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Therapeutic effects of the combination of moderate-intensity endurance training and MitoQ supplementation in rats with isoproterenol-induced myocardial injury: The role of mitochondrial fusion, fission, and mitophagy

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ABSTRACT

Introduction: Mitochondrial dysfunction causes myocardial disease. This study investigated the effects of MitoQ alone and in combination with moderate-intensity endurance training (EX) on cardiac function and content and mRNA expression of several proteins involved in mitochondrial quality control in isoproterenol (ISO)-induced heart injuries

Methods: Seven groups of CTL, ISO, ISO-EX, ISO-MitoQ-125, ISO-MitoQ-250, ISO-EX+MitoQ-125, and ISO-EX+MitoQ-250 were assigned. Rats were trained on a treadmill, and the MitoQ groups received MitoQ in drinking water for 8 weeks, starting one week after the induction of heart injury. Arterial pressure and cardiac function indices, mRNA expression, protein content, oxidant and antioxidant markers, fibrosis, and histopathological changes were assessed by physiograph, Real-Time PCR, immunofluorescence, calorimetry, Masson's trichrome, and H&E staining, respectively.

Results: The impacts of MitoQ-125, EX+MitoQ-125, and EX+MitoQ-250 on arterial pressure and left ventricular systolic pressure were higher than MitoQ-250 or EX alone. ± dp/dt max were higher in ISO-EX+MitoQ-125 and ISO-EX+MitoQ-250 than ISO-MitoQ-125 and ISO-MitoQ-250 groups, respectively. Histopathological scores and fibrosis decreased in ISO-EX, ISO-MitoQ-125, ISO-EX+MitoQ-125, and ISO-EX+MitoQ-250 groups. The restoration of MFN2, PINK-1, and FIS-1 changes was higher in ISO-EX+MitoQ-125 and ISO-EX+MitoQ-250 than ISO-EX, ISO-MitoQ-125 and ISO-MitoQ-250 groups. The expression of MFN2 and PINK-1 was lower in ISO-MitoQ-125 and ISO-EX+MitoQ-125 than ISO and CTL groups. The expression of FIS-1 in ISO-EX and ISO-EX+MitoQ-250 increased compared to CTL and ISO groups. MDA decreased in ISO-MitoQ-125 and ISO-EX+MitoQ-125 groups.

Conclusion: Exercise and MitoQ combination have additive effects on cardiac function by modulating cardiac mitochondria quality. This study provided a possible therapy to treat heart injuries.

1. Introduction

Cardiovascular diseases (CVDs) are one of the leading causes of disability and mortality in developed and developing countries [1]. Myocardial ischemia, which may lead to myocardial injuries is one of the common causes of heart failure [2]. Finding new appropriate interventions for the diagnosis and treatment of myocardial injuries and heart failure will increase the survival and life quality of these patients [3]. It has been demonstrated that injection of Isoproterenol (ISO) in rats, a synthetic catecholamine and β-adrenergic agonist, causes hemodynamic disturbance, cardiomyocyte damage, calcium overload, oxygen and nutrient reduction and disproportionate production of radical oxidative species that ultimately leads to myocardial necrosis and fibrosis, similar to those that occur in patients with cardiac ischemia [4,5].

Low physical activity is known as one of the main risk factors for

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cardiovascular diseases (CVDs). The beneficial effects of exercise on the cardiovascular system are well-known in physiological and pathological conditions [6]. Studies have shown that exercise training reduces the adverse outcomes of heart disorders and improves heart function and left ventricular contractility after ischemia [7–9]. Moderate-intensity endurance training increases cardiac output in aged rats and causes physiological hypertrophy of the heart [10]. Exercise training increases contractile capacity, elevates ventricular fibrillation threshold, and reduces heart rate [11].

Recently, the mitochondrion has been considered a new target in the treatment of different diseases [12]. In the heart, mitochondria are involved in the main functions of cells, such as catabolic and anabolic processes, energy production, calcium regulation, reactive oxygen species (ROS) homeostasis, and cell survival. Mitochondrial dysfunction plays an important role in the pathology of cardiovascular diseases due to the disruption of the electron transport chain, increased ROS production, and excessive increase in calcium load, which ultimately lead to cell death [13,14].

Fusion and fission contribute to mitochondrial quality control processes, including mitochondrial biogenesis and mitophagy [15]. The fission process prepares the damaged mitochondria for mitophagy, and the mitochondria are then replaced by new mitochondria. Dynamin-related protein 1 (Drp1), a member of the large GTPase family, and its corresponding adapters in mitochondria are the main components of mitochondrial fission. After post-translational changes in Drp1, it transfers from the cytosol to the outer membrane of the mitochondria, where it binds to the mitochondrial fission adapter protein (FIS-1), and, then, the mitochondrial fission process initiates with the participation of other proteins. The damaged components of mitochondria that have been separated by fission are eventually eliminated by mitophagy. Mitophagy is a type of selective organ autophagy that prevents abnormal accumulation of mitochondria, preventing cardiomyocyte dysfunction or death [16].

Mitophagy is regulated by different proteins, including PTEN-induced putative kinase 1 (PINK-1) [17]. It has been shown that mitochondrial PINK-1 and mitophagy decreases in heart failure induced by transverse aortic ligation (TAC) [18].

The fusion phenomenon allows the re-entry of fragmented mitochondria that are still alive into the mitochondrial network [19]. Mitochondrial fusion is regulated by two members of the large GTPase family, mitofusin-1 (MFN1) and mitofusin-2 (MFN2) [16,20]. The genetic deletion of mitofusins leads to the accumulation of dysfunctional mitochondria and heart damage. Impaired mitochondrial fusion leads to fatal heart failure in rodents [15].

Discovering strategies that target mitochondria can be useful in the treatment of various diseases. One of the strategies could be exercise. Exercise improves mitochondrial function by stimulating the synthesis of new mitochondria and the induction of mitophagy [21]. Various agents, such as MitoQ (mitoquinone mesylate), also exert their therapeutic effects by targeting mitochondria [14]. MitoQ, which is a mitochondrial-targeted antioxidant supplement, scavenges free radicals, reduces oxidant production and lipid peroxidation, and removes peroxide nitrite [14,22]. MitoQ, as a mitochondrial antioxidant, is ~800-fold more effective than untargeted antioxidants [23]. It condenses on the matrix surface of the inner mitochondrial membrane and exerts antioxidative effects by oxidizing ubiquinol to ubiquinone [23]. It has been shown that chronic administration of MitoQ ameliorates mitochondrial ROS production in the skeletal muscles of middle-aged men [24]. Numerous experiments in in vitro and in vivo laboratory models have shown that MitoQ has a beneficial impact on cardiovascular diseases. MitoQ alleviates oxidative stress-induced cell damage and death due to ischemia-reperfusion (IR) induced by hydrogen peroxide in vitro [14,22,25]. Treatment with MitoQ for eight weeks reduces systolic blood pressure and cardiac hypertrophy in spontaneous hypertensive mice [26]. Clinical studies have also indicated that MitoQ improves venous and arterial function by conserving endothelial integrity and reducing mitochondrial-derived oxidative stress in the elderly [27].

A study found that exercise increases the peak power of skeletal muscles through the regulation of oxidative stress, and mitochondrial biogenesis is not influenced by MitoQ supplementation [28]. However, the effects of the combination of exercise and MitoQ supplementation on the regulation of oxidative stress and mitochondrial quality and function in the pathological conditions of heart injury are unknown.

Given the beneficial effect of exercise and MitoQ on CVDs, the impairment of mitochondrial quality in heart diseases, and the positive effect of exercise and MitoQ on mitochondrial quality, in this study, the effects of MitoQ alone and in combination with moderate-intensity endurance exercise were investigated on cardiomyocyte damage, inflammation, fibrosis, cardiac function, the mRNA expression and protein levels of MFN2, FIS-1, and PINK-1, and oxidant and antioxidant indices in ISO-induced heart injuries in male Wistar rats.

2. Material and methods

2.1. Materials

In this experimental study, 49 male Wistar rats ($n = 7$ in each group) were provided by Kerman University of Medical Science. The animals (220–250 gr) were kept in conventional conditions (12 h light and 12 h darkness) with free water and normal food access. The animals were randomly divided into seven groups: control (CTL), isoproterenol (ISO), ISO-Exercise (ISO-EX), ISO-MitoQ at doses of 125 and 250 mg/kg (ISO-MitoQ-125 and ISO-MitoQ-250), ISO-EX+MitoQ-125, and ISO-EX+MitoQ-250. The CTL and ISO groups received saline (as a vehicle) and ISO (85 mg/kg) subcutaneously for two consecutive days, respectively [29]. Isoproterenol (Sigma, UK), MitoQ powder (MitoQ Ltd, New Zealand), RNA isolation kit (Bio Basic, Canada), cDNA synthesis kit (Parstous Biotechnology, Iran) Master Mix Green (Amplicon, Denmark), Mitofusin 2 polyclonal antibody (No.: bs-2988R, Bioss, USA), rabbit polyclonal antibody for FIS-1 (No: TX111010, GeneTex, USA), Anti-PINK1 antibody (No.: GTX107851, GeneTex. USA), and goat anti-rabbit IgG (H+L) antibody (FITC) (No.: orb688925, Biorbyt, United Kingdom) were used in this study. The experimental protocol was approved by the Ethics Committee of Kerman University of Medical Sciences (ethical code: IR.KMU.REC.1400.292).

2.2. Exercise training protocol

Exercise training was initiated 1 week after the injection of ISO. The animals were trained on a motor-driven treadmill for 8 weeks, 5 d/wk, 50 min/d at 60–75% V_{max}. The speed and duration were gradually increased during the training protocol. Before the main training program, the rats were adapted to exercise training at 10 m/min for 10 min per session for 3 days [30].

2.3. MitoQ treatment

MitoQ was given to animals at doses of 125 and 250 μM in their drinking water one week after injures was induced for eight weeks [31].

2.4. Recording of hemodynamic parameters

One day after the last exercise training session or the last administration of MitoQ, the rats were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg). A catheter filled with heparin saline was placed in the right femoral artery to record the hemodynamic parameters, including systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR). Cardiac function indices and contractility indicators, including left ventricular systolic pressure (LVSP), LV end-diastolic pressure (LVEDP), the maximum rate of rise in the LV pressure (contraction velocity; +dp/dt max), and the maximum rate of

reduction in the LV pressure (relaxation velocity; -dp/dt max) were recorded by insertion of another catheter into the left ventricle (LV) through the right carotid [32]. The artery and ventricular cannulas were connected to pressure transducers and then to an 8-channel PowerLab system (ADInstruments, New South Wales, Australia). The animals were ventilated through a tracheal cannula if necessary.

2.5. Histopathological examination

To evaluate histopathological changes and fibrosis, under deep anesthesia, the animals' hearts were removed and fixed in 10% paraformaldehyde and embedded in paraffin. Then 5–6 μm sections of paraffin blocks were prepared and stained with hematoxylin and eosin and/or Masson's trichrome. The total histopathological scores were observed and calculated by a pathologist, who was blind to the groups, based on four indices: congestion and hemorrhage, hypereosinophilic bundles, leukocyte infiltration, and cardiomyocytes necrosis, which were categorized as severe (+++), moderate (++), mild (+), and normal (0). Each animal was assigned one score, with a total of 12 histological scores [33]. The myocyte diameter was determined using a micrometer that was attached to the ocular part of the microscope (Olympus CX33, Japan). The average diameter of 10 fibers was reported as myocyte diameter [34]. The percentage of fibrosis in the total area in each image was measured using Image J software (NIH Image, Bethesda, MD).

2.6. Total RNA extraction and Real-Time PCR

After homogenizing of heart tissue, total RNA was extracted using a total RNA Mini-Preps Kit (Bio Basic, Canada) according to the manufacturer's instructions. RNA concentration and purity were quantified using NanoDrop ND-2100 (Thermo Fisher Scientific, USA). Complementary DNA (cDNA) synthesis was performed using the Easy cDNA synthesis kit (Parstous Biotechnology, Iran). Expression of mRNAs was quantified by the StepOnePlus instrument (Applied Biosystems, USA) using RealQ Plus 2 × Master Mix Green (Amplicon, Denmark). Small nucleolar 18 S rRNAs were used as the internal control. The primer sequences for MFN2, FIS-1, PINK-1, and 18 S rRNA are reported in Table 1.

2.6.1. Assessment of the protein levels of MFN2, FIS-1, and PINK-1 by immunofluorescence staining

The heart tissues were embedded in formaldehyde 10% for 24 h. Then, 5-μm-thick slices were provided from the paraffin-embedded hearts, dewaxed in xylene, and dehydrated in a graded series of ethanol. The slices were placed in TBS 1X (T5912-Sigma) for 20 min and subjected to Triton 0.3% (Sigma-T8787) for 30 min to permeabilize the cell membrane. Then 10% goat serum (Sigma-G9023) was added to the samples for 45 min to prevent a secondary antibody reaction. The samples were incubated overnight in diluted MFN2, PINk-1, and FIS-1 primary antibodies (1/100 with PBS) at 2 to 8 °C followed by goat anti-rabbit IgG (H+L) secondary antibody (FITC, green) (1/150) at 37 °C in the incubator (AriaTeb, Iran) for 90 min in dark conditions. Then the samples were stained with DAPI (D9542, Sigma) dye, and their fluorescent pictures were taken with an Olympus fluorescent microscope.

Table 1
Sequences of primers used for RT-PCR.

Genes	Forward Sequence-Forward	Reverse Sequence-Reverse
MFN2	AGTCGGTTGGAAGTCACTGT	TGTACTCGGGCTGAAAGGAG
FIS1	CTGTTACAGACTGAGCCCCA	TGAGGCCTGTCACCTTTCTT
PINK-1	CCATGGGCAGGAACACTATT	CCTACACACAGCCCTCACCT
18 s rRNA	GCAATTATTCCCCATGAACG	GGCCTCACTAAACCATCCAA

2.6.2. Determination of oxidative stress indices

Superoxide dismutase (SOD) activity was determined using the use instructions of the Randox kit (UK; Cat NO.RS504). In brief, the cardiac tissue (100 mg) was homogenized in a solution of 100 mM PBS and then centrifuged for 20 min at 4 °C at 4000–6000 rpm. The absorbance density was measured at 560 nm. For measuring the activity of glutathione peroxidase (GPX), 100 mg of heart tissue was homogenized in 200 μl assay buffer. The homogenate was centrifuged for 15 min at 10000 x g at 4 °C. Then, the supernatant was separated to determine the activity of GPX. The glutathione peroxidase assay was used according to the method described by Paglia and Valentine. GPX activity was determined indirectly by a coupled reaction with glutathione reductase [35]. The thiobarbituric acid reaction was used to quantify malondialdehyde (MDA), as a lipid peroxidation index. For this, 100 mg homogenized tissue was lysed in a KCl (1.5%) solution, and centrifuged at 1200 rpm (10 min). The absorbance of the MDA-TBA product was measured at 535 nm [36].

2.7. Statistical analysis

The data are presented as mean ± SEM in the tables and figures. After checking normality with the Kolmogorov-Smirnov test, the One-way ANOVA test was used for comparisons among groups followed by Tukey's post hoc test. The categorical data (histopathological scores) were analyzed by nonparametric tests. *P*-value < 0.05 was considered significant. Fold changes in gene expression were calculated using the $2^{-\Delta\Delta Ct}$ method.

3. Results

The body gain weight (BWG) was lowest in the ISO-MitoQ-250 group compared to other groups ($P < 0.05$). Heart weight/body weight (HW/BW) was higher in the ISO, ISO-MitoQ-125, ISO-MitoQ-250, ISO-EX+MitoQ-125 and ISO-EX+MitoQ-250 groups than in the CTL group ($P < 0.05$). There was an increase in left ventricular weight/body weight (LVW/BW) in the ISO-MitoQ-250 and ISO-EX+MitoQ-125 groups compared to the CTL and ISO groups ($P < 0.05$). Lung weight/body weight ratio was higher in the ISO-MitoQ-250 group compared to the CTL and ISO groups ($P < 0.05$ to $P < 0.01$) (Table 2).

Hematoxylin and eosin (H&E) staining indicated that histopathological scores, including congestion and hemorrhage, hypereosinophilic bundles, leukocyte infiltration, and cardiomyocyte necrosis increased in the ISO group compared to the CTL group ($P < 0.001$). EX, MitoQ-125, EX+MitoQ-125, and EX+MitoQ-250 could ameliorate the histopathological changes ($P < 0.05$ to $P < 0.001$) (Fig. 1A and C). The percentage of fibrosis increased in rats that were treated with ISO ($P < 0.01$). MitoQ-

Table 2
Effects of 8 weeks of MitoQ treatment with exercise training on the body, lung and heart weight indices in the study groups.

Variables Groups	BWG (g)	HW/BW (mg/g)	LVW/BW (mg/g)	LW/BW (mg/g)
CTL	20 ± 7.6	2.58 ± 0.08	2.12 ± 0.05	5.51 ± 0.16
ISO	21.3 ± 3.3	2.86 ± 0.04*	2.39 ± 0.06	5.4 ± 0.09
ISO-EX	34.3 ± 4.3	2.7 ± 0.1*	2.14 ± 0.05	5.64 ± 0.14
ISO-MitoQ-125	16 ± 5.3	2.86 ± 0.05*	2.29 ± 0.14	5.62 ± 0.31
ISO-MitoQ-250	8.8 ± 3.2*#	2.83 ± 0.08*	2.36 ± 0.09	6.29 ± 0.51**#
ISO-EX+MitoQ-125	25.3 ± 6.32	3.22 ± 0.13*#	2.62 ± 0.12*##	5.39 ± 0.13
ISO-EX+MitoQ-250	18.2 ± 9.2	2.8 ± 0.04*	2.49 ± 0.10*#	5.04 ± 0.24

BWG: body weight gain, LVW: left ventricular weight, HW: heart weight, LW: lung weight, CTL: control, ISO: Isopropanol, Ex: Exercise. *P < 0.05, vs. CTL, # $P < 0.05$, ## $P < 0.01$ vs. ISO. n = 7 in each group.

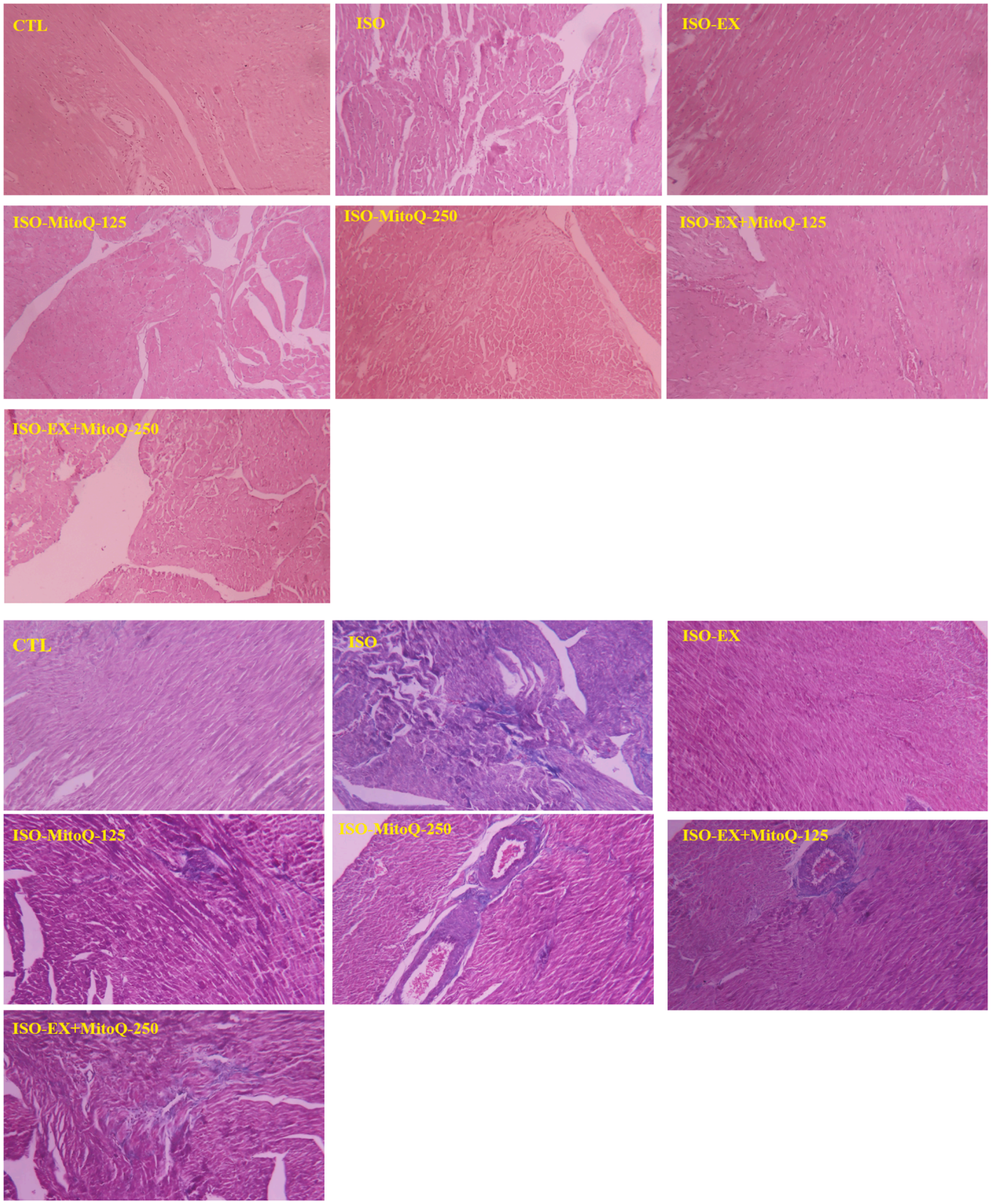


Fig. 1. Effects of 8 weeks of MitoQ treatment with exercise training on histopathological changes and fibrosis in the heart, stained with hematoxylin and eosin (H&E) and Masson's trichrome (×100 magnification) A: Histopathological changes in one rat of each group, B: fibrosis in one rat of each group, C: Quantification of the total histopathological score, D: Quantification of fibrosis, E: Cardiomyocyte diameter (Magnification =100 ×). CTL: Control, ISO: Isopropanol, Ex: Exercise. * $P < 0.05$, **$P < 0.01$, *** $P < 0.001$ vs. CTL, # $P < 0.05$, ## $P < 0.01$ ### $P < 0.001$ vs. ISO. $n = 5$ in each group.

125, EX, and EX+MitoQ-125 decreased the fibrosis ($P < 0.05$ to $P < 0.001$) (Fig. 1B and D).

Cardiomyocyte diameters were higher in the ISO, ISO-EX-MitoQ-125 and ISO-EX-MitoQ-125 groups ($P < 0.05$) than in the normal rats. MitoQ-125 decreased cardiomyocyte diameters compared to the CTL group ($P < 0.05$) (Fig. 1E).

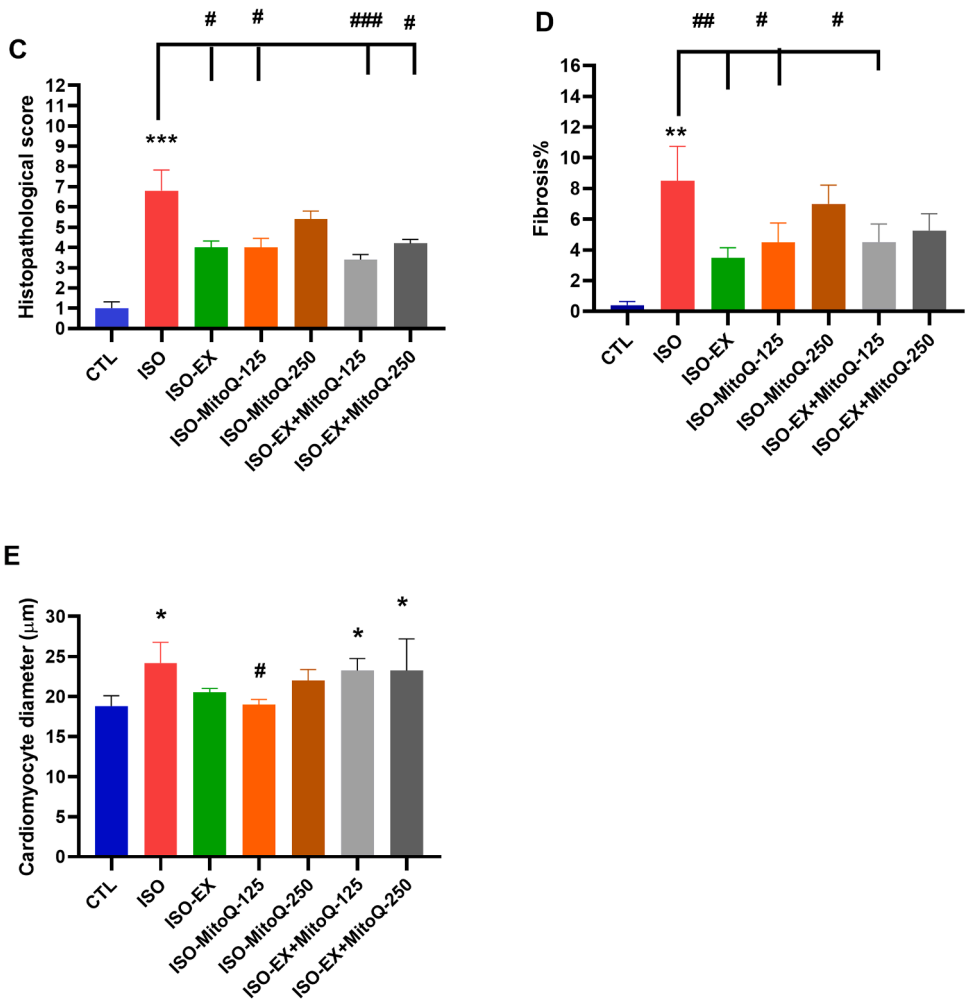


Fig. 1. (*continued*).

SBP ($P < 0.001$) and DBP ($P < 0.01$) decreased in the ISO-treated rats compared to the CTL animals. SBP and DBP increased in the ISO-MitoQ-125, ISO-EX+MitoQ-125, and ISO-EX+MitoQ-250 groups compared to the ISO group ($P < 0.05$ to $P < 0.001$). The impact of EX+MitoQ-250 was higher on SBP and DBP than on MitoQ-250 alone ($P < 0.05$). SBP and DBP were higher in the ISO-MitoQ-125 group than in the ISO-MitoQ-250 group ($P < 0.05$). Heart rate was not different between groups (Fig. 2).

LVSP also was lower in the ISO group than in the CTL group ($P < 0.001$). MitoQ-125, MitoQ-250, EX, EX+MitoQ-125, and EX+MitoQ-250 could normalize its value ($P < 0.05$ to $P < 0.001$) (Fig. 3A). Although LVEDP was higher in the ISO group than in the CTL group ($P < 0.05$), none of the interventions were able to significantly change it (Fig. 3B).

+max dp/dt decreased in the ISO group ($P < 0.01$) (Fig. 4A). EX, MitoQ-125, MitoQ-250, EX+MitoQ-125, and EX+MitoQ-250 were able to increase +max dp/dt value ($P < 0.05$ to 0.001). The combination of exercise and MitoQ-125 and MitoQ-250 increased +max dp/dt compared to MitoQ-125 and MitoQ-250 alone, respectively ($P < 0.01$). EX and the combination of EX and MitoQ-125 or 250 increased -dp/dt max compared to the ISO group ($P < 0.001$).

According to Real-Time PCR data (Fig. 5), the expression of MFN2 was significantly higher in the ISO group compared to the CTL group ($P < 0.05$). EX did not have any effect on the expression level of MFN2 in the ISO-treated animals. MFN2 expression decreased in the ISO-MitoQ-125, ISO-MitoQ-250, and ISO-EX+MitoQ125 groups compared to the ISO group ($P < 0.05$ to $P < 0.001$). MFN2 levels in the ISO-MitoQ-250 and ISO-EX+MitoQ-250 groups ($P < 0.001$) were relatively similar to the CTL group level. MitoQ at both doses decreased the effects of EX on MFN2 expression.

The expression levels of FIS-1 in ISO-EX and ISO-EX+MitoQ-250 increased compared to the CTL and ISO groups ($P < 0.05$ to $P < 0.001$). MitoQ-125 modified the effect of exercise on FIS-1 expression ($P < 0.01$). The expression of PINK-1 increased in the ISO-EX groups compared to the CTL and ISO groups ($P < 0.05$). MitoQ-125 decreased and MitoQ-250 increased PINK-1 expression in the ISO-treated rats ($P < 0.01$ and $P < 0.001$, respectively). In the ISO-EX+MitoQ-125 group, its expression level was lower than in the ISO-EX group ($P < 0.01$).

3.1. The protein levels of MFN2, FIS-1, and PINK-1

The immunofluorescence analysis indicated that the protein level of MFN2 was significantly lower in the ISO group compared to the CTL group ($P < 0.001$). EX, MitoQ-125, MitoQ-250, EX+MitoQ-125, and EX+MitoQ-250 were able to significantly increase MFN2 levels ($P < 0.001$) (Fig. 6A). The effects of the combination of exercise and MitoQ-125 or MitoQ-250 were higher than exercise or MitoQ alone ($P < 0.01$).

The protein level of FIS-1 was higher in the ISO group than in the CTL group ($P < 0.001$) (Figure B). Its level decreased in the ISO-EX, ISO-MitoQ-125, ISO-MitoQ-250, ISO-EX+MitoQ-125, and ISO-EX+MitoQ-250 groups ($P < 0.01$ to $P < 0.001$). FIS-1 levels were lower in the ISO-EX+MitoQ-125 and ISO-EX+MitoQ-250 groups than in the ISO-Ex, ISO-

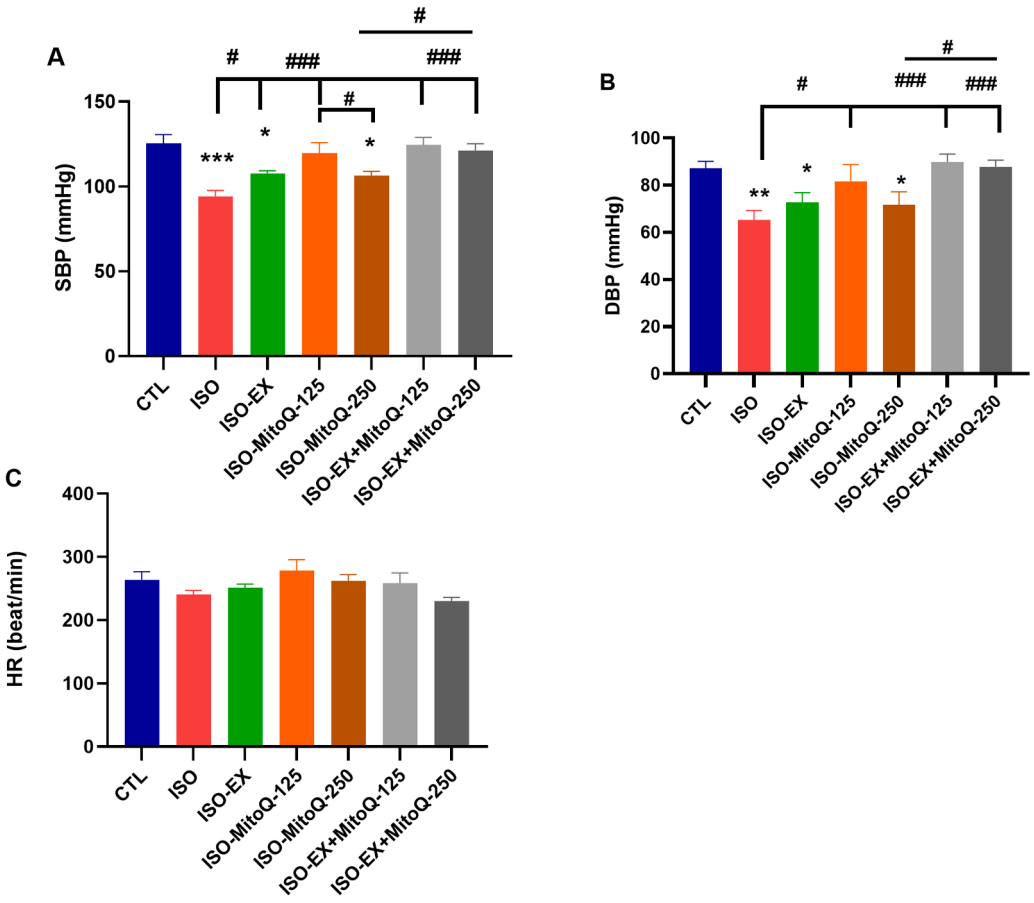


Fig. 2. Effects of 8 weeks of MitoQ treatment with exercise training on A: SBP (systolic blood pressure), B: DBP (diastolic blood pressure), and C: HR (heart rate) in the study groups. CTL: Control, ISO: Isopropanol, Ex: Exercise. *$P < 0.05$, * * $P < 0.01$, * ** $P < 0.001$ vs. CTL, # $P < 0.05$, ## $P < 0.01$ ### $P < 0.001$ vs. ISO and ISO-MitoQ-250. $n = 7$ in each group.

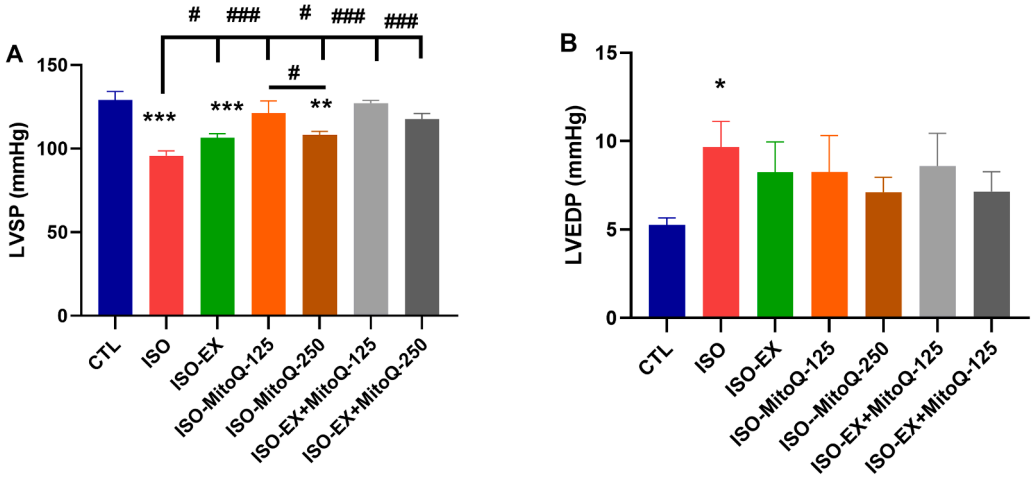


Fig. 3. Effects of 8 weeks of MitoQ treatment with exercise training on A: LVSP (left ventricular systolic pressure), B: LVEDP (left ventricular end-diastolic pressure) in the study groups. CTL: Control, ISO: Isopropanol, Ex: Exercise. * $P < 0.05$, * * $P < 0.01$, * ** $P < 0.001$ vs. CTL, # $P < 0.05$, ## $P < 0.01$ ### $P < 0.001$ vs. ISO and ISO-MitoQ-250. $n = 7$ in each group.

MitoQ-125, and ISO-MitoQ-250 groups, respectively ($P < 0.01$ to $P < 0.001$).

The protein level of PINK-1 decreased in the ISO group compared to the CTL group ($P < 0.001$). Its levels increased in the ISO-EX, ISO-MitoQ-125, ISO-MitoQ-250, ISO-EX+MitoQ-125, and ISO-EX+MitoQ-250 groups ($P < 0.01$ to $P < 0.001$). The effects of the combination of exercise and MitoQ-125 or MitoQ-250 were higher than those of exercise or MitoQ-125 or MitoQ-250 alone ($P < 0.01$ to $P < 0.001$) (Fig. 6C).

3.2. Effect of different treatments on MDA, SOD, and GPX

The results showed that MDA levels increased in the ISO group

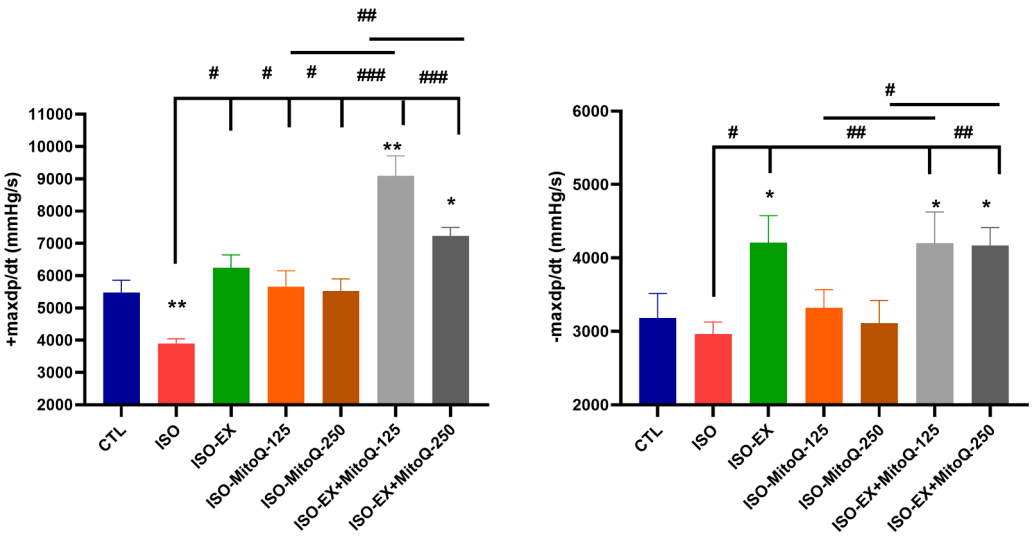


Fig. 4. Effects of 8 weeks of MitoQ treatment with exercise training on A: +dp/dt max (Maximum rate of increase in left ventricular pressure during systole), B: -dp/dt max (maximum rate of decrease in left ventricular pressure during diastole) in the study groups. CTL: Control, ISO: Isopropanol, Ex: Exercise. * $P < 0.05$, * * $P < 0.01$, * ** $P < 0.001$ vs. CTL, # $P < 0.05$, ## $P < 0.01$ ### $P < 0.001$ vs. ISO, ISO-MitoQ-125 and ISO-MitoQ-250. $n = 7$ in each group.

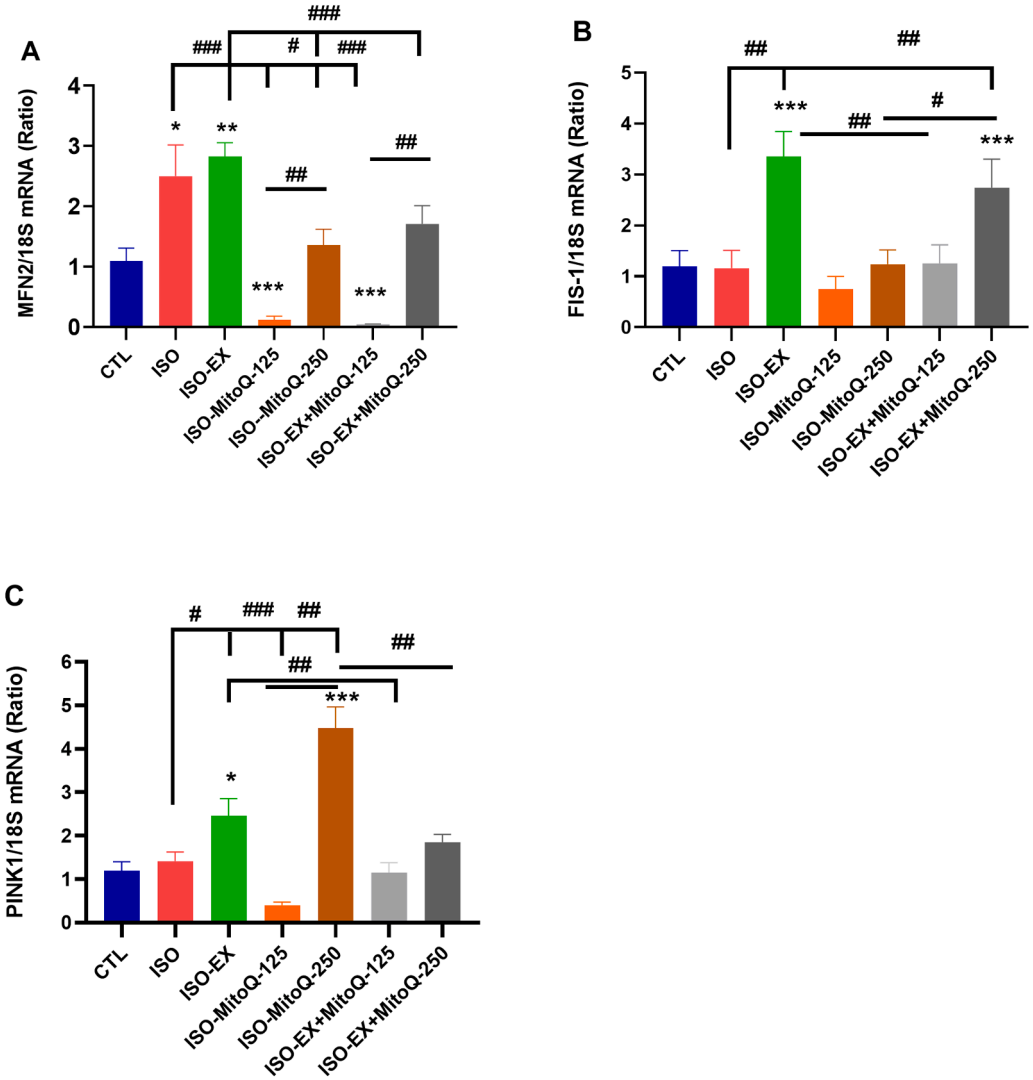


Fig. 5. Effects of 8 weeks of MitoQ treatment with exercise training on mRNA expression of A: MFN2 B: FIS-1 and C: PINK-1 in the study groups. * $P < 0.05$, * * $P < 0.01$, * ** $P < 0.001$ vs. CTL, # $P < 0.05$, ## $P < 0.01$ ### $P < 0.001$ vs. ISO, ISO-MitoQ-125 and ISO-MitoQ-250. $n = 7$ in each group.

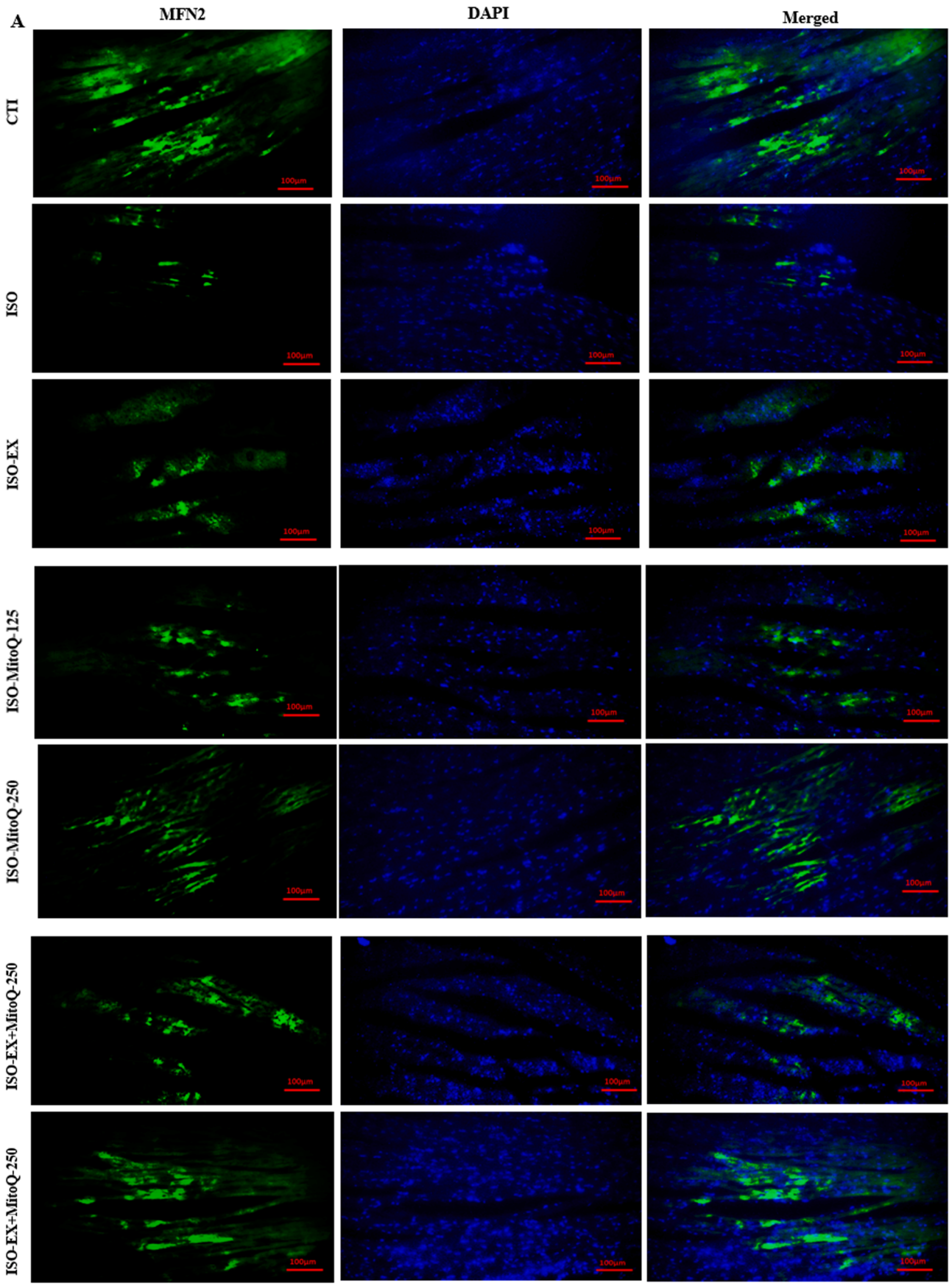
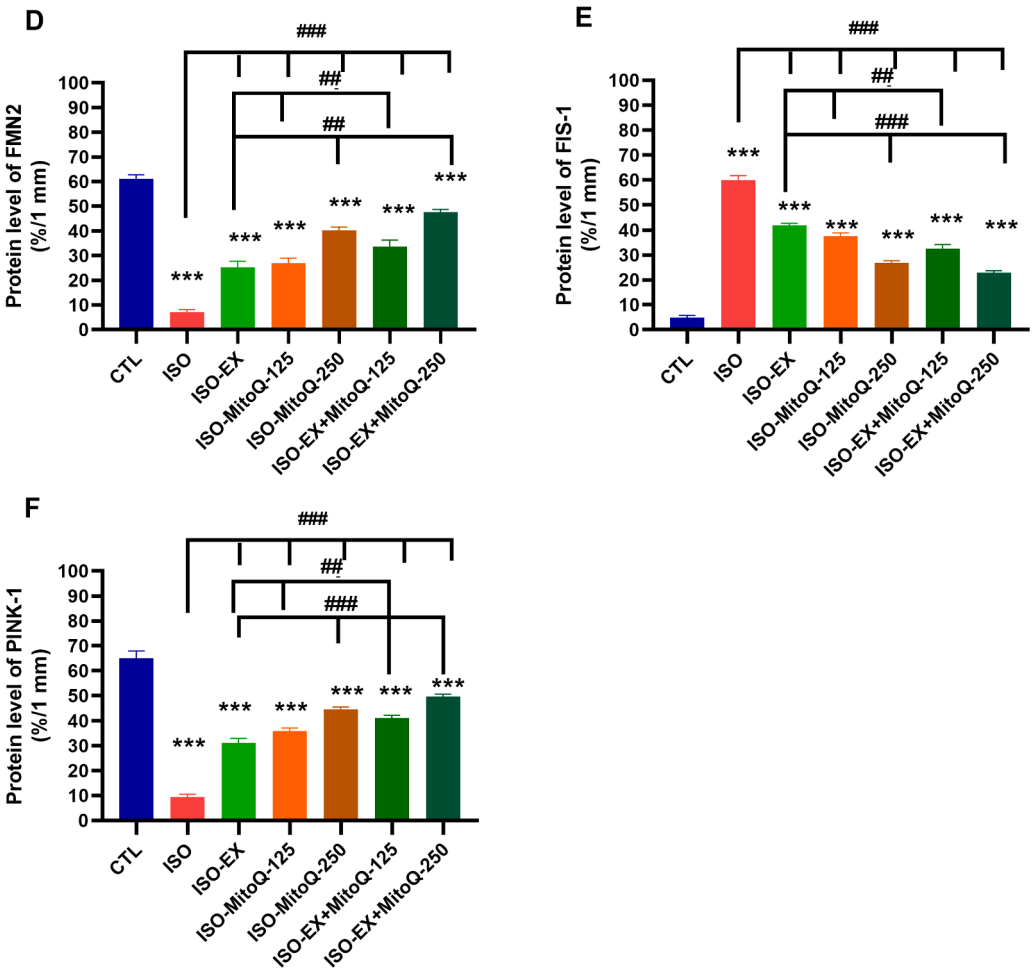


Fig. 6. Effects of 8 weeks of MitoQ treatment with exercise training on protein levels of A: MFN2 B: FIS-1 and C: PINK-1 in the study groups. * ** $P < 0.001$ vs. CTL, ## $P < 0.01$ ### $P < 0.001$ vs. ISO, ISO-EX+MitoQ-125 and ISO- EX+MitoQ-250. $n = 7$ in each group.



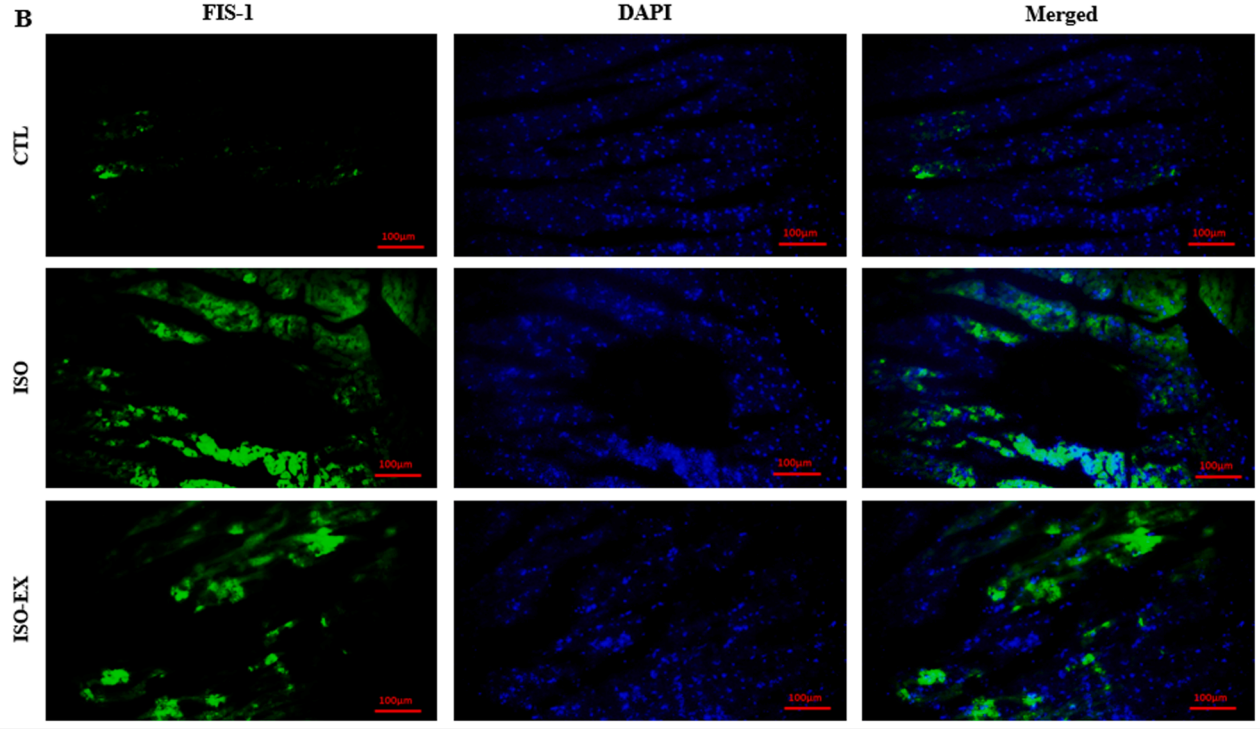
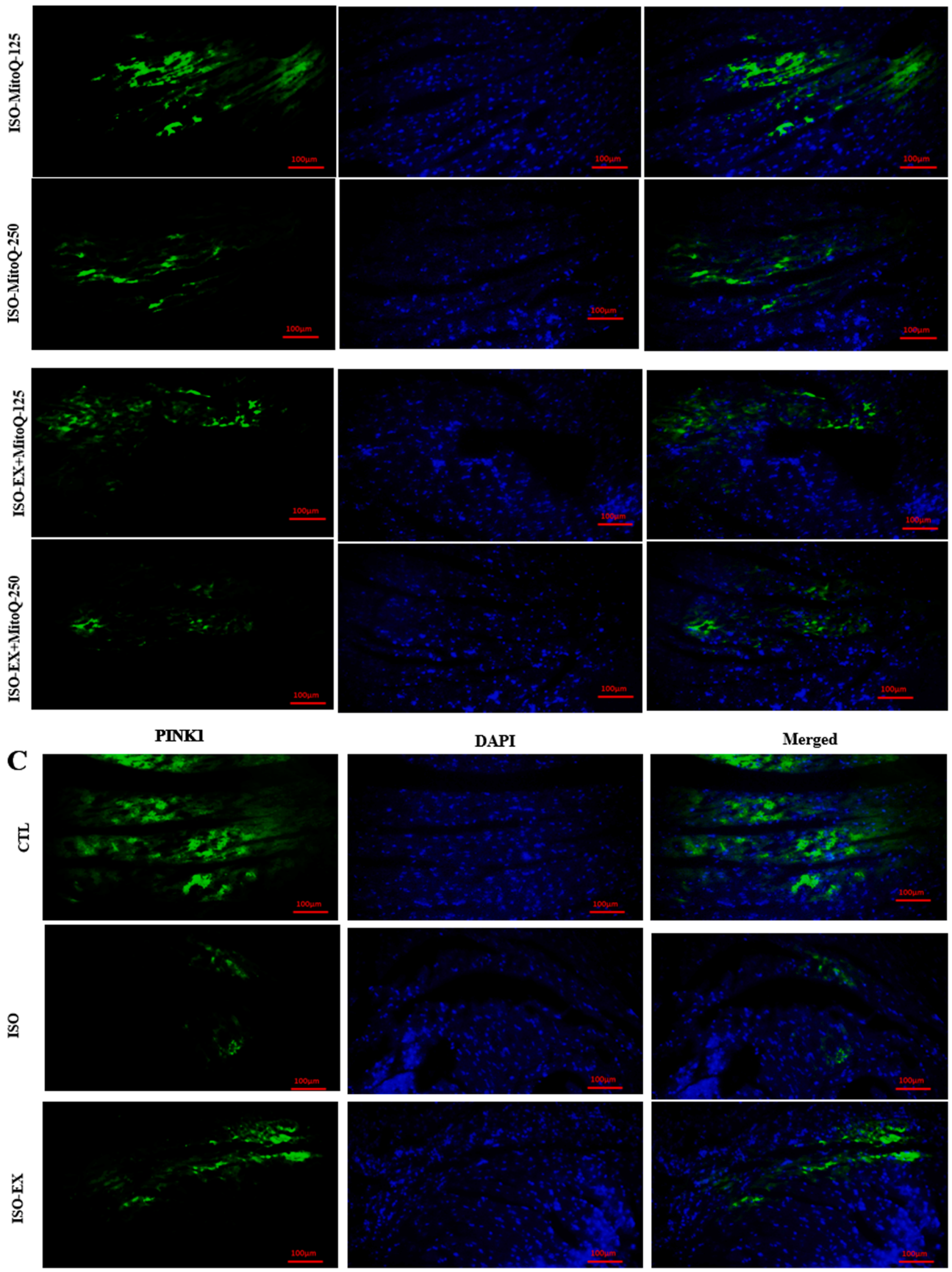


Fig. 6. (*continued*).



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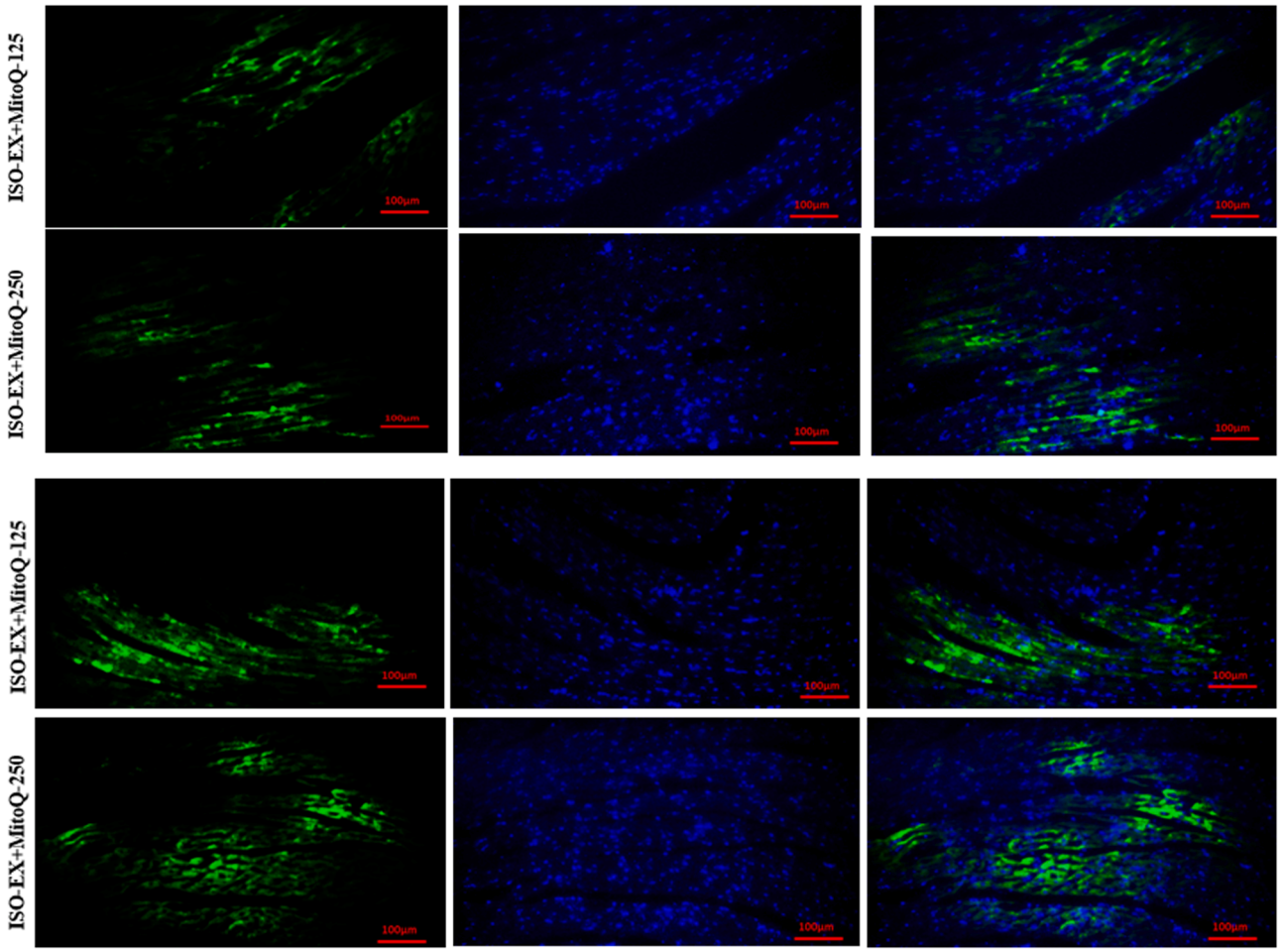

Fig. 6. (*continued*).

($P < 0.01$). MitoQ-125 and the combinations of exercise and MitoQ-125 and MitoQ-250 were able to recover its increase ($P < 0.05$) (Fig. 7A). SOD and GPX levels were lower in the ISO group compared to the CTL group ($P < 0.001$) (Fig. 7B, C). The GPX level increased in the ISO-EX and ISO-EX+MitoQ-250 group compared to the ISO group ($P < 0.01$). Exercise and the combination of exercise and MitoQ-250 could restore the reduction of SOD levels in group ISO-treated animals ($P < 0.01$ to $P < 0.001$). The MDA/GPX ratio was higher in the ISO group than in CTL group ($P < 0.001$). Its ratio decreased in ISO-EX, ISO-MitoQ-125, ISO-EX+MitoQ-125, and ISO-EX+MitoQ-250 (Fig. 7D) ($P < 0.01$).

4. Discussion

This study evaluated the impacts of the combination of moderate-intensity endurance training and MitoQ supplementation, which is a mitochondrion-targeted antioxidant, on heart function, hemodynamic indices, and expression of some mRNAs and their protein contents that regulate pathways involved in mitochondrial quality. The main findings were that MitoQ and exercise training improved cardiac function, and their combination had greater effects on cardiac performance. The combination of MitoQ and EX normalized the reduction of SBP and DBP that had happened in the ISO group. The recovered changes in the protein levels of MFN2, FIS-1, and PINK-1 showed that exercise and MitoQ at both doses (125 and 250 mg/kg) had noticeable effects on mitochondrial quality. Adding MitoQ to the exercise training protocol potentiated the impact of exercise training on the protein levels of MFN2, FIS-1, and PINK-1, which are involved in the fusion, fission, and mitophagy processes. Heart injuries and fibrosis induced by ISO were alleviated by EX, MitoQ-125, and their combination. MitoQ-125 had a more positive influence on heart injuries, fibrosis, and cardiac function probably through stabilizing of oxidant/antioxidant status.

Previous clinical and experimental studies have demonstrated that exercise and MitoQ have useful impacts on CVDs [12,37–41]. This study also revealed that exercise and MitoQ have beneficial effects on cardiac function, as the ± max dp/dt, and LVDP improved in the ISO groups that were treated with EX or MitoQ. Furthermore, the combination of EX and MitoQ has additive effects on cardiac function indices and blood pressure compared to EX or MitoQ alone. Another study also revealed that the combination of EX and MitoQ has additive impacts on cardiac function indices, alleviating blood pressure in subjects with hypertension [37].

The pathological remodeling of the heart following myocardial infarction, ischemia, and ischemia-reperfusion is attributed to mitochondrial dysfunction. It partly occurs due to extra ROS production, which is produced in the cytosol and mitochondria [42]. Isoproterenol, a β-adrenergic agonist, which is frequently used for induction of experimental heart injuries, has deleterious impacts on the heart, partly through mitochondrial dysfunction and excessive production of ROS [43,44]. The findings of a recent study also indicated that ISO disrupted the balance of oxidative stress and mitochondrial fission, fusion, and mitophagy. The control of mitochondrial quality that is attained by eliminating damaged mitochondria and accelerating the biogenesis of new mitochondria reduces the harmful consequence of injuries in various organs, including those of the cardiovascular system. It has been reported that the increase in expression of mitochondrial fusion-fission proteins protects cardiomyocytes from excessive Ca^{++} influx, and

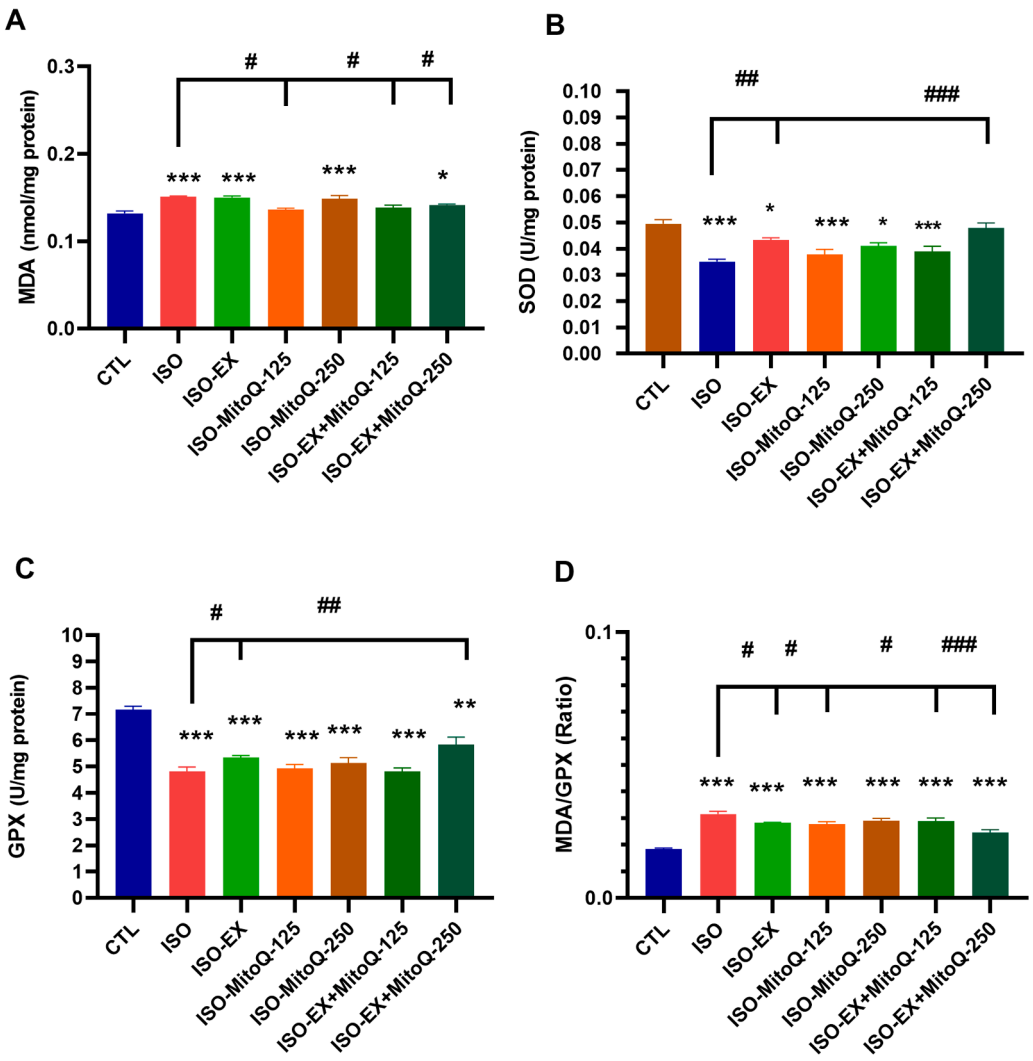

Fig. 7. Effects of 8 weeks of MitoQ treatment with exercise training on A: MDA (malondialdehyde), B: GPX (glutathione peroxidase), C: SOD (superoxide dismutase), D: MDA/GPX ratio * $P < 0.05$, * *$P < 0.01$, * ** $P < 0.001$ vs. CTL, # $P < 0.05$, ## $P < 0.01$ ### $P < 0.001$ vs. ISO, ISO-EX+MitoQ-125 and ISO- EX+MitoQ-250. $n = 7$ in each group.

oxidative damage and diminishes cardiac remodeling following myocardial infarction [45,46].

Consistent with this study, it has been shown that moderate-intensity exercise training reduces CVD prevalence and mortality and improves ventricular contractility following myocardial ischemia [38]. The study indicated that exercise possibly promotes fusion and mitophagy through an increase in MFN2 and PINK-1 levels. The decreased MFN2 and PINK-1 in the heart injured with ISO may cause mitochondrial and cardiac dysfunction. Other investigations have also reported that the expression of FMN2 decreases in various experimental models of heart hypertrophy and failure and angiotensin II-induced myocyte hypertrophy [47–50]. In addition to a disturbance in mitochondrial fusion, the reduction of FMN2 probably promotes cardiac dysfunction by dysregulation of cytoplasmic Ca^{++} content, as FMN2 stimulates Ca^{++} uptake by mitochondria by tethering the mitochondria and the endoplasmic reticulum (ER) [51]. The increase in MFN2 levels improves heart performance following appropriate intervention. Intermittent aerobic exercise training improves energy access and reduces oxidative stress damage by increasing the expression of MFN2 and OPA1 [52]. The findings also indicated that EX improved the oxidant/antioxidant balance.

The reduced protein levels of PINK-1 and, subsequently, mitophagy have been associated with heart failure in the transverse aortic contraction model [53]. Exercise potentiates the fission and fusion process and stimulates mitophagy with an increase in PINK-1 expression [54]. PINK1/parkin signaling is the main targeting pathway that induces mitochondrial autophagy following aerobic exercise in cardiovascular diseases. It has been suggested that an increase in mitophagy induced by EX probably has protective effects against inflammation and ROS production during exercise [55]. MFN2 also promotes autophagosome formation, the fusion of autophagosomes and lysosomes, and, subsequently, mitophagy [56].

Moreover, exercise possibly improves mitochondrial dysfunction with a reduction of FIS-1 levels. Higher FIS-1 levels can stimulate autophagy [57], cytochrome c release, apoptosis, and Ca^{++} release from ER calcium stores, and inhibit fusion GTPase, MFN1/2, and Opa1 [58,59]. Exercise decreases FIS-1 protein levels, probably secondary to the improvement of mitochondrial structure and function.

In a mouse model with severe hypertension, MitoQ improved cardiac contractile function and mitochondrial network integrity [60]. In the animal model of IR, MitoQ maintained mitochondrial and heart function by reducing ROS [61]. Our study also indicated that MitoQ improved cardiac function, and reduced fibrosis, cardiomyocyte damage, and inflammation possibly by ameliorating the changes in MFN2, Fis-1, and PINK-1 protein levels. It has been demonstrated that the balance between fission and fusion is a critical aspect of maintaining heart function

[62]. MitoQ-125 also decreased the level of MDA and equilibrated oxidant/antioxidant ratio. MitoQ reduces mitochondrial damage and improves mitochondrial quality by removing free radicals and peroxide nitrite, reducing oxidant production and lipid peroxidation [14]. In contrast to this study, previous studies reported MitoQ administration increases the activity of antioxidant enzymes, including SOD and GPX by stimulating the Nrf2 nuclear translocation. However, the results of various studies are not similar, which may be related to different physiological and pathophysiological conditions and tissue type [63, 64].

The results indicated that when MitoQ was added to the training protocol, the modification of FIS-1, MFN2, and PINK-1 protein content was augmented compared to EX or MitoQ alone. The improved cardiac function and decreased cardiomyocyte damage can be attributed to improvement in mitochondrial quality and the accumulation of healthy mitochondria in response to combination therapy. It has been demonstrated that the chronic pre-treatment of MitoQ reduced the harmful effects of high-intensity exercise on genes by reducing ROS production in human muscles [65]. Therefore, the balanced action of mitochondrial fission and fusion possibly improved the function of the mitochondrial network, and the combination of exercise and MitoQ improved the function of the mitochondria and, consequently, the efficiency of the heart.

Despite the positive effects of MitoQ-250 on contractility, lusitropic indices, and the restoration of FIS-1, MFN2, and PINK-1, the results indicated that it had a smaller effect on some harmful consequences of heart damage, including fibrosis, inflammation, and bleeding, than MitoQ-125. Similar to other studies, this study also indicated that MitoQ, especially at the high dose, reduced weight gain probably due to increased metabolic mechanism [66]. The increase in cardiomyocyte diameter, HW/BW and LVW/BW of rats that received the combination of exercise training and MitoQ suggested an increase in cardiac performance may be as a result of the physiological hypertrophy of the heart.

The discrepancy between mRNA expression and protein levels could be explained by the various regulating pathways involved in mRNA expression and stability, transcription, translation, half-lives of mRNA, the regulation of mRNA expression by proteins, and protein synthesis and degradation in different treatments. The correlation between the expression levels of protein and mRNA is relatively low [67,68]. However, the protein content determines the cell's function.

5. Conclusion

In summary, our results suggested that the modification of fission, fusion, and mitophagy by the combination of moderate-intensity endurance exercise and MitoQ intervention have added beneficial effects on cardiac function, hemodynamic indices, and mitochondrial quality in ISO-induced cardiac injuries.

Author contribution

HN, SA and FR designed the research. SA trained the animals and performed Real-Time PCR. FR recorded hemodynamic parameters and collected tissue samples. EJ observed and interpreted the histopathological results. FR analyzed the data and wrote the manuscript in collaboration with SA and HN. All authors read and approved the final version of the manuscript.

CRediT authorship contribution statement

Najafipour Hamid: Conceptualization, Writing – review & editing. **Aminizadeh Soheil:** Conceptualization, Investigation, Methodology, Writing – review & editing. **Jafari Elham:** Data curation, Investigation, Methodology. **Rostamzadeh Farzaneh:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2023.116020.

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