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Research Paper

Effect of High-intensity Interval Training Combined With Silymarin on Oxidative Stress Levels in Heart and Lung Endothelial Tissues of Rats Exposed to Diazinon

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ABSTRACT

Background and Aim: Diazinon is an organophosphorus insecticide that causes oxidative stress. Physical activity and consumption of antioxidants improve the antioxidant status of the body. The present study aimed to investigate the effect of high-intensity interval training combined with silymarin extract on malondialdehyde (MDA) and superoxide dismutase (SOD) indices of heart and lung endothelial tissues in male rats exposed to diazinon.

Materials and Methods: In this experimental study, 36 male Sprague-Dawley rats with a weight range of 150 to 200 g and an average age of 8 weeks were randomly assigned to the following groups: Control (C), sham (Sh), diazinon (D), silymarin extract (SL), high-intensity interval training (TH), and high-intensity interval training with silymarin extract (TH+SL). For eight weeks, rats in groups 3 to 6 received 1.5 mg/kg diazinon intraperitoneally five days a week. Groups 5 and 6 performed high-intensity interval training (HIIT) exercises five times a week, and groups 4 and 6 received 50 mg/kg silymarin extract intraperitoneally five days a week. HIIT was conducted at an intensity of 85% to 110% of VO_2 max, with speeds ranging from 15 to 45 m/min over the 8-week period. At the end of the study, MDA levels and *SOD* gene expression were measured in the heart and lung endothelial tissues. Data analysis was performed using the Shapiro-Wilk test and one-way analysis of variance with Tukey's post hoc test in SPSS software version 22, with a significance level set at $P \leq 0.05$.

Results: Exposure to diazinon significantly increased MDA and decreased SOD in the heart and lung endothelial tissues ($P<0.05$). However, HIIT and consumption of silymarin alone led to a reduction in MDA levels and an increase in SOD levels in heart and lung endothelial tissues of diazinon-exposed rats ($P<0.05$). Meanwhile, the combined intervention of HIIT with silymarin had an even greater effect on reducing MDA and enhancing SOD in the heart and lung endothelial tissues of rats exposed to diazinon ($P<0.05$).

Conclusion: Regular HIIT and silymarin supplementation, both alone and in combination, can decrease oxidative indices and increase antioxidant defenses in heart and lung endothelial tissues affected by diazinon exposure.

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Introduction

Organophosphate compounds are widely used in agriculture, industry, horticulture, veterinary medicine, and households [1]. Most organophosphorus compounds are rapidly or almost completely absorbed through the skin, mouth, respiratory mucus, and digestion, with widespread distribution throughout the body. Due to their easy access and high toxicity, these compounds account for a significant rate of accidental poisonings and suicides, resulting in approximately 100,000 poisoning cases worldwide annually [2]. In Iran, these compounds are among the leading causes of poisoning-related fatalities [1, 2].

Diazinon is an organophosphorus insecticide that enters the body through the skin, respiratory tract, or digestive system and is quickly converted into an active metabolite in the liver and kidney [3]. The severity of the destructive effect caused by diazinon depends on the dose, duration of contact, absorption method, cell structure, and stability in the body [4]. Studies have reported oxidative damage, reduced activity of antioxidant enzymes, and apoptosis induction as consequences of diazinon poisoning [3, 5]. Besides inhibiting acetylcholinesterase, organophosphates generate free radicals [6]. The oxidation of organophosphates, especially diazinon, leads to the production of reactive oxygen species (ROS) and lipid peroxidation, facilitating cellular damage [7].

Free radicals, formed during lipid, nucleic acid, and protein oxidation, are implicated in various chronic diseases, including cardiovascular conditions and certain cancers. Oxidative stress is the result of an imbalance between the production of free radicals and ROS, resulting in damage to many macromolecules. It occurs when ROS levels exceed the body's antioxidant capacity, causing harm to cellular components, like DNA, proteins, and lipids, ultimately leading to pathophysiological disorders [8]. The body's antioxidant system includes enzymatic and non-enzymatic antioxidants that can be affected by exercise and nutrition. Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), while non-enzymatic antioxidants include vitamins A, C, and E, glutathione, etc. which reduce the production and formation of free radicals, thus preventing their destructive effects [9]. Regular exercise is recommended to maintain health and reduce the risk of various diseases; however, prolonged exercise can also stimulate ROS production and activate the body's antioxidant defense system [10].

Although evidence suggests that intense exercise can produce free radicals and cause cell damage, it is generally believed that regular, moderate physical activity improves the body's antioxidant status and reduces free radical production [10]. Many studies have been conducted on the effect of aerobic exercises on oxidative stress indicators, with many indicating that endurance sports activity leads to a reduction in oxidative stress [11]. This is while recent evidence has shown that high-intense interval training (HIIT) leads to more metabolic and physiological adaptations related to changes in oxidative stress compared to regular aerobic training [12]. HIIT is an ideal activity to achieve greater improvement in physiological variables because it allows a person to perform high-intensity activity for a longer period. The main difference between this training method and continuous training is that in continuous training, submaximal activity is performed continuously for a long period, while HIIT consists of repeated short-to-medium-duration efforts at intensities above the aerobic threshold [13].

In this regard, the results of studies have shown that HIIT reduces oxidative stress indicators and, conversely, increases antioxidant indicators [14, 15]. However, findings vary; for instance, Songstad et al. reported in a study on the effects of HIIT on malondialdehyde (MDA) levels in the liver tissue of male rats that six weeks of HIIT did not significantly affect tissue MDA concentration [16]. In addition to sports activities, antioxidant foods can have anti-oxidative stress effects. In this context, silymarin extract is the active compound derived from *Silybum marianum*, consisting of a group of chemicals known as flavonolignans [17]. Silymarin has significant antioxidant properties; it reduces free radicals, inhibits lipid peroxidation, and increases SOD activity in erythrocytes [17].

Considering the above information and the widespread consumption of organophosphates in agriculture, along with their side effects on various body tissues, as well as the contradictory results from studies related to oxidative stress and HIIT exercises, there is a notable lack of research examining the combined effects of silymarin extract and HIIT exercise on the oxidative stress indicators in the body when exposed to organophosphorus toxins. The present study was conducted to investigate the effect of eight weeks of HIIT exercise along with the consumption of silymarin extract on the oxidative stress indicators in the heart and lung endothelial tissues of male rats exposed to diazinon.

Materials and Methods

In this experimental study, 36 male Sprague-Dawley rats with a weight range of 150 to 200 g and an average age of 8 weeks were purchased from the Center for Breeding and Reproduction of Laboratory Animals of Islamic Azad University, Marvdasht Branch, and transferred to the specialized sports physiology laboratory of this academic unit. After a week of adaptation to the laboratory environment, rats were divided into six groups of six animals, which included control (C), sham (Sh), diazinon (D), silymarin extract (SL), high-intensity interval training (HIIT) (TH) and HIIT with silymarin extract consumption (TH+SL). All rats were kept in clean and sterilized transparent cages under standard conditions, with a 12-hour light and dark cycle and a temperature range of 19-22 ˚C. The animals were fed with a standard rodent laboratory diet and had unlimited access to tap water. Over the course of 8 weeks, rats in groups 3 to 6 received diazinon at a dosage of 1.5 mg/kg intraperitoneally five days a week [3]. Groups 5 and 6 performed HIIT exercises five times a week, while groups 4 and 6 received 50 mg/kg silymarin extract intraperitoneally five days a week [17].

Sampling method and measuring gene expression values

Forty-eight hours after the last training session, the rats were anesthetized with ketamine at a dose of 95 mg/kg and xylazine at a dose of 5 mg/kg intraperitoneally using an insulin syringe, and their heart tissue was isolated by laboratory experts. Then, it was immediately frozen in liquid nitrogen and kept at -70 ˚C. For molecular investigations at the level of gene expression, RNA was extracted from the heart tissue according to the manufacturer's protocol (Cinagen, Iran). For this purpose, the heart tissue was frozen with liquid nitrogen and then, the tissue was crushed and homogenized by mechanical methods. The initial step in extracting RNA from animal cells involved lysing the cell walls with a lysing buffer known as RB Buffer. A total of 350 µL of RB Buffer was added to the sample (the cell sediment obtained from centrifugation) (10 µL of mercaptoethanol-β had already been added to the buffer for every 1 mL) and left at room temperature for 5 minutes.

In the next step, the filter column was placed in a collection tube and the sample mixture was transferred to the filter column and centrifuged at 14000 rpm for 2 minutes. After centrifugation, the clear solution was transferred from the collection tube to a new microcentrifuge tube. Then, an equal volume of 350 µL of 70% ethanol was added, and the mixture was vortexed thoroughly. The RB Mini Column was placed in the collection tube, and the sample containing ethanol was transferred to the RB Mini Column and centrifuged at 14000 rpm for 1 minute, and the solution in the collection tube was discarded.

In the next step, 500 µL of wash buffer 1 was added to the RB Mini Column and centrifuged at 14000 rpm for 1 minute, after which the solution was discarded. Next, the RB Mini Column was centrifuged with 750 µL of wash buffer 2 at 14,000 rpm for 1 minute, and the solution in the collection tube was discarded. This step was repeated twice. The column was then centrifuged for 3 minutes at 14,000 rpm. Then, the RB Mini Column was placed in the elution tube, and 50 µL of RNase-free ddH_2O was added to the RB Mini Column, allowing it to sit for 1 minute before centrifuging for 2 minutes at 14,000 rpm. The solution inside the elution tube was the extracted RNAs, which were kept at -70 °C. The concentration and purity of the RNA sample were quantitatively assessed using the property of light absorption at a wavelength of 260 nm, applying the Equation 1:

1. $C\ (\mu g/\mu L)=A260\times \varepsilon\times d/1000$

After extracting RNA with very high purity and concentration from all studied samples, complementary DNA (cDNA) synthesis was performed according to the protocol of the fermentase kit (K1621). The synthesized cDNA was then used to conduct the reverse transcription reaction. First, the designed primers related to the genes were examined, and then the expression of the genes was examined using the quantitative reverse transcription (q-RT) polymerase chain reaction (PCR) method.

Also, to assess the expression of SOD, catalase, and *MDA* genes for the cell groups, RealQ 2x Master Mix Green Dye (AMPLQON, Denmark) was used according to the kit's instructions. The real-time PCR device was set to run in two steps. After completing the operation of the device and observing the graphs based on the increase in the number of the desired fragments and the amount of fluorescence emission, the ΔΔCt method was used to measure the change in expression of the desired gene compared to the reference gene *B2m* and the control group. Subsequently, the expression rate was calculated using the formula ΔΔCt-2 (Equation 2) [18].

2. $\Delta C_t=C_{t\ interets}-C_{t\ B2m}$

$C_t=\Delta C_{t\ Treat}-\Delta C_{tUn\ TreatΔΔ}$

High-intense interval training (HIIT) protocol

First, to familiarize the rats with the treadmill, they walked on a 5-channel treadmill for three days, 5 minutes each day, at a speed of 8 m/min. Next, to obtain the maximum speed, the rats first warmed up for 5 minutes at a speed of 8 m/min, and then, two meters were added to the speed of the treadmill every 3 minutes until the speed reached 18 m/min Following that, the speed was increased by 3 meters per minute every 2 minutes until the rats were unable to continue running, defined as a state in which they hit the end of the treadmill three times within a minute due to fatigue [19].

After establishing the maximum running speed, HIIT was conducted at an intensity of 85% to 110% of VO_2 max. This involved seven attempts lasting 1 minute at a speed of 31 m/min, with active rest intervals consisting of six attempts at a speed of 15 m/min during the first week (Table 1). The regimen was gradually intensified, reaching 10 one-minute attempts at a speed of 45 m/min and active rest intervals consisting of 9 one-minute attempts at a speed of 23 m/min by the eighth week, with an average weekly increase of 2 m/min [19, 20].

Preparation of silymarin extract

To prepare silymarin extract, first, the seeds of native and cultivated milk thistle plants were placed in a freezer at -20 ˚C for 24 hours. They were then ground into a fine powder using a mill, and the resulting powder was passed through a 45-mesh sieve. Ten grams of the resulting powder were placed into a Soxhlet extractor and extracted with ether for 12 hours. Then, fat-free powders were extracted using acetonitrile, methanol, acetone, ethanol, and ethyl acetate in an ultrasound bath for 30 minutes. The obtained extracts were concentrated and dried in a vacuum. The resulting extract was dissolved in 10 mL of HPLC methanol and injected directly into the HPLC device. The USP26 method was used to measure flavolignans, and a solution with a concentration of 1 mg/mL of standard silymarin powder was prepared in methanol. Subsequently, after concentrating the extract with normal saline, rats received a dose of 50 mg/kg of body weight per day intraperitoneally [17]. Also, the sham group (Sh) received only physiological serum in the same volume as the other groups, administered intraperitoneally five times a week for eight weeks. To induce stress, the treadmill was kept fixed on the conveyor belt during each session. A gentle blow on the tail with a soft brush was used to encourage the rats to continue running on the treadmill.

Data analysis

To check the normality of the distribution of the results, the Shapiro-Wilk test was used, and to analyze the results, a one-way analysis of variance with Tukey's post hoc test was conducted using SPSS software, version 26. A significance level of $P \geq 0.05$ was considered ($P \geq 0.05$).

Table 1. HIIT protocol in rats

Training / Week	HIIT					
	Speed at Low Intensity (m/min)	Number of Low-intensity Intervals	Low-Intensity Interval Intensity (VO_2 max)	Speed at High Intensity (m/min)	The Number of Intense Intervals	Intense Interval Intensity (VO_2 max)
1st	15	6	50	31	7	85
2nd	16	6	50	33	7	85
3rd	17	7	50	35	8	90
4th	18	7	50	37	8	95
5th	19	8	55	39	9	100
6th	21	8	55	41	9	100
7th	22	9	55	43	10	110
8th	23	9	55	45	10	110

Results

The results of one-way ANOVA showed a significant difference in MDA levels in heart tissue (F=206.176, P=0.001) and lung endothelial (F=213.819, P=0.001), as well as in SOD levels in heart tissue (F=21.936, P=0.001) and lung endothelial tissue (F=16.970, P=0.001) among the research groups. The results of Tukey's post hoc test indicated that MDA levels in heart tissue (P=0.001) and lung endothelial tissue (P=0.001) of the D groups were significantly higher than those in the C and Sh groups. Meanwhile, the MDA levels in the heart tissue of the HIIT, SL, and TH+SL groups were significantly lower than in the D group (P=0.001). Additionally, MDA levels in the TH+SL group were significantly lower than in the TH (P=0.018) and SL) (P=0.004 groups alone. In the lung endothelial tissue, MDA levels in the TH and TH+SL groups were significantly lower than in the diazinon (D) group (P=0.001). The MDA levels in the lung endothelial tissue of the TH+SL group were significantly lower than in the TH (P=0.022) and SL groups (P=0.002) alone (Figure 1).

Also, the results of Tukey's post hoc test showed that *SOD* gene expression levels in the D group were significantly lower than in the C and Sh groups (P=0.001). However, SOD levels in heart and lung endothelial tissues in the TH, SL, and TH+SL groups were significantly higher than in the D group (P=0.001). Also, the SOD levels in the TH and TH+SL groups were higher than in the SL group alone for both heart (P=0.001) and lung endothelial tissues (P=0.004) (P=0.001). SOD levels in the TH+SL group in lung endothelial tissue were significantly higher than in the TH group (P=0.005) (Figure 2).

Discussion

The results of the present study showed that exposure to diazinon increases MDA levels and decreases SOD levels in heart and lung endothelial tissues of rats. On the other hand, HIIT has a significant effect on reducing MDA and increasing SOD levels in heart and lung endothelial tissues of rats exposed to diazinon. The main mechanism of diazinon toxicity, like other organophosphates, is the inhibition of acetylcholinesterase activity by its metabolites. Also, diazinon can induce oxidative stress and produce ROS [21]. As an indicator of oxidative stress, MDA is the last product of lipid breakdown. Increased MDA levels indicate lipid peroxidation and cell membrane damage [22]. On the other hand, SOD and CAT enzymes are the first lines of cell defense against oxygen-free radicals. SOD converts superoxide anions to H_2O_2 and O_2, which are subsequently converted to H_2O and O_2 by the CAT enzyme [23].

In this regard, studies have shown that diazinon poisoning causes a decrease in antioxidant indices and an increase in oxidative stress in different tissues [3, 24]. However, other studies have reported increased activity of SOD and CAT enzymes in different tissues [25]. This discrepancy may be attributed to factors such as the type, breed, and species of the animals, the type of poison and tissue, the route of administration of the toxic substance, as well as the dosage and duration of treatment.

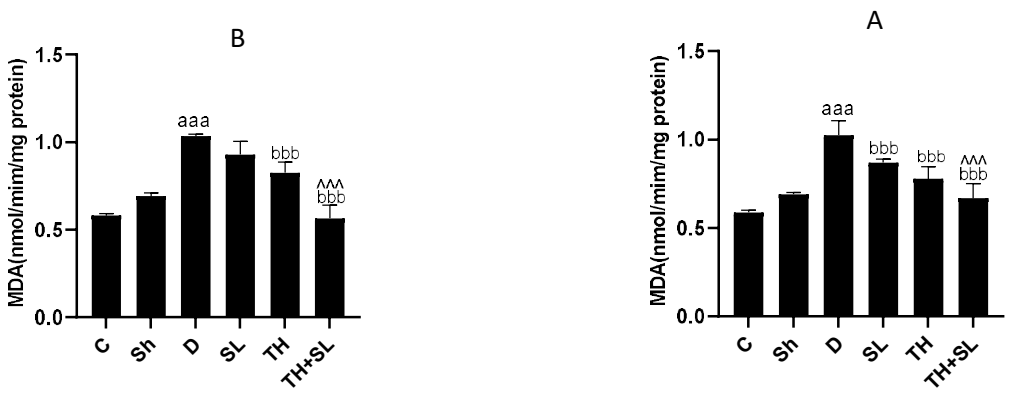

Figure 1) MDA levels in the research groups heart tissue (A) and lung endothelial tissue (B)

Abbreviations: C: Control; Sh: Sham; D: Diazinon; SL: Silymarin; TH: High-intensity interval training; TH+SL: High-intensity interval training with silymarin.

[aaa]Significant increase compared to the C and Sh groups (P=0.001), [bbb]Significant decrease compared to the D group (P=0.001), [^^^]Significant decrease compared to the TH and SL groups (P<0.05).

Journal of Vessels and Circulation
Qom University of Medical Sciences

Figure 2) MDA levels in the heart tissue (A) and lung endothelial tissue (B)

Journal of Vessels and Circulation
Qom University of Medical Sciences

Abbreviations: C: Control; Sh: Sham; D: Diazinon; SL: Silymarin; TH: High-intensity interval training; TH+SL: High-intensity interval training with silymarin.

[aaa]Significant decrease compared to the C and Sh groups (P=0.001), [bbb]Significant increase compared to the D group (P=0.001), [bb]Significant increase compared to the D group (P=0.01),[^^^]Significant increase compared to the SL group (P<0.05),[***]Significant increase compared to the TH group (P=0.005).

Additionally, the results of this study showed that HIIT increases the SOD levels and decreases MDA levels in the heart and lung endothelial tissues of rats poisoned with diazinon. In this regard, Ezabadi et al. showed that performing eight weeks of endurance training increases the antioxidant enzymes SOD and decreases MDA in rats poisoned with diazinon [3]. However, no study was found investigating the effects of HIIT under diazinon poisoning, which limited comparisons of the present study with similar research. Nonetheless, some studies have investigated the effect of intense training along with poisoning with various poisons on the heart tissue. For example, Ezabadi et al. showed that HIIT caused a significant increase in SOD and CAT levels and a decrease in MDA levels in the heart tissue of male rats exposed to doxorubicin [3]. Azhdari et al. also showed that HIIT increased antioxidant indices and reduced oxidative stress in the heart tissue of rats poisoned with cadmium [26].

On the other hand, numerous studies have shown that a single session of intense physical activity can increase lipid peroxidation [27]. In another study, performing an endurance training session increased the level of lipid peroxidation in the cardiac muscle of exercised rats [28]. The findings from these studies are inconsistent with the present research. This inconsistency may stem from differences in the intensity or duration of the exercise used in the various studies. Short-term training (a single session of intense training) has been associated with an increase in free radicals, while long-term training (four weeks, eight weeks, and beyond) is associated with cellular adaptation that increases antioxidants [29].

Studies have shown that the stimulation of exercise to induce antioxidant enzymes is rooted in the increase in ROS production caused by muscle contraction and activity [30]. ROS, as a secondary peak, activates redox transcription factors, such as activator protein 1 (Ap-1) and the nuclear factor of kappa B (NF-KB). These factors are located in the promoter region of genes encoding zinc (Zn)-SOD, copper (Cu)-SOD, manganese (Mn)-SOD, catalase, and GPX [31]. It seems that a series of factors are effective in reducing the concentration of MDA following the training period, and the improvement of oxidative stress cannot be attributed to the improvement of the antioxidant status. The resistance of the cell membrane, especially of red cells, against ROS increases after exercise and may contribute to this issue [32]. In any case, it seems that the activation of cell signaling pathways leads to an increase in the expression of enzymatic antioxidants, such as SOD, ultimately reducing lipid peroxidation and MDA levels [32].

The results of the present study showed that the consumption of 50 mg/kg silymarin has a significant effect on reducing MDA levels in heart tissue and increasing SOD levels in the heart and lung endothelial tissues of rats exposed to diazinon. In this regard, according to Beydilli et al., the consumption of silymarin extract for four periods reduces oxidative stress and increases serum antioxidants in rats poisoned with diazinon [33]. Habotta et al. showed that the consumption of silymarin extract increased the antioxidant indices in testicular tissues of rats poisoned with thiamethoxam [34]. El-Houseiny et al. also showed that the consumption of silymarin increases antioxidant indices and decreases MDA levels in fish exposed to fluoride [35], which is consistent with

the results obtained in the present study. Pharmacological and medical studies have shown that silymarin can enter the cell nucleus due to its similarity with steroid hormones, and by acting on RNA polymerase I enzymes and rRNA transcription, it can improve the formation of ribosomes to increase the synthesis of structural proteins [36]. It is also possible that silymarin directly reduces oxidative stress from H_2O_2 and free radicals produced by H_2O_2. Thus, it can be concluded that silymarin may possess antioxidant effects, leading to an optimal balance between oxidative stress caused by toxicity and its mitigation [37].

Regarding the interactive effect of HIIT and silymarin consumption, the results of the present study showed that HIIT and 50 mg/kg silymarin consumption leads to increased *SOD* gene expression levels and decreased MDA levels in the heart and lung endothelial tissues of rats poisoned with diazinon. Research shows that calcium modulation, following a reduction in oxidative stress and caspase-3 activity, along with an increase in vascular endothelial growth factor (VEGF) in the heart and lung tissues of rats, could partially inhibit the apoptotic effects of diazinon [38]. Also, the consumption of silymarin extract, through the activation of mitogen-activated protein kinase, leads to an increase in the expression of fat metabolism proteins, which have fat-reducing effects in the blood. This reduction in fat levels further decreases oxidative stress and inflammatory factors, such as nuclear transcription factor kappa B (NF-κB), while increasing the anti-inflammatory cytokine interleukin (IL)-10 and inhibiting IL-1β, tumor necrosis factor (TNF)-α, IL-6, and IL-8, thereby inducing anti-inflammatory and anti-apoptotic effects [39]. Therefore, it seems that these two interventions could synergistically strengthen each other's effects in reducing oxidative stress under diazinon poisoning through different pathways.

Regarding the increase in antioxidant capacity, it can be stated that HIIT increases the number of mitochondria and affects prostanoid metabolism, the activity of xanthine oxidases, and macrophage function [40]. The consumption of silymarin reduces lipid peroxidation and increases antioxidant indices [41]. Nevertheless, researchers have reported the effect of silymarin supplementation on different tissues and its role in improving the antioxidant system in conjunction with physical activities, particularly in cases of poisoning with different toxins [42, 43]. However, no study was found on the antioxidant effects of silymarin consumption on heart and lung endothelial tissues under diazinon poisoning. Therefore, comparisons of the present study with similar studies are limited. However, some studies have investigated the simultaneous effect of silymarin and sports exercises, showing that silymarin reduces oxidative stress indicators caused by intense physical activities [37, 44].

Among the strengths of the study are the controllability of the training duration, and the availability of the necessary equipment and facilities. However, the limitations o include enforced training and the lack of control over the effect of anesthetic drugs on the subjects. Given the increase in antioxidant levels and the reduction in oxidative stress in the studied tissues of rats following HIIT and silymarin consumption, it seems that androgen receptors in heart and lung endothelial tissues align with slow fibers of high contraction, resulting in improved antioxidant activity. Therefore, in the case of diazinon poisoning, it is recommended to use these two interventions simultaneously due to their synergistic effects. Furthermore, there is a need for more studies employing immunohistochemical analyses and the expression of effector genes in heart and lung tissues.

Conclusion

Eight weeks of HIIT and the consumption of silymarin can each have significant effects on increasing the levels of the oxidative index (MDA) in the heart and lung endothelial tissues of rats exposed to diazinon, as well as on increasing the gene expression levels of certain indicators of the antioxidant defense system (SOD). Nevertheless, performing HIIT and taking silymarin supplements simultaneously may yield more favorable effects on oxidative and antioxidant indicators affected by diazinon poisoning than either intervention alone. Therefore, based on the results of the present study, it is possible to use the benefits of HIIT and the consumption of silymarin extract, both separately and simultaneously, as a control strategy against diazinon exposure.

Ethical Considerations

Compliance with ethical guidelines

The current research was conducted according to the guidelines of the National Institute of Health (NIH). This study was approved by the Ethics Committee in Biomedical Research at the Islamic Azad University, Marvdasht Branch (Code: IR.IAU.M.REC.1401.003). This study was also approved by the Ethics Committee in Biomedical Research at the Islamic Azad University, Mahalat Branch.

Journal of Vessels and Circulation
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Authors' contributions

Conceptualization, methodology, data collection, analysis and research: Amir Hosseinzadeh, and Bahram Abedi; Writing the original draft: Amir Hosseinzadeh; Review and editing: Amir Hosseinzadeh, Bahram Abedi, and Lida Moradi; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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References

[1] Salehi M, Jafari M, Asgari A, Saleh Moghaddam M, Salimian M, Abbasnejad M, et al. [Study of diazinon Effect on antioxidant enzymes and lipid peroxidation in rat's brain (Persian)]. Razi J Med Sci. 2010; 17(70):15-23. [Link]

[2] Rahimi Anbarkeh F, Jalali M, Nikravesh MR, Soukhtanloo M. Protective effects of alpha-lipoic acid on diazinon-induced renal toxicity in rats: An immunohistochemistry study. Toxin Rev. 2022; 41(1):1-10. [DOI:10.1080/15569543.2020.1812659]

[3] Ezabadi A, Peeri M, Azarbayjani MA, Hosseini SA. The effects of resistance training and berberine chloride supplementation on oxidative stress markers in the cerebellum tissue of diazinon-poisoned rats. Middle East J Rehabil Health Stud. 2019; 6(3):e92870. [DOI:10.5812/mejrh.92870]

[4] Fattahi E, Khoskafa A. An investigation of the effects of optional exercise and salvia officinalis extracts on pancreatic tissue injuries in rats poisoned by diazinon. J Babol Univ Medical Sci. 2015; 17(8):48-54. [DOI:10.22088/jbums.17.8.48]

[5] Abdel-Daim MM, Abushouk AI, Bahbah EI, Bungău SG, Alyousif MS, Aleya L, et al. Fucoidan protects against subacute diazinon-induced oxidative damage in cardiac, hepatic, and renal tissues. Environ Sci Pollut Res Int. 2020; 27(11):11554-64. [DOI:10.1007/s11356-020-07711-w] [PMID]

[6] Saraei F, Sadoughi M, Kaka G, Sadraie SH, Foaddodini M. Study of the effects of diazinon on fetal liver in BALB/c Mice. I Iran Red Crescent Med J. 2016; 18(4):e28076. [DOI:10.5812/ircmj.28076] [PMID]

[7] Rafieian-Kopaei M, Baradaran A, Rafieian M. Oxidative stress and the paradoxical effects of antioxidants. Res Med Sci. 2013; 18(7):629. [PMID]

[8] Kaldur T, Unt E, Ööpik V, Zilmer M, Eha J, Paapstel K, et al. The acute effects of passive heat exposure on arterial stiffness, oxidative stress, and inflammation. Medicina. 2016; 52(4):211-6. [DOI:10.1016/j.medici.2016.06.001] [PMID]

[9] Nasiru S, Bulama I, Abdurrahman J, Abubakar N, Salisu A, Salisu B, et al. Neurobiochemical roles of low molecular weight antioxidants on oxidative stress biomarkers and severity of ischemic stroke in wistar rats. J Neurol Neurol Disord. 2018; 4(1):101-11. [DOI:10.15744/2454-4981.4.401]

[10] Olher RR, Rosa TS, Souza LHR, Oliveira JF, Soares BRA, Ribeiro TBA, et al. Isometric exercise improves redox balance and blood pressure in hypertensive adults. Med Sci Sports Exerc. 2020; 52(5):1187-95. [DOI:10.1249/MSS.0000000000002223] [PMID]

[11] Katoli M, Daloii A. The effect of boldenone and aerobic training with jujube extract and gallic acid on glutathione peroxidase and catalase in heart tissue of male wistar rats. Tradit Integr Med. 2017; 2(1):15-23. [Link]

[12] Larsen S, Danielsen JH, Søndergård SD, Søgaard D, Vigelsoe A, Dybboe R, et al. The effect of high-intensity training on mitochondrial fat oxidation in skeletal muscle and subcutaneous adipose tissue. Scand J Med Sci Sports. 2015; 25(1):e59-69. [DOI:10.1111/sms.12252] [PMID]

[13] Costa EC, Hay JL, Kehler DS, Boreskie KF, Arora RC, Umpierre D, et al. Effects of high-intensity interval training versus moderate-intensity continuous training on blood pressure in adults with pre-to established hypertension: A systematic review and meta-analysis of randomized trials. Sports Med. 2018; 48(9):2127-42. [DOI:10.1007/s40279-018-0944-y] [PMID]

[14] Poblete Aro CE, Russell Guzmán JA, Soto Muñoz ME, Villegas González BE. Effects of high intensity interval training versus moderate intensity continuous training on the reduction of oxidative stress in type 2 diabetic adult patients: CAT. Medwave. 2015; 15(7):e6212. [DOI:10.5867/medwave.2015.07.6212] [PMID]

[15] Rohani H, Safarimosavi S, Mohebbi H. [The comparison of 12 weeks continuous training at fatmax and anaerobic threshold intensities and high intensity interval training on antioxidant enzymes and lipid peroxidation index in prediabetic patients (Persian)]. Med J Tabriz Univ Med Sci. 2020; 42(2):168-76. [DOI:10.34172/mj.2020.033]

[16] Songstad NT, Kaspersen KH, Hafstad AD, Basnet P, Ytrehus K, Acharya G. Effects of high intensity interval training on pregnant rats, and the placenta, heart and liver of their fetuses. Plos One. 2015; 10(11):e0143095. [DOI:10.1371/journal.pone.0143095] [PMID]

[17] Roghani M, Baluchnejadmojarad T. [Effect of chronic administration of Silymarin on oxidative stress markers in renal tissue of diabetic Rats (Persian)]. J Gorgan Univ Med Sci. 2012; 14(2):10-6. [Link]

[18] Roozbehi M, Gaeini A, Nouri R, Kordi MR. [Interaction effect of stanozolol and endurance training on oxidant and antioxidant capacity in liver tissue of healthy male wistar rats (Persian)]. Stud Med Sci. 2019; 30(7):537-47. [Link]

[19] Kheyrdeh M, Nora M, Fashi RJ, Rakhshanizadeh A. [The effect of eight weeks of high intensity interval training with Citrus Aurantium extract consumption on oxidative stress and antioxidant levels of soleus muscle in elderly rats (Persian)]. Res Sport Sci Med Plants. 2020; 1(1):29-38. [Link]

[20] Yazdanparast Chaharmahali B, Azarbayjani MA, Peeri M, Farzanegi Arkhazloo P. The effect of moderate and high intensity interval trainings on cardiac apoptosis in the old female rats. Rep Health Care. 2018; 4(1):26-35. [Link]

[21] Izadi F, Jafari M, Bahdoran H, Asgari A, Divsalar A, Salehi M. [The role of N-acetyl cysteine on reduction of diazinon-induced oxidative stress in rat liver and kidney (Persian)]. J Rafsanjan Univ Med Sci. 2014; 12(11):895-906. [Link]

[22] Tahmasebi K, Jafari M, Salehi M. [Modulation of diazinon-induced oxidative stress by vitamins E And C in rat brain (Persian)]. J Guilan Univ Medi Sci. 2017; 26(J101):66-73. [Link]

[23] Limón-Pacheco J, Gonsebatt ME. The role of antioxidants and antioxidant-related enzymes in protective responses to environmentally induced oxidative stress. Mutat Res. 2009; 674(1-2):137-47. [DOI:10.1016/j.mrgentox.2008.09.015] [PMID]

[24] Abdel-Daim MM, Abushouk AI, Alkhalf MI, Toraih EA, Fawzy MS, Ijaz H, et al. Antagonistic effects of Spirulina platensis on diazinon-induced hemato-biochemical alterations and oxidative stress in rats. Environ Sci Pollut Res Int. 2018; 25(27):27463-70. [DOI:10.1007/s11356-018-2761-0] [PMID]

[25] Birdane YO, Avci G, Birdane FM, Turkmen R, Atik O, Atik H. The protective effects of erdosteine on subacute diazinon-induced oxidative stress and inflammation in rats. Environ Sci Pollut Res Int. 2022; 29(15):21537-46. [DOI:10.1007/s11356-021-17398-2] [PMID]

[26] Azhdari A, Hosseini SA, Farsi S. The interactive effect of high intensity interval training (HIIT) and selenium consumption on superoxide dismutase (SOD) and glutathione peroxidase (GPX) in the heart tissue of cadmium-poisoned rats (Cd). Int J Appl Exerc Physiol. 2019; 8(2):1-11. [Link]

[27] Feizi Y, Afzalpur ME, Abtahi-Eivary SH. [Effect of 2-weeks Coenzyme Q10 supplementation on malondialdehyde and catalase serum levels following moderate and severe acute resistance training in inactive female students (Persian)]. Intern Med Today. 2019; 25(4):256-69. [DOI:10.32598/hms.25.4.256]

[28] Ji LL, Kang C, Zhang Y. Exercise-induced hormesis and skeletal muscle health. Free Radic Biol Med. 2016; 98:113-22. [DOI:10.1016/j.freeradbiomed.2016.02.025] [PMID]

[29] Al-abdaly Y, Al-Kennany E, Al-Hamdany E. Concomitant occurrence of oxidative stress with sustanon in male rat. Basrah J Vet Res. 2018; 17(3):136-47. [Link]

[30] Karabulut AB, Kafkas ME, Kafkas AS, Onal Y, Kiran TR. The effect of regular exercise and massage on oxidant and antioxidant parameters. Indian J Physiol Pharmacol. 2013; 57(4):378-83. [Link]

[31] Bellezza I, Riuzzi F, Chiappalupi S, Arcuri C, Giambanco I, Sorci G, et al. Reductive stress in striated muscle cells. Cell Mol Life Sci. 2020; 77(18):3547-65. [DOI:10.1007/s00018-020-03476-0] [PMID]

[32] Groussard C, Maillard F, Vazeille E, Barnich N, Sirvent P, Otero YF, et al. Tissue-specific oxidative stress modulation by exercise: A comparison between MICT and HIIT in an obese rat model. Oxid Med Cell Longev. 2019; 2019:1965364. [DOI:10.1155/2019/1965364] [PMID]

[33] Beydilli H, Yilmaz N, Cetin ES, Topal Y, Celik OI, Sahin C, et al. Evaluation of the protective effect of silibinin against diazinon induced hepatotoxicity and free-radical damage in rat liver. Iran Red Crescent Med J. 2015; 17(4):e25310. [DOI:10.5812/ircmj.17(4)2015.25310] [PMID]

[34] Habotta OA, Ateya A, Saleh RM, El-Ashry ES. Thiamethoxam-induced oxidative stress, lipid peroxidation, and disturbance of steroidogenic genes in male rats: Palliative role of Saussurea lappa and Silybum marianum. Environ Toxicol. 2021; 36(10):2051-61. [DOI:10.1002/tox.23322] [PMID]

[35] El-Houseiny W, Abd El-Hakim YM, Metwally MM, Ghfar SSA, Khalil AA. The single or combined Silybum marianum and co-enzyme Q10 role in alleviating fluoride-induced impaired growth, immune suppression, oxidative stress, histological alterations, and reduced resistance to Aeromonas sobria in African catfish (Clarias gariepinus). Aquaculture. 2022; 548:737693. [Link]

[36] Yalinbaş EE, Akcilar R, Kocak H, Çi□rki□noğ MS, Meti□nere M, Kaçar H. Antioxidative effects of silymarin in a neonatal rat model of necrotizing enterocolitis. Osmangazi Tıp Dergisi. 2023; 45(1):28-34. [DOI:10.20515/otd.1161399]

[37] Moein A, Zarghami Khameneh A. [The effect of silymarin supplementation on the serum levels of interleukin-6 and C-reactive protein following a single bout of aerobic exercise in healthy men (Persian)]. Daneshvar Med. 2018; 26(1):39-50. [Link]

[38] Alzahrani S, Ezzat W, Elshaer R, Abd El-Lateef A, Mohammad H, Elkazaz A, et al. Standarized Tribulus terrestris extract protects against rotenone-induced oxidative damage and nigral dopamine neuronal loss in mice. J Physiol Pharmacol. 2018; 69(6):979-4. [Link]

[39] Kovac JR, Pan M, Arent S, Lipshultz LI. Dietary adjuncts for improving testosterone levels in hypogonadal males. Am J Mens Health. 2016; 10(6):NP109-17. [DOI:10.1177/1557988315598554] [PMID]

[40] Simioni C, Zauli G, Martelli AM, Vitale M, Sacchetti G, Gonelli A, et al. Oxidative stress: Role of physical exercise and antioxidant nutraceuticals in adulthood and aging. Oncotarget. 2018; 9(24):17181-98. [DOI:10.18632/oncotarget.24729] [PMID]

[41] Seyed A, Farsi S, Hosseini SA, Kaka G. [Antioxidant effects of swimming training and curcumin in withdrawal period of alcohol overdose in rats (Persian)]. J Fasa Univ Med Sci. 2018; 8(3):901-10. [Link]

[42] Shirali S, Barari AR, Hosseini SA. [The effects of endurance training and administration of silymarin supplementation on oxidative enzyme of SOD and heat shock proteins 70 in plasma of unathletes men students (Persian)]. Jundishapur Sci Med J. 2016; 14(6):703-12. [Link]

[43] Aghaali G, Sojasi GF, Norouzi HR. Effects of 8 week strenuous endurance and high intensity interval training on thioredoxin reductase-1 enzyme and malondialdehyde in lung tissue of male wistar rats. J Nurse Physician War. 2022; 9(33):26-36. [Link]

[44] Hasani M, Behpour N, Karimi M, Darabi F. [The effect of silybummarianum consumption along with a period of increasing exercises on the oxidative response to an acute exercise session in young wrestlers (Persian)]. Qom Univ Med Sci J. 2020; 13(12):34-44. [DOI:10.29252/qums.13.12.34]

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