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The comparison toxicity effects of lead and cadmium exposure on hematological parameters and organs of mice

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ABSTRACT

The hematopoietic system is very susceptible to lead (Pb) and cadmium (Cd) poisoning, because the metal is bound mostly to erythrocytes and blood plasma. It can cause fragility and damage to blood cells and accumulate in tissues. It leads to cause damage to tissue or organs. The aim of this research was to investigate the comparison toxicity effects of lead and cadmium exposure on hematological parameters (red blood cell count/RBC, hemoglobin concentration/HGB, hematocrit/HCT, mean corpuscular volume/MCV, mean corpuscular hemoglobin concentration/MCHC, mean corpuscular hemoglobin/MCH, platelet count/PLT, differential count percentage of lymphocytes, monocytes, and granulocytes), and weight of organs (liver, spleen, and kidney) in mice. Research was conducted using twenty five male mice, which were grouped into five treatments: P1 = control, P2 = Pb 50 mg/L, P3 = Pb 100 mg/L, P4 = Cd 50 mg/L, and P5 = Cd 100 mg/L. The results showed that to compare the control group, Lead and cadmium exposure are significantly shifted to decrease in RBC, HGB, HCT, percentage of granulocytes, body weight and liver weight; on the contrary increase PLT and percentage of lymphocytes. Lead exposure can increase the value of MCHC and MCH, whereas cadmium exposure actually reduces the value of MCHC and MCH in comparison to the control group.

Key words : *Heavy metal, Hematological parameters, Organs of mice*

Introduction

Lead (Pb) and cadmium (Cd) are included in 10 of heavy metals that endanger human health according to the World Health Organization (WHO). Even the International Agency for Research on Cancer (IARC) has been classified Pb and Cd as probably carcinogenic to human. Lead is used as a production material for batteries, solder cables, anti-rust coatings, and paint-making materials. Whereas cadmium is widely used as a pigment in the paint industry, stabilizers in the manufacture of PVC, in the manufacture of batteries and ceramics, as well as in iron coatings to be stainless steel (ATSDR, 2012; Wani *et al.,* 2015; Andjelkovic *et al.,* 2019; Fahruddin and Tanjung, 2019).

Heavy metals can enter the human body through by food, inhalation, and food plants (Eshmat *et al.,* 2014; Ashar *et al.,* 2016; Adhim *et al.,* 2017; Meidivanto *et al.,* 2018). The toxicity of heavy metals in humans is closely related to their accumulation in tissues (Eka and Mukono, 2017; Sulastri et *al.,* 2019; Wijaya *et al.,* 2019. Candra *et al.*, (2019) mentioned that concentration Cd inmantis shrimp

(*Harpiosquillaharpax*) which in the eastern region of Java Sea was 1.5-1.6 mg/kg BW. This value exceeds the threshold allowed for human consumption which is 1.0 mg/kg BW, which can be detrimental to health if consumed regularly. According to Xu *et al.,* (2018) reported there was increasing Pb and Cd levels in the blood and DNA damage which was responsible for the decrease in the enzyme 8-hydroxydeoxyguanosine (8-OHdG) in children that living in areas contaminated with heavy metals.

The hematopoietic system is very susceptible to Pb and Cd poisoning, because the metal is bound mostly to erythrocytes and blood plasma so that it can cause fragility and damage to blood cells. Pb and Cd diffuse and accumulate in several tissues, such as bone marrow, muscle, brain, kidney, heart, spleen and liver, it leads to cause damage to these organs. Accumulation heavy metals will trigger oxidative stress which causes the formation of reactive oxygen species (ROS) due to the deactivation of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPOD) which function as antioxidants (Abraham *et al.,* 2002; Flora *et al.,* 2012; Wani *et al.,* 2015).

Some studies showed that the administration of $Pb(NO_3)_2$ and $CdCl_2$ causes high levels of Pb and Cd in the liver (Sugiharto, 2004), increases malondialdehyde (MDA) levels, swollen, and necrosis in mice liver cells (Sugiharto *et al.,* 2019b), reduce blood serum SOD levels in mice (Sugiharto *et al.,* 2019a), shift to percentages of glomeruli (especially in normal, swollen, and contracted glomeruli), Bowman's capsule diameters, and glomeruli - Bowman's ratio (Sugiharto *et al.,* 2020). This is supported Sharma and Singh (2014) which states that lead acetate can reduce endogenous antioxidant enzymes SOD, CAT and can increase lipid peroxidation in kidney organs. The mechanism of Pb toxicity in reducing antioxidant activity is by activating glutathione (GSH) and antioxidant enzymes such as SOD and CAT (Flora *et al.,* 2012). Hayati *et al.,* (2017) also stated that heavy metal Cd can cause cell damage to gonads and liver of *Barbodes sp*. When specifically compared between Pb and Cd in inhibiting the ATP-ase enzymes, respiration activity, the á-glycerophosphate dehydrogenase enzyme in the tissue of aquatic organisms, Cd has a more toxic effect (Wani *et al.,* 2015).

The aim of this research was to investigate the comparison toxicity effects of lead and cadmium exposure on hematological parameters (red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), platelet count (PLT), differential count percentage of lymphocytes, monocytes, and granulocytes), and weight of organs (liver, spleen, and kidney) in mice.

Materials and Methods

Animals and Materials

The research used male mice (*Mus musculus*, strains Balb/C), aged 8-10 weeks from the Faculty of Pharmacy, Airlangga University, lead acetate and cadmium sulfate was obtained from local chemical stores, Eppendorf micropipette, Micros 60 Hematology analyzer spectrophotometer, ABX Minidil LMG, ABX Minilyse LMG, was carried out in Molecular Genetic Laboratory, Faculty of Sciences and Technology, Airlangga University, Surabaya.

The use of animal subjects for the research have been approved by Ethics Committee of the Faculty of Veterinary Medicine, Airlangga University (certificate no. 2.KE.151.07.2019).

Lead and Cadmium Exposure

Twenty fivemale mice were acclimated for seven days and then randomly gathered into five treatment groups:

P1: 0.3 mL of distilled water (control)
P2: 0.3 mL of lead solution 50 mg/L
P3: 0.3 mL of lead solution 100 mg/L
P4: 0.3 mL of cadmium solution 50 mg/L
P5: 0.3 mL of cadmium solution 100 mg/L

The lead treatment was given every morning (08:00 to 09:00 hours) and were administered orally for 30 days using injection syringe with a round tip (a cannula).

Tissue preparations

On the last day of treatment, the mice were sacrificed. An aliquot blood samples (with anticoagulant EDTA) were collected by intra-cardiac puncture after anesthetic administration. Organs (liver, spleen, and kidney) were removed and weighed. In addition, the body weight of mice was also recorded at the beginning and at the end of the treatment, to see the possibility of weight loss due to Pb and Cd treatments.

Hematological analysis

Measurement of the hematological parameters of the spectrophotometric method was carried out with an ABX Micros 60 Hematology analyzer. The reagents used were ABX Minidil LMG (2.1 mL) and ABX Minilyse LMG (alphalize, 0.52 mL). The following hematological parameters were examined: red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), platelet count (PLT), differential count percentage of lymphocytes, monocytes, and granulocytes.

Statistical Analysis

The statistical analyses were performed using SPSS 16.0. ANOVA and Duncan's Multiple Range Test (DMRT) at 5 % significance level were applied.

Results and Discussion

Lead and cadmium have a significant decrease in RBC, HGB, HCT, percentage of granulocytes, body weight and liver weight, but increasePLT and percentage of lymphocytes. Lead exposure can increase the value of MCHC and MCH, whereas cadmium exposure actually reduces the value of MCHC and MCH (Table 1).

The use of heavy metals, especially lead and cadmium, has increased from year to year. In our study, after oral administration in mice, Pb and Cd were absorbed by the intestine. They were distributed via erythrocytes because they have a high affinity for the sulfhydryl group (-SH) or bound to metallothionein. The hematopoietic system is very susceptible to Pb and Cd poisoning, because the metal is bound mostly to erythrocytes and blood plasma so that it can cause fragility and damage to blood cells. It leads to deficiency of glucose-6-phosphate dehydrogenase (G6PD) enzyme as a glutathione producer that functions to protect hemoglobin and erythrocyte membrane from oxidative stress. It can reduce life span of erythrocytes and increased erythrocyte membrane fragility, resulting in a decrease in the number of erythrocytes, and anemia (ATSDR, 2012, Wani*et al.*, 2015).

Decreasing of RBC, HGB, and HCT values indicate the possibility of anemia symptoms due to Pb and Cd treatment. This is supported by a decrease in the value of MCHC and MCH due to Cd treatment but an increase due to Pb treatment, and also decrease in the value of MCV due to the treatment concentration 100 mg/L of Pb and Cd.Likewise, the increase in PLT value and the percentage of lymphocytes due to Pb and Cd treatment. All data indicated symptoms of anemia by various mechanisms, such as destruction of RBC due to lack of enzymes, heavy metal toxicity,vitamin B12 deficiency, lack of

Table 1. The comparison toxicity effects of lead and cadmium exposure

Groups	Control	Pb 50 mg/L	Pb 100 mg/L	Cd 50 mg/L	Cd 100 mg/L
RBC (10^6/mm^3)	9.88 ± 0.9 ^{c}	8.13 ± 0.9 ^{ab}	8.05 ± 0.2 ^{a}	9.23 ± 0.7 ^{abc}	9.59 ± 1.0 ^{abc}
HGB (g/dL)	16.1 ± 1.7 ^{b}	14.1 ± 1.1 ^{a}	13.6 ± 0.6 ^{a}	15.2 ± 0.4 ^{ab}	15.0 ± 0.7 ^{ab}
HCT (%)	46.0 ± 3.9 ^{b}	40.4 ± 4.1 ^{ab}	37.7 ± 1.2 ^{a}	45.1 ± 1.9 ^{b}	45.0 ± 3.3 ^{b}
MCV (fL)	48.0 ± 2.0 ^{ab}	49.7 ± 0.6 ^{b}	47.0 ± 1.7 ^{ab}	49.0 ± 1.7 ^{ab}	45.7 ± 2.9 ^{a}
MCHC (g/dL)	34.1 ± 0.8 ^{a}	35.1 ± 1.1 ^{ab}	36.0 ± 0.7 ^{b}	33.7± 1.1 ^{a}	34.1 ± 0.6 ^{a}
MCH (pg)	16.3 ± 0.3 ^{ab}	17.4 ± 0.7 ^{b}	16.9 ± 1.0 ^{ab}	16.5 ± 1.1 ^{ab}	15.6 ± 1.1 ^{a}
% Lymphocytes	67.3 ± 8.3 ^{a}	75.6 ± 2.9 ^{b}	70.2 ± 16.0 ^{a}	74.6 ± 4.7 ^{a}	72.9 ± 7.5 ^{a}
% Monocytes	15.8 ± 3.8	17.5 ± 1.6	21.8 ± 2.5	16.5 ± 2.0	14.7 ± 3.4
% Granulocytes	16.87 ± 2.9 ^{b}	6.87 ± 1.4 ^{a}	8.00 ± 2.5 ^{b}	8.83 ± 2.8 ^{a}	12.40 ± 2.3 ^{ab}
PLT (10^3/mm^3)	0.85 ± 0.1 ^{ab}	0.73 ± 0.1 ^{a}	1.08 ± 0.1 ^{a}	0.97 ± 0.3 ^{ab}	0.87 ± 0.2 ^{ab}
Liver (g)	2.26 ± 0.6 ^{c}	2.03 ± 0.2 ^{bc}	2.19 ± 0.3 ^{c}	1.78 ± 0.2 ^{ab}	1.54 ± 0.3 ^{a}
Spleen (g)	0.36 ± 0.15	0.29 ± 0.06	0.34 ± 0.09	0.33 ± 0.13	0.26 ± 0.09
Kidney (g)	0.46 ± 0.09	0.45 ± 0.08	0.45 ± 0.08	0.41 ± 0.10	0.45 ± 0.05
BW (g)	2.19 ± 0.03 ^{c}	1.76 ± 0.02 ^{c}	1.25 ± 0.01 ^{bc}	0.61 ± 0.02 ^{b}	0.15 ± 0.02 ^{a}

RBC = red blood cell count, HGB = hemoglobin concentration, HCT = hematocrit, MCV = mean corpuscular volume, MCHC = mean corpuscular hemoglobin concentration, MCH = mean corpuscular hemoglobin, PLT = platelet count, BW = body weight. Statistical analysis was performed by one-way ANOVA and Duncan's test. The different letters show significant differences in the Duncan's test ($p<0.05$).

absorption Fe ions, kidney failure, and liver damage (Sim *et al.,* 2017; Maner and Moosavi, 2019).Lead inhibits heme biosistesis through inhibition of the enzyme coproporphyrinogen, ä-amino laevulinic acid dehydrogenase (ä-ALAD) and ferrochelatase. Inhibition of these enzymes further causes a decrease in hemoglobin levels in the blood(Richard *et al.,* 2006). Poisoning of Pb and Cd increasing serum levels of ä-amino laevulinic acid (ä-ALA), decreasing to hematocrit, HGB, and MCV (Abraham *et al.,* 2002; Ros and Mwanri, 2003).Cadmium exposure reduces gastrointestinal uptake of Fe, which can result in anemia if dietary intake of Fe is low (ATSDR, 2012).

The accumulation of Pb and Cd in the body, can be stored in soft tissue (liver, kidney, spleen), increased levels of reactive oxygen species (ROS) and oxidize lipids found in cell membranes. In our previous study, it was found that lead exposure increased the number of necrotic cells and the swollen cells, concomitantly decreasing the normal cells in the liver cells, increased MDA levels (Sugiharto *et al.,* 2019b), decreased SOD enzyme activity (Sugiharto *et al.,* 2019a), and also shift to percentages of glomeruli (especially in normal, swollen, and contracted glomeruli), Bowman's capsule diameters, and glomeruli - Bowman's ratio (Sugiharto *et al.,* 2020). According to Kim *et al.,* (2018) showed that cadmium (Cd) induced nephrotoxicity in rats, so in histopathology studies showed hydropic swelling and hypertrophy of the proximal tubular cells in the renal cortex after Cd treatment. Nisar *et al.,* (2011) showed that administration of lead, the glomeruli appear larger in size and the changes in the proximal convoluted like pyknosis, necrosis, and hydropic. It suggested to degenerative effect on tissues, lead to several response in physiology, and decreasing in organ weight (liver, kidney, spleen) or body weight of mice. This is consistent with Andjelkovic *et al.,* (2019) that exposure Pb and Cd induced toxic effects in blood, liver, and kidney of rats by oxidative stress mechanisms.

Conclusion

Lead and cadmium exposure are significantly shifted to decrease in RBC, HGB, HCT, percentage of granulocytes, body weight and liver weight to compare the control group; on the contrary increase PLT and percentage of lymphocytes to compare the control group. Lead exposure can increase the value of MCHC and MCH, whereas cadmium exposure actually reduces the value of MCHC and MCH to compare the control group.

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