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The effect of aerobic exercise and saffron supplementation on antioxidant capacity in diabetic rats

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Abstract

The purpose of the present research was to study the effect of aerobic exercise and saffron supplementation on antioxidants capacity in diabetic rats. 50 male rats (age: 10 weeks; weight: 325±32.7 grams) were randomly divided into healthy-control (HC), diabetic-control (DC), diabetic-exercise (DE), diabetic-saffron (DS), and diabetic-saffron-exercise (DSE) groups (n = 10 per group). Except for the HC group, all the other groups induced with diabetes by injecting streptozotocin. For a period of 4 weeks, the DS and DSE groups received 30 mg/kg of daily saffron supplementation through oral gavage. The DE and DSE groups ran on the treadmill at a specific speed for 4 weeks, 4 session per week, and 30 minutes per session. After 24 hours of last session of exercise and saffron gavage, rats' hepatic was extracted and their Total antioxidant capacity (TAC) and Glutathione (GSH) were measured. Results showed that there is a significant difference between the five groups in the levels of GSH and TAC ($P = 0.000$) and GSH level had the largest and lowest increase in DSE and DC respectively ($P = 0.032$). TAC level had the largest and lowest increase in DSE and DC respectively ($P = 0.005$). Thus, it can be concluded that a combination of saffron supplementation and low-intensity aerobic exercise is effective for improving the antioxidant capacity of diabetic rats.

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Introduction

Diabetes is the most important metabolic disease in humans and according to the International Diabetes Federation (IDF), presently 246 million people around the world are affected by this disease and this number is expected to increase to 380 million people by 2025 (Fedration, 2008). This issue becomes more dramatic knowing that during the past 20 years this disease has become much more prevalent among children and adolescents. Many pharmacological and non-pharmacological (changes in lifestyle) strategies have been provided for controlling this disease. In diabetes, reactive oxygen species (ROS) are produced in various tissues due to several mechanisms, such as non-enzymatic glycosylation reactions, electron transport chain in the mitochondria, and membrane-bound nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Evans, *et al.,* 2005). There is much evidence supporting the close association between oxidative stress and diabetes evolution, suggesting that oxidative stress secondary to hyperglycemia and hyperlipidemia occurs before the appearance of clinical manifestations of late diabetes complications and plays a key role in the pathogenesis of the disease. Therefore, insulin resistance and pancreatic β-cell dysfunction, which are indications diabetes, are modulated by ROS (Evans, *et al.,* 2003; Evans *et al.,* 2005; Urakawa *et al.,* 2003). Under diabetic condition, chronic hyperglycemia may induce large amounts of ROS that are responsible for the progressive dysfunction of β cells, worsening insulin resistance and further promoting relative insulin deficiency (Robertson, *et al.,* 2003).

At present, the main effective treatment for diabetes mellitus is using insulin and hypoglycemic medicines, but these compounds have many adverse complications. Although for long herbal medicines and their derivatives have been discussed in the treatment of diabetes mellitus and its complications, however, no valid research evidence has been found to confirm the definite effectiveness of most of these medicines (Shapiro & Gong, 2002). Saffron is a small perennial plant from the Iridaceae family. Besides being an extensively consumed condiment, saffron has many pharmacological effects and is considered as a powerful medicine. It has been reported that small nutriment consumption of saffron (100 mg daily or 30 mg of hydro-ethanolic saffron extract) can have considerable pharmacological benefits for humans (Agha Hosseini *et al.,* 2008; Akhondzadeh, *et al.,* 2004; Verma & Bordia, 1998). Recent studies have shown that saffron extract has antineoplastic and anti-mutagenesis properties and inhibits nucleic acid synthesis in malignant cells. There are many compounds present in saffron extract, such as crocetin, water-soluble carotenoid, crocin (de-, tri-, and picrocrocin), and safranal (Hosseinzadeh & Talebzadeh, 2005); further, the anti-carcinogenic effects of carotenoids is almost completely acknowledged (Ochiai *et al.,* 2007). However, the physiological effects of saffron on other issues such as diabetes remain unknown. Since in saffron, crocin, crocetin, and safranal quench free radicals and have antioxidant properties (Assimopoulou, *et al.,* 2005; Chen *et al.,* 2008; Kanakis, *et al.,* 2007), saffron supplementation may be able to prevent the increase in oxidative stress and development of type I diabetes.

On the one hand, the results of research studies in this area indicate that physical exercise is associated with increased production of free radicals which is mainly due to increased 0_2 uptake by active tissues (Bloomer & Goldfarb, 2004; Urso & Clarkson, 2003). A research in this regard showed that free radicals increase in biological tissues following acute or chronic exercise which is accompanied by damage to the tissues (Bloomer & Goldfarb, 2004). On the other hand, some researchers have reported that regular exercise will lead to alterations in antioxidant capacity, protects cells against the detrimental effects of oxidative stress, and consequently prevents damage to the cells (Urso & Clarkson, 2003). The results of Tuomilehto *et al.* (2001) showed that prevalence of diabetes in men and women with high cardiovascular risk can change as a result of lifestyle interventions. They also reported a 58% reduction in the overall prevalence of diabetes (Tuomilehto *et al.,* 2001). Therefore, it can be expected that aerobic exercises will prevent the development of diabetes.

Assuming that consuming saffron, performing aerobic exercises, and both these treatments affect the oxidative stress of diabetes-induced rats, the researcher aimed to study the effect of aerobic exercise and saffron supplementation on the antioxidants capacity such as Glutathione (GSH) which is an index of antioxidant capacity of the body and exists in high concentrations in the liver, and total plasma antioxidant capacity (TAC) in rats induced with diabetes using streptozotocin.

Materials and methods

Subject selection

50 male rats (age: 10 weeks; weight: 325±32.7 grams) were studied in the present research. The subjects were transported to a physiology laboratory with an ambient temperature of 22 ± 2 in a light-regulated space (12-hour circadian rhythm) and underwent a 10-day period of adaptability. The animals were free to access food and water during this period. On the tenth day, after a night of fasting, the subjects were anesthetized with chloroform and were subjected to 60 μg/kg intraperitoneal injection of streptozotocin (made by Sigma Co.) dissolved in citrate buffer. 7 days after the injection, blood samples were collected via tail vein puncture in order to measure the level of blood sugar and rats with a blood sugar level above 300 mg/dl entered the experiment.

Supplementation and exercise prescription

The studied rats were randomly divided into healthy-control (HC), diabetic-control (DC), diabetic-exercise (DE), diabetic-saffron (DS), and diabetic-saffron-exercise (DSE) groups (n = 10 per group). For a period of 4 weeks, the DS and DSE groups received 30 mg/kg of daily saffron supplementation through oral gavage. The DE and DSE groups ran on the treadmill at a specific speed for 4 weeks, 4 session per week, and 30 minutes per session. Treadmill grade set up at zero, 5, and zero degree on first 2 weeks, third week and the last week respectively. Electric shock was applied to stimulate the animal to run. After four weeks of exercise and saffron supplementation in the experimental groups, the concentration of hepatic antioxidants was measured in all the five groups. The measured factors included GSH which is an indicator of antioxidant capacity of the body and exists in high concentrations in the liver, and ferric reducing antioxidant power (FRAP) as an indicator of TAC.

Statistical methods

Descriptive statistics were used to describe the data of each group of rats and one-way ANOVA was applied in order to compare the data between the groups. In case of a significant difference between groups, Tukey's post hoc test would be applied to determine the source of variance. All the calculations were done at $\alpha \leq 0.05$ level and using SPSS 16.

Results

The results of 1-sample Kolmogorov-Smirnov test showed that the data distribution related to GSH and TAC was normal between the groups ($P > 0.05$). The results of one-way ANOVA showed a significant difference between the studied groups in the level of GSH ($F = 24.574, P = 0.000$), and TAC ($F = 8.486, P = 0.000$). The diagrams of the level of GSH and TAC are presented in figures 1 and 2 respectively.

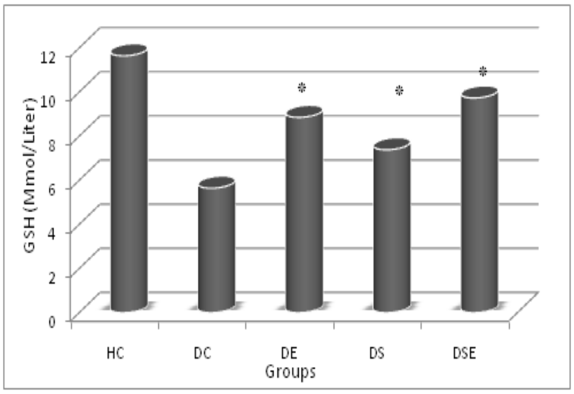

Fig. 1. GSH concentration in groups, * significant difference with diabetic control group (P ≤ 0.05).

The results of Tukey's post hoc test revealed that after four weeks of exercise, the concentration of GSH significantly decreased in the DSE ($P = 0.002$), DE ($P = 0.003$), and DS ($P = 0.001$) groups in comparison with the DC group. Further, the concentration of TAC significantly increased in the DSE group in comparison with the DC group ($P = 0.004$).

Discussion

The purpose of the present research was to study the effect of aerobic exercise and saffron supplementation on antioxidants capacity in diabetic rats. The results showed that the concentration of antioxidants decreased significantly in the rats that were induced with diabetes via streptozotocin injection; yet after oral administration of saffron, conducting the exercise period, and a combination of these treatments, the antioxidant capacity of diabetes-induced rats significantly increased. The increase was greater in the group that performed exercises and took supplements together and the concentration of their hepatic antioxidants reached that of the rats in the HC group.

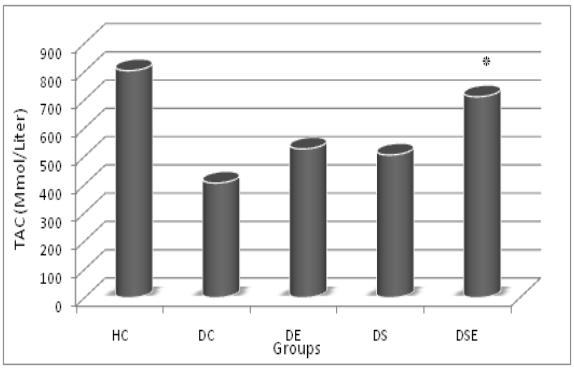

Fig. 2. TAC concentration in groups, * significant difference with diabetic control group (P ≤ 0.05).

Apparently, diabetes is accompanied by many autoimmune diseases in the body, causing disruptions in the electron transport chain and finally increasing free radicals due to oxidative stress while at the same time decreasing the level of blood antioxidants (Chessler & Lernmark, 2000). Moreover, the results of some studies suggest that the function of beta cells is disrupted as a result of prolonged exposure to high levels of glucose and free fatty acids or both (Evans *et al.,* 2003; Evans *et al.,* 2005). Especially β cells are sensitive to reactive oxygen species because they are low in free-radical quenching (antioxidant) enzymes such as catalase, glutathione peroxidase, and superoxide dismutase (Evans, 2007; Evans, *et al.,* 2002). The results of the present research is consistent with the results of previous research indicating that development of diabetes and subsequent disruptions in production and release of insulin will lead to other disruptions in the body and will, in particular, reduce body's antioxidant capacity (Larsen, *et al.,* 2002).

Regarding improvement in the body's antioxidant capacity after administration of saffron, the results of the present research are consistent with some of the findings of previous research. Previously, researchers had specifically reported the effect of saffron in boosting the immune system (Chen *et al.,* 2008). Crocin, crocetin, and safranal are the main compounds of saffron (Liakopoulou-Kyriakides & Kyriakidis, 2002). It has been reported previously that crocin and crocetin are effective in quenching free radicals and have antioxidant properties (Assimopoulou *et al.,* 2005; Chen *et al.,* 2008; Kanakis *et al.,* 2007). Thus, considering the fact that radical quenching activity is strongly related to anti-aging effect, it has been suggested that saffron extract can be used as a supplement in foods and drinks as well as pharmaceutical and cosmetic products (Assimopoulou *et al.,* 2005). For instance, it has been reported that daily consumption of 100 mg saffron dissolved in milk for 6 weeks improves plasma antioxidant capacity in patients diagnosed with coronary heart disease. In the mentioned research, 10 healthy volunteers and 10 patients consumed saffron, while 10 patients formed the control group by taking milk. Lipoprotein sensitivity to oxidative damage decreased by 35.8% in patients and by 42.3% in healthy volunteers, while it did not change significantly in the control group (Verma & Bordia, 1998). The results of a research in this regard showed that due to antioxidant effect, crocetin protects liver cells of rats against the detrimental effects of aflatoxins (Wang, *et al.,* 1991); further, saffron and crocetin can prevent ischemia-induced renal oxidative damage in rats (Hosseinzadeh, *et al.,* 2005). Saleem *et al.* (2006) showed that in the middle cerebral artery occlusion (MCAO) model of acute cerebral ischemia in rats, saffron has an antioxidant effect and can inhibit neuronal cell death; further, it can prevent the degeneration of neurobehavioral activities of the animal (grip strength, spontaneous motor activity, and motor coordination). Finally, the mentioned researchers suggested that saffron may be helpful for local brain ischemia due to its antioxidant properties (Saleem *et al.,* 2006). Thus, it can be argued that the compounds present in saffron extract probably increase blood antioxidants as well as non-enzymatic antioxidants and increase the total capacity for defense against oxidative stress and free radicals in diabetic rats.

In previous studies, Kretzschmer *et al.* (1991) reported an increase in plasma glutathione following endurance exercises in human samples (Kretzschmar, *et al.* 1991). The results of Mena *et al.* (1991) confirmed the effectiveness of a progressive exercise period for improving the activity of the antioxidant enzymes of erythrocytes (Mena *et al.,* 1991). Moreover, most studies carried out on laboratory animal samples such as rats unanimously indicate the improvement in the activity of antioxidant enzymes in muscles (Powers, *et al.,* 1999). Although no research was found to had dealt with the effect of exercise on the activity of hepatic non-enzymatic antioxidants, it was revealed that exercise increases body's total antioxidant capacity; thus, it can be argued that the results of the present research confirm the findings of the previous research (Powers *et al.,* 1994). The results of some studies showed that the weight of some organs or tissues (heart, liver, kidneys, and muscles) increases in diabetic rats, indicating the effect of exercise in morphological and physiological adjustments for maintaining hemostasis (Eaton, *et al.,* 1999; Hansen *et al.,* 2005). The results of Tuomilehto *et al.* (2001) showed conduction of more than 4 hours of training each week without any change in the weight of subjects significantly decreases the chance of being affected by diabetes (Tuomilehto *et al.,* 2001).

Conclusion

The results of the present research showed that the combination of oral saffron supplementation along with exercise has a greater effect on improving the antioxidant capacity of diabetic rats which can be attributed to a sum of the abovementioned reasons for the effect of exercise and saffron supplementation on body's antioxidant capacity. It is thus recommended that saffron supplementation be used as an effective tool for improving the results of exercise in diabetic patients, especially in cases where the point is to improve antioxidant capacity and prevent the incidence of autoimmune diseases as well as damage to tissue sells as a result of oxidative stress due ROS production by mitochondria.

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