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Interactive effect of saffron aqueous extract and aerobic training on glutathione peroxidase and malondialdehyde in men with type 2 diabetes

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ABSTRACT

Article History
Received:
15-December-2017
Revised:
26 February 2017
Accepted:
09 March 2017

Aims: Type 2 diabetes is a common metabolic disease in the world. Nonpharmaceutical and pharmaceutical strategies have been proposed for the control and treatment of diabetes. Nutrition and exercise are nonpharmaceutical strategies for the prevention and control of diabetes. Heavy acute exercises may increase oxidative stress. Thus, it is necessary to adopt nutritional strategies to help diabetic athletes.

Methods: In this semiexperimental research study, 24 men with type 2 diabetes were divided randomly into four groups including saffron extract (SE), aerobic training (AT), aerobic training plus saffron extract (AT+SE), and control groups. The saffron extract was used at a 3 mg/kg dose. Aerobic exercise was performed three days a week, for eight weeks, at 55% to 70% of maximum heart rate. At the end of the intervention period, levels of malondialdehyde (MDA) and glutathione peroxidase (GPX) were measured. Data were analyzed using the one-way ANOVA, Tukey's, and paired t test.

Results: The serum MDA decreased significantly in the SE and AT+SE groups ($p = 0.001$ and $p = 0.026$, respectively). Saffron extract consumption or aerobic training alone did not significantly influence erythrocyte GPX activity ($p = 0.12$ and $p = 0.14$, respectively). However, erythrocyte GPX activity increased significantly in the AT+SE group ($p = 0.041$).

Conclusions: Antioxidant compounds of saffron are effective in the reduction and inhibition of tissue damages after physical activities. Aerobic training plus saffron extract can decrease and increase, respectively, the levels of MDA and erythrocyte GPX activity in men with type 2 diabetes.

key words:
Saffron;
Type 2 diabetes;
Aerobic training;
GPX;
MDA

Introduction

Diabetes is a common metabolic disease in the world [1]. The occurrence of type 2 diabetes increases with dietary changes and decreased physical activity. Exercise and physical activity contribute to the prevention of complications of diabetes [2]. As the prevalence of diabetes continues to rise worldwide, it becomes increasingly important to identify high-risk populations and to implement

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strategies to delay or prevent the onset of diabetes. Today, the role of exercise and physical activity in the prevention or treatment of insulin resistance and type 2 diabetes, and in the balance of hormonal and metabolic factors contributing to those conditions, has been recognized [3]. Epidemiological studies show that regular exercise is associated with a decrease in cardiovascular risk [4]. Despite numerous findings on the relationship between regular physical activity and reduction in cardiovascular damage, the impact of heavy and acute physical activity on the cardiovascular system are unclear. Reactive oxygen species (ROS) are created in many tissues under diabetic conditions. Evidence supports an association between oxidative stress and the development of diabetes. One of the biological changes during physical activity is the increase in metabolism and the production of free radicals [5]. Free radicals have great potential to react with molecules and damage macromolecules. There are systems in the body to confront damages caused by free radicals known as antioxidant defense system [6]. The antioxidant defense system, which is built of several nonenzymatic and enzymatic compounds, is effective in the reduction or prevention of tissue damages after physical activities [5]. Crocus sativus L., commonly known as saffron, of the family Iridaceae, is a natural antioxidant, cultivated in Iran, India, and Spain. The dried stigmas of saffron are used in traditional medicine. The most powerful active compounds of saffron are safranal, crocin, crocetin, and picrocrocin. Crocin and crocetin are the main carotenoids and bioactive constituents of saffron and have a wide spectrum of biological properties [7]. Crocin has shown antidiabetic effects in mice [8]. Crocetin reduces insulin resistance caused by palmitate and tumor necrosis factor alpha (TNF-α) and inhibits leptin expression in the adipose tissue [8,9]. Type 2 diabetes is also increasing in prevalence in adolescents and children, with increasing sedentary and obesity as key contributors. Antioxidant compounds of saffron are effective in the reduction and inhibition of tissue damages after physical activity. Varma and Bordia suggested that saffron has cardioprotective effects and decreases contractility and heart rate in stressful conditions [9]. Crocin and safranal were shown to inhibit lipid peroxidation in skeletal muscle [10], hippocampus [11], and kidneys [12] during oxidative injury in rats. Glutathione peroxidase (GPX) is one of the enzymes of the antioxidant defense system. Lipid peroxidation is a process that may cause peroxidative tissue damage in cancer, inflammation, diabetes, and aging. The membrane polyunsaturated fatty acids are peroxidized in a free radical–mediated process. Malondialdehyde (MDA) is produced as a result of lipid peroxidation and is considered an indicator of membrane damage and vascular injury [6]. Studies have reported decreases, increases, or no changes in MDA and GPX after aerobic and anaerobic exercises. For example, Haghighi et al compared the effects of two types of vigorous exercise on MDA in smokers. They reported that vigorous exercise led to a significant increase in MDA levels [13]. Rahbani reported that ethanolic extract of saffron led to a significant increase and decrease in GPX and MDA, respectively [14]. In previous studies, the effects of endurance exercise on GPX and MDA have not been explored adequately [13,14]. In spite of saffron utilization as a spice in many societies, few studies in the literature have dealt with the interaction between endurance exercises and saffron extract on oxidative stress. Thus, the aim of the present study was to investigate the effects of saffron extract, aerobic training, or aerobic training plus saffron extract on GPX activity and MDA levels in patients with type 2 diabetes.

Methods and Materials

The research population comprised male patients with type 2 diabetes referred to the Valiasser Hospital in Rasht city. Participants were aged 40 to 50 years and their medical history and health condition was evaluated using questionnaires. Those who had chronic obstructive pulmonary disease, pulmonary embolism, angina, hypertension, liver or kidney disease, hyperthyroidism, or hypothyroidism were excluded. Written informed consent was obtained from all the subjects. Twenty-four eligible patients were included in the study. The participants were randomly divided into four equal groups ($n = 6$) including the saffron extract (SE), aerobic training (AT), aerobic training plus saffron extract (AT+SE), and control group (neither saffron extract nor exercise). This study was a semi-experimental research. The groups were homogenized by height, weight, and body mass index (BMI). The BMI (weight [kg] divided by height [m] squared) is a measure of adiposity that applies to adult men and women. The diet was evaluated by a 24-hour diet recall questionnaire. The subjects were instructed not to change their diets during the study period.

After 12 hours of fasting, blood was collected in vacuum tubes containing no additives, and the serum was separated by centrifugation at 2000 g for 20 min at room temperature to assess GPX and MDA levels. Plasma lipid peroxide concentration was assessed by the thiobarbituric acid procedure [16]. Erythrocyte GPX activity was evaluated by the method of Valentine [15,16,17], which is expressed in MU/molHb. In addition to measuring these factors, blood pressure, heart rate, subcutaneous fat percentage, and VO2 max were measured. Fat percentage was measured by the bioelectrical impedance analysis (InBody 770).

Subjects in the SE and AT+SE groups were administered saffron extract at 3 mg/kg/d. Ideal doses for saffron have not been set for any condition, although studies have been done using a dose of 30 mg/kg per day. Ingredients in supplements may vary widely. This makes it very hard to set a standard dose.

Dry stigmas of saffron (*Crocus sativus L.*) were collected from Torbat-e Heydarieh (Khorasan province, northeastern Iran). The stigmas were powdered using a pounder. Then, the resulting powder was soaked in distilled water and its extract was received using a distiller. For this aim, 100 g of dried stigma powder was soaked in 1000 ml of distilled water and was boiled for 10 minutes at 100°C. Then, the supernatant was passed through a filter and kept in a bain-marie at 55°C for a week until gradual evaporation of its water; and the powdered extract was acquired [15,17].

Aerobic training was performed at 55% to 70% of maximum heart rate, at Malavan Sports Hall in Rasht (for eight weeks, three days a week, every day 75 minutes). Twenty-four hours after the last exercise session, blood samples were taken again from the groups and their MDA levels and erythrocyte GPX activity were measured.

The Kolmogorov-Smirnov test was used to evaluate the distribution of the data. After ensuring the normality of the data distribution, the parametric tests were used to analyze the data. Therefore, a paired t test was carried out to assess the within-group differences, and a one-way analysis of variance (ANOVA) followed by Tukey's HSD post hoc test, when applicable, to assess the between-group differences and then if there were significant differences, was used to identify its value. Data analysis was performed using SPSS software version 12. A significance level of $\alpha = 0.05$ was considered for all calculations.

Results

In Table 1, demographic characteristics of the participants, expressed as mean ± standard deviation (SD), are presented.

Analysis of changes in GPX in the four groups after the 8-week period showed that SE (3 mg/kg/d) or AT had no significant effect on GPX levels. However, AT+SE changed GPX levels significantly ($p = 0.041$) (Table 2 and Figure 1). Between-group comparisons showed no significant difference.

The results of paired t test showed that both SE and AT+SE caused significant decreases in plasma MDA levels in type 2 diabetic men ($p < 0.001$ and $p = 0.026$, respectively). Between-group comparisons, however, showed no significant difference (Table 3 and Figure 2).

Table 1. Demographic characteristics of participants

Variable	Group	Control	Saffron	Aerobic training	Saffron + aerobic training	p value (inter-group)
Number		6	6	6	6	
Age, y		40.3 ± 4.9	43.7 ± 1.75	46.5 ± 1.64	43.8 ± 1.72	
Height, cm		174.8 ± 3.7	175.6 ± 5.6	174.6 ± 3.8	174.5 ± 1.87	
Weight, kg	pre-test	86.7 ± 11.7	75 ± 16.16	81.5 ± 4.1	79.5 ± 6.3	
	post-test	86.5 ± 1.1	75 ± 15.40	79 ± 3.8	77.3 ± 6	0.063
p value (intra-group)		0.793	0.611	0.232	0.032*	
BMI, kg/m^2	pre-test	27.06 ± 1.04	25.5 ± 2.4	24.06 ± 3.4	28.3 ± 3.1	
	post-test	25.9 ± 0.71	25.4 ± 2.4	24.1 ± 3.5	28.2 ± 2.9	0.074
p value (intra-group)		0.810	0.575	0.365	0.013*	
VO_{2max}, ml·kg^{-1}·min^{-1}	pre-test	28 ± 3.03	30 ± 2.7	33.7 ± 2.5	32.8 ± 4.1	
	post-test	36.3 ± 2.9	37 ± 2	34 ± 2	32.7 ± 3.9	*0.001
p value (intra-group)		0.363	0.102	< 0.001*	< 0.001*	

**p < 0.05 level of significance*

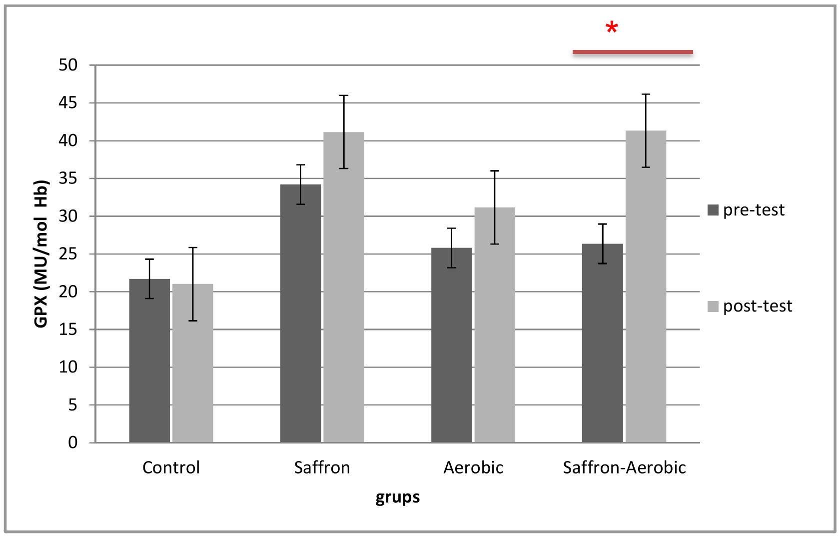

Figure 1. Comparison of pre-test and post-test GPX in the four groups

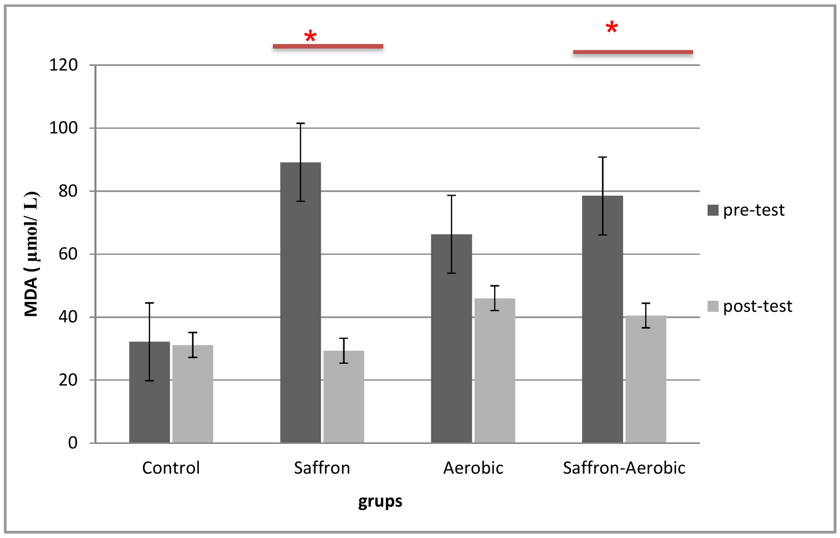

Figure 2. Comparison of pre-test and post-test MDA in the four groups

Table 2. Comparison of pre-test and post-test GPX levels in groups

Group		Paired differences Mean ± SD	t	p value
Control	pre-test	21.75 ± 1.84	−1.007	0.36
	post-test	21.01 ± 1.81		
Saffron	pre-test	34.23 ± 1.90	1.875	0.12
	post-test	41.16 ± 1.87		
Aerobic training	pre-test	25.81 ± 1.10	1.755	0.14
	post-test	31.16 ± 1.62		
Saffron + aerobic training	pre-test	26.36 ± 1.36	2.732	0.041*
	post-test	41.13 ± 1.14		

$p < 0.05$ level of significance

Discussion

The effect of SE on plasma MDA levels

The results showed that saffron extract consumption at a dose of 3 mg/kg/d, for eight weeks, caused a significant decrease in the plasma levels of MDA in type 2 diabetic men. In a similar study, Altinoz et al investigated the effect of crocin (5 mg/kg, dissolved in normal saline) on serum concentrations of MDA in diabetic rats and showed that, after 21 days, serum levels of MDA in the crocin

Table 3. Comparison of pre-test and post-test MDA levels in groups

Group		Paired differences Mean ± SD	t	p value
Control	pre-test	32.16 ± 11.33	−1.96	0.852
	post-test	31.16 ± 12.82		
Saffron	pre-test	89.16 ± 17.26	−6.32	0.001*
	post-test	29.33 ± 20.20		
Aerobic training	pre-test	66.33 ± 31.99	−1.94	0.11
	post-test	46 ± 20.21		
Saffron + aerobic training	pre-test	78.5 ± 17.16	−3.13	0.026*
	post-test	40.5 ± 26.61		

**p < 0.05 level of significance*

group decreased significantly compared with the control group [18]. Similarly, Piri et al investigated the effect of saffron extract on concentrations of MDA in streptozotocin-induced diabetic rats. They reported that saffron extract led to a significant decrease in MDA [19]. Also, Rahbani et al investigated the effects of the ethanolic extract of saffron on concentrations of MDA and GPX in liver tissue in streptozotocin-induced diabetic rats [14]. At the end of the study, MDA levels decreased and GPX activities increased significantly. Kakar et al showed that oxidative stress occurs at the beginning of diabetes and increases with the development of the condition. In uncontrolled diabetes, glucose oxidation in the phosphogluconate pathway leads to an increased formation of NADPH, which can increase lipid peroxidation [20]. Also, it is suggested that the decrease in the antioxidant state leads to a significant increase in free radical activity in diabetes.

MDA is the most important parameter related to the lipid peroxidation in many illnesses. Saffron is a natural antioxidant with various active ingredients such as safranal, crocin, and crocetin. Verma and Bordia opined that the consumption of saffron can prevent the increased oxidative stress and the development of diabetes [8,9,21]. The protective effect of saffron extract is probably multifactorial, and further extensive study is needed to identify the mechanisms of action of saffron extract and its constituents on MDA in different conditions [9,21].

The effect of AT and AT+SE on MDA

The results showed that eight-week AT had no significant effect on MDA levels, but AT+SE led to a significant decrease in the amount of MDA in type 2 diabetic men. These results are consistent with the findings of Afzalpour et al, on 29 patients, who showed that two types of exercise (acute resistance exercise and acute aerobic exercise) did not change MDA in the exercise groups compared with the control group [22]. Also, Piri et al investigated the effects of SE and AT+SE on MDA concentrations in streptozotocin-induced diabetic rats and reported that both SE and AT+SE led to significant decreases in MDA levels [19]. Haghighi et al investigated the effects of a bout of exhaustive aerobic exercise with two different intensities on serum concentrations of MDA in smokers and showed that serum levels of MDA increased significantly in the exercise groups compared with the control group [13].

Although the mechanisms underlying vascular complications in type 2 diabetes are complex, free radical reactions associated with ROS are thought to be one of the factors involved. Exercise is associated with increased production of free radicals. Several studies have shown that the amount of free radicals in biological tissues is increased after chronic or acute exercise, which is associated with tissue damage. Free radical reactions cause the denaturation of membrane proteins, peroxidation and oxidation of membrane lipids, and disturb membrane permeability [23]. Saffron is a natural antioxidant with various active ingredients such as safranal, crocin, and crocetin. Antioxidant compounds of saffron are effective in the prevention or alleviation of tissue damages after activities [21]. In contrast to our finding, Mohammadi et al showed that swimming training, an hour a day for eight weeks, was able to significantly decrease MDA levels in streptozotocin-induced diabetic rats [24]. This inconsistency could be a result of the use of animal subjects, or the type, duration, and intensity of the exercise.

Our findings showed that saffron supplementation prevented from increases in inflammatory, stress oxidative, and muscle damage markers induced by a bout of endurance training. Recently, a reduction in sperm lipid peroxidation marker (MDA) and DNA fragmentation was reported following 8 weeks of training and saffron supplementation (21,24).

The effects of SE, AT, and AT+SE on GPX activity

The results showed that neither SE nor AT had a significant effect on GPX levels. Similar to the results of our study, Afzalpour et al investigated the effect of acute resistance and aerobic exercises on

GPX levels in healthy active men and found no significant differences in GPX activity between the exercise and control groups following resistance and aerobic exercises [22]. In this study, AT+SE led to a significant increase in the amount of GPX in type 2 diabetic men Similar to our study, Piri et al investigated the effect of SE and AT+SE on concentrations of GPX in streptozotocin-induced diabetic rats and reported that both treatments led to significant increases in GPX activity [19]. We did not find significant changes in serum levels of GPX after saffron supplementation. This may have been due to the lower dose of saffron (3 mg/kg/d). Higher doses of saffron have been tested in animal studies. Conversely, the results of a study by Kalkhoran et al on diabetic male rats showed that SE, AT, and AT+SE led to significant decreases in GPX [25]. This inconsistency can be attributed to differences in subjects and exercise type, duration, and intensity.

In conclusion, data from this study demonstrate that saffron intake may beneficially protect the myocardium from injuries. The consumption of saffron extract alone or in combination with aerobic training leads to a significant decrease in the amount of MDA in type 2 diabetic men. Also, SE+AT led to a significant increase in the amount of GPX in type 2 diabetic men. Therefore, in order to get better results from endurance training, it is proposed that saffron supplement be used as an effective factor, especially in the cases aiming at the improvement of antioxidant capacity.

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