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Treadmill Running and Antioxidants Supplementation Increases Cardiac Apelin and Oxidative Stress-related Biomarkers in Rats

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Abstract

Purpose: The American Heart Association (AHA) published a statement on the importance of air pollution in the development of cardiovascular disease. Apelin is a multifunctional neuropeptide that plays an important regulatory role in cardiac dysfunction. We have investigated the cardioprotective effects of treadmill running and/or curcumin supplementation on the cardiac apelin and oxidative stress-related biomarkers such as total antioxidant capacity (TAC) and malondialdehyde (MDA) in male rats exposed to lead acetate.

Material and Methods: In this study Forty male Wistar rats were randomly divided into 5 groups: (1) lead acetate; (2) curcumin; (3) treadmill running; (4) treadmill+ curcumin; and (5) sham-operate groups. The rats in groups 1 to 4 received lead acetate (20 mg/kg). Also, groups 3 and 4 underwent treadmill running, 15 to 22 m/min for 25 to 64 minutes, 5 times a week, for 8 weeks, while groups 2 and 4 received curcumin solution (30 mg/kg) intraperitoneally. Rats in group 5 only received curcumin solvent (ethyl oleat).

Results: Lead administration resulted in a significant decrease in apelin, heart mass, TAC levels, and significantly increased MDA. Furthermore, treadmill running and\or curcumin supplementation resulted in a significant increase in apelin, heart mass, TAC levels and a significant decrease in MDA levels.

Discussion and Conclusion: These results suggest a cardioprotective effect of antioxidants and regular endurance training in ameliorating lead-induced cardiotoxicity.

Key words: Apelin, Antioxidant, Cardiovascular Disease, Exercise training, Lead

Introduction

Air pollution is known to induce a broad range of physiological, biochemical, and behavioral abnormalities in humans [1, 2], and recently, the American Heart Association (AHA) published a statement on the importance of air pollution in the development of cardiovascular disease [3]. Lead is a persistent and common environmental contaminant, and like other commonly found, persistent toxic metals such as mercury, arsenic, and cadmium, it damages cellular material and alters cellular genetics [4]. The mechanism all of these toxic metals have in common involves oxidative damage. Toxic metals increase production of free radicals and decrease availability of antioxidant reserves to respond to the resultant damage [4]. Recent studies have reported lead's potential for inducing oxidative stress and evidence is accumulating in support of an important role for oxidative stress in pathophysiology of lead poisoning [2].

Apelin is a multifunctional neuropeptide that regulates body fluid homeostasis, food intake and respiratory and biological rhythm [5, 6]. It has been detected in various tissues, including the heart, lung, testes, ovaries, mammary glands, brain, liver, skeletal muscle, and kidney [6]. In the cardiovascular system, apelin has been detected in rat and human vascular endothelial cells. It is well known that apelin is an autocrine/ paracrine factor in cardiovascular tissues and one of the most potent positive inotropic substances identified to date. Apelin may play an important regulatory role in human cardiac dysfunction and might be an acute inotropic agent in patients with ischemic heart failure [6].

Under normal and resting conditions, reactive oxygen species (ROS) are removed from the cell

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preventing any subsequent damage [7]. However, under more extreme conditions, the body's endogenous antioxidant system is not able to effectively remove excessive ROS production [7]. It has been suggested that increasing the circulating levels of antioxidants will help to prevent the accumulation of free radicals inside our cells, thus reducing oxidative stress [7]. Supplementation with antioxidants, either through an increased consumption in the diet or from supplementation, has become extremely popular as a means to improve one's health [7]. Some plant products have also been reported to cause augmentation of myocardial antioxidants. A more recent discovery is Curcumin {1,7-bis(4-hydroxy-3 methoxyphenyl)-1,6- heptadiene-3,5-dione} (diferuloyl methane), the principle colouring agent present in the rhizomes of Curcuma longa (zingiberaceae) [8]. It has been reported as an efficient antioxidant, anti-inflammatory, and hypoglycemic agent [9, 10]. It also acts as a scavenger of oxygen free radicals and some cardiovascular protective effects have been demonstrated [9]. Because ROS have been implicated in the development of various pathological conditions, curcumin has the potential to control these diseases though its antioxidant activity [8-10].

There has been a great deal of attention paid to the role of lipid peroxidation and antioxidant effects of physical training in recent years. Many studies have reported that acute submaximal exercise increases exercise-induced lipid peroxidation. On the other hand, Dabidi Roshan et al. recently reported regular physical training caused an increase in the antioxidant system and a reduction in lipid peroxidation [11, 12]. While the concept that regular exercise favorably changes established cardiovascular risk factors is well established [13], the effect of treadmill running on cardiovascular apelin activity is yet unknown. Zhang et al. investigated the effect of swimming exercises for 9 weeks on apelin present in cardiovascular tissues of spontaneously hypertensive rats, and revealed that an improvement in systolic blood pressure in response to training was accompanied by an up-regulation of apelin expression in the aorta and myocardium in spontaneously hypertensive rats [6]. Despite this, limited data is available with respect to concomitant effects of regular aerobic training and antioxidant supplementation, particularly apelin level and oxidative/antioxidant biomarkers in left ventricle tissue during chronic exposure to air pollution. Hence, the aim of the current study was to examine the effects of treadmill running and herbal supplementation of curcumin on left ventriclar apelin and oxidative stress-related biomarkers (MDA and TAC) in male rats exposed to lead acetate.

Material and Methods

Forty adult, male, Wistar rats, (initial body weight 240 ± 20 g) were obtained from the Laboratory of Animal Bearing and Multiplying at the Pasture Institute of Iran. Each rat was housed in a single standard cage of polycarbonate (20 × 15 × 15), made at the Pasture Institute of Iran, in a large air-conditioned room with controlled temperature of 22 ± 2ºC, 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 g of body weight for each rat. Water was available ad libitum.

We replicated 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 [11, 12]. 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. Rats were familiarized with the laboratory environment and running on the treadmill, and then were randomly assigned into 5 experimental groups of eight rats each. The groups were defined as follows: group 1- these animals were exposed to lead acetate(Pb) at a concentration of 20 mg/kg in the form of a water solution (for intraperitoneal [ip] injection), 3 days a week, for 8 weeks; group 2 - Curcumin (Cum) this group similarly received lead acetate, as well as curcumin 30 mg/kg 5 days weekly for 8 weeks (ip); group 3 - treadmill running (Pb + treadmill) - these rats similarly received lead acetate, and performed progressive running exercise of 15 to 22 m/min for 25 to 64 minutes, 5 times a week; group 4 - treadmill and Curcumin (Pb + treadmill + Cum); the rats in this group performed a training protocol of running on the treadmill similar to that in group 3, and received lead acetate and curcumin

supplementation as well; group 5 - the sham-operate or control group (sham); these rats received 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 ip 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 [11, 12]. All groups were anesthetized with ketamine and Xaylozine and decapitated after 12 to 14 hours of overnight fast. Blood samples were collected 24 hours after the last dose of treatment. These blood samples were initially centrifuged by a refrigerated centrifuge at 3000 rpm for 15 minutes within 30 minutes of collection and were stored at -80ºC for subsequent TAC assay. The body cavities were then opened and the heart was quickly excised from the aortic root. Heart tissues were weighed and the left ventricle was placed into Petri dishes containing cold isolation medium (0.1 mol/L K_2HPO_4, 0.15 mol/L NaCl, pH 7.4) to remove the blood and were frozen immediately in liquid nitrogen and stored at -80ºC for subsequent analysis of apelin and MDA. Left ventricular tissue was squashed in liquid nitrogen, homogenized in a lysis buffer (5 ml/g of tissue) with a protease inhibitor cocktail for mammalian cell and tissue extracts (SigmaeAldrich, St. Louis, U.S.A) 100 ul/1 ml, and 10 mM tris base (Sigma-Aldrich, St. Louis, U.S.A), pH 7.4 and was centrifuged at 1600 g at 4 ºC for 15 min. Left ventricular supernatant was diluted 1:30. Plasma was diluted 1:10 and the fluids were used in an Apelin-13 kit (Phoenix peptides, Burlingame, California, USA), following the manufacturer's instructions, as previously described by Andersen and et al. (2009) [14]. The kit detects apelin-13 with 100% cross reactivity, and has an inter-assay variation of <14%. All samples were analyzed in duplicate. The coefficient of variation was calculated based on the duplicate analyses. All samples were analyzed in the same assay to avoid inter-assay variations. The detection of apelin was also controlled with a diluted apelin peptide. Control experiments for non-specific colour reactions were performed. Absorbance was read at 450 nm on an ELISA plate reader (ELx808 Ultraplate Reader, Biotek Instruments, Inc., UK). All samples were processed in the same assay to avoid inter-assay variations. Lipid peroxidation (MDA) levels in the left ventricle tissue were measured with the thiobarbituric-acid reaction using the method of Ohkawa et al. The quantification of thiobarbituric acid reactive substances was determined at 532 nm by comparing the absorption to a standard curve of MDA equivalents generated by acid catalyzed hydrolysis of 1, 1, 3, 3 tetramethoxypropane. The values of MDA in the left ventricle were expressed as nmol/mg tissue.

Serum TAC was measured using a commercially available kit (Randox Laboratories, Crumlin, UK) as previously described by Dabidi-Roshan et al (2011) and Asali et al (2011) [11, 12]. In this method, the most potent radical, hydroxyl radical, is produced. First, a ferrous ion solution is mixed with hydrogen peroxide. The sequentially produced radicals such as the brown colored dianisidinyl radical cations, produced by the hydroxyl radical, are potent radicals. The anti-oxidative effect of the sample against the potent free radical reactions is then measured. The assay has excellent precision, which has been shown to be higher than 97%. The results are expressed in mmol/mL. In accordance with the protocols of Daniel et al. (2004) and Dabidi Roshan et al. (2011), and Asali et al.(2011), we analyzed the lead acetate concentration using a spectrophotometer method only in the lead acetate group [8, 11, 12].

Statistical analysis was performed using a commercial software package (SPSS version 16.0 for Windows). Results are expressed as mean $\pm$ SE. Data for heart apelin and oxidative stress-related biomarkers were normally distributed after log-transformation. A one-way analysis of variances (ANOVA) was used to detect statistical differences between the groups. A post-hoc test (Tukey test) was performed to determine differences in the various markers between groups. The differences were considered significant at $P < 0.05$.

Results

Data in Table 1 show changes in left ventriclar apelin levels, and oxidative stress-related biomarkers in the rats exposed to lead acetate. Intra-peritoneal administration of lead acetate (20 mg/kg) caused a decrease in the levels of left ventricular apelin, by 38%, as compared to the sham group ($P < 0.001$) (Figure 1). Furthermore, the administration of lead acetate for 8 weeks resulted in a decrease in TAC levels by 27%, and an increase in MDA levels by 71%, in comparison to the sham group (both $P < 0.01$) (Figures 2 and 3).

Treadmill running and/ or curcumin + treadmill + lead treatment significantly decreased apelin, TAC, and MDA levels ($P < 0.001$, < 0.001, and $P < 0.01$, respectively) (Figures 1 - 3). Treatment with curcumin resulted in significant increases in MDA and TAC levels (Figures 2 and 3), and insignificant changes in apelin levels and heart mass (Figures 1 and 4). However, curcumin + treadmill + lead treatment was more effective than curcumin + lead and treadmill + lead alone.

Mean values of body mass, heart mass, and heart/body mass ratio for the five groups are shown in Table 2. After lead acetate administration (20 mg/kg), decreases in body mass and heart mass were detected as compared to the other groups ($P < 0.05$). In addition, curcumin + treadmill + lead treatment during chronic exposure to lead acetate caused preservation in body mass and heart mass (Figure 4). However, only in the curcumin + treadmill + lead treatment group significant increases in heart mass and heart-to-body mass ratio were observed as compared to rats in the lead acetate group ($P < 0.01$).

Discussion and Conclusion

We demonstrated that apelin levels are elevated in rats after treatment with curcumin and treadmill running by reversing oxidative stress-related biomarkers (MDA and TAC) by exposure to lead acetate. Animal and human studies suggest that apelin is a cardioprotective peptide, both in vivo and in vitro, and that it acts through accepted cardioprotective mechanisms and may play a role in the pathogenesis of heart failure [15]. A meta-analysis from the Cochrane Database has confirmed that exercise training is highly effective in patients with coronary artery disease (CAD) [13]. Regular exercise favorably changes established cardiovascular risk factors such as hyperlipidemia, hypertension and diabetes mellitus [13]. Zhang et al. (2006) revealed that long-term swimming training reduced pathogenesis related to hypertension and reversed the down-regulation of

Table 1: Effect of treadmill running and Curcumin on left ventriclar apelin, heart mass, TAC and MDA levels in rats during chronic exposure to lead acetate.

groups	Markers		
	Apelin (pg/mg)	TAC (µmol/ml)	MDA (nmol/mg)
curcumin resolvent (sham)	3.463±.357	385.75±8.89	26.83±2.85
Treadmill Running + lead	4.847±.655	11.75±13.87	18.41±3.28
training + curcumin + lead	6.047±.879	450.38±19.74	13.36±2.54
lead acetate	2.132±.518	279.86±18.33	46.09±9.22
curcumin + lead	4.363±.537	411.71±14.80	35.83±6.50

(Mean ± SEM for 8 Rats); Abbreviations: Total Antioxidant Capacity (TAC) and malondialdehyde (MDA)

Table 2: Effect of treadmill running and Curcumin on body mass, heart mass and heart-body mass ratio in rats during chronic exposure to lead acetate.

groups	Markers of Mass		
	body mass(g)	heart mass(g)	heart-body mass ratio
curcumin resolvent (sham)	342 ± 34	1.070±.152	3.12±0.13
Treadmill Running + lead	328 ± 20	1.236± 0.114	3.76±0.12
training + curcumin + lead	343 ± 43	$‡1.273± 0.136	$‡3.72±0.07
lead acetate	317 ± 24	1.063±0.104	3.35±0.07
curcumin + lead	322 ± 23	1.067±0.161#	3.29±0.26#

Values are mean±standard deviation; ‡ significantly different from sham group ($P < 0.001$), $ significantly different from lead ($P < 0.001$), # significantly different between combined (training + lead + curcumin) group with training and or curcumin groups ($P < 0.05$).

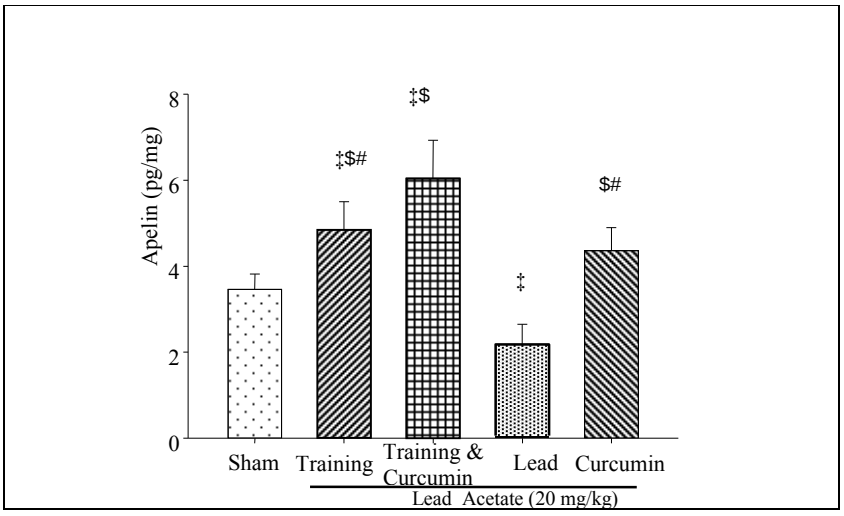

Figure 1: Left ventricle apelin levels during chronic exposure to lead acetate. Abbreviation; Sham (Curcumin resolvent or ethyl oleate), Training (Treadmill Running + Lead), Training & Curcumin (Training + Curcumin + Lead), Lead (lead acetate), Curcumin (Curcumin+ lead). Data are presented as the mean±SD for 8 Rats;
‡ significantly different from sham group ($P < 0.001$), $ significantly different from lead group ($P < 0.001$), # significantly different between combination (training + lead + curcumin) and with training and or curcumin groups ($P < 0.05$).

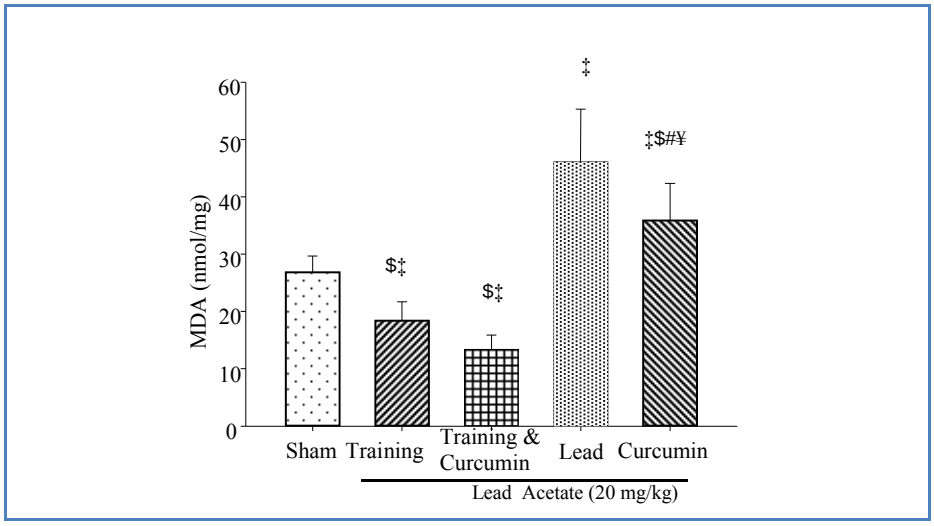

Figure 2: Left ventriclar malondialdehyde (MDA) levels in rats during chronic exposure to lead acetate. Abbreviations; Sham (Curcumin resolvent or ethyl oleate), Training (Treadmill Running + Lead), Training & Curcumin (Training + Curcumin + Lead), Lead (lead acetate), Curcumin (Curcumin+ lead). Data are presented as mean±SD;
‡ significantly different from sham group ($P < 0.001$), ¥ significantly different between training and curcumin groups ($P < 0.001$), $ significantly different from lead group ($P < 0.001$), # significantly different between combination (training + lead + curcumin) group with training and/ or curcumin groups ($P < 0.05$).

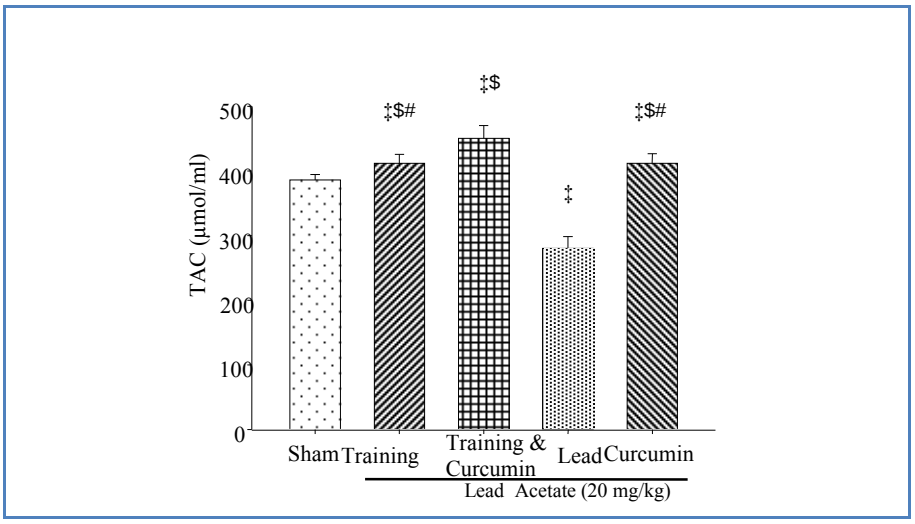

Figure 3: Serum total Antioxidant Capacity (TAC) levels in rats during chronic exposure to lead acetate. Abbreviation; Sham (Curcumin resolvent or ethyl oleate), Training (Treadmill Running + Lead), Training & Curcumin (Training + Curcumin + Lead), Lead (lead acetate), Curcumin (Curcumin+ lead). Data are presented as the mean±SD for 8 Rats; ‡ significantly different from sham group ($P < 0.001$), $ significantly different from lead ($P < 0.001$), # significantly different between combination(training + lead + curcumin) group with training and or curcumin groups ($P < 0.05$).

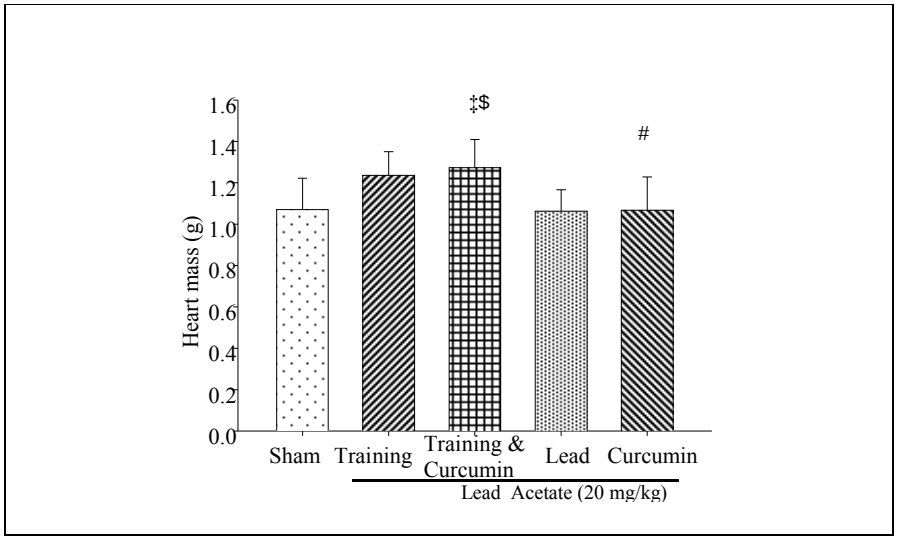

Figure 4: Heart mass changes in rats during chronic exposure to lead acetate. Abbreviation; Sham (Curcumin resolvent or ethyl oleate), Training (Treadmill Running + Lead), Training & Curcumin (Training + Curcumin + Lead), Lead (lead acetate), Curcumin (Curcumin+ lead). Data are presented as the mean±SD for 8 Rats;
‡ significantly different from sham group ($P < 0.001$), $ significantly different from lead ($P < 0.001$), # significantly different between combination(training + lead + curcumin) group with training and or curcumin groups ($P < 0.05$).

the cardiovascular apelin induced by hypertension. This suggests that the effects of exercise training on hypertension could be mediated by up-regulating cardiovascular apelin [5]. In addition, training reduces peripheral inflammatory markers associated with endothelial dysfunction in patients with heart failure [11]. Although exercise acutely increases oxidative metabolism and thereby induces oxidative stress, there is evidence that long-term physical activity increases antioxidant defenses through up-regulation of antioxidant enzymes [11]. Furthermore, this antioxidant effect of exercise reduces the susceptibility of LDL to oxidation, which in turn prevents endothelial injury and inflammation [11]. Frederico et al.(2009) reported that 12 weeks of treadmill training increased antioxidant enzymes and decreased oxidative damage and injury in the myocardium through

reducing MDA and the elevation of CK-MB induced by isoproterenol [16]. Our results also corroborate these findings.

In the current study, we observed that exposure to lead caused a decrease in apelin levels and heart mass. However, curcumin and treadmill running treatment did not change the heart mass as compared to the sham-operate group. This may reflect an important role of air pollution and lead exposure in cardiovascular damage versus lead-induced myocardial damage. Lead is an environmentally persistent toxin that causes neurological, hematological, gastrointestinal, reproductive, circulatory, and immunological pathologies [17]. Recent studies suggest that one of the mechanisms by which lead can exert some of its toxic effects is through the disruption of the delicate pro-oxidant/antioxidant balance that exists within mammalian cells. In vivo studies have suggested that lead exposure is capable of generating ROS and thus alters antioxidant defense systems in animals [11, 12]. In addition, ROS alters cellular membranes and tissue, resulting in cardiovascular, neurological, and genetic damage [4]. Thus, the potential exists for biological mechanisms linking pollutants such as lead with cardiovascular disease through inflammation and oxidative stress [11, 12]. Results of the current study suggest that changes in oxidative stress biomarkers (eg. increase in MDA and decrease in TAC) in male rats exposed to lead acetate underlie oxidative damage in the heart muscle.

Conversely, treatment by curcumin and treadmill running restored oxidative stress-related biomarkers (MDA and TAC) in rats exposed to lead acetate, suggesting a reversal of lead-induced cardiotoxicity. This confirms the free radical scavenging property of curcumin (Table 1; Figure 1). Curcumin may thus have a preventive effect on cardiac hypertrophy and heart failure [9]. Li and coworkers observed that rodents treated with oral curcumin were markedly resistant to cardiac hypertrophy produced by banding of the aorta, and the progression of heart failure was reduced [18]. The increase in antioxidant levels leads to the scavenging of excess free radicals and thereby may contribute to a decrease in oxidative damage, whereas a decrease in the levels of antioxidants should lead to an increase in oxidative damage [7]. Our data indicate a protective effect of curcumin against cardiotoxicity. Sreejayan, et al. revealed that the presence of phenolic groups in the structure of curcumin is fundamental in explaining its ability to eliminate oxygen-derived free radicals from the medium largely responsible for the peroxidation of cell lipids [11]. Indeed, curcumin inhibits lipid peroxidation by scavenging free radicals, interacting with the oxidative cascade, quenching oxygen, inhibiting oxidative enzymes such as cytochrome P450, and chelating metal ions such as Fe2+. In addition, curcumin inhibits peroxidation of membrane lipids and helps maintain cell membrane integrity and function [19, 20]. Curcumin treatment has been shown to yield an increase in cardiac glutathione content, and this treatment may augment the action of these naturally occurring sulphhydryl groups in maintaining membrane integrity and helping promote the non-enzymatic detoxification of hydroxyl radicals and lipid peroxides [19].

In summary, these results demonstrate that administration of lead decreases apelin levels, heart mass, and TAC, and leads to an increase in MDA. Furthermore, curcumin supplementation and/or treadmill running has useful effects on reducing the toxicity of lead through increasing apelin and reducing biomarkers linked to cardiac damage. Finally, simultaneous use of curcumin and treadmill running are more effective than either alone.

References

1- Park SK, O'Neill MS, Vokonas PS, Sparrow D, Wright RO, Coull B, et al. (2008).Air pollution and heart rate variability: Effect modification by chronic lead exposure. Epidemiology 19(1):111-20.

2- Gurer H, Ercal N (2000). Can antioxidants be beneficial in the treatment of lead poisoning? Free Radic Biol Med 15;29(10):927-45.

3- Chuang KJ, Chan CC, Su TC, Lee CT, Tang CS (2007). The effect of urban air pollution on inflammation, oxidative stress, coagulation, and autonomic dysfunction in young adults. Am J Respir Crit Care Med 15;176(4):370-6.

4- Patrick L (2006). Lead toxicity part II: The role of free radical damage and the use of antioxidants in the pathology and treatment of lead toxicity. Altern Med Rev 11(2):114-27.

5- Zhang J, Ren CX, Qi YF, Lou LX, Chen L, Zhang LK, et al. (2006). Exercise training promotes expression of apelin and APJ of cardiovascular tissues in spontaneously hypertensive rats. Life Sci 15;79(12):1153-9.

6- Castan-Laurell I, Vitkova M, Daviaud D, Dray C, Kovacikova M, Kovacova Z, et al. (2008). Effect of

hypocaloric diet-induced weight loss in obese women on plasma apelin and adipose tissue expression of apelin and APJ. Eur J Endocrinol 158(6):905-10.

7- Kerksick C, Willoughby D. (2005). The antioxidant role of glutathione and N-acetyl-cysteine supplements and exercise-induced oxidative stress. J Int Soc Sports Nutr 9;2:38-44.

8- Daniel S, Limson JL, Dairam A, Watkins GM, Daya S (2004). Through metal binding, curcumin protects against lead- and cadmium-induced lipid peroxidation in rat brain homogenates and against lead-induced tissue damage in rat brain. J Inorg Biochem. 98(2):266-75.

9- Wongcharoen W, Phrommintikul A (2009). The protective role of curcumin in cardiovascular diseases. Int J Cardiol 3;133(2):145-51.

10- Pongchaidecha A, Lailerd N, Boonprasert W, Chattipakorn N (2009). Effects of curcuminoid supplement on cardiac autonomic status in high-fat-induced obese rats. Nutrition 25(7-8):870-8.

11- Roshan VD, Assali M, Moghaddam AH, Hosseinzadeh M, Myers J (2011). Exercise training and antioxidants: Effects on rat heart tissue exposed to lead acetate. Int J Toxicol 30(2):190-6.

12- Asali M, Dabidi Roshan V, hosseinzadeh S, Mahjoub S, Hajizadeh Moghaddam A (2011). The Role of Exercising and Curcumin on the Treatment of lead-induced Cardiotoxicity in Rats. Iranian Journal of Health and Physical Activity 2 (1): 1-5.

13- Niessner A, Richter B, Penka M, Steiner S, Strasser B, Ziegler S, et al. (2006). Endurance training reduces circulating inflammatory markers in persons at risk of coronary events: Impact on plaque stabilization? Atherosclerosis 186(1):160-5.

14- Andersen CU, Markvardsen LH, Hilberg O, Simonsen U (2009). Pulmonary apelin levels and effects in rats with hypoxic pulmonary hypertension. Respir Med 103(11):1663-71.

15- Chandrasekaran B, Dar O, McDonagh T (2008). The role of apelin in cardiovascular function and heart failure. Eur J Heart Fail 10(8):725-32.

16- Frederico MJ, Justo SL, Da Luz G, Da Silva S, Medeiros C, Barbosa VA, et al. (2009). Exercise training provides cardioprotection via a reduction in reactive oxygen species in rats submitted to myocardial infarction induced by isoproterenol. Free Radic Res 43(10):957-64.

17- Gholamhosseini B, Khaki A, Khaki AA, Kachabi H, Radsaeed F. (2009). Ultra structure study of lead acetate cytotoxic effects on heart tissue in rabbit. Ofogh-e-Danesh. GMUHS Journal. (in Persian) 14(4): 5-12.

18- Li HL, Liu C, de Couto G, Ouzounian M, Sun M, Wang AB, et al. (2008). Curcumin prevents and reverses murine cardiac hypertrophy. J Clin Invest 118(3):879-93.

19- Venkatesan N (1998). Curcumin attenuation of acute adriamycin myocardial toxicity in rats. Br J Pharmacol 124(3):425-7.

20-. Kalpana C, Sudheer AR, Rajasekharan KN, Menon VP (2007). Comparative effects of curcumin and its synthetic analogue on tissue lipid peroxidation and antioxidant status during nicotine-induced toxicity. Singapore Med J 48(2):124-30.