RESEARCH ARTICLE

Pretreatment Hepatoprotective Effect of Regular Aerobic Training Against Hepatic Toxicity Induced by Doxorubicin In Rats

Fatemeh Zolfagharzadeh[1], Valiollah Dabidi Roshan[2]*

Abstract

Background: Doxorubicin is an anthracycline antibiotic commonly used to treat a variety of cancers as a most effective antitumor. However, its clinical use is associated with the toxic effects in numerous healthy tissues. Here we investigated the pretreatment effect of regular aerobic exercise on oxidative stress in rats acutely exposed to DOX-induced hepatotoxicity. Materials and Methods: Forty-eight Wistar male rats were randomly divided into 2 groups: control and training. The training protocol included treadmill running between 25 to 54 min/day and 15 to 20m/min, 5 days/week for 6 weeks. At the end of the exercise training protocol, rats from the control and trained groups were again randomly separated into 3 subgroups: DOX10mg/kg, DOX20mg/kg and saline. All treatments were carried 24 h after the last exercise bout and animals were sacrificed 24 h after DOX and saline injections. Results: Administration of DOX (10 and 20 mg.kg^{-1}) resulted in imbalance in biomarkers related to oxidants and antioxidants in liver tissue, as compared to control groups. Six weeks of pretreatment training led to a significant increase in nitric oxide (NO), superoxide dismutase (SOD) and glutathione peroxidase (GPX) as compared to the control+DOX 10 mg/kg group. Training before DOX 20 mg/kg administration also led to a significant increase in NO and SOD, and a significant decrease in malondialdehyde (MDA). In addition, there was a significant difference between DOX 10 mg/kg and DOX 20 mg/kg treatments in MDA levels, only. Conclusions: The results of the present study indicate that pretreatment with aerobic exercise induces positive adaptations and has a potential protective effect against doxorubicin (DOX)-induced hepatotoxicity with doses of 10 and 20 mg.kg.

Keywords: Oxidative stress - pretreatment aerobic training - doxorubicin - rat hepatotoxicity

Asian Pacific J Cancer Prev, **14 (5)**, 2931-2936

Introduction

Cancer is a major public health problem for many people around the world in the industries countries (Siegel et al., 2012). Conventional drug therapy for many common and chronic disorders, including cancers, has limited efficacy and potentially life- threatening side effects (Mahmud et al., 2012). Doxorubicin (DOX) is a powerful and highly efficacious drug and shows a broad range of antitumor activity in many kinds of cancers (Vishwanatha et al., 2012). However, the clinical use of DOX is often limited because of its undesirable serious toxic side effects on various organs such as cardiac, liver, and etc. (Ashrafi and Dabidi Roshan, 2012). Several mechanisms have been proposed to account for the DOX-induced cardiotoxic and hepatotoxic side effects including free radical, lipid peroxidation, mitochondrial damage and cellular toxicity (Ashrafi and Dabidi Roshan, 2012). In contrast, several researchers have reported that an organism is generally protected from damage caused by free radicals by means of its antioxidant defense system and administration of DOX modulates glutathione and glutathione-dependent antioxidant systems (Ashrafi and Dabidi Roshan, 2012). The cellular antioxidant defense system operates mainly via antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and apart from nonenzymatic antioxidants. Previous studies showed that one of the major functions of antioxidant is the detoxification of reactive species and toxic oxygen metabolites generated by the endogenous and exogenous metabolism pathways (Choi et al., 2013). For example, Glutathione peroxidase mediates scavenging of intermediates such as hydrogen peroxide and hydroperoxides. Therefore, up-regulation of antioxidant and down-regulation of oxidants is increasingly important in oxidative stress related diseases and their care.

On the other hand, drug-induced hepatotoxicity is continuously emerging as a significant cause of liver

*[1]Exercise Physiology, Azad University of Sari, Sari, Mazandaran, [2]College of Physical Education and Sport Sciences, Department of Sport Physiology, University of Mazandaran, Babolsar, Iran *For correspondence: v.dabidi@umz.ac.ir, vdabidiroshan@yahoo.com*

disorders, representing today a leading cause of acute liver failure in economically developed countries (Mrzljak et al., 2013). During DOX therapy, the liver receives, accumulates and metabolizes high concentrations of DOX. Hence, it is expectable that the liver is one of the most affected organs by DOX therapy. Indeed, almost 40% of patients suffered liver injury after DOX treatment (Carvalho et al., 2009). Thus, protection of normal tissue and organs during acute and chronic DOX exposure is still a matter of special concern. Therefore, it is necessary to search for complementary and alternative strategies against DOX-induced hepatotoxicity. Although, there is evidence that acute exercise resulted in oxidative stress and cardiac damage (Ashrafi and Dabidi Roshan, 2012), 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 (Wonders et al., 2009; Ashrafi et al., 2012; Shirinbayan and Dabidi Roshan., 2012), To the best of our knowledge, we are the first to investigate the hepatoprotective effects of prior (pretreatment) short-term treadmill running aerobic exercise on doxorubicin-induced hepatotoxicity with various dosages (10 and 20 mg/kg) of the DOX drug in liver 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 hepatotoxicity is 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 liver tissue. Therefore, the purpose of this study was to determine the pretreatment effects of 6 weeks treadmill running on malondialdehyde (MDA), nitric oxide (NO), superoxide dismutase (SOD) and glutathione peroxidase (GPX) in rats that have been acutely exposed to DOX-induced hepatotoxicity with various dosages (10 and 20 mg/kg).

Materials and Methods

Experimental design and laboratory environment

The experimental protocol of the current study approved by department of physiology, university of Mazandaran 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-weeks-old, initially weighing 269±4 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 h 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 aerobic exercise protocols

Animals were habituated to treadmill running for 5 days (10 min exercise/day at 10 m/min, 0% grade). 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 and 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 sedentary and trained groups. Exercise training protocol was performed on treadmill with zero slopes between 25 to 54 min/session and 15 to 20 m/min, 5 days/week for 6 weeks. We replicated the aforesaid exercise training protocol that was previously reported by Ashrafi et al. (2012).

Subject's 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) and saline treatment. Thus, rats finally were distributed into control+saline (C+S, n=8), control+DOX10 (C+DOX10 mg/kg, n=8), control+DOX20 (C+DOX20 mg/kg, n=8), training+saline (T+S, n=8), training+DOX10 (T+DOX10 mg/kg, n=8) and training+DOX20 (T+DOX20 mg/kg, n=8) groups.

Doxorubicin treatment

DOX was obtained from EBEWE Pharma Ges.m.b.H.Nfg. KG (A-4866 unterach, Austria) as a vial of ph. Eur. In order to bring the drug concentration of 10 and 20 mg/kg, it was dissolved in 0.9% saline for administration. The dose 20 $mg.kg^{-1}$ of DOX is human clinical doses that are pharmacologically scaled for use in rats (Ogawa et al., 2011). Saline was used as the vehicle and the placebo treatment and was used to form saline solution (0.9% NaCl ip).

Liver tissue collection and preparation

All groups were anesthetized with ketamine and xylozine and decapitated after 10 to 12 h overnight fasting. Abdominal cavity was opened and the liver was quickly excised from the hiatus of liver. Liver tissue were weighed and placed into Petri dishes containing cold isolation medium (0.1 mol/L K2HPO4, 0.15 mol/L NaCl, pH 7.4) to remove the blood and were frozen immediately in liquid nitrogen and stored at -80ºC for subsequent analysis of MDA, NO, SOD and GPx. Liver tissue was squashed in liquid nitrogen, homogenized in a lysis buffer (5 ml/g of tissue) with a protease inhibitor cocktail for mammalian cell and tissue extracts (Sigma-Aldrich, St. Louis, U.S.A) 100 ul/1 ml, and 10 m Mtris base (Sigma-Aldrich, St. Louis, U.S.A), pH 7.4 and centrifuged at 1500 g at 4ºC for 15 min. The homogenates were diluted with cold 20 mM Tris-HCl and centrifuged (10 min at 58C, 3000 g). Biochemical measurements on activity of the GPx enzyme were conducted using GPx-

340 kit (OXIS, Portland, OR, USA). In the supernatants, activity of GPx was estimated by spectrophotometry. All samples were processed in the same assay to avoid interassay variations. Superoxide dismutase (SOD) activity was determined spectrophotometrically using the method described by Dabidi Roshan et al (2012). In brief, for total SOD (tSOD) activity the adequate amount of protein (2 mg tissue wet weight) was incubated at 258C with 1 mM N,Nbis(2-(bis(carboxymethyl)amino)-ethyl) glycine (DTPA) in 50 mM Tris_HCl, pH 8.2, in 1 ml final volume. Reaction was started with 0.3 mM pyrogallol, in which the auto-oxidation rate was recorded at 420 nm. Furthermore, the NO concentration was determined by first reducing the nitrate to nitrite using nitrate reductase (Sigma-Aldrich, St. Louis, USA). Lipid peroxidation levels in the homogenate tissue were measured with the thiobarbituric acid reaction using the method of Ohkawa et al. (1979). The Thiobarbituric acid-reactive substances (TBARS) were quantified at 532 nm by comparing the absorption to a standard curve of malondialdehyde (MDA) equivalents generated by acid catalysed hydrolysis of 1,1,3,3 tetramethoxypropane.

Statistical analysis

All data have been expressed as mean±SD. Statistical analysis was performed using a commercial software package (SPSS version 20.0 for Windows). Data of the biomarkers related to the liver oxidative stress 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

Mean Values from malondialdehyde (MDA), nitric oxide (NO), superoxide dismutase (SOD) and glutathione peroxidase (GPX) in rats that have been acutely exposed to DOX-induced hepatotoxicity with various dosages (10 and 20 mg/kg), are shown in Tables 1 and 2. After DOX10 mg/kg administration, there was a significant increase (33.8%) in MDA levels, an insignificant increase (20%) in NO levels and an insignificant decrease in SOD and GPX levels (7.39% and 9.21%, respectively) as compared to control+saline group. However, DOX20 mg/kg treatment groups was showed a significant increase in MDA and NO levels (70.99% and 35%, respectively) and a significant decrease (24.85%) in SOD levels and an insignificant decrease (23.56%) in GPx levels, in comparison with control+saline group.

Data are presented as the Mean±SD for 8 Rats, Abbreviations: Malondialdehyde (MDA), Nitric oxide (NO), Superoxide dismutase (SOD) and Glutathione peroxidase (GPX), C+DOX10 (Control+Doxorubicin 10 mg/kg), C+DOX20 (Control+Doxorubicin 20 mg/kg).

Data are presented as the Mean±SD for 8 Rats, Abbreviations: Malondialdehyde (MDA), Nitric oxide (NO), Superoxide dismutase (SOD) and Glutathione peroxidase (GPX), T+DOX10 (Training+Doxorubicin 10 mg/kg), T+DOX20 (Training+Doxorubicin 20 mg/kg).

In contrast, the 6 weeks of treadmill running aerobic training led to an insignificant decrease (17.45 %) in

Table 1. Effect of DOX Treatment Various Dosages (10 and 20 mg/kg) on Biomarkers Related to Oxidative Stress in Liver Tissue in the Various Groups

Markers and groups	Control+Saline	C+DOX10	C+DOX20
MDA(nmol/mg protein)	22.13±3.44	29.45±3.86	37.84±5.41
NO(nmol/mg protein)	0.20±0.02	0.24±0.03	0.27±0.03
SOD(u/mg protein)	104.67±7.55	96.93±6.36	78.66±6.82
GPx (μm.mg protein)	11.84±3.18	10.75±1.57	9.05±1.86

*Data are presented as the Mean±SD for 8 Rats; Malondialdehyde (MDA), Nitric oxide (NO), Superoxide dismutase (SOD) and Glutathione peroxidase (GPX), C+DOX10 (Control+Doxorubicin 10 mg/kg), C+DOX20 (Control+Doxorubicin 20 mg/kg)

Table 2. Effect of the Prior (pretreatment) Aerobic Exercise and DOX Treatment on Biomarkers Related to Oxidative Stress in Liver Tissue in the Various Groups

Markers and groups	Training+Saline	T+DOX10	T+DOX20
MDA(nmol/mg protein)	20.49±2.86	24.31±3.12	31.12±3.57
NO(nmol/mg protein)	0.24±0.02	0.29±0.03	0.32±0.03
SOD(u/mg protein)	120.18±5.33	117.67±2.65	110.09±4.48
GPx (μm.mg protein)	17.90±4.58	15.65±3.27	13.29±3.15

*Data are presented as the Mean±SD for 8 Rats; Malondialdehyde (MDA), Nitric oxide (NO), Superoxide dismutase (SOD) and Glutathione peroxidase (GPX), T+DOX10 (Training+Doxorubicin 10 mg/kg), T+DOX20 (Training+Doxorubicin 20 mg/kg).

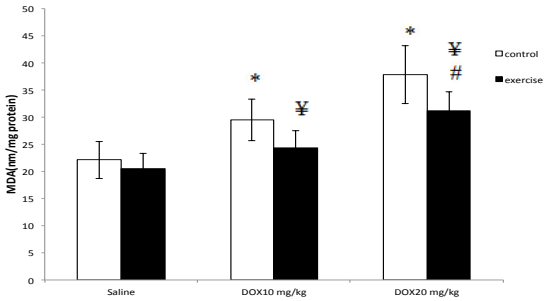

Figure 1. Malondialdehyde (MDA) Levels after Six Weeks of Pretreatment Aerobic Training and DOX Treatment. *Significantly different to control+saline group (P<0.05), #significantly different to similar control group (P<0.05), ¥ significantly different between DOX10 and DOX20 treatment groups (P<0.05)

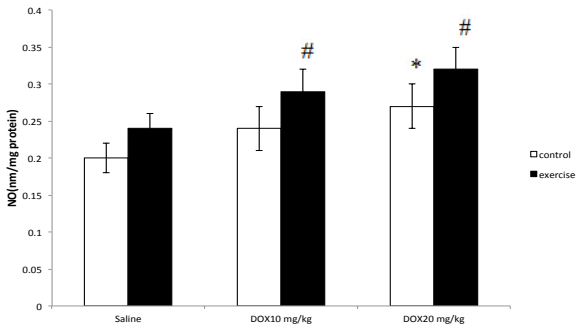

Figure 2. Nitric Oxide (NO) Levels after Six Weeks of Pretreatment Aerobic Training and DOX Treatment. *Significantly different to control+saline group (P<0.05), #significantly different to similar control group (P<0.05)

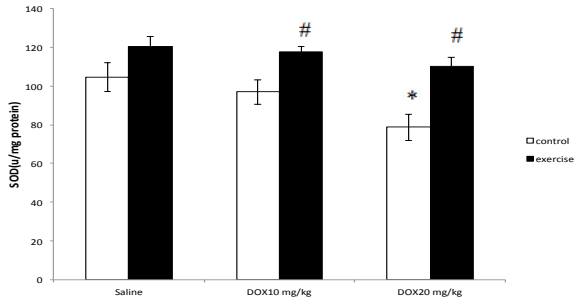

Figure 3. Superoxide Dismutase (SOD) Level after Six Weeks of Pretreatment Aerobic Training and DOX Treatment. *Significantly different to control+saline group (P<0.05), #significantly different to similar control group (P<0.05)

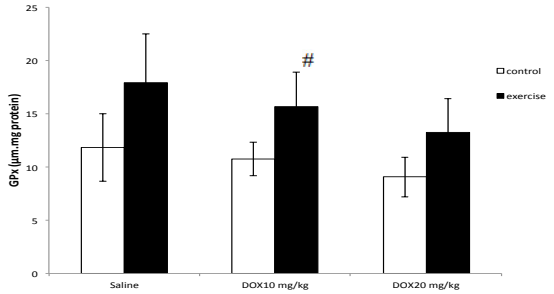

Figure 4. Glutathione Peroxidase (GPX) Levels after Six Weeks of Pretreatment Aerobic Training and DOX Treatment. #significantly different to similar control group (P<0.05)

MDA levels in T+DOX 10 mg.kg^{-1} group and a significant decrease (17.76%) in T+DOX 20 mg.kg^{-1} group as comparison to similar control group (Figure 1). However, the 6 weeks of treadmill running aerobic training led to a significant increase in NO (20.83% and 18.52%) and SOD (21.40% and 39.96%) levels in T+DOX 10 mg.kg^{-1} and T+DOX 20 mg.kg^{-1}, as comparison to similar control groups (Figure 2 and 3). Also, after of the prior (pretreatment) short-term aerobic exercise, GPx value has a significant increase (45.58%) in T+ DOX10 mg.kg^{-1} group, as comparison to the control+DOX 10 mg.kg^{-1} group (Figure 4). However, pretreatment of treadmill running exercise with DOX20 mg.kg^{-1} administration resulted in an insignificant increase (46.85%) in GPx value, as comparison to the control+DOX 20 mg.kg^{-1} group. While, after the 6 weeks training, there was no significant difference between the training+doxorubicin 10 mg/kg and training+doxorubicin 20 mg/kg groups in GPx, SOD and NO levels, a significant difference was detected in MDA levels between the aforesaid groups (Figure 4).

Discussion

The present study was designed to determine pretreatment effects of moderate-term aerobic training before the various dosages (10 and 20 mg/kg) of doxorubicin (DOX) on biomarkers related to oxidative stress in liver tissue. The primary novel finding in present study was that, administration of DOX 20 mg/kg induced hepatotoxicity in rats, which was detected by an increase in malondialdehyde (MDA) and nitric oxide (NO) levels, and a decrease in superoxide dismutase (SOD) and glutathione peroxidase (GPX) values in liver tissue. This result indicates that there is a potent relationship between oxidative stress and DOX-induced hepatotoxicity. The main findings of our study was that pretreatment of treadmill running training prevented the Dox-induced hepatotoxicity in the aforesaid biomarkers levels. In the present study, a significant decrease in the MDA and No levels and a significant increase in SOD and GPx values in rats treated with training+DOX treated groups, suggests the protective and pretreatment effect of aerobic exercise in DOX-induced oxidative stress in liver tissue. These findings are consistent with data from randomized controlled trials that reported increase in DOX-induced cardiotoxicity and hepatotoxicity (Injac et al., 2008; Ana et al., 2009; Rašković et al., 2011; Henninger et al., 2012; Mahmud et al., 2012). Furthermore, the current study provided additional support to understand how regular physical exercise, particularly treadmill running training, could contribute to improve of liver resistance against free radical-induced oxidative stress induced by DOX administration.

The liver is a vital organ that plays a major role in metabolism and has a number of important functions in the body including glucose storage in the form of glycogen, plasma protein synthesis and detoxification. During DOX therapy, the liver receives, accumulates and metabolizes high concentrations of DOX. Hence, it is expectable that the liver is one of the most affected organs by DOX therapy. Despite, this effect, the most important cause of DOX toxicity in the liver seems to be oxidative stress (Carvalho et al., 2009). Two different ways of free radical formation by DOX have been described. The first way implicates the formation of a semiquinone free radical by the action of several NADPH-dependent reductases that produce a one-electron reduction of the DOX to the corresponding DOX semiquinone. In the presence of oxygen, redox cycling of DOX -derived quinone–semiquinone yields superoxide radicals. In the second way, DOX free radicals come from a non-enzymatic mechanism that involves reactions with iron (Kalender et al., 2005). DOX not only increases free radical production in the tissue, but also decreases its ability to detoxify reactive oxygen species (Kalender et al., 2005). In the present study, decrease in SOD and GPx values and an increase in MDA and No levels in liver tissue indicate that DOX have hepatotoxic effects. We believe that the changes observed in the antioxidant defenses are a result of DOX induced hepatotoxicity. In contrast, in this study, we showed that the regular exercise training can limited DOX-induced oxidant/antioxidant imbalanced and aerobic regular exercise before administration of DOX may be considered as a potentially useful candidate to protect liver tissue against oxidative stress. These antioxidant enzymes are involved in the reduction of reactive oxygen species (ROS) and peroxides produced in the living organism thus play a vital role in the maintenance of a balanced redox status (Mahmud et al., 2012). The restoration of the SOD activity toward a normal value indicates that the pretreatment of aerobic training can help in cellular defense mechanisms by preventing cell

membrane oxidation. Therefore, in the present study, the hepatoprotection of the regular aerobic training against DOX- induced hepatotoxicity in rats could be attributed to its ability to restore the antioxidant enzymes SOD and GPx of liver cytosol or to the free radical scavenging activity of the pretratment. Liver plays an important role during exercise through glucose release to the bloodstream and gluconeogenesis, and mitochondria are clearly important in exercise performance due to aerobic energy production (Lima et al., 2013). While, current data demonstrate that exercise training protects the liver against Dox-induced oxidative stress, the mechanism(s) by which exercise training protects oxidative stress remain unclear. There were three possible pathways to explain the protective effects of regular endurance exercise against DOX-induced oxidative stress. At present, the principal mechanism of Dox-induced oxidative stress is believed to be increased oxidant production by the mitochondria. In addition, mitochondria are also the major sites for the production of ROS. (Ascensao et al., 2005; Kavazis et al., 2010). ROS can also activate signal-transduction pathways to induce a stress-resistance response that protects against some of the toxic outcomes of ROS generation (Powers et al., 2010). In contrast, exercise seems to increase the oxygen consumption rate by 10-20 fold as reported earlier and might have released the above factors and thereby induced oxidant activity (Husain, 2002). Indeed, exercise training has been reported to produce adaptive responses to oxidative stress, as studied primarily on skeletal muscles, but also in the liver (Lima et al., 2013).

The metabolic adaptations to exercise are not restricted to the working muscle; exercise is also a major challenge to other organs such as cardiac muscle, stomach or brain (Lima et al., 2013). This is particularly relevant to the liver due to its central role in the maintenance of energy supply to the exercising muscle (Hoene et al., 2010). Studies aiming to evaluate the effects of the acute exercise on oxidative stress in the liver have shown increased lipid peroxidation (Korivi et al., 2012), and decreased antioxidant defenses (Lima et al., 2013). Additionally, it is known that strenuous exercise causes a number of marked metabolic changes that may impair mitochondrial function in several ways, one major factor being mitochondrial ROS formation (Radak et al., 2004). Interestingly, mitochondrial dysfunction appears to be a key issue during exhaustive exercise, and may cause oxidative damage and tissue injury to liver, among others organ (Lima et al., 2013).

In conclusion, the results of the present study indicate that pretreatment of aerobic exercise induces positive adaptations in liver tissue of rats, characterized by increases in the antioxidant defense and decrease in the oxidants biomarkers. Therefore, it has a potential hepatoprotective effect against doxorubicin (DOX)-induced hepatotoxicity with dosages 10 and 20 mg.kg. However, further studies are required to examine other mechanisms, which are responsible for the hepatoprotective efficacy of this regular exercise protocols and protect against exercise-induced stress.

Acknowledgements

The authors thanks College of Physical Education and Sport Sciences, department of sport physiology, University of Mazandaran, Babolsar, Iran, for their cooperation.

References

Ascensao A, Magalhaes J, Soares J, et al (2005). Endurance training attenuates doxorubicin induced cardiac oxidative damage in mice. *Int J Cardiol*, **100**, 451-60.

Ashrafi J, Dabidi Roshan V (2012). Is Short-term Exercise a Therapeutic Tool for Improvement of Cardioprotection Against DOX-induced Cardiotoxicity? An Experimental Controlled Protocol in Rats. *Asian Pac J Cancer Prev*, **13**, 4025-30.

Ashrafi J, Dabidi Roshan V, Mahjoub S (2012). Cardioprotective effects of aerobic regular exercise against doxorubicin-induced oxidative stress in rat. *AJPP*, **6**, 2380-8.

Carvalho C, Santos RX, Cardoso S, et al (2009). Doxorubicin: The Good, the Bad and the Ugly Effect. *Current Medicinal Chemistry*, **16**, 3267-85.

Choi J, Park KH, Kim SZ, et al (2013). The ameliorative effects of L-2-oxothiazolidine-4-carboxylate on acetaminophen-induced hepatotoxicity in mice. *Molecules*, **18**, 3467-78.

Dabidi RV, Ranjbar S, Hosseinzadeh M, et al (2012). Left ventricular oxidant and antioxidant markers induced by lifestyle modification in rats exposed to lead acetate. *Eur J Sport Sci*, **12**, 485-490.

Henninger C, Huelsenbeck J, Huelsenbeck S, et al (2012). The lipid lowering drug lovastatin protects against doxorubicin-induced hepatotoxicity. *Toxicology and Applied Pharmacology*, **261**, 66-73.

Hoene M, Weigert C (2010). The stress response of the liver to physical exercise. *Exerc Immunol Rev*, **16**, 163-83.

Husain K (2002). Exercise conditioning attenuates the hypertensive effects of nitric oxide synthase inhibitor in rat. *Molecular and Cellular Biochemistry*, **231**, 129-37.

Injac R, Perse M, Obermajer N, et al (2008). Potential hepatoprotective effects of fullerenol C60(OH)24 indoxorubicin-induced hepatotoxicity in rats with mammary carcinomas. *Biomaterials*, **29**, 3451-60.

Kalender Y, Yel M, Kalender S (2005). Doxorubicin hepatotoxicity and hepatic free radical metabolism in rats. The effects of vitamin E and catechin. *Toxicology*, **209**, 39-45.

Kavazis A, Smuder A, Kisuk Min, et al (2010). Short-term exercise training protects against doxorubicin-induced cardiac mitochondrial damage independent of HSP72. *Am J Physiol Heart Circ Physiol*, **299**, 1515-24.

Korivi M, Hou CW, Huang CY, et al (2012). Ginsenoside-rg1 protects the liver against exhaustive exercise-induced oxidative stress in rats. *Evid Based Complement Alternat Med*, **2012**, 932165.

Lima FD, Stamm DN, Della-Pace ID, et al. (2013). Swimming training induces liver mitochondrial adaptations to oxidative stress in rats submitted to repeated exhaustive swimming bouts. *PLoS One*, **8**, 55668.

Ludke AR, Al-Shudiefat AA, Dhingra S, Jassal DS, Singal PK (2009). A concise description of cardioprotective strategies in doxorubicin-induced cardiotoxicity. *Can J Physiol Pharmacol*, **87**, 756-63.

Mahmud Z, Bachar S, Qais N (2012). Antioxidant and hepatoprotective activities of ethanolic extracts of leaves of premna esculenta roxb. against carbon tetrachloride-induced liver damage in rats. *J Young Pharm*, **4**, 228-34.

Mrzljak A, Kosuta I, Skrtic A, et al. (2013). Drug-induced liver injury associated with noni (Morinda citrifolia) juice and phenobarbital. *Case Rep Gastroenterol*, **7**, 19-24.

Ogawa K, Seta R, Shimizu T, et al (2011). Plasma adenosine triphosphate and heat shock protein 72 concentrations after aerobic and eccentric exercise. *Tokyo Metropolitan Institute of Gerontology EIR*, **17**, 136-49.

Ohkawa H, Ohishi N, Yagi K (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, **95**, 351-8.

Powers SK, Duarte J, Kavazis AN, Talbert EE (2010). Reactive oxygen species are signalling molecules for muscle adaptation. *Exp Physiol*, **95**, 1-9.

Radak Z, Chung HY, Naito H, et al (2004). Age-associated increase in oxidative stress and nuclear factor kappaB activation are attenuated in rat liver by regular exercise. *FASEB J*, **18**, 749-50.

Rašković A, Stilinović N, Kolarović J, et al (2011). The protective effects of silymarin against doxorubicin-induced cardiotoxicity and hepatotoxicity in rats. *Molecules*, **16**, 8601-13.

Shirinbayan V, Dabidi Roshan V (2012). Pretreatment effect of running exercise on HSP70 and DOX-induced cardiotoxicity. *Asian Pac J Cancer Prev*, **13**, 5849-55.

Siegel R MPH, Naishadham D MA, Jemal A (2012). Cancer statistics. *CA, A Cancer J Clinicians*, **62**, 10-29.

Viswanatha SA, Gulliaya S, Thippeswamy A, et al (2012). Cardioprotective effect of curcumin against doxorubicin-induced myocardial toxicity in albino rats. *Indian J Pharmacol*, **44**, 73-7.

Wonders KY, Hydock DS, Schneider CM, et al (2008). Acute exercise protects against doxorubicin cardiotoxicity. *Integr Cancer Ther*, **7**, 147-54.