Effect of Saffron Extract and Aerobic Exercises on Troponin T and Heart-Type Fatty Acid Binding Protein in Type 2 Diabetes Patients

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Abstract

Objective: Exercise and nutrition as life style modification strategies for the prevention and control of diabetes are considered. The aim of present study was to assess the effects of saffron consumption and aerobic exercises on serum levels of Heart-type fatty acid binding protein (HFABP) and Troponin T in type2 diabetes (T2DM) patients.

Materials and Methods: This study was a quasi-experimental research. Therefore, 24 T2DM men were selected and randomly divided in four groups (1. control, 2. saffronextract, 3. aerobic exercises, 4. compound aerobic exercisesand saffronextract).Saffronextract with 100 mg/daywas used. Aerobic exercises, three days a week, for eight weeks,with 55-70% of maximum heart ratewere performed. At the end, levels ofHFABP) and Troponin Twere measured. Data were analyzed by Paired T-test, One-way ANOVA and Tukey tests.

Results: The serum Troponin T increased significantly in saffron extract, aerobic exercises and compound saffron extract -aerobic exercises in T2DM men(*P*-value:0.024, *P*-value: 0.013, *P*-value:0.005 respectively). Saffron extract consumption (100 mg/day) and aerobic exercises did not significantly influence the serum HFABP (*P*-value: 0.365, *P*-value: 0.188 respectively). But serum HFABP decreased significantly in compound saffron extract -aerobic exercises group (*P*-value: 0.003).

Conclusion: Raised cardiac Troponin T and HFABPconcentration are accepted as the standard biochemical markers for the diagnosis of cardiac injury. Saffron intake maybeneficially protect the myocardium from injuries. Compound saffron extract -aerobic exercises can decrease levels of Troponin T and HFABP in T2DM men.

Keywords: Saffron, Aerobic exercises, Type 2 diabetes, Heart-type fatty acid binding protein, Troponin T

Introduction

Diabetes mellitus is one of the most common metabolic diseases. Diabetes mellitus causes adverse effects such as retinopathy, nephropathy, cardiovascular problems, gastrointestinal disorders and the skeletal muscle atrophy (1). The occurrence of type 2 diabetes (T2DM) is increasing with the decreased physical activity and dietary

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changes .While herbal medicine was traditionally discussed in the treatment of diabetes and its complications, but reliable evidences were not found about their effectiveness (2-4). Physical activity contributes to the prevention of special complications of diabetes (2). Today, sport and physical activity for prevention or treatment of insulin resistance T2DM are important (5,6). Despite numerous findings on the relationship between regular physical activity and reduction of cardiovascular damage, but heavy and acute physical activity impact on the cardiovascular system are unclear. Epidemiological studies showed that regular exercise is associated with decrease cardiovascular risks (7).

Heart-type fatty acid binding protein (HFABP) is a low molecular weight cytoplasmic protein in the myocardium. With myocardium injuries, low molecular weight cytoplasmic proteins including HFABP are released into the circulation. So HFABP is a diagnostic marker for cardiac injury (8). Cardiac Troponin T is cardiac regulatory protein that control the calcium mediated interaction between actin and myosin. The measurement of serum Troponin T is superior in terms of sensitivity and specificity to cardiac muscle enzyme measurements in the identification of cardiac muscle damage. Raised cardiac Troponin T concentration is now accepted as the standard biochemical marker for the diagnosis of cardiac injury (9).

Saffron (*Crocus sativus L.*), dependent to the Iridaceae family, is planted in Iran, Spain, Greece and India. The dried stigmas of Saffron are used as a food seasoning and in herbal medicine for therapeutic purposes. Stimulant, expectorant, antispasmodic are some of the saffronattributes, which have been demonstratein herbal medicine (10). Recent studies have demonstrated the anti-inflammatory (11), learning improvement (12), anti-tumoreffect (13), anti-hypertensive (14) and calcium channel inhibitory (15) properties of saffron.

The phytochemical compounds of saffron include carotenoids (crocin, crocetin), glycoside (picrocrocin) and a fugaciousoil component (safranal). Crocetin and Crocin, which are main carotenoids and bioactive constituents of saffron, have a wide spectrum of biological activities (16). Crocin has an anti-diabetic effect on mice (17). Antioxidant compounds of saffron are effective in prevention and reduction of tissue damages (10). Joukar et al, suggested that saffron has cardioprotective effects which is applied by stability and even consolidation of antioxidant system. Also, saffron cause decrease of contractility and heart rate in stressful conditions (10). There are controversial findings about troponin Tafter aerobic and anaerobic exercises. For example, Löwbeer et al (2007), indicated that the implementation of exercise 5 to 7 times a week reduced Troponin T in football players (18). Scharhag et al (2004), observed that Troponin T remained without significant changes after two standardized endurance exercises in healthy endurance athletes (19). Conversely, Koller et al, (2008) reported that severe exercise led to a significant increase in Troponin T and I(20). Moghadasi et al, investigated the effects of physical exercise on HFABP levels. Studies on athletes showed that two months of aerobic exercises for 45 minutes per session, three times a week, has no significant effect on HFABP levels (21). In previous studies, the effect of resistance exercise on Troponin T and HFABP have not been explored adequately. Thus, the aim of present study was to assess the effects of saffron consumption and aerobic exercises on serum levels of HFABP and Troponin T in T2DM patients.

Materials and Methods

Study method

This study was a quasi-experimental research. The studied samples were 40 -50 years old male T2DM patients, who referred to the Valiasser Hospital, Rasht. They were invited to participate in the study. About 50 patientswanted to participate in this study.

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After interviewing volunteers, 24 men were selected. Inclusion criteria: personal consent to participate in the studyT2DM, lack of chronic obstructive pulmonary disease, lack of pulmonary embolism and angina, no history of hypertension, liver or kidney disease, hypothyroidism and hyperthyroidism. First, study participants gave written consent. The diet was controlled by 24 hours diet questionnaire. The participants were asked not to change their diets during the study period, in addition taking antioxidant supplements such as tea, vitamins C and E or taking medication during the study period was controlled. Before intervention , after 12 hours of fasting, blood was collected in a sitting status in vacuum tubes containing no additives, serum immediately separated by centrifugation at 2000g for 20 min at room temperature and their serum HFABP and Troponin T were measured. For analysis of serum Troponin T, a third generation assay was applied using an Electrochemiluminescence method employed by the Elecsys automated batch analyzer 2010 (Roche Diagnostics, Switzerland, Basel).HFABP levels were measured by a sandwich enzyme linked immunosorbent assay (Markit-M H-FABP, Dainippon Pharmaceutical Co. Ltd.).In addition, heart rate, blood pressure, subcutaneous fat percentage, body mass index (BMI) and VO2max were measured. Then, subjects were randomly divided in to four groups: 1- control (no exercise and without taking the saffron extract) 2- consumption of aqueous extract of saffron, 3- aerobic exercises, 4- consumption of saffron extract and aerobic exercises. The control group received no intervention. Subjects in saffron extract, and compound saffron extract -aerobic exercises groups used saffronextract100 mg per day. Aerobic exercise training groups were present in the Sport Hall of Malavan Javan in Rasht, three times a week, Every day 75 minutes, for eight weeks (a total of 24 sessions) for aerobic exercises with an intensity of 55 to 70 percent under the supervision of a trainer. After two months, a day after the last session, blood samples were taken and their serum HFABP and troponin T levels were measured.

Preparation of Saffron extract

Saffron (Crocus sativus L.) stigmas were collected from Torbat-e Heydarieh (Khorasan province, Northeast of Iran). First, stigmas of saffron is powdered using a pounder; then the resulting powder was dissolved in distilled water and its extract was generated using a distiller. For this purpose, 100 grams of dried stigmas powder were poured in a glass tank and after the addition of 1000 ml of distilled water were boiled for 10 minutes at 100 °C. Then, the supernatant was passed through a filter and kept in 55 °C for a week until step-by-step evaporation of its water and powdered extract was obtained (22).

Statistical analysis

Kolmogorov–Smirnov test was used to evaluate the distribution of the data related to each of the variables. After ensuring the normal data, the parametric tests were used to analyze the data. Therefore, Paired t-test was used to assess the intragroup differences and One-way analysis of variance (ANOVA) was also used to assess the intergroup differences and then if there were significant differences, Tukey's HSD post hoc test was used to identify its value. To analyze the data, SPSS version 12.0 was used. A significance level of $P \leq 0.05$ was considered for all calculations

Results

Table 1 describes the characteristics of subjects. Analysis of changes in HFABP in the four study groups after two months showed that compound aerobic exercises-saffron extract has significant effect on HFABP levels in T2DM men (Table 2).

Paired T-test results showed that the significance level is less than 0.05, so the mean difference indicates that compound aerobic exercises - saffron extract, increases

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Table 1. Demographic characteristics of participants

Variable	Group	Control	Saffron	Aerobic exercise	Saffron-aerobic exercise	*P*-value (inter-group)
Number		6	6	6	6	
Age (years) Mean ± SD		40.3± 4.9	43.7± 1.75	46.5± 1.64	43.8 ± 1.72	
Height (cm) Mean ± SD		174.8± 3.7	175.6 ± 5.6	174.6 ± 3.8	174.5± 1.87	
Weight (kg) Mean ± SD	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/m2) Mean ± SD	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	
maximum oxygen consumption Mean ± SD	pre-test	28 ± 3.03	30± 2.7	33.7 ± 2.5	328 ± 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	*0.00	*0.00	

BMI: body mass index

the amount of HFABP in T2DM men (*P*-value:0.003). As shown in Table 4, significant difference was seen in the Troponin T in the saffron extract (*P*-value:0.024), aerobic training (*P*-value:0.013) and compound aerobic exercises - saffron extract (*P*-value: 0.005) groups. (Table 3).

Data analysis with one-way ANOVA showed that there was no significant difference in the amount of HAFBP Between groups (*P*-value:0.224) (figure 1).

There was a significant difference in the level of troponin T Between groups (*P*-value: 0.003) (figure 2).

Also, Tukey post hoc test showed a significant difference between saffron with control group and train-saffron with control group (*P*-value:0.005 and *P*-value:0.007)

Table 2. Comparison of pre-test and post-test HFABP levels within groups

Group		Paired differences Mean±SD	T	*P*-value
Control	pre-test post-test	5.41±1.28 6.57±2.45	0.971	0.376
Saffron	pre-test post-test	14.15±7.77 11.83±6.65	-0.997	0.365
Aerobic exercise	pre-test post-test	15.75±9.64 9.08±3.85	-1.524	0.188
Saffron-aerobic exercise	pre-test post-test	11.16±3.81 9.16±4.57	-5.477	0.003

Table 3.Comparison of pre-test and post-test Troponin T levels within groups

Group		Paired differences Mean±SD	T	*P*-value
Control	pre-test post-test	33.49±26.31 36.33±24.8	1.086	0.327
Saffron	pre-test post-test	36.41±30.46 21.6±19.22	-3.218	0.024
Aerobic exercise	pre-test post-test	38.61±35.48 29.92±30.06	-3.755	0.013
Saffron-aerobic exercise	pre-test post-test	34.25±21.91 19.85±14.67	-4.732	0.005

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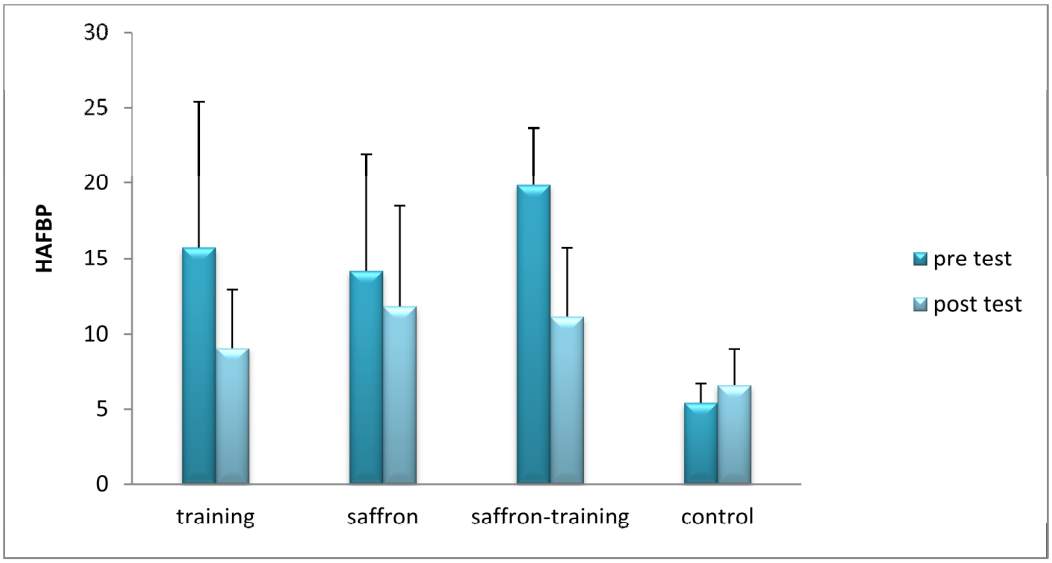


Figure 1. Compare Between groups in HAFBP level

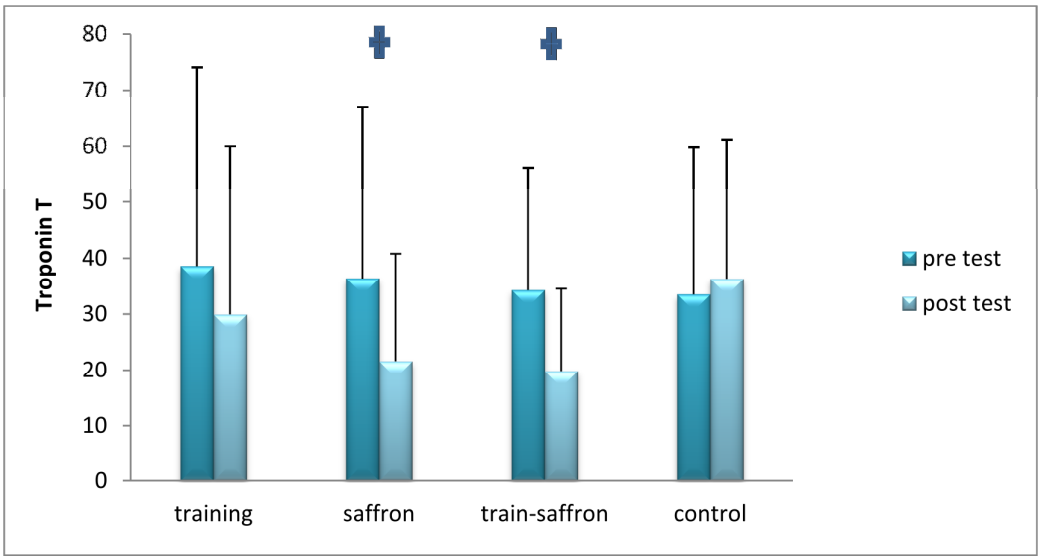


Figure 2. Compare Between groups in troponin T level
Significant difference with control group

Discussion

The aim of present study was to investigate the effects of saffron extract, aerobic exercises and saffron extract-aerobic exercises on serum HFABP and Troponin T in T2DM patients.

The effect of the saffron extract on Troponin T

The results showed that saffron extract consumption for eight weeks at a dose of 100 mg/day caused a significant decrease in the amount of Troponin T in T2DM men. No research was found about the effects of the saffron extract on Troponin T. Most studies about saffron focused on its antioxidant properties. It is believed that saffron has antioxidant properties and also the active ingredient such as crocin and Safranal (23-25). Verma and Bordia are believed that the use of saffron can prevent the increased oxidative

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stress and the development of type 1 diabetes (26). The mechanism of the effect of the saffron extract on Troponin T is unclear. However, more research is required to determine the effect of the saffron extract on Troponin T.

The effect of aerobic exercise and compound saffron extract -aerobic exercises on Troponin T

The results showed that eight weeks aerobic exercises and compound saffron extract - aerobic exercises caused a significant decrease in the amount of Troponin T levels in T2DM men. Several studies have examined the effects of physical exercise on Troponin T levels but there are no reports about interaction between saffron extract and exercise training on Troponin T.

In previous studies, the effect of resistance exercise on Troponin T were not explored adequately, however, it was reported the increases, decreases or no changes on Troponin T after aerobic and anaerobic exercises. For example, a similar study, Löwbeer et al (2007), indicated that the implementation of exercise at 70% maximal oxygen consumption for 45 minutes per session and 5 to 7 times a week reduced Troponin T in football players (18). Conversely, Scharhag et al (2004), observed that Troponin T remained without significant changes after two standardized endurance exercise trials in healthy endurance athletes (19). Also, Rahnama et al (2011), indicated that severe training for 90 minutes per session with carbohydrate supplement, did not change Troponin I in football players. They Concluded that short-term and extreme sports such as football do not effect on cardiac Troponin I (27). Similarly, the results of a study by Rajayi et al (2011) on 15 patients showed that three types of exercises (strength, endurance and combination) did not change Troponin T. Also, O'Hanlon et al, (2010) investigated the effect of long-term heavy exercise on serum concentrations of Troponin I and showed that after marathon, serum levels of Troponin I, in the exercise group compared to the control group, increased significantly (28). Similarly, Shave et al. (2010) investigated the effect of short-term heavy exercise on serum concentrations of Troponin I in active men and showed that after 30 min of running by 75 to 90 percent of maximum heart rate, serum levels of Troponin I in the exercise group compared to the control group, increased significantly (29). Finally, Koller et al, (2008) compared the effects of three types of severe exercise (Marathon, running 100 km, Mountain Biking) on Troponin T and I. They reported that severe exercise led to a significant increase in Troponin T and I in three groups (20). In this study, saffron extract, aerobic exercises and compound saffron extract-aerobic exercises has significant effect on Troponin T levels in T2DM men. The results of more studies are not consistent with our results because of different samples and lack of the saffron extract consumption, type, duration and severity of the exercise, the initial level of Troponin T, age of the participants, their gender and medications that impress exercise effects. Saffron (*Crocus sativus* L.) is a natural antioxidant with various active compounds include crocin, crocetin, and safranal (25). Antioxidant compounds saffron, is effective in prevention and reduction of tissue damages after activities (25). Joukar et al, suggested that saffron has cardio-protective effects on heart, which is applied by stability and even consolidation of antioxidant system and also, it is feasible by decreasing contractility and heart rate in stressful conditions (10). However, it seems that more studies are needed to understand the mechanisms of the extract.

Comparison of the effect of saffron extract, aerobic exercise, and combination of saffron extract- aerobic exercise on the Troponin T

Results of a comparison between the effects of interventions made in Troponin T showed that no significant difference is present between the interventions in decreasing Troponin T, but

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all three interventions caused a significant decrease in the amount of Troponin T compared to the control group. The effect of each of these interventions was described earlier.

The effect of saffron extract, aerobic exercise and compound saffron extract - aerobic exercises on HFABP

The results showed that eight weeks aerobic exercises or saffron extract (100 mg/day) has no significant effect on HFABP levels, but saffron extract-aerobic exercises led to a significant decrease in the amount of HFABP in T2DM men. We did not find significant changes in serum level of HFABP after saffron supplementation and this may be due to the lower dose of saffron (100 mg/day). Higher doses of saffron were examined on animal studies. H-FABP is a sensitive marker for injured myocardium (30). The first function of FABP is the facilitation of intracellular long-chain fatty acid transport (31), other roles include regulation of gene expression by interceding fatty acid signal translocation to peroxisome proliferator activated receptors (PPARs) (32) and protection of cardiac myocytes (33). The cellular expression of FABPs is regulated at the transcriptional level, as a result of changes in lipid metabolism that induced by diabetes (34), resistance exercises and ischemia (35). H-FABP is abundant in the cytosol and released readily into circulating blood following myocardial damage. Thus, H-FABP levels appear to be a useful marker of ongoing myocardial damage in T2DM men with heart failure (36). In this study, increased concentrations of free fatty acids may be an effective factor in reducing levels of H-FABP in the compound saffron extract -aerobic exercises group. There are no reports about interaction between saffron extract and resistance exercises on HFABP. Moghadasi et al, investigated the effects of physical exercise on HFABP levels. The results of the research by Moghadasi et al, (2013) on athletes women, showed that two months of aerobic exercise for 45 minutes per session, three times a week, has no significant effect on serum HFABP (21).

Conclusions

In conclusion, data from this study demonstrated that saffron intake may protect the myocardium from injuries. The consumption of saffron extract, aerobic exercise and compound saffron extract - aerobic exercises had a significant effect on the levels of Troponin T in T2DM men. Also, the compound saffron extract-aerobic exercises led to a significant decrease in the amount of HFABP in T2DM men.

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