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The effect of saffron extract on catalase (CAT) and glutathione peroxidase (GPX) antioxidants in young male futsal players

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Abstract

Purpose: Saffron is one of the most important medicinal plants that has been used as an antioxidant supplement in sports. The aim of this study was to investigate the effect of saffron extract on catalase (CAT) and glutathione peroxidase (GPX) antioxidants in young futsal players.

Method: 24 young male futsal players were randomly divided into two experimental and control groups (12 people in each group). The experimental group used 1500 mg of saffron supplement per day for seven days, while the control group received placebo. A simultaneous futsal game was held for the two groups (two teams of 6 people per match) after the end of the intervention period. CAT and GPX were measured before the intervention, immediately after the match, and 30 minutes after the match. Anthropometric characteristics were assessed in resting conditions. Data analysis was performed using repeated measures variance test. A significance level was considered at $P<0.05$.

Results: The results of the hockey study showed that immediately after the match in the experimental group, a significant increase in GPX levels ($P=0.031$) and a significant decrease in CAT levels ($P=0.041$) were observed, but this significance was not observed 30 minutes after the match.

Conclusion: According to the results of this study, it can be said that short-term consumption of saffron improves antioxidant defense in young male futsal players. Studies with long-term follow-up should be considered to confirm these findings.

Keywords: saffron, antioxidant indices, player, young male.

Introduction

Men's futsal is one of the fastest growing sports worldwide, with over 12 million players in 100 countries. However, there is less information in the literature about the physical demands of male futsal players than female futsal players (Castillo et al., 2022). (W.-D. Chang et al., 2020). Several studies have shown that the injury rate in futsal is 2 to 6 times that of soccer, which is considered a high-injury sport (A Pourrahim et al., 2021). Immediate and short-term changes induced by a futsal match on oxidative, inflammatory, muscle damage and antioxidant markers (K. Kaliarntas et al., 2023). From a physical perspective, futsal performance has been associated with high levels of agility, muscular strength, repeated running ability and maximal oxygen uptake (60 mL/kg/min) (Á Lopez-Samanes et al., 2021). Speed, jump, and agility tests were sport-specific functional tests that validated the competitive performance of basketball and football athletes. They could assess the strength, speed, and agility of the lower limbs for a specific sport. Increased oxygen consumption during exercise causes a large FR leak in the mitochondria, which then leads to an antioxidant response (W Souissi et al., 2020). When the production of free radicals overcomes the body's antioxidant defense system, oxidative stress occurs (T. M. Delarosa et al., 2023). Oxidative stress reduces strength and performance; mechanistically, reactive oxygen species (ROS) can accelerate skeletal muscle fatigue by reducing calcium sensitivity (H Arazi et al., 2021). However, the antioxidant system of these cells can effectively protect them from severe damage. The primary enzymatic antioxidant system present in cells includes glutathione peroxidase (GPX), glutathione peroxidase (SOD) and catalase (CAT) (M Dekany et al., 2008). In this regard, Fatoros et al. (2010) investigated the level of oxidative stress and antioxidant status of 20 soccer players during recovery; they provided the first data on oxidative stress conditions after a soccer game. The findings showed that oxidative stress is strongly exacerbated by the game and, as part of the exercise-induced inflammatory response, is associated with a significant deterioration in anaerobic performance for 72 h. This has led to the use of alternative products to classical antioxidants that are able to modulate redox responses without reducing some of the beneficial sports adaptations. Hundreds of natural polyphenols are found in edible plants. Some polyphenols may enhance athletic performance by reducing exercise-

induced muscle damage and facilitating adaptation to regular exercise (M Gelabert-Rebato et al. 2019).

Due to the side effects and adverse effects of chemical drugs, the desire to use medicinal plants has increased significantly in the world today. Among these plants, chamomile and saffron can be mentioned (N Mirazi et al.2019). Saffron is best known for its anti-inflammatory, antioxidant, anticancer, neuroprotective, antiallergic and antimicrobial properties (Saara Ahmad et al.2022). This is due to the content of biologically active compounds including essential oils and several polyphenols (NAN Hanafy et al.2022). Polyphenols with antioxidant properties are among these nutrients that are very important for increasing endurance capacity by increasing mitochondrial biogenesis and fatty acid uptake and reducing oxidative stress. Polyphenols also help maintain the immune system by eliminating free radicals, such as superoxide anions (Katsuhiko Suzuki 2021). There are five main groups of phenolic compounds in chamomile extract: coumarins, phenylpropanoids, flavones, flavonols, and flavanones (Z. Asadi et al., 2020). According to previous studies, most of the therapeutic effects of saffron have been investigated, and so far no study has measured the effect of saffron consumption on antioxidant levels after a futsal game, especially in men. Therefore, the aim of this study was to investigate the effect of saffron extract on catalase (CAT) and glutathione peroxidase (GPX) antioxidants in young futsal players.

Methods

This quasi-experimental study used a randomized, double-blind, placebo-controlled trial. Twenty-four young male professional futsal players from Malayer University (age: 23.27 ± 4.34 years, weight: 61.14 ± 5.41 kg, and body mass index: 22.35 ± 2.45 kg/m2) voluntarily participated in this study (Table 1). G*Power software was used to determine the sample size. The inclusion criteria for the subjects in the study were: male gender with an age range of 20 to 30 years, no use of anti-inflammatory drugs, no allergy to saffron, no diet, and no smoking.

The exclusion criteria for this study were: having any respiratory disease or joint and bone injuries, taking some antidepressants such as fluoxetine, as well as alcohol, aspirin, warfarin, and heparin (due to drug interactions with chamomile capsules), not completing the course of taking supplements, having any underlying disease, and intolerance to saffron, taking stimulants, taking painkillers, and anti-inflammatory drugs. All participants provided signed informed consent after being informed about the test procedures. At baseline, study variables such as weight, height, body mass index (BMI), and body fat percentage (FFM) were measured using a Body composition 365 x_contact device made in South Korea, and blood was drawn from all subjects to determine the subject's biochemical indices Comment (GPX and CAT).

The day before the subjects came to measure the variables, they were advised to fast and not consume caffeine or heavy exercise to reduce the error in measuring muscle and fat percentages by the body analyzer. Then, the subjects were randomly divided into two experimental groups (n=12) and a placebo group (n=12). For 7 days, the experimental group used 500 mg of dry saffron extract three times a day, and the control group used a placebo with the same color and containing 500 mg of breadcrumbs made by the Karaj Medicinal Plants Research Institute. To minimize the effect of the intervention variable, supplementation was performed in a double-blind manner. After one week, the subjects came to the laboratory as on the first day. Then, the subjects competed in two futsal matches with two 20-minute halves in four teams of 6 people and were only allowed to drink water during the match. In order to measure biochemical variables, blood samples were taken from all subjects twice, immediately after the end of the race and 30 minutes after the race.

Table 1: Anthropometric characteristics of participants

Group	Age (year)	Weight (kg)	Body Fat (%)	BMI (kg/m^2)	Height (cm)
supplemental group	23.16±3.48	8.23±61.39	5.37±17.66	3.08±21.34	4.81±164.45
Placebo group	24±4.42	8.29±61.47	5.22±18.29	2.05±21.47	4.21±164.21

Blood collection and statistical method

Before blood sampling, the subjects were advised not to do any heavy physical activity for 72 hours before the test protocol as well as blood sampling and initial measurements and to consume antioxidants as much as possible. In this study, blood samples were taken from the subject's arm vein in three stages before supplement consumption, immediately after the game, and thirty minutes after the game, 5 cc of blood was taken from the subject's arm vein. Then, the blood samples were placed in tubes containing separating gel and centrifuged for 10 minutes at 4°C at a speed of 3000-3500 rpm. Glutathione peroxidase (GPX) and catalase (CAT) enzyme levels were measured by ELISA using a German Zelbio research kit for human samples with a sensitivity of 1 and 0.5 units per milliliter, respectively. Data are presented as mean ± standard deviation. The normality of each variable was examined using the Shapiro-Wilk test. Then, changes in each of the indices during different measurement stages were examined using repeated-measures analysis of variance (for comparing data within groups) and Bonferroni post hoc test at a significance level of $\alpha=0.05$ using SPSS statistical software version 26.

Results

The study found that, in the comparative analysis, for both CAT and GPX variables, the results of the Mauchly's test indicate the establishment of the hypothesis of sphericity ($p=0.84$) and ($p= 0.069$).

Therefore, to check the difference between the measured times, Sphericity Assumed corrective tests were used. The results of this test showed that there is a significant difference between the two times of blood plasma measurements before supplementation and immediately after the competition, (p=0.31) and (p=0.41) respectively. These results are also shown in Table 2, Fig1 and Fig2.

Table 2: Results of analysis of variance with repeated measures

Group	Mean Square	F	Sig.
CAT	17.54	3.89	0.031*
GPX	41.68	3.78	0.041*

* Significant at P≤0.05 level

Figure 1 shows the changes in CAT levels in the two experimental and placebo groups before, immediately, and 30 minutes after the race; the results of Figure 1 showed that there was a significant difference immediately after the race.

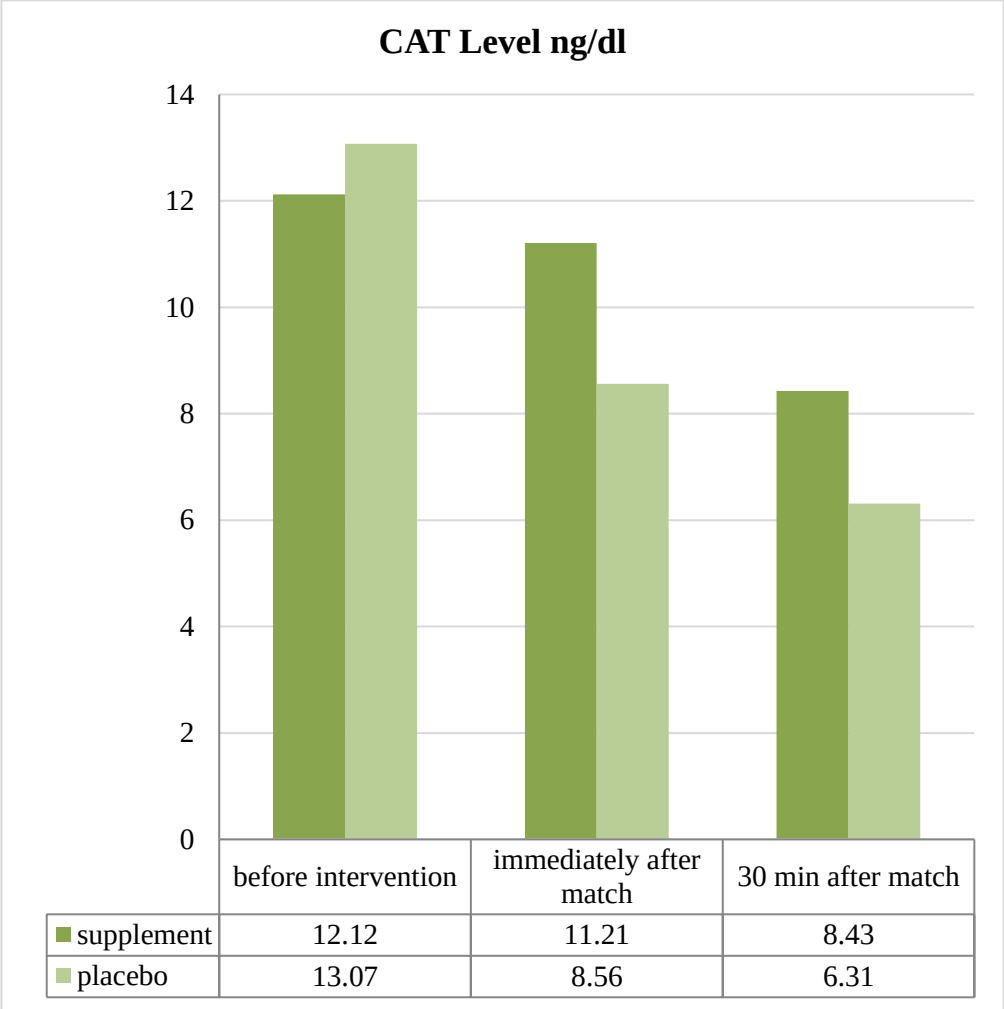

Fig 1. Changes in CAT levels before, immediately after, and 30 minutes after the competition.

Figure 2 shows the changes in GPX levels in the two experimental and placebo groups before, immediately, and 30 minutes after the race; the results of Figure 2 showed that there was a significant difference immediately after the race.

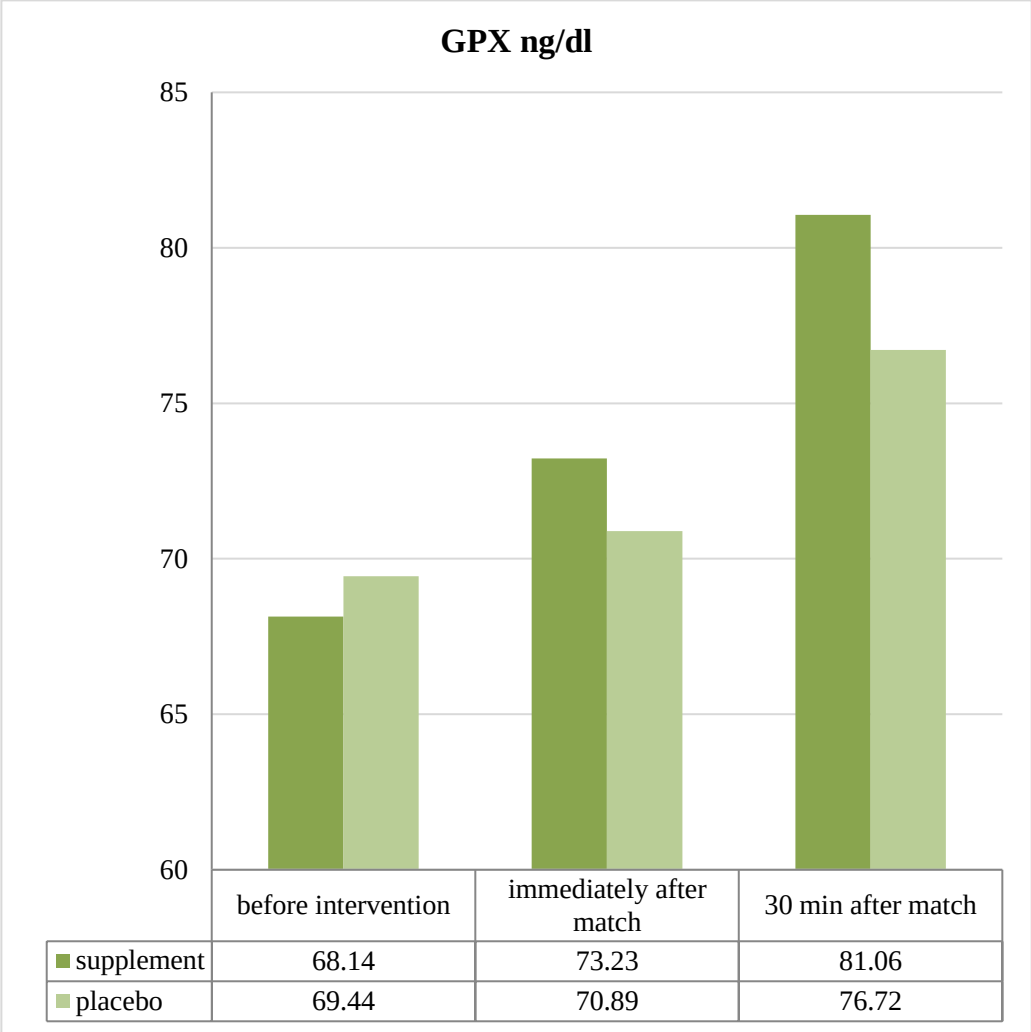

Fig 2. Changes of GPX levels before, immediately, and 30 minutes after the competition.

Discussion

This study was designed to investigate the effect of saffron extract on the antioxidants catalase (CAT) and glutathione peroxidase (GPX) in young futsal players. The main findings of this study showed a decrease in catalase enzyme levels immediately after the match and 30 minutes after the match in both the supplement and placebo groups. The difference between the two groups was significant at the first measurement, i.e. immediately after the match. A study by Casey et al. in 2012 on soccer players and the decrease in catalase after the match showed that acute exercise leads to the production of ROS and consequently to the intensification of lipid peroxidation, which increases MDA levels, probably by exhausting the antioxidant defense system. This is reflected in the results of the present study. Hodgson et al. (1975) showed in a study that following intense and high-intensity aerobic exercise, the activity of antioxidant enzymes decreases during the increase in reactive oxygen species, and oxidative stress may be related to their greater use against free radicals and, on the other hand, due to the limitation of antioxidant enzymes by reactive oxygen species. In a study, Tartebian et al. (2013) showed that catalase gene expression increased significantly after exercise in female athletes, which was not consistent with the results of the present study. Given that the amount of free radical production depends on the intensity, duration of the exercise, and adaptation to the exercise, differences in the intensity and type of exercise are likely the reason for this difference. Hajizadeh et al. (2013) tested the effect of 16 weeks of intense cycling training on 75 subjects in the off-season on oxidative stress and antioxidant capacity of male road cyclists. The results showed that an increase in ROS and a decrease in antioxidant capacity occurred after this type of exercise.

The present study showed an increase in glutathione peroxidase enzyme levels immediately after the competition and 30 minutes after the competition in both the supplement and placebo groups, and the difference between the two groups was significant in the first measurement, i.e. immediately after the competition. Feizi et al. (2022) showed in a study on inactive girls that no significant change in GPX

enzyme activity was observed immediately after performing two intense and moderate resistance training protocols, but after two weeks of antioxidant supplement consumption, a significant increase in the activity of this enzyme was observed. In a review study, Yilaghi Ashrafi et al. (2016) showed that performing an intense exercise activity leads to a non-significant increase in glutathione peroxidase gene expression or no significant change in it, and stated that this type of contradiction can be attributed to the difference in researchers' reports of the two terms adaptation and subsequent adaptation response, so that some researchers have used stimulation protocols to examine the response of stress indices to a training session after adaptation to exercise, while some other researchers have reported resting levels of this index after adaptation to exercise. Exercise training changes the antioxidant activity of tissues based on the type of exercise protocol, exercise volume, and the presence of rest periods between exercise programs. Intense exercise with insufficient rest leads to stimulation of neutrophils. Neutrophils can produce reactive oxygen species (ROS), which ultimately lead to the creation or increase of oxidative stress and consequently to the decrease of antioxidant defense (Rami M et al., 2018). The reason for these discrepancies may be related to the effects of aerobic exercise in increasing resistance to oxidative stress and reducing the need for the CAT enzyme for antioxidant defense and activating other antioxidant pathways. Considering that in the present study, a smaller decrease in catalase was observed in the supplement group, it probably means that the use of chamomile as a polyphenol supplement was effective in helping the antioxidant system.

In the present study, saffron extract probably prevented the onset of the degradation process in muscle tissue through its antioxidant effect. The increase in plasma GPX indicates that the oxidation and antioxidant balance is partially shifted towards antioxidation. Maintaining the oxidation and antioxidation balance in a state that favors antioxidation is beneficial for the overall health of the body. Therefore, the increase in plasma GPX is considered a positive outcome in this study, which showed a significant increase in the saffron supplement group

compared to the placebo group after the race. According to the results, the supplement dose, duration of intervention, and individual characteristics are likely to be important confounding factors that are suggested to be considered in future studies.

Conclusion

Current findings indicate that the use of saffron as a nutritional supplement in futsal sport may reduce the adverse effects of oxidative stress generated during the match and maintain player performance at an optimal level.

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Conflicts of Interest:
The authors declare no conflict of interest related to this study.

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