International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **6(9),** 1-6, September **(2017)** *Int. Res. J. Biological Sci.*

In-vivo study of stress oxidative and liver damage in rats exposed to acetate lead

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Available online at: www.isca.in, www.isca.me
Received 21st June 2017, revised 2nd September 2017, accepted 8th September 2017

Abstract

Lead (Pb) is a very toxic divalent heavy metal that occurs and diffuses into nature and the environment through human activities. The aim of this study was to evaluate the structure and liver function in rats exposed to lead. Our experimentation is carried out on Ten female Wistar rats were divided into two groups (n=5). The first group of rats received normal diet and water (controls) and the second group of rats received normal diet and acetate lead-contaminated water (100 mg/kg b.w) for 70 days. On which we measured some biochemical parameters. Results of our study showed that, in comparison with the control rats, lead exposure caused, a significant reduction ($p<0.001$) in the body weight and a significant augmentation ($p<0.001$) in relative liver weight. In addition, Result showed that in Pb-intoxicated rats, an increase in serum transaminases and Alkaline phosphatase activities and a decrease of GOT and GPT activities in liver when compared with normal animal group. Results revealed also that acetate lead treatment in rats affected antioxidant defense system by decreasing GSH level and GST activity and increasing MDA concentration. Also, the results clearly showed that lead causes alterations of hepatic tissue in comparison with controls. In Conclusion, Results demonstrated the toxic effect of high-dose of lead by causing oxidative stress and damage in hepatic tissue.

Keywords: Acetate lead, Liver, GOT, GPT, Stress oxidative.

Introduction

Lead is a chemical element that exists in nature. It is recognized that the heavy metal ions present a great danger to the ecosystem in very low doses because of their high toxicity, stability and accumulation and transfer in food chains[1]. Lead is considered among the main environmental pollutants, along with cadmium and mercury, which is one of the most harmful effects on human well-being. Lead has been known and used since 5000 BC. It is widely used in industry such as the manufacture of metal products, as well as in paints and pesticides[2]. Lead interacts with bio systems causing dysfunction at the level Molecular and cellular processes as well as in the cell signaling process. Lead contamination in mammals causes toxicity and numerous biochemical, physiological and behavioral dysfunctions[3]. Previous studies have shown several health problems as cardiovascular disease and high blood pressure appears due to exposure to lead at low doses[4]. Lead poisoning causes an alteration of biological functions by interactions with molecules and other cellular constituents. In mammals, lead nitrate causes severe intoxication which leads to a wide alteration in the physiology, neurology and behavior of the animal[5]. One of the major toxicity mechanisms of lead is presented by replacing Essential ions such as Na^+, Ca^{2+} and Mg^{2+} which disrupt their metabolism and also modify various other processes such as apoptosis, cell adhesion and release of neurotransmitters[6]. Another mechanism of toxicity of heavy metals a was attributed to oxidative stress[7]. Toxic heavy metals increase the production and release of free radicals and reduction in enzymatic and non-enzymatic antioxidant system activity to respond to the oxidative stress damaged[8]. The objective of our study was to explore the effect of acetate lead given orally in water on the oxidative stress and damage in liver of rats.

Materials and methods

Animals and handling: In our study we used females rats wistar with initial weight between 224–230 g were obtained from the Animal service of Pasteur institute, Algeria. They were placed in two groups of 5 rats in each and kept in animal's house of Department of cellular and molecular biology, University of El Oued, Algeria. The animals are carried in a laboratory place for adaptation with conditions of temperature (23 ± 2 C°), humidity (65.3%) and photoperiod (12 hours of light / 12 hours of black). Access to Standard diet and water is free for animals ad libitum during the experiments, according to previous work[9,10]. The realization of the experimental part is respect to the ethical approval.

Experimental design: After a period of adaptation, the animals, at the age of 8 weeks, were divided into two groups (n=5). The first group of rats received standard diet and water (controls) and the second group of rats received standard diet and acetate lead-contaminated water. Acetate lead used (100 mg/kg b.w) as $Pb(C_2H_3O_2)_2$ in water for 70 days in rats. Evaluate the body weight was controlled during the experiment.

Blood collection and tissue preparation: After of 10 weeks of lead exposed, rats were fasted for 16 hrs, anaesthetized with chloroform by inhalation. After the rats were sacrificed, the blood was collected in tubes without anticoagulants for biochemical analysis. The serum was obtained by centrifuging the blood at 3000 rpm for 10 min and then stored at −20C^{o}, and used for evaluation of liver indices, including serum transaminases and alkaline phosphatase activities. The liver was rapidly excised, weighed and stored at - 20°C until use. Part of the liver from each rat was removed immediately and preserved in a sample bottle containing 10% formalin solution. The liver was treated by the paraffin technique. Liver of each group are cut 5 µm thick and then rinsed in hematoxylin and eosin for histological analysis.

Determination of biochemical marker: Measurement of transaminase (GPT and GOT) and alkaline phosphatase (ALP) activities were carried out by commercial kits from Spinreact (Girona, Spain) (ref: GPT-1001171, GOT-1001161and ALP-1001131).

Antioxidants measurement: Preparation of homogenates: One gram of liver from each rat of the different experiment groups was used. After milling and homogenizing the tissues in 9 ml of buffer solution of TBS (50 mMTris, 150 mMNaCl, pH 7.4). The tissue suspension was centrifuged at 9000 rpm for 15 min at 4°C, the supernatant obtained was stored at -20°C until use for the oxidative stress marker assay.

Determination of lipid peroxidation[11]: Liver lipid peroxidation levels was measured as malondialdehyde (MDA) which measured according to the technique of Sastre et al.[13]. The method is based on the reaction between the carbonyl compounds of malondialdehyde with thiobarbituric acid (TBA) to give absorbent pink chromophores at 532 nm. MDA level was expressed asnmol of MDA/mg prot.

Level of Reduced Glutathione (GSH) assay[12]: Liver Reduced Glutathione (GSH) level was determined by a colorimetric method according to the technique described by Ellman, the measurement of optical density results from the formation of thionitrobenzoic acid (TNB) from the reduction of The 5, 5'dithiodis-2-nitrobenzoic acid (DTNB), which is called the Ellman reagent with the SH groups exist in GSH, which has an absorbance at 412nm. Total GSH level was expressed as nmol GSH/mg prot.

Activity of Glutathione-S-transferase (GST) assay[13]: The activity of Glutathione-S-transferase (GST) in Liver was measured spectrophotometrically by the method of Habig et al. based on the formation kinetics of a complex between a GST substrate: 1-chloro-2-4-dinitrobenzene (CDNB) and reduced Glutathione (GSH).

The complex formed can be visualized by increasing the optical density at a 340 nm. The GST activity was expressed as nmol CDNB /min/mg prot.

Statistical analysis: The statistical evaluation is carried out by ANOVA followed by the student's T test. The values are given as mean and standard deviations (ES) for two groups of 5 rats each. Statistical significance was defined as $P < 0.05$.

Results and discussion

Body weight and relative liver weight: Pb $(C_2H_3O_2)_2$ treatment at a dose (100 mg/kg b.w) caused a decrease ($p<0.001$) in body weight and induced a severe hepatomegaly when compared to the control group (Table-1).

Table-1: Initial body weight, final body weight and relative liver weight in control and Pb treatment rats.

Parameters	Control (n=5)	Pb (n=5)
Initial body weight (g)	225±5.06	225.2±5.69
Final body weight (g)	291±3.07	198±2.32***
Relative liver weight (g/g tissu)	2.519±0.057	3.206±0.246**

Significantly different from Control group: ** $p<0.01$, ***$p<0.001$. The results are presented by mean ± SEM, n=number of observations.

Serum and liver enzymes Activities: Seen from Table-2, Pb induced a significant rise ($p < 0.05$) in serum GOT, GPT and ALP ($p < 0.01$) activities and a significant decrease in liver GOT ($p < 0.05$) and liver GPT ($p < 0.01$) activities when compared to the corresponding control values.

Table-2: Transaminases and ALP activities in serum and liver of control and Pb treatment rats.

Parameters	Control (n=5)	Pb (n=5)
Serum GOT (U/l)	205.8±1.3	218.7±2.7*
Serum GPT (U/l)	89.5±4.33	98.5±7.79*
Serum ALP (U/l)	40.16±6.07	74.1±4.25**
Liver GOT (U/l)	55.8±2.90	40.75±6.37*
Liver GPT (U/l)	1140.2±30.74	890.52±40.79**

Significantly different from control group: * $p<0.05$, ** $p<0.01$. The results are presented by mean ± SEM, n=number of observations.

Stress oxidative parameters: As showed in Figure-1 and Figure-2, that the concentration of hepatic MDA is highly significant increased ($p<0.01$) and the concentration of GSH in the liver is significantly decreased ($p<0.001$) in the lead-exposed groups compared to control. On the other hand, the results presented in Figure-3 showed that the exposure to Pb led to a significant decrease ($p<0.001$) in liver GST activity compared to the control group.

Histopathological studies: Microscopic observation of histological sections of liver from the control rat revealed normal structure with clear hepatic cells (Figure-4A). However, histological sections of the lead-exposed rat liver revealed degeneration represented by necrosis, hemorrhage, inflammatory and sinusoidal dilatation (Figure-4B).

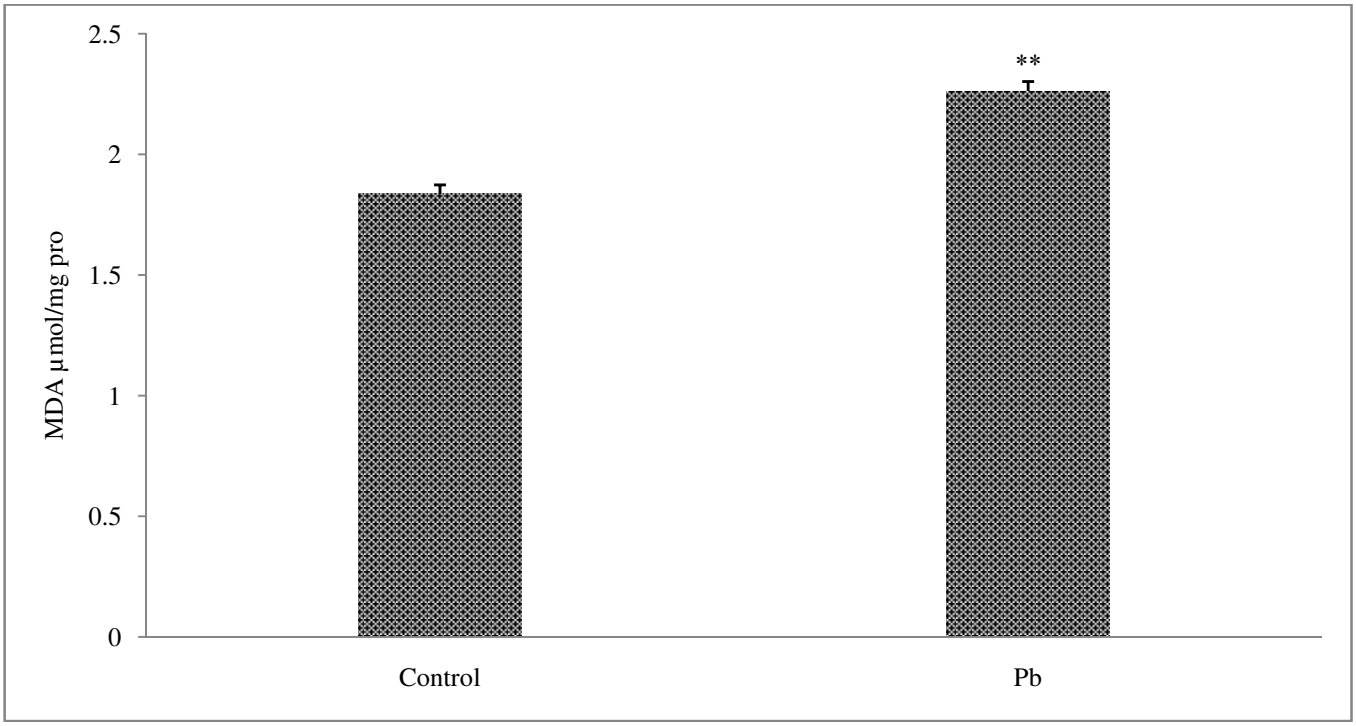

Figure-1: Liver MDA concentration in rats of control (C) and Pb treatment group. Means± SE from 5 rats in each group. Significance from Control: **p<0.01.

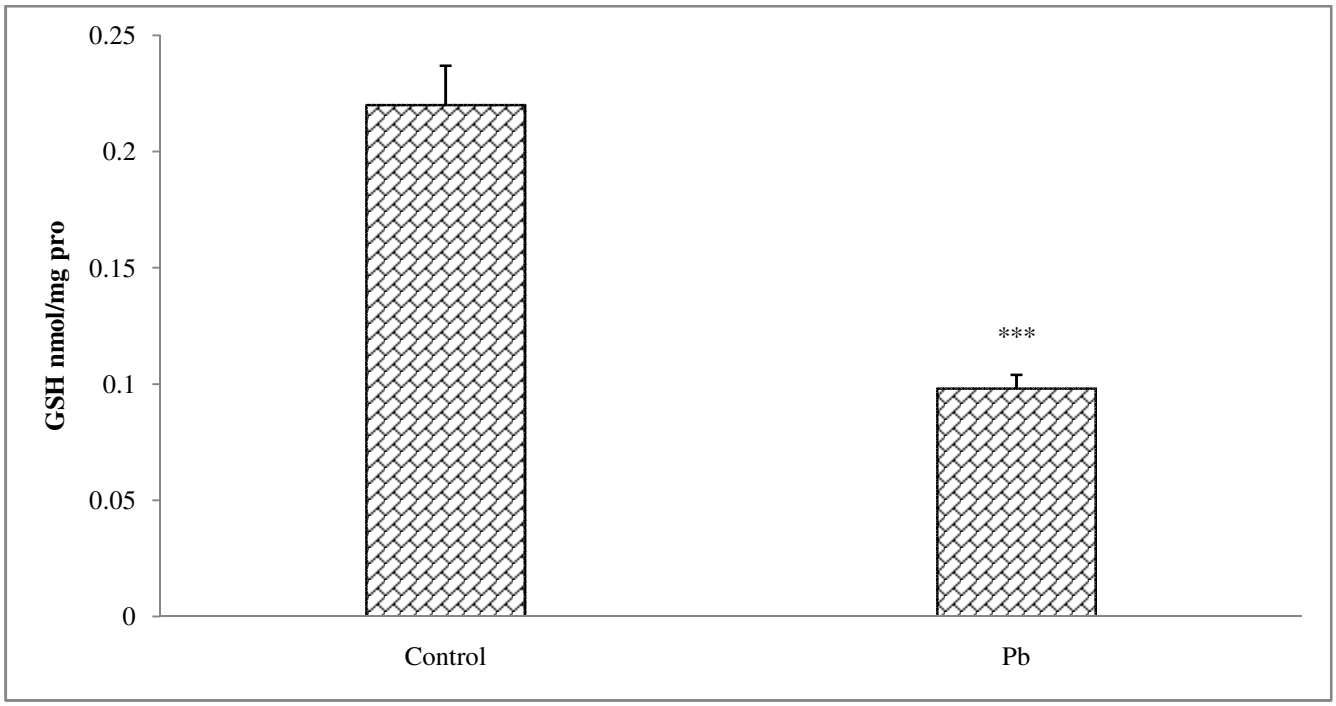

Figure-2: Liver GSH concentration in rats of control (C) and Pb treatment group. Means ± SE from 5 rats in each group. Significance from Control: ***p<0.001.

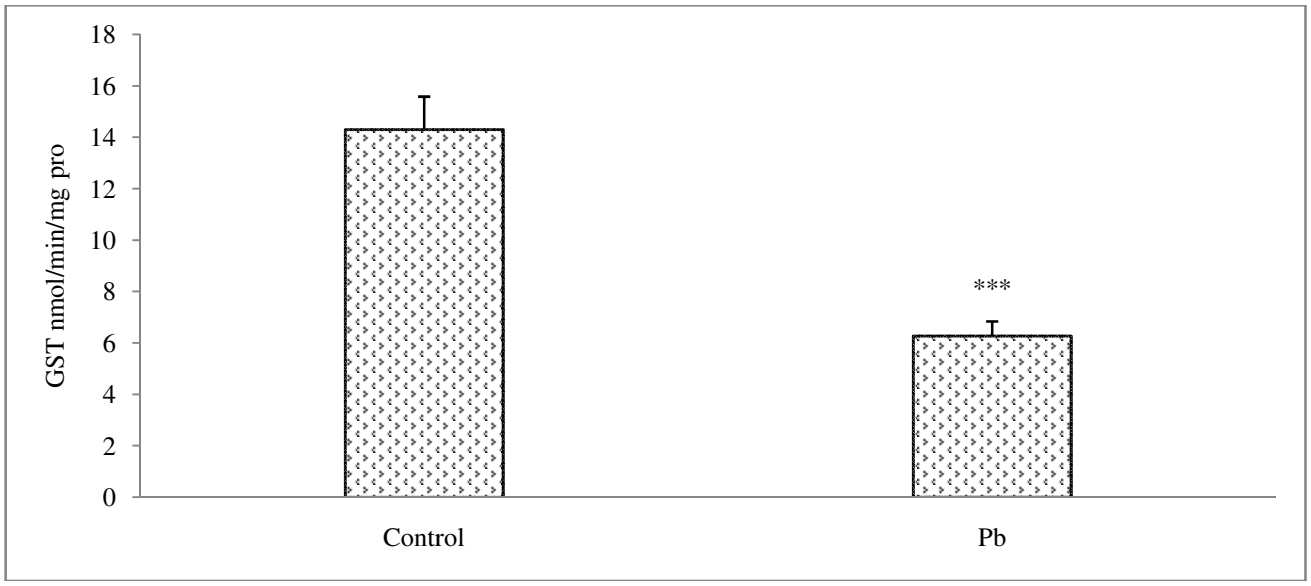

Figure- 3: Activity of GST in liver tissues of control (C) and Pb treatment group. Means ± SE from 5 rats in each group. Significance from Control: ***p<0.001.

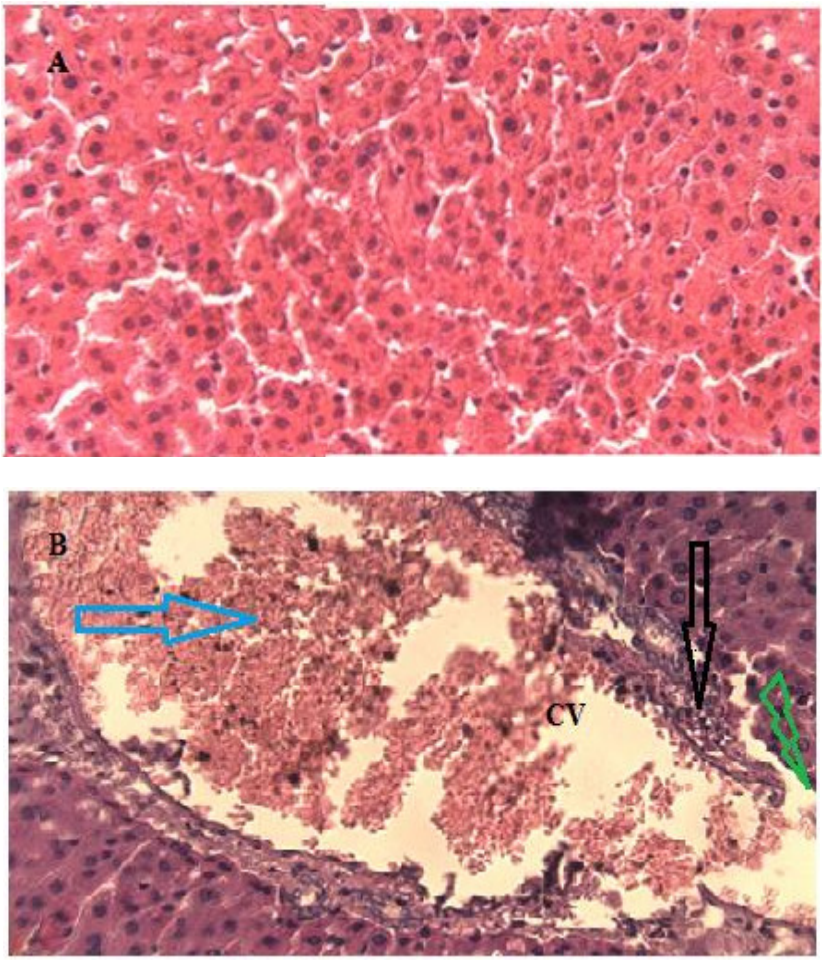

Figure-4: The histological analysis of liver in rats of control group showing normal architecture (Figure-4A, ×400). Acetate lead treated in liver rats showing necrosis and hemorrhage (blue arrow), inflammatory (black arrow) and sinusoidal dilatation (green bolt) (Figure-4B, ×400).

Discussion: In our study, we observed that for 70 days of contamination of rats by lead acetate resulted in a fall in body weight. Similar results have been indicated by the work of Reckziegel et al.[14]. However, this weight loss probably due to anorexia induced by the ingestion of this heavy metal following continuous exposure over a long period[15]. The elevation of the relative weight of the liver of the Pb group indicated in our study may be due to liver necrosis due to the accumulation of lipid in hepatic cells[16]. This is in line with the presence of liver necrosis reported in our results caused by the toxicity of lead acetate. Levels of the biomarkers of liver damage in serum and liver (GOT and GPT) were found to be changed compared to control. Our results are consistent with some previously published reports[17]. From the results of our study it is seen that administration of lead to rats caused a sharp increase in lipid peroxidation rates. Our results also corroborate well with study of Mudipalli[18] who demonstrated that acetate lead treatment increases the rate of Malondialdehyde (MDA) in liver. Oxidative stress is a damage caused by an increase in free radicals and oxidants such as reactive oxygen species[19]. According, the Pb may cause oxidative stress By two routes, though linked either by the generation of reactive oxygen species such as hydroperoxides and hydrogen peroxide, or by the exhaustion of the antioxidantreserves[20], the increase in lipid peroxidation could result to the reduction of liver antioxidant activities such as GST and GSH detected in these study. In line with some studies, the liver GSH level was significantly reduced in Pbtreatment rats when compared to the control group. Glutathione (GSH) is a non-enzymatic antioxidant that contributes to the defense system in the body against oxidative stress induced by reactive oxygen species[21]. It is a tripeptide possessing a sulfhydryl (SH) group widely distributed in all tissues and organs. Its sulfhydryl (SH) group can interact with free radicals which limits their undesirable effect, these elements (GSH) can also be incorporated into enzymatic detoxification reactions against ROS as cofactor of several enzymes[22]. The decrease in GSH levels presented in our results probably due to its use in scavenging free radicals generated by lead, also the Pb can decrease the level of GSH by its fixation to the SH groups which affects the activity antioxidant[23,24]. Meanwhile oxidative stress is very sensitive to environmental pollutants, liver cells can react with these pollutants by activating the antioxidant system and stimulating detoxification responses to minimize damage caused by heavy metals. The involvement of antioxidant enzymes such as GST as protective factors of cells and organs against toxic agents and oxidative stress[25]. Thus, evaluation of the activity of this enzyme (GST) can provide important information on the state and level of oxidative stress of the cells. We have shown that the lead used in this study causes a severe decline in the enzymatic antioxidant activity of. Similar results have been described by Sarkar et al.[26]. According to the results, lead treatment produces histological damage in the liver including focal necrosis, central venous dilation, haemorrhage and inflammation. These findings are in support with study of Shalan[27]. These alterations may be due to excessive ROS production and subsequent of lipid peroxidation induced by lead acetate[28].

Conclusion

This study shows the toxicity and harmful effect of 100mg / kg b.w of lead on structure and liver function in rats. From the study, it can be further concluded that excess lead water exposure produce toxic effects on liver tissue.

Acknowledgements

We thank the Biology Laboratory members for help in the course of this work.

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