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# The Effects of Endurance Training and Purslane (Portulaca oleracea) Seed Consumption on Cytochrome-C and Malondialdehyde in the Heart Tissues of Rats Poisoned with H2O2

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**ABSTRACT**

**Introduction:** Oxygenated water intake could increase cell death markers through increasing the free radicals. However, sport activities and antioxidant substances may prevent some of the symptoms caused by free radical production. The present study aimed to investigate the effects of endurance training (ET) and purslane (Portulaca oleracea; PO) seed consumption on cytochrome-C and malondialdehyde (MDA) in the heart tissues of rats poisoned with H2O2.

**Methods:** In total, 45 male rats were randomly divided into nine groups of five, including control, 50 mg/kg of PO, 200 mg/kg of PO, 400 mg/kg of PO, ET, ET with 50 mg/kg of PO, ET with 200 mg/kg of PO, ET with 400 mg/kg of PO, and healthy control. During eight weeks, groups 1-8 received H2O2 (1 mmol/kg) intraperitoneally three times per week, and groups 5-8 ran on treadmill three days per week.

**Results:** ET and PO significantly reduced cytochrome-C and MDA (P=0.001), while the interactive effects of ET and PO on the reduction of cytochrome-C (P=0.52) and MDA (P=0.08) were not considered significant. In addition, the administration of 200 mg/kg (P=0.01) and 400 mg/kg of PO (P=0.001) significantly decreased cytochrome-C, while 400 mg/kg of PO had more significant effects on the reduction of cytochrome-C compared to 200 mg/kg of the substance (P=0.01). Moreover, 400 mg/kg of PO significantly reduced MDA (P=0.001).

**Conclusion:** According to the results, ET and PO could improve cytochrome-C and MDA in the heart tissues of the rats poisoned with H2O2.

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## Introduction

Cardiovascular diseases are significant health concerns across the world, which are predicted to account for over 40% of deaths by 2020 (1). Apoptosis is considered to be a major cause of cardiovascular disease, especially heart failure (2). This process plays a key role in the regulation of the balance between cell generation and cell death in various tissues, especially somatic tissues such as the heart muscle. Apoptosis initiates with the fragmentation of chromatins and densification of the cell cytoplasm and terminated by the crimping of the nucleus and cell membranes and production of vacuoles containing apoptotic particles (3).

The imbalance between the production of free radicals and antioxidant defense system is referred to as oxidative stress (4). Free radicals are found in several environmental sources, including photochemical air pollution, electromagnetic/particle radiation, tobacco smoke, and drugs (5). Oxidative stress occurs when the balance between peroxidants and antioxidants is shifted in favor of peroxidants (6). Despite the beneficial health effects of physical activity, oxidative stress is caused by the increased production of reactive oxygen species (ROS) during physical exercise due to increased oxygen consumption and metabolism (7).

Under normal circumstances, free radicals are the byproducts of the metabolism of oxygen in

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the body, which may cause damage to cell membranes and react with the genetic materials that may give rise to numerous diseases (8). Reactive free radicals affect several important cellular components (e.g., DNA, proteins, and membrane lipids), thereby leading to tissue damage. The damage caused by physical exercise is gradual and largely depends on the intensity, timing, and duration of the activity (9). The production of free radicals during physical activity is involved in the development of muscle injury and spread of inflammation after exercise, which in turn increase cellular damage. Endurance training (ET) prevents the manifestation of some of the symptoms induced by free radical production, while the damage caused by free radicals could improve the tissue antioxidant defense through enhancing the activity of antioxidant substances (10). The antioxidant system comprises of a set of biological antioxidants and antioxidant enzymes, which neutralize ROS *in-vivo*, including endogenous enzymatic antioxidants (e.g., superoxide dismutase [SOD] and catalase [CAT]). Among ROS, the hydroxyl radical group could cause fat peroxidation, some of the products of which may be variable concentrations of cytochrome-C and malondialdehyde (MDA), which are considered to be the indicators of oxidative stress.

Dietary factors are believed to play a critical role in the development of various human diseases, such as cardiac and metabolic disorders, atherosclerosis, hyperlipidemia, thrombosis, diabetes, and hypertension (11). In this regard, special attention has been paid to *Portulaca oleracea* (PO) (12), also known as the purslane plant, which belongs to the Portulacaceae family. PO is an annual plant with a succulent stem, reciprocating leaves, and small yellow flowers. This plant grows extensively in Iran and is used as an oral and traditional medicine (12).

PO has notable antiseptic, antispasmodic, diuretic, antioxidant, blood purifying, analgesic, and anti-inflammatory properties, while exhibiting protective functions against heart attack and strengthening the immune system (13). Furthermore, PO is an abundant source of various antioxidants, such as vitamins A, B1, C, and E, beta-carotene, and other essential amino acids (14). To date, several studies have been focused on the protective effects of PO. For instance, Ahangarpour et al. (2016) reported that administrating 200 mg/kg of PO for three weeks could attenuate the aging alternations induced by D-gal, as well as aging in the female reproductive system (15). In addition, Khodadadi et al. (2018) have claimed that 100, 200, and 400 mg/kg of PO could exert protective effects on cardiac dysfunction caused by subclinical hyperthyroidism induced by levothyroxine sodium in rats (16). Hozayen et al. (2011) have also concluded that aqueous PO extract could improve the adverse changes in renal function through increasing antioxidant activities and reducing peroxidation (17).

Considering the lack of observational studies regarding the simultaneous effects of ET and PO seed consumption on cytochrome-C and MDA in $H_2O_2$ poisoning, the present study aimed to investigate the effects of ET and PO consumption on cytochrome-C and MDA in the heart tissues of rats poisoned with $H_2O_2$.

## Materials and Methods

This experimental study was conducted on 45 male Wistar rats aged eight weeks, which were purchased and transferred to the laboratory. The sample size was determined based on a previous study in this regard (8, 9). The rats were preserved in standard conditions for one week to adapt to the new environment. In terms of ethical considerations, the researchers received the required introduction letters from the Islamic Azad University, Central Tehran Branch in Tehran, Iran (ethics code: 10121404981020).

### *Grouping*

On the eighth day, the rats were randomly divided into nine groups of five, including control, 50 mg/kg of PO, 200 mg/kg of PO, 400 mg/kg of PO, ET, ET with 50 mg/kg of PO, ET with 200 mg/kg of PO, ET with 400 mg/kg of PO, and healthy control. For eight weeks, groups 1-8 were administered with $H_2O_2$ (1 mmol/kg) intraperitoneally three times per week (18), groups two, three, four, and 6–8 received the PO daily at fixed doses via gastric intubation (19), and groups 5-8 ran on a treadmill three days per week (20).

### *ET Protocol*

To preform ET, the rats ran on a treadmill in the first week at the speed of 8 m/min and slope of 10 degrees for 30 minutes. In the second week, the speed was 12 m/min with the same slope and time, and in the third week, the speed was

set at 16 m/min at the same slope for 45 minutes. In the fourth week, the running speed was 20 m/min at the same slope for 45 minutes, and in weeks 5-8, the rats were trained at the speed of 20 m/min with the slope of 10 degrees for 60 minutes per day (20).

### *PO Extract Preparation*

The aqueous extract of PO was prepared in this study. After the preparation and grinding of PO, and 10 milliliters of saline per each gram of the powder was added to the beaker and boiled for 20 minutes. After cooling, the extract was filtered through a clean cloth, followed by Buchner's filter paper and funnel. At the next stage, the extract was heated again to concentrate until high viscosity was achieved. The final extract was transferred to several plates and incubated at the temperature of 70°C until completely dried. Afterwards, 500 milligrams of the dry substance was dissolved in 50 milliliters of saline, and 50, 200, and 400 mg/kg of the solution was injected to the rats (19).

### *Tissue Sampling*

Forty eight hours after the last ET session and PO consumption, the rats were anesthetized with 10% ketamine (50 mg/kg) and 2% xylazine (10 mg/kg) after approximately five minutes. Following that, the heart tissues of the animals were extracted by specialists, and after setting in a cryotube, they were placed in liquid nitrogen and stored at the temperature of -70ºC for further investigation. Cytochrome-C and MDA were measured using the ELISA assay and CUSABIO laboratory kits (catalogue No. CSB-E14281r and CSB-E08558r), respectively.

### *Statistical Analysis*

Data analysis was performed using independent-samples t-test, two-way analysis of variance (ANOVA), and Bonferroni post-hoc test (P≤0.05).

## Results

Figures 1 and 2 depict the levels of cytochrome-C and MDA in the nine research groups. The results of independent-samples t-test indicated that the levels of cytochrome-C (P=0.001) and MDA (P=0.001) significantly increased in the control group compared to the healthy control group. In addition, the results of two-way ANOVA (Table 1) showed that ET (P=0.001) and PO consumption (P=0.001) significantly reduced cytochrome-C. However, the interactive effects of ET and PO consumption on the reduction of cytochrome-C were not considered significant (P=0.52).

According to the results of Bonferroni post-hoc test (Table 2), 50 mg/kg of PO had no significant effect on the reduction of cytochrome-C (P=0.99), while 200 mg/kg (P=0.01) and 400 mg/kg of PO (P=0.001) significantly reduced cytochrome-C. Moreover, 400 mg/kg of PO had a more significant effect on the reduction of cytochrome-C compared to 200 mg/kg of PO (P=0.01).

The results of two-way ANOVA (Table 1) indicated that ET (P=0.001) and PO (P=0.001) could significantly reduce MDA, while the interactive effects of ET and PO on the reduction of MDA were not considered significant (P=0.39). Furthermore, the results of Bonferroni post-hoc test (Table 2) showed that 50 mg/kg (P=0.99) and 200 mg/kg of PO (P=0.42) had no significant effects on MDA, while 400 mg/kg of PO could significantly reduce MDA (P=0.001).

**Table 1.** Results of Two-way ANOVA on Effects of ET and PO on Cytochrome-C and MDA Levels

| Variable | Source | Sum of Squares | Mean Square | F | P-value | Partial Eta Squared |
|---|---|---|---|---|---|---|
| Cytochrome- C | ET | 17.85 | 17.85 | 369.63 | 0.001 | 0.92 |
| | PO | 2.29 | 0.76 | 15.86 | 0.001 | 0.59 |
| | Interaction of ET and PO | 0.11 | 0.03 | 0.76 | 0.52 | 0.06 |
| MDA | ET | 891007.27 | 891007.27 | 318.98 | 0.001 | 0.90 |
| | PO | 169913.56 | 56637.85 | 20.27 | 0.001 | 0.65 |
| | Interaction of ET and PO | 8528.85 | 2842.95 | 1.01 | 0.39 | 0.08 |

*ET: endurance training; PO: Portulaca oleracea*

**Table 2.** Results of Bonferroni Post-hoc Test on Effects of PO Seeds (50, 200, and 400 mg/kg) on Cytochrome-C and MDA

| Variable | Factor | 200 mg/kg | 400 mg/kg | No Consumption |
|---|---|---|---|---|
| Cytochrome- C | 50 mg/kg | M=0.19<br>P=0.34 | M=0.50<br>P=0.001 | M=-0.13<br>P=0.99 |
| | 200 mg/kg | ------- | M=0.31<br>P=0.01 | M=-0.32<br>P=0.01 |
| | 400 mg/kg | ------- | ------- | M=-0.63<br>P=0.001 |

| | | | | |
|---|---|---|---|---|
| MDA | 50 mg/kg | M=21.59<br>P=0.99 | M=145.80<br>P=0.001 | M=-22.58<br>P=0.99 |
| | 200 mg/kg | ------- | M=124.20<br>P=0.001 | M=-44.17<br>P=0.42 |
| | 400 mg/kg | ------- | ------- | M=-168.38<br>P=0.001 |

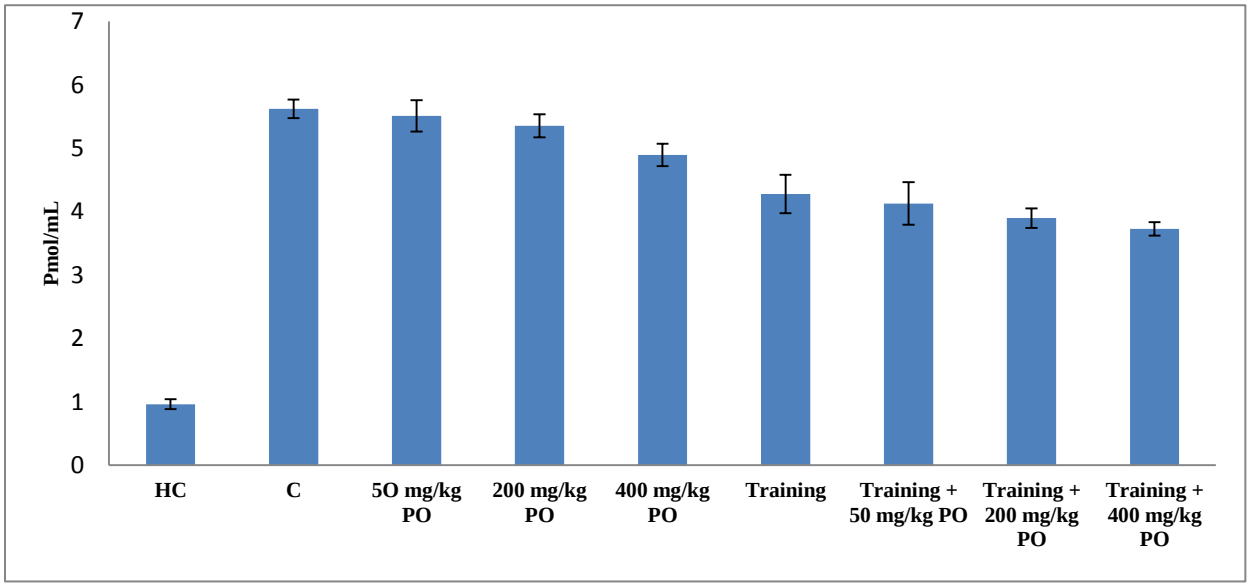


**Figure 1.** Cytochrome-C Level in Heart Tissues of Rats in nine Study Groups
(HC: healthy control, C: control, PO: *Portulaca oleracea*)

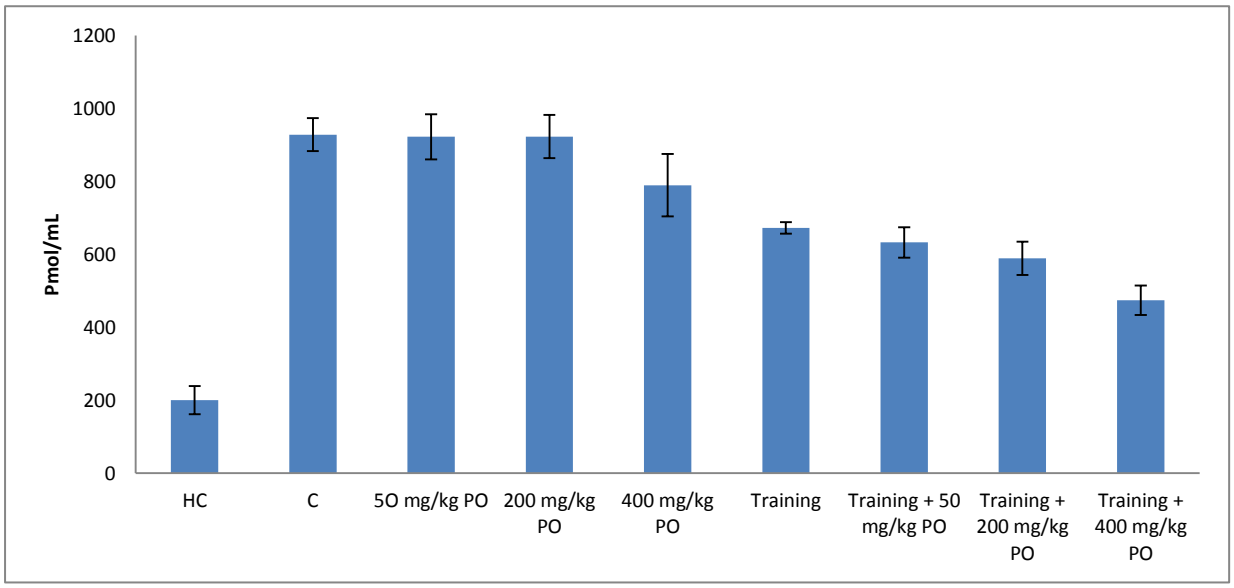


**Figure 2.** MDA Level in Heart Tissues of Rats in nine Study Groups

## Discussion

According to the results of the present study, $H_2O_2$ poisoning significantly increased cytochrome-C and MDA in the heart tissue of the rats, while eight weeks of ET significantly reduced cytochrome-C and MDA in the heart tissue of the $H_2O_2$-poisoned rats. In this regard, Balcı et al. (2012) examined the effects of gender, ET, and acute exhaustion exercise on oxidative stress in the cardiac and skeletal muscles of rats, reporting that gender was a determinant of the changes in the MDA, nitric oxide (NO), and glutathione levels in the heart

tissues and skeletal muscles following exhaustive exercise or ET (8).

In another research, Ashrafi et al. examined the antioxidant protective effects of ET on the heart tissues of Wistar rats, claiming that ET significantly increased the levels of apelin, NO, and SOD, while slightly decreasing MDA (9). Moreover, Fakoory et al. investigated the effects of eight weeks of aerobic training on peroxidant and antioxidant indices in women with type II diabetes, reporting that after eight weeks of aerobic training, MDA levels decreased, while SOD and CAT levels significantly increased (4).

Most of the studies in this regard have confirmed the positive effects of sports activities on the improvement of cytochrome-C and MDA. Furthermore, the intensity of these activities is considered to be a significant influential factor in the changes in antioxidant enzyme, and most findings have demonstrated the anti-oxidative effects of physical exercise (21). With respect to the mechanism of the antioxidant effects of physical exercise, it has been suggested that exercise may influence oxidative stress processes through several mechanisms, including oxygen sitting at the electron transfer chain, prostanoid metabolism, xanthine oxidase and macrophage activity, and increased catecholamine activity (21).

According to the findings of the current research, eight weeks of PO consumption significantly reduced cytochrome-C and MDA in the heart tissues of the rats poisoned with $H_2O_2$. Consistent with our findings, eight weeks of PO consumption was reported to significantly decrease MDA and increase SOD and CAT in women with type II diabetes (4). In addition, the results obtained by Wang et al. (2010) indicated that PO had a significant effect on the reduction of MDA (22).

PO contains high levels of alpha-linoleic acid, beta-carotene, flavonoids, coumarin monoterpene and alkaloid glycosides, antioxidants, and omega-3. Plants containing omega-3 and omega-6 fats could inhibit lipid peroxidation by breaking down the existing oxidizing structure using cytochrome P450 and neutralizing free radicals, the effects of which are associated with these compounds in plants, especially omega-3 and linoleic acid (23).

According to the present study, 400 mg/kg of PO could significantly reduce MDA, while the concentrations of 200 and 400 mg/kg significantly reduced cytochrome-C. In addition, 400 mg/kg of PO had more significant effects on the reduction of cytochrome-C compared to the concentration of 200 mg/kg. Therefore, it could be concluded that the effects of PO on cytochrome-C and MDA were dose-dependent, and the effects significantly increased at higher doses of PO. Previous findings have also denoted that the phenolic compounds in the PO seed could inhibit hydrogen peroxide peroxidation activities in fatty acids, thereby decreasing MDA. Beta-cyanine is an effective compound in PO, which reduces oxidative stress. Several reports have confirmed the antioxidant effects of this plant and its inhibiting properties against lipid peroxidation and membrane vulnerability to free radicals (24).

Regarding the effects of PO on cytochrome- C, Zheng et al. suggested that Portulacerebroside A (PCA), which is a novel cerebroside isolated from PO, could reduce the viability of human liver cancer HCCLM3 cells. Additionally, PCA has been reported to markedly elevate the percentage of apoptotic cells, phosphorylation of *p38* mitogen-activated protein kinase and c-Jun N-terminal kinases, release of mitochondrial cytochrome-C and apoptosis inducing factor to the cytosol, and activation of caspase-9 and caspase-3. Furthermore, previous findings have emphasized that PO could be a proper candidate for cancer treatment (25).

With respect to interactive effects, our findings indicated that eight weeks of ET combined with PO seed consumption had no interactive effects on the reduction of cytochrome-C and MDA in the heart tissues of the $H_2O_2$-poisoned rats. Therefore, it seems that ET and PO seed differently affect cytochrome-C and MDA and have no synergistic effects.

Some of the limitations of the present study were the lack of control of the calorie content received by the rats and enormity of the oxidative stress pathway factors. Therefore, it is suggested that further investigations in this regard consider these factors in relation to oxidative stress and cell death and assess the effects of ET on various tissues in order to achieve more accurate results.

## Conclusion

According to the results, ET and PO seed consumption could improve cytochrome-C and MDA in the heart tissues of the $H_2O_2$-poisoned rats. Moreover, the effects of PO seed on cytochrome-C and MDA were dose-dependent.

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