Sport Sciences for Health (2019) 15:133–139
https://doi.org/10.1007/s11332-018-0501-y

ORIGINAL ARTICLE

Biochemical changes in oxidative stress markers following endurance training and consumption of purslane seed in rats with hydrogen peroxide-induced toxicity

Rahman Soori[1] · Valiollah Shahedi[1] · Ali Akbarnejad[1] · Siroos Choobineh[1]

Received: 22 June 2018 / Accepted: 12 September 2018 / Published online: 25 September 2018

Abstract
The current study examined fluctuations in oxidative stress markers following endurance training (ET) and consumption of purslane seeds (Ps) in rats after receiving H_2O_2. Fifty-four adult male Wistar rats were assigned to nine experimental groups: (1) control (intoxicated-no treatment); (2) ET; (3) ET + Ps 50 mg/kg/day; (4) ET + Ps 200 mg/kg/day; (5) ET + Ps 400 mg/kg/day; (6) Ps 50 mg/kg/day; (7) Ps 200 mg/kg/day; (8) Ps 400 mg/kg/day; (9) control (non-intoxicated, intact). The first eight groups were given 100 mg/kg of H_2O_2 to induce oxidative stress. Groups 2–5 were given ET for a period of 8 weeks. Heart and lung tissues were then exposed to evaluate the oxidative stress markers. Catalase, glutathione peroxidase, malondialdehyde, and superoxide dismutase enzymes were measured using ELISA kits. A marked improvement in enzyme concentration was observed in both tissues. It was more pronounced in the groups receiving higher doses of Ps + ET. The findings provide evidence that purslane seed supplementation has antioxidant potential alongside endurance training and improved the ability to cope with oxidative stress.

Keywords Oxidative stress · Purslane seed · Endurance training · Hydrogen peroxide

Introduction

Oxidative stress arises from an imbalance in antioxidants and excessive generation of reactive oxygen species (ROS) and reactive nitrogen species [1]. Oxidative stress is known to contribute to conditions such as heart failure, coronary artery disease, neurodegenerative diseases, chronic kidney disease, amyotrophic lateral sclerosis and cerebrovascular disease [2–4]. Catalase (CAT), glutathione peroxidase (GPX), malondialdehyde (MDA) and superoxide dismutase (SOD) reciprocate against the cytotoxic effects of reactive oxygen metabolites in pathophysiological conditions [5]. The activation of oxidative stress can cause tissue damage, subclinical inflammation by damage to cellular components and contribute to the degradation of the cell membrane and DNA [6]. Thus, oxidative stress must be reduced to prevent or treat diseases for which the root cause is elevated oxidative stress.

It has been proven that regular physical activity significantly affects the antioxidant capacity of the body and induces positive stress [7–9]. This includes resistance [10] and aerobic [11] exercise. One mechanism of activation of oxidative stress is stress itself [12]. It has been documented that a single bout of exercise can increase antioxidant enzyme activity and induce oxidative stress in humans [13] and animals [14]. Studies on humans have shown a significant improvement in oxidant/antioxidant balance after physical exercise through an increase in the endogenous antioxidant defense system [15, 16]. These responses to exercise may be related to redox signaling that activates the pathways involved in antioxidant enzyme gene transcription to increase resistance to cellular stress [17, 18].

In recent years, natural products and herbal remedies have been used as alternative medicines to treat and prevent disease [19, 20]. Pharmacological studies have demonstrated that there is a link between the presence of free radicals and degenerative disease, and the role of free radical scavengers has been explained [21]. Dietary phytochemicals and natural plants are potential therapeutic agents with valuable

✉ Rahman Soori
soori@ut.ac.ir

[1] Department of Exercise Physiology, Faculty of Physical Education and Sport Sciences, University of Tehran, Tehran, Iran

therapeutic properties as well as nontoxic and cost effective [22]. *Portulaca oleracea*, commonly known as purslane, is a plant from the Portulacaceae family that has been used for therapeutic purposes [20]. This plant is an excellent source of antioxidants such as vitamins A, C, E and β-carotene [23, 24]. Studies have shown that the consumption of purslane seeds in combination with 8 weeks of resistance exercise can prevent exercise-induced oxidative stress, realign the pro-oxidant and antioxidant balance [25], and improve indicators associated with liver damage [26], and high blood pressure [27].

The current study aimed at developing an exercise protocol along with the consumption of purslane seed to ameliorate oxidative stress induced by H_2O_2. This study assessed the effect of exercise and purslane seed consumption on oxidative stress induced by H_2O_2 and investigated the fluctuation of oxidative stress markers such as CAT, GPX, MDA, and SOD in the heart and lung tissues of rats.

Research design and method

Experimental animals and hydrogen peroxide toxicity induction

Fifty-four adult male Wistar rats weighing 180–220 g that were 8 weeks of age were purchased from the animal center of Tehran University and kept in polycarbonate laboratory cages in the animal room. They were reared at 22 ± 2 °C and 55% ± 5% moisture under a 12:12-h light:dark cycle. During the research process, the animals were fed with commercial pellets and were provided with tap water ad libitum. All animals were acclimatized for 1 week prior to the start of the experiment.

The rats were assigned to one of nine experimental groups: (1) control (intoxicated-no treatment); (2) ET; (3) ET + Ps 50 mg/kg/day; (4) ET + Ps 200 mg/kg/day; (5) ET + Ps 400 mg/kg/day; (6) Ps 50 mg/kg/day; (7) Ps 200 mg/kg/day; (8) Ps 400 mg/kg/day [28]; (9) control (non-intoxicated, intact). The first eight groups were given 100 mg/kg of body weight of H_2O_2 to induce oxidative stress [29, 30]. Group 9 did not receive H_2O_2 and was used to show the level of enzymes in normal intact rats. All procedures involving animal experiments were approved and carried out in strict accordance with US Institute of Animal Research guidelines for the care and with the use of laboratory animals and by the Animal Care and Use Committee of the University of Tehran.

Preparation of purslane seeds

Purslane seeds were purchased from a grocery shop in Tehran (Iran). The seeds were washed and air dried at room temperature for 7 days. The dry seeds were powdered and dissolved in distilled water. Plant identification and information were determined according to the method of Dehghan et al. [20] with voucher specimen no.15-04979.

Exercise training protocol

Endurance training was considered for this study. After a week of acclimatization, groups 2–5 were given endurance training on a six-channel motor driven treadmill, 5 days weekly, 30 min/day, for a period of 8 weeks at the running speed of 23 m/min following the previously published protocol by Mallikarjuna et al. [31]. All animals of these four exercise groups completed 8-week period of exercise training protocol. The running program was scheduled between 8.00 and 10.00 a.m.

Tissue sampling and determination of oxidative stress markers

All rats from the ET, control and sedentary treatment groups were euthanized at the end of 8 weeks of treatment. Animals were first anesthetized with ketamine/xylazine (80/8 mg/kg IP) and killed by cervical dislocation. Killing was performed at this time and all efforts were made to minimize suffering. The heart and lung tissues were removed, washed with PBS and snap-frozen in liquid nitrogen prior to protein extraction. The following enzyme-linked immunosorbent assay (ELISA) was performed using the following commercial kits (Cusabio; USA): CSB-E08558r for MDA, CSB-E12146r for GPX, CSB-E13439rr for CAT and CSB-EL022397RA for SOD.

Statistical analysis

All data have been presented as a mean ± standard deviation. The Shapiro–Wilks test was conducted to determine whether or not the data were normally distributed. The data were analyzed in SPSS 22.0 and considered to be statistically significant at $p < 0.05$. Two-way, repeated-measures analysis of variance (ANOVA) was used to compare differences between groups. The Bonferroni post hoc test was used to check for significant differences between ET, Ps, and combined Ps + ET.

Results

The results showed that CAT activity in the heart tissue after consumption of purslane seed ($F = 16.35$, $p = 0.001$, $\eta^2 = 0.754$) and ET ($F = 6.42$, $p = 0.022$, $\eta^2 = 0.287$) increased significantly after 8 weeks in a dose-dependent manner (Fig. 1). The interaction of ET and purslane seed

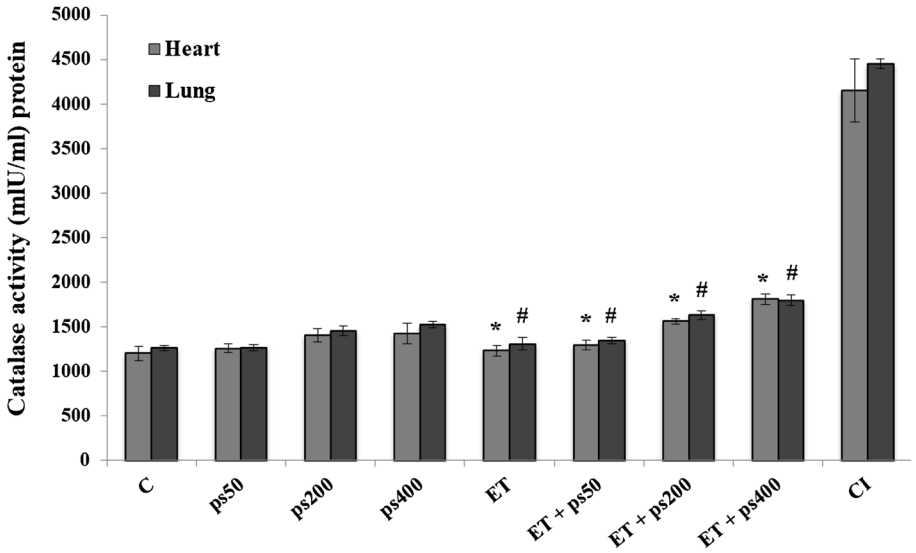

Fig. 1 CAT activity (mlU/ml) in heart and lung tissues after 8 weeks of ET and purslane seed consumption after H_2O_2 toxicity induced in rats. Data were expressed as mean ± SEM. C: intoxicated control, ps50: 50 mg purslane seed, ps200: 200 mg purslane seed, ps400: 400 mg purslane seed, ET: ET, ET + ps50: ET + 50 mg purslane seed, ET + ps200: ET + 200 mg purslane seed, ET + ps400: ET + 400 mg purslane seed, CI: non-intoxicated control intact, *$p < 0.05$ compared to intoxicated control group in heart tissue, #$p < 0.05$ compared to intoxicated control group in lung tissue

was also significant ($F = 12.08$, $p = 0.001$, $\eta^2 = 0.694$) in rats after oxidative stress was induced by H_2O_2. Exercise significantly increased the CAT concentration in the lung tissue ($F = 35.98$, $p = 0.001$, $\eta^2 = 0.692$) (Fig. 1). Purslane seed also significantly increased the concentration of CAT in the lung tissue ($F = 57.60$, $p = 0.001$, $\eta^2 = 0.915$) in a dose-dependent fashion (Fig. 1). The interaction of ET and purslane seed was significant ($F = 4.40$, $p = 0.019$, $\eta^2 = 0.452$) in rats after oxidative stress was induced by H_2O_2.

The GPX activity in heart tissue indicated that purslane seed extract ($F = 9.48$, $p = 0.001$, $\eta^2 = 0.640$) and ET ($F = 15.88$, $p = 0.001$, $\eta^2 = 0.498$) after 8 weeks of treatment significantly increased GPX concentration in heart tissue in a dose-dependent manner (Fig. 2). The interaction of ET and purslane seed also was significant ($F = 0.277$, $p = 0.841$, $\eta^2 = 0.049$) in rats after oxidative stress was induced by H_2O_2. ET significantly increased GPX concentration in the lung tissue ($F = 102.12$, $p = 0.001$, $\eta^2 = 0.865$) (Fig. 2). Purslane seed significantly increased the concentration of GPX in the lung tissue ($F = 47.186$, $p = 0.001$, $\eta^2 = 0.898$) in a dose-dependent fashion (Fig. 2).

The results showed that feeding purslane seed extract ($F = 0.824$, $p = 0.500$, $\eta^2 = 0.134$) and endurance exercise ($F = 3.51$, $p = 0.079$, $\eta^2 = 0.180$) had no effect on the

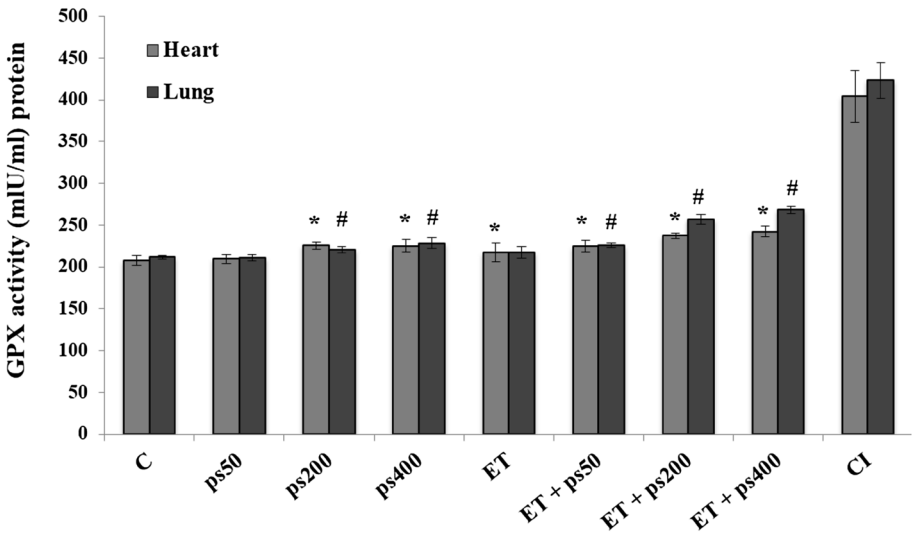

Fig. 2 GPX activity (mlU/ml) in heart and lung tissues after 8 weeks of ET and purslane seed consumption after H_2O_2 toxicity induced in rats. Data were expressed as mean ± SEM. C: intoxicated control, ps50: 50 mg purslane seed, ps200: 200 mg purslane seed, ps400: 400 mg purslane seed, ET: ET, ET + ps50: ET + 50 mg purslane seed, ET + ps200: ET + 200 mg purslane seed, ET + ps400: ET + 400 mg purslane seed, CI: non-intoxicated control intact, *$p < 0.05$ compared to intoxicated control group in heart tissue, #$p < 0.05$ compared to intoxicated control group in lung tissue

MDA concentration in the heart tissue (Fig. 3). The interaction of endurance exercise and purslane seed also had no effect ($F=0.371$, $p=0.775$, $\eta^2=0.065$) in rats after oxidative stress was induced by H_2O_2. ET significantly decreased the MDA concentration in the lung tissue ($F=7.96$, $p=0.012$, $\eta^2=0.332$) (Fig. 3). Purslane seed also significantly decreased the concentration of MDA in the lung tissue ($F=4.35$, $p=0.020$, $\eta^2=0.450$), but not in a dose-dependent manner (Fig. 3). The interaction of endurance exercise and purslane seed was not significant ($F=0.873$, $p=0.476$, $\eta^2=0.141$) in rats after oxidative stress was induced by H_2O_2.

SOD activity in the heart tissue showed that purslane seed extract ($F=8.90$, $p=0.001$, $\eta^2=0.626$) and endurance exercise ($F=52.45$, $p=0.001$, $\mu=0.766$) after 8 weeks of treatment significantly increased catalase concentration in heart tissue in a dose-dependent manner (Fig. 4). The interaction of endurance exercise and purslane seed was not significant ($F=10$, $p=0.001$, $\eta^2=0.652$) in rats after oxidative stress was induced by H_2O_2. ET significantly increased the SOD concentration in the lung tissue ($F=146.83$, $p=0.001$, $\eta^2=0.902$) (Fig. 4). Purslane seed also significantly increased the concentration of SOD in the lung tissue ($F=48.95$, $p=0.001$, $\eta^2=0.902$) in a dose-dependent manner (Fig. 4). The interaction of endurance exercise and purslane seed was also significant ($F=4.40$, $p=0.019$, $\eta^2=0.452$) in rats after oxidative stress was induced by H_2O_2.

Discussion

The findings of the present study highlighted the beneficial effects of 8 weeks of ET and the consumption of purslane seed on the oxidative stress markers in rats who had been exposed to H_2O_2 compared to pre-test value. The effect of exercise on human oxidative stress and its effect on skeletal muscle function have been the subject of several studies [32, 33]. Paradoxically, it is clear that H_2O_2 and superoxide radicals generated from contracting human skeletal and cardiac muscles during ET may cause oxidative damage to cellular constituents [8]. Amplified production of trigger peroxidation of muscle membrane lipids [34] disrupts the integrity of the sarcolemma and releases intramuscular creatine kinase into the blood [35]. The intensity of ET of greater than normal duration or intensity of the adaptation level of subjects had a greater effect on the induction of oxidative stress [36].

Regular physical activity has been shown to increase ROS-induced lipid peroxidation and oxidation protein resistance [37], while acute physical exercise has been shown to cause oxidative stress [38] on rats. The beneficial effects of endurance training on antioxidant defense mechanisms of different tissues have been proven in mice and rat [39, 40]. It also has been reported that CAT [38] and glutathione S-transferase activities [41] of the heart tissue do not change after ET or acute exercise in rats. Although some enzymes are unaltered, an increase in enzyme activity has been found in rats after acute treadmill training [42, 43]. A decrease in SOD activity in untrained rats and no change in SOD

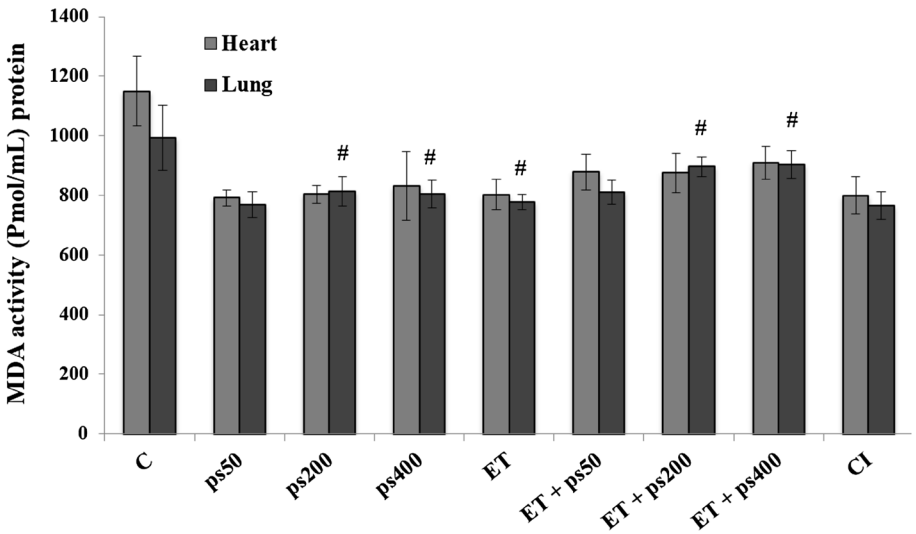

Fig. 3 MDA (Pmol/ml) in heart and lung tissues after 8 weeks of ET and purslane seed consumption after H_2O_2 toxicity induced in rats. Data were expressed as mean ± SEM. C: intoxicated control, ps50: 50 mg purslane seed, ps200: 200 mg purslane seed, ps400: 400 mg purslane seed, ET: ET, ET + ps50: ET + 50 mg purslane seed, ET + ps200: ET + 200 mg purslane seed, ET + ps400: ET + 400 mg purslane seed, CI: non-intoxicated control intact, $*p<0.05$ compared to intoxicated control group in heart tissue, $\#p<0.05$ compared to intoxicated control group in lung tissue

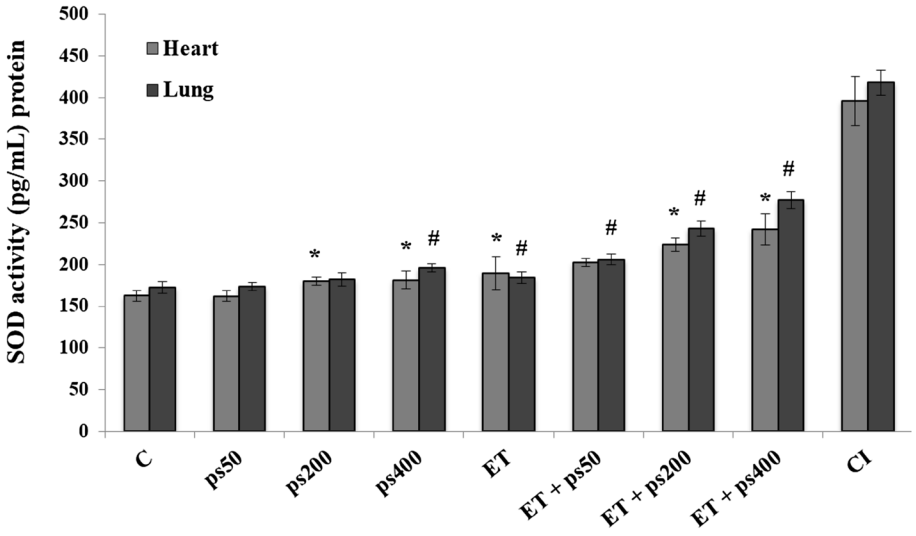

Fig. 4 SOD (Pg/ml) in heart and lung tissues after 8 weeks of ET and purslane seed consumption after H_2O_2 toxicity induced in rats. Data were expressed as mean ± SEM. C: control intoxicated, ps50: 50 mg purslane seed, ps200: 200 mg purslane seed, ps400: 400 mg purslane seed, ET: ET, ET + ps50: ET + 50 mg purslane seed, ET + ps200: ET + 200 mg purslane seed, ET + ps400: ET + 400 mg purslane seed, CI: non-intoxicated control intact. *$p < 0.05$ compared to intoxicated control group in heart tissue, #$p < 0.05$ compared to intoxicated control group in lung tissue

activity in trained rats have been observed after acute exercise and suggest a positive effect for acute exercise [38]. In addition, increased MDA, SOD and GPX levels have also been recorded at high physical fitness levels in healthy older adults [44]. In agreement with previous studies, regular ET in the current study improved antioxidant activity at 8 weeks after exposure to H_2O_2. An increase in physical training had a greater effect on the increase in oxidative stress markers than did weak physical training.

The most important finding of the present study was that oxidative stress markers developed upon consumption of purslane seeds to alleviate oxidative stress in rats with induced H_2O_2 toxicity compared to pre-test value. The beneficial effects may due to the presence of unsaturated fatty acids and beta-sitosterol, as identified by GC–MS analysis [20]. It has been proven that unsaturated fatty acids can improve the LDL level, glucose tolerance, lipid profile [45] and cholesterol synthesis [46], and increase insulin function [47]. Moreover, increased oxidative stress markers in the heart and lung tissues activate beta-sitosterol, a phytosterol, which is present in purslane seeds. High doses of purslane seed have been correlated with an increase in the enzymatic capacity of rat heart and lung tissues. The purslane plant has been demonstrated to be a rich source of essential biochemical and high amounts of trace minerals [48, 49] which have remarkable effects on body dysfunction.

Another important finding of the present study is the synergistic influence of exercise and purslane seed consumption, which was more pronounced for the pattern of release of all oxidative stress enzymes in the heart and lung tissues compared to pre-test value. A significant interaction between this herbal plant and physical activity was found in the present study. Purslane seed has the highest protein and fatty acid content of all five species when compared to cereals such as corn, wheat and barley [50]. This was the motivation behind the selection of this plant seed for consumption in conjunction with physical training to pharmacologically activate enzymes that fight oxidative stress. It is possible that enzymes released from both tissues in response to physical training may have eclipsed the consumption of purslane seed. Numerous dietary antioxidants, including vitamins C and E, and carotenoids, may have contributed to cellular protection against free radicals or ROS. The vitamins in this seed have direct antioxidant properties and promote chain-breaking antioxidants in cell membranes [51].

Conclusion

The enzyme activity levels in the heart and lung tissues of rats after induced H_2O_2 toxicity were compared with pre-test levels. Although the adaptive changes in antioxidant enzymes due to the endurance training were limited, the results of the present study clearly demonstrate that purslane seed consumption combined with 8 weeks of ET improved the activities of all enzymes in both tissues. These improvements reveal the potential role in both tissues for antioxidant defense. The results indicate that rat hearts and lungs had sufficient antioxidant enzyme capacity to defend against oxidative stress induced by H_2O_2 and may be able to protect and

defend themselves against free radical attacks. Although the mechanistic interpretation of our results is prevented by the use of human, it suggests that the consumption of an appropriate antioxidant alongside effective physical training may help human subjects to maintain better antioxidant defenses.

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Compliance with ethical standards

Conflict of interest None of the authors have financial or other conflicts of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed consent For this type of study, formal consent is not required.

References

1. Nita M, Grzybowski A (2016) The role of the reactive oxygen species and oxidative stress in the pathomechanism of the age-related ocular diseases and other pathologies of the anterior and posterior eye segments in adults. Oxid Med Cell Longev 2016:3164734
2. Borchi E, Bargelli V, Stillitano F, Giordano C, Sebastiani M, Nassi PA, d'Amati G, Cerbai E, Nediani C (2010) Enhanced ROS production by NADPH oxidase is correlated to changes in antioxidant enzyme activity in human heart failure. Biochim Biophys Acta 1802:331–338
3. Barber SC, Shaw PJ (2010) Oxidative stress in ALS: key role in motor neuron injury and therapeutic target. Free Radic Biol Med 48:629–641
4. Cachofeiro V, Goicochea M, De Vinuesa SG, Oubiña P, Lahera V, Luño J (2008) Oxidative stress and inflammation, a link between chronic kidney disease and cardiovascular disease: new strategies to prevent cardiovascular risk in chronic kidney disease. Kidney Int 74:S4–S9
5. Dieterich S, Bieligk U, Beulich K, Hasenfuss G, Prestle J (2000) Gene expression of antioxidative enzymes in the human heart: increased expression of catalase in the end-stage failing heart. Circulation 101:33–39
6. Kao MP, Ang DS, Pall A, Struthers AD (2010) Oxidative stress in renal dysfunction: mechanisms, clinical sequelae and therapeutic options. J Hum Hypertens 24:1–8
7. Gomez-Cabrera MC, Domenech E, Vina J (2008) Moderate exercise is an antioxidant: upregulation of antioxidant genes by training. Free Radic Biol Med 44:126–131
8. Powers SK, Nelson WB, Hudson MB (2011) Exercise-induced oxidative stress in humans: cause and consequences. Free Radic Biol Med 51:942–950
9. Radak Z, Chung HY, Koltai E, Taylor AW, Goto S (2008) Exercise, oxidative stress and hormesis. Ageing Res Rev 7:34–42
10. Rahimi R (2017) Effect of resistance exercise on oxidative DNA damage and lipid peroxidation in trained and untrained men. Sport Sci Health 13:225–232
11. Georgakouli K, Manthou E, Fatouros IG, Georgoulias P, Deli CK, Koutedakis Y, Theodorakis Y, Jamurtas AZ (2017) Enhanced erythrocyte antioxidant status following an 8-week aerobic exercise training program in heavy drinkers. Alcohol, Fayetteville
12. Holbrook NJ, Ikeyama S (2002) Age-related decline in cellular response to oxidative stress: links to growth factor signaling pathways with common defects. Biochem Pharmacol 64:999–1005
13. Sacheck JM, Milbury PE, Cannon JG, Roubenoff R, Blumberg JB (2003) Effect of vitamin E and eccentric exercise on selected biomarkers of oxidative stress in young and elderly men. Free Radic Biol Med 34:1575–1588
14. Hollander J, Fiebig R, Gore M, Ookawara T, Ohno H, Ji L (2001) Superoxide dismutase gene expression is activated by a single bout of exercise in rat skeletal muscle. Pflügers Archiv 442:426–434
15. Arikawa AY, Thomas W, Gross M, Smith A, Phipps WR, Kurzer MS, Schmitz KH (2013) Aerobic training reduces systemic oxidative stress in young women with elevated levels of F2-isoprostanes. Contemp Clin Trials 34:212–217
16. Miyazaki H, Oh-ishi S, Ookawara T, Kizaki T, Toshinai K, Ha S, Haga S, Ji LL, Ohno H (2001) Strenuous endurance training in humans reduces oxidative stress following exhausting exercise. Eur J Appl Physiol 84:1–6
17. Jackson MJ, McArdle A (2011) Age-related changes in skeletal muscle reactive oxygen species generation and adaptive responses to reactive oxygen species. J Physiol 589:2139–2145
18. Bouzid MA, Filaire E, McCall A, Fabre C (2015) Radical oxygen species, exercise and aging: an update. Sports Med 45:1245–1261
19. Dehghan F, Hajiaghaalipour F, Yusof A, Muniandy S, Hosseini SA, Heydari S, Salim LZA, Azarbayjani MA (2016) Saffron with resistance exercise improves diabetic parameters through the GLUT4/AMPK pathway in-vitro and in-vivo. Sci Rep 6:25139
20. Dehghan F, Soori R, Gholami K, Abolmaesoomi M, Yusof A, Muniandy S, Heidarzadeh S, Farzanegi P (2016) Purslane (*Portulaca oleracea*) seed consumption and aerobic training improves biomarkers associated with atherosclerosis in women with Type 2 diabetes (T2D). Sci Rep 6:37819
21. Lipinski B (2011) Hydroxyl radical and its scavengers in health and disease. Oxid Med Cell Longev 809696:9
22. Arshadi S, Azarbayjani MA, Hajaghaalipor F, Yusof A, Peeri M, Bakhtiyari S, Stannard RS, Osman NA, Dehghan F (2015) Evaluation of *Trigonella foenum-graecum* extract in combination with swimming exercise compared to glibenclamide consumption on type 2 diabetic rodents. Food Nutr Res 59:29717
23. Liu L, Howe P, Zhou YF, Xu ZQ, Hocart C, Zhan R (2000) Fatty acids and beta-carotene in australian purslane (*Portulaca oleracea*) varieties. J Chromatogr A 893:207–213
24. Uddin MK, Juraimi AS, Hossain MS, Nahar MA, Ali ME, Rahman MM (2014) Purslane weed (*Portulaca oleracea*): a prospective plant source of nutrition, omega-3 fatty acid, and antioxidant attributes. Sci World J 2014:951019
25. Jouybari MF, Farzanegi P, Barari AR (2014) The effect of 8-week aerobic exercise with purslane supplementation consumption on peroxidant and antioxidants indicators in women with type 2 diabetes. J Shahid Sadoughi Univ Med Sci 22:928–939
26. Salehi A, Farzanegi P (2015) Effect of 8 weeks of resistance training with and without Portulacalo seeds on some of liver injury markers in women with diabetes type 2. Urmia Med J 25:968–978
27. Esmaillzadeh A, Zakizadeh E, Faghihimani E, Gohari M, Jazayeri S (2015) The effect of purslane seeds on glycemic status and lipid profiles of persons with type 2 diabetes: A randomized controlled cross-over clinical trial. J Res Med Sci 20:47–53
28. Ahmed OM, Hozayen WG, Sree HTA (2015) Effects of ethanolic purslane shoot and seed extracts on doxorubicin-induced hepatotoxicity in albino rats, vol 2. World Academy of Science, Engineering, Istanbul
29. El-Azime ASA, Hussein EM, Ashry OM (2014) Synergestic effect of aqueous purslane (*Portulaca oleracea* L.) extract and fish oil on radiation-induced damage in rats. Int J Radiat Biol 90:1184–1190

30. Garcia J, Martinez-Ballarin E, Robinson M, Allue J, Reiter R, Osuna C, Acuna-Castroviejo D (2000) Protective effect of beta-carbolines and other antioxidants on lipid peroxidation due to hydrogen peroxide in rat brain homogenates. Neurosci Lett 294:1–4
31. Mallikarjuna K, Shanmugam KR, Nishanth K, Wu MC, Hou CW, Kuo CH, Reddy KS (2010) Alcohol-induced deterioration in primary antioxidant and glutathione family enzymes reversed by exercise training in the liver of old rats. Alcohol (Fayetteville NY) 44:523–529
32. Samjoo I, Safdar A, Hamadeh M, Raha S, Tarnopolsky M (2013) The effect of endurance exercise on both skeletal muscle and systemic oxidative stress in previously sedentary obese men. Nutr Diabetes 3:e88
33. Steinbacher P, Eckl P (2015) Impact of oxidative stress on exercising skeletal muscle. Biomolecules 5:356–377
34. Goldfarb AH (1999) Nutritional antioxidants as therapeutic and preventive modalities in exercise-induced muscle damage. Can J Appl Physiol 24:249–266
35. Pal S, Chaki B, Chattopadhyay S, Bandyopadhyay A (2017) High intensity exercise induced oxidative stress and skeletal muscle damage in post-pubertal boys and girls: a comparative study. J Strength Cond Res 32:1045–1052
36. Bogdanis GC (2012) Effects of physical activity and inactivity on muscle fatigue. Front Physiol 3:142
37. Radak Z, Sasvari M, Nyakas C, Kaneko T, Tahara S, Ohno H, Goto S (2001) Single bout of exercise eliminates the immobilization-induced oxidative stress in rat brain. Neurochem Int 39:33–38
38. Gul M, Demircan B, Taysi S, Oztasan N, Gumustekin K, Siktar E, Polat MF, Akar S, Akcay F, Dane S (2006) Effects of endurance training and acute exhaustive exercise on antioxidant defense mechanisms in rat heart. Comp Biochem Physiol Part A Mol Integr Physiol 143:239–245
39. Kanter MM, Hamlin RL, Unverferth DV, Davis HW, Merola AJ (1985) Effect of exercise training on antioxidant enzymes and cardiotoxicity of doxorubicin. J Appl Physiol (Bethesda, Md: 1985) 59:1298–1303
40. Venditti P, Di Meo S (1996) Antioxidants, tissue damage, and endurance in trained and untrained young male rats. Arch Biochem Biophys 331:63–68
41. Gül M, Atalay M, Hänninen O (2003) Endurance training and glutathione-dependent antioxidant defense mechanism in heart of the diabetic rats. J Sports Sci Med 2:52
42. Somani S, Frank S, Rybak L (1995) Responses of antioxidant system to acute and trained exercise in rat heart subcellular fractions. Pharmacol Biochem Behav 51:627–634
43. Terblanche S (1999) The effects of exhaustive exercise on the activity levels of catalase in various tissues of male and female rats. Cell Biol Int 23:749–753
44. Bouzid MA, Hammouda O, Matran R, Robin S, Fabre C (2015) Influence of physical fitness on antioxidant activity and malondialdehyde level in healthy older adults. Appl Physiol Nutr Metab 40:582–589
45. Lu J, Borthwick F, Hassanali Z, Wang Y, Mangat R, Ruth M, Shi D, Jaeschke A, Russell JC, Field CJ, Proctor SD, Vine DF (2011) Chronic dietary n-3 PUFA intervention improves dyslipidaemia and subsequent cardiovascular complications in the JCR:LA-cp rat model of the metabolic syndrome. Br J Nutr 105:1572–1582
46. Nestel PJ (2000) Fish oil and cardiovascular disease: lipids and arterial function. Am J Clin Nutr 71:228S–231S
47. Oh JY (2010) Serum cystatin C as a biomarker for predicting coronary artery disease in diabetes. Korean Diabetes J 34:84–85
48. Jimoh F, Oladiji A (2005) Preliminary studies on *Piliostigma thonningii* seeds: proximate analysis, mineral composition and phytochemical screening. Afr J Biotechnol 4(12):1439–1442
49. Prakash D, Pal M (1991) Nutritional and antinutritional composition of vegetable and grain amaranth leaves. J Sci Food Agric 57:573–583
50. Miller T, Wing J, Huete A (1984) The agricultural potential of selected C4 plants in arid environments. J Arid Environ 7(3):275–286
51. Powers SK, Jackson MJ (2008) Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. Physiol Rev 88:1243–1276