



Disponible en ligne sur

ScienceDirect
www.sciencedirect.com

Elsevier Masson France

EM|consulte
www.em-consulte.com



ORIGINAL ARTICLE

Effect of *Crocus Sativus Linnaeus* (saffron) supplementations combined with circuit resistance training on total creatine kinase, lactate dehydrogenase and creatine kinase MB levels in young untrained men



Effets de la supplémentation en Crocus Sativus Linnaeus (safran) combinée à l'entraînement en musculation sur les niveaux de créatine kinase totale et MB, et de lactate déshydrogénase chez des jeunes adultes non entraînés

A. Ghanbari-Niaki^a, A. Saeidi^a, S. Kolahdouzi^a,
M. Aliakbari-Baydokhty^a, S. Ardeshiri^a, A.B. Abderrahman^b,
F. Rhibi^b, F. Malekian^d, H. Zouhal^{c,*}

^a Exercise Biochemistry Division, Faculty of Sport Sciences, University of Mazandaran, Babolsar, Mazandaran, Iran

^b Laboratory of Biomonitoring of the Environment, Faculty of Science of Bizerte, University of Carthage, Tunisia

^c Movement, Sport and Sciences laboratory (M2S), UFR-APS, University of Rennes, CS 24414, 2, avenue Charles-Tillon, 35044 Rennes cedex, France

^d Southern University Agricultural Land Grant Campus, Baton Rouge, LA, USA

Received 29 December 2017; accepted 10 October 2018

Available online 23 December 2018

KEYWORDS

Circuit resistance training;
Saffron supplements;
Lactate dehydrogenase;
Creatine kinase

Summary Cardiovascular diseases are among the main cause of deaths in developing countries. This study aims to investigate the effect of circuit-resistance training with saffron supplementation on some cardiovascular risk factors in young untrained men. Twenty-three healthy untrained men participated in a randomized controlled, double blind study and were divided in two groups: placebo–training (PT, $n = 11$) and saffron stigma (sargoal)–training (ST, $n = 12$) groups. Participants were trained in a circuit-resistance training program (12 stations, 30 second/station) for 5 days/week for the duration of 2 weeks. The ST group received

* Corresponding author.

E-mail address: hassane.zouhal@univ-rennes2.fr (H. Zouhal).

supplement in form of capsules (250 mg) twice per day (morning and immediately after each training session) while the PT group received water. Blood samples were taken 48 hours before and after the last training session to determine creatine kinase (CK), CK-MB, and lactate dehydrogenase (LDH) concentrations. Statistical analysis showed that there were no significant differences in cardiovascular risk factor ($P > 0.05$) except for plasma levels of LDH ($P = 0.025$) between the two groups. In conclusion, the results of the present study indicated that the 2 weeks circuit-resistance program was able to induce significant changes in measurement variables and supplementation by saffron somewhat reinforced the effect of circuit-resistance training program.

© 2018 Elsevier Masson SAS. All rights reserved.

MOTS CLÉS

Circuit-training ;
Supplémentation en
safran ;
Lactate
déshydrogénase ;
Créatine kinase

Résumé Les maladies cardiovasculaires sont parmi les principales causes de décès dans les pays en développement. Ce travail vise à étudier les effets de l'entraînement en musculation sous forme de circuit (CRT) combiné à la supplémentation en safran sur certains facteurs de risque cardiovasculaires chez les jeunes hommes non entraînés. Essai contrôlé randomisé et en double aveugle. Au total, vingt-trois hommes non entraînés en bonne santé ont participé à cette étude et ont été divisés en deux groupes différents : un groupe entraîné et consommant de l'eau (PT, $n = 11$) et un autre groupe entraîné et consommant du safran (ST, $n = 12$). Les participants ont suivi le programme de CRT (12 stations, 30 secondes/station, 5 jours/semaine, pendant 2 semaines) et ont reçu un supplément sous forme de capsule (250 mg) deux fois par jour (matin et immédiatement après chaque séance d'entraînement). Des échantillons sanguins ont été prélevés 48 heures avant et après la dernière séance d'entraînement pour déterminer les concentrations en créatine kinase (CK), CK-MB et lactate déshydrogénase (LDH). L'analyse statistique a montré qu'il n'y avait pas de différences significatives entre les deux groupes concernant les facteurs de risque cardiovasculaire ($p > 0,05$), sauf pour la LDH ($p = 0,025$). En conclusion, les résultats de la présente étude indiquent que seulement deux semaines d'entraînement sous forme de CRT sont capables d'induire des variations significatives concernant les concentrations plasmatiques de CK, CK-MB et de LDH. Les résultats suggèrent aussi que la supplémentation en safran a, en quelque sorte, renforcé l'effet du programme d'entraînement.

© 2018 Elsevier Masson SAS. Tous droits réservés.

1. Introduction

Cardiovascular diseases are among the main cause of deaths in developing countries [1]. Cardiovascular diseases such as myocardial infarction are as a result of heart and blood vessels dysfunction and it is mostly diagnosed with proprietary enzymes such as lactate dehydrogenase (LDH), creatine kinase (CK), and its isoenzyme CK-MB [2]. Changes in these serum enzymes have been assumed as an indicator to identify the tissue and cell dysfunction [3,4]. CK-MB isoenzyme is one of the most valid clinical indicator of heart cells damages [3–5]. Studies have shown that diagnosis of myocardial infarction and damages by CK-MB serum level as a golden indicator [3–6].

In order to accurately assess myocardial damage, previous studies cited CK-MB/CK serum higher than 3–5% as a sign of CK-MB leakage from myocardial cells into bloodstream, and over 20% as a risk to damage myocardial cells [3–6].

Physical activity reduces the risk of heart disease. It is well known that regular exercise prevents the risk of chronic diseases such as cardiovascular diseases, diabetes, cancer, hypertension, obesity, depression, osteoporosis premature

death, and heart failure [2,6,7]. While, most studies have focused on the impact of physical activity and exercise on the mentioned enzymes, few have addressed the effect of exercise combined with a healthy diet/supplement with antioxidant properties on CK-MB or CK-MB/CK [2–4,6,7].

For example, Yu et al. [8] observed a significant increase in serum CK levels in response to creatine supplementation combined with two weeks of resistance exercise in athletes [8]. However, Rawson et al. [9] found no significant changes in blood enzymes activity in response to daily consumption of 20 grams of creatine supplementation for five days followed by an acute bicycle ergometer exercise in young males. [9]. In another study it was concluded that supplement consumption prevented muscle weakness in older adults [10].

In recent years, consumption of medicinal plants has been suggested for athletes and those who engaged in different types of physical activity. Therefore, researchers recently have turned to experiment natural supplements due to their multiple properties and lack of side effects [1,11]. Saffron has antioxidant properties that increase glutathione levels and helps prevent lipid oxidation [11]. The

Table 1 Descriptive characteristics of subjects.

Group	ST	PT
Age (years)	21.50 ± 1.93	21.91 ± 2.34
Height (cm)	175.92 ± 5.31	178.18 ± 4.75
Weight (kg)	67.42 ± 8.46	69.91 ± 9.40
BMI (kg/m ²)	21.75 ± 1.96	22.00 ± 2.96

Data are represented as mean + SD. Placebo plus training: PT; Saffron plus training: ST; Body mass index: BMI.

result of another study on saffron showed that consumption of 200 and 400 mg saffron per week lead to a significant increase in creatinine, blood urea and sodium [12]. On the other hand, the effect of saffron on damaged tissues has not been studied up to this date. Therefore, the goal of this investigation was to study the effect of circuit resistance training along with saffron supplementation on some cardiovascular risk indices in inactive men was studied.

2. Materials and methods

2.1. Subjects and anthropometrical measurements

The present study has a functional and quasi-experimental design. Twenty-three young male volunteers from University of Mazandaran participated in this study. Before the implementation of the study, all the steps and procedures were explained to the participants and after obtaining full knowledge from protocols and completing a medical questionnaire, a written informed consent was obtained. The inclusion criteria included: non-addiction to drugs and alcohol, lack of regular exercise for at least 6 months, no history of renal, hepatic, cardiovascular diseases and diabetes, and no injury or physical problem of any kind. Participants were matched based on weight, height and body mass index (BMI) (Table 1) and randomly divided into two groups by a person independent of the trial with group allocation provided in sequentially numbered sealed envelopes. The two groups consisted of: 1) placebo plus training (PT, $n = 11$) and saffron supplement plus training (ST, $n = 12$).

2.2. Experimental and exercise protocol

Participants were familiarized with the gym and equipment and received instruction in proper lifting technique for the prescribed exercises during the first week. Following the familiarization week one repetition maximum (1-RM) of the prescribed movements was determined using the Brzycki equation (Brzycki, 1993) [13]: (displaced weight (kg)/1.0278) - number of repetition up to failure \times 0.0278).

After measuring 1-RM, all subjects performed two sets of 12 station including; squat, chest press, leg press, standing military press, knee extension, seated cable rowing, knee curl, biceps curl, standing calf raise, triceps press, back extension, and abdominal crunch at the intensity of 40% of 1-RM for 30 second in a station with 3 min rest between each set, over 2 weeks (5 sessions per week) [1]. Each exercise session started with 10 min warm-up and cool-down.

Table 2 The main components of the saffron stigma evaluated by GC-MS analysis.

Saffron stigma	
42.15%	Safrenal
36.16%	Octadecanoic acid
9.9%	Polyphenol
8.56%	Linoleic acid

2.3. Preparing and consuming of saffron supplement

Saffron leaves were collected in December, around the gardens of Beydokht of Mashhad, East of Iran, and dried in the shade for 10 days. Saffron leaves were further dried in an oven for 48 hours at a temperature of 32 °C and powdered.

The isolation of the volatile compounds was performed in an ultrasound water bath Sonorex (Berlin, Germany) Super RK 255H type (300 9 150 9 150 mm internal dimensions), at the fixed frequency of 35 kHz. The temperature of the sonicated water bath was 25 °C. The sample flask was charged with 5 g of saffron. The solvent system extractant was 50 mL of diethyl ether. Each saffron sample was sonicated two times for 15 min (two fractions per saffron sample). For each sonication a new volume of the solvent extractant was added in the sample flask. After the end of each sonication the whole organic extract (100 mL) was collected. The whole organic extract was concentrated to 1/3 using a rotary evaporator type Heidolph Laborota 4000 Efficient (Schwabach, Germany). The temperature of the water bath was 20 °C. The organic extract was placed into a volumetric cylinder and it was further concentrated by a gentle flow of nitrogen up to a 5 mL volume and a minor quantity of anhydrous magnesium sulphate was added (Anastasaki et al., 2009) [14] (Table 2).

The subjects consumed 500 mg capsule of the saffron stigma (ST) or placebo (water Hoffmann) in two phases (250 mg after breakfast and 250 mg immediately after the exercise, with 100 ml of water) per day (2003). Capsules were made in biochemistry laboratory of university of Mazandaran.

2.4. Dietary habits control

All participants were resided in Navab dormitory and ate the same foods. They were asked to have their normal nutrition habits during the experiment and to eat the same food for pretest and posttest two days before blood sampling process. Subjects were also asked to complete a two-day food diaries prior to both the baseline and endpoint blood sampling. The amount of nutrients consumed was calculated using the method described by McCance, (2002) [15] (Table 3).

Participants were requested to comply with the following conditions prior to each blood sample:

- avoid use of non-prescription medications and supplements;

Table 3 Food intake for 2 days before the pretest and posttest blood sampling in two groups.

	ST	PT		
	Pretraining	Posttraining	Pretraining	Posttraining
Total (kcal/day)	2225 ± 190	2396 ± 103	2203 ± 157	2287 ± 98
Total protein (g/day)	111 ± 16	112 ± 17	112 ± 14	110 ± 18
Protein (g/kg BW/day)	1.05 ± 0.45	1.2 ± 0.4	1.1 ± 0.4	1.0 ± 0.3
Total protein (% energy)	18.3 ± 4.1	19.6 ± 3.5	18.4 ± 4.2	19.7 ± 4.9
Total CHO (g/day)	295 ± 19	304 ± 23	285 ± 20	289 ± 25
Total CHO (% energy)	48.5 ± 6.9	50.8 ± 7.1	47.4 ± 7.2	50.5 ± 7.0
Total fat (g/day)	81.5 ± 21	77.3 ± 27	83.5 ± 23	78 ± 26
Total fat (% energy)	31.9 ± 7.6	28.8 ± 6.9	32.5 ± 6.9	29 ± 6.4

Data are represented as mean + SD. Placebo plus training: PT; Saffron plus training: ST; Carbohydrate: CHO.

- avoid any strenuous exercise other than the exercise prescribed as part of the study for at least 72 h before the test;
- avoid coffee, dark tea, bananas, cereal and heavy or greasy foods at least 24 h before the test, and 4) match their diet in the 48 hours prior to each blood sample.

2.5. Laboratory measurements

Fasting blood samples were collected from the antecubital vein of the participants in the morning at 10:00 am to 12 noon in two separate phases: 48 hours before and 48 hours after the last exercise session. Blood samples were centrifuged at 3000 rpm for 10 minutes. The plasma was isolated and used for analysis of LDH through photometric method (DGKC) using Pars Azmoon quantitative diagnostic kits test (Tehran, Iran) with a coefficient of variation and sensitivity of 1.2% and 5% international unit per liter for the measurement method. Similarly, plasma and CK-MB were used through the same instrument with a coefficient of variation and sensitivity of 70% and 1 international units per liter.

2.6. Statistical analysis

The results were analyzed using SPSS version 20 at the significant level of $P < 0.05$. After confirming normal distribution of data using Kolmogorov-Smirnov test, results of the study and Levene's test showed normal distribution of data and homogeneity of variances ($P > 0.05$). Repeated measures ANOVA was used to compare the two groups and paired-samples *t*-test was used to determine intragroup changes.

3. Results

Results showed that in ST group LDH decreased significantly in comparison to that of PT ($P = 0.025$). Intragroup analysis showed a significant reduction after training period in ST group ($P = 0.005$). There was no significant difference in PT group ($P > 0.05$) (Fig. 1A).

No significant changes were observed concerning the plasma levels of total CK between ST and PT groups ($P = 0.734$). But analysis of intra-group changes showed

significant decrease in PT ($P < 0.001$) and ST ($P = 0.002$) groups (Fig. 1B).

Changes of plasma levels of CK-MB ($P = 0.623$) were not significant between the two groups. But intra-group analysis showed a significant decrease between the data before and after exercise in PT ($P = 0.003$) and ST ($P < 0.001$) groups (Fig. 1C).

The results showed no significant differences in CK-MB/CK between the two groups ($P = 0.343$), but in the intra-group, CK-MB/CK significantly dropped in PT group (Fig. 1D, $P = 0.018$).

4. Discussion

Reducing muscle damage and improving muscle performance is the most important objective of exercise training that is also affected by food and nutrition. The results of this study showed that short term circuit resistance training with or without saffron supplement reduces muscle damage indices in the young untrained subjects. Also, these data revealed that consumption of saffron with exercise training is more effective to reduce muscle damage markers such as LDH (Fig. 1A).

The exact mechanism of these changes is unclear. But it may be related to antioxidant properties of saffron. Studies have shown that dried stigma of the saffron plants are rich in carotenoids crocin, crocetin and safranal. Flavonoids in saffron are able to neutralize free radicals, particularly superoxide [11,12,16]. Also, the same scientists suggested that the effect of analgesic and anti-inflammatory properties of saffron extract compared to conventional anti-inflammatory pain relieving medications have been approved in laboratory mice [11]. Thus, in this study it is possible that the existence of antioxidants in saffron inhibited accumulation of free radical during exercise, and eventually led to more reduction in LDH levels in plasma in comparison with exercise training alone. Previously, it was observed that using antioxidant supplementation with exercise can delay oxidative damage in the blood and skeletal muscle, which is caused by exercise and thereby, reduces oxidative stress [14].

The antioxidant compounds in saffron include crocin, safranal, carotenoids, flavonoids, crocin and crocetin [10]. These antioxidant compounds of saffron may prevent lipid peroxidation of the cell wall and increase the antioxidant

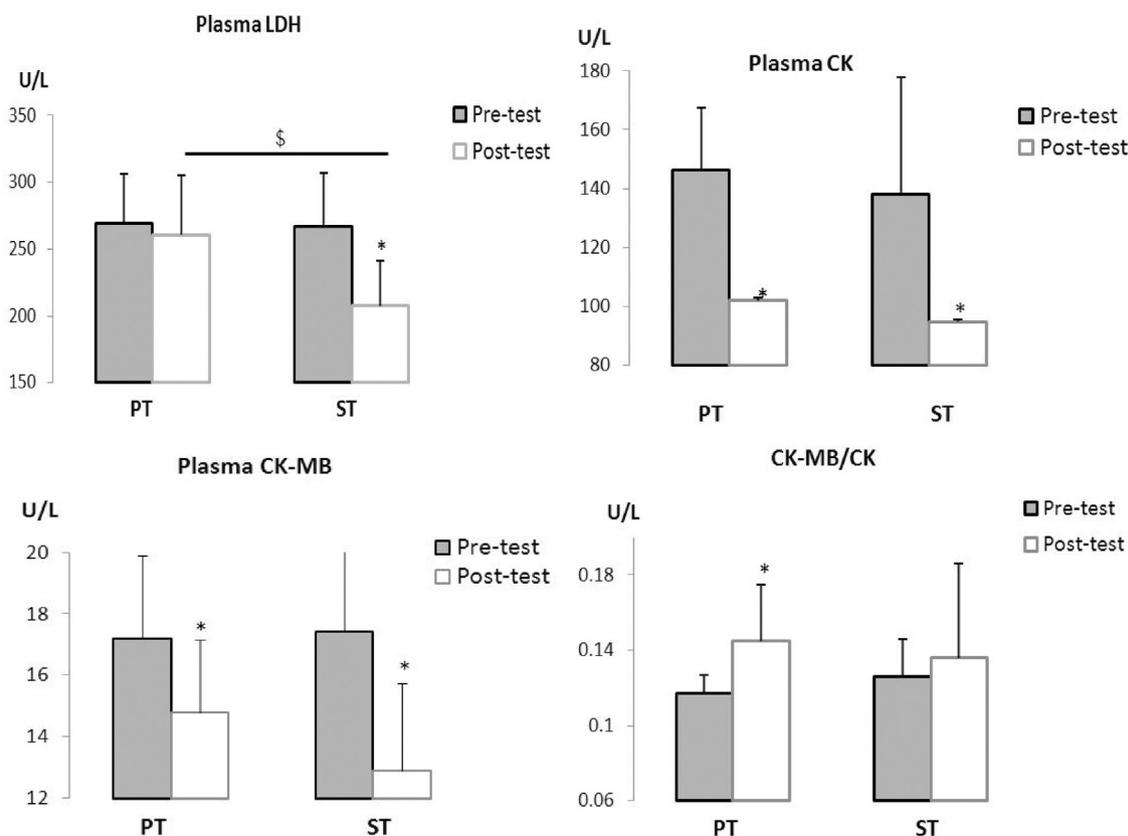


Figure 1 Differences between groups (PT and ST) and within group in plasma levels of LDH, CK, CK-MB, CK-MB/CK. *: Significant difference between before and after training ($P < 0.05$). \$: Significant differences between groups ($P < 0.05$).

capacity of the body [17]. Therefore, positive properties of saffron may be attributed to its antioxidant effects.

Possible mechanism of saffron in reducing the levels of these enzymes, probably decreases membrane lipid peroxidation, and reduces damage to phospholipids membrane of muscle cells perhaps by eliminating free bases and increasing the body's antioxidant capacity. Therefore, it prevents the leakage and penetration of these intracellular enzymes into the cell [18,19].

In line with current study, previous studies have suggested that consumption of saffron during resistance exercise reduces fibrinogen and viscosity and showed the reduction of these indices can be associated to the reduction in CK and LDH [1]. In consistent with this findings, studies have shown that exercise-induced oxidative stress and acute inflammatory phase response that causes tissue damage can be reduced by antioxidant supplementations [11,12,20]. By destructing the cell lipid membrane, lipid peroxidation increases the leakage of CK and LDH from the cells [11,12,20].

Preventive effects of antioxidants such as vitamin E and C [21] have not been able to prevent markers of muscle damage similar to the current study. Thus, it is likely that saffron has other bioactive component that may affect plasma membrane to reduce muscle damage. Therefore, further investigation on different compounds showed that saffron is pure of unsaturated fatty acids such as linoleic and octadecanoic. These fatty acids are involved in cell membrane structure and by strengthening against the created

stress, cardiovascular diseases such as atherosclerosis and heart attack is reduced [21]. Thus, it is possible that saffron through reinforcement of plasma membrane prevents exercise-induced cell damage. One limitation of this study was the examination of cell membrane changes during exercise training, which needed muscle biopsy. Therefore further investigation is needed.

Resistance training reduces cardiovascular risk indices such as CK and CK-MB. In line with results from this study, another study has shown that resistance exercise increases the antioxidant enzymes (glutathione and Total antioxidant capacity) thereby, reduces free radicals and prevents damage occurring to the cell wall and an increase in CK [22]. Muscle damage increases due to stress, stiffness of arteries and atherosclerosis and these enzymes appear at the plasma level. Reduction in levels of these enzymes can have a variety of reason including reduces levels of fibrinogen and viscosity caused by a period of circuit resistance training, which reduce stress in vessels and prevent an increase in levels of the enzymes [1]. Other reasons may include: increase levels of HDL, reduces levels of LDL, VLDL, triglycerides and cholesterol [23], decreases level of some hematological and rheological factors after a period of training [23,24].

In the present study the subjects were inactive. In a study by Brancaccio et al. it was observed that less reduction in levels of serum enzymes after physical exercise was observed in athletes compared to non-athletes, slow-twitch compared to fast-twitch fibers, white people compared to black people, and the elderly compared to the young [4].

Based on previous studies, the level of physical preparation is an important factor in research related to oxidative stress since physical preparation and doing regular exercise speeds up muscle recovery capacity for the energy production by the oxidation processes [25]. It has been reported that regular physical activity reduces oxidative stress indices and levels of antioxidant enzymes in the muscles [25,26]. Regular physical activity can, at least, prevent age-related endothelial damage [25,26].

Even though in this study the period of training was short, but it seems that short run circuit training may have improved cell plasma structure in untrained individuals. When it was combined with antioxidant supplements such as saffron the plasma membrane structure was more strengthened. Therefore, to understand the exact mechanism of these effects, future research should be conducted.

5. Conclusion

The results from this study showed that low intensity circuit resistance training represent an appropriate exercise for inactive individuals for improving cardio and skeletal muscle damages. Moreover, saffron supplementation may reinforce the effect of circuit resistance training on some cardiovascular risk factors. Further studies are needed to clarify the mechanism of these effects.

6. Practical Implications

Crocus Sativus (saffron) especially saffron stigma (sargol) could be useful for athletes and non-athletes who want to improve their anti-inflammatory capacity.

Circuit resistance training is a one of training strategies to provide a better condition for cardiovascular functions.

Saffron supplementations with circuit resistance training reduce some cardiovascular risk factors.

Disclosure of interest

The authors declare that they have no competing interest.

Acknowledgments

We would like to thank the participants for their valuable cooperation in this project and to Dr. Azarmeidokht Ghahari, the chief of Babolsar pathobiology lab for blood analysis, and all participants for take part in research study.

References

- [1] Ghanbari-Niaki A, et al. Effects of circuit resistance training with *Crocus Sativus* (saffron) supplementation on plasma viscosity and fibrinogen. *Ann Appl Sport Sci* 2015;3(2):1–10.
- [2] McPherson RA, Pincus MR. Henry's clinical diagnosis and management by laboratory methods e-book. In: Elsevier Health Sciences; 2017.
- [3] Christenson RH, Azzazy HM. Biochemical markers of the acute coronary syndromes. *Clin Chem* 1998;44(8):1855–64.
- [4] Brancaccio P, Maffulli N, Limongelli FM. Creatine kinase monitoring in sport medicine. *Br Med Bull* 2007;81(1):209–30.
- [5] Chanoit G, et al. Exercise does not induce major changes in plasma muscle enzymes, creatinine, glucose and total proteins concentrations in untrained beagle dogs. *Transboundary Emerg Dis* 2002;49(4):222–4.
- [6] Souza C, Garcez-Leme L. Evaluation of myocardial alterations using the enzymatic profile of elderly long-distance runners. *Eur Rev Aging Phys Act* 2006;3(2) [p 91].
- [7] Thirumalai T, et al. Intense and exhaustive exercise induce oxidative stress in skeletal muscle. *Asian Pac J Trop Dis* 2011;1(1):63–6.
- [8] Yu P, Deng Y. Potential cytotoxic effect of chronic administration of creatine, a nutrition supplement to augment athletic performance. *Med Hypotheses* 2000;54(5):726–8.
- [9] Rawson ES, Gunn B, Clarkson PM. The effects of creatine supplementation on exercise-induced muscle damage. *J Strength Cond Res* 2001;15(2):178–84.
- [10] Abdullaev F. Plant-derived agents against cancer, in *Pharmacology and therapeutics in the new millennium*. India: Narosa Publishing House, New Delhi; 2001. p. 345–54.
- [11] Meamarbashi A, Abedini F. Preventive effects of purslane extract on delayed onset muscle soreness induced by one session bench-stepping exercise. *Isokinetics Exerc Sci* 2011;19(3):199–206.
- [12] Modagheh M-H, et al. Safety evaluation of saffron (*Crocus sativus*) tablets in healthy volunteers. *Phytomedicine* 2008;15(12):1032–7.
- [13] Brzycki M. Strength testing—predicting a one-rep max from reps-to-fatigue, *Journal of Physical Education. Recreation Dance* 1993;64(1):88–90.
- [14] Nieman DC, et al. Influence of vitamin C supplementation on oxidative and immune changes after an ultramarathon. *J Appl Physiol* 2002;92(5):1970–7.
- [15] McCance R. Food Standards Agency; AFRC Institute of Food Research McCance and Widdowson's The composition of foods. In: Cambridge: royed society of chemistry; 2002.
- [16] Gliottoni RC, et al. Effect of caffeine on quadriceps muscle pain during acute cycling exercise in low versus high caffeine consumers. *Int J Sport Nutr Exerc Metab* 2009;19(2):150–61.
- [17] Farahmand SK, et al. Saffron ameliorates antioxidant enzymes and suppresses lipid peroxidation and nitric oxide formation in aged male rat liver. *Bogerontology* 2013;14(1):63–71.
- [18] Charles DJ. Antioxidant properties of spices, herbs and other sources. In: Springer Science & Business Media; 2012.
- [19] Tuberoso CI, et al. Antioxidant activity, cytotoxic activity and metabolic profiling of juices obtained from saffron (*Crocus sativus* L.) floral by-products. *Food Chem* 2016;199:18–27.
- [20] Thompson D, et al. Prolonged vitamin C supplementation and recovery from demanding exercise. *Int J Sport Nutr Exerc Metab* 2001;11(4):466–81.
- [21] Sawyer BG. Tietz fundamentals of clinical chemistry and molecular diagnostics. Elsevier; 2015.
- [22] Sheikholeslami DV, Ahmadi FKG. Changes in antioxidant status and cardiovascular risk factors of overweight young men after six weeks supplementation of whey protein isolate and resistance training. *Appetite* 2012;59(3):673–8.
- [23] Prabhakaran B, et al. Effect of 14 weeks of resistance training on lipid profile and body fat percentage in premenopausal women. *Br J Sports Med* 1999;33(3):190–5.
- [24] Kilibic-Toprak E, et al. Hemorheological responses to progressive resistance exercise training in healthy young males. *Med Sci Monit* 2012;18(6):pCR351.
- [25] Tiidus PM. Estrogen and gender effects on muscle damage, inflammation, and oxidative stress. *Can J Appl Physiol* 2000;25(4):274–87.
- [26] Clarkson PM, Thompson HS. Antioxidants: what role do they play in physical activity and health? *Am J Clin Nutr* 2000;72(2):637S–46S.