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Punica Granatum Juice Effects on Oxidative Stress in Severe Physical Activity

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ABSTRACT

Aim: The aim of this study was to investigate Punica granatum juice effects on oxidative stress in young healthy males during severe physical activity. **Methods:** Our subjects were selected from healthy males at 18 - 24 years. They were enrolled and randomly distributed into control and supplemented groups. 240 ml of Punica granatum juice and tap water were given to supplement and control groups daily for two weeks, respectively. Fasting blood samples were taken at the starting and the end of two weeks of intervention. Subjects were given once severe physical activity and then fasting blood samples were taken. Fasting blood samples were used for testing of oxidative and antioxidative factors. Data were analyzed using descriptive statistical tests, paired samples t-test, and independent samples t-test. **Results:** The levels of arylesterase, superoxide dismutase, glutathione peroxidase and total antioxidant capacity after severe physical activity in supplement group were significantly increased ($p<0.05$), while the content of malondialdehyde showed a significantly decrease in comparison to control group ($p<0.05$). **Conclusion:** Our findings indicate that regular intake of Punica granatum juice significantly modulates oxidative stress and thus protects against severe physical activity oxidative injury in young healthy males.

Key words: Punica granatum, Oxidative stress, Physical activity, Males

1. INTRODUCTION

Severe physical activity considered as source of oxidative stress in multiple tissues due to raised production of reactive oxygen species (ROS) that disrupts the balance between oxidant and antioxidant levels in normal condition. Increased oxidative stress can lead to damage to macromolecules like proteins, lipid, DNA and enzymatic systems (1-4). Physical activity plays an important role in free radical processing at an obscure way. Nevertheless, recent evidence suggests that overconsumption of oxygen can result in subsequent increase in the free radical activities (5). An increase in aerobic physical activity by enhancing electron transportation in body could affect oxidative stress (1, 6). There are several sources to produce active mediator like H_2O_2 and hydroxyl radicals, mitochondria likely have important role to in this production (6, 7). In the condition of physical activity, oxygen expenditure may be enhanced 200 fold in activating muscle fibers compared to resting time (8), leading to production of superoxide (9). Natural antioxidant ingredients of fruits and vegetables could act as protective factors against oxidative damage (10). According to recent studies, the antioxidant features of many fruits and vegetable stems from their flavonoid and related polyphenol components (11, 12). Daily human's consumption of these compounds is approximately between a few hundred milligrams and one gram (13, 14). However, there are several studies which have indicated the bioavailability of the antioxidants in many fruits and plants (15, 16). Fresh Punica granatum (PG) or pomegranate juice includes 85 % water, 10 % total sugars, and 1.5 % pectin, ascorbic acid and polyphenolic flavonoids (17, 18). It has been shown that intake of pomegranate juice as a dietary supplement, may play an important role in oxidative stress reduction and also could ameliorate the antioxidant status (19-22). Pomegranate extracts, as a potential natural antioxidant, contribute to free radicals scavenging and damage reduction induced by oxidative stress and lipid peroxidation in macrophage (23) and lead to improvements in plasma antioxidant status (21). The aim of this study was to evaluate the efficacy of PG juice supplementation in improving antioxidant function in young healthy males during severe physical activity.

2. MATERIALS AND METHODS

2.1. Study design

Study was conducted in Ardabil University of Medical Sciences, Iran in 2010 - 2011. The proposal was approved by Medical Ethical Committee of the Ardabil University of Medical Sciences and recorded in Iranian Registry of Clinical Trials with identification code of IRCT201012255144N2. 28 healthy men, 18-24 year old, were recruited and randomly divided into two groups, supplement and control. The aims of current investigation were explained to the subjects and a writ-

ten consent was taken prior to fill in a questionnaire. Subjects with certain diseases, non-athletes, smoking, and consumption of other supplements were excluded. After getting consent, general information, anthropometric factors (height and weight), and fasting blood samples, one cup (240 ml) pomegranate juice and tap water were given to supplement and control groups daily for two weeks, respectively. Fasting blood samples were drawn from participants at the end of two weeks of intervention by venipuncture and sera separated immediately. Subjects were given once severe physical activity and then fasting blood samples were taken. Collected sera were kept frozen at – 80 °C until analyzed.

2.2. Measurement of biochemical factors

2.2.1. Paraoxonase 1 (PON1)

Serum PON1 activity in high salt, No salt conditions toward paraoxon was determined as described by Furlong et al. in 1988 (7) with little modification. The basal assay mixture included 760 µL of assay buffer [0.132 M Tris – HCl (pH 8.5), 1.32 mM CaCl2], 40 µL serum and 200 µL of 6 mM freshly prepared paraoxon. The rate of hydrolysis at 37 °C was determined at 40 nm for 5 min. PON1 activity was calculated using molar extinction coefficient of 18.05×10^3 per M^{-1} cm^{-1} and was expressed as IU/L, where IU is the number of µmol of paraoxon hydrolyzed per min. For NaCl- stimulate assay 2.63 M NaCl was added into previous mixture.

2.2.2. Arylestrase

Phenylacetate was used as a substrate to measure the arylesterase activity. 10 µL of a 1:40 dilution of serum was mixed with 200 µL of substrate [3.26 mM phenylacetate in 9 mM Tris- HCl (pH 8) 0.9 mM CaCl2]. The rates of hydrolysis at 25 °C were determined at 270 nm for 4 min. The molar extinction coefficient of 1310 M^{-1} Cm^{-1} was used for calculation of activity, and units were expressed as micromoles of phenylacetate hydrolyzed per minutes.

2.2.3. Serum total antioxidant capacity (TAC)

Serum TAC was measured using by colorimetric method using Randox kit (Randox laboratories ltd., UK).

2.2.4. Malondialdehyde (MDA)

MDA levels were assessed utilizing the thiobarbituric acid reactive substances (TBARS) method. Samples were heated with 0.6% thriobarbituric acid under acidic condition and after cooling, the colored product was extracted into n-butanol. The pink color absorbance was measured at 530 nm. MDA standards were prepared with 1, 1, 3, 3 - tetraethoxypropane.

2.2.5. Glutathione

Total serum glutathione levels were assayed using Glutathione Assay Kit (Cayman Chemical Co., USA).

2.2.6. Superoxide dismutase (SOD) and Glutathione peroxidase (GPX) activity

Erythrocyte antioxidant enzymes activity of SOD and GPX were determined by spectrometric method using Ransod and Ransel kit, respectively (Randox laboratories ltd., UK). For the accuracy of assessment, duplicate assay were performed.

2.3. Dietary method

The food intake was estimated for energy and nutrients by 24-hour dietary recalls three days (2 week days and 1 weekend day) during the study. Mean daily dietary intake and food composition were estimated using Nutritionist IV software.

2.4. Data analysis

Data were analyzed using descriptive statistical tests, and paired, independent samples t-test. Statistically significant level for all tests was considered as $p<0.05$.

3. RESULT

There was no significant relationship among age, weight, calorie, and nutrients intake in both groups during the study (table 1). In supplement group, serum levels of arylesterase, SOD, GPX, TAC, standardized arylestrase activity, and glutathione were significantly higher in compared to control group at the end of two weeks of intervention ($P< 0.05$). But the serum level of MDA in supplement group was significantly lower than control group ($P< 0.05$) (Ttables 2 and 3). Results obtained showing that the after severe physical activity in supplement group, serum levels of arylestrase, SOD, GPX, TAC, and

variables	groups		P value	variables	groups		P value
	Supplement M±SD	Control M±SD			Supplement M±SD	Control M±SD	
Age (year)	19±1	20±0.89	0.06	V. B1 (mg)	1.56±0.28	1.49±0.36	0.58
Weight (kg)	68±9	73±8	0.2	V. B2 (mg)	1.37±0.29	1.36±0.35	0.95
BMI (kg/m2)	22.25±2.92	23.17±1.88	0.33	V. B3 (mg)	24.17±7.9	23.15±3.6	0.66
Height (cm)	178±5	177±6	0.74	V. B6 (mg)	1.33±0.48	1.05±0.3	0.07
Cal (Kcal/day)	2487±267	2505±263	0.88	V. B9 (µg)	120±67	116±52	0.85
Protein (g)	97±13	93±12	0.5	V. C (mg)	52±36	56±27	0.74
CHO (g)	325±2	350±72	0.25	Ca (mg)	611±232	625±269	0.88
Fiber (g)	13±5	12±3	0.76	Fe (mg)	20±5	24±8	0.09
Total fat (g)	84±12	88±16	0.47	Zn (mg)	4.2±1.06	3.49±1.13	0.09
SF (g)	16±3	19±6	0.08	Cu (mg)	0.93±0.26	0.87±0.14	0.47
PUFA (g)	14±5	13±4	0.86	Mg (mg)	156±60	147±33	0.61
MUFA (g)	20±3	23±6	0.14	Se (µg)	43±8	42±11	0.88
Ch (mg)	306±125	342±148	0.49	P (mg)	934±174	896±140	0.52
V. A (RE)	834±811	605±238	0.14	K (mg)	2046±656	1871±274	0.36
V. E (mg)	13.21±2.83	15.46±2.99	0.74	V. B12 (µg)	2.9±1	3.1±1.5	0.07
V. B5 (mg)	3.4±0.78	3.1±0.79	0.43				

Table 1. Relationship among of age, weight, calorie, and nutrients intake in both groups during the study.. CHO = carbohydrate, RE = retinol equivalent, BMI = body mass index, V = Vitamin, V.B9= Folic acid, Ca = Calcium, Mg = Magnesium, Cal = Calorie, Ch = Cholesterol, MUFA = Mono unsaturated fat (g), PUFA = Poly unsaturated fat, SF= Saturated fat, K = Potassium, P = Phosphorous, Se = Selenium, Fe = Iron, Cu = Copper, Zn = Zinc,

variables	Measurement stage	supplement group	control group	P value
Glutathione peroxidase (U/gHb)	1	42.21±2.77	40.58±2.41	0.11
	2	45.46±2.01	40.15±1.81	0.001
	3	46.06±2.27	42.4±2.77	0.001
Superoxide dismutase (U/gHb)	1	1556.14±169.36	1469.50±177.91	0.20
	2	1720.14±136.52	1486.71±161.67	0.001
	3	1774.57±140.82	1542.79±161.6	0.001
Arylesterase (U/L)	1	129.36±36.93	123.92±40.61	0.71
	2	186.61±47.61	127.23±33.84	0.001
	3	175.98±41.8	128.42±26.67	0.001
PON1(no salt) (U/L)	1	52.98±23.22	59.77±21.20	0.43
	2	65.25±17.01	60.59±20.11	0.51
	3	63.04±18.32	58.4±23.61	0.57
PON1(high salt) (U/L)	1	245.73±91.1	286.84±183.99	0.46
	2	266.51±76.99	281.07±134.30	0.73
	3	266.83±75.05	299.26±110.28	0.37
Standardized Arylesterase activity	1	2.66±0.82	2.15±0.79	0.10
	2	3.43±1.01	2.41±0.63	0.003
	3	3.2±0.65	2.51±0.41	0.003
Standardized PON1 activity (no salt)	1	1.08±0.42	1.03±0.36	0.76
	2	1.21±0.37	1.16±0.42	0.77
	3	1.16±0.37	1.14±0.44	0.94
Standardized PON1 activity (high salt)	1	5.02±1.83	4.83±2.95	0.84
	2	4.87±1.46	5.29±2.40	0.58
	3	4.86±1.44	5.91±2.17	0.14

Table 2. The comparison of antioxidants enzyme activities before and after severe physical activity in both groups. 1. Before supplementation, 2. After supplementation (Before severe physical activity), 3. After severe physical activity

variables	Measurement stage	supplement group	control group	P value
Total antioxidant capacity (mmol/l)	1	0.7564±0.14	0.6736±0.09	0.07
	2	0.8686±0.12	0.6643±0.07	0.001
	3	0.8129±0.09	0.6393±0.1	0.001
Glutathione (mmol/l)	1	0.2093±0.04	0.1843±0.04	0.14
	2	0.2479±0.08	0.1764±0.03	0.005
	3	0.2179±0.07	0.2036±0.03	0.49
MDA(nmol/ml)	1	0.1993±0.04	0.1950±0.04	0.78
	2	0.1671±0.03	0.2029±0.03	0.002
	3	0.2614±0.05	0.3393±0.1	0.02
Cholesterol(mg/dl)	1	139.86±25.13	147.28±26.89	0.46
	2	130.07±16.98	141.85±25.83	0.17
	3	140.28±24.58	137.07 ±13.40	0.67
HDL-Cholesterol (mg/dl)	1	49.32±6.91	53.18±5.77	0.12
	2	55.05±6.02	52.97±5.46	0.35
	3	55.36±7.24	51.35±6.22	0.13
LDL-Cholesterol (mg/dl)	1	69.83±12.99	67.76±26.61	0.79
	2	65.66±9.68	65.48±22.69	0.98
	3	70.25±18.87	72.9±23.79	0.75
Triglyceride (mg/dl)	1	87.78±39.73	100±38.16	0.37
	2	72.36±30.95	93.85±29.03	0.07
	3	84.28±20.48	78.14±24.49	0.48

Table 3. The comparison of lipid profile, antioxidant and oxidant parameters before and after severe physical activity in both groups 1. Before supplementation, 2. After supplementation (Before severe physical activity), 3. After severe physical activity

standardized arylestrase activity were significantly increased, whereas serum level of MDA was significantly decreased in comparison with control group ($P< 0.05$). There were no significant differences between the two groups in the serum levels of other biochemical factors. The data also supported that there was no significant effect of pomegranate juice on serum lipid profiles.

4. DISCUSSION

Based on the results of this study, administration of PG juice supplementation to supplement group for two weeks can prevent the increase serum levels of MDA and lipid peroxidation. Also, this study shows that the severe physical activity could increase the rate of arylesterase, SOD, GPX and MDA. It has been documented that physical activity can cause increase in ROS generation in skeletal muscle (24), in consistency with our study. It has been shown that the administration of antioxidant supplementation may play a positive role on metabolism in physical activity (25). PG has been widely consumed as fresh and juice. Pomegranate juice has been extensively used in ancient cultures for various medical features (26). The results reveal significantly elevated serum levels of arylestrase, SOD, and GPX in pomegranate juice receiving group in comparison with controls at the end of 2 weeks intervention. Moreover, the amount of MDA was decreased in supplement group with pomegranate juice in comparison to controls group after following severe physical activity. Free radicals causing oxidative stress are an inevitable byproduct of mitochondrial metabolism and have been proposed to exert repetitive damage to individual cells of the body promoting increased prevalence of disease and aging (27). However, in regard to physical activity, antioxidants were incapable of further extending exercise-induced lifespan extension in rats (28). Fenercioglu et al showed that the substantial antagonizing effects of PG extract, as a polyphenol-rich antioxidant supplement, on oxidative stress and lipid peroxidation (29). The feeding of PG seed pulp did not have any significant effect on serum triglyceride and lipoproteins in animal models (30). Fazli et al showed that the drinking consumption of 100 ml of pomegranate juice per day for 14 days caused significant reduction in lipid peroxidation and rising of the total serum antioxidant capacity in 15-17 years old girls (31). The result of study from Hzji-Mahmoudi et al revealed that the 100 grams pomegranate per day for ten days significantly increase plasma antioxidant capacity in thirty healthy volunteers (32). Trombold *et al* displayed enrolled active sportsmen in order to evaluate the muscular damage after an intense eccentric exercise. It is well known that an intense eccentric exercise produces oxidative stress at local muscular level and pain for several days. Trial results confirmed that PG improved muscular strength recovery after 2 days from the sport activity (33). Previous work in animal models has shown reduction in post exercise inflammation (34) or oxidative stress (35) to attenuate myofiber damage and in situ muscle force during electrical stimulation, respectively. Schmidt et al showed that an increased level of oxidative stress was associated with high levels of physical exertion of training in a cold environment at moderate altitude. The antioxidant mixture contain PG extract tested did not attenuate the mean oxidative stress levels in the entire group of test subjects (36), which is inconsistent with this study. It seems that this difference can be attributed to the types of exercise and supplements. This study showed that the antioxidant activities increased in supplement group in comparison to control group at the end of two weeks and after severe physical activity. Therefore, it is suggested that there is a synergy between endogenous antioxidant enzyme and exogenous antioxidant mixture in diet to reduce

oxidative stress, rather than individual antioxidants. In order to protection against the destructive effects of oxidative stress, our bodies have a complex system of endogenous antioxidant protection in the form of enzymes such as SOD, catalase, and GPX (37). Supplementation with antioxidants, either through an increased consumption in the diet or from supplementation, has become extremely popular as a means to improve one's health or increase physical performance. It has been suggested that increased circulating levels of certain antioxidants will help to prevent the accumulation of free radicals inside our cells thus reducing oxidative stress (38, 39). Our findings showed that PG juice has a significant effect on antioxidant enzyme activities after severe physical activity.

5. CONCLUSION

We found that PG juice supplements prevent the rise of MDA and induce endogenous antioxidant defense by physical activity. Results showed that the interpretation of accurate relationship between physical activity and oxidative stress status may depend on the indicators studied and the combination of the antioxidant mixture ingested. An analogous relationship between biochemical factors of oxidative stress in blood and severe physical activity indicate that the important antioxidant diet source may be beneficial before exercise. There is not a general effects on all indicators so two possibilities could be suggested; 1) there is not direct relationship between some indicators and particular types of oxidative stress or 2) there is not a sufficient level of oxidative stress production to cause raise in all indicators in this research. This study demonstrated that daily PG supplementation during two weeks improves antioxidant activities before and after a severe physical activity.

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CONFLICT OF INTEREST: NONE DECLARED.

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