

Hepatoprotective, Antihyperlipidemic, and Anti-inflammatory Activity of *Moringa oleifera* in Diabetic-induced Damage in Male Wistar Rats

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ABSTRACT

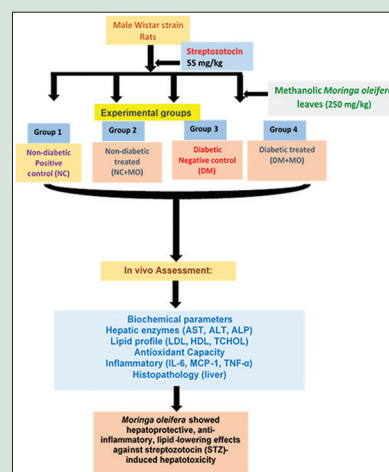
Background: The number of individuals with diabetes is increasing daily, and diabetes is presently estimated to affect about 422 million adults worldwide. Conventional drugs used to treat diabetes are not without severe side effects, accessibility, and affordability. This study elucidates the potential effects of *Moringa oleifera* (MO) leaves extract to manage and treat diabetes induced in male Wistar rats.

Materials and Methods: Adult male Wistar rats were randomly divided into four groups ($n = 12/\text{group}$): NC – nondiabetic rats (positive control), MO – nondiabetic-treated rats, DM – diabetic rats (negative control), DM + MO – diabetic-treated rats. Hepatic enzymes and biochemical parameters as well as antioxidant capacity and inflammatory cytokine levels were assessed. Levels of low-density lipoprotein, high-density lipoprotein, and total cholesterol were evaluated. **Results:** Oral administration of methanolic extract of MO (250 mg/kg) to diabetic rats for 42 days showed a significant reduction in hepatic enzyme markers and normalized lipid profile parameters in the serum compared to normal control group. Treatment also increased the level of antioxidant capacity and alleviated inflammatory biomarkers of the liver. Histology sections of the liver tissue showed protective effect of MO in treated rats. **Conclusions:** MO showed hepatoprotective, anti-inflammatory, and lipid-lowering effects against streptozotocin-induced hepatotoxicity. Histological section demonstrated specific alterations in the liver of the diabetic and nondiabetic male Wistar rats while MO treatment revealed improvement in liver alterations.

Key words: Diabetes, hepatic enzymes, inflammatory cytokines, lipid profile, *Moringa oleifera*, streptozotocin

Abbreviations Used: IL 1: Interleukin 1, IL 6: Interleukin 6, MCP-1: Monocyte chemoattractant protein, TNF- α : Tumor Necrotic factor alpha, ROS: Reactive oxygen species, MO: *Moringa oleifera*, STZ: Streptozotocin, SRC: Standard rat chow, ALP: Alkaline phosphatase, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ORAC: Oxygen radical

absorbance capacity, LDL: Low density lipoprotein, HDL: High density lipoprotein, CHOL: Total cholesterol.



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INTRODUCTION

The number of individuals with diabetes is increasing daily, and diabetes is presently estimated to affect about 422 million adults worldwide.^[1] Diabetes is associated with liver cirrhosis, inflammation, apoptosis, and microvascular and macrovascular complications of the liver.^[2] This is linked with significant increase in inflammatory cytokines C-reactive protein, interleukin-1 (IL-1), interleukin 6 (IL 6), tumor necrotic factor-alpha (TNF- α), and chemokine monocyte chemoattractant protein-1 (MCP-1). Reports have shown hyperglycemic effects from uncontrolled hepatic glucose output and reduced glucose uptake by skeletal muscle coupled with reduced glycogen synthesis.^[3] Moreover, there is increasing evidence from experimental and clinical studies indicating oxidative stress as a major player in diabetic pathophysiology leading to dyslipidemia, impaired glucose tolerance, β -cell dysfunction,

and ultimately resulting to liver malfunction.^[4,5] Conventional drugs metformin and glimepiride are used to treat diabetes but with severe side effects, lack of accessibility, and affordability while unconventional therapy is known to play significant roles in treating and managing diabetes with

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minimal side effects.^[6,7] In diabetic state, there is increased generation of reactive oxygen species (ROS) which triggers a chain of reaction leading to the peroxidation of lipids, lipoprotein modifications, and several cellular mutations of biomolecules.^[8,9] This effect leads to oxidative stress which is an important risk factor in the pathogenesis of cardiovascular and chronic diseases such as diabetic and associated complications.^[10]

Moringa oleifera (MO) is the most cultivated species of the Moringaceae family.^[11] It has been reported in folk medicine for its use in the treatment and management of diabetes.^[12] Interestingly, studies have shown that MO leaves are rich sources of antioxidant due to the presence of various phytochemicals such as polyphenolics, carotenoids, α -tocopherol, ascorbic acid, and several amino acids.^[13-15] The bioavailability of these metabolites in MO has been directly linked to various biological profiles, especially its scavenging activity which helps ameliorate the damaging effects caused by oxidative stress in diabetic rats.^[16-18] Other notable metabolites with medicinal importance from the extracts of MO leaves include glycosides such as niazirin, niazirinin, niaziminin A and B, and 4-(α -1-rhamnopyranosyloxy)-benzylglucosinolate.^[19-22] Scientists have reported the potency of various parts of this plant in animal model for their medicinal properties such as antidiabetic, analgesic, antiviral, antiulcer, anti-inflammatory, anticancer, antimicrobial, antioxidant, antifertility, cardioprotective, anticonvulsant, antiallergic, and antihelminthic activities.^[23-26] These biological and pharmacological activities may help reduce cell death and cell proliferation. Sreelatha *et al.*^[27] reported antiproliferative effect of MO leaf extract on human cancer cells. MO has also been reported to elicit hepatoprotective properties in rats.^[28,29]

Previous scientific reports on MO in the treatment of diabetes are limited in scope and do not extensively elucidate its action in the liver; hence, the need for this study to examine specific alterations in the liver and elucidate the hepatoprotective, anti-inflammatory, and lipid-lowering effects of MO in diabetic and nondiabetic male Wistar rats.

MATERIALS AND METHODS

Animal care

Healthy male adult Wistar strain rats weighing between 200 and 250 g were purchased from Charles River (Margate, UK) and housed at the Primate Unit, Stellenbosch University (Tygerberg Campus, South Africa) where animal treatment took place. For the duration of the study, the rats were housed in stainless steel cages of five rats per cage in a temperature-controlled animal facility of $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and humidity $55\% \pm 5\%$ with alternating 12 h light/12 h dark cycles. The rats had access to fresh water and standard rat chow (SRC) from Aquanuro, Malmesbury, South Africa *ad libitum*.

Ethical approval

The study received approval from the Faculty of Health and Wellness Sciences, Research Ethics Committee of Cape Peninsula University of Technology, South Africa (CPUT/HW-REC 2014/AO8). All animals in the study received humane care according to the principles of laboratory animal care of the National Institutes of Health Guide for the care and use of laboratory animals of the National Academy of Science and published by the National Institutes of Health (publication no. 80-23, revised 1978).

Plant material

MO fresh leaves were collected and identified by Mr A.A. Adeyemo of the Forestry Research Institute of Nigeria. A voucher specimen was deposited in the herbarium with specimen number FHI-110287. MO leaves were washed with water, dried, and pulverized. The leaf powder was extracted with n-hexane, and dried marc was extracted with methanol at 45°C for 24 h. The filtrate was filtered using Whatman no. 1

filter paper and then evaporated in a rotatory evaporator under reduced pressure at 35°C . The methanolic extract was stored at -4°C until use.

Induction of diabetes

Diabetes was induced in rats by injecting intraperitoneally, freshly prepared streptozotocin (STZ; 55 mg/kg body weight in 0.1 M cold citrate buffer, pH 4.5) according to the procedure of Jaiswal *et al.*^[18] Blood sample was collected from the rat tail, and fasting blood glucose level was measured using a glucometer (Accu-Check, Roche, Germany). The rats were considered diabetic when blood glucose level was ≥ 18 mmol/L. MO administration commenced on the 3rd day after the rats were confirmed diabetic, and this was considered the 1st day of treatment. The rats were randomly assigned into four groups of twelve rats ($n = 12$).

Experimental protocol

Forty-eight rats were randomly divided into four groups as follows:

- Group I: (Positive control) consisting of nondiabetic rats, which were treated with vehicle and SRC only
- Group II: (*Moringa* treated + nondiabetic) nondiabetic-treated rats received only MO extract at a dose of 250 mg/kg and SRC
- Group III: (Negative control) were diabetic and received the vehicle and SRC without receiving the plant extract
- Group IV: (*Moringa* treated + diabetic) diabetic-treated rats received only MO extract at a dose of 250 mg/kg and SRC.

MO methanolic extract was dissolved in the vehicle and administered orally via oral gavage daily for 6 weeks to the treated groups while positive control groups and negative control groups received the vehicle and SRC daily for the duration of the study. The rats were weighed at the beginning of the experiment and weekly. At the end of study, animals were weighed, fasted overnight, and anesthetized intraperitoneally with sodium pentobarbital injection (60 mg/kg) to ensure rapid and painless death of rats.

Serum preparation

Whole blood was collected from the rat's abdominal aorta into a serum clot activator tube. Serum was prepared by centrifugation at 4000 g for 10 min at 4°C and stored at -80°C for biochemical analysis.

Liver homogenate preparation

Liver was excised, rinsed in phosphate-buffered saline, and weighed, after which it was homogenized and centrifuged at 15,000 rpm for 10 min at 4°C and stored at -80°C for analysis.

Determination of weight parameter and glucose level

Liver weight was measured, and relative kidney weight was calculated by dividing the kidney weight by the total body weight. Glucose level was determined using kit from Randox Laboratories Limited (Crumlin, United Kingdom).

Analysis of liver enzymes activity

Liver enzyme activities, namely, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), were determined. AST activity is based on the reaction of α -oxoglutarate with L-aspartate to form oxaloacetate and L-glutamate. The oxaloacetate formed further reacts with NADH to form L-malate and NAD^+ . ALT activity was estimated kinetically based on the reaction of α -oxoglutarate and L-alanine catalyzed by ALT to form L-glutamate and pyruvate. The indicator reaction used pyruvate for the kinetic estimation of NADH

consumed. The pyruvate formed further reacts with NADH to form L-lactate. Measurement of ALP is based on the reaction of p-nitrophenyl phosphate which was hydrolyzed by ALP in the serum in the presence of magnesium ion to yield p-nitrophenol (yellow). The color intensity was proportional to the activity of ALP in the serum. The complex formed was measured at 405 nm. All protocols were followed according to the manufacturer's manual.

Determination of lipid profile levels

Lipid profiles such as low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol (CHOL) were analyzed. All assays were analyzed using diagnostic kits (Randox Crumlin, United Kingdom) using Randox Daytona automated analyzer (Randox Laboratories Limited, Crumlin, United Kingdom). Protocols were followed according to the manufacturer's standard operating procedures.

Antioxidant capacity

Antioxidant capacity was measured in the liver by oxygen radical absorbance capacity (ORAC) assay according to the method of Ou *et al.*^[30] Values were expressed as $\mu\text{mol trolox equivalents/g tissue}$.

Analysis of inflammatory cytokines in the liver

The levels of inflammatory cytokines IL-6, MCP-1, and TNF- α were evaluated in the liver homogenates using MILLIPLEX[®] MAP rat cytokine magnetic bead-based Luminex kit (Merck Millipore, Billerica, MA, USA). Assays were performed strictly according to the manufacturer's protocol (Merck Millipore) and read on the Bio-plex[®] platform (Bio-Rad Laboratories, Hercules, CA, USA). The Bio-Plex Manager[™] version 6.0 software was used for bead acquisition and analysis of median fluorescent intensities.

Histopathological analysis

The liver specimens excised from all experimental animals were washed and fixed in 10% buffered formalin. Tissues were processed by embedding in paraffin and sectioned (5 μm thickness). After deparaffinization, the paraffin blocks were stained with hematoxylin and eosin. The examination of stained slides and photomicrographing of slides was performed by a pathologist who was blinded to the study.

Statistical analysis

Values are represented as mean $\pm$ standard deviation. GraphPad Prism version 5.0 for Windows (San Diego, CA, USA) was used to analyze data using ANOVA with Bonferroni's multiple comparison test. $P < 0.05$ was considered statistically significant.

RESULTS

Effect of *Moringa oleifera* on weight parameters and serum glucose level

MO's effect on liver weight, relative liver weight, and serum glucose level are shown in Table 1. As shown in Table 1, the liver weight and the relative liver weight of animals injected with STZ were heavier compared to the NC, nondiabetic rats (positive control), MO, and nondiabetic-treated rats, respectively. However, significant ($P < 0.05$) decrease in liver weight and relative liver weight was observed in MO-treated rats when compared to diabetic control group. Elevated serum glucose level decreased significantly ($P < 0.05$) in groups administered with MO when compared to diabetic control.

Hepatic enzyme activities

Table 2 shows the activities of liver enzyme in nondiabetic rats and diabetic rats. From the results, a significant elevation in the activities of AST, ALT, and ALP in diabetic groups was observed when compared to nondiabetic rats (positive control). Treatment with MO led to a reduction in the activities of hepatic enzymes in all treated groups.

Effect of *Moringa oleifera* on lipid profile and antioxidant capacity in the liver of rats

Table 3 illustrates the effect of MO on LDL, HDL, total CHOL, and antioxidant capacity in the liver of rats. Induction of diabetes with STZ elevated the level of LDL and CHOL in diabetic rats (negative control) when compared to nondiabetic rats (positive control). However, after the treatment of nondiabetic rats and diabetic rats with MO, LDL and CHOL levels reduced significantly compared to diabetic rats (negative control). HDL level increased significantly in nondiabetic-treated rats compared to nondiabetic and nontreated rats (positive control). There was no significant elevation ($P > 0.05$) in the liver antioxidant capacity (ORAC) of MO-treated diabetic rats compared to the negative control but significantly increased ($P < 0.05$) in nondiabetic-treated rats when compared to the positive control.

Table 1: Weight parameters and serum glucose level

Parameters	NC	MO	DM	DM+MO
Liver weight (g)	8.50 $\pm$ 0.46	7.48 $\pm$ 0.43 ^{a,c}	9.35 $\pm$ 0.68 ^a	8.49 $\pm$ 0.47 ^c
Relative liver weight (g/100 g)	2.62 $\pm$ 0.10	2.45 $\pm$ 0.10 ^{a,c}	4.30 $\pm$ 0.32 ^a	3.98 $\pm$ 0.10 ^{a,b,c}
Glucose (mmol/L)	7.25 $\pm$ 1.27	5.38 $\pm$ 0.96 ^c	30.96 $\pm$ 2.11 ^a	27.61 $\pm$ 1.77 ^{a,b,c}

Values represent mean $\pm$ SD of 12 animals. ^a $P < 0.05$ values are significant compared with nondiabetic control. ^b $P < 0.05$ values are significant compared to nondiabetic treated group. ^c $P < 0.05$ values are significant compared with diabetic control. NC: Nondiabetic rats (positive control); MO: Nondiabetic-treated rats; DM: Diabetic rats (negative control); DM + MO: Diabetic-treated rats; SD: Standard deviation

Table 2: Hepatic enzyme activities

Parameters (U/L)	NC	MO	DM	DM+MO
AST	195.70 $\pm$ 9.02	171.33 $\pm$ 5.03 ^{a,c}	270.30 $\pm$ 9.29 ^a	240.00 $\pm$ 13.89 ^{a,b,c}
ALT	48.50 $\pm$ 10.19	34.00 $\pm$ 8.08 ^c	123.3 $\pm$ 33.13 ^a	100.00 $\pm$ 24.49 ^{a,b}
ALP	79.71 $\pm$ 10.44	61.29 $\pm$ 8.10 ^c	345.57 $\pm$ 47.12 ^a	190.60 $\pm$ 27.66 ^{a,b,c}

Values represent mean $\pm$ SD of 12 animals. ^a $P < 0.05$ values are significant compared with nondiabetic control. ^b $P < 0.05$ values are significant compared to nondiabetic treated group. ^c $P < 0.05$ values are significant compared with diabetic control. NC: Nondiabetic rats (positive control); MO: Nondiabetic-treated rats; DM: Diabetic rats (negative control); DM + MO: Diabetic-treated rats; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; SD: Standard deviation

Table 3: Lipid profile and antioxidant capacity in the liver of rats

Parameters	NC	MO	DM	DM+MO
LDL (mg/dl)	0.10 $\pm$ 0.03	0.06 $\pm$ 0.02 ^c	0.14 $\pm$ 0.07	0.05 $\pm$ 0.02 ^c
HDL (mg/dl)	0.64 $\pm$ 0.03	0.73 $\pm$ 0.04 ^{a,c}	0.63 $\pm$ 0.06	0.67 $\pm$ 0.07
CHOL (mg/dl)	1.60 $\pm$ 0.14	1.36 $\pm$ 0.28 ^c	1.92 $\pm$ 0.27	1.26 $\pm$ 0.19 ^c
ORAC ($\mu\text{molTE/g}$)	13.48 $\pm$ 0.76	14.87 $\pm$ 0.52 ^c	11.84 $\pm$ 0.95	12.49 $\pm$ 1.12 ^b

Values represent mean $\pm$ SD of 12 animals. ^a $P < 0.05$ values are significant compared with nondiabetic control. ^b $P < 0.05$ values are significant compared to nondiabetic treated group. ^c $P < 0.05$ values are significant compared with diabetic control. NC: Nondiabetic rats (positive control); MO: Nondiabetic-treated rats; DM: Diabetic rats (negative control); DM + MO: Diabetic-treated rats; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; CHOL: Cholesterol; ORAC: Oxygen radical absorbance capacity; SD: Standard deviation

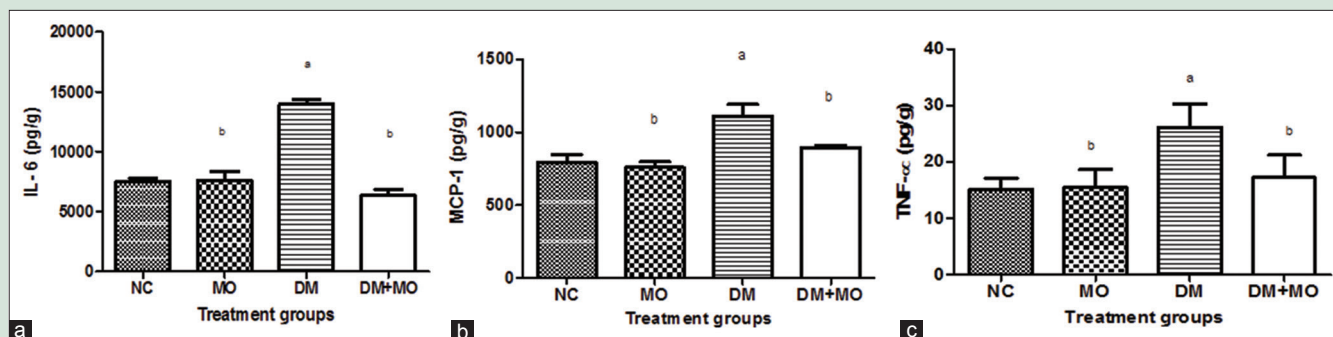


Figure 1: (a-c) Effect of *Moringa oleifera* on hepatic inflammatory biomarkers in diabetic and nondiabetic rats. Each bar represents mean (standard deviation) of 12 rats. ^a $P < 0.05$ values are significant compared with nondiabetic control. ^b $P < 0.05$ values are significant compared with diabetic control. NC: Nondiabetic rats (positive control), MO: Nondiabetic-treated rats; DM: Diabetic rats (negative control), DM + MO: Diabetic-treated rats

Moringa oleifera effect on inflammatory biomarkers in the liver of diabetic and nondiabetic rats

Figure 1a-c illustrates MO protective effect on inflammatory biomarkers in the liver of diabetic and nondiabetic rats. Concentrations of inflammatory cytokines IL-6, TNF- α , and chemokine MCP-1 were elevated in diabetic groups. MO's treatment reduced significantly ($P < 0.05$) inflammatory cytokines in the liver of diabetic rats.

Histopathological effect of *Moringa oleifera* on the hepatic structure of rats

Histopathological liver specimens in nondiabetic-treated rats showed no visible lesions when compared to nondiabetic control [Figure 2a and b]. In diabetic rats, liver pathology revealed severe hepatocyte necrosis (1) at the centrilobular zone with large focus of massive hemorrhage into the liver parenchyma showing undefined boundaries, (2) degenerating nucleus, and (3) when compared to the diabetic-treated rats as shown in Figure 2c. After treating diabetic rats with MO, liver section showed moderate portal congestion and very mild periportal cellular reaction, an improvement [Figure 2d].

DISCUSSION

The liver plays a vital role in the regulation of carbohydrate metabolism. It supplies glucose to other organs that require glucose as energy source and is one of the organs affected by diabetes. Attention has recently been focused on the use of therapeutic agents from plant origin in the treatment and management of diabetes as a result of severe side effects of contemporary antidiabetic drugs.^[31] In this study, diabetes was induced by intraperitoneal injection of STZ which caused hepatotoxicity and damage in the liver of diabetic rats. Medicinal plants including MO have attracted attention for their therapeutic properties and diabetes management. The hepatoprotective, antihyperlipidemic, and anti-inflammatory action of MO as well as its ability to modulate hepatic enzymes and biochemical parameters were evaluated in diabetic-induced rats.

Glucose homeostasis (balance between insulin and glucagon to maintain a stable blood glucose level) is essential for the utilization of glucose by the liver, muscles, and adipose tissues.^[32] Excess glucose concentration causes hyperglycemia and liver damage. Antidiabetic and glucose-lowering effect of MO has been reported.^[33,34] In this study, diabetic-induced condition led to an increased serum glucose level (hyperglycemia). MO treatment significantly reduced glucose level which indicated an improvement in impaired glucose metabolism.

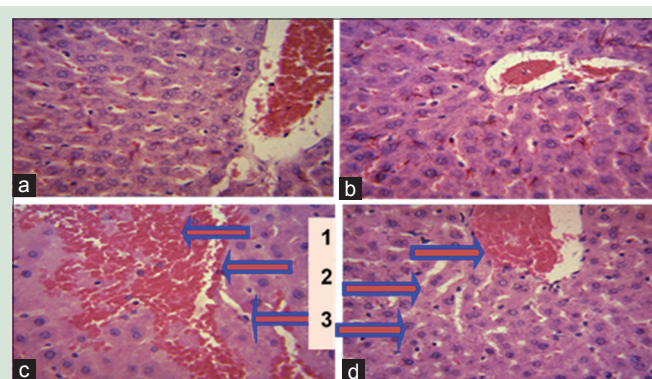


Figure 2: (a-d) Histopathological effect of *Moringa oleifera* on hematoxylin and eosin stained liver sections of nondiabetic and diabetic rats, (H and E, $\times 400$). (a) Nondiabetic rats (positive control), (b) nondiabetic-treated rats, (c) diabetic rats (negative control), and (d) diabetic-treated rats

MO has high glucose-lowering effect and can be used as an antidiabetic component. Our study confirms glucose-lowering effect of MO as previously reported.^[35]

We investigated the functional activities of liver enzymes AST, ALT, and ALP which are markers of hepatic damage. AST is an important biomarker for the diagnosis of myocardial infarction and amino acid metabolism.^[36] We observed a significantly ($P < 0.05$) higher activity of AST in serum of STZ-diabetic-induced rats. Administration of MO reduced the activity of this enzyme.^[37] Result suggests hepatic damage as shown by elevated AST activity.

ALT predominantly found in the liver is a biomarker for hepatocellular injury. Elevated level is seen in diabetic state. Results from the current study showed a significantly higher ALT activity in diabetic groups when compared to nondiabetic-treated rats. The reduction of ALT concentration after MO treatment as seen in this study confirms observation by Fakurazi *et al.*^[29]

Increased activity of ALP is indicative of bile ducts obstruction and a biomarker for hepatic damage. ALP activity in diabetic groups was significantly elevated. Treatment with MO resulted in a significant reduction in ALP activity in diabetic rats compared to diabetic control, confirming previous studies.^[38,39] These enzyme activities (AST, ALT, and ALP) were elevated in nontreated diabetic group but reduced significantly in MO diabetic-treated group. Studies revealed that exposure to STZ

increased serum biomarkers associated with hepatic damage. Protective action of MO significantly reduced hepatic enzymes activities.

The liver plays a pivotal role in lipid metabolism. CHOL is essential in Vitamin D synthesis and hormone metabolism. Normal range is beneficial but when exceeded it becomes harmful to cellular integrity. Excess CHOL forms plaque in artery walls, narrowing it and reducing the rate of blood circulation leading to a condition known as arteriosclerosis.

In STZ-induced diabetic rats, CHOL levels were elevated. However, after treatment with MO, CHOL level was reduced significantly when compared to controls. Furthermore, CHOL level in nondiabetic-treated rats was reduced after MO administration. Our results are in agreement with Chumark *et al.*^[40] who reported hypolipidemic activity of MO leaves extracts, indicating that MO significantly lowered CHOL level. Manohar *et al.*^[41] also reported similar results in which MO caused reduction in serum CHOL level.

Lipoproteins help transfer lipids around the body in the extracellular fluid.^[42] LDL is synthesized in the liver by the action of lipolytic enzymes with increased concentrations leading to CHOL build-up in the arteries.^[43] High level of LDL was observed in diabetic rats when compared to nondiabetic rats. Interestingly, after treatment with MO, LDL levels of diabetic rats and nondiabetic rats reduced significantly when compared to the control. Many other studies have alluded to the fact that MO has hypoglycemic and hypolipidemic effects which are in tandem with findings from this study.^[12,44,45]

HDL mediates the reverse transport of CHOL from the extrahepatic tissues to the liver where the CHOL is converted to bile acid and excreted.^[46] HDL level increased significantly in nondiabetic-treated rats compared to nondiabetic rats (positive control).

Treatment with MO led to an increase in liver antioxidant capacity (ORAC) when compared to control groups. ORAC of MO-treated diabetic liver was restored to near normal values. Similar study by Jaiswal *et al.*^[18] confirms this result.

The concentrations of three cytokines (IL-6, TNF- α) and one chemokine (MCP-1/CCL2) were quantified in the liver homogenate of diabetic and nondiabetic rats in the present study [Figure 1a-c]. Cytokines are messenger molecules of the immune system, mediating cellular movement among lymphocytes, macrophages, dendritic cells, and other inflammatory cells. Inflammation is one of the markers of oxidative stress. Generation of ROS leads to the upregulation of pro-inflammatory cytokines which have been reported in diabetic state in rats.^[47,48] TNF- α activates endothelial cells attached to corresponding proteins and inflammatory responses. TNF- α has also been implicated in diabetic hepatic disease through diverse biological activities including impairment of insulin secretion and induction of apoptosis and necrosis.^[49,50] MO has beneficial effects in various pathological conditions through its antioxidative and anti-inflammatory properties. Concentrations of IL-6, TNF- α , and MCP-1 increased significantly ($P < 0.05$) in STZ-induced rats, and a decrease was observed after MO treatment compared to diabetic control. As a result of MO's potent anti-inflammatory activity, it can be said to show profound influence on inflammatory-associated diseases and resultant symptoms. On the basis of our study, it can be postulated that MO can exert its hepatoprotective effect and anti-inflammatory action against these cytokines and chemokine.

The present study demonstrates STZ-induced hepatotoxicity and that the liver and relative liver weights of diabetic rats were heavier compared to nondiabetic groups. However, after administration of MO to nondiabetic and diabetic rats, a decrease in liver and relative weights was observed. Increase in both weights in diabetic rats may be due to enlargement of tubular cells lining and fatty infiltration of the liver.^[51]

Histopathological sections of the liver are shown in Figure 2a-d. Nondiabetic-treated rats showed no visible lesions when compared to nondiabetic control [Figure 2a and b]. Liver specimens from diabetic rats revealed severe hepatocyte necrosis at the centrilobular zone with large focus of massive hemorrhage into the liver parenchyma, with undefined boundaries when compared to the diabetic-treated rats as shown in Figure 2c. This may have accounted for the increase in the weight of the liver. After treatment of diabetic rats with MO, liver section showed moderate portal congestion and very mild periportal cellular reaction [Figure 2d]. MO treatment restored the normal histology of the liver compared to that of the diabetic-positive control group, and this was corroborated by similar previous study.^[52] Furthermore, histological section showed severe hepatocyte necrosis in the liver of the diabetic rats which were cleared in MO-treated diabetic rats.

The main limitation of our study was identifying the specific metabolic pathway for the action of MO. Further studies to investigate the metabolic action of MO in the liver are recommended.

CONCLUSIONS

MO methanolic leaves extracts showed hepatoprotective, anti-inflammatory, and lipid-lowering effect against hyperglycemia and liver damage induced by STZ in rats. MO may be able to lower the risk of cardiovascular diseases with its ability to lower the levels of CHOL and LDL but increase HDL.

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Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES

1. World Health Organization. Global Report on Diabetes. Geneva: World Health Organization. 2016-. Available from: <http://www.who.int/diabetes/global-report/en/>. [Last accessed on 2016 Dec 12].
2. Selvakumar D, Natarajan P. Hepato-protective activity of *Moringa oleifera* Lam leaves in carbon tetrachloride induced hepato-toxicity in albino rats. *Pharmacogn Mag* 2008;4:97-8.
3. Surya S, Salam AD, Tomy DV, Carla B, Kumar RA, Sunil C. Diabetes mellitus and medicinal plants-a review. *Asian Pac J Trop Dis* 2014;4:337-47.
4. Ghosh S, Bhattacharyya S, Rashid K, Sil PC. Curcumin protects rat liver from streptozotocin-induced diabetic pathophysiology by counteracting reactive oxygen species and inhibiting the activation of p53 and MAPKs mediated stress response pathways. *Toxicol Rep* 2015;2:365-76.
5. Tangvarasittichai S. Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. *World J Diabetes* 2015;6:456-80.
6. Chan JC, Malik V, Jia W, Kadowaki T, Yajnik CS, Yoon KH, *et al.* Diabetes in Asia: Epidemiology, risk factors, and pathophysiology. *JAMA* 2009;301:2129-40.
7. Palleria C, Leporini C, Maida F, Succurro E, De Sarro G, Arturi F, *et al.* Potential effects of current drug therapies on cognitive impairment in patients with type 2 diabetes. *Front Neuroendocrinol* 2016;42:76-92.
8. Mbikay M. Therapeutic potential of *Moringa oleifera* leaves in chronic hyperglycemia and dyslipidemia: A review. *Front Pharmacol* 2012;3:24.
9. Miller YI, Choi SH, Wiesner P, Fang L, Harkewicz R, Hartvigsen K, *et al.* Oxidation-specific epitopes are danger-associated molecular patterns recognized by pattern recognition receptors of innate immunity. *Circ Res* 2011;108:235-48.

10. Forbes JM, Cooper ME. Mechanisms of diabetic complications. *Physiol Rev* 2013;93:137-88.
11. Paliwal R, Sharma V, Pracheta J. A review on horse radish tree (*Moringa oleifera*): A multipurpose tree with high economic and commercial importance. *Asian J Biotechnol* 2011;3:317-28.
12. Bhattacharya A, Naik MR, Agrawal D, Rath K, Kumar S, Mishra SS. Anti-pyretic, anti-inflammatory, and analgesic effects of leaf extract of drumstick tree. *J Young Pharm* 2014;6:1-5.
13. Sultana B, Anwar F. Flavonols (kaempferol, quercetin, myricetin) contents of selected fruits, vegetables and medicinal plants. *Food Chem* 2008;108:879-84.
14. Saini RK, Manoj P, Shetty NP, Srinivasan K, Giridhar P. Dietary iron supplements and *Moringa oleifera* leaves influence the liver hepcidin messenger RNA expression and biochemical indices of iron status in rats. *Nutr Res* 2014;34:630-8.
15. Rady MM, Mohamed GF. Modulation of salt stress effects on the growth, physico-chemical attributes and yields of *Phaseolus vulgaris* L. plants by the combined application of salicylic acid and *Moringa oleifera* leaf extract. *Sci Hortic (Amsterdam)* 2015;193:105-13.
16. Luqman S, Srivastava S, Kumar R, Maurya AK, Chanda D. Experimental assessment of *Moringa oleifera* leaf and fruit for its antistress, antioxidant, and scavenging potential using *in vitro* and *in vivo* assays. *Evid Based Complement Alternat Med* 2012;2012:519084.
17. Gupta R, Mathur M, Bajaj VK, Katariya P, Yadav S, Kamal R, *et al.* Evaluation of antidiabetic and antioxidant activity of *Moringa oleifera* in experimental diabetes. *J Diabetes* 2012;4:164-71.
18. Jaiswal D, Rai PK, Mehta S, Chatterji S, Shukla S, Rai DK, *et al.* Role of *Moringa oleifera* in regulation of diabetes-induced oxidative stress. *Asian Pac J Trop Med* 2013;6:426-32.
19. Mishra G, Singh P, Verma R, Kumar S, Srivastav S. Traditional uses, phytochemistry and pharmacological properties of *Moringa oleifera* plant: An overview. *Der Pharm Lett* 2011;3:141-64.
20. Fernandes EE, Pulwale AV, Patil GA, Moghe AS. Probing regenerative potential of *Moringa oleifera* aqueous extracts using *in vitro* cellular assays. *Pharmacognosy Res* 2016;8:231-7.
21. Waterman C, Cheng DM, Rojas-Silva P, Poulev A, Dreifus J, Lila MA, *et al.* Stable, water extractable isothiocyanates from *Moringa oleifera* leaves attenuate inflammation *in vitro*. *Phytochemistry* 2014;103:114-22.
22. Stohs SJ, Hartman MJ. Review of the safety and efficacy of *Moringa oleifera*. *Phytother Res* 2015;29:796-804.
23. Rathi BS, Bodhankar SL, Baheti AM. Evaluation of aqueous leaves extract of *Moringa oleifera* Linn for wound healing in albino rats. *Indian J Exp Biol* 2006;44:898-901.
24. Oluduro AO. Evaluation of antimicrobial properties and nutritional potentials of *Moringa oleifera*. *Malays J Microbiol* 2012;8:59-67.
25. Karthivashan G, Arulselvan P, Tan SW, Fakurazi S. The molecular mechanism underlying the hepatoprotective potential of *Moringa oleifera* leaves extract against acetaminophen induced hepatotoxicity in mice. *J Funct Foods* 2015;17:115-26.
26. Sabale V, Patel V, Paranjape A, Arya C, Sakarkar SN, Sabale PM. Plant review *Moringa oleifera* (Drumstick): An overview. *Pharmacogn Rev* 2008;2:7-13.
27. Sreelatha S, Jeyachitra A, Padma PR. Antiproliferation and induction of apoptosis by *Moringa oleifera* leaf extract on human cancer cells. *Food Chem Toxicol* 2011;49:1270-5.
28. Chattopadhyay S, Maiti S, Maji G, Deb B, Pan B, Ghosh D. Protective role of *Moringa oleifera* (Sajina) seed on arsenic-induced hepatocellular degeneration in female albino rats. *Biol Trace Elem Res* 2011;142:200-12.
29. Fakurazi S, Hairuszah I, Nanthini U. *Moringa oleifera* Lam prevents acetaminophen induced liver injury through restoration of glutathione level. *Food Chem Toxicol* 2008;46:2611-5.
30. Ou B, Hampsch-Woodill M, Prior RL. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *J Agric Food Chem* 2001;49:4619-26.
31. Wadkar KA, Magdum CS, Patil SS, Naikwade NS. Antidiabetic potential and Indian medicinal plants. *J Herb Med Toxicol* 2008;2:45-50.
32. Gylfe E, Gilon P. Glucose regulation of glucagon secretion. *Diabetes Res Clin Pract* 2014;103:1-10.
33. Toma A, Makonnen E, Debella A, Tesfaye B. Antihyperglycemic effect on chronic administration of butanol fraction of ethanol extract of *Moringa stenopetala* leaves in alloxan induced diabetic mice. *Asian Pac J Trop Biomed* 2012;2:606-10.
34. Luangpiom A, Kourjampa W, Junaimaung T, Taxonomic A, Kaen K. Anti-hyperglycemic properties of *Moringa oleifera* Lam. aqueous leaf extract in normal and mildly diabetic mice. *Br J Pharmacol Toxicol* 2013;4:106-9.
35. Sánchez-Muñoz MA, Valdez-Solana MA, García-Arenas G, Avitia-Domínguez C, Téllez-Valencia A, Pardo-Vázquez JP, *et al.* *Moringa oleifera* Lam improves hyperglycemia induced mitochondrial alterations in liver Wistar rats. *Free Radic Biol Med* 2014;76:S117.
36. Aldous SJ. Cardiac biomarkers in acute myocardial infarction. *Int J Cardiol* 2013;164:282-94.
37. Manna P, Das J, Ghosh J, Sil PC. Contribution of type 1 diabetes to rat liver dysfunction and cellular damage via activation of NOS, PARP, I κ B α /NF κ B, MAPKs, and mitochondria-dependent pathways: Prophylactic role of arjunolic acid. *Free Radic Biol Med* 2010;48:1465-84.
38. Efiog EE, Igile GO, Mgbeje BI, Otu EA, Ebong PE. Hepatoprotective and anti-diabetic effect of combined extracts of *Moringa oleifera* and *Vernonia amygdalina* in streptozotocin-induced diabetic albino Wistar rats. *Glob J Hematol Endocrinol* 2013;1:5-10.
39. Zvinorova PI, Lekhanya L, Erlwanger K, Chivandi E. Dietary effects of *Moringa oleifera* leaf powder on growth, gastrointestinal morphometry and blood and liver metabolites in Sprague Dawley rats. *J Anim Physiol Anim Nutr (Berl)* 2015;99:21-8.
40. Chumark P, Khunawat P, Sanvarinda Y, Phornchirasilp S, Morales NP, Phivthong-Ngam L, *et al.* The *in vitro* and *ex vivo* antioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of *Moringa oleifera* Lam. leaves. *J Ethnopharmacol* 2008;116:439-46.
41. Manohar VS, Jayasree T, Kishore KK, Rupa LM, Dixit R. Evaluation of hypoglycemic and antihyperglycemic effect of freshly prepared aqueous extract of *Moringa oleifera* leaves in normal and diabetic rabbits. *J Chem Pharm Res* 2012;4:249-53.
42. Baynes J, Dominiczak MH. Medical Biochemistry. 4th ed. China: Saunderson Elsevier Health Sciences; 2014. p. 214.
43. Ganji SH, Kamanna VS, Kashyap ML. Niacin and cholesterol: Role in cardiovascular disease (review). *J Nutr Biochem* 2003;14:298-305.
44. Ezuruike UF, Prieto JM. The use of plants in the traditional management of diabetes in Nigeria: Pharmacological and toxicological considerations. *J Ethnopharmacol* 2014;155:857-924.
45. Sugunabai J, Jayaraj M, Karpagam T, Varalakshmi B. Antidiabetic efficiency of *Moringa oleifera* and *Solanum nigrum*. *Int J Pharm Pharm Sci* 2014;6 Suppl 1:40-2.
46. Khovidhunkit W, Kim MS, Memon RA, Shigenaga JK, Moser AH, Feingold KR, *et al.* Effects of infection and inflammation on lipid and lipoprotein metabolism: Mechanisms and consequences to the host. *J Lipid Res* 2004;45:1169-96.
47. Azam S, Huda AF, Shams K, Ansari P, Mohamed MK, Hasan MM, *et al.* Anti-inflammatory and anti-oxidant study of ethanolic extract of *Mimosa pudica*. *J Young Pharm* 2015;7:234-41.
48. Navarro-González JF, Mora-Fernández C. The role of inflammatory cytokines in diabetic nephropathy. *J Am Soc Nephrol* 2008;19:433-42.
49. Kumar B, Gupta SK, Srinivasan BP, Nag TC, Srivastava S, Saxena R, *et al.* Hesperetin rescues retinal oxidative stress, neuroinflammation and apoptosis in diabetic rats. *Microvasc Res* 2013;87:65-74.
50. Ayepola OR, Brooks NL, Oguntibeju OO. Kolaviron improved resistance to oxidative stress and inflammation in the blood (erythrocyte, serum, and plasma) of streptozotocin-induced diabetic rats. *ScientificWorldJournal* 2014;2014:921080.
51. Assy N, Kaita K, Mymin D, Levy C, Rosser B, Minuk G. Fatty infiltration of liver in hyperlipidemic patients. *Dig Dis Sci* 2000;45:1929-34.
52. Adedapo AA, Mogbojuri OM, Emikpe BO. Safety evaluations of the aqueous extract of the leaves of *Moringa oleifera* in rats. *J Med Plants Res* 2009;3:586-91.