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The Effects of Cherry Juice Supplementation on Antioxidant Capacity, Hydrogen Peroxide and Creatine Kinase Following an Exhaustive Aerobic Exercise in Non-Athlete Men

Mohammad Reza ASAD[1], Hamid ARAZI[2], Narjes POORMOHAMMAD[3], Roghayyeh GHOLIZADEH[4]
[1]Department of Physical Education and Sport Sciences, Payame Noor University, Tehran, IRAN
[2]Department of Exercise Physiology, Faculty of Sport Sciences, University of Guilan, Rasht, IRAN
[3]Department of Physical Education and Sport Sciences, Payame Noor University, Tehran, IRAN
[4]Department of Exercise Physiology, Faculty of Sport Sciences, University of Mohaghegh Ardebilli, Ardebil, IRAN
Email:asad_mohammadreza@yahoo.com, hamidarazi@yahoo.com, NarjesPoormohammad@yahoo.com, r.gholizadeh60@yahoo.com

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Abstract

Introduction: Oxidative stress is a condition in which the reactive oxygen species production exceeds the antioxidant system capacity to neutralize these peroxidases. In these situations, proteins, lipids, and nucleic acids are damaged. In this regard, the cherry can be noted as a food antioxidant which leads an increasing antioxidant capacity and reducing inflammation and damage muscle. Therefore, The purpose of this study was to determine the effect of cherry juice supplementation on total antioxidant capacity (TAC), creatine kinase (CK), hydrogen peroxide (H_2O_2) in non-athlete men after an exhaustive aerobic exercise. Method: In this quasi-experimental research, ten untrained (UT) men were randomly selected. Then, they were divided into two equal groups: supplement group (cherry juice) and placebo group (commercial Cherry juice diluted with natural water). After eight days of supplementation period, all subjects were participated in aerobic exercise protocol (Bruce test run to the point of exhaustion) on the treadmill. Primary blood samples in the baseline were taken. The second was immediately after the Bruce test, third and fourth were six and twenty-four hours later were taken (5 ml). For analysis of the results. Analysis of variance with repeated measures was used at the significant level. Result: A significant effect of short-term cherry juice supplementation on TAC, H_2O_2, and CK was observed ($p \leq 0.05$). Conclusions: In general, it can be concluded that probably eight days of cherry juice supplementation probably cannot prevent the adverse effects of oxidative stress caused by acute aerobic exercise.

Keywords: Cherry juice supplementation, Antioxidants, Oxidative stress, Exhaustive exercise.

Introduction

Today, researchers believe that participation in physical activities can be effective in improving the health of athletes or even people with diseases (Edge et al.,2006; Helgerud et al.,2006) While participating in these activities, especially exhaustive activities is accompanied to releasing free radicals (free radicals) and depletion of antioxidant resources that Lipid peroxidation and oxidative stress indices of blood and urine rises(increases), in fact, free radicals can cause inflammatory responses (Scott and Jackson Malcolm 2008). Although the production of free radicals is necessary to some extent (partly) for the body's physiological processes, increase its useless, that often introduced in the form of reactive oxygen species (ROS), is harmful to the body and lead to oxidative stress (Hamedi Nia, 2002). On the other hand, some research results have shown that oxidative stress is involved in the pathogenesis of many diseases, including atherosclerosis, hypertension, ischemic heart disease, diabetes, cancer, rheumatoid arthritis and inflammatory, degenerative disease of the nervous system and also in the aging process (Meagher and Rader, 2001). although, one way to deal with the adverse effects of oxidative stress caused by intense and heavy exercise is using natural and edible antioxidant supplements (Williams et al., 1990). In this regard, the cherry can be noted as a food antioxidant rich in vitamins A and c, phenolic compounds, anthocyanin and melatonin Which leads an increasing antioxidant capacity and reducing inflammation and damage muscle (Barrett et al., 2005). As Dimitriou et al., 2015) studies showed that consumption of cherry juice before and after marathon reduced developing symptoms of inflammation of the upper respiratory tract caused by trauma, hyperventilation caused by exercise and also other causes of infectious and non-infectious in runners. Also, the results of some studies suggest that consumption of cherries and its products are effective in reducing oxidative stress(Bell et al., 2014), However, few studies are at hands regarding the role of Cherry in increasing antioxidant properties and reducing oxidative stress and muscle damage caused by doing aerobic exercise. So that, Bell et al. (2014)'s study showed that consumption of 30 ml cherry concentrate for seven days decreasing lipid peroxidation and inflammatory cascade in cyclists. Traustadóttir and colleagues (2015) showed that the consumption of 240 ml sour cherry juice for 14 days in older adults improves the antioxidant defense (Traustadóttir et al., 2009). Also, Howatson and colleagues (2010) showed that consumption of cherry juice for five days before the Marathon, increased antioxidant capacity, reduced inflammation and lipid peroxidation.

Due to limited and contradictory studies on cherry supplementation on antioxidant properties and muscle damage caused by aerobic exercise, the question is whether short-term consumption of cherry juice can reduce oxidative damage caused by moderately severe aerobic exercise by Increasing the antioxidant capacity and at least reduce the adverse effects of oxidative stress and its characteristics?

Therefore, this study aimed to determine the effect of cherry juice on total antioxidant capacity, creatine kinase and hydrogen peroxide in non-athlete men following an exhaustive aerobic exercise.

Methods

This study was conducted as quasi-experimental design in two groups (experimental and placebo) with repeated measures (four stage) as double-blind. The study participant consisted of untrained and employed men that among theme 30 volunteers announced to participate in this research project.

All subjects participated in a coordination meeting and were aware of goals and methods of measurement by the researcher, then informed consent form and completed the 24-hour recall diet, then underwent medical examinations. Volunteers had not used natural and industrial supplements in the past month. Ten days before starting the study, anthropometric measurements, height, weight, and BMI, maximal oxygen consumption (VO_2max) were measured. Then from among 30 volunteers, ten people with an average age of 41.9±2.9 were selected and randomly divided into two equal groups of Cherry juice supplementation (710 ml two times daily for seven days) and placebo (710 ml commercial Cherry juice diluted with city water that is similar but it has significantly declined nutrients).in this study, Cherry juice obtained Natural cherry that had no commercial additives.

In this study, levels of TAC, H_2O_2, and CK were measured through blood sampling. At each sampling time, approximately five-milliliter blood samples were taken from the left brachial vein. Values of TAC, H_2O_2, and CK were measured by using the ELISA test and spectrophotometer (made in America Biotech). Used kits were from Padgin Medicine Company. Blood samples for determination of blood parameters were used without the addition of anticoagulant for preparation of serum. All measurements in the same condition (9-10 o'clock in the morning, the temperature of 28-26 °C and humidity 50%) were performed. In addition, the subjects 48 hours Before the test, they avoided doing any heavy physical activity and also the meal before the test had no fruit and antioxidants, tablets or supplements. Initial blood samples were obtained at baseline before making of Cherry juice from in all subjects.

On the day of testing, all subjects warmed up their body with stretching and exercising after 30-minute rest interval. Then, they ran to the point of exhaustion on the treadmill (Bruce test run to the point of exhaustion) and Second Blood samples were taken immediately after exercise protocol. third and fourth blood sampling were measured 6 and 24 hours after the test, respectively. The normal state of the data (mean and standard deviation) was conducted by using Shapiro-Wilk test; For analysis of the results. repeated variance analysis was used. All operations and statistical analysis were performed at the significant level (P <0/05) by Using SPSS version 20.

Results

Subject's characteristics are presented in table 1. Average antioxidant capacity, creatine kinase, hydrogen peroxide in cherry juice and placebo groups are presented in Table 2.

Table 1. Subjects' characteristics

Variable	Supplement	Placebo
Age (year)	40.04±9.35	41.80±1.06
Height (cm)	173.92±5.5	170.25±4
Weight (kg)	86.80±9.31	91.20±9.56
BMI (kg/m^2)	28.86±3.72	31.37±3.72
VO_2max (ml/kg/min)	39.43±2.79	39.34±1.44

× Values are reported as mean±SD.

Table 2. Changes in total antioxidant capacity, creatine kinase and hydrogen peroxide in different stages (M±SD)

Variables	Group	Before	Immediately	After 6 hours	After 24 hours
Total antioxidant	Placebo	0.1391±0.8600	0.1337±0.8800	0.1047±0.9420	0.1742±0.8220
Capacity(U/ml)	Cherry juice	0.08526±0.7580	0.1456±0.8240	0.1298±0.8780	0.1291±0.8240
Hydrogen	Placebo	16.51±39.20	9.56±20	11.97±43.60	14.71±17
Peroxide(mμ)	Cherry juice	15.67±30.40	3.27±15.20	10.47±33.20	6.74±17
Creatine	Placebo	27.973±61	24.382±84	38.895±84.40	21.064±91.20
Kinase(U/ml)	Cherry juice	24.674±62.60	61.063±95.20	107.934±134.20	44.136±109

According to results, the sustainability of antioxidant capacity, creatine kinase, hydrogen peroxide were not significantly different in cherry juice and placebo conditions after supplementation of cherry juice.

Discussion

According to the results, short-term use of cherry juice supplement did not significantly change the total antioxidant capacity, hydrogen peroxide and creatine kinase in non-athletic men.

Howatson et al (2010) showed that the average of antioxidant capacity increased by only 10% compared to control group in athletes. In addition, Su et al. (2008) reported that there was no reduction in total antioxidant capacity following by exhausting aerobic exercise (Su et al., 2008). Sacheck et al. (2003) also found that oxidative capacity did not decrease after 45 minutes of jogging in healthy young men. In addition, other researchers have reported that inhibiting free radicals, stimulating second-phase enzymes, reducing the formation of oxidative increases in DNA and lipid peroxidation and also, mutagenic inhibition by environmental and carcinogenic agents, including anthocyanin and phenolic compounds in cherry juice (Kong et al., 2003). In this regard, Traustadóttir et al. (2015), in their study on older adults found that cherry juice reduces oxidative damage in nucleic acids. Also, Bell et al (2014) reported cellular disorders, caused by oxidative stress, reduce in cycling men after taking cherry juice. While in the study of Shadmanfard et al (2012), TAC and MDA reduction was observed after an exhausting aerobic exercise period. In connection with the justification of these differences, can mention the difference in the type of activity and demographic characteristics (type, race, age, gender, health status and physical fitness) (Su et al., 2008); because in the present study, the subjects were non-athletic men who were able to complete the Bruce test in four steps. Also, Baradaran and et al (2012) stated that exercise increases the intensity of plasma H_2O_2 concentration in female athletes. In this case, muscle damage may be increased in this process (Córdova et al., 2010); some studies have reported about increasing plasma H_2O_2 concentration levels, as the intensity of exercise increases, the production and releasing of this free radical also increases (Groussard et al., 2003). However, the findings contradict to results of some previous studies. These contradictions in studies probably indicate that hydrogen peroxidation levels may also be affected by inflammatory

markers, some researchers have pointed to the relationship between LDH and free radicals, and it has been reported that increased LDH enzymes have an inhibitory role in increasing of free radicals (Bloomer and Cole, 2014).

Other possible mechanisms of herbal supplements, such as cherry juice, can be the direct accumulation of free radical species which are responsible for cell damage (Martinez, 2009). In fact, cherries form a complex resistant to the attack of free radicals (Sarma and Sharma, 1999) and rejuvenating the androgenic enzymes of antioxidant defense systems (Shih et al., 2007) and finally the mix of genetic sequence of all these things is one of these mechanisms (Traustadóttir et al., 2009; Bell et al., 2014).

Anthocyanin is responsible for the antioxidant properties of cherries that poorly absorbed in the body (Bitsch et al., 2004; Charron et al., 2009) and quickly repel from the human system (Felgines et al., 2003; Kurilich et al., 2005). Therefore, the direct removal of free radicals a lonely will not be responsible for immediately reducing H_2O_2 after exercise. In addition, experiments show that the formation of resistant complexes of oxidative damage that formed with a mixture of cherries anthocyanin is shown only by DNA, not by measuring the amount of H_2O_2 from blood sampling (Sarma and Sharma, 1999). The redox regulation of Endogenous antioxidant defense seems to be a prominent mechanism of protection against H_2O_2 peroxidation

On the other hand, the findings of the present study suggest that insignificant blood creatine kinase changes after aerobic exercise (Bruce test) is consistent with the results of some studies.

In a study, Howatson et al. (2010) examined the consumption of cherry juice for 8 days on 20 Morton runners. The results showed aerobic exercises does not have an effect on the amount of creatine kinase in the blood. In this regard, Hindell et al. (2001) did not achieve significant increases in subjects Ck after 20-minute protocol of the step test (Hindell et al., 2001) and also in another study by Walsh et al (2001), which examined 30 minutes of cycling, they could not find significant increases in subjects Ck. The researchers say that this intensity of exercise has not been able to cause muscular damage, and in fact, the test should be performed in more intensely. On the other hand, in a study Tokmakidis et al., 2003, examined the effect of ibuprofen after hamstring muscle contractions on CK and delayed muscle soreness. The results showed CK decrease in ibuprofen group (Tokmakidis et al.,2003). Also, the Tartibian and Azizbeigi (2008) observed the inhibitory effect of naproxen after 48 hours on CK. They believe Probably naproxen reduces the release of CK into the blood by preventing increased muscle damage. The contradiction of this finding with the results of some previous studies may be due to variations in the range of training programs, the intensity, and duration of activities, the type of supplementation, the time of blood transfusion, and the level of readiness of subjects.

Indeed, the unreasonable increase in creatine kinase after the Bruce test could possibly result in minor damage to the sarcolemma, which leads to the entry of creatine kinase into the outer space of the cell. The peak levels of creatine kinase vary from person to person and do not necessarily indicate muscle damage (Jordan, 2007; Brenner et al.,1990). According to Brenner et al. (1999) study, the increase in creatine kinase levels does not occur in parallel with the increase in cytokines, why so the mechanism involved in cytokines diffusion may be different from muscle damage. Changes in creatine kinase are depended on muscle mass, severity, duration and volume of exercise, and whether the subject is familiar with exhausting activity is different (Jordan, 2007). Perhaps the difference in protocol type used that in studies is the most important factor in the difference in creatine kinase changes. As noted earlier,

muscle damage causes CK to be released into the bloodstream. The more and wider damage, cause the greater amount of CK released. For example, marathon is much more than extreme and persistent extravagant activities produce creatine kinase (Nieman et al.,2005).

In general, according to the findings of this study, it can be concluded that probably eight days of cherry juice supplementation cannot prevent the adverse effects of oxidative stress caused by intense aerobic activity. However, by considering the previous studies and the benefits of cherry fruit as a source of antioxidant properties, more accurate results are needed in further studies on more doses and longer periods of consumption of cherry juice on the oxidative stress caused by aerobic exercises in the number of more samples from the athlete and non-athlete population.

Conflict of Interest

The authors have not declared any conflicts of interest.

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