Research Article

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

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ABSTRACT

Background: Increasing use of pesticides is one of the health hazards. Physical activity and medicinal plants appear to be a strategy to mitigate the adverse effects of these substances. Objective: The purpose of this study was to investigate the effect of resistance training and berberine chloride on apoptosis and oxidative stress markers in the heart tissue of diazinon-infected rats. Methods: In this study, 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 ≤ 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 ≤ 0.05) concentrations of GSH; 2.5 mg/kg of berberine consumption had a significant (P ≤ 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...
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 radicals are produced 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 aliphatic 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, antianxia, 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 non-pharmacological 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 and cardiac muscles, may disrupt their homeostasis, thereby, increase the cellular damage or death, inflammation, 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
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 (22 ± 2 °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 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).

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...
Co.). Masson’s trichrome stain kit (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 magnification ×10 of light microscopy. 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 = 0.99) (Fig. 1). Meanwhile, 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). 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).
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).
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 =
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 significant reduction of 8-OHDG [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
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 neurotrophic 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-OHDDG and caspase-3, and increased GSH; and 2.5 mg / kg of berberine consumption had a significant effect on reduction of 8-OHDDG. 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-OHDDG. 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 cardiac disturbances [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
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 is dose 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 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.

Author contributions
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 تحقیقی

اثرات ضد آپوپتوزی و آنتی اکسیدانی تمرین مقاومتی با بربرین کلراید بر سمت سلول های قلبی ناشی از دیازینون در موش صحرایی

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چکیده

مقدمه: تمرین مقاومتی، درمان ناشی از خطرات تهدیدکننده سلامتی و تحقیقات اخیر اثبات کرده است که افزایش مقاومت پیری باعث کاهش افزایش نرخ آپوپتوز شده است. هدف از اجرای این مطالعه، بررسی اثرات تمرین مقاومتی و بربرین کلراید بر نشانگران آپوپتوزی در بافت قلب موش در حیطه مسمومیت با دیازینون می‌باشد.

ماده و روش: در این مطالعه، 60 رنگ موش به صورت تصادفی به ارزش گروه داشته و 4 گروه دریافتی به صورت تصادفی در این روش داشتند. گروه کنترل سالم، گروه عادی، گروه دیازینون و گروه دیازینون به همراه بربرین کلراید را تشییع کردند. دیازینون با حفظ وزن بدن، بربرین با حفظ وزن بدن و دیازینون به همراه بربرین با حفظ وزن بدن مصرف گردید. دیازینون (25 میلی گرم/کیلو گرم وزن بدن در هر کیلو گرم وزن بدن) در 3 هفته هر گروه داده شد. همچنین، 15 میلی گرم بربرین به همراه دیازینون در هر کیلو گرم وزن بدن به مصرف گردید.

میتالپک و آنتی اکسیدانی تمرین مقاومتی: تمرین مقاومتی می‌تواند به کاهش افزایش نرخ آپوپتوز و در نتیجه افزایش حیاتیت و حفظ قابلیت عملیاتی بدنی کمک کند. در این مطالعه، ارتباط بین اثرات آپوپتوز و آنتی اکسیدانی تمرین مقاومتی با بربرین کلراید بر نشانگران آپوپتوز بود.

نتایج: اثرات تمرین مقاومتی و بربرین کلراید بر نشانگران آپوپتوز و فشار اکسیداتیو بافت موش در مقایسه با گروه کنترل سالم و گروه دیازینون، کاهش معنی‌داری در میزان میتالپک و آنتی اکسیدانی تمرین مقاومتی داشت.

اثرات مقاومت: گروه تمرین مقاومتی با بربرین کلراید، بهبودی در کاهش آپوپتوز و حفظ سلامت بافت قلب موش داشت.

مصرف بربرین/کیلو گرم: به نظر می‌رسد که مصرف بربرین/کیلو گرم در هر کیلو گرم قلب موش به بهبودی در کاهش آپوپتوز و حفظ سلامت بافت قلب موش بینجامد.