

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

Alireza Barari^{1*}, Saeed Shirali², Shirin Amini³, Asieh Abbassi Daloui⁴,
Parvane Golizade Gangraj⁵

1. Associate Professor, Department of Sport Physiology, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran.
2. Assistant Professor, Department of Laboratory Sciences, Hyperlipidemia Research Center, Faculty of Paramedicine, Jundishapur University of Medical Sciences, Ahvaz, Iran.
3. PhD student, Department of Nutrition, Student Research Committee, Faculty of Paramedicine, Jundishapur University of Medical Sciences, Ahvaz, Iran.
4. Assistant Professor, Department of sport Physiology, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran.
5. Msc, Department of sport Physiology, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran.

*Correspondence:

Alireza Barari, Department of Sport Physiology, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran.
Tel: (98) 911 127 7793
Email: alireza54.barari@gmail.com

Received: 17 May 2017

Accepted: 15 August 2017

Published in October 2017

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

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 saffron attributes, which have been demonstrated in herbal medicine (10). Recent studies have demonstrated the anti-inflammatory (11), learning improvement (12), anti-tumor effect (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 fugacious soil 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 T after 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 patients wanted to participate in this study.

After interviewing volunteers, 24 men were selected. Inclusion criteria: personal consent to participate in the study, T2DM, 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 into 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 saffron extract 100 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

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) | | 40.3± 4.9 | 43.7± 1.75 | 46.5± 1.64 | 43.8 ± 1.72 | |
| Mean ± SD | | | | | | |
| Height (cm) | | 174.8± 3.7 | 175.6 ± 5.6 | 174.6 ± 3.8 | 174.5± 1.87 | |
| Mean ± SD | | | | | | |
| Weight (kg) | pre-test | 86.7 ± 11.7 | 75 ±16.16 | 81.5 ± 4.1 | 79.5± 6.3 | |
| Mean ± SD | 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 | |
| Mean ± SD | 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 | pre-test | 28 ± 3.03 | 30± 2.7 | 33.7 ± 2.5 | 328 ± 4.1 | |
| Mean ± SD | 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 | 5.41±1.28 | | |
| | post-test | 6.57±2.45 | 0.971 | 0.376 |
| Saffron | pre-test | 14.15±7.77 | | |
| | post-test | 11.83±6.65 | -0.997 | 0.365 |
| Aerobic exercise | pre-test | 15.75±9.64 | | |
| | post-test | 9.08±3.85 | -1.524 | 0.188 |
| Saffron-aerobic exercise | pre-test | 11.16±3.81 | | |
| | post-test | 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 | 33.49±26.31 | | |
| | post-test | 36.33±24.8 | 1.086 | 0.327 |
| Saffron | pre-test | 36.41±30.46 | | |
| | post-test | 21.6±19.22 | -3.218 | 0.024 |
| Aerobic exercise | pre-test | 38.61±35.48 | | |
| | post-test | 29.92±30.06 | -3.755 | 0.013 |
| Saffron-aerobic exercise | pre-test | 34.25±21.91 | | |
| | post-test | 19.85±14.67 | -4.732 | 0.005 |

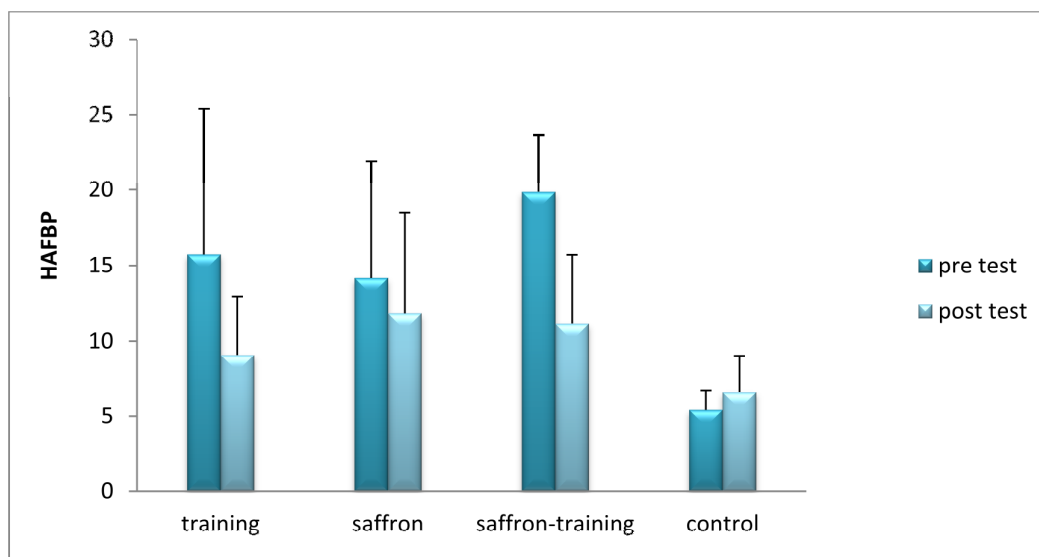
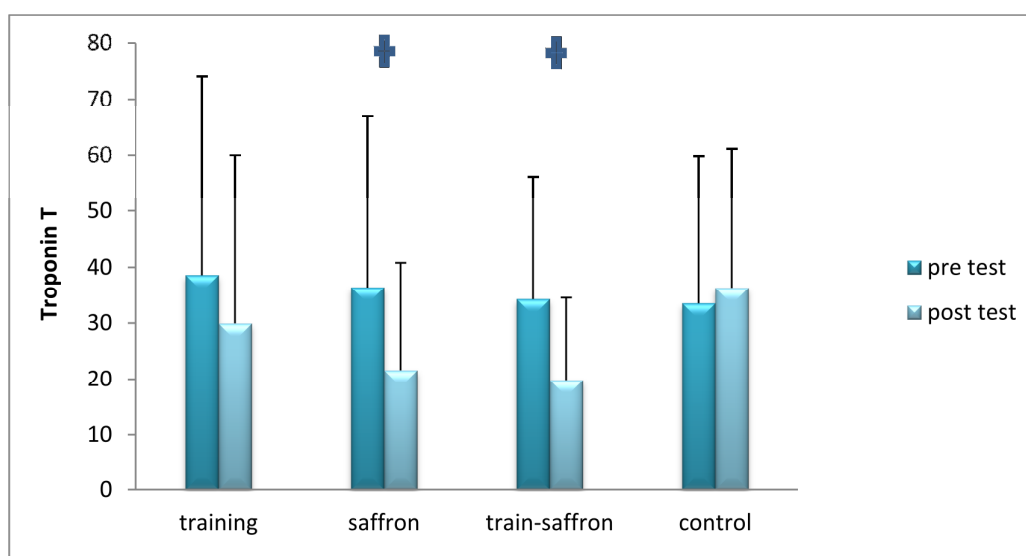


Figure 1. Compare Between groups in HAFBP level

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

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

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.

References

1. Shapiro K, Gong WC. Natural products used for diabetes. *Journal of the American Pharmacists Association* 2002;42:217-26.
2. Stewart K. Exercise training: can it improve cardiovascular health in patients with type 2 diabetes? *British journal of sportsmedicine* 2004;38:250-2.
3. Ebrahimi E, Shirali S, Talaei R. The Protective Effect of Marigold Hydroalcoholic Extract in STZ-Induced Diabetic Rats: Evaluation of Cardiac and Pancreatic Biomarkers in the Serum. *Journal of Botany* 2016.
4. Shirali S, Hosseini SA, Yadollahpour A, Babaali H, Babaali S, Saki S. A Comparative Study on the Effects of Incretin and Metformin on Lipid Profile and Oxidative Stress in Diabetic Rats. *International Journal of Pharmaceutical Research And Allied Sciences* 2016;5:227-33.
5. Ghasem nian A, Kurdi M, Gaini A, Gaorbanian B, M. H. Changes in plasma visfatin and insulin resistance in overweight and obese adolescents non-athletes during 8 weeks of interval endurance rope. *Journal of Zanzan University of Medical Sciences* 2014;11:11-22.
6. Hosseini SA, Gorjian M, Rasouli L, Shirali S. A Comparison between the Effect of Green Tea and Kombucha Prepared from Green Tea on the Weight of Diabetic Rats. *Biosciences Biotechnology ResearchASIA* 2015;20:141-5.
7. Chen Y, Serfass RC, Mackey-Bojack SM, Kelly KL, Titus JL, Apple FS. Cardiac troponin T alterations in myocardium and serum of rats after stressful, prolonged intense exercise. *Journal of Applied Physiology* 2000;88:1749-55.

8. Okamoto F, Sohmiya K, Ohkaru Y, Kawamura K, Asayama K, Kimura H, et al. Human heart-type cytoplasmic fatty acid-binding protein (H-FABP) for the diagnosis of acute myocardial infarction. Clinical evaluation of H-FABP in comparison with myoglobin and creatine kinase isoenzyme MB. *Clinical chemistry and laboratory medicine* 2000;38:231-8.
9. Ammann P, Pfisterer M, Fehr T, Rickli H. Raised cardiac troponins. *Bmj* 2004;328:1028-9.
10. Joukar S, Najafipour H, Khaksari M, Sepehri G, Shahrokhi N, Dabiri S, et al. The effect of saffron consumption on biochemical and histopathological heart indices of rats with myocardial infarction. *Cardiovascular toxicology* 2010;10:66-71.
11. Hosseinzadeh H, Younesi HM. Antinociceptive and anti-inflammatory effects of *Crocus sativus* L. stigma and petal extracts in mice. *BMC pharmacology* 2002;2:1-8.
12. Pitsikas N, Sakellariadis N. *Crocus sativus* L. extracts antagonize memory impairments in different behavioural tasks in the rat. *Behavioural brain research* 2006;173:112-5.
13. Abdullaev F, Espinosa-Aguirre J. Biomedical properties of saffron and its potential use in cancer therapy and chemoprevention trials. *Cancer Detection and prevention* 2004;28:426-32.
14. Fatehi M, Rashidabady T, Fatehi-Hassanabad Z. Effects of *Crocus sativus* petals' extract on rat blood pressure and on responses induced by electrical field stimulation in the rat isolated vas deferens and guinea-pig ileum. *Journal of ethnopharmacology* 2003;84:199-203.
15. Boskabady M, Shafei M, Shakiba A, Sefidi HS. Effect of aqueous-ethanol extract from *Crocus sativus* (saffron) on guinea-pig isolated heart. *Phytotherapy Research* 2008;22:330-4.
16. Hosseinzadeh H, Motamedshariaty V, Hadizadeh F. Antidepressant effect of kaempferol, a constituent of saffron (*Crocus sativus*) petal, in mice and rats. *Pharmacologyonline* 2007;367:70-2.
17. Shirali S, Zahra Bathaie S, Nakhjavani M. Effect of Crocin on the Insulin Resistance and Lipid Profile of Streptozotocin-Induced Diabetic Rats. *Phytotherapy Research* 2013;27:1042-7.
18. Löwbeer C, Seeberger A, Gustafsson SA, Bouvier F, Hulting J. Serum cardiac troponin T, troponin I, plasma BNP and left ventricular mass index in professional football players. *Journal of Science and Medicine in Sport* 2007;10:291-6.
19. Scharhag J, Urhausen A, Herrmann M, Schneider G, Kramann B, Herrmann W, et al. No difference in N-terminal pro-brain natriuretic peptide (NT-proBNP) concentrations between endurance athletes with athlete's heart and healthy untrained controls. *Heart* 2004;90:1055-6.
20. Koller A, Sumann G, Griesmacher A, Falkensammer G, Klingler A, Fliri G, et al. Cardiac troponins after a downhill marathon. *International journal of cardiology* 2008;129:449-52.
21. Moghadasi M, Nuri R, Ahmadi N. effects of 8 weeks high intensity aerobic exercise on serum retinol binding protein 4 levels infemale athletes. *Brazilian Journal of Biomotricity* 2013;7.
22. H SS. Extracts of medicinal plants and extraction of active ingredients and methods Identify and evaluate them. *Mani*;1992.
23. Kanakis CD, Tarantilis PA, Tajmir-Riahi HA, Polissiou MG. Crocetin, dimethylcrocetin, and safranal bind human serum albumin: stability and antioxidative properties. *Journal of Agricultural and Food Chemistry* 2007;55:970-7.
24. Shirali S, Bathaie S, Nakhjavani M, Ashoori M. Effects of saffron (*Crocus Sativus* L (.aqueous extract on serum biochemical factors in streptozotocin-induced diabetic rats. 2012.
25. Bathaie S, Mokarizade N, Shirali S. An overview of the mechanisms of plant ingredients in the treatment of diabetes mellitus. *Journal of Medicinal Plants* 2012;1:4-24.
26. Verma S, Bordia A. Antioxidant property of Saffron in man. *Indian journal of medical sciences* 1998;52:205-7.
27. Rahnama N, Faramarzi M, Gaeini AA. Effects of intermittent exercise on cardiac troponin I and creatine kinase-MB. *International journal of preventive medicine* 2011;2.
28. O'Hanlon R, Wilson M, Wage R, Smith G, Alpendurada FD, Wong J, et al. Troponin release following endurance exercise: is inflammation the cause? a cardiovascular magnetic resonance study. *Journal of Cardiovascular Magnetic Resonance* 2010;12:1.
29. Shave R, Baggish A, George K, Wood M, Scharhag J, Whyte G, et al. Exercise-induced cardiac troponin elevation: evidence, mechanisms, and implications. *Journal of the American College of Cardiology* 2010;56:169-76.
30. Adams JE, Schechtman KB, Landt Y, Ladenson JH, Jaffe AS. Comparable detection of acute myocardial infarction by creatine kinase MB isoenzyme and cardiac troponin I. *Clinical chemistry* 1994;40:1291-5.
31. Tanaka T, Hirota Y, Sohmiya K-I, Nishimura S, Kawamura K. Serum and urinary human heart fatty acid-binding protein in acute myocardial infarction. *Clinical biochemistry* 1991;24:195-201.
32. Kleine AH, Glatz JF, Van Nieuwenhoven FA, Van der Vusse GJ. Release of heart fatty acid-binding protein into plasma after acute myocardial infarction in man. *Lipid Metabolism in the Healthy and Disease Heart*: Springer; 1992. p. 155-62.
33. Setsuta K, Seino Y, Takahashi N, Ogawa T, Sasaki K, Harada A, et al. Clinical significance of elevated levels of cardiac troponin T in patients with chronic

- heart failure. The American journal of cardiology 1999;84:608-11.
34. Tsutamoto T, Wada A, Maeda K, Hisanaga T, Maeda Y, Fukai D, et al. Attenuation of compensation of endogenous cardiac natriuretic peptide system in chronic heart failure prognostic role of plasma brain natriuretic peptide concentration in patients with chronic symptomatic left ventricular dysfunction. Circulation 1997;96:509-16.
35. Wodzig KWH, Kragten JA, Hermens WT, Glatz JF, van Dieijen-Visser MP. Estimation of myocardial infarct size from plasma myoglobin or fatty acid-binding protein. Influence of renal function. Clinical Chemistry and Laboratory Medicine 1997;35:191-8.
36. Yoshimoto K, Tanaka T, Somiya K, Tsuji R, Okamoto F, Kawamura K, et al. Human heart-type cytoplasmic fatty acid-binding protein as an indicator of acute myocardial infarction. Heart and vessels 1995;10:304-9.