RESEARCH COMMUNICATION

Is Short-term Exercise a Therapeutic Tool for Improvement of Cardioprotection Against DOX-induced Cardiotoxicity? An Experimental Controlled Protocol in Rats

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Abstract

Background and Objective: Cardiotoxicity and oxidative stress is a life-threatening side effect of doxorubicin (DOX). We investigate the effects of short-term exercise as therapeutic tool for improvement of cardioprotection against DOX-induced cardiotoxicity in the rat. Methods: Wistar males (weighing 257±28 g) were divided into six groups: (1) control+placebo (2) control+DOX 10 mg.kg^{-1} (3) control+DOX 20mg.kg^{-1} (4) training+placebo (5) training+ DOX10 mg.kg^{-1} (6) training+DOX 20mg.kg^{-1}. Cardiotoxicity was induced by DOX (10 and 20 mg.kg^{-1}). The rats in groups 4, 5 and 6 experienced treadmill running of 25 to 39 min.day^{-1} and 15 to 17 m.min^{-1}, 5 days/wk for 3 wk. At the end of the endurance training program, rats in the 1 and 4 groups, in the 2 and 5 groups and in the 3 and 6 groups received saline solution, DOX 10 mg.kg^{-1} and DOX 20 mg.kg^{-1}, respectively. Result: DOX administration (10 and 20 mg.kg^{-1}) caused significant increase in MDA and Apelin, an insignificant increase in NO and a significant decrease in SOD, as compared to the C+P group. Three weeks of the pretreatment endurance exercise resulted in a significant increase of Apelin and SOD, an insignificant increase of NO and an insignificant decrease of MDA, as compared to the C+P group. Furthermore, after three weeks of endurance training and DOX treatment with 10mg.kg^{-1} and 20mg.kg^{-1}, a significant increase in apelin and SOD, and a significant decrease in MDA were detected in comparison to C+DOX10 and/or C+DOX20 groups. There was a significant difference between DOX10 mg.kg^{-1} and DOX20 mg.kg^{-1} treatments in MDA levels only. Conclusion: Pretreatment exercise may improve myocardial tolerance to DOX-induced cardiotoxicity by inhibition of oxidative stress and up-regulation of antioxidants in heart tissue.

Keywords: Cardiac toxicity - doxorubicin - oxidative stress - aerobic training - rat model

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Introduction

Doxorubicin (DOX) is a broad spectrum anthracycline antibiotic used to treat a variety of malignancies. However, its utility is limited by DOX-induced cardiotoxicity, mainly due to free radical induced myocardial injury, lipid peroxidation, mitochondrial damage and cellular toxicity (De Beer et al., 2001; In Duk et al., 2002; Li et al., 2007; Kanu et al., 2010; Verma and Vinayak, 2012; Vishwanatha et al., 2012). Several researchers have reported that an organism is generally protected from damage caused by free radicals by means of its antioxidant defence system. DOX modulates glutathione and glutathione-dependent antioxidant systems (Verma and Vinayak, 2012). The cellular antioxidant defence system operates mainly via antioxidant enzymes, such as apelin, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and apart from nonenzymatic antioxidants. The antioxidant enzymes neutralize highly reactive free radicals, and thus prevent uncontrolled generation of ROS (Mates, 2000). Therefore, decrease in DOX toxicity by enhancement in the activity of antioxidant enzymes is proposed to help in prevention of malignant growth by DOX chemotherapy. In contrast, oxidative stress and release of free radicals as well as endogenous antioxidant deficits have been suggested to play a major role in Dox-induced cardiotoxicity and heart damage (Ioanna et al., 2007; Hitesh et al., 2011; Vishwanatha et al., 2012). DOX is significantly toxic to most tissues and organs, but it's particularly toxic to heart tissue and constitutes a major cause of morbidity and mortality in cancer patients (El-Sayed et al., 2011). In fact, the weakness of the heart to oxidative damage may be in part explained by the fact that heart demonstrates a slow turnover and relatively lower levels of antioxidant enzyme activity when compared to most other tissues such as liver (Ascensao et al., 2005; Babaei et al., 2008; Xin et al., 2011).

In recent years, by understanding the free radical mechanism of DOX-induced cardiotoxicity, it has become possible to develop effective strategies to prevent or modify expected damage. To date, a number of pharmaceutical agents have been tested, to assess their

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potential to reduce the risk of doxorubicin cardiotoxicity (Abdel-Moneim et al., 2009; Cecen et al., 2011; Hitesh et al., 2011; Raschi et al., 2010; Viswanatha et al., 2011; Vishwanatha et al 2012).

On the other hand, there is a growing interest in the usage of endurance regular training as a non-drug therapeutics protective strategy against problems related to cardiovascular health such as Dox-induced cardiotoxicity (Ascensão et al., 2012). Although, there is evidence that acute exercise resulted in oxidative stress and cardiac damage (Teixeira de Lemos et al., 2011), it seems probable that regular endurance exercise training could constitute an excellent tool either to prevent and/or to treat several diseases. Also, while most recent studies have focused on the treatment effect of endurance exercise in induce DOX cardiotoxicity (Hydock et al., 2008; Kavazis et al., 2010; Ascensão et al., 2012). We are the first to investigate the cardioprotective effects of prior (pretreatment) short-term treadmill running endurance exercise on doxorubicin-induced cardiotoxicity with various dosages (10 and 20 mg/kg) of the DOX drug in heart tissue. These new insights would consist in the recognition of regular training as a non-drug therapeutics protective strategy against DOX treatment.

The hypothesis proposed was that if DOX cardiotoxicity are related to free radical formation and oxidative stress, an enhancement in antioxidant/oxidation ratio after regular endurance exercise may protect against DOX-induced toxicity in the heart. Therefore, the purpose of this study was to determine pretreatment effects of three weeks of aerobic training on biomarkers related to the cardiac oxidative damage including; Apelin, malondialdehyde (MDA), nitric oxide (NO), superoxide dismutase (SOD) in rats that have been acutely exposed to DOX-induced cardiotoxicity.

Materials and Methods

Experimental Design and Laboratory Environment

The experimental protocol of current study approved by department of physiology, university of Mazandaran and were performed according to guiding procedures in the Care and Use of Animals, prepared by the Council of the American Physiological Society. The experiments were carried out with forty-eight Wistar male rats (8-week-old, initially weighing 257 ± 28 g), which were obtained from the laboratory of animal bearing and multiplying at the Pasture institute of Iran. Rats were housed in standard cages of polycarbonate (20 × 15 × 15 cm), made at the Pasture institute of Iran, in a large air-conditioned room with a controlled temperature of 22 ± 2°C, light- dark cycles of 12 : 12 hours and humidity of 50 ± 5%. The pollutant standard index (PSI) was in the acceptable range as determined by the Iranian meteorological organization. Rats were fed with a standard rat chow provided by Pars Institute for animals and poultry with a daily regimen of 10 g per 100 g body weight for each rat. Water was available ad libitum.

Familiarization and Exercise Training Protocols

Rats in all groups were adapted to the treadmill by running for 5 days. The familiarization protocol was designed as once a day for 10 min.session^{-1} at a speed of 10 m.min^{-1} at a slope of 0 degree. Because rats are more active in darkness, the front portion of the treadmill lines was covered with a dark thick paper to darken this area. At the rear of the lines, an electric grid provided a stimulus for running. An electric stimulus (30 V, 0.5 A) was manually turned on for less than 2 s when the animals stayed on the electric grid for longer than 10 s. Rats quickly learned to stay on the belt and avoid shock, except for one rat, which would not stay on the moving belt, and thus was quickly removed from familiarization process. Following this familiarization period, they were randomly assigned into control and trained groups. Exercise training protocol was performed on treadmill with zero slope between 25 to 39 min.session^{-1} and 15 to 17 m.min^{-1}, 5 days/wk for 3wk (Table 1). We replicated the aforesaid exercise training protocol that was previously reported by Dabidi Roshan et al. (2011).

Table 1. The Regular Physical Activity Program during 3-weeks

Weeks	Training days / Parameters of training	1	2	3	4	5
1	Speed (m/min)	15	15	15	15	15
	Time (min)	25	26	27	28	29
2	Speed (m/min)	16	16	16	16	16
	Time (min)	30	31	32	33	34
3	Speed (m/min)	17	17	17	17	17
	Time (min)	35	36	37	38	39

Subject Classification

At the end of the exercise training protocol, rats from the control and trained groups were again randomly separated into subgroups; the DOX (10, 20 mg.kg^{-1}) and placebo treatment. Thus, the control rats were distributed into control + placebo (C+P, n = 8), control + DOX (C + DOX 10 mg.kg $^{-1}$, n = 8) and control + DOX (C + DOX 20mg.kg^{-1}, n = 8) groups and the trained rats into trained + placebo (T + P, n = 8), trained + DOX (T + DOX 10 mg.kg^{-1}, n = 8) and trained + DOX (T + DOX 20 mg.kg^{-1}, n = 8) groups.

Doxorubicin treatment

Doxorubicin hydrochloride (EBEWE Pharma Ges.m.b.H.Nfg.KG) was dissolved in saline and administered by i.p injection at two dosages of 10 mg.kg^{-1} (Wonders et al., 2009) and 20 mg.kg^{-1} (Ascensão et al., 2006), and control animals received saline with comparable volume. Both treatments were carried 24 h after the last exercise bout and animals were sacrificed 24 h after DOX and placebo injections.

Heart Tissue collection and preparation

All groups were anesthetized with ketamine and xylazine and decapitated after 10 to 12 hours overnight fasting. The Thoracic cavity was opened and the heart was quickly excised from the aortic root. Heart tissues were weighed and it was placed into Petri dishes containing cold isolation medium (0.1 mol.L^{-1} K2HPO4, 0.15 mol.L^{-1} NaCl, pH 7.4) to remove the blood and

Table 2. Effect of Endurance Training and DOX Treatment on Apelin, NO and MDA Levels in the Various Groups

markers	C+P	C+DOX10	C+DOX20	T+P	T+DOX10	T+DOX20
Apelin(pg/mg protein)	3.64±0.46	5.13±0.33	5.43±0.44	7.12±0.51	6.08±0.57	5.73±0.92
NO(nmol/mg protein)	0.245±0.022	0.267±0.023	0.270±0.032	0.274±0.023	0.270±0.032	0.292±0.037
MDA(nmol/g protein)	29.63±1.89	44.72±3.67	58.66±7.97	22.35±4.25	35.45±6.74	47.16±10.15
SOD(u/mg protein)	92.34±2.9	84.32±4.42	75.31±6	112.22±3.06	103.42±4.07	98.75±2.75

were frozen immediately in liquid nitrogen and stored at -80ºC for subsequent analysis of Apelin, NO, SOD and MDA. Heart tissue was squashed in liquid nitrogen, homogenized in a lysis buffer (5 ml.g^{-1} of tissue) with a protease inhibitor cocktail for mammalian cell and tissue extracts (Sigma-Aldrich, St. Louis, U.S.A) 100 ul.l^{-1} ml, and 10 m Mtris base (Sigma-Aldrich, St. Louis, U.S.A), pH 7.4 and centrifuged at 1500 g at 4 ºC for 15 min. Heart supernatant was diluted 1:30. Plasma was diluted 1:10 and the fluids were used in an Apelin-13 ELISA kits (Phoenix peptides, Burlingame, California, USA), following the manufacturer's instructions.

Biochemical Analysis the assay kit was very specific and detects apelin-13 with 100% cross reactivity. It has an inter-assay variation less than 14% and intra assay coefficient of variation less than 10%. Apelin-13 in the mentioned sample was measured using ELISA kits too (Rat Apelin, ELISA, USCN LIFE Science Inc., Wuhan, P. R. China, USCN, Life Science Inc, Sensitivity 0.128 ng.ml^{-1} and Intra CV: 5%).

Lipid peroxidation (MDA) levels, as important marker in oxidative stress in the heart tissue, were measured with the thiobarbituric-acid reaction using the method of Daniel et al (2004). The quantification of thiobarbituric acid reactive substances was determined at 532 nm by comparing the absorption to a standard curve of MDA equivalents generated by acid catalyzed hydrolysis of 1, 1, 3, 3 tetramethoxypropane. The values of MDA in heart tissue were expressed as nmol.g^{-1} tissue. The NO concentration was determined by first reducing the nitrate to nitrite using nitrate reductase (Sigma). Superoxide dismutase (SOD) activity was determined spectrophotometrically using the method described by Dabidi Roshan et al (2011). In brief, for total SOD (tSOD) activity the adequate amount of protein (2 mg tissue wet weight) was incubated at 25 ºC with 1 m MN,Nbis (2-(bis(carboxymethyl)amino)-ethyl) glycine (DTPA) in 50 m MTris_HCl, pH 8.2, in 1 ml final volume. Reaction was started with 0.3 m Mpyrogallol, in which the auto-oxidation rate was recorded at 420 nm.

Statistical Analysis

All data have been expressed as mean ± SD. Statistical analysis was performed using a commercial software package (SPSS version 16.0 for Windows). Data of the biomarkers related to the cardiac oxidative damage were normally distributed after log-transformation. A one-way analysis of variance (Statistics software, Stat Soft, Inc., Tulsa, OK) was used to detect statistical differences between groups. A post-hoc test (Tukey test) was performed to determine differences in the various biomarkers between groups. Differences were considered statistically significant at p-value<0.05.

Results

Table 2 shows changes in Apelin, NO, MDA and SOD levels following doxorubicin treatment in the various groups. Rats treated with Doxorubicin (10 and 20 mg.kg^{-1}) in control group, showed a significant increase in MDA (51% and 96%, respectively), a significant increase in Apelin (41% and 49%, respectively), a significant decrease in SOD (9% and 18%, respectively) and an insignificant increase in NO (8% and 12%), as compared to C+P group. Although, there was no significant difference between DOX10mg.kg^{-1} and DOX20 mg.kg^{-1} treatments in Apelin and NO levels, there was a significant difference between DOX10 mg.kg^{-1} and DOX20mg.kg^{-1} treatments in MDA and SOD levels.

Three weeks of the endurance regular training led to a significant increase of heart Apelin and SOD levels (95% and 21%, respectively), insignificant increase in NO, an insignificant decrease in MDA, as compared to C+P group (P <0.05) (Table 2). However, after three weeks of aerobic training and DOX treatment with 10mg.kg^{-1}, a significant increase in SOD (23%), and an insignificant increase in Apelin and NO (18% and 1%, respectively), and an insignificant decrease in MDA (12%) were detected in comparison to C+DOX10 group (P <0.05). In contrast, three weeks of aerobic training and DOX treatment with 20 mg.kg^{-1} resulted in an insignificant increase in Apelin and NO (6%and 8%, respectively), a significant increase in and SOD (31%), and a significant decrease in MDA (17%) in comparison to C+DOX20 group (P <0.05).

Changes between heart tissue Apelin, MDA, NO and SOD levels, are shows in Figure 1, 2, 3 and 4, respectively.

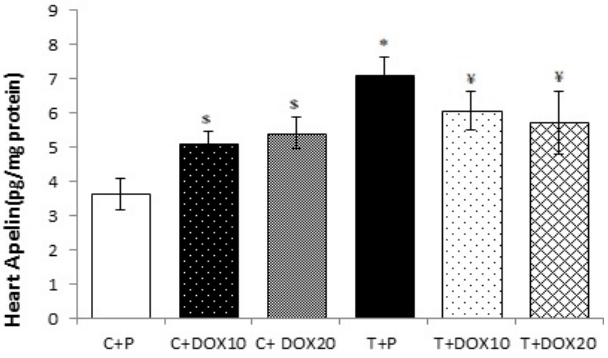

Figure 1. Apelin Level After Three Weeks of Aerobic Training and DOX Treatment. Abbreviations; C+P (Control+Placebo), C+DOX10 (Control + Doxorubicin 10 mg.kg^{-1}), C+DOX20 (Control + Doxorubicin 20 mg.kg^{-1}), T+P (Training+Placebo), T +DOX10 (Training + Doxorubicin 10 mg.kg^{-1}), T +DOX20 (Training + Doxorubicin 20 mg.kg^{-1}). Data are presented as the mean±SD for 8 Rats.*Significantly different to similar control group (P<0.05), $ significantly different to the C+P group (P<0.05), ¥Significantly different to T+P group (P<0.05)

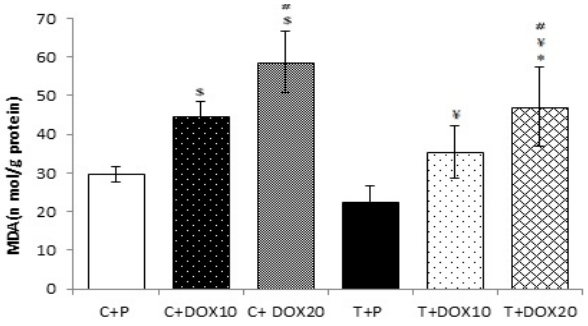

Figure 2. Malondialdehyde(MDA)Level After Three Weeks of Aerobic Training and DOX Treatment. Abbreviations; C+P (Control+Placebo), C+DOX10(Control + Doxorubicin 10 mg.kg^{-1}), C+DOX20 (Control + Doxorubicin 20 mg.kg^{-1}), T+P (Training+Placebo), T +DOX10 (Training + Doxorubicin 10 mg.kg^{-1}), T +DOX20 (Training + Doxorubicin 20 mg.kg^{-1}).Data are presented as the mean±SD for 8 Rats.*significantly different to similar control group (P<0.05), $ significantly different to the C+P group (P<0.05), #significantly different to dose 10 mg.kg^{-1}in it's group (P<0.05), ¥ significantly different to the T+P group (P<0.05)

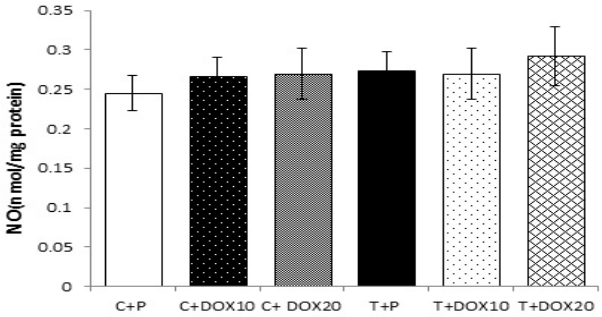

Figure 3. Nitric Oxide (NO) Level After Three Weeks of Aerobic Training and DOX Treatment. Abbreviations; C+P (Control+Placebo), C+DOX10 (Control + Doxorubicin 10 mg.kg^{-1}), C+DOX20 (Control + Doxorubicin 20 mg.kg^{-1}), T+P (Training+Placebo), T +DOX10 (Training + Doxorubicin 10 mg.kg^{-1}), T +DOX20 (Training + Doxorubicin 20 mg.kg^{-1}). Data are presented as the mean±SD for 8 Rats

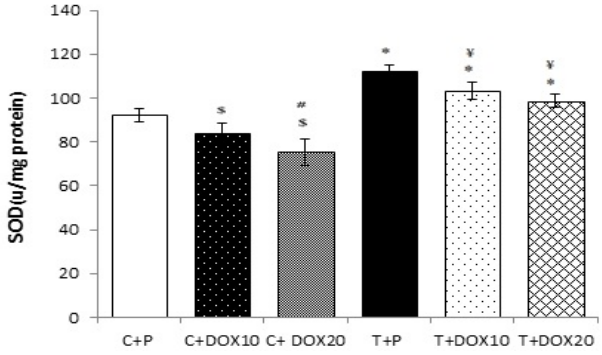

Figure 4. Superoxide Dismutase (SOD) Level After Three Weeks of Aerobic Training and DOX Treatment. Abbreviations; C+P (Control+Placebo), C+DOX10(Control + Doxorubicin 10 mg.kg^{-1}), C+DOX20 (Control + Doxorubicin 20 mg.kg^{-1}), T+P (Training+Placebo), T +DOX10 (Training + Doxorubicin 10 mg.kg^{-1}), T +DOX20 (Training + Doxorubicin 20 mg.kg^{-1}). Data are presented as the mean±SD for 8 Rats. .*Significantly different to similar control group (P<0.05), $ significantly different to the C+P group (P<0.05), #significantly different to dose 10 mg.kg^{-1} in it's group (P<0.05), ¥ significantly different to the T+P group (P<0.05)

After three weeks of aerobic training and Doxorubicin treatment, both 10 and 20 mg.kg^{-1}, a significant decrease in Apelin (14% and 19%, respectively), a significant decrease in SOD(8% and 12%, respectively), a significant increase in MDA (58% and 111%, respectively) were detected, as compared to T+P group (P <0.05). However, there was no significant difference between DOX10 mg.kg^{-1} and DOX20mg.kg^{-1} treatments in Apelin, NO and SOD levels. Furthermore, there was a significant difference between DOX10 mg.kg^{-1} and DOX20mg.kg^{-1} treatments in MDA levels.

Discussion

There are several reports stating that the clinical utility of DOX is marred by an increased risk of myocardial injury, which is mainly caused by reactive oxygen species from DOX disposition (Kavazis et al., 2010; Menna et al., 2010). Because of the relatively lower levels of the antioxidant defenses in the cardiomyocytes, heart is more susceptible to oxidative damage than other tissues (Ascensao et al., 2005; Babaei et al., 2008; Xin et al., 2011). While, previous researchers have reported role physical activity as a nonpharmacological strategy in various cancers, we are the first to investigate the pretreatment effect of short-term endurance training before the various dosages (10 and 20 mg.kg^{-1}) of Doxorubicin (DOX) on markers of related to cardiotoxicity in heart tissue. Our study demonstrated, although, 10mg.kg^{-1} of DOX induced oxidant/antioxidant imbalance in rats heart tissue, a significant increase in MDA and Apelin, an insignificant increase in NO, and a significant decrease in SOD; were detected following 20mg.kg^{-1} of DOX. The results indicate that there is a potent relationship between oxidative stress and DOX-induced cardiotoxicity. In addition, these data suggest that the increased oxidative stress production by DOX could be blocked by the pretreatment with endurance regular exercise, with improve antioxidants and dysfunction markers. Also, data of the current study provided additional support to understand how regular physical exercise, particularly treadmill running training, could contribute to augmentation of cardiac muscle resistance against oxidative stress-based cardiotoxicity induced by DOX administration.

Free radicals are continuously produced in vivo and there are number of protective antioxidant enzymes (superoxide dismutase, catalase, glutathione S-transferase, glutathione peroxidase and antioxidant glutathione) for dealing with these toxic substances. The delicate balance between the production and catabolism of oxidants is critical for maintenance of the biological function. Two lines of evidence can be emphasized from the present study. First and considering cardiac stress marker, namely MDA, endurance regular training decreased the rise of cardiac disturbances induced by acute single doses of DOX administration, particularly, in dose of 10mg.kg^{-1}. Second, and according to changes observed in cardiac SOD response and in part, apelin in both control and trained rats hearts treated with DOX, it is likely that these markers might be considered as essential cellular defense against free radical-based cardiotoxicity caused by DOX, providing enhanced tolerance to trained myocardium at least in the first 48 h after the end of training period.

The other important finding in the present study was that after three weeks of aerobic training and DOX administration (10 and 20mg.kg^{-1}), an insignificant increase in apelin and a significant increase in SOD activity in heart tissue, were found, as compared to C+DOX (10 and 20mg.kg^{-1}) groups. Moreover, although current data demonstrate that exercise training protects the heart against Dox-induced damage (Ascensao et al., 2005; Chicco et al., 2006); the mechanism(s) by which exercise training protects cardiomyocytes remain unclear. There were two possible pathways to explain the protective effects of regular endurance exercise against DOX-induced cardiotoxicity. At present, the principal mechanism of Dox-induced cardiotoxicity is believed to be increased oxidant production by the mitochondria (Ascensao et al., 2005; Chicco et al., 2005; Chicco et al., 2006; Kavazis et al., 2010). Our data indicate that Dox administration, in particularly with 20mg.kg^{-1}, increased ROS production in cardiac tissue. In addition, an interesting finding in the present study that may provide further insight into the effects of Dox on the myocardium was a slightly increase in SOD content in the heart of the Dox-treated rats, as compared to C+P group. In contrast, regular endurance exercise lead to significant increase in the apelin and SOD activity and decrease in lipid peroxidation in heart tissue of T+DOX treated groups.

In summary, our study suggests that Dox treatment is associated with oxidant/antioxidant imbalance in heart tissue. In addition, the present investigation provides new insights into the biochemical mechanisms by which pretreatment of endurance exercise training through its potent antioxidant properties, protects cardiac muscle tissue against the toxicity induced by DOX. Thus, our study suggests that moderate-term aerobic regular exercise training may be considered as a potentially useful candidate for improve myocardial tolerance against DOX-induced oxidative damage.

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