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## Blood, Serum Glucose and Renal Parameters in Lead-Loaded Albino Rats and Treatment with Some Chelating Agents and Natural Oils

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**Abstract:** This study investigated lead impact on blood, serum glucose and kidney function and the efficacy of treatment with chelating agents meso-2,3-dimercaptosuccinic acid (DMSA), calcium disodium ethylenediaminetetraacetic acid (CaNa<sub>2</sub>-EDTA) and natural olive and garlic oils to reduce lead toxicity in albino rats. Oral administration of 1000 or 2000 ppm lead acetate significantly decreased red blood cell count, hemoglobin level and hematocrit value at 20, 40 and 60 days compared with control groups (rats administered deionized water and 1000 or 2000 ppm sodium acetate). Serum glucose was also decreased significantly with both doses of lead acetate administration. In contrast, serum urea, uric acid and creatinine were significantly increased. Garlic or olive oils (1 ml/kg body weight/day, each) alleviated the previous changes in blood, serum glucose and renal parameters, whereas DMSA (50 mg/kg body weight/day) or CaNa<sub>2</sub>-EDTA (100 mg/kg body weight/day) reversed such changes to near the control levels. Although chemical therapy is more efficient in reducing lead toxicity, natural products with their fewer side effects are still preferable.

**Key Words:** Lead acetate, blood, kidney, albino rats, therapy, chelating agents, natural oils

### Introduction

Lead is a natural element and widespread in the environment. This heavy metal is still mined and added to many products including paints, eye cosmetics, gasoline, water pipes and health care supplies. The two major routes of lead entry into the body are the alimentary and respiratory tracts (1). Irrespective of the exposure pathway, lead enters the bloodstream and is primarily distributed among three compartments: blood and soft and mineralizing tissues (2,3).

Lead poisoning may affect numerous organ systems and is associated with a number of morphological, biochemical and physiological changes, including kidney dysfunction, abnormal glucose metabolism, nervous system disturbances, impairment of liver function and hematological disorders (4-6).

Exposure to lead significantly decreased red blood cell counts, hemoglobin levels and hematocrit values in rats (7). Intramuscular injection of lead acetate (10 mg/kg

body weight) daily for 7 days in rats abolished heme biosynthesis as evidenced by decreased blood hemoglobin (8). It was reported that anemia accompanying lead poisoning is in part the result of various inhibitory effects of lead on heme biosynthesis (9). The rate of red blood cell destruction was also increased. In rabbits, exposure to lead at different doses in drinking water significantly decreased red blood cell count, hemoglobin concentration and hematocrit value (10).

Regarding lead effect on carbohydrate metabolism, it was shown that the rate of glucose metabolism in 4-6-week-old calves given lead acetate (15 mg/kg body weight) orally for 7 to 8 days was less than half the rate in controls (11). The effects of lead on glucose metabolism in the spinal cord in 9 rats exposed to lead for 4 weeks and 10 control rats were determined (12). Glucose metabolism was shown to be significantly lower in the lead-exposed rats compared to control animals. Daily oral administration of lead acetate (40 mg/kg body weight) to rabbits caused significant decrease in serum

glucose levels throughout the experimental periods studied compared with those of the controls (13).

Alterations in renal parameters upon lead exposure were also addressed. Increased levels of blood urea were encountered beginning with week 14 in calves which fed from the 4<sup>th</sup> – 20<sup>th</sup> week of age on a milk powder diet containing 40 mg lead acetate per kg dry substance (14). Oral administration of lead acetate in the diet of mice at concentration 0.5% (W/W) for 1 month induced a significant increase in serum urea and creatinine in comparison with the control group (15). The effect of chronic lead exposure on kidney function in male and female rats was investigated (5). Lead acetate was administered orally at the rate of 0.3 and 0.6%. The treatment continued for 15, 30, 45, 60 and 90 days, and the results showed an increase of creatinemia and uremia on the 30<sup>th</sup> day of the experiment in both sexes.

Efforts have been focused on using chelating agents including meso-2,3-dimercaptosuccinic acid (DMSA) and calcium disodium ethylenediaminetetraacetic acid (CaNa<sub>2</sub>-EDTA) to protect both human and laboratory animals from lead toxicity (16-18). However, not much data are available on natural products therapy (13,19). The present study was undertaken to investigate lead impact on blood, serum glucose and renal parameters and to examine the ability of natural oils (olive and garlic) to combat lead toxicity in albino rats in comparison with synthetic chemical substances (EDTA and DMSA). The findings could be useful to understand lead toxicity and useful protection.

## Materials and Methods

### Experimental animals and dosing

Animals used in the present study were adult male albino rats, weighing 100-120 g. They were purchased from the breeding unit of the Biology Department, Faculty of Science, Islamic University of Gaza. Rats were left for 1 week before experimentation to adapt to laboratory conditions. They were kept in plastic cages with wire mesh covers. The cages were freshly spread with wood saw to absorb urine. Animals were housed 6 to a cage under normal environmental conditions of temperature and humidity with an alternating 12-hour light/dark cycle. Commercial standard diet and water were continuously and regularly supplied *ad libitum* throughout the experimental period. Diet and water were

lead-free. Two sublethal doses of lead acetate (1000 and 2000 ppm) were used (20,21). For each dose of lead acetate, animals were divided into four main groups. The first group represented the control group and was subdivided into two subgroups which were orally administered deionized water and sodium acetate (1000 or 2000 ppm) daily for 60 days. The second group of animals was orally administered lead acetate (1000 or 2000 ppm) daily for 60 days. In the third group of animals, lead acetate was discontinued for 5 days. The fourth group was divided into two subgroups. Animals of one of the subgroups were intraperitoneally injected with 1 ml/kg body weight olive or garlic oils and those of the other subgroup were intraperitoneally injected with 50 mg/kg body weight DMSA or 100 mg/kg body weight CaNa<sub>2</sub>-EDTA daily for 5 days.

Analytical grade lead acetate and sodium acetate were obtained from Riedel-deHaën Laborchemikalien GmbH & Co. Garlic (*Allium sativum* L.) oil was purchased from a local pharmacy, imported from El Captain Company (CAPPHARM), El about-Cairo, Egypt. Virgin olive (*Olea europaea* L.) oil was obtained from a private olive farm. The doses of garlic and olive oils were based on other studies (13,19). The doses of DMSA and CaNa<sub>2</sub>-EDTA were based on previous work (22,23).

### Blood sampling and processing

At each sampling date, 6 animals were taken from each group and subgroup/time interval except for control subgroups, which included 8 animals each. Animals were decapitated and blood was collected into two tubes. The first tube contained calcium EDTA for complete blood count (CBC) analysis. Blood sample in the other tube was left for a short time to allow clotting. Clear serum samples were obtained by centrifugation at 3000 r.p.m. for 20 min. and then kept in the refrigerator for glucose, urea, uric acid and creatinine assay.

### Measurement of primary blood indices, glucose, urea, uric acid and creatinine

Primary blood indices (red blood cells, hemoglobin and hematocrit) were obtained from CBC analysis by a full-automated Sysmex Hematology Instrument. Serum glucose was determined using Trinder method (24). Serum urea measurement was based upon the cleavage of

urea with urease (25). Serum uric acid was determined (26). Serum creatinine was measured without protein precipitation (27).

### Data analysis

Data were computer analyzed using SPSS 8.0 for Windows (Statistical Package for the Social Sciences Inc, Chicago, Illinois). Means were compared by independent-samples t-test. Percentage change was also calculated.

## Results

### Hematological parameters

#### Red Blood Cells (RBCs)

Table 1 illustrates RBC count in control and experimental groups of animals. No significant change was observed in RBC count among control groups ( $P>0.05$ ). In the animal group given lead acetate (1000 ppm), the average values of RBC count were decreased, at rates of 6.2, 13.9 and 13.2% after 20, 40 and 60 days, respectively, compared to controls. Such decreases were significant after 40 and 60 days. In the animal group given 2000 ppm lead acetate, RBC count showed larger decreases, at rates of 14.7, 17.5 and 20.4% compared to control levels. In general, these changes were highly significant ( $P<0.01$ ). Despite discontinuation of lead acetate exposure for 5 days in both groups (1000

and 2000 ppm), RBC count still maintained highly significant decreases at rates of 13.0 and 18.5% compared to controls. Intraperitoneal injection of olive or garlic oils to animals given 1000 ppm lead acetate improved RBC count (particularly garlic oil), with recorded decreases of 10.6 and 7.9% compared to control levels. DMSA and  $\text{CaNa}_2\text{-EDTA}$  returned RBC count to near control values, exhibiting decreases of only 3.2 and 4.2%, respectively. In animals given 2000 ppm lead acetate, olive or garlic oil also improved RBC count, recording decreases of 13.0 and 11.2%, respectively. However, such change was still significant ( $P<0.05$ ). Again, DMSA and  $\text{CaNa}_2\text{-EDTA}$  were able to effectively return RBC count to near the control values.

#### Hemoglobin (Hb)

Hemoglobin content of control and experimental groups of animals is presented in Table 2. The change in Hb content of sodium acetate and deionized water groups was not significant. The effect of lead acetate on Hb content was parallel to its action on RBC count. After administration of 1000 ppm lead acetate, Hb content showed decreases of 4.4, 9.7 and 10.3% after 20, 40 and 60 days, respectively, compared to controls. On the other hand, in animals administered 2000 ppm lead acetate, the decreases were 7.8, 11.8 and 14.9%, respectively. In general, the previous changes were highly significant ( $P<0.01$ ). Even when lead acetate (1000 and 2000 ppm)

Table 1. Effect of lead acetate (1000 and 2000 ppm) administered daily in drinking water on red blood cells count (count  $\times 10^6$  cell/ $\mu\text{l}$ ) of control and experimental groups of albino rats.

Dose	Control groups						Experimental groups							
	Deionized water			Sodium acetate			Lead acetate			Lead discontinuation	Natural oils		Chemicals	
	20	40	60	20	40	60	20	40	60		Olive oil	Garlic oil	DMSA	$\text{CaNa}_2\text{EDTA}$
1000 ppm	6.80 $\pm 0.19$	6.85 $\pm 0.10$	6.95 $\pm 0.17$	6.50 $\pm 0.20$	6.78 $\pm 0.19$	6.88 $\pm 0.26$	6.38 $\pm 0.13$	5.90 $\pm 0.14$	6.03 $\pm 0.21$	5.98 $\pm 0.15$	6.14 $\pm 0.25$	6.33 $\pm 0.27$	6.65 $\pm 0.29$	6.58 $\pm 0.27$
% Change				-4.4	-1.0	-1.0	-6.2	-13.9	13.2	-13.0	-10.6	-7.9	-3.2	-4.2
P				$>0.05$	$>0.05$	$>0.05$	$>0.05$	$<0.01$	$<0.05$	$<0.01$	$<0.05$	$>0.05$	$>0.05$	$>0.05$
2000 ppm	6.80 $\pm 0.19$	6.85 $\pm 0.10$	6.95 $\pm 0.17$	6.65 $\pm 0.17$	6.98 $\pm 0.21$	7.03 $\pm 0.21$	5.80 $\pm 0.22$	5.65 $\pm 0.19$	5.53 $\pm 0.15$	5.60 $\pm 0.17$	5.98 $\pm 0.23$	6.10 $\pm 0.22$	6.48 $\pm 0.27$	6.45 $\pm 0.31$
% Change				-2.2	+1.9	+1.2	-14.7	-17.5	-20.4	-18.5	-13	-11.2	-5.7	-6.1
P				$>0.05$	$>0.05$	$>0.05$	$<0.05$	$<0.01$	$<0.01$	$<0.01$	$<0.05$	$<0.05$	$>0.05$	$>0.05$

Eight experimental animals were used for each control group and six animals for each of the other groups/time interval.

All values are expressed as Mean  $\pm$  SE.

P value:  $>0.05$  non significant,  $<0.05$  significant,  $<0.01$  highly significant and  $<0.001$  more highly significant.

Table 2. Effect of lead acetate (1000 and 2000 ppm) administrated daily in drinking water on blood hemoglobin content (g/dl) of control and experimental groups of albino rats.

Dose	Control groups						Experimental groups							
	Deionized water			Sodium acetate			Lead acetate			Lead discontinuation	Natural oils		Chemicals	
	20	40	60	20	40	60	20	40	60		Olive oil	Garlic oil	DMSA	CaNa <sub>2</sub> EDTA
(Day)			(Day)			(Day)								
1000 ppm	14.2	14.4	14.6	13.8	14.1	14.2	13.6	13	13.1	13.1	13.4	13.5	14.2	14.0
% Change	±0.4	±0.2	±0.2	±0.4	±0.4	±0.5	±0.2	±0.3	±0.2	±0.2	±0.3	±0.3	±0.4	±0.4
P				>0.05	>0.05	>0.05	>0.05	<0.01	<0.01	<0.01	<0.05	>0.05	>0.05	>0.05
2000 ppm	14.2	14.4	14.6	14.0	14.2	14.3	13.1	12.7	12.4	12.4	12.9	13.2	14.0	14.1
% Change	±0.4	±0.2	±0.2	±0.4	±0.4	±0.4	±0.2	±0.4	±0.3	±0.3	±0.4	±0.4	±0.5	±0.5
P				>0.05	>0.05	>0.05	<0.05	<0.01	<0.01	<0.01	<0.05	<0.05	>0.05	>0.05

Eight experimental animals were used for each control group and six animals for each of the other groups/time interval.

All values are expressed as Mean ± SE.

P value: >0.05 non significant, <0.05 significant, <0.01 highly significant and <0.001 more highly significant.

was stopped, Hb content still recorded decreases of 8.9 and 14.1%, respectively, compared to controls (P<0.01). Intraperitoneal injection of olive or garlic oil to rats given 1000 ppm lead acetate returned Hb content towards normal with decreases of 7.0 and 6.1%, respectively, compared to controls. This protective effect was more pronounced in rats injected with DMSA or CaNa<sub>2</sub>-EDTA, where Hb content returned to nearly control levels. In animals given 2000 ppm lead acetate, olive or garlic oil also improved Hb content, registering decreases of 10.1 and 8.3%, respectively. This change was still significant compared to controls (P<0.05). Again, DMSA and CaNa<sub>2</sub>-EDTA were able to return Hb content to close to control values.

### Hematocrit (Hct)

Table 3 demonstrates Hct values of control and experimental animals. There were no significant changes among control groups. In animals given 1000 ppm lead acetate, Hct was gradually decreased, at rates of 9.3, 14.4 and 15.1% after 20, 40 and 60 days, respectively, compared to control values (P<0.05). Animals administered 2000 ppm lead acetate recorded highly significant decrease (P<0.01) in Hct, at rates of 13.7, 16.3 and 17.8%, respectively, compared to controls. Upon lead acetate (1000 and 2000 ppm) discontinuation,

Hct still exhibited decreases at rates of 14.9 and 17.0%, respectively, compared to control values (P<0.01). Intraperitoneal injection of olive or garlic oil to animals treated with 1000 ppm lead acetate alleviated lead effect, registering Hct decreases of 8.9 and 6.7%, respectively, compared to control values (P>0.05). Upon DMSA and CaNa<sub>2</sub>-EDTA therapy, Hct exhibited levels close to control levels. Olive and garlic oils also ameliorated Hct in animals given 2000 ppm lead acetate, showing decreases of 12.4 and 10.2%, respectively. However, Hct values were still significantly lower than controls. On the other hand, treatments with DMSA and CaNa<sub>2</sub>-EDTA returned Hct values to near control values.

### Serum Glucose

The mean values of serum glucose of both control and experimental animals are shown in Table 4. There was no significant change in glucose level among sodium acetate and deionized water groups. Oral administration of lead acetate at a dose of 1000 ppm generally caused a highly significant decrease (P<0.01) in glucose levels, with decreases of 22.9, 20.7 and 27.7% after 20, 40 and 60 days, respectively, compared to control values. The dose of 2000 ppm lead acetate generally provoked a more highly significant decrease (P<0.001) in glucose levels, showing decreases of 28.1, 29.9 and 29.6% after 20,

Table 3. Effect of lead acetate (1000 and 2000 ppm) administrated daily in drinking water on blood hematocrit value (%) of control and experimental groups of albino rats.

Dose	Control groups						Experimental groups							
	Deionized water			Sodium acetate			Lead acetate			Lead discontinuation	Natural oils		Chemicals	
	20	40	60	20	40	60	20	40	60		Olive oil	Garlic oil	DMSA	CaNa <sub>2</sub> EDTA
1000 ppm	38.6	39.0	39.0	37.5	38.3	38.8	35.0	33.4	33.1	33.1	35.4	36.2	37.9	37.7
	±1.0	±1.2	±1.2	±1.7	±1.8	±2.0	±1.2	±1.4	±1.3	±1.2	±1.4	±1.5	±2.2	±1.9
% Change				-2.7	-1.7	-0.5	-9.3	-14.4	-15.1	-14.9	-8.9	-6.7	-2.5	-2.9
P				>0.05	>0.05	>0.05	>0.05	<0.05	<0.05	<0.01	>0.05	>0.05	>0.05	>0.05
2000 ppm	38.6	39.0	39.0	37.6	39.8	40.1	33.3	32.6	32.1	32.3	34.1	34.9	37.0	36.6
	±1.0	±1.2	±1.2	±1.7	±2.0	±2.8	±1.7	±1.1	±1.1	±1.2	±1.4	±1.4	±1.5	±1.4
% Change				-2.4	2.1	2.5	-13.7	-16.3	-17.8	-17	-12.4	-10.2	-4.8	-5.7
P				>0.05	>0.05	>0.05	<0.05	<0.01	<0.01	<0.01	<0.05	<0.05	>0.05	>0.05

Eight experimental animals were used for each control group and six animals for each of the other groups/time interval.

All values are expressed as Mean ± SE.

P value: >0.05 non significant, <0.05 significant, <0.01 highly significant and <0.001 more highly significant.

Table 4. Effect of lead acetate (1000 and 2000 ppm) administrated daily in drinking water on serum glucose levels (mg/dl) of control and experimental groups of albino rats.

Dose	Control groups						Experimental groups							
	Deionized water			Sodium acetate			Lead acetate			Lead discontinuation	Natural oils		Chemicals	
	20	40	60	20	40	60	20	40	60		Olive oil	Garlic oil	DMSA	CaNa <sub>2</sub> EDTA
1000 ppm	105.7	105.2	107.2	108.2	104.2	109.2	81.5	83.5	77.5	76.0	90.5	94.8	111.0	98.0
	±2.5	±2.8	±3.4	±3.9	±4.5	±3.3	±4.2	±2.2	±2.7	±3.6	±3.8	±4.3	±6.0	±3.2
% Change				2.4	-1.0	+1.9	-22.9	-20.7	-27.7	-28.4	-14.7	-10.7	4.6	-7.6
P				>0.05	>0.05	>0.05	<0.01	<0.01	<0.001	<0.001	<0.01	<0.05	>0.05	>0.05
2000 ppm	105.7	105.2	107.2	106.2	104.5	108.7	76.0	73.8	75.5	74.5	90.3	93.5	102.8	103.5
	±2.5	±2.8	±3.4	±3.4	±4.4	±4.8	±3.3	±3.9	±3.1	±4.3	±3.0	±3.3	±3.9	±4.0
% Change				+0.5	-0.7	+1.4	-28.1	-29.9	-29.6	-29.8	-14.9	-11.9	-3.1	-2.4
P				>0.05	>0.05	>0.05	<0.001	<0.01	<0.001	<0.001	<0.01	<0.05	>0.05	>0.05

Eight experimental animals were used for each control group and six animals for each of the other groups/time interval.

All values are expressed as Mean ± SE.

P value: >0.05 non significant, <0.05 significant, <0.01 highly significant and <0.001 more highly significant.

40 and 60 days, respectively, when compared to control levels. After cessation of lead acetate (1000 and 2000 ppm), glucose levels still exhibited decreases of 28.4 and 29.8%, respectively, compared to control levels (P<0.001). When rats treated with 1000 ppm lead acetate were injected with olive or garlic oils, glucose level showed decreases of 14.7 and 10.7%, respectively,

compared to controls. This prophylactic effect was more pronounced with DMSA or CaNa<sub>2</sub>-EDTA therapy, with glucose levels approaching those of controls. In animal groups given 2000 ppm lead acetate, olive and garlic oils also improved glucose levels, showing decreases of 14.9 and 11.9%, respectively. DMSA and CaNa<sub>2</sub>-EDTA therapy returned glucose levels to close to control levels.

**Non-protein nitrogen constituents**

**Serum Urea**

Table 5 shows serum urea concentration of control and experimental animal groups. No significant change in serum urea was found between animal groups given deionized water and sodium acetate. Lead acetate at 1000 ppm dose generally caused highly significant increase ( $P < 0.01$ ) in urea, at rates of 24.3, 42.1 and 27.9% after 20, 40 and 60 days, respectively, compared to controls. Lead acetate at 2000 ppm also provoked a prominent increase in urea, at rates of 27.0, 44.1 and 47.4% after 20, 40 and 60 days, respectively, compared to control levels ( $P < 0.01$ ). Discontinuation of lead acetate (1000 and 2000 ppm) administration only slightly altered this change, with recorded increases of 31.5 and 43.6%, respectively, compared to control values. Olive or garlic oil treatment in animals administered 1000 ppm lead acetate still registered a significant increase in urea, at rates of 19.5 and 15.4%, respectively. This ameliorating effect was more obvious with DMSA or  $\text{CaNa}_2\text{-EDTA}$  treatment, with an increase in urea of only 5.4% compared to controls. For 2000 ppm lead acetate-treated animals, olive and garlic oils offered some protection, recording increases of 24.2 and 22.2%, respectively, compared to controls ( $P < 0.05$ ). With DMSA and  $\text{CaNa}_2\text{-EDTA}$  treatment, urea concentrations returned

to close to control values, with increases of only 0.7 and 2.7%, respectively ( $P > 0.05$ ).

**Serum Uric Acid**

The average values of serum uric acid level in both control and experimental animals are presented in Table 6. There was no significant change in uric acid concentration among control groups. Administration of 1000 ppm lead acetate provoked a significant increase in uric acid, with recorded increases of 18.8, 38.7 and 22.9% after 20, 40 and 60 days, respectively, compared to controls. These values were 28.1, 29.8 and 41.7% with 2000 ppm lead acetate. Elevation in uric acid persisted on withdrawal of lead acetate (1000 and 2000 ppm), with increases of 26.8 and 39.9%, respectively, compared to controls ( $P < 0.05$ ). Administration of olive or garlic oil to animals given 1000 ppm lead acetate lowered uric acid concentrations (particularly with garlic oil), with recorded increases of 14.9 and 8.9%, respectively, compared to controls. DMSA or  $\text{CaNa}_2\text{-EDTA}$  treatment returned uric acid nearly to control levels. In animals given 2000 ppm lead acetate, olive and garlic oils also reduced uric acid concentration (particularly garlic oil), to increases of 25.0 and 14.9%, respectively. Again, DMSA and  $\text{CaNa}_2\text{-EDTA}$  returned uric concentrations to close to normal values.

Table 5. Effect of lead acetate (1000 and 2000 ppm) administered daily in drinking water on serum urea concentrations (mg/dl) of control and experimental groups of albino rats.

Dose	Control groups						Experimental groups							
	Deionized water			Sodium acetate			Lead acetate			Lead discontinuation	Natural oils		Chemicals	
	20	40	60	20	40	60	20	40	60		Olive oil	Garlic oil	DMSA	$\text{CaNa}_2\text{-EDTA}$
1000 ppm	37.0	36.3	38.5	36.8	38.0	38.8	46.0	51.5	49.3	49.0	44.5	43.0	39.3	39.3
	$\pm 2.5$	$\pm 1.8$	$\pm 2.1$	$\pm 2.2$	$\pm 2.0$	$\pm 2.4$	$\pm 2.6$	$\pm 3.5$	$\pm 1.5$	$\pm 1.7$	$\pm 1.9$	$\pm 2.1$	$\pm 1.9$	$\pm 1.7$
% Change				-0.7	+4.8	+0.7	+24.3	+42.1	+27.9	+31.5	+19.5	+15.4	+5.4	+5.4
P				>0.05	>0.05	>0.05	<0.05	<0.01	<0.01	<0.01	<0.05	<0.05	>0.05	>0.05
2000 ppm	37.0	36.3	38.5	38.0	39.0	41.3	47.0	52.3	56.8	53.5	46.3	45.5	37.5	36.3
	$\pm 2.5$	$\pm 1.8$	$\pm 2.1$	$\pm 2.0$	$\pm 1.8$	$\pm 2.6$	$\pm 1.9$	$\pm 1.7$	$\pm 2.1$	$\pm 1.6$	$\pm 2.4$	$\pm 2.3$	$\pm 2.0$	$\pm 1.7$
% Change				+2.7	+7.6	+7.1	+27.0	+44.1	+47.4	+43.6	+24.2	+22.2	+0.7	-2.7
P				>0.05	>0.05	>0.05	<0.05	<0.01	<0.01	<0.001	<0.05	<0.05	>0.05	>0.05

Eight experimental animals were used for each control group and six animals for each of the other groups/time interval.

All values are expressed as Mean  $\pm$  SE.

P value: >0.05 non significant, <0.05 significant, <0.01 highly significant and <0.001 more highly significant.

Table 6. Effect of lead acetate (1000 and 2000 ppm) administrated daily in drinking water on serum uric acid concentrations (mg/dl) of control and experimental groups of albino rats.

Dose	Control groups						Experimental groups							
	Deionized water			Sodium acetate			Lead acetate			Lead discontin- uation	Natural oils		Chemicals	
	20	40	60	20	40	60	20	40	60		Olive oil	Garlic oil	DMSA	CaNa <sub>2</sub> EDTA
1000 ppm	1.60	1.68	1.75	1.65	1.63	1.78	1.90	2.33	2.15	2.13	1.93	1.83	1.78	1.80
	±0.14	±0.12	±0.13	±0.16	±0.13	±0.19	±0.12	±0.13	±0.10	±0.11	±0.10	±0.10	±0.15	±0.12
% Change				+3.1	-3	+1.7	+18.8	+38.7	+22.9	+26.8	+14.9	+8.9	+6.0	+7.1
P				>0.05	>0.05	>0.05	>0.05	<0.01	<0.05	<0.05	<0.05	>0.05	>0.05	>0.05
2000 ppm	1.60	1.68	1.75	1.70	1.63	1.68	2.05	2.18	2.48	2.35	2.10	1.93	1.63	1.76
	±0.14	±0.12	±0.13	±0.16	±0.22	±0.22	±0.11	±0.13	±0.14	±0.21	±0.11	±0.10	±0.13	±0.20
% Change				+6.3	-3.0	-4.0	+28.1	+29.8	+41.7	+39.9	+25.0	+14.9	-3.0	+4.8
P				>0.05	>0.05	>0.05	<0.05	<0.05	<0.01	<0.05	<0.05	>0.05	>0.05	>0.05

Eight experimental animals were used for each control group and six animals for each of the other groups/time interval.

All values are expressed as Mean ± SE.

P value: >0.05 non significant, <0.05 significant, <0.01 highly significant and <0.001 more highly significant.

### Serum Creatinine

Table 7 shows serum creatinine concentration of control and experimental animal groups. No significant change in serum creatinine was found among control groups. Lead acetate intake at dose of 1000 ppm caused gradual significant increase in creatinine, at rates of 18.0, 21.2 and 25.5% after 20, 40 and 60 days, respectively, compared to controls. These values reached 24.0, 40.4 and 56.4% with intake of 2000 ppm lead acetate ( $P < 0.01$ ). This effect was maintained when lead acetate (1000 and 2000 ppm) was ceased, with increases of 30.8 and 55.8%, respectively, compared to controls. With olive or garlic oil treatment of animals given 1000 ppm lead acetate, creatinine levels improved (particularly with garlic oil), with increases of 15.4 and 9.6%, respectively, compared to controls. This ameliorating effect was more obvious with DMSA or CaNa<sub>2</sub>-EDTA treatment, with creatinine concentrations approached those of controls. In 2000 ppm lead acetate-treated animals, olive and garlic oils also reduced creatinine, recording increases of 19.2 and 15.4%, respectively, compared to controls ( $P < 0.05$ ). Again, DMSA and CaNa<sub>2</sub>-EDTA were more efficient, returning creatinine concentrations to nearly control values.

### Discussion and Conclusion

Lead is known to be a toxic agent, and blood lead level (BLL) is a convenient and direct indicator of such toxicity.

In the first part of this study BLL was determined (28). Increment in BLL following lead acetate administration was significant, time- and dose-dependent, and persisted even when lead acetate intake was ceased. This coincides with the lead toxic effect on the current tested parameters. The observed decrease in RBC count depends on dose and duration of lead acetate intake and was even maintained when lead intake was stopped for a period. The decrease in RBC count observed here is in agreement with that recorded previously (6,29). A shortening of erythrocyte survival time was observed in the rats exposed to lead (7). The precise mechanism underlying lead toxicity on RBC is still to be defined. However, lead could affect the rat erythrocyte membrane and decrease their mobility (30). In addition, lead may induce oxidative stress in RBCs (31).

The current data showed that hemoglobin content and hematocrit value were significantly decreased in parallel to the decrease in RBC count in experimental animals with lead acetate inoculation at doses of 1000 or 2000 ppm. Similar decreases in hemoglobin concentration and hematocrit value were reported in lead-treated animals (10,32,33). The decrease in hemoglobin concentration may coincide with higher BLLs. The Environmental Protection Agency estimated that the threshold BLL for a decrease in hemoglobin is 50 µg/dl (34). Lead may inhibit the body's ability to make hemoglobin by interfering with several enzymatic steps in the heme pathway. Specifically, lead decreases heme biosynthesis by inhibiting



Table 7. Effect of lead acetate (1000 and 2000 ppm) administrated daily in drinking water on serum creatinine concentrations (mg/dl) of control and experimental groups of albino rats.

Dose	Control groups						Experimental groups							
	Deionized water			Sodium acetate			Lead acetate			Lead discontinuation	Natural oils		Chemicals	
	20	40	60	20	40	60	20	40	60		Olive oil	Garlic oil	DMSA	CaNa <sub>2</sub> EDTA
1000 ppm	0.50 ±0.03	0.52 ±0.04	0.55 ±0.04	0.54 ±0.05	0.51 ±0.05	0.59 ±0.05	0.59 ±0.03	0.63 ±0.04	0.69 ±0.03	0.68 ±0.04	0.60 ±0.03	0.57 ±0.03	0.54 ±0.04	0.55 ±0.04
% Change				+8.0	-1.9	+7.3	+18.0	+21.2	+25.5	+30.8	+15.4	+9.6	+3.9	+5.8
P				>0.05	>0.05	>0.05	>0.05	<0.05	<0.05	<0.05	<0.05	>0.05	>0.05	>0.05
2000 ppm	0.50 ±0.03	0.52 ±0.04	0.55 ±0.04	0.53 ±0.04	0.55 ±0.05	0.57 ±0.05	0.62 ±0.03	0.73 ±0.03	0.86 ±0.04	0.81 ±0.04	0.62 ±0.03	0.60 ±0.02	0.53 ±0.03	0.51 ±0.04
% Change				+6.0	+5.8	+3.6	+24.0	+40.4	+56.4	+55.8	+19.2	+15.4	+1.9	-1.9
P				>0.05	>0.05	>0.05	<0.05	<0.01	<0.01	<0.01	<0.05	<0.05	>0.05	>0.05

Eight experimental animals were used for each control group and six animals for each of the other groups/time interval.

All values are expressed as Mean ± SE.

P value: >0.05 non significant, <0.05 significant, <0.01 highly significant and <0.001 more highly significant.

aminolevulinic acid dehydratase and ferrochelatase activity (35,36).

From the previous results one can say that lead may induce anemia both by interfering with heme biosynthesis and by diminishing RBC survival. Several authors pointed out different types of anemia developed in animals exposed to lead (7,10). Treatment of animals with olive or garlic oil resulted in some improvement in the RBC count, hemoglobin concentration and hematocrit value. However, the prophylactic effect of garlic oil on blood parameters was more pronounced than that with olive oil. It was pointed out that garlic oil contains natural sulfur compounds which act as anti-lead active substances (37). This implies the antioxidant action of garlic sulfhydryl groups on RBCs. On the other hand, chemical treatment of animals with DMSA or CaNa<sub>2</sub>-EDTA exerted substantial improvements in all hematological parameters studied and returned their levels to near those of controls. A similar result recorded that treatment of lead-intoxicated rats with DMSA reversed lead-induced alterations in RBCs (31). The therapeutic role of DMSA was attributed to its antioxidant/chelating action.

Our data revealed a significant decrease in serum glucose level upon lead acetate administration. A similar result was obtained previously (13). Lead appears to exert a direct or indirect effect on serum glucose of

intoxicated rats. In this context, lead might be regarded as a risk factor in the abnormal glucose metabolism seen in some kinds of neurodegenerative disorders (11,12). Many environmental and occupational agents including lead have been shown to cause detrimental effects on endocrine function related to glucose metabolism (38). Treatment experiments with natural oils revealed improvement in serum glucose level particularly in rats injected with garlic oil. This result coincides with that reported (13). The protection action of garlic against lead toxicity could be attributed to the antioxidant action of its sulfhydryl groups. On the other hand, detoxification action of olive oil against lead toxication was not clear. The potential action of DMSA and CaNa<sub>2</sub>-EDTA in returning serum glucose to about its normal level coincides with their function as chelating agents (17,18,22).

The observed elevation in serum urea concentration in response to lead acetate administration is in agreement with previous studies (14,39). Urea is the principal end product of protein catabolism. Enhanced protein catabolism together with accelerated amino acid deamination for gluconeogenesis is probably an acceptable postulate to interpret the elevated levels of urea. On the other hand, the elevated serum urea levels may be due to the previously reported destruction of

RBCs. Lead intoxication also significantly augments the uric acid concentration. The elevation in uric acid was reported by other authors (40,41). Uric acid is the end product of the catabolism of tissue nucleic acid, i.e. purine and pyrimidine bases metabolism (42). The increments in uric acid concentrations may be due to degradation of purines or to an increase of uric acid levels by either overproduction or inability of excretion (42). Increase in creatinine concentrations is in agreement with previous reports (5,43). However, such increase may indicate impairment in kidney function. About 50% of kidney function must be lost before a rise in the serum concentration of creatinine can be detected (44). Therefore, urea, uric acid and creatinine could be considered as suitable prognostic indicators of renal dysfunction in case of lead exposure (45-47). Treatment of animals with natural oils alleviated such toxic effect of lead, but garlic oil was more effective than olive oil. The assumption of oxidative stress as a mechanism in lead toxicity suggests that antioxidant action of garlic sulfhydryl groups might play a role in the treatment of

lead poisoning. Chemical treatment was more efficient than natural treatment. The potential therapeutic action of DMSA and  $\text{CaNa}_2\text{-EDTA}$  could be attributed to their chelating ability.

In conclusion, lead could have toxic effects which manifest in hematological disorders, disturbance of glucose metabolism and impairment of kidney function. Although chemical therapy exhibited a more potent role in ameliorating lead toxicity, natural compounds may be preferable in reducing lead toxicity in the exposed population as they have little or probably no side effects.

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