



# *Origanum majorana* L. Extract Protects Against Isoproterenol-Induced Cardiotoxicity in Rats

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Received: 21 October 2020 / Accepted: 15 March 2021 / Published online: 30 March 2021  
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## Abstract

Coronary artery diseases are the major causes of disabilities and death worldwide. Evidence from the literature has demonstrated that *Origanum majorana* L. (marjoram) acts as an antioxidant, anti-inflammatory, antiplatelet, and assists in hormonal regulation. However, there is limited scientific evidence describing the signaling pathways associated with the marjoram's positive effect on cardiac injury. Therefore, we aimed to understand the mechanistic protective effects of marjoram on isoproterenol (ISO)-induced myocardial injury in rats. Sprague Dawley rats were randomly assigned into six groups. Marjoram was administered by oral gavage and isoproterenol was administered subcutaneously (ISO; 85 mg/kg). Heart weight, cardiac enzymes, inflammatory, and oxidative stress biomarkers were measured. The ISO-induced cardiac injury was confirmed by the significant increase in the levels of cardiac enzymes ( $P$  value  $< 0.05$ ), whereas pre-treatment with marjoram normalized these cardiac injury parameters. We also determined that marjoram had a protective effect against ISO-induced increase in C-reactive protein (CRP), IL-6, IL-13, and TNF- $\alpha$ . Additionally, marjoram significantly decreased cardiac thiobarbituric acid reactive substances (TBARS) levels ( $P$  value  $< 0.05$ ) and protected against ISO-induced oxidative stress. We have demonstrated that marjoram decreased both cardiac oxidative stress and inflammation, thus establishing the beneficial effects of marjoram on ISO-induced cardiac injury in rats.

**Keywords** Marjoram extract · Isoproterenol · Cardiovascular disease · Myocardial injury · Oxidative stress · Fibrosis · Inflammation

## Abbreviations

CVDs	Cardiovascular diseases
ACUC	Animal Care and Use Committee
IL	Interleukin
CRP	C-reactive protein
CK	Creatine kinase
LDH	Lactate dehydrogenase
AST	Aspartate transaminase
ALT	Alanine transaminase
TNF- $\alpha$	Tumor necrosis factor-alpha

SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive substances
GPx	Glutathione peroxidase
ISO	Isoproterenol

## Introduction

Cardiovascular disease (CVD) describes a group of conditions affecting the cardiovascular system which includes congestive heart failure (HF), hypertension, coronary artery disease, heart attack, arrhythmia, stroke, and congenital heart diseases. CVDs are still the leading cause of death especially in low-income countries [1, 2]. Since the 1990s, CVDs were the number one leading cause of death worldwide, and the number of deaths was increased by 12.5% [1]. This increase is mainly due to the increased number of the aging population as well as the changes in the epidemiology of CVDs [3].

Isoproterenol (ISO) is a synthetic catecholamine and  $\beta$ -adrenergic receptor ( $\beta$ -AR) agonist. Persistent activation

Handling Editor: Y. James Kang.

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of  $\beta$ -AR using a high dose of ISO produces complex structural and biochemical changes causing cellular damage and necrosis through the induction of oxidative stress, increase in the myocyte size, and induction of fetal genes via different signaling mechanisms [4–6]. Hence, experimental induction of HF by high doses of ISO in animals is a well-established model that reveals morphological and pathophysiological changes in the cardiac muscle compared to those appearing in the human cardiac pathologies [7–9].

Numerous lines of evidence have revealed the beneficial effects of the standard drugs including beta-blockers, aldosterone receptor antagonists, and angiotensin-converting enzyme inhibitors in the treatment of CVDs and cardiac fibrosis [10–12]. However, scientific researchers are still looking for a potential molecular target that could retard the signaling pathways of cardiac fibrosis.

Marjoram (*Origanum majorana L.*) is an aromatherapeutic perpetual herb mainly found in countries including Turkey, Egypt, and other Eastern Mediterranean countries. Marjoram is characterized by its strong and pleasant odor, along with its spicy flavor [13, 14]. Marjoram leaves are comprised of acids (carnosic, ursolic, and oleanolic acids), flavonoids (apigenin, diosmetin, and luteolin), cis-sabine hydrate, hydrocarbons (P-cymene and c-terpinene), phenolic glycosides, phenolic terpenoids, and tannins [15, 16]. The marjoram composes of 22% fibers, 14% proteins, 8% moisture, 6–24% ash, 1.8% essential oils, in addition to vitamins, especially A and C. Phenolic acid and monoterpene that is found in the essential oil are the main chemical component in the marjoram [14]. Moreover, thymol and carvacrol are another phenolic component found in marjoram's essential oil and are accountable for many biological activities [17]. It has been validated from the literature that marjoram has anti-inflammatory, anti-cancer, antioxidant, antiplatelet, and cardiac protective effects [14]. However, limited scientific evidence is currently available characterizing marjoram. Thus, we set out to examine the mechanistic protective effects of this herb on inflammatory and toxicity factors following ISO-induced myocardium injury in rats. *Ramadan and colleagues* previously reported that ISO treatment causes significant changes on both hematological and oxidative indices, while marjoram presenting a protective effect through its antioxidant properties [15]. Moreover, polyphenols originated in plant-derived foods, where evidence has suggested that natural polyphenols have numerous protective effects against CVDs [18]. Previous work from our laboratory demonstrated that marjoram reduced ovarian inflammatory biomarkers and improved the levels of ovarian antioxidant enzymes [19]. The current study aims to understand the mechanistic protective effects of marjoram on ISO-induced myocardial injury in adult Sprague Dawley rats.

## Methods and Materials

### Animal Groups and Treatments

Adult Sprague Dawley rats (180–250 g) were housed in metal cages (5 rats/cage) under hygienic conditions at room temperature (25 °C) with a regular 12-h light/dark cycle (light on at 8 am). Rats were allowed for one week to acclimatize before manipulation began. Animals had free access to a commercial pellet diet and water ad libitum. All procedures involving animals were performed in accordance with the regulation of the Animal Care and Use Committee (ACUC) at Jordan University of Science and Technology (ACUC-Grant Approval # 20,190,451, Irbid, Jordan). The current research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the European Community guidelines. The study was conducted under the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies [20].

Animals were randomly assigned to 6 groups (10 rats in each group). The study groups are as follows:

- 1) *Control group*: healthy rats that were not exposed to ISO induction or marjoram treatment),
- 2) *Marjoram low-dose group*: marjoram (50 mg/kg) was given by oral gavage daily for three weeks,
- 3) *Marjoram high-dose group*: marjoram (100 mg/kg) was given by oral gavage daily for three weeks,
- 4) *ISO-treated group*: rats were injected subcutaneously with ISO (85 mg/kg) every 24 h for the last 2 days (days 29 and 30),
- 5) *ISO-50 marjoram-treated group*: marjoram (50 mg/kg) was given by oral gavage daily for three weeks, and ISO injected subcutaneously (85 mg/kg) every 24 h for the last 2 days (day 29 and 30),
- 6) *ISO-100 marjoram-treated group*: marjoram (100 mg/kg) was given by oral gavage daily for three weeks, and ISO injected subcutaneously (85 mg/kg) every 24 h for the last 2 days (day 29 and 30).

### Drugs Preparation and Administration

Marjoram liquid certified aqueous extract (G-072319-MAOMB-PK; Hawaii Pharm LLC, Honolulu, HI, USA) was freshly prepared and given to the rats using oral gavage for three weeks. The dose and duration of marjoram treatment were according to previously published literature [15, 21]. Also, we used two different concentrations of marjoram, 50 and 100 mg/kg body weight, to

provide a comparative analysis and to explore whether the potential to achieve the ameliorative effect of marjoram is dose-dependent or not. We used a certified non-alcohol Organic Marjoram (*Origanum majorana L.*) dried berries extract (Origin: Egypt) which has been extracted using a cold maceration extraction method (dried material/solvents ratio is 1:3 w/v). The doses were given after mixing the extract with deionized water according to the animal body weight [22]. While giving the treatment, the prepared solution was stored in plastic tubes covered by aluminum foil for 24 h at room temperature.

The ISO solution (Cat # I6504; Sigma-Aldrich, St. Louis, MO, USA) was prepared freshly and injected subcutaneously to rats according to their body weights at the last two days of the experimental period. ISO doses were freshly prepared by dissolving 40 mg of ISO in 32 ml deionized water to achieve a stock solution which was diluted by preparing a 15 ml solution consisting of 1 ml of stock solution and 14 ml of deionized water. Then, the proper doses of ISO were taken from the liquid stock solution to administer the adequate dose (85 mg/kg) according to the animal weight. The ISO dose and administration protocol was performed according to previously published literature [23–25]. Animals were observed for any toxicity signs or stifling for two hours after each administration protocol. Figure 1 represents the scheduled outline for the study of the experimental timeframe.

## Animal Heart Dissection

Following the treatment period, animals were sacrificed by decapitation. Fresh blood was collected immediately into a two-serum clot activator gel tube, centrifuged at 5000 rpm for 10 min to separate serum. After that, the serum was eluted, collected in Eppendorf tubes, and frozen at  $-80^{\circ}\text{C}$  for further biochemical analysis.

Hearts of the sacrificed rats were surgically removed, washed immediately with phosphate-buffered saline (PBS; Cat# P4417; Sigma-Aldrich Corp, MI, USA) on crushed ice, dried, weighed, and then cut off into two identical halves.

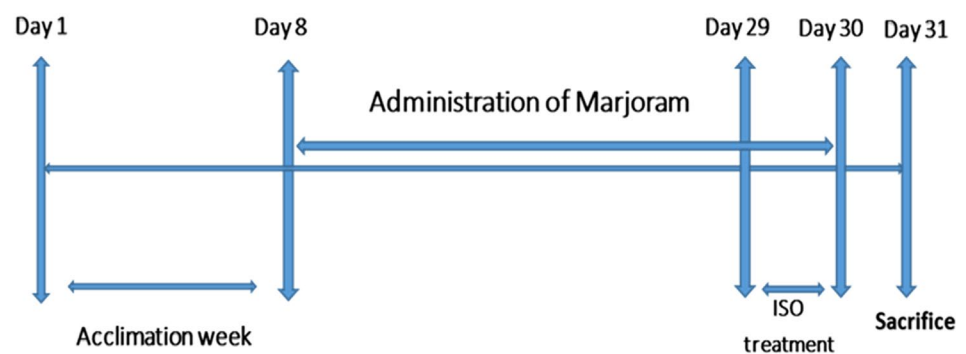
Pre-labeled Eppendorf tubes were used to collect the excised organs and then stored at  $-80^{\circ}\text{C}$  until the time of tissue homogenization. Two animals per group were saved for histological studies. The hearts of these animals were preserved and fixed in 10% formaldehyde.

## Biochemical Tests

The heart tissues were homogenized in 2 ml of phosphate buffer saline (PBS) prepared by dissolving one tablet (Cat# P4417; Sigma-Aldrich Corp, MI, USA) in 200 ml of deionized water to produce 0.01 M phosphate buffer, pH 7.4, at  $25^{\circ}\text{C}$ , containing protease inhibitor cocktail tablet (Cat#S8820; Sigma-Aldrich Corp, MI, USA). Protease inhibitor tablet contained 2 mM 4-benzenesulfonyl fluoride hydrochloride (AEBSF), 0.3 mM Aprotinin, 30 mM Bestatin, 1 mM EDTA, 1 mM leupeptin, and 14 mM E-64. Homogenized tissues were incubated for 15 min on the ice, centrifuged (15,000 rpm for 15 min at  $4^{\circ}\text{C}$ ) to remove insoluble materials. The supernatant homogenates were taken and divided into pre-labeled Eppendorf tubes, stored at  $-80^{\circ}\text{C}$  for analysis according to ELISA protocol and clinical chemistry analysis procedure.

Total protein concentration was estimated using a commercially available kit (BCA Protein Assay Kit (Cat# ab102536); Abcam, USA). The activities of antioxidant enzymes (superoxide dismutase (SOD; Cat # ab65354, Abcam, USA), glutathione peroxidase (GPx; Cat # ab102530, Abcam, USA), and thiobarbituric acid reactive substances (TBARS; Cat# STA-330, cell Biolab, INC, USA)), and the inflammatory biomarkers (tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ; Cat# ab46070, Abcam, USA), interleukin-6 (IL-6; Cat# ab100772, Abcam, USA), interleukin-13 (IL-13; Cat# MBS355408, MyBioSource Inc, San Diego, USA), and C-reactive protein (CRP; Cat# ab256398, Abcam, USA)) were determined in all groups using enzyme-linked immunosorbent assay (ELISA). All procedures were performed according to the kit manufacturer's instructions. Furthermore, the levels of the cardiac enzymes (aspartate transaminase (AST), alanine transaminase (ALT), lactate

**Fig. 1** Representation of the experimental procedures. Animals were acclimated for one week, and then they were treated with marjoram by oral gavage for three weeks. On day 29, isoproterenol was administered for 2 days (days 29 and 30). Animals were killed by decapitation on day 31. ISO: isoproterenol



dehydrogenase (LDH), and creatine kinase (CK) were measured at Jordan University of Science and Technology Health Center (JUST Center) by using a commercially available kit (Beckman Coulter, USA). ELISA plates were scanned at the kit's specified wavelengths using a microplate reader (ELx800, Bio Tek Instruments, plate reader, Highland Park, Winooski, USA).

## Microscopic Examination

Hematoxylin and eosin stain (H&E) was used to evaluate the myocardial injury. Cardiac tissues were fixed in 10% formaldehyde for 24 h, dehydrated in alcohol, cleared with xylene, and fixed in pure paraffin wax. Finally, tissues were sectioned and stained with H&E stain (Cat# ab245880, Abcam, USA) according to the manufacture's instructions. The procedure was performed by deparaffinization, hydration, nuclear staining by hematoxylin, and differentiation was made by acid alcohol until sections turned blue. The sections stained by eosin stain were washed, dehydrated, cleared, and then finally read using a high-resolution light microscope (BEL photonics, ITALY) at 40× original magnification by BEL capture program.

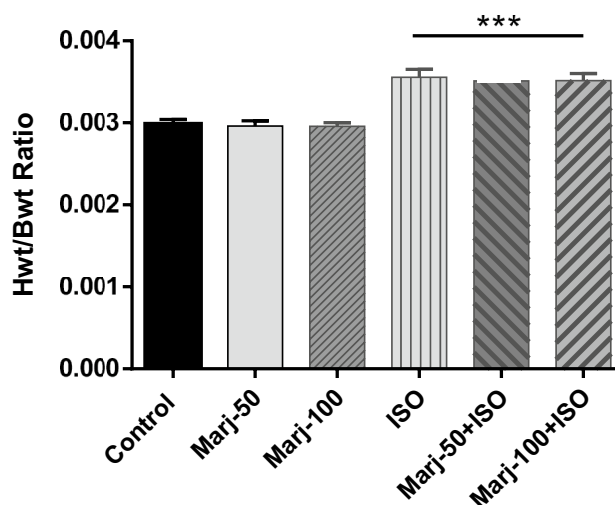
## Statistical Analysis

All statistics were performed using the GraphPad Prism software V7.00 "GraphPad Software, USA". Data analysis was performed using one-way ANOVA followed by Tukey's multiple comparisons test. A  $P$  value  $< 0.05$  was considered statistically significant. All values were represented as mean  $\pm$  SEM (standard error mean).

## Results

### Effect of Marjoram Extract on Heart/Body Weight Ratio and Cardiac Enzyme Levels

Figure 2 represents the heart weight-to-body weight ratio (Hwt/Bwt) at the end of the study. The Hwt/Bwt ratio was significantly higher in ISO-treated group compared to the control (Fig. 2;  $P$  value  $< 0.0001$ ). The data showed that pre-administration of marjoram at two different concentrations (50 and 100 mg/kg) did not prevent the increase in Hwt/Bwt ratio due to ISO administration as compared to the control group. On the other hand, administration of both concentrations of marjoram alone did not affect the Hwt/Bwt when compared to control group. Following the treatment period, the data demonstrated a significant increase in the levels of serum AST, CK, and LDH in ISO-treated group compared to the control



**Fig. 2** The heart weight-to-body weight ratio. The ratio was significantly higher in ISO-treated group compared to the control. Marj-50: Marjoram-50 mg/kg; Marj-100: Marjoram-100 mg/kg; Isoproterenol: ISO 85 mg/kg; Marj-50+ISO: Marjoram-50 mg/kg + ISO; Marj-100+ISO: Marjoram-100 mg/kg + ISO. ISO: isoproterenol; Hwt/Bwt: heart weight-to-body weight ratio. Each point represents the mean  $\pm$  SEM. Level of significance was detected when  $P < 0.05$ . \*\*\* indicates significance to the control group

( $P$  value  $< 0.05$ ; Table 1). Interestingly, we also observed that pre-treatment with marjoram (at two different doses 50 and 100 mg/kg) protected against the ISO-induced increase in the serum levels of AST, CK, and LDH (Table 1).

### Effect of Marjoram Extract Administration on Cardiac Inflammatory Biomarkers

To assess the effect of marjoram administration on the cardiac inflammatory biomarkers, we measured the levels of serum CRP, cardiac IL-6, IL-13, and TNF- $\alpha$  at the end of the study. Relative to the control group, there was a significant increase in the levels of all measured inflammatory biomarkers (CRP, IL-6, IL-13, and TNF- $\alpha$ ) in the ISO-treated group ( $P$  value  $< 0.05$ ; Fig. 3a–d, respectively). Interestingly, the pre-treatment of marjoram prevented the ISO-induced increase in the cardiac levels of the inflammatory biomarkers (Fig. 3a–d). Administration of marjoram only at two different concentrations (50 and 100 mg/kg) did not affect the levels of inflammatory biomarkers compared to the control group.

### Effect of Marjoram Extract Administration on Cardiac Oxidative Parameters

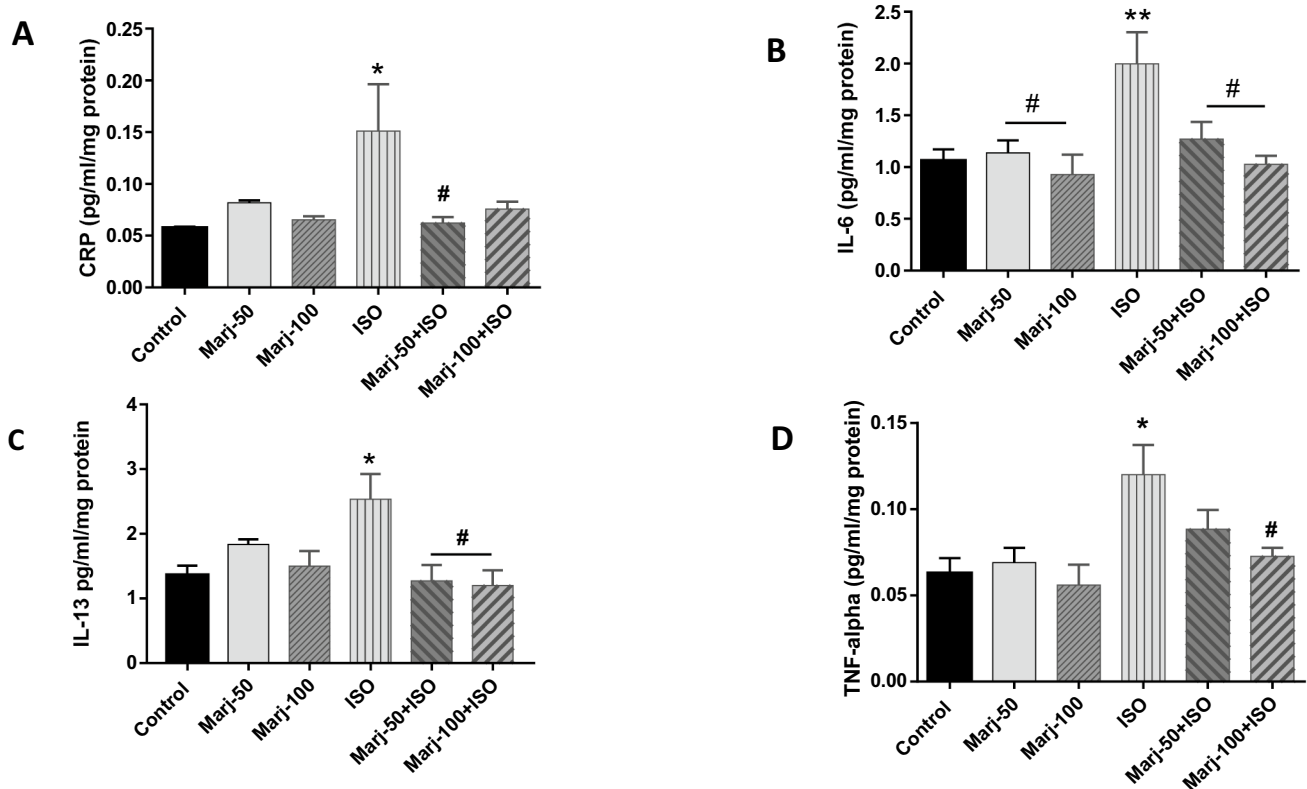
We evaluated the effects of marjoram extract on the cardiac oxidative stress parameters, by measuring the levels of cardiac superoxide dismutase (SOD), glutathione peroxidase

**Table 1** Effect of Marjoram extract administration on cardiac enzyme levels

Groups/parameter	Control	Marjoram-50	Marjoram-100	ISO	Marjoram-50+ISO	Marjoram-100+ISO
Aspartate transaminase (AST); (U/L)	202.0 ± 14.01	245.6 ± 21.44	200.8 ± 9.61 <sup>#</sup>	358.6 ± 29.88**	274.2 ± 40.32	253.8 ± 27.15
Alanine transaminase (ALT); (U/L)	49.62 ± 4.65	55.17 ± 8.57	55.56 ± 6.78	71.30 ± 7.87	61.12 ± 4.00	59.88 ± 4.137
Lactate dehydrogenase (LDH); (U/L)	1180 ± 98.87	1249 ± 70.30	1422 ± 129.5	1879 ± 80.84*	1464 ± 155.3 <sup>#</sup>	1233 ± 99.62 <sup>#</sup>
Creatine kinase (CK; heart); (U/L)	417.8 ± 117.6	826.3 ± 101.1	732.3 ± 96.18 <sup>#</sup>	1203 ± 29.82*	949.3 ± 101.0	945.2 ± 61.03

ISO isoproterenol. Values are expressed as mean ± SEM

\* $P < 0.05$  compared to control group. <sup>#</sup> $P < 0.05$  compared to ISO-treated group



**Fig. 3** Levels of inflammatory biomarkers. **a** C-reactive protein: CRP; **b** Interleukin-6: IL-6; **c** Interleukin 13: IL-13; and **d** Tumor necrosis factor-alpha: TNF- $\alpha$ . Marj-50: Marjoram-50 mg/kg; Marj-100: Marjoram-100 mg/kg; Isoproterenol: ISO 85 mg/kg; Marj-50+ISO: Mar-

joram-50 mg/kg + ISO; Marj-100+ISO: Marjoram-100 mg/kg + ISO. Each point represents the mean ± SEM. Level of significance was detected when  $P < 0.05$ . \* Significant to control group; <sup>#</sup>significant to ISO-treated group

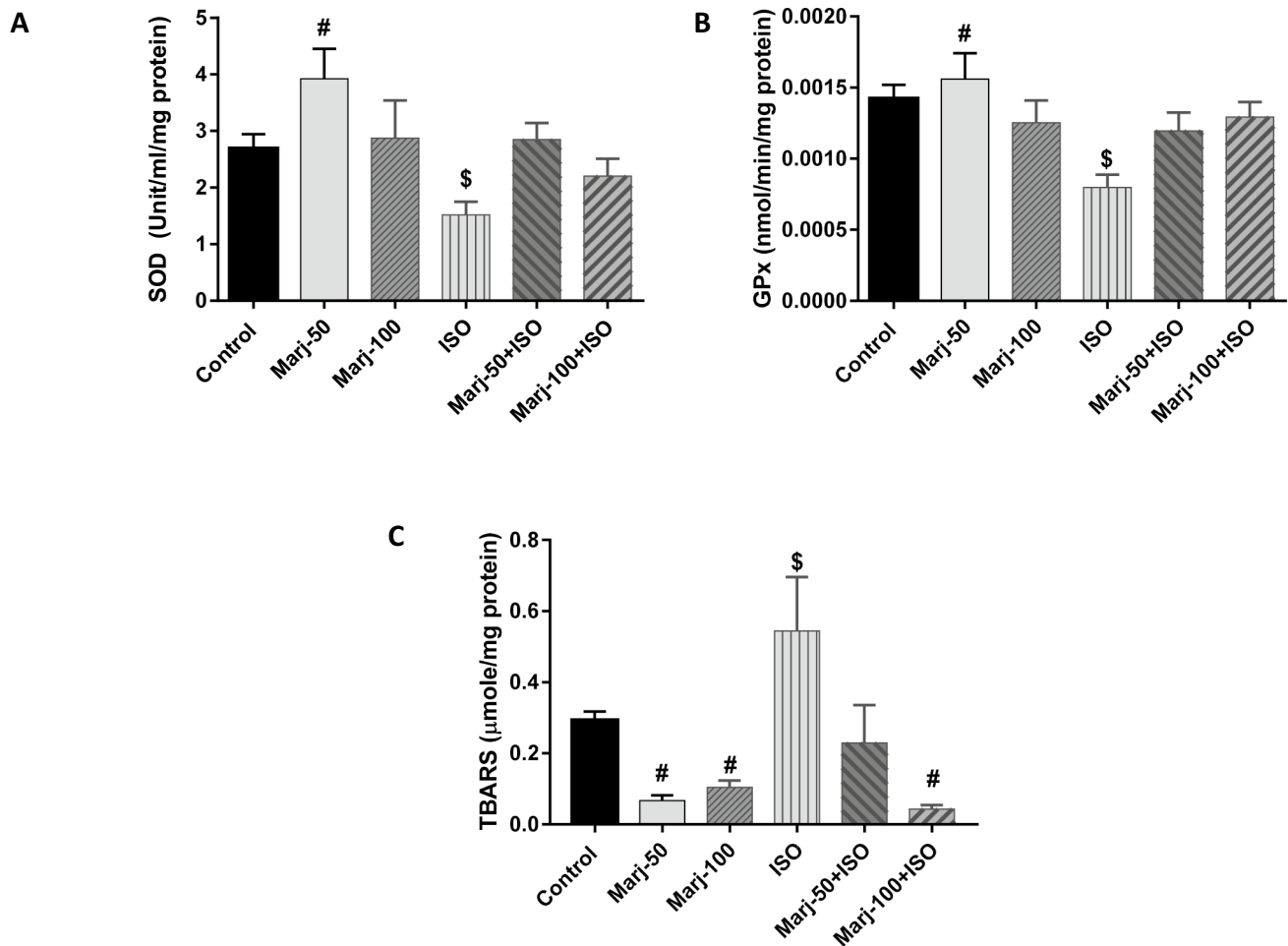
(GPx), and thiobarbituric acid reactive substances (TBARS). The data indicated that administration of marjoram increased the levels of SOD and GPx and reduced the cardiac TBARS (Fig. 4). Conversely, we found a decrease in the levels of cardiac SOD and GPx in the ISO-treated group compared to the control group (Fig. 4). The levels of SOD and GPx were significantly elevated in the marjoram (50 mg/kg)-treated group relative to ISO group ( $P$  value  $< 0.05$ ; Fig. 4). Interestingly, pre-administration of marjoram extract prevented

the ISO-induced increase in the TBARS level suggesting a valuable and effective marjoram's antioxidant property.

## The Hematoxylin and Eosin Staining

Figure 5 shows ventricular sections for different study groups as detected by hematoxylin and eosin (H&E) staining. The heart tissue of the normal control group rats





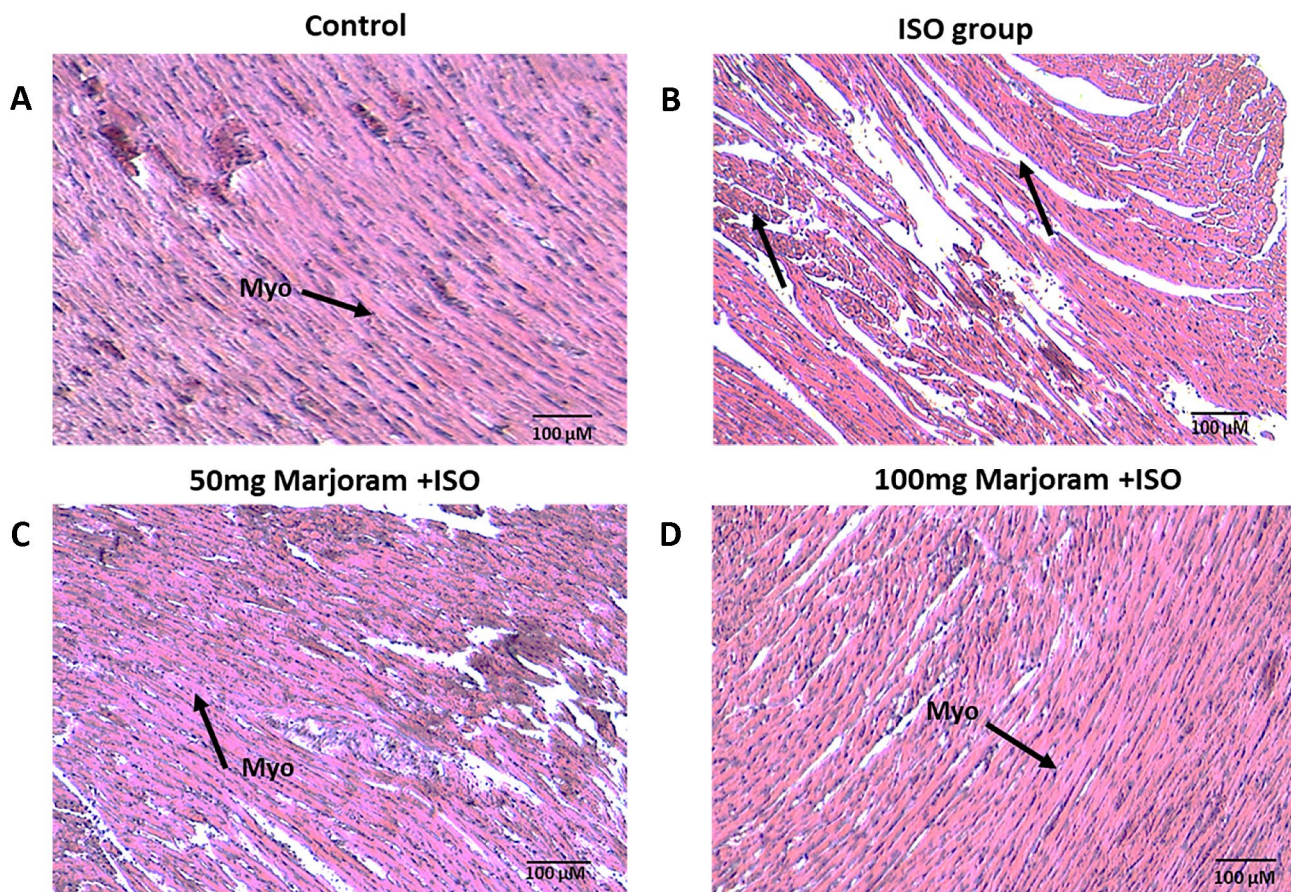
**Fig. 4** Levels of oxidative stress biomarkers. **a** Superoxide dismutase: SOD; **b** Glutathione peroxidase: GPx; and **c** Thiobarbituric acid reactive substances: TBARS. Marj-50: Marjoram-50 mg/kg; Marj-100: Marjoram-100 mg/kg; Isoproterenol: ISO 85 mg/kg; Marj-50+ISO:

Marjoram-50 mg/kg+ISO; Marj-100+ISO: Marjoram-100 mg/kg+ISO. Each point represents the mean±SEM. Level of significance was detected when  $P < 0.05$ . <sup>#</sup>significant to ISO-treated group; <sup>\$</sup>significant to Marj-50-treated group

showed normal myocardial branching, normal-sized blood vessels, centrally located oval to elongated nuclei, and normal myocardial fibers (Fig. 5a). In the ISO-treated group, severe damage in the cardiomyocytes was observed, illustrated by fragmentation of fibers, loss of nuclei, increased eosinophils, and lymphocytic infiltration (Fig. 5b). In contrast, the marjoram (50 mg/kg)-treated group showed less severity of lesions, lower degeneration, moderate myocardial loss, and a moderate degree of lymphocytic infiltration, in comparison with the ISO-treated group (Fig. 5c). The animals treated with a high dose of marjoram (100 mg/kg) showed very mild changes as compared to controls. Thus, we observed that marjoram treatment was capable to maintain the hearts with close to normal structure with only mild degeneration of cardiomyocytes fibers following ISO-induced cardiac injury (Fig. 5d).

## Discussion

Isoproterenol (ISO), a beta-adrenergic agonist, causes severe oxidative stress in the myocardium cells, which leads to cardiac injury and the development of cardiac fibrosis. Different hypotheses were proposed to identify the mechanisms of ISO-induced myocardium injury; however, the generation of oxidative stress in the heart due to catecholamines effect is still the most significant theory. Experimental induction of cardiac injury by high doses of ISO provides a standard model (catecholamine-mediated cardiac injury model) to investigate new potential cardioprotective agents. Upon the administration of a high dose of ISO [26], we showed an increase in the heart weight-to-body weight ratio (Hwt/Bwt) in the ISO-treated group



**Fig. 5** The stained cardiac sections. **a** Hematoxylin and eosin staining of the left ventricular (LV) of a normal rat. **b** H&E staining of LV of ISO (85 mg/kg)-treated rat. **c** H&E staining of LV of rat treated with marjoram (50 mg/kg) and ISO (85 mg/kg). **d** H&E staining of LV of rat treated with marjoram (100 mg/kg) and ISO (85 mg/kg).

The black arrows in **a**, **c**, and **d** represent normal striated cardiomyocytes with centrally located nuclei. However, the arrows in **b** represent severe damage in cardiomyocytes by fragmentation of fibers and loss of nuclei. Myo: myocardium. The scale bar is 100  $\mu\text{m}$  and the sections were viewed at 40 magnification

compared to the control. Many studies showed a relationship between ISO and the increase in the Hwt/Bwt ratio [27, 28]. Additionally, the results of the current research showed that pre-administration of marjoram at two different concentrations (50 and 100 mg/kg) could not prevent the increase in Hwt/Bwt ratio due to ISO treatment, suggesting that the occurrence of cardiac dilation or remodeling could not be prevented by marjoram.

To assess the degree of myocardial injury, several cardiac biomarkers and enzymes were measured, such as AST, LDH, and CK [29]. Many studies approved the role of the cardiac enzyme creatine kinase (CK-MB) to detect myocardial ischemia [30]. The administration of ISO (85 mg/kg) significantly increased the level of CK. On the other hand, the pre-treatment of marjoram (at two different doses 50 and 100 mg/kg) prevented the ISO-induced increase in the cardiac levels of CK. This is consistent with previous studies that documented the effect of marjoram in reducing the ISO-induced increase in the total CK levels [31]. On the

other hand, we reported a non-significant increase in the levels of cardiac CK in marjoram (50 and 100 mg/kg)-treated rats with the absence of ISO administration compared to the control. It is noteworthy to mention that the levels of cardiac CK in aforementioned groups were still within the normal range and the difference could be illuminated due to the influence of groups' intervariability. Moreover, we reported a significant increase in the levels of serum AST and LDH in the ISO-treated group. This is consistent with several studies showing treatment with ISO increased the levels of LDH and AST enzymes [32, 33]. Interestingly, our current study discovered that pre-treatment with marjoram was capable to reduce the ISO-induced increase in the LDH, AST, and ALT serum levels. Hence, the release of cardiomyocyte enzymes in the ISO-treated rats demonstrated that changes in the plasma membrane structure or cell permeability could be attributed to the chronic beta-adrenergic stimulation by high doses of ISO. This is also supported by the changes in the cardiomyocytes structures following

ISO treatment. Following confirmation of the ISO-induced cardiac injured rat model, we then looked for a mechanistic approach to explain the protective effect of marjoram against ISO-induced injury on cardiomyocytes.

An increase in the levels of inflammatory biomarkers is commonly associated with cardiac injury [34]. In our study, we detected the role of marjoram in preventing the ISO-induced increase in the cardiac levels of CRP. CRP is a protein released by the liver a few hours after tissue injury [29]. We found that the levels of CRP, IL-6, IL-13, and TNF- $\alpha$  significantly increased upon ISO treatment which confirmed previously published studies [35, 36]. This indicated an inflammatory process caused by ISO toxicity. Interestingly, pre-treatment of marjoram prevented the ISO-induced increase in the levels of CRP, IL-6, IL-13, and TNF- $\alpha$ , which supported the anti-inflammatory activity of marjoram components and consistent with previous studies [37, 38]. Marjoram has the anti-inflammatory activity of essential oil which decreases the production of tumor necrosis factor-alpha (TNF- $\alpha$ ) and interleukin (IL-6) [13, 16]. It is well known from the literature that the marjoram's anti-inflammatory effect is ascribed mainly to the presence of sabinene hydrate and terpineol [39]. Additionally, the essential oils present in marjoram decreased the IL-6 and TNF- $\alpha$  in lipopolysaccharide-activated THP-1 human macrophage cells [39]. Elevated IL-6 is associated with an increased risk of a future cardiac injury, whereas elevated TNF- $\alpha$  is associated with increased risk of other coronary events [40]. Furthermore, increased levels of inflammatory biomarkers are commonly associated with myocardial infarction [41]. Collectively, we proposed a potential protective effect of marjoram against ISO-induced cardiac injury via its anti-inflammatory effect.

Oxidative stress is an imbalance between antioxidants and free radicals, with this imbalance potentially leading to damage of the components of the cells, including nucleic acids, proteins, amino acids, and lipids [10]. Ramadan and colleagues (2012) showed that ISO treatment caused significant changes in both hematological and oxidative indices, while marjoram provided a protective effect through its antioxidant property [42, 43]. Other studies investigating oxidative stress in ISO-induced myocardial infarction revealed elevated levels of TBARS (lipid peroxidation marker) and decreased SOD and GPx levels [44]. In our study, we showed changes in the cardiac oxidative stress biomarkers upon ISO treatment, indicated by a decrease in the antioxidant enzyme activities (SOD and GPx) as well as an increase in TBARS levels. Specifically, the SOD and GPx activities increased significantly in the treated group with 50 mg/kg marjoram as compared to the ISO-treated group. Hence, ISO treatment causes a significant reduction in the levels SOD and GPx compared to marjoram administration only. However, the pre-treatment with marjoram

before challenging the animals with ISO administration was capable to raise the levels of both enzymes in a non-statistical significant manner. This could be justified by different aspects, including the needs for larger doses and longer duration of pre-treatment with marjoram. Moreover, the marjoram-treated groups (50 and 100 mg/kg marjoram) both showed a significant reduction in the level of TBARS compared to ISO group. These results are consistent with previous studies of others [45, 46] involving the effect of marjoram on oxidative stress biomarkers. On the other hand, the marjoram administration in both concentrations was capable to reduce the TBARS levels compared to control healthy rats. This is suggesting that marjoram provides antioxidant protective effect on both healthy and ISO-challenged rats. Thus, we found that marjoram significantly prevented the ISO-induced increase in TBARS levels. Marjoram acts as an antioxidant via scavenging the free radicals due to its hydrogen donating ability [47]. The high polyphenolic compounds especially thymol and carvacrol are the responsible constituents for marjoram antioxidant properties [48, 49]. Another proposed mechanism is the prevention of lipid peroxidation due to the presence of hydroxycinnamic acid and flavonoids compounds, thus protecting cells from damage [48, 50]. Additionally, the essential oil presents in the marjoram extract is proposed to be responsible for the plant's antioxidant properties due to the presence of ursolic acid, carnolic acid, and carnolic constituents [51]. Therefore, our data show another potential protective effect of marjoram against ISO-induced cardiac injury via its antioxidant properties.

## Conclusions

The data presented in the current study revealed that the use of marjoram remarkably reduced cardiac oxidative stress and inflammation, which established the beneficial effects of marjoram being administered to ISO-induced cardiac injured rats. This could open a new therapeutic approach to protect against stress-induced cardiac injury. Until supplementary validation of the applicability of these findings is also observed in clinical trials, we recommend future intensive comparative studies to elucidate the dose–response effectiveness and identification of marjoram's chemical composition that provides the specific molecular target of this aromatherapeutic perpetual herb.

**Acknowledgment** This work was supported by the Deanship of Research at Jordan University of Science and Technology to AR [Project Number: 451/2019]. We would like to thank Professor Bradley McConnell (*Department of Pharmacological and Pharmaceutical Sciences, University of Houston*) for proofreading the final version of the manuscript.



**Authors' contribution** AR: conceptualization; AR and MA designed the study; AR provided the plant; AR and MA conducted the experimental work; AR and MA drafted the manuscript; AR and MA conducted the field research; AR contributed new reagents or analytic tools; AR and MA performed data analysis and interpreted the results; AR and MA contributed to the discussion. All authors read and approved the final manuscript.

**Funding** Financial support was via grant number 451/2019 from the Deanship of Research at the Jordan University of Science and Technology to AR.

**Data Availability** The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

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