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Beetroot Juice Supplementation and Aerobic Training Improve Antioxidant Capacity in Female Athletes

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Background: The purpose of this study was to investigate whether beetroot juice can alter total antioxidant capacity (TAC) in female soccer players.

Methods: In this randomized clinical trial, female soccer players (n=20) were selected randomly and assigned into two groups: experimental (beetroot juice, n= 10) and control (placebo, n = 10). Subjects did their soccer training for six weeks (3 sessions per week for 90 min) with a consumption of 200 ml juice 2 hours before they started their training. Blood samples were collected and transferred to the laboratory. After separating the serum from the plasma, serum was put on the total antioxidant capacity' kits and total antioxidant capacity was investigated in the pretest and posttest.

Results: Findings showed that the experimental group had a significant increase in TAC after taking beetroot juice consumption (P<0.001) compared to baseline. Also, compared to the control group, there was a significant difference between experimental and control groups in TAC changes (P<0.001).

Conclusion: The present study provides evidence that 6 weeks of beetroot juice consumption positively affects TAC in female soccer players.

Introduction

Under normal conditions, about 2-5% of mitochondrial oxygen is converted to the free radical oxygen species such as superoxide, Hydrogen peroxide, hydroxyl and related radicals to the electron leakage from electron transport chain (ETC) [1]. The oxidative stress is a process created by free radicals on the surface of the cell membrane and makes damage to the membrane of the cell and membrane of the intracellular organelles in particular mitochondria. The lipid cell membrane damage causes membrane lipid peroxidation and hardening of the cell wall, as a result many vital cell actions are influenced. In order to suppress oxidative stress in the body, cell is well equipped with an antioxidant defense system such as superoxide dismutase, glutathione peroxidase and catalase, which are the first cellular defense barrier against various types of activated oxygen radicals. Exercise can have positive or negative effects on oxidative stress, which depends on exercise load, type of exercise and level of exercise. In addition, it seems that oxidative stress is involved in muscle tiredness and leads to overtraining [2]. Soccer trainings simultaneously increase oxidative stress and activity of erythrocyte Superoxide dismutase enzyme, which reduces the amount of lipid peroxidation of the erythrocyte membrane[3]. High intensity soccer trainings, especially to the fatigue limit, has been shown to increase the production of free radicals in the working muscle more than triple the resting levels. Recent studies suggest that antioxidant supplementations are used for preventing oxidative stress among patients, active individuals and athletes [4-5]. Evidence showed that beetroot, in addition to its lipid reduction potential, can decrease reactive oxygen species and increase resistance of plasma lipoprotein to oxidation that leads to the prevention of diseases such as atherosclerosis [6-8] and act as a useful strategy for improving recovery after exercise. Among natural antioxidant nutrients, Beetroot contains phytochemicals (polyphenols and

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betalain) and compared to other vegetables, the content of polyphenols in beetroot is high [9]. Polyphenols reduce the levels of reactive oxygen species created through exercise and counteract with oxidative stress created during exercise [10]. In addition, numerous studies have shown that betalain can act through antioxidant defense mechanisms; improves the redox balance and overcomes oxidative stress damage [11]. As beetroot juice is more popular among athletes, so the purpose of this study was to investigate whether beetroot juice can alter total antioxidant capacity (TAC) in female soccer players.

Subjects and methods

Subjects

Twenty young experienced female soccer players were recruited from soccer team of Razi University, Kermanshah, Iran. Written permission was given to subjects after explaining procedures and targets of the study. Inclusion criteria to the study were:

- They must not be pregnant.
- They must not have kidney disorder or kidney stone.
- They must not be addicted or use alcoholic drinks.

Exclusion criteria included beetroot allergy, vomiting, diarrhea or other chronic diseases (diabetes, liver disease, etc.) during study. The study was approved by the sciences ethics committee at medical sciences, Kermanshah, Iran (Ethical code: IR.KUMS.REC.1397.655). Subjects were asked not to use supplementations (Vitamin E, Vitamin C and vitamin A), energetic beverages, vegetables and fruits which contains antioxidant such as (Tomato, Watermelon) during study. A list of nitrate-containing vegetables (celery, lettuce, cabbage …) was given to subjects not to use them throughout the research. Subjects had the same diet and they used the food that provided by central canteen of Razi University.

Description of the tool

Data was collected from female soccer players by a short questionnaire that designed by researcher before trial. This data included type of family, family income per month, menstrual cycle and type of food diet (do they have Vegetarian diet or Carnivorous diet).

Supplementation protocol

For six weeks (18 session), subjects either consumed a dose of concentrated beetroot beverage (200 ml) or an equal amount of placebo (red carmoisine food color and a little dose of stevia that dissolved in 200 mL water) 2 hour before they started their soccer training [12].

The design of the study

Study was conducted in a randomized and placebo-controlled way. All subjects reported to the laboratory 48 hour before trial and they were divided randomly into two groups: experimental and control (n=10). Then, anthropometric characteristics were measured (Table 1). Blood samples were collected 24 hour before and after the protocol. None had any known cardiovascular diseases or any other contraindication to the study procedures. Subjects were asked to continue their current training programs throughout the study period.

Anthropometric measurements

Height, weight and BMI were measured before the test. Height measurement was done by using semiautomatic height measurement equipment (HD; STDK, Tokyo, Japan).Weight and body composition were measured by using a bioelectrical impedance analysis body composition analyzer (Inbody220; Biospace, Seoul, Korea) (Table 1).

Exercise training method

The training programs were the routine trainings held three times per week for 90 min. After the general warm-up, players performed special warm-ups; including (front and back running, dribbling between obstacles, pass and shoot types). Next, they performed offensive, defensive trainings which supervised by the coach. Then, the coach assessed the players' skills by grouping the players and setting up the competition and outlining their strengths and weaknesses. At the end of the training session the cool down was done.

Blood collection and TAC analysis method

Blood samples (10ml) were collected from antecubital vein and transferred to the laboratory by a laboratory specialist. Blood separation was performed by centrifugation at 3000 rpm for 15 min. After separating the serum from the plasma, serum was put on the Zellbio total antioxidant capacity' kits. Optical Density (OD) was measured by the Elisa Reader. We calculated the difference by the below formula. Then, TAC in the pretest and posttest was measured.

OD=OD2-OD1

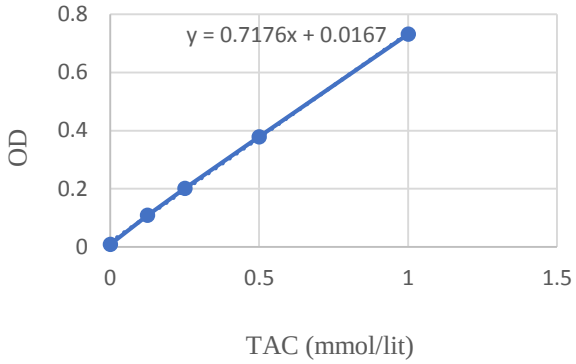

Figure1. Total Antioxidant Capacity (TAC) (mmol/lit)

Statistical analysis

Data generated were analyzed using statistical package for social sciences (SPSS version 22). Comparisons within groups in the pretest and posttest were done by paired sample T-test and comparison between experimental and control groups were done by student t-test. Statistical significance (p) was set at 0.05.

Results

Anthropometric measurements:

Table 1. Anthropometric measurements (Weight, Height, BMI) (Mean ± SD).

Variable	Experimental	Control
Age (year)	23.20±0.91	23±0.81
Weight(kg)	58.56±4.88	61.18±2.48
Height(cm)	161.05±5.10	162.60±1.89
BMI (kg/m2)	22.57±1.54	23.13±0.58

According the questionnaire with regard to type of family, (83.3%) in experimental group and (80%) in control group were from nuclear family. Regarding family monthly income, experimental group (36.67%) and in control group (46.67%) had income of 2 million toman. In relation to menstrual cycle, majority of girls in the experimental group (100%) had regular menstrual cycle and in control group (90%) had regular menstrual cycle. Regarding type of food diet (Vegetarian diet or Carnivorous diet), experimental group (100%) and control group (100%) have carnivorous diet. As vegetables contain antioxidant, they were asked not to use vegetable (like tomato, coriander, parsley, garlic, spinach…) throughout the study.

Laboratory investigations:

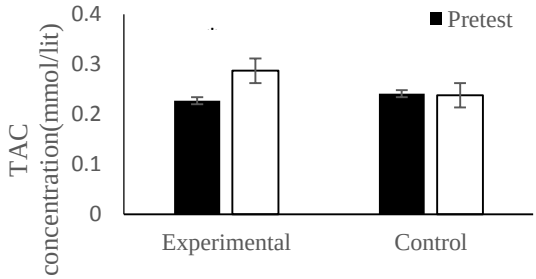

Figure 2. TAC concentration between experimental and control groups.

Table 2. Difference of pre and post TAC in the experimental and control groups.

	Group	Pre	Post	t-value	P value
TAC (mmol/lit)	Experimental	0.227±0.021	0.287±0.010	-12.68	0.0001*
	Control	0.241±0.043	0.238±0.045	1.88	0.09

*Significance: p ≤0.05

As seen, there was a significant increase in TAC after taking beetroot juice consumption (P<0.05) (Table 2).

Table 3. Comparison of TAC score between experimental and control group

	TAC(mmol/lit)
Experimental	0.060±0.015
Control	-0.002±0.004
t	12.63
P value	0.0001
*Significance: p ≤0.05	

(Table 3) indicates results of student t-test. Results show there were significant difference in (TAC) between experimental and control subjects (P<0.05).

Discussion

The present study showed that 6 weeks beetroot juice consumption increased total antioxidant capacity in female soccer players. Limited studies have investigated the effect of beetroot juice on the antioxidant capacity and there is no study that investigates effect of beetroot juice consumption on the total antioxidant capacity in female soccer players. Kojaska (2009) examined effects of beetroot juice consumption on the Wistar rats. Their research results showed that 28 days of beetroot juice consumption reduced oxidative stress and increased antioxidant enzymes (superoxide dismutase, glutathione peroxidase, catalase and glutathione reductase) concentrations, which is consistent with the result of present study[13]. Lu (2009) examined the effects of beetroot juice consumption in the mice. Their research results showed that 30 days beetroot juice consumption reduced oxidative stress and increased the activity of antioxidant enzymes such as (superoxide dismutase, catalase and glutathione) which is consistent with the results of this study [14]. El Gamal (2014) investigated the effects of beetroot extract consumption in Wistar rats. The results of their study showed that 28 days use of beetroot extract reduced oxidative stress and increased antioxidant activity of catalase enzyme[15]. Ruth (2015) investigated benefits of acute and chronic beetroot juice consumption on antioxidant capacity in the healthy individuals. Both acute and chronic consumption of beetroot juice increased antioxidant capacity. These results are consistent with results of present study[16]. Studies have provided evidence that beetroot is the best source of antioxidants that protects cellular components against oxidation in both laboratory and non-laboratory conditions. It actually suggests that beetroot juice may be a useful strategy for treating diseases that are caused by oxidative stress, such as liver damage and cancer. However, there is still little research that limits any clinical recommendations for beetroot juice consumption [17]. In addition, soccer trainings lead to the production of large amounts of reactive oxygen species, which can reduce time to fatigue and affect on performance. The body's defense system has also tackled with these compounds, but sometimes faced defeat. Beetroot is one of the antioxidant materials that strengthen antioxidant system. In fact, consumption of beetroot juice can be attributed to the achievements of this study. Beetroot juice, by increasing total antioxidant capacity in female soccer players, leads to delay fatigue and improve performance.

Conclusion

In general, given that soccer trainings lead to produce free radicals and reactive oxygen species that damage to the cells and decreases performance in soccer players. So, nutritional interventions and antioxidant supplements can be a good solution for protecting defense system against oxidative stress caused by trainings. The present study could justify increase of total antioxidant capacity in experimental group. Due to the high antioxidant capacity of beetroot juice, this beverage is suggested as one of the best beverages to female soccer players for improving total antioxidant capacity in their body.

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Conflict of interest

None of authors have conflict of interests.

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