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Environmental Pollution and the Reproductive System: Beneficial Effect of Treadmill Running and Curcumin Supplementation

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Abstract

Introduction: The effect of environmental lead (Pb) on the male reproductive system has been a major area of concern for several years. The current study was carried out to evaluate the protective effect of curcumin(CCM) and moderate treadmill running on the reproductive system during exposed to lead acetate (Pb).

Material and Methods: 50 male Wistar rats were divided into five groups (10 rats/group): 1)sham-operated, 2)Pb, 3)exercise + Pb, 4)CCM + Pb, and 5)exercise + CCM + Pb. The 2 to 5 groups were exposed to lead acetate (20 mg/kg). In addition, the 4 and 5 groups received CCM solution (30mg/kg), while the 1 group received ethyl oleate, intra-peritoneally. The rats in the training groups performed treadmill running consisting of 15 to 22 m/min for 25 to 64 min, 5 times a week for 8 weeks. Motility and morphological profile of sperm, the testis and epididymal MDA and the reproductive hormone were assessed by standards methods.

Results: there are no significant differences were found between reproductive hormones concentration in all groups. However, Compared with other groups, mean values of testis and epididymis lipid peroxidation was higher in Pb group but not significant. Moreover, lead administration was corrupted motility and morphology of sperm in Pb group, but intervention of moderate treadmill running and CCM supplement have an ameliorating effect on this parameters.

Discussion and Conclusions: The results of this study shows that regular moderate treadmill running and using curcumin supplement are suitable guideline to inhibit the negative effects of Environmental pollution on reproductive system.

Keywords: Air pollution, Reproductive Parameters, Endurance Training, Antioxidants.

Introduction

Lead poisoning has been among the most studied health problems over the years. Despite the tremendous amount of data accumulated, the known mechanisms of lead toxicity are incapable of explaining some of the toxic effects of lead [1,2]. One current theory as to how lead exerts its toxic effects suggests that lead-induced oxidative stress may be a possible contributor to the pathogenesis of lead poisoning [1,2]. Some in vitro studies pointed to elevated production of reactive oxygen species (ROS) by lead treatment [2]. These findings were further supported by in vivo studies in lead-exposed animals and workers, where increased lipid peroxidation and altered antioxidant defense systems were found [2].

Recent evidences suggest that lead acetate(Pb)exposure enhances intracellular reactive oxygen species (ROS) production and lipid peroxidation, which may lead to tissue damage [3,4]. Testicular tissue is a major target for metal-induced oxidative damage because of its high content of polyunsaturated membrane lipids [5,6]. The sperm plasma membrane contains a high amount of unsaturated fatty acids . Therefore, it is particularly susceptible to peroxidative damage. The lipid peroxidation destroys the structure of lipid matrix in the membranes of spermatozoa, and it is associated with loss of motility and the defects of membrane integrity [7]. One aspect of lead-associated endocrine disruption is reproductive dysfunction. Reproductive toxicity associated with lead poisoning has been considered primarily an occupational disease of adults [8]. Some epidemiological studies have suggested that lead exposure during pregnancy results in a shorter

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gestational period and increased infant mortality [8]. Therefore, we believe that antioxidants should be considered as a component of an effective treatment for lead poisoning [6]. Trends on applying nutritional antioxidants in diseases related to oxidative stress have gained immense interest in recent years [5]. Plant products such as Curcumin(CCM) are known to exert their protective effects by scavenging free radicals, modulating antioxidant defense system and is considered to be an effective antioxidant against oxidative tissue damage [5]. CCM is a yellow coloured phenolic pigment yield from the rhizome of turmeric (family *Zingiberaceae*) . The most important feature of CCM is that it has no side effects despite being a therapeutic agent with multiple beneficial functions [5,9,10].

Physical exercise, associated with oxidative stress, depends on the type and intensity of exercise. However, studies have demonstrated that regular moderate training improves the antioxidant defense and the oxidative capacity in skeletal muscle [11]. Despite the great efforts expended, the causes of oxidant–antioxidant imbalance during exercise are still unclear [11]. Beneficial effects of training on antioxidant defense mechanisms in various tissues have been reported in treadmill training [11]. Physical exercise is a double-edged sword, when practiced strenuously it causes oxidative stress and cell damage; in this case antioxidants should be given. But when practiced in moderation, it increases the expression of antioxidant enzymes and thus should be considered an antioxidant [12].

Despite the fact that Pb can induce oxidative stress, most studies have only identified effects of exercise and/or antioxidants on the reproductive system health, without considering air pollutants and/ or after acute exposure to air pollution. Moreover, there is less information with respect to simultaneous effects of lifestyle, including regular endurance training and CCM antioxidant supplementation, or both, on the reproductive system during chronic exposure to Pb. Thus, the aim of this study was to investigate the effect of moderate endurance training and CCM supplementation on the lead acetate – induced reproductive toxicity of male rats.

Material and Methods

The experimental protocol was approved by Department of Physiology, University of Mazandaran and was performed according to guiding procedures in the Care and Use of Animals, prepared by the Council of the American Physiological Society. Forty male Wistar rats, 8 weeks of age (initial body weight of 240 ± 20 g), were obtained from the Laboratory of Animal Bearing and Multiplying at the Pasture Institute of Iran. Each rat was housed in single standard cages of polycarbonate (20×15×15), made at the Pasture Institute of Iran, in a large air-conditioned room with controlled temperature of 22±2 °C, light- dark cycles of 12:12 hours and humidity of 50±5%. The pollutant standard index (PSI) was in the acceptable range as determined by the Iranian Meteorological Organization. Rats were fed with a standard rat chow provided by Pars Institute for animals and poultry with a daily regimen of 10 / 100 gr body weight for each rat. Water was available ad libitum.

Rats were familiarized with the laboratory environment and running on the treadmill, then were randomly assigned into five experimental groups of 8 rats each. The groups were defined as follows: Group1 – the animal were exposed to lead acetate(Pb) at a concentration of 20 mg/kg in the form of a water solution for intraperitoneally injection(i.p.), 3 days weekly for 8 wks; Group 2 – Curcumin (CCM) similarly received lead acetate, as well as curcumin 30 mg/kg 5 days weekly for 8 wks (i.p.); Group 3 – endurance training (Pb + training) - the rats in this group similarly received lead acetate, and in addition they performed progressive running training of 15 to 22 m/min for 25 to 64 min, 5 times a week; Group 4 – training and curcumin (Pb + training + Cum); the rats in this group performed a physical training protocol similar to that in Group 3, and in addition received lead acetate and curcumin supplementation; Group 5 –the sham-operate or control group; these rats received water and ethyl oleate, in the same manner and for the same duration of time as other groups.

Lead acetate (Sigma) was solubilized in Milli-Q water, and curcumin was solubilized in 50% ethanol. In order to perform intra peritoneal (i.p) injections, curcumin was solubilized in ethyl oleate and was injected at a dose of 30 mg/kg. Curcumin was protected from light throughout the experiment [13]. We are replicating a previously-reported lead dosing regimen that caused oxidative stress so that the doses of Curcumin and lead acetate were 30 and 20 mg/kg respectively [13]. At the end of the 8-wk exposure period, all groups were anesthetized with ketamine

and Xylazine and decapitated after 12-14 hours overnight fasting. Blood samples were collected 24 h after the last dose of treatment. These blood samples were initially centrifuged by a refrigerated centrifuge at 3,000 rpm for 15 minutes within 30 minutes of collection and then stored at -80°C before the reproductive hormone assay. The abdominal cavities were then opened and the testes and epididymis of each rat were removed and weighed. left epididymis of each rats was removed within 2 min of death, finely minced, and immediately placed in 3 ml Tyrode's solution[0.8 g NaCl, 0.02 g KCl, 0.02 g CaCl2 (anhydrous), 0.01 g MgCl2 (anhydrous),0.005 g NaH2PO4 (anhydrous), 0.01 g NaHCO3, 0.1 g glucose; volume adjusted to 100 ml with distilled water] at 37°C for 20 min to ensure an even distribution of sperm [14].The suspension was filtered through nylon mesh [14].

Sperm Motility and Sperm Morphology

To determine the percentage of motile sperm, a drop of sperm suspension was placed on a slide, covered with a coverslip, and the percentage of motile spermatozoa was estimated by light microscopy at a magnification of (40×14). At least 200 spermatozoa were estimated per suspension droplet. Moreover, to examine the sperm morphology, a drop of the sperm suspension was placed on a glass slide and a smear was prepared. The smear was fixed in ethanol for 1 h, stained with haematoxylin and eosin., washed, dried, and examined with a light microscope at 100×magnification. At least 500 spermatozoa were counted and the percentage of abnormal sperm determined. Morphologic abnormalities of spermatozoa Include: Headless, Flattened head, Pinhead, Bent neck, Bent tail and sperm with multiple abnormalities (bent neck and tail) [15].

Tissues MDA assay

Oxidative status in testicular and epididymal tissues could be estimated from the concentrations of malondialdehyde(MDA) [16]. To measure this index, Testes and epididymis tissues frozen at −80 ◦C were thawed and homogenized in 2 ml oflysis buffer (50mM Tris, 150mM NaCl adjusted to pH 7.4); the homogenates were centrifuged at 9000rpm for 15 min; the supernatants were saved; and the protein concentrations was measured, according to the method of Bradford [17]. Using bovine serum albumin as standard. As a marker of lipid peroxidation (LPO) production, the level of LPO in testes and epididymis was measured by the method of as thiobarbituric acid reactive substances (TBARS). Since malondialdehyde (MDA) is a degradation product of peroxidized lipids, the development of pink color with the absorption characteristics (absorption maxima at 532 nm) of TBA–MDA chromophore is taken as an index of LPO. The concentration of TBARS was expressed as n/moles of malondialdehyde/mgof protein tissue [16].

Statistical Analysis

Statistical analysis was performed using a commercial software package (SPSS version 16.0 for Windows). Results are expressed as means ± SE. A two-way ANOVA was used to detect statistical differences between groups. A post-hoc test was performed to determine differences in the various markers between groups. The differences were considered significant at $p \leq 0.05$.

Results

Effects of lead acetate (Pb) on motility and morphology of sperm

The motility and morphologywere examined in each group of rats after 8wk of Pb acetate exposure (Table 1).There was no significant differences were found between all groups, however the mean values of sperm morphology in sham-operate, curcumin+Pb, training+Pband training+curcumin+ Pb groups was 1.03%, 2.42%, 3.48% and 5.82% higher than Pb group, respectively.

Table1.Effect of exercise training and curcumin(CCM) on Motility and Morphology of the Spermatozoa in ratschronically exposure to lead acetate (Pb)

Groups / Variable	Sham-operate	Pb	CCM+Pb	Training+Pb	Training+ CCM+Pb
Percent motile spermatozoa	47.16±3.95	40.69±8.82	46.69±8.28	45.90±10.76	47.45±8.38
Percent normal spermatozoa	87.34±2.77	86.31±4.27	88.73±3.91	89.79±2.97	92.13±4.20

Data are presented as the mean±SD, *n* = 8.

Effects of lead acetate (Pb) on the weight of reproductive organs

Testes and epididymis to body weight ratios were compared in each group with the Control(Table2). Relative weight of testis and epididymis was unaltered in all the experimental animals.

Effects of lead acetate (Pb) on hormonal Levels

To determine if the effects of lead acetate exposure on the reproductive physiology of the rats occurred via the hypothalamic-pituitary-gonadal axis, plasma levels of LH, FSH, and testosterone were examined following lead administration. There was no significant difference in any of the hormonal levels in each groups, as compared to the control animals (Table 3).

Effect of lead acetate on the testicular and epididymal lipid peroxidation (LPO)

Testicular and epididymal LPO levels are shown in Table 4.There was no significant differences were found between all groups, however the mean values of Testicular LPO in sham-operate, curcumin+Pb, training+Pb and training+curcumin+Pbgroups was 15.87%, 47.94%, 53.97% and 23.85%lower than lead group respectively. As well as the mean values of epididymal LPO in sham-operate, curcumin+Pb, training+Pband training+curcumin+Pbgroups was 1.29%, 3.32%, 5.38% and 6.39% lower than lead acetate group respectively. Figure 1 shows the effect of training and curcumin on Testicular and epididymal LPO levels in rats exposed to lead acetate.

Table2. Effect of exercise training and curcumin(CCM)on the testes and epididymis/body weight ratios in rats chronically exposure to lead acetate (Pb)

Groups / Variable	Sham-operate	Pb	CCM+Pb	Training+Pb	Training+ CCM+Pb
testes/body weight ratios	0.0039±0.0008	0.0048±0.00051	0.0046±0.00083	0.0049±0.00031	0.0046±0.00029
epididymis/body weight ratios	0.00169±0.00028	0.00196±0.00005	0.00188±0.00019	0.00189±0.00014	0.00174±0.00015

Data are presented as the mean±SD, *n* = 8.

Table3. Effect of exercise training and curcumin(CCM)on reproductive hormones in rats chronically exposure to lead acetate (Pb)

Groups / Hormone	Sham-operate	Pb	CCM+Pb	Training+Pb	Training+ CCM+Pb
Testosterone(ng/ml)	4.11±2.02	3.82±2.76	5.47±3.98	3.82±2.41	4.38±3.38
FSH (mIu/ml)	0.1143±0.0378	0.1000±0.00	0.2000±0.1825	0.1571±0.0534	0.1429±0.0534
LH (mIulml)	0.1714 ±0.1112	0.1571±0.0786	0.2142±0.1911	0.2428±0.1397	0.2571±0.1923

Data are presented as the mean±SD, n = 8; Abbreviations: FSH= Follicle Stimulating Hormone; LH= Luteinizing hormone.

Table4. Effect of exercise training and curcumin(CCM)on the testes and epididymis MDA(nmol/mg protein) in rats chronically exposure to lead acetate (Pb)

Groups / Variable	Sham-operate	Pb	CCM+Pb	Training+Pb	Training+ CCM+Pb
Testes MDA	0.0466±0.0090	0.0540±0.0125	0.0365±0.0191	0.0353±0.0059	0.0434±0.0217
Epididymis MDA	0.2087±0.0768	0.2114±0.0522	0.2046±0.0514	0.2006±0.0171	0.1987±0.0214

Data are presented as the mean±SD, *n* = 8; Abbreviations: MDA= malondialdehyde

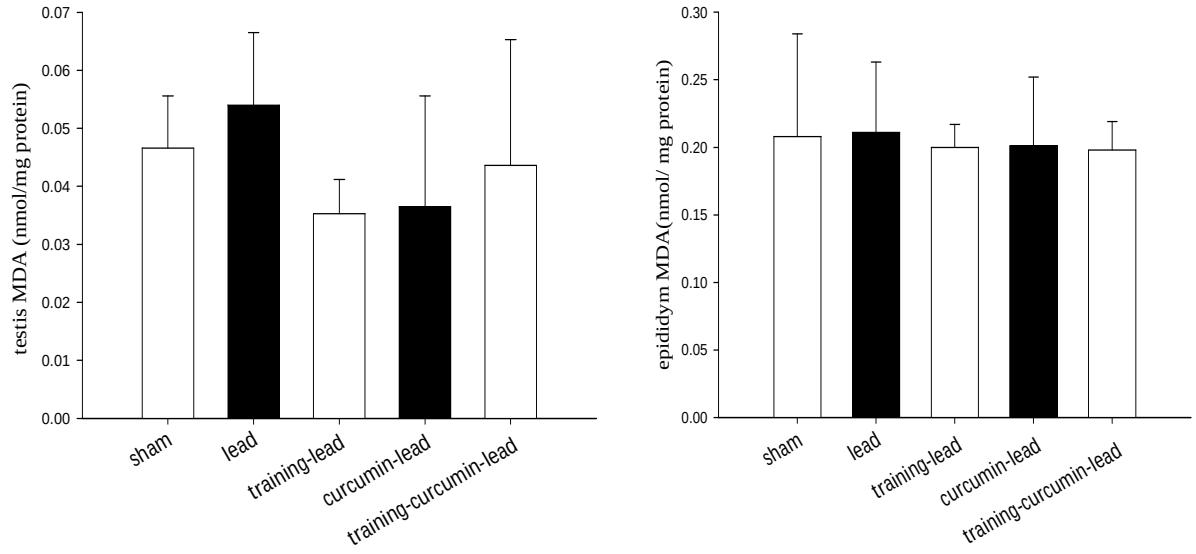

Figure 1. Testis and epididymis MDA concentrations in experimental animals.Data are presented as the mean±SD, $n = 8$.

Discussion and Conclusion

The purpose of the present study is to find out the ameliorating effect of endurance training and curcumin supplementation on lead-induced oxidative damage in reproductive system of adult male Wistar rats. Our data shows that the mean values of testicular and epididymal LPO in sham-operate, curcumin+Pb, training+Pb and training+curcumin+Pb groups was lower than lead group. As well as There was no significant differences were found between motility and morphology of all groups, however the mean values of sperm morphology in sham-operate, curcumin+Pb, training+Pband training+curcumin +Pb groups was higher than Pb group.

Over the years, several studies have investigated the effects of lead exposure on various aspects of health, in particular the reproductive system. Often the results from these studies are conflicting and contradictory, due, in part, to the use of different species of animal models [14]. Adding to the confusion of the data is the finding that the severity of lead toxicity appears to be dependent on the age of the animal at exposure, the length of exposure, the dose administered, and the body burden of lead [14,18].

The results indicated that animals treated with lead acetate (Pb) showed a decrease in sperm motility and morphology, however this reduce not significant. Similar to these observations Pinon-Lataillade et al (1995) reported that sperm motility of the animals treated with Pb was not changed than that of the other groups [19]. The decrease in sperm motility can be due to indirect effects of lead, such as increase of ROS generation in sperm cells. The effects of ROS may involve lipid oxidation, in particular, membrane lipids that are required to give the plasma membrane fluidity, which is essential for sperm motility, and structural integrity, and ultimately, for sperm viability [18]. Another mechanism by which ROS production can influence sperm motility is by a decreased phosphorylation of axonemal proteins, required for sperm movement [18]. A third hypothesis was proposed by Armstrong and colleagues (1999), and may involve ROS induced reduction in ATP levels, leading to a decreasein sperm motility, without a decline in mitochondrial membrane potential [20]. On the other hand, direct effects of lead on sperm motility must also be considered. It has been demonstrated that metals inhibit microtubule sliding [18]. Lead may also interfere with calcium binding to calmodulin, as already reported for cadmium [18] affecting protein tyrosine phosphorylation of some sperm components and therefore reducing sperm motility without altering

viability [18,20]. Thus, further studies of both direct and indirect mechanisms of lead-induced damage are still needed. One of the most commonly observed effects of lead on sperm is an increase in cells presenting an abnormal morphology [18]. In agreement with the data found in the literature, in the present study, a decrease in the percentage of normal sperm in rats injected with 2 mg/kg Pb of the experiment was observed.

Pb treatment increased the lipid oxidation levels, as determined by measuring the testicular MDA levels (table 4), however it is not significant. Lead is well known to produce oxidative damage in enhancing lipid peroxidation(LP) [1,13]. LP inactivates cell constituents by oxidation or cause oxidative stress by undergoing radical chain reaction ultimately leading to loss of membrane integrity [3,21]. In the current study, treatment with Pb resulted in an increase in LP as indicated by the increase in testicular MDA and the decrease in sperm motility and increase abnormal sperm.

Other authors suggest that a principal mechanism of action of lead toxicity at the level of the hypothalamic-pituitary axis or a combined defect involving the gonad and hypothalamic-pituitary sites [22]. However, our data indicate that lead has no significant effects on the reproductive system via the hypothalamic-pituitary-gonadal axis. Previous studies have also reported that there is no change in androgen levels in animals exposed to lead [14]. While Pb on its own reduced the sperm motility, morphology and testicular MDA, co-treatment with curcumin reversed all of these effects. Oxidative damage caused by Pb exposure can be prevented by free radical scavengers or antioxidants, which further strengthens the hypothesis that free radicals play a key role in Pb toxicity [3,6,13]. Antioxidants are the frontline of defense against free radicals [5]. The antioxidant mechanism of curcumin is due to its specific conjugated structure of two methoxylated phenols and an enol form of β-diketone [5]. This structure is responsible for free radical trapping ability as a chainbreaking antioxidant [10,23]. Curcumin prevents free radical generation by competing with peroxidant metals for cell binding sites, which decrease the possibility of free radical formation or by maintaining the activities of antioxidant enzymes like SOD and catalase [5,13,23]. It also protects free radical induced damage by defending sulfhydryl groups against oxidation [5].

In addition, while animals performed endurance training, the toxic effects of Pb are reduced. We demonstrated that decreased oxidative damage in the testis is associated with improved sperm quality induced by endurance training. In one study Srinivasulu et al (2008) reported that regular exercise might protect the male reproductive system against diseases that involve oxidative stress [24]. Physical exercise appears to increase accumulation of free radicals in response to increased oxygen utilization [11]. Elevated metabolic rates, resulting from exercise, may dramatically increase maximal oxygen consumption to 20 times more than that at steady state and 100-fold at the muscle fiber level [25]. Evidence indicates that aerobic physical exercise-generated ROS, such as superoxide anion and hydrogenperoxide, are able to cause cellular damage and inflammation [11]. Despite this, elaborated oxidative defense systems may be induced, counteracting oxidative damage, as a result of regular physical-exercise programs [11]. Physical exercise, associated with oxidative stress, depends on the type and intensity of exercise. However, studies have demonstrated that endurance training improves the antioxidant defense and the oxidative capacity in skeletal muscle [11,12,26]. Beneficial effects of training on antioxidant defense mechanisms in various tissues have been reported in treadmill training [11,12,26].

In conclusion, the present results demonstrate that the reproductive system is induced by lead acetate-induced stress. Therefore, the reproductive system is sensitive to stress. Moreover, our study results shows that regular moderate endurance running and using curcumin turmeric supplementation are suitable guideline to inhibit the negative effects of lead on reproductive system. However, study to establish the effect of stress on the reproductive system is in progress.

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