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Effects of aerobic exercise and pomegranate extract on antioxidant markers in women postmenopausal with type 2 diabetes

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Original Article

Abstract

Introduction: The aim of this study was to evaluate the effects of aerobic exercise and pomegranate extract on antioxidant markers in women postmenopausal with type 2 diabetes.

Methods: The statistical population of this study was the postmenopausal diabetic women of Babol City, North of Iran, who were referred to the Diabetes Association of the city. Among them, 33 subjects were selected randomly and divided into four groups: Control-Water (CW), Control-Pomegranate extract (CPE), Training-Water (TW) and Training-Pomegranate extract (TPE). The experimental group training plan consisted of 6-week aerobic exercise training, three times a week for at least 45 minutes per session. Consumer groups of pomegranate extract drank 150 ml of pomegranate extract every day for 6 weeks. Before and after this interventional study, blood sampling was performed from the brachial vein in a sitting position in a fasting state. The antioxidant markers of GPX, SOD, GSH and TAC were measured in the specimens.

Results: The results showed that aerobic exercise training with the consumption of pomegranate extract significantly increased the levels of glutathione peroxidase and superoxide dismutase, plasma glutathione and Total Antioxidant Capacity (TAC) in women with type 2 diabetes compared to the control group ($P < 0.001$). Women with type 2 diabetes in Training-Pomegranate extract groups had the highest levels of the antioxidants.

Conclusion: Our finding showed that aerobic exercise training associated with the consumption of pomegranate extract improve the body's antioxidant defense system by increasing the levels of plasma antioxidants.

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Introduction:

Diabetes mellitus is a metabolic disorder that is widespread in the world associated with an increase in blood glucose, insulin inadequate secretion or dysfunction (1). It consists almost 95% of diabetes cases and is caused by the inability of the muscle cells to respond to insulin, which hereditary and environmental factors affect it and it is more common during the aging and is mostly associated with obesity, inactivity and reducing the metabolism

of the whole body especially in postmenopausal women (2).

According to studies, Iran is among the countries that are greatly exposed to increased risk of diabetes (3). There is much evidence that suggests the role of oxidative stress and the subsequent production of free radicals in diabetic patients and the role of these factors in diabetes pathogenesis (4). It has been found that hyperglycemia with increased production of reactive oxygen species lead to oxidative stress in cells (5). The researches have shown that the enzymatic and non-enzymatic defense system removing free radicals in diabetic cell become weak and the rate of lipid per oxidation will increase in cells (6). Free radicals are highly reactive molecules that are produced naturally in the body's metabolic reactions. High levels of free radicals damage cellular proteins, membrane lipids, and nucleic acids and eventually cause cell death. Free radicals play an important role in the pathogenesis of many chronic diseases, including atherosclerosis, myocardial failure, autoimmune diseases, cell damage and diabetes (7,8). In diabetic patients, in addition to oxidative stress, antioxidant defense of the body is impaired. Although currently the use of insulin and oral hypoglycemic drugs are the main and effective treatment of oxidative stress etiology of diabetes, it has numerous side effects. Therefore, it is very important to study the factors that decrease these side effects in these patients. One way to help these patients is physical activity of moderate intensity like aerobic activity that results in reducing the symptoms of this disease (9).

Physical activity and exercise increases the body's use of glucose by muscle cells and more active fat metabolism, it reduces blood glucose and recovers blood lipids (10). Also, the risk of type 2 diabetes in people who have regular physical activity is two times lower than sedentary individuals (11). Also, a lot of efforts have been done in the identification of compounds useful in the treatment of diabetes with fewer side effects (12).

Nowadays, the traditional treatment of diabetes using some plants or herbal extracts is considered around the world (13). Studies have shown that consumption of nutrients rich in antioxidant compounds are useful in animals and human (14,15). Pomegranate fruit is rich in flavonoid. Metabolites in various parts of pomegranate fruit contains a variety of sugars, organic acids, alkaloids, polyphenols, flavonoids, anthocyanins, essential fatty acids and vitamins (16). Several studies were performed to evaluate the effect of different parts and active ingredients in pomegranate such as Gallic acid, uric acid, olenolic acid in diabetes mellitus and its side effects. (17-19).

Inactivity is an independent risk factor for insulin resistance and type 2 diabetes. Several studies have shown that exercise alone has several benefits, including reducing oxidative stress in people with diabetes. On the other hand, the use of antioxidant agents will have a significant role in reducing the consequences of diabetes (20).

Oxidative stress plays an integral part of the aging process and results from the overproduction of free radicals such as reactive oxygen species (ROS), which overwhelm the body's antioxidant defense mechanisms. Normally, antioxidants neutralize ROS and thus help to prevent over exposure from oxidative stress (21,22). However, as the body ages, antioxidant levels decline, leaving the human body susceptible to a variety of age-related pathologies. This decline combined with a gradual loss of estrogen in the female reproductive system is highly associated with the various sequelae of menopause. The elevation of cytokines and pro-oxidant makers suggests that there is a high degree of oxidative stress in the postmenopausal state (23).

Regarding the effects of aerobic exercises and natural antioxidant agents on the reduction of oxidative stress and complications of diabetes, and since there has been no research on the simultaneous effect of aerobic training with intensity and consumption of pomegranate extract on oxidative stress, so given the importance of the subject, this study intends to examine the issue whether aerobic exercise with moderate intensity associated with the consumption of pomegranate extract significantly affects plasma oxidative stress markers in older women with type 2 diabetes.

Methods:

The research method was semi-experimental and with pre-test, post-test, and control group. The research population included postmenopausal diabetic women (aged 45 to 60, height 1.57±0.6, BMI 27.86±3.64) of Babol City who were invited with the coordination of the Diabetes Association of this county. When the volunteers were interviewed and their written consents were obtained and, considering the conditions of volunteers for participating in the training program, 33 persons were randomly selected and put into four groups: control-water (CW, n=7), control- pomegranate extract (CPE, n=9), training-water (TW, n=9) and training- pomegranate extract (TPE, n=8). The exercises were conducted at the Health Club under the supervision of the Diabetes Association in Babol City. All qualified subjects submitted their letters of consent and the relevant questionnaire one week before the start of the research and expressed their readiness to start the training program. The subjects also took part in an introductory session to get familiar with the program. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

The training group participated in the endurance running program for six weeks and three sessions per week. The training program started with 10 minutes warm-up. The training time started from 25 minutes and each day, two minutes were added to it until the activity time reached 45 minutes. From that point on, the duration was kept constant. The intensity of the activity started from 60% of heart rate reserve and 5% percent was added each week until the time when the intensity reached 75% of heart rate reserve. After that, the intensity of exercise was kept constant. At the end of these training sessions, the participants cooled down for 10 minutes (24).

Subjects in the Control-Pomegranate extract (CPE) and Training–Pomegranate extract groups consumed 150 ml of pomegranate extract per day for 6 weeks (approximately 18pm) (25,26). Training-pomegranate extract group used pomegranate extract an hour after exercise (27,28).

Two days before and after the training program blood samples were taken from the participants' brachial veins in a sitting position in a fasting state (12 hours prior to the test). Their physical and physiological measures were also documented. The subjects were asked not to change their diets during the research.

Superoxide Dismutase (SOD) and glutathione peroxidase (GPX) activities were measured using kits manufactured by Cayman Chemical Company (Michigan 48108, USA) according to the procedures. Plasma Glutathione (GSH) was measured using Ellman's assay (29). The general thiol reagent, 5-5'-dithiobis [2-nitrobenzoic acid] (DTNB, Ellman's Reagent) reacts with GSH to form the 412 nm chromophore, 5-thionitrobenzoic acid (TNB) and GS-TNB. The GS-TNB is subsequently reduced by glutathione reductase and β-nicotinamide adenine dinucleotide phosphate (NADPH), releasing a second TMB molecule and recycling the GSH; thus amplifying the response. Any oxidized GSH (GSSG) initially present in the reaction mixture or formed from the mixed disulfide reaction of GSH with GS-TNB is rapidly reduced to GSH. STAT FAX 2100 Microplate Reader was used for reading all absorbance. Total antioxidant capacity (TAC) was measured according to the method of Benzie and Strain (30).

Descriptive statistics was used to classify the data; Kolmogorov-Smirnov test was applied to check whether the data are normally distributed. Statistical analysis was performed using a one-way analysis of variance, Turkey's post hoc test. It should be noted that the findings are expressed in the form of mean±standard deviation. SPSS program (version 18) was used to calculate the data and the significance difference was set up at the level of $P \leq 0.05$.

Results:

The descriptive indicators of subjects at the beginning are provided in table 1. The weight of the experimental group after the intervention period had a non-significant reduction. Also, the BMI of the test subjects in the experimental group after the intervention period showed a non-significant decrease.

In table 2, the results related to GPX, SOD, GSH and TAC are provided in the form of indices of mean and standard deviation in different groups. Data obtained from GPX activity showed a significant difference between groups ($P<0.001$). Tamhany follow up test (due to lack of homogeneity of variance) revealed that plasma GPX in group TPE had a significant increase compared to CW, CPE and TW groups after intervention.

Table 1. Physical chahracteristics of the participants (mean±SD)

Groups		CW (No=7)	CPE (No=9)	TW (No=9)	TPE (No=8)
Age (years)		49.50±8.06	56.50±3.85	50.50±5.65	51.50±7.69
Height (m)		1.57±0.04	1.59±0.06	1.57±0.06	1.57±0.08
Weight (kg)	Before	66.78±9.20	69.77±15.09	73.38±10.34	69.68±10.73
	After	66.14±8.64	68.06±15.46	68.61±12.09	67.50±10.71
BMI (kg/m^2)	Before	26.77±3.29	27.09±4.57	29.45±3.51	28.16±3.22
	After	26.52±2.97	26.42±4.79	27.44±3.78	27.26±3.12

Table 2. Mean and SD of GPX, SOD, GSH and Total antioxidant capacity in different research groups

Index		CW	CPE	TW	TPE
GPX (U/L)	Before	525.29±189.07	497.56±192.67	475.72±111.03	414.78±71.10
	After	515.29±181.38	643.04±138.22	629.44±77.62	746.88±90.24
SOD (U/ml)	Before	178.43±24.13	180.11±36.78	174.11±19.40	176.50±28.72
	After	177.14±20.78	210.55±26.62	213.88±21.03	218.13±22.02
GSH (μmole/L)	Before	5.57±4.52	5.66±4.00	5.89±2.84	5.88±3.13
	After	5.85 ±2.41	8.33±3.53	9.33±2.60	10.63±2.26
TAC (μmole/L)	Before	470.85±128.83	520.00±94.20	482.22±135.62	498.75±118.61
	After	475.71±131.41	585.55±71.95	563.33±150.74	664.37±125.62

The findings also indicate a significant difference between the groups in SOD activity levels ($P<0.001$). Using LSD follow up tests meaningful increase was observed in groups CPE, TW and TPE compared to CW ($P<0.001$). The analysis of TAC data showed a significant difference between groups ($P<0.001$). LSD follow up test showed that plasma TAC in TPE group significantly increase compared to CW ($P<0.001$), CPE ($P<0.001$) and TW ($P=0.002$) groups after intervention. Meanwhile, the TAC rate in groups CPE and TW showed a significant increase compared to control group ($P=0.026$ and $P=0.006$). GSH results also showed a significant difference between groups ($P<0.001$). LSD follow up test showed that plasma GSH in TPE group had significant increase compared to CW and CPE groups after research protocol ($P<0.001$ and $P=0.034$). The GSH level after a period of moderate aerobic exercise showed a significant increase compared to CW ($P<0.001$).

Conclusion:

The results showed that the average plasma TAC, superoxide dismutase (SOD), glutathione peroxidase (GPX) and the average plasma glutathione (GSH) in the aerobic training group associated with pomegranate extract (TPE), revealed meaningful increase compared to the Control-Water (CW), Control-Pomegranate extract (CPE) and Training-Water (TW) groups after intervention. Meanwhile, the level of GPX showed a significant increase compared to the control group after a period of pomegranate extract consumption, but there were no significant differences compared to aerobic exercise (TW) group.

Increase in the levels of glutathione peroxidase after exercises has been shown in some researches (16) which reveal the increase in glutathione reserves as glutathione oxidase coenzyme. Also, it has been realized that superoxide dismutase is produced as the first line of defense against

Reactive oxygen species (ROS) by antioxidant enzyme system during exhaustive exercise (18,17).

In another study, it was shown that endurance training causes 33 percent increase in the production of glutathione in muscle (16). In athletes, this adjustment reduces cell damage caused by free radical production due to exercise (31). In the current study, moderate-intensity aerobic exercise significantly increased levels of antioxidant enzymes. The findings of this study are consistent with the results of Ngala et al. (2013), Pittaluga et al. (2015), Delavar et al. (2017) which showed that acute and endurance exercise reduces oxidative stress because of training. (32-34).

In addition, in this study, moderate-intensity aerobic exercise caused a significant reduction in weight, waist and hip circumference. Loss of appetite and fat absorption reduction from the gut due to pancreatic lipase activity could be a reason to lose weight loss. The main mechanism which influences on indicators of oxidative stress after exercise is the status of exercise (type, intensity and duration of exercise). An acute exercise increases temporarily, the production of reactive oxygen and nitrogen species interestingly but it provides the necessary stimulus to endogenous antioxidant defenses (35). Prolonged exercise increases the body's metabolism which results in increased oxygen consumption and free radicals causing an increase in antioxidant enzymes (16). Menopause is a gradual process that occurs over a period of years in females who are typically between 45–55 years of age. Previous studies have shown that Oxidative stress levels are reduced in postmenopausal women with exercise training regardless of hormone replacement therapy status (36).

However, more studies are needed to explore the mechanism. Given that one of the most important reasons for the increase in oxidative stress factors is the tissue's high oxygen supply, oxidative stress response to exercise is influenced by factors such as health status, age, sex, race, genetics, fitness level, individual differences, different responses of tissues, muscle fibers and its types, intensity and duration of exercise and the reduction of anti-oxidative stress food intake in daily nutrition (37). Most importantly, the diversity of lipid per oxidation markers and methods of its measurement and sensitivity in different researches could also lead to different results. Additionally, regular exercise of moderate intensity changes oxidative homeostasis of cells and tissues by reducing oxidative damage and increasing resistance to oxidative stress. Studies have shown that exercise can increase the antioxidant enzymes, causes oxidative stress in pancreatic tissue to reduce, prevent the destruction of beta cells and improve pancreatic beta cell mass and performance; all of these improve the quality of life of patients after the exercise. Therefore, according to the results of the research, the increase in the GPX and SOD antioxidant enzymes in diabetic patients following a moderate-intensity aerobic exercise improves the antioxidant status of these patients. Considering the results of this study, women with type 2 diabetes who had exercised with pomegranate extract had the highest average superoxide dismutase, TAC, plasma glutathione peroxidase and glutathione average, and older women with type 2 diabetes without the use of pomegranate extract and exercise, had the lowest levels of superoxide dismutase, TAC, glutathione peroxidase, and average plasma glutathione. Few studies in humans showed that pomegranate has anti-atherosclerotic, anti-inflammatory, anti-hypertensive and antioxidant effect (38). Aviram et al. study in healthy subjects showed that the consumption of pomegranate extract has increased paraoxonase enzyme activity by 20% and reduced LDL oxidation by 90 percent (39). Mertenz, Talcott et al. study also showed that the consumption of pomegranate polyphenol by healthy people their serum antioxidant capacity would increase, if they did not affect the production of reactive oxygen species (40).

In the current study, the antioxidant enzymes in the group consuming pomegranate extract were significantly higher than the group that did not consume pomegranate extract, in other words, the pomegranate extract supplementation consumption caused a significant increase in the amount of antioxidant enzymes. The results of the present study are consistent with the results of Fazli et al. (2009), Gil et al. (2000), Sohrab et al. (2017), Guo et al. (2008) and Taheri Rouhi et al. (2017) following the consumption of pomegranate polyphenols, although in their studies, the production of reactive oxygen species was not

affected, and this is due to the fact that in recent study, the rate of oxidative stress after moderate-intensity aerobic exercise and consumption of pomegranate extract have decreased (41-45). Thus, a significant increase in antioxidant enzymes was seen following the consumption of pomegranate extract for six weeks. It may be taken into account that such a diet control may play a major role in antioxidant level response to exercise and supplements. Therefore, an increase in plasma antioxidant enzyme rate after training in the exercise group with pomegranate consumption can be related to the combined impacts of exercises with pomegranate extract hence the findings of various studies have shown that the consumption of food containing phenolic compounds can have a positive role in human health. However, in this study, levels of antioxidant enzymes and other factors of oxidative stress not measured. In addition, the lack of control over the strict diet (measured energy intake and consumption) is one of the limitations of this study.

In conclusion, our finding showed that aerobic exercise training with the consumption of pomegranate extract improve the body's antioxidant defense system by increasing the levels of plasma antioxidants in diabetic women. Regarding the weakening of the antioxidant system in diabetic individuals, maybe moderate-intensity aerobic training and the use of pomegranate extract can help improve the antioxidant defense system in these individuals.

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تأثیر تمرین هوازی و عصاره انار بر شاخص‌های آنتی‌اکسیدانی در زنان یائسه دیابتی نوع ۲

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مجله پزشکی هرمزگان سال بیست و یکم شماره دوم ۹۶ صفحات ۱۳۷-۱۲۹

چکیده

مقدمه: *هدف از این تحقیق، تأثیر تمرین هوازی و عصاره انار بر شاخص های آنتی اکسیدانی در زنان یائسه دیابتی نوع ۲ بود.*

روش کار: *جامعه آماری این تحقیق زنان دیابتی یائسه شهرستان بابل (سن بین ۴۵ تا ۶۰ سال؛ قد ۶/۰±۱/۵۷ متر، وزن ۲۲/۶ ±۶۹/۹۰ کیلوگرم و شاخص توده بدن ۳/۶۴ ± ۲۷/۸۶ کیلوگرم بر مجذور متر) بودند که با هماهنگی انجمن دیابت این شهرستان معرفی شده و از بین آنها ۳۳ نفر به عنوان آزمودنی انتخاب شدند و به طور تصادفی به چهار گروه کنترل، عصاره انار، تمرین و تمرین – عصاره انار تقسیم شدند؛ برنامه تمرینی گروه تجربی شامل ۶ هفته تمرین هوازی، سه جلسه در هفته و حداقل ۴۵ دقیقه در هر جلسه بود. گروه های مصرف کننده عصاره انار ۱۵۰ میلی لیتر عصاره انار هر روز به مدت ۶ هفته نوشیدند. دو روز قبل و بعد از دوره تمرینی در وضعیت ناشتایی (۱۲ساعت) نمونه‌گیری خونی از ورید بازویی در حالت نشسته انجام گرفت. سطوح شاخص های GPX، SOD، GSH و TAC آزمودنی ها اندازه‌گیری شد.*

نتایج: *نتایج نشان داد که تمرین هوازی همراه با مصرف عصاره انار سطوح گلوتاتیون پراکسیداز و سوپراکسید دیسموتاز، گلوتاتیون و آنتی اکسیدانت تام پلاسما (TAC) در زنان دیابتی نوع ۲ را در مقایسه با گروه کنترل به طور معنی‌داری افزایش داد (P= ۰/۰۰۱)، زنان دیابتی نوع ۲ در گروه تمرین – عصاره انار بیشترین میزان آنزیم‌های آنتی‌اکسیدانی را دارا بودند.*

نتیجه‌گیری: *یافته‌های تحقیق نشان داد که ورزش هوازی همراه با مصرف عصاره انار سیستم دفاع آنتی‌اکسیدانی بدن را با افزایش سطح آنتی‌اکسیدان پلاسما بهبود می‌بخشد. با توجه به تضعیف سیستم آنتی‌اکسیدانی در افراد دیابتی، احتمالاً تمرینات هوازی با شدت متوسط و مصرف عصاره انار می‌تواند به بهبود سیستم دفاع آنتی‌اکسیدانی در این افراد کمک نماید.*

کلیدواژه‌ها: *ورزش هوازی، عصاره انار، آنتی اکسیدان، دیابت نوع ۲*

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