Original Article

# Antioxidant Effects of Aerobic Training and Crocin Consumption on Doxorubicin-Induced Testicular Toxicity in Rats

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## Abstract

**Objective:** Doxorubicin (DOX) treatment has been reported to increase the risk of serious toxicity in testis, therefore the aim of the present study was to investigate the antioxidant effects of training and Crocin on doxorubicin-induced testicular toxicity in rats.

**Materials and methods:** In this experimental study, 42 Wistar rats were randomized into seven groups of six rats, including 1) Control, 2) DOX, 3) DOX + 10 mg/kg/d (day) Crocin, 4) DOX + 50 mg/kg/d Crocin, 5) DOX + high intensity interval training (HIIT), 6) DOX + HIIT with 10 mg/kg/d Crocin and 7) DOX + HIIT with 50 mg/kg/d Crocin. During eight weeks, rats in groups 3, 4, 6, and 7 administered Crocin daily at specific doses by gavage, and groups 5 to 7 performed HIIT(2-8rep×2min at 80-110% $V_{max}$) 5 day/w. Also, groups 2 to 7 administered 2 mg/kg/w DOX intraperitoneal. The testes were removed and glutathione peroxidase ($GP_X$), total antioxidant capacity (TAC) and protein carbonyl (PC) were analyzed using ELISA methods, one-way analysis of variance along with Bonferroni's post hoc test were used for analysis in SPSS ($P \leq 0.05$).

**Results:** The results of the present study showed that doxorubicin induced oxidative stress in testicular tissue by decreasing the level of GPX and TAC and increasing PC level ($P \leq 0.05$); TAC and GPX improved in all groups except groups 2 and 5, respectively, and their increase in the group 7 was significantly higher compared to other groups ($P \leq 0.05$). Increased PC levels were significantly reduced in the groups 5, 6 and 7.

**Conclusion:** The increase in antioxidant levels in the concurrent Crocin and training group seems to be dose-dependent, but the oxidative stress in both Crocin and training groups of 10 and 50 mg/kg/d is associated with a decrease, but its modulation in the Crocin consumption group alone depends on the dose.

**Keywords:** Crocin; Doxorubicin; Exercise; Testis

## Introduction

Doxorubicin (DOX) is an anthracycline and anti-cancer antibiotic commonly used to treat various cancers such as leukemia and testicular, breast, lung, ovarian and uterine cancers (1, 2). Although it can be used effectively against malignant tumors (2), treatment with DOX has been reported to increase the risk of serious

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dose-dependent toxicity in other non-target tissues such as testicles, kidneys, heart, and brain (3). DOX can significantly inhibit spermatogenesis, which ultimately leads to infertility (4).

Nowadays, the main proposed mechanisms responsible for DOX-induced testicular dysfunction include oxidative stress, lipid peroxidation, and cellular apoptosis (3, 5). On the other hand, the findings of recent studies strongly suggest oxidative stress as one of the main reasons (6). Experiments on testicular tissue in DOX-treated rats showed increased levels of malondialdehyde (MDA), decreased levels of superoxide dismutase (SOD), glutathione peroxidase and glutathione (GSH), and increased cell apoptosis through deoxyribonucleic acid (DNA) fragmentation (5). Mohamed et al. showed that MDA and testosterone levels were significantly increased in the DOX group compared to the control group. Also, a significant decrease in Catalase (CAT) and SOD levels was observed in the DOX group compared to the control group (7). Although DOX has beneficial effects on cancer cells, its toxic effects on healthy cells such as testicular tissue are worrying. Therefore, non-pharmacological strategies such as exercise and antioxidant supplements are of significant importance in aiding DOX-induced tissue toxicity (8).

In recent years, a number of studies have examined the effect of exercise on markers of male reproductive function in testes using oxidative stress markers (9). Although studies in this area are not yet abundant, they point out that exercise plays an important role in improving male reproductive function markers in healthy and infertile individuals, especially by reducing inflammatory markers and oxidative stress (10). In fact, homeostasis in the cell and especially at the organism level is the balance between the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and antioxidant reactions to maintain the proper level of ROS and minimize their specific reactions with vital biological molecules. These antioxidants fall into three categories: One is enzymatic antioxidants such as CAT and GPx, which remove hydrogen peroxide. Another class is biological metal chelators. Finally, low weight hydrophilic or hydrophobic molecular materials such as glutathione, ascorbic acid (vitamin C), uric acid, tocopherols (vitamin E), carotenoids, coenzyme Q, bilirubin, and some amino acids (such as cysteine, methionine, or tyrosine). These compounds are often referred to as "free radical scavengers". However, at least some of these compounds are also effective protectors against non-ROS / RNS radicals such as peroxynitrate or hypochlorite. The total antioxidant activity of this "non-specific" set of antioxidants is often referred to as the TAC (11).

Protein carbonylation, as one of the most irreversible changes of oxidative protein modifications, is also considered as the main symptom of oxidative stress disorders. PC measurements are often performed to assess oxidative stress in cell damage, aging, and several age-related disorders (12).

Research has also shown the supportive effects of exercise on DOX toxicity in various tissue such as cardiovascular and testicular (8, 13). The study by Magalhães et al. showed that twelve weeks of endurance training in male rats injected with DOX significantly reduced MDA compared with the inactive group (9).

High Intensity Interval Training (HIIT) is a modern type of aerobic training that is defined as repeated attempts to perform high-intensity exercise with intermittent rest or low-intensity activity as recovery periods. Recently, HIIT has been reported to have significant protective effects on various tissues compared to conventional endurance training (13). Also, the findings of Maleki and Tartibian showed that 24 weeks of HIIT led to a significant improvement in semen oxidative stress and inflammation markers, semen parameters and sperm DNA integrity in infertile men (10), while Songstad et al. did not observe a significant difference in total antioxidant capacity (TAC) and MDA of liver and heart tissue after 6 weeks of high intensity interval training (14).

On the other hand, natural products, which are widely distributed in plant foods and medicinal plants, provide molecules for human nutrition, health and reproductive system (15-17). Crocin (an active ingredient in saffron), has antioxidant properties that can be effective in eliminating free radicals (18). Previous studies have shown that Crocin is an antioxidant with therapeutic effects including anti-cancer, anti-inflammatory and an enhancer of spermatogenesis (15, 19).

Research by Roshankhah et al. showed that 50 mg/kg Crocin reduces oxidative stress produced by busulfan administration on fertility of male rats (20). Potnuri et al. also showed 10 mg/kg Crocin attenuates cyclophosphamide induced testicular toxicity by preserving glutathione redox system (15). Therefore, in this study, one of the relatively lowest and highest

doses used in the articles was used as an antioxidant.

Research suggests that supplementation together with physical activity affects the adaptations of physical activity. Some recent studies have reported that supplementation is not always desirable and can stop the adaptations resulting from the activity (21).

We hypothesized that high-intensity physical activity and Crocin consumption each independently modulated the destructive effects of doxorubicin on testicular tissue, but we were not aware of their simultaneous protective effects. Therefore, the aim of this study was to investigate the separate and interactive effects of HIIT and Crocin on testicular tissue toxicity induced by doxorubicin injection in rats.

## Materials and methods

***Animals:*** In this experimental study, 42 Wistar rats weighting approximately 220±20 gr were purchased from the Laboratory Animal Breeding Research Center of Ahvaz Jundishapur University of Medical Sciences. After transfer to the laboratory of the histology department of Jundishapur University of Medical Sciences were kept under standard conditions (temperature 25±2° C, relative humidity 50±5%, 12:12-hour light-dark cycle and free access to water and food) for a seven-day adaptation period. Excluding criteria included: observation of any disease or boredom in rat, significant weight loss, and food allergies to consumption of Cronin, any allergies or reactions to doxorubicin injection, any toxication, and Inability to perform exercises.

***Ethical Consideration:*** All procedures performed on animals in accordance with the guidelines of the National Ethics Committee in Biomedical Research, the Principles of the 2008 Helsinki Declaration, and the Code of Ethics, IR.SSRC.REC.1399.026 of the Sport sciences research Institute.

***Study groups and treatment of animals:*** Animals were randomly divided into seven groups of six rats including 1) healthy control (Control), 2) doxorubicin (DOX), 3) doxorubicin + 10 mg/kg/d Crocin (DOX+Cr10), 4) doxorubicin + 50 mg/kg/d Crocin (DOX+Cr50), 5) doxorubicin + high intensity interval training (DOX+HIIT), 6) doxorubicin + HIIT and 10 mg/kg/d Crocin (DOX+HIIT+Cr10) and 7) doxorubicin + HIIT and 50 mg/kg/d Crocin (DOX+HIIT+Cr50).

Normal saline was administered in control group and DOX+HIIT group. Crocin (Sigma-Aldrich Co (St. Louis, MO, USA)) dissolved in normal saline was administered at certain doses in DOX+Cr10, DOX+Cr50, DOX+HIIT+Cr10, and DOX+HIIT+Cr50 groups. All administrations were carried out at the same time and continued for 8 weeks with a volume of 1 mL by gavage.

2 mg/kg doxorubicin hydrochloride (Sobhan oncology, Rasht, Iran) dissolved with normal saline was administered Intraperitoneal seven times on Fridays (48 hours after the last training session and 24 hours before the next session) (22).

***Exercise training Procedure:*** The exercises were performed on the treadmill at 10 a.m. Before starting the main protocol, rats in the training groups were trained for two weeks (10 days) for five to 10 minutes each time to get acquainted with the training protocol. The maximum training speed was determined after performing an incremental test. In this way, the rats first started moving at a speed of 11.6 m/min, and every two minutes, their speed increased by 1.6 m/min to a speed of 20 m/min, then in case of progression, 3.2 m/min was added every minute to the speed to the point of fatigue (the fatigue criterion was 5 times of rats' contact with the end of the runway per minute) (23).

HIIT protocol consisted of three parts: warm-up and cool-down (5 minutes at a maximum velocity of 50 to 60 percent) and main training (Table 1). HIIT was performed for 5 day/week for 8 weeks (24). Twenty-four hours after the last training session at the end of the eighth week, the rats underwent surgery to measure the studied parameters.

***Animals' dissection and sampling:*** Rats were anesthetized with 10% ketamine (50 mg/kg body weight) and 2% Xylazine (10 mg/kg body weight) after about 5 minutes. Then, their testicular tissue was extracted by specialists.

**Table 1:** Training intensities through the entire protocol for training groups

| Weeks | First | Second | Third | Forth | Fifth | Sixth | Seventh | Eighth |
|---|---|---|---|---|---|---|---|---|
| Duration of HII (rep×min) | 2×2 | 4×2 | 6×2 | 8×2 | 8×2 | 8×2 | 8×2 | 8×2 |
| Intensity of HII(% $V_{max}$) | 80 | 90 | 100 | 110 | 110 | 110 | 110 | 110 |
| Duration of LII (rep×min) | 1×2 | 3×2 | 5×2 | 7×2 | 7×2 | 7×2 | 7×2 | 7×2 |
| Intensity of LII(% $V_{max}$) | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| Duration(min/day) | 16 | 24 | 32 | 40 | 40 | 40 | 40 | 40 |

HII: High Intensity Interval; LII: Low Intensity Interval; rep×min: repetition × minute; %Vmax: percent of maximal velocity; min/day: Minute per day

After that, homogenize 50mg of tissue. Add 500µL of Repa buffer to the tissue and lubricate the tissue with it. Then put the samples in the refrigerator for an hour. After that, centrifuge the samples for 100 minutes at 10000 RPM. Transfer the supernatant to a new microtube and store it in the -70C freezer for further studies.

***Measurement of oxidant and Antioxidant capacity:*** The levels of $GP_X$ (Catalog number: ZB-$GP_X$-96A Sensitivity: 5 U / mL) and TAC (Catalog number: ZB-TAC-96A; Sensitivity: 0.1mM) were measured by ELISA method and using special kits of Zellbio GmbH (Germany) and levels of PC (Catalog Number: STA-310) were measured by ELISA method using special kits of Cell Biolabs Inc. (USA).

***Statistical analysis:*** To investigate the normality of the distribution of findings, the Shapiro-Wilk test and to analyze the findings, one-way analysis of variance (ANOVA) with Bonferroni's post hoc test in SPSS software version 26 was used ($P<0.05$).

## Results

***Oxidant and antioxidant capacity:*** The Mean ± SD levels of PC, GPx and TAC in the testicular tissue of rats are reported in Table 2.

***PC levels:*** The levels of PC in the testicular tissue were a significant different between groups (F= 13.29, P=0.0001, η coefficient=0.7). The results of Bonferroni's post hoc test for PC changes in Figure 1 showed that doxorubicin consumption had a significant increase in comparing with Control (P = 0.0001), DOX + HIIT (P = 0.024), DOX + Cr10 (P = 0.007), DOX + Cr50 (P = 0.0001), DOX + HIIT+ Cr10 (P = 0.0001), and DOX + HIIT+ Cr50 (P = 0.0001). The PC levels also decreased in DOX + Cr50, DOX + HIIT+ Cr10, and DOX + HIIT+ Cr50, so that there was not any statically different with control group ($P\geq0.05$). Different between control group with DOX + HIIT (P = 0.003) and DOX + Cr10 (P = 0.01) groups was significant. There was no significant difference between other groups.

***GPx levels:*** The results of one-way analysis in Figure 2 showed that there was a significant different in $GP_X$ levels between groups (F= 11.78, P=0.0001, η coefficient=0.70). Doxorubicin consumption had a significant decrease in comparing with Control (P = 0.0001), DOX + HIIT (P = 0.021), DOX + Cr10 (P = 0.001), DOX + Cr50 (P = 0.006), DOX + HIIT+ Cr10 (P = 0.0001), and DOX + HIIT+ Cr50 (P = 0.006). Different between control group with DOX + HIIT (P = 0.034) was significant. DOX + HIIT+ Cr50 only increased the $GP_X$ levels compared to the DOX + HIIT (P = 0.047).

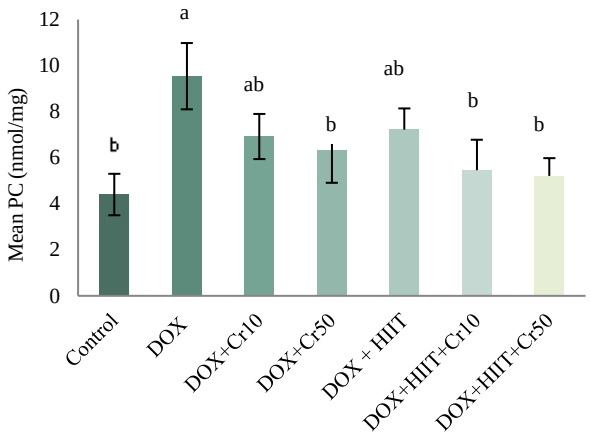


**Figure 1:** Effect of Crocin and HIIT on PC level. DOX (Doxorubicin), DOX+ Cr10 (Doxorubicin + Crocin 10 mg/kg/d), DOX+ Cr50 (Doxorubicin + Crocin 50 mg/kg/d), DOX+ HIIT (Doxorubicin+ HIIT), DOX+ HIIT + Cr10 (Doxorubicin+ HIIT+ Crocin 10 mg/kg/d), and DOX+ HIIT + Cr50 (Doxorubicin+ HIIT+ Crocin 50 mg/kg/d) during 8 weeks of study (n=6, for each group). Values are expressed as mean±SD. Significance differences of groups when compared to control group: a; p<0.05. Significant differences of groups when compared to DOX group: b; p<0.05.

***TAC levels:*** There was a significant different in TAC levels between groups (F= 7.02, P=0.0001, η coefficient=0.55).

**Table 2:** Mean ±standard deviation of PC, GPx, and TAC in the seven research groups

| Variable | Group | | |
|---|---|---|---|
| | PC (nmol/mg) | GPX (U/mL) | TAC (mM) |
| Health Control | 4.40±0.90 | 70.55±13.51 | 2.06±0.58 |
| Doxorubicin | 9.54±1.44 | 26.85±6.83 | 0.89±0.41 |
| Doxorubicin + 10 mg/kg Crocin | 6.92±0.98 | 56.46±3.96 | 1.26±0.52 |
| Doxorubicin + 50 mg/kg Crocin | 6.31±1.40 | 52.15±11.84 | 1.60±0.40 |
| Doxorubicin + HIIT | 7.22±0.92 | 49.23±8.78 | 1.27±0.31 |
| Doxorubicin + HIIT and 10 mg/kg Crocin | 5.46±1.32 | 63.87±13.05 | 1.28±0.29 |
| Doxorubicin + HIIT and 50 mg/kg Crocin | 5.21±0.77 | 69.80±13.56 | 2.13±0.35 |

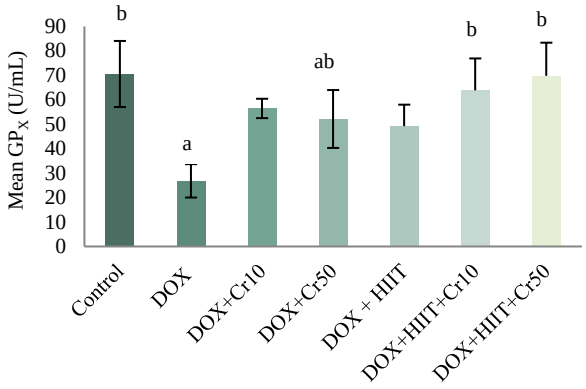


**Figure 2:** Effect of Crocin and HIIT on GPX level. DOX (Doxorubicin), DOX+ Cr10 (Doxorubicin + Crocin 10 mg/kg/d), DOX+ Cr50 (Doxorubicin + Crocin 50 mg/kg/d), DOX+ HIIT (Doxorubicin+ HIIT), DOX+ HIIT + Cr10 (Doxorubicin+ HIIT+ Crocin 10 mg/kg/d), and DOX+ HIIT + Cr50 (Doxorubicin+ HIIT+ Crocin 50 mg/kg/d) during 8 weeks of study (n=6, for each group). Values are expressed as mean±SD. Significance differences of groups when compared to control group: a; p<0.05. Significant differences of groups when compared to DOX group: b; p<0.05; Significant differences of groups when compared to DOX+ HIIT + Cr50 group: c; p<0.05.

Figure 3 showed that doxorubicin consumption had a significant decrease in comparing with Control (P = 0.001), and DOX + HIIT+ Cr50 (P = 0.0001). Different between control group with DOX + Cr10 (P = 0.048) was significant. Also DOX + HIIT+ Cr50 had significant improvement compare with DOX + HIIT (P = 0.025), DOX + Cr10 (P = 0.022), DOX + HIIT+ Cr10 (P = 0.029). There was not significant different between other groups.

## Discussion

The results of the present study showed that doxorubicin injection was associated with a significant increase in PC compared to all groups, while concurrent use of Crocin 10 and 50 mg/kg/d and training, as well as Crocin 50 mg/kg/d group receiving doxorubicin injection caused the PC values to be modulated. However, 10 mg/kg/dof Crocin and training alone did not have such an effect (Figure 1). Another result of the study was the decrease in GPX antioxidant enzyme function in the DOX-treated group compared to all other groups. Due to the significant difference between the training group and the control group in GPX, it seems that training alone seems to have less effect on eliminating the adverse effects of doxorubicin on the status of this antioxidant than the other groups (Figure 2).

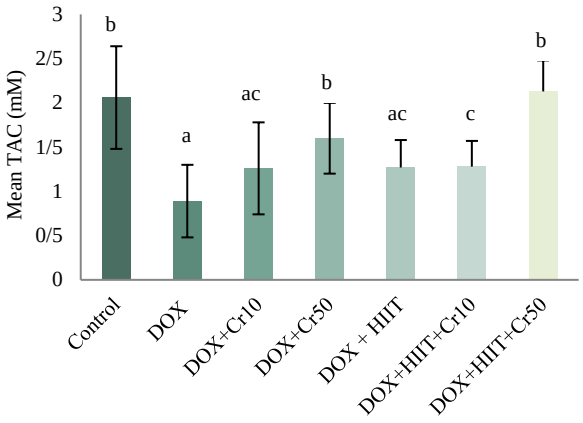


**Figure 3:** Effect of Crocin and HIIT on TAC level. DOX (Doxorubicin), DOX+ Cr10 (Doxorubicin + Crocin 10 mg/kg/d), DOX+ Cr50 (Doxorubicin + Crocin 50 mg/kg/d), DOX+ HIIT (Doxorubicin+ HIIT), DOX+ HIIT + Cr10 (Doxorubicin+ HIIT+ Crocin 10 mg/kg/d), and DOX+ HIIT + Cr50 (Doxorubicin+ HIIT+ Crocin 50 mg/kg/d) during 8 weeks of study (n=6, for each group). Values are expressed as mean±SD. Significance differences of groups when compared to control group: a; p<0.05. Significant differences of groups when compared to DOX group: b; p<0.05; Significant differences of groups when compared to DOX+ HIIT + Cr50 group: c; p<0.05.

However, the decreasing effect of doxorubicin injection on TAC was significant only in comparison with the control group and concurrent use of Crocin 50 mg/kg/d and training. Also, due to the lack of significant difference between the Crocin 10 mg/kg/d and the control groups, it can be concluded that Crocin 10 mg/kg/d alone could not attenuate the negative effects of doxorubicin on TAC and the effectiveness of other interventions was favorable (Figure 3); especially the concurrent use of 50 mg/kg/d Crocin and training on increasing TAC was significant compared to other interventions, showing the potential effects of taking this dose with training on the total antioxidant capacity.

Previous studies have also shown that 10 mg/kg DOX leads to irregularities in the germinal center of the epithelium, the basement membrane of the seminal vesicles, degeneration, depletion and destruction of germ cells, as well as a decrease in the diameter of the seminal vesicles and the germinal epithelium; this histopathological damage disrupts spermatogenesis (25). In this study, an increase in the protein oxidation

index of PC in the DOX-treated group indicates an increase in ROS in the testicular tissue, Studies also show that oxidative stress is a mechanism of DOX-induced toxicity in testicular tissue.

Despite the effects of ROS on testicular tissue, consumption of Crocin at a dose of 50 mg/kg/d resulted in a significant reduction in PC in the testicular tissue of rats poisoned with doxorubicin.

The findings of the present study showed the effect of Crocin in preventing oxidative damage to testicular tissue in doxorubicin-poisoned rats. Also in the present study, doses of 10 and 50 mg/kg/d Crocin had a significant effect on maintaining GPx levels and a dose of 50 mg/kg/d on TAC levels, which show dose-dependent effects of Crocin.

Crocin is a water-soluble carotenoid derived from the saffron plant and has several medicinal functions, including antioxidant effects. With seven double bonds, Crocin is more effective than many antioxidants, including alpha-tocopherol, and with polyphenols, it is even more effective than white wine (26).

Consistent with the present study, Bakhtiari et al. showed that the use of intraperitoneal injection of Crocin at a dose of 200 mg/kg/d in rats poisoned with cyclophosphamide (15 mg/kg/w) as a drug used in chemotherapy, reduced MDA levels in testicular tissue, indicating the antioxidant effects of Crocin (27).

Putnori et al. also studied the effect of Crocin at doses of 10 and 20 mg/kg/d for eight weeks on cyclophosphamide (15 mg/kg/w) toxicity. The results showed that cyclophosphamide significantly reduced $GP_X$ compared to the control group; also both doses of Crocin combined with cyclophosphamide injection increased the decreased levels of $GP_X$, which confirms the findings of the present study on the effectiveness of a dose of 10 mg of Crocin on $GP_X$ (15).

Kamali et al. investigated the effect of intraperitoneal injection of Crocin (200 mg/kg/d) on paraquat-induced oxidative stress (a compound for annual weed control) on testicular tissue in adult mice. The results showed that paraquat significantly reduces SOD levels and increases MDA levels. In contrast, Crocin consumption in the peritoneally injected paraquat group upregulated SOD levels through bcl2 expression and decreased MDA levels (28).

Roshankhah et al. also investigated the effect of Crocin at doses of 12, 25 and 50 mg/kg/d on oxidative stress induced by busulfan (a cancer treatment drug) in adult rats. Their results showed that busulfan significantly decreases TAC levels and increases MDA. Consumption of Crocin at all doses in the healthy group and in the busulfan-treated groups increased TAC and decreased MDA. In the present study, only Crocin at a dose of 50 mg/kg/d and not 10 mg/kg/d maintained TAC levels (20).

According to what was stated, it seems that values higher than 10 mg/kg/d are required to cause favorable changes in TAC levels as a result of the production of free radicals due to cancer treatment drugs. Likely, that one of the reasons for not observing any change in PC levels at a dose of 10 mg/kg/d Crocin is the lack of the same effect on TAC.

Studies show that saffron has modulatory effects on the immune system through two pathways of MAPK and its members (such as P38, c-Jun and N-terminal kinase) and the NF-κB signaling pathway as two important molecular targets to develop potentially known inflammatory and anti-inflammatory factors (29).

Saffron plant and its main bioactive components such as Crocin with effect on different parts of the signaling pathway of various transcription factors such as NF-κB and activator protein 1 as well as their downstream signaling pathways reduce factors such as MDA and increase SOD and CAT activity (29).

The results of the present study showed that eight weeks of HIIT significantly increased $GP_X$, decreased PC and non-significantly increased TAC compared to the DOX-treated group. Also, training alone had no advantage in the modulation of $GP_X$, TAC and PC compared to other interventions on the testicular tissue of doxorubicin-poisoned rats.

In line with the results of the present study, Kalantari et al.'s research on healthy mice showed that four weeks of swimming training alone could not reduce MD levels in the testicular tissue of male mice and only simultaneous training and alpha tocopherol use significantly reduced MD levels (30). Darash et al.'s study by examining the effect of moderate-intensity continuous training (MICT) and Crocin consumption on testicular tissue in DOX-injected rats showed that the training group had no certain superiority over other groups in reducing MDA and increasing SOD and CAT activity(31).

The study by Nikbin et al. also showed that aerobic activity in chlorpyrifos (CPF)-injected rats increased SOD activity in two weeks of activity, but in the fourth and sixth weeks, SOD activity decreased in the CPF-injected groups or groups without aerobic activity. Also, MDA-induced lipid peroxidation in testicular tissue improved with training in the CPF-

injected group, which was not statistically significant (32). Overall, the results of this study, as in the present study, did not show significant improvement for most CPF-induced injuries in trained rats.

Hossein and Somani investigated the effect of six and a half weeks of activity on ethanol-induced oxidative stress on the testicular tissue of Fisher mice. The results of their study showed that ethanol increased oxidative stress and decreased antioxidant activity, and training was associated with increased SOD and CAT activity and decreased MDA, which is not consistent with some results of this study (33).

In this study, TAC values increased in the training group, but the increase in $GP_X$ was not very favorable. Also, training had less decreasing effects on increased PC levels compared to other interventions.

Part of the discrepancy observed in the results may be related to the measurement of two different oxidative stress indicators, MDA and PC, each of which examines the resulting oxidative stress in two different sections (lipid peroxidation versus protein oxidation). Doxorubicin may also cause more oxidative stress (an increase of ~3.5 nmol/mg MDA versus 9.5 nmol/mg PC) in testicular tissue compared to ethanol, while these increased values lead to less effective interventions. The antioxidant results of training were almost similar in both studies.

Magalhães et al. investigated the effect of 60-minute endurance activity on oxidative damage induced by doxorubicin injection on testicular tissue in male rats in four groups: healthy control, doxorubicin control, training + saline and training + doxorubicin. Rats ran on a treadmill at moderate intensity five times a week for twelve weeks. The duration of injection was seven weeks, starting from the fifth week after the start of training at a dose of 2 mg/kg/week. The results showed a significant increase in MDA and a non-significant increase in PC due to doxorubicin injection, which is not consistent with the results of this study. Doxorubicin + training also increased MDA and PC compared to saline + training, but they were not different from the healthy control group, whereas, in the present study, DOX + training alone could not modulate PC values compared to the control group (9).

Observing contradictory results and non-reduction of PC values by training in the present study can be due to two reasons: first, different duration of doxorubicin injection in two studies (10 injections vs. 14 with the same dose) and so the increasing effect of additional doses on PC values in the present study caused training not to modulate the effect of oxidative stress. Second, the different intensity and volume of training in the two studies may be two factors influencing the results. Due to its nature, aerobic training leads to the production of free radicals, which may affect the amount of oxidative stress depending on the intensity, volume, age, type of tissue, etc (34).

Studies have yielded conflicting results from the oxidative stress of different training intensities on testicular tissue and the parameters of the male reproductive system. Some studies show that HIIT increases the production of free radicals more than continuous activity and can even impair male reproductive function in testicular tissue (5). Nonetheless, by equalizing the volume in the aerobic training protocol and intensity manipulation, Paes et al. showed that HIIT not only does not produce more free radicals than moderate-intensity training, but also increases antioxidant capacity and antioxidant SOD and GPx production (35).

Studies by Hajizadeh Maleki and Maleki et al. also showed a greater protective effect of HIIT on free radical production, antioxidant capacity and reproductive parameters of infertile men (10, 36).

Research showed that exercise promotes a variety of cellular and mitochondrial defense/remodeling systems that may be a reference for activating regeneration/clearance of autophagic forms. Compatibility in mitochondrial network remodeling including signaling mechanisms related to redox homeostasis, activating or deactivating the apoptotic death pathway, mitochondrial dynamics (biogenesis, fusion and fission), and activity-stimulated quality control may indicate dependent or non-dependent vital processes in reducing DOX toxicity (37).

The results of the present study also showed that training along with Crocin consumption at both doses of 10 and 50 mg/kg/d moderated the destructive effects of ROX on testicular tissue by reducing PC levels.

Also, the interactive effects of training and 50 mg/kg/d Crocin consumption on increasing the antioxidant TAC and GPx were significant, which shows the dose-dependent effects of consumption on these variables.

In confirmation of these findings, Darash et al. showed that concurrent MICT and use of Crocin at 50 mg/kg/d was associated with the most decreasing effects on MDA and the most increasing effects on SOD and CAT activity in the testicular tissue of rats compared to DOX-treated groups (31). Regarding the

results of Daresh et al.'s study and the present study, doxorubicin injection in rats elevated lipid peroxidation due to increased MDA and elevated protein oxidation due to increased PC. In both of these studies, the interactive effect of high-intensity and moderate-intensity aerobic training and Crocin use at a dose of 50 mg/kg/d was associated with the greatest reduction in oxidative stress induced by doxorubicin. Also, the increase in mean antioxidant levels of SOD, CAT, $GP_X$ and TAC due to the interaction of both training methods and 50 mg/kg/d Crocin consumption in the DOX-injected groups was higher than other interventions, although the modulating effect of HIIT and 50 mg/kg/d Crocin consumption on TAC and CAT (unpublished results) was Crocin dose-dependent.

Some antioxidants can counteract the adverse effects of free radicals, but their concurrence with activity is still shrouded in mystery.

We know that ROS increases during aerobic physical activity and the amount of increase depends on the intensity of the activity. The relationship between physical activity and ROS is inversely related to performance improvement in the form of an upside-down bell, meaning that a certain amount of activity can produce amounts of ROS that lead to improved performance and maximum adaptation. Supplementation stops physiological responses before ROS reaches a level where it can achieve maximal adaptive responses. On the other hand, if supplements are taken when the ROS concentration is associated with a decreased physiological response (overtraining) due to increased activity or physical activity, it can delay fatigue to improve performance and its resulting adaptations (21).

According to the results of this study, the rate of ROS production through DOX and physical activity had an adaptable response to Crocin supplementation, which caused the interaction of training and Crocin consumption to achieve effective physiological responses to oxidative and antioxidant stress.

The interactive effects of HIIT and Crocin consumption on oxidant / antioxidant redox balance have also been investigated in the heart and liver tissue of DOX-treated rats, indicating favorable interactions on decreasing MDA and increasing CAT and SOD (32, 33). This study showed for the first time that concurrent Crocin supplementation, especially at a dose of 50 mg/kg/d and HIIT training, with a synergistic effect, improves antioxidant defense indicators and reduces oxidative pressure in the testicular tissue of rats.

The present study had several limitations. The samples of this study were healthy rats. Oxidative and antioxidant factors such as MDA, SOD and CAT and sperm quality factors were not measured. It is suggested that a study with the same interventions be performed on cancer rats and different training protocols or intensities be evaluated.

## Conclusion

The clinical findings of this study showed a significant increase in carbonyl protein in the testicular tissue of rats in the doxorubicin-treated group compared to other groups, which could affect important functions such as reduced sperm quality (motility, morphology, DNA integrity and sperm count). Significant modulatory effects in the interaction of training and Crocin consumption showed the potential dose-dependent effects of Crocin consumption in favor of a higher dose of Crocin.

Therefore, saffron extract and physical activity can be used as a safe intervention in patients undergoing chemotherapy that are toxic to male organs. The results of this study may provide a perspective for the proper treatment of male patients by improving oxidative and antioxidant indices to use exercise and saffron extract to increase the effectiveness of treatment.

## Conflict of Interests

Authors have no conflict of interests.

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