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The Antioxidant Effects of Continuous Training with Crocin Consumption on Doxorubicin-induced Hepatotoxicity in Rats

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ABSTRACT

Introduction: Doxorubicin has been reported to cause liver damage, while physical exercise and crocin consumption could improve antioxidant defense. The present study aimed to investigate the antioxidant effects of continuous training with crocin consumption on the liver tissues of doxorubicin-poisoned rats.

Methods: This experimental study was conducted on 40 rats, which were divided into five groups of eight, including unhealthy control (Dox), crocin consumption, continuous training, continuous training with crocin consumption, and healthy control (saline). For eight weeks, groups 1-4 received 2 mg/kg of doxorubicin peritoneally seven times every Friday throughout the study period. Groups 1-4 received 10 mg/kg of crocin peritoneally every day, groups three and four performed five sessions of continuous training per week, and group five were only injected with 0.9% normal saline.

Results: Doxorubicin induction could significantly decrease superoxide dismutase (SOD) and catalase (CAT), while increasing *malondialdehyde* (MDA). Continuous training and crocin consumption could significantly increase SOD and CAT in the doxorubicin-poisoned rats ($P<0.05$). However, continuous training with crocin consumption had no interactive effects on the increasing of SOD and CAT in the doxorubicin-poisoned rats ($P>0.05$), while continuous training and crocin consumption could significantly reduce MDA in the doxorubicin-poisoned rats. Moreover, continuous training with crocin consumption had interactive effects on the reduction of MDA in the liver tissues of the doxorubicin-poisoned rats ($P<0.05$).

Conclusion: According to the results, continuous training with crocin consumption had interactive effects on the reduction of MDA in the liver tissues of doxorubicin-poisoned rats, while it had no interactive effects on the increasing of SOD and CAT.

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Introduction

The liver occupies about 3-5% of the body mass, and some of its most important activities include metabolizing and detoxifying chemical drugs, such as anti-inflammatory drugs, painkillers, chemotherapeutic and antidepressant agents, and contaminants that may cause cellular oxidative stress (1, 2). Doxorubicin is a drug that is used in chemotherapy and is considered to be a highly effective anthracycline antibiotic, which is known with the trademark of adriamycin. Doxorubicin is prescribed alone or in combination with other drugs for the treatment of various neoplasms (3). However, the production of various reactive oxygen species (ROSs) and induction of apoptosis in healthy organs (especially the liver) have been reported to occur during the course of treatment with this drug, limiting its use and increasing its challenges (4).

Oxidative stress and inflammation are considered to be the major causes of liver diseases, which ultimately lead to various types of cell death, such as apoptosis, necrosis, necroptosis, and autophagy, as well as vascular injury in the liver. Liver toxicity occurs in response to toxic reactions and chemotherapeutic drugs not only in the hepatocytes, but also in the endothelial cells, Kupffer cells, and satellite cells (5). Therefore, use of chemotherapeutic drugs such as doxorubicin may cause liver damage due to the induced toxicity, with the patterns of damage including necrosis, steatosis, fibrosis, cholestasis,

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and liver vascular injury (6). In this regard, some findings have indicated that regular physical exercise a few days per week at moderate intensity could enhance the antioxidant defense of the body and its physiological adaptation (7). However, other studies have suggested that physical exercise may increase the production of free radicals and their balance with antioxidants (8).

Another approach involves the use of herbal antioxidants in non-pharmacological treatments. Crocin is a water-soluble carotenoid, which constitutes approximately 3.5% of the dried stigma of saffron (9). Numerous studies have shown that the crocin found in saffron is a potential antioxidant owing to its carotenoid structure and is used in clinical therapies (10). The main therapeutic properties of this compound include anti-inflammatory, antioxidant, anticancer, and antitumor effects, which have received special attention from medical researchers (11).

Considering the inadequate studies regarding the interactive effects of antioxidants on physical exercise and crocin antioxidant supplementation with the liver tissues of doxorubicin-poisoned rats, the present study aimed to investigate the antioxidant effects of continuous training along with crocin consumption on the liver tissues of doxorubicin- poisoned rats.

Materials and Methods

This experimental study was conducted on 40 Wistar rats with the mean age of eight weeks and mean weight of 200-220 grams, which were obtained from Ahvaz Jondi Shapur Laboratory of Experimental and Proliferation Animals, Iran and maintained in clean and transparent cages in standard conditions at an ambient temperature (23+3°C) within a 12-hour light-dark cycle at 35±5% humidity. The animals had adequate ventilation and unlimited access to water and food for two weeks prior to the tests in order to adapt to the laboratory conditions.

The adaptation training program was performed on a rodent treadmill at the speed of 8-10 m/min and slope of 0°C for 5-10 minutes during 10 days. After two weeks of adaptation to the environment and training, the rats were randomly divided into five groups of eight, including unhealthy control (Dox), crocin consumption, continuous training, continuous training with crocin consumption, and healthy control (saline).

There were some limitations in the study of the incompatibility of the trained rats and mortality induced by doxorubicin injection, and these animals were excluded from the study and replaced by other rats. This study was performed in accordance with the ethical guidelines of the Ministry of Science and Research as adopted from Marvdasht Islamic Azad University (code: IR.IAU.M.REC.1398.013).

In this experimental study, 40 Wistar rats weighing approximately 220±20 grams were purchased and transferred to the laboratory in standard conditions. The animals went through an adaptation phase for seven days. In total, 40 rats were divided into five groups of eight, including unhealthy (Dox), crocin consumption, continuous training, continuous training with crocin consumption, and healthy (saline).

During the study, all the rats (n=32) were peritoneally administered with 2 mg/kg of doxorubicin (Belgian Abve Company), which was dissolved in normal saline. The drug was administered seven times every Fridays (48 hours after the last training session and 24 hours before the next session). The rats in groups 2-4 received 10 mg/kg of crocin (Sigma-Aldrich Co., St. Louis, MO, USA) via oral gavage daily, and the healthy and doxorubicin groups received the same amount of normal saline via gavage (12, 13). In order to similarize the conditions of the subjects and neutralize the effects of injection on the animals in group five, equal amounts of saline (0.9% sodium chloride) was administered. The animals in groups three and four performed continuous training five sessions per week. In order to initiate and perform the continuous training protocol in the present study, the rats ran 10 minutes on an animal treadmill at the speed of 5 m/min and slope of 0° for one week.

The main part of the continuous training was conducted using an animal treadmill in a one-hour session per day five days a week for eight weeks. The first week of the main training began with 40% of the maximum running speed. From the second to the fourth week, the speed reached 50-55% of the maximum running speed, and from the fifth to the eighth day, it reached 60% of the maximum running speed (14). The maximum running speed was measured using the incremental exercise test protocol. Initially, the rats started running at the speed of 10 meters per minute, and to feel fatigue, the running speed increased 1.7 meters per minute every two minutes until the rats were exhausted.

Exhaustion was considered when the rats touched the bottom of the canal five times in one minute.

The duration of each exercise training session was one hour, with the warm-up program performed at the beginning of each training session, consisting of five minutes of running at 7 m/min. Moreover, cooling down was performed at the end of the exercise through the stepwise reduction of the speed to 7 m/min at the end of each training session (15). It is also notable that in order to investigate the effects of doxorubicin on the study variables, the remaining eight rats were assigned to the healthy control group, and 24 hours after the last training session at the end of the eighth week, the rats underwent surgery in order to measure the studied parameters. To this end, the rats were anesthetized by 10% ketamine and 2% xylazine after approximately five minutes. Following that, their liver tissues were extracted by specialists. A cryotube was inserted into liquid nitrogen, and preserved at the temperature of -70˚C for further examination. Finally, catalase (CAD), superoxide dismutase (SOD), and malondialdehyde (MDA) levels were measured using ELISA, ZellBio GmbH (ZB-CAT-96A at 0.5 kU/l sensitivity), ZB-SOD-96A at 1KU / L sensitivity), and ZB-MDA-96A kits (0.1 µM sensitivity), respectively.

To investigate the normality of the data distribution, the Shapiro-Wilk test was used. To analyze the findings, independent sample t-test and two-way analysis of variance (ANOVA) were applied (P≥0.05).

Results

The levels of SOD, CAT, and MDA are presented in Figures 1-3, respectively.

The results of independent sample t-test (Table 1) indicated that SOD (P=0.001) and CAT levels (P=0.001) significantly decreased in the control group compared to the healthy control group. However, the MDA levels significantly increased (P=0.001).

According to the results of two-way ANOVA (Table 1), eight weeks of continuous training and crocin consumption could significantly increase SOD in the liver tissues of the doxorubicin-poisoned rats. On the other hand, continuous training concurrent with crocin consumption had no interactive effects on the increased SOD in the liver tissues of the doxorubicin-poisoned rats.

According to our findings, eight weeks of continuous training and crocin consumption could significantly increase CAT in the liver tissues of the doxorubicin-poisoned rats, while continuous training concurrent with crocin consumption had no interactive effects on the increasing of CAT in the liver tissues of the doxorubicin-poisoned rats. Furthermore, eight weeks of continuous training and crocin consumption could significantly decrease MDA in the doxorubicin-poisoned rats, while continuous training along with crocin consumption had interactive effects on the reduction of MDA in the liver tissues of the doxorubicin-poisoned rats.

Table 1. Results of Two-way ANOVA and Independent Sample T-test on Effects of Doxorubicin, Continuous Training, and Crocin Consumption on SOD, CAT, and MDA

Parameter	Independent Sample T-test		Two-way ANOVA								
			Training			Crocin Consumption			Interactive Effects		
	t	P-value	F	P-value	Effect Size	F	P-value	Effect Size	F	P-value	Effect Size
SOD	25.24	0.001*	105.91	0.001€	0.79	32.93	0.001¥	0.54	0.21	0.65	0.007
CAT	26.75	0.001*	251.07	0.001€	0.90	91.45	0.001¥	0.76	3.25	0.08	0.10
MDA	35.16	0.001*	933.15	0.001¥	0.97	597.99	0.001¥	0.95	269.82	0.001£	0.90

*Significant difference between healthy control group and control group; €: significant effect of continuous training on increased CAT and SOD and decreased MDA; ¥: significant effect of crocin on increased CAT and SOD and decreased MDA; £: significant interaction of continuous training and crocin on decreased MDA

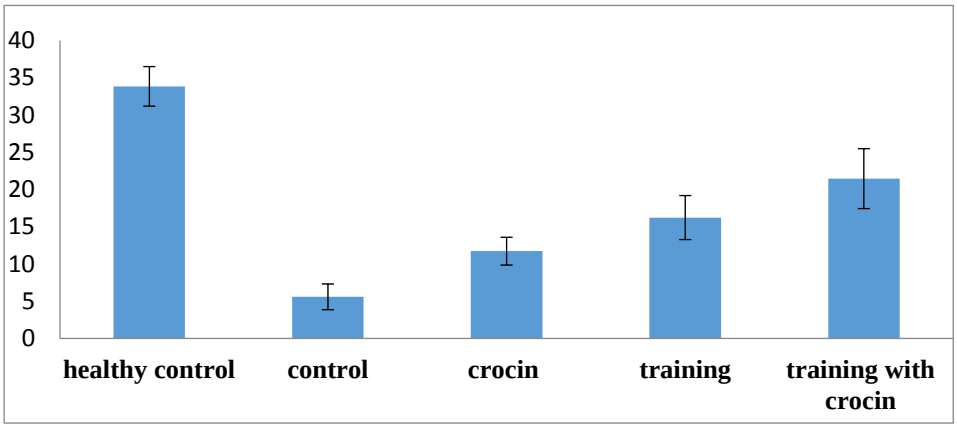

Figure 1. SOD (mg of protein) Levels in Study Groups

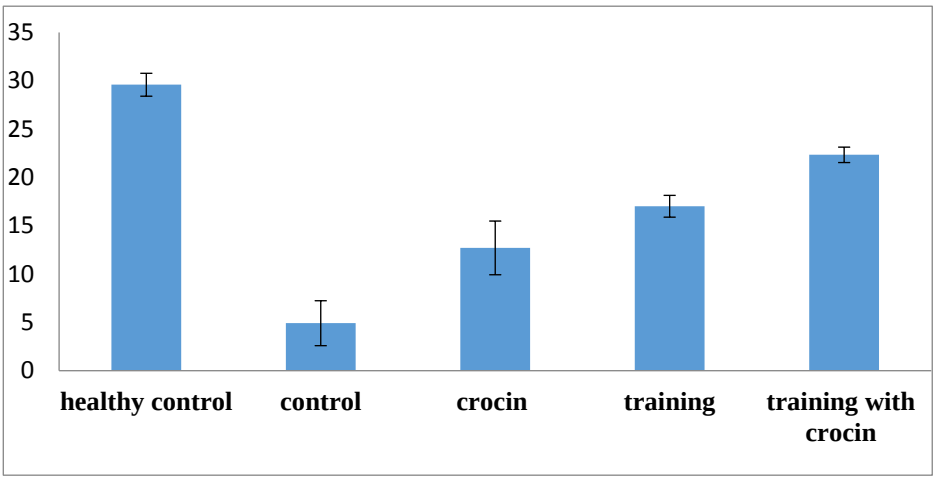

Figure 2. MDA (nmol/mg protein) Levels in Study Groups

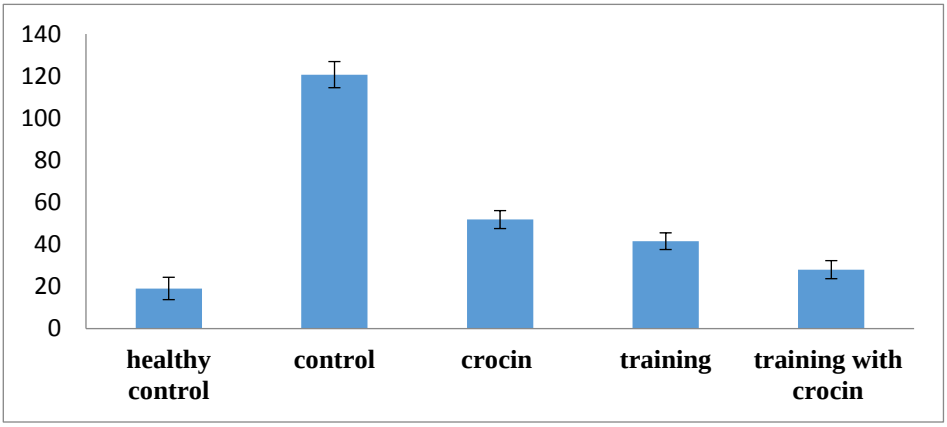

Figure 3. CAT (mg of protein) Levels in Study Groups

Discussion

The present study aimed to investigate the antioxidant effects of continuous training and crocin consumption on the liver tissues of doxorubicin-poisoned rats. According to the findings, doxorubicin induction significantly

increased MDA and significantly decreased SOD and CAD in the liver tissues of the rats. Consistent with our findings, doxorubicin has been reported to significantly increase MDA and significantly reduce SOD, which is an influential factor in oxidative stress and liver toxicity in rats (16). Furthermore, previous studies have denoted that the acute induction of various doses of doxorubicin could cause a marked decrease in CAT (17).

According to the results of the present study, eight weeks of continuous training significantly decreased MDA concentrations and significantly increased SOD and CAT concentrations in the liver tissues of the doxorubicin-poisoned rats. Moderate-intensity exercise is beneficial to health and disease prevention through reducing the production of oxidative stress (18). In addition, physical activity enhances the antioxidant defense and reduces lipid peroxidation in middle-aged and elderly individuals. Moderate-intensity exercise also has beneficial effects on the reduction of oxidative stress (19).

Several factors contribute to the induction of oxidative stress during exercise, including the type, intensity, and duration of exercise, individual characteristics, gender, nutrition, and genetics (20). Following three weeks of aerobic training, we observed a significant increase in the levels of glutathione peroxidase (GPX), as well as a significant decrease in the MDA and carbonyl protein levels in the liver of the doxorubicin-induced rats. Aerobic exercise has been shown to regulate hepatotoxicity indices and could be considered as a non-pharmacological approach for disease treatment (21).

In another study in this regard, the effects of three and six weeks of aerobic training on the liver tissues of doxorubicin-induced rats were compared, and no significant differences were reported between the training sessions and CAT. However, a significant increase was observed in CAT, indicating the reduction of oxidative stress in the liver tissues (22). Moreover, six weeks of aerobic training with doxorubicin induction significantly increased nitric oxide and SOD, while significantly decreasing the MDA, indicating the adaptation and protection of exercise effects on cytotoxicity (23).

Concerning the consistency of our findings with the results of the previous studies in this regard, it could be concluded that continuous exercise reversed the imbalance between the pre-oxidant and oxidant reactions due to doxorubicin induction in the liver tissues of the rats. One possible mechanism is the beneficial effects of exercise and training on the ROS and activation of the NF-KB and MAPK pathways in cells, leading to the increased production of antioxidant enzymes, such as SOD, glutathione reductase (GR), and CAT (24).

The activation of these pathways activates antioxidant enzymes such as manganese superoxide dismutase in the mitochondria (MnSOD or SOD2), as well as the copper-zinc superoxide dismutase in the cytosol and nucleus (Cu Zn SOD or SOD1), which convert peroxidase anion into hydrogen peroxide (H_2O_2), GR, and CAT, transferring hydrogen to water (25). On the other hand, researchers have recommended the strategy of using herbal medicines and supplements along with chemotherapy. Evidence suggests that crocin consumption could reduce the adverse effects of chemotherapy with doxorubicin.

In the present study, eight weeks of using 10 mg/kg of daily crocin significantly decreased the MDA concentration and significantly increased the SOD and CAT concentrations in the liver tissues of the doxorubicin-induced rats. Consistent with our findings, a similar study suggested that crocin supplementation significantly reduced the MDA and significantly increased SOD and CAT antioxidant enzymes in the cardiac tissues of doxorubicin-poisoned rats. In addition, crocin was reported to have protective effects against the damage induced by this drug (26).

According to the literature, the consumption of crocin in the rats exposed to hepatic ischemia-reperfusion could increase cardiac tissue antioxidant activity through the improvement of THE SOD, CAT, and GPX enzymes and protecting the heart against ischemia-reperfusion injury (27). This is in line with the findings of the current research, which indicated that crocin traps free radicals with its specific carotenoid structure, thereby acting as a potential antioxidant.

According to the results of the present study, continuous training along with crocin supplementation in the doxorubicin-induced rats decreased MDA, while no significant changes were observed in the SOD and CAT antioxidant enzymes. Regarding the antioxidant effects of

physical exercise and crocin consumption on intoxication with doxorubicin, our findings could be compared to no studies. Therefore, further investigations are required to obtain more accurate data on the mechanism of the interactive effects of exercise and crocin consumption.

Conclusion

According to the results, performing regular exercise for eight weeks could improve the antioxidant/oxidant balance and decrease doxorubicin-induced toxicity through increasing the SOD and CAT levels and decreasing the MDA oxidant index. In addition, crocin supplementation as an antioxidant alone improved the antioxidant/oxidant balance in favor of reducing the oxidative stress induced by doxorubicin, while concurrent regular exercise and crocin supplementation had no effects on the antioxidant system and only reduced MDA due to doxorubicin induction in the liver tissues of the rats, which requires further investigation.

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