Full Length Research Article
Advancements in Life Sciences – International Quarterly Journal of Biological Sciences

ARTICLE INFO

Date Received: 27/08/2024;
Date Revised: 03/11/2025;
Available Online: 28/12/2025;

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How to Cite:
Saeed M, Ali AW, Maqsood Q, Tariq MR, Saeed A, Ahmad A, Muzzafar MA, Rasheed AA. (2025). Synergistic effect of *Moringa oleifera* leaf extract on glycemic control and lipid profile: a preclinical evaluation. Adv. Life Sci. 12(4): 836-844.

Keywords:
Moringa oleifera; Antioxidant; Anti-diabetic; Phytonutrients; Aqueous Extraction

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Synergistic effect of *Moringa oleifera* leaf extract on glycemic control and lipid profile: a preclinical evaluation

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Abstract

Background: The global incidence of Type-2 diabetes is escalating, driven by lifestyle changes, urbanization, and dietary habits, particularly in developing countries. This has led to a growing demand for novel, natural therapeutic agents to effectively manage this chronic condition. *Moringa* freeze-dried extract powder (MFDP), a plant widely recognized for its nutritional profile and medicinal properties, has shown promise in treating metabolic disorders.

Methods: The *Moringa oleifera* leaf extract was analyzed using advanced analytical technique, revealing substantial concentrations of bioactive compounds including flavonoids, polyphenols, and quercetin. In addition, its antidiabetic and anti-hyperlipidemia effect were evaluated through rat trials, enabling assessment of glucose regulation and lipid profile improvement. This study also assessed the impact of freeze-drying on the physicochemical composition of MFDP, considering regional and environmental variation to ensure local relevance of the findings.

Results: In diabetic rats, the MFDP significantly ($p \leqslant 0.05$) reduced blood glucose levels (random: 343 ± 0.02 mg/dL to 171 ± 0.03 mg/dL; fasting: 243.21 ± 0.03 mg/dL to 98.2 ± 0.02 mg/dL) and HbA1c levels (5.4 ± 0.15 %). It also improved lipid profiles by lowering total cholesterol (85.17 ± 5 mg/dL), triglycerides (94.11 ± 10.4 mg/dL), and LDL concentrations (27.82 ± 7.1 mg/dL) while increasing HDL levels (41.14 ± 2.5 mg/dL). Additionally, MFDP treatment enhanced liver glycogen (18.07 ± 3 mg/g to 43.68 ± 2.5 mg/g), indicating improved hepatic glucose utilization.

Conclusion: The findings underscore the dual therapeutic potential of MFDP in managing metabolic disorders like diabetes and hypercholesterolemia. Given the increasing prevalence of these conditions, especially in regions like South Asia, further research is needed to explore the molecular mechanisms underlying these beneficial effects and to validate the use of *Moringa oleifera* as a natural therapeutic agent.

Introduction

Moringa oleifera is globally recognized for its broad therapeutic applications, earning it the name "Miracle Tree". This resilient plant exhibits resistance to pathogens and fungi, and it can thrive even in poor soil conditions. It is also known by several alternative names that reflect diverse roles in health, nutrition, and trade, such as "Arbour Vitae", "Elixir Plant" and "Versatile Botanical Resource". Diabetes is a widespread metabolic condition affecting approximately 463 million adults globally as of 2019, representing a significant and growing public health challenge. This condition is characterized not only by insulin resistance but also by myocardial infarction, neurological dysfunction, and visual impairment [1]. Despite the significant advancements in pharmacological treatments for hyperglycemia, certain conventional medication may still cause adverse side effects, including renal dysfunction and gastric disorders. Considering these challenges, the exploration of natural resources such as *Moringa oleifera,* well-known for its therapeutic properties and nutritional benefits, emerges as a promising strategy to mitigate diabetes. This approach offers potential therapeutic benefits with fewer adverse effects [2]. The current study seeks to provide valuable insight into optimizing the plant extracts and evaluating their impact on conditions, such as hyperglycemia and hypercholesterolemia [3]. Tomita [4] stated that the extraction of nutraceutical components from mulberry leaves is influenced by several crucial factors, such as temperature, time duration, water-to-leaf ratio, which directly affect the recovery of bioactive compounds. Drawing on the extraction principles established in mulberry leaf studies, our research applies similar extraction parameters to *Moringa oleifera* to elucidate its effect on cholesterol profiles and glycemic control. The current study highlights the capacity of MFDP to successfully managing diabetes. Unlike organic solvents such as methanol, ethanol or acetone, which may leave toxic residues or alter the stability of bioactive compounds, the water was selected as the extraction solvent due to its safety profile, cost-effectiveness, and environment friendly attributes, particularly for subsequent *in vivo* application. Furthermore, this research establishes the optimal duration for maceration to extract bioactive compounds from *Moringa oleifera*, thereby contributing to the development of more efficient extraction methods.

Methods

Acquisition of Raw Material

The *Moringa oleifera* leaf powder was procured from Virsa Agri Farms (Faisalabad), with guaranteed quality. The analytical reagents and HPLC grade reagents were procured from Sigma-Aldrich (Tokyo, Japan) and Merck KGaA (Darmstadt, Germany). Reference standard, testing kits, and bioassay were purchased from Sigma-Aldrich (Japan) and Merck (Germany).

Physicochemical Properties of *Moringa oleifera* Leaf Powder (MLP)

The analysis of MLP entailed assessing its qualitative and quantitative content, mineral composition and bioactive compounds [5]. The trials were conducted three times to ensure reproducibility.

Preparation of *Moringa oleifera* Aqueous Leaf Extract (MLE)

According to the methodology outlined by Siddhuraju et al. and Tchabo et al. [6,7], the extraction procedure was carried out. A beaker containing 100 g of MLP was prepared, and 1000 mL of deionized water was added. The brewing times of 25 hours (T_1), 50 hours (T_2), 75 hours (T_3) and 100 hours (T_4) were selected to systematically evaluate the effect of extraction duration on the yield of bioactive compounds, as extraction time can significantly influence phytochemical recovery.

Phytochemical Characterization of MLE

The phytochemical analysis involving, total flavonoid content (TFC), total antioxidant capacity (TAC), total phenolic content (TPC) and quality characteristics were performed using spectrophotometric assays. TPC was quantified using the Folin-Ciocalteu method and expressed as mg gallic acid equivalents (GAE)/g extract. TFC was measured using the aluminum chloride colorimetric assay and expressed as mg quercetin equivalents (QE)/g extract. TAC was evaluated using the phosphomolybdenum method and expressed as mg ascorbic acid equivalents (AAE)/g extract. All procedures followed standardized protocols described by Mehdi et al. and Luqman et al. [8, 9], with slight modifications.

Measurement of Quercetin Using High Performance Liquid Chromatography (HPLC)

The bioactive compound quercetin, which is believed to be the most effective component to control hyperglycemia and associated risks in MLE, was quantified using HPLC. Samples were sonicated with 80% methanol, centrifuged and filtered. Analysis was conducted on Shimadzu LC-20AT system with an SPD-20A UV detector and C18 column (250 x 4.6 mm, 5 µm, 30°C). The mobile phase consisted of water with 0.1% formic acid (A) and acetonitrile (B) in isocratic mode (60:40, v/v) at 1.0 ml/minute. Injection volume was 20

µL and detected at 370 nm. For extraction, 100 mg of MFDP was sonicated with 10 ml of 80 % methanol for 40 min, centrifuged (10000 x g, 10 min), and filtered (0.22 µm PTFE). Samples were protected from light throughout. Quantification was performed using an external calibration curve of quercetin standard (10-100 µg/ml; $R^2 > 0.99$). Peak identity was verified by retention- time matching and standard spiking. Results were expressed as mg quercetin/ g dry weight [10].

Lyophilization of MLE

After filtration of MLE, the liquid was subjected to freezing at -80 ˚C. The solidified solution was then subjected to lyophilization using freeze-dryer (Labconco Freezone) under vacuum conditions in order to create the freeze-dried powder. The freeze-dried extract powder was then stored at a temperature of -20˚C for future use [11].

Antioxidant Activity Assay of MFDP

The determination of FRAP, 0.1 g of sample was dissolved in 10 ml distilled water. An aliquot of 150 µL of this solution was mixed with 3 mL of FRAP reagent. The FRAP reagent consisted of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6, Tripyridyl-s-triazine) in 40 mM HCl, and 20 mM ferric chloride hexahydrate ($FeCl_3.6H_2O$) in 10:1:1 (v/v/v) ratio. The reaction mixture was incubated at 37 °C for 30 minutes, and the absorbance was measured at 593 nm using a UV-Visible spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) [12].The results were expressed as µmol Fe^{2+} equivalents per gram of sample.

Functional Group Analysis In MFDP

Approximately 1 mg of MFDP was placed directly on ZnSe crystal surface of Fourier Transform Infrared (FTIR) spectrometer (Agilent Cary 630, Benchtop). Background correction was performed using a clean ZnSe crystal prior to each measurement. The spectra were recorded in the wavelength range of 550 to 4000 cm^{-1}, at a resolution of 4 cm^{-1}, averaging 32 scans per sample [13]. The spectra obtained were recorded and analyzed to identify the characteristic functional groups present in MFDP.

Physicochemical Analysis of Optimized MFDP

The physicochemical analysis was performed by the method as detailed above.

Efficacy Study

The potential anti-diabetic effect of MFDP in male albino rats was investigated. Diabetes was induced in 30 rats (5 groups, having 6 rats in each group) using alloxan (at a dosage of 130 mg/kg body weight), and animals were categorized into a non-diabetic control group (N_0) and diabetic groups (D_0, D_1, D_2, D_3), which received MFDP at doses of 0 mg/kg ,100 mg/kg, 200 mg/kg, 300 mg/kg body weight, respectively. Prior to the experiment, all rats underwent a one-week acclimatization period under controlled conditions, including a standardized room temperature (25°C) and a 12-hour light/dark cycle.

Ethical Approval

The experimental procedures conducted in this research were approved by the Institutional Review Board and the Doctoral Program Coordination Committee (DPCC) at the University of the Punjab under the reference number R/3606/DPCC. Ethical permission was obtained before the research was conducted, and the procedures followed the guidelines of Good Laboratory Practice (GLP).

Serum Biochemical Analysis of Blood Profile

Albino rats that were fasted overnight. For terminal collection, animal was anesthetized with urethane (1.0-1.3 g/kg intraperitoneal. of 25% solution) and cardiac puncture performed under deep anesthesia. Daily glucose in live animal was measured from tail vein using fluoride/oxalate (or immediate plasma separation) and the GOD-PAP (glucose oxidase-peroxidase with phenol/4-aminoantipyrie chromogen) method. Fasting blood glucose (FBG) and Random blood glucose (RBG) levels were assayed per kit IFU within 1 hour of collection. To determine the HbA1c rat-validated method (affinity/ion-exchange HPLC or species-validated immunoassay) was used [14], insulin level was measured using ELISA technique. The total cholesterol (TC) and triglyceride (TG) were measured using CHOD-PAP (Cholesterol oxidase-phenol amino phenazone) method and GPO-PAP (glycerol-3-phosphate oxidase-peroxidase with PAP chromogen) method, respectively; high-density lipoprotein cholesterol (HDL-C) was determined after ApoB precipitation with CHOD-PAP on the supernatant, and Low-density lipoprotein cholesterol (LDL-C) was measured using a direct enzymatic assay kit (Abcam ab65390). For liver glycogen, tissues were digested in 30 % KOH (boiling water bath, 10 min), glycogen precepted with ethanol, and quantified by phenol-sulfuric acid assay. The blood glucose level was measured daily. HbA1c, insulin levels, and liver glycogen were measured at two-weeks intervals. Whereas lipid profile, liver functioning enzymes were performed at the end of study (week 8) [15].

Hepatic Functioning Tests

Biochemical kits and an automatic serum analyzer (Bio-Ray 310 diagnostic) were used to measure the levels of alanine amino-transferase (ALT) and aspartate aminotransferases (AST) [16].

Histopathological Study of Liver

The liver was preserved in a 10 % neutral buffered formalin solution for histological analysis immediately following the decapitation of the animals. Thin slices of 5 µm thickness were prepared and mounted on glass plates. The staining procedure employed in this study involved the use of periodic acid-Schiff (PAS) method and examined under a light microscope (MCX 100 & Micros Austria) [17].

Statistical analysis

The study employed a Randomized Complete Block Design (RCBD) and conducted two-way ANOVA analysis using SPSS version 23 and the R programming language version 4.3.1, commonly used for statistical analysis. The factors compared in the analysis were the dosage of MFDP on various parameters such as blood glucose levels, HbA1c, and lipid profile (total cholesterol, triglycerides, HDL, LDL). ANOVA was used to determine the level of significance of the differences between the means of the different dosages and time interval. Significant differences were considered at $p \leq 0.05$ [18].

Results

Proximate Composition and Mineral Content Analysis

The MLP demonstrated notable levels of moisture (8.4 ± 0.01 %), crude protein (21.15 ± 0.03 %), crude fiber (19.5 ± 0.03 %), fat (1.8 ± 0.01 %), ash (6.19 ± 0.02 %), and NFE concentration (28.5 ± 0.04 %). Figure 1 illustrates the mineral content, showing the presence of calcium (1500 ± 2 mg/100g), magnesium (350 ± 1 mg/100g), potassium (1200 ± 0.05 mg/100g), sodium (70 ± 0.02 mg/100g), and phosphorus (100 ± 1 mg/100g). The current study showed significant differences in calcium, phosphorus, and potassium concentrations in comparison with the results of Khan et al. [19]. Variations in mineral contents may influence *Moringa oleifera* bioactivity, as higher levels of calcium, magnesium, and potassium are associated with improved glycemic control and cardiovascular benefits. These variations highlight the potential impact of regional and environmental factors on the plant's therapeutic efficacy.

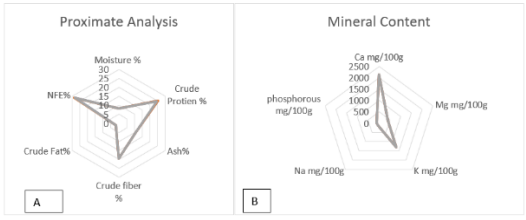

Figure 1: Graphical representation of mean values of quality characteristics of MLP.

Phytochemical Characterization of MLE

In Table 1, it is evident that T_1 had the lowest TAC value while T_3 had the highest, which is consistent with the findings of Nobossé et al. [20]. The trend of TPC, TFC, and TAC of MLE is visually represented in the lollipop graph in Figure S-1 (Supplementary File). While all treatment results significantly differed for TPC and TFC, only the means of T_1 and T_3 showed a significant difference (p-value ≤0.05) for TAC.

Treatment with water	TAC (mg AAE/ g)	TPC (mg GAE/ g)	TFC (mg RE/ g)
T_1	26.8 ±0.01^d	15.7±0.01^d	5.5±0.01^d
T_2	31.2±0.02^c	20.4±0.03^c	6.5±0.02^c
T_3	35.1±0.05^a	26.5±0.01^a	8.8±0.01^a
T_4	33.4±0.01^b	24.7±0.02^b	7.8±0.01^b

Table 1: Phytochemical Characterization of MLE Over Different Time Duration (T_1: 25 Hours, T_2: 50 Hours, T_3: 75 Hours, And T_4: 100 Hours). Values are expressed as means ± SD, Differences among treatments were determined by one-way ANOVA followed by Tukey's HSD test (P≤0.05). Letters (a, b, c, d) in superscript within each column indicate statistically significant differences. *TAC= Total antioxidant capacity TPC= Total phenolic content, TFC= Total flavonoid content.

In contrast to Jarial et al. [21] findings, who observed a TFC value ranging from 12.46-145.22 (µg RE/g) in *Moringa oleifera* leaf extracts , the differences could be because of topographical changes and climatic circumstances on *Moringa oleifera* varieties. It is clear from comparing the mean values of each quality attribute that T_3 (75 hours) had a higher mean value than any of the other treatments (T_1, T_2, T_4). The increased TPC and TFC at 75 hours may be attributed to prolonged solvent penetration and cell wall disruption, while the decline at 100 hours could result from oxidative degradation or compound leaching.

Quantification of Quercetin Through HPLC

The chromatogram separation for quercetin is presented in Figure S-2 (Supplementary File). The quantification of quercetin in MLE at different maceration times revealed variations in concentration, with the highest yield observed at 75 hours of extraction. HPLC analysis showed that the quercetin content increased with maceration time, peaking at 75 hours before slightly decline at 100 hours. This suggests that prolonged extraction enhances flavonoid release up to an optimal duration, beyond which degradation or saturation may occur. Detailed concentration values and corresponding peak areas for different maceration times are provided in Table S-1 (Supplementary file). It has already been reported by Khalid et al. [22], that the water and ethanolic extract (30% : 70 %) of *Moringa oleifera* contained varying concentration of quercetin depending on the plant's life stage and maceration method.

Phytochemical Screening of MFDP Using FTIR

The absorption peaks at 1680 cm^{-1}, 1500 cm^{-1}, and 1429 cm^{-1} were attributed to the stretching vibrations of C–C, C=O, and C=C aromatic bonds, respectively. Additionally, OH bending vibrations of phenols were detected at 1359 cm^{-1}, as shown in Figure 2. In the IR spectra of the MFDP, the presence of alcohol and phenol was confirmed by the appearance of peaks at 3400.58 and 2840.77 cm^{-1}, respectively. The absorption peaks of the current study align with Kainat et al. [23], who reported similar bands at 1678 cm^{-1},1498 cm^{-1} and 1430 cm^{-1}, confirming aromatic structure. Likewise, OH-related peaks 3400 cm^{-1} and 2838 cm^{-1} matched closely, validating the presence of alcohol and phenol.

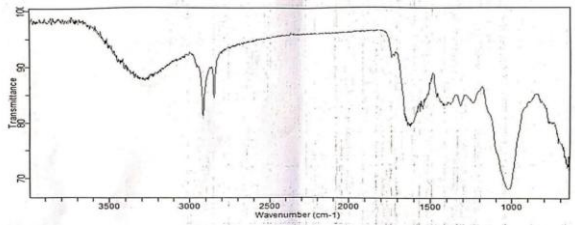

Figure 2: FTIR spectrum of MFDP, highlighting characteristics functional groups.

Physicochemical Analysis of MFDP

The moisture content in MFDP decreased compared to MLP, with lower fiber content. Conversely, MFDP had higher protein, ash content, calcium, magnesium, potassium, sodium, and phosphorus concentrations than MLP, as shown in Table 2.

Physicochemical Analysis	MLP	MFDP
Moisture %	8.4 ± 0.01	3.91 ± 0.01
Fiber %	19.5 ± 0.03	9.31 ± 0.01
Ash %	6.19 ± 0.02	10.7 ± 0.02
Crude Protein %	21.15 ± 0.03	25.11 ± 0.02
Ca mg/100g	1500 ± 2	3100 ± 0.08
Mg mg/100g	350 ± 1	480 ± 0.05
K mg/100g	1200 ± 0.05	1630 ± 0.05
Na mg/100g	70 ± 0.02	217.7 ± 0.05
Phosphorus mg/100g	100 ± 1	440 ± 0.5

Table 2: Comparative nutritional analysis of MLP and MFDP.

That is in line with the observation of Nastić et al. [24], that different methodologies, either drying or maceration in leaves extraction, have different impacts on the extraction of phytonutrients.

Antioxidant Activity Profile of MFDP

As shown in Table 3, The FRAP value 6.64 ±0.05 (µmol Fe^{2+}E)/g was higher than the reported 4.9 (µmol Fe^{2+}E)/g, reflecting a moderately stronger ferric potential.

Physicochemical analysis	Measurement
Vitamin C mg AAE/g	18.37 ± 0.03
FRAP (µmol Fe^{2+}E)/g	6.64 ± 0.05

Table 3: Antioxidant analysis of MFDP on dry weight basis. Values are expressed as means ± SD.

Anti-Diabetic Effect of MFDP

The statistical significance of physical parameters (feed intake, water intake, body weight gain, and changes concerning weeks) was analyzed by ANOVA, as shown in Figure S-3 (Supplementary File). It was observed that all three variables showed a decreasing trend as the concentration of MFDP increased.

Random Blood Glucose Level and Fasting Blood Glucose Level

In the study, both the control group rats and diabetic rats experienced an increase in random blood glucose (RBG) levels. After 8 weeks, the diabetic control group (D_0) exhibited a significant elevation in RBG (343 ± 0.02 mg/dL) compared to the non-diabetic control (N_0: 105 ± 0.01 mg/dL). Treatment with MFDP produced a marked reduction in RBG in all groups. At 100 mg/kg (D_1), RBG decreased to 179.67 ± 0.02 mg/dL, while 200 mg/kg (D_2) and 300 mg/kg (D_3) showed further reductions (181.67 ± 0.01 mg/dL and 171 ± 0.03 mg/dL, respectively). The reduction was dose-dependent, with the highest dose (D_3) approaching near-normal values. FBG followed a similar trend. Diabetic control (D0) had significantly higher FBG (243.21 ± 0.03 mg/dL) compared to N_0 (97.83 ± 0.01 mg/dL). MFDP treatment lowered FBG to 151.71 ± 0.01 mg /dL (D_1), 112.45 ± 0.03 mg/dL (D_2), and 98.2 ± 0.02 mg/dL (D_3). Notably, the highest dose (D_3) normalized FBG to levels comparable with the non-diabetic group.

HbA1c

Table 4 displays the HbA1c level was markedly elevated in the diabetic control (12.7 ± 0.20 %) versus the normal control (5.6 ± 0.15 %). Treatment with MFDP substantially reduced HbA1c, with values of 5.9 ± 0.18 % (D_1), 5.6 ± 0.16 % (D_2), and 5.4 ± 0.15 % (D_3). These values were statistically indistinguishable from the non-diabetic control, indicating near-complete glycemic normalization.

Insulin Level

Diabetes induction led to a significant reduction in insulin levels (7.6 ±1 µIU/L) compared to non-diabetic rats (13.92 ± 0.8 µIU/L). MFDP treatment restored insulin secretion in a dose-dependent manner, with 12.5 ± 0.9 µIU/L (D_1), 15.5± 0.9 µIU/L (D_2), and 16.4 ± 1.0 µIU/L (D_3) [25].

Liver Glycogen Level

In the current investigation, the experimental group D_0 glycogen level (18.07 ±0.03 mg/g) was significantly lower that of the normal rat control group N_0 (45.25 ±0.01 mg/g). MFDP treatment significantly improved glycogen content to 32.32 ± 2.5 mg/g (D_1), 38.45 ± 2.5 mg/g (D_2), and 43.68 ± 2.5 mg/g (D_3), with the highest dose nearly restoring normal glycogen levels.

Effect of MFDP on Lipid Profile

Following the results presented in Table 4, the highest recorded value of TC was 120.55 ± 6 mg/dL in the D_0 group relative to N0 (83.05 ± 5 mg/dL). MFDP treatment progressively reduced TC to 90.22 ± 5 mg/dL (D_1), 87.63 ± 5 mg/dL (D_2), and 85.17 ± 5 mg/dL (D_3), with values approaching those of the non-diabetic group.

High-Density Lipoprotein Level

The study has demonstrated that the minimum value for HDL was seen in D_0. Nevertheless, an increase was noted in alternative therapies. This therapeutic effect may be attributed to Beta-sitosterol, a prominent compound found in *Moringa oleifera* leaves, renowned for its potential to enhance the HDL levels due to its superior absorption compared to cholesterol. Beta-sitosterol has been reported in literature to positively influence lipid profiles by promoting the lipid metabolism and reducing cholesterol absorption. This finding underscore potential of *Moringa oleifera* in lipid metabolism [26].

Low-Density Lipoprotein Level

Table 4 demonstrates significant fluctuations in LDL cholesterol was elevated in the diabetic group (55.49 ± 5.8 mg/dL) versus normal controls (35.34 ± 5.7 mg/dL). MFDP reduced LDL to 33.02 ± 7.4 mg/dL (D_1), 30.92 ± 6.8 mg/dL (D_2), and 27.82 ± 7.1 mg/dL (D_3), all of which were lower than diabetic group.

Triglyceride Level

The finding of the current study demonstrated that the diabetic control group (D_0) exhibited the highest triglyceride level at 140.9 ± 5.4 mg/ dL, While treatment with MFDP at increasing doses (D_1: 100 mg/kg, D_2: 200 mg/kg and D_3: 300 mg/kg) progressively reduced triglyceride level to 99.13 ± 7.5 mg/dL, 96.12 ± 6.8 mg/dL, 94.11 ± 10.4 mg/dL, respectively. This dose dependent reduction in triglyceride may be attributed to enhanced lipase enzyme activation and improved triglyceride metabolism, consistent with previous studies demonstrating the lipid -lowering effect of *Moringa oleifera* bioactive compounds [27].

Hepatic Functioning Enzyme Activity

The finding suggested a significant decrease in liver functioning enzyme (ALT, AST and ALP) in diabetic rats, treated with MFDP at dose of 300 mg/kg (D_3) compared to the base line levels (D_0). Detailed findings are presented in Table 5 highlighting the potential hepatoprotective effects of MFDP in diabetes management.

Histopathology Results

Diabetic liver cells displayed disorganization around central veins, while other liver cells showed improvement with increasing doses of MFDP as depicted in Figure 4 [28]. Histopathological evaluation of liver tissue revealed distinct alterations across the experimental groups. The normal control group exhibited preserved hepatic structure with well arranged hepatocyte cords, normal sinusoidal spacing and uniformly stained cytoplasm, indicating healthy liver morphology. In contrast, the diabetic control group showed severe hepatic injury characterized by hepatocellular ballooning, sinusoidal dilation, inflammatory infiltration and focal necrotic areas, reflecting the damaging effects of sustained hyperglycemia. Treatment with MFDP produced a dose-dependent improvement in hepatic structure. Rats receiving 100 mg/kg MFDP displayed partial restoration, although mild vacuolation and disorganization of hepatic cells were evident. The 200 mg/kg of MFDP dose demonstrated clearer improvement with reduced fatty changes, fewer inflammatory cells and more defined hepatic central vein. Notably, the 300 mg/kg MFDP treated group showed near-normal hepatic structure, with minimal cytoplasmic vacuolation and well- preserved cellular organization, closely resembling the normal control. These findings confirm that MFDP exert a hepatoprotective effect that increase proportionally with dosage.

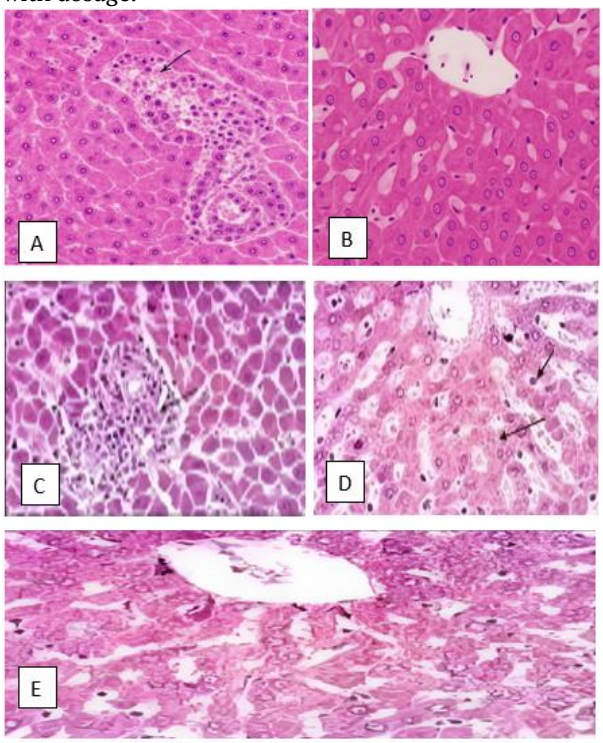

Figure 4: Illustration of hepatic histological alterations using MFDP
A: Positive control group hepatic histopathological variation (D_0)
B: Variations in the negative control group's hepatic histopathology (N_0)

C: Diabetic group hepatic histopathological variation with 100 mg/kg body weight (D_1)
D: Diabetic group hepatic histopathological variation with 200 mg/kg body weight (D_2)
E: Diabetic group hepatic histopathological variation with 300 mg/kg body weight (D_3)

Investigational groups	RBG Week (mg/dL) ± S. D	FBG Weeks (mg/dL) ± S. D	HbA1c (%) ± S. D	Insulin (µIU/L) ± S. D	Liver glycogen (mg/g) ± S. D	Total Cholesterol (TC) mg/dL ± S. D	HDL mg/dL ± S. D	LDL mg/dL ± S. D	Triglycerides (TGL) mg/dL ± S. D
N_0	105 ± 0.01	97.83 ± 0.01	5.6 ± 0.15	13.92 ± 0.8	45.25 ± 2.5	83.05 ± 5	41.52 ± 1.3	35.34 ± 5.7	93.9 ± 3.5
D_0	343 ± 0.02	243.21 ± 0.03	12.7 ± 0.20	7.6 ± 1	18.07 ± 3	120.55 ± 6	37.04 ± 2.3	55.49 ± 5.8	140.9 ± 5.4
D_1	179.67± 0.02	151.71 ± 0.01	5.9 ± 0.18	12.5 ± 0.9	32.32 ± 2.5	90.22 ± 5	39.76 ± 2.01	33.02 ± 7.4	99.13 ± 7.5
D_2	181.67 ± 0.01	112.45 ± 0.03	5.6 ± 0.16	15.5 ± 0.9	38.45 ± 2.5	87.63 ± 5	40.13 ± 2.4	30.92 ± 6.8	96.12 ± 6.8
D_3	171 ± 0.03	98.2 ± 0.02	5.4 ± 0.15	16.4 ± 1.0	43.68 ± 2.5	85.17 ± 5	41.14 ± 2.5	27.82 ± 7.1	94.11 ± 10.4

Table 4: Antidiabetic effects of MFDP at different concentrations after 8th week. *N_0: Non-diabetic control, D_0: Diabetic control, D_1: Diabetic group treated with 100 mg/kg MFDP, D_2: Diabetic group treated with 200 mg/kg MFDP, D_3: Diabetic group treated with 300 mg/kg MFDP.* Values are presented as mean ± SD (n=6).

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	Percentage Difference between Treatments and Control		
				AST (U/L)	ALT (U/L)	ALP (U/L)
N_0	90.32 ± 3.5	72.03 ± 2.8	71.97 ± 2.6	0	0	0
D_0	98.4 ± 4.1	77.54 ± 3.2	76.55 ± 3.0	8.08	5.51	4.58
D_1	88.77 ± 3.4	72.63 ± 2.7	72.22 ± 2.5	-1.55	0.6	0.25
D_2	86.44 ± 3.2	71.22 ± 2.6	71.12 ± 2.4	-3.88	-0.81	-0.85
D_3	85.77 ± 3.1	69.65 ± 2.5	70.66 ± 2.3	-4.55	-2.38	-1.31

Table 5: Liver Functioning Enzyme Values (AST= aspartate aminotransferases, ALT= alanine transaminase, ALP=alkaline phosphatase) and the distinction between treatment and control values. *N_0: Non-diabetic control, D_0: Diabetic control, D_1: Diabetic group treated with 100 mg/kg MFDP, D_2: Diabetic group treated with 200 mg/kg MFDP, D_3: Diabetic group treated with 300 mg/kg MFDP.* Values are presented as mean ± SD (n=6).

Discussion

This study investigated the therapeutic potential of MFDP on alloxan induced diabetic rats, with a particular focus on its antidiabetic and antihyperlipidemic effects. MFDP produced a statistically significant ($p \leq 0.05$) improvement in glycemic parameters, lipid profiles, and hepatic enzyme activity, with a clear dose-responsive trend was observed for the highest dose (300 mg/kg, D_3) showing the greatest therapeutic outcomes. Phytochemical characterization confirmed that MFDP was rich in flavonoids, phenolics, quercetin and other bioactive compounds, and the optimized extraction conditions (75 hours) maximized their yield. The observed hypoglycemic effect, evident from fasting and random blood glucose levels, aligns with prior evidence that *Moringa oleifera* constituents- particularly quercetin- modulate glucose metabolism by enhancing insulin secretion and suppressing hepatic gluconeogenesis. The dose-dependent increase in insulin levels and liver glycogen content suggests that MFDP not only preserve pancreatic Beta-cell function but also promotes glycogenesis, improving glucose homeostasis. Additionally, the significant decrease in HbA1c levels in MFDP-treated rats indicates a sustained improvement in long term glycemic control. These results are particularly notable when compared with the untreated diabetic group, where glycemic dysregulation remained unmitigated. Furthermore, the increase in HDL levels concurrent with a reduction in LDL, triglycerides, and total cholesterol levels in MFDP groups underscore the compound's potential in mitigating diabetic dyslipidemia- a critical risk factor for cardiovascular complications in diabetic patients. Our findings are in strong agreement with a growing body of literature that supports the antidiabetic and lipid-lowering potential of *Moringa oleifera*, particularly in preclinical models. For instance, Nada et al. [29], reported that methanolic extracts of *Moringa oleifera* significantly reduced blood glucose levels and improved lipid profiles in streptozotocin-induced diabetic rats, consistent with the glycemic reduction observed in our MFDP treated groups. Notably, our study has demonstrated reduction in fasting blood glucose at the highest MFDP dose, which slightly surpassed results reported for solvent-based extractions, supporting the efficiency of our optimized aqueous extraction approach. Similarly, Alijazzaf et al. [30] , emphasized the role of phenolic compounds in modulating insulin secretion and sensitivity. The increased insulin levels and hepatic glycogen storage observed in current study indicated that MFDP may enhances insulin signaling and glucose utilization. The higher FRAP value together with increased TPC and TAC suggests that the MFDP possesses substantial antioxidant potential. This highlights the fact that antioxidant activity cannot be fully evaluated by a single assay [23, 31]. These differences likely stem from variation in extraction method, maceration time, plant genotype, and agroclimatic conditions. The decision to employ lyophilization and aqueous maceration for 75 hours in current study appears to have maximized the recovery and preservation of thermolabile bioactives, particularly quercetin and phenolic acids. Moreover, a study by Fahey [32], highlighted the regional variability in the nutrient and phytochemical content of *Moringa*

oleifera. Our mineral analysis supports this observation, with the MFDP showing higher levels of calcium and potassium compared to other published reports. This coincided with a comparatively higher ash content in MFDP, which is attributable to the freeze-drying process minimizing non-nutritive inorganic residue accumulation while preserving bioavailable minerals. The improved lipid metabolism observed in our study, especially the increase in HDL and reduced LDL levels, is consistent with the findings of Mbikay [33], who attributed Moringa's hypolipidemic effects to its sterol constituents and their ability to modulate intestinal cholesterol absorption pathways. However, the use of aqueous extract with freeze drying not only maintained efficacy but also provided a safer alternative to organic solvents. Together, these comparisons strongly position MFDP as a regionally adapted, eco-friendly, and biochemically potent formulation, offering efficacy comparable to prior studies that used less sustainable methods or solvent based extraction. The implications of this study are multifaceted. First, it established MFDP as a promising dual-action therapeutic agent, capable of modulating both glucose and lipid metabolism. Second, the use of *Moringa oleifera* in a regionally optimized and lyophilized form (MFDP) enhances its suitability for therapeutic application in South Asian populations, where both the disease burden and the availability of the plant are high. The findings also reinforce the need to consider regional and environmental factors in the phytochemical profile of medicinal plants. This study provides strong evidence that MFDP exhibits antihyperglycemic, and antihyperlipidemic making it a viable natural alternative in the management of metabolic disorders such as Type-2 diabetes. Future studies should focus on studies to uncover pathways through which MFDP modulates glucose and lipid metabolism (e.g., AMPK, GLUT4 expression) and also conduct the clinical trials to validate MFDP's efficacy and safety in human subjects.

CONCLUSION:

This study underscores the profound antioxidant, anti-diabetic, and anti-hypercholesterolemia activities of locally produced MFDP prepared from optimized extract (75-hour maceration). It has excellent antioxidant properties, as indicated by high levels of total phenolic content, total flavonoid content, total antioxidant capacity, and quercetin. The efficacy study also indicated that MFDP reduced glucose levels conferred an improved insulin response and modulated lipid profiles favorably. Using a locally grown plant highlights the value of sustainable and regionally tailored treatments, providing a cost effective, sustainable, and culturally relevant option for managing metabolic disorders. Moreover, MFDP safety profile supported by the rat model's lack of toxicity and adverse effects, points to the substance's potential for secure long-term use. However, while these findings are promising, they are limited to preclinical (animal) models and further human trials are required to validate the therapeutic potential of MFDP. Overall, this research lays a robust foundation for future studies to explore the full therapeutic potential of MFDP in metabolic disorders induced by diabetes. Incorporating MFDP into commonly consumed food items in Pakistan may offer promising prospects for developing natural and effective interventions.

Acknowledgments

We acknowledge *Dr. Hafiz Roy Umair, Fahmir Pharma Pvt. Ltd,* for facilitation regarding resources and writing assistance for the article.

Conflict of Interest

The authors declare no conflict of interest regarding this manuscript.

Author Contributions

MS and SWA collaboratively designed and conducted the research. Both authors contributed to data analysis, interpretation, and manuscript preparation. SWA also served as the corresponding author and provided critical revisions. QM supported data curation, visualization and manuscript preparation. MRT conducted statistical analysis and validation. AS and AA helped in proof reading the manuscript and review. MAM and AAR offered technical support and editing. All authors reviewed and approved the final manuscript.

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