Hemodynamic and cardiac functions in rats exposed to lead toxicity, the possible effect of vitamin C (ascorbic acid)

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Abstract: Background: Lead is one of the environmental pollutants of high risk to public health. In this study we tried to find if there is a relationship between blood lead level and cardiac function after myocardial ischemia reperfusion injury. In addition, this study tried to evaluate the effect of ascorbic acid [vitamin C (VC)] on cardiovascular parameters in the lead-exposed rats. **Methods:** Twenty four Wister male rats (initially weighing 150 to 170 gm) were divided into three groups; ***Group I (Control rats)*** was given distilled water every day. ***Group II (lead-exposed rats)*** received orally lead acetate solution (60mg/kg body weight), once daily. ***Group III*** (***lead-exposed + VC-treated rats)*** received lead acetate 60 mg /kg, once daily and concurrently supplemented with Vitamin C, 40mg/kg, every other day. Water, lead and vitamin C were all administered by using intra-gastric intubation for 8 weeks. All rats were subjected to an *in vivo* measurement of arterial blood pressure, ECG recording, blood samples collection for determination of lead content in the blood, blood picture, plasma total cholesterol, and HDL, in addition, atherogenic index was calculated. *In vitro* study of isolated hearts to record the intrinsic activity of the heart under baseline condition, responses of the heart to ischemia and reperfusion and determination of cardiac weights. Also glutathione peroxidase activity, and malondialdehyde level were measured in the cardiac tissue. **Results:** Lead exposure increased the concentration of lead in the blood from the pre-exposure level of 2.07 ±0.52µg/dl to 25.24 ±1.86 µg/dl after 8 weeks of exposure in lead-exposed rats. In addition, plasma total cholesterol was significantly increased together with reduction in plasma HDL, both values nearly reversed with the administration of vitamin C. Also decreased level of the MDA and increasing glutathione peroxidase activity in cardiac tissue were observed in lead exposed group. RBCs count, hemoglobin content, Haematocrite value, mean corpuscle volume (MCV), mean corpuscle hemoglobin (MCH), of lead exposed animals were significantly decreased, as compared to control rats. Vitamin C attenuated these hematological changes. ECG showed shortened the QT interval with significant increase in QRS voltage in lead exposed rats. Significant elevation of arterial blood pressure was observed in lead exposed rats as compared to control rats, As regards isolated heart perfusion, baseline chronotropy and inotropy were increased, but myocardial flow rate decreased in lead exposed rats. Vitamin C administration diminished the cardiac toxicity of lead as it normalized the mean arterial pressure, QT interval, as well as, HR regarding the baseline values and after 30 minutes of ischemia reperfusion. **Conclusion:** chronic Lead exposure has toxic effects which disturb the heart function, while natural antioxidant (Vitamin C) may be preferable in reducing Lead toxicity in the exposed rats, suggesting that lead chelating agents having antioxidant properties are preferred in treating cardiovascular disorders accompanying lead toxicity.

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1.Introduction

Cardiovascular disease is the leading cause of mortality and a primary contributor to the burden of disease worldwide [1]. Environmental toxicants, including lead and other metals, are potentially preventable exposures that may explain population variation in cardiovascular disease rates [2-3].

Lead is considered as an environmental pollutant of high risk to public health [4-5]**.** All humans have lead in their body as a result of exposure to exogenous sources [6]. This exposure occurs during the manufacture of ammunition, batteries, sheet lead, solder, ceramic glazes, caulking, bronze plumbing, military equipment, drinking water and some surgical equipment [7-9]. Vegetables are polluted by lead from the air and a considerable amount of lead contamination is found in cereals and leafed vegetables [10]**.**

Blood lead level is the most reliable indicator of lead intoxication. The Center for disease control and prevention redefined the reference value for elevated blood lead level from >25 µg/dl to >10µg/dl [11]**.**

Lead affects the central nervous system [6]**,** and the renal system [12]**,** and population studies have demonstrated an association between lead exposure and hemoglobin disorders and anemia, and peripheral vascular disease [13]**.** In addition, Experimental and

epidemiological studies suggest a close relationship between lead exposure, hypertension and cardiovascular disease [14].

It was reported that, vitamins B6, C and E, selenium, have been shown, in a number of animal studies, to interrupt or minimize the damaging effects of lead and improve the effects of pharmaceutical chelating agents [15].

Vitamins are essential to maintain normal metabolic processes and homeostasis within the body. Vitamin C is an antioxidant that scavenge free radicals [16]. Moreover, the treatment of lead-injected mice with vitamin C inhibited cytotoxic effects of lead on the testes [17]. Vit C reduced lipid peroxidation and oxidative stress result from arsenic [18], Ozone [19], and cadmium [20], toxicities. Combined treatment with vitamin C and vitamin E exhibited a good protection against thioacetamide [21] and radiation induced oxidative stress [22].

Therefore, ***the aim of this study*** was to determine the effects of lead acetate on the hemodynamics and responsiveness of the isolated heart to ischemia reperfusion in male rat. In addition, it evaluates the effect of ascorbic acid [vitamin C] as one of the antioxidant on cardiovascular parameters in the lead-exposed rats.

2. Materials and Methods:

Experimental animals

After approval of the ethics committee in Ain Shams Faculty of Medicine, twenty four male white albino rats initially weighing 150 to 170 gm were provided by the ***Vacsera Animal House (Helwan)*** and housed in a standard animal facility under controlled environmental conditions, with free access to standard laboratory rat diet and water.

Experimental protocols

Rats were randomly assigned to three main experimental groups (8 animals per group):

- ***Group I (Control rats)*** was given 0.5ml distilled water intra-gastrically every day for 8 weeks.
- ***Group II (lead-exposed rats)*** received orally 0.5ml lead acetate (***PbAc***) solution (60mg/kg body weight) for 8 weeks, intra-gastrically once daily [23]. *Lead acetate was made soluble in water by the addition of one or two drops of acetic acid.* [24].
- ***Group III (lead-exposed + VC-treated rats)*** received 60 mg PbAc/kg body weight, once daily and were concurrently supplemented with 40 mg Vitamin C/kg body weight, every other day 30 min before lead acetate administration using intra-gastric intubation for 8 weeks [23].

Chemicals:

Chemicals such as lead acetate, Vit C and all compounds of Krebs-Henseleit solution were obtained from EL NASR PHARMACEUTICAL CHEMICALS CO.

Experimental procedures

Blood pressure measurement:

The rat tail was placed inside the tail cuff and the cuff was inflated and released a few times to allow the animal to be conditioned to the procedure. Systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure were recorded by a tail sphygmomanometer (NIBP200A, Biopac systems Inc; USA).

On the day of sacrifice, overnight fasted rats were weighed and injected intraperitoneally with 5000 IU/Kg B.W heparin sodium (Nile Company, Egypt). Fifteen minutes later, the rats were anaesthetized with intraperitoneal injection of thiopental sodium (Pharco Pharmaceuticals, Egypt), in a dose of 40 mg/kg B.W.

ECG Recording:

Needle electrodes were placed under the skin of the 4 limbs of the anaesthesized animal, near the paws and connected to an ECG recorder (Cardimax Fx-2111, Fukuda Denshi Co., Ltd., Japan). Measurements were made for heart rate, R-wave voltage, duration of P-R interval, QRS complex and Q-T interval.

Blood Sampling:

After recording of the ECG, blood samples were collected from retro-orbital plexus with the help of capillary tube into ***3 plastic tubes***. One tube containing EDTA (Ethylene-Diamine-Tetra-Acetic acid) for determination of blood picture. The second tube was heparinized plastic tube for determination of blood lead concentration. The third tube was heparinized plastic tube which was centrifuged at 4000 rpm for 10 minutes to separate plasma. The plasma was stored at -20°C for later determination of lipid profile.

Isolated Heart Study:

Preparation of the isolated heart:

After collection of the blood samples, a V-shaped incision was made between the upper abdomen and the base of the neck and the attachment of the diaphragm to the ribs was transected. The heart was raised with the fingers and the great vessels were cut out about 5mm distal to the base of the heart. The heart was immediately placed in ice-cold modified Krebs-Henseleit Bicarbonate (KHB) buffer solution for fast cardioplegia.

Then the isolated hearts transferred immediately to a constant pressure Langendorff rat isolated heart set up. The isolated spontaneously beating hearts were continuously perfused with Krebs-Henseleit solution (at 37°C, pH=7.4, gassed with 95% O2 + 5%CO and perfusion pressure of 70 mmHg) with compositions of (in mM/l): NaCl, 118; KCl, 7.4;

NaHCO3, 25.0; KH2PO4, 1.2; CaCl, 2.5; MgSO4.7H2O, 1.2; and Glucose, 11.0.

15 minutes period was allowed in order for the heart to reach a steady state condition prior to any treatment [25].

Ischemia/Reperfusion (I/R) Technique:

Total global ischemia was induced by stopping of the perfusion fluid delivered to the heart by a clamp for 30 minutes, afterwards the heart was reperfused for an additional 30 minutes to record the reperfusion values of cardiac activities at 5, 15 and 30 minutes.

Measurements:

The values of different cardiac activities were calculated from the recordings as follows:

- **The heart rate** (HR, beats per minute) was calculated by the formula:
  - HR= 3000 divided by the distance in (mm) between two successive peaks of tension.
  - For irregular HR, 15000 was divided by the distance between 5 successive peaks.
- ***The peak tension (PT, g)***, which is the amplitude of the recorded contraction force, was measured in mm and equivalent force in g was obtained from calibration curve. The peak tension was, also, calculated per left ventricular weight (PT/LV, g/100mg).
- ***The myocardial flow rate (MFR, ml/min)*** was calculated per minute. MFR was, also, calculated per left ventricular weight (MFR/LV, ml/min/100mg).

Determination of Cardiac Weights:

Following heart perfusion, hearts were washed with normal saline, dried by filter paper and cleaned from fat and fibrous tissue. They were wrapped in parafilm and frozen at -80○C. Cardiac chamber weights (atria, right ventricle and left ventricle) and whole heart weight were expressed as absolute value in (mg) as well as relative values (absolute weight/ body weight ratio, cardiac indices) in mg/g.

Biochemical analysis

Plasma lipid profile

Plasma total cholesterol, and HDL-cholesterol were determined by an enzymatic colorimetric method [26] using kits supplied by Bio-diagnostic, Egypt.

Atherogenic index (AI)

Atherogenic index (AI) was calculated by the following equation AI=(total cholesterol−HDL-cholesterol)/HDL-cholesterol [27].

Determination of lead in blood:

Lead content of whole blood was measured using an atomic absorption spectrophotometer (Shimadzu 680A, with graphite furnace, Shimadzu, Japan) and expressed as micrograms per deciliter (µg/dl) [28].

Measurement of myocardial oxidative stress

Left ventricular homogenate (10%, w/v) was prepared with 0.1 M phosphate buffer solution (PBS) and centrifuged at 12,000 g for 10 min. The supernatant was used to determine glutathione peroxidase activity [29] and MDA levels [30] using kits supplied by Bio-diagnostic, Egypt.

Statistical Analysis:

Student`s-t for paired data, 1-Way ANOVA (Analysis of variance), for all statistical analysis tests, a probability of $P < 0.02$ is considered statistically significant and Mann-Whitney Test**.** All statistical data, statistical significance were performed by using SPSS (Statistical Program for Social Science) statistical Package (SPSS Inc.) version 20.

3. Results

No mortality was observed for rats exposed to lead or treated with vitamin C over the entire period of the study.

Body weight*:* as shown in Table 1 and figure 1 Administration of lead acetate showed a significant decrease ($P< 0.05$) in body weight gain, and hence, slower growth in body weight is encountered in this lead exposed group compared to the control rats.

Biochemical analysis: as shown in Table 2, 3

The blood lead concentration of treated rats after 8weeks was significantly higher than controls, while this elevated value was significantly decreased upon vitamin C administration, although it still higher than control rats.

The plasma total cholesterol level was increased significantly in lead exposed group as compared to control group. On the other hand, the value of cholesterol of rats treated with Vit C are significantly less than those of lead exposed group.

A significant decrease in ***plasma HDL levels*** were recorded in lead exposed group as compared with the control. Meanwhile, vitamin C treated rats showed significant increase HDL than those of in lead exposed group.

There was significant decrease in ***atherogenic index***, in group treated with vitamin C as compared to control. Meanwhile it was elevated significantly in lead exposed group.

Regarding ***Cardiac tissue Malondialdehyde,*** lead exposed rats showed significant increases in left ventricular MDA compared to vitamin C treated rats and to control rats. ***In addition, Cardiac glutathione Peroxidase activity, increased upon*** vitamin C administration to lead exposed rats, but its activity decreased significantly.

Table (1): Mean ± SEM values of *Initial body weight (gm)*, *Final body weight (gm) and Body weight gain (gm)* in the different studied groups.

	Control	*Lead- exposed rats*	*Lead- exposed +VC-treated rats*
Initial body weight (gm)	163.85 ±3.39	170.24 ±5.11	167.84 ±4.04
Final body weight (gm)	201.60 ±4.49	192.16 ±3.93	199.00 ±5.14
Body weight gain (gm)	37.75 ±1.07	21.92 ±0.89 [a]	31.16 ±0.93

a: Significance from control rats (group I) calculated by LSD at P < 0.05.
b: Significance from lead exposed rats calculated by LSD at P < 0.05.

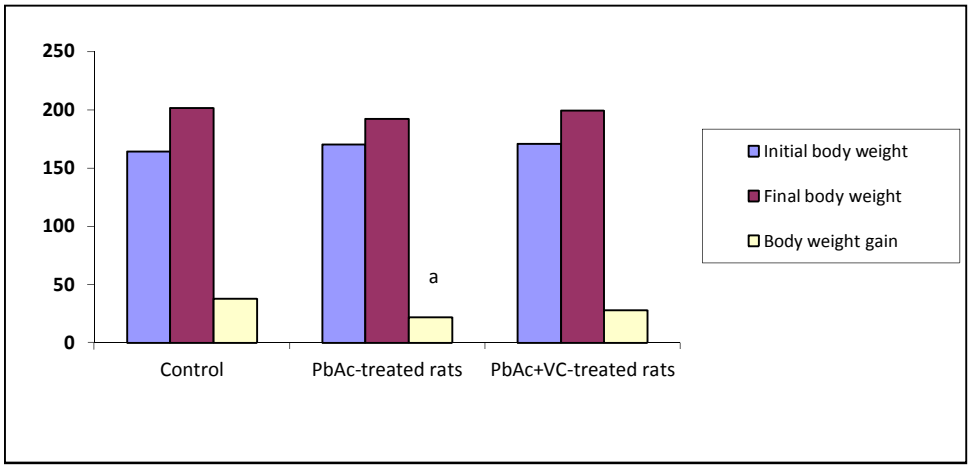

Figure (1): Mean ± SEM values of *Initial body weight (gm)*, *Final body weight (gm) and Body weight gain (gm)* in the different studied groups.

Table (2): Mean ± SEM values of blood lead concentration and plasma levels of total cholesterol, HDL, and calculated atherogenic index in the studied groups.

	Control	*Lead- exposed rats*	*Lead- exposed +VC-treated rats*
Lead concentration (ug/dl)	2.07 ±0.52	25.24±1.86 [a]	5.07 ±0.84 [ab]
Total cholesterol (mg%)	76.76 ± 2.889	93.44± 4.455 [a]	78.92 ±5.190 [b]
HDL (mg%)	63.43 ±4.182	37.44±3.398 [a]	53.93±5.132 [b]
Atherogenic index	1.11 ±0.112	4.30 ±0.238 [a]	2.58 ±0.150 [ab]

a: Significance from control rats (group I) calculated by LSD at P < 0.05.
b: Significance from lead exposed rats (group II) calculated by LSD at P < 0.05.

Table (3): Mean ± SEM values of Cardiac tissue malondialdehyde (MDA) level and glutathione peroxidase activity (GPx) in the studied groups.

	Control	*Lead- exposed rats*	*Lead- exposed +VC-treated rats*
Cardiac MDA (umol/g tissue)	5.3 ± 0.2	9.6 ± 0.5 [a]	6.6 ± 0.4 [b]
Cardiac GPx (mU/mg protein)	7.5 ± 0.3	4.2 ± 0.3 [a]	6.4 ± 0.2 [b]

a: Significance from control rats (group I) calculated by LSD at P < 0.05.
b: Significance from lead exposed rats (group II) calculated by LSD at P < 0.05.

Blood picture in different experimental groups: as shown in Table 4

In lead exposed group, the levels of RBC count, Hb, HV, MCV, MCH, were significantly decreased, while total WBC count was increased in lead exposed group as compared to the control group. No significant changes were observed in mean corpuscle hemoglobin concentration (MCHC) and platelet count between different groups. Vitamin C treated group showed significant higher RBC count, Hb, and HV as compared to the lead exposed group.

Arterial blood pressure changes: as shown in Table 5, Figure 2

In table (5), lead exposed group showed significant increase in systolic blood pressure (SBP) diastolic and mean arterial blood pressure (MAP). These significant elevations nearly normalized in vitamin C treated rats (group III).

Table (4): Blood picture in different experimental groups:

	Control	*Lead-exposed rats*	*Lead-exposed +VC-treated rats*
Haematocrite value (%)	38.67 ±0. 999	26.67 ±0. 882[a]	32.17 ±1.138[ab]
Total Hb (g/dL)	12.83 ±0.873	7.83±0.60 [a]	11.33 ±0.714[b]
RBCs Count X $10^6/mm^3$	8.16 ±0.542	6.00 ±0.365[a]	7.83±0.477[b]
MCV (FL)	41.16 ±1.013	36.17 ±1.249 [a]	37.33 ±1.605
MCH (pg)	18.33 ±0.843	14.50 ±1.118[a]	15.33 ±0.954[a]
MCHC (g/dL)	34.33 ±1.605	32.16 ±1.222	33.83 ±1.536
WBCs count X $10^3/mm^3$	7.83 ±0.872	16.83 ±1.249[a]	14.50±0.991
Platelets	294±81	311±97	308±91

a: Significance from control rats (group I) calculated by LSD at P < 0.05.
b: Significance from lead exposed rats (group II) calculated by LSD at P < 0.05.

Table (5): Mean ± SEM values of systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) of the studied groups.

	Control	*Lead-exposed rats*	*Lead-exposed +VC-treated rats*
SBP (mm/Hg)	110.16 ±4.094	139.66 ±2.716[a]	124.83 ±3.081[ab]
DBP (mm/Hg)	77.66 ±1.646	100.83 ±2.495[a]	91.16 ±2.845[ab]
MAP (mm/Hg)	92.83 ±1.701	112.00 ±1.861[a]	99.00 ±2.863[b]

a: Significance from control rats (group I) calculated by LSD at P < 0.05.
b: Significance from lead exposed rats (group II) calculated by LSD at P < 0.05.

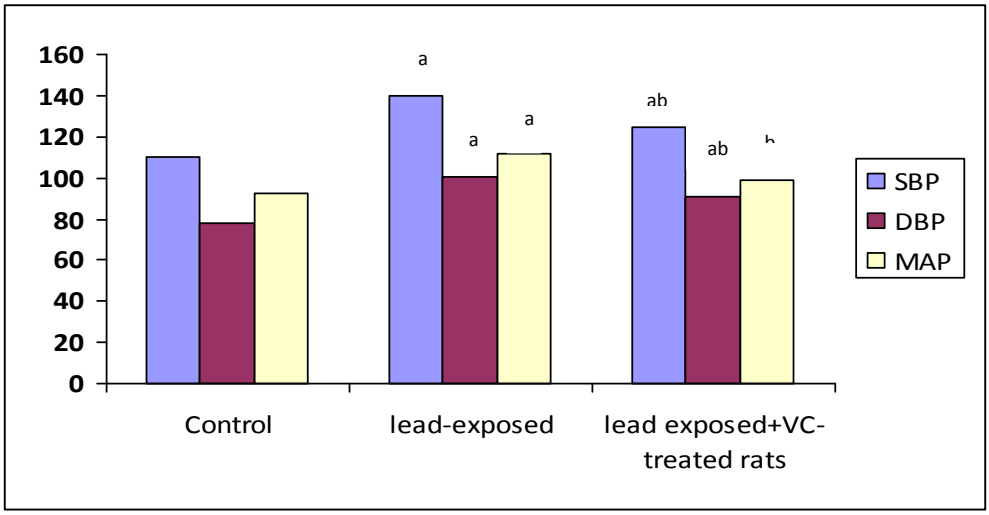

Figure (2): Mean ± SEM values of systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) of the studied groups.

Cardiac weights: as shown in Table 6

The lead exposed rats exhibited significant increase in absolute weights of whole heart (WH) and left ventricle (LV), as well as in their cardiac indices (WH/BW and LV/BW). This hypertrophic response is an expected physiological adaptation to elevated blood pressure. On the other hand, vitamin C treated rats were associated with significant decrease in the left ventricular mass and cardiac indices compared to the control group.

Table (6): Mean ± SEM values of body weight (gm), and absolute cardiac weights (mg) and cardiac indices (mg/g) of left ventricle (LV), whole heart (WH) in the studied groups.

	Control rats	*Lead-exposed rats*	*Lead-exposed +VC-treated rats*
BW (gm)	201.60 ±4.49	192.16 ±3.93	199.00 ±5.14
WH (mg)	663.42±29.54	704.08±25.00 [a]	688.34±31.58
WH/BW (mg/g)	2.71±0.06	3.04±0.09 [a]	2.74±0.12 [b]
LV (mg)	477.98±22.86	533.89±18.71 [a]	495.71±12.35[b]
LV/WH (mg/mg)	2.04±0.05	2.29±0.07 [a]	2.10±0.05 [b]

a: Significance from control rats (group I) calculated by LSD at P < 0.05.
b: Significance from lead exposed rats (group II) calculated by LSD at P < 0.05.

ECG changes: as shown in Table 7

Lead exposed group was associated with significant increase in heart rate and QRS voltage together with significant shortening in Q -T interval in lead exposed group as compared to control group. On the other hand, vitamin C treated rats showed no significant changes in ECG findings compared to lead exposed rats.

Table (7): Mean ± SEM values of some electrocardiographic parameters in the studied groups:

	Control rats	*Lead-exposed rats*	*Lead-exposed +VC-treated rats*
Heart rate(bpm)	325 ±12.11	357 ±15.07 [a]	339 ±13.13
QRS voltage (µv)	290 ±20	334 ±30 [a]	340 ±20
Q-T interval (msec)	84.54 ±3.11	77.15 ±3.60 [a]	78.77 ±1.75

a: Significance from control rats (group I) calculated by LSD at P < 0.05.
b: Significance from lead exposed rats (group II) calculated by LSD at P < 0.05.

Results of ischemia reperfusion of Isolated hearts

***Chronotropic activity*:** Tables (8) and figure (4)

Baseline preischemia values of the HR of hearts isolated from lead exposed rats showed significant increase as compared to the control and vitamin C treated rats. However in reperfusion, there was a significant decrease in heart rate as compared to the control rats, but this decrease was significantly attenuated in vitamin C treated rats. There was a significant decrease in the chronotropic activity in reperfusion as compared to the initial preischaemic values in all studied groups.

Table (8): Baseline value of Heart rate (bpm) and effect of 30 minutes of ischemia-reperfusion of hearts isolated hearts from different studied groups.

	Baseline value (bpm)	**Reperfusion**		
		5 min (bpm)	**15 min** (bpm)	**30 min** (bpm)
Control rats	205 ±10.21	148±7.91*	152±9.24*	146±9.64*
Lead-exposed rats	223±12.34[a]	131±7.88*[a]	128±7.11*[a]	116±7.59*[a]
Lead-exposed +VC-treated rats	196±10.61[b]	159±8.55*[b]	150±8.97*[b]	132±8.11*[ab]

**: Significance from preischemia baseline value calculated by Student`s t- test for paired data at P<0.05*
a: Significance from control rats (group I) calculated by LSD at P < 0.05.
b: Significance from lead exposed rats (group II) calculated by LSD at P < 0.05.

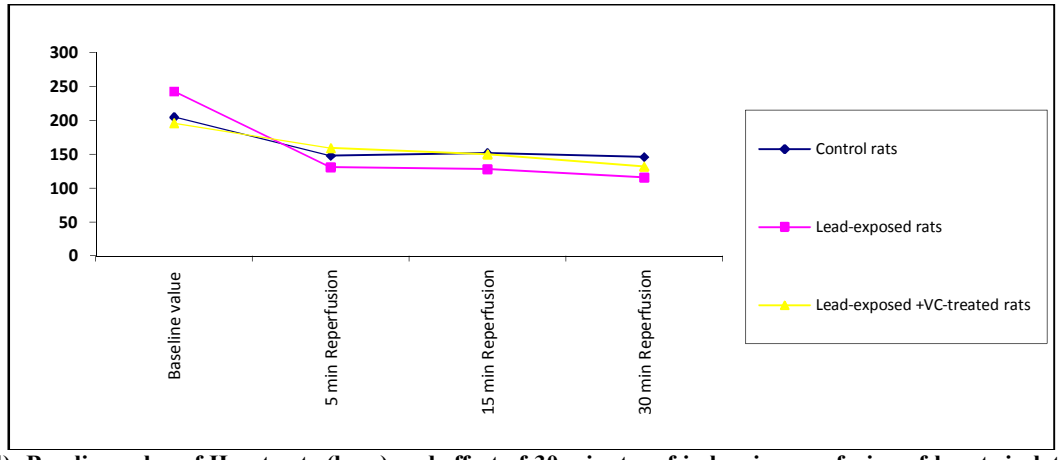

Figure (4): Baseline value of Heart rate (bpm) and effect of 30 minutes of ischemia-reperfusion of hearts isolated hearts from different studied groups.

Myocardial flow rate: Tables (9) and figure (5)

All groups showed significant decrease in MFR and MFR/LV at 5, 15, 30 minutes of reperfusion compared to their respective initial baseline preischemia values. Significant reduction in the preischemia baseline value of MFR was found in lead exposed group as compared to the control group. MFR and MFR/LV reperfusion values showed significant decrease in lead exposed group after 15 and 30minutes compared to control group. In vitamin C treated group, basal MFR and MFR/LV was nearly near to the control and ameliorated the decrease observed in lead exposed group, also vitamin C significantly increase MFR/LV at 15 and 30 minutes compared to lead exposed group.

Table (9): Baseline value of Myocardial flow rate (MFR, ml/min) and MFR per left ventricular weight (ml/min/100mg), and effect of 30 minutes ischemia-reperfusion of hearts isolated from different studied groups.

		Baseline value	Reperfusion		
			5 min	15 min	30 min
Control rats	**MFR** (ml/min)	7.18 ±0.47	5.99±0.52*	5.64±0.57*	6.05±0.71*
	MFR /LV mass (ml/min/100mg)	2.02 ±0.12	1.86±0.11	1.73±0.09*	1.61±0.09*
Lead-exposed rats	**MFR** (ml/min)	6.22 ±0.55 [a]	5.44±0.42*	5.01±0.67*[a]	4.39±0.38*[a]
	MFR /LV mass (ml/min/100mg)	1.97±0.11	1.73±0.15*	1.49±0.13*[a]	1.41±0.08*[a]
Lead-exposed +VC-treated rats	**MFR** (ml/min)	6.92 ±0.41	5.92±0.73*	5.61±0.52*	5.01±0.50*
	MFR /LV mass (ml/min/100mg)	1.99 ±0.10	1.84±0.15*	1.66±0.12*[b]	1.58±0.11*[b]

**: Significance from preischemia baseline value calculated by Student`s t- test for paired data at P<0.05*
a: Significance from control rats (group I) calculated by LSD at P < 0.05.
b: Significance from lead exposed rats (group II) calculated by LSD at P < 0.05.

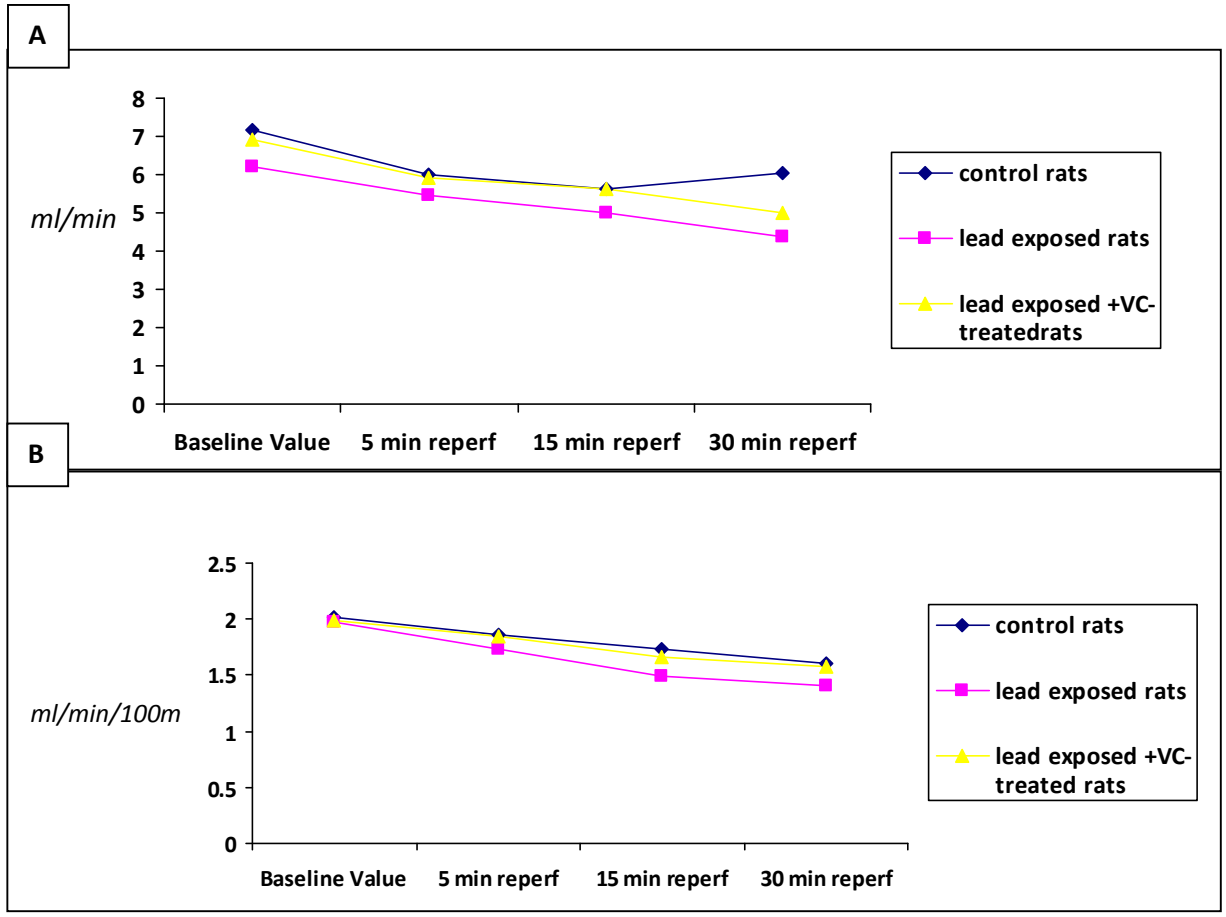

Figure (5): Baseline value of A. Myocardial flow rate (MFR, ml/min) and B. MFR per left ventricular weight (ml/min/100mg), and effect of 30 minutes ischemia-reperfusion of hearts isolated from different studied groups.

***Inotropic activity*:**Tables (10) and figure (6)

Regarding Peak developed tension (PT) and peak developed tension per left ventricular weight (PT/LV), all groups showed significant decrease in PT and PT/LV at 5, 15, 30 minutes of reperfusion compared to their respective initial baseline preischemia values. Significant The preischemia baseline value of PT and PT/LV were found to be significantly elevated in lead exposed group as compared to the control group. The PT and PT/LV

reperfusion values showed significant decrease in lead exposed rats compared to control rats. In vitamin C treated rats, the reperfusion values were not differ significantly from normal control rats.

Table (10): Baseline value of Peak developed tension (PT, gm) and PDT per left ventricular weight, PT /LV mass (g/100mg), and effect of 30 minutes ischemia-reperfusion of hearts isolated from different studied groups.

		Baseline value	Reperfusion		
			5 min	15 min	30 min
Control rats	**PT (gm)**	6.18 ±0.42	4.10 ±0.35*	4.77 ±0.45*	4.38 ±0.43*
	PT /LV mass (g/100mg)	1.64±0.12	1.27±0.08*	1.02±0.09*	1.07±0.11*
Lead-exposed rats	**PT (gm)**	6.98±0.54	3.79±0.33*	4.50±0.44*	3.71±0.43*
	PT /LV mass (g/100mg)	1.93±0.11[a]	0.75±0.07*[a]	0.82±0.08*[a]	0.59±0.06*[a]
Lead-exposed +VC-treated rats	**PT**	6.15±0.42[b]	3.80±0.43*	4.67±0.53*	4.14±0.48*[b]
	PT /LV mass (g/100mg)	1.54±0.13[b]	0.94±0.09*	1.19±0.10*	1.03±0.08*[b]

**: Significance from preischemia baseline value calculated by Student`s t- test for paired data at P<0.05*
a: Significance from control rats (group I) calculated by LSD at P < 0.05.
b: Significance from lead exposed rats (group II) calculated by LSD at P < 0.05.

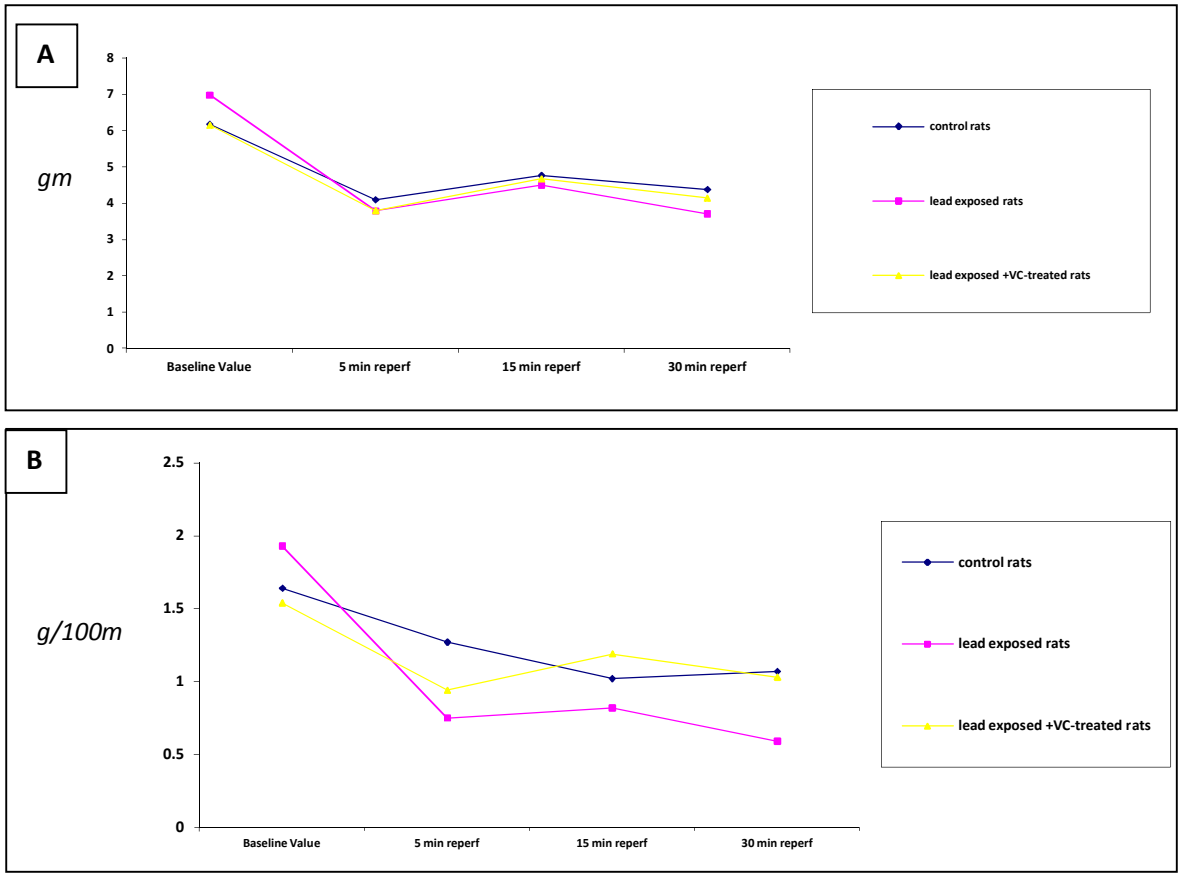

Figure (6):
Baseline value of A. Peak developed tension (PT, gm) and B. PDT per left ventricular weight (g/100mg), and effect of 30 minutes ischemia-reperfusion of hearts isolated from different studied groups.

4. Discussion

The **body weight** gain of intoxicated rats with lead was significantly lower than that of the healthy normal group. The obtained results are in agreement with the findings in previous study which suggested that failure of optimal body weight gain in lead exposed rats may be explained by impairing zinc

status in zinc-dependent enzymes which are necessary for many metabolic processes [31].

The elevated Blood lead concentration in lead exposed group, and its significant decrease with vitamin C intervention in group III. Both effects in agreement with other study [32]**.** Vitamin C might have significant chelation capacity for lead [33]**.** Another explanation is the ability of vitamin C to decrease the intestinal absorption of lead, possibly by reducing ferric iron to ferrous iron in the duodenum, vitamin C increases the availability of iron, which competes with lead for intestinal absorption [34]**.** In addition to acting as an antioxidant and anti absorption agent, vitamin C also has an inhibiting effect on lead uptake on a cellular level [35]**.** Moreover ***Jiao et al.,*** [36] reported that natural antioxidant treatment as vitamin C reduces the blood lead level by metal ion chelating mechanism.

It was noticed in the present study, a significant increase in plasma cholesterol, and a significant decrease of HDL-C of the rats treated with lead. Studies in both humans and animals indicate that lipid metabolism is altered in chronic lead exposure [37]**.** It was concluded that the increase in the levels of triglycerides in lead treated animals may indicate the breakdown of fatty acids. HDL helps to scavenge cholesterol from extra hepatic tissues. Decreased HDL concentration can contribute to the increased cholesterol levels [38]. There is evidence linking increased serum cholesterol and LDL levels to a higher risk for developing coronary heart diseases [39]**.** However other studies found was no difference in the prevalence of HDL-cholesterol level between workers with blood lead level (BLL) > 40 µg/dl and Workers with BLL ≤ 40 µg/dl [40-41].

Obvious oxidative stress was observed in this study by elevation of MDA and decrease in glutathione peroxidase activity. Selenium is essential for GPx activity, and lead forms a complex with selenium, thereby decreases its activity [42]**.** Previous studies were demonstrated that lead exposed animals showed increased lipid peroxidation or decrease in antioxidant defence mechanism [43]. A number of researchers have also shown enhanced rate of lipid peroxidation in brain of lead exposed rats [44]**.** It was reported also that the level of lipid peroxidation was directly proportional to lead concentrations in brain regions [45]**.**The significant decrease in cardiac tissue MDA and increased glutathione peroxidase activity with vitamin C treatment concomitant with lead intoxication, this could be attributed to the antioxidant activity of vitamin C.

Vitamin C is a known **free-radical scavenger** and has been shown to inhibit lipid peroxidation in liver and brain tissue of lead-exposed animals**.** In lead-exposed rats, a minimal 500 mg/L concentration in drinking water was able to reduce ROS levels by 40 percent [46]. The ameliorating effect of vitamin C on these parameters was observed in other studies where the toxic effects of lead on heme production were reversed by a vitamin C dose of 100 mg/kg 57 [47]**.** ***In this study,*** the significant correcting effect of vitamin C on RBCs count, hemoglobin content, haematocrite value, MCV, MCH, and in the blood of lead exposed animals consistent with human findings in previous study [48]. However Total WBCs increased significantly in lead intoxicated rats, as previously described by Choi and Kim, [49]

It was reported that low blood lead levels (about 15 µg/dl) is sufficient to inhibit the activity of aminolevulinic acid dehydratase (ALAD) [50]**.** Failure of normal functioning of ALAD to convert 2 molecules of ALA into prophobilinogen decreases heme formation. This in turn stimulates ALA synthetase, the first enzyme of heme biosynthesis by negative feedback inhibition. As a result of this, there is an increased accumulation of ALA and decreased formation of porphobilinogen [51]**.** A number of studies have shown that accumulation of ALA induces ROS generation [52]**.** *It was reported that* simultaneous supplementation of vitamin E or vitamin C to lead treated erythrocytes prevent the inhibition of δ-aminolevulinic dehydratase activity and lipid oxidation [53]**.** ***Ali et al.,*** [54] also noticed that hematological parameters were reduced due to lead acetate-treatment but when Vitamin C was given along with lead the values tend to be normal.

The increased arterial blood pressure was observed in this study in lead exposed rats as compared to control. This finding in agreement with ***Ghiasvand et al.,*** [41] who showed that high diastolic blood pressure was more common in workers with BLL > 40 µg/dl than in workers with BLL ≤ 40 µg/dl. Moreover, the result in this study also could be supported by similar result [55]**,** but some studies have shown that there were opposite effects [56]. The reduced blood pressure with vitamin C may be attributed to its antioxidant property. There is an inverse relationship with blood pressure and plasma vitamin C. Vitamin C has a lowering effect on blood pressure, especially on systolic pressure more than a diastolic pressure. Increased consumption of ascorbic acid raises serum ascorbic levels and could decrease the risk of death [57]**.**

It was reported that chronic exposure to low levels of lead has been shown to cause hypertension in both humans and animals [58-59]. lead-induced hypertension can be explained by inhibition of sodium pump [60]**,** lowering the Ca^{+2} binding capacity in intracellular Ca stores, leading to an increase intracellular Ca^{+2} concentration [61],

decreased plasma levels of bradikinin [58], increased cardiovascular sensitivity to catecholamines [59], increasing oxidative stress with increased free radicals which may directly raise the arterial blood tension, or indirectly by increased amount of Ca^{2+} in endothelial cells [62-63], reduction of the bioavailability of nitric oxide [64-65], increasing the release of endothelin [66], and increased activity of renin-angiotensin system [67].

A rise of arterial blood tension under lead influence may be followed by the overgrowth of heart muscle and enlarged left ventricular mass which was observed in this study.

Studying the isolated hearts, lead exposed rats showed baseline positive chronotropy and inotropy, but the coronary flow was decreased significantly as compared to the control. However, the positive inotropy and chronotropy was reversed in reperfusion, but the coronary flow still decreased on reperfusion injury.

In the current study, the observed initial positive inotropy and chronotropy in lead exposed rats may be attributed to the increase in Ca^{+2} influx and elevation of intracellular Ca^{+2}, such as activation of protein kinase C that activates Ca^{+2} channel opening [61], inhibition of Na^{+} -K^{+}, ATP_{ase}, [68]. It is already known that sympathetic nerve terminals continue to release catecholamines in isolated preparations [69]. Previous reports on rats exposed to 60 ppm lead acetate indicated increased myocardial inotropism [70].

However, there was a significant reduction in contractility and heart rate following reperfusion. Some reports have indicated that acute lead administration to perfused rat hearts attenuates positive inotropic responses [71]. Moreover, In rat papillary muscles, acute 100 mM lead in the bath, has a negative inotropic effect and reduces myosin ATPase activity [72]. Lead action contributing to calcium channel blocking has also been reported in rat myocytes [73]. Depressed contractility of the heart muscle may also be a result of the interaction between lead and calcium in cardiomiocytes and decreased production of ATP [70].

Regarding myocardial flow rate, there was a significant reduction in lead exposed rats. It has been demonstrated that lead causes constriction of blood vessels through a direct effect on the endothelium and thus reduces blood flow [74]. It may be that lead tends to reduce coronary flow *per se* by mechanisms such as inhibition of nitric oxide synthesis or increase of endothelin or rennin-angiotensin system activation.

Conclusion

From the above study, it was concluded that, Lead Acetate have toxic effects which disturb the heart function, while natural antioxidant (Vitamin C) may be preferable in reducing Lead acetate toxicity in the exposed rats as they have probably no side effects. Also this study suggested that lead chelating agents that have antioxidant properties are preferred in treating cardiovascular disorders accompanying lead toxicity.

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