Tissue distribution and hemato-biochemical alteration induced by lead acetate in male albino rats

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Abstract*:* The present study was conducted to evaluate the effect of two sub lethal doses of lead acetate (1/10 and 1/20) of LD_{50} on blood picture, liver and kidney functions, and measuring the lead concentration in different tissues (blood, liver , kidney, brain and spleen). Male albino rats were ingested orally with the two tested doses respectively, 5 days a week for 30 days. Samples were taken every 10 days. The results indicated that the lead level was increased by the time of experiment. The maximum concentration was in kidney followed by spleen, liver, blood and brain. There was significant induction in liver function enzymes (ALT, AST) and (ALP).The ingestion of lead acetate significant reduce the RBCs count, Hemoglobin contents, PLT count and HCT % while WBCs count was increased. Also, urea, creatinine and uric acid concentrations were significantly increased.

Keywords*:* Lead acetate, Toxicity, Liver, Kidney, Hematology.

1. Introduction

Exposure to lead (Pb) is unavoidable as it occurs through many routes including contaminated air, water, soil, food and consumer products. Lead is a wide-spread environmental pollutant, which has been implicated in toxic processes that affect several organ systems in man and other mammals **(Correa *et al.*, 2004)**. It is well accepted that lead intoxication is associated with significant increase in the lipid peroxidation and a decrease in the levels of endogenous antioxidants in liver, kidney, lung , heart, and brain, anemia, hepato-renal dysfunction, hypertension, abnormal vascular function, genotoxicity and immunosuppression **(Piasek et al. 1989, Hertz-Picciotto and Croft 1993: Reichlmayer-Lais and Kirchgessner 1997: Zhao *et al.*, 2004)**.

Lead (Pb) is a toxic metal that induces a broad range of physiological, biochemical and neurological dysfunctions in humans **(Sherfving and Bergdahl 2007)** . Although atmospheric (Pb) pollution due to tetraethyl lead from gasoline has been improved over the last two decades, humans are still exposed to (Pb) via contaminated foods and water through industrial activities **(Barbosa *et al.*, 2005)**. Precise mechanisms of (Pb) toxicity as well as the protective measures against (Pb) toxicity still remains to be solved.

The lead (Pb) is a persistent toxic **(Annabiet *et al.*, 2007 and Reglero *et al.*, 2009)**, as other xenobiotics induces to different health risks since the fetal stage until senescence. On the other hand, although lead is one of the most useful metals, it is also one of the most toxic ones **(Shotyk K and Roux 2005)** . Also, both occupational and environmental exposure remain a series problem in many developing and industrializing countries **(Yucebilgic *et al.*, 2003)**. Several reports have indicated that lead (Pb) can cause neurological, hematological, gastrointestinal, reproductive, circulatory, and immunological pathologies, all of them related to the dose and the amount of time of lead (Pb) exposure **(Ademuyiwa *et al.*, 2007 and Park 2006)**. Also, the health risks occasioned by exposure to lead are considered a global public health problems .

The liver plays a major role in lead's metabolism, and it is in special risk due to the oxidative action of this xenobiotic; given the unquestionable evidence that lead-induced lipid peroxidation of cellular membranes, plays a crucial role in the mechanisms of hepatotoxic action of these xenobiotics **(Sivaprasad *et al*; 2004)**. On the other hand, lead (Pb) is also known to affect the kidney, which is another important target **(Garcon *et al.*, 2007)**.Lead (Pb) produces oxidative damage in the kidney as evidenced by enhancing lipid peroxidation **(EL Nekeety *et al.*, 2009 and Farrag *et al*; 2007)**.

Lead (Pb) is an important heavy metal pollutant in the environment. The nervous system, kidney and liver are the most susceptible organs to lead (Pb) deposition, Showing that this pollutant has no single target system **(Perez *et al.*, 2014)**. The aim of the current study was to evaluate the effect of two sub lethal doses of lead acetate 1/10 and 1/20 LD_{50} on blood picture, liver and kidney function and

measure it`s concentrations in different tissues (blood, liver, kidney, brain and spleen)

2. Materials and Methods

2.1. Chemicals : Lead acetate trihydrate (99%) purity was purchased from el Gmohoria chemicals company, Alexandria, Egypt

2.2. Experimental animals and design:

Male adult wistar rats (70-80 days of age, 150 ± 10 g body weight) were housed and allowed to acclimatize under standard laboratory conditions for one week before initiation of treatment, feeding on a commercial diet and water ad libitum.

The rats were randomly divided into three groups, (18 rats/group) and the three groups were treated with 0, 20 and 40 mg/kg body weight of lead acetate respectively 5 days/week for 30 days . The doses administered represent a (1/20 and 1/10) of the oral LD 50 of lead acetate.

The lead acetate doses were dissolved in 0.2 ml distilled water, one dose was ingested orally via a syringe with a feeding gavage needle. All studies were conducted in accordance with good laboratory practice standards and oral toxicity guideline for pesticide testing **(Organization for Economic Cooperation and Development, OECD, 1995)**.

After the exposure and treatment periods, rats were sacrificed by cervical dislocation 6 rats every 10 days, The brain, liver, kidney and spleen immediately isolated in ice and kept in 4^{o}C for determination of the levels of lead in each tissue. After sacrifice, blood was immediately collected from vain into heparinized vials and stored at 4^{o}C to determine blood picture, and lead levels. Some of which was centrifuged at 3000 rpm to obtain the plasma which was kept frozen at -20^{o}C until used for analysis

2.3. Hematological parameters : The blood picture parameters measured with XS-800i automated call counter (Sysmex, Japan). The hematological profile included red blood cells (RBCs), white blood cells (WBCs), hemoglobin contents (Hb), hematocreet (HT) % , platelets count (PLT).

2.4. Biochemical measurements :

Stored plasma samples were analyzed for the activities of (ALT), (AST), AIP, urea, creatinine and uric acid. All measured by fully-automated Cobas C311 chemistry analyzer (Roche diagnostics .Ltd, Switzerland)

2.4.1 .Lead measurement : The tissue samples from blood, liver, kidney, brain and spleen were obtained from all group of animals for determination of lead residues. The samples were weighed, digested and processed for lead measurement using and atomic absorption spectrophotometer Shimadzu model (AA-6650)

2.4.2 .Statistical analysis : Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. **Student t-test** was used for comparing means between treated and control group **(Snedecor and Cochran, 1989).**

3. Results and Discussion:

Table 1 illustrate the content of lead in blood, liver, kidney, brain and spleen after treated with 1/10 and 1/20 LD_{50} of lead acetate respectively for 30 days. It was showed that a significant increase in lead level was detected in blood of rats treated with 1/10 LD_{50} after 10 , 20 and 30 days of treatment. Where it was as twice as the lead concentration in blood for both control group and treated group of rats with 1/20 LD_{50}.Lead level significantly increased in different tissues by increasing the dose administration. Similarly, **Manal *et al*. (2013)** recorded that there was a significant increase of lead concentration in blood with increasing intrapertioneal dose administration of lead acetate (25, 50 and 100 mg/kg b.w) to rats for 7 days in comparison to control group. The present study showed that the most affected organ was kidney followed by spleen, liver, blood and brain. In comparison with control group the level of kidney lead was about 10.15 fold increase in the case of high dose 1/10 after 30 days of treatment. While it was about 3.6 fold increase in the case of low dose 1/20 LD_{50} .These results were agreed with that of **Velaga *et al*., (2014)**, They showed a significant ($p<0.05$) increase in metal content of brain regains, liver and kidney after exposure to lead acetate 2000 ppm for 2 weeks.

Table (1): Lead contents in different tissues of rats treated with tow sub lethal doses of LD_{50} lead acetate

	Control (n=6)	1/10 LD_{50}	1/20 LD_{50}
Blood (µg/dL)			
10 days	16.05 ± 0.01	30.07** ± 0.02	16.28** ± 0.11
20 days	18.05 ± 0.01	41.99** ± 0.07	19.07** ± 0.02
30 days	21.97 ± 0.05	51.83** ± 0.02	24.50** ± 0.18
Liver (µg/g)			
10 days	0.43 ± 0.01	1.13** ± 0.06	0.47* ± 0.03
20 days	0.43 ± 0.01	1.38** ± 0.04	0.49** ± 0.01
30 days	0.43 ± 0.04	4.91** ± 0.07	0.90** ± 0.01
Kidney (µg/g)			
10 days	0.61 ± 0.32	4.08** ± 0.01	0.93 ± 0.01
20 days	0.32 ± 0.01	4.58** ± 0.31	0.97** ± 0.01
30 days	0.66 ± 0.01	6.70** ± 0.24	2.41**± 0.03
Brain (µg/g)			
10 days	0.11 ± 0.03	0.27** ± 0.04	0.18** ± 0.01
20 days	0.17 ± 0.06	0.36** ± 0.03	0.25* ± 0.05
30 days	0.12 ± 0.03	0.38** ± 0.04	0.35** ± 0.04
Spleen (µg/g)			
10 days	0.14 ± 0.0	0.64** ± 0.04	0.17** ± Q0.02
20 days	0.15 ± 0.02	1.12** ± 0.14	0.56** ± 0.02
30 days	0.16 ± 0.01	3.04** ± 0.11	0.86 **± 0.04

Data were expressed in mean ± SD and was compared using **Student t-test**
*: Statistically significant at $p \leq 0.05$
**: Statistically significant at $p \leq 0.01$

Table (2):The effect of lead acetate on the hematological parameters in male albino rats

	Control(n=6)	1/10 LD_{50}	1/20 LD_{50}
RBCs*10^6/µL			
10 days	6.25 ± 0.60	5.34* ± 0.23	5.47 ± 0.69
20 days	8.50 ± 0.31	5.40** ± 0.22	5.69** ± 0.11
30 days	8.66 ± 0.72	5.27** ± 0.31	5.61** ± 0.31
WBCs*10^3/µL			
10 days	8.0 ± 1.0	12.56** ± 0.29	11.73** ± 0.20
20 days	8.76 ± 0.44	15.78* ± 6.75	13.16 ± 5.74
30 days	9.30 ± 0.40	13.42* ± 2.58	12.86 ± 3.71
Hb g/dL			
10 days	12.07 ± 0.27	10.47** ± 0.39	10.81** ± 0.16
20 days	13.55 ± 0.23	11.92** ± 0.46	12.52** ± 0.38
30 days	13.63 ± 0.22	10.58** ± 0.39	11.84** ± 0.10
HCT %			
10 days	40.77 ± 1.11	30.93** ± 1.90	31.40** ± 0.18
20 days	58.37 ± 2.50	45.47** ± 0.49	52.37* ± 4.05
30 days	55.57 ± 2.38	48.97** ± 0.76	50.73* ± 3.03
PLT*10^3/ µL			
10 days	887.7 ± 47.19	444.0** ± 0.89	721.0** ± 38.65
20 days	934.3 ± 58.38	758.0** ± 75.43	656.3** ± 94.05
30 days	833.7 ± 117.7	619.7** ± 83.94	684.0* ± 101.9

Data were expressed in mean ± SD and was compared using **Student t-test**
*: Statistically significant at $p \leq 0.05$
**: Statistically significant at $p \leq 0.01$

Table (2) show the effect of lead acetate on the hematological parameters in male albino rats. It was a significant decreases in RBC's count, Hemoglobin (Hb) content hematocrit (HT) value and platelet count in both 1/10 & 1/20 LD_{50} at the three times of experiment while it was a significant increase in WBCs count in rats treated with 1/10 LD_{50} after 10, 20, 30 days of exposure while it was significant increase in 1/20 LD_{50} only after 10 days of exposure the significant increase was increasing with higher dose of lead acetate (1/10 LD_{50}).

These results are in agreement with that reported by **Abdel-Moneim *et al.*, (2015)**. They found that rats intraperitoneally treated with lead acetate at 25 mg/kg once a day for 7 days showed a significant decreased of hemoglobin (Hb) content, hematocrit (Ht) value and platelet (PLT) count, while pb^{2+}related leukocytosis was accompanied by absolute neutrophilia, monocytosis, lymphopenia, and eosinopenia.

Also, **Abdou HM and Hassan MA (2014)** found that the Hb and PCV values and the RBCs and PLT counts were significantly decrease ($p<0.05$) in rats treated with lead acetate at a dose of 25 mg/kg b.w/day for 5 days in compared with control.

Furthermore, the results of the present study are in agreement with those described by **Kim *et al.*, (1996) and Simsek *et al.*, (2009)**. However, **Topashka-Ancheva *et al.*, (2003)** showed that lead could damage the erythrocytes membrane resulting in hemolysis or decrease of blood iron level which may be the reason of decreasing the concentration of HB and PCV. These hematological alterations might be also due to the effect of lead on the activity of δ-aminoevulinic acid dehydratase (ALAD) which acts as key enzyme of heme synthesis.

The effect of lead acetate on liver function enzymes in male albino rats was illustrated in table (3). The results showed an increase in serum alanine aminotransferase (ALT), aspartate

Table (3): Changes in the concentration of liver function enzymes of plasma ALT, AST and AIP of control and treated rats with lead acetate

	Control (n=6)	1/10 LD_{50}	1/20 LD_{50}
ALT U/L			
10 days	42.87 ± 3.28	58.53** ± 3.68	53.67** ± 6.0
20 days	33.33 ± 1.37	46.67** ± 3.14	42.70** ± 2.78
30 days	47.67 ± 5.24	63.33** ± 1.37	53.12** ± 3.1
AST U/L			
10 days	142.6 ± 27.52	206.6**± 16.60	190.2** ± 16.57
20 days	181.33 ± 30.48	234.0**± 23.26	208.7± 8.16
30 days	324.0 ± 29.77	353.0 ± 10.08	323.7 ±50.69
ALP U/L			
10 days	126.3 ± 12.79	210.7**± 21.21	126.3 ± 7.28
20 days	112.3 ± 17.58	158.0** ± 7.32	155.7** ± 4.59
30 days	121.3 ± 14.35	169.0* ± 8.85	122.0 ± 10.88

Data were expressed in mean ± SD and was compared using **Student t-test**
*: Statistically significant at $p \leq 0.05$
**: Statistically significant at $p \leq 0.01$

aminotransferase (AST) and alkaline phosphatase (ALP) activity in both 1/10 and 1/20 LD_{50} at all times of the experiment. It was significant at $p<0.01$ in ALT activity after treated with 1/10 LD_{50} lead acetate in comparison with control after 10,20 and 30 days where it is cleared that the treated rats with 1/20 LD_{50} caused a significant stimulation in ALT, AST, and ALP activities after 20 days relative to control. These results are in agreement with previous study which reported that rats ingested orally with sub lethal doses of lead acetate (1/20, 1/40 and 1/60) of orally LD_{50} respectively one dose was ingested every two days during the experimental period (14 weeks) induced significant stimulation in ALT, AST and ALP activities **(Nabil *et al.*, 2012)**.

Also, Ibrahim et al (2005) showed that the activities of serum levels of ALT, AST and ALP were significantly elevated after lead acetate (500 mg/kg diet) for one month to albino mice. Many studies have demonstrated similar results that lead changes the liver function by increased AST, ALT and ALP enzymes. **(Soliman *et al.*, 2015)** and **Wang *et al.*, (2012)**.

Hepatic cell participate in a variety of metabolic activities and contain a lot of enzymes. Among these, AST, ALT and

ALP are commonly employed as biological markers for hepatic injury and efficacy of hepatoprotective interventions **(Rosser & Gores 1995)**. It is well known that changes in plasma activity of liver enzymes can be a result of many conditions including cell necrosis, improved of increased synthesis and alterations in the permeability of enclosing cell membrane **(Boyd 1985)**.

In addition, **Yuan *et al.*, (2014)** suggested that low-dose exposure to Pb and Cd can cause significant hepatic and renal apoptosis and finally impair their function.

The effect of lead acetate on male albino rats in kidney function represented in Table (4). It was clear that the level of urea, creatinine and uric acid were significantly increased in rats treated with lead acetate compared to the control ones reflecting renal impairment.

Table (4):Changes in the concentration of kidney function parameters in control and treated rats with lead acetate

	Control (n=6)	1/10 LD_{50}	1/20 LD_{50}
Urea mg/dL			
10 days	27.67 ± 2.25	34.33* ± 2.73	29.67 ± 4.93
20 days	29.50 ± 0.45	43.67* ± 2.73	40.33** ± 6.95
30 days	30.0 ± 1.79	46.67* ± 11.91	32.33 ± 1.86
Creatinine mg/dL			
10 days	0.41 ± 0.03	0.49 ± 0.06	0.46 ± 0.13
20 days	0.49 ± 0.02	0.61** ± 0.03	0.54** ± 0.04
30 days	1.17 ± 0.10	2.07* ± 0.81	1.21 ± 0.30
Uric acid mg/dL			
10 days	2.02 ± 0.11	3.18* ± 1.03	3.03** ± 0.54
20 days	2.37 ± 0.18	3.33** ± 0.28	2.66 ± 0.54
30 days	2.57 ± 0.27	4.67** ± 0.81	3.70** ± 0.54

Data were expressed in mean ± SD and was compared using **Student t-test**
*: Statistically significant at $p \leq 0.05$
**: Statistically significant at $p \leq 0.01$

In the current study, the induced elevation of urea, creatinine and uric acid due to lead acetate administration indicated that the kidney function was affected. This result agreed with that reported by **Abdou and Hassan (2014)** they showed that the levels of urea and creatinine significantly increased in female rats treated with lead acetate (25 mg/kg b.w) for 5 days. Also, **Ibrahim *et al*., (2005)** found that oral administration of lead acetate in the diet of mice at concentration of 0.5 % (w/w) for one month significantly elevated the serum level of urea and creatinine. **Wang *et al*., (2012)** reported that the levels of hepatic and renal markers such as alanine aminotransferase, aspartate aminotransferase, urea, uric acid, and creatinine were significantly ($P<0.05$) increased following lead acetate administration (500 mg Pb/L) orally for 8 weeks. Also, **Ozsoy *et al*., (2011)** reported that rats treated with lead acetate for 60 days showed a significant decrease in HB, HCT and RBC, a significant increase in WBC, AST, ALT and creatinine compared to controls. Further studies should be conducted to investigate the hazards of environmentally relevant, low-dose exposure to lead and the present study advises people to prevent exposure to the lead compound to avoid injurious hazard risk.

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