The effects of endurance training and pumpkin seed consumption on oxidative stress and DNA damage markers in the cardiac muscle of rats poisoned with H_2O_2

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Abstract

Introduction: Oxidative stress can impair the antioxidant protective capacity of the body, which is associated with decreased capacity of the body's internal defense system. There are some evidences that training and pumpkin seed (PS) consumption by different mechanisms have a protective effect on myocardial tissue by inhibiting oxidative stress. The aim of the present study was to investigate the effects of endurance training (ET) and PS consumption on oxidative stress and DNA damage markers in the cardiac tissue of rats poisoned with H_2O_2.
Materials and methods: In this experimental study, 42 healthy male Wistar rats were randomly divided into 7 groups, including: 1) control, 2) H_2O_2, 3) H_2O_2+ET, 4) H_2O_2+1mg/kg PS, 5) H_2O_2+2 mg/kg PS, 6) H_2O_2+ET+ 1 mg/kg PS, and 7) H_2O_2+ET+2 mg/kg PS. During eight weeks, groups 2 to 7 received 1 mg/kg of H_2O_2 (manufactured by Sigma Aldrich Co.) peritoneally; also, groups 3, 6, and 7 ran for 30 minutes on a treadmill at a speed of 23 m/min for 5 sessions per week. Independent sample t-test and two-way ANOVA with Bonferroni's *post- hoc* tests were used to analyze the data ($P<0.05$).
Results: H_2O_2poisoning significantly decreased ATP and increased cytochrome-C, MDA and PAB ($P=0.001$); on the other hand, ET and PS consumption alone significantly increased ATP and decreased cytochrome-C and MDA ($P=0.001$); but the interactive effects of ET and PS consumption on increase of ATP and decrease of cytochrome-C, MDA and PAB were not significant ($P>0.05$). In addition, although consumption of 1 mg/kg PS had no significant effect on increase of ATP and decrease of cytochrome-C and MDA ($P>0.05$), 2 mg/kg PS significantly increased ATP and decreased cytochrome-C, MDA and PAB ($P<0.05$).
Conclusion: It seems that ET and PS consumption alone can be a good strategy to reduce the adverse effects of toxicity by inducing oxidative stress; however the effects of PS are dose-dependent.

Keywords: Training, Pumpkin Seed, Oxidative stress, Heart, H_2O_2

Introduction

Oxidative stress is commonly considered as the starting point for various diseases and has an important role in the pathophysiology of various cardiovascular diseases such as atherosclerosis, hypertension, heart failure and dysfunction of myocardial ischemia reperfusion (1). In addition, it causes excessive production of reactive oxygen species (ROS), which is an

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important event in the development of cardiovascular disease (2, 3). Oxidative stress is caused by an imbalance in the body's redox state, in which an increase in free radicals results in tissue damage. It has been shown that ROS, such as superoxide ($O_2-•$) and hydroxyl (•OH) radicals and hydrogen peroxide (H_2O_2), are formed during the redistribution of blood and oxygen following myocardial ischemia or hypoxia. H_2O_2 is an important tool for studying the effects of oxygen-derived free radicals on blood redistribution in myocardial ischemia. It can easily pass through the cell membrane and become toxic •OH radicals in the presence of iron ions (4). In a biological environment, oxidants, which capture electrons, and reducers, which lose electrons, are known as oxidants and antioxidants, respectively. The redox state of the cell describes the oxidant / antioxidant balance (PAB) and plays an important role in the fluctuations of various metabolic processes and cell signaling (5). Internal sources of reactive oxygen species include mitochondria, peroxisomes, inflammatory cells (neutrophils, eosinophils, and macrophages), flavins, adrenalines and dopamine, quinones, NADPH cytochrome complex enzymes, P450 oxidases and xanthine oxidase; also external sources comprise environmental pollution, optical beams and rays as well as chemical compounds such as anticancer drugs, cigarette smoke, alcohol, etc. (6). Oxidative stress and subsequent changes in signaling pathways can have different pathophysiological effects at different stages of life. ROS damages the cell membrane by inducing lipid peroxidation. Malondialdehyde (MDA) is an important product of lipid peroxidation and probably reflects the extent of cell damage (7); on the other hand, elevated levels of H_2O_2 lead to decreased respiratory capacity, decreased ATP renewal and bioenergetic analysis, as well as decreased ATP (7). Exercise is one of the factors that can improve oxidative stress. In addition, regular exercise has been shown to play an important role in enhancing the antioxidant system's efficiency and mitochondrial function against toxicity. In observational studies and clinical trials, exercise has been associated with a decrease in oxidative stress (8) and it has been found that increased ROS and free radical production during exercise can have both positive and negative physiological effects (9). Taking antioxidants is one of the other factors by which the body can counteract oxidative stress. The use of herbal supplements due to their anti-inflammatory and antioxidant properties is one of the ways to enhance antioxidant defense. Pumpkin seed (PS) has many benefits to maintain and promote health; however, the antioxidant or anti-inflammatory activity of its extract has not been extensively studied. Pumpkin belongs to the *cucurbitaceae* family, an edible plant often used as a functional food or herbal medicine. PSs contain enriched unsaturated fatty acids, phytoestrogens and antioxidants such as vitamin E, carotenoids and auxiliary elements such as zinc that have potential medicinal, nutritional and cosmetic properties (10). Despite the aforementioned points, limited studies have been conducted on the interactive effects of endurance training (ET) and PS consumption on DNA damage and oxidative stress markers; also, no integrated data are available and the effects of ET and PS extract have most often been investigated separately. Therefore, the aim of this study was to investigate the concurrent effects of ET and PS extract on cytochrome- C, ATP, MDA and PAB levels in the cardiac tissue of male Wistar rats poisoned with H_2O_2.

Material and methods

In this experimental study, 42 healthy adult male Wistar rats weighing approximately 200-220 g (10- 12 weeks of age) were purchased and seven days after familiarized to a new laboratory environment, were randomly assigned to 7 groups of 6 rats, including: 1) control, 2) H_2O_2, 3) H_2O_2+ ET, 4) H_2O_2+ 1mg/kg PS, 5) H_2O_2+ 2

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mg/kg PS, 6) H_2O_2+ ET + 1 mg/kg PS, and 7) H_2O_2+ ET + 2 mg/kg PS.

For eight weeks, groups 2 to 7 received 1 mg/kg H_2O_2 (manufactured by Sigma Aldrich Co.) peritoneally; also, groups 3, 6, and 7 ran for 30 minutes on treadmill at a speed of 23 m/min for 5 sessions per week. Groups 4- 7 also received ethanolic extract of PS by gavage at specified doses (12). PSs were powdered by electric milling and immersed in 80% ethanol at 1: 10 ratio in two one-hour stages. Then, it passed through a 0.2 mm paper filter and the remaining material was placed in a percolation system to allow to evaporate the ethanol. Afterwards, every 50 mg of the remaining dried extract powder was dissolved in 0.1 ml of distilled water and fed to rats by gavage method (12).

Forty eight hours after the last ET session and PS consumption, rats were anesthetized at the end of 8 weeks and after complete anesthesia, perfusion was performed to extract blood from the tissues. To collect the heart tissue specimens, rats were sacrificed by cervical dislocation and the heart tissue was extracted. Levels of ATP, cytochrome-C, MDA, and PAB were determined by ELISA using a specific kit (Minneapolis, MN, USA) according to the manufacturer's guide. Independent sample t-test was used to investigate the effects of induction of H_2O_2 poisoning and two-way ANOVA with Bonferroni's *post- hoc* tests were used to evaluate the effect of ET and PS consumption ($P<0.05$).

Results

The levels of ATP, cytochrome-C, MDA, and PAB in the heart tissue of rats in the seven study groups are presented in Figures 1 to 4, respectively. The results of independent sample t-test showed that the levels of ATP ($P=0.001$) in the H_2O_2 group were significantly lower than the control group, however the levels of cytochrome-C ($P=0.001$), MDA ($P=0.001$) and PAB ($P=0.001$) were significantly higher.

The results of two-way ANOVA showed that ET ($F=29.90$, $P=0.0001$, $\mu=0.49$) and PS consumption ($F=10.60$, $P=0.0001$, $\mu=0.41$) significantly increased ATP, however ET with PS consumption had no interactive effect on increase of ATP ($F=0.29$, $P=0.746$, $\mu=0.01$). ET ($F=123.78$, $P=0.0001$, $\mu=0.80$) and PS consumption ($F=12.66$, $P=0.0001$, $\mu=0.45$) significantly decreased cytochrome-C, but ET with PS consumption had no interactive effects on cytochrome-C ($F=1.59$, $P=0.21$, $\mu=0.09$). ET ($F=125.56$, $P=0.0001$, $\mu=0.80$) and PS consumption ($F=33.48$, $P=0.0001$, $\mu=0.69$) significantly decreased MDA, however ET with PS consumption had no interactive effects on MDA ($F=1.58$, $P=0.22$, $\mu=0.09$). Also, PS consumption ($F=35.87$, $P=0.0001$, $\mu=0.70$) significantly decreased PAB, however the effect of ET ($F=3.49$, $P=0.07$, $\mu=0.10$) had no significant effect on PAB and interaction of ET and PS consumption ($F=2.36$, $P=0.11$, $\mu=0.13$) was not significant on decrease of PAB.

The results of Bonferroni's *post- hoc* test showed that 1 mg/kg PS had no significant effect on increase of ATP ($P=0.11$), decrease of cytochrome-C ($P=0.86$) and MDA ($P=0.08$), however 2 mg/kg PS significantly increased ATP ($P=0.001$); decreased cytochrome-C ($P=0.002$) and MDA ($P=0.001$). Also, 1 mg/kg and 2 mg/kg PS significantly decreased PAB ($P=0.001$) as well as 1 mg/kg and 2 mg/kg PS had the same effects on decrease of PAB ($P=0.06$).

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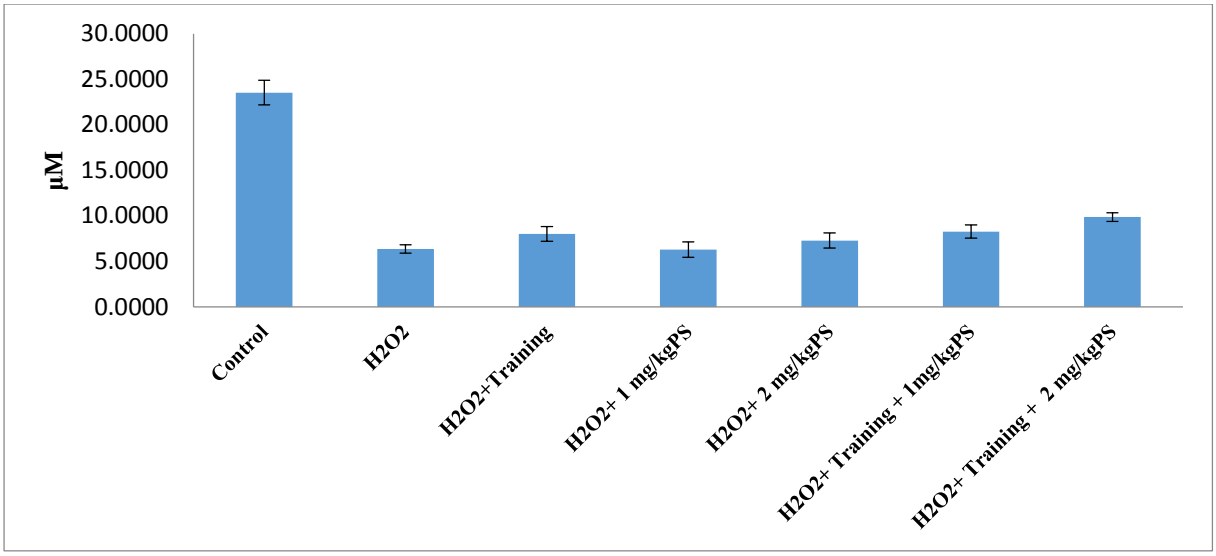


Figure 1. ATP levels in the heart tissue of rats in seven groups of the study.

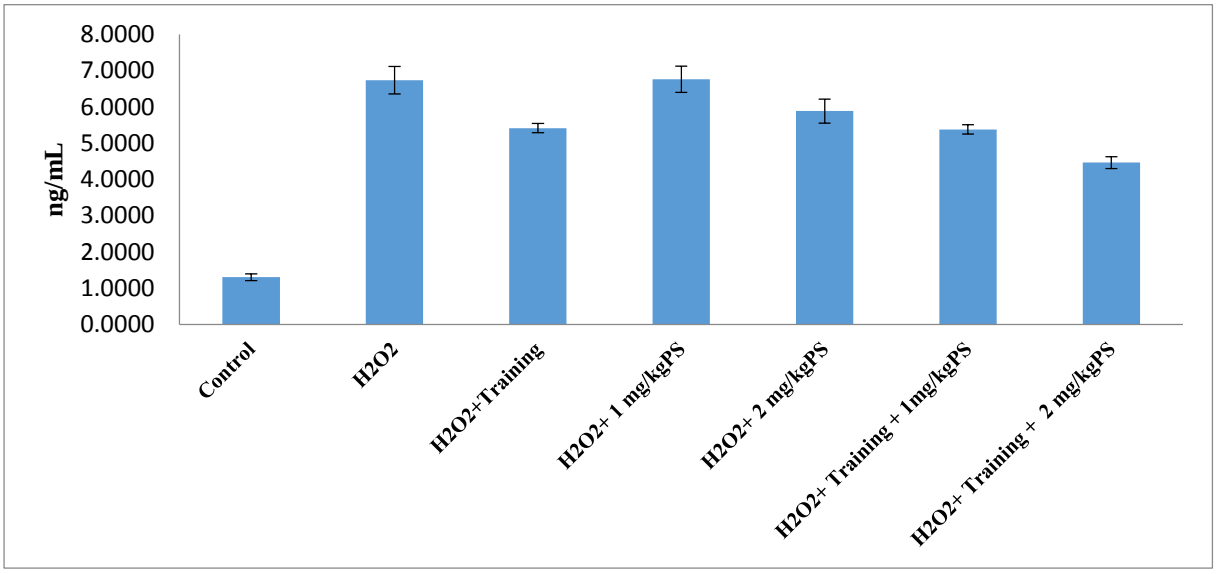


Figure 2. Cytochrome-C levels in the heart tissue of rats in seven groups of the study.

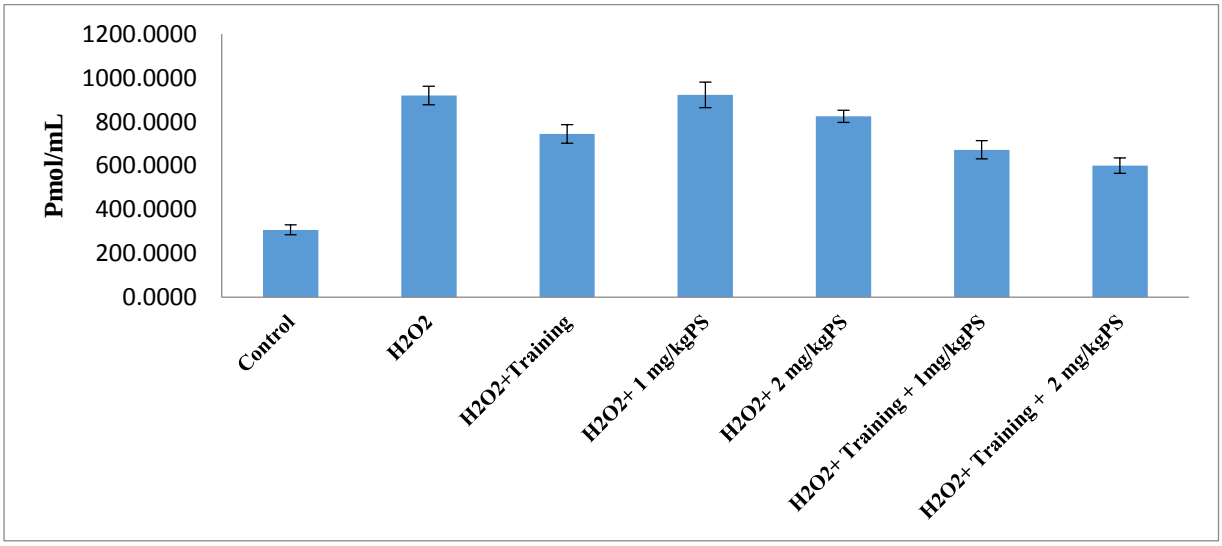


Figure 3. MDA levels in the heart tissue of rats in seven groups of the study.

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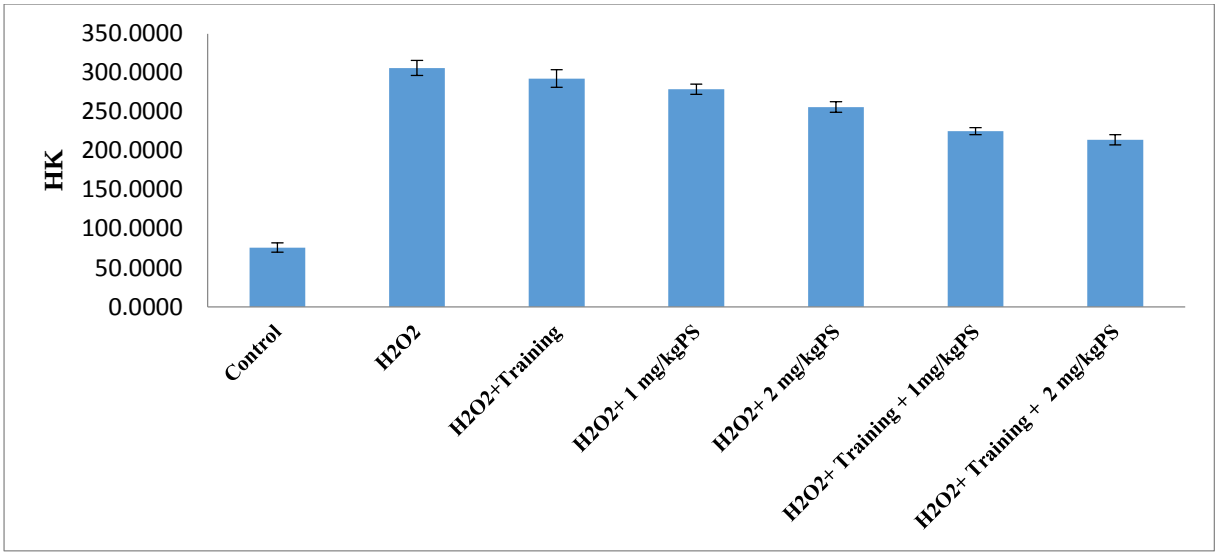


Figure 4. PAB levels in the heart tissue of rats in seven groups of the study.

Discussion

The results of present study showed that H_2O_2 poisoning significantly decreased ATP and increased cytochrome-C, MDA and PAB in the heart tissue of rats. The most vulnerable ROS targets have been reported to be proteins, lipids, and DNA (13). ROS can oxidize proteins that damage their structure, disrupt their functional activities and also affect gene transcription (14). Some potential ROS-producing factors have also been identified in muscle cells. These include nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX), phospholipase A2 (PLA2), xanthan oxidase (XO), and lipoxygenases. In addition to intracellular sources, ROS has also been shown to be produced from non-muscle sources. High intensity exercise that requires a great deal of energy or power increases the levels of anti-inflammatory cytokines, cytokine inhibitors, and chemokines; consequently, it results in increased plasma levels of TNF-α, interleukin (IL) -1β (15). Inflammation is the primary response of the immune system to removing pathogens or other stimuli in order to restore cells to normal or replace damaged tissue with scarring (16). In fact, when cells of the same organ are damaged, the cells of the immune system activate and stimulate the production of free radicals to destroy the damaged structures. However, the free radicals produced by the body's immune system against damaged organs oxidize and damage neighboring healthy cells and cause inflammation.

The present study showed that eight weeks of ET significantly increase ATP and reduced cytochrome-C and MDA in the cardiac tissue of rats poisoned with H_2O_2. Consistent with the findings of the present study, Seyedghomi *et al.* showed that high-intensity ET was able to reduce cytochrome-C, which could be due to improved mitochondrial membrane stability and reduced permeability (17). However, in contrast to the findings of the present study, eight weeks of high intensity ET significantly increased MDA in the cardiac tissue of rats (18). The reasons for inconsistency of the findings of this study with the present study may be the type of training (interval vs. continuous) as well as the intensity of training (low intensity in the present study). In this regard, it has been suggested that prevention through physical fitness and increased cardiovascular fitness in adapting to regular physical activity, by affecting PI3K, can prevent myocyte stress (19). It has also been shown that the CaMEK gene plays an important role in protecting cardiomyocytes against H_2O_2 damage and autophagy occurs through the ERK1 / 2 pathway (20).

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It is well-established that maintaining health and preventing chronic and age-related disorders requires a proper lifestyle, including regular exercise. Regular physical activity against stressful conditions, in almost all body tissues, induces biochemical and metabolic adaptation responses, such as increasing the capacity to cope with conditions in which ROS production is increased. In addition, aerobic physical activity is recommended as an auxiliary therapy to reduce the side effects of some pathologies (21).

It has been reported that natural antioxidants can also help prevent H_2O_2 disorders. The results of the present study showed that eight weeks of PS consumption significantly increased ATP and decreased cytochrome-C, MDA and PAB in the cardiac tissue of rats poisoned with H_2O_2. Also, 1 mg/kg PS had no significant effect on the increase of ATP and decrease of cytochrome-C and MDA, but 2 mg / kg PS significantly increased ATP, decreased cytochrome-C, MDA and PAB ($p\leq0.05$). The findings of the present study indicate that the effects of PS on DNA damage markers and oxidative stress can be dose dependent. Consistent with the findings of the present study, three weeks of PS consumption significantly decreased MDA and increased CAT in rats poisoned with carbon tetrachloride (22). Also, similar to the present study, consumption of 40 to 100 mg/kg of pumpkin oil resulted in decreased MDA in the cardiac tissue. Phenolic compounds have been shown to have proven protective effects against genotoxic degradation effects of cancers by sweeping free radicals and enhancing the host's antioxidant defense system.

There is ample evidence that some herbal compounds such as vitamins, flavonoids, polyphenols, carotenoids, catechins and herbal steroids may act as mutagenic inhibitors. PS and seed oils are rich sources of protein, phytosterols, polyunsaturated fatty acids including linolenic, linoleic, vitamins (A and E) and antioxidants such as carotenoids, lutein, tocopherol, gamma, chlorophyll and elements such as zinc and selenium (10). The PS also contains some zinc. Zinc contained in PSs acts as an antioxidant against the attack of free radicals and prevents the oxidation and formation of free radicals (10). Concerning the interactive effects, the results of the present study showed that ET and PS had no interactive effects on increase of ATP and decrease of cytochrome-C, MDA and PAB. Regarding to review of literature, no study was found to evaluate the concurrent effect of training and PS consumption on ATP, cytochrome-C, MDA and PAB to compare the results with the present study. Therefore, lack of sufficient information about the effect of ET and PS on DNA damage and oxidative stress markers in the muscle tissue is one of the limitations of the present research. Another limitation of the present study is the inability to measure cytochrome-C, MDA and PAB by ELISA and western blotting. Since the effects of PS were dose dependent in the present study, it is recommended to investigate the effect of ET with higher doses of PS consumption in future studies to obtain more information in this regard.

Conclusion

Based on the results of the present study, it seems that ET and PS alone can be a suitable strategy to reduce the effects of toxicity by inducing oxidative stress. Also, the effects of PS are dose dependent; however ET and PS consumption do not have interactive effects on DNA damage and oxidative markers in the cardiac tissue of rats poisoned with H_2O_2.

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