International Journal of Phytomedicine 7 (2015) 219-230
http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Fatty acids Profile, Nutritional Values, Anti-diabetic and Antioxidant Activity of the Fixed Oil of *Malva parviflora* Growing in Egypt

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Abstract

In the current study, the fixed oil obtained from *Malva parviflora* seeds was tested for its physicochemical properties, fatty acids composition and nutritional value. The oil yield from the seed was 3.3%. The fatty acid composition was represented by 9 compounds, among which coriolic acid was the major fatty acid (31.60%), followed by pentadecanoic acid (30.05%). The antidiabetic and antioxidant activity of *Malva parviflora* seeds fixed oil (MPO) were also assessed. Diabetic rats were induced by injection of STZ (55mg/kg, i.p.) and diabetes was confirmed 48h after induction, and then allowed for 7 days to stabilize blood glucose level. MPO (1/100 LD_{50}; 157mg/kg.b.w. and 1/50 LD_{50}; 314mg/kg.b.w) was administered daily for 28 days. Treated diabetic rats significantly reduced elevated blood glucose, TC, TG, LDL-C, hepatic and renal TBARs and HP levels. The treatment also resulted in improved plasma insulin, HDL-C and GSH, SOD and CAT levels. These results clearly suggest that MPO may effectively normalize the impaired antioxidant status in streptozotocin induced diabetes as well as exert rapid protective effects against lipid peroxidation and ultimately reduce the risk of liver and renal complications.

Keywords: Fatty acids, *Malva parviflora*, oil seed, antidiabetic, antioxidant, STZ, GSH, SOD and CAT.

Introduction

Malvaceae, or mallow family, is a family of flowering plants containing over 200 genera comprising about 2,300 species. There is a large number of economically important members of this family. Some are best known as fiber plants, such as cotton (*Gossypium* spp.), kenaf (*Hibiscus cannabinus*) and Chinese jute (*Abutilon theophrasti*), whereas others are food plants, such as okra (*Abelmoschus esculentus*or *Hibiscus esculentus*) and several edible *Malva* spp. (*M. nicaensis*, *M. parviflora*, *M. sylvestris* and *M. verticillata* var. *crispa*) [1]. Diabetes mellitus is a multi-systemic disorder which affects almost every cell in the body and considered one of the most important health problems worldwide [2, 3]. As the global epidemic of diabetes continues to expand, the prevalence of diabetes mellitus increased from 1.4% to 25.5% over the last twenty years [4]. In hyperglycemic condition reactive oxygen species increased the oxidative stress mainly due to over production of oxygen free radicals [5]. Antioxidants play an important role in scavenging the free radical, damage the reactive oxygen species and protect the human body from oxidative stress [6]. Medicinal plants are a rich source of various phytochemical constituents which act by a variety of mechanism to cure the diabetes [7]. Among of these plants *Malva parviflora* L. (family Malvaceae) which is a herb native to Africa, Asia and Europe. Its common name is cheeseweed and locally known as Sonchal. Pharmacologically, it has been reported to be antibacterial, anti-inflmmatory and antifungal [8, 9]. Traditionally *M. parviflora* is used for the treatment of inflammation, pain and liver injuries [10]. The plant contains phenolic and flavonoid compounds [11]. Traditional importance and phytochemical profile of the plant appeal its use as antidiabetic and antioxidant. Some natural products have antioxidant activity that could mitigate obesity-related complications, including atherosclerosis and some cancers [12–15]. No detailed study on the chemical composition of the oil of the seeds of *M. parviflora* growing in Egypt has been performed so far. The aim of this study was to determine the oil content including the fatty acid content and the nutritional value of seeds to evaluate its potential for nutritional and medicinal applications in treatment of diabetic complications in STZ-induced diabetic rats.

Materials and Methods

Plant material

Plant materials of *M. parviflora* seeds were collected from Horbit Village, El-Sharkyea Governorate, Egypt. The plant material was identified, authenticated by *Dr.* Reem Hamdy, Faculty of Sciences, Cairo University, Egypt. Voucher specimens No. (3-1-2015) were kept in the department of Pharmacognosy, Faculty of Pharmacy, October 6th University. The seeds were cleaned, dried under direct sunlight and powdered by a mechanical grinder.

Extraction of Fixed oil

After being cleaned by hand carefully to remove the foreign materials such as other seeds, stones and small stalks, *M. parviflora* seed were dried at 50 ^{0}C for 12h in an oven, and then crushed into powder in a grinder with a size range of 0.55-1.0mm. The resulted powder was kept in a vacuum dessicator until use. *M. parviflora* powdered sample was mixed with hexane (1:10, *m/V*) at (60-80 ^{0}C) using a Soxhlet apparatus [16]. This process of extraction was continuous for 6h, the hexane removed by distillation, then concentrated by rotatory evaporator and air-dried at temperature of 40±2 ^{0}C.

Materials

Boron fluoride, methanol, pentane and hexane were obtained from Sigma-Aldrich, USA.

Preparation of BF_3 - Methanol Reagent

One liter of reagent grade methanol, in a 2-liter flask, was cooled in an ice Bath. With the flask still in the bath, 125 grams of BF_3 is bubbled through a glass tube into the methanol in fume hood, and the gas was not allowed to flow so fast that white fumes emerge from the flask (The BF_3 must be flowing through the glass tube before it is placed in the methanol and until it is removed from it and may be drawn into the gas cylinder valve system) This reagent has an excellent shelf life and has been used up to 4 months after preparation [17].

Preparation of FAME (fatty acid methyl ester)

Seeds (400mg) were dried overnight at 50ºC and ground into powder with a mortar and pestle, after which 0.6ml of dichloromethane and 4.0ml of 0.5N sodium methoxide were added. Acidic catalyzed esterification using the boron triflouride-methanol complex (14%w/ v) was added according to the method described by [17, 18]. The tube was shaken and heated for 30min. at 50^{0}C. The reaction was stopped by adding 5.0 ml of water containing 0.2ml of glacial acetic acid. The esterified fatty acids were extracted with 3.0ml petroleum ether (40-60^{0}C). The clear fraction was kept at -20 ^{0}C until further analysis.

Separation conditions of Fatty Acids on GC/MS

Instrument

HP 6890 Series Gas Chromatograph System with an HP 5973 Mass Selective Detector was used.

The FAME in hexane (1µL) was injected into the column with a split ratio of 100:1. The injector and detector temperature were set at 200 and 250°C, respectively. He_2 was used as the carrier gas at a flow rate of 1.5mL/min. Separation was carried out on a TR-FAME (Thermo 260 M142 P) (30mm x 0.25mm ID) with a film thickness of 0.25υm film) (70% Cyanopropyl – Polysilphenylenesiloxane) capillary column. The column temperature was programmed from 100 to 160 ^{0}C at 2 ^{0}C/min and then to 250 ^{0}C at 4 ^{0}C/min and finally held at 250 °C for 20 min. The weights of the individual FAME were calculated on the basis of their relative peak area compared with that of internal standard, and then they were corrected using the corresponding GC response factors for each fatty acid.

Refractive index

It was done using refractometer at a constant temperature (20 ^{0}C) according to Wolff [18].

Saponification index

It was determined according to Lion [19].

Determination of Total Flavonoids

The total flavonoid content was estimated using aluminum chloride colorimetric assay. The 0.5 ml of oil sample solution in methanol (5mg/100ml) were mixed with 2 ml of distilled water, 150 µl of 10% aluminum chloride and 2ml of 1M sodium hydroxide were added and left at room temperature for 15min. Absorbance of the mixtures was measured at 510nm (UV-Visible Ultraspec 2000 Spectrophotometer, England) and total flavonoid contents were calculated as rutoside equivalents from a calibration curve of rutoside. The calibration curve was prepared in the same manner using 0.0156-1 mg/ml of rutoside solution in methanol, Zhishen et al [20].

Determination of Total Phenol

The total phenol content was carried out by Folin-Ciocalteu test according to Singleton & Rossi [21] with some modifications. One ml of the oil (dissolved in 60% acetone, 5 mg/100 ml) was mixed with 200µl Folin-Ciocalteu reagent and 1 ml of aqueous Na_2CO_3. The mixtures were left at room temperature for 30 min. and the phenol content was determined by colorimetric method at 715 nm. The calibration curve was prepared using gallic acid solutions at concentractions of 1- 0.0156 mg/ml in 60% acetone. Total phenol content was expressed in terms of gallic acid equivalent (mg g-1).

Determination of Total Protein

Total protein was determined according to Kjeldahl [22], the sample was heated with sulphric acid, which decomposes the organic substance by oxidation to liberate the reduced nitrogen as ammonium sulfate. The solution is then distilled with sodium hydroxide, which converts the ammonium salt to ammonia. The amount of ammonia present, and thus the amount of nitrogen present in the sample is determined by back titration.

Determination of Total Carbohydrates

Total soluble sugars were determined according to Duboies et al. [23], using the phenol-sulfuric method.

Determination of Total Lipid

Lipid extraction was done according to Folch et al.,[24] by using chloroform-methanol (2:1v/v), the associated non-lipids were removed by washing the lipid phase three times with methanol-water (1:1v/v), the lipids in chloroform were dried over anhydrous sodium sulfate, then the solvent was removed by heating at 80 C under vacuum.

Determination of Vitamin A and C.

It was done according to A.O.A.C. [25] and Hussain et al., [26], respectively.

Biochemical studies

Determination LD_{50} of Malva parviflora seeds fixed oil

Preliminary experiments were carried out on groups of 4 rats. Compound was administrated orally in different doses to find out the range of doses which cause zero and 100 % mortality of animals. A range doses was determined for each compound.

LD_{50} was determined by oral administration of *Malva parviflora* seeds fixed oil in different doses 7000, 10000, 15000, 17000, 20000 and 25000mg/kg b.w. according to the method of Spearman and Karber [27].

Animals 60 male albino rats weighing around 180-200gms were divided into 6 groups, 10 rats in each group. They were acclimatized to animal house conditions. Animals were provided with standard diet and water *adlibtum*. Animals were kept under constant environmental condition and observed daily throughout the experimental work.

After administration of the tested *Malva parviflora* seeds fixed oil, animals were observed individually every hour during the first day and every day for 21days. Behavior and clinical symptoms of the animals were noted throughout of the experiment.

The LD_{50} was then calculated by the application of the following formula:

$$LD_{50} = D_m - \frac{\sum (Z \cdot d)}{n}$$

D_m= the dose which killed all the rats in the group.
Z = half the sum of the dead rats from 2 successive experiments.
d = the difference between 2 successive doses.
n = number of animals in each group.

Antidiabetic and antioxidant activity of Malva parviflora seeds fixed oil

Animals

Male albino rats weighing around 180±10gms were purchased from Faculty of Veterinary Medicine, Cairo University. The reason that potentiates us to use rats for testing is because they are available and they provide enough blood and organs; liver and kidney to make all biochemical analysis. All the rats were given a period of acclimatization for 15 days before starting the experiment. Animals were provided with standard diet and water *adlibtum* and were kept on a 12h light/12h dark cycle, in a room with the temperature regulated at 21–25^{o}C and humidity roughly at 56%. All protocols were performed in accordance with the Institutional Animal Ethical Committee (IAEC) as the directions of Faculty of Pharmacy October 6th University, Egypt.

Induction of diabetes

Streptozotocin-induced diabetes has been described as a useful experimental model to study the activity of hypoglycemic agents [28, 29]. After an overnight fasting (deprivation of food for 16 hours and allowed free access to water), diabetes was induced in rats by intra-peritoneal injection of STZ (Sigma, St. Louis, Mo. dissolved in 0.1M sodium citrate buffer pH 4.5 at a dose of 55mg/kg body weight) [30]. After 6 h of STZ injection, rats were received 5 % dextrose solution for the next 24h to prevent STZ induced fatal hypoglycemia as a result of massive pancreatic insulin release after its administration [31]. Diabetes was confirmed 48h after induction by measurement of tail vein blood glucose levels using glucose oxidase-peroxidase method. Diabetic rats were kept 7 days under standard laboratory condition for the stabilization of blood glucose levels [32]. After a week time for the development of diabetes, the rats with moderate diabetes having glucosuria and hyperglycemia (blood glucose range of above 15.6mM/L) were considered as diabetic rats and were used for the further experiments. The change in the body weight of the experimental animals was observed throughout the treatment period.

Experimental set up

The rats were divided into seven groups with eight rats in each.

Group I: normal control rats receiving 1% tween 80, orally (5mL/kg.b.w.).

Group II: Normal control rats treated with *Malva parviflora* seeds fixed oil1/100LD_{50} (157mg/kg.b.w.) suspended in 5ml, 1% tween 80, orally.

Group III: Normal control rats treated with *Malva parviflora* seeds fixed oil 1/50LD_{50} (314mg/kg.b.w.) suspended in 5ml, 1% tween 80, orally.

Group IV: Diabetic control (STZ-induced diabetic rats, given 1% tween 80, orally (5mL/kg.b.w.)

Group V: STZ-induced diabetic rats treated with *Malva parviflora* seeds fixed oil 1/100LD_{50} (157mg/kg.b.w.) suspended in 5ml, 1% tween 80, orally.

Group VI: STZ- induced diabetic rats treated with *Malva parviflora* seeds fixed oil 1/50LD_{50} (314mg/kg.b.w.) suspended in 5ml, 1% tween 80, orally.
Group VII: STZ-induced diabetic rats treated with glibenclamide (5mg/kg.b.w.) [33].
Malva parviflora seeds fixed oil and glibenclamide were suspended 1% tween 80 and administered orally to its respective group animals for 28 days. The fasting blood glucose level and body weight were estimated every week (0, 7, 14, 21 and 28 day). At the end of the fourth week, the fasting rats were sacrificed by cervical decapitation and the blood was collected using sodium fluoride as an anticoagulant for the determination of blood glucose. The liver and kidneys were dissected out, washed in ice-cold saline, patted dry and weighed.

Blood sampling and Biochemical assays

At the end of the study, all mice were sacrificed blood was collected, centrifuged, and plasma was used freshly for estimation of plasma glucose [34], insulin [35], triacylglycerols [36], total cholesterol [37], HDL- cholesterol [38] and LDL-cholesterol [39] formula (LDL-cholesterol = total cholesterol – triacylglycerols/5– HDL-cholesterol). A portion from liver and kidney was blotted, weighed and homogenized with methanol (3 volumes). It was used for the estimation of TBARs [40] and hydroperoxides [41]. Another portion of the tissues was homogenized with phosphate buffer saline and used for the estimation of GSH [42], Catalase (CAT) [43] and SOD [44].

Measurement of lipid peroxidation

A thiobarbituric acid reactive substances (TBARS) assay kit (ZeptoMetrix) was used to measure the lipid peroxidation products and malondialdehyde (MDA) equivalents [45]. In brief, liver and renal tissues were homogenized with 0.1 mol/l sodium phosphate buffer (pH 7.4). One hundred microliters of homogenate were mixed with 2.5 ml reaction buffer (provided by the kit) and heated at 95 C for 60 min. After the mixture had cooled, the absorbance of the supernatant was measured at 532 nm using a spectrophotometer. The lipid peroxidation products were expressed in terms of MDA equivalents.

Measurement of antioxidant enzymes

Superoxide dismutase (SOD) and Catalase (CAT) activities were determined using assay kits. Briefly, liver and renal tissues were weighed and homogenized with appropriate buffers (provided by the kits). The homogenates were then determined following the procedures provided by the respective manufacturers. The superoxide dismutase assay kit utilizes a tetrazolium salt for detection of superoxide radicals generated by red formazan dye reduction produced [46]. One unit (U) of SOD activity is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. The catalase assay kit utilizes the peroxidative function of CAT for determination of enzyme activity [47]. The method is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H_2O_2. The generated formaldehyde is assayed spectrophotometrically with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole as the chromogen. One unit (U) of CAT activity is defined as the amount of enzyme that will cause the formation of 1.0nmol of formaldehyde per minute at 25 C.

Statistical analysis

All data were expressed as mean ± SD. All analyses utilized SPSS 13.0 statistical package for Windows (SPSS, 13.0 software, Inc., Chicago, IL, 2009)[48]. A one-way analysis of variance (ANOVA) was employed for comparisons of means of the different groups. A p-value 0.05 was accepted as statistically significant. Diabetic positive control rats were compared with normal control rats. Experimental groups were compared with positive and normal controls. Also, diabetic rats treated with different doses of *Malva parviflora* seeds fixed oil as well as glibenclamide were compared with each other.

Results and discussion

Determination of the physicochemical properties of the oil

Physicochemical properties of the seed oil of *Malva parviflora* are an important criterion of purity of the oil. Refractive index is the ratio of the sine of the angle of incidence and the sine of the angle of refraction of a light beam of given wavelength passing from air into the oil at constant temperature (20 C), the result of determination was 1.223.Saponification index is the number of mg of KOH required to form 1 gm of ester, the result was 11.564.
It is the first report about physicochemical properties of the seed oil of *Malva parviflora*, as far as we know.

Fatty acid profile

The oil content of the dried seed was found to be (3.3%) , 9 fatty acids were identified, the major fatty acid was coriolic acid comprising (31.60%) of the total fatty matter, followed by pentadecanoic acid (30.05%), whereas eicosanoic acid was the lowest fatty acid comprising (0.47%). Our study differs from that done by Moghis et al [49], as his study revealed the predominance of vernolic acid followed by coriolic acid, also the presence of epoxy fatty acids, whereas it was in agreement with our study in that it revealed the presence of cyclopropene fatty acids e.g. malvalic acid in the seed fixed oil. The cyclopropenoid fatty acids manifest a number of unusual properties; because the cyclopropane ring is a very reactive and highly strained. The main imputes leading to the discovery of cyclopropenoid fatty acids came from the food and agricultural industries. Cyclopropenoid fatty acids have been investigated extensively owing to their biological effects on animals and their anti-carcinogenic properties [50]. The fatty acids composition demonstrates high amounts of

polyunsaturated fatty acids (52.86%) of the total fatty acids, the variation of seed oil composition may be attributed to various factors including the geographical origin of samples. The results were compiled in Table (1).

Table (1): GC/MS of seed oil of *Malvaparviflora* seeds fixed oil

Fatty acids	Constituent	Seed oil %
C15:0	Pentadecanoic acid	30.05
C18:0	Stearic acid	14.07
C18:1	Malvalic acid	0.78
C18:1	Octadecenoic acid	16.61
C18:2	Coriolic acid	31.60
C18:3	9,12,15-octadecatrienoic acid	1.69
C20:0	Cyclopropaneoctanoic acid, 2-octyl-methyl ester	2.55
C20:0	Eicosanoic acid	0.47
C20:1	Methyl 2- octylcyclopropene-1-octanoate	2.18
*SFA		47.14
*PUFA		52.86

SFA: saturated fatty acids, PUFA: poly unsaturated fatty acids

Determination of Total Phenols and Total Flavonoids

Total phenols were determined colorimetrically and were found to be (31.2mg/100g), while total flavonoids were (14.8mg/100g).

Determination of Total Protein &Total Carbohydrates and Total Lipids

Total protein was (16.5%), total carbohydrates were (34.60%) and total lipid was (14%) w/w.
These results support the use of the seeds as a herbal nutrition supplement.

Determination of Vitamin A and C

Vitamin A found to be (80mg/100g) and vitamin C as (14mg/100g). The recommended dietary allowances values developed by the Food Nutrition Board (FNB) for vitamin A is 600 µg and vitamin C is 75mg; respectively [51]. These results suggest the use of the seeds as vitamins supplement also these vitamins supplementation triggers oxidative stress in normal tissue [52-53].

LD50 of Malva parviflora seeds fixed oil

The results are given in Table (2) shows that oral administration of *Malva parviflora* seeds fixed oil in doses of 7000, 10000, 15000, 17000, 20000 and 25000mg/kg b.w. resulted in mortalities of 0, 1, 3, 7, 9 and 10 respectively. The dose of *Malva parviflora* seeds fixed oil that killed half of the rat (LD_{50}) was 15700mg/kg.b.w.

Toxic symptoms: Malva parviflora seeds fixed oil administrated rats exhibit an increase in heart rate and rapid respiration. There is a general depression in activity of rats. The mucous of the eye became brownish in cooler and the skin and toes bluish. The temperature of the rats extremities dropped with the toes and tail being cool.

Effect of Malva parviflora seeds fixed oil on body weight, blood glucose and insulin levels

Administration of STZ significantly (P<0.01) reduced body weight in diabetic rats compared to normal control rats, Table (3). In diabetic rats, treatment of both doses of *Malva parviflora seeds oil* and glibenclamide significantly (P<0.01) increased body weight compared to diabetic control rats. The blood glucose level was significantly (P<0.01) increased but decreased the level of insulin (P<0.01) after the administration of STZ compared to the normal control rats. Oral treatment of *Malva parviflora* seeds *oil* 157 and 314mg/kg.b.w. and glibenclamide (5mg/kg) decreased significantly (P<0.01) blood glucose level as well as the level of plasma insulin was significantly increased (P<0.01) in diabetic rats compared to diabetic control rats as shown in Table (4).

Effect of Malva parviflora seeds oil on lipid profiles

In STZ-induced diabetic rats, TC, TG and LDL levels were increased and HDL level was decreased significantly (P<0.01) compared to normal control rats. In diabetic rats, administration of *Malva parviflora* seeds *oil*157 and 314mg/kg.b.w. dose showed significant reduction (P<0.01) of TC, TG and LDL levels compared

to diabetic control rats. Also, a significant increase (P<0.01) level of HDL was observed in diabetic rats treated with both doses of *Malva parviflora seeds oil* and glibenclamide compared to diabetic control rats, Table (5).

Antioxidant activity of Malvaparviflora seeds oil

The concentrations of TBARs and hydroperoxides in liver and kidney of control and experimental groups of rats were complied in Table (6). The levels of TBARs and hydroperoxides in diabetic rats were significantly higher (P<0.01) than control rats, whereas diabetic rats-treated with *Malva parviflora seeds oil*157 and 314mg/kg.b.w. and glibenclamide restored the altered values close to that of the normal. The antioxidant activity of *Malva parviflora* seeds oil in liver and kidney was studied in diabetic rats and the data were compiled in Table (7). The present results showed significant decrease (P< 0.01) in GSH, SOD and CAT levels when compared to normal control rats. These altered above antioxidant levels were reversed significantly (P<0.01) to near normal levels after the administration of *Malva parviflora* seeds fixed oil(157and 314mg/kg.b.w.) dose and glibenclamide(5mg/kg) dose compared to diabetic control rats.

Table (2): Determination of LD_{50} of *Malva parviflora* seeds fixed oil given orally in adult rats

Group Number	Dose (mg/kg)	No. of animals/group	No. of dead animals	(Z)	(d)	(Z.d)
1	7000	10	0	0.5	3000	1500
2	10000	10	1	2.0	5000	10000
3	15000	10	3	5.0	2000	10000
4	17000	10	7	8.0	3000	24000
5	20000	10	9	9.5	5000	47500
6	25000	10	10	0	00	00

$$LD_{50} = Dm - \frac{\sum (Z \cdot d)}{n}$$

$$LD_{50} = 25000 - \frac{93000}{10} = 15700 \text{mg/ kg. b.w.}$$

Table (3):Effect of *Malva parviflora seeds oil*(MPO) on body weight in normal and diabetic rats

Groups/days Treatment/Dose (mg/mL)	Intervals				
	Zero time	**7 days**	**14 days**	**21 days**	**28 days**
Normal Control	185.48± 2.50	186.80± 2.90	188.20± 1.80	197.45 ± 2.45	203.30 ± 5.00
Control + MPO (157mg/kg)	180.60 ± 3.70	183.70± 2.60	190.60 ± 2.15	193.20 ± 4.00	205.00± 3.69
Control +MPO (314mg/kg)	185.50 ± 1.80	184.60 ± 3.10	187.50 ± 4.70	194.20 ± 5.49	203.50 ± 4.08
Diabetic Control	184.00± 2.30	170.60± 4.00$^{@}$	165.40± 3.54$^{@a}$	157.00 ± 3.45$^{@a}$	146.80± 2.74$^{@a}$
Diabetic + MPO (157mg/kg)	186.40± 2.47	174.70 ± 2.60^{a}	180.80 ± 3.00$^{@b}$	184.00± 2.40$^{@b}$	188.25 ± 1.45$^{@b}$
Diabetic +MPO (314mg/kg)	187.80± 3.80	184.35± 3.25$^{@b}$	189.76± 2.60$^{@b}$	193.60± 5.00$^{@b}$	197.30 ± 2.60$^{@b}$
Diabetic+Glibenclamide(5mg/kg)	183.00± 4.15	185.00± 2.87$^{@b}$	186.40± 2.40$^{@b}$	190.25± 1.90$^{@b}$	194.60± 3.15$^{@b}$

Values are given as mean ± SD for groups of eight animals each. Values are statistically significant at $^{@}P$<0.01. Diabeticcontrol rats were compared with normal control rats. Experimental groups were compared with diabetic control. a: significant from normal control; b: significant from diabetic control; c: significant from diabetic + MPO (157mg/kg) supplement group; d: significant from diabetic +MPO (314mg/kg) supplemented group.

Table (4): Effect of *Malva parviflora* seeds fixed oil (MPO)on plasma glucose and insulin levels in control and experimental groups of rats

Groups Treatment/Dose (mg/mL)	Glucose (mg/dl)	Insulin (μU/ml)
Normal Control	110.45±5.25	4.86 ±0.45
Control + MPO (157mg/kg)	107.35±4.11	4.45 ± 0.33
Control +MPO (314mg/kg)	113.25±5.25	4.76 ± 0.40
Diabetic Control	410.60±11.60$^{@a}$	2.17 ±0.18$^{@a}$
Diabetic + MPO (157mg/kg)	202.4 ±9.84$^{@ab}$	3.40±0.25$^{@ab}$
Diabetic +MPO (314mg/kg)	188.67±12.37$^{@abc}$	3.65±0.38$^{@ab}$
Diabetic +Glibenclamide(5mg/kg)	163.25 ±7.49$^{@abcd}$	3.90 ±0.22$^{@abc}$

Values are given as mean ± SD for groups of eight animals each. Values are statistically significant at $^{@}P<0.01$. Diabetic control rats were compared with normal control rats. Experimental groups were compared with the diabetic control rats. a: significant from normal control;b: significant from diabetic control; c: significant from diabetic + MPO (157mg/kg) supplement group; d: significant from diabetic +MPO (314mg/kg) supplemented group.

Table (5):Effect of *Malva parviflora* seeds oil (MPO) on plasma triglyceride (TG), total Cholesterol (TC), HDL-cholesterol (HDL-C) and LDL- cholesterol (LDL-C) in control and experimental groups of rats.

Groups Treatment/Dose (mg/mL)	TG (mg/dl)	TC (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
Normal Control	82.40±6.25	90.23±3.6	33.25±2.69	31.1± 3.50
Control + MPO (157mg/kg)	83.69±6.15	92.70±2.44	31.45±2.29	33.7±2.47
Control +MPO (314mg/kg)	87.10±5.69	96.86±2.97	34.70±1.48	33.03±3.57
Diabetic Control	198.74±11.80$^{@a}$	216.10±4.70$^{@a}$	20.80±3.79$^{@a}$	134.69±5.60$^{@a}$
Diabetic + MPO (157mg/kg)	117.35±9.45$^{@ab}$	144.50±6.25$^{@ab}$	30.95±3.45$^{@b}$	57.5±2.87$^{@ab}$
Diabetic +MPO (314mg/kg)	110.90±8.00$^{@ab}$	137.35±4.34$^{@ab}$	34.60±2.64$^{@b}$	48.83±3.70$^{@ab}$
Diabetic +Glibenclamide(5mg/kg)	105.23±6.59$^{@abc}$	130.50±1.81$^{@abc}$	30.70±2.57$^{@b}$	48.43±2.47$^{@ab}$

Values represent the mean ± SE (n=8). Diabetic control rats were compared with normal control rats. Experimental groups were compared with the diabetic control rats. LDL-C (mg/dl) = TC-HDL-[TG / 5], $^{@}$Significantly different from control group at *p< 0.01*. a: significant from normal control; b: significant from diabetic control; c: significant from diabetic + MPO (157mg/kg) supplement group.

Table (6): Effect of *Malva parviflora* seeds fixed oil(MPO) on liver and kidney TBARs and HP in control and experimental groups of rats.

Groups Treatment/Dose (mg/mL)	Liver		Kidney	
	TBARs mM/g. tissue	**HP mM/g. tissue**	**TBARs mM/g. tissue**	**HP mM/g. tissue**
Normal Control	45.76± 7.1	0.39± 0.040	48.76± 4.11	0.26± 0.04
Control + MPO (157mg/kg)	43.30± 3.45	0.37± 0.077	43.74± 3.15	0.24± 0.06
Control +MPO (314mg/kg)	42.77± 0.04	0.38± 0.020	45.70± 2.73	0.25± 0.01
Diabetic Control	121.15±5.90$^{@a}$	0.65± 0.053$^{@a}$	91.32± 3.62$^{@a}$	0.46± 0.03$^{@a}$
Diabetic + MPO (157mg/kg)	43.58± 6.88$^{@b}$	0.40± 0.011$^{@b}$	44.80± 5.17$^{@b}$	0.25± 0.17$^{@b}$
Diabetic +MPO (314mg/kg)	39.54± 2.56$^{@b}$	0.35± 0.026$^{@b}$	43.75± 4.60$^{@b}$	0.21± 0.03$^{@b}$
Diabetic +Glibenclamide(5mg/kg)	44.60± 3.25$^{@b}$	0.43± 0.040$^{@b}$	49.86± 4.81$^{@b}$	0.35± 0.04$^{@b}$

Values represent the mean ± SE (n=8). Diabetic control rats were compared with normal control rats. Experimental groups were compared with the diabetic control rats. $^{@}$Significantly different from control group at *p< 0.01*. a: significant from normal control; b: significant from diabetic control.

Table (7):Effect of *Malva parviflora* seeds fixedoil (MPO) on liver and kidney GSH, SOD and CAT in control and experimental groups of rats.

Groups Treatment/Dose (mg/mL)	Liver			Kidney		
	GSH µmole/100g. tissue	SOD µmole/100g. tissue	CAT µmole/100g. tissue	GSH µmole/100g. tissue	SOD µmole/100g. tissue	CAT µmole/100g. tissue
Normal Control	375.35± 12.36	136.70± 11.25	110.90± 6.57	154.78± 12.64	122.40± 9.00	94.30± 7.65
Control + MPO (157mg/kg)	366.45± 17.80	135.66± 9.87	115.34± 8.22	150.90± 9.40	125.74± 7.52	91.66± 8.06
Control +MPO (314mg/kg)	380.90± 18.11	139.81± 15.43	117.60± 6.00	157.64± 14.30	127.06± 13.20	96.50± 9.00
Diabetic Control	174.30± 15.68$^{@a}$	63.42±17.55$^{@a}$	52.11±7.60$^{@a}$	53.16±6.55$^{@a}$	73.20±8.50$^{@a}$	35.49±3.74$^{@a}$
Diabetic + MPO (157mg/kg)	325.40± 17.69$^{@ab}$	121.00±13.40$^{@ab}$	117.54±7.15$^{@b}$	132.76±8.70$^{@ab}$	115.40±10.90$^{@b}$	85.16±5.90$^{@b}$
Diabetic +MPO (314mg/kg)	368.73± 13.42$^{@abc}$	134.66±9.64$^{@b}$	129.80±11.25$^{@ab}$	165.11±13.90$^{@b}$	132.85±11.74$^{@b}$	90.44±6.11$^{@b}$
Diabetic+Glibenclamide(5mg/kg)	310.60± 19.84$^{@abd}$	115.40±8.11$^{@abd}$	113.00±6.60$^{@b}$	142.50±7.84$^{@abd}$	117.45±9.33$^{@bd}$	80.15±4.84$^{@b}$

Values represent the mean ± SE (n=8). Diabetic control rats were compared with normal control rats. Experimental groups were compared with the diabetic control rats. Activity is expressed as: 50% of inhibition of epinephrine auto oxidation per min for SOD; µmoles of hydrogen peroxide decomposed per min. for catalase; $^{@}$significantly different from control group at $p< 0.01$.a: significant from normal control;b: significant from diabetic control; c: significant from diabetic + MPO (157mg/kg) supplement group; d: significant from diabetic +MPO (314mg/kg) supplemented group.

Antidiabetic and antioxidant activity of *Malva parviflora* seeds fixed oil (MPO)

Diabetes mellitus is one of the most widespread diseases in developed countries. Diabetes mellitus is a disorder that is characterized by hyperglycemia, (high blood sugar). Diabetes mellitus Type 1 (T1DM) results from the auto-immune destruction of the insulin-producing beta cells in the pancreas and the subsequent lack of insulin. The literature quotes over 900 plants supposed to be useful in treatment traditionally [54] and some of them have also received scientific and medical evaluation to assess their efficacy. The present study was conducted to evaluate nutritional value as well as the beneficial effects of *Malva parviflora* seeds oil on antioxidant status in STZ-induced diabetic rats.

In acute toxicity study, *Malva parviflora* seeds fixed oil did not show significant toxicity signs when observed for the parameters during the first four hours and followed by daily observations for 21 days and no mortality was also observed, also, *Malva parviflora* seeds oil was found to be safe at (7000, 10000, 15000 and 17000mg/kg b.wt) as shown in Table (2).

STZ [2-deoxy-2- (3-methyl-3-nitrosoureido)-D-glucopyranose] is commonly used to induce experimental diabetes in animals [55]. STZ -induced diabetes may be due to vitiate glucose oxidation and reduction of insulin biosynthesis and secretion. The toxicity of STZ is due to DNA alkylation of its methyl nitrosourea moiety mainly at 6 *O*-position of guanine [56]. The transfer of methyl group from STZ to the DNA molecule causes damage which results in fragmentation of DNA and functional defects of the beta cells. Moreover, STZ is potential to act as an intracellular nitric oxide (NO) donor and generates reactive oxygen species (ROS). The synergistic action of both NO and ROS may also contribute to DNA fragmentation and other deleterious changes caused by STZ [57].

Single dose of STZ (55mg/kg.b.w. rats) in sterile citrate buffer (e.g. pH 4.5, 0.1M) was administered intraperitoneally [58]. The rats were allowed to resume feeding and drinking of 5% glucose solution for 30 minutes after STZ administration to avoid exposure of rats to a sudden drop in the level of insulin. Diabetes develops gradually and was assessed after a seven days. In the present study, the plasma glucose level of about 13.8mM indicates the induction of diabetes mellitus. Glibenclamide is often used as an insulin stimulant in many studies and also used as a standard antidiabetic drug in STZ-induced moderate diabetes to compare the antidiabetic properties different hypoglycemic compounds [59-61].

This model permits the evaluation of the effect of MPO in an animal without the interference of the side effects induced by STZ [62]. In our study, elevated blood glucose level and decreased insulin level were observed in STZ-induced diabetic rats as shown in Table (4). Oral administration of MPO and glibenclamide to the diabetic rats significantly reduced blood glucose level with compared to diabetic control rats. Also, the decreased insulin level was noticed in diabetic rats compared to normal control rats which directly support and represent STZ-mediated β-cells destruction or damage. In diabetic rats, treatment of MPO and glibenclamide increased the insulin level compared to diabetic control rats. Hence, the hypoglycemic activity of MPO may be due to presence of cyclopropene and high percentage of unsaturated fatty acids [62], as well as adequate percentages of vitamins A and C which show its protective action against STZ-mediated damage to the pancreatic β-cells and also possibly because of regeneration of damaged beta cell or increased insulin release or secretion.

Muscle wasting is an unintentional loss of body weight due to accelerated muscle proteolysis, resulting in loss of body cell mass. Insulin is an important regulator of protein synthesis and proteolysis in skeletal muscle. Insulin resistance or deficiency produces impaired muscle protein turnover and muscle wasting. The uncontrolled diabetes is associated with severe muscle wasting [63]. In this study, STZ-induced diabetes is characterized by severe loss in body weight [64] and this was also seen in the present study MPO and glibenclamide administration controlled this loss in body weight Table (3). However, it did not normalize the body weight completely as it remained lesser than normal control rats. The decrease in body weight observed in diabetic rats might

be the result of protein wasting due to unavailability of carbohydrate for utilization as an energy source [65].

In the present study, increases in plasma TC, TG and LDL levels as well as the reduction of HDL level were observed in STZ-induced diabetic rats as shown in Table (5). It is interesting to note that MPO did not only lower the TC, TG and LDL levels, but also enhanced the cardio protective lipid HDL of the diabetic rats after 30 days of treatment. This may be due to presence of the high percentage of unsaturated fatty acids and cycolpropene fatty acids, as well as presence of suitable percentages of vitamins A and C. The increase in HDL-cholesterol is a desirable feature. In addition, the reduction in TC, TG and LDL-cholesterol levels could be beneficial in preventing diabetic complications as well as improving lipid metabolism in diabetics [66]. This would definitely reduce the incidence of coronary events being the major cause of morbidity and deaths in diabetes subjects [67]. HDL-C transports cholesterol from peripheral tissues to the liver, thereby reducing the amount stored in tissues and decreasing the likelihood of getting atherosclerotic plagues [68].

This study indicated that MPO significantly reduced TC, TG and LDL levels as well as increased HDL level, as shown in Table (5), which could be due to stimulating effect on insulin secretion from pancreatic β-cells Table (4). The possible mechanism by which MPO can exert lipid lowering activities may be explained by decreasing the cholesterol biosynthesis, particularly by decreasing the activity of hydroxyl-3-methylglutaryl-coenzyme A (HMG–CoA) reductase or by reducing the NADPH required for cholesterol synthesis and/or by stimulating glucose utilization [69]. In our study, it has been proposed that MPO and glibenclamide acted in a similar way by increasing insulin production in STZ-induced diabetic rats and lowering TG level by activation of the enzyme lipoprotein lipase, because insulin activates it [70].

Several studies have demonstrated the involvement of free radicals in the genesis of diabetes mellitus and their role in the induction of lipid peroxidation during diabetes [71]. It has been reported that in diabetes mellitus, oxygen free radicals are generated by stimulating H_2O_2 *in vitro* as well as *in vivo* and in pancreatic β-cells [71]. The increased lipid peroxidation in the diabetic animals may be due to the observed remarkable increase in the concentration of TBARs and HPs (lipid peroxidative markers) in the liver and kidney of diabetic rats [72]. Moreover, drastic reduction of in vivo antioxidant enzymes level in various tissues was reported in diabetic condition [72]. In our study, decreased levels of liver and kidney GSH, SOD and CAT as well as increased level of TBARs and HPs were observed in liver, renal and heart tissues of STZ-induced diabetic rats compared to normal control rats as complied in Tables (6 and 7). The reduction of above enzymes directly reflects the oxidative stress in diabetic rats and these enzyme levels changes may be due to generation of free radicals by auto-oxidation of glucose, glycosylation in hyperglycemic condition as well as STZ mediated generation of ROS by its NO donor property to the intracellular molecules. In the present study, increased GSH, SOD and CAT levels as shown in Tables (6 and 7) as well as reduced TBARs and HPs levels were noticed in diabetic rats after the administration of both doses of MPO and glibenclamide in liver and kidney [73]. The above actions represent the antioxidant property of MPO in diabetic condition and hence, MPO possesses a potential to reduce or prevent the diabetic complications due to presence of cyclopropene and poly unsaturated fatty acids as well as vitamins A and C [74 and 75].

Nutritional evaluation and antioxidant effect of Malva parviflora seeds fixed oil (MPO) against STZ-induced diabetic complications in rats has not been reported earlier to our knowledge, and this study is perhaps the first observation of its kind. Conclusively, our observations have clearly demonstrated that the Malva parviflora seeds oil (MPO) has significant antioxidant and anti-hyperglycaemic activity due to presence of cyclopropene and polyunsaturated fatty acids as well as vitamins A and C. MPO possessed a capability to inhibit the lipid peroxidation and activate the antioxidant markers (GSH, SOD and CAT) in diabetes. Also, the ability of *Malva parviflora* seeds oil (MPO) to reduce oxidative stress may help to prevent diabetic complications. More studies are needed to prove its medicinal and biological importance which may pave the way for possible therapeutic applications.

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