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ORIGINAL RESEARCH ARTICLE

Effect of *Dunaliella salina* on myocardial ischemia-reperfusion injury through KEAP1/NRF2 pathway activation and JAK2/STAT3 pathway inhibition

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Abstract

Myocardial ischemia-reperfusion injury (MIRI) is a life-threatening vascular emergency, in which oxidative stress and excessive inflammation reactions play significant roles. *Dunaliella salina* is rich in natural beta (β)-carotene, which is considered an antioxidant and anti-cancer compound, preventing night blindness, delaying aging, and regulating immunity. Due to its unique nutritional and functional components, *D. salina* has the potential to be developed as a nutritional health food. At present, it is unknown whether *D. salina* can rescue MIRI. The present study aimed at investigating whether *D. salina* has protective effect on MIRI and at exploring its potential mechanism. We developed a Langendorff perfused heart model in mice. The mice were given *D. salina* by gavage at a dose of 500 mg/kg for 7 days consecutively. It was found that *D. salina* could improve left ventricle function and reduce the rate of malignant arrhythmia and infarct size ($P < 0.01$). Furthermore, *D. salina* administration increased superoxide dismutase and decreased malondialdehyde content in myocardial tissue ($P < 0.01$). Importantly, real-time polymerase chain reaction and Western blot results showed that *D. salina* caused nuclear factor (erythroid-derived 2)-like 2 (NRF2) activation and enhanced the expression of antioxidative genes, such as heme oxygenase-1 (HO-1) and NAD(P)H quinone oxidoreductase 1 (NQO1) ($P < 0.05$ and $P < 0.01$, respectively). At the same time, the phosphorylation of Janus kinase (JAK) and signal transducer and activator of transcription 3 (STAT3) was inhibited ($P < 0.05$ and $P < 0.01$, respectively), and proinflammatory cytokines interleukin-1 beta (IL-1β) and IL-6 decreased with *D. salina* administration ($P < 0.05$). In conclusion, *D. salina* has a protective effect on MIRI, which may be mediated by promoting kelch-like ECH-associated protein 1 (KEAP1)/NRF2 pathway and inhibiting JAK2/STAT3 signaling pathway.

Keywords: *Dunaliella salina*; Myocardial ischemia-reperfusion injury; KEAP1/NRF2; JAK2/STAT3

1. Introduction

Ischemic cardiomyopathy is a special type or the late stage of coronary heart disease. With the increasing incidence of coronary heart disease, the threat from ischemic

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cardiomyopathy to human health has grown[1]. The first principle of treatment is to restore blood supply to the ischemic myocardium as soon as possible[2]. However, reperfusion might further aggravate myocardial injury. This is known as myocardial ischemia-reperfusion injury (MIRI)[3]. A number of mechanisms are involved in it, causing serious new dysfunction, including excessive inflammatory response, calcium ion (Ca^{2+}) overload, endoplasmic reticulum, and mitochondrial damage[4,5]. MIRI leads to an inflammatory response, resulting in inflammatory cell infiltration and accumulation of proinflammatory factors[5]. In addition, excessive oxidative stress has also been reported to play an important role in MIRI. Previous research has identified the oxidative stress theory as one of the main etiologies of MIRI, which usually originates in either the excessive production of reactive oxygen species (ROS) or the decline of scavenging capacity[6,7].

Dunaliella salina, a halophilic green microalgae, rich in natural beta (β)-carotene, is considered an antioxidant and anti-cancer compound, preventing night blindness, delaying aging, and regulating immunity. In view of its unique nutritional and functional components, *D. salina* has the potential to be developed as a nutritional health food and authorized as additives for both humans and animals[8]. Hence, *D. salina* is considered a powerful antioxidant and has excellent effect in preventing oxidative stress[9,10]. As shown in mice, *D. salina* also has preventive effect on cornea injury induced by ultraviolet radiation, which may be due to the increased activity of antioxidant defense system and the inhibition of lipid peroxidation[11]. A study has also shown that *D. salina* could exert therapeutic potential against hepatic encephalopathy through its antioxidant, anti-inflammatory, and cytoprotective effects[12]. Oral supplementation with *D. salina* has also shown to improve cardiac dysfunction in obese rats[8]. In addition, *D. salina* has shown to ameliorate heart dysfunction associated with aging in rats induced by D-galactose[10]. Whether or not *D. salina* could alleviate MIRI, the involved mechanism remains to be elucidated.

The kelch-like ECH-associated protein 1 (KEAP1)/nuclear factor (erythroid-derived 2)-like 2 (NRF2) signaling pathway is considered one of the most pivotal endogenous antioxidative system[13]. In brief, NRF2 and KEAP1 bind together in the cytoplasm physiologically. When stimulated, NRF2 detaches from KEAP1, translocates to the nucleus, and then promotes the expression of genes associated with antioxidant activities, such as heme oxygenase-1 (HO-1) and quinone oxidoreductase 1 (NQO1)[6]. Recent studies have shown that suppressing oxidative stress response could ameliorate ischemia/reperfusion (I/R) injury through NRF2/KEAP1/antioxidant response element (ARE) pathway activation[14]. At the same time, the inflammatory damage during MIRI should not be underestimated. The Janus kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3) pathway is involved in various cardiovascular complications, including cardiomyocyte apoptosis, endoplasmic reticulum stress, oxidative stress, and inflammation[15]. The rapid activation of JAK2/STAT3 pathway after I/R enhances the transcription of inflammatory cytokines, which are closely related to myocardial damage[5,16]. The inhibition of JAK2/STAT3 pathway could reduce inflammatory damage in rats suffering from MIRI[17]. The present study aimed at investigating the effect of *D. salina* on MIRI and the status of KEAP1/NRF2 and JAK2/STAT3 signaling pathways underlying *D. salina* against MIRI.

2. Material and methods

2.1. Experimental animals

C57BL/6J mice (male, 21–25 g) were obtained from Laboratory Animal Center of Henan University of Science and Technology (Luoyang, China; license number: SCXK 20190008). The mice were randomly divided into three groups (n = 8 per group) after 1 week of adaptive feeding: (i) Control group; (ii) I/R group; and (3) I/R + *D. salina* group (500 mg/kg). All mice in the I/R + *D. salina* group were given *D. salina* by gavage for 7 days consecutively, whereas the other two groups of mice were given saline in the same way. All mice were kept in a controlled environment (temperature, 22 ± 2°C; humidity, 50 ± 5%; 12 h light/dark cycle). The mice had unrestricted access to food and water.

The study was approved by the Animal Care and Ethics Committee of Henan University of Science and Technology (Luoyang, China) and was performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals[18].

2.2. Langendorff perfused heart model

The mice were given intraperitoneal injection of ethyl urethane (1 g/kg) for anesthesia and heparin (500 IU/kg) for heparinization. The hearts were removed from the thoracic cavity and attached on the Langendorff apparatus. Perfusion was maintained at a constant pressure with warmed (37.5°C) perfusate, and the hearts were immersed in a water-jacketed temperature-controlled glass chamber at 37.5°C, so as to ensure normothermia throughout the perfusion protocol. A latex balloon connected to the sensor was inserted into the left ventricle of each heart through left atrium incision. In the meantime, the heart rate, left ventricular systolic pressure (LVSP), and electrocardiograph

(ECG) were continuously sampled using the BL-420S biological and functional experimental system.

2.3. Creatine kinase (CK) and lactate dehydrogenase (LDH) activity measurement

The CK and LDH activity of the perfusate were measured using a commercial test kit (Nanjing Jiancheng Biotechnology, China).

2.4. Triphenyltetrazolium chloride (TTC) staining

Myocardial infarction area was detected by TTC staining. Each heart was removed and frozen at −20°C for 20 min. The whole heart was cut into 5–6 pieces of 2 mm tissue slides, which were placed in 1% 2,3,5-TTC (lot number: abs47011070, Absin) to stain evenly. After washing in phosphate-buffered saline (PBS), the heart pieces were fixed in 10% formalin for 24 h[19] and then photographed. All the non-infarct areas (red) and infarct areas (white) were measured and calculated from each sample in the corresponding group by the image analysis software Image-Pro. The percentage of infarct area was calculated according to the following formula:

Myocardial infarct area (%) = Myocardial infarct area/ total myocardial area × 100%.

2.5. Hematoxylin and eosin (H&E) staining

At the end of reperfusion, part of the left ventricle tissue was immediately placed in 4% paraformaldehyde for more than 24 h. After embedding the myocardial tissues in paraffin, the tissues were sliced, dewaxed, stained with hematoxylin, re-stained with eosin, dehydrated, sealed, and then observed under a microscope and photographed.

2.6. Superoxide dismutase (SOD) activity and maleic dialdehyde (MDA) content in myocardial homogenate

At the end of reperfusion, the hearts were taken off the Langendorff apparatus and placed in an icy plate. The left ventricles were sliced to about 50 mg and homogenized at 4°C in saline (1:9). The homogenates were centrifuged at 3000 rpm for 15 min, and then, the supernatant was taken out for copper zinc (Cu/Zn) SOD activity and MDA content assay. The activity levels of Cu/Zn SOD and the content of MDA were determined by enzyme-linked immunosorbent assay (ELISA) using the assay kit (Nanjing Jiancheng Biotechnology, China) and following the manufacturer's instructions.

2.7. RNA extraction and quantitative polymerase chain reaction (qPCR)

RNA was extracted from the myocardium using TRIzol® reagent (Invitrogen), and copy DNA (cDNA) was synthesized by reverse transcription using a high-capacity complementary reverse transcription kit (Applied Biosystems): denatured at 70°C for 10 min, amplified at 37°C for 60 min, and inactivated at 94°C for 10 min. The SYBR™ Green PCR Master Mix kit (CWBio) was used for qPCR amplification. The messenger RNA (mRNA) levels of *NQO1*, *HO1*, *IL-1β*, and *IL-6* were detected. qPCR was performed in the StepOnePlus qPCR system (Applied Biosystems), that is, predenaturation at 94°C for 20 s, then 94°C for 15 s and 60°C for 1 min for 40 cycles. Genes were quantified by the $2^{-\Delta\Delta Cq}$ method, and the internal reference gene was glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene. The primer sequences are shown in Table S1.

2.8. Western blot analysis

Total proteins were extracted from the left ventricle with radioimmunoprecipitation assay lysis buffer at 4°C. Equal amounts of protein (30 μg) were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis and transferred to polyvinylidene difluoride membranes (EMD Millipore). The membranes were sealed with 5% bovine serum albumin containing 0.05% Tween-20 (TBST) for 2 h at room temperature and then incubated overnight with the following primary antibody at 4°C: NRF2 (1:500 Proteintech 163961AP), KEAP1 (1:500 Proteintech 105032AP), JAK2 (1:1000 CST 3230S), phos-JAK2 (1:1000 CST 3774S), STAT3 (1:1000 CST 12640S), phos-STAT3 (1:1000 CST 9145S), and GAPDH (1:1000 BOSTER. BM3896). After the primary antibody incubation overnight, the membranes were incubated with secondary antibody for 1 h at room temperature. Protein bands were developed with excellent chemiluminescent substrate (ECL; catalog no. 180–501, Tanon). GAPDH was used as the internal reference of total protein, and ImageJ 1.80 was used for band quantification.

2.9. Statistical analysis

Data were expressed as mean ± standard error of the mean. Statistical analysis was conducted with GraphPad Prism 8.0. One-way analysis of variance was used for comparing multiple groups with one factor. $P < 0.05$ was considered statistically different.

3. Results

3.1. *D. salina* pre-treatment improved cardiac function after myocardial ischemia reperfusion in mice

The drug treatment and building steps of isolated MIRI are shown in Figure 1A. The cardiac function was monitored during myocardial ischemia reperfusion. *D. salina* pre-

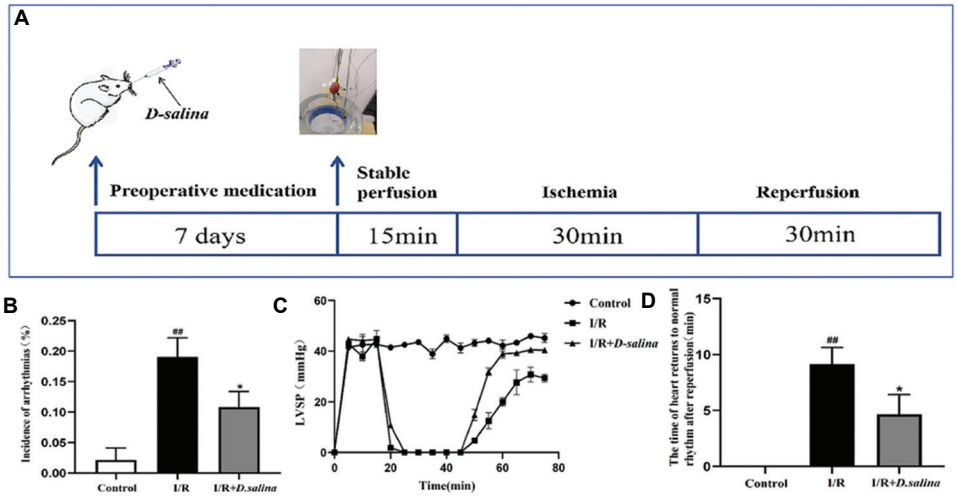


Figure 1. *D. salina* improved the cardiac function of mice after myocardial ischemia reperfusion. (A) A schematic diagram of myocardial ischemia reperfusion model. (B) *D. salina* decreased the incidence of malignant arrhythmias. (C) *D. salina* improved LVSP. (D) *D. salina* shortened the recovery time of the heart returning to normal rhythm after reperfusion. Data were expressed as mean ± SEM, n = 8 per group. $^{\#\#}P < 0.01$, compared with the control group; $^{**}P < 0.01$, $^{*}P < 0.05$, compared with the I/R group.
D. salina: *Dunaliella salina*; I/R: Ischemia/Reperfusion; LVSP: Left ventricular systolic pressure; SEM: Standard error of the mean.

treatment significantly reduced the incidence of malignant arrhythmias ($P < 0.01$; Figure 1B), increased LVSP (Figure 1C), and shortened the recovery time of the heart returning to normal rhythm after reperfusion (Figure 1D) in mice ($P < 0.05$).

3.2. *D. salina*-alleviated myocardial injury after ischemia reperfusion in mice

To investigate the effect of *D. salina* on MIRI, CK and LDH levels were evaluated in coronary effluent. The CK and LDH levels were both significantly elevated after I/R ($P < 0.01$), but *D. salina* pre-treatment significantly reversed CK and LDH levels (Figure 2A and B). Furthermore, TTC staining showed that the myocardial infarction area increased in the I/R group ($P < 0.01$) but significantly decreased in the I/R + *D. salina* group ($P < 0.01$, Figure 2C and D). H&E staining revealed that the myocardial tissue structure in the control group was arranged normally without inflammatory cell infiltration. In the I/R group, the myocardial tissue showed disordered myocardial fiber rupture and significant infiltration of inflammatory cells, but *D. salina* treatment reversed this structural disorder (Figure 2E).

3.3. *D. salina*-enhanced antioxidant activity after myocardial ischemia reperfusion in mice

To elucidate the protective mechanism of *D. salina* against MIRI, the levels of SOD and MDA in cardiac tissue homogenate were measured by ELISA, and reverse transcription (RT)-qPCR and Western blot were used to determine the expression associated with NRF2/KEAP1 signaling. The levels of SOD were significantly downregulated after I/R, while *D. salina* pre-treatment partly restored SOD content ($P < 0.01$) (Figure 3A). On the contrary, the levels of MDA were elevated in the I/R group ($P < 0.01$) but reduced significantly in the I/R + *D. salina* group ($P < 0.01$) (Figure 3B). In addition, the expression of *HO-1* and *NQO1* genes was significantly upregulated with *D. salina* pretreatment ($P < 0.05$ and $P < 0.01$, respectively, Figure 3C and D). After I/R, KEAP1 protein expression decreased in trend, and NRF2 protein expression decreased significantly ($P < 0.01$) (Figure 3E–G), but HO-1 expression showed an opposite trend ($P < 0.05$) (Figure 3E and H). *D. salina* pretreatment decreased KEAP1 protein expression and increased NRF2 and HO-1 protein expression significantly ($P < 0.05$; Figure 3E–H).

3.4. *D. salina* reduced inflammatory response after myocardial ischemia reperfusion in mice

To determine if inflammatory response is involved in the protective effect of *D. salina* against MIRI, the expressions of inflammation-related factors and JAK2/STAT3 signaling pathway were measured using molecular biological techniques. The results showed that the gene expression of proinflammatory cytokines *IL-1β* and *IL-6* both increased after I/R ($P < 0.05$ and $P < 0.01$, respectively), while *D. salina* significantly decreased the expression of both *IL-6* and *IL-1β* ($P < 0.05$; Figure 4A and B). Western blot results showed that JAK2/STAT3 signaling pathway was activated after I/R and the protein expression of phospho-

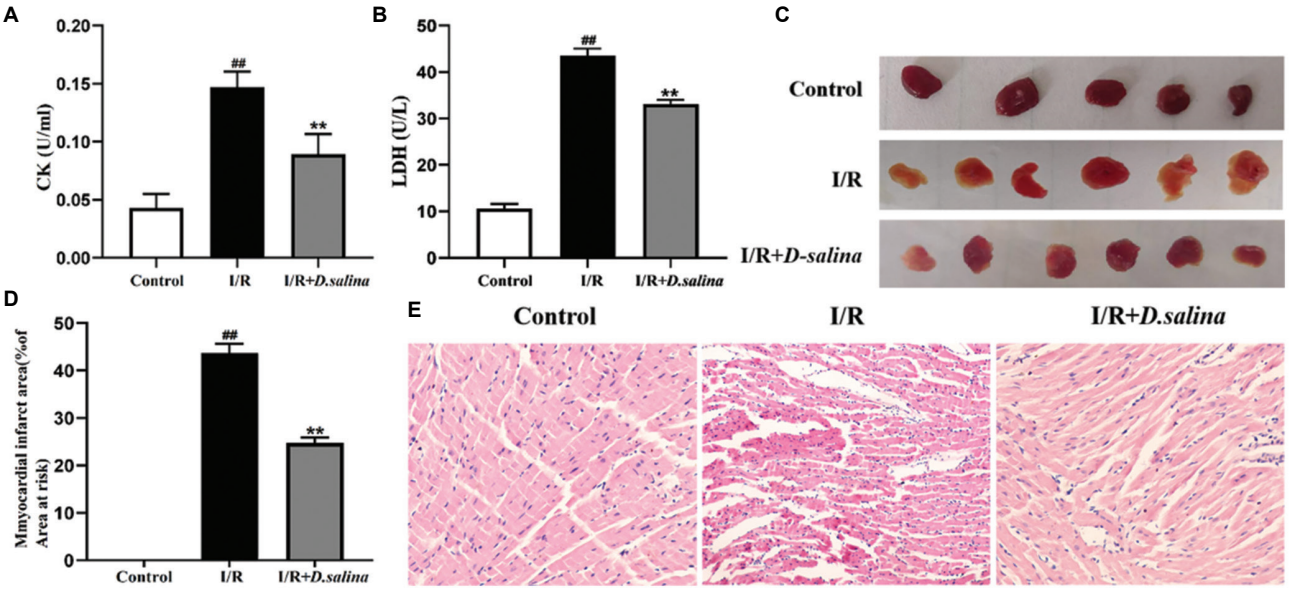


Figure 2. *D. salina* protected against MIRI in mice. (A and B) CK and LDH activity in coronary effluent. (C and D) Representative images of TTC staining and averaged infarct size. (E) Representative pictures of H&E-stained cardiac tissue sections. Data were expressed as mean ± SEM, n = 8 per group. $^{\#\#}P < 0.01$, compared with the control group; $^{**}P < 0.01$, compared with the I/R group.
CK: Creatine kinase; D. salina: *Dunaliella salina*; H&E: Hematoxylin and eosin; I/R: Ischemia/Reperfusion; LDH: Lactate dehydrogenase; SEM: Standard error of the mean; TTC: Triphenyltetrazolium chloride.

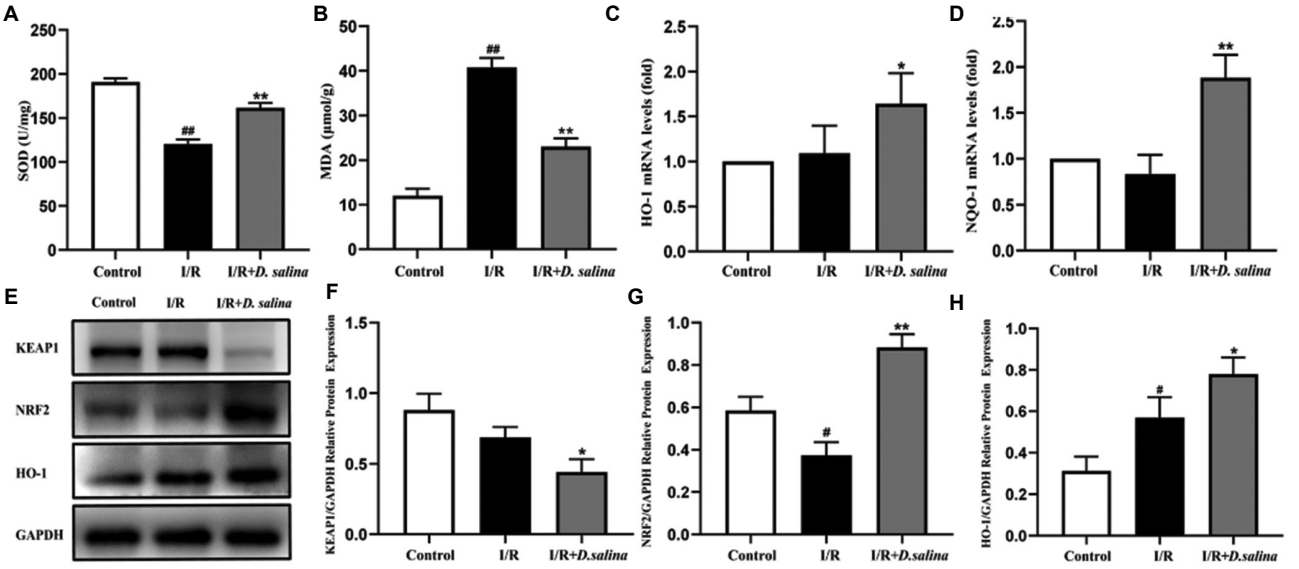


Figure 3. *D. salina* enhanced antioxidant activity after myocardial ischemia reperfusion in mice. (A) SOD activity and (B) MDA content in myocardial homogenate. mRNA levels of (C) *HO-1* and (D) *NQO1*. (E) Representative Western blot images. (F) Quantity analysis of KEAP1, (G) NRF2, and (H) HO-1. Data were expressed as mean ± SEM, n = 8 per group. $^{\#\#}P < 0.01$, $^{\#}P < 0.05$, compare with the control group; $^{**}P < 0.01$, $^{*}P < 0.05$, compared with the I/R group.
D. salina: *Dunaliella salina*; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; HO-1: Heme oxygenase-1; I/R: Ischemia/Reperfusion; KEAP1: Kelch-like ECH-associated protein 1; MDA: Maleic dialdehyde; mRNA: messenger RNA; NRF2: Nuclear factor (erythroid-derived 2)-like 2; NQO1: NAD(P)H quinone oxidoreductase 1; SEM: Standard error of the mean; SOD: Superoxide dismutase.

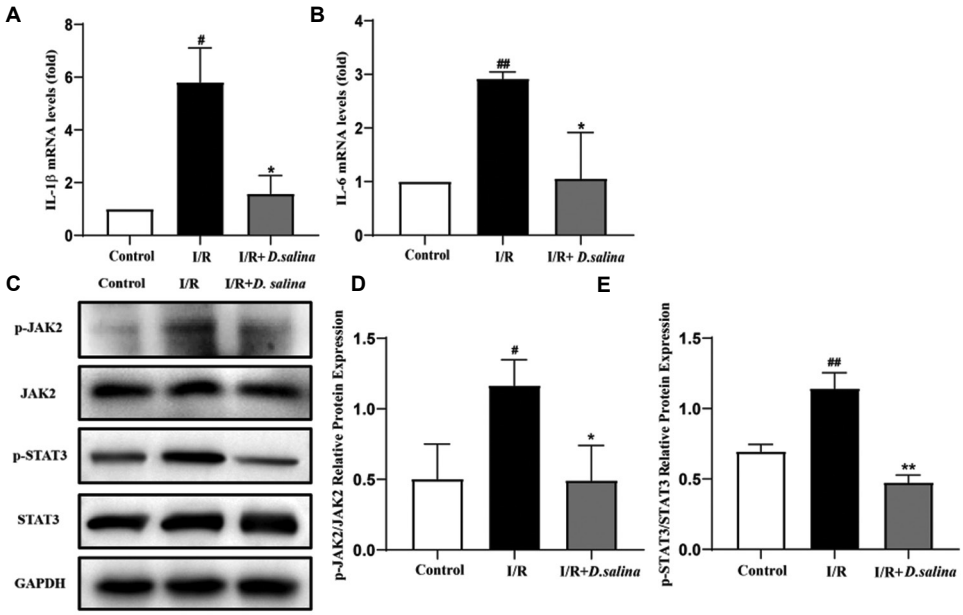


Figure 4. *D. salina* reduced inflammatory response after myocardial ischemia reperfusion in mice. Gene expression of inflammatory cytokines (A) *IL-1β* and (B) *IL-6*. (C) Representative Western blot images of JAK2/STAT3 signaling pathway. Quantity analysis of (D) p-JAK2 and (E) p-STAT3. Data were expressed as mean ± SEM, n = 8 per group. $^{\#\#}P < 0.01$, $^{\#}P < 0.05$, compared with the control group; $^{**}P < 0.01$, $^{*}P < 0.05$, compared with the I/R group. *D. salina*: *Dunaliella salina*; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; I/R: Ischemia/Reperfusion; IL: Interleukin; JAK2: Janus kinase 2; mRNA: messenger RNA; p-JAK2: phospho-JAK2; p-STAT3: phospho-STAT3; SEM: Standard error of the mean; STAT3: Signal transducer and activator of transcription 3.

JAK2 (p-JAK2) and p-STAT3 in the I/R + *D. salina* group decreased significantly ($P < 0.05$ and $P < 0.01$, respectively, Figure 4C–E).

4. Discussion

The present study demonstrated that *D. salina* pre-treatment provides protection against MIRI and the effect may be mediated through the enhancement of KEAP1/NRF2 endogenous antioxidant system and the weakening of JAK2/STAT3 inflammatory signaling pathway. The results indicated the potential of *D. salina* as a promising candidate drug in the prevention of MIRI. The present study is the first study to show that the protective effect of *D. salina* on MIRI may be related to KEAP1/NRF2 and JAK2/STAT3 signaling pathways.

The Langendorff perfused heart is a classical *in vitro* model used to examine cardiac function, such as LVSP and heart rate, without the influence of complex internal environment[19]. The results showed that *D. salina* pre-treatment could increase LVSP and decrease the incidence of malignant arrhythmias within 30 min of reperfusion. In addition, CK and LDH are often used as indicators of myocardial injury[20]. The results showed that the levels of CK and LDH decreased significantly after pretreatment with *D. salina*. TTC staining results also showed that the preconditioning of *D. salina* could reduce myocardial infarction area. In addition, H&E staining showed that *D. salina* preconditioning reduced myocardial damage at the morphological level. Taken together, these results suggest that *D. salina* pretreatment could protect the heart from MIRI.

Enormous ROS can consume endogenous antioxidant substances and enzymes, cause peroxidation of the lipid bilayer of the cell membrane, and lead to cell injury[21]. SOD can effectively scavenge ROS[22], while MDA, a marker of lipid peroxidation, indicates the degree of lipid peroxidation[23,24]. In the *D. salina* pre-treatment group, SOD activity increased and the content of MDA significantly reduced in the myocardial tissue, indicating that *D. salina* can significantly reduce the oxidative stress level. The KEAP1/NRF2 signaling pathway is an important endogenous antioxidant defense system[25]. NRF2 regulates the transcription of antioxidant-related genes by binding to ARE[26]. NRF2 and KEAP1 bind together in the cytoplasm under normal physiological conditions. When stimulated, KEAP1 degrades, and NRF2 is released from the cytoplasm. Then, NRF2 nuclear translocation occurs, and NRF2 binds with MAF to form heterodimers[27]. *D. salina* administration upregulated NRF2 expression and its downstream antioxidative enzymes, such as HO-1 and NQO1, which may be related to the degradation of KEAP1.

The upregulation of HO-1 and NQO1 can counteract excessive oxidative stress in myocardium caused by MIRI[28]. The present study suggested that *D. salina* has protective effect against MIRI, which may potentially be due to the enhancement of KEAP1/NRF2 endogenous antioxidant function.

The JAK/STAT signaling pathway is vital in the progression of MIRI, especially in the treatment of inflammatory injuries. JAK-STAT signaling molecules bind to specific nucleotide sequences in the promoter regions of cytokines and inflammatory cytokines (including IL-1β and IL-6) to induce gene expression. The activated JAK catalyzes tyrosine phosphorylation of the receptor, thus providing a binding site for the Src homology 2 (SH2) domain of STATs. The phosphorylated STAT3 transferred from the cytoplasm to the nucleus results in the release of proinflammatory factors[5,29,30]. Xu *et al*. reported that baicalin can substantially decrease the expression of *IL-1β* and *IL-6* genes, and Zhang *et al*. found that oxymatrine reduces myocardial damage by downregulating JAK2/STAT3 pathway and reducing IL-1β and IL-6 release[5,31]. Similar to these studies, we found that the activation of JAK2/STAT3 pathway induced by I/R was inhibited after pretreatment with *D. salina* and the release of IL-1β and IL-6 also reduced.

In conclusion, *D. salina* exerts potent protective effect against MIRI, which may be related to the activation of KEAP1/NRF2 pathway and the inhibition of JAK2/STAT3 pathway, thus reducing inflammatory damage. The current findings suggested that *D. salina* may serve as a candidate drug for MIRI. However, there are still some limitations to the present study; for instance, it is unknown how *D. salina* regulates these signaling pathways and which components of *D. salina* are responsible for them. Further studies are needed to elaborate the exact composition and direct targets of *D. salina*.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

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Writing – review & editing: Jingxia Du

Ethics approval and consent to participate

This study was approved by the Animal Care and Ethics Committee of Henan University of Science and Technology (Licence number: SCXK 2021-0009) and in accordance with the guide for the care and use of laboratory animals of National Institutes of Health Guidelines.

Consent for publication

Not applicable.

Availability of data

The original contributions presented in the study are included in the article or Supplementary File. Further inquiries can be directed to the corresponding author.

References

1. Kwon SP, Hwang BH, Park EH, *et al*., 2021, Nanoparticle-mediated blocking of excessive inflammation for prevention of heart failure following myocardial infarction. *Small*, 17: e2101207.
2. Wang J, Chen Z, Dai Q, *et al*., 2020, Intravenously delivered mesenchymal stem cells prevent microvascular obstruction formation after myocardial ischemia/reperfusion injury. *Basic Res Cardiol*, 115: 40.
3. Lieder HR, Braczko F, Gedik N, *et al*., 2021, Cardioprotection by post-conditioning with exogenous triiodothyronine in isolated perfused rat hearts and isolated adult rat cardiomyocytes. *Basic Res Cardiol*, 116: 27.
4. Yadid M, Lind JU, Ardoña HAM, *et al*., 2020, Endothelial extracellular vesicles contain protective proteins and rescue ischemia-reperfusion injury in a human heart-on-chip. *Sci Transl Med*, 12: eaax8005.
5. Xu M, Li X, Song L, 2020, Baicalin regulates macrophages polarization and alleviates myocardial ischaemia/reperfusion injury via inhibiting JAK/STAT pathway. *Pharm Biol*, 58: 655–663.
6. Ucar BU, Ucar G, Saha S, *et al*., 2021, Pharmacological protection against ischemia-reperfusion injury by regulating the Nrf2-Keap1-ARE signaling pathway. *Antioxidants* (*Basel*), 10: 823.
7. Sugamura K, Keaney JF Jr., 2011, Reactive oxygen species in cardiovascular disease. *Free Radic Biol Med*, 51: 978–992.
8. El-Baz F, Aly HF, Abd-Alla HI, 2020, The ameliorating effect of carotenoid rich fraction extracted from *Dunaliella salina* microalga against inflammation-associated cardiac dysfunction in obese rats. *Toxicol Rep*, 7: 118–124.

9. Madkour FF, Abdel-Daim MM, 2013, Hepatoprotective and antioxidant activity of *Dunaliella salina* in paracetamol-induced acute toxicity in rats. *Indian J Pharm Sci*, 75: 642–648.
10. El-Baz F, Jaleel GAA, Saleh DO, *et al*., 2018, Protective and therapeutic potentials of *Dunaliella salina* on aging-associated cardiac dysfunction in rats. *Asian Pac J Trop Bio*, 8: 403–410.
11. Li J, Ichikawa T, Villacorta L, *et al*., 2009, Nrf2 protects against maladaptive cardiac responses to hemodynamic stress. *Arterioscler Thromb Vasc Biol*, 29: 1843–1850.
12. El-Baz FK, Elgohary R, Salama A, 2021, Amelioration of hepatic encephalopathy using *Dunaliella salina* microalgae in rats: Modulation of hyperammonemia/TLR4. *Biomed Res Int*, 2021: 8843218.
13. Younis NS, Abduldaium MS, Mohamed ME, 2020, Protective effect of geraniol on oxidative, inflammatory and apoptotic alterations in isoproterenol-induced cardiotoxicity: Role of the Keap1/Nrf2/HO-1 and PI3K/Akt/mTOR pathways. *Antioxidants* (*Basel*), 9: 977.
14. Zheng Y, Lu H, Huang H, 2020, Desflurane preconditioning protects against renal ischemia-reperfusion injury and inhibits inflammation and oxidative stress in rats through regulating the Nrf2-Keap1-ARE signaling pathway. *Drug Des Devel Ther*, 14: 1351–1362.
15. Fang X, Liu Y, Lu J, *et al*., 2018, Protocatechuic aldehyde protects against isoproterenol-induced cardiac hypertrophy via inhibition of the JAK2/STAT3 signaling pathway. *Naunyn Schmiedebergs Arch Pharmacol*, 391: 1373–1385.
16. Song M, Cui X, Zhang J, *et al*., 2022, Shenlian extract attenuates myocardial ischaemia-reperfusion injury via inhibiting M1 macrophage polarization by silencing miR-155. *Pharm Biol*, 60: 2011–2024.
17. Zhang WY, Zhang QL, Xu MJ, 2019, Effects of propofol on myocardial ischemia reperfusion injury through inhibiting the JAK/STAT pathway. *Eur Rev Med Pharmacol Sci*, 23: 6339–6345.
18. Clark JD, Gebhart GF, Gonder JC, *et al*., 1997, Special report: The 1996 guide for the care and use of laboratory animals. *ILAR J*, 38: 41–48.
19. Zhang J, Huang L, Shi X, *et al*., 2020, Metformin protects against myocardial ischemia-reperfusion injury and cell pyroptosis via AMPK/NLRP3 inflammasome pathway. *Aging* (*Albany NY*), 12: 24270–24287.
20. Brewster LM, Haan YC, Zwinderman AH, *et al*., 2020, CK (creatine kinase) is associated with cardiovascular hemodynamics: The HELIUS study. *Hypertension*, 76: 3 73–380.
21. Suwanjang W, Abramov AY, Charngkaew K, *et al*., 2016, Melatonin prevents cytosolic calcium overload, mitochondrial damage and cell death due to toxically high doses of dexamethasone-induced oxidative stress in human neuroblastoma SH-SY5Y cells. *Neurochem Int*, 97: 34–41.
22. Takayanagi R, Inoguchi T, Ohnaka K, 2011, Clinical and experimental evidence for oxidative stress as an exacerbating factor of diabetes mellitus. *J Clin Biochem Nutr*, 48: 72–77.
23. Zhou Q, Song J, Wang Y, *et al*., 2020, Remifentanil attenuates cardiac dysfunction, lipid peroxidation and immune disorder in rats with isoproterenol-induced myocardial injury via JNK/NF-KB p65 inhibition. *Ann Transl Med*, 8: 551.
24. Guleken Z, Kuruca SE, Ünübol B, *et al*., 2020, Biochemical assay and spectroscopic analysis of oxidative/antioxidative parameters in the blood and serum of substance use disorders patients. A methodological comparison study. *Spectrochim Acta A Mol Biomol Spectrosc*, 240: 118625.
25. Kong L, Deng J, Zhou X, *et al*., 2021, Sitagliptin activates the p62-Keap1-Nrf2 signalling pathway to alleviate oxidative stress and excessive autophagy in severe acute pancreatitis-related acute lung injury. *Cell Death Dis*, 12: 928.
26. Shaw P, Chattopadhyay A, 2020, Nrf2-ARE signaling in cellular protection: Mechanism of action and the regulatory mechanisms. *J Cell Physiol*, 235: 3119–3130.
27. Gañán-Gómez I, Wei Y, Yang H, *et al*., 2013, Oncogenic functions of the transcription factor Nrf2. *Free Radic Biol Med*, 65: 750–764.
28. Zhang Z, Peng L, Fu Y, *et al*., 2021, Ginnalin A binds to the subpockets of keap1 Kelch domain to activate the Nrf2-regulated antioxidant defense system in SH-SY5Y cells. *ACS Chem Neurosci*, 12: 872–882.
29. Geng Z, Fan WY, Zhou B, *et al*., 2019, FNDC5 attenuates obesity-induced cardiac hypertrophy by inactivating JAK2/STAT3-associated inflammation and oxidative stress. *J Transl Med*, 17: 107.
30. Liu X, Ye L, Bai Y, *et al*., 2008, Activation of the JAK/STAT-1 signaling pathway by IFN-gamma can down-regulate functional expression of the MHC class I-related neonatal Fc receptor for IgG. *J Immunol*, 181: 449–463.
31. Zhang M, Wang X, Wang X, *et al*., 2013, Oxymatrine protects against myocardial injury via inhibition of JAK2/STAT3 signaling in rat septic shock. *Mol Med Rep*, 7: 1293–1299.