DOI: http://doi.org/10.21698/simi.2019.fp35

TOXIC EFFECTS OF NON-ACUTE LEAD EXPOSURE ON ANIMAL MODEL

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Abstract
Lead, a high-risk environmental pollutant and extensively used by industry, is one of the most widely outspread toxic metal today. Lead toxicity is a public health problem both for the children and for the adults. Lead does not have any useful functions in the body, instead it produces only harmful effects once it gets into the body. In this study, we investigated the toxicity of lead in an animal model of non-acute exposure. Experimental groups received treatment of aqueous solution lead acetate at different doses and time of administration. Toxic effects of lead were investigated on haematological and behaviour of treated rats. We noticed the disturbances of both haematological parameters and behaviour. Our results indicated that non-acute exposure to lead induced toxic effects in the blood, and central nervous system of adult Wistar rats.

Keywords*: non-acute lead toxicity, haematology, anxiety, rat*

Introduction

For centuries, lead (Pb) toxicity has been one of the most significant causes of toxicant-induced neurologic morbidity related to irreversible damage of various tissues. Lead poisoning has been reported in almost every country, generally in industrial areas with storage battery manufacturing and ore mining, and it is more common in developing countries. The Institute for Health Metrics and Evaluation estimated that 540 000 deaths in 2016 result from lead poisoning, the highest burden was in low- and middle-income countries (WHO 2018). In Romania, lead poisoning represents 14.37% of the total occupational diseases registered in the last 10 years, representing therefore a major health concern (Stoia et al 2009).
Lead, a heavy metal with not apparently biological function, is widespread and non-biodegradable pollutant of great concern to human health (Andjelkovic et al 2019, Meghea et al 2009). While acute lead poisoning related to occupational exposure is quite uncommon, chronic lead toxicity is still a major problem. It is still present in many commercial products, and the two main routes of lead exposure are ingestion and inhalation. Lead poisoning can affect many organs and tissues, being associated with a number of morphological, biochemical, hematological, physiological, and behavioral changes (Sun et al 2017). The critical target for lead intoxication is the

hematopoietic system, but lead toxicity is linked to toxic effects on the nervous system also (Abadin et al 2007, Flora et al 2012). On the central nervous system, the lead exposure is associated with several neurobehavioral and psychological alterations.

In this study, we investigated the toxicity of alone in an animal model of non-acute exposure. The effects of the exposure to lead were investigated on hematological parameters and the consequences of its toxicity on rat behaviour.

Materials and Methods

Animals. Male adult Wistar rats from Animal Husbandry of "Victor Babes" National Institute of Pathology (Bucharest, Romania). The experiment was performed on male rats weighing approximately 320-350 g. The animals were maintained in optimal conditions: temperature 22±2˚C, humidity 55±10%, artificial ventilation, lighting 12/12, light/dark cycle. Rats received standard chow and drinking water *ad libitum*. All animals were kept under a rigorous cleaning and hygiene program and were monitored daily. The experiments were done in accordance with recognized principles of Laboratory Animal Care in the framework of EU Directive 2010/63/EU for animal experiments. The study was approved by the Ethics Committee from "Victor Babes" National Institute of Pathology and by the National Sanitary Veterinary and Food Safety Authority through project authorization, no. 449/02.04.2019 (Bucharest, Romania).

Study design. Healthy male rats were divided into five groups of 6 animals each, one control group and four experimental groups. Intoxication of experimental animals was done with lead acetate (Chemical Company, Iasi, Romania) dissolved in distilled water, and the treatment of all animals was performed by an oral gavage (150 µL per animal), as follows: group control, treated with water only; group intoxicated 400mg/kg body weight/day for 4 weeks (Pb400); group intoxicated 200mg/kg body weight/day for 4 weeks (Pb200); group intoxicated 100mg/kg body weight/day for 4 weeks (Pb100); group intoxicated 200mg/kg body weight/day for 6 weeks (Pb200*). Animals were sacrificed by cervical dislocation at the end of the experiment.

Haematology analysis. For the haematological tests blood was collected by retro-orbital puncture, and was immediately analysed for assessing the complete count of white blood cell (WBC), lymphocytes (LY), neutrophils (NE), monocytes (MO), red blood cell (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and platelets (PLT), using the fully automated Hemavet 950 analyser (Drew Scientific, Miami Lakes, USA).

Behaviour test. The light/dark box test was assessed using a device divided into a light chamber connected by a door to a dark chamber. Animals were placed initially in the bright chamber, and their behaviour was recorded for 20 minutes. Before each animal test the box was cleaned with 5% ethanol.

Statistical analysis. Data was analysed using Microsoft Excel. Results are presented as mean ± standard deviation (SD) (n=6). For statistical analysis, the Student's t-test (two-tailed, assuming equal variance) was used for statistical evaluation of the differences between the experimental groups. P-value <0.05 was considered to indicate a statistically significant difference.

Results and Discussion

Toxic effects of lead were investigated on haematological parameters, and behaviour of treated Wistar rats.

Higher doses of lead in 4 weeks treated animals produced a decrease in the WBC number compared to the control group and to a lower dose. The change in absolute lymphocyte and neutrophil count had a similar trend. Animals from the group lead-poisoning for 6 weeks, presented statistically increased absolute counts of WBC ($p<7.82E-05$), NE ($p<0.0035$) and LY ($p<4.25E-05$) in comparison with controls (Fig. 1). Monocytes count was not affected by the treatment (data not shown).

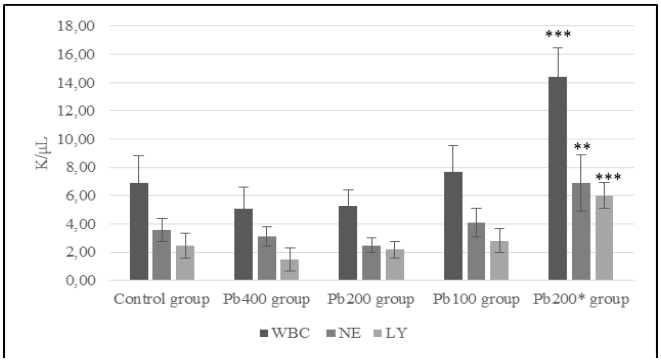

Figure 1. Effect of lead on WBC, NE, LY counts in rats after non-acute exposure.

Lead administration for 4 weeks did not affect the levels of red blood cells, but lead treatment for 6 weeks led to a significant statistical increase in RBC count ($p<1.121E-05$) (Fig. 2).

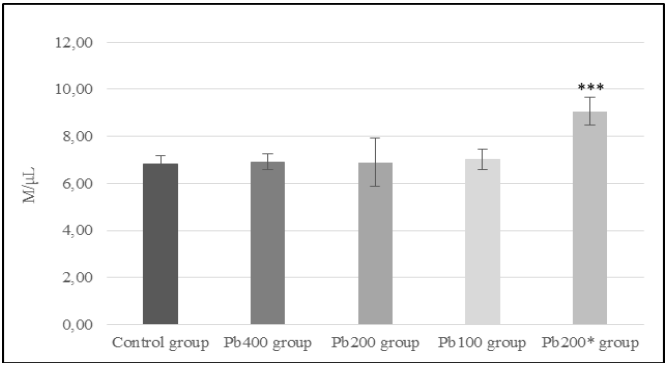

Figure 2. Effect of lead on RBC count in rats after non-acute exposure.

Regarding hemoglobin and hematocrit, the animals treated 4 weeks with lead have presented proximate values to controls (Figure 3). Instead, in group lead-poisoning for 6 weeks, there was a statistically significant increase in the number of hematocrit ($p<1.11E-05$).

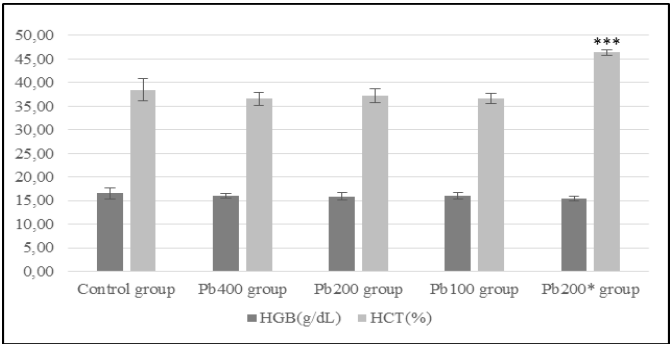

Figure 3. Effect of lead on HGB concentration and HCT percentage in rats after non-acute exposure.

The most pronounced effects were observed on MCV, MCH, and MCHC. Significant changes in MCH were noticed in both 4 weeks Pb-treated groups (Pb400 group where $p<0.0259$; Pb100 group where $p<0.0178$) when compared to the controls. Pb100 group present also a significant decrease of MCH ($p<0.0152$). The 6 weeks' treatment affects all three parameters, MCV ($p<0.0116$), MCH ($p<2.3E-08$), and MCHC ($p<4.74E-07$) (Fig. 4).

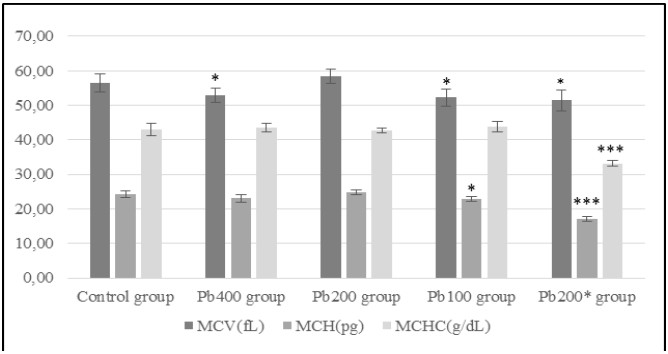

Figure 4. Effect of lead on MCV, MCH, and MCHC in rats after non-acute exposure.

In all experimental groups, regardless of the dose or time of administration, we noticed an increase in platelets count in contrast to control, statistically significant for Pb400 group ($p<0.0020$), Pb100 group ($p<0.0105$), and Pb200* group ($p<0.0004$) (Figure 5).

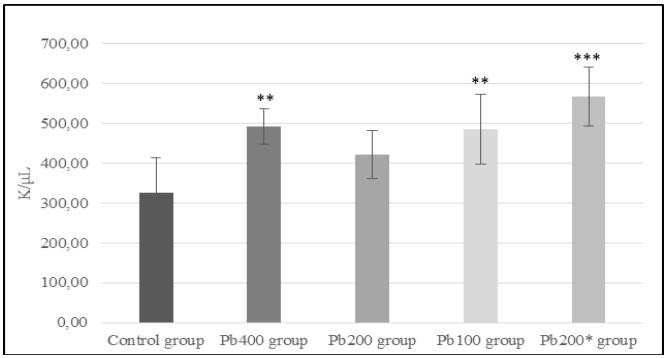

Figure 5. Effect of lead on PLT count in rats after non-acute exposure.

Lead toxic effects on haematological parameters seem to be leukopenia lead treatment, and leukocytosis for 6 weeks' treatment. All experimental groups present thrombocytosis.

The light/dark box test showed that the total time spent in the dark chamber in controls is around 81% during a time period of 20 minutes, while the animals treated with lead spent around 61-65% (Figure 6).

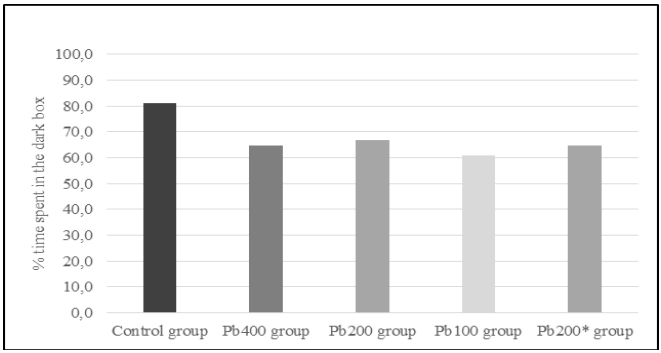

Figure 6. Behavioural response in the light/dark box indicated by the % of total time spent by each group of animals in the dark chamber.

Exposure to lead causes disturbances in behaviour both in humans and animals. These behavioural disturbances have been associated with alteration of the activity of monoaminergic neurotransmitters in the central nervous system, lead intoxication causing an increase in anxious behaviour. The light/dark box test evaluates the axiogenic effects caused by the exposure to lead (Sansar et al. 2012). The time spent by the animal in each room provides a measure of anxious or fear-induced inhibition of normal exploration activity.

Assessment of animal behaviour using the light/dark box test suggests that lead exposure may possibly induce the increasing of anxiety.

Conclusions

Our results showed that non-acute exposure to lead could induce toxic effects in the blood, and central nervous system of adult Wistar rats, a longer exposure has affected more haematological parameters. Future studies of our group will be focused on other parameters and mechanisms implicated in lead toxicity in order to develop more mechanism-specific drugs that address pathological processes and molecular targets of lead intoxication.

Acknowledgements

Funded by Ministry of Research and Innovation in Romania, under Program 1 – The Improvement of the National System of Research and Development, Subprogram 1.2 – Institutional Excellence – Projects of Excellence Funding in RDI, Contract No. 7PFE/16.10.2018 and grant COP A 1.2.3, ID: P_40_197/2016.

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