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The Effect of Regular Treadmill Exercise on Heart Histopathological Changes and Biochemical Alterations in Cardiac Ischemia-Reperfusion Injury in Rat

Efecto del Ejercicio Regular en Cinta Rodante Sobre los Cambios Histopatológicos y las Alteraciones Bioquímicas Cardíacas en la Lesión por Isquemia-Reperfusión Cardíaca en Ratas

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SUMMARY : Restoring blood flow to ischemic heart muscle can induce more damage to myocardium. The purpose of this study was to evaluate effects of regular increasing long-term aerobic exercise on cardiac Ischemia/Reperfusion (I/R) injury in rats. For this purpose, 40 male Wistar rats were randomly allocated in 4 equal groups including: control, I/R, I/R with two weeks of aerobic exercise, and I/R with eight weeks of regular increasing aerobic exercise groups. Aerobic exercise was performed 5 times per week on treadmill at speed of 10-25 m/min for 10-30 min with the slope of 5 degrees. For induction of I/R injury, left descending coronary artery was clamped for 30 min, thereafter blood flow was restored for 2 h. Blood samples were collected for measurement of serum cardiac biomarkers including: creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), cardiac troponin I (cTnI) and tumor necrosis factor alpha (TNF-α). Finally, all animals were euthanized for histopathological examination and assessment of myocardial antioxidant status. Aerobic exercise significantly decreased the CK-MB, LDH, cTnI and TNF-α serum levels, which were increased following I/R injury and significantly increased the cardiac levels of SOD, CAT and GPx, which were reduced in these animals, as well as significantly decreased MDA level ($P<0.05$). Microscopically, aerobic exercise significantly alleviated myocardial degenerative and necrotic changes and significantly decreased the number of apoptotic cells ($P<0.05$), which were increased due to I/R injury. The results showed regular increasing long term aerobic exercise protects the cardiac muscle of rats following I/R injury.

KEY WORDS: Apoptosis; Exercise; Heart; Ischemia-reperfusion; Rat; Serum biomarkers.

INTRODUCTION

Myocardial ischemia, resulting from the occlusion of major coronary arteries, is a leading cause of myocardial infarction (MI) (Ferdinandy *et al*., 2007). Following an acute MI, immediate reperfusion of the injured myocardium is considered the most effective intervention to reduce infarct size and severity while improving clinical outcomes (Foadoddini *et al*., 2014). However, the process of restoring blood flow to ischemic heart tissue can paradoxically cause further myocardial damage, a phenomenon referred to as myocardial reperfusion injury. This paradox underscores the complex relationship between ischemic tissue survival and the additional damage caused by reperfusion itself (Foadoddini *et al*., 2011). Ischemia-reperfusion (I/R) injury has been attributed to the excessive production of free radicals which damage lysosomal membranes and activate previously inactive enzymes. This cascade ultimately disrupts cellular integrity, leading to the loosening and breaking of hydrogen bondswithin cell membranes. Consequently, the detachment of membrane-bound enzymes impairs cellular function. Moreover, DNA damage caused by free radicals results in cell death via both apoptosis and necrosis (Van Dijk *et al*., 2009). Although the exact mechanisms underlying I/R injury remain incompletely understood, one major factor is the focal amplification of inflammatory cells during reperfusion. These cells release large quantities of reactive oxygen species (ROS), including superoxide anions and hydrogen peroxide, as well as

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inflammatory cytokines and enzymes such as myeloperoxidase and proteases. This exacerbates membrane destruction, intracellular component leakage, and mitochondrial permeability. Increased mitochondrial permeability leads to membrane potential loss, reduced ATP production, and mitochondrial swelling, ultimately triggering cellular damage (Gottlieb, 2003). The resulting oxidative stress induces DNA damage, protein oxidation, lipid peroxidation, and apoptosis (Moens *et al*., 2005). Given the global prevalence of coronary artery disease and the myocardial damage associated with I/R injury, identifying effective strategies to protect the heart from this phenomenon is critically important. Researchers have explored various pharmaceutical and exercise-based interventions to minimize oxidative stress and mitigate myocardial damage caused by I/R (Bezzerides & Rosenzweig, 2011). Among these, regular aerobic exercise has emerged as a practical and sustainable approach for protecting the heart (Bayat *et al*., 2012). Regular aerobic exercise is widely recognized as a key strategy for treating and preventing cardiovascular diseases, as it reduces their associated complications (Ignarro *et al*., 2007). Evidence suggests that aerobic exercise enhances mitochondrial function by reducing ROS levels (Tofighi *et al*., 2015). Additionally, it has been shown to decrease apoptosis and improve antioxidant capacity in patients with chronic heart failure (Linke *et al*., 2005). While the precise mechanisms through which exercise protects the heart from I/R injury remain under investigation, numerous pathways have been proposed. Research indicates that even a single exercise session can confer myocardial protection against I/R injury (Brown *et al*., 2005a). Epidemiological studies in humans have demonstrated that exercise reduces mortality associated with myocardial damage by mitigating subsequent I/R injury (Brown & Moore, 2007). Laboratory studies on animals have further revealed that regular aerobic exercise, such as running or swimming, protects the heart from I/R-induced damage (Powers *et al*., 2008). For example, Brown *et al*. (2005b), found that short-term exercise reduced myocardial damage in rats subjected to I/R injury. Similarly, Chicco *et al*. (2007), reported that a brief five-day exercise regimen reduced infarct size in rats undergoing I/R. While substantial evidence supports the protective effects of regular aerobic exercise on the heart, questions remain regarding the optimal exercise model, including intensity, duration, frequency, and type, for real-world applications. The effects of progressively increasing, time-dependent aerobic exercise on I/R injury are not yet fully understood. Moreover, to date, no studies have comprehensively examined the protective effects of short-term versus long-term aerobic exercise on myocardial I/R injury. This study aims to address this gap by comparing the impacts of short-term and long-term aerobic exercise on myocardial I/R injury. The investigation focuses on serum biomarkers indicative of myocardial damage and corresponding histopathological changes to assess the protective effects of aerobic exercise regimens.

MATERIAL AND METHOD

All research protocols were approved by the Committee on the Rights of Laboratory Animals, and ethical standards for laboratory animal care and use were strictly followed.

Study Design

The study included 40 male Wistar rats, each weighing 200 ± 25 g and aged approximately 10 weeks. The animals were procured from the Iran Pasteur Institute and acclimatized to laboratory conditions for one week before the study commenced. All rats were housed under identical conditions, including a 12-hour light/dark cycle, a temperature of 21 ± 2 °C, and unrestricted access to food and water. The rats were randomly divided into the following four groups:

Control Group: Rats in this group underwent no physical intervention or cardiac manipulation. *I/R Group:* Rats were subjected to ischemia for 30 minutes followed by two hours of cardiac reperfusion. *I/R + Two Weeks of Exercise Group:* Rats underwent aerobic exercise for the last two weeks of an eight-week period, followed by ischemia for 30 minutes and two hours of reperfusion. *I/R + Eight Weeks of Exercise Group:* Rats participated in eight weeks of regular, progressive aerobic exercise before ischemia for 30 minutes and two hours of reperfusion.

Exercise Protocol

Aerobic exercise was performed five times per week on a treadmill with a 5° incline. Initially, rats were acclimatized to the treadmill conditions by being placed in the equipment for 10 min during the first two sessions. For the next three sessions, the rats began light running at speeds of 5–10 m x min. During the first two weeks, the rats ran at

Table I. Pattern of regular aerobic exercise for the studied rats.

	Week				
Exercise intensity	Accustoming	1st and 2nd	3rd and 4th	5th and 6th	7th and 8th
Speed (meters per minute)	5-10	10	15	20	25
Duration (minutes)	10	15	20	25	30
Slope (percent)	5	5	5	5	5

a speed of 10 m × min for 15 min per session. Over the following two weeks, the speed and duration were progressively increased, reaching 25 m × min and 30 min per session. This protocol was consistent throughout the study, with each session including a 5-min warm-up and a 5-min cool down at 5–10 m × min (Table I). The exercise regimen was designed and regulated following the recommendations of the American College of Sports Medicine (Thompson, 2010).

Induction of Injury

To eliminate the acute effects of exercise, ischemia-reperfusion injury was induced 48 h after the final exercise session. Rats were fasted for two hours prior to the procedure. Anaesthesia was induced via intraperitoneal injection of ketamine (5 mg/kg, 10 %) and xylazine (5 mg/kg, 2 %). Rats were also heparinized with an intraperitoneal injection of heparin (1000 IU/kg). The surgeries were performed based on the method described by Zhang *et al*. (2018). Briefly, the animals were mechanically ventilated with room air to maintain arterial pH, PCO2, and PO2 within normal physiological ranges. After 10 minutes of stabilization, a thoracotomy was performed through the left fifth intercostal space, and the pericardium was opened. A 6-0 silk suture was placed around the proximal left anterior descending (LAD) coronary artery for 2–3 mm, using a small polytetrafluoroethylene tube to form a snare. LAD occlusion was induced by tightening the snare for 30 minutes, which was confirmed by the appearance of epicardial cyanosis. Reperfusion was achieved by loosening the snare for two hours, and successful reperfusion was verified by epicardial hyperaemia.

Biochemical Tests

Following the induction of ischemia-reperfusion, blood samples were collected from the retro-orbital plexus to measure biomarkers indicative of myocardial tissue damage. These included lactate dehydrogenase (LDH), serum creatine kinase-MB (CK-MB), cardiac troponin I (cTnI), and tumor necrosis factor-alpha (TNF-α). The blood samples were centrifuged at 4000 rpm × 10 min to separate the serum. The serum cTnI levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (JAJ International Inc., USA), while TNF-a levels were assessed using a commercial ELISA kit from Assaypro, USA. The levels of CK-MB and LDH were determined using commercially available kits (Randox Laboratories Ltd, UK), following the manufacturers' protocols. After blood collection, all rats were euthanized by cervical dislocation. Heart tissue samples were quickly excised, rinsed with cold saline, and homogenized to prepare 10 % tissue homogenates in 1.15 % (w/v) potassium chloride (KCl) solution. The homogenates were centrifuged at 7000 rpm × 10 min at 4 °C. Malondialdehyde (MDA), an indicator of lipid peroxidation, was measured in the tissue homogenates using the thiobarbituric acid reactive substances (TBARS) method, as described by Fraga *et al*. (1988). Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities were assessed using commercially available kits (NanjingJiancheng Bioengineering Institute, China), following the manufacturer's instructions. The tissue MDA levels were expressed as nmol/mg protein, and antioxidant enzyme activities were recorded as conventional units per mg protein.

Lipid Peroxidation Assay

Lipid peroxidation was measured colorimetrically via the TBARS method (Fraga *et al*., 1988). Briefly, 1.0 mL of heart tissue homogenate was mixed with 2.0 mL of a reagent containing thiobarbituric acid, trichloroacetic acid, and hydrochloric acid (37 % TBA, 0.25 M HCl, and 15 % TCA) in a 1:1:1 ratio. The mixture was incubated in a boiling water bath for 15 min, cooled, and centrifuged at 3500 *g* × 10 min at room temperature. The absorbance of the supernatant was measured at 535 nm, and MDA levels were calculated and expressed as nmol/100 mg tissue.

Superoxide Dismutase (SOD) Activity Assay

SOD activity was determined using the method of Nishikimi *et al*. (1972). Briefly, 5 μg of total protein from the heart homogenate was mixed with sodium pyrophosphate buffer, phenazine methosulfate (PMS), and nitro-blue tetrazolium (NBT). The reaction was initiated by adding nicotinamide adenine dinucleotide (NADH) and incubating the mixture at 30 °C for 90 s. The reaction was stopped by adding glacial acetic acid, and the absorbance of the resulting solution was measured at 560 nm. One unit of SOD activity was defined as the amount of enzyme required to inhibit color formation by 50 % in one minute.

Catalase (CAT) Activity Assay

CAT activity was assessed using the method described by Claiborne.21 A reaction mixture containing 1.95 mL of phosphate buffer (0.05 M, pH 7.0), 1.0 mL of hydrogen peroxide (0.019 M), and 0.05 mL of 10 % homogenate was prepared, making a final volume of 3.0 mL. The decomposition of hydrogen peroxide was monitored by measuring the absorbance changes at 240 nm. CAT activity was expressed as units per minute (Claiborne, 1985).

Glutathione Peroxidase (GPx) Activity Assay

GPx activity was measured following the method of Rotruck *et al*. (1973). The reaction involved the oxidation of reduced glutathione (GSH) to oxidized glutathione (GSSG) by hydrogen peroxide. A mixture containing 0.2 mL of 0.8 mM ethylenediamine tetraacetic acid (EDTA), 0.1 mL of 10 mM sodium azide, 0.1 mL of 2.5 mM hydrogen peroxide, and 0.2 mL of homogenate was incubated at 37 °C × 10 min. The reaction was terminated by adding 0.5 mL of 10 % trichloroacetic acid (TCA), and the mixture was centrifuged at 2000 rpm × 15 min. The supernatant was then mixed with 3.0 mL of 0.8 mM disodium hydrogen phosphate and 0.1 mL of 0.4 mM dithiobis nitrobenzoic acid (DTNB). The absorbance of the resulting color was measured at 420 nm. GPx activity was expressed as μg protein/min/μM oxidized glutathione.

Histopathological Study

The remaining portions of the rats' hearts were fixed in 10 % buffered formalin, and 5 μm sections were prepared for routine hematoxylin and eosin (H&E) staining and TUNEL staining (terminal deoxynucleotidyl transferase dUTP nick end labeling). Histological sections were assessed using a semi-quantitative scale in a double-blind manner, based on the method proposed by Joukar *et al*. (2012). The severity of myocardial injury was graded on a scale of 0 to 4, evaluating parameters such as inflammation, hemorrhage, necrosis, and degenerative changes (0: no damage, 1: minimal damage, 2: mild damage, 3: moderate damage, 4: severe damage).

Microscopic observations were performed to measure and count apoptotic cells. For each section, five random microscopic fields were analyzed at 40x magnification using a Nikon optical microscope (model ECLIPSE E200, Japan). The number of apoptotic cells was calculated as a proportion of the total number of cells counted. Apoptotic cells were identified and quantified using the TUNEL assay, following the manufacturer's protocol (In Situ Cell Death Detection Kit, POD, Roche, Germany).

Briefly, sections were deparaffinized, dehydrated, and washed in distilled water. Tissues were treated with 20 μg/mL protein kinase for 15 min at room temperature (Boehringer Mannheim, Mannheim, Germany). Endogenous peroxidase activity was blocked by incubation with 3 % hydrogen peroxide in methanol for 30 min at 37 °C. The sections were then incubated with terminal deoxynucleotidyl transferase for 60 min at 37 °C. Deoxyuridine triphosphate (dUTP) conjugated with digoxigenin was added to the 3'-OH ends of fragmented DNA. Labeled nucleotides were detected using an anti-digoxigenin antibody conjugated to peroxidase. Sections were stained with diaminobenzidine (DAB), and counterstained with hematoxylin to visualize the nuclei.

Statistical Analysis

Statistical analyses were performed using SPSS software version 22. Quantitative data were expressed as mean ± standard deviation (SD). Differences between groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. For histopathological data, the Kruskal-Wallis test, followed by the Mann-Whitney U test, was applied to detect significant differences and compare ranked outcomes of heart tissue damage. A p-value of less than 0.05 ($P < 0.05$) was considered statistically significant.

RESULTS

The impact of aerobic exercise on serum levels of creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), cardiac troponin I (cTnI), and tumor necrosis factor-alpha (TNF-α) in ischemia-reperfusion (I/R) injury is presented in Table II.

In the I/R group, significant elevations were observed in serum levels of CK-MB, LDH, cTnI, and TNF-α compared to the control group ($P < 0.01$). However, in the I/R group subjected to eight weeks of regular, progressive aerobic exercise, a significant reduction was noted in these elevated biomarker levels ($P < 0.05$). In contrast, two weeks of aerobic exercise did not result in

Table II. Effect of regular and increasing aerobic exercise on the serum values of LDH, cTnI, CK-MB and TNF-α in the rats.

Groups	Evaluated factors			
	LDH (IU/L)	CK-MB	cTnI (ng/ml)	TNF- α (pg/ml)
Control group	253.71±33.48	86.53±3.47	0.07±0.01	20.95±5.75
I/R	417.57±54.11[a]	184.94 ±8.66[a]	0.71±0.08[a]	55.61±10.92[a]
I/R + 2-week aerobic exercise	399.48±55.33[a]	172.11±7.[54a]	0.60±0.05[a]	51.34±8.27[a]
I/R + 8-week regular and increasing aerobic exercise	310.72±49.71[b]	112.9±6.32[b]	0.38±0.04[b]	35.16±5.44[b]

CK-MB: creatine kinase-MB, LDH: lactate dehydrogenase, cTnI: cardiac troponin I, TNF-α: tumor necrosis factor alpha. a: stands for significant difference of groups in comparison with the control group (P<0.01); b: stands for significant difference with the I/R group (P<0.05).

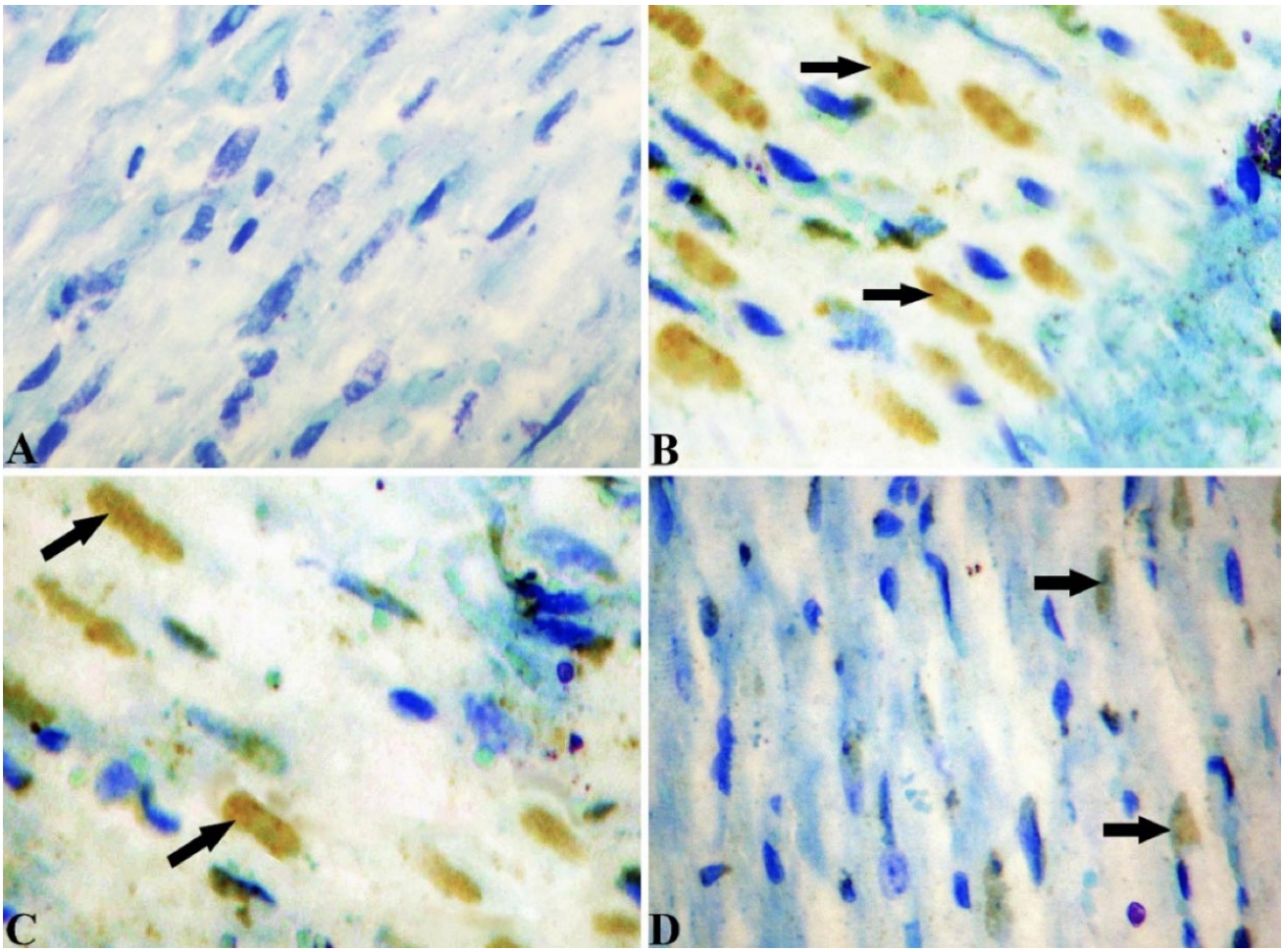

Fig. 1. Microscopic view of rats' heart tissue (TUNEL staining, 100X magnification). A (Control group): healthy heart tissue without pathological changes. B (I/R group): numerous apoptotic cells are seen in light brown color (arrows). C (I/R group with 2-week of aerobic exercise): apoptotic cells are observed in light brown color (arrows). D (I/R group with 8-week of regular and increasing aerobic exercise): fewer number of apoptotic cells are observed in light brown color.

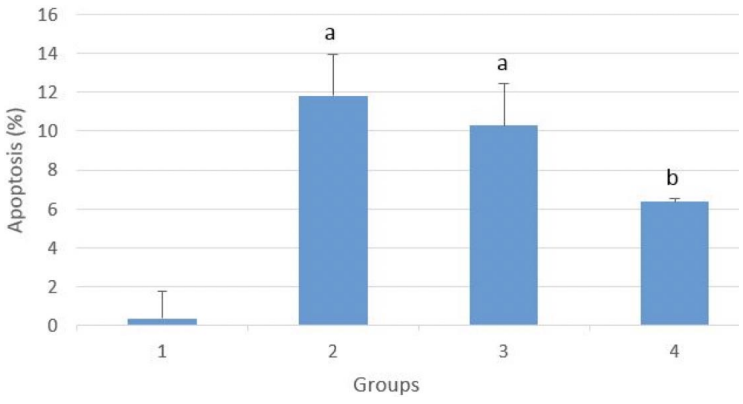

Fig. 2. Effect of regular and increasing aerobic exercise on the severity of apoptosis in the studied rats' myocardial tissue. a: stands for significant difference of groups in comparison with the control group (P<0.01); b: stands for significant difference with the I/R group.

significant changes in the elevated serum values of these biomarkers. Histopathological examination using TUNEL staining revealed apoptotic cells as light brown to dark brown. A significant increase in the number of apoptotic myocardial cells was detected in the I/R group compared to the control group ($P < 0.01$). However, in the I/R group subjected to eight weeks of aerobic exercise, the number of apoptotic myocardial cells was significantly reduced compared to the I/R group ($P < 0.05$). Conversely, two weeks of exercise showed no significant effect on the number of apoptotic cells. Figure 1 illustrates representative TUNEL staining images, while Figure 2 depicts the comparative intensity of apoptosis across the experimental groups.

Table III. Effect of regular and increasing aerobic exercise on the rat's heart antioxidant status.

Groups	Parameters			
	MDA (nmol/mg protein)	SOD (U/mg protein)	CAT (µmol of H_2O_2 consumed/mg protein)	GPx (µmol of glutathione oxidized/mg protein)
Control	8.11±1.54	10.18±0.72	18.36±0.43	2.53±0/14
I/R	13.27±2.65[a]	6.33±0.61[a]	11.25±0.35[a]	1.88±0.10[a]
I/R + 2-week aerobic exercise	11.88±2.74[a]	5.92±0.50[a]	12.16±0.38[a]	1.96±0.11[a]
I/R + 8-week regular and increasing aerobic exercise	9.46±1.83[b]	8.00±0.72[b]	15.78±0.49[b]	2.25±0.12[b]

MDA: creatine kinase-MB, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase. a: denotes significant difference in comparison with control group (P<0.01), b: denotes significant difference in comparison with I/R group (P<0.05).

The activities of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), and malondialdehyde (MDA) levels in myocardial tissues are presented in Table III.

In the I/R group, the activities of SOD, CAT, and GPx in myocardial tissues were significantly reduced, whereas MDA levels were significantly elevated compared to the control group (P < 0.01). However, in the I/R group subjected

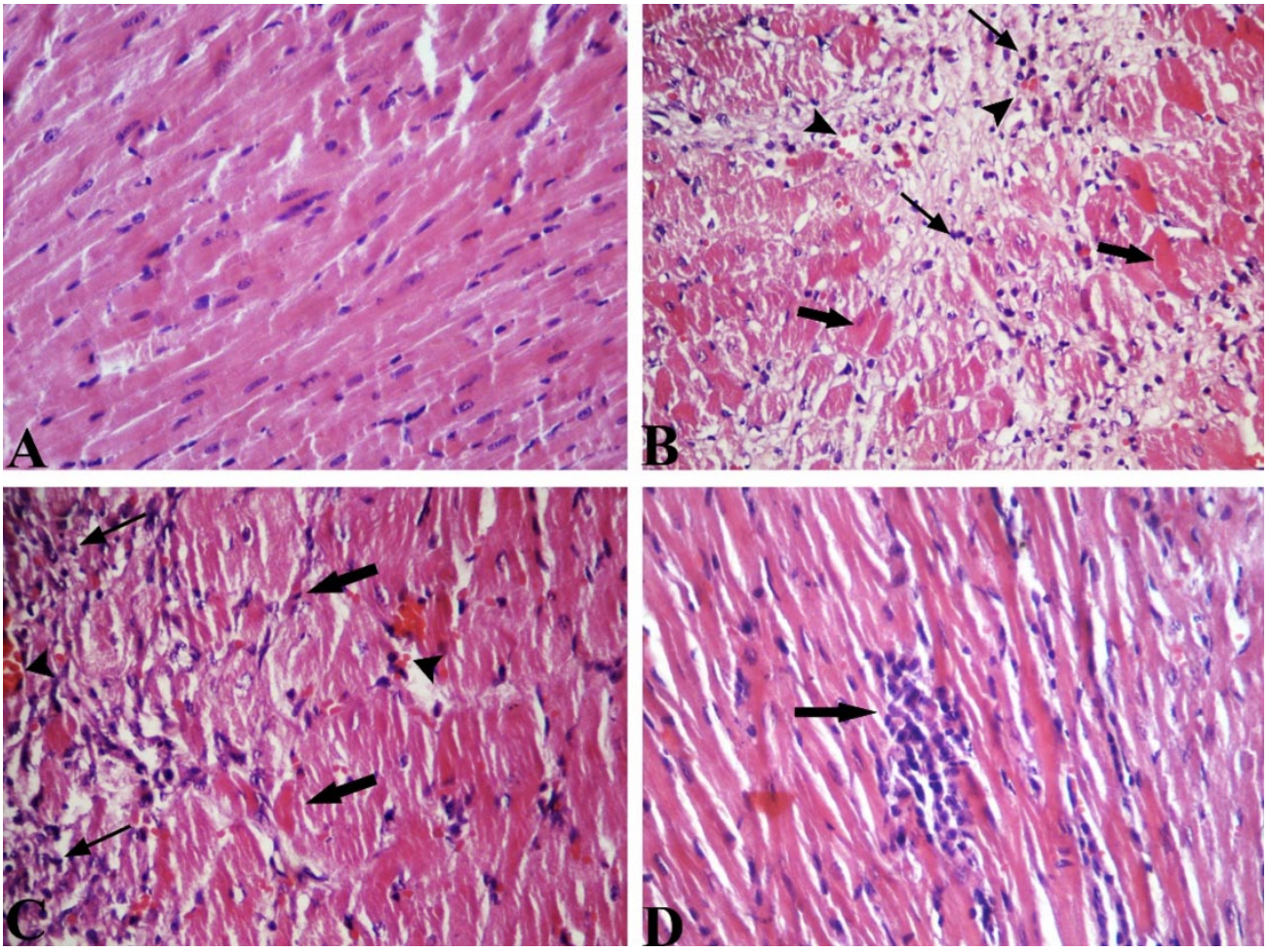

Fig. 3. Microscopic view of the rats' heart tissue (Hematoxylin-Eosin staining, 40X magnification). **A.** (Control group): healthy heart tissue without any pathological changes. **B.** (I/R group): extensive necrosis and degenerative changes along with severe necrotic changes in heart muscle fibers (thick arrows) as well as diffuse infiltration of inflammatory cells (thin arrows), hyperemia, interstitial edema, focal hemorrhage (arrowheads) are obvious. **C.** (I/R group with 2-week aerobic exercise): degenerative changes and cardiomyocyte necrosis (thick arrows), inflammatory cell infiltration (thin arrows), hyperemia, focal hemorrhage (arrowheads) are seen. **D.** (I/R group with 8-week regular and increasing aerobic exercise): focal accumulation and mild inflammatory cells infiltration (arrows) are seen among the myocytes.

to eight weeks of aerobic exercise, the activities of SOD, CAT, and GPx were significantly enhanced ($P < 0.05$), while MDA levels were significantly reduced compared to the I/R group. In contrast, two weeks of aerobic exercise did not significantly affect the activities of antioxidant enzymes or MDA levels in the myocardial tissues of rats. Table IV.

Table IV. Effect of aerobic exercise on heart ischemia-reperfusion injury in the studied rats.

Groups	degenerative and necrotic changes	degree of inflammation	Degree of bleeding	Kruskal-Wallis test result
Control	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	
I/R	3.82 ± 0.54^{b}	3.26 ± 0.12^{b}	2.55 ± 0.10^{b}	
I/R+2-week aerobic exercise	3.25 ± 0.17^{b}	3.10 ± 0.10^{b}	2.43 ± 0.17^{b}	P<0.001
I/R+8-week regular and increasing aerobic exercise	0.50 ± 0.12^{a}	0.42 ± 0.09^{a}	0.22 ± 0.05^{a}	

a,b: non-similar characters indicate the significance of difference between the groups.

Histopathological evaluation revealed that the myocardial tissue of the control group was intact and exhibited normal structural characteristics. In contrast, the I/R group demonstrated significant pathological changes, including degenerative and necrotic alterations of myocardial fibers, inflammatory cell infiltration, red blood cell extravasation, and interstitial edema. However, in the I/R group with eight weeks of aerobic exercise, pathological alterations, particularly necrosis and leukocyte infiltration, were markedly reduced. The remaining damage was primarily limited to mild interstitial edema and vacuolation of myocytes, particularly in endocardial regions. Notably, two weeks of aerobic exercise did not mitigate the pathological damage observed nking the groups and comparing their outcomes.

DISCUSSION

Heart muscle fibers contain key enzymes, such as lactate dehydrogenase and creatine kinase-MB (Haleagrahara *et al*., 2011). In the present study, ischemia-reperfusion (I/R) damage caused a significant increase in the activities of these enzymes. Regular, progressively increasing aerobic exercise over an eight-week period significantly prevented this enzyme activity enhancement due to I/R damage, suggesting that such exercise reduces heart muscle injury. Furthermore, serum levels of cardiac troponin I (cTnI) in rats subjected to I/R damage were notably elevated. cTnI, a protein found on actin filaments, regulates the speed and contractile force of cardiac cells. This highly sensitive marker is predominantly secreted into the bloodstream after heart damage or fatigue and can rise during severe cardiac diseases. In healthy individuals, cTnI levels are typically undetectable (Neumayr *et al*., 2002; Babuin & Jaffe, 2005). This protein's unique amino acid sequence differentiates it from related isoforms (Ng *et al*., 2005). The exercise protocol used in this study—regular and progressively increasing aerobic exercise significantly reduced serum cTnI levels in rats affected by I/R damage. Existing research suggests that cTnI levels reflect the extent of heart tissue damage (Powers *et al*., 1998). Thus, the findings of this study underscore the protective effect of aerobic exercise on heart tissue during ischemic damage. Marefati *et al*. (2016), demonstrated that moderate-intensity interval exercise significantly reduced serum cTnI levels in rats with ischemia-induced heart damage, reinforcing the cardioprotective role of such exercise protocols. Research also indicates that long-term exhaustive physical activity in humans lasting over an hour can elevate cardiac troponins, creatine kinase (CK), and its isoenzymes (CK-MB) in the serum (Neumayr *et al*., 2002; Dawson *et al*., 2003). Notably, endurance athletes and professional soccer players exhibit increased serum cTnI levels, indicative of heart tissue damage (Owbeer *et al*., 2007). While the precise cause of cTnI elevation following intense or prolonged exercise remains unclear, oxidative stress, hypoxia, or cytosolic leakage due to unstable ischemic heart tissue have been proposed as contributing factors (Sato *et al*., 2004). Additionally, cTnI elevation may result from the hemodynamic and physiological pressures on the heart during exercise. However, in healthy athletes, such increases are often observed without pathological significance during endurance activities (Soozani *et al*., 2010). Although the mechanisms underlying the protective effects of exercise on the heart remain a significant area of research, regular and consistent exercise enhances myocardial tolerance to ischemia-reperfusion damage (Lee *et al*., 2000; Gatta *et al*., 2012). Mechanisms of exercise-induced cardioprotection include vascular development, thermal stress adaptation, and improved antioxidant capacity. Studies have demonstrated that long-term exercise enhances the activity of antioxidant enzymes, which play a crucial role in protecting the heart (French *et al*., 2006). Moreover, ischemia followed by reperfusion leads to severe oxidative damage in rats' heart muscles (Kevin *et al*., 2005; Mokni *et al*., 2013). The generation of reactive oxygen species (ROS) or the depletion of antioxidants induces oxidative stress and, subsequently, myocardial injury (Sawyer *et al*., 2002). Free radical-scavenging enzymes,

such as superoxide dismutase, catalase, and glutathione peroxidase, neutralize oxygen radicals (e.g., superoxide ions and hydrogen peroxide) and prevent the formation of hydroxyl radicals, serving as a defense system against oxidative stress (Sawyer *et al*., 2002). In this study, it was observed that the reduction in the activities of superoxide dismutase, catalase, and glutathione peroxidase in rats with I/R-induced damage was significantly improved through long-term, regular aerobic exercise. Tofighi *et al*. (2015), also confirmed the protective effects of prolonged exercise on I/R-induced heart damage by demonstrating improved antioxidant enzyme activity. Such findings demonstrate that long-term and regular aerobic exercise enhances the antioxidant myocardial protective system, reducing oxidative stress. This study also reveals that lipid peroxidation is directly associated with the extent of cellular membrane damage in heart muscle fibers and the deactivation of enzymes. These results align with the findings of Gupta *et al*. (2013), who reported similar observations regarding lipid peroxidation in ischemia-reperfusion heart damage. Previous studies have suggested that the damage to heart tissues following ischemia and subsequent reperfusion is primarily caused by lipid peroxidation induced by free radicals (Zhou *et al*., 2006). Malondialdehyde (MDA), a key end-product of lipid peroxidation, serves as an indicator of oxidative damage. Its elevated levels are attributed to increased free radical production and decreased antioxidant enzyme activity. This study demonstrates that long-term, regular aerobic exercise significantly reduces the elevated levels of MDA caused by ischemia-reperfusion. The reduction in MDA levels can be linked to increased activity of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. These findings highlight the protective effects of aerobic exercise in eliminating free radicals and mitigating myocardial damage caused by oxidative stress. Additionally, this study shows that ischemia-reperfusion significantly increases serum levels of tumor necrosis factor-alpha (TNF-α) and induces extensive apoptotic changes in myocardial cells, indicating severe heart tissue damage. However, long-term, regular aerobic exercise significantly reduces TNF-α levels and apoptosis in myocardial cells, further confirming the cardioprotective effects of exercise against ischemia-reperfusion damage. Microscopic observations in this study revealed severe pathological changes in the myocardium of rats in the I/R group, including cardiomyocyte necrosis, interstitial edema, leukocyte infiltration, and bleeding. These findings emphasize the severity of myocardial damage caused by ischemia-reperfusion. Notably, aerobic exercise was shown to significantly prevent such pathological changes, underscoring its protective role. The generation of myocardial TNF-α during severe ischemia, with or without reperfusion, has been well-documented (Irwin *et al*., 1999). Additionally, research has shown that myocardial infarction leads to apoptosis in both infarcted and non-infarcted myocardium. Studies suggest that regulated TNF-α production in physiological responses contributes to cardiomyocyte immunity, typically observed during exercise (Gu *et al*., 2014). However, excessive TNF-α production during pathological conditions, such as ischemia, promotes myocardial infarction and apoptosis in cardiomyocytes (Sack *et al*., 2002). Chronic overproduction of TNF-α in the heart has been associated with functional impairments and the induction of apoptosis (Szabolcs *et al*.,1996). Link *et al*. (2005), demonstrated that exercise reduces apoptosis and improves antioxidant capacity in cardiomyocytes, thereby mitigating chronic heart failure. Similarly, Tofighi *et al*. (2015), found that aerobic exercise reduces oxidative stress in ischemic heart tissues by lowering lipid peroxides and enhancing the antioxidant defense system. Reactive oxygen species (ROS) are potent mediators of apoptosis. The expression of Bax, a pro-apoptotic protein, and the activation of apoptotic enzymes such as caspase-3 and caspase-9 are heightened in response to oxidative stress, leading to cardiomyocyte apoptosis (Cesselli *et al*., 2001). However, endurance exercise has been shown to enhance antioxidant enzyme activity, particularly superoxide dismutase, which reduces Bax expression and caspase activity while promoting the expression of Bcl-2, an anti-apoptotic protein. As a result, endurance exercise acts as a protective mechanism for maintaining heart function and preventing oxidative damage (Siu *et al*., 2004).

CONCLUSION

This study, supported by previous research, highlights that long-term, regular aerobic exercise strengthens the antioxidant defense system and protects myocardial tissues from oxidative damage caused by ischemia-reperfusion. Exercise prevents myocardial damage in both necrotic and apoptotic forms, reducing oxidative stress and improving overall heart function. Based on these findings, long-term, regular aerobic exercise is strongly recommended as a preventive strategy to mitigate myocardial oxidative damage caused by ischemia-reperfusion.

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HEJAZI, S.; DOUSTAR, Y.; MALEK-MOHAMMADI, K.; BOYDAK, M.; ASADABADI, S.; REZVANDOUST, M-A. & MOHAJERI, S. Efecto del ejercicio regular en cinta rodante sobre los cambios histopatológicos y las alteraciones bioquímicas cardíacas en la lesión por isquemia-reperfusión cardíaca en ratas. *Int. J. Morphol., 4(3)*:903-912, 2026.

RESUMEN: Restablecer el flujo sanguíneo al músculo cardíaco isquémico puede inducir mayor daño al miocardio. El objetivo de este estudio fue evaluar los efectos del ejercicio aeróbico regular y progresivo a largo plazo sobre la lesión por isquemia/reperfusión (I/R) cardíaca en ratas. Para ello, se asignaron aleatoriamente 40 ratas Wistar macho a 4 grupos iguales: control, I/R, I/R con dos semanas de ejercicio aeróbico e I/R con ocho semanas de ejercicio aeróbico regular y progresivo. Se realizó ejercicio aeróbico 5 veces por semana en cinta rodante a una velocidad de 10-25 m/min durante 10-30 minutos con una pendiente de 5 grados. Para inducir la lesión por isquemia/reperfusión (I/R), se ocluyó la arteria coronaria descendente izquierda durante 30 minutos, y posteriormente se restableció el flujo sanguíneo durante 2 horas. Se tomaron muestras de sangre para medir biomarcadores cardíacos séricos, incluyendo: creatina quinasa-MB (CK-MB), lactato deshidrogenasa (LDH), troponina I cardíaca (cTnI) y factor de necrosis tumoral alfa (TNF-α). Finalmente, todos los animales fueron sacrificados para examen histopatológico y evaluación del estado antioxidante miocárdico. El ejercicio aeróbico disminuyó significativamente los niveles séricos de CK-MB, LDH, cTnI y TNF-α, que se incrementaron tras la lesión por I/R, e incrementó significativamente los niveles cardíacos de SOD, CAT y GPx, que se redujeron en estos animales, así como disminuyó significativamente el nivel de MDA ($P<0,05$). Microscópicamente, el ejercicio aeróbico alivió significativamente los cambios degenerativos y necróticos del miocardio y disminuyó significativamente el número de células apoptóticas ($P<0,05$), que se incrementaron debido a la lesión por isquemia-reperfusión (I/R). Los resultados mostraron que el ejercicio aeróbico regular y progresivo a largo plazo protege el músculo cardíaco de las ratas tras la lesión por I/R.

PALABRAS CLAVE: Apoptosis; Ejercicio; Corazón; Isquemia-reperfusión; Rata; Biomarcadores séricos.

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