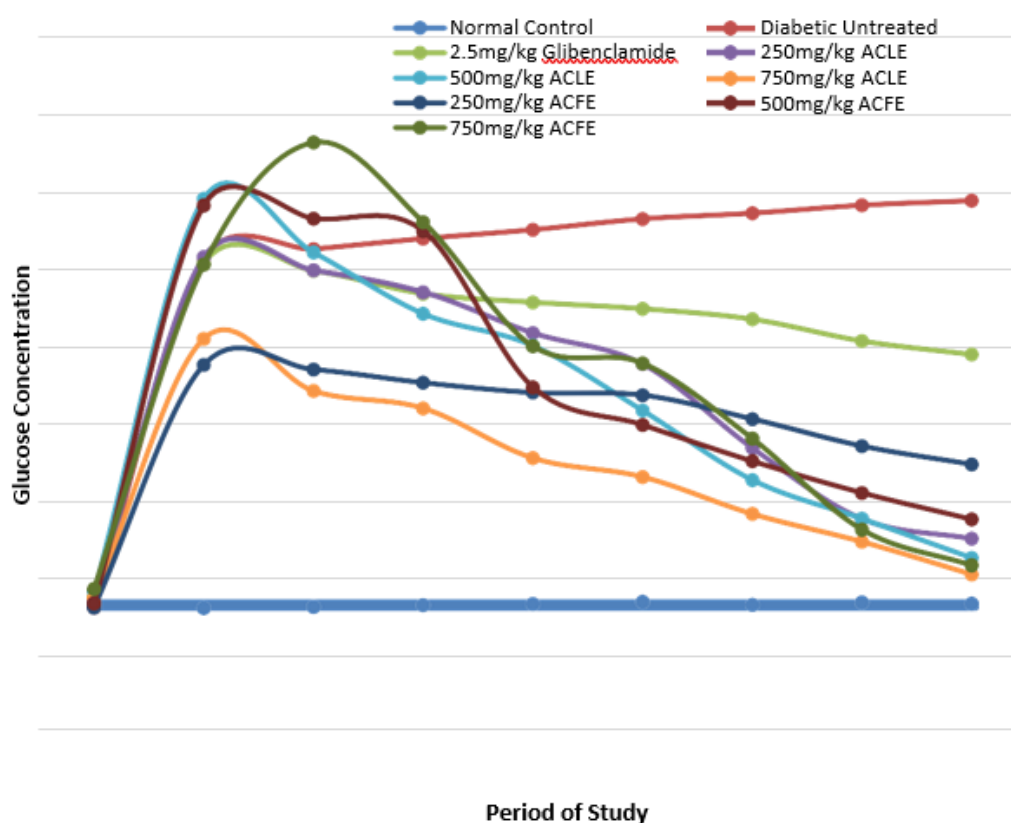


Fig. 1. Effect of *Averrhoa carambola* leaf and fruit ethanol extracts on glucose level of alloxan-induced diabetic rats

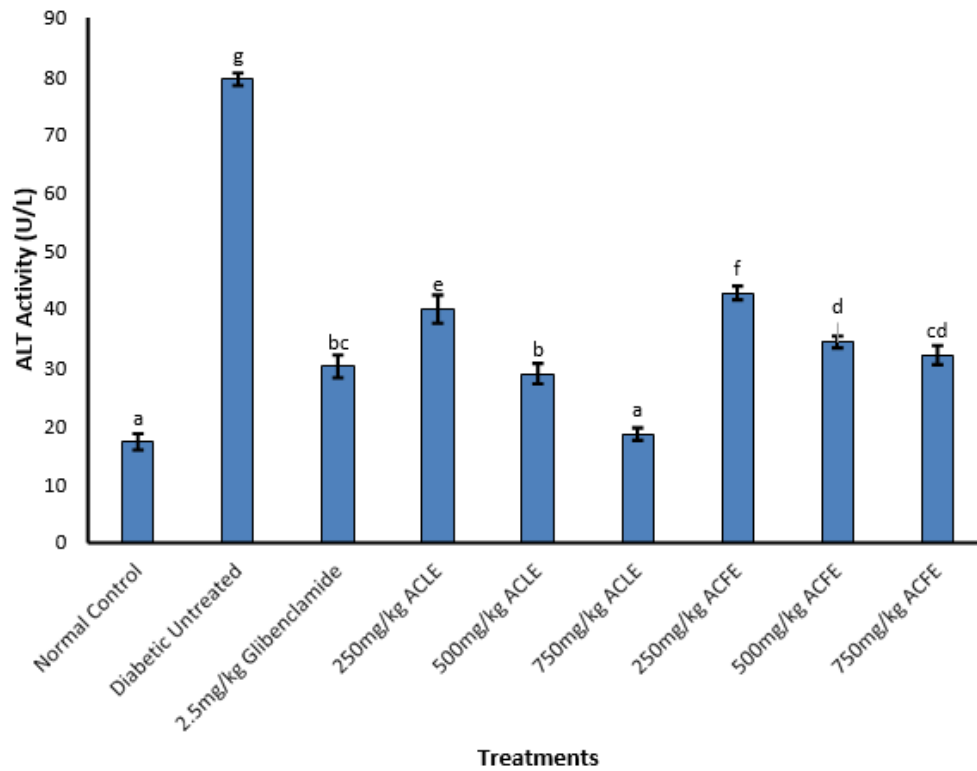


Fig. 2. Effect of *Averrhoa carambola* leaf (ACLE) and fruit (ACFE) ethanol extracts on Alanine Transaminase (AST) of alloxan-induced diabetic rats
Bars with the same superscript are not statistically different ($p < 0.05$)

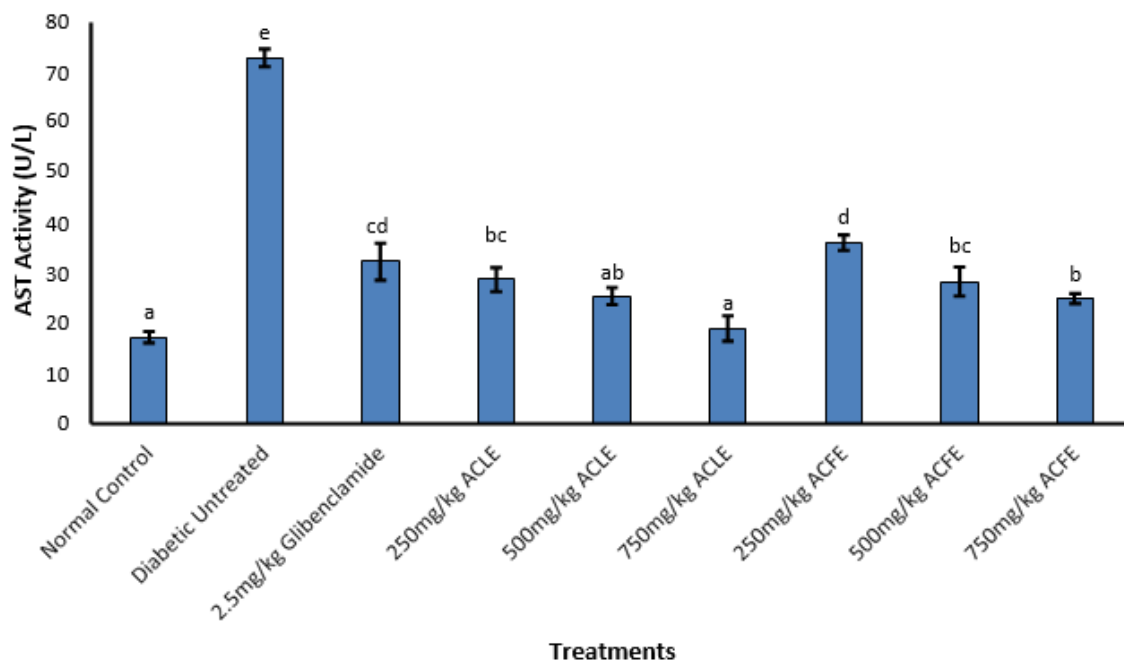


Fig. 3. Effect of *Averrhoa carambola* leaf (ACLE) and fruit (ACFE) ethanol extracts on Aspartate Transaminase (AST) of alloxan-induced diabetic rats
Bars with the same superscript are not statistically different ($p < 0.05$)

The diabetic untreated group showed a significant increase in AST activity compared to normal control, standard drug as well as the extracts treated groups Fig. 3. In comparison with the standard drug, the reduction in AST activity of 500mg/kg ACLE and 750mg/kg ACLE and 750mg/kg ACFC were significant while no significant difference was seen in 250mg/kg ACLE and 500mg/kg ACFE treated groups.

With the increase in concentration of the extracts, a reduction in ALP activity was

observed Fig. 4. Reduction to normal level was observed in the group treated with the highest concentration of leaf extract. No significant difference was seen in ALP activity of the groups treated with 500mg/kg ACLE and 750mg/kg ACFE but the ALT activity was significantly reduced compared to that of 250mg/kg ACLE and 2.5mg/kg glibenclamide (standard drug). In comparison with the standard drug, 250mg/kg and 500mg/kg ACFE exhibited a high increase in ALP activity but the increase exhibited by the diabetic untreated group was more significant.

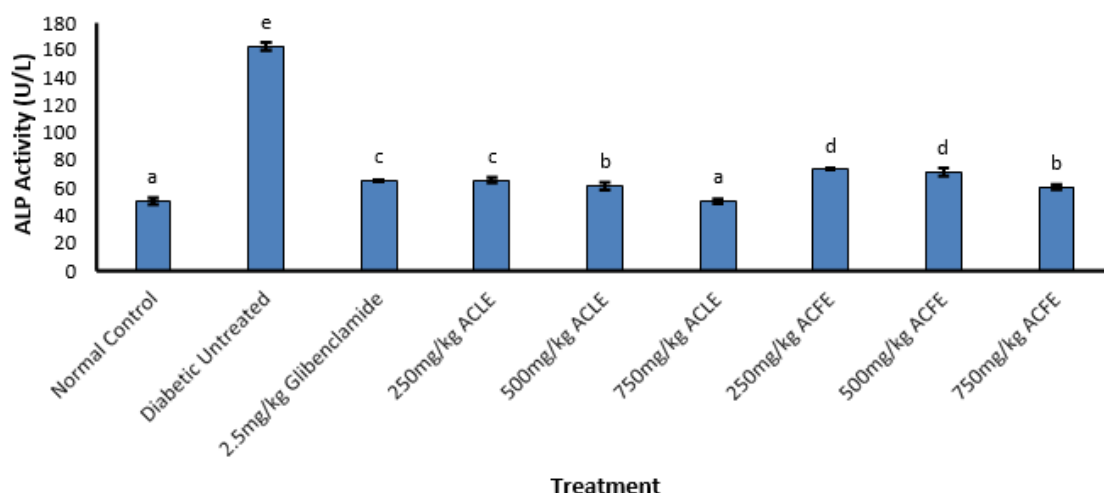


Fig. 4. Effect of *Averrhoa carambola* leaf (ACLE) and fruit (ACFE) ethanol extracts on Alkaline Phosphatase (ALP) of alloxan-induced diabetic rats
Bars with the same superscript are not statistically different ($p < 0.05$)

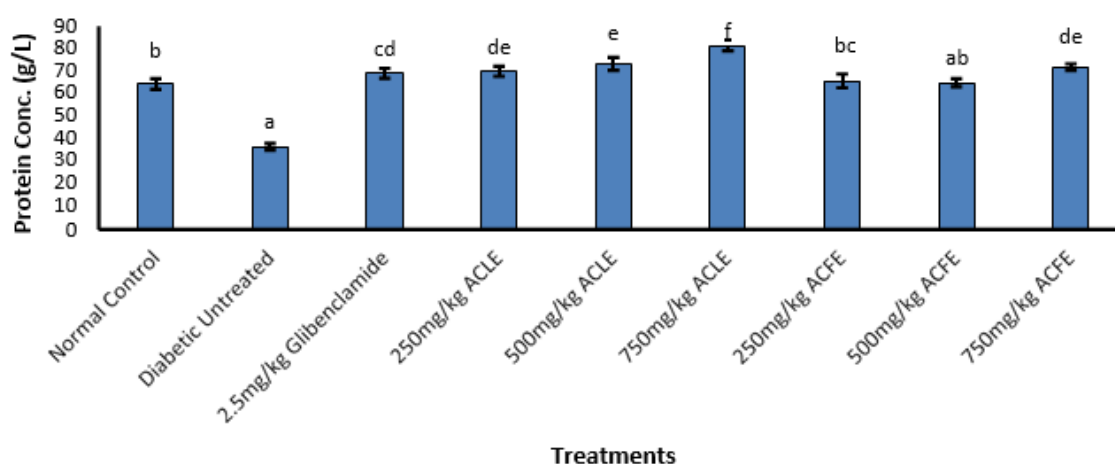


Fig. 5. Effect of *Averrhoa carambola* leaf (ACLE) and fruit (ACFE) ethanol extracts on Total Protein concentration of alloxan-induced diabetic rats
Bars with the same superscript are not statistically different ($p < 0.05$)

There was a significant reduction in total protein concentration of the diabetic untreated animals compared to normal control, 2.5mg/kg glibenclamide (standard drug) and extracts treated groups Fig. 5. In comparison with 2.5mg/kg glibenclamide, a dose-dependent significant increase was observed in leaf extracts groups whereas 250mg/kg and 500mg/kg of ACFE had a significant reduction. No significant difference was seen in 250mg/kg ACLE and 750mg/kg ACFE. An increase in leaf and fruit extract concentration resulted to an increase in protein concentration.

With the increase in concentration of the extracts an increase in albumin concentration was observed across all the treated groups as well as the normal control while a significant reduction was seen in the diabetic untreated group Fig. 6. In comparison with 2.5mg/kg glibenclamide (standard drug) the leaf extract increased significantly with an increase in extract doses whereas no significant difference was observed in the groups treated with 250mg/kg and 500mg/kg ACFE. Although all the treated groups showed significant effects, 750mg/kg ACLE was more effective.

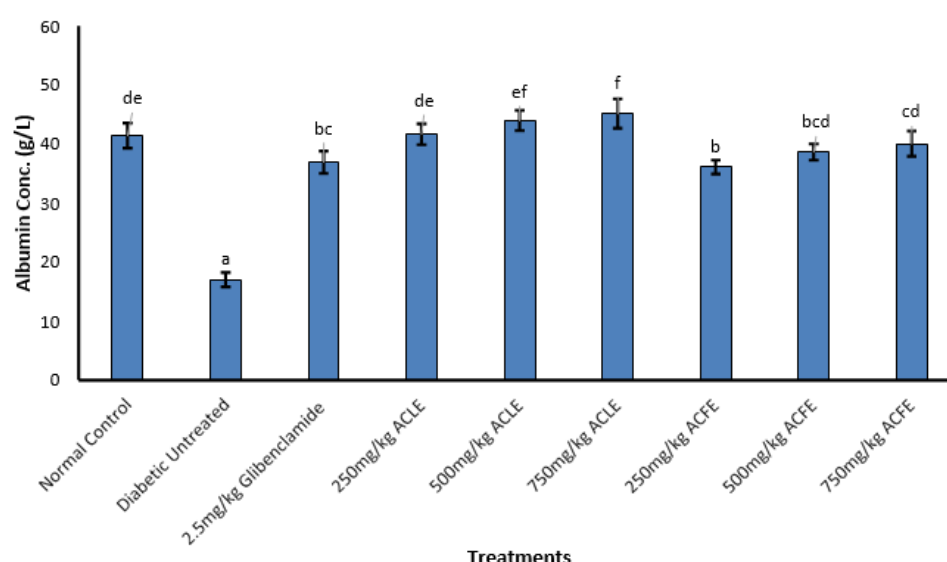


Fig. 6. Effect of *Averrhoa carambola* leaf (ACLE) and fruit (ACFE) ethanol extracts on albumin concentration of alloxan-induced diabetic rats
Bars with the same superscript are not statistically different ($p < 0.05$)

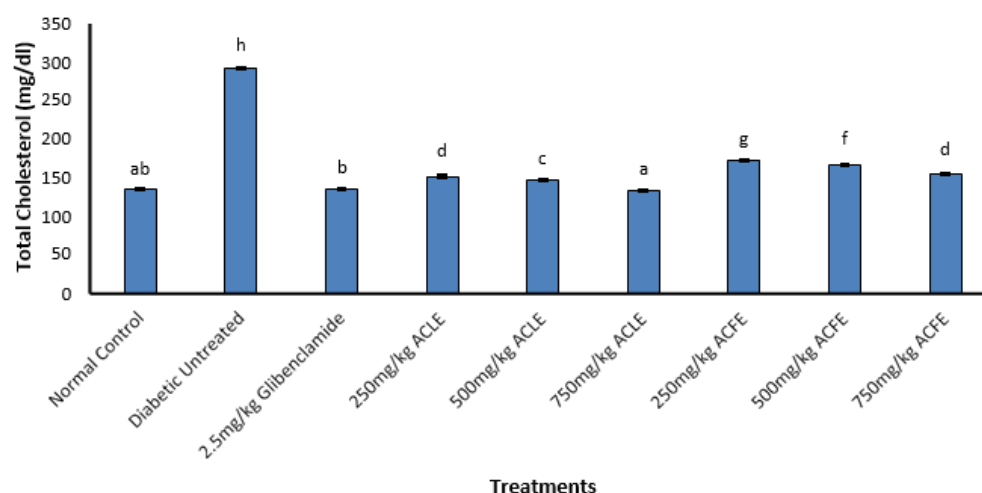


Fig. 7. Effect of *Averrhoa carambola* leaf (ACLE) and fruit (ACFE) ethanol extracts on Total Cholesterol level of alloxan-induced diabetic rats
Bars with the same superscript are not statistically different ($p < 0.05$)

With the increase in concentration of the extracts, a reduction in total cholesterol level was observed across all the extract-treated groups in comparison with the diabetic untreated group Fig. 7. The cholesterol concentrations of animals treated with 250mg/kg and 500mg/kg of ACFE were significantly higher ($p<0.05$) than those treated with 500mg/kg and 750mg/kg of leaf extract. Furthermore, no significant difference was seen in the groups treated with 250mg/kg ACLE and 750mg/kg ACFE.

From the result presented in Fig. 8, the diabetic untreated group showed a significant increase in

triglyceride concentration compared to normal control, standard drug as well as the treated groups. In comparison with the 2.5mg/kg glibenclamide (standard drug), a significant increase was seen in the extract-treated groups except the group that received 750mg/kg ACLE which exhibited a significant decrease in TG concentration. The TG concentration of the normal control was found to be significantly higher ($p<0.05$) than that of the standard drug and group treated with 750mg/kg of ACLE respectively but was significantly lower than that of other treatment groups.

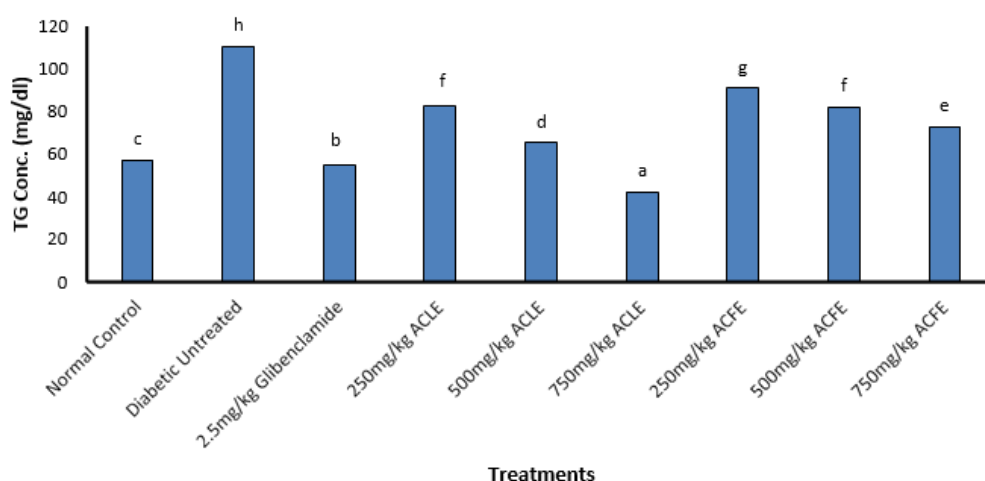


Fig. 8. Effect of *Averrhoa carambola* leaf and fruit ethanol extracts on Triglyceride (TG) concentration of alloxan-induced diabetic rats

Bars with the same superscript are not statistically different ($p<0.05$)

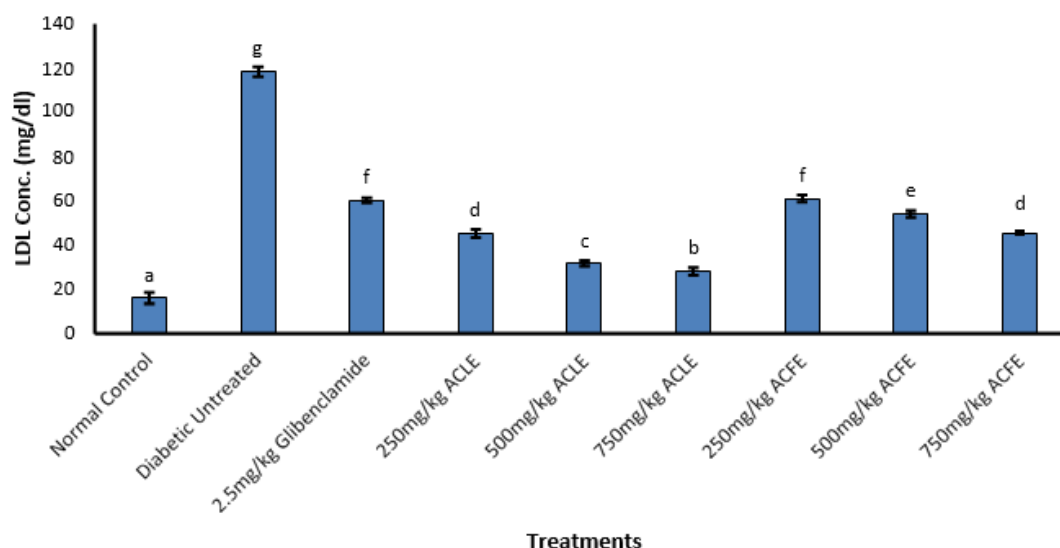


Fig. 9. Effect of *Averrhoa carambola* leaf and fruit ethanol extracts on Low Density Lipoprotein (LDL) concentration of alloxan-induced diabetic rats

Bars with the same superscript are not statistically different ($p<0.05$)

All the treatment groups in Fig. 9 had LDL concentrations that was significantly higher than the normal control with a concentration-dependent decrease within the treatment groups. No significant difference was established between group D treated with 250mg/kg ACLE and group I treated with 750mg/kg ACFE.

From the result presented in Fig. 10, the diabetic untreated group showed a significant decrease in HDL concentration in comparison with normal control, 2.5mg/kg glibenclamide (standard drug) and the extracts treated groups. No significant difference was seen in the HDL concentration of 750mg/kg ACLE in comparison with 2.5mg/kg

glibenclamide but HDL concentration of other treatment groups was significantly lower than the normal control.

The result on urea concentration is presented in Fig. 11. The diabetic untreated group showed a significant increase in urea concentration compared to the urea level of the normal control, 2.5mg/kg glibenclamide (standard drug) and all the extracts treated groups. In comparison with 2.5mg/kg glibenclamide (standard drug), different doses of ACLE and 250mg/kg of ACFE had a significant reduction whereas, 750mg/kg ACFE increased significantly. Reduction to normal level was seen in the group treated with 500mg/kg ACFE.

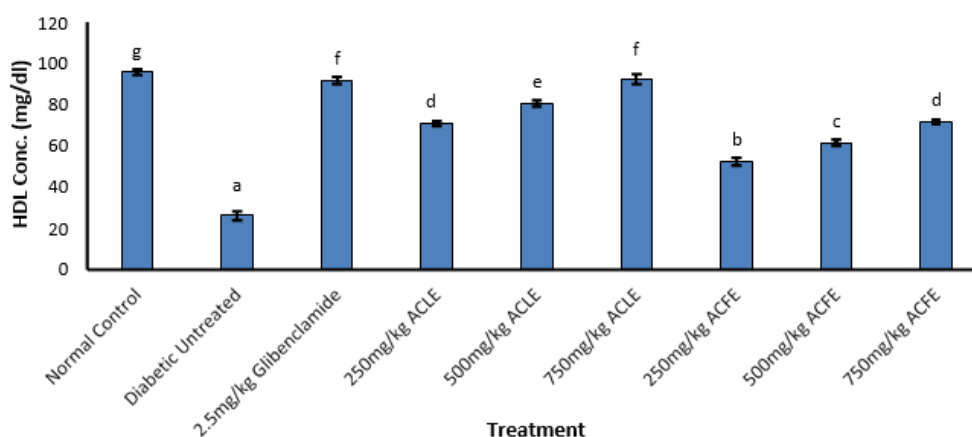


Fig. 10. Effect of *Averrhoa carambola* leaf (ACLE) and fruit (ACFE) ethanol extracts on High Density Lipoprotein (HDL) of alloxan-induced Diabetic rats
Bars with the same superscript are not statistically different ($p < 0.05$)

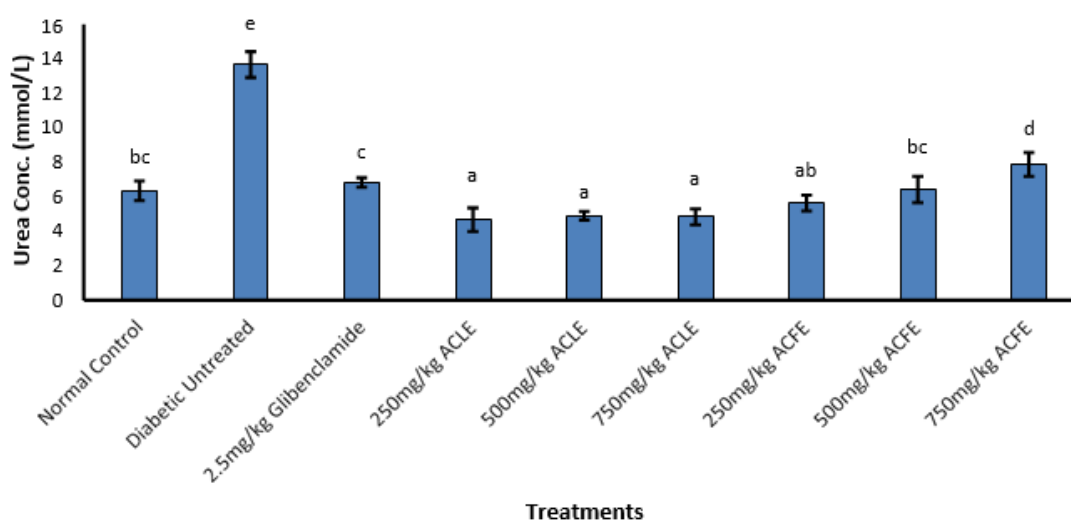


Fig. 11. Effect of *Averrhoa carambola* leaf and fruit ethanol extracts on urea concentration of alloxan-induced diabetic rats
Bars with the same superscript are not statistically different ($p < 0.05$)

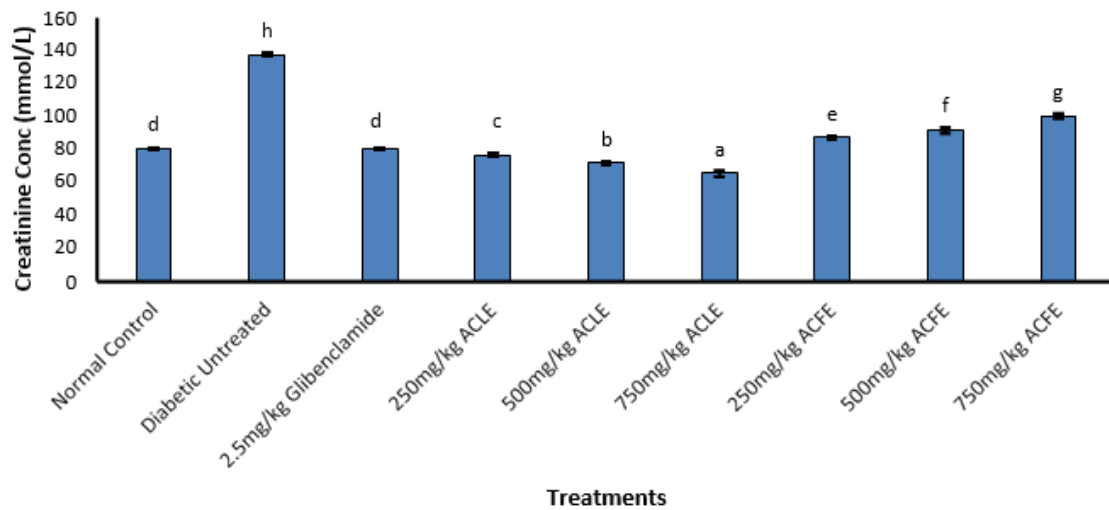


Fig. 12. Effect of *Averrhoa carambola* leaf and fruit ethanol extracts on creatinine concentration of alloxan-induced diabetic rats

Bars with the same superscript are not statistically different ($p < 0.05$)

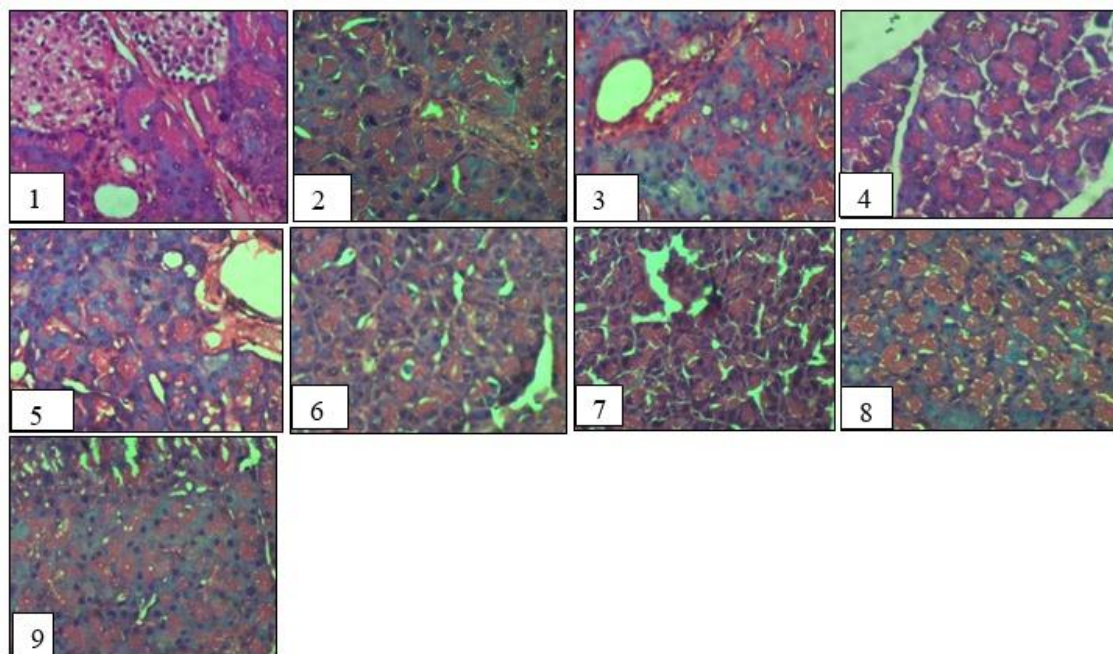


Fig. 13. Effect of *Averrhoa carambola* leaf and fruit ethanol extracts on histopathology of the pancreas of different groups of alloxan- induced diabetic rats

The creatinine concentration increased significantly in the diabetic untreated group in comparison with the normal control, 2.5mg/kg glibenclamide and extracts treated groups as presented in Fig.12. However 250mg/kg, 500mg/kg and 750mg/kg ACLE had significant reduction in creatinine level in comparison with 2.5mg/kg glibenclamide while 250mg/kg, 500mg/kg and 750mg/kg of ACFE increase

significantly. No significant different was seen in the group treated with 2.5mg/kg glibenclamide and the normal control.

The following were observed from the histopathology examination of the pancreas carried out on rats treated with *Averrhoa carambola* leaf and fruit extracts as shown in Fig. 13. **Group 1:** Normal parenchymal and islet cells

and blood capillaries was observed (H&E X 200).

Group 2: Showed prominent nuclei, marked haemorrhagic necrosis, dilated and decreased β islet cells (H&E X 200). **Group 3:** Mild inflammatory response and vacuolation was seen (H&E X 200). **Group 4:** Showed improved islet cells and pancreatic acini. Features are (H+) reparative changes in pancreatic parenchymal and islet cells (H&E X 200). **Group 5:** Normal islet and pancreatic acini was observed. Features were moderate (H+) reparative changes in pancreatic architecture (H&E X 200). **Group 6:** Showed normal islet cells, normal blood capillaries and pancreatic acini. Over all features improvement in pancreatic parenchymal architecture (H&E X 200). **Group 7:** Photomicrograph of the pancreas showing normal islet cells and pancreatic acini, features mild (+) reparative changes in pancreatic parenchyma (H&E X 200). **Group 8:** Photomicrograph of the pancreas showing intact islet cells and intact acini. Features (H+) cellular and reparative changes of beta islet cells (H&E X 200). **Group 9:** Photomicrograph of the pancreas showing normal islet cells and pancreatic acini. Features mild (+) reparative changes in pancreatic parenchyma (H&E X 200).

4. DISCUSSION

Alloxan is a well-known diabetogenic agent that selectively destroys insulin-producing beta cells in the pancreas, leading to hyperglycemia [21]. In this study, the plant extracts administration resulted in a significant reduction in blood glucose levels in the treatment groups compared to the diabetic untreated group. This suggest that *Averrhoa carambola* extracts may contain bio-active compounds with hypoglycemic properties. The possible mechanism by which plants extract brings about its hypoglycemic effect may be by increasing pancreatic secretion of insulin from beta-cells, enhancing peripheral glucose uptake or reducing hepatic glucose production [22]. The plants antioxidant and anti-inflammatory potential might also play a role in protecting beta cells from oxidative stress, thus aiding in the regulation of blood sugar levels [23]. This implies that *Averrhoa carambola* leaf and fruit extracts may promote beta-cells generation and have a protective effect on beta-cells from glucose toxicity. The result of this experiment aligns with findings suggesting that *Averrhoa carambola* can be used as generic medicine to balance glucose level [24].

Liver enzymes markers like ALT and AST are often elevated in diabetic models due to liver

damage. However, in treatment groups with *Averrhoa carambola* extracts this marker were observed to have significantly reduced ($p < 0.05$) in comparison with the untreated group indicating protection against liver injury. Several studies has reported the hepatic effect of *Averrhoa carambola* leaf extract, one of the researchers in his work titled hepatoprotective effect of *Averrhoa carambola* fruit extract on carbon tetrachloride-induced hepatotoxicity in mice stated that the ability of *Averrhoa carambola* fruit extract to reduce the level of liver marker enzymes in treated groups could be due to the presence of antioxidants and polyphenols which may reduce hepatic oxidative damage and inflammation, thereby improving liver function [25]. These results are in line with previous studies, which showed a significant reduction in liver marker enzymes in groups treated with *Averrhoa carambola* extracts compared to the untreated group [26, 27].

In diabetes, protein and albumin levels are often reduced due to liver dysfunction or nephropathy. The observed increase in protein and albumin levels in the treated groups suggests that *Averrhoa carambola* extracts may help in normalizing protein synthesis, improving liver function and overall metabolic health. This improvement could also be linked to the plant's extract ability to mitigate oxidative stress and inflammatory responses which are commonly elevated in diabetic conditions and impact protein metabolism. Also in this study titled anti-hyperglycemic activity of alcoholic extract of *Averrhoa carambola* leaves in alloxan-induced diabetic rats has shown that improved liver function and protein synthesis could be attributed to the antioxidant and anti-inflammatory properties of the plant which mitigate oxidative damage often seen in diabetic organs [28].

Hyperlipidemia is commonly associated with diabetes, and plant extracts like *Averrhoa carambola* have been shown to lower cholesterol and triglyceride levels which is in line with this present work [29]. The presence of phytosterols and saponins in the plant extract has been discovered to inhibit cholesterol absorption or reduce lipid synthesis [30,31]. Also another study titled antihyperlipidemic and hepatoprotective activities of aqueous extract of *Averrhoa carambola* has shown that reduction in cholesterol and triglycerides in rats treated with *Averrhoa carambola* extract might be due to the inhibition of the HMG-CoA reductase enzyme, which is responsible for cholesterol biosynthesis [32]. Also previous study has shown that

flavonoids and alkaloids present in *Averrhoa carambola* could also be responsible for this lipid lowering effect [33]. HDL plays a critical role in reverse cholesterol transport, reducing atherogenic risk factors. It is often referred to as good cholesterol because it helps remove excess cholesterol from the blood stream [34]. An increase in HDL was observed, indicating improved lipid metabolism and reduced cardiovascular risk. The increase in HDL level in treatment groups suggests that the plant extracts have a favorable impact on lipid metabolism.

Elevated creatinine and urea levels in hyperglycemia are often indicative of underlying complications, primarily involving kidney function [35]. The groups treated with fruit extracts showed a slight increase in creatinine and urea levels, though not significantly higher than the diabetic untreated group. Elevated creatinine often indicates renal stress, but this result suggest that *Averrhoa carambola* did not cause significant kidney damage compared to untreated diabetic group. Some studies indicates that the antioxidant properties of certain plants extracts can prevent or reduce diabetic nephropathy [36] while creatinine level were slightly elevated.

Histopathological examination of pancreatic section from the alloxan induced diabetic control group revealed typical features of beta cells and disorganized islet architecture. These findings align with the expected cytotoxic effects of alloxan on the pancreatic beta cells, which lead to a marked reduction in insulin production and hyperglycemia [37]. In contrast, diabetic rats treated with *Averrhoa carambola* leaf and fruit extract showed a slight improvement in pancreatic function indicating potential recovery. The pancreatic islets in this groups also exhibited a more preserved structure with reduced evidence of beta cell destruction. The degree of improvement in the number of beta cells was more pronounced in the groups treated with leaf extract suggesting that the phytochemical constituents present in the leaf may have more protective effect. This improvement could be attributed to the antioxidant and anti inflammatory properties of the bioactive compounds found in the plant such as flavonoid, polyphenols and ascorbic acid which are known to combat oxidative stress, a key distributor to beta cell damage in diabetes.

5. CONCLUSION

The overall effect of the plant extract on alloxan induced diabetic rats shows a promising potential

in managing hyperglycemia, dyslipidemia, and liver function, while maintaining protein synthesis and albumin levels. The slight elevation in creatinine warrants further investigation. These results support the therapeutic value of the plant extract, particularly for its hypoglycemic, lipid-lowering and hepatoprotection properties, while emphasizing the need on renal monitoring. Further research on the extract on dose optimization and long term safety is necessary for clinical translation.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (Chat GPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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