

Interactive effect of Saffron extracts and acute resistance exercise on serum Paraoxonase-1 activity and C-reactive protein

Mahboobe Ajam Zibad¹, Mohammad Esmaeil Afzalpour¹, Seyed Hossein Abtahi², Marziyeh Saghebjo¹, Mohammad Hossein Zadeh³

¹Faculty of Physical Education & Sport Sciences, University of Birjand, Birjand, Iran

²Department of Biochemistry, Gonabad University of Medical Sciences, Gonabad, Iran

³Faculty of Physical Education & Sport Sciences, Islamic Azad University of Neyshabur, Iran

Abstract. Acute and heavy exercises may increase oxidative stress and thereby suppress antioxidant defense system; thus it is necessary to take nutrition strategies to help athletes. The aim of this study was to evaluate the effect of 4-week consumption of saffron extract on the serum activity of PON1 enzyme and CRP in healthy young women following one session of acute resistance training. This study was a semi-experimental research. Therefore, 30 young non-athlete healthy women were divided randomly into three groups including resistance training, resistance training plus saffron extract and control groups. Following the supplementary period (4 weeks, 30 mg per day), the subjects performed a session of acute resistance training at %80 of 1RM (one repetition maximum). At the end of the supplementary period, blood samples were taken before and after the training. Kruskal-Wallis and Mann-Whitney U-tests were used. Significance was considered below 0.05 ($p < 0.05$). Results of the present study indicated that PON1 activity increased significantly in resistance training plus saffron extract groups and resistance training ($p = 0.04$, $p = 0.00$ respectively), while mean difference of PON1 activity did not show significant difference between above-mentioned groups. Indeed, we observed that 4 weeks of saffron extract consumption (30 mg/day) did not significantly influence the serum CRP ($p < 0.36$). But serum PON1 activity increased significantly. Nonetheless, this elevation was not beyond the changes induced by resistance exercise alone. Based on the current results we cannot consider this dose of saffron extract influential on anti-oxidative enzyme PON1 in healthy inactive women.

Key words: *Saffron extract, resistance training, Paraoxonase-1 enzyme, C-reactive protein*

Introduction

Physical activity is an inseparable part of human life that covers a wide range: from daily activities to severe athletic activities. One of the prominent biological changes during physical activity is the increase in metabolism and the production of free radicals (FR) or types of reactive oxygen species (ROS) (1). Free radicals have copious energy to react with other molecules and damage macromolecules like proteins, lipids and carbohydrates (2). There are special systems in the human body to confront damages caused by FR known as anti-oxidative defense system (3). Anti-oxidant defense system, which is made of different enzymatic and non-enzymatic compounds, is effective in prevention or reduction of tissue damages after activities (4). Enzymatic component of anti-oxidative defense system includes some enzymes such as glutathione peroxidase (GPX), paraoxonase (PON), catalase (CAT), superoxide dismutase (SOD) and so on (4-5).

Paraoxonase family enzymes (PON1, PON2, PON3) have been widely studied in human medical sciences. Apparently they have important endogenous effects against acute and chronic oxidative pressure caused by athletic activities or disease like Atherosclerosis (6). PON1 is an esterase enzyme dependent to calcium contains 354 amino acids that sit on HDL. Its molecules' weight is 45 kDa and is generally made in the liver (7). PON2, same as PON1, has anti-oxidative features but it can play its anti-oxidative role on cell surface and to the out causing enzymes inside the cell (6). PON3 has not been recognized in physiologic tissue but has been shown as lactones that sit on HDL in rabbits (8).

It is believed that different factors may affect PON1 density and activity such as genetic, age, nutrition, smoking and exercise (8).

As pointed out above, acute physical activities can significantly affect the body's antioxidant defense system, but available findings are not in agreement regarding the amount and severity of the effects on the activity of PON1 enzyme.

Some studies have shown an increase in the activity level of PON1 after training exercise of %80 VO₂ max intensity or after a hard training session (9). Shadmanfar et al, (2012) reported a significant increase in PON1 after a exhaustive training session (10).

On the contrary, a decrease in PON1 activity has been reported subsequent to an acute and hard training session (11). Tsakiris et al (2009) reported a significant decrease in PON1 activity in 10 basketball players after an acute training session (12). Decrease in PON1 was also noted in another study by Motta on 10 inactive dogs (13).

Beside the reported decreases and increases in PON1 activity after intense training, absence of significant changes have also been reported following 8 weeks of aerobic training with VO₂max intensity levels of %80-85 and %60-65 (6). Özdag (2010) and Robinson (2011) found no significant change in PON1 respectively after short-term soccer among men and after a one-session 30-minute aerobic exercise %60 and %80 VO₂max (14-15). Also, Bnitez did not find any change in PON1 in marathon athletes after 4 hours of running (16). Review of literature leads one to conclude that resistance training, although comprising a notable portion of what is performed by athletes, has been less concentrated on than other kinds of sports training. In general, and assuming that PON1 activity decreases after acute training, it is vital to take precautions against undesirable aftermaths and to help the body's defense system.

The fact that saffron is anti-inflammatory (17) and also because acute exercise training causes inflammation quite often, led the present researchers to the decision that they should study, beside PON1 activity, CRP reacting protein, which is among the best predicting inflammatory indices of cardiovascular diseases (11), so that the supplementary effect of saffron might be better discussed. CRP is a member of pentraxins that plays an important role immunologic responses. Recent research shows a significant relationship among PON1 and CRP in cardiovascular patients (18), who studied patients with myocardial infarct and found lower PON1 activity and higher CRP density than those of healthy people.

Weight reported an increases in CRP in 70 male and 20 female marathon runners (19). Based on Scarhag during bicycling for 4 hours at intensity of 70 lactates, CRP concentration increased for 3 or 4 times (20). Vincent reported that the amount of inflammatory cytokines (CRP, TNF- α and IL -6) are increased after exercise (21). Jafari studied the effect of acute heavy training on CRP and fibrinogen and reported that CRP increased and fibrinogen decreased significantly (22). These results do not match with the findings of Cosio, because he showed that physical exercise did not affect CRP of old diabetic people, while it increased the TNF- α and IL -6 (23). One and two sessions of resistance and endurance exercises at the same time (3) and one session of circular resistance exercise do not have any significant effect on CRP level, either (24).

As mentioned above, there are no enough reports about interaction between saffron extract, exercise performance and PON1 activity. Studies on PON1 have been carried out more frequently by the use of vitamin E, C, pomegranate juice and such supplements and the results of most of these studies verify the improvement in PON1 activity (10). However, saffron is an herbal supplement endemic to southern Khorasan that can be considered prior to medicinal complements. Thus, this paper has been designed and run to study the effects of consuming saffron extracts for 4 weeks on serum PON1 activity and CRP in inactive women after one session of heavy resistance training.

Material and Method

This study was a semi-experimental research. The researched subjects were female students of Islamic Azad University - Gonabad branch, Iran. At first, 30 patients were gathered in a targeted way through public call. Participants were aged 19 to 23 years and their health status and medical history were evaluated by the use of health questionnaires. Those who suffered from heart disease, breath impairment, pulmonary hypertension, diabetes and joint problems, tobacco, and those who attended regular physical activities were omitted. The participants were randomly divided into three equal groups (n=10) including control group, acute resistance training plus saffron extract (RT+SE), and acute resistance training (RT) groups. Before starting the training, the subjects gave written informed consent. Samples were homogenized by weight, height and body mass index (BMI). The diet was controlled by 24 hours diet questionnaire, so that taking antioxidant supplements such as vitamins E and C, pomegranate juice, green tea, or taking medication during the study period was

controlled. Since physical fitness could affect the response to exercise, physical activity was assessed by the use of an oral questionnaire so that all participants would be equal in this regard.

The research protocol was developed during 4 weeks. Subjects in RT+SE group used a capsule containing 30 mg per day, while the control group and RT group did not take any Saffron extract. RT protocol contained a circular resistance training at 85% of one repetition maximum (1RM). 9 movements (bench press, leg press, seated boat, overhead press, knee extension, arm extension, arm flexion, and lifting the heel) in 3 sets were performed. Duration of each movement was 30 seconds with 120 seconds rest time between each set. The session lasted about 50 to 55 minutes including 15 to 20 min warm-up, 30 min main body, and 10 min cool down. It should be noted that before starting the main program, training group subjects went to the gym to get familiar with the training protocol and it was taken the 1RM for 9 movements using by the Bersizky method. All dependent variables were measured in the two phases including 4 weeks after taking saffron extract, and immediately before and after resistance training session. 5 ml of blood was taken from the brachial vein in the fasting state (12 hours fasting). All measurements were performed in the same conditions 8 to 9 AM, with temperature of 26 to 28 ° C and 50% humidity. Blood samples were placed in hemolytic tubes and were centrifuged to 3000 RPM, and the obtained serum was used to measure PON1 and CRP.

Measurement of PON1. PON1 enzyme was measured by spectra photometric method (kinetic), using commercial kit of Paraoxonase Assay Kit manufactured by Turkey. First, some of the samples (30 ml) were mixed with the amount reagent kit (630 ml) and was placed in a photometer machine by a wavelength of 405. Then, the absorbing difference obtained in 3 sequenced minutes. The average difference in absorption in these 3 minutes was multiplied by Kit (1294) factor and was recorded as U/L (units per liter).

Measurement of CRP. CRP levels were measured by ELISA method in high-sensitivity with Rider Elisa machine, using commercial kit (intraassay CV%: 5/9, sensitivity: 10 ng/ml) made by Canada.

Instructions to make Saffron extract. In this study, 52 g of pure saffron (no white and waste part) was milled to have powder and then was soaked in 85-degree alcohol for 48 hours. Afterwards, the gained liquid, using rotary machines (Boss 114 R - Made in Switzerland), was concentrated in vacuum condition, and the alcohol was removed. Concentrated liquid was divided into the glass pelt and was dried in 40 ° in the fore (Avan Behdad, made in Iran). Dried material was taken out as powder and put into capsules using a digital scale (Sartorius Model S230LA made in Germany with the precision of 0/0001 g). Each capsule contained 30 mg of dried saffron extract (25). During 4 weeks, each day, subjects consumed a capsule. The other two groups, namely acute resistance training (RT) and the control group did not receive any supplementary, saffron or the like, during the protocol. Moreover, they attended the classes at different times and the RT group had their training exclusively since it was intended that the groups did not know if others were given supplements so that this could help the researchers reduce the chance for interfering and/or disturbing factors to leave an effect.

Statistical analysis. For the analysis of the data, the Kolmogorov - Smirnov test was carried out. Then the Paired t-test, Kruskal- Wallis and Mann-Whitney U-test were used at a significance level of 0.05 for extraction of results. Whole analysis was performed using SPSS software version 16.

Results

In Table I, the characteristics of participants including mean and standard deviation are given. Table II shows PON1 activity and CRP levels at different stages of the research. Based on the results of t- test, immediately after acute RT, both RT with and without consumption of SE, PON1 activity has increased (0.05, $p < 0.04$), but CRP levels have not shown significant changes ($p < 0.31$, $p < 0.33$) (table II).

At the beginning, demographic indices of the subjects were compared and it was found that the apparent differences among the groups are not statistically significant. With regard to age, weight, and BMI, p was calculated 0.26, 0.21, and 0.06, respectively.

After it is established of normal distribution of data by Kolmogrov-Smirnov test, non-parametric tests including Kroskal Wallis and Mann Whitney U test were selected.

Kroskal Wallis results about changes in PON1 (table III) suggest that there is significant difference between PON1 change in different groups ($p < 0.006$). To find precise difference between groups, Mann-Whitney U-test was carried out. Table (4) gives the summarized results.

Table I. Demographic characteristics of participants (mean variance deviation)

Variables	Groups		
	RT + SE	RT	Con
Age (year)	20/40 ± 1/89	22/3 ± 1/41	20/8 ± 1/39
Weight (kg)	68/60 ± 12/16	56/30 ± 5/59	55/70 ± 8/98
BMI (kg/m ²)	26/04 ± 3/51	22/07 ± 4/24	21/28 ± 3/74

* Rt=resistance training, SE= Saffron Extract, Con= control

Table II. Results of paired t-test about comparison of PON1 activity and CRP level between pre and post- tests

Variables	Groups	before RT	after RT	(P w)
PON1 (U/L)	Con	95/15 ± 64/40	97/25 ± 64/72	0/27
	RT	47/25 ± 15/25	79/50 ± 51/63	0/04(*)
	RT+SE	102/12 ± 47/03	108/25 ± 50/74	0/05(*)
CRP (ng/ml)	Con	0/56 ± 0/07	0/56 ± 0/07	0/31
	RT	1/2 ± 0/37	1/4 ± 0/61	0/31
	RT+SE	1/2 ± 0/36	1/4 ± 0/61	0/33

* RT=resistance training, SE= Saffron Extract, Con= control

Table III. Results of Kroskal Wallis test about comparison of changes in PON1 activity and CRP level between groups

Variables	Mean difference (MD)			df	P value
	Con	RT	RT+ SE		
PON1 (U/L)	7/85	19/19	14/88	2	0.006(*)
CRP (ng/ml)	1/5	1/02	1/31	2	0.36

* RT=resistance training, SE= Saffron Extract, Con= control; * Significance difference at p<0.05.

The results listed in Table IV show that the difference of changes in PON1 between control group and both RT (P < 0.001) and RT+ SE groups (P < 0.04) is significant. In other words, there are significant increases in PON1 activity between the pre-test and post-test in both acute RT and acute RT + SE groups. However, lack of significant difference in PON1 changes between acute RT and acute RT+SE groups (p< 0.54), indicates that an acute RT and consumption of the SE have a similar effect on serum PON1 activity in healthy young women. In other words, consuming SE for 4 weeks did not affect serum PON1 activity beyond the influence of an acute RT. Moreover, the results of the Kruskal-Wallis test (Table III) suggests that neither acute RT nor acute RT+SE, affected serum CRP significantly (p < 0.36).

Table IV. Results of Mann-Whitney U-test about paired comparison of PON1 activity between groups

Variable	groups	Level of significance
PON1	Con - RT	0.001 (*)
	Con - RT+ SE	0.04 (*)
	RT - RT+ SE	0.54

RT=resistance training, SE= Saffron Extract, Con= control; * Significance difference at p<0.05

Discussion

There was a significant increase in PON1 activity after an acute resistance exercise at intensity of 85% 1RM, while consuming saffron extracts for 4 weeks at a dose of 30 mg/day, did not have more effect on PON1 activity than acute resistance exercise.

Shademan Fard, similarly indicated that consuming pomegranate juice did not change PON1 activity following of an exhaustive exercise, while acute training exercise raise PON1 significantly (10).

Conversely, Teskarish (2009) found that intensive exercise without supplementation of vitamin E reduced PON1 in basketball players, while PON1 activity did not show significant changes in extreme sports activities after one month of vitamin E supplementation. They concluded that vitamin E can reduce free radicals in the exercise and supplementation group that signifies antioxidant system enhancement (12). Motta (2009) have reported lower PON1 activity after performing intensive workouts without supplementation of vitamin E, while it improved after intensive exercise alone with antioxidant supplements (13). We did not find significant changes in PON1 activity after saffron supplementation and this may have been due to the lower dose of saffron (30 mg/day). In most of studies different doses have been evaluated and it is found that they are more effective in higher doses (26). Higher dose of saffron has been tested on animal studies and because of saffron intake more than 1 to 3 g/day can induce poisoning state in human, it is impossible to test high doses of it in humans.

On the other hand, limited studies showed that consuming 30 mg/day saffron may reduce depression in humans (25), therefore the highest possible dose to be taken was 30 mg/day. Based on available data, it may have been reduce the amount of memory and depression, but it was not enough to strengthen the anti-oxidation system. Also, it should be considered that individual differences, type of saffron extracts, way of extracting saffron, and other factors may affect obtained results in different studies, hence it will show clearer results in the future if these factors are controlled.

Another finding was that a single session of acute resistance exercise significantly increased PON1 activity. In previous studies, the effect of acute resistance exercise on PON1 have not been explored adequately, however, it is reported an increases, decreases or not-changes in PON1 activity after acute aerobic and anaerobic exercises. For example, PON1 activity enhancement have been reported after 2 hours of intensive exercise on a treadmill (9), aerobic exercise at intensity of 75% VO_2 max in the elite swimmers and sedentary (27), and aerobic exercise at 60% VO_2 max in trained athletes (28). However, it has been monitored no significant changes in PON1 after a short exercise session by football players (14), aerobic exercise at two intensities of 60 and 80% VO_2 max (15), marathon competition (29), and 4 hours running by marathoners (16). It seems that very heavy, intensive and relatively long exercise trainings have potential to depress PON1 activity. Thomas et al. (2002), Tskaris et al. (2009) and Mata et al. (2009) have reported PON1 activity reduction after intensive weight training (11-13). Lack of consistent findings on PON1 could have several reasons, including differences in the duration, intensity and type of exercise. Most of findings about PON1 are related to aerobic and anaerobic exercises, since the present study evaluate effects of acute resistance exercise; and due to few studies on resistance training, we cannot compare researches properly. Also, differences in fitness level of participants may induce inconsistency results.

Another noteworthy finding of the current research is the lack of a significant effect of a single session of acute training exercise, whether by itself or in combination with a dose of 30 mg/day saffron extract on the CRP blood serum of young healthy women. It has been shown that increase in mechanical pressure, activation of endothelial cells, severity, duration, and type of training exercises and the seriousness of muscle damage due to the training can enhance CRP and cause inflammation. However, herbal and nutritional supplements can help harness this inflammation. Dragger (2012) reported lack of significant change in the amount of CRP following an acute training exercise (29). Nor did taking BAA amino acid supplement half an hour prior to an acute training exercise bring about a significant change in the amount of CRP in soccer players (17). Consistent as these findings may be with the present study, the amount of increase in CRP has been reported somewhat differently in the elementary stages of an 8-week aerobic training accompanied by omega-3 supplement, and in acute exercise on a steep road after 14 days of taking caffeine in non-athlete men. An amazing finding is the decrease in inflammatory indices such as CRP, which has been clearly documented in several studies. Some researchers believe that CRP is more highly influenced by the severity and duration of the exercise activity, while others believe it is ground level that matters so that the greater the ground level of inflammatory indices, the greater the impression of training and taking antioxidant supplements on the indices. Altogether, it can be stated that a great number of factors such as physical structure; estrogen; smoking; frequency, duration and acuteness of exercise training; and nutrition do affect the response from CRP to the training (7). On the whole, CRP did not change significantly after one session of acute resistance training, hence a reasonable analysis of the possible effect of taking saffron supplement

on this factor cannot be made. This might be related to sampling time and CRP half-life, sampling was done right after the training session and it usually takes some time for CRP to respond to training. On the other hand, it is probable that the training protocol was not acute enough to cause inflammation. One influential subject in giving supplements is the dose. Professional athletes and those involved in acute training are often advised to take antioxidants so that the protective property of these materials prevent the accumulation of free radicals and oxidation pressure (30). A lot of studies stress the idea of taking natural oral antioxidants. There is ample evidence that such supplementation enhances exercise performance and also reinforces antioxidant defense and lowers the chance of oxidative harm caused by acute exercise activities (30)

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Corresponding author

Mahboobe Ajam Zibad

Faculty of Physical Education & Sport Sciences

University of Birjand, Birjand, Iran

E-mail address: ajam.mahboobeh@gmail.com

Received: June 21, 2015

Accepted: October 25, 2015