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

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A B S T R A C T

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.

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
Malondialdehyde (MDA) is produced as a result of inflammation, diabetes, and aging. The membrane polyunsaturated fatty acids are substrates of the peroxidative process that may cause peroxidative tissue damage and heart rate in stressful conditions [9]. Crocetin and crocin have shown cardioprotective effects and decreases contractility and heart rate in stressful conditions [9]. 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.

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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).

<p>| Table 1. Demographic characteristics of participants |
|-----------------|-----------------|-----------------|------------------|------------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Control</th>
<th>Saffron</th>
<th>Aerobic training</th>
<th>Saffron + aerobic training</th>
<th>p value (inter-group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
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<tr>
<td>Age, y</td>
<td>40.3 ± 4.9</td>
<td>43.7 ± 1.75</td>
<td>46.5 ± 1.64</td>
<td>43.8 ± 1.72</td>
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<td>0.793</td>
</tr>
<tr>
<td>Height, cm</td>
<td>174.8 ± 3.7</td>
<td>175.6 ± 5.6</td>
<td>174.6 ± 3.8</td>
<td>174.5 ± 1.87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>pre-test 86.7 ± 11.7</td>
<td>75 ± 16.16</td>
<td>81.5 ± 4.1</td>
<td>79.5 ± 6.3</td>
<td></td>
<td>0.063</td>
</tr>
<tr>
<td></td>
<td>post-test 86.5 ± 1.1</td>
<td>75 ± 15.40</td>
<td>79 ± 3.8</td>
<td>77.3 ± 6</td>
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</tr>
<tr>
<td>p value (intra-group)</td>
<td>0.793</td>
<td>0.611</td>
<td>0.232</td>
<td>0.032*</td>
<td></td>
<td></td>
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<tr>
<td>BMI, kg/m²</td>
<td>pre-test 27.06 ± 1.04</td>
<td>25.5 ± 2.4</td>
<td>24.06 ± 3.4</td>
<td>28.3 ± 3.1</td>
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<td>post-test 25.9 ± 0.71</td>
<td>25.4 ± 2.4</td>
<td>24.1 ± 3.5</td>
<td>28.2 ± 2.9</td>
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<tr>
<td>p value (intra-group)</td>
<td>0.810</td>
<td>0.575</td>
<td>0.365</td>
<td>0.013*</td>
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<tr>
<td>VO₂max, ml·kg⁻¹·min⁻¹</td>
<td>pre-test 28 ± 3.03</td>
<td>30 ± 2.7</td>
<td>33.7 ± 2.5</td>
<td>32.8 ± 4.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>post-test 36.3 ± 2.9</td>
<td>37 ± 2</td>
<td>34 ± 2</td>
<td>32.7 ± 3.9</td>
<td></td>
<td>*0.001</td>
</tr>
<tr>
<td>p value (intra-group)</td>
<td>0.363</td>
<td>0.102</td>
<td>&lt;0.001*</td>
<td>&lt; 0.001*</td>
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</table>

*p < 0.05 level of significance
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
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

<table>
<thead>
<tr>
<th>Group</th>
<th>Paired differences</th>
<th>t</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre-test</td>
<td>32.16 ± 11.33</td>
<td>−1.96</td>
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<tr>
<td>post-test</td>
<td>31.16 ± 12.82</td>
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<td>Saffron</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre-test</td>
<td>89.16 ± 17.26</td>
<td>−6.32</td>
<td>0.001*</td>
</tr>
<tr>
<td>post-test</td>
<td>29.33 ± 20.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerobic training</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre-test</td>
<td>66.33 ± 31.99</td>
<td>−1.94</td>
<td>0.11</td>
</tr>
<tr>
<td>post-test</td>
<td>46 ± 20.21</td>
<td></td>
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<tr>
<td>Saffron + aerobic training</td>
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<tr>
<td>pre-test</td>
<td>78.5 ± 17.16</td>
<td>−3.13</td>
<td>0.026*</td>
</tr>
<tr>
<td>post-test</td>
<td>40.5 ± 26.61</td>
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</table>

*p < 0.05 level of significance
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.

References
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18. Altinzo E, Oner Z, Elbe H, Vardi N. Neuro-


