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Malva parviflora seed oil; Isolation, gas chromatographic profiling and its cardioprotective activity against myocardial infraction in animal model

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ABSTRACT

Myocardial infarction (MI), one of the most perilous types of cardiac illness, carries a significant burden of mortality and morbidity. This study aims to shed light on the impact of *Malva parviflora* seed oil (MPSO) on plasma cardiac function tests, levels of cardiac inflammatory mediators, and the expression of cardiac miRNA140-5p and miRNA208b genes in a rat model of MI induced by isoproterenol (ISO). The methods involved the extraction of MPSO using hexane, with the determination of fatty acid contents accomplished through GC spectrometry. Adult albino rats, weighing 185 ± 7 g, were divided into five groups (n = 6): normal control rats, ISO-treated rats, ISO-treated rats with MPSO (157 and/or 314 mg/kg, respectively), and ISO-treated rats with omega (100 mg/kg, respectively) for a duration of four weeks. The results revealed that *Malva parviflora* seeds yielded 3.3 gm/100 dry seeds. Among the nine fatty acid components identified, coriolic acid was the most abundant (31.60 %), followed by pentadecanoic acid (30.05 %). The cardioprotective potential of MPSO was assessed in rats subjected to ISO-induced cardiac injury. Following 24 h of ISO treatment, rats displayed elevated levels of plasma cardiac troponin I (cTnI), troponin T (cTnT), lactate dehydrogenase (LDH), and B-Type Natriuretic Peptide (BNP), as well as cardiac BcL-2, P53, toll-like receptor 4 (TLR4), NF-κB (Nuclear factor kappa-light-chain-enhancer of activated B cells), thiobarbituric acid reactive substances (TBARs), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), miRNA-140-5p, and miRNA-208b gene expression. Histopathological examination confirmed cardiac injury in ISO-treated rats. Furthermore, MPSO mitigated the elevation of cardiac enzymes and TBARS, as well as cardiac inflammatory mediators, while concurrently downregulating the expression levels of miRNA-140-5p and miRNA-208b genes. Conversely, the enhancement of cardiac GSH, SOD, and CAT activity demonstrated the antioxidant capabilities of MPSO against ISO-induced cardiac injury. Histopathological findings underscored MPSO's protective effect on cardiac tissue against oxidative damage in ISO-treated rats. In nutshel, the findings of this study unveil the cardioprotective and free radical scavenging attributes of MPSO in rats with ISO-induced cardiac damage. MPSO appears to provide cardiac protection against free radicals and inflammation induced by xenobiotics, potentially owing to its rich content of polyphenols, flavonoids, polyunsaturated fatty acids (PUFAs), and cyclopropenoid fatty acids.

1. Introduction

Myocardial infarction (MI) stands as one of the most devastating forms of heart disease, marked by a disturbingly high mortality and morbidity rate (Li et al., 2015). According to the World Health Organization (WHO), MI accounts for nearly 50 % of all cardiovascular disease (CVD)-related fatalities (Lu et al., 2018). MI results from an insufficient supply of oxygenated blood to the myocardium through a

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coronary artery, leading to an inequality in the demand for oxygen and nutrients, ultimately culminating in myocardial injury (Reed et al., 2017, Abdu et al., 2020). Various reports have substantiated that oxidative stress, inflammatory responses, necrosis, and obesity are among the primary culprits contributing to MI (Panda et al., 2014, Wong et al., 2017, Ouyang et al., 2019).

The intricate relationship between oxidative stress and MI, along with the significant roles played by various plant compounds with antioxidant properties, has been extensively elucidated by numerous researchers (Hussein, 2011, Hussein 2012, El-gizawy and Hussein, 2017). Natural remedies endowed with antioxidant attributes hold promise in mitigating the repercussions of obesity-related issues, including atherosclerosis and certain malignancies (H Jr and AG 2010, El Gizawy et al., 2019, Elgizawy et al., 2021, Mohamad et al., 2022). Among these potential remedies, Malva parviflora L., a herb naturally distributed across Africa, Asia, and Europe, stands out. It is known by various names, with "Butter plant" being a common moniker and "Sonschal" being its regional appellation (Tosif et al., 2021). Fixed oil *of Malva parviflora* had antidiabetic efficacy in rats at dosages of 157 and 314 mg/kg body their weight, as reported by El-Gizawy and Hussein in their 2015 study. Additionally, hepatoprotective, antibacterial, anti-inflammatory, antioxidant, and antifungal properties of Malva parviflora was proved by serval authors (Kahramanoğlu and Wan, 2020, Nazari et al., 2020, Dybka-Stępień et al., 2021). The plant is rich in phenolics, flavonoids, tannins, alkaloids, steroids, and mucilage, with mucilage finding applications in pharmaceuticals, food, nutraceuticals, and cosmetics (Ododo et al., 2016). Surprisingly, the potential of *Malva parviflora* seed oil, both in isolated form and as a dietary component, in the context of cardiovascular disease remains largely unexplored. Furthermoreas much as we are aware of, no published studies have delved into the cardioprotective benefits offered by *Malva parviflora* seed oil. Therefore, the main goal of this research was two fold: firstly, to isolate and characterize *Malva parviflora* seed oil, and secondly, to investigate its potential cardioprotective effects in the context of ISO-induced cardiac toxicity in rats. This investigation will assist the researcher in covering the crucial ground that many others were unable to investigate while evaluating MPSO as a promising novel drug in the treatment of cardiotoxicity. Thus, a novel explanation for the association between miRNA140-5p and miRNA208b gene signaling and cardiotoxicity may be developed.

2. Materials and methods

2.1. Plant material

Seeds of *Malva parviflora* were gathered from Horbit Village in the Egyptian Governorate of Al-Sharqia. Dr. Wafaa Amer, a professor of taxonomy at the Faculty of Science, recognized and verified the plant material. The voucher specimens with number 20220725 were stored at Faculty of Pharmacy in October 6th University's, Department of Pharmacognosy. After being thoroughly cleaned, the seeds were dried in the sun and then ground into a powder using a machine. The supplier of isoproterenol was Sigma Aldrich in the USA. We purchased methanol, pentane, hexane, and boron fluoride from Sigma-Aldrich in the United States.

2.2. Extraction of fixed oil

The *Malva parviflora* seed was very carefully cleaned by hand to remove all impurities, including other seeds, stones, and tiny stalks. It was baked at 50 °C for 12 h to dry it, and after that, it was ground into a powder with a particle size of 0.55–1.0 mm in a grinder. In a vacuum desiccator, the *Malva parviflora* seed powder was stored until use. Using a Soxhlet apparatus (Ajayi et al., 2006), *Malva parviflora* seed powder was stirred with hexane (1:10, m/V; 60–80 °C) for 6 h. After that, with the help of distillation method, hexane was extracted, and concentrated in a rotatory evaporator, and then allowed to air dry at 40 ± 2 °C.

2.3. BF_3-Methanol reagent preparation

A two-liter flask containing one liter of 100 % methanol was then chilled in an ice bath. 125 g of BF_3 are bubbled *via* a glass tube into the methanol, kept in fume hood. This reagent has a long shelf life and is excellent for usage up to 4 months after manufacturing (Metcalfe and Schmitz, 1961).

2.4. Fatty acid methyl ester preparation (FAME)

Malva parviflora seed powder (0.5 g) were taken into a a Quickfit® conical flask and followed by addition of 0.6 mL dichloromethane (0.6 mL), 4.0 mL sodium methoxide (0.5 N), and 14 % w/v boron trifluoride to catalyze esterification (Metcalfe and Schmitz, 1961, Hooper et al., 2004). After shaking the conical flask, it was heated to 50 °C for half an hour. 5.0 mL of water containing of glacial acetic acid (0.2 mL) was then added to the reaction mixture to quinch the reaction. 3.0 mL of petroleum ether were used to extract the esterified fatty acids at 40–60 °C. For further analysis, the clear extracted fraction was kept in storage at a temperature of −20 °C. A Gas Chromatograph System of HP 6890 series with Mass Selective Detector HP 5973 was used for the separation and estimation of FAME. The refractive sponification index (RSI) was calculated at 20 °C using methods documented by Ali et al. (Ali et al., 2022) and Min et al. (Min et al., 2013), respectively. The methods described by Zeynali et al. (Zeynali et al., 2023), and Ghiassi et al. (Tarzi et al., 2012) were used to determine the total flavonoid and phenolic concentrations, respectively. The technique developed by Kang et al. (Kang et al., 2020) was used to compute total protein, total carbohydrate, and total lipids.

3. Biochemical studies: Cardio protective and antioxidant activity of *M. parviflora* seed fixed oil

3.1. Experimental design

A total of thirty adult albino rats weighing 160 ± 10 g were purchased from the cancer institute at Cairo University. The October 6th University's Applied Health Sciences Technology ethical committee authorized the study protocol (NO. 20220524). The animals were housed in polypropylene cages with a natural light–dark cycle and humidity levels that complied with industry standards. There was water and regular pellets accessible, provided by the Cantacuzino Institute in Bucharest, Romania. As shown in Table 1, the rats were split into four groups of six rats each at random.

After the initial ISO dosages, the rats were fasted for 48 h. The blood was then collected using NaF as an anticoagulant agent. Then, fresh plasma was used to estimate level of BNP, LDH, cTnI, and cTnT in

Table 1
Treated animal grouping and description.

Groups	Group name	Treatment description
I	Normal control	3 mL of distilled water orally for 30 days
II	Isoproterenol (ISO)	Rats exposed with ISO (85 mg/k.b.w.) suspended in 3 mL of distilled water subcutaneously at the 29th and 30th day (Eldourghamy et al., 2023).
III	ISO + MPSO (157 mg)	Rats exposed with ISO + MPSO (157 mg) suspended in 3 mL of distilled water orally for 30 days (El-Gizawy and Hussein, 2015).
IV	ISO + MPSO (314 mg)	Rats exposed with ISO + MPSO (314 mg) suspended in 3 mL of distilled water orally for 30 days (El-Gizawy and Hussein, 2015).
V	ISO + Omega 3 (100 mg/kg.b.w.)	Rats exposed with ISO + Omega 3 suspended in 3 mL of distilled water orally for 30 days (Eldourghamy et al., 2023).

accordance with the kit manufacturer's instructions (Abcam, Cambridge, UK). The first part was homogenized to produce a 25 % W/V homogenate using ice-cold saline in a glass homogenizer (Universal Lab. Aid MPW-309, mechanikaprecyzyjna, Poland). Two distinct aliquots of homogenate were divided. According to the manufacturer's instructions, RayBio®, Abcam®, Elabscience®, and Elabscince® rat ELISA kits were used to quantify NF-κB, TLR4, BcL-2, and P53, in the first aliquot. The Tsikas technique (Tsikas, 2017) was used to determine the amount of MDA in the second aliquot after it had been deproteinized with 12 % (ice-cold) trichloroacetic acid and centrifuged at 1000*g*. The cytosolic fraction of the heart was created after centrifugation at 10500*g* for 15 min at 4 °C in a cooling ultra-centrifuge, and the diagnostic kit technique based on the approaches of Sinha (1972), Kakkar et al. (1984), and Owen and Butterfield (2010), were used to evaluate the activity of CAT, GSH, and SOD, respectively, in the clear supernatant.

3.2. Quantitative real-time PCR

Using a Sepasol-RNA1Super (Nakarai Tesque) and following the manufacturer's instructions, total RNA was extracted from cardiac tissues. Sections (10–15 g) of the recovered RNA were then subjected to real-time quantitative PCR testing. A two-step RT-PCR was used to evaluate the expression of the genes. As previously mentioned (Clotman et al., 2002), the levels of miRNA-140-5p and miRNA-208b gene expression were measured using quantitative real-time PCR. PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, and 0.4 M of specific primers made up the PCR reaction mixture (Table 2). 50 cc of the single-plex reaction mixture was used for conducting the assays. The reaction conditions were pre-incubated at 50 °C for two minutes and 95 °C for ten minutes, followed by 40 cycles of 95 °C for 15 s and 60 °C for one minute each. Automatic measurments were taken. The quantitative RT-PCR data is displayed as a control. GAPDH mRNA was employed as an internal control as shown in Table 2.

3.3. Histological assessment

After being fixed in 10 % neutral formalin solution, the second slice of cardiac tissues was dried in graded alcohol and embedded in paraffin. The tiny slices were mounted on glass slides (H&E) for light microscopic examination using Bancroft and Steven's approach (Bancroft and Steven, 1983).

3.4. Statistic evaluation

Three distinct determinations were made for the quantities of oil fatty acids and PCR analysis of gene expression, six independent readings were recorded for ELISA and spectrophotometric measurements, and the results were displayed as mean standard deviation (±SD). All the data were analyzed by using software SPSS/20, one-way of variance analysis (ANOVA) and the multiple comparison test 'Bonferroni'. The significance of a statistic was assessed using a $P < 0.05$ value.

Table 2
Primers used in real-time PCR.

Gene	Primer sequence
miR-140-5p	F: 5′-CTGTGTCCTGCCAGTGGTTTT-3′ R:5′-CGGTATCCTGTCCGTGGTTCT-3′
miR-208b	F: 5′-ACACTCCAGCTGGGATAAGACGAACA-3 R: 5′-TGGTGTCGTGGAGTCG-3
GAPDH	F: 5′-TCGGAGTCAACGGATTTGGT-3′ R: 5′-TTCCCGTTCTCAGCCTTGAC-3′

4. Results

4.1. Evaluation of the oil's physicochemical characteristics

Malva parviflora seed oil's physicochemical characteristics are a crucial indicator of the oil's purity. The refractive and saponification index of MPSO's were found to be 1.223 and 1.564 respectively at 20 °C. Table 3 provided an illustration of the fatty acid composition of MPSO. 100 g of dry *Malva parviflora* seed has 3.3 gm of oil. Additionally, the composition of fatty acids shows significant levels of saturated and polyunsaturated fatty acids, representing 47.14 and 52.86 % of the total fatty acids content, respectively. Coriolic acid (31.60 %), which was the major compound of the total fixed oil, followed by 30.05 % pentadecanoic acid.Colorimetric analysis revealed that the total phenols were (31.2 mg/100 g) and the total flavonoids were (14.8 mg/100 g) of dry seed. Additionally, there were 16.5, 34.60, and 14.0 gm of total protein, total carbs, and total lipid per 100 g of dry seed, correspondingly.

4.2. Effect of PMSO (157 and 314 mg/kg.b.w.), and omega 3 (100 mg) on plasma cTnI, cTnT, LDH and BNP in rats

Table 4 illustrates plasma cTnI, cTnT, LDH, and BNP levels. When the comparision are made to the normal rats in group (I), our data demonstrate a substantial rise ($p < 0.05$) in plasma cTnI, cTnT, LDH, and BNP levels of 145.83, 56.41, 45.04, and 73.58 %, respectively. After 4 weeks, ISO-treated rats receiving PMSO (157 mg/kg.b.w.) had 49.15, 21.31, 21.45, and 25.80 % lower plasma cTnI, cTnT, LDH, and BNP levels, respectively, compared to ISO-administrated rats ($p < 0.05$). Moreover, administration of PMSO (314 mg/kg.b.w.), the levels of cTnI, cTnT, LDH and BNP were significantly decreased by 54.24, 31.15, 27.81 and 44.32 %, respectively, as compared to the ISO-treated rats ($p < 0.05$). On the other hand, Rats receiving Omega 3 (100 mg) showed significant decreased cTnI, cTnT, LDH and BNP levels by 42.37, 21.31, 27.43 and 42.08 %, correspondingly, relative to ISO-exposed rats after 4 weeks ($p < 0.05$).

4.3. Effect of PMSO (157 and 314 mg/kg.b.w.), and omega 3 (100 mg) on cardiac BcL-2, P53, toll-like receptor 4 (TLR4) and NF-κB in rats

Cardiac BcL-2, P53, TLR4, and NF-κB levels were increased by 105.03, 83.35, 217.5, and 198.85 %, respectively, compared to normal rats (Table 5). When ISO-exposed rats were treated with PMSO (157 mg/kg.b.w.), cardiac BcL-2, P53, TLR4, and NF-κB levels were reduced by 20.95, 23.57, 36.61, and 30.86 %, respectively, compared to ISO-treated rats ($p < 0.05$). Furthermore, when rats were given ISO followed by PMSO (314 mg/kg.b.w.), their cardiac BcL-2, P53, TLR4, and NF-κB levels were reduced by 47.63, 38.80, 51.57, and 42.03 %, respectively, as compared to ISO-exposed rats ($p < 0.05$). Furthermore, when

Table 3
MPSO characterization by GC/MS.

Fatty Acids	Component	% Seed oil
C15:0	Pentadecanoic acid	30.05 ± 0.76
C18:0	Stearic acid	14.07 ± 0.32
C18:1	Malvalic acid	0.78 ± 0.04
C18:1	Octadecenoic acid	16.61 + 0.07
C18:2	Coriolic acid	31.60 ± 0.25
C18:3	9,12,15-octadecatrienoic acid	1.69 ± 0.06
C20:0	Cyclopropane octanoic acid, 2-octyl-methyl ester	2.55 ± 0.04
C20:0	Eicosanoic acid	0.47 ± 0.01
C20:1	Methyl 2-octylcyclopropene-1-octanoate	2.18 ± 0.03
*SFA		47.14 ± 0.83
**PUFA		52.86 ± 1.07

The data displayed are the mean ± standard deviation (SD) of the number of observations for each treatment.
* SFA stands for saturated fatty acids.
** PUFA stands for poly unsaturated fatty acids.

Table 4
Effect of MPSO on cTnI, cTn T, LDH and BNP in ISO treated rats.

Group No.	Group name	cTnI (ng/mL)	cTnT (ng/mL)	LDH (U/L)	BNP (pg/mL)
I	Normal control	0.24 ± 0.04[a]	0.39 ± 0.03[a]	392.45 ± 14.03[a]	61.78 ± 3.34[a]
II	Isoproterenol (ISO)	0.59 ± 0.07[e]	0.61 ± 0.05[d]	569.20 ± 22.25[d]	107.24 ± 8.60[e]
III	ISO + MPSO (157 mg)	0.30 ± 0.04[c]	0.48 ± 0.03[c]	447.09 ± 17.96[c]	79.50 ± 4.01[d]
IV	ISO + MPSO (314 mg)	0.27 ± 0.03[a]	0.42 ± 0.04[b]	410.92 ± 19.5[b]	59.71 ± 5.76[b]
V	ISO + Omega 3 (100 mg/kg.b.w.)	0.34 ± 0.02[d]	0.48 ± 0.0[c]	413.06 ± 25.47[b]	62.11 ± 4.95[c]

The data displayed are the mean ± standard deviation (SD) of the number of observations for each treatment.
For four weeks daily, the tested MPSO was administered orally at 157 and 314 mg/kg.b.w.
The parameters' high significant levels falls into the range of a < b < c < d.
Superscript alphabet 'a' represents significantly lower value than superscript 'b'. While in the same fashion superscript 'b' are significantly lower than superscript 'c & d'at level $p < 0.05$.
The data, those are followed by the same superscript afterward are not meaningfully unalike at level $p \leq 0.05$.

Table 5
Effect of MPSO on cardiac BcL-2, P53, TLR4 and NF-κB in ISO treated rats.

Group No.	Group name	Bcl2 (ng/mg tissue)	P53 (ng/mg tissue)	TLR4 (ng/mg tissue)	NF-κB (ng/mg tissue)
I	Normal control	7.20 ± 0.39[a]	20.85 ± 2.23[d]	0.80 ± 0.04[a]	4.38 ± 0.26[a]
II	Isoproterenol (ISO)	14.82 ± 1.88[c]	38.23 ± 13.30[a]	2.54 ± 0.32[d]	13.09 ± 0.67[e]
III	ISO + MPSO (157 mg)	10.53 ± 1.06[b]	29.08 ± 2.50[b]	1.61 ± 0.08[b,c]	9.05 ± 0.70[d]
IV	ISO + MPSO (314 mg)	7.76 ± 0.70[a]	23.29 ± 2.93	1.23 ± 0.10[b]	6.41 ± 0.19[b]
V	ISO + Omega 3 (100 mg/kg.b.w.)	7.66 ± 0.94[a]	25.88 ± 1.06[b]	1.40 ± 0.13[b]	7.94 ± 0.59[c]

The data displayed are the mean ± standard deviation (SD) of the number of observations for each treatment.
For four weeks daily, the tested MPSO was administered orally at 157 and 314 mg/kg.b.w.
The parameters' high significant levels falls into the range of a < b < c < d.

compared to ISO-exposed rats, treatment with Omega 3 (100 mg) reduced cardiac BcL-2, P53, TLR4, and NF-κB levels by 48.31, 32.30, 44.18, and 39.54 %, respectively ($p < 0.05$).

4.4. Effect of PMSO (157 and 314 mg/kg.b.w.), and omega 3 (100 mg) on cardiac MDA, GSH, SOD and CAT in rats

Comparing ISO-treated rats to control rats, the cardiac MDA levels were drastically elevated by 86.95 % (p 0.05), while the level of cardiac GSH, SOD and CAT were decreased by 73.29, 57.88, and 50.76 respectively in ISO-administered rats. When compared to ISO-treated rats, to the rats treated with ISO plus PMSO (157 and 314 mg/kg.b.w.) and ISO and omega 3 (100 mg), the level of cardiac MDA were considerably decreased ($p < 0.05$) by 24.34, 39.62, and 37.67 %, respectively. The cardiac GSH, SOD, and CAT levels were considerably higher in MI-induced rats treated with PMSO (157 mg/kg.b.w.) than in ISO-treated rats ($p < 0.05$) by 145.78, 35.58, and 32.29 %, respectively. Additionally, compared to rats exposed to ISO, PMSO (314 mg/kg b.w.) treatment significantly raised the levels of cardiac GSH, SOD, and CAT by 224.57, 120.25, and 104.34 %, respectively. Omega-3 supplementation also significantly enhanced the levels of cardiac GSH, SOD, and CAT by 172.77, 90.64, and 77.02 %, respectively, in comparison to rats that had undergone a MI ($p < 0.05$) (Table 6).

4.5. Effect of PMSO (157 and 314 mg/kg.b.w.), and omega 3 (100 mg) on cardiac miRNA140-5p and miRNA208b gene expression in rats

Figs. 1 and 2 show that cardiac miRNA140-5p and miRNA208b gene expression levels in MI-induced rats were significantly higher by 216.98 and 491.84 %, respectively than those in control rats. When compared to MI-induced rats, the administration of PMSO (157 mg/kg b.w.) results in a substantial decrease ($p < 0.05$) in the expression of the cardiac miRNA140-5p and miRNA208b genes by 32.44 and 17.24 %, respectively. Additionally, when compared to ISO-treated rats, the administration of PMSO (314 mg/kg b.w.) results in a significant douwn regulation in the expression of the cardiac miRNA140-5p and miRNA208b genes by 58.63 and 53.96 %, respectively. Omega 3 (100 mg) treatment to rats with MI also results in a significant reduction ($p < 0.05$) in the expression of the cardiac miRNA140-5p and miRNA208b genes by 50.29 and 44.31 %, respectively, when compared to ISO-administered rats.

4.6. Compared to the control group, distinct groups of rat cardiac tissues underwent histological analysis

The longitudinal muscle fibers of the cardiac myocytes in the normal group are branching and anastomosing (Fig. 3a). In the ISO-exposed groups, cardiac muscle is also inaccurately portrayed. Significantly more space now separates cardiomyocytes from one another (star). Both edoema between cardiac muscle fibers (e) and signs of enlarged and congested blood vessels (cbv) are seen. There are focal areas of deterioration and myocyte cytolysis (turn arrows). Areas of discontinuity (d) appear when cardiomyocytes lose their sarcoplasmic striations. Apoptotic muscle fibers can be seen, with hyper acidophilic cytoplasm and pyknotic nuclei in some cases (Fig. 3b). Cardiomyocytes from rats treated with ISO + MPOS (157 mg) had intact morphology and central oval vesicular nuclei (*). There are no cytoplasmic vacuoles present, but few clogged dilated blood vessels (cdbv) and mild atrophy in focal areas (a) were seen in Fig. 3c. Additionally, MI-induced rats treated with MPSO (314 mg/k.g.b.w.) showed longitudinal cardiac muscle fibers

Table 6
Effect of MPSO on cardiac TBARs, GSH, SOD and CAT in ISO-treated rats.

Group No.	Group name	MDA (nmol/mg tissue)	GSH (mg/mg tissue)	SOD (U/mg tissue)	CAT (μm H_2O_2/min/mg Tissue)
I	Normal control	23.67 ± 2.14[a]	15.54 ± 1.49[d]	9.14 ± 0.51[d]	3.27 ± 0.21[d]
II	Isoproterenol (ISO)	44.25 ± 3.18[d]	4.15 ± 0.42[a]	3.85 ± 0.52[a]	1.61 ± 0.16[a]
III	ISO + MPSO (157 mg)	33.46 ± 3.84[c]	10.20 ± 1.03[b]	5.22 ± 0.53[b]	2.13 ± 0.16[b]
IV	ISO + MPSO (314 mg)	26.72 ± 1.91[a]	13.47 ± 1.36[c]	8.48 ± 3.38[c]	3.29 ± 0.36[d]
V	ISO + Omega 3 (100 mg/kg.b.w.)	27.58 ± 1.85[a]	11.32 ± 1.57[b]	7.34 ± 0.30[b]	2.85 ± 0.28[c]

The data displayed are the mean ± standard deviation (SD) of the number of observations for each treatment.
For four weeks daily, the tested MPSO was administered orally at 157 and 314 mg/kg.b.w.
The parameters' high significant levels falls into the range of a < b < c < d.
Superscript alphabet 'a' represents significantly lower value than superscript 'b'. While in the same fashion superscript 'b' are significantly lower than superscript 'c & d'at level $p < 0.05$.
The data, those are followed by the same superscript afterward are not meaningfully unalike at level $p \leq 0.05$.

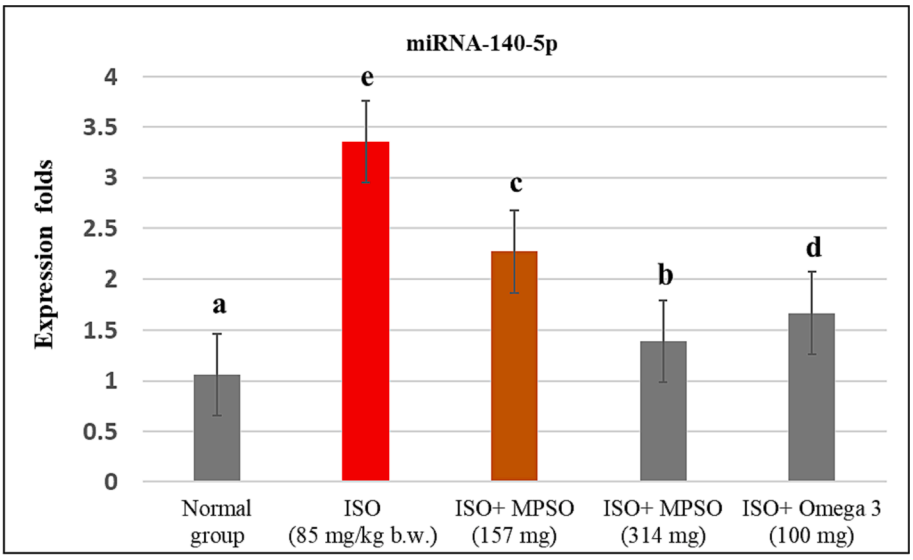

Fig. 1. Effect of MPSO on cardiac miRNA140-5p gene expression in Isoproterenol treated mice. When the data followed by the same letter, there is no significantly different at $p < 0.05$.

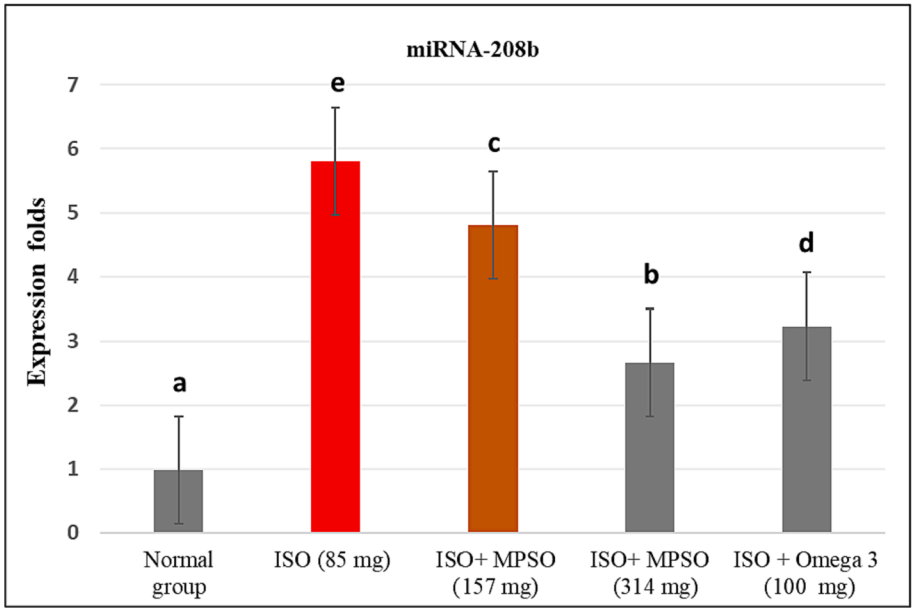

Fig. 2. Effect of MPSO on cardiac miRNA208b gene expression in Isoproterenol treated mice. When the data followed by the same letter, there is no significantly different at $p < 0.05$.

(MF) with regular, densely connected oval nuclei in the center. There are no spots of bleeding visible. Muscle fibers of various sizes were detected, ranging from thick to thin. No vacuoles were seen among cardiac muscle fibers. Few congested blood vessels were observed. (Fig. 3d). Moreover, administration of ISO then Omega (100 mg) shows a good picture of cardiomyocytes with normal histological structure. There were no spaces detected between muscle fiber. No leucocytes and lymphocytes were found. Mild disorganization was noticed amongst cardiac muscle fibers (Fig. 3e).

5. Discussion

The most prevalent form of myocardial infarction (MI), commonly recognized as atherosclerosis or Infarctive cardiac disease, represents the primary cause of heart attacks (Li et al., 2015). In recent years, plant-derived fixed oils have garnered substantial pharmacological significance due to their therapeutic potential encompassing antiviral, antioxidant, antidiabetic, anticancer, anti-inflammatory, bactericidal, and anti-atherosclerotic properties (Chen et al., 2002, Collins et al., 2003, Kinniry et al., 2006, Zanwar et al., 2011).

Our findings underscore the nutritional value of *Malva parviflora* seed fixed oil and its remarkable cardioprotective attributes against ISO-induced Myocardial infarction in rats. The seed is a rich source of various essential fatty acids, such as coriolic acid and pentadecanoic acid. We also found epoxy fatty acids, which is consistent with El-Gizawy and Hussein (2015) findings about the existence of cyclopropene fatty acids, particularly malvalic acid. Their findings were primarily vernolic acid, followed by coriolic acid. These cyclopropenoid fatty acids possess distinct properties due to the highly reactive and strained nature of the cyclopropane ring. Researchers have mostly concentrated on the biological evaluation of cyclopropenoid fatty acids in animals and their possible anti-carcinogenic qualities since their discovery, which

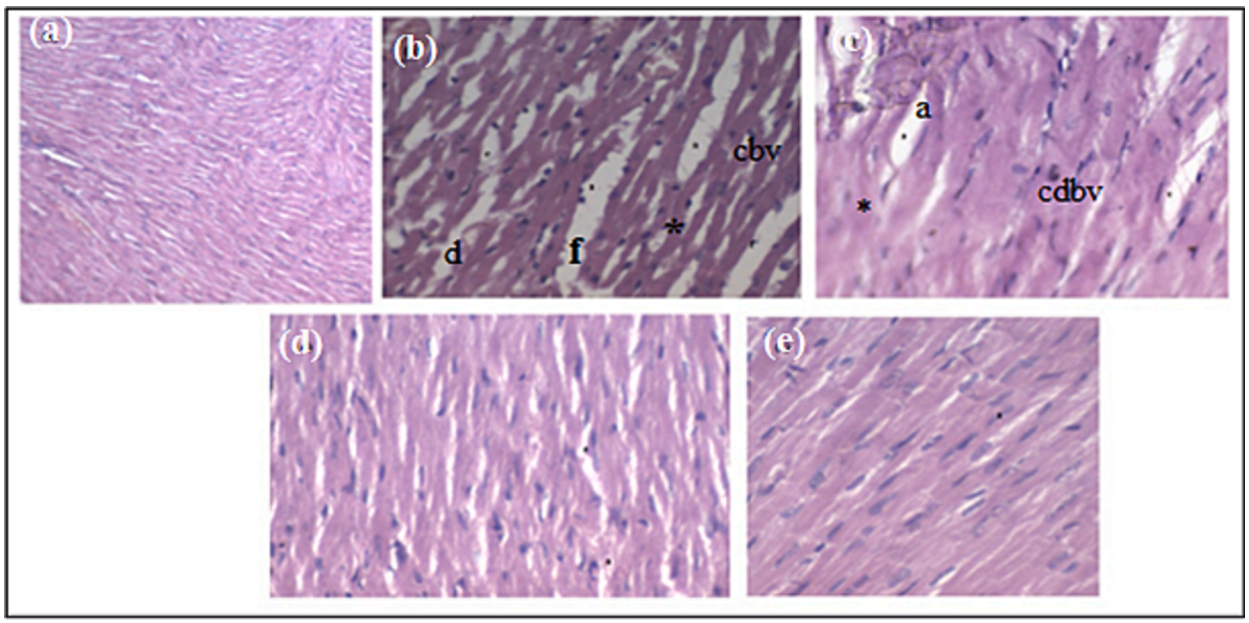

Fig. 3. Histological sections stained with hematoxylin and eosin (H&E; 200X) showing the hearts tissues of the mice of different groups in comparison to the control group. (a) Group 'I' reoresents normal control; (b) Group 'II' represents ISO (85 mg/kg.b.w.) administered group (c) Group 'III' represents ISO + MPSO (157 mg) treated group; (d) Group 'IV' represents ISO + MPSO (314 mg) treated group; (e) Group 'V' represents ISO + Omega 3 (100 mg) treated group. **Abbreviations**: e = edema in-between cardiac muscle fibers, f = fatty changes, a = trophy in focal areas.

came from the food and agricultural industries (Msalilwa et al., 2020).

In our study, ISO-induced acute MI was associated with elevated plasma levels of cardiac troponin I (cTnI), cardiac troponin T (cTnT), LDH, and BNP. Supramaximal dosages of isoproterenol have been shown to cause subendocardial myocardial ischemia, hypoxia, and necrosis in the past(Karthick and Prince, 2006, Roger et al., 2006, Ganapathy et al., 2014). In our rat model, *Malva parviflora* seed oil (MPSO) mitigated the elevation of cTnI, cTnT, LDH, and BNP levels, which could be explained by the existence of cyclopropenoid fatty acids, polyphenols, flavonoids, and PUFAs.

Our results are consistent with several studies showing that PUFAs can improve cTnI, cTnT, LDH, and BNP levels in ISO-treated rats (Anandan et al., 2007, Sokoła-Wysoczańska et al., 2018). These improvements may also be related to changes in coronary artery lumen. Moreover, the presence of malvalic acid in MPSO effectively ameliorated ISO-induced cardiac toxicity in the myocardium. Our findings align with those of a study (Priscilla and Prince, 2009) who, demonstrated the reduction of cardiac enzymes in serum following malvalic acid treatment in ISO-challenged rats, indicating the cardioprotective potential of cyclopropenoid fatty acids.

ISO-induced cardiotoxicity in our animal model showed distinctive pathophysiological features, including myocardial injury and increased levels of pro-inflammatory cytokines such as BcL-2, P53, TLR4, and NF-κB, as well as clear signs of oxidative stress. In contrast, MPSO effectively reduced the stimulation of TLR4 and NF-κB inflammatory signaling pathways in the context of acute cardiac damage, consistent with our study's observations. TLR4 stimulation typically activates NF-κB, leading to the generation of pro-inflammatory cytokines (Roy et al., 2016) and the activation of NADPH oxidase (NOX), resulting in the generation of reactive oxygen species (ROS) (Hsieh et al., 2016). Oxidative anxiety plays a pivotal role in NF-κB activation, which in turn can drive inflammation (Biswas, 2016). Elevated levels of pro-inflammatory cytokines are commonly observed in myocardial insults (Karin and Ben-Neriah, 2000), but in the MPSO-treated group, we observed a substantial reduction in these cytokine levels, suggesting potential anti-inflammatory properties contributing to its cardioprotective effects.

Our results highlight the presence of PUFAs, polyphenols, and malvalic acid in MPSO. Several studies have demonstrated the preventive action of dietary polyphenols in hypertension, cardiovascular disease (CVD), and diabetes treatment (Pandey and Rizvi, 2009). Natural polyphenols possess potent antioxidant properties and have been associated with a reduced risk of ischemic heart disease (Ginter and Simko, 2012). Additionally, malvalic acid has been shown to exhibit significant antioxidant activity (Maksoud et al., 2019, El Gizawy et al., 2021). Our study reveals a substantial reduction in myocardial necrosis, which can be attributed to the antioxidant properties of MPSO.

In our study, ISO treatment led to a significant increase in reactive oxygen species (ROS) production, resulting in myocardial damage and decreased levels of cardiac catalase (CAT), superoxide dismutase (SOD) and andglutathione (GSH). Numerous investigations have explored the role of ROS in cardiac damage (González-Montero et al., 2018). We observed that ISO treatment decreased SOD levels in heart tissue, increased plasma levels of cardiac damage markers (cTnI, cTnT, LDH, and BNP), in line with earlier studies (Ghorab et al., 2010, Li et al., 2012). MPSO significantly improved cardiac GSH, CAT, and SOD activity, concurrently reducing cardiac malondialdehyde (MDA) levels. Mitochondria, a prominent source of intracellular ROS, are susceptible to ROS-mediated oxidative damage in the heart. Increased ROS production due to excessive synthesis can lead to cell membrane damage, fatty acid oxidation, and TCA deactivation, altering TCA cycle enzyme activity and resulting in free radical-induced cardiac injury. Numerous investigations have demonstrated that abnormalities in the structure and composition of the mitochondrial membrane are important factors in myocardial infarction. (Gao et al., 2008, Kornfeld et al., 2015). These results suggest that MPSO exerts free radical scavenging activity, providing cardioprotection against ISO-induced damage, a hypothesis supported by histopathological analysis demonstrating the normal appearance of rat cardiac tissue.

The presence of PUFAs, polyphenols, and cyclopropenoid fatty acids in MPSO may mechanistically underpin its effects on downregulating cardiac miRNA140-5p and miRNA208b genes by depleting cardiac inflammatory mediators and TBARs (thiobarbituric acid reactive substances) and activating cardiac antioxidant biomarkers (GSH, SOD, and CAT). Dietary polyphenols have been shown in numerous studies to have the ability to reduce hypertension and cardiovascular disease. (CVD), and diabetes (Hussein, 2012a,b, Borik and Hussein, 2021,

Hussein et al., 2021, Soliman et al., 2022). Natural polyphenols possess potent antioxidant properties and have been linked to a reduced risk of ischemic heart disease (Boshra and Hussein, 2016, Gobba et al., 2018, Mosaad et al., 2022, Mostafa et al., 2023). Our findings suggest that MPSO may have therapeutic applications as a cardioprotective agent, in addition to its cytoprotective properties in preventing cardiac injury induced by ISO treatment.

Furthermore, our results unveiled a substantial increase in the expression of cardiac miRNA140-5p and miRNA208b genes in ISO-induced rats compared to normal rats. Elevated levels of miRNA140-5p and miRNA208b have been associated with cardiac dysfunction and rehospitalization in individuals with dilated cardiomyopathy. These miRNAs are triggered in a number of inflammatory disorders, including as hypertension, myocardial infarction, ischemia/reperfusion injury, myocarditis, and aortic valve illnesses (Xu et al., 2020). Our investigations suggest that miRNA140-5p and miRNA208b may play roles in regulating ROS production, which aligns with our findings. Thus, these miRNAs might have dual functions in modulating cellular signaling processes, both upstream and downstream of ROS.

6. Conclision

In conclusion, following 4 weeks of therapy with MPSO, a substantial decrease in free radical scavenging (TBARs) levels has been observed, protecting the heart from ISO-induced damage. Increased cardiac GSH, SOD, and CAT levels and a reduction in levels of cTnI, cTnT, LDH, BNP, BcL-2, P53, TLR4, and NF-κB in rat cardiac tissue provided evidence of this protection. The protective effect of MPSO on rat cardiac tissues exposed to ISO stress was confirmed as well by histological analysis. The high levels of polyphenols, flavonoids, PUFAs, and cyclopropenoid fatty acids in MPSO could possibly be responsible for its cardio-protective effects. Each of these components plays a role in protecting the heart tissue from ISO-induced damage.

Limitations of study

MPSO is enriched in polyphenols, flavonoids, PUFAs, and cyclopropenoid fatty acids. It possesses cardioprotective properties as a result of its anti-inflammatory and antioxidant actions. The current work demonstrated that treatment of isoproterenol-injected rats with MPSO attenuates myocardial dysfunction by regulating cardiac inflammatory mediators and oxidative stress biomarkers. MPSO may be used as a cardioprotective agent which needs further investigation.

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CRediT authorship contribution statement

Mohammed A. Hussein: Conceptualization, Validation, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition. **Mohammad Shahidul Islam:** Writing – original draft, Writing – review & editing, Funding acquisition. **Ali A. Ali:** Conceptualization, Methodology. **Mohamed S Mansour:** Methodology, Data curation. **Mohamed Bondok:** Methodology, Data curation. **Mohamed A. Salem:** Data curation. **Ahmed S. Amein:** Methodology, Validation, Data curation. **Heba A. El-gizawy:** Conceptualization, Validation, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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