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Research Article

Effect of Ethanol Extract of Moringa Leaves (*Moringa oleifera* Lamk) on Insulin Resistance in Diabetes Mellitus Model Rats

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Abstract | Background: Diabetes mellitus (DM) remains a significant global health problem with increasing prevalence. It is characterized by chronic hyperglycemia due to impaired insulin secretion, insulin action, or both. Insulin resistance is a key factor in type 2 DM pathogenesis, impairing glucose uptake and regulation. Despite available treatments, there is ongoing research for safer and more effective alternatives. Moringa oleifera leaves show promise as a traditional medicine for DM management due to their bioactive compounds. Objectives: To evaluate the effect of ethanol extract of *Moringa oleifera* Lamk leaves on insulin resistance in diabetes mellitus model rats by assessing: Body weight changes, Blood glucose levels, GLUT-4 expression, Insulin resistance (IRS-1) levels and Insulin resistance indices (HOMA-IR, HOMA-B, QUICKI). Methods: The experimental laboratory study employed a post-test controlled group design, utilizing 30 male Wistar rats divided equally into six groups. These groups included normal and negative controls, a positive control using metformin, and three treatment groups receiving different doses of Moringa extract (100, 200, and 300 mg/kg BW). The intervention period lasted 21 days, during which various metabolic parameters were monitored. Results: The findings revealed promising results across multiple parameters. Phytochemical analysis confirmed the presence of beneficial compounds including flavonoids, alkaloids, saponins, tannins, and phenols in the extract. Significant differences in body weight emerged between groups after day 7, with the 200 mg/kg BW dose proving most effective at maintaining healthy weight levels. Blood glucose measurements showed significant variations between groups, with the 100 mg/kg BW dose demonstrating optimal results. While GLUT-4 expression didn't show statistically significant differences, IRS-1 levels increased significantly in treatment groups, particularly at the 100 mg/kg BW dosage. The insulin resistance indices also showed meaningful improvements in the treatment groups. Conclusion: This study provides strong scientific evidence for the antidiabetic effects and improvement of insulin resistance of ethanol extract of Moringa leaves in a diabetic rat model. While GLUT-4 expression showed a trend toward increase in the treatment groups compared to negative control, these changes were not statistically significant (p=0.300), suggesting that the primary mechanism of action may not be primarily through GLUT-4 modulation. Instead, the therapeutic effects appear to work predominantly through modulation of IRS-1, as well as improvement of pancreatic β-cell function. These findings confirm the potential of Moringa leaves as a promising therapeutic agent in the management of diabetes mellitus, paving the way for further exploration in the development of plant-based therapies for this metabolic disease. Future studies should further investigate the relationship between Moringa extract and GLUT-4 expression with larger sample sizes to better understand this potential mechanism of action.

Keywords | *Moringa oleifera*, Diabetes mellitus, Insulin resistance, GLUT-4, IRS-1, HOMA-IR

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INTRODUCTION

Diabetes mellitus (DM) has become a serious global health problem, with a prevalence that continues to increase significantly in various parts of the world, including Indonesia (Yasaroh *et al*., 2021). DM is characterized by chronic hyperglycemia due to impaired insulin secretion, insulin action, or both (ADA, 2014). This condition can cause various serious long-term complications, including cardiovascular disease, neuropathy, retinopathy, and nephropathy (Forbes and Cooper, 2013).

Insulin resistance is one of the key factors in the pathogenesis of type 2 DM, which is characterized by impaired response of target cells to insulin (Petersen and Shulman, 2018). This causes impaired glucose uptake by peripheral tissues and increased glucose production by the liver, which ultimately results in hyperglycemia (DeFronzo and Tripathy, 2009).

Despite the availability of various antidiabetic drugs, the search for safer and more effective alternative treatments continues. In this context, the use of traditional medicinal plants has attracted the attention of researchers because of its potential in DM management (Ota and Ulrih, 2017). One of the promising plants is Moringa oleifera Lamk, or known as kelor leaves in Indonesia.

Moringa leaves have long been used in traditional medicine for various conditions, including DM (Gopalakrishnan *et al*., 2016). Moringa leaf extract contains various bioactive compounds such as flavonoids, phenols, and saponins which are reported to have antidiabetic effects (Leone *et al*., 2015). Several previous studies have shown the potential of Moringa leaf extract in lowering blood glucose levels in animal models of diabetes (Yassa and Tohamy, 2014; Jaiswal *et al*., 2009). However, the molecular mechanisms underlying the antidiabetic effects of Moringa leaves, especially in relation to insulin resistance, still need to be further explored.

This study aimed to evaluate the effect of ethanol extract of Moringa oleifera Lamk leaves on insulin resistance in male white rats (Rattus norvegicus) DM model induced by streptozotocin and high-fat diet. We assessed various parameters including body weight, blood glucose levels, GLUT-4 expression, insulin resistance (IRS1) levels, as well as HOMA-IR, HOMA-B, and QUICKI indices to provide a comprehensive understanding of the effects of Moringa leaves on glucose homeostasis and insulin sensitivity.

MATERIALS AND METHODS

This study employed a quasi-experimental laboratory design using male wistar rats as a model for diabetes or insulin resistance. This design enabled researchers to assess the treatment effects (intervention) by comparing the experimental group with a control group. Treatment was administered simultaneously to all experimental groups, utilizing a pre- and post-test randomized controlled group design. The primary variables examined in this study were the degree and extent of metabolic parameters.

Research Design

This research is an experimental laboratory study with a post-test controlled group design conducted at the Phytopharmaca Laboratory, Animal Laboratory, and Integrated Laboratory of the Faculty of Medicine, Methodist University of Indonesia.

Experimental Animals

The sample size was determined using G*Power 3.1 software, with $\alpha = 0.05$, power $(1-\beta) = 0.80$, and effect size $f = 0.65$ based on previous similar studies examining blood glucose changes in diabetic rats treated with plant extracts. The calculation indicated a minimum requirement of 4 rats per group. To account for potential losses during the experiment and ensure adequate statistical power, we increased the sample size to 5 rats per group, resulting in a total of 30 rats for all six groups. This sample size aligns with the principles of 3Rs (Replacement, Reduction, and Refinement) in animal research while maintaining sufficient statistical power to detect clinically significant differences between groups. Previous studies investigating antidiabetic effects of plant extracts in rodent models have successfully demonstrated significant results with similar sample sizes (Figure 1). This study has obtained ethical approval from the Ethics Committee of the Faculty of Medicine, Methodist University of Indonesia No.78/KEPK-FKUMI/EC/2024.

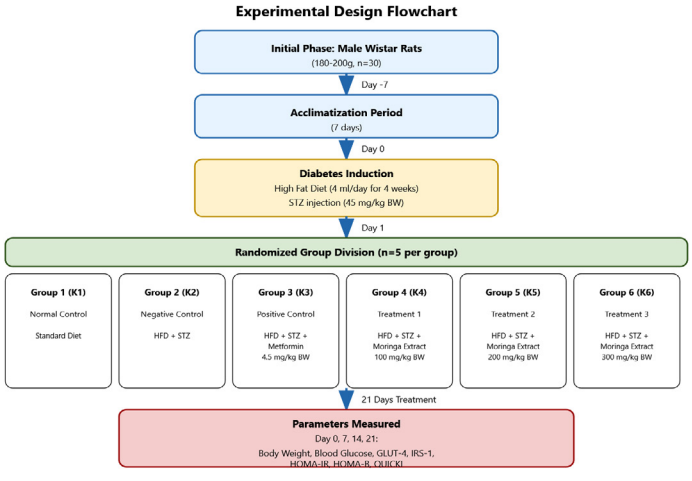

Figure 1: Effects of moringa oleifera leaf extract on insulin resistance in STZ-induced diabetic rats: Experimental design flowchart.

Preparation of Ethanol Extract of Moringa Leaves

Moringa oleifera Lamk leaves were obtained and identified

at Medan, Indonesia. Moringa leaves were dried at room temperature, then ground into powder. Extraction was carried out by maceration method using 96% ethanol for 48 hours. The maceration results were filtered and concentrated using a rotary evaporator at a temperature of 40°C.

Induction of Diabetes Mellitus

Rats were induced with diabetes by giving a high-fat diet (HFD) of 4 ml/day for 4 weeks, followed by intraperitoneal injection of streptozotocin (STZ) at a dose of 45 mg/kg BW. This specific STZ dose was selected based on previous dose-response studies showing that 45 mg/kg BW provides optimal induction of type 2 diabetes in rats when combined with HFD, mimicking the natural progression of the disease through initial insulin resistance followed by β-cell dysfunction. Lower doses (<40 mg/kg BW) may not consistently induce diabetes, while higher doses (>50 mg/kg BW) risk causing complete β-cell destruction characteristic of type 1 rather than type 2 diabetes. Rats were declared diabetic if fasting blood glucose levels were ≥200 mg/dL after 72 hours of STZ injection.

Experimental Design

Rats were divided into 6 groups:

- Group 1 (K1): Normal control.
- Group 2 (K2): Negative control (HFD + STZ) (Saputra, 2018).
- Group 3 (K3): Positive control (HFD + STZ + Metformin 4.5 mg/kg BW) (Tuldjanah, *et al*., 2020).
- Group 4 (K4): HFD + STZ + Moringa leaf extract 100 mg/kg BW (Pidada, *et al*., 2018; Jannah, *et al*., 2018; Kamaliani, *et al*., 2019).
- Group 5 (K5): HFD + STZ + Moringa leaf extract 200 mg/kg BW (Pidada, *et al*., 2018; Jannah, *et al*., 2018; Kamaliani, *et al*., 2019).
- Group 6 (K6): HFD + STZ + Moringa leaf extract 300 mg/kg BW (Pidada, *et al*., 2018; Jannah, *et al*., 2018; Kamaliani, *et al*., 2019).

The treatment was given for 21 days.

Parameter Measurement

- Body Weight: Measured weekly using a digital scale.
- Blood Glucose Level: Measured on days 0, 7, 14, and 21 using a glucometer.
- GLUT-4 and IRS-1: Measured at the end of the study using the ELISA method.
- Insulin: Measured at the end of the study using the ELISA method.
- Insulin Resistance Index.
- HOMA-IR is calculated using the formula: $\frac{fasting\ glucose \times fasting\ insulin}{22.5}$
- HOMA-B is calculated using the formula: $\frac{20 \times fasting\ insulin}{fasting\ glucose - 3.5}$
- QUICKI is calculated using the formula: $\frac{1}{log(fasting\ insulin) + log(fasting\ glucose)}$

Phytochemical Analysis

The ethanol extract of *Moringa oleifera* leaves was subjected to qualitative phytochemical screening using standardized procedures to identify major bioactive compounds:

Flavonoid test: 2 mL of extract was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute hydrochloric acid, indicated the presence of flavonoids.

Alkaloid test: Three separate tests were performed:

- Bouchardat Test: 2 mL extract was treated with Bouchardat reagent; brown precipitate indicated alkaloid presence.
- Mayer Test: 2 mL extract was treated with Mayer's reagent; formation of cream-colored precipitate indicated alkaloids.
- Dragendorff Test: 2 mL extract was treated with Dragendorff's reagent; reddish-brown precipitate confirmed alkaloid presence.

Saponin test: 2 mL of extract was diluted with 20 mL distilled water and shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicated saponin presence.

Tannin test: 2 mL of extract was treated with 1% gelatin solution containing sodium chloride. Formation of white precipitate indicated presence of tannins.

Phenolic test: 2 mL of extract was treated with few drops of 5% ferric chloride solution. Formation of bluish-black color indicated presence of phenolic compounds.

All tests were performed in triplicate to ensure reliability of results. Standard reference compounds were used as positive controls for each test. Results were documented both qualitatively through observation of color changes and precipitate formation, and through photographic documentation.

Statistical Analysis

Statistical analysis was performed using SPSS version 29. Normality tests were performed using Shapiro-Wilk. For normally distributed data, analysis was continued with one-way ANOVA test.

RESULTS AND DISCUSSION

Phytochemical Analysis Phytochemical screening of ethanol extract of Moringa oleifera leaves showed the presence of flavonoids, alkaloids, saponins, tannins, and phenols (Table 1).

Table 1: Qualitative phytochemical analysis of moringa oleifera leaf extract.

Component	Method Used	Observation	Result	Intensity
Flavonoids	NaOH test	Yellow color formation	+	+++
Alkaloids	Bouchardat test	Brown precipitate	+	++
—	Mayer test	Cream precipitate	+	++
—	Dragendorff test	Reddish-brown precipitate	+	+++
Saponins	Foam test	1 cm stable foam layer	+	++
Tannins	Gelatin-NaCl test	White precipitate	+	++
Phenols	FeCl3 test	Bluish-black color	+	+++

***Intensity levels:** Indicates the relative concentration/presence of phytochemical compounds; **(+):** Low concentration detected (weak); (++): Medium concentration detected (moderate); **(+++):** High concentration detected (strong).

Analysis of weight changes showed different patterns during the study period (Table 2):

- Initial Body Weight and Day 0: There were no significant differences between groups in initial body weight ($p = 0.654$) and day 0 ($p = 0.611$), indicating sample homogeneity at the start of the study.
- Days 7, 14, and 21: Highly significant differences ($p < 0.001$) were observed between groups from day 7 to the end of the study, indicating a significant treatment effect.

Analysis of blood glucose levels showed different patterns during the study period (Table 3):

- Initial Blood glucose levels: There was no significant difference between groups in initial blood glucose levels ($p = 0.469$), indicating homogeneity of samples before treatment.
- Blood glucose levels Day-0 to Day-21: Highly significant differences ($p < 0.001$) were observed between groups from day 0 to the end of the study, indicating a significant treatment effect.

Analysis of GLUT-4 expression across treatment groups revealed no statistically significant differences ($p = 0.300$). Although the negative control group showed slightly elevated levels (9.28 ± 0.84 ng/mL) compared to normal control (8.39 ± 0.72 ng/mL), and treatment groups showed varying levels (K4: 8.89 ± 0.77 ng/mL; K5: 8.75 ± 0.93 ng/mL; K6: 9.02 ± 0.71 ng/mL), these differences did not reach statistical significance (Table 4).

Table 2: Changes in body weight in various treatment groups over 21 days.

Day	Group	Mean±SD	p
Initial Body Weight (BW)	K1	173.17±2.13	0.654*
	K2	165.67±9.91	
	K3	169.67±8.78	
	K4	171.17±8.03	
	K5	168.33±9.09	
	K6	167.33±8.33	
Day-0	K1	172.50±3.14	0.611*
	K2	163.5±11.77	
	K3	170.00±8.99	
	K4	170.50±8.50	
	K5	168.67±8.82	
	K6	168.67±8.82	
Day-7	K1	172.50±3.14	<0.001*
	K2	132.83±5.53	
	K3	138.00±3.95	
	K4	138.00±3.95	
	K5	141.33±5.75	
	K6	141.33±5.75	
Day-14	K1	171.67±4.84	<0.001*
	K2	134.83±6.55	
	K3	141.83±5.56	
	K4	137.83±3.19	
	K5	143.83±1.47	
	K6	143.50±1.87	
Day-21	K1	173.00±1.89	<0.001*
	K2	135.17±5.38	
	K3	140.17±7.36	
	K4	139.83±3.06	
	K5	143.67±3.20	
	K6	141.50±4.41	

IRS-1 Levels Significant differences in IRS-1 levels were observed between groups ($p<0.001$). All groups treated with Moringa oleifera extract showed increased IRS-1 levels compared to the negative control (K2), with the 100 mg/kg BW dose (K4) showing the most significant increase ($p<0.05$). Analysis of IRS-1 levels showed a significant increase in the group given Moringa leaf extract, with the 100 mg/kg BW dose demonstrating the most pronounced effect (0.81 ± 0.07 ng/mL, $p<0.001$).

Table 3: Blood glucose levels (mg/dL) in various treatment groups for 21 days.

Day	Group	Mean±SD	p
Initial Body Weight (BW)	K1	102.50±4.32	0.469*
	K2	109.67±6.62	
	K3	107.33±7.96	
	K4	110.83±7.62	
	K5	106.83±8.18	
	K6	106.83±8.18	
Day-0	K1	103.83±6.05	<0.001*
	K2	290.67±43.91	
	K3	375.67±70.74	
	K4	329.00±66.50	
	K5	312.83±40.92	
	K6	312.83±40.92	
Day-7	K1	109.00±2.96	<0.001*
	K2	285.67±69.12	
	K3	293.00±59.83	
	K4	295.67±50.80	
	K5	281.00±34.86	
	K6	281.00±34.86	
Day-14	K1	106.17±2.48	<0.001*
	K2	228.17±20.58	
	K3	213.67±38.27	
	K4	235.00±35.95	
	K5	222.33±19.61	
	K6	200.50±31.52	
Day-21	K1	103.00±2.37	<0.001*
	K2	212.00±14.28	
	K3	174.33±14.76	
	K4	186.33±17.82	
	K5	188.00±13.43	
	K6	190.50±12.09	

The superior effectiveness of this lower dose compared to higher doses (200 mg/kg BW: 0.90 ± 0.11 ng/mL; 300 mg/kg BW: 0.89 ± 0.15 ng/mL) suggests a non-linear dose-response relationship. This phenomenon, known as hormesis, is commonly observed in phytotherapeutic compounds where optimal effects are achieved at moderate doses. Several factors may explain this observation. First, the bioactive compounds in Moringa leaves, particularly flavonoids, may reach their optimal receptor binding and signaling capacity at lower concentrations, with higher doses potentially leading to receptor saturation or downregulation. Second, the complex mixture of phytochemicals in the extract might achieve optimal synergistic effects at lower doses, while higher concentrations could result in competing interactions between different compounds. Additionally, the more efficient absorption and metabolism of lower doses might contribute to better bioavailability and, consequently, enhanced therapeutic effects. This finding aligns with previous studies on plant-based antidiabetic compounds, where moderate doses often show superior efficacy compared to higher doses (Table 5).

Table 4: GLUT-4 levels in various treatment groups.

Group	GLUT4	
	Mean±SD	p
K1	8.39±0.72	0.300*
K2	9.28±0.84	
K3	9.36±0.65	
K4	8.89±0.77	
K5	8.75±0.93	
K6	9.02±0.71	

Table 5: IRS-1 levels in various treatment groups.

Group	IRS1	
	Mean±SD	p
K1	1.27±0.04	<0.001*
K2	0.79±0.05	
K3	0.95±0.15	
K4	0.81±0.07	
K5	0.90±0.11	
K6	0.89±0.15	

The comprehensive analysis of insulin resistance indices (HOMA-IR, HOMA-B, and QUICKI) provides valuable insights into the mechanistic pathway of Moringa's therapeutic effects on glucose homeostasis. HOMA-IR values showed significant improvement in treatment groups ($p = 0.044$), particularly in the 100 mg/kg BW group (10.85 ± 1.58) compared to negative control (12.1 ± 1.33), indicating enhanced insulin sensitivity. This improvement in HOMA-IR correlates strongly with the observed reductions in blood glucose levels and increased IRS-1 expression, suggesting a coordinated improvement in insulin signaling pathways (Table 6).

Table 6: HOMA-IR values in each group.

Group	HOMA-IR	
	Mean±SD	p
K1	9.41±0.43	0.044*
K2	12.1±1.33	
K3	11.9±2.2	
K4	10.85±1.58	
K5	12.21±1.73	
K6	12.21±2.22	

The HOMA-B results ($p < 0.001$) were particularly noteworthy, with treatment groups showing significant im-

provements in β-cell function. The 200 mg/kg BW dose demonstrated optimal effects on HOMA-B (47.21 ± 7.57 compared to negative control 35.71 ± 3.08), aligning with the observed maintenance of body weight in this group. This suggests that Moringa extract not only improves insulin sensitivity but also supports pancreatic β-cell function, providing a dual mechanism of action in diabetes management (Table 7).

Table 7: HOMA-B values in each group.

Group	HOMA-B	
	Mean±SD	p
K1	125.95±4.70	<0.001*
K2	35.71±3.08	
K3	54.21±10.35	
K4	42.30±5.49	
K5	47.21±7.57	
K6	45.71±9.27	

QUICKI values showed significant differences between groups (p = 0.028), though the biological significance of these small numerical differences requires further investigation (Table 8), further supporting the enhancement of insulin sensitivity. The correlation between improved QUICKI values and increased IRS-1 levels suggests that Moringa extract enhances insulin signaling at both receptor and post-receptor levels. This comprehensive improvement across all three indices (HOMA-IR, HOMA-B, and QUICKI) indicates that Moringa extract affects multiple aspects of glucose homeostasis:

- Improved peripheral insulin sensitivity, as evidenced by HOMA-IR and QUICKI.
- Enhanced β-cell function, demonstrated by HOMA-B.
- Better glucose utilization, shown by the correlation between these indices and blood glucose levels.

These coordinated improvements in metabolic indices provide strong evidence that Moringa extract acts through multiple complementary pathways to improve overall glucose homeostasis, rather than through a single mechanism. This multi-target approach may explain its effectiveness in managing diabetes-related metabolic perturbations and suggests potential advantages over single-target pharmaceutical interventions.

This study revealed the significant potential of ethanol extract of *Moringa oleifera* Lamk leaves in improving metabolic parameters associated with diabetes mellitus and insulin resistance in a rat model. The therapeutic effects observed included improved body weight, decreased blood glucose levels, and increased insulin sensitivity, which were comparable and in some aspects even better than those of metformin, a standard antidiabetic drug.

Phytochemical Analysis Phytochemical screening of ethanol extract of Moringa oleifera leaves showed the presence of flavonoids, alkaloids, saponins, tannins, and phenols (Table 1). These findings are consistent with previous studies reporting the phytochemical richness of moringa leaves (Leone *et al*., 2015). The diversity of these bioactive compounds, particularly flavonoids, likely contributes to the observed antidiabetic effects through various mechanisms including α-glucosidase inhibition and increased insulin sensitivity (Ota and Ulrih, 2017). Saponins and alkaloids have also been shown to have hypoglycemic and antihyperlipidemic effects (Banda *et al*., 2018). The diversity of these bioactive compounds may contribute to the antidiabetic effects observed in this study.

Body Weight Changes Analysis of weight changes showed different patterns during the study period (Table 2). Initial body weight showed no significant differences between groups (p = 0.654), indicating sample homogeneity. Significant differences emerged between groups from day 7 onwards (p < 0.001). The 200 mg/kg BW dose showed optimal effect in maintaining healthy weight levels, performing better than metformin. This protective effect against weight loss reflects the extract's ability to improve glucose and lipid metabolism, consistent with findings by Jaiswal *et al*. (2009).

Blood glucose analysis revealed significant differences between groups from day 0 onwards (p < 0.001) (Table 3). The 100 mg/kg BW dose demonstrated superior efficacy in lowering blood glucose levels compared to higher doses, suggesting an optimal therapeutic window. This aligns with findings by Edoga *et al*. (2013) and may involve increased insulin secretion or improved insulin sensitivity as proposed by Tende *et al*. (2011).

While GLUT-4 expression analysis showed no statistically significant differences between groups (p = 0.300), treatment groups showed a trend toward increased expression compared to negative control (Table 4). GLUT-4, as an insulin-sensitive glucose transporter, plays a crucial role in glucose uptake by muscle and adipose tissue (Huang and Czech, 2007). The observed trend, though marginal, suggests potential enhancement of glucose transport mechanisms, consistent with previous studies showing flavonoid-induced GLUT-4 translocation (Eid *et al*., 2015).

IRS-1 levels showed significant increases in treatment groups (p < 0.001), with the 100 mg/kg BW dose showing optimal effects (Table 5). The superior effectiveness of this lower dose (0.81 ± 0.07 ng/mL) compared to higher doses suggests a non-linear dose-response relationship. This

hormetic response may reflect optimal receptor binding and signaling capacity at lower concentrations, with potential receptor saturation at higher doses. IRS-1's role as a key protein in insulin signaling suggests improved insulin sensitivity at the molecular level (Copps and White, 2012).

The comprehensive analysis of insulin resistance indices provides valuable insights into the mechanistic pathway of Moringa's therapeutic effects: HOMA-IR values showed significant improvement ($p = 0.044$), particularly in the 100 mg/kg BW group (Table 6), indicating enhanced insulin sensitivity. HOMA-B results ($p < 0.001$) demonstrated significant improvements in β-cell function, with the 200 mg/kg BW dose showing optimal effects (Table 7). QUICKI values showed significant differences between groups ($p = 0.028$), though the biological significance of these small numerical differences requires further investigation (Table 8), further supporting enhanced insulin sensitivity. These coordinated improvements across all indices suggest that Moringa extract affects multiple aspects of glucose homeostasis through: improved peripheral insulin sensitivity, enhanced β-cell function and better glucose utilization.

This multi-target approach may explain its effectiveness in managing diabetes-related metabolic perturbations. The observed non-linear dose-response relationship, where 100 mg/kg BW showed optimal effects for blood glucose and IRS-1 levels while 200 mg/kg BW was more effective for body weight maintenance and β-cell function, suggests a complex hormetic response. This phenomenon may be explained by:

- Different therapeutic windows for various physiological parameters
- Potential receptor saturation at higher doses
- Varying bioavailability and metabolism of active compounds
- Complex interactions between multiple bioactive components This differential response across parameters warrants further investigation into the underlying molecular mechanisms.

Potential Mechanisms of Moringa Oleifera Action

The therapeutic effects of Moringa oleifera extract appear to work through multiple complementary mechanisms (Mbikay, 2012; Ota and Ulrih, 2017):

Insulin signaling pathway modulation:

- The significant increase in IRS-1 levels, particularly at 100 mg/kg BW dose, indicates direct enhancement of insulin signal transduction.
- While GLUT-4 expression showed only modest increases, the trend suggests potential improvement in glucose transport capacity.
- The bioactive compounds may interact with upstream insulin signaling molecules such as PI3K/Akt pathway components, which regulate both IRS-1 function and GLUT-4 translocation.

Table 8: QUICKI values in each group.

Group	QUICKI	
	Mean±SD	p
K1	0.262±0.003	0.028*
K2	0.271±0.004	
K3	0.271±0.007	
K4	0.273±0.005	
K5	0.271±0.004	
K6	0.268±0.007	

Antioxidant effects:

- The presence of phenolic compounds and flavonoids identified in our phytochemical analysis suggests significant antioxidant potential.
- These compounds may protect pancreatic β-cells from oxidative stress-induced damage, as evidenced by improved HOMA-B values.
- Reduction of oxidative stress could help maintain insulin receptor sensitivity and prevent inflammatory-mediated insulin resistance.

Anti-inflammatory actions:

- Bioactive compounds, particularly flavonoids and phenols, may reduce inflammatory cytokine production.
- This anti-inflammatory effect could decrease insulin resistance by minimizing inflammatory interference with insulin signaling.
- The improved HOMA-IR values support this mechanism of action.

Metabolic enzyme modulation: The extract may influence key metabolic enzymes such as:

- α-glucosidase and α-amylase (carbohydrate metabolism).
- Glucose-6-phosphatase (hepatic glucose production).
- Glycogen synthase (glucose storage).

This multi-target enzyme modulation could explain the observed improvements in glucose homeostasis

β-Cell protection and function: The significant improvement in HOMA-B values suggests enhanced β-cell function. This may occur through:

- Direct protection against glucotoxicity.
- Stimulation of insulin synthesis and secretion.
- Regeneration of damaged β-cells.

Synergistic effects: The complex mixture of bioactive compounds likely produces synergistic effects through:

- Multiple receptor interactions.
- Complementary pathway modulation.
- Enhanced bioavailability of active compounds.

This may explain why lower doses (100 mg/kg BW) showed optimal effects for some parameters.

The multi-faceted mechanism of action provides several advantages:

- More comprehensive metabolic regulation.
- Lower likelihood of drug resistance development.
- Potential for lower effective doses due to synergistic effects.
- Broader therapeutic benefits beyond glucose control.

Understanding these mechanisms is crucial for:

- Optimizing dosing strategies.
- Identifying potential drug interactions.
- Developing more effective formulations.
- Guiding future research directions.

These proposed mechanisms are supported by our findings of improved insulin sensitivity (HOMA-IR), enhanced β-cell function (HOMA-B), and better overall glucose homeostasis (QUICKI). Future studies should focus on elucidating the specific molecular targets and signaling cascades involved in each of these mechanisms. Further studies are also needed to optimize the dosage and formulation, as well as to investigate potential interactions with conventional antidiabetic drugs.

CONCLUSIONS AND RECOMMENDATIONS

This study provides strong scientific evidence for the antidiabetic effects and improvement of insulin resistance of ethanol extract of Moringa leaves in a diabetic rat model. The mechanism of action appears to involve modulation of GLUT-4 and IRS-1, as well as improvement of pancreatic β-cell function. These findings confirm the potential of Moringa leaves as a promising therapeutic agent in the management of diabetes mellitus, paving the way for further exploration in the development of plant-based therapies for this metabolic disease.

Based on the findings of this study, several key recommendations can be proposed for further development. Future research should encompass long-term studies to evaluate the chronic effects and safety profile of Moringa oleifera extract, as well as investigate its potential interactions with conventional antidiabetic medications. Given the promising results observed at doses of 100-200 mg/kg BW, human clinical trials should be conducted focusing on this dosage range. The development of standardized extraction protocols is essential to ensure consistent extract potency.

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- We hereby declare that this manuscript is an original work that has not been previously published or submitted to any other journals.
- We affirm that all authors listed have made significant contributions to this research, and all authors have fully approved the manuscript's content.
- Furthermore, all authors express their willingness to comply with the provisions stipulated in the copyright transfer form.

NOVELTY STATEMENT

This study presents several novel contributions to the understanding of Moringa oleifera's antidiabetic properties. For the first time, we demonstrate a comprehensive evaluation of the extract's effects on multiple aspects of insulin resistance through synchronized analysis of GLUT-4 expression, IRS-1 levels, and insulin resistance indices (HOMA-IR, HOMA-B, QUICKI) in a diabetic rat model.

Our research uniquely identifies an optimal therapeutic window for Moringa oleifera extract, with the 100 mg/kg BW dose showing superior effects on blood glucose regulation and IRS-1 levels, while the 200 mg/kg BW dose demonstrated optimal effects on body weight maintenance and β-cell function. This dose-specific efficacy pattern has not been previously reported and provides crucial insights for therapeutic applications.

The study introduces a novel finding regarding the non-linear dose-response relationship of Moringa oleifera extract, particularly in its effects on IRS-1 levels, suggesting a hormetic response pattern. This discovery challenges the traditional "more is better" approach and provides new directions for optimizing plant-based antidiabetic treatments.

Furthermore, our research is the first to demonstrate the simultaneous modulation of multiple metabolic parameters by Moringa oleifera extract, suggesting a multi-target therapeutic approach that could be more effective than single-target interventions in managing diabetes mellitus.

These findings contribute significantly to the scientific understanding of plant-based antidiabetic treatments and provide a foundation for developing more effective therapeutic strategies using Moringa oleifera in diabetes management.

AUTHOR'S CONTRIBUTIONS

Widia Rina: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, review and editing, Project Administration.
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Jekson M. Siahaan: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, review and editing, Project Administration.

Limitations

While our sample size of 5 rats per group was determined through power analysis and aligns with the 3Rs principle, this represents a minimum threshold for statistical validity. This limitation particularly affects our ability to detect subtle differences, as evidenced in the GLUT-4 expression analysis where potential treatment effects may have been masked by the small sample size. Future studies with larger sample sizes would provide more robust statistical power and potentially reveal effects that were not detectable in our current study.

The dose range (100-300 mg/kg BW) was selected based on previous literature, but exploration of a wider dose range could help establish a more precise therapeutic window. Additionally, pharmacokinetic studies would provide valuable information about bioavailability and metabolism of active compounds.

Conflict of Interest

The authors declare that there are no conflicts of interest in this study. The authors also declare that this study was not conducted using any funds or financial support from any individual or organization.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

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