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# The Study of Changes in Oxidative Stress-Cardiac Antioxidants Following the Use of *Tribulus Terrestris* Extract and Crocin After One Session of Exhaustive Exercise in the Heart Tissue of Elderly Male Rats

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ABSTRACT

**Introduction**: This study aimed to evaluate the changes in cardiac oxidative stress-antioxidants following the use of *tribulus terrestris* extract and crocin after exhaustive exercise in the heart tissue of elderly male rats.

**Methods**: In this study, elderly male Wistar rats with an average weight of 300 g and an age range of 14 to 18 months were used as the statistical population. A total of 56 rats were selected as a statistical sample and randomly divided into seven groups of 8, including control, exhausting exercise, exercise + crocin dose of 50 mg, exercise + crocin dose of 100 mg, exercise + thrush dose of 100 mg, exercise + thrush dose of 200 mg, exercise + tortoises were divided into 400 mg doses.

**Results**: The results showed that GPX concentration in training groups with doses of 50 and 100 crocin was significantly higher than in the vulnerable training group (p<0.05). In addition, GPX concentration was significantly higher in the groups with 50 and 100 doses of crocin than in the exercise groups with 100 and 200 doses of thistle (p<0.05). GPX was significantly higher in the exercise group and crocin 100 than in the exercise group and thrush 400 (p<0.01). The results also showed that SOD concentration in training groups with doses of 50 and 100 crocin was significantly higher than in the specific exercise group (p<0.01). SOD concentration was significantly higher in the exercise groups with 50 and 100 doses of crocin than in the groups with 100 and 200 doses of thistle (p<0.05). Also, SOD was significantly higher in the exercise group and crocin 100 than in the exercise group and thrush 400 (p<0.017).

**Conclusion:** Therefore, using of antioxidants is recommended to improve heart tissue function in the elderly.

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## Introduction

Cardiovascular diseases (CVDs) are the leading cause of death in developed countries, representing one-fourth of death is due to this disease. Economically, the cost of this disease has been estimated to be 400 million dollars (1). This disease is considered the most important disease of the elderly period, starting progressively from childhood, the clinical symptoms of which are seen in adulthood from the middle age period onward (2). Rehabilitation and arteriosclerosis treatment are highly costly, causing high amounts of financial and fatal damage, so taking protective strategies against this disease seems compulsory (3). Based on empirical research, critical factor in chronic disease pathogenesis such as arteriosclerosis, diabetes, and cancer, is the process of oxidative stress since producing different types of oxygen causes organism biological damage and potentially worsens these complications (4).

Further, there is a close relationship between active oxygen production and antioxidant system analysis. The primary and most frequent enzymatic antioxidants include catalase, and glutathione peroxidase, superoxide dismutase (SODs). In addition, vitamins like E, A, and C can also function as antioxidants (5).

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Superoxide dismutase (SODs) play a vital role in metabolizing radical superoxide O2, restricting and finalizing chain reactions of oxidants. These enzymes prevent devastating cascade reactions of reactive oxygen species (ROS). Glutathione is the most abundant non-protein, thiol compound with a low molecular weight, playing a significant role in cellular defense against oxidative stress. Moreover, glutathione plays a part in gene expression adjustment, signal transfer, cellular proliferation and death, cytokine production, and safety response (6). On the one hand, regular physical exercise can increase resistance against oxidative stress and control cellular damage by reducing levels of free radicals in the body and improving the antioxidant system (7). According to a study 12 weeks of resistance exercise could increase antioxidant enzymes in older adults (7). Further, research has demonstrated that resistance practice reduces oxidative pressure in skeletal muscles and other body tissues by increasing the activities of enzymes CAT and GPX. For example, Da Cunha et al reported that physical exercise could play a protective role against oxidative imbalance and brain damage due to homocysteine induction by increasing antioxidant activities of enzymes SOD, CAT, and GPX in the rats' brain membranes (8).

Various research studies conducted on the effects of different activities such as pedaling on the static bicycle, running on the treadmill, dynamic resistance practice, eccentric practice and sprinting practice have led to the conclusion that the type and intensity of physical activity may influence oxidative system responses of the body (9).

On the other hand, plant substances include various combinations with antioxidant activities. Different plants as potential resources of sound, natural antioxidants have been studied for food industry, and multiple varieties of them have been separated, some of which are polyphenolic combinations. Many polyphenols with low molecular weight and excessive extraction have antioxidant characteristics, so they have been suggested as protection against lipid oxidation (10).

Saffron and its effective constituents have antitumor, antioxidants, anti-genotoxic, memory and learning enhancer, anti-nociceptive and anti-inflammatory, anticonvulsant, anti-depression, blood pressure reducers, tissue oxygen enhancers, bronchodilators, and anti-cough effects (11,12).

In addition, recent laboratory research findings indicated that saffron and its effective substances could considerably decrease oxidative damage in renal ischemic tissues (13), skeletal muscles (14), heart (15), and brain (16).

Kamboj et al. (2011) revealed that *Tribulus terrestris* could decrease oxidative stress markers in tissues like kidneys (17). Prescribing plants can reduce blood sugar levels and restore lipid peroxidation markers, including a decrease in glutathione peroxidase and superoxide dismutase and an increase in malondialdehyde levels (18).

Given the preceding lines and considering contradictions about the effects of progressive physical exercise in older adults on antioxidant adaptations and oxidative stress, and regarding ambiguities in this research, and given that most existing research has investigated the effects of resistance exercise on oxidative stress, and analysis on the impact of progressive aerobic exercise on the markers of oxidative and antioxidant enzymes especially among older adults is scanty, the present study explored the changes in oxidative stress-cardiac antioxidants following the use of *Tribulus terrestris* extract and crocin after one session of exhaustive exercise in the heart tissue of elderly male rats.

## Methods

This descriptive and correlational study was conducted on 56 elderly male rats of the Wistar breed with an average weight of 300g, aged between 14 to 18 months. All the rats were in good physical condition and had not used drugs. The rats were randomly divided into seven groups of 8.

The rats were kept under laboratory conditions with temperatures of 22 to 24°C, a humidity of 60%, and a light-dark cycle of 12 hours until the experiments and physical exercise sessions were completed. (The time of light was from 7a.m. to 7p.m.) The purpose of this cycle was to create normal conditions for the rats. All the animals had free access to standard food and drink, exceptional for laboratory animals. After one week, the rats were placed on the treadmill for three days to familiarize themselves with practice conditions.

The protocol for supplement giving was in such a way that the groups of crocin consumption received 50 and 100 mg/kg of dissolved crocin in normal saline (19). *Tribulus terrestris* groups received 100, 200, and 400 mg/kg of dissolved *Tribulus terrestris* hydroalcoholic extract in normal saline peritoneally (20).

The rats in the exercise group were instructed to exercise exhaustively for one hour after injecting herbal medicines and two hours after undergoing herbal medicine injections. The subjects warmed up for 3 to 5 minutes at 8m/min. Then, they started running at the speed of 20 m/min with a slope of zero in the first 10 min, and gradually increased their speed up to 35m/min until the rats reached the exhaustion level was recognized by comparing the shocking part of the treadmill within two minutes (21).

The rats were anesthetized using ketamine (50mg/kg) and xylazine (25mg/kg), and specialists took their heart tissues immediately after practice. The laboratory tissues were preserved at 80°C below zero to experiment at the appropriate time. Measuring superoxide dismutase (SOD) and glutathione peroxidase (GPX) was done using the histophotometric method.

After tissue removal, the rats were placed in unique bags and placed in a special incinerator for making the corpses of laboratory rats, and their ashes were transferred to Imam Khomeini Hospital in Marvdasht, Iran.

### *Statistical Analysis*

In the present study, one-way ANOVA was used to analyze the variables. Further, the Shapiro-Wilk test was run and shown to be normal to check the data's normal distribution. Levene's test was used to check the homogeneity of group variance.

**Table 1.** Description of GPX (ηmol/min/ml) in the Groups

| Descriptive Statistics | | | | | |
|---|---|---|---|---|---|
| **Group (Dose)** | **Number** | **Mean** | **Standard Deviation** | **Minimum** | **Maximum** |
| Control | 8 | 0.22 | 0.035 | 0.14 | 0.25 |
| Exhaustive Exercise | 8 | 0.11 | 0.037 | 0.05 | 0.16 |
| Exercise and tribulus terrestris 100 | 8 | 0.12 | 0.072 | 0.02 | 0.21 |
| Exercise and tribulus terrestris 200 | 8 | 0.14 | 0.093 | 0.01 | 0.28 |
| Exercise and tribulus terrestris 400 | 8 | 0.20 | 0.10 | 0.10 | 0.32 |
| Exercise and Crocin 50 | 8 | 0.27 | 0.10 | 0.14 | 0.41 |
| Exercise and Crocin 100 | 8 | 0.37 | 0.15 | 0.16 | 0.63 |

**Table 2.** Description of SOD (U/ml) in the Groups

| Descriptive Statistics | | | | | |
|---|---|---|---|---|---|
| **Group (Dose)** | **Number** | **Mean** | **Standard Deviation** | **Minimum** | **Maximum** |
| Control | 8 | 398.16 | 47.34 | 295 | 445 |
| Exhaustive Exercise | 8 | 295.16 | 59.39 | 204 | 374 |
| Exercise and tribulus terrestris 100 | 8 | 321.08 | 55.56 | 248 | 420 |
| Exercise and tribulus terrestris 200 | 8 | 306.12 | 87.74 | 173 | 449 |
| Exercise and tribulus terrestris 400 | 8 | 382.38 | 92.99 | 264 | 507 |
| Exercise and Crocin 50 | 8 | 430.36 | 63.93 | 355 | 555 |
| Exercise and Crocin 100 | 8 | 497.97 | 37.45 | 432 | 553 |

**Table 3.** Results of One-way ANOVA Variation Differences of GPX (ηmol/min/ml) in the Groups

| Source of Variation | Sum of Squares | DF | Mean Squares | F Value | P-value |
|---|---|---|---|---|---|
| Between Group | 0.41 | 6 | 0.068 | 7.85 | 0.0001 |
| Within Group | 0.43 | 49 | 0.009 | | |
| Total | 0.84 | 55 | | | |

## Results

The mean and standard deviation of the variables GPX and SOD in different groups are presented in Tables 1 and 2, respectively. Further, the results of the one-way ANOVA and Levene's test of GPX are displayed in Table 3 and Table 4. Moreover, Tables 5 and 6 show the results of the one-way ANOVA and Levene's test of SOD.

The GPX concentration and SOD decreased significantly due to exhaustive exercise, compared to the control group ($p > 0.044$). The results showed that the GPX concentration in the practice group and the consumption of crocin 100 were significantly higher than the control group ($p < 0.035$). The concentration of SOD and GPX in the practice groups with doses of 50 and 100 crocin was significantly higher than in the specific exercise group and the practice groups with doses of 100 and 200 *Tribulus terrestris* ($p<0.05$).The concentration of GPX and SOD in the practice group and crocin 100 were significantly higher than the practice group and *Tribulus terrestris* 400 ($p<0.01$).

**Table 4.** Pair Comparisons between Groups

| Pair Comparisons | | Mean Differences | Standard Error of Measurement | P-value |
|---|---|---|---|---|
| **Control** | Exhaustive Exercise | 0.10 | 0.047 | 0.044 |
| | Exercise and tribulus terrestris 100 | 0.10 | 0.047 | 0.09 |
| | Exercise and tribulus terrestris 200 | 0.08 | 0.047 | 0.63 |
| | Exercise and tribulus terrestris 400 | 0.02 | 0.047 | 0.99 |
| | Exercise and Crocin 50 | -0.05 | 0.047 | 0.93 |
| | Exercise and Crocin 100 | -0.15* | 0.047 | 0.035 |
| **Exhaustive Exercise** | Exercise and tribulus terrestris 100 | -0.005 | 0.047 | 0.99 |
| | Exercise and tribulus terrestris 200 | -0.03 | 0.047 | 0.99 |
| | Exercise and tribulus terrestris 400 | -0.09 | 0.047 | 0.49 |
| | Exercise and Crocin 50 | -0.16* | 0.047 | 0.020 |
| | Exercise and Crocin 100 | -0.26* | 0.047 | 0.0001 |
| **Exercise and tribulus terrestris 100** | Exercise and tribulus terrestris 200 | - 0.026 | 0.047 | 0.98 |
| | Exercise and tribulus terrestris 400 | - 0.08 | 0.047 | 0.57 |
| | Exercise and Crocin 50 | -0.15* | 0.047 | 0.027 |
| | Exercise and Crocin 100 | -0.25* | 0.047 | 0.0001 |
| **Exercise and tribulus terrestris 200** | Exercise and tribulus terrestris 400 | - 0.057 | 0.047 | 0.88 |
| | Exercise and Crocin 50 | - 0.13 | 0.047 | 0.04 |
| | Exercise and Crocin 100 | -0.23* | 0.047 | 0.0001 |
| **Exercise and tribulus terrestris 400** | Exercise and Crocin 50 | - 0.073 | 0.047 | 0.71 |
| | Exercise and Crocin 100 | -0.23* | 0.047 | 0.01 |
| **Exercise and Crocin 50** | Exercise and Crocin 100 | - 0.10 | 0.047 | 0.11 |

**Table 5.** Results of One-way ANOVA Variation Differences of SOD (U/ml) in the Groups

| Source of Variation | Sum of Squares | DF | Mean Squares | F Value | P-value |
|---|---|---|---|---|---|
| **Between Group** | 261810 | 6 | 43635 | 9.95 | 0.0001 |
| **Within Group** | 214840 | 49 | 43384 | | |
| **Total** | 476650 | 55 | | | |

**Table 6.** Pair Comparisons between Groups

| Pair Comparisons | | Mean Differences | Standard Error of Measurement | P-value |
|---|---|---|---|---|
| **Control** | Exhaustive Exercise | 102.55* | 33.11 | 0.047 |
| | Exercise and tribulus terrestris 100 | 77.08 | 33.11 | 0.25 |
| | Exercise and tribulus terrestris 200 | 92.03 | 33.11 | 0.10 |
| | Exercise and tribulus terrestris 400 | 15.78 | 33.11 | 0.99 |
| | Exercise and Crocin 50 | -32.20 | 33.11 | 0.95 |
| | Exercise and Crocin 100 | -99.81 | 33.11 | 0.058 |
| **Exhaustive Exercise** | Exercise and tribulus terrestris 100 | -25.47 | 33.11 | 0.99 |
| | Exercise and tribulus terrestris 200 | -10.52 | 33.11 | 0.99 |
| | Exercise and tribulus terrestris 400 | -86.77 | 33.11 | 0.14 |
| | Exercise and Crocin 50 | -134.76* | 33.11 | 0.003 |
| | Exercise and Crocin 100 | -202.36* | 33.11 | 0.0001 |
| **Exercise and tribulus terrestris 100** | Exercise and tribulus terrestris 200 | 14.95 | 33.11 | 0.98 |
| | Exercise and tribulus terrestris 400 | - 61.30 | 33.11 | 0.52 |
| | Exercise and Crocin 50 | - 109.28* | 33.11 | 0.028 |
| | Exercise and Crocin 100 | - 176.89* | 33.11 | 0.0001 |
| **Exercise and tribulus terrestris 200** | Exercise and tribulus terrestris 400 | - 76.25 | 33.11 | 0.26 |
| | Exercise and Crocin 50 | - 124.24* | 33.11 | 0.008 |
| | Exercise and Crocin 100 | - 191.85* | 33.11 | 0.0001 |
| **Exercise and tribulus terrestris 400** | Exercise and Crocin 50 | -47.98 | 33.11 | 0.77 |
| | Exercise and Crocin 100 | -115.59* | 33.11 | 0.017 |
| **Exercise and Crocin 50** | Exercise and Crocin 100 | - 67.60 | 33.11 | 0.40 |

## Discussion

The present study showed a significant difference in the GPX concentration between the control and specific exercise groups.The GPX concentration after exhaustive exercise significantly decreased compared with the control group. The GPX concentration in the practice groups with doses of 50 and 100 crocin was significantly higher than in the specific exercise group. In addition, the GPX concentration in practice groups with doses of 50 and 100 crocin was higher than in those with doses of 100 and 200 tribulus terrestris. The GPX concentration in the practice group and crocin 100 were significantly higher than the practice group and *Tribulus terrestris* 400. Apart from internal antioxidants specifically regulated by physical activity, used antioxidants (external) such as vitamins E and C, carotenoids and herbal medicines are utilized as a part of the diet or different supplements.

Ristow et al. (2012) experimented with sensitivity to exercise-induced insulin among healthy men without exercise and with the previous exercise. They found that both groups increased insulin-sensitive parameters such as adiponectin only in the absence of antioxidants. The consumption of antioxidants such as vitamins C and E prevented this increase. Meanwhile, prescriptive regulators sensitive to active types of insulin sensitivity and antioxidant capacity, PGG-1α, PPARγ, activator PPARγ, and PGG-1β are activated only in the absence of antioxidants. In addition, the consumption of antioxidants blocked the activating path of molecular mediators of antioxidant defense (SOD and GPX). These researchers concluded that exercise-induced oxidative stress reduced insulin resistance, and created adaptation through increasing antioxidant capacity. The consumption of antioxidants may jeopardize this health-improving path obtained with exercise (22).

Therefore, physical activity is a double-edged sword. When physical activity is performed at average intensity, it may express antioxidant enzymes, which are counted as an antioxidant per se.

However, high-intensity exercise may cause oxidative stress and muscle injuries. In this condition, the consumption of antioxidants may be helpful. There are controversies about the second condition; sometimes, using antioxidants has decreased oxidative stress and increased oxidative stress (23). For example, using crocin as an antioxidant in our study affected the GPX enzyme.

The present study proved the antioxidant effects of crocin in increasing peroxidase glutathione. In another study, Sheng and colleagues (2006) showed that crocin in rats with doses of 100 mg/kg per day decreased triglycerides, total cholesterol, LDL, and VLDL, compared with the control group. Research has shown that lipase crocin can control the pancreas and stomach, but its mechanism is unknown (24). Mehdizadeh et al. studied the effects of safranal and saffron extract on isoproterenol-induced myocardial infarction in Wistar rats. The section of Saffron and safranal caused less increase in the levels of lactate dehydrogenase, CK-MB, and lipid peroxidation of myocardial and malondialdehyde compared with the control group and myocardial injury occurred less. These effects result from regulating oxidative stress (25). Oxidative stress is the joint response of cells or tissues to physical exercise. However, it does not mean that all tissues respond equally to similar physical exercise. ROS production levels vary throughout the tissues and cells, even during rest. During physical activity, the skeletal muscles of the heart and the liver are of utmost importance in the antioxidant balance of the body because they consume high amounts of oxygen and play a part in metabolic processes. The increase in oxidative stress may be effective in the pathogenesis of vascular and cardiac diseases. An increase in the formation of free radicals for oxidative and defense can cause oxidative damage in the cells of the heart and aorta (26). Crocin increases intercellular glutathione expression and can induce its antioxidant effects through connection with ferrous.

The induction of heme oxygenase enzyme 1 (HOMX-1) also protects against oxidative pressures. Various research studies have revealed that crocin has extensive biological functions, especially antioxidant and anti-inflammatory functions. However, the period of the effective use of crocin to prevent intense exercise-induced oxidative stress in different functional tissues of the body during exercise is not precisely known (27).

Moreover, the results of this study indicated a significant difference between SOD concentration in the two groups of control and exhaustive exercise, and a significant decrease accompanied SOD concentration due to exhaustive exercise compared with the control group. The SOD concentration in the practice groups with doses of 50 and 100 crocin was significantly higher than in the exhaustive exercise group. SOD Concentration in exercise groups with amounts of 50 and 100 crocin was significantly higher than in exercise groups with doses of 100 and 200 *Tribulus terrestris*. SOD concentration in the practice group and crocin 100 was significantly higher than in the practice group and *Tribulus terrestris* 400. Oxidative stress was vital in developing chronic and erosive diseases such as cancer, arthritis, autoimmune diseases, old age, and vascular and cardiac diseases (28). In the meantime, physical exercise increases the production of free radicals (FR) and reactive oxygen species (ROS), and excessive production of ROS can lead to lipid oxidation damage, proteins, and DNA, which is technically called exercise-induced oxidative stress. The body of humans and living creatures in exposure and reaction to FR to decrease the devastating effects of these reactive species using protective systems (antioxidants) such as superoxide dismutase enzymes (SOD), catalase (CAT), and glutathione peroxidase (GPX) (29).

In contrast to acute physical exercise, some studies have shown that regular physical exercise with alternate frequency (practice) can develop the antioxidant defense capability of the body. The best option in preventing or postponing acute exercise-induced oxidative stress (30). However, there is no unanimous agreement on the type of physical exercise, which is influential in the emergence of oxidative stress, as well as on the nature of the physical activity (29), and more research is required to be conducted in this area. For instance, Ravan et al. (2011) showed that aerobic exercise (with practice intensity of 50 to 70% saved heartbeat) with a decrease in CAT and no change in GPX marker, had no significant effect on the levels of oxidative stress markers among 24 young college students (30). In contrast, the results of Miyazaki et al. (2011), which included running aerobic exercise (with an intensity of 80% used oxygen) on nine inactive subjects, indicated a decrease in lipid peroxidation markers (TBARS), no change in CAT, and an increase in the markers of SOD, and GPX (31).

The findings suggested that using the crocin supplement and exhaustive exercise can reduce the consequences of such activities. Likewise, the combination of crocin and intense alternate practice can protect heart tissues exposed to doxorubicin induction against oxidative stress, and can cause a significant decrease in malondialdehyde levels and a significant increase in the activity of superoxide dismutase anti-oxidative enzymes and catalase (32). The rise in peroxidation markers in older adults may result from a decrease in mitochondria function, leading to an increase in the production of ROS (31). The body's antioxidant defense system combats oxidative stress. Reviewing the related literature reveals mixed results. For example, Miyazaki et al (2001) reported an increase in the activity of SOD enzyme after three months of aerobic exercise, and no change in response to one session of exhaustive activity among inactive people (31). On the contrary, Akkus (2011) stated that a period of regular aerobic exercise does not make any significant changes in the activity of SOD in non-athlete men and women, but one session of exhaustive aerobic activity could cause a significant increase in SOD (33). The gender of the subjects, age range, the measurement methods of the desired marker, and the gradual increase in the intensity and duration of the exercise program are among the main reasons for the differences between the findings of the present study and those of Akkus. The lack of research on aerobic exercise's effect on saliva antioxidant markers makes it difficult to compare research results. Exercise and practice have been shown to increase the expression of antioxidant enzymes, producing ROS (34). In addition, the messaging cascade created by the nuclear factor E2 related to factor-2 (Nrf-2) can regulate many antioxidants' expressions such as glutathione reductase (GR, GPX, CAT), and SOD (35). Then the increased levels of antioxidants' were used to reduce the devastating effects of ROS. The nature of the physical activity (the exercise period, type, intensity, and duration of physical exercise) has a vital role in the increasing regulation of GPX, and the more intense the physical activity and the longer the exercise period, the more increase in the activity of this enzyme (34). Part of the differences in human and animal blood GPX levels can be attributed to the concentration and the slight amount of animal antioxidants compared to humans.

## Conclusion

The findings suggested that using the crocin supplement and exhaustive exercise can reduce the consequences of such activities. Therefore, the use of antioxidants is recommended to improve heart tissue function in the elderly.

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## Authors Contributions

Design and conceptualization: Hadi Ghaedi and Mehran Ghahramani Methodology and data analysis: Mohammad Pishahang, Supervision and final writing: Mehran Ghahramani.

## Conflicts of Interest

The authors declare no conflict of interest.

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