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# Umbelliferone prevents isoproterenol-induced myocardial injury by upregulating Nrf2/HO-1 signaling, and attenuating oxidative stress, inflammation, and cell death in rats

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## ABSTRACT

The role of oxidative injury and inflammatory response in cardiovascular diseases and heart failure has been well-acknowledged. This study evaluated the protective effect of umbelliferone (UMB), a coumarin with promising radical scavenging and anti-inflammatory activities, on myocardial injury induced by isoproterenol (ISO) in rats. Rats received 50 mg/kg UMB orally for 14 days and 85 mg/kg ISO twice at an interval of 24 h. Administration of ISO elevated serum troponin I, creatine kinase-MB and lactate dehydrogenase, and caused histopathological alterations, including degeneration, fatty vacuolation, myolysis, and atrophy of myocardial fibers. Malondialdehyde (MDA), nitric oxide (NO), nuclear factor-kappaB (NF-κB) p65, tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-1β were increased, whereas reduced glutathione (GSH), superoxide dismutase (SOD), and catalase were decreased in ISO-administered rats. UMB effectively ameliorated myocardial injury, alleviated cardiac function markers, MDA, NO, NF-κB p65, and the inflammatory mediators, and enhanced cellular antioxidants. Bax, caspase-3, and 8-OHdG were decreased, and Bcl-2 was increased in ISO-administered rats treated with UMB. In addition, UMB upregulated nuclear factor-erythroid factor 2-related factor 2 (Nrf2) and heme oxygenase (HO)-1 in the heart of ISO-administered rats. In conclusion, UMB can protect the myocardium from oxidative injury, inflammatory response, and cell death induced by ISO by upregulating Nrf2/HO-1 signaling and antioxidants.

## 1. Introduction

Myocardial infarction (MI) is an immense health problem and a leading cause of death worldwide [1]. The pathophysiological mechanism of MI is the disturbance of homeostasis between the blood supply and the demand for blood, which eventually leads to cardiomyocyte death [2]. Several factors, including overproduction of reactive oxygen species (ROS), inflammatory mediators, and endothelial dysfunction contribute to the development and progression of MI [3]. Animal models have shown a demonstrable value for understanding the mechanism(s)

underlying human MI and evaluating novel cardioprotective agents [4]. Isoproterenol (ISO) is a non-selective β-adrenergic agonist used in inducing MI in rodents [5]. The primary mechanism behind the cardiotoxicity of ISO is an excessive generation of free radicals via the auto-oxidation of catecholamines, which has been implicated as one of the critical causative factors [6]. Cardiac oxidative stress induced by ISO can provoke inflammation and cell death associated with reduced cardiac performance and dysfunction [7]. Nitric oxide (NO) is a messenger molecule that plays a role in the modulation of the coronary vessel tone, cardiomyocyte contractility, fibroblasts, and smooth muscle cell

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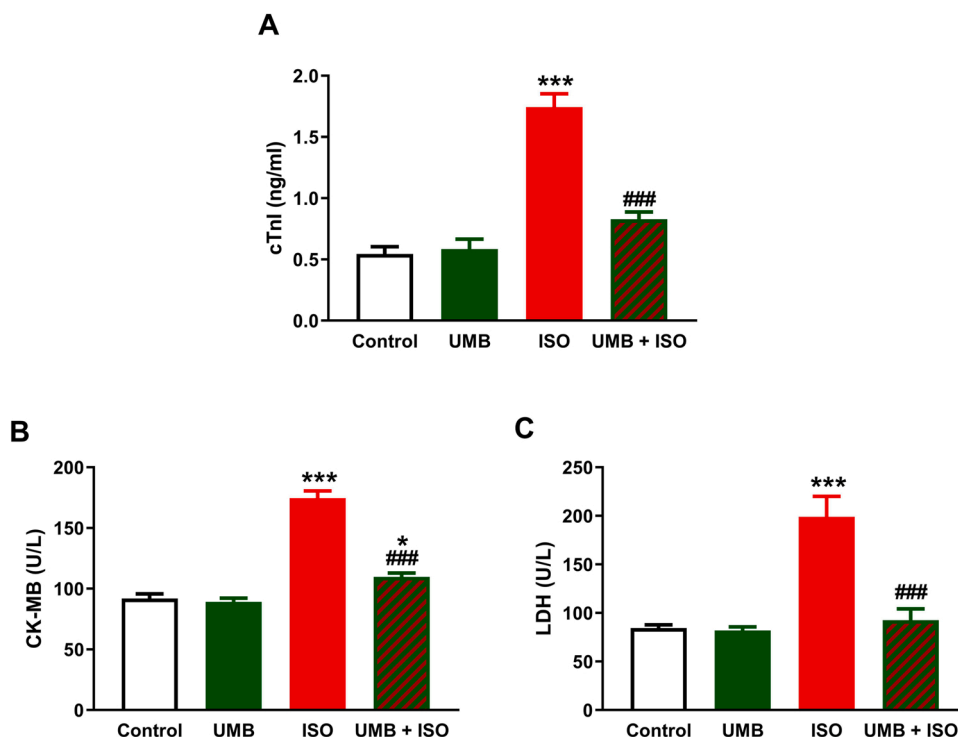


Fig. 1. UMB prevented ISO-induced myocardial dysfunction in rats. UMB ameliorated serum cTnI (A), CK-MB (B), and LDH in ISO-treated rats. Data are mean  $\pm$  SEM, ( $n = 8$ ). \* $P < 0.05$  and \*\*\* $P < 0.001$  versus Control, and ### $P < 0.001$  versus ISO.

proliferation. However, overproduction of NO via inducible NO synthase (iNOS) activation can cause endothelial dysfunction and the release of inflammatory mediators that finally lead to cardiac dysfunction [8]. Therefore, mitigation of oxidative stress and inflammation could represent a potent strategy for preventing cardiomyocyte injury and MI.

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a key cytoprotective factor that regulates antioxidant defenses and prevents oxidative damage [9]. Nrf2 is located in the cytoplasm and inactivated by Keap1 under normal cellular conditions. Upon exposure to ROS, Nrf2 dissociates from Keap1 and binds DNA within the nucleus at the antioxidant response element (ARE) to promote the expression of heme oxygenase 1 (HO-1) and other antioxidants [9]. The role of Nrf2 activation in attenuating cardiomyocyte and endothelial injury has been reported in ischemia/reperfusion (I/R) injury [10], lipid- and high glucose-induced endothelial dysfunction [11–13], ISO-induced myocardial injury [14], and cyclophosphamide cardiotoxicity [15]. In addition, activation of Nrf2 protected mice against pathological cardiac hypertrophy and heart failure by preventing oxidative stress, effects that were abolished in Nrf2<sup>-/-</sup> mice [16].

Umbelliferone (UMB) or 7-hydroxycoumarin is a natural product of the coumarin family found in many plants, such as, coriander, carrot, and garden angelica [17]. UMB exhibited potent antioxidant and anti-inflammatory activities and prevented cell injury in experimental models of liver fibrosis, nephrotoxicity, hepatotoxicity, testicular dysfunction in diabetic and heavy metal-treated animals, and hyperammonemia [18–22]. Activation of Nrf2 has been suggested to contribute to the therapeutic effects of UMB [18,21–23]. UMB ameliorated cardiac function and decreased lipid peroxidation (LPO) and infarct size in ISO-administered rats as reported by Jagadeesh et al. [24, 25]. However, the mechanism underlying the protective effect of UMB on myocardial injury induced by ISO is not fully understood. We assumed that UMB can prevent ISO-induced cardiac injury via activating Nrf2/HO-1 signaling, and mitigating oxidative stress, inflammatory response, and cell death. Therefore, this study investigated the protective efficacy of UMB against ISO-induced oxidative stress, inflammation,

and cardiac injury, pointing to the possible role of Nrf2/HO-1 signaling.

## 2. Materials and methods

### 2.1. Animals and treatment

Thirty-two male Wistar rats, 180–200 g were included in this study. The rats were accommodated in standard cages under standard conditions and allowed free access to food and water. The rats were divided into 4 groups ( $n = 8$ ) as follows:

Group I (Control): received 0.5% carboxymethyl cellulose (CMC) orally for 14 days.

Group II (UMB): received 50 mg/kg UMB (Sigma, USA) [18–22] suspended in 0.5% CMC for 14 days.

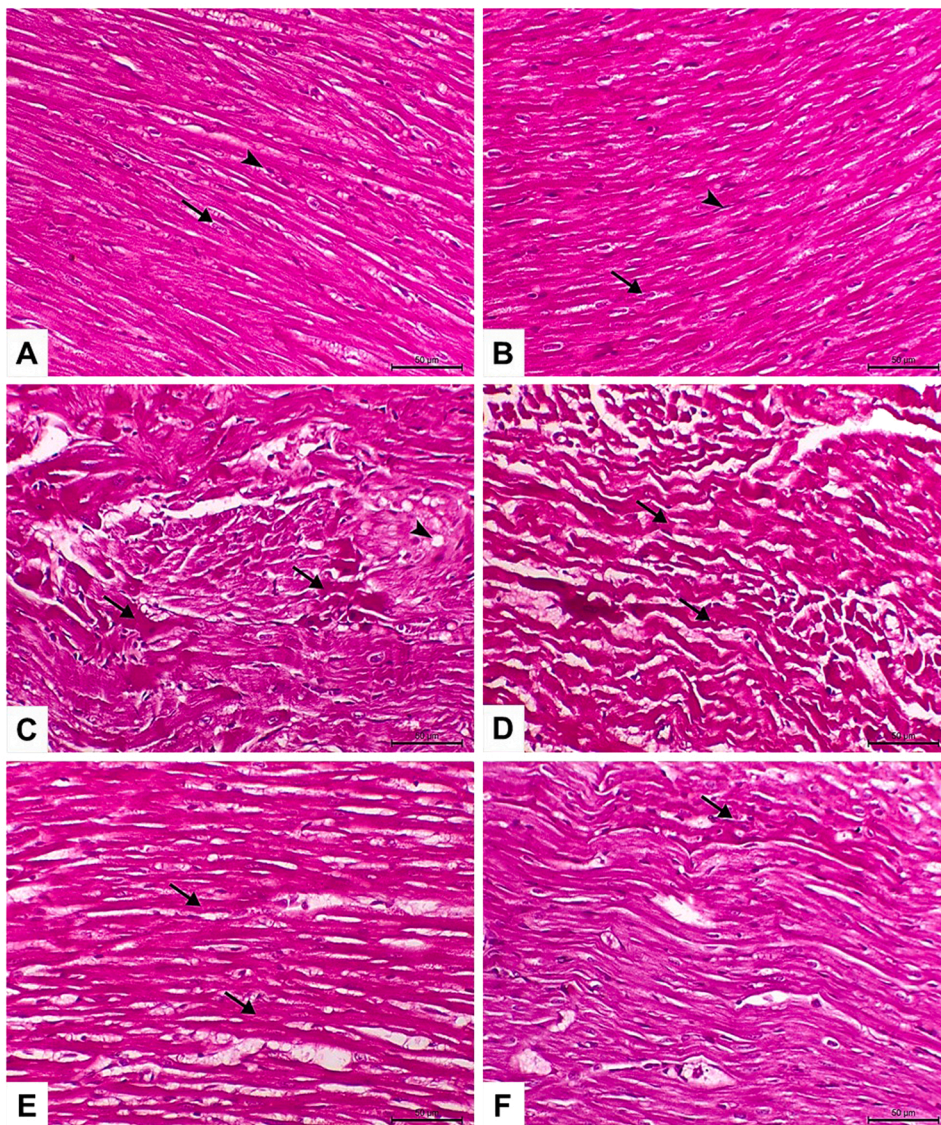
Group III (ISO): received 0.5% CMC orally for 14 days and 85 mg/kg ISO [26] (Sigma, USA) subcutaneously (s.c.) at days 13 and 14.

Group IV (UMB + ISO): received 50 mg/kg UMB [18–22] suspended in 0.5% CMC orally for 14 days and 85 mg/kg ISO subcutaneously (s.c.) at days 13 and 14.

ISO was dissolved in physiological saline and rats in groups I and II received s.c. injection of saline at days 13 and 14. At the end of the experiment, blood samples were collected under ketamine (90 mg/kg)/xylazine (10 mg/kg) anesthesia and serum was separated by centrifugation at 3000 rpm for 15 min for investigating cardiac troponin I (cTnI), creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH). The heart was removed and samples from the left ventricle were homogenized (10% w/v) in cold Tris-HCl buffer (pH = 7.4), centrifuged and the clear supernatant was separated. The protein content was assayed according to Lowry et al. [27]. Other samples from the left ventricle were fixed in 10% neutral-buffered formalin (NBF).

### 2.2. Determination of cTnI, CK-MB, and LDH

Serum cTnI was determined using ELISA kit (Kamiya, USA), and activities of CK-MB and LDH were assayed using Spinreact (Spain) kits. All assays were conducted following the manufacturers' instructions.



**Fig. 2.** Photomicrographs of sections in the heart of (A) control rats and (B) UMB-treated rats showing normal myocardial fibers (arrow) separated with interstitial cells (arrowhead), (C–D) ISO-administered rats showing myocardial degeneration associated with fatty vacuolation (arrowhead), marked sarcoplasmic eosinophilia (arrows) [C], myocardial degeneration associated with myolysis and atrophy of the myocardial fibers (arrows) [D], marked decrease in myocardial degeneration and myolysis (arrows) [E], and (F) ISO-administered rats pre-treated with UMB showing marked decrease in myocardial degeneration and myolysis. (H&E - X200 - Scale bar = 50 µm).

### 2.3. Determination of oxidative stress markers and antioxidants

Malondialdehyde (MDA), a LPO marker, and NO were assayed in the heart homogenate according to the methods described by Ohkawa et al. [28] and Green et al. [29], respectively. Reduced glutathione (GSH) [30], glutathione disulfide (GSSG) [31], superoxide dismutase (SOD) [32], and catalase (CAT) [33] were assayed using reagents prepared in the laboratory and following standard methods. 8-hydroxy-2-deoxyguanosine (8-OHdG) and HO-1 were assayed using specific ELISA kits (MyBioSource, USA), following the provided instruction.

### 2.4. Determination of pro-inflammatory cytokines, Bax, and Bcl-2

Levels of TNF- $\alpha$ , IL-6, and IL-1 $\beta$  were assayed in the heart tissue using specific ELISA kits supplied by R&D Systems (USA), whereas Bax and Bcl-2 were determined using MyBioSource (USA) ELISA kits. All assays were performed following the manufacturers' instructions.

### 2.5. Histopathology and immunohistochemistry (IHC)

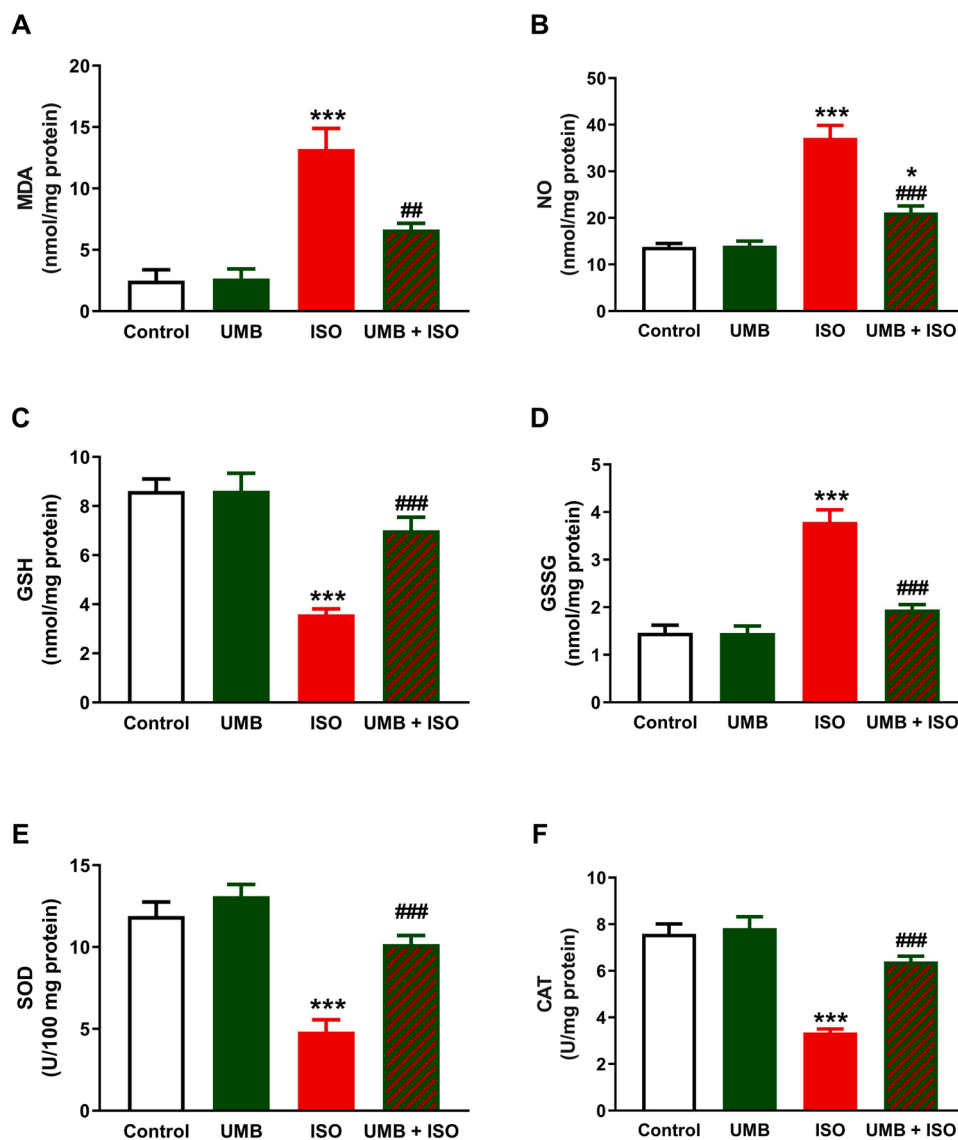
Heart samples fixed in 10% NBF for 24 h were processed for routine paraffin embedding and 5- $\mu$ m sections were cut and stained with hematoxylin and eosin (H&E) [34]. The stained sections were examined

under a light microscope.

Changes in NF- $\kappa$ B p65, Nrf2, Bax, caspase-3, and Bcl-2 were evaluated in the heart of rats using IHC. Briefly, the sections (5- $\mu$ m) were dewaxed, and antigen retrieval was carried out using 0.05 M citrate buffer (pH 6.8) followed by treatment with 0.3% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and protein block. The sections were probed with anti-Nrf2 (Invitrogen, USA), anti-NF- $\kappa$ B p65 (Santa Cruz Biotechnology, USA), Bax (Abcam, USA), Bcl-2 (Abcam, USA) or caspase-3 (Invitrogen, USA). After overnight incubation at 4 °C, the slides were washed with PBS and incubated with the secondary antibody for 30 min at room temperature. The slides were visualized with DAB kit, counterstained with Mayer's hematoxylin and the color intensity was measured by ImageJ (NIH, USA).

### 2.6. Statistical analysis

All values are presented as the mean  $\pm$  standard error of the mean (SEM). The statistical comparisons were performed using one-way ANOVA followed by Tukey's post-hoc test on GraphPad Prism 8. A P value < 0.05 was considered statistically significant.



**Fig. 3.** UMB attenuated cardiac oxidative stress in ISO-intoxicated rats. Pre-treatment with UMB decreased cardiac MDA (A), NO (B), and GSSG (D), and increased GSH (C), SOD (E), and CAT (F) in ISO-administered rats. Data are mean  $\pm$  SEM, ( $n = 8$ ). \* $P < 0.05$  and \*\*\* $P < 0.001$  versus Control. ## $P < 0.01$  and ### $P < 0.001$  versus ISO.

### 3. Results

#### 3.1. UMB prevents ISO-induced myocardial dysfunction in rats

The protective efficacy of UMB against myocardial injury was assessed by assaying cardiac function markers (Fig. 1) and histopathological alterations (Fig. 2). Cardiac injury in ISO-induced rats was manifested by the significant elevation in cTnI (Fig. 1A), CK-MB (Fig. 1B), and LDH (Fig. 1C) ( $P < 0.001$ ). Pre-treatment with UMB ameliorated serum cTnI, CK-MB, and LDH ( $P < 0.001$ ) in ISO-administered animals. Oral supplementation of UMB did not affect these markers in normal rats.

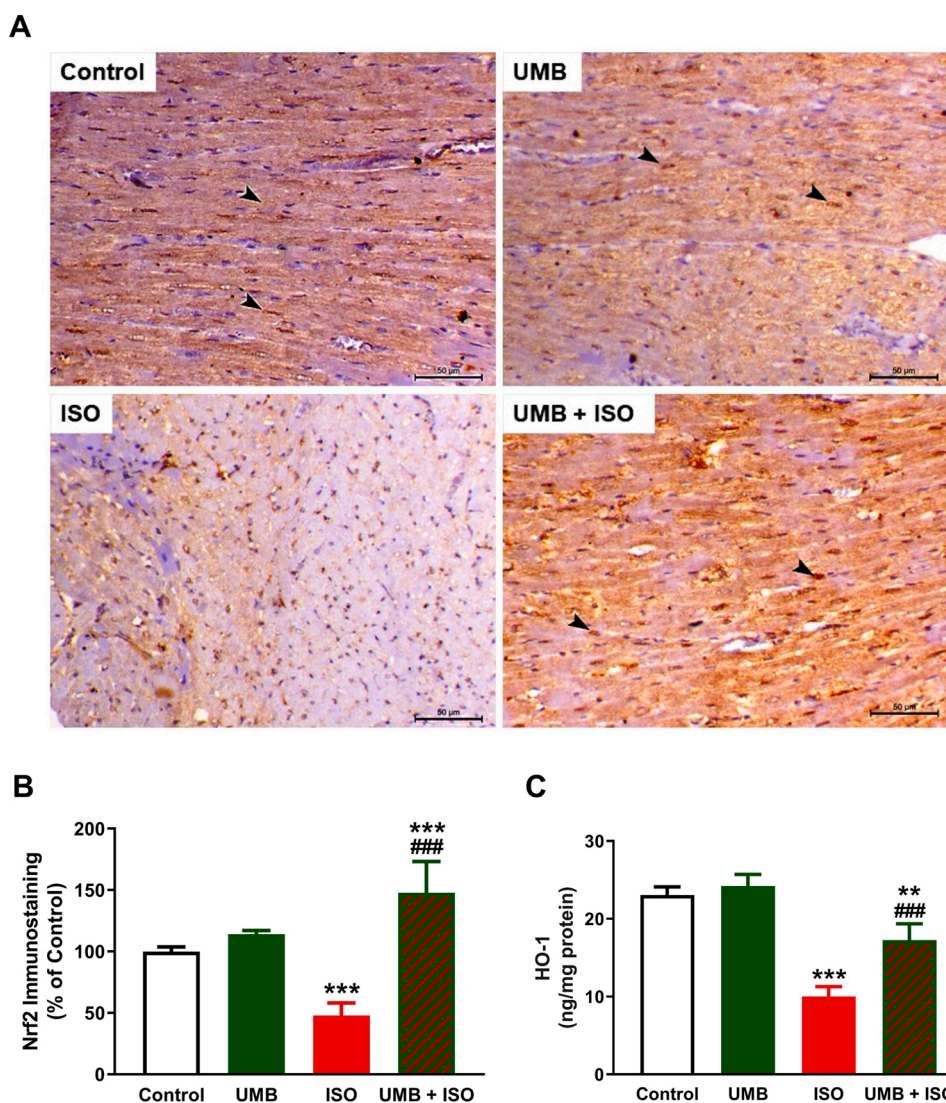
Examination of sections in the heart of normal (Fig. 2A) and UMB-treated rats (Fig. 2B) revealed normal tissue architecture with normal myocardial fibers separated with interstitial cells. In contrast, ISO administration caused myocardial degeneration associated with fatty vacuolation and marked sarcoplasmic eosinophilia (Fig. 2C), myolysis, and atrophy of the myocardial fibers (Fig. 2D). ISO-intoxicated rats pre-treated with UMB showed marked decrease in myocardial degeneration and myolysis as depicted in Fig. 2E–F.

#### 3.2. UMB attenuates cardiac oxidative stress in ISO-intoxicated rats

The ability of UMB to suppress ISO-induced cardiac oxidative stress was evaluated through the measurement of MDA, NO, and antioxidants. Administration of ISO induced a significant increase ( $P < 0.001$ ) in myocardial MDA (Fig. 3A) and NO (Fig. 3B) as compared to the control rats. The cellular antioxidant GSH was decreased (Fig. 3C), whereas its oxidized form, GSSG (Fig. 3D), was elevated significantly ( $P < 0.001$ ) in ISO-intoxicated rats. The antioxidant enzymes, SOD (Fig. 3E) and CAT (Fig. 3F), were remarkably ( $P < 0.001$ ) suppressed following ISO administration. UMB effectively prevented cardiac oxidative stress in ISO-treated rats by decreasing MDA, NO, and GSSG, and enhancing GSH, SOD and CAT.

#### 3.3. UMB activates Nrf2 signaling in ISO-induced rats

The expression of Nrf2 and HO-1 was determined in the heart of ISO-and/or UMB-treated rats as shown in Fig. 4. The results revealed significant downregulation of Nrf2 (Fig. 4A–B) and HO-1 (Fig. 4C) ( $P < 0.001$ ). UMB significantly upregulated both Nrf2 and HO-1 in the



**Fig. 4.** UMB activated Nrf2/HO-1 signaling in ISO-administered rats. (A) Photomicrographs of sections in the heart of control rats and UMB-treated rats showing marked immunostaining of Nrf2 within the myocardial fibers (arrowheads), ISO-administered rats showing decreased immunostaining of Nrf2, and ISO-administered rats pre-treated with UMB showing marked increase in the expression of Nrf2 within the myocardial fibers (arrowheads). (X200 – Scale bar = 50  $\mu$ m). (B) Image analysis of Nrf2 immunostaining showing significant upregulation in the heart of ISO-intoxicated rats treated with UMB. (C) UMB increased HO-1 expression in the heart of ISO-administered rats. Data are mean  $\pm$  SEM, ( $n = 8$ ). <sup>\*\*</sup> $P < 0.01$  and <sup>\*\*\*</sup> $P < 0.001$  versus Control, and <sup>###</sup> $P < 0.001$  versus ISO.

heart of ISO-treated rats. Of note, normal rats received UMB showed no changes in cardiac Nrf2 and HO-1.

### 3.4. UMB prevents cardiac inflammation in ISO-induced rats

The expression of NF- $\kappa$ B p65 was demonstrated using IHC as depicted in Fig. 5A and 5B. Administration of ISO provoked a significant increase in the levels of NF- $\kappa$ B p65 in the heart of rats when compared with the control group ( $P < 0.001$ ). Although UMB had no effect in normal rats, it decreased NF- $\kappa$ B p65 in ISO-treated rats ( $P < 0.001$ ). The anti-inflammatory activity of UMB was supported by the findings of the inflammatory cytokines TNF- $\alpha$  (Fig. 5C), IL-6 (Fig. 5D), and IL-1 $\beta$  (Fig. 5E). All assayed pro-inflammatory mediators were significantly increased in ISO-intoxicated rats ( $P < 0.001$ ). UMB effectively ameliorated these cytokines in ISO-intoxicated rats ( $P < 0.001$ ).

### 3.5. UMB attenuates apoptosis in the heart of ISO-induced rats

To evaluate the protective effect of UMB against ISO-induced cardiac apoptosis, we assayed the expression levels of Bcl-2, Bax, and caspase-3 as well as the levels of 8-OHdG in the heart of rats.

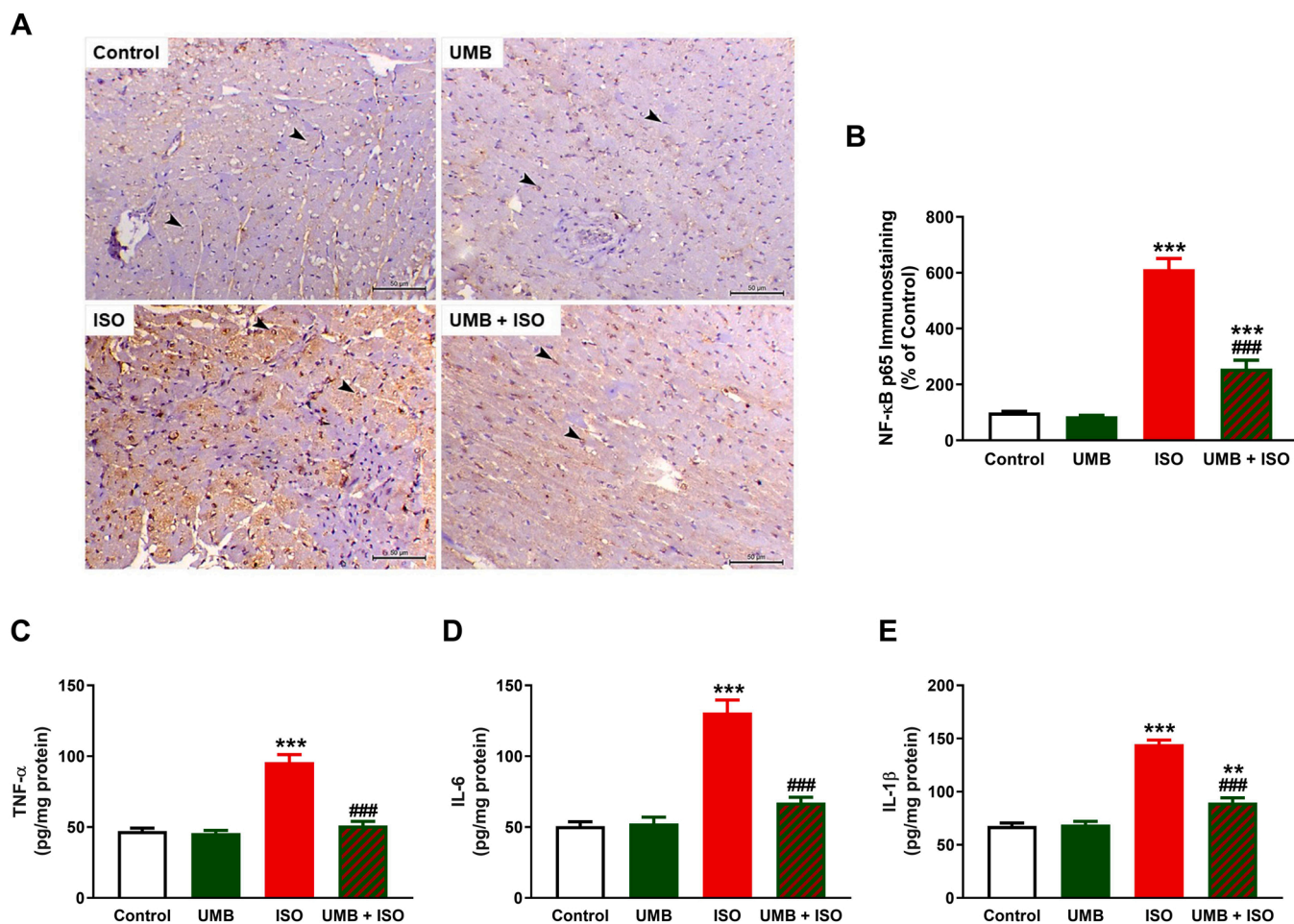
ISO provoked down-regulation of Bcl-2 as demonstrated by IHC (Fig. 6A) and ELISA (Fig. 7A). In contrast, Bax was significantly increased in the heart of ISO-intoxicated rats ( $P < 0.001$ ) (Figs. 6A and

7B). The expression of caspase-3 was upregulated ( $P < 0.001$ ) in ISO-intoxicated rats (Fig. 6A). Pre-treatment with UMB effectively down-regulated the expression of Bax and caspase-3, and increased Bcl-2 in ISO-administered rats (Figs. 6 and 7). In addition, UMB ameliorated the levels of cardiac 8-OHdG in ISO-treated rats ( $P < 0.001$ ) as represented in Fig. 7C. Oral supplementation of UMB to normal rats had no effect on the expression of all apoptosis and anti-apoptosis mediators.

## 4. Discussion

Myocardial infarction is a serious clinical problem implicated in heart damage, and the therapeutic methods are inadequate and have more adverse effects [35]. Oxidative stress is implicated in the pathological mechanism of MI [36] and agents with antioxidant properties could exert beneficial effects. This study evaluated the effect of UMB on ISO-induced oxidative stress, inflammatory response, and myocardial injury in rats, pinpointing the possible involvement of Nrf2/HO-1 signaling. The results revealed that UMB has a potent cardioprotective effect mediated through upregulation of Nrf2, enhancement of cellular antioxidants and prevention of oxidative stress, inflammatory response, and apoptotic cell death.

ISO-induced myocardia injury is a reliable model to investigate the ability of therapeutic candidates to prevent the progression of MI. ISO promotes myocardial structural and functional changes similar to the

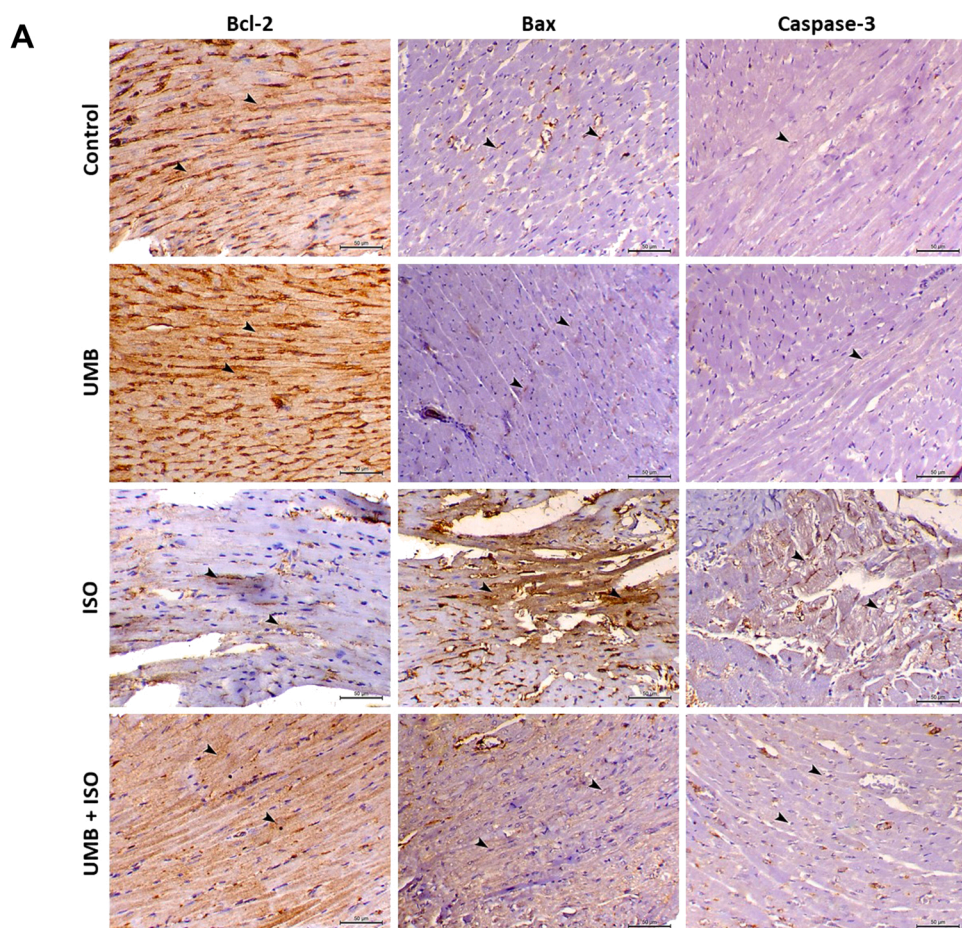


**Fig. 5.** UMB prevented cardiac inflammation in ISO-induced rats. (A) Photomicrographs of sections in the heart of control rats showing mild cytoplasmic NF- $\kappa$ B p65 within the myocardial fibers (arrowheads indicate positive immunostaining within the interstitial cells); UMB-treated rats showing mild immunostaining of NF- $\kappa$ B p65 within the myocardial fibers (arrowheads); ISO-administered rats showing marked increase the nuclear NF- $\kappa$ B p65 within the myocardial fibers (arrowheads); and ISO-administered rats pre-treated with UMB showing marked decrease in the expression of NF- $\kappa$ B p65 within the myocardial fibers (arrowheads indicate interstitial expression). (X200 – Scale bar = 50  $\mu$ m). (B) Image analysis of NF- $\kappa$ B p65 immunostaining in the heart of rats showing significant upregulation in ISO-intoxicated rats and significant decrease in rats treated with UMB. (C–E) UMB decreased cardiac TNF- $\alpha$  (C), IL-6 (D), and IL-1 $\beta$  (E) in ISO-administered rats. Data are mean  $\pm$  SEM, ( $n = 8$ ). \*\* $P < 0.01$  and \*\*\* $P < 0.001$  versus Control, and ### $P < 0.001$  versus ISO.

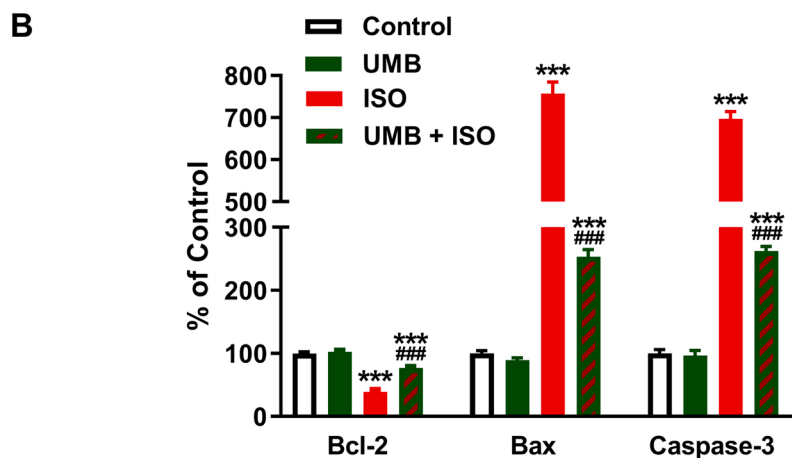
changes observed in acute MI in human [14]. ISO-induced myocardial damage was evidenced by the elevated serum levels of cTnI, CK-MB, and LDH along with the histopathological alterations, including myocardial degeneration, fatty vacuolation and sarcoplasmic eosinophilia, myolysis, and atrophy of the myocardial fibers. Accordingly, previous studies have demonstrated elevated serum cTnI, CK-MB and LDH in ISO-induced acute MI in rodents [14,37–39]. These biomarkers of cardiac function increase in serum as a consequence of cardiomyocyte injury because of cell apoptosis or necrosis [40]. Catecholamine-induced cardiac injury involves water accumulation due to increased membrane permeability and cell death [41]. UMB prevented ISO-induced cardiac injury evidenced by the ameliorated serum cardiac function markers and the histological architecture. These findings introduce new information that UMB has a protective effect against ISO-induced cardiac injury. Few studies have demonstrated the cardioprotective efficacy of UMB. Jagadeesh et al. have reported the protective effect of UMB on hyperlipidemia and cardiac hypertrophy induced by ISO in rats [24,25]. Pre-treatment with UMB for 8 days ameliorated serum troponin T, CK-MB, and plasma lipid peroxidation, and decreased the infarct size in ISO-treated rats [24,25].

Given the role of oxidative injury in mediating the detrimental effects of ISO on the heart, we assumed that UMB prevented cardiac injury through suppression of oxidative injury and enhancement of

antioxidants. Excessive production of free radicals through auto-oxidation of catecholamines has been suggested as an important contributor to ISO-induced cardiac injury [36]. In this study, MDA, NO and GSSG were increased whereas GSH, SOD and CAT were declined, demonstrating oxidative stress. Increased lipid peroxides and decreased antioxidant defenses have been reported in the heart of rats received ISO [14,37,38,42,43]. The generated lipid peroxides have a negative impact on the membrane fluidity and permeability, and can inactivate membrane-bound enzymes and covalently modify cellular proteins, leading to cell death [44]. NO can interact with the hydroxyl radicals to produce the potent oxidant peroxynitrite that induces DNA damage and cell death [45]. Therefore, attenuation of free radical generation and its subsequent LPO could help mitigating the cardiotoxic effect of ISO. UMB effectively suppressed cardiac MDA and NO, and enhanced GSH and antioxidant enzymes, demonstrating a potent antioxidant efficacy. The ability of UMB to suppress ROS generation, LPO and nitrate stress, and upregulate antioxidants has been demonstrated in several studies from Mahmoud's lab [18–22]. In rat models of cyclophosphamide hepatotoxicity, diabetes, lead-induced testicular injury, hepatic fibrosis, and hyperammonemia, UMB effectively decreased LPO and NO, and enhanced GSH, SOD and CAT in different organs [18–22]. Thus, the antioxidant properties of UMB played a key role in its cardioprotective effect in ISO-treated rats.



**Fig. 6.** UMB attenuated apoptosis in the heart of ISO-induced rats. (A) Photomicrographs of sections in the heart of rats stained with anti-Bcl-2, anti-Bax and anti-caspase-3 antibodies. Control and UMB-treated rats showed marked cytoplasmic immunostaining of Bcl-2 within the myocardial fibers (arrowheads), and mild immunostaining of Bax and caspase-3 within the myocardial fibers (arrowheads). ISO-administered rats exhibited a marked decrease in the expression of Bcl-2 within the myocardial fibers (arrowheads) and marked increase in Bax and caspase-3 within the myocardial fibers (arrowheads). ISO-administered rats pretreated with UMB showed marked increase in the expression of Bcl-2 within the myocardial fibers (arrowheads), and decreased expression of Bax and caspase-3. (X200 – Scale bar = 50  $\mu$ m). (B) Image analysis of Bcl-2, Bax, and caspase-3 immunostaining. Data are mean  $\pm$  SEM, (n = 8). \*\*\*P < 0.001 versus Control, and ###P < 0.001 versus ISO.



Besides its radical-scavenging properties, we assumed that upregulation of Nrf2/HO-1 signaling is involved in mediating the cardioprotective effect of UMB. Nrf2 is a redox-sensitive transcription factor that is activated in response to increased ROS and electrophilic chemicals to promote the transcription of genes encoding antioxidant enzymes, including HO-1 and SOD [9]. Here, Nrf2 and HO-1 were downregulated in the heart of ISO-intoxicated rats, an effect that could be attributed to the prolonged and excessive ROS generation. UMB upregulated Nrf2 and HO-1 in ISO-intoxicated rats, pointing to the key role of this signaling pathway in attenuating oxidative stress. The ability of UMB to activate Nrf2 signaling has been previously reported in the testes of lead-intoxicated rats [21] and liver of cyclophosphamide-intoxicated rats [18]. The role of Nrf2 in mediating

the antioxidant effect of UMB has been supported by the study of Li et al. [46]. UMB protected the liver against methyl glyoxal toxicity by activating Nrf2, whereas siRNA-induced depletion of Nrf2 inhibited the hepatoprotective effect of UMB [46].

Inflammation is implicated in MI and previous studies have demonstrated elevation in pro-inflammatory cytokines following the administration of ISO [14,37,47]. Here, ISO-treated rats exhibited significant upregulation in cardiac NF- $\kappa$ B p65, TNF- $\alpha$ , IL-6, and IL-1 $\beta$ , demonstrating the development of an inflammatory response. The inflammatory status could be connected to the excessive production of ROS which activate NF- $\kappa$ B and the release of inflammatory mediators and subsequently can trigger mitochondrial dysfunction and cell apoptosis [48]. Activation of NF- $\kappa$ B can promote further oxidative



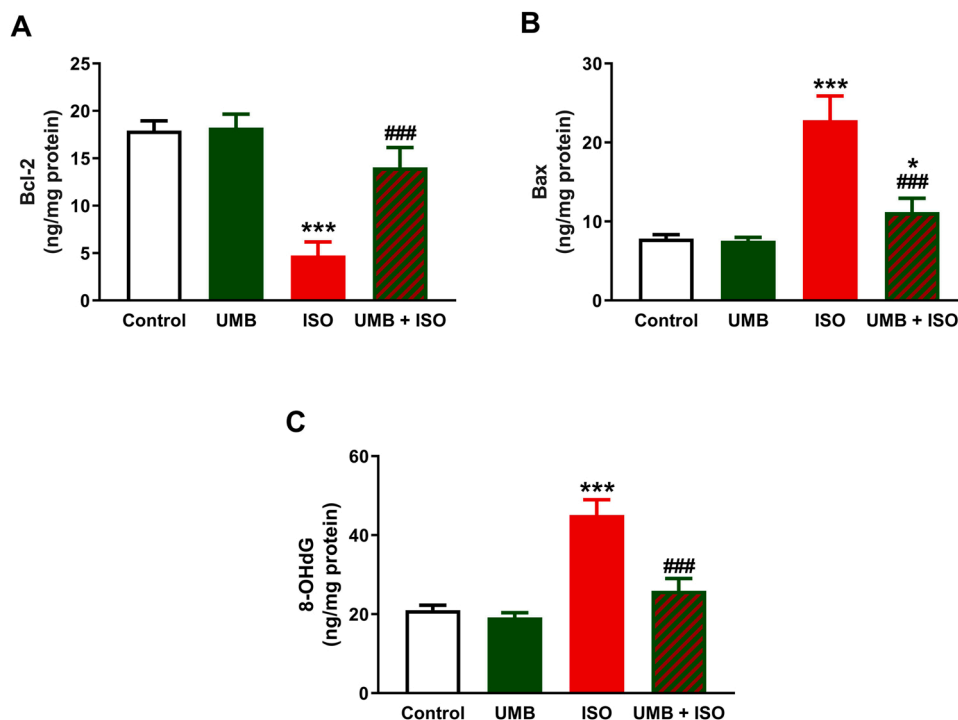


Fig. 7. UMB increased cardiac Bcl-2 (A), and decreased Bax (B) and 8-OHdG in ISO-administered rats. Data are mean  $\pm$  SEM, ( $n = 8$ ). \* $P < 0.05$  and \*\*\* $P < 0.001$  versus Control, and ### $P < 0.001$  versus ISO.

damage and cardiac dysfunction [49]. The inflammatory response and oxidative stress orchestrate cardiomyocyte death via apoptosis in ISO-treated animals [14,37,39,49]. In this context, ISO-treated rats in this study exhibited apoptotic cell death manifested by the down-regulation of Bcl-2 and increased Bax and caspase-3 as well as the provoked oxidative DNA damage. In ISO-treated animals, apoptosis is thought to be elicited via the increased generation of ROS and pro-inflammatory cytokines leading to the release of mitochondrial cytochrome *c* and activation of caspase-3 along with diminishing anti-apoptosis factors [50]. ISO can also provoke the accumulation of  $Ca^{2+}$  in cardiomyocytes and subsequently generates ROS and activates endonuclease leading to DNA fragmentation [51,52].

UMB suppressed NF- $\kappa$ B, inflammatory cytokines, Bax, and caspase-3 and boosted Bcl-2 in the heart of ISO-administered rats. The protective role of UMB against inflammatory response and apoptotic cell death has been previously demonstrated. UMB protected the spermatogenic cells against lead-induced oxidative DNA damage, inflammation, and apoptosis by upregulating Nrf2/HO-1 signaling [21]. In experimental hyperammonemia [22], liver fibrosis [19], and diabetes [20], UMB prevented inflammation and apoptotic cell death. Through upregulation of Nrf2/HO-1 signaling and suppression of inflammation and oxidative damage, UMB protected rats against cyclophosphamide hepatotoxicity [18]. These findings added support to the anti-inflammatory and anti-apoptosis properties of UMB which could be a direct consequence of enhanced antioxidant defenses via Nrf2/HO-1 signaling.

## 5. Conclusion

The findings of this study showed the involvement of Nrf2/HO-1 signaling in the protective effect of UMB against cardiac injury induced by ISO. UMB ameliorated circulating cardiac function markers, prevented histopathological alterations, attenuated oxidative stress, and enhanced antioxidants in the heart of ISO-administered rats. In addition, UMB downregulated ISO-induced cardiac NF- $\kappa$ B, pro-inflammatory cytokines, oxidative DNA damage, and apoptosis. These beneficial effects were associated with upregulation of Nrf2. Therefore, UMB may

represent a promising cardioprotective agent against acute MI, pending further investigations to explore other mechanism(s) underlying the cardioprotective effect.

## 6. Limitations of the study

This study reveals the cardioprotective effects of UMB on ISO-induced myocardial injury; however, it has some limitations. Although UMB increased the antioxidant defenses and decreased MDA and NO, we didn't measure its effect on the generation of ROS. While our experiment clearly demonstrated upregulation and downregulation of some proteins as shown by immunohistochemistry and ELISA, we did not validate it by RT-PCR to show the relationship between mRNA and protein levels. The lack of electrocardiography to detect pathological changes and support the biochemical and histological findings could be considered as a limitation.

## Ethics approval

The study was conducted in agreement with the guidelines of the National Institutes of Health (NIH Publication no. 8523, revised 2011), and approved by the Ethics Committee of Al-Hussein Bin Talal University.

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## CRediT authorship contribution statement

**Osama Y. Althunibat:** Data curation, Resources. **Maisa Siddiq Abduh:** Resources, Data curation, Funding acquisition, Investigation. **Mohammad H. Abukhalil:** Conceptualization, Methodology, Formal

analysis, Supervision, Writing – original draft. **Saleem H. Aladaileh**: Data curation, Investigation, Methodology. **Hamza Hanieh**: Resources. **Ayman M. Mahmoud**: Conceptualization, Formal analysis, Investigation, Data curation Methodology, Validation, Supervision, Writing – original draft, Writing – review & editing.

### Declaration of Competing Interest

All authors declare no conflict of interests in relation to the manuscript.

### Data availability

The manuscript contains all data supporting the reported results.

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