

Pre-supplementation of *Crocus sativus* Linn (saffron) attenuates inflammatory and lipid peroxidation markers induced by intensive exercise in sedentary women

Mohammad Hosseinzadeh¹, Hossein TaheriChadorneshin^{2*}, Mahboobe Ajam-Zibad³, Seyed-Hosein Abtahi-Eivary⁴

¹Department of Sport Sciences, University of Farhangian, Tehran, Islamic Republic of Iran.

²Department of Sport Sciences, University of Bojnord, Bojnord, Islamic Republic of Iran.

³Exercise Physiology, University of Gonabad, Gonabad, Islamic Republic of Iran.

⁴Department of Clinical Biochemistry, Gonabad University of Medical Sciences, Gonabad, Islamic Republic of Iran.

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ABSTRACT

Antioxidant and anti-inflammatory actions of *Crocus sativus* Linn (saffron) in relation to intensive exercise need to be studied. This study aimed to investigate the effect of four weeks of saffron supplementation on serum levels of tumor necrosis factor alpha (TNF- α), as a marker of inflammation, malondialdehyde (MDA), as a marker of lipid peroxidation, and levels of Lactate dehydrogenase (LDH) and creatine kinase (CK), as two markers of muscle damage, in sedentary women following intensive exercise. After a 4-week period of supplementation (30 mg/day), the healthy inactive young women performed one bout of acute resistance exercises at 85 % of one-repetition maximum. Using commercial kits, TNF- α and MDA levels were measured using ELISA method. In addition, LDH and CK were measured by photometric method. Data were statistically analyzed by one-way ANOVA ($P < 0.05$). Our finding showed a significant increases in serum LDH ($P = 0.015$), CK ($P = 0.001$), TNF- α ($P = 0.001$) and MDA ($P = 0.001$) levels following one bout of resistance exercise. However, saffron supplementation prevent from increases in LDH ($P = 0.999$), CK ($P = 0.935$), TNF- α ($P = 0.898$) and MDA ($P = 0.617$) levels induced by one bout of resistance exercise. Although, intensive resistance exercise increases muscle damage enzymes, inflammatory and lipid peroxidation markers, however, pre-supplementation of saffron returns these markers to baseline levels. Therefore, it can be concluded that saffron supplementation acts as anti-oxidative and anti-inflammation agent, making it advisable to athletes and coaches involved in intensive resistance exercises.

INTRODUCTION

Resistance exercise has several health promoting affects. Paradoxically, it is also clear that contracting skeletal muscles generate free radicals that results in damage to various body tissues (Powers and Jackson, 2008). Free radicals attack to membrane lipids and eventually led to the production of malondialdehyde (MDA) (Powers and Jackson, 2008; Gadruni *et al.*

et al., 2015). Destruction of cells membrane results in releasing of lactate dehydrogenase (LDH) and creatine kinase (CK) into serum (Gadruni *et al.*, 2015; Pareja-Blanco *et al.*, 2016). In this context, an increase in serum MDA, LDH, and CK levels has been reported following one bout of resistance exercise at 70 % of one-repetition maximum (1-RM) (Gadruni *et al.*, 2015; Pareja-Blanco *et al.*, 2016), resistance elastic-band exercise (Gadruni *et al.*, 2015), anaerobic power testing (Hammouda *et al.*, 2012), and running-based anaerobic sprint tests (Deminice *et al.*, 2013). In addition to oxidative damage, free radical increases expression of tumor necrosis factor alpha (TNF- α) which in turn results in inflammatory conditions (Fatouros *et al.*, 2010; Townsend *et al.*, 2015; Vincent *et al.*, 2014).

* Corresponding Author

Hossein TaheriChadorneshin, Hossein TaheriChadorneshin, 4 Km of Bojnord-Esfarāyen Highway, University of Bojnord, Bojnord, North Khorasan, Islamic Republic of Iran. Tel: +985832284610, Fax: +985832284610, E-mail: h.taheri@ub.ac.ir

It has been indicated that one bout of intensive running on treadmill (Yamashita *et al.*, 1999), eccentric resistance exercise (Vincent *et al.*, 2014 and traditional resistance exercise at 70-80 % 1-RM (Fatouros *et al.*, 2010; Townsend *et al.*, 2015) results in serum TNF- α levels. Because of oxidative and inflammatory conditions induced by intensive resistance exercise, athletes and sports researchers sought a way to prevent or reduce these conditions (Altinoz *et al.*, 2016; Hajizadeh Maleki *et al.*, 2016; Dehghan *et al.*, 2016). Athlete's awareness of the benefits of taking different antioxidant supplements has caused a significant rise in the consumption of such substances. On the other hand, the unfavorable results of taking chemical supplements have attracted sport researchers and experts' attention toward herbal supplements (Altinoz *et al.*, 2016; Hajizadeh Maleki *et al.*, 2016; Dehghan *et al.*, 2016; Khazdair *et al.*, 2015).

Saffron (*Crocus sativus* Linn.) is an herb commonly cultivated in Iran (Khorasan province) and has important health promoting properties (Altinoz *et al.*, 2016; Hajizadeh Maleki *et al.*, 2016). Recent data from phytochemistry and pharmacological studies have demonstrated that this plant include crocin, crocetin, picrocrocin and a volatile oil component (safranal) (Dehghan *et al.*, 2016; Khazdair *et al.*, 2015). It has been reported that saffron extracts act as anti-atherosclerotic agent through reduction in plasma viscosity and fibrinogen (Ghanbari-Niaki *et al.*, 2015). Moreover, saffron improves diabetic parameters through activating in AMP-activated protein kinase (AMPK)/Glucose transporter type 4 (GLUT4) pathways (Dehghan *et al.*, 2016). In addition, saffron is used in the traditional medicine due to its antioxidant and anti-inflammatory properties (Altinoz *et al.*, 2016). In this context, it has been showed that saffron supplementation reduced MDA and 8-Isoprostane levels and increased superoxide dismutase (SOD) and catalase (CAT) activity in seminal plasma (Hajizadeh Maleki *et al.*, 2016). Also, it has been reported that saffron supplementation reduce MDA levels and xanthine oxidase enzymes (Altinoz *et al.*, 2016). Furthermore, an increase in total antioxidant capacity (TAC) and glutathione levels have been demonstrated following saffron supplementation in diabetic rats (Hosseini *et al.*, 2014).

Considering the aforementioned literature, we hypothesized that saffron extract and its active constituents can attenuate inflammatory and stress oxidative markers induced by intensive exercise. In reality, the effect of saffron supplementation on inflammatory and oxidative damage following intensive resistance exercise is not well elucidated. Therefore, the purpose of present study was to investigate the effect of 4-week saffron supplementation on serum levels of TNF- α , MDA, LDH and CK in sedentary women following intensive resistance exercise.

MATERIALS AND METHODS

Participants

Forty healthy and sedentary women (19-23 years old) participated in this quasi-experimental study. At first, a health questionnaire was applied to get assured of their health and illness

history. Those who had a heart diseases, high blood pressure, diabetes, arthritis, drug addiction or involved in regular physical activity were excluded. Each subject signed a formal letter of consent prior to beginning the investigation. Finally, the subjects were randomly assigned to the four equal groups: control (C), supplementation (S), resistance exercise (RE), and resistance exercise + supplementation (RE+S) groups. Anthropometric characteristics of subjects are presented in table 1.

Table 1: Anthropometric and demographic characteristics of subjects in groups.

Groups	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m ²)	WHR
C	21±1	55.70±9	161±9	21.28±4	0.94±0.05
S	21±1	56.40±6	160±4	21.50±4	0.95±0.31
RE	22±1	56.30±6	160±5	22.07±4	0.98±0.03
RE+S	20±2	68.60±12	162±6	22.04±3	0.97±0.06

Abbreviations: C; Control, S; Supplementation, RE; Resistance exercise, RE+S; Resistance exercise + supplementation.

Preparing saffron supplement

The dried saffron stigma was obtained from Bahraman Saffron Co (Mashhad, Iran). In this study, 52 grams of pure stigma (without the white waste) were milled in a porcelain mortar. Then, milled stigmas were steeped by 1800 ml ethanol (80 %) for 48 hour. The resulting liquid was clear by the filter. Finally, ethanol was dried by evaporation at a temperature of 35-40 °C (Hajizadeh Maleki *et al.*, 2016; Akhondzadeh *et al.*, 2005). The obtained extract was placed into the capsule. Each capsule contained 30 mg dried extract of saffron. Subjects in the S and IR+S groups received one capsule of saffron extract (30 mg/day, for 4 weeks) (Zibad *et al.*, 2015; Akhondzadeh *et al.*, 2005), whereas the C and RE groups received no supplement. Previous studies have used high dose of saffron supplement (Hajizadeh Maleki *et al.*, 2016; Ghanbari-Niaki *et al.*, 2015). However, other studies have used a dose of 30 mg/day without any side effects (Zibad *et al.*, 2015; Akhondzadeh *et al.*, 2005). Hence, we used this dose for our research.

Nutritional and medicinal control

At the beginning of the protocol, information on use of medications/supplements was obtained through self-reported questionnaires. Their diet was also taken into account by applying 24-hour dietary recall questionnaire so that meal subjects consisted of 60 % carbohydrate, 30 % fat and 10 % protein. Furthermore, the use of anti-oxidant supplements and drug-taking was controlled over the research period. Subjects were asked to maintain their normal diet during the period of study and consumed a similar high-carbohydrate meal three day before intensive resistance exercise.

Resistance exercise protocol

At first, subjects in RE and RE+S groups attended in body-building club and one-repetition maximum (1-RM) was

determined (Zibad *et al.*, 2015; Brzycki, 1993). According to participants, weight was chosen so that the participants lifted it at least once and up to 10 times. Finally, 1-RM determined by Brzycki equation as below (Brzycki, 1993):

$$1\text{-RM} = \text{Weight} \div [1.0278 - (0.0278 \times \text{Number of repetitions})]$$

Then, subjects in exercising groups performed one bout of circular resistance exercise at 85 % of 1-RM at 8 stations (bench press, leg press, seated rows, overhead press, seated leg press, arm extension, arm curls and calf raises), each for 3 sets (Zibad *et al.*, 2015). 30 seconds was allocated to the activity rest in each station and 120 seconds for the intervals. At the beginning and end of resistance exercise protocol, warm-up and cool-down were performed (Zibad *et al.*, 2015).

Biochemical estimations

Twelve-hour fasting blood was taken from antecubital vein before and immediately after resistance exercise (Zibad *et al.*, 2015). The blood samples were put into tubes and centrifuged at 3000 rpm for 10 min at 4 °C (Zibad *et al.*, 2015). We used the commercially 96-well ELISA kits to measure the serum contents of TNF- α (Diacclone SAS., France) and MDA (Biocore Diagnostik Ulm, German). The sensitivities of the TNF- α and MDA kits were 8 pg/ml and 0/1 μ M, respectively. Also, serum LDH and CK levels were determined by photometric method (Pars Azmun Co, Karaj, Iran).

The sensitivities of the LDH and CK kits were 5 IU and 4 IU, respectively. The assays were carried out according to the manufacturer's instructions.

Statistical analysis

Data were analyzed using a commercially available statistics software package (SPSS, version 16.0, Chicago, USA). All data values are presented as means \pm SD for the all groups. Normality and equality of variances were confirmed by Shapiro-Wilk's and Levene's tests, respectively. Data were analyzed using a one-way ANOVA and Tukey's post-hoc comparison to test the differences between groups in post-exercise stage. The significance level was set at $P < 0.05$.

RESULTS

The results indicated that the serum MDA levels, as a marker of lipid peroxidation, increased significantly in RE group ($P=0.001$). As for TNF- α , a pro-inflammatory marker, a significant increase was found in RE group ($P=0.001$). Furthermore, one bout of resistance exercise significantly increased LDH ($P=0.015$) and CK ($P=0.001$) levels, as two markers of muscle damage, in sedentary women.

However, saffron supplementation reduced serum MDA ($P=0.007$), TNF- α ($P=0.019$), LDH ($P=0.001$) and CK ($P=0.004$) levels in S group ($P=0.007$). Also, saffron supplementation lowered serum MDA ($P=0.617$), TNF- α ($P=0.898$), LDH

($P=0.999$) and CK ($P=0.935$) levels induced by resistance exercise in RE+S group.

Table 2: Means and standard deviation of dependent variables in different groups.

Dependent variables	Groups	Pre- exercise	Post- exercise
MDA (mol/L/ μ)	C	44.6 \pm 12.66	46.52 \pm 13.10
	S	24.21 \pm 6.52	23.70 \pm 6.48 *
	RE	48.61 \pm 1.10	83.5 \pm 18.10 *
	RE+S	26.12 \pm 5.41	52.00 \pm 14.29
TNF- α (pg/ml)	C	25.40 \pm 8.06	24.8 \pm 13.54
	S	10.23 \pm 6.38	9.99 \pm 4.02 *
	RE	29.40 \pm 8.69	63.90 \pm 19.58 *
	RE+S	12.70 \pm 4.67	25.5 \pm 7.26
LDH (U/L)	C	107.5 \pm 15.23	105.46 \pm 14.3
	S	56.70 \pm 12.48	58.20 \pm 7.96 *
	RE	109.9 \pm 16.52	139.20 \pm 40.20 *
	RE+S	62.61 \pm 10.48	106.60 \pm 6.50
CK (U/L)	C	9.6 \pm 1.26	9.10 \pm 1.15
	S	7.70 \pm 2.48	6.50 \pm 2.06 *
	RE	8.9 \pm 1.14	14.60 \pm 2.67 *
	RE+S	6.14 \pm 1.97	10.10 \pm 1.97

The asterisk (*) indicate a significant difference from C group. Abbreviations are the same as are denoted in the legend of Table. 1.

DISCUSSION

Key factor involving in stress oxidative is intensity of exercise (Powers and Jackson 2008). In reality, resistance exercise increases free radical production through ischemia/reperfusion mechanism (Gadruni *et al.*, 2015; Townsend *et al.*, 2015; Cakir-Atabek *et al.*, 2010). Cellular integrity disruption induced by lipid peroxidation is thought to result in releasing of CK and LDH into serum. In this regard, a significant increase in serum MDA, LDH and CK was shown in present study that represents lipid peroxidation and muscle damage. In contrast to our findings, no change in serum MDA has reported in active subjects following acute resistance exercise (Cakir-Atabek *et al.*, 2010). Furthermore, Dixon and colleague have revealed that acute resistance exercise (8 circuit resistance exercises, 3 sets of 10 repetitions at 10 repetitions maximum for each exercise) had no significant effect on serum MDA in resistance-trained collegiate men (Dixon *et al.*, 2006). These contradictions in results may be due to different levels of physical fitness of subjects. It has been reported that enhanced antioxidant systems in active subjects scavenge free radicals produced during intensive resistance exercise (Cakir-Atabek *et al.*, 2010). In addition, Bloomer and coworkers have showed no significant change in serum MDA following 30 minutes of continuous cycling at 70% of maximal oxygen uptake (VO_2 max) (Bloomer *et al.*, 2005). Other reasons for this disagreement may be due to the type of performed activity (endurance activity versus resistance activity). Besides, Rosene *et al.*, in a study no change in serum CK and LDH (at 12, 24, and 48 hours post-exercise) have attributed to low number of movements, repetitions and muscle groups (knee extension with 150 % 1 RM) (Rosene *et al.*, 2009). Our findings are consistent with another study in terms of the significant increases in serum MDA, LDH

and CK following short-term maximal exercise (Hammouda *et al.*, 2012), resistance elastic-band exercise (Gadruni *et al.*, 2015), and repeated-sprint exercise (Deminice *et al.*, 2013). The response of these markers following exercise depend on the type and duration of exercise (Gadruni *et al.*, 2015; Hammouda *et al.*, 2012), nutrition and supplementation status (Deminice *et al.*, 2013), and number of movements, repetitions and muscle groups (Gadruni *et al.*, 2015; Pareja-Blanco *et al.*, 2016). Collectively, these factors will determine the amount and the period of their releasing into serum after injury induced by exercise (Koch *et al.*, 2014).

In addition, results of the present study demonstrate that intensive resistance exercise increased serum TNF- α . Our finding is consistent with other studies that showed an increase in serum TNF- α following running-based anaerobic sprint test (Deminice *et al.*, 2013), eccentric resistance exercise (Vincent *et al.*, 2014) and traditional resistance exercise (Fatouros *et al.*, 2010; Townsend *et al.*, 2015). It has revealed that stress oxidative and accumulation of intracellular calcium induced by resistance exercise results in activation of nuclear factor kappa B (NF- κ B) (Deminice *et al.*, 2013). In turn, activation of NF- κ B increases expression of TNF- α which subsequently release into the serum (Fatouros *et al.*, 2010; Townsend *et al.*, 2015). In contrast, in two studies have reported that 50 to 60 minutes exercise on ergometer bicycle at moderate intensity (70 to 75 % VO₂max) had no significant effect on plasma TNF- α level in healthy young moderately trained men (Brandauer *et al.*, 2013; Ullum *et al.*, 1994). These contradictions in results may be due to differences in type and exercise intensity.

In contrast, our findings showed that saffron supplementation prevent from increases in inflammatory, stress oxidative, and muscle damage markers induced by one bout of intensive resistance exercise. Recently, a reduction in sperm lipid peroxidation markers (MDA and 8-Isoprostane) and DNA fragmentation has been reported following 16-week low-to-intensive cycling training and saffron supplementation (Hajizadeh Maleki *et al.*, 2016). Also, xanthine oxidase enzymes and MDA levels increased after swimming exercise, but there was a lower increase in crocin (active constituent of saffron) treated groups (Altinoz *et al.*, 2016). Furthermore, it has been concluded that a combination of saffron supplementation and low-intensity aerobic exercise is effective for improving the TAC levels of diabetic rats (Hosseini *et al.*, 2014).

Another study has pointed out that saffron supplementation through increase in serum paraoxonase/arylesterase 1 level prevents from peroxidation of lipids in serum following one bout of resistance exercise (Ajam Zibad *et al.*, 2015). It appears that decreased levels of inflammatory and stress oxidative markers following saffron supplementation is due to direct scavenging of free radicals (Hajizadeh Maleki *et al.*, 2016) and increase in antioxidant system (Altinoz *et al.*, 2016; Hajizadeh Maleki *et al.*, 2016).

In this regard, it has been reported that saffron supplementation scavenging reactive oxygen species in seminal plasma (Hajizadeh Maleki *et al.*, 2016). In the context of antioxidant system, it has been shown that saffron (Hajizadeh

Maleki *et al.*, 2016) and crocin (Altinoz *et al.*, 2016) supplementation increased TAC through increases in SOD, CAT activity (Hajizadeh Maleki *et al.*, 2016) and glutathione levels (Altinoz *et al.*, 2016).

CONCLUSION

Although, intensive resistance exercise increases muscle damage enzymes, inflammatory and lipid peroxidation markers, however, pre-supplementation of saffron returns these markers to baseline levels. Therefore, it can be concluded that saffron supplementation act as anti-oxidative and anti-inflammation agent, making it advisable to athletes and coaches involved in intensive resistance exercises.

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