

Original article

Antidiabetic and Antioxidant Activities of Ethanolic Extract of Dried Flowers of *Moringa oleifera* in Streptozotocin-induced Diabetic Rats

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SUMMARY

This study was undertaken to determine the antidiabetic and antioxidant effects of oral administration of ethanolic extract of *Moringa oleifera* flower on streptozotocin-induced diabetic rats at 100, 200, and 300 mg/kg b.w.

Thirty (30) male experimental albino rats were grouped randomly into six groups: groups A, B, and F are the control, diabetic control and reference drug groups, while C-E received 100, 200, and 300mg/kg b.w of the extract, respectively. Blood samples and organs were collected to assay for blood glucose level and antioxidant enzymes.

Levels of blood glucose, serum lipids and lipid peroxidation as well as aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) activities were significantly reduced ($p < 0.05$) in STZ-induced diabetic rats orally administered ethanolic extract of *M. oleifera* flower. However, the body weight; catalase and superoxide dismutase activities were significantly increased ($p < 0.05$) when compared with the controls.

M. oleifera flower ethanolic extract administered orally therefore exhibited improved lipid metabolism, glucose-lowering potential and is hence beneficial in preventing diabetic complications as a result of lipid peroxidation and oxidative systems in streptozotocin-induced diabetic rats. It could thus be employed therapeutically in managing diabetes mellitus.

Key words: ethanolic extract, antidiabetic, antioxidant enzymes, *Moringa oleifera*, streptozotocin

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INTRODUCTION

Diabetes mellitus, a complex multisystem and metabolic disorder is characterized by relative or absolute defects in insulin secretion and disturbances in carbohydrate, protein and lipid metabolism (1). The world population with diabetes is rising yearly and by the year 2030, it is expected to hit 439 million (2). Diabetes mellitus has been classified into two major types: type 1 is also referred to as insulin-dependent diabetes mellitus (IDDM) and type 2 is referred to as non-insulin-dependent diabetes mellitus (NIDDM). Type I diabetes, an autoimmune disease, normally presents with local inflammatory reactions in and around islets, which results in the selective destruction of β -cells that secrete insulin. Peripheral insulin resistance and impaired insulin secretion are seen in type 2 diabetes (3).

Diabetes mellitus has been shown to be associated with oxidative stress, leading to an increased production of reactive oxygen species (ROS) such as hydroxyl radical ($\text{OH}\cdot$), hydrogen peroxide (H_2O_2), and superoxide anion radical ($\text{O}_2\cdot^-$) or diminished availability of endogenous antioxidants. In addition to oxygen free radical generation, non-enzymatic protein glycosylation, formation of peroxides and auto-oxidation of glucose have contributed to the involvement of oxidative stress in the pathogenesis of diabetes mellitus (4, 5). Lipid peroxidation (LPO), a key marker of oxidative stress induced by free radicals results in the oxidative worsening of poly-unsaturated fatty acids that consequently results in massive membrane damage and dysfunction (6).

The various endogenous antioxidants in cells help to prevent or mend the damage caused by free radicals, and also serve to regulate signaling pathways that are redox-sensitive. Superoxide radicals are inactivated through conversion to hydrogen peroxide and molecular oxygen by the superoxide dismutase, and hydrogen peroxide is itself converted to water by catalase and peroxidases causing membrane destabilization, damages to DNA and proteins, and oxidation of low-density lipoprotein (LDL); ROS are implicated in mutagenesis, cellular aging, carcinogenesis, and coronary heart disease (6). Moreover, increased LPO in diabetics can be due to an upsurge in oxidative stress in the cell due to a weakened anti-oxidant protective systems (7).

Plants are known to be rich in natural antioxidants such as carotenoids, tocopherols, flavonoids,

vitamin C and several other phenolic compounds (8). Ethnobotanical information has it that plants numbering more than 800 are in use traditionally for treating diabetes owing to their potency, fewer side effects, and cheapness (9). Through their normoglycemic effect, ability to improve lipid metabolism, antioxidant defense system and capillary function, botanical products can enhance glucose metabolism and the overall condition of individuals with diabetes (10).

Moringa oleifera, a tropical highly treasured plant, is found in several countries of the tropical and subtropical regions. It is a perennial softwood tree with low quality timber belonging to the single genus family Moringaceae. It is a small, rapidly developing ornamental tree which is believed to be of Indian origin. Several parts of the tree including its root, pods, bark, and leaves are of importance in traditional medicine for treating human diseases, whereby the leaves are enriched in A and C vitamins. The therapeutic effects of *M. oleifera* include: antibiotic (11), anticancer (12), antiulcerogenic (13), analgesic (14), antiurolithiatic (15) and larvicidal (16). Parts of the plant including the roots, seed, leaves, bark, fruit, immature pods and flowers act as cardiac and circulatory stimulants, possess antitumor (17), antipyretic, anti-inflammatory, antiepileptic and antiulcer properties (18). It is also known to be antispasmodic (19), diuretic (20), anti-hypertensive (21), antihyperlipidaemic (22), hepatoprotective, antioxidant, antidiabetic, (23), antifungal and antibacterial (24). Highly appreciable levels of four natural antioxidants of dietary origin: vitamins A, C and E and phenolics are found in *M. oleifera*. Studies have estimated 46 antioxidants in *Moringa* which are important to cells in combating free radicals (25-27). This study was therefore aimed at investigating the effect of ethanolic extract of dried flowers of *M. oleifera* in streptozotocin-induced diabetes in rats.

MATERIALS AND METHODS

Chemicals used

Streptozotocin (STZ) was a product of Sigma Chemical Company (USA). The assay kits for cholesterol, LDL, HDL, triglyceride, ALT, AST were products of Randox Laboratory Ltd, Co-Atrim (UK). Glibenclamide was a product of TuyilPharmaceuticals Limited, Nigeria. All other reagents used were of analytical grades.

Plant material

The freshly harvested flower of *M. oleifera* was a gift from the University of Ilorin Moringa Plantation in Ilorin, Kwara State, Nigeria, and was identified at the Herbarium Unit, Plant Biology Department, University of Ilorin, Nigeria, where herbarium specimen was deposited with Voucher Specimen Number: U.I.H 002/1008.

Phytochemical screening

A preliminary phytochemical screening of the flowers of *M. oleifera* was conducted using a standard method of analysis as described by Sofowora (28).

Extract preparation

Fresh flowers of *M. oleifera* were air-dried at 40°C using Uniscope SM9053 laboratory Oven, Surgifriend Medicals, England. The dried flowers (161 g) were then pulverized using an electric blender (Crown Star Blender CS-242B, Trident (H.K) Ltd, China) after which the resulting powder was extracted in 95% ethanol for 72 hours at 37°C with continuous shaking. This extract was then filtered using Whatman No 1 filter paper and the filtrate obtained was concentrated in a water bath at 40°C. The extract was stored in a refrigerator at -4°C. Calculated amounts were reconstituted in distilled water to give the required doses of 100, 200, and 300 mg/kg body weights used for oral administration.

Experimental animals

Thirty male experimental albino rats (*Rattus norvegicus*) of weight 151±5.00 g obtained from the Animal Holding Unit of Biochemistry Department, University of Ilorin, Ilorin, Nigeria were acclimatized for 2 weeks before the experiment. The animals were housed in clean wooden cages, fed standard diets and were allowed unhindered access to water with regulated 12 h light/dark cycle.

Induction of diabetes

Diabetes was induced via intra-peritoneal injection of 45mg/kg body weight of freshly prepared streptozotocin (STZ). STZ was prepared in citrate buffer (0.1M, pH 4.5). Prior to diabetes induction, the

animals were fasted overnight and the overnight fasted-rats were confirmed diabetic by measuring blood glucose concentration 96 h after injection with STZ using Accu-Chek glucometer. Rats belonging to the non-diabetic control group were injected with just citrate buffer. Blood sample was obtained from tail puncture of the rats for the fasting blood glucose determination, and animals with blood glucose values greater than 200 mg/dL were considered diabetic; blood glucose level was checked every day for 7 days. Treatment was commenced on the fifth day post-STZ injection and was considered the first day of treatment. For a period of 21 days, the treatment persisted.

Animal grouping

Group A: Control rats received 1 ml of distilled water once daily.

Group B: Diabetic control rats received 1 ml of distilled water once daily.

Group C: STZ-induced diabetic rats administered 100 mg/kg body weight ethanolic extract of *M. oleifera* flower once daily.

Group D: STZ-induced diabetic rats treated with 200 mg/kg body weight ethanolic extract of *M. oleifera* flower once daily.

Group E: STZ-induced diabetic rats treated with 300 mg/kg body weight ethanolic extract of *M. oleifera* flower once daily.

Group F: STZ-induced diabetic rats treated with standard antidiabetic drug (glibenclamide 5 mg/kg body weight) once daily.

Collection of blood samples and serum preparation

After the 21-day treatment, the animals were anaesthetized with ethyl ether and sacrificed by simple incision of the jugular vein, the blood samples were collected into clean, dry, sample bottles for serum analyses. Blood samples were left undisturbed to clot at room temperature for 30 minutes and then centrifuged at 1000 rpm for a period of 15 minutes using Uniscope Laboratory centrifuge (Model SM800B, Surgifriend Medicals, Essex, England). After centrifugation, the supernatants (sera) were aspirated using a Pasteur's pipette. The sera, thus obtained, were appropriately labelled and stored in a freezer at -5°C until required for further analysis.

Preparation of tissue homogenate

Tissues (liver, kidney, and pancreas) were isolated from the dissected rats. The isolated tissues were cleansed with blotting paper to remove blood stains, weighed and stored immediately in ice cold 0.25M sucrose solution. Specific weight of the liver, pair of kidney, and pancreas were then subjected to homogenization using mortar and pestle in ice-cold 0.25M sucrose solution (1:5w/v). At a speed of 4000 rpm, the homogenates were centrifuged for 10 minutes and the supernatants were siphoned into appropriately labeled sample bottles and stored in the freezer (-20°C) until needed for further analysis.

Assay of biochemical parameters

Serum total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL-C), high density lipoprotein (HDL-C), alanine transaminase (ALT) and aspartate aminotransferase (AST) were determined using the standard kit of Randox laboratories, Co-Atrim, UK. Malondialdehyde, catalase and superoxide dismutase were estimated using the methods described by Jiang et al. (29), Aebi (30) and Misra and Fridovich (31), respectively. Alkaline phosphatase activity was estimated using the method described by Wright et al. (32). All spectrophotometric measurements were carried out in a Camspec UV-Visible spectrophotometer. The determinations were replicated 5 times (n = 5).

STATISTICAL ANALYSIS

Results were presented as mean of replicates \pm SD. Means were analyzed using a one-way analysis of variance (ANOVA), followed by the Duncan Multiple Range Test to determine significant differences in all the parameters. Differences with values of $p < 0.05$ were considered statistically significant (33).

RESULTS

Phytochemical screening

Table 1 shows the result of preliminary phytochemical screening of *M. oleifera* flowers extract. Flavanoids, alkaloids, tannins, phenolics, terpenoids, cardiac glycoside, cardenolides and dienolides were present.

Table 1: Phytochemical constituents of *Moringa oleifera* flower

Phytochemical	Inference(+/-)
Anthraquinones	-
Tannins	+
Phenolics	+
Saponins	-
Terpenoids	+
Alkaloids	+
Steroids	-
Cardiac glycoside	+
Flavonoids	+
Triterpenes	-
Cadenolides and Dienolides	+

Key: + Presence of constituents, - Absence of constituents

Blood glucose

Table 2 shows the effect of ethanolic extract of *M. oleifera* on fasting blood glucose levels of rats induced with diabetes via STZ injection. Blood glucose level was significantly elevated ($p < 0.05$) after induction of diabetes in STZ-induced diabetic rats relative to the control group. However, a significant decrease ($p < 0.05$) was noted at 100, 200, 300 mg/kg of ethanolic extract of *M. oleifera* flower which produced more than 70% reduction in glucose level within 2 weeks of administration.

Body weight

Presented in Table 3 is the effect of *M. oleifera* ethanolic extract on weight gain of rats induced with diabetes via STZ injection. A significant reduction ($p < 0.05$) in body weight was noticed in the diabetic rats. STZ diabetic rats lost 35.6 g of body weight by the 21st day post-induction of diabetes, while non-diabetic animals gained 79.20 g during this period. Glibenclamide-treated diabetic rats also gained weight, but this weight gain (66 g) was less than that of the control animals. Diabetic rats administered ethanolic extract of *M. oleifera* flower and glibenclamide showed significantly increased ($p < 0.05$) body weight gain to near-control level.

Serum lipid profile

Table 4 shows the effects of administration of ethanolic extract of *M. oleifera* on serum lipid profile of

Table 2: Effects of ethanolic extract of *M. oleifera* on fasting blood glucose level of STZ- induced diabetic rats

Group	Blood glucose (mg/dL)			
	After Induction	Week 1	Week 2	Week 3
A	76.20 ± 6.87 ^a	77.67 ± 5.86 ^a	73.00 ± 3.72 ^a	83.80 ± 3.83 ^a
B	269.67 ± 18.04 ^b	290.20 ± 25.40 ^c	297.67 ± 63.01 ^b	326.40 ± 28.9 ^b
C	331.00 ± 18.00 ^b	179.00 ± 11.52 ^b	83.33 ± 5.08 ^a	85.00 ± 8.10 ^a
D	287.20 ± 19.19 ^b	170.67 ± 0.58 ^b	78.00 ± 4.00 ^a	71.00 ± 6.20 ^a
E	336.40 ± 15.90 ^b	162.00 ± 12.00 ^b	82.33 ± 2.83 ^a	70.80 ± 7.16 ^a
F	266.20 ± 21.00 ^b	158.00 ± 10.51 ^b	78.33 ± 1.45 ^a	77.60 ± 7.60 ^a

Values are means (n=5) ± SD. (Values with different superscript are significantly different at p<0.05).

Group A: Control; Group B: Diabetic Control; Group C: Diabetic + (100 mg/kg body weight); Group D: Diabetic + (200 mg/kg body weight); Group E: Diabetic + (300 mg/kg body weight); Group F: Diabetic + (5 mg/kg body weight of glibenclamide).

STZ-induced diabetic rats. The values of TG, HDL-C, LDL-C, TC and atherogenic index of STZ-induced diabetic rat significantly increased ($p < 0.05$) while groups treated with ethanolic extract of *M. oleifera* flower restored their values to near-control levels. Serum lipid profile of STZ-induced diabetic rats treated with ethanolic extract of *M. oleifera* flower was not significantly different ($p > 0.05$) from the control except group E which had a significantly elevated ($p < 0.05$) serum lipid profile when compared with control.

Antioxidant parameters

Table 5 presents the effect of oral administration of ethanolic extract of *M. oleifera* flower on liver, kidney and pancreas malondi-aldehyde concentration in the rats induced with diabetes via STZ injection. There was a significant decrease ($p < 0.05$) in liver, kidney and pancreas malondialdehyde concentration of all STZ-induced diabetic rats treated with ethanolic extract of *M. oleifera* flower.

Table 6 shows the effects of oral administration of ethanolic extract of *M. oleifera* flower on liver, kidney and pancreas SOD activity of rats induced with diabetes using STZ. A significant decrease ($p < 0.05$) in the liver, pancreas and kidney SOD activities of all STZ-induced diabetic groups relative to the control was observed. However, there was a significant elevation ($p < 0.05$) in the liver, kidney and pancreas SOD activity of STZ-induced diabetic rats treated with ethanolic extract of *M. oleifera* flower.

Table 3: Effects of ethanolic extract of *M. oleifera* on the body weight of STZ-induced diabetic rats

Group	Changes in body weight (g)
A	79.20 ± 3.83 ^a
B	35.6 ± 4.62 ^b
C	56.4 ± 3.08 ^a
D	60.4 ± 2.06 ^a
E	53.8 ± 3.27 ^a
F	66.0 ± 3.8 ^a

Values are means (n=5) ± SD. (Values with different superscript are significantly different at p<0.05).

Group A: Control; Group B: Diabetic Control; Group C: Diabetic + (100 mg/kg body weight); Group D: Diabetic + (200 mg/kg body weight); Group E: Diabetic + (300 mg/kg body weight); Group F: Diabetic + (5 mg/kg body weight of glibenclamide).

Table 7 presents the effects of the oral administration of ethanolic extract of *M. oleifera* flower on liver, kidney and pancreas catalase (CAT) activity of STZ-induced diabetic rats. There was a significant decrease ($p < 0.05$) in catalase activity in the liver, kidney and pancreas of all STZ-induced diabetic groups with respect to the control, while there was a significant increase ($p < 0.05$) in catalase activity of the liver, pancreas and the kidney in all STZ-induced diabetic rats administered ethanolic extract of *M. oleifera* flower.

Table 4: Effects of ethanolic extract of *M. oleifera* on serum lipid profile of STZ-induced diabetic rats

Group	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	TG (mg/dL)	Atherogenic Index (LDL/HDL)
A	145.6 ± 3.4 ^a	45.8 ± 1.8 ^a	74.8 ± 4.9 ^a	124.94 ± 4.9 ^a	1.64 ± 0.15 ^a
B	287.8 ± 20.6 ^b	54.6 ± 5.0 ^c	192.7 ± 12.4 ^b	202.9 ± 18.1 ^b	3.53 ± 0.20 ^b
C	161.4 ± 15.3 ^a	46.5 ± 2.8 ^{ab}	79.5 ± 5.4 ^a	142.8 ± 6.0 ^a	1.86 ± 0.14 ^a
D	152.8 ± 5 ^a	46.5 ± 0.5 ^{ab}	79.5 ± 3.0 ^a	133.8 ± 13.1 ^a	1.71 ± 0.09 ^a
E	153.1 ± 7.7 ^a	49.7 ± 2.9 ^b	76.3 ± 6.6 ^a	134.5 ± 10.1 ^a	1.54 ± 0.10 ^a
F	154.3 ± 12.0 ^a	45.3 ± 0.5 ^a	84.2 ± 6.6 ^a	123.8 ± 11.8 ^a	1.86 ± 0.15 ^a

Values are means (n=5) ± SD. (Values with different superscript are significantly different at p<0.05).

Group A: Control; Group B: Diabetic Control; Group C: Diabetic + (100 mg/kg body weight); Group D: Diabetic + (200 mg/kg body weight); Group E: Diabetic + (300 mg/kg body weight); Group F: Diabetic + (5 mg/kg body weight of glibenclamide).

Table 5: Effect of ethanolic extract of *M. oleifera* flower on malondialdehyde concentration in the liver, kidney and pancreas of STZ-induced diabetic rats

Group	MDA (nmol/mg × 10 ⁻⁵)		
	Liver	Kidney	Pancreas
A	0.89 ± 0.30 ^a	0.53 ± 0.05 ^a	0.65 ± 0.02 ^a
B	6.32 ± 0.51 ^b	1.18 ± 0.04 ^c	1.50 ± 0.14 ^c
C	2.71 ± 0.21 ^a	0.39 ± 0.03 ^{ab}	1.07 ± 0.16 ^{ac}
D	1.74 ± 0.16 ^a	0.59 ± 0.05 ^a	0.83 ± 0.04 ^a
E	2.36 ± 0.2 ^a	0.74 ± 0.04 ^a	0.24 ± 0.01 ^b
F	1.16 ± 0.08 ^a	0.21 ± 0.02 ^b	0.38 ± 0.03 ^b

Values are means (n=5) ± SD. (Values with different superscript are significantly different at p<0.05).

Group A: Control; Group B: Diabetic Control; Group C: Diabetic + (100 mg/kg body weight); Group D: Diabetic + (200 mg/kg body weight); Group E: Diabetic + (300 mg/kg body weight); Group F: Diabetic + (5 mg/kg body weight of glibenclamide).

Table 6: Effect of administration of ethanolic extract of *M. oleifera* flower on the activity of superoxide dismutase of STZ-induced diabetic rats

Group	SOD (U/mg protein)		
	Liver	Kidney	Pancreas
A	1.40 ± 0.09 ^a	24.43 ± 2.25 ^a	17.34 ± 0.75 ^a
B	0.93 ± 0.02 ^c	5.69 ± 0.37 ^d	7.13 ± 0.58 ^c
C	1.13 ± 0.07 ^b	17.64 ± 1.60 ^b	8.20 ± 0.82 ^{bc}
D	1.17 ± 0.05 ^b	18.55 ± 1.56 ^b	12.11 ± 1.02 ^b
E	1.13 ± 0.04 ^b	18.68 ± 1.61 ^b	10.07 ± 0.63 ^b
F	1.20 ± 0.05 ^b	11.00 ± 1.05 ^c	13.14 ± 0.84 ^{ab}

Values are means (n=5) ± SD. (Values with different superscript are significantly different at p<0.05).

Group A: Control; Group B: Diabetic Control; Group C: Diabetic + (100 mg/kg body weight); Group D: Diabetic + (200 mg/kg body weight); Group E: Diabetic + (300 mg/kg body weight); Group F: Diabetic + (5 mg/kg body weight of glibenclamide).

Table 7: Effect of administration of ethanolic extract of *M. oleifera* flower on the activity of catalase in the liver, kidney and pancreas of STZ-induced diabetic rats

Catalase (U/mg)			
Group	Liver	Pancreas	Kidney
A	7.52 ± 3.70 ^a	8.56 ± 3.44 ^{ab}	5.15 ± 0.15 ^a
B	1.65 ± 0.41 ^c	3.13 ± 0.59 ^c	1.92 ± 0.04 ^b
C	4.69 ± 1.15 ^b	6.49 ± 1.77 ^b	2.89 ± 0.53 ^{ab}
D	3.75 ± 1.94 ^b	6.91 ± 2.61 ^b	2.32 ± 0.36 ^{ab}
E	4.35 ± 1.63 ^b	5.38 ± 1.62 ^{bc}	2.97 ± 0.15 ^{ab}
F	3.89 ± 0.95 ^b	9.18 ± 1.39 ^a	3.17 ± 0.005 ^{ab}

Values are means (n=5) ± SD. (Values with different superscript are significantly different at p<0.05).

Group A: Control; Group B: Diabetic Control; Group C: Diabetic + (100 mg/kg body weight); Group D: Diabetic + (200 mg/kg body weight); Group E: Diabetic + (300 mg/kg body weight); Group F: Diabetic + (5 mg/kg body weight of glibenclamide).

Serum enzymes

Table 8 presents the effects of oral administration of ethanolic extract of *M. oleifera* flower on alanine aminotransferase (ALT) activity in serum and liver of rats induced with diabetes using STZ. Serum alanine aminotransferase activity of all STZ-induced diabetic groups was not significantly different (p > 0.05) from that of the control. There was also no significant difference (p > 0.05) in the liver alanine aminotransferase activity of all STZ-induced diabetic groups except group B which had a significant increase (p < 0.05) with respect to the control.

Table 8: Effect of administration of ethanolic extract of *M. oleifera* flower on alanine aminotransferase activity of STZ-induced diabetic rats

ALT (IU/L)		
Group	Serum	Liver
A	0.025±0.001 ^{ab}	0.427±0.03 ^{ab}
B	0.027±0.001 ^b	2.64±0.05 ^c
C	0.023±0.001 ^{ab}	0.12±0.07 ^a
D	0.022±0.001 ^a	0.15±0.01 ^a
E	0.025±0.001 ^{ab}	0.91±0.03 ^b
F	0.023±0.002 ^{ab}	0.13±0.010 ^a

Values are means (n=5) ± SD. (Values with different superscript are significantly different at p<0.05).

Group A: Control; Group B: Diabetic Control; Group C: Diabetic + (100 mg/kg body weight); Group D: Diabetic + (200 mg/kg body weight); Group E: Diabetic + (300 mg/kg body weight); Group F: Diabetic + (5 mg/kg body weight of glibenclamide).

Table 9: Effect of administration of ethanolic extract of *M. oleifera* flower on aspartate aminotransferase activity of STZ-induced diabetic rats

AST (IU/L)		
Group	Serum	Liver
A	0.035±0.001 ^{ab}	0.58±0.02 ^a
B	0.0375±0.002 ^b	3.54±0.15 ^b
C	0.0314±0.003 ^{ab}	0.16±0.11 ^a
D	0.03±0.001 ^a	0.21±0.01 ^a
E	0.034±0.002 ^{ab}	0.13±0.04 ^a
F	0.032±0.002 ^{ab}	0.18±0.01 ^a

Values are means (n=5) ± SD. (Values with different superscript are significantly different at p<0.05).

Group A: Control; Group B: Diabetic Control; Group C: Diabetic + (100 mg/kg body weight); Group D: Diabetic + (200 mg/kg body weight); Group E: Diabetic + (300 mg/kg body weight); Group F: Diabetic + (5 mg/kg body weight of glibenclamide).

The effects of oral administration of ethanolic extract of *M. oleifera* flower on aspartate amino-transferase activity in serum and liver of rats induced with diabetes via STZ injection are shown in Table 9. Serum AST activity of all STZ-induced diabetic groups was not significantly different (p > 0.05) from that of the control. Liver AST activity of all STZ-induced diabetic groups was not significantly different (p>0.05) from that of the control except group B which had a significant elevation (p<0.05). However, there was a significant decrease (p<0.05) in the liver and serum aspartate amino-transferase activity of rats treated with ethanolic extract of *M. oleifera* flower.

Table 10: Effect of administration of ethanolic extract of *M. oleifera* flower on alkaline phosphatase activity of STZ-induced diabetic rats

ALP		
Group	Serum	Liver
A	23.76 ± 2.90 ^{ab}	143.95 ± 11.11 ^{ab}
B	28.47 ± 2.32 ^c	251.03 ± 18.00 ^c
C	25.52 ± 2.02 ^b	136.25 ± 10.99 ^{ab}
D	26.46 ± 1.78 ^{ab}	179.53 ± 15.41 ^b
E	21.82 ± 2.06 ^a	97.06 ± 7.37 ^a
F	18.61 ± 1.85 ^a	136.91 ± 10.10 ^{ab}

Values are means (n=5) ± SD. (Values with different superscript are significantly different at p<0.05).

Group A: Control; Group B: Diabetic Control; Group C: Diabetic + (100 mg/kg body weight); Group D: Diabetic + (200 mg/kg body weight); Group E: Diabetic + (300 mg/kg body weight); Group F: Diabetic + (5 mg/kg body weight of glibenclamide).

Table 10 displays the effects of oral administration of ethanolic extract of *M. oleifera* flower on alkaline phosphatase activity of STZ-induced diabetic rats. There was a significant decrease (p < 0.05) in the serum and liver alkaline phosphatase of the STZ-induced diabetic groups when compared to the control. Serum and liver alkaline phosphatase activity of the untreated STZ-induced diabetic group was significantly increased (p < 0.05), while ALP activity in the groups treated with ethanolic extract of *M. oleifera* flower was significantly decreased.

DISCUSSION

Phytochemical constituents

Results of the phytochemical screening shows the presence of alkaloids and flavonoids which have been implicated as possible bioactive agents leading to toxicological and antidiabetic effects (34). Basically, flavonoids are best known for their antidiabetic properties which agree with the report of Soliman (35) that *M. oleifera* flowers possess antioxidant and antidiabetic activity owing to the presence of flavonoids. Although the hypoglycemic effect of terpenoids appear to involve stimulation of pancreatic β -cells and subsequent secretion of preformed insulin (36), one or more other chemical constituent(s) of the flower especially is/are also likely to have played a prominent part in the hypoglycemic action of the flower

extract and may be involved in the alterations of biochemical parameters in this present study.

Blood glucose and antidiabetic studies

Diabetes mellitus is a chronic disease characterized by high blood glucose levels as a result of absolute or relative deficiency of circulating insulin levels. In this study, rats induced with diabetes via STZ injection exhibited a significant increase (p < 0.05) in blood glucose level which corresponds to the report of Ghada (35) and Tendelet al. (37) on streptozotocin-induced diabetes. According to Ghada (35) and Tendelet al. (37), the elevated glucose level may be attributed to destruction of pancreatic β -cells by STZ, causing a notable reduction in insulin release by pancreatic β -cells. Studies have shown that a variety of plant part extracts are able to effectively reduce blood glucose level in STZ-induced diabetic rats (38). Ethanolic extract of *M. oleifera* flower demonstrated glucose-lowering effect in hyper-glycaemic rat possibly through the stimulation of β -cells and/or as a result of its insulin-like activity. The possible mechanism of hypoglycaemic action of the ethanolic extract of *M. oleifera* flower may be via potentiation of insulin secretion from β -cells of the islets of Langerhans or as a result of improved blood glucose transport to peripheral tissues (39).

Antioxidants provide defence against degenerative diseases such as cancer, Alzheimer's diseases, and coronary heart disease (6). Antioxidants expressed their protective actions through the repression of ROS formation, inhibition of enzymes and chelation of elements required for free-radical production, scavenging reactive species, and up regulation of antioxidant defenses (10). The antioxidant enzyme superoxide dismutase (SOD) has been described as the first line of defense enzymes against free radicals (35). In this study, the significant elevation in activity of liver superoxide dismutase (SOD) with a significant elevation in liver, pancreas and kidney malondialdehyde (MDA) concentration of rats induced with diabetes via STZ injection can be attributed to large free radicals produced by the hyperglycaemic condition. It was observed that treatment with ethanolic extract of *M. oleifera* flowers significantly increased SOD and significantly decreased MDA levels in STZ-induced diabetic rats. This shows that the ethanolic extract possesses the potential to lower reactive free radical levels which may ameliorate oxidative dama-

ge and this may be traceable to the rich presence of flavonoids which have antioxidant property (35). *M. oleifera* is a rich source of anti-oxidants (25-27) which are largely responsible for its antioxidant activity (40-41).

Serum lipid profile

Concentrations of total cholesterol, TG, HDL/LDL ratio and coronary risk indices (CRI) are indicators of susceptibility to cardiovascular diseases (CVDs) (42). The marked reduction of these parameters in STZ-induced diabetic rats administered the extract compared to those administered glibenclamide demonstrates the potential of ethanolic extract of *M. oleifera* flower to reduce the risk of CVDs. Treatment of diabetes mellitus with the extract could therefore prevent and/or reduce the risk of cardiovascular complications of diabetes. Diabetics usually have smaller, densely oxidized LDL molecules, which may elevate atherogenicity even if the absolute LDL cholesterol concentration is not increased (42). This study also shows significantly elevated levels of triglyceride in diabetic rats in relation to the controls. Triglyceride levels were significantly reduced following the treatment with ethanolic extract of *M. oleifera* flower. Thus, by suppressing diabetes-induced elevation in lipid levels, ethanolic extract of *M. oleifera* flower may be useful in the reduction of the risk of cardiovascular complications of diabetes.

Enzymes studied

Elevated levels of aminotransferases in the serum are a common sign of liver and kidney damage and are observed more often among diabetics than in the general population (43). This study shows that the

levels of liver aminotransferase (ALT and AST) increased significantly in STZ-induced diabetic untreated rats as shown in Tables 7 and 8, respectively. The increase in aminotransferase levels may be attributed to cellular damage in the liver induced by diabetes. Cell damage results to increased permeability leading to the leakage of cytosolic enzymes into the blood (44). However, ethanolic extract of *M. oleifera* flower produced a marked significant decrease of the elevated AST and ALT activities. This decrease may be attributed to the hepatoprotective and antioxidant activity of a number of flavonoids in the ethanolic extract of *M. oleifera* flower. This agrees with the report of Ghada (35) where it was reported that the effect of STZ-diabetes on ALP activity revealed a significant increase in ALP activity of STZ-induced untreated rats. A marked decrease of serum and liver ALP activity of STZ-induced rats after treatment with ethanolic extract of *Moringa-oleifera* indicates its protective effect over liver and improvement in liver function efficiency.

CONCLUSION

The present study showed that ethanolic extract of *M. oleifera* flower possesses hypo- and normoglycemic properties in rats induced with diabetes via STZ injection. This indicates that the ethanolic extract of *M. oleifera* contains biologically active components. Different doses of the plant extract used in this study demonstrated hypolipidemic and antioxidant abilities of the flower. This suggests its efficacy in the maintenance of glucose homeostasis and may be used as a therapeutic alternative in the management of diabetes mellitus. It also has beneficial effect on various metabolic and enzymatic functions of the liver in STZ-induced diabetic rats.

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Antidijabetična i antioksidativna svojstva etanolskog ekstrakta suvih cvetova *Moringa oleifera* kod miševa sa dijabetesom izazvanim streptozotocinom

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SAŽETAK

Cilj ove studije bio je utvrđivanje antidijabetičnih i antioksidativnih efekata oralno datog etanolskog ekstrakta cvetova *Moringa oleifera* kod miševa sa dijabetesom izazvanim streptozotocinom u dozi od 100, 200, i 300 mg/kg telesne mase.

Trideset (30) eksperimentalnih belih miševa muškog pola nasumično je podeljeno u šest grupa: A, B i F su označavale kontrolnu, dijabetičnu kontrolnu i referentnu grupu koja je primala lek, dok su grupe označene od C do E primile dozu ekstrakta od 100, 200 i 300 mg/kg telesne mase. Uzorci krvi i organa su sakupljeni zbog analiza nivoa glukoze i antioksidativnih enzima.

Nivoi glukoze u krvi, serumskih lipida i lipidne peroksidacije, kao i aspartata aminotransferaze (AST), alkalne fosfataze (ALP) i alanin aminotransferaze (ALT) bili su značajno smanjeni ($p < 0,05$) kod miševa sa dijabetesom izazvanim streptozotocinom kojima je oralno dat etanolski ekstrakt cveta *M. oleifera*. Međutim, telesna masa i aktivnost katalaze i superoksid dismutaze bili su značajno povećani ($p < 0,05$) kada se uporede sa kontrolnim grupama.

Etanolski ekstrakt cveta *M. oleifera* dat oralno poboljšao je metabolizam masti, uticao na smanjenje nivoa glukoze i pokazao blagotvorna svojstva u preveniranju komplikacija dijabetesa, što je rezultat lipidne peroksidacije i oksidativnih sistema kod miševa sa dijabetesom izazvanim streptozotocinom. It tog razloga se može koristiti u terapeutske svrhe za lečenje dijabetesa melitusa.

Ključne reči: etanolski ekstrakt, antidijabetični, antioksidativni enzimi, *Moringa oleifera*, streptozotocin

