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Research Article

The Effect of Pomegranate-Black Carrot Juice on Serum and Erythrocytes of Sedentary Subjects Exposed to Exhausting Exercise

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Background. In this study, the effects of pomegranate-black carrot juice mixture on serum and erythrocytes of sedentary individuals who had exhaustion test were investigated. *Methods*. A total of 20 men voluntarily participated in the study. Blood samples were obtained from participants on three conditions. First, before the study, blood samples of participants were collected (baseline). Second, the same participants performed in the 20-meter shuttle run test for 1 week each day and were subjected to oxidative stress. Lastly, the same participants were given a mixture of pomegranate-black carrot juices (100 ml/100 ml) for a week, 45 minutes prior to the 20-meter shuttle run test, and the stress + supplement was performed. Blood samples were taken at the end of each process. *Results*. In the erythrocytes, while the oxidative stress condition malondialdehyde (MDA) level and carbonic anhydrase (CA) enzyme activity levels increased compared to the baseline, reduced glutathione (GSH) level, glutathione reductase (GR), and glutathione S-transferase (GST) enzyme activity levels decreased. In stress + supplement conditions, while GSH and GR levels increased according to oxidative stress conditions, CA and MDA levels decreased. While the lactate dehydrogenase (LDH) level of the oxidative stress condition increased compared to the baseline, the LDH level of the stress + supplement decreased compared to the oxidative stress condition. *Conclusions*. Our results showed that the level of oxidative stress in subjects exposed to the exhaustion test decreased with the mixture of pomegranate-black carrot juices.

1. Introduction

Regular physical exercise plays an important role in the prevention of diseases such as cancer, obesity, depression, hypertension, and diabetes. Heavy physical exercises increase oxygen consumption through metabolism and accelerate the formation of reactive oxygen species (ROS) [1]. With the increase in oxygen uptake during exercise, free radical products are formed in the cell [2]. With the increase in exercise intensity, it causes more production of superoxide radicals [3], which are converted to hydrogen peroxide (H_2O_2) by enzyme superoxide dismutase (SOD). H_2O_2 can be detoxified into water and oxygen by catalase (CAT) and glutathione peroxidase (GPx). H_2O_2 is converted to hydroxyl radical via the Fenton reaction [4, 5]. After an acute exercise, the concentration of oxidative stress markers immediately increases in plasma [6, 7]. Severe physical exercise increases the production of ROS, negatively affecting the antioxidant system, and causes severe muscle pain. Furthermore, it causes an increase in the activity of

enzymes such as lactate dehydrogenase (LDH) and creatine kinase (CK) in plasma [8, 9]. Levels of reactive oxygen species in cells are tried to be balanced by neutralization by catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), vitamins, reduced glutathione (GSH), and flavonoids. Although elevated ROS levels as a result of acute and chronic exercises decrease glutathione and vitamin reserves in the cellular antioxidant defense system, enzymatic antioxidants try to adapt to this condition. ROS production increases in the muscle, liver, and heart tissues as a result of physical exercise in humans and animals. It has been reported that the activity of SOD, CAT, and GPx enzymes increased as a result of the physical exercise, reducing the damage caused by ROS [10].

Pomegranate juice contains 85% water, 10% sugar, 1.5% pectin, ascorbic acid, and polyphenols [11]. In addition, pomegranate juice contains active substances against free radicals, such as anthocyanins, ellagitannins, quercetin, kaempferol, luteolin, glycosides, ellagic acid, and gallic acid. Pomegranate has many beneficial effects on human metabolism, such as antioxidant and antiproliferative activities, anti-inflammatory, and antiobesity effects [12–14]. Pomegranate juice has been reported to reduce oxidative stress in the blood of endurance-based athletes [15]. Carrots have many flavonoid-derived substances such as quercetin, luteolin, kaempferol, and myricetin. Black carrots have higher amounts of flavonoids than other carrots [16, 17]. There are many bioactive components in the chemical component of black carrots. The most important of these are carotenoids, anthocyanins, polyacetylenes, and falcarindiols [18]. These components in black carrot have many biochemical effects such as antioxidant, antiallergic, antimicrobial, anti-inflammatory, antitumor, and antiatherosclerotic activities [19].

Nutrition is of strategic importance in exercise programs developed for athletes. Today, carbohydrates and proteins from macronutrients are often given more importance, whereas micronutrients are considered to be of less importance. Pomegranate and black carrot juices contain many biochemical micronutrients, such as flavonoids and anthocyanins. Many pharmacological effects of these substances are mentioned above. Studies in the literature have shown that a fruit juice is usually given in order to reduce oxidative stress due to heavy exercise [20–25]. The use of black currant, cherry, grape, watermelon, blueberry, pomegranate, and banana has been reported in detail in the studies on athletes [20–25]. It has been reported that drinking pomegranate juice before Olympic-style weightlifting sessions reduces malondialdehyde levels and improves the enzymatic reactions of catalase and glutathione peroxidase [26]. In another study, a significant improvement in the vascular diameter and regulation of blood flow of pomegranate seed extracts was observed in the sprinting ability test [27].

It has been found that the mixture of two or more fruit juices is not used in such studies. The aim of this study was to investigate the effects of pomegranate-black carrot juices on oxidative stress in sedentary subjects who were exposed to the exhaustion exercise test.

2. Materials and Methods

2.1. Subjects. This research was conducted in accordance with the pretest and posttest models. As a result of the power analysis, the minimum sample size required to detect a significance difference using this test should be at least 7, considering type I error (alpha) of 0.05, power (1-beta) of 0.8, effect size of 1.36, and two-sided alternative hypothesis (H1). However, this number was set at 20 to obtain stronger results. The study included 20 male sedentary individuals aged 19– 22 years. Ethical approval of the study was obtained from Firat University, Noninvasive Research Ethics Committee Directorship (date: 26.10.2017, decision no: 14/35). The inclusion criteria for the study were the absence of any disability or disease of the participants. It was also noted that there was no obstacle for them to participate in the tests. The inability to continue the exercises or the disability of the participants was determined as exclusion criteria.

2.2. Collection of Blood Samples. Blood samples were obtained from participants on three conditions (baseline, after 1-week exhaustion tests (oxidative stress) and after 1-week stress + supplement). Blood samples of sedentary individuals were collected in overnight fasting for baseline conditions after one day from applications (oxidative stress period and stress + supplement). The same participants performed in the 20-meter shuttle run test for 1 week each day and were subjected to oxidative stress. Blood samples were taken from participants for oxidative stress. Lastly, the same participants were given a mixture of pomegranate-black carrot juices (100 ml/100 ml) for a week, 45 minutes prior to the 20-meter shuttle run test, and the stress + supplement was performed. Blood samples were taken at the end of each process. At the end of the administration period, blood samples were collected and centrifuged. The erythrocytes and serum obtained were stored in a deep freezer at −50°C.

2.3. Supplementation of Black Carrot and Pomegranate Juices. In the study, pomegranate grown in the Adıyaman region was used. Pomegranate fruit has a dark red color. In the Adıyaman region, this pomegranate is known as Hicaz pomegranate. Pomegranates and black carrots were cleaned by washing with distilled water. After the pomegranates were separated from their shells, the juice was obtained in the juicer. Black carrot juice was obtained by the same method. After the second condition, the mixture of pomegranate-black carrot juices (100 ml/100 ml) for a week 45 minutes prior to the 20-meter shuttle run test was given to participants. In our previous study, the content of pomegranate juice was examined and phenolic acid 490.75 mg/kg, anthocyanin 137.1 mg/L, ellagic acid 175 mg/100 g, total flavonoids 63 mg/kg, and total antioxidants 1530 mg/kg were determined [28]. In the literature, total anthocyanins 837 mg/100 g, total phenolics 7.98–291.48 mg/100 g, flavonoids 3.00–111.70 mg/100 g, flavonols 51.6 mg/100 g, and falcarinol 1.55 mg/100 g were reported in the content of black carrot juice [29].

2.4. Exhaustion Exercise Test. Exhaustion exercise test was applied in the gymnasium of Adıyaman University, School of Physical Education and Sports. The exhaustion exercise test was performed with a 20-meter shuttle run. The characteristics of this test are explained as follows: The test starts at a slow running speed (8.5 km/h), and the subject must have reached the opposite line with each beep. Sedentary individuals run on the 20-meter runway, and with the signal, the subjects touch the line with one foot. The running speed is increased by 0.5 km/h per minute. In other words, the time between the signals decreases by 0.14 seconds per minute. If the individual misses a signal, but catches the rhythm again with the other signal, the test continues. If the individual fails to reach the line, that is, 3 meters ahead of the runway in two consecutive signals, the test is ended. The stage at which the individual is stopped corresponds to the test result and indicates the degree of endurance of the individual [30].

2.5. Preparation of the Hemolysate. Fresh blood samples were collected in tubes containing EDTA and without EDTA (for analyses serum LDH) and then centrifuged (15 min, 2.500 × g), and plasma and buffy coat (leucocytes) were removed. The packed red cells were washed three times with saline, hemolyzed with 5 volume of ice-cold water, and then centrifuged (10.000 × g, for 30 min) to remove the ghosts and intact cells.

2.6. Analyses of Erythrocytes MDA and Reduced GSH, GST, and GR. The MDA, GSH, and GST concentrations were determined using a microplate reader spectrophotometer system (ThermoTM Varioskan Flash, Thermo Fisher Scientific Inc., Vantaa, Finland). The MDA levels were measured based on the relative production of reactive substances of thiobarbituric acid [31]. Results were represented as nmol/mg protein. The reduced GSH activity was measured by its reaction with 5.5-dithiobis 2-nitro-benzoic (DTNB) acid to form a compound that absorbs at 412 nm [32]. The reduced GSH levels are expressed nmol/mg protein. For determining GST activity, first 20 mM 1-chloro-2,4-dinitrobenzene was prepared in 96% ethanol, and this solution was used as the substrate. Reductive glutathione (0.002 M) was used as the cofactor in the reaction [33]. In brief, 10 μL of the supernatant, 100 μL of phosphate buffer (0.1 M, pH 6.5), 100 μL of GSH mixture, and finally 10 μL of 1-chloro-2,4-dinitrobenzene were transferred into the wells of the microplate. After this process, the well plates were placed in the microplate reader system, and the change in absorbance was recorded at 344 nm for 2 min at 25°C. Specific GST and GR activities were calculated as EU/L. Analyses of GR activity was determined by the modified method [34].

2.7. Analyses of Erythrocyte CA Enzyme Activity. The CA activity was assayed by following the change in absorbance of 4-nitrophenyl acetate to 4-nitrophenolate ion at 348 nm over a period of 3 min at 25°C using a spectrophotometer (Shimadzu UV-Vis Spectrophotometer, UV-1800) according to the method described by Verpoorte [35]. The enzymatic reaction, in a total volume of 3.0 mL, contained 1.4 mL 0.05 M Tris-SO_4 buffer (pH 7.4), 1.0 mL 3 mM 4-nitrophenyl acetate, 0.5 mL H_2O, and 0.1 mL enzyme solution. A reference measurement was obtained by preparing the same cuvette without enzyme solution. Specific CA activity was calculated as EU/L.

2.8. Protein Determination. Quantitative protein determination was spectrophotometric ally measured at 595 nm according to Bradford's method, with bovine serum albumin being used as a standard [36].

2.9. Determination of Serum LDH. The technique was used to evaluate LDH based on the reaction of pyruvate to L-lactate. LDH results were expressed as U/l. LDH levels in serum samples were measured in Japanese branded kits.

LDH is a hydrogen transfer enzyme that catalyzes the oxidation of L-lactate to pyruvate via NAD^+ as a hydrogen acceptor. The LDH activities of the samples were measured at 340 nm absorbance. LDH activity was measured with ARCHITECT c8000 branded device. LDH levels were determined in Adıyaman University Medical School Biochemistry Laboratories.

2.10. Statistical Analysis. SPSS package program was used for data analysis. Results are expressed as mean ± SD. The comparison of the measurements was carried out using repeated measures ANOVA followed by the Bonferroni test. Statistical significance was accepted as $p < 0.05$.

3. Results

Erythrocyte biochemical parameters are shown in Table 1. The erythrocyte MDA concentration of measurement 2 was significantly higher than that of the baseline ($p < 0.05$). The MDA level of measurement 3 decreased compared to that of measurement 2 ($p < 0.05$). While the GSH level of measurement 2 decreased compared to that of the baseline ($p < 0.05$), the GSH level of measurement 3 increased compared to that of measurement 2 and baseline ($p < 0.05$). The GR and GST enzyme activity levels in measurement 2 decreased compared to those of the baseline ($p < 0.05$). However, the GR and GST enzyme activity levels of measurement 3 increased compared to those of measurement 2 ($p < 0.05$). While the measurement 2 CA enzyme activity level increased compared to that of the baseline ($p < 0.05$), the measurement 3 CA enzyme activity level decreased compared to that of measurement 2 ($p < 0.05$).

Serum LDH levels are shown in Figure 1. While the LDH level of measurement 2 increased compared to that of the baseline ($p < 0.05$), the LDH level of measurement 3 decreased compared to that of measurement 2 ($p < 0.05$).

4. Discussion

In this study, MDA levels were increased in red blood cells of sedentary individuals who were exposed to exhaustion exercise. Blood MDA levels have been reported to increase in many exercise studies. Kanter and Child observed increases in plasma MDA levels after excessive exercise programs

Table 1: Summary of general changes in erythrocyte biochemical parameters in subjects.

Parameters	Baseline	Measurement 2	Measurement 3
MDA (nmol/mg protein)	0.330 ± 0.015	0.393 ± 0.020^{a}	0.344 ± 0.012^{x}
GSH (nmol/mg protein)	447.600 ± 7.980	417.500 ± 10.230^{a}	557.800 ± 38.490^{ax}
GR (EU/L)	1.929 ± 0.269	1.119 ± 0.250^{a}	2.553 ± 0.870^{x}
CA (EU/ml)	1.034 ± 0.220	2.678 ± 0.450^{a}	1.878 ± 0.820^{ax}
GST (EU/L)	6.630 ± 1.290	3.860 ± 0.450^{a}	5.470 ± 1.770^{x}

Statistical significance compared to the baseline: a: $p < 0.05$. Statistical significance compared to measurement 2: x: $p < 0.05$. Measurement 2: after 1-week (each day) exhaustion tests (oxidative stress). Measurement 3: after 1-week (each day) exhaustion tests + supplement.

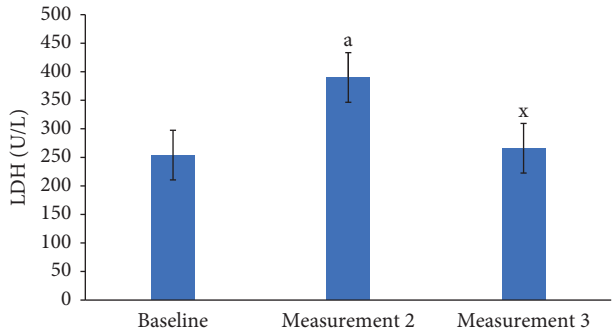

Figure 1: Serum LDH levels of subjects. Statistical significance compared to the baseline: a: $p < 0.05$. Statistical significance compared to measurement 2: x: $p < 0.05$. Measurement 2: after 1-week (each day) exhaustion tests (oxidative stress). Measurement 3: after 1-week (each day) exhaustion tests + supplement.

[37, 38]. In another study, the effects of pomegranate juice on oxidative stress parameters were examined in athletes exposed to endurance exercises. This study reported that consumption of pomegranate juice reduced the levels of MDA that increased after exhaustion exercise [15]. In addition, in another study conducted in recent years, pomegranate juice has been reported to reduce high MDA levels in healthy young men who have undergone heavy exercises [39]. LDH is known to be a marker of muscle damage. Increased LDH concentration in serum is used as an indicator of damage in the muscle membrane and other tissue structures [40]. In order to detect muscle damage after resistance and aerobic exercises, the rate of change in serum LDH levels should also be known [41]. In addition, serum LDH levels were increased in the stress group. Serum LDH levels have been reported to increase in many exercise studies. In aerobic and resistance exercise studies conducted by Kobayashi in 2005, it was reported that there was an increase in serum LDH levels, which resulted in immunological hormonal changes [42]. Serum LDH levels were studied before and after the run in the London Marathon in 2002, and an increase in LDH levels was reported after the run [43]. In another study, there was an increase in LDH levels after 10 weeks of regular speed endurance training application for basketball players [44]. In previous studies, there were dramatic increases in serum LDH levels after exercise [45]. In the present study, it was determined that elevated LDH levels decreased as a result of administration of the mixture of pomegranate and black carrot juices. We believe that the decrease in high MDA and LDH levels in the diet group can be attributed to the positive effects of many biochemical molecules in the fruit juice mixture.

GSH is a tripeptide molecule that inhibits the action of oxidant molecules. It is also used as a cofactor by some peroxidase enzymes. The GSH molecule is catalyzed by the GR enzyme. GR is activated when the intracellular levels of GSH, which have a significant reducing role in oxidation/reduction reactions, are activated and complete the formation of GSH. GSH reacts with free radicals in the cell and acts as an electron donor in the reduction of peroxides by GPx. A decrease in the GSH level and GSH/GSSG ratio and an increase in GSSG levels are determinants of oxidative stress [46]. In our study, GSH levels were found to be lower in the exhaustion exercise group than in the control group, but it was found to be significantly higher in the diet group. GST enzyme protects the cell against reactive oxygen metabolism. GST is the enzyme that catalyzes conjugation of electrophilic compounds with reduced GSH. In addition, GSH has important roles in defense against oxidative damage and peroxidative products of DNA and lipids [47]. GSH is a biomolecule with antioxidant effect against oxidative stress in mammalian cells. It has been reported that GSH levels decrease with acute physical exercise in young men and women [48]. In addition, there was a significant increase in the levels of TBARS (thiobarbituric acid reactive substances) and conjugated dienes from oxidative parameters in male rats exposed to strenuous exercise, whereas a decrease in SOD, CAT, GPx, and GST enzyme activities from antioxidant enzyme parameters [49]. In a study evaluating the antioxidant effects of pomegranate juice consumption in humans, 14 healthy people were given pomegranate juice for a period of 15 days. This study reported that pomegranate juice decreased plasma MDA levels and significantly increased GSH levels in erythrocytes. In addition, pomegranate juice has been reported to improve the antioxidant mechanism [50]. In another study, it was reported that pomegranate juice consumed for 3 months increased GSH levels in serum [51]. Guo et al. reported that consumption of pomegranate juice did not affect plasma GSH levels [52]. The difference between the results of these cited studies and our study can be attributed to the fact that the majority of GSH in the plasma is from the liver and not from erythrocytes [53]. The increase in GSH levels in erythrocytes may be due to induction of the catalytic activity or expression of GSH syntheses and glutamate cysteine ligase enzymes [54]. The decrease in the levels of GSH molecules and GR and GST enzymes, which have a metabolic link between each other, as a result of oxidative stress in

metabolism after exhaustion exercise is an important result obtained in this study. A decrease in the activity levels of GR and GST enzymes leads to a decrease in the levels of GSH, which is catalyzed by GR and GST enzymes. The increase in the levels of GSH molecules and GR and GST enzyme activities after consumption of the mixture of pomegranate and black carrot juices in sedentary individuals shows that it decreases oxidative stress in the metabolism of these individuals.

The literature review showed no study on the effects of dietary supplements on CA enzyme activity levels in sedentary individuals exposed to exhaustion exercise. The activity of CA enzyme, which is one of the most important parameters of the study, was observed to be significantly increased in the sedentary group exposed to exhaustion physical exercise. The activity of CA enzyme was found to be decreased in the sedentary group that was given the mixture of pomegranate and black carrot juices. Carbonic anhydrase is a very important enzyme for human metabolism. It regulates acid and base balance in many tissues and blood. It is produced in many tissues in metabolism and catalyzes the conversion of CO_2 to bicarbonate. In addition, this enzyme acts in many metabolic events such as lipogenesis, gluconeogenesis, urea synthesis, calcifications, and tumor formation [55]. In the study, it can be said that the increase in the levels of the CA enzyme activity in the sedentary group exposed to exhaustion physical exercise is caused by the effort of this enzyme to reduce the increased CO_2 concentration in the blood as a result of oxidative stress. In the diet group, the activity of this enzyme decreased. Many biochemical substances, such as polyphenol, found in the content of pomegranate and black carrot juices, reduce oxidative stress in the sedentary group exposed to exhaustion exercise thanks to their antioxidant activity, thus reducing the over activity of the CA enzyme. In addition, our study showed that the mixture of pomegranate-black carrot juices decreased oxidative stress by increasing GSH, GST, and GR levels in erythrocytes of the stress + diet group. Regarding the activity of CA in erythrocytes of athletes and sedentary individuals exercised, in a study of the CA enzyme levels in erythrocytes of athletes who trained continuously for 6 weeks, a 50% increase in the CA enzyme activity was reported in cold weather and high altitude (>1600 m). In the report, this is explained by the increased activity of the CA enzyme in order to achieve a CO_2 hydration/bicarbonate dehydration balance. However, it has been reported that CA enzyme activity change near the initial level at 4–6 weeks [56]. There are many polyphenols in the biochemical components of carrot and pomegranate juices. In our previous study, The PJ content was determined by that researcher as phenolic acid 490.75 mg/kg, anthocyanin 137.1 mg/L, ellagic acid 175 mg/100 g, total flavonoids 63 mg/kg, and total antioxidants 1530 mg/kg. It has been reported that pomegranate juice inhibits lipid peroxidation against lead toxicity and has positive effects on enzyme activities. In addition, it has been reported that the pomegranate juice used in the study has a high antioxidant capacity [28]. Carrots have chemical components such as quercetin, luteolin, kaempferol, myricetin, carotenoids, anthocyanins, polyacetylenes, and falcarindiols, and these components are known to have antioxidant, antiallergic, antimicrobial, anti-inflammatory, antitumor, and anti-atherosclerotic activities [15, 18]. In recent years, polyphenols, which have attracted attention with their possible effects on exercise, have also been found to have many positive effects on performance, training, adaptation, and immune functions [57–61].

As in every study, there are some limitations in this study. The limitation of this study is that the research group consisted of only sedentary men. Another limitation is the absence of a washout period between treatments. However, not following the performance change is another limitation. As a result of current research, it showed that the level of oxidative stress in sedentary participants exposed to the exhaustion test decreased with the mixture of pomegranate-black carrot juices. Based on this, this supplement can be recommended to reduce the oxidative stress. It is thought that this situation may be important in increasing sportive performance.

Data Availability

All data generated or analyzed during this study are included within this published article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References

[1] M. Gomez-Cabrera, E. Domenech, and J. Viña, "Moderate exercise is an antioxidant: upregulation of antioxidant genes by training," *Free Radical Biology and Medicine*, vol. 44, no. 2, pp. 126–131, 2008.

[2] M. J. Jackson, D. Pye, and J. Palomero, "The production of reactive oxygen and nitrogen species by skeletal muscle," *Journal of Applied Physiology*, vol. 102, no. 4, pp. 1664–1670, 2007.

[3] F. McArdle, D. M. Pattwell, A. Vasilaki, A. McArdle, and M. J. Jackson, "Intracellular generation of reactive oxygen species by contracting skeletal muscle cells," *Free Radical Biology and Medicine*, vol. 39, no. 5, pp. 651–657, 2005.

[4] B. Halliwell, "Reactive oxygen species in living systems: source, biochemistry, and role in human disease," *The American Journal of Medicine*, vol. 91, no. 3, pp. 14S–S22, 1991.

[5] D. M. Patwell, A. McArdle, J. E. Morgan, T. A. Patridge, and M. J. Jackson, "Release of reactive oxygen and nitrogen species from contracting skeletal muscle cells," *Free Radical Biology and Medicine*, vol. 37, no. 7, pp. 1064–1072, 2004.

[6] T. Ashton, C. C. Rowlands, E. Jones et al., "Electron spin resonance spectroscopic detection of oxygen-centred radicals in human serum following exhaustive exercise," *European Journal of Applied Physiology*, vol. 77, no. 6, pp. 498–502, 1998.

[7] V. Pialoux, R. Mounier, E. Rock et al., "Effects of acute hypoxic exposure on prooxidant/antioxidant balance in elite endurance athletes," *Int J Sports Med*, vol. 30, no. 02, pp. 87–93, 2009.

[8] D. A. Leaf, M. T. Kleinman, M. Hamilton, and T. J. Barstow, "The effect of exercise intensity on lipid peroxidation," *Medicine &amp Science in Sports &amp Exercise*, vol. 29, no. 8, pp. 1036–1039, 1997.

[9] M. Antoncic Svetina, D. Sentija, A. Cipak, D. Milicic, A. Meinitzer et al., "Ergometry induces systemic oxidative stress in healthy human subjects," *Tohoku Journal of Experimental Medicine*, vol. 221, pp. 43–48, 2010.

[10] L. L. Ji, "Exercise and oxidative stress: role of the cellular antioxidant systems," in *Exercise and Sport Science Reviews*, J. O. Holloszy, Ed., Williams and Wilkins, Baltimore, MD, USA, pp. 135–166, 1995.

[11] M. Aviram, L. Dornfeld, M. Rosenblat et al., "Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: studies in humans and in atherosclerotic apolipoprotein E-deficient mice," *American Journal of Clinical Nutrition*, vol. 71, no. 5, pp. 1062–1076, 2000.

[12] N. P. Seeram, L. S. Adams, S. Henning et al., "In vitro antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice," *The Journal of Nutritional Biochemistry*, vol. 16, no. 6, pp. 360–367, 2005.

[13] L. S. Adams, N. P. Seeram, B. B. Aggarwal, Y. Takada, D. Sand, and D. Heber, "Pomegranate juice, total pomegranate ellagitannins, and punicalagin suppress inflammatory cell signaling in colon cancer cells," *Journal of Agricultural and Food Chemistry*, vol. 54, no. 3, pp. 980–985, 2006.

[14] F. Lei, X. N. Zhang, W. Wang et al., "Evidence of anti-obesity effects of the pomegranate leaf extract in high-fat diet induced obese mice," *International Journal of Obesity*, vol. 31, no. 6, pp. 1023–1029, 2007.

[15] E. Fuster-Munoz, E. Roche, L. Funes, P. Martınez-Peinado, J. Sempere, and N. Vicente-Salar, "Effects of pomegranate juice in circulating parameters, cytokines, and oxidative stress markers in endurance-based athletes: a randomized controlled trial," *Nutrition*, vol. 32, no. 5, pp. 539–545, 2016.

[16] T. Bahorun, A. Luximon-Ramma, A. Crozier, and O. I. Aruoma, "Total phenol, flavonoid, proanthocyanidin and Vitamin C levels and antioxidant activities of Mauritian vegetables," *Journal of the Science of Food and Agriculture*, vol. 84, no. 12, pp. 1553–1561, 2004.

[17] D. Marinova, F. Ribarova, and M. Atanassova, "Total phenolics and total flavonoids in Bulgarian fruits and vegetables," *Journal of the University of Chemical Technology and Metallurgy*, vol. 40, pp. 255–260, 2005.

[18] S. A. Arscott and S. A. Tanumihardjo, "Carrots of many colors provide basic nutrition and bioavailable phytochemicals acting as a functional food," *Comprehensive Reviews in Food Science and Food Safety*, vol. 9, no. 2, pp. 223–239, 2010.

[19] A. V. Rao and L. G. Rao, "Carotenoids and human health," *Pharmacological Research*, vol. 55, no. 3, pp. 207–216, 2007.

[20] M. D. Cook, S. D. Myers, S. D. Blacker, and M. E. T. Willems, ""New Zealand black-currant extract improves cycling performance and fat oxidation in cyclists," *European Journal of Applied Physiology*, vol. 115, no. 11, pp. 2357–2365, 2015.

[21] G. Howatson, P. G. Bell, J. Tallent, B. Middleton, M. P. McHugh, and J. Ellis, "Effect of tart cherry juice (Prunus cerasus) on melatonin levels and enhanced sleep quality," *European Journal of Nutrition*, vol. 51, no. 8, pp. 909–916, 2012.

[22] S. Lafay, C. Jan, K. Nardon et al., ""Grape extract improves antioxidant status and physical performance in elite male athletes," *Journal of Sports Science and Medicine*, vol. 8, no. 3, pp. 468–480, 2009.

[23] R. A. Shanely, D. C. Nieman, P. Perkins-Veazie et al., "Comparison of watermelon and carbohydrate beverage on exercise induced alterations in systemic inflammation, immune dysfunction, and plasma antioxidant capacity," *Nutrients*, vol. 8, no. 8, p. 518, 2016.

[24] Y. McLeay, M. J. Barnes, T. Mundel, S. M. Hurst, R. D. Hurst, and S. R. Stannard, "Effect of New Zealand blueberry consumption on recovery from eccentric exercise induced muscle damage," *Journal of the International Society of Sports Nutrition*, vol. 9, no. 1, pp. 19–12, 2012.

[25] D. C. Nieman, N. D. Gillitt, W. Sha et al., ""Metabolomics-based analysis of banana and pear ingestion on exercise performance and recovery," *Journal of Proteome Research*, vol. 14, no. 12, pp. 5367–5377, 2015.

[26] A. Ammar, M. Turki, O. Hammouda et al., "Effects of pomegranate juice supplementation on oxidative stress biomarkers following weightlifting exercise," *Nutrients*, vol. 9, no. 8, pp. 819–913, 2017.

[27] E. J. Roelofs, A. E. Smith-Ryan, E. T. Trexler, K. R. Hirsch, and M. G. Mock, "Effects of pomegranate extract on blood flow and vessel diameter after high-intensity exercise in young, healthy adults," *European Journal of Sport Science*, vol. 17, no. 3, pp. 317–325, 2017.

[28] E. Annac, M. Uckun, A. Ozkaya et al., "The protective effects of pomegranate juice on lead acetate-induced neurotoxicity in the male rat: a histomorphometric and biochemical study," *Journal of Food Biochemistry*, vol. 46, no. 4, Article ID e13881, 216 pages, 2022.

[29] S. Akhtar, A. Rauf, M. Imran, M. Qamar, and M. Riaz, "Black carrot (Daucus carota L.), dietary and health promoting perspectives of its polyphenols: a review," *Trends in Food Science and Technology*, vol. 66, pp. 36–47, 2017.

[30] V. Cinar, R. Mogulkoc, and A. K. Baltaci, "Calcium supplementation and 4-week exercise on blood parameters of athletes at rest and exhaustion," *Biological Trace Element Research*, vol. 134, no. 2, pp. 130–135, 2010.

[31] Z. A. Placer, L. L. Cushman, and B. C. Johnson, "Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems," *Analytical Biochemistry*, vol. 16, no. 2, pp. 359–364, 1966.

[32] M. S. Moron, J. W. Depierre, and B. Mannervik, "Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver," *Biochimica et Biophysica Acta (BBA)-General Subjects*, vol. 582, no. 1, pp. pp67–78, 1979.

[33] W. H. Habig, M. J. Pabst, and W. B. Jakoby, "Glutathione S-transferases," *Journal of Biological Chemistry*, vol. 249, no. 22, pp. 7130–7139, 1974.

[34] I. Carlberg and B. Mannervik, "Glutathione reductase," *Methods in Enzymology*, vol. 113, pp. 484–490, 1985.

[35] J. A. Verpoorte, S. Mehta, and J. T. Edsall, "Esterase activities of human carbonic anhydrases B and C," *Journal of Biological Chemistry*, vol. 242, no. 18, pp. 4221–4229, 1967.

[36] M. M. Bradford, "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding," *Analytical Biochemistry*, vol. 72, no. 1-2, pp. 248–254, 1976.

[37] M. M. Kanter, G. R. Lesmes, L. A. Kaminsky, J. La Ham-Saeger, and N. D. Nequin, "Serum creatine kinase and lactate dehydrogenase changes following an eighty kilometer race," *European Journal of Applied Physiology and Occupational Physiology*, vol. 57, no. 1, pp. 60–63, 1988.

[38] R. B. Child, D. M. Wilkinson, and J. L. Fallowfield, "Effects of a training taper on tissue damage indices, serum antioxidant capacity and half-marathon running performance," *International Journal of Sports Medicine*, vol. 21, no. 5, pp. 325–331, 2000.

[39] M. Mazani, A. S. Fard, and A. N. Baghi, "Effect of pomegranate juice supplementation on matrix metalloproteinases 2 and 9 following exhaustive exercise in young healthy males," *Journal of Pakistan Medical Association*, vol. 64, no. 7, pp. 785–790, 2014.

[40] D. Foschini and J. Prestes, "Acute hormonal and immune responses after a bi-set strength training," *Fitness and Performance Journal*, vol. 6, no. 1, pp. 38–44, 2007.

[41] P. M. Clarkson and M. J. Hubal, "Exercise-induced muscle damage in humans," *American Journal of Physical Medicine and Rehabilitation*, vol. 81, pp. 52–69, 2002.

[42] Y. Kobayashi, T. Takeuchi, T. Hosoi, H. Yoshizaki, and J. A. Loeppky, "Effect of a marathon run on serum lipoproteins, creatine kinase, and lactate dehydrogenase in recreational runners," *Research Quarterly for Exercise and Sport*, vol. 76, no. 4, pp. 450–455, 2005.

[43] J. E. Smith, G. Garbutt, P. Lopes, and D. T. Pedoe, "Effects of prolonged strenuous exercise (marathon running) on biochemical and haematological markers used in the investigation of patients in the emergency department," *British Journal of Sports Medicine*, vol. 38, no. 3, pp. 292–294, 2004.

[44] B. Mohammed, K. Bachir, S. Nour-Eddine, and B. Adel, "Study of LDH adaptations associated with the development of Speed endurance in basketball players U19," *International Journal of Applied Exercise Physiology*, vol. 7, no. 3, pp. 2322–2337, 2018.

[45] P. L. Wolf, J. A. Lott, G. J. Nitti, and R. Bookstein, "Changes in serum enzymes, lactate, and haptoglobin following acute physical stress in international-class athletes," *Clinical Biochemistry*, vol. 20, no. 2, pp. 73–77, 1987.

[46] E. Camera and M. Picardo, "Analytical methods to investigate glutathione and related compounds in biological and pathological processes," *Journal of Chromatography B*, vol. 781, no. 1-2, pp. 181–206, 2002.

[47] R. Van der Oost, J. Beyer, and N. P. Vermeulen, "Fish bioaccumulation and biomarkers in environmental risk assessment: a review," *Environmental Toxicology and Pharmacology*, vol. 13, no. 2, pp. 57–149, 2003.

[48] A. B. Karabulut, M. E. Kafkas, S. Savas, M. Hazar, and R. T. Kiran, "Effect of exhaustive exercise on oxidative stress and adenosine deaminase activities in women compared to men," *Journal of US-China Medical Science*, vol. 8, no. 3, pp. 150–155, 2011.

[49] T. Thirumalai, S. V. Therasa, E. K. Elumalai, and E. David, "Intense and exhaustive exercise induce oxidative stress in skeletal muscle," *Asian Pacific Journal of Tropical Disease*, vol. 1, no. 1, pp. 63–66, 2011.

[50] C. M. Matthaiou, N. Goutzourelas, D. Stagos, E. Sarafoglou, and A. Jamurtas, "Pomegranate juice consumption increases GSH levels and reduces lipid and protein oxidation in human blood," *Food and Chemical Toxicology*, vol. 73, pp. 1–6, 2014.

[51] O. Rozenberg, A. Howell, and M. Aviram, "Pomegranate juice sugar fraction reduces macrophage oxidative state, whereas white grape juice sugar fraction increases it," *Atherosclerosis*, vol. 188, no. 1, pp. 68–76, 2006.

[52] C. Guo, J. Wei, J. Yang, J. Xu, W. Pang, and Y. Jiang, "Pomegranate juice is potentially better than apple juice in improving antioxidant function in elderly subjects," *Nutrition Research*, vol. 28, no. 2, pp. 72–77, 2008.

[53] R. Abbas, R. S. Kombu, R. A. Ibarra, K. K. Goyal, H. Brunengraber, and J. R. Sanabria, "The dynamics of glutathione species and ophthalmate concentrations in plasma from the VX2 rabbit model of secondary liver tumors," *HPB Surgery*, vol. 2011, Article ID 709052, pp. 1–8, 2011.

[54] J. Ø. Moskaug, H. Carlsen, M. C. Myhrstad, and R. Blomhoff, "Polyphenols and glutathione synthesis regulation," *The American Journal of Clinical Nutrition*, vol. 81, no. 1, pp. 277–283, 2005.

[55] M. Ilies, C. T. Supuran, A. Scozzafava et al., "Carbonic anhydrase inhibitors: sulfonamides incorporating furan-thiophene- and pyrrole-carboxamido groups possess strong topical intraocular pressure lowering properties as aqueous suspensions," *Bioorganic and Medicinal Chemistry*, vol. 8, no. 8, pp. 2145–2155, 2000.

[56] T. Murat, S. Esra, E. Deniz et al., "Comparison of blood carbonic anhydrase activity of athletes performing interval and continuous running exercise at high altitude," *Journal of Enzyme Inhibition and Medicinal Chemistry*, vol. 34, no. 1, pp. 219–225, 2019.

[57] D. C. Nieman, D. A. Henson, S. J. Gross et al., "Quercetin reduces illness but not immune perturbations after intensive exercise," *Medicine and Science in Sports and Exercise*, vol. 39, no. 9, pp. 1561–1569, 2007.

[58] D. C. Nieman, A. S. Williams, R. A. Shanely et al., "Quercetin's influence on exercise performance and muscle mitochondrial biogenesis," *Medicine and Science in Sports and Exercise*, vol. 42, no. 2, pp. 338–345, 2010.

[59] V. Somerville, C. Bringans, and A. Braakhuis, "Polyphenols and performance: a systematic review and meta-analysis," *Sports Medicine*, vol. 47, no. 8, pp. 1589–1599, 2017.

[60] S. R. Mcanulty, D. C. Nieman, L. S. Mcanulty, W. S. Lynch, F. Jin, and D. A. Henson, "Effect of mixed flavonoids, n-3 fatty acids, and vitamin C on oxidative stress and antioxidant capacity before and after intense cycling," *International Journal of Sport Nutrition and Exercise Metabolism*, vol. 21, no. 4, pp. 328–337, 2011.

[61] A. Hernandez, A. Cheng, and H. Westerblad, ""Antioxidants and skeletal muscle performance: "common knowledge, experimental evidence," *Frontiers in Physiology*, vol. 3, no. 46, pp. 1–6, 2012.