Research Paper

Effect of Aerobic Exercises on Reduced and Oxidized Glutathione in Aortic Endothelial Cells of Rats Exposed to Arsenic

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ABSTRACT

Background and Aim: Arsenic is an environmental pollutant that can damage tissue by producing free radicals. However, regular aerobic exercise is essential in enhancing antioxidant defense and resistance to oxidative stress. This study aimed to evaluate the effect of aerobic exercise on reduced glutathione (GSH) and oxidized glutathione (GSSG) and aortic endothelial cells of heart tissue in rats exposed to arsenic.

Materials and Methods: In this experimental study, 24 male Wistar rats with a weight ranging 220-240 g and a mean age of 6-8 weeks were divided into 3 groups of 8: healthy control, toxic control, and toxic aerobic exercise. Rats receiving arsenic were prescribed 25 ppm arsenic daily in oral water for 8 weeks. The exercise program consisted of 8 weeks of aerobic exercise, five sessions per week with an intensity of 75%-80% of maximum oxygen consumption. Eventually, 24 hours after the last exercise session, the rats were anesthetized and killed, and the target tissue was removed for examination. The Kolmogorov-Smirnov statistical test and one-way analysis of variance with Tukey post hoc test were used to analyze the findings in SPSS software, version 22 (P≥0.05).

Results: Exposure to arsenic significantly reduced GSH and GSSG levels in heart tissue and aortic endothelial cells (P≥0.05). In contrast, aerobic exercise increased GSH and GSSG levels in heart tissue and aortic endothelial cells in arsenic-poisoned rats (P≥0.05).

Conclusion: Aerobic exercise effectively reduces oxidative stress and increases antioxidant defense against arsenic toxicity in heart disorders.
1. Introduction

Arsenic is one of the most dangerous environmental pollutants, and its toxicity causes considerable damage [1]. Arsenic compounds are present in the body as inorganic (arsenite) and organic (arsenate) forms. Groundwater, soil, contaminated rice, and some seafood are sources of arsenic [2]. Prolonged exposure to arsenic can increase mortality and cause cancers of the skin and internal organs, including the heart, liver, lungs, kidneys, and bladder. Arsenic can be absorbed through the skin, respiratory system, and digestive tract [3]. Arsenic can interfere with redox reactions and produce large amounts of reactive oxygen species (ROS) through a chain reaction that causes oxidative stress [4]. Various studies have reported oxidative damage, decreased activity of enzymatic antioxidants, and induction of apoptosis due to arsenic toxicity [2, 4]. It induces oxidative stress by reducing delta-amino acid dehydratase activity and increasing malondialdehyde (MDA) levels. Malondialdehyde, a peroxidation product, is a membrane lipid marker of cardiac oxidative damage [5].

On the other hand, the antioxidant system plays an important role in the damage caused by free radicals. The antioxidant defense system contains non-enzymatic and enzymatic antioxidant factors, including superoxide dismutase (SOD), catalase enzyme (CAT), and glutathione [6]. Glutathione is a non-enzymatic antioxidant found in the cytosol and mitochondria. This protein is responsible for detoxifying the cell, antioxidant defense, and modulating cell proliferation. Glutathione can be found in reduced glutathione (GSH) and oxidized glutathione (GSSG) forms. Reduced glutathione is one of the body’s most important antioxidants, and excessive release of free radicals reduces GSH levels or increases GSSG in the cell [7]. Cardiac and myocardial tissue is one of the most vulnerable tissues to chronic oxidative damage due to oxidative stress [8]. Reactive oxygen species (ROS), including superoxide, peroxide, and hydroxyl, are the major causes of heart muscle damage and cause many changes, including impaired contractile function, arrhythmia, and altered gene expression. ROS has also been shown to impair endothelial cell integrity. Increased oxidative stress has been observed in heart failure, which causes inflammation and endothelial dysfunction and plays an important role in congestive heart failure [9].

Exercise is recommended to maintain good health and reduce the risk of various diseases, but prolonged exposure to exercise stimulates the production of reactive oxygen species and the antioxidant defense system [10]. Although some evidence supports the production of free radicals and cellular damage after strenuous exercise, regular and moderate physical activity improves the body’s antioxidant status and reduces the production of free radicals [10]. Many studies have been conducted on the effect of aerobic exercise on oxidative stress markers, many of which have shown that endurance exercise reduces oxidative stress [11]. In this regard, Ishaqi et al. reported that aerobic exercise could reduce lipid peroxidation and increase antioxidant defense [12]. Also, Rezaei et al. showed that aerobic exercise increases antioxidant enzymes in heart tissue [13].

In contrast, Rinaldi et al., in a study of the effect of eight weeks of aerobic exercise on the treadmill on cardiac SOD in rats, observed an increase in this index following aerobic exercise. However, no significant changes in levels were reported [14]. Intensity, duration, and type of exercise seem to have different effects on antioxidant activity. It has also been observed that following exercise, the cell’s defense system tries to balance or increase antioxidant enzymes against oxidative stress. As a result, regular and continuous exercise makes people more resistant to oxidative stress and provides a healthier life. Therefore, considering the destructive effects of arsenic in the living environment on health and also considering the benefits of aerobic exercise on cardiovascular health, this study aimed to investigate the effect of aerobic exercise on oxidative stress indices of GSH and GSSG in heart tissue and aortic endothelial cells of rats poisoned with arsenic.

2. Materials and Methods

In this experimental study, 24 male Wistar rats with a weight range of 220-240 g and a mean age of 6-8 weeks were purchased and transferred to the Sports Physiology Laboratory of Islamic Azad University, Mahallat Branch. The present study was performed according to the National Institute of Health (NIH) instructions and was approved by the Ethics Committee of the Islamic Azad University of Arak (Code: IR.IAU.ARAK.REC.1398.012). All rats were kept in clean and sterile cages under standard conditions with a 12:12 h dark:light cycle at a temperature of 19°C-22°C. Animals with a standard rodent laboratory diet (crude protein 19.50%-20.50%, 5.3%-4.5% fat, 4%-4.5% fiber, 0.95%-1% calcium, 0.65%-0.7% phosphorus, 0.55%-5% salt, 1.15% lysine, methionine 0.33%, threonine 0.72%, tryptophan 0.25%, calories 16.16-17 mJ/kg) and tap water were fed ad libitum. After one week of adaptation to the laboratory environment, the rats were randomly divided into...
3 groups of 8: healthy control, toxic control, and toxic aerobic exercise. To induce oxidative stress, the drinking water of groups 2 and 3 was exposed to arsenic (25 ppm) for 8 weeks daily [15].

Aerobic Exercise Protocol

In the first stage, to get acquainted and reduce stress, the rats ran on a treadmill at a speed of 5-10 m/min for 10 minutes. In order to warm up and cool down before and after the exercises, aerobic exercises were performed on a treadmill for 2 minutes at a speed of 10 m/min. The exercise protocol started for 8 weeks and 5 sessions per week with an intensity of 10 m/min for 15 minutes with a slope of 5% in the first week and gradually increased to 25 m/min with a slope of 5% for 30 minutes in the last week. The protocol was as follows: in the first 2 weeks, 10 m/min for 15 minutes with a slope of 5%; in the second 2 weeks, 15 m/min for 20 minutes with a slope of 5%; in the third 2 weeks, 20 m/min for 25 minutes and a slope of 5% and; in the fourth 2 weeks, 25 m/min for 30 minutes with a slope of 5% [16].

Tissue sampling and measurement steps

Twenty-four hours after the last intervention and after 10-12 hours of fasting, the rats were anesthetized by intraperitoneal injection of ketamine (90 mg/kg) and xylazine (10 mg/kg). The chest was opened, and the heart and aortic tissue were immediately removed and frozen using liquid nitrogen to be sent to a laboratory. The frozen tissues were homogenized at 4°C in the next step. So 50 mg of ventricular muscle was homogenized by dissolving in 1 mL of ice cell lysis buffer. Then, the solution was centrifuged at 1000 rpm for 1 minute at 4°C to remove the supernatant. It was then stored in at -80°C nitrogen tank in the refrigerator for long-term use.

Components of the glutathione cycle

Because GSSG is considered an indicator of oxidative stress and GSH is an indicator of cellular antioxidant capacity, the ratio of GSSG to GSH is a helpful indicator for determining the state of oxidative stress. Reduced and oxidized glutathione (GSH/HSSG) were measured by ELISA method and the Zell BioGmbH kit (ZB-10034C) produced in Germany was used [8].

Statistical analysis

The Kolmogorov-Smirnov test was used to evaluate the normal distribution of results, and a one-way analysis of variance (ANOVA) with Tukey post hoc test was used to analyze the results in SPSS software version 22 (P≤0.05).

3. Results

The one-way analysis of variance test results showed a significant difference in the levels of GSH (P=0.000, F=253.95) and GSSG (P=0.001, F=240.98) of aortic endothelial cells between the study groups. The results also showed a significant difference between the three groups in GSH (P=0.001, F=205.59) and GSSG (P=0.001, F=351.64) levels of cardiac tissue. The results of the Tukey post hoc test showed that GSH and GSSG levels of aortic endothelium in the toxic control group were significantly reduced compared to the healthy control group (P=0.001). However, GSH and GSSG levels in the aerobic exercise group were significantly higher than in the toxic control group (P=0.001) (Figure 1). GSH and GSSG levels of cardiac tissue in the toxic control group were significantly lower than in the healthy control group (P=0.001). While tissue GSH and GSSG levels in the aerobic exercise group were significantly higher than in the toxic control group (P=0.001) (Figure 2).

4. Discussion

The present study showed that arsenic exposure reduces antioxidant levels and increases oxidative stress in rats’ cardiac tissue and aortic endothelial cells. However, aerobic exercise significantly increases GSH and GSSG in cardiac and endothelial tissues of arsenic-poisoned rats. Studies have shown that arsenic increases oxidative stress. Arsenic metabolism is one of the reasons for the change in the activity of antioxidant enzymes, especially glutathione peroxidase (GPX) [17]. Arsenic, by various mechanisms, decreases glutathione and the activity of glutathione-dependent enzymes, including GPX, and the reduction of GSH facilitates the accumulation of arsenic and causes oxidative stress. Increased oxidative stress led to decreased SOD activity, and increased production of superoxide radicals inhibits catalase activity. Increased H2O2 level indicates a decrease in catalase activity after arsenic consumption [17].

In a study on albino Swiss mice, Singh et al. showed that arsenic toxicity reduced SOD and GSH but increased lipid peroxidation and carbonyl protein content in various tissues [18]. Rasolifard et al. showed that sodium arsenite consumption significantly reduced the activity of CAT, GPX, and SOD enzymes and increased MDA in the liver tissue of rats [17]. Arsenic also has high toxicity and is naturally considered stress, and free radicals play a pivotal role in the pathophysiological mechanisms of
cardiac disorders [19]. Direct and indirect observations have also shown that oxidative stress is a major cause of myocardial damage, and further evidence suggests that ROS is involved in cardiac dysfunction. For example, Bugger et al. in a study, showed that ROS plays an important role in ischemia-blood reperfusion and also increases significantly after a few minutes of reperfusion, causing many changes, including weakening of contractile responsiveness, arrhythmias, and gene expression [20]. In addition, ROS and disorders of calcium homeostasis severely damage the myocardium because ROS production in conditions such as arsenic toxicity increases intracellular calcium by releasing calcium from the sarcoplasmic reticulum, opening ryanodine receptors, and damaging calcium dehydration in cardiomyocytes as well as endothelial connective tissue disorders [21].

Exercise and physical activity are among the most well-known factors in preventing and treating cardiovascular disease. Studies have shown that aerobic exercise directly affects cardiovascular vessels, including oxygen supply to the heart, endothelial function, coagulation factors, and inflammatory markers [22]. Aerobic exercise also increases the heart’s ability in glycolysis, increasing ATP to compensate for the decrease in oxidative phosphorylation concentration in myocardial ischemia [23]. Another protective effect of aerobic exercise on the heart is its ability to increase the enzyme cyclooxygenase-2. It is one of the most important enzymes synthesizing prostaglandins and inflammation in humans [24].

On the other hand, aerobic exercise causes angiogenic processes. It increases the diameter of blood vessels in skeletal and cardiac muscles, thereby improving blood flow in the organs and vascular endothelial function and releasing nitric oxide, which causes the arteries to dilate [25]. The results of studies have shown that if aerobic exercise is done regularly, it can systematically reduce oxidative stress in heart disease [12]. In this regard, Stockelman et al. reported that aerobic exercise improves endothelial function and reduces myocardial ischemia. It seems that during aerobic exercise, increasing the moderate tension on the arterial walls leads to improved endothelial function, which facilitates the synthesis and release of nitric oxide activity [26]. In another study, Kanter et al. examined the effect of low-intensity aero-

**Figure 1.** Concentrations of GSH and GSSG in Aortic endothelial cells of different groups
Each group consisted of 8 rats, and the data were expressed as Mean±SD.
*** Significant decrease in the toxic control group (TC) compared to the healthy control group (HC).
&&& Significant increase in the toxic aerobic exercise group (EA) compared to the toxic control group (TC).

**Figure 2.** Concentrations of GSH and GSSG in cardiac tissue in different groups
Each group consisted of 8 rats, and the data were expressed as Mean±SD.
*** Significant decrease in the toxic control group (TC) compared to the healthy control group (HC).
&&& Significant increase in the toxic aerobic exercise group (EA) compared to the toxic control group (TC).
bic exercise in rats and concluded that aerobic exercise significantly reduces oxidative stress and cell apoptosis and increases antioxidant activity in cardiac tissue [27].

In the current study, the effect of regular aerobic exercise on oxidative stress in the cardiac and aortic endothelial cells of rats exposed to arsenic toxicity was investigated. However, no study was found to investigate the effect of aerobic exercise on oxidative stress of cardiac and endothelial tissues under arsenic toxicity, so comparing the present study with similar studies was limited. However, studies have examined the effect of aerobic exercise on the toxicity of various toxins. For example, Ishaqi et al. found that 6 weeks of aerobic exercise reduced lipid peroxidation levels and increased antioxidant defense in rats exposed to environmental dust [12]. Habibian et al. studied the supportive effect of aerobic exercise on oxidative stress caused by the head in the cerebellum of rats. They concluded that aerobic exercise reduces oxidative stress and brain health by increasing brain-derived neurotrophic factor [28]. Also, Ezabadi et al. reported that resistance exercise reduces oxidative stress in the cerebellar tissue of diazinon-poisoned rats [29], which is consistent with the results of the present study.

Due to increased GSH and GSSG levels in cardiac tissue and aortic endothelial cells of rats following aerobic exercise, cardiac androgen receptors appear to be in line with slow-twitch fibers, resulting in improved antioxidant activity. It seems that cardiac function reporting methods such as electrocardiography, examining apoptotic indices, and measuring oxidative stress levels are the limitations of the present study. However, confirmation of this subject requires further research, and researchers are advised to study and compare the effect of resistance exercise or combination exercise on antioxidant and ROS indices of slow-twitch and fast-twitch fibers and heart and myocardium.

5. Conclusion

Based on the present study results, we concluded that arsenic toxicity could lead to oxidative stress in cardiac tissue and endothelial cells, which can be significantly reduced with regular aerobic exercise. Therefore, regular aerobic exercise can act as a therapeutic and supportive strategy to resist oxidative stress caused by environmental pollutants. However, several points in this topic should be studied in the future.

### Ethical Considerations

#### Compliance with ethical guidelines

The study was approved by the Ethics Committee of the Islamic Azad University, Arak Branch (Code: IR.IAU.ARAK.REC.1398.012)

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#### Authors' contributions

All authors equally contributed to preparing this article.

#### Conflict of interest

The authors declared no conflict of interest.

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