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Research Article

A Comparative approach to the effects of aqueous leaf and root extracts of *Moringa oleifera* on selected biochemical, haematological, and gut microbiota parameters of alloxan-induced diabetic Wistar rats

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Background: Diabetes mellitus is one of the metabolic diseases known all over the world. This syndrome features symptoms like hyperglycaemia, oxidative stress, and associated organ dysfunctions. *Moringa oleifera* is a commonly known medicinal plant explored by many for its healing activities. This study seeks to find out the comparative effects of the aqueous root and leaf extracts of *M. oleifera* on some biochemical, haematological, and gut microbiota parameters in alloxan-induced diabetic Wistar rats.

Methods: About thirty-six (36) adult Wistar rats were used in the study. These rats were randomly divided into six (6) groups, viz the normal control, diabetic control, diabetic treated with leaf extract, diabetic treated with root extract, diabetic treated with both leaf and root extracts, and diabetic treated with metformin, the standard drug. 150 mg/kg body weight of alloxan monohydrate was intraperitoneally given to the rats to induce diabetes. The experimental rats were exposed to treatments orally for 21 days. The study adopted standard methods for the estimation of the biochemical and haematological parameters, including fasting blood glucose, lipid profile, liver enzyme activities. Faecal sampling and culture-based methods were used to estimate the gut microbiota composition.

Results: The results of the study show that both the extracts from the leaves and roots of *M. oleifera* lowered blood glucose levels and lipid profile status at $p < 0.05$ level of significance. Similarly, the haematological and antioxidant parameters were normalised compared with diabetic controls, with the leaf extract exhibiting moderately higher potency. The results of the gut microbiota analysis showed that both the leaf and root extracts of *M. oleifera* exerted the modulation of bacterial diversity, reduction of pathogenic taxa and enrichment of beneficial commensals, with the combined extract showing synergistic effects.

Conclusion: The findings revealed that the leaf and root extracts of *M. oleifera* exert potent blood-sugar lowering and microbiota-modulating effects, hence support their use as a complementary therapeutic agent for the management of diabetes mellitus.

Keywords: *Moringa oleifera, diabetes mellitus, biochemical parameters, haematological parameters, gut microbiota*

Introduction

There is no doubt that diabetes mellitus is one of the widely known and most ptevalent metabolic disorders. It is mainly associated with symptoms like chronic hyperglycaemia as a result of impairment in the secretion or action of insulin or both (Owo & Beresford, 2020; Yameny, 2024). Several studies have shown that long-term diabetes mellitus results in poor metabolism. For instance, Owo et al. (2025) and Chen et al. (2025), chronic diabetes mellitus results from abnormal metabolisms of carbohydrates, lipids, and proteins. The consequences of this metabolic dysfunction are oxidative stress, cardiovascular problems, and organ damages. Be that as it may, the dependence on synthetic antidiabetic drugs for a long time has been associated with several adverse effects and also the high cost of purchase of these drugs has impacted their availability. Hence, these problems have provoked the urgent search for natural remedies that would replace these synthetic antidiabetic drugs (Blahova et al., 2021). One of the highly celebrated herbs for the treatment of several diseases is *Moringa oleifera* due to its rich nutritional and phytochemical content (Shivangini et al., 2022). The leaves and roots of the plant contain various biologically-active compounds like flavonoids, alkaloids, terpenoids, polyphenols, and tannins. *M. oleifera* has been known for its antioxidant, hypoglycaemic, and antimicrobial potential due to the presence of these biologically-active compounds (Adekanmi et al., 2020; Abhang et al., 2024). Metabolic obstructions associated with diabetes mellitus are the leading reasons for diabetes-associated gut dysbiosis. This condition arises when there is an imbalance in the normal intestinal microbial community in individuals with long-term diabetes mellitus (Gradisteanu Pircalabioru et al., 2021), leading to the reduction in microbial diversity, overgrowth of pathogenic bacteria, and reduction in the population of beneficial microbes (Harsch & Konturek, 2018; Bielka et al., 2022). A key problem of this microbial imbalance is the reduced production of short-chain fatty acids (SCFAs), which are normally produced by gut microorganisms during the fermentation of dietary fibres (Wang et al., 2019; Vinelli et al., 2022). Short-chain fatty acids (SCFAs) carry out important functions in the body. Some of them are strengthening of the intestinal barrier, alteration of immune and inflammatory responses, and balancing of glucose and lipid metabolism (He et al., 2020). In diabetic individuals, reduced SCFA levels affect gut barrier function, increase intestinal permeability ("leaky gut"), and facilitate the translocation of bacterial lipopolysaccharides (LPS) into systemic circulation. This process triggers chronic low-grade inflammation, that is metabolic endotoxaemia, further worsening hyperglycaemia and insulin resistance (Ghosh et al., 2020; Di Vincenzo et al., 2024). Furthermore, bioactive compounds obtained from medicinal plants can directly cause microbial growth by inhibiting the actions of bacterial enzymes, disrupting cell wall and membrane integrity, and modulating quorum-sensing pathways, hence modulating the composition and activity of the gut microbiota (Bouyahya et al., 2022). In addition, these bioactive compounds can indirectly modulate the gut microbiota by affecting the immune responses of the hosts, which in turn can disrupt the gut microbial environment. Collectively, these mechanisms suggest that phytochemicals may offer several approaches to the management of diabetes mellitus through gut microbiota modulation (Chen et al., 2022; Wang et al., 2024). Despite that several studies have explored the leaves and roots of *M. oleifera* for their antidiabetic potential, comparative evidence between them, particularly in relation to biochemical, haematological, and gut microbiota parameters in diabetic models, remains underexplored. It is on this basis that this study aimed to evaluate comparatively the effects of aqueous leaf and root extracts of *M. oleifera* on biochemical, haematological, and gut microbiota parameters in alloxan-induced diabetic Wistar rats.

Materials and methods

Plant collection and preparation

The fresh leaves and roots of *M. oleifera* were collected from Oil Mill Main Market in Port Harcourt, Rivers State, Nigeria. The plant samples were verified and authenticated by experts from the Department of Plant Science and Biotechnology, Rivers State University, Port Harcourt, and were later prepared for experimental use. After collection, the leaves and roots were cleaned with distilled water, air-dried at room temperature, and then pounded into a fine powder. The aqueous extracts were prepared by cold maceration in distilled water, filtered and finally concentrated by means of a rotary evaporator.

Experimental animals

Thirty-six healthy adults male Wistar rats, each weighing between 150 and 200 g, were obtained from the Rivers State University Animal House, Port Harcourt. The rats were housed in a standard laboratory with proper ventilation and constant electricity supply. These rats were allowed to get used to their new environment for a period of two weeks, with unrestricted feed and water.

Induction of diabetes

Diabetes was induced in the experimental rats. About 150 mg/kg body weight of alloxan monohydrate was injected into the rats intraperitoneally with a syringe. After three days, their blood was collected and measured for blood glucose levels using a calibrated glucometer. Hyperglycaemia was observed and rats with fasting blood glucose levels of 200 mg/dL and above were considered diabetic and used for the study.

Experimental design

The experimental Wistar rats were randomly divided into six groups (n = 6):

Group 1: Normal control (distilled water)
Group 2: Diabetic control (untreated)
Group 3: Diabetic + aqueous leaf extract (200 mg/kg)
Group 4: Diabetic + aqueous root extract (200 mg/kg)
Group 5: Diabetic + combined leaf + root extracts (100 mg/kg each)
Group 6: Diabetic + metformin (5 mg/kg)

The rats received oral experimental treatments every day for a period of 21 days to ascertain uniformity or consistency in dosage and exposure.

Biochemical analyses

Fasting blood glucose was measured weekly. Serum lipid profile (TC, TG, HDL, LDL), liver enzymes (ALT and AST), and oxidative stress markers (MDA and SOD) were determined using standard biochemical kits.

Haematological analyses

Blood samples were collected via retro-orbital puncture and analysed for Hb, RBC, and WBC counts using an automated haematology analyser.

Gut microbiota analysis

Fresh faecal samples were collected aseptically, serially diluted, and cultured on selective media for enumeration of *Lactobacillus, Bifidobacterium*, and *Enterobacteriaceae* (*E. coli*).

Statistical analysis

All results were expressed as mean ± SEM. Differences among groups were analysed using one-way ANOVA, and significant pairwise variations were further examined using Tukey's multiple comparison post hoc test. All analyses were carried out using SPSS version 20.0 (IBM Corp., Armonk, NY, USA), and results were confirmed statistically significant at $p < 0.05$.

Results

Effects of *M. oleifera* extracts

The effects of aqueous leaf and root extracts of *M. oleifera* on selected biochemical parameters are presented in Table 1. All groups tolerated the experimental protocol. Alloxan-induced diabetes resulted in significant hyperglycaemia and notable biochemical alterations in the diabetic control rats compared with the normal control group. Treatment with aqueous leaf extract, root extract, combined leaf and root extracts, and metformin produced significant improvements in biochemical indices relative to the diabetic control. Precisely, fasting blood glucose showed a consistent and significant increase in the diabetic control group throughout the course of the experiment ($p < 0.001$) and was progressively reduced in all treatment groups; the leaf-extract group and the combined-extract group showed earlier and larger reductions in fasting glucose compared with the root-extract group (all treatment groups vs diabetic control, $p < 0.05$; combined vs root, $p < 0.05$). The induction of diabetes with alloxan led to a sharp fall in serum insulin levels, but treatment with *M. oleifera* extracts improved insulin concentrations significantly. The combined and leaf extract groups showed the most significant hyperinsulinemic effect ($p < 0.05$) compared with the diabetic control. The induced-diabetes was observed to alter lipid balance. This led to an elevation in serum total cholesterol, LDL, and triglycerides while reducing serum HDL. Both the leaf and root extracts reversed these changes in lipid parameters, with the combined and leaf-treated rats exerting lower total cholesterol and triglyceride levels, and elevated HDL levels ($p < 0.05$). Elevated liver enzymes (ALT and AST) in the diabetic controls were brought closer to normal values after treatment, particularly in the combined extract

group ($p < 0.05$). The findings further showed some level of improvement in oxidative stress markers as well, with lowered MDA and elevated SOD levels in all the groups exposed to treatments ($p < 0.05$). The combined and leaf extracts demonstrated the strongest protective effects, with results closely matching those of the metformin-treated group.

Table 1. Effects of aqueous leaf and root extracts of *M. oleifera* on selected biochemical parameters in alloxan-induced diabetic Wistar rats.

Group	FBG (mg/dL)	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	ALT (U/L)	AST (U/L)	MDA (nmol/mL)	SOD (U/mL)
Normal Control	90 ± 4.2	125 ± 5.1	98 ± 4.3	62 ± 2.5	40 ± 2.2	28 ± 2.1	35 ± 2.8	2.1 ± 0.2	18.5 ± 1.1
Diabetic Control	312 ± 12.5	228 ± 8.7	176 ± 7.5	28 ± 2.0	122 ± 5.4	71 ± 3.5	89 ± 4.2	6.7 ± 0.3	9.3 ± 0.8
Leaf Extract	142 ± 6.8*	148 ± 6.1*	112 ± 5.2*	52 ± 2.8*	55 ± 2.7*	34 ± 2.5*	41 ± 3.1*	3.0 ± 0.2*	16.1 ± 0.9*
Root Extract	176 ± 7.4*	165 ± 6.5*	124 ± 5.7*	46 ± 2.6*	68 ± 3.1*	39 ± 2.6*	52 ± 3.2*	3.8 ± 0.3*	14.3 ± 0.8*
Leaf + Root Extract	126 ± 5.9*	132 ± 5.4*	105 ± 4.9*	58 ± 2.4*	48 ± 2.6*	31 ± 2.3*	38 ± 2.9*	2.5 ± 0.2*	17.2 ± 1.0*
Metformin (5 mg/kg)	118 ± 5.5*	128 ± 5.0*	101 ± 4.6*	60 ± 2.7*	45 ± 2.4*	29 ± 2.2*	36 ± 2.8*	2.3 ± 0.2*	18.0 ± 1.1*

* Significant versus diabetic control at $p < 0.05$.

Effects of *M. oleifera* extracts on selected hematological parameters

The results showing the effects of *M. oleifera* extracts on selected hematological parameters in alloxan-induced diabetic Wistar rats are revealed in Table 2. Alloxan-induced diabetes produced significant haematological alterations compared with the normal control group, including reductions in haemoglobin concentration (Hb), red blood cell (RBC) counts, as well as alterations in total white blood cell (WBC) count. Treatment with aqueous leaf and root extracts partially restored these parameters. The leaf extract and combined extract groups showed statistically significant increases in Hb and RBC compared with the diabetic control ($p < 0.05$), indicating improvement in anaemia-like changes associated with diabetes. Total WBC counts, which were elevated in diabetic controls, were normalized in extract-treated groups; neutrophil-to-lymphocyte ratios also improved in treated animals, suggesting reduced systemic inflammation. Platelet counts remained within physiological ranges across groups, with no treatment-related thrombocytopenia observed.

Table 2. Effects of *M. oleifera* extracts on selected hematological parameters in alloxan-induced diabetic Wistar rats

Group	Hb (g/dL)	RBC ($\times 10^6$/µL)	WBC ($\times 10^3$/µL)
Normal Control	14.2 ± 0.6	6.8 ± 0.3	8.1 ± 0.4
Diabetic Control	9.6 ± 0.5	4.1 ± 0.2	4.3 ± 0.3
Leaf Extract	13.1 ± 0.5*	6.2 ± 0.2*	7.6 ± 0.3*
Root Extract	12.3 ± 0.4*	5.7 ± 0.2*	6.8 ± 0.3*
Leaf + Root Extract	13.8 ± 0.6*	6.6 ± 0.3*	7.9 ± 0.4*
Metformin (5 mg/kg)	14.0 ± 0.5*	6.7 ± 0.3*	8.0 ± 0.3*

* Significant versus. diabetic control at $p < 0.05$.

Effects of *M. oleiferia* extracts on gut microbiota composition

Table 3. Effects of *M. oleifera* extracts on gut microbiota composition in alloxan-induced diabetic Wistar rats

Group	*Lactobacillus* ($\times 10^6$)	*Bifidobacterium* ($\times 10^6$)	*E. coli* ($\times 10^6$)
Normal Control	6.2 ± 0.3	5.7 ± 0.2	2.1 ± 0.1
Diabetic Control	2.3 ± 0.2	1.9 ± 0.1	6.5 ± 0.3
Leaf Extract	5.1 ± 0.3*	4.6 ± 0.2*	3.2 ± 0.2*
Root Extract	4.6 ± 0.2*	4.1 ± 0.2*	3.7 ± 0.2*
Leaf + Root Extract	5.8 ± 0.3*	5.3 ± 0.2*	2.6 ± 0.2*
Metformin (5 mg/kg)	6.0 ± 0.3*	5.5 ± 0.2*	2.4 ± 0.1*

* Significant versus. diabetic control at $p < 0.05$.

Table 3 shows the results of the effects of *M. oleifera* extracts on gut microbiota composition. Gut microbiota analysis showed that diabetes reduced beneficial bacterial populations (e.g., *Lactobacillus, Bifidobacterium*) while increasing pathogenic strains (*E. coli*). Treatment with *M. oleifera* extracts restored the balance, with the combined extract group showing the greatest modulation.

Discussion

Effects of *M. oleifera* extracts

The present study demonstrates that aqueous leaf and root extracts of *M. oleifera* ameliorate several biochemical derangements induced by alloxan in Wistar rats, with the leaf extract and the combined leaf and root extract producing the greatest effects. The hypoglycaemic action observed is consistent with reports attributing the glucose-lowering effects of *M. oleifera* to multiple mechanisms, such as the enhancement of insulin secretion, preservation or partial regeneration of β-cell function, increased peripheral glucose uptake, and inhibition of intestinal glucose absorption (Alam et al., 2022; Saini et al., 2025). The greater potency of the leaf extract may reflect its higher concentration of flavonoids, phenolic compounds, and other insulin-mimetic phytoconstituents, while the combined extract could provide complementary phytochemicals from roots and leaves that act additively or synergistically. Improvements in lipid profile (reduced total cholesterol, LDL, and TG; raised HDL) likely stem from antioxidant-mediated prevention of lipid peroxidation, modulation of hepatic lipid metabolism (e.g., inhibition of HMG-CoA reductase activity or upregulation of reverse cholesterol transport mechanisms), and improved glycaemic control (García-Carrasco et al., 2015; Afiaenyi et al., 2023). The normalisation of liver and kidney markers suggests hepatoprotective and renoprotective effects, plausibly via antioxidant, anti-inflammatory, and membrane-stabilizing activities of the phytochemicals (Algheshairy et al., 2025). The lowering of MDA and increase in SOD as observed in the results showed that the extracts reduced oxidative stress, which is an established mediator of diabetic complications, hence led to the protection of the tissues and improving metabolic outcomes (Mthiyane et al., 2022). The comparable actions l of the combined extract and metformin in several indices buttresses the possibility of using the *M. oleifera* extracts as therapeutic agents in the management of diabetes mellitus and other metabolic disorders, as well as and supports their further mechanistic exploration.

Effects of *M. oleifera* extracts on selected hematological parameters

Diabetes-associated anaemia and haematological alterations are multifactorial, arising from oxidative damage, altered erythropoiesis, renal dysfunction, and chronic inflammation (Williams et al., 2023). The results in Table 2 shows the partial restoration of RBC count and Hb by the extracts, especially the leaf and combined treatments. This suggests that the extracts of *M. oleifera* are capable of mitigating mechanisms driving these changes, especially via antioxidant protection of red blood cells (Sarkar et al., 2025), enhancement of erythropoietic signalling (Nova et al., 2020), and attenuation of systemic inflammation (de Oliveira Junior et al., 2024). The WBC counts appeared normal, hence further supports an anti-inflammatory action for the extracts (Nurudhin et al., 2021). The systemic benefits of the extracts beyond glycaemic control are due to the moderate improvements in the haematological parameters. This aligns with some previous studies that explain the haematoprotective effects of the biologically-active compounds present in *M. oleifera* (Ajugwo et al., 2017; Nurhayati et al., 2023).

Effects of *M. oleifera* extracts on gut microbiota composition

The result showing the effect of the extracts of *M. oleifera* on the composition of gut microbiota in Table 3 revealed that alloxan-induced diabetes mellitus caused an alteration in the normal population of gut microbiota. These changes lead to a decline in the population of beneficial bacteria and an elevation in the population of potentially harmful bacteria (Kumar et al., 2025). The imbalance in the gut microbiota composition is reversed to normal when treated with the extracts of *M. oleifera* extracts by increasing the population of beneficial microbial species like *Lactobacillus* and *Bifidobacterium*, and lowering the population of harmful Enterobacteriaceae such as *E. coli* (Kustyawati et al., 2024; Elbermawi et al., 2022). These changes show that the extracts of *M. oleifera* exert both prebiotic and antimicrobial activities. These activities result from its rich polyphenolic, flavonoid, and oligosaccharide concentrations that promote the growth of beneficial microbes while reducing the pathogenic ones. The gut environment became more favourable for healthy microbial growth due to normal glucose levels decline in oxidative stress (Husien et al., 2024). The most significant effects were produced by the combined extract, due to its diverse phytochemicals, which influenced a wider range of beneficial bacteria. This rebalancing of the microbiota may have caused the observed metabolic improvements through mechanisms such as enhanced short-chain fatty acid production, high intestinal barrier function, and modulation of immune responses (Bouyahya et al., 2022). Be that as it may, these findings buttress the growing knowledge that herbal treatments may partly improve metabolism by acting via the gut microbiome (Dai et al., 2023).

Conclusion

This study demonstrates that aqueous leaf and root extracts of *M. oleifera* ameliorated alloxan-induced biochemical and haematological perturbation and beneficially modulated gut microbiota composition in diabetic Wistar rats, with the leaf extract and the combined leaf and root formulation producing the most robust effects. The data support the potential use of parts of *M. oleifera* as complementary agents in diabetes management and highlight the importance of considering microbiota-mediated mechanisms in future nutraceutical development and clinical studies.

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Author contributions

Conceptualisation: Austin Achinike Okwelle
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Validation: Enyohwo Dennis Kpomah
Project administration: Owo Gogo James
All authors read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Conflict of interests

The authors declare that there no competing interests exist.

Ethics approval

The study protocol was reviewed and approved by the Faculty of Natural and Applied Sciences Ethics Committee of Ignatius Ajuru University of Education, Rumuolumeni, Port Harcourt, Rivers State, Nigeria. All animal handling and experimental procedures adhered to international ethical standards, with efforts made to minimise pain and distress and to limit animal use.

AI tool usage declaration

Grammerly was used solely to enhance the grammatical quality of the manuscript. All aspects of the study, including experimental design, data collection, statistical analyses, interpretation, and conclusions, were independently conducted by the authors.

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