



Original Article

Effects of Aerobic Exercise and Ethanolic Extract of Purslane Seed on Markers of Oxidative Stress and DNA Damage in Cardiac Tissue of Rats Poisoned with Hydrogen Peroxide

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ABSTRACT

Background and objectives: Oxidative stress is the most important pathophysiological cause of diseases. Studies have shown that exercise and supplementation with medicinal plants have limited side effects. The aim of the present study was to evaluate effects of aerobic exercise and ethanolic extract of purslane seed on ATP, 0-6-Methylguanine DNA methyltransferase (MGMT), malondialdehyde (MDA) and prooxidant-antioxidant balance (PAB) levels in the heart tissue of rats poisoned with hydrogen peroxide.

Methods: In this experimental trial, 72 male Wistar rats were randomly divided into nine groups: (1) control + H₂O₂, (2) aerobic exercise, (3) aerobic exercise and 50 mg/kg purslane seed extract, (4) aerobic exercise and 200 mg/kg purslane seed extract, (5) aerobic exercise and 400 mg/kg purslane seed extract, (6) 50 mg/kg purslane seed extract, (7) 200 mg/kg purslane seed extract, (8) 400 mg/kg purslane seed extract, and (9) healthy control. Oxidative stress was induced by intraperitoneal injection of 1 mmol/kg hydrogen peroxide three times a week for eight weeks. Aerobic exercise was performed three sessions a week for eight weeks, and the purslane seed extract was intraperitoneally injected daily at the mentioned doses.

Results: Aerobic exercise and purslane seed extract alone or combined significantly increased ATP, MGMT and significantly reduced MDA and PAB levels in cardiac tissue of rats exposed to hydrogen peroxide ($P < 0.05$). Moreover, the effect of purslane seed extract was dose dependent.

Conclusion: It seems that aerobic exercise and purslane seed extract supplementation have synergistic cardioprotective effects under oxidative stress.

Keywords: Exercise, Purslane Seed, MGMT, ATP, MDA, PAB, Heart.

INTRODUCTION

Heart disease is one of the leading causes of mortality in the world (1). Studies have shown that oxidative stress, inflammation and cell death are the most important pathophysiological causes of diseases, such as heart failure and cardiomyopathy (2). It has been shown that increase in oxidative stress in turn raises the level of reactive oxygen species (ROS) in the heart tissue (1–3). Generation of energy in the mitochondria from electron transfer chain pathway is associated with H⁺ production, which increases the ROS levels, thereby increasing the pro-oxidant-antioxidant balance (PAB) protein and oxidative stress. This ultimately causes dysfunction in proton carriers, DNA damage and cell death (4–6).

The overexpression of O-6-methylguanine DNA methyltransferase (MGMT) that has a high affinity to free electrons, could indicate a decrease in O₂⁻ levels (7,8). Studies have shown that under oxidative stress conditions, exercise reduces malondialdehyde (MDA) and improves antioxidant capacity and performance of electron transport chain proteins (8–10). In addition to exercise, the use of medicinal plants has been widely examined for this purpose mainly due to their limited side effects. *Portulaca oleracea* from the *Oleracea* family is a medicinal plant with favorable components including omega-3 fatty acids, alpha-linolenic acid, flavonoids, coumarins and betalin, which could protect cells against free radicals and lipid peroxidation. According to some studies, this plant also has anti-atherosclerotic effects (11,12). It is believed that the antioxidant-effects of this medicinal plant can reduce left ventricular blood pressure, MDA (13,14) and inflammatory factors (15). On the other hand, the simultaneous use of medicinal plants along with exercise activities have been suggested as a complementary therapy for treatment of heart disease (11).

Although the results of most studies on the effect of exercise on oxidative stress markers are contradictory (6,8,9,11), it has been well-established that combination therapy with exercise and healthy diet can improve oxidative stress. In the present study, we investigate effects of aerobic exercise and *P. oleracea* (purslane) seed extract supplementation on mitochondrial function and oxidative stress markers in the heart tissue of H₂O₂-poisoned rats.

MATERIAL AND METHODS

In this clinical trial, 72 male Wistar rats (weighting 180 to 200 g) were purchased from Pasteur Institute of Iran. The animals were housed in the laboratory of the University of Tehran for one week to adapt to the environment. All animals were treated in accordance with the ethical codes of working with laboratory animals. The animals were kept in standard temperature (20 to 24 °C) and under 12-hour dark and 12-hour light cycles, with free access to food and water.

First, the rats were randomly divided into nine groups of eight including (1) control+H₂O₂ (C+H₂O₂), (2) aerobic exercise (EX+H₂O₂), (3) aerobic exercise and 50 mg/kg purslane seed extract (EX+PS50+H₂O₂), (4) aerobic exercise and 200 mg/kg purslane seed extract (EX+PS200+H₂O₂), (5) aerobic exercise and 400 mg/kg purslane seed extract (EX+PS400+H₂O₂), (6) 50 mg/kg purslane seed extract (PS50+H₂O₂), (7) 200 mg/kg purslane seed extract (PS200+H₂O₂), (8) 400 mg/kg purslane seed extract (PS400+H₂O₂), and (9) control (C) groups.

Rats in the experimental groups received peritoneal injection of 1mmol/kg H₂O₂ for eight weeks, three times a week (16). Groups 2 to 5 performed aerobic exercise three sessions a week for eight weeks, and rats receiving purslane seed extract were given intraperitoneal injections on a daily basis. The control group was considered to investigate the effects of H₂O₂ on the research variables in the heart tissue and received intraperitoneal normal saline injections.

Dry purslane seeds were obtained from the Research Institute of Medicinal Plants. The seeds were powdered with an electric mill and then steeped in 80% ethanol at 1:10 ratio in two one-hour steps. The mixture was then passed through a 0.2 mm paper filter. The remaining material was placed in the percolation device to evaporate ethanol (11) and finally diluted with normal saline for injection to rats.

The exercise groups 2-5 performed daily aerobic exercise on the treadmill for eight weeks. Rats were trained on the treadmill at speed of 8 m/min and 10° slope for 30 minutes in the first week, at 12 m/min with the same slope and duration in the second week, at 16 m/min with the same slope for 45 minutes in the third week, and at 20 m/min with the same slope for 45 minutes in the fourth week. From

the fifth week until the end of the study period, the rats were trained at 20 m/min and 10° slope for 60 minutes (17).

Forty eight hours after the last exercise session and after 10-12 hours of fasting, biopsy was performed. First, the rats were anesthetized by intraperitoneal injection of ketamine (90 mg/kg) and xylazine (10 mg/kg). After cleaving the thoracic cavity, the heart tissue was carefully separated, washed with distilled water and weighed. Then, it was immediately placed at -70 °C until measurement of research variables. All sampling procedures began at 8:00 and completed at 11:30. It should be noted that all rats were sacrificed as quickly as possible with minimum pain.

The levels of ATP (Cat NO: KA1661; ABNOVA; Germany), MGMT (Cat NO: DL-MGMT-Ra; DEVELOP; China) and MDA (Cat NO: CSB-E08558r; CASOBIO; China) were measured using commercial ELISA kits. The Guimarães-Ferreira paper was used for measuring PAB (18).

Data were reported as mean and standard deviation. Normal distribution of data was assessed using the Shapiro-Wilk test. Independent sample t-test was used to determine the effect of H₂O₂ on the C and C+H₂O₂ groups. The main effect of exercise, purslane seed extract and their combination were assessed using two-way analysis of

variance and the Bonferroni's post hoc test. All statistical analyses were performed in SPSS (version 19) and at significance level of 0.05.

RESULTS

The cardiac level of ATP ($P=0.0001$, $t_{18}=32.760$) and MGMT ($P=0.0001$, $t_{18}=13.716$) in the C group were significantly higher than in the C+H₂O₂ group. Also, the cardiac level of MDA ($P=0.0001$, $t_{18}=54.833$) and PAB ($P=0.0001$, $t_{18}=32.834$) in the C group was significantly lower than in the C+H₂O₂ group. Aerobic exercise ($F_{1,72}=37.948$, $P=0.0001$, $\eta=0.345$) and purslane seed extract ($F_{1,72}=147.610$, $P=0.0001$, $\eta=0.860$) each significantly increased cardiac ATP concentration in the H₂O₂-poisoned rats. Combination of aerobic exercise and purslane seed extract supplementation also significantly increased cardiac ATP level in the H₂O₂-poisoned rats ($F_{3,72}=16.35$, $P=0.0001$, $\eta=0.405$). According to the Bonferroni's post hoc test, the effect of purslane seed extract on ATP level was dose dependent. Compared to the control group, supplementation with purslane seed extract significantly increased cardiac ATP levels at doses of 200 ($P=0.0001$) and 400 mg/kg ($P=0.0001$) but not at 50 mg/kg ($P=0.99$). This effect was more profound at dose of 400 mg/kg compared to 200 mg/kg ($P=0.0001$) (Figure 1).

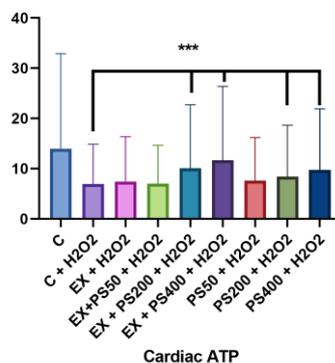


Figure 1- ATP concentration in the heart tissue of H₂O₂-poisoned rats in different study groups.

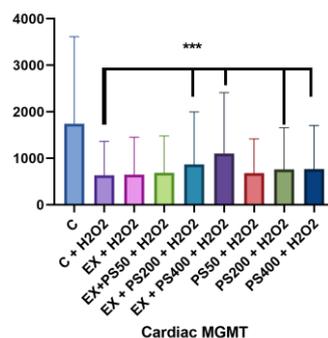


Figure 2- MGMT concentration in the heart tissue of H₂O₂-poisoned rats in different study groups

Aerobic exercise ($F_{1,72}=77.957$, $P=0.0001$, $\eta=0.520$) and purslane seed extract ($F_{3,72}=85.994$, $P=0.0001$, $\eta=0.782$) significantly increased cardiac MGMT concentrations in the H_2O_2 -poisoned rats. Combination of aerobic exercise and purslane seed extract also significantly increased cardiac MGMT concentration in the H_2O_2 -poisoned rats ($F_{3,72}=21.143$, $P=0.0001$, $\eta=0.468$). Compared to the control group, purslane seed extract significantly increased cardiac MGMT concentrations at doses of 200 mg/kg ($P=0.0001$) and 400 mg/kg ($P=0.0001$) but not at 50 mg/kg ($P=0.99$). This effect was more profound at 400 mg/kg compared to 200 mg/kg purslane seed extract ($P=0.0001$) (Figure 2).

Aerobic exercise ($F_{1,72}=606.307$, $P=0.0001$, $\eta=0.894$), purslane seed extract ($F_{3,72}=750.344$, $P=0.0001$, $\eta=0.969$) and their combination ($F_{3,72}=180.794$, $P=0.0001$, $\eta=0.883$)

significantly reduced cardiac MDA concentration in the H_2O_2 -poisoned rats. Compared to the control group, supplementation with purslane seed extract could significantly reduce ($P=0.0001$) MDA concentration at all tested doses with the highest effect observed at 400 mg/kg (Figure 3).

Aerobic exercise ($F_{1,72}=665.628$, $P=0.0001$, $\eta=0.902$), purslane seed extract ($F_{3,72}=407.634$, $P=0.0001$, $\eta=0.944$) and their combination ($F_{3,72}=60.373$, $P=0.0001$, $\eta=0.716$) significantly reduced PAB concentration in the cardiac tissue of H_2O_2 -poisoned rats. The effect of purslane seed on the reduction of cardiac PAB was dose dependent. Consumption of purslane seeds at 200 mg/kg ($p=0.0001$) and 400 mg/kg ($p=0.0001$) resulted in decreased cardiac PAB. Also, a dose of 400 mg/kg had a greater effect on cardiac PAB reduction than 200 mg/kg (Figure 4).

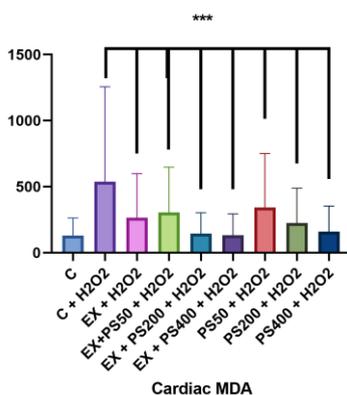


Figure 3- MDA concentration in the heart tissue of H_2O_2 -poisoned rats in different study groups

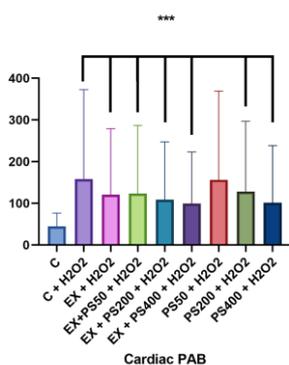


Figure 4-PAB concentration in the heart tissue of H_2O_2 -poisoned rats in different study groups

DISCUSSION

In our study, exposure to H₂O₂ caused a significant reduction in cardiac ATP and MGMT levels as well as a significant increase in cardiac MDA and PAB levels of rats. The heart tissue carries out the most oxidative activity with a large number of mitochondria compared to other tissues. Therefore, most cardiac tissue damages such as pathological hypertrophy and myocardial infarction are associated with increased ROS levels (19). Previous studies have indicated that increased ROS is associated with alterations in biological macromolecules including lipids, proteins and DNA. With the oxidation of cell membranes and proteins, MDA, lipid hydroxy peroxides, isoprostanes and thiobarbituric acid reactive substances increase, which in turn increase PAB and carbonyl protein but decrease MGMT, which ultimately leads to cell death (6,19,20). In the present study, aerobic exercise caused a significant increase in ATP and MGMT as well as a significant decrease in MDA and PAB levels in the cardiac tissue of rats exposed to H₂O₂. Studies showed that regular and long-term exercise can increase the expression of cellular antioxidants (8,21,22). In other words, upregulation of factors involved in antioxidant capacity following aerobic exercise is a protective mechanism against DNA damage caused by increased ROS levels. Researchers believe that MGMT as a marker for enhancing DNA regeneration ability, is directly linked to increased total antioxidant capacity, mitochondrial superoxide dismutase, protein kinase and catalase (21).

In the present study, aerobic exercise significantly decreased the cardiac level of MDA and PAB, which could contribute to DNA repair. In line with our findings, a previous study showed that eight weeks of endurance exercise with treadmill can improve total antioxidant capacity as well as CAT and reduce MDA level in muscle tissue of rats (21). However, another study reported that eight weeks of running wheel exercise did not increase antioxidant profiles in young adult rats (22). This inconsistency could be related to the higher intensity of training in our study compared to the mentioned study. A previous study indicated that 20 weeks of running wheel had significant effects on expression of antioxidants and DNA repair capacity in

young adult rats (22). It seems that the duration of training period and type of training as well as the method of measuring variables should be considered as important factors when analyzing the results.

In our study, supplementation with purslane seed extract significantly increased the concentration of ATP and MGMT and decreased cardiac MDA and PAB levels in rats poisoned with H₂O₂ in a dose-dependent manner. It has been shown that consumption of purslane seed extract can decrease MDA and improve TBARS, glutathione reductase, superoxide dismutase and lipid profile in cardiovascular patients (23). For example, 100 µg/ml of hydroalcoholic extract of purslane seed had anti-inflammatory and antioxidant effects on human blood cells (24). In line with our results, a previous study reported that supplementation with 100, 200 and 400 mg/kg of hydroalcoholic extract of purslane seed reduced MDA and left ventricular developed pressure in rats with hyperthyroidism in a dose-dependent manner (14). However, a previous study reported that consumption of 10 g/day purslane seed had no significant effect on serum antioxidant and MDA in diabetic rats (23). The difference in the study findings could be due to the prescribed doses of purslane seed.

In our study, combination of aerobic exercise and purslane seed extract supplementation significantly increased ATP and MGMT and significantly decreased MDA and PAB in cardiac tissue of rats exposed to H₂O₂. It seems that both interventions exert their favorable effects through similar pathways such as increased total antioxidant capacity, mitochondrial superoxide dismutase, protein kinase, catalase and reduced oxidative stress (6,11,21-24). In a previous study, consumption of purslane seed extract combined with aerobic exercise significantly reduced inflammatory factors in the heart tissue of rats poisoned with H₂O₂, while higher doses of purslane seed extract had more favorable effects on reducing inflammatory factors (13).

Consistent with our results, it has been demonstrated that 7.5 g/day purslane seed extract along with aerobic exercise at 50-70% of heart rate reserve could reduce cardiac damage markers in diabetic (24) and middle-aged women (25).

CONCLUSION

The results of this study show that exercise and purslane seed extract supplementation have synergistic cardioprotective effects under oxidative stress condition induced by hydrogen peroxide.

In addition, the protective effect of purslane is dose dependent.

It is recommended to conduct further studies in this regard while measuring other variables

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including aortic blood pressure and electrocardiogram.

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CONFLICT OF INTEREST

All authors declare that there is no conflict of interest.

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