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Research Article

Antiapoptotic and antioxidant effects of resistance training with berberine consumption on diazinon induced cardiotoxicity in rats

Reza Niazi, Maghsoud Peeri*, Mohammad Ali Azarbayjani

Department of Exercise Physiology, Central Tehran Branch, Islamic Azad University, Tehran, Iran

ARTICLE INFO	ABSTRACT
Keywords:	Background: Increasing use of pesticides is one of the health hazards. Physical
Antioxidant	activity and medicinal plants appear to be a strategy to mitigate the adverse effects
Apoptosis	of these substances. Objective: The purpose of this study was to investigate the
Berberine	effect of resistance training and berberine chloride on apoptosis and oxidative
Diazinon	stress markers in the heart tissue of diazinon-infected rats. Methods: In this study,
Resistance training	80 rats were randomly assigned into 8 groups of 10 animals in each group,
	including: 1) healthy control, 2) sham, 3) diazinon, 4) diazinon + resistance
	training, 5) diazinon + berberine (2.5 mg/kg) 6) diazinon + berberine (15 mg/kg),
	7) diazinon + resistance training and berberine (2.5 mg/kg), and 8) diazinon +
	resistance training and berberine (15 mg/kg). During 4 weeks, each groups
	received a certain amount of diazinon poison, berberine chloride and performed
	three sessions per week of resistance training. Results: In the heart tissue of
	diazinon-poisoned rats, resistance training had a significant (P \leq 0.05) effect on
	the increased concentrations of MDA, caspase-3, 8-OHDG and GSH; 15 mg/kg of
	berberine consumption had a significant effect on decreased concentrations of
	ROS, MDA, 8-OHDG, caspase-3 and increased ($P \le 0.05$) concentrations of GSH;
	2.5 mg/kg of berberine consumption had a significant (P \leq 0.05) effect on reduced
	8-OHDG. Also, 15 mg/kg of berberine consumption compared to 2.5 mg/kg of
	berberine consumption had a greater effect on reduced 8-OHDG. Conclusion: It
	seems that berberine consumption along with resistance training has interactive
	protective effects against oxidative stress and cell death in the heart tissue of
	diazinon-poisoned rats.

1. Introduction

Different pesticides are used to cope with agricultural pests to increase crop production. Organophosphate pesticides are used as insecticides in the industry and agricultural farms. [1, 2]

Diazinon is the most important member among organophosphorus family that is

Abbreviations: (ROS) reactive oxygen species; (MDA) malondialdehyde

^{*} Corresponding author: <u>m.peeri@iauctb.ac.ir</u>

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absorbed through skin, digestive system and respiratory system, and is rapidly metabolized to diazooxone in the liver. The half-life of diazinon may last for more than a month in aerosol mineral soils, but is rapidly degraded in aquatic environments [6].

In the normal physiological state, some free produced radicals are via activity and metabolism of cells, which are neutralized by antioxidants in the body such as superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH). There is a balance between the two processes in the body. Therefore, any factor that disrupts this redox balance through high release of free radicals or reduction of antioxidants may result in oxidative stress, which consequently leads to pathological changes in the cell surface [3, 4].

Studies have shown that some organophosphates produce oxidative stress and peroxidation of lipids in mammals by producing free radicals [1, 5]. On the other hand, the heart continuously performs blood supply to all tissues and organs. The heart muscle is unable to store oxygen for use, however, continuous oxygen and other nutrients must be provided for proper heart function. Moreover, the amount of antioxidant enzymes in heart tissues is less compared to other tissues. Therefore, the heart tissues are more susceptible to oxidative stress [13].

Increased sensitivity to oxidative stress in insulin resistance tends to reduce the expression of antioxidant enzymes in the tissues. In addition, the heart tissues particularly vulnerable to oxidative damage, have a larger oxidative capacity and relatively weak enzymatic capacity compared to other organs [6, 7].

It seems that the heart tissue is one of the organs affected by poisons with the mechanism of increasing cardiac apoptosis; hence, antioxidant foods consumption can delay the

damage caused by the use of pesticides. In this regard, berberine is a plant alkaloid that has been used as a drug in the ancient Chinese, Indian, and Middle Eastern medicine due to its antimicrobial and anti-radiation properties. Berberine is an isoquinoline alkaloid which has anxiolytic, analgesic, anti-inflammatory, antimania, anti-depressant effects. Additionally, it has protective effects on memory [8].

Furthermore, Berberine has also been reported to have beneficial effects on functioning of the nervous system, and it can protect the neurons from various brain damage [10]. On the other hand, among different therapeutic ways to effectively cope with the side effects of cardiovascular injuries, physical activity is recommended as a multifactorial nonpharmacological remedy with no adverse effects.

It has now been widely recognized that an intense physical activity induces major homeostatic changes in the body's internal environment, which means it offers challenges to cells to survive under stress conditions [16]. On the other hand, increased mechanical and metabolic requirements through exercise in several organs and tissues, especially skeletal muscles, may and cardiac disrupt their thereby, increase the cellular homeostasis. damage death. inflammation, or and immunological changes in the blood [17, 18].

Now days, the potential value of resistance training in the field of development of health and fitness has been perceived [11]. Through recognizing the intensity and duration of exercise serve as an agent, that can modify apoptosis because increased apoptosis may cause excessive cell loss, affect the function of the tissues, and disrupt the process of tissue recovery.

The lack of research on the interactive effects of berberine consumption along with resistance training on the improvement of apoptosis and

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oxidative stress in the diazinon-poisoned conditions, this study aimed to investigate the effect of resistance training and berberine chloride on apoptosis and oxidative stress markers in the heart tissue of diazinon-infected rats.

2. Materials and Methods

2.1. Animal

In this experimental study, 80 male wistar rats weighing 250 ± 50 gr and 10-12 weeks' old were purchased from the Iran Pasteur Institute Center and transferred to the Animal Center of Iran University of Medical Sciences. To adapt to the environment, rats were kept under standard conditions of temperature 1 ($22 \pm 2 \circ C$) and 12 hours of light / dark alternate cycles for one week. Then, animals were divided into 8 groups (10 in each group) including Healthy Sham. Diazinon, Diazinon Control. +Resistance Training (Diazinon + RT), Diazinon + Berberine (2.5 mg/kg) (Diazinon + 2.5 B) Diazinon + Berberine (15 mg/kg) (Diazinon + 15 B), Diazinon + Resistance Training and Berberine (2.5 mg/ kg) (Diazinon + RT + 2.5 B), and Diazinon + Resistance Training and Berberine (15 mg/kg) (Diazinon + RT + 15 B).

2.2. Diazinon injection

The diazinon (manufactured by American Sigma Company code 454258-250MG) was diluted with 9% normal saline solution and was intraperitoneally injected at dose rat of 1.5 mg/kg in all groups; and berberine chloride (manufactured by American Sigma Company code B3251) was diluted with 9% normal saline solution and was peritoneally injected at given dose rat in all groups.

2.3. Resistance training protocol

Exercise groups rats performed resistance exercise training three times per week, while

rats in control groups has no exercise activity. Resistance training included 3 sessions per week of climbing a vertical ladder (up to 1 meter on the 26th staircase) with a gradient of 80 degrees along with weightlifting. Each training session consisted of 2 sets with 6 repetitions, each requiring 8-12 active moves. The rest between each repetition was 60 seconds and between each set was 2-3 minutes. The training load started at 10% of the total body weight and reached 50% of body weight by end of protocol. The body weight of the rats was carefully measured at the start and end of each training week with a digital scale.

2.4. Tissue collection

All rats were anesthetized 48 hours after the last training session with intraperitoneal injection of ketamine (100 mg/kg) and xylazin (10 mg/kg). After complete anesthesia, perfusion was performed to remove blood from tissues. Heart tissues was removed, washed again, and kept in formalin until laboratory analysis.

2.5. Tissue variable measurements

Heart reactive oxygen species (ROS) (serial number CSB-EL020063RA), malondialdehyde (MDA) (serial number CSB-E08558r), GSH (serial number CSB-E12146r), caspase-3 (serial number CSB-E08857r) and 8-Hydroxy-Desoxyguanosine (serial number CSB-E10526r) was measured by the ELISA method using the American CUSABIO kit.

To investigate the histology of the heart, the heart tissues were isolated and washed with physiological serum and placed in the dishes with 10% formalin. The fixed samples were then embedded in paraffin wax. Serial sections were cut (5-µm thickness), stained with haematoxylin and eosin, and examined by using a light microscope (Olympus BX51; Olympus

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(Histogenotech Co., Iran), according to the manufacturer's instructions, to evaluate ventricular collagen fibers. Briefly, the section stain in Biebrich scarlet-acid fuchsin solution for 10-15 minutes. After washing the sample in distilled water, differentiate in phosphomolybdic-phosphotungstic acid solution for 10-15 minutes was done. The sections directly were transferred to aniline blue solution and stain for 5-10 minutes and then Rinsed in distilled water and differentiated in 1% acetic acid solution for 2-5 minutes. Dehydration very quickly was done through 95% ethyl alcohol, absolute ethyl alcohol, cleared in xylene and Mounted with resinous mounting medium. The collagen fibers will be stained blue and the nuclei will be stained black and the background is stained red. Image Tools Software (ver.3, Microsoft, Texas, USA) was used to measure the collagen deposition in five sections and four fields per section (an overall of 20 fields in each animal sample) were analyzed at microscopy.

trichrome

stain

kit

magnification $\times 10$ of light Photomicrographs were prepared.

2.6. Data analyses

Data were expressed as mean ± SD. The Shapiro-wilk test was used to determine the normality of data. The one-way and two-way ANOVA along with Bonferroni's post hoc test were used to determine the changes between groups. The changes consider as significant changes when P was ≤ 0.05 . All statistical analyses performed using SPSS software version 24.

3. Results

The results of one-way ANOVA showed a significant difference in the levels of ROS (P = 0.001), MDA (P = 0.001), GSH(P = 0.001), caspase-3 (P = 0.001), and 8-Hydroxy-Desoxyguanosine (P = 0.001) in the heart tissue of rats in the healthy control, sham and diazinon groups.

The results of Bonferroni's post hoc test showed that ROS levels in the diazinon group were significantly higher than the healthy control and sham groups (P = 0.001) (Fig. 1); also, there was no significant difference between the healthy control group and sham group (P = 0.07) (Fig. 1); MDA levels in the diazinon group were significantly higher than the healthy control and sham groups (P = 0.001) (Fig. 1). Also, there was no significant difference between the healthy control and sham groups (P =0.99) (Fig. 1); 8-OHDG levels in the diazinon group were significantly higher than the healthy control and sham groups (P = 0.001) (Figure 1). There was no significant difference between the healthy control group and sham group (P = 0.60)(Fig. 1); levels of caspase-3 in the diazinon group were significantly higher than the healthy control and sham groups (P = 0.001) (Fig. 1). Meanwhile, there was no significant difference between the healthy control group and sham group (P = 0.99) and GSH levels in the diazinon group were significantly lower than the healthy control and sham groups (P = 0.001) (Fig. 1). Also, there was no significant difference between the healthy control group and sham group (P = 0.06) (Fig. 1).

The results of two-way ANOVA showed that resistance training had significant effect on increased MDA (P = 0.001) and caspase-3 (P =(0.001) and decreased 8-OHDG (P = (0.001)), and GSH (P = 0.001) levels in the heart tissue of diazine-poisoned rats (Fig. 1); yet it did not have a significant effect on ROS (P = 0.40). Berberine consumption had a significant effect on decreased ROS (P = 0.001), MDA (P =0.001), 8-OHDG (P = 0.001), caspase-3 (P = (0.001) and increased GSH (P = (0.001)) in the heart tissue of diazine-poisoned rats (Fig. 1).



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Fig. 1. Heart ROS (A), MDA (B), 8-OHDG (C), caspase-3 (D), GSH (E) concentration in different groups of study. The rats are 10 in each group. Data was expressed as mean ± SD. The animal groups are Healthy Control, Sham, Diazinon, Diazinon + Resistance Training (Diazinon + RT), Diazinon + Berberine (2.5 mg / kg) (Diazinon + 2.5 B) Diazinon + Berberine (15 mg / kg) (Diazinon + 15 B), Diazinon + Resistance Training and Berberine (2.5 mg / kg) (Diazinon + RT + 2.5 B), and Diazinon + Resistance Training and Berberine (15 mg / kg) (Diazinon + RT + 15 B).

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Fig. 2. Histological profile from heart tissue of rats. G1: diazinon + resistance training and 2.5 mg / kg of berberine group (damage up to about 75%); G2) diazinon + resistance training and 15 mg / kg of berberine group (damage up to about 50%); G3: diazinon group (damage greater than 75%); G4: diazinon + resistance training group (damage up to about 75%); G5: diazinon + 2.5 mg / kg of berberine (damage up to about 75%; G6: diazinon + 15 mg / kg of berberine (damage up to about 75%); G7: healthy control group (no damage to heart) and G8: sham group (no damage to heart).



Fig. 3. Histopathological analysis of left ventricular (LV) tissue sections stained with Masson trichrome. Interstitial fibrosis with Masson trichrome stain were measured after 8 week from training in G1: diazinon + resistance training and 2.5 mg / kg of berberine group (damage up to about 30%); G2) diazinon + resistance training and 15 mg / kg of berberine group (damage up to about 20%); G3: diazinon group (damage greater than 45%); G4: diazinon + resistance training group (damage up to about 45%); G5: diazinon + 2.5 mg / kg of berberine (damage up to about 55%; G6: diazinon + 15 mg / kg of berberine (damage up to about 35%); G7: healthy control group (no damage to heart) and G8: sham group (no damage to heart). blue = fibrous collagen, and red = cardiomyocytes 200X magnification.

In addition, concurrent consumption of berberine along with resistance training had interactive effects on decreased ROS (P = 0.001), MDA (P = 0.001), caspase-3 (P = 0.001) and increased GSH (P = 0.04) in the heart tissue of rats poisoned with diazinon (Fig. 1).

The results of Bonferroni's post hoc test showed that in heart tissue of rats poisoned with

diazinon, 15 mg / kg of berberine consumption had a significant effect on decreased ROS (P = 0.001), MDA (P = 0.001), 8-OHDG (P = 0.001), caspase-3 (P = 0.001) and increased GSH (P = 0.001); 2.5 mg / kg of berberine consumption had a significant effect on decreased 8-OHDG (P = 0.008) (Fig. 1). However, there was no significant effect on ROS (P = 0.99), MDA (P =

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0.99), caspase-3 (P = 0.07), and GSH (P = 0.59) (Fig. 1). Also, 15 mg / kg of berberine consumption compared to 2.5 mg / kg of berberine consumption had a greater effect on reduction of 8-OHDG (P = 0.001) (Fig. 1).

The results of microscopic examination of tissue samples showed that cardio myocytes were not damaged in the healthy control and sham groups. However, in rats received diazinon, cardio myocytes were subjected to cell death. Cell death observed in the form of cellular partially vacuolated cytoplasm, fragmented cell nucleus, undisclosed nucleic membrane, lost cell membrane, and destroyed intercellular bindings.

The results showed that cardiomyocytes had the least damage in the diazinon + resistance training and 15 mg / kg berberine consumption (damage up to about 50%) (Fig. 1b) and the highest cardiomyocyte damage was observed in the diazinon treated group (damage greater than 75%) (Fig. 2c). Cardiomyocytes were also damaged in the diazinon + resistance training groups and 2.5 mg / kg berberine consumption, diazinon + resistance training, diazinon + 2.5 mg / kg of berberine consumption and diazinon + 15 mg / kg of berberine up to about 75% (Fig. 2a, d, e, f, g, h).

Myocardial fibrotic remodeling, as reflected by trichrome staining of myocardial sections, was significantly more pronounced in the G3, when compared to G7 and G8 groups (P<0.05). This increase in G4 and G5 groups was lower than G3 group (Fig. 2). Semi-quantitative scoring of the staining showed a significant difference in LV fibrosis between the experimental groups (Fig. 3).

4. Discussion

In the present study, although resistance training did not have a significant effect on ROS, it has a significant effect on increase in MDA, as well as reduction in 8-OHDG and GSH in the heart tissue of diazinon poisoned rats. Reported studies have suggested that the effects of regular exercise are beneficial. However, acute and intense sport activity through the activation of several pathways leads to the production of free radicals and ROS [39]. Increased ROS in the heart impairs endothelial function, decreases vascular dilatation and increases vascular contraction and, as a result, increases hypertension. [12] In this regard, increased production of free radicals, especially MDA, as one of the indicators of lipid peroxidation in the red blood cell membrane, following intense endurance exercise as reported previously [13, 14]. In fact, MDA changes depend on the severity and duration of exercise, the amount of which after short and severe exercises increased [15], and conversely, after 9 months of regular exercise decreased [32].

Consistent with the findings of this research, 14 weeks of resistance training led to a 8-OHDG significant reduction of [43]. therefore, participating in resistance training, increased strength and muscle hypertrophy, reduced oxidative stress and increased antioxidant enzymes activity [43]; thus, prolonged resistance training does not only seem to result in the deletion of the mitochondrial genome, but also reduces the amount of damage to the DNA of cell. Also, contrary to the findings of the present study, 6 weeks of resistance training can increase the levels of GSH irrespective of the severity of resistance training [51].

Given that resistance training has a recovery interval between movements and exercises between each course, the long recovery period of 30-90 seconds in low-intensity exercises with high volumes and a long-term recovery time of 2-5 minutes in the high and low intensity

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resistance training can be effective in generating responses and ultimately possible adaptations.

It seems that part of the mechanism of exercise activity in reducing oxidative stress relates to other protective mechanisms of exercise, including increased neurotrophic factors, including the brain-derived neutrophic factor (BDNF) and the relative improvement of cerebral blood flow during ischemia.

In the present study, resistance training had a significant effect on increased levels of caspase-3 in the heart tissue of diazinon-poisoned rats. In line with the findings of the present study, it was reported that sport activity, especially severe and eccentric, led to an increase in the cell death [16-18].

The type of exercise activity can be an important factor in the changes in apoptotic indices; in this vein, Bax gene expression and Bax to Bcl2 ratio, which is one of the caspase-3 production factors, was reported to reduce in the rats running for 12 weeks on the treadmill compared to the control group [19].

One of the effects of sports activities is the increase of mitochondrial biogenesis, which can play an important role in the pathway of causing agents and thus preventing apoptosis [20]. Also, the results of this study showed that 15 mg / kg of berberine consumption had a significant effect on decreased ROS, MDA, 8-OHDG and caspase-3, and increased GSH; and 2.5 mg / kg of berberine consumption had a significant effect on reduction of 8-OHDG. Besides, 15 mg/kg of berberine consumption compared to 2.5 mg/kg of berberine consumption had a greater effect on the reduction of 8-OHDG. Furthermore, 150 mg / kg of berberine consumption has a significant effect on reducing MDA and also decreases pathologic changes in diabetic rats [21]; leads to improvement of disturbances cardiac [38], and improves apoptosis process [47].

It has also been shown that berberine consumption increases GSH, which can cope with excess free radicals and overcome oxidative stress [52, 53].

According to the findings of this study, it seems that the anti-oxidative and apoptotic effects of berberine consumption are dose dependent, so that in present study, the use of 15 mg/kg of berberine had a greater effect than 2.5 mg/kg of berberine. It has been argued that berberine can act as an indirect antioxidant and direct detoxifying agent in the cell. Also, berberine directly neutralizes a variety of oxidative species and protects cells from oxidative damage by stimulating the synthesis of SOD and promoting the activity of various antioxidant enzymes, including CAT and GSH-Px [22].

Moreover, Berberine can easily cross the brain-blood barrier and after transferring to nerve cells, is slowly eliminated, which shows a direct effect of it on nerve cells in different areas of the brain, including the cortex. Although the precise neuro-protective mechanism of berberine is not well defined, [23]. In this regard, the results of the examination of tissue samples showed that 2.5 mg/kg and 15 mg/kg consumption of berberine reduced the pathological damage in the heart tissue of diazinon poisoned rats.

Concerning the interactive effects in the present study, concurrent consumption of berberine along with resistance training had interactive effects in reducing ROS, MDA, caspase-3 and GSH. The production of reactive oxygen species (ROSs) is increased during high intensity exercise through involvement of active muscle cell mitochondria [24]. Nonetheless, in the present study, resistance training alone did not have a significant effect on reduction of ROS, berberine consumption along with resistance training had a decreasing effect on ROS, which can be

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attributed to the beneficial effects of berberine and may be associated with affecting the pathways for the production of free radicals in special physiological conditions in specific tissues. Consumption of berberine helps to repair DNA damage to proteins and lipids by the production of antioxidants.

In sum, protein damage by reactive oxygen species in natural environments or exposure to toxins affects the function of receptors, enzymes and transfection proteins, and is also involved in secondary degradation of other biomolecules by deactivating defensive antioxidants enzymes and regenerative enzymes [27, 28].

Examination of tissue samples showed that cardio myocytes had the least damage with 15 mg/kg barbrin consumption with resistance training group (damage up to 50%). In the group receiving 2.5 mg/kg berberine with resistance training, the damage to cardio myocytes was about 75 percent. Therefore, it seems that the interactive effect of berberine consumption and resistance training isdose dependent, so that consumption of berberine with a higher dose has more protective effects on the heart tissue of diazinon poisoned rats than the lower dose.

5. Conclusion

In this study, although diazinon poisoning led to an increase in oxidative stress and apoptosis markers and cardio myocyte damage, berberine consumption along with resistance training had interactive effects against oxidative stress and cell death in the heart tissue of rats poisoned

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with diazinon; therefore, these findings strongly suggest that berberine consumption along with resistance training has protective effect under oxidative pressure conditions by increasing the antioxidant enzyme activity and by decreasing the peroxidation of lipids.

Therefore, berberine consumption along with resistance training can be implemented as an effective agent for controlling oxidative stress and protecting DNA cell.

All authors equally contributed to the writing and revision of this paper.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical Considerations

Researchers received introduction letters from Sport Sciences Research Institute of Iran with ethics code IR.SSRI.REC.1397.38

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مقاله تحقيقاتي

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چکیدہ	اطلاعات مقاله
مقدمه : افزایش استفاده از آفتکشها یکی از خطرات تهدیدکننده سلامتی میباشد. فعالیتهای بدنی و	گلواژگان:
گیاهان دارویی به عنوان یک راهکار سودمند برای کاهش اثرات این مواد توصیه شدهاند. هدف : هدف از	آنتىاكسي <i>د</i> ان
اجرای این مطالعه، بررسی اثر تمرین مقاومتی و بربرین کلراید بر نشانگران آپوپتوز و فشار اکسیداتیو بافت	آپوپتوز
قلب رتهای مسموم شده با دیازینون بود. روش بررسی : در این مطالعه ۸۰ سر رت نر به صورت تصادفی	ې ې ې ې.
به هشت گروه ده تایی شامل کنترل سالم، شم، دیازینون، دیازینون تمرین مقاومتی، دیازینون ۲/۵ میلیگرم	
بربرین به ازای هر کیلوگرم از وزن بدن، دیازینون ۱۵ میلیگرم بربرین به ازای هرکیلوگرم وزن بدن،	دياريىون
دیازینون تمرین مقاومتی ۲/۵ میلیگرم بربرین به ازای هر کیلوگرم از وزن بدن و دیازینون تمرین مقاومتی	تمرين مقاومتي
۱۵ میلیگرم بربرین به ازای هر کیلوگرم وزن بدن تقسیم شدند. طی چهار هفته هر گروه دیازینون و بربرین	
کلرایدرا دریافت نموده و هفتهای سه جلسه تمرین مقاومتی انجام دادند. نتایج : در بافت قلب موشهای	
صحرایی مسموم با دیازینون، تمرین مقاومتی اثر معنیداری در کاهش غلظت افزایش یافته MDA، کاسپاز	
۳، ۸-OHDG و GSH اثر معنیداری (P ≤ ۰/۰۵) داشت. ۱۵ میلیگرم بر کیلوگرم مصرف بربرین موجب	
کاهش معنیدار غلظت ROS، MDA، GHDG، ۳ و کاسپاز ۳ شد، اما غلظت GSH را افزایش داد	
OHDG-۸ میلیگرم در کیلوگرم مصرف بربرین تأثیر معنیداری (P ≤ ۰/۰۵) در کاهش OHDG-۸	
داشت. همچنین، ۱۵ میلیگرم بربرین کلراید به ازای هر کیلوگرم وزن بدن در مقایسه با ۲/۵ میلیگرم به	
ازای هر کیلوگرم وزن بدن اثر بیشتری در کاهش ۸-OHDG داشت. نتیجهگیری : به نظر میرسد مصرف	
بربرین همراه با تمرین مقاومتی دارای اثرات محافظتی در برابر استرس اکسیداتیو و مرگ سلولی در بافت	
قلب موش های مسموم با دیازینون دارد.	

مخففها: (ROS) reactive oxygen species; (MDA) malondialdehyde)

* نويسنده مسؤول: <u>m.peeri@iauctb.ac.ir</u>

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