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The effects of lead acetate exposure on blood component and Kidney: The mechanism of oxidative stress

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ABSTRACT

Lead (Pb) may induce oxidative stress and it can increase the production of free radicals. Lead exposure increased of malondialdehyde (MDA) and reduced superoxide dismutase (SOD) level in mice blood serum and increased lead levels in the liver. It can induce several responses in physiological and biochemical functions of the body, especially to biochemical of blood components and kidney. The objective of this research was to evaluate the effect of lead acetate exposure on levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), hemoglobin (Hb) concentration, the number of red blood cells (RBC), also glomeruli kidney in mice. Research was conducted using eighteen male mice, which were grouped into three treatments: P1 (control), P2 (Pb 75 mg/kg BW), P3 (Pb 150 mg/kg BW). The results showed that to compare the control group, lead exposure significantly shift to enzyme ALT levels, Hb concentration, RBC numbers, percentages of glomeruli (especially in normal, swollen, and contracted glomeruli), Bowman's capsule diameters, and glomeruli – Bowman's ratio. On the contrary, the AST levels and glomeruli diameter are insignificantly compared to the control group.

Key words: *Blood component, Kidney, Lead, Oxidative stress*

Introduction

Heavy metal pollution is one of the greatest threats to human health. Lead (Pb) is one of heavy metal that induces a wide range of behavioral, physiological and biochemical effects in human. Contamination of lead generally occurs through absorption via the digestive and respiratory systems. Lead is absorbed into the bloodstream and distributed to body organs, such as the liver, kidneys, lungs, and brain. The indicators of the occurrence of lead poisoning in tissues are blood components and kidney histology (Sharma *et al.,* 2011; Gillis *et al.,* 2012; Kim *et al.,* 2018).

Several studies suggested that lead is responsible for reactive oxygen species (ROS) production. Lead has a high affinity for the sulfhydryl group (-SH). It can reduce endogenous antioxidant activity by binding to the functional group -H in several enzymes. Sugiharto *et al.,* (2019a) reported that lead exposure in mice at 75 mg/kg BW reduced SOD levels and shifted the percentage of leukocyte cells (especially in granulocytes, monocytes, and lymphocytes). Lead exposure increased concentration of MDA blood serum and lead levels in the liver of mice (Sugiharto *et al.,* 2019b). Administration of

lead-acetate in mice at 150 mg/kg BW for 40 days, significantly reduced the endogenous antioxidant enzyme, SOD and catalase (CAT), increased lipid peroxidation in kidney organs, and inactivated glutathione (GSH) (Flora *et al.*, 2012; Patra *et al.*, 2011; Sharma and Singh, 2014). It suggested that the degenerative effect on tissues (especially blood component, liver, and kidney) according to the oxidation and antioxidant enzymes due to the reactive oxygen species (ROS) production.

Sugiharto *et al.*, (2018) also reported that lead exposure increased the number of necrotic cells and the swollen cells, concomitantly decreasing the normal cells in the liver cells. It may affect to the biochemical of blood serum, such as enzyme AST, enzyme ALT, Hb concentration, and the number of RBC. AST and ALT were normally found in a variety of tissues including liver, heart, muscle, kidney, and the brain. They are released into the serum when any one of these tissues is damaged (Huang *et al.*, 2006). Poisoning of lead increasing serum levels of δ-amino laevulinic acid (δ-ALA), decreasing to hematocrit, Hb concentration, and mean corpuscular values (Abraham *et al.*, 2002; Ros and Mwanri, 2003). 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. Kim *et al.*, (2018) showed that heavy metal such as 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.

The aim of this research was to investigate the effects of lead acetate exposure on enzyme AST, enzyme ALT, Hb concentration, the number of RBC, and kidney injury especially glomerular diameter and Bowman capsule ratio 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 was obtained from local chemical stores, ASAT kit (tris solution, L-aspartate, L-alanine, 2-oxoglutarate, LDH, and NADH). centrifuge (Eppendorf 5424R), Eppendorf micropipette, ABX Pentra 400 spectrophotometer, microplate reader (Multiskan Go – Thermo scientific), 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.100.06.2018)

Lead Exposure

Eighteen mice were acclimated for seven days and then randomly gathered into three treatment groups normalized to per kg body weight (BW):

P1: 0.4 mL of distilled water (control)
P2: 0.4 mL of lead solution 75 mg/kg BW
P3: 0.4 mL of lead solution 150 mg/kg BW

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). On the last day of the treatment, the mice were sacrificed, and blood samples were taken using intra-cardiac technique. The blood samples were centrifuged at 3,000 rpm for 10 minutes at 10 °C by centrifuge to harvest the serum. Kidney organs also were taken for histopathology analysis by Hematoxylin Eosin (HE) staining (Sugiharto *et al.*, 2018).

AST and ALT Assay

The AST and ALT assay was performed using ABX Pentra 400. Briefly, 10 mL serum sample mixed with 1,000 mL R1 (Tris, LDH, L-aspartate for AST and L-alanine for ALT) and incubated for five minutes. Then, the tube solution mixed with 250 mL R2 (2-oxoglutarat, NADH) and incubated for one minute. Absorbance measured at λ 365 nm on ABX Pentra 400.

Hb concentration

Measurement of Hb concentration was carried out using the Cyanmethemoglobin method. Briefly, 10 µL blood samples was diluted with 1,000 µL Drabkin's solution and then incubated for five minutes. Then, it taken 100 µL to microplate with 2 replications. The absorbance of the solution was measured at λ 540 nm with microplate reader.

The Number of RBC

The number of erythrocytes was performed using an improved *Neubauer haemocytometer.* Briefly, blood samples was sucked by erythrocyte pipette from Thoma to 0.5 and diluted until the 101 with

Hayem's solution, then filled into the haemocytometer. Incubated for 3 minutes and observed in the microscope.

Glomeruli and Glomeruli – Bowman's Ratio Observation

Observation of glomeruli, glomeruli and Bowman capsule ratio were carried out at 50 glomeruli for each treatment by micrometres in the microscope. Swollen glomeruli showed by an enlarged diameter of Malpighi or hypertrophy of glomeruli cells, and contracted glomeruli has characteristics decreasing diameter of Malpighi or the atrophy of glomeruli cells. The glomeruli and Bowman's capsule diameter measuring in average of vertically and horizontally. Then the glomeruli ratio is calculated by dividing glomeruli diameter to Bowman's capsule diameter (Kim *et al.,* 2018).

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 is known to induce toxicity in wide range of target organs and accumulate in the liver, kidney, lung, brain, and lung. According to Flora *et al.* (2012), the main mechanism of lead toxicity is via oxidative stress by inducing the generation of ROS and it can be detected by increasing MDA levels and decreasing SOD levels. Lead has a high affinity for the sulfhydryl group (-SH). It leads to the increase in free radicals production and reduced endogenous antioxidants, it suggested that the degenerative effect on tissues according to the oxidation and antioxidant enzymes due to the ROS production. ROS can reduce polyunsaturated fatty acids and produced MDA. This compound is a highly reactive aldehyde that causes stress in cells and forms covalent bonds with a protein advanced lipoxidation end-products (ALE). Aldehyde production can be used as a biomarker to measure the level of oxidative stress in an organism. The level of lipid peroxidation can be estimated by the amount of MDA in the tissues. In our previous study, it was found that administration of lead can increase blood serum MDA levels and decrease SOD (Sugiharto *et al.,* 2019a; Sugiharto *et al.,* 2019b). It suggested to induce several physiological and biochemical responses in the cells and tissues (Table 1).

Sugiharto *et al.,* (2018) reported that lead exposure increased the number of necrotic cells and the swollen cells, concomitantly decreasing the normal cells in the liver cells. AST and ALT were normally found in a variety of tissues including liver, heart, muscle, kidney, and the brain. They are released into the serum when any one of these tissues is damaged. However, it must be emphasized that higher-than-normal levels of these enzymes should not be automatically equated to liver disease (Huang *et al.,* 2006). ALT is normally found in the liver and a fairly specific indicator of liver damaged. In this study, lead exposure was more significantly to increase in ALT levels to compare of AST levels due to liver damage.

Lead is bound 90% in the circulation system and it can affect the hematopoietic system. Poisoning of lead increasing serum levels of δ-amino laevulinic acid (δ-ALA), decreasing to haematocrit, Hb concentration, and mean corpuscular values (Abraham

Table 1. Effect of Lead Exposure on Blood Component and Kidney

Groups	Control	Pb 75 mg/kg BW	Pb 150 mg/kg BW
AST (U/L)	160.4 ± 35.8 ^{a}	181.6 ± 54.3 ^{a}	184.0 ± 45.7 ^{a}
ALT (U/L)	55.6 ± 18.3 ^{a}	77.4 ± 8.6 ^{ab}	84.4 ± 21.9 ^{b}
Hb (g/dL)	13.7 ± 1.0 ^{b}	11.7 ± 1.5 ^{a}	12.5 ± 0.9 ^{ab}
RBC (x 10^{6}/mm^{3})	9.26 ± 0.6 ^{b}	8.03 ± 1.0 ^{a}	8.52 ± 0.6 ^{ab}
Normal glomeruli (%)	66.0 ± 11.4 ^{b}	40.0 ± 10.0 ^{a}	32.0 ± 4.5 ^{a}
Swollen glomeruli (%)	28.0 ± 8.4 ^{a}	34.0 ± 11.4 ^{ab}	42.0 ± 13.0 ^{b}
Contracted glomeruli (%)	6.0 ± 8.9 ^{a}	26.0 ± 11.4 ^{b}	26.0 ± 11.4 ^{b}
Glomeruli diameter (µm)	19.9 ± 2.7 ^{a}	20.7 ± 1.8 ^{a}	19.5 ± 1.3 ^{a}
Bowman's capsula diameter (µm)	23.9 ± 3.0 ^{a}	27.7 ± 3.7 ^{b}	26.8 ± 6.7 ^{ab}
Glomeruli and Bowman's capsule ratio	0.83 ± 0.02 ^{b}	0.75 ± 0.05 ^{a}	0.73 ± 0.03 ^{a}

Note: The different letters show significant differences in the Duncan's test ($p<0.05$)

et al., 2002; Ros and Mwanri, 2003). According to Sugiharto (2004), administration of $Pb(NO_3)_2$ decreased Hb concentration and the number of erythrocytes of rats. Lead poisoning has been found to be the cause of anaemia because lead inhibits porphobilinogen synthase, ferrochelatase, and prevents heme synthesis (Wani *et al.,* 2015). In this study, lead exposure significantly decreased Hb concentration and the number of RBC to compare the control group.

Heavy metals induced oxidative stress and suggested increment possibility of the kidney degenerative. It is well established that oxidative stress is an important mechanism nephrotoxicity. Heavy metal, including lead, increased lipid peroxidation in kidney and it lead induced nephropathy, loss of proximal tubules and interstitial fibrosis (Wani *et al.,* 2015). According to Kim *et al.,* (2018) showed that histopathological studies showed hydropic swelling and hypertrophy of the proximal tubular cells in the renal cortex after Cd treatment. The apoptotic pathway is closely related to the pathogenesis of cadmium-induced nephrotoxicity. Nisar *et al.,* (2011) showed that the glomeruli appear larger in size and the changes in the proximal convoluted like pyknosis, necrosis, and hydropics after lead exposure. Momeni and Eskandari (2017) reported sodium arsenite-treated showed a significant decrease in the diameter of glomerulus and proximal tubule, glomerular area, compared to the control group. On the contrary, no significant difference was found in kidney weight, area and diameter of Bowman's capsule as well as the diameter of distal tubule. In this study, Bowman's capsule diameter was increased and glomeruli–Bowman's ratio was decreased significantly to compare the control group. This condition was also supported by the fact that percentage of normal glomeruli was decreased, but the percentage of contracted and swollen glomeruli were increased.

Conclusion

Lead exposure are significantly shifted to increase enzyme ALT levels, RBC numbers, percentages of glomeruli (especially swollen and contracted glomeruli), and Bowman's capsule diameters to compare the control group; however lead exposure decreased Hb concentration, percentages of normal glomeruli and glomeruli – Bowman's ratio to compare the control group.

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