ejpmr, 2017,4(07), 154-162

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EVALUATION OF ANTIOXIDANT AND HEPATOPROTECTIVE ACTIVITIES OF MORINGA (MORINGA OLEIFERA) LEAVES IN DIABETIC RABBITS

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Article Received on 09/05/2017 **Article Revised on 28/05/2017** **Article Accepted on 18/06/2017**

ABSTRACT

Background*:** Because of the potential antioxidant, anti-inflammatory, and immune-modulating properties of Moringa (*Moringa oleifera*), there is an increased interest in its potential role in the prevention and control of diabetes mellitus (DM). ***Objective*:** This study evaluated the potential antidiabetic efficacy of *Moringa oleifera* leaves (MOL) powder against streptozotocin (STZ)-high-fat diet (HFD) induced diabetic rabbits. ***Material and methods*:** Forty rabbits were divided into four groups of ten animals each. Control group, received no any treatment; diabetic group, injected STZ (35 mg/ kg BW, i.p) and fed on HFD; MOL50 group, received MOL, 5% of the diet and MOL100, received MOL, 10% of diet. ***Results: The STZ - HFD induced diabetic rabbits exhibited a significant increase in blood glucose, lipid peroxidation, liver enzymes and renal functions as well as decrease in insulin, antioxidant enzymatic activities and catalase mRNA expression compared with the control group. However, concurrent administration of MOL normalized the levels of all these parameters compared with the diabetic group. ***Discussion and conclusion*:** MOL ameliorated the adverse biochemical effect of DM, likely by increasing insulin secretion and sensitivity, improving the antioxidant status and decreasing the lipid peroxidation and insulin resistance.

KEYWORDS: Antioxidant status, *Moringa oleifera*, Streptozotocin, Hepatoprotection.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by increased blood glucose levels resulting from defects in insulin secretion, insulin action or both (Danescu et al., 2009) Chronic hyperglycemia is related to microvascular and macrovascular complications that can lead to visual impairment, blindness, kidney disease, heart disease, and stroke (Francisco *et al.*, 2010). Currently available therapies for diabetes include insulin and many oral antidiabetic agents have adverse effects as hypoglycemia, gastrointestinal problems. Therefore, the search for more effective and safer hypoglycemic agents is one of the important areas of investigation (Saxena, 2004).

Moringa is the cultivated species of the genus Moringa of the family Moringaceae. Many health benefits were reported because of supplementation with Moringa leaves or seeds or their extract (Yassa *et al.*, 2014). It is cultivated throughout tropical and sub-tropical areas of the world (Ramachandran *et al.*, 1980). The antioxidant and antidiabetic activity of Moringa oleifera leaves (MOL) powder indicated potential benefits as a potent antidiabetic in streptozotocin-induced diabetic rats (Yassa *et al.*,2014). Moringa oleifera possess antitumor, antioxidant, antidiuretic, anti inflammatory, hepatoprotective and antidiabetic properties that were reflected by decrease the oxidative stress and lipid peroxidation levels and increase the antioxidant activity (Sadek, 2014). Therefore, the aim of the present study was to evaluate the antidiabetic and antioxidant potential effect of MOL against STZ-HFD induced diabetic rabbits.

MATERIAL AND METHODS

Chemicals

Streptozotocin was purchased from Sigma-Aldrich (Sigma Chemical Co., St. Louis, MO, USA.). Kits for determination of all liver functions, kidney functions, lipid profile and antioxidant parameter were purchased from the Biodiagnostic Company, Cairo, Egypt. All chemicals used were of analytical grade.

Moringa leaves Preparation

Moringa leaves were obtained from Egyptian Society of Moringa in National research Centre. The Moringa leaves were harvested and air-dried under a shed until

they were crispy to touch while retaining their greenish coloration. The leaves were milled to obtain a powder.

Gas chromatography–mass spectrometry (GC-MS) analysis

Trace GC Ultra-ISQ mass spectrometer with a direct capillary column TG–5MS (30 m × 0.25 mm × 0.25 µm) was injected by 10 µl of MOL methanolic extract. The column oven temperature was started 60 °C and then increased by 5 °C/min. till reach 280 °C. The injector and detector (MS transfer line) temperatures were kept at 250 °C. Helium flow rate of 1 ml/min. was used as carrier gas for 37.83 minutes. The solvent delay was 2 min. and diluted samples of 1 µl were injected automatically using auto-sampler AS3000 coupled with GC in the splitless mode. The ion source and quadrupole temperatures were set at 200 and 150°C, respectively. The mass spectra of the identified components were determined by comparison to NIST 11 mass spectral database.

Animal

Forty white male New Zealand rabbits weighing 1-1.1 kg. They were obtained from the Faculty of Agriculture, Alexandria University, Egypt. Animals were kept in the laboratory house for 2 weeks prior to the initiation of the experiment for acclimatization. Rabbits were maintained on a basal diet that stated in Table 1. All animals were accessed water *ad libitum*.

Induction of diabetes mellitus

Rabbits were fed with high-fat diet, prepared using 20% fat (20% sheep tallow) Samah, (2014) for 2 weeks then were injected intraperitoneally with STZ by 35 mg/kg b.w. STZ was prepared by dissolving in sodium citrate buffer (PH 4.5) and injected immediately to avoid degradation (Atanasovska *et al*., 2014) then rabbits were fed with HFD till the end of the experiment. Ten days after STZ injection, rabbits were screened for measuring blood glucose levels. Overnight fasted (8 h) animals blood samples were taken from ear vein and kept without anticoagulant at room temperature for one hour then centrifuged and serum glucose level was measured. Rabbits having serum glucose more than 200 mg/dl were considered as diabetic and included in the experiment.

Experimental design

Rabbits were allocated into four groups of ten animals each as following; Group I: Control rabbits received basal normal diet. Group II: Diabetic rabbits fed basal diet (35 mg/kg body weight) and HFD 20%, Group III (MOL50): Diabetic rabbits treated with 50 g of Moringa leaves powderper kg diet, and Group IV (MOL100): Diabetic rabbits treated with 100 g Moringa leaves powderper kg diet (Odetola et al., 2014).

Sample collection and preparation

Blood samples were collected from the ear vein of each at the 2nd, 4th and 8th weeks after 8 h fasting .The blood samples were collected into labelled sterile vacuum tube without anticoagulant and left to coagulate at room temperature, after centrifugation at 3000 rpm for 15 min., the non-hemolyzed serum samples were collected in clean sterilized rubber stopper glass vials and stored at -20 °C until used for biochemical analysis of serum glucose, total protein, albumin, alanine aminotransferase(ALT, EC 2.6.1.2), aspartate aminotransferase (AST, EC 2.6.1.1), triacylglycerol, total cholesterol, urea, and creatinine.

At the end of the 2nd month the sacrificed rabbits were eviscerated and liver was harvested from the carcass and washed by phosphate buffered saline (PBS) solution, PH 7.4 containing 0.16 mg /ml heparin to remove any red blood cells and clots. The tissue was homogenized in 5 ml cold PBS per gram tissue (1:5 dilution). All samples were centrifuged at 4000 rpm for 15 min. at 4°C. The supernatants were collected and stored at -20°C until biochemical analysis of catalase activity andmalondialdehyde (MDA) and Total antioxidant capacity (TAC) levels.

Up to 30 mg of tissue were placed and ground thoroughly with a mortar and pestle. Then the tissue powder was transferred immediately into a 1.5 ml microcentrifuge tube containing 300 ul of lysis buffer supplemented with β-mercaptoethanol and thoroughly mixed by vortex for 10s then the homogenate was submitted to the RNA purification protocol, cDNA synthesis and amplification protocol of catalase gene in presence of housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Table 2). The primer sequences of selected genes were designed with Primer3 (http://primer3.ut.ee/) and BLAST. (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch).

Biochemical analysis

Serum glucose determined by kit manufactured by Biodiagnostic Company according to Trinder, (1969). Insulin hormone levels were evaluated by insulin ELISA kits manufactured by DRG International company according to (Flier *et al*., 1979). The homeostasis model assessment of insulin resistance (HOMA-IR) was determined according to (Matthews et al. 1985). Serum creatinine level was determined according to (Bartles et al., 1972), urea was determined according to Fawacett and Scott (1960). AST and ALT were determined according to the method of Reitman and Frankel (1957).

Serum total protein evaluated according to Gornal *et al* (1949)., albumin according to (Doumas et al., 1971), globulin according to Coles (1974). Serum total cholesterol levels were determined by the kit manufactured by the bio-diagnostic company according to Richmond (1973)., high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were determined according to friendewald (1972),and triacylglycerol (TAG) were evaluated according to Fassati and Prencip (1982).

The supernatants collected from centrifugation of liver homogenates were subjected to determination of catalase (Aebi 1984), total antioxidant capacity (Koracevic 2001), and MDA (Ohkawa et al., 1979).

RT-PCR

Purification of total mRNA was done by the kit of Thermo-Scientific Company (Boom et al. 1990). Furtherly, cDNA samples were synthesized by the kit of Thermo-Scientific Company (Wiame et al. 2000). Amplification of the reverse transcription reaction product occurred by the kit manufactured by Thermo-Scientific Company (Longo et al. 1990).

Statistical analysis

Data was analyzed by one-way analysis of variance (ANOVA), with Duncan's multiple range tests for significant between means by SPSS software package v.20. A p value of less than 0.05 was considered statically significant.

RESULTS

The data illustrated in Table (3) and presented in Figure (1) and revealed the chemical composition of MOL that analyzed by GC–MS led to identification of some antioxidant agents as; eugenol (45.15%), caryophyllene (12.48%) , ascorbic acid (6.87%), phenol (5.06%), octadecenoc acid (4.41%), heptadecyne (2.45%), cyclopropanoctonic (2.53%), humulene (1.85%), pentadecanoic (1.27%), heptatriacotanole (0.94%) , quercetin (0.73%) and retinol (0.65%), respectively.

The serum glucose levels of diabetic rabbits were significantly higher (P< 0.05) than the control group and decreased significantly (P< 0.05) in all treated diabetic rabbits. The insulin level was significantly decreased in diabetic rabbits in relation to normal rabbits. Administration of MOL significantly increased the level of insulin when compared with the diabetic group. The HOMA-IR is a marker of insulin resistance was significantly increased in diabetic rabbits in relation to control group and decreased significantly in treated diabetic groups compared to diabetic group (Table 4).

Table (5) revealed the effect of MOL administration on liver enzymes in serum of diabetic rats. Serum ALT and AST activities were significantly increased (P<0.05) in diabetic control rabbits compared to normal control group. Moreover, treatment of diabetic animals with MOL induced potential improvement (P<0.05) of these altered parameters.

The mean value of blood urea and creatinine in the serum of diabetic rabbits was significantly (< 0.05) increased compared with the negative control group. Treating diabetic rabbits in MOL50 and MOL100 had significantly (< 0.05) improvement in all kidney functions parameters in relation to the control group. The kidney functions were more ameliorated by treating with the higher dose of MOL (Table 5).

Table (6) showed that Protein, albumin, globulin level decreased significantly in diabetic group compared to healthy control group and a significant improvement in total protein levels in case of treatment with MOL.

The obtained data in Table (7) showed a significant increase (P<0.05) in levels of serum total cholesterol, TAG, HDL-C, LDL-C, and VLDL-C in diabetic rabbits than the healthy control group. The treatment of diabetic rabbits with MOL improved (P<0.05) of elevated values. Treating rabbits with 100 g of MOL in the diet was more efficient than the lower dose.

The data presented in Table (8) indicated that MDA in liver tissue homogenate of diabetic rabbits showed a highly significant (P<0.05) elevation as compared with normal rabbits. The treatment of diabetic rabbits with MOL induced a potential improvement (P<0.05) of elevated values. The higher dose of MOL was more effective than the lower dose.

The data illustrated in Table (8) revealed that the total antioxidant capacity in liver tissue homogenate of diabetic rabbits showed a highly significant (P<0.05) decrease as compared with normal animals. The treatment of diabetic animals with MOL induced significant increase (P<0.05) of total antioxidant capacity. The higher dose of MOL was more effective than the lower dose.

Catalase activities significantly were decreased (P<0.05) in diabetic rabbits than the healthy control group. The diabetic rabbits treated with MOL showed a significant increase (P<0.05) in catalase activity compared to non-treated diabetic group (Table 8).

Catalase mRNA expression levels of diabetic rabbits treated with MOL significantly increased (P<0.05) compared to the diabetic control group. Moreover, treatment of diabetic animals with MOL100 seemed to be more effective than MOL50 of catalase mRNA expression improvement of diabetic rabbits (Fig. 2).

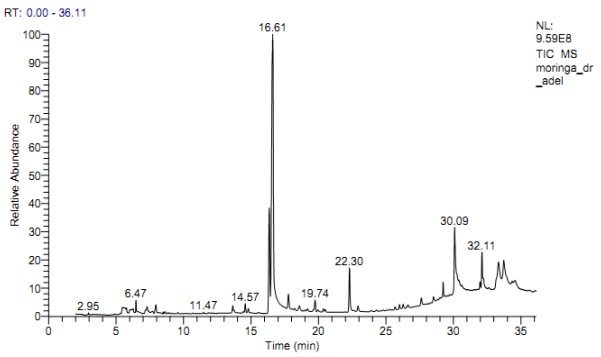

Fig. 1: GC-MS chromatogram of MOL methanolic extract.

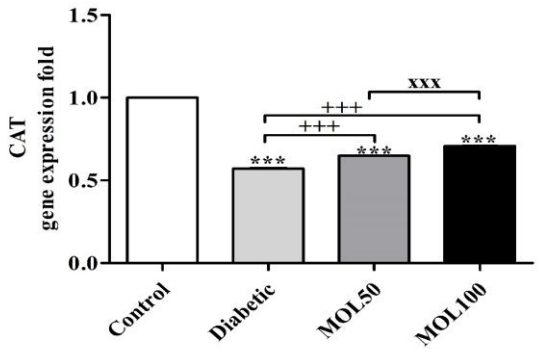

Fig. 2: Effect of Moringa leaves on catalase mRNA expression in rabbits. $^{***}p < 0.001$ vs. control. $^{+++}p < 0.001$ vs. Diabetic. $^{xxx}p < 0.001$ vs. MOL100.

1Each kg of vitamin and mineral mixture contained: Vit A 2 000 000 IU; E 10 mg; B1 400 mg; B2 1200 mg; B6 400 mg; B12 10 mg; D3 180000 IU; Colin chloride 240 mg; Pantothenic acid 400 mg; Niacin 1000 mg; Folic acid 1000 mg; Biotin 40 mg; Mn 1700 mg; Zn 1400 mg; Fe 15 mg; Cu 600 mg; Se 20 mg; I 40 mg and Mg 8000 mg. GAE: Gallic acid equivalents.

Table 1: Ingredients of basal diet.

Ingredient	g
Yellow corn	7.5
Wheat bran	24
Barley	20
Clover hay	22
Soybean meal (44% CP)	23.5
Limestone	1.15
Di-calcium phosphate	0.5
DI-Methionine	0.2
Anti-aflatoxin+Anti-coccidian	0.5
Vitamin and minerals premix	0.30
NaCl	0.35
Total	100

Table 2: nucleotide sequence of primers used in qPCR.

Gene symbol	Gene description	Gen Bank Accession #	Sequences (5'->3')
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	NM_001082253	F: ATCTCGCTCCTGGAAGATGG R: CAAAGTGGATGTTGTCGCCA
CAT	Catalase	XM_002709045	F: GCCCTGGTCAGTCTTGTAGT R: CCATCGGCATATGAACGGAT

Table 3: GC-MS analysis of Moringa leaves methanolic extract.

No.	Compound Name	RT (Minutes)	Area %	Molecular Formula
1	Eugenol	16.61	45.15	$C_{10}H_{12}O_2$
2	Caryophyllene	16.35	12.48	$C_{15}H_{24}$
3	Hexadecanoic acid	30.09	6.87	$C_{38}H_{68}O_8$
4	Phenol	22.30	5.06	$C_{12}H_{14}O_3$
5	Octadecenoic acid	32.11	4.41	$C_{19}H_{36}O_2$
6	Heptadecyne	33.34	2.45	$C_{17}H_{32}O$
7	Cyclopropanoctonic	33.73	2.53	$C_{22}H_{38}O_2$
8	Humulene	17.77	1.85	$C_{15}H_{24}$
9	Pentadecanoic	29.24	1.27	$C_{17}H_{34}O_2$
10	Heptatriacotanole	27.62	0.94	$C_{37}H_{76}O$
11	Quercetin	34.59	0.73	$C_{18}H_{16}O_7$
12	1,1-Cyclobutanedicarboxamide	22.31	0.65	$C_{20}H_{28}O$

Table 4: Effect of Moringa leaves on glucose, insulin and HOMA-IR.

Weeks	Group	Glucose (mg/dl)	Insulin (µU/mL)	HOMA-IR
		Mean±SE	Mean±SE	Mean±SE
2nd week	Control	93.67±4.06c	12.27±0.02a	2.84±0.12c
	Diabetic	202.33±5.21a	10.13±0.05c	5.06±0.10a
	MOL50	161.00±6.43b	10.84±0.37b	4.44±0.16b
	MOL100	147.33±2.33b	11.27±0.03c	4.11±0.05b
4th week	Control	95.33±3.48c	12.21±0.02a	2.87±0.10c
	Diabetic	256.67±8.09a	8.96±0.06c	5.68±0.14a
	MOL50	145.33±3.48b	10.80±0.15b	3.85±0.03b
	MOL100	136.67±3.18b	10.98±0.10b	3.71±0.05b
8th week	Control	98.00±4.73c	12.31±0.01a	2.98±0.14d
	Diabetic	315.67±3.48a	7.21±0.10d	5.63±0.02a
	MOL50	214.33±3.53b	9.13±0.05b	4.83±0.05b
	MOL100	199.67±5.93b	8.82±0.10c	4.33±0.08c

Means bearing different letters within the same column within each period are significant at (P<0.05).

Table 5: Effect of Moringa leaves on liver enzymes and kidney function.

Weeks	Group	Urea (mg/dl)	Creatinine (mg/dl)	ALT (U/L)	AST (U/L)
		Mean±SE	Mean±SE	Mean±SE	Mean±SE
2nd week	Control	20.00±1.53b	0.74±0.04b	21.33±2.40b	22.33±1.76c
	Diabetic	32.00±1.73a	1.87±0.15a	32.67±2.33a	40.33±1.45a
	MOL50	29.33±0.88a	1.50±0.12a	31.00±2.31a	28.67±0.88b
	MOL100	26.67±2.03a	1.43±0.24a	27.33±1.76ab	28.33±1.76b
4th week	Control	20.33±1.45c	0.84±0.06b	21.33±1.45b	27.00±2.08b
	Diabetic	47.33±2.33a	2.23±0.18a	30.67±2.03a	40.00±1.73a
	MOL50	40.67±2.33ab	1.70±0.28a	24.67±2.73ab	27.00±2.31b
	MOL100	35.00±3.46b	1.92±0.08a	21.67±2.33b	22.67±2.33b
8th week	Control	30.33±0.88b	0.87±0.12c	18.33±1.20c	23.67±1.76c
	Diabetic	61.00±1.15a	3.20±0.17a	50.00±1.73a	61.00±2.08a
	MOL50	49.00±9.54a	2.84±0.12ab	39.00±1.15b	43.00±1.53b
	MOL100	47.33±2.73a	2.67±0.18b	32.00±2.08b	40.00±1.15b

Table 6: Effect of Moringa leaves on serum proteins.

Weeks	Group	Protein (g/dl)	Albumen (g/dl)	Globulin (g/dl)	A/G
		Mean±SE	Mean±SE	Mean±SE	Mean±SE
2nd week	Control	7.23±0.05a	4.13±0.02a	3.11±0.03a	1.32±0.00c
	Diabetic	5.92±0.06d	3.32±0.02c	2.60±0.04b	1.27±0.02d
	MOL50	6.25±0.05c	3.87±0.03b	2.38±0.03c	1.63±0.01a
	MOL100	6.73±0.05b	4.09±0.02a	2.64±0.03b	1.55±0.01b
4th week	Control	7.24±0.03a	4.13±0.03a	3.11±0.01a	1.33±0.01b
	Diabetic	5.77±0.03d	3.23±0.03d	2.54±0.01b	1.31±0.02b
	MOL50	6.09±0.03c	3.77±0.02c	2.32±0.01d	1.62±0.01a
	MOL100	6.28±0.02b	3.92±0.03b	2.36±0.01c	1.66±0.03a
8th week	Control	7.22±0.02a	4.14±0.02a	3.08±0.01a	1.34±0.01c
	Diabetic	5.61±0.02d	3.13±0.01d	2.48±0.01b	1.26±0.00d
	MOL50	5.83±0.02c	3.62±0.02c	2.19±0.01c	1.64±0.02b
	MOL100	5.94±0.02b	3.77±0.03b	2.17±0.01c	1.73±0.02a

Means bearing different letters within the same column within each period are significant at (P<0.05).

Table 7: Effect of Moringa leaves on lipid profile.

Weeks	Group	Cholesterol(mg/dl)	TAG (mg/dl)	HDL-C(mg/dl)	LDL-C(mg/dl)	VLDL-C (mg/dl)
		Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE
2nd week	Control	130.33±5.78b	104.33±7.69c	43.95±0.07a	65.70±5.16c	20.88±1.54c
	Diabetic	170.33±4.98a	142.00±2.08a		115.93±4.97a	28.42±0.42a
	MOL50	141.33±3.76b	127.33±2.33b	32.21±0.31c	83.63±3.29b	25.47±0.48b
	MOL100	137.00±2.52b	118.00±1.53bc	38.92±0.36b	74.32±2.91bc	23.71±0.26b
4th week	Control	136.00±4.58c	93.33±2.03c	43.62±0.15a	73.67±5.09c	18.71±0.38c
	Diabetic	209.67±10.11a	187.33±2.33a	22.02±0.21d	150.14±9.88a	37.45±0.46a
	MOL50	157.67±2.40b	137.67±3.53b	29.99±0.31c	100.17±2.96b	27.54±0.70b
	MOL100	154.33±3.84bc	132.33±2.03b	33.18±0.20b	94.71±4.37b	26.49±0.41b
8th week	Control	162.67±3.84d	107.00±2.52d	43.85±0.08a	97.42±3.64d	21.45±0.52d
	Diabetic	253.33±3.76a	212.33±2.40a	19.99±0.09d	190.85±3.75a	42.48±0.48a
	MOL50	219.33±2.60b	165.67±3.84b	28.33±0.36c	157.75±2.27b	33.18±0.75b
	MOL100	204.67±4.37c	150.00±2.08c	30.82±0.26b	143.85±3.76c	30.01±0.41c

Means bearing different letters within the same column within each period are significant at (P<0.05)

Table 8: Effect of Moringa leaves on lipid peroxidation, catalase activity and total antioxidant capacity in rabbit's liver.

Group	Catalase (U/mg protein)	TAC (mM/g tissue)	MDA (nmol/g tissue)
	Mean±SE	Mean±SE	Mean±SE
Control	4.21±0.07a	2.63±0.08a	63.67±1.76d
Diabetic	0.46±0.02c	1.30±0.14d	124.33±2.03a
MOL50	1.75±0.05c	1.95±0.02c	88.00±1.73b
MOL100	2.78±0.02b	2.35±0.04b	76.00±2.65c

Means bearing different letters within the same column are significant at (P<0.05)

DISCUSSION

DM is a chronic disease which affects the metabolism of proteins, carbohydrates, and lipids. The major characteristic is hyperglycemia because of abnormal secretion of insulin in the pancreas (Type I) or inefficient action of insulin in the target tissues (Type II) (Baynes, 2001). STZ is used to develop animal models of diabetes by exerting a cytotoxic effect on pancreatic β-cells possibly by generating lipid peroxides and excess reactive oxygen species (ROS), causing DNA damage either by alkylation or peroxynitrite formation leading to cell death and drop in insulin level (Rupérez *et al*., 2008).

The GC-MS analysis of MOL cleared that it is rich in antioxidant agents as eugenol, caryophyllene, ascorbic acid, and phenol (45.15%, 12.48%, 6.87%, and 5.06%, respectively) area in the chromatogram. Gupta *et.al*., (2010) investigated that the phytochemical screening of MOL revealed the presence of secondary metabolites such as eugenol, ascorbic acid, and caryophyllene those possess antioxidant and hypoglycemic activities by inhibiting the accumulation of fat peroxide products in red blood cells and maintaining the activities of the body's antioxidant enzymes at normal levels. Also, eugenol can be directly incorporated into cell membranes, which prevents its lipid peroxidation. The obtained data revealed that the injection of STZ resulted in significant increase in serum glucose levels and significant decrease in the level of insulin compared to normal control group. These results came in agreement with those obtained by (Sadek and Shaheen, 2014; Ghanema and Sadek, 2012; Sadek 2011; Taha et al. 2013a and 2013b) who reported that, a significant increase in serum glucose and a decrease in the level of insulin were recorded in STZ induced diabetic group compared to normal control one. Additionally, our obtained results showed that HOMA-IR was significantly increased in the diabetic group indicating induction of insulin resistance. these results were in accordance with data reported by Yogendra *et al*., (2014) who mentioned that there was a significant elevation in HOMA-IR in diabetic controls. On the other hand, treatment of diabetic rabbits with MOL significantly decreased serum glucose and increased the level of insulin when compared with the diabetic group. Also, HOMA-IR decreased significantly in all treated with MOL diabetic groups. These results come in harmony with those obtained by Anyanwu *et al*., (2014) who reported that due to its antioxidant activity that protected the pancreas cells against the oxidative stress induced by STZ. Treated STZ induced diabetes rats with the MOL revealed a safe and an excellent antidiabetic activity due to its content of antioxidant compounds such as eugenol, ascorbic acid and phenol (Mbikay, 2012). Diabetic rabbits showed also an increase in MDA and decreased catalase and total antioxidant capacity in the liver tissue homogenate in relation to control group.. The treatment with MOL improved these parameters and nearly restored them to their normal levels. these results come in accordance with those obtained by (Ghiridhari *et al*., 2011) who reported that medication with MOL gives diabetic patients better antioxidant activity as MOL contains phytochemicals, such as eugenol, caryophyllene, and phenol. Treatment of diabetic rabbits with MOL significantly increased the levels of catalase mRNA expression in the liver tissue homogenate in relation to the diabetic group.

The obtained data revealed that diabetic rabbits exhibited in elevation of ALT and AST activities compared with control group. These obtained data indicated the hepatocellular damage induced by STZ. Treatment with MOL revealed significant improvement of liver enzymes levels especially in higher dose 10% these come in agreement with those obtained by Fakurazi *et al*., (2008) as Moringa may preserve the structural integrity of hepatocytes membranes and subsequently preventing enzyme leakage into plasma. The kidney functions as creatinine, urea levels were increased in the positive control group because of diabetic nephropathy which is considered a major complication of DM. These results came in accordance with those obtained by (Gross *et al*., 2005). On the other hand, treatment of diabetic group with MOL decreased the level of serum creatinine and serum urea as compared with the untreated diabetic group. The present findings agreed with (Ndong *et al*., 2007) who stated that MOL restored the normal renal function and histology of kidney with no pathological changes due to its content of very powerful antioxidant such as ascorbic acid, eugenol, and phenol that have scavenging effect on the free radicals. The obtained data revealed that STZ significantly increased the serum TAG, total cholesterol, LDL-C, and VLDL-C levels and decreased the HDL-C level in diabetic group compared to control one. The present findings came in accordance with those obtained by (Kumar *et al*., 2013; Sadek et al 2013) who reported that STZ induced diabetic rats had hyperlipidemia with significant elevation of TAC, total cholesterol, LDL-C, and VLDL-C and significantly decreased HDL-C due to insulin deficiency result in failure to activate lipoprotein lipase causing hypertriglyceridemia. On the other hand, our study revealed that treatment of diabetic groups with MOL significantly decreased serum TAG, total cholesterol, VLDL-C, and LDL-C and increased the serum HDL-C when compared with untreated diabetic group. These results come in harmony with those obtained by Mai *et al*., (2007) who reported that phenolic compounds as octadecenoic acid, eugenol, ascorbic acid, caryophyllene, and phenol in MOL may play important role for inhibition of α-glucosidase, pancreatic cholesterol esterase activity, as well as bile acid binding and inhibiting the formation of cholesterol micellization.

CONCLUSION

From the obtained results, we can conclude that MOL possessed antihyperglycaemic, antihyperlipidemic, and hepatoprotective effects against DM alterations. Furthermore, MOL up-regulates the gene expression of catalase enzyme in DM due to the presence of biologically active antioxidant compounds. Ultimately, we recommend the patients suffering from DM to use MOL as a food supplement in their diet.

Competing Interests

The authors have no conflict of interest.

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