18783-55026-1-PB_1.pdf

by Hasni Syahida

BERKALA KEDOKTERAN

doi: http://dx.doi.org/10.20527/jbk.v20i1.18783

RESEARCH ARTICLE

OPEN ACCES

Effect of Heavy Metal Polluted River Water on Body Weight and Hemoglobin Level in White Rats (*Rattus novergicus*)

Franciscus Xaverius Hendriyono[1], Wivina Riza Devi[1], Azma Rosida[1], Taufiq Hidayat[1]

[1]Department of Clinical Pathology, Faculty of Medicine, Universitas Lambung Mangkurat, Banjarmasin, Indonesia
Coresspondence Author: dr.azmarosida@ulm.ac.id

Abstract:

Contamination of heavy metal on the South Kalimantan River has become a well thrived issue. In the Barito River watershed, it is reported that there are heavy metals in the form of Pb, Hg and Cd. Heavy metals can affect physiological anatomical changes and the hematopoietic system. This study aims to determine the impact of heavy metal contamination on changes in body weight and hemoglobin levels. The research method is a true laboratory experiment using a pre-post-test group design. The subjects used were healthy male white rats (Rattus norvegicus), aged 2-6 months and weighing 250-300 gr. The research group was divided into two groups, the control group (K) was a group of experimental animals that were given distilled water while the treatment group (P) was a group of experimental animals that were given water contaminated with heavy metals (Pb, Cd, and Hg) ad libitum for 30 days. The results showed that there was no effect of giving Pb metal; 0.01 mg/l = 0.006 mg/kgBW, Cd; 0.003 mg/l = 0.018 mg/kgBW, Hg; 0.001 mg/l = 0.0006 mg/kgBW and Pb+Cd+Hg; 0.006 mg/kgBB + 0.018 mg/kgBB + 0.0006 mg/kgBB on body weight of white rats. There was a significant decrease in Hb levels in white rat hemoglobin after administration of Pb metal; 0.01 mg/l = 0.006 mg/kgBB. Pb metal in water can influence changes in decreasing Hb levels during sub-acute administration. It can be concluded that there was a significant difference in hemoglobin levels before and after giving water containing Pb/lead metal, whereas in the other groups there was no significant difference, as did the Hb levels.

Keywords: Heavy Metal; Body Weight; Hemoglobin

Introduction

River is a live resource for people around it. Many kind of activities such as such as the disposal of industrial, household and agricultural waste cause a decrease in river water quality. Heavy metal pollution in rivers in the South Kalimantan area is a growing issue. Herliwati et al's research found the presence of heavy metals in shrimp from the Barito River estuary indicating that there was bioaccumulation of lead and cadmium metals that exceeded the tolerance limits according to JECFA (Joint FAO/WHO Expert Committee on Food Additives).[1] Research by Rahmadinah et al reports that the quality of the river in Sungai Paring Village shows moderate to poor polluted conditions, this is in line with data from the South Kalimantan Regional Environmental Agency (BLHD) 2021.[2,3]

Heavy metals consist of 2 types, essential and non-essential. Essential heavy metals such as copper (Cu), selenium (Se), iron (Fe) and zinc (Zn) are needed to maintain the human body's metabolism. On the other hand, non-essential heavy metals have no function in the human body, and are even so dangerous that they cause poisoning (toxicity) in humans, including: lead (Pb), mercury (Hg), arsenic (As) and cadmium (Cd). Several studies have reported that heavy metal toxicity in experimental mice can cause a decrease in several hematological parameters, one of the consequences of a decrease in hematological parameters is a decrease in hemoglobin or anemia. [4,5,6,7]

Anemia or lack of blood is a serious problem because it causes sufferers to experience tiredness and lethargy, which will have an impact on creativity and productivity. Apart from that, anemia also increases susceptibility to disease in adulthood and gives birth to a generation that experiences nutritional disorders. The incidence of anemia in Indonesia is still quite high. Based on 2018 Riskesdas data, the prevalence of anemia in adolescents is 32%. Based on the data, examination results in South Kalimantan in 2018 showed that 52.98% of adolescent girls experienced anemia, while in 2019 adolescent girls' anemia reached 42,45%. Apart from teenage girls, pregnant women are also at risk of anemia. Anemia in pregnant women in South Kalimantan in 2021 is 19,6%.[8]

This research is aimed to find out the effect of heavy metal pollution on body weight change and Hemoglobin level.

Research Method

Research Design

The research used True Experiment Method with pre-post-test control group design. Research subject used male rats (Rattus norvegicus), 2-4 months old, healthy with 250-300 gram in weight. Research groups were divided into 2 groups: control group (K) was a group of experimental animal fed with aquades ad libitum; and treatment group (P) was a group of experimental group fed with heavy metal polluted water. Treatment period was 30 days. Based on the number of groups, the minimum number of repetitions of research subjects determined using the Federer formula was 16 per group plus 10% risk factor for death, so the total research subjects used were 32 white rats (Rattus norvegicus).

Research Variable and Operational Definition

Independent variable in this research was heavy metal polluted water while dependent variables were body weight and hemoglobin level. Heavy metal polluted water is water that added by heavy metal with following concentrations: Pb: 0,01 mg/l; Cd: 0,003 mg/l; Hg : 0,001 mg.l; and Pb + Cd + Hg : 0,01 mg/ l + 0,003 ml/l + 0,001 ml/l.[9]

Feeding was done ad libitum, and once a week the rats were weighted. Rats weight is the result of weighting using scale in gram (g). Hemoglobin level (Hb) is rats hemoglobin levels measured using portable hemoglobin measurement, before and after treatment.

Treatment Proccess

The white rats (Rattus norvegicus) were selected with some certain requirement: male, 2-6 months old, 250-300 gram, and healthy. Before treatment, the rats were acclimatized for one week in separate cages to provide the same physical and psychological conditions. Each cage was only filled with 3-4 animals and given special food for experimental animals. Then the experimental animals were randomly grouped into two groups, the control group (K) given distilled water ad libitum and the treatment group (P) given water contaminated with heavy metals ad libitum. The rats before and after treatment had their body weight measured and hemoglobin checked.

Measurement of Body Weight and Hemoglobin Levels

Body weight was checked using a scale in gram units and blood glucose levels are checked using the enzymatic method, using a hemoglobinometer, units mg/dL. The data collection process was carried out based on the parameters measured in the study: assessing body weight and hemoglobin levels before and after treatment within 30 days.

Data Analysis

The data were analysed using statistic test in SPSS program. The hemoglobin level variable was subjected to a normality test to determine the normality of the data distribution using the Shapiro-Wilk test because $n \leq 50$. Then proceed with a homogeneity test using Levene's test to determine whether the variance of several data populations is the same or not. Data was normally distributed and homogeneous if the confidence level value is 95%, $p > 0.05$ so that the data can be analyzed using the independent parametric t-test. The independent t-test was used to determine differences in body weight and hemoglobin levels between control and treatment group data. If the data is not normally distributed then the research data is analyzed using nonparametric test analysis with the Mann-Withney test.

Results

The research group consisted of 4 groups: the control group (K) and the treatment group (P1-P4), K was the group given distilled water, P1 was the rat group given water contaminated with the heavy metal Pb; 0.01 mg/l = 0.006 mg/kgBW, P2 was a group of mice that were given water contaminated with the heavy metal Cd; 0.003 mg/l = 0.018 mg/kgBW, P3, the group of mice given water contaminated with the heavy metal Hg; 0.001 mg/l = 0.0006 mg/kgBW, P4 was a group of mice that were given water contaminated with the heavy metal combination Pb+Cd+Hg; 0.006 mg/kgBB + 0.018 mg/kgBB + 0.0006 mg/kgBB.

All treatment groups were given contaminated water orally with sondase while the control group was given distilled water ad libitum for 28 days. After the 29th day, the mice were fasted at night and the next day they were euthanised to check research parameters for body weight and hemoglobin levels.

Table 1 The Mean of Body Weight and Hemoglobin Levels's Measurement in Research Group

Group	Body Weight (gram)			Hemoglobin (g/dL)		
	Pre	Post	p	Pre	Post	p
Control	217,8	216,2	>0,05	18,25	22,78	<0,05
Pb Group	212,4	215	>0,05	20,72	18,38	<0,05
Cd Group	212,6	231,4	>0,05	20,4	19,64	>0,05
Hg Group	211	213	>0,05	18,1	19,9	>0,05
Pb+Cd+Hg Group	212	226,6	>0,05	20,2	19,18	>0,05

Discussion

This study used doses of heavy metals that were safe to consume via bottled water according to SNI 2009 with a subchronic exposure time (28 days) according to BPOM regulations. The results of subchronic administration of the heavy metals Pb, Cd, Hg orally once a day were behavioral responses to the rats' appetite which was reflected through weight observations carried out during 28 days of observation. In the control and treatment groups there was no significant difference in the weight of the mice. This is different from Sani et all's research. 2021, rats who received Pb and Cd at a dose of 1 mg/kg BW or those given Pb (2mg/kgBW) + Cd (1mg/kgBW) and Cd (2 mg/kgBW) + Pb (1mg/kgBW) experienced a decrease in BW.[10]

In the control group there was a significant difference in the increase in Hb levels before and after 28 days, while in the group given Pb there was a decrease in meaningful Hb levels. In contrast to other treatment groups there was no difference in meaningful Hb levels before and after administration of Cd, Hg and all heavy metals. This is in accordance with the meta-analysis results of Capitặo C et al (2022) which found a relationship between Hb and Pb of -0.750; 95% Confidence Interval (CI) 0.583- 0.874.

Heavy metals can inhibit the synthesis of haem and globin at certain amounts. Heavy metal poisoning inhibits the pyrimidine 5' nucleotidase enzyme which causes the accumulation of denatured RNA in red blood cells. This RNA will give a basophilic stippling picture on peripheral blood examination using Romanowsky staining. Red blood cells become hypochromic or develop hemolytic anemia. [11]

Exposure to heavy metals can also inhibit enzymes, such as hexokinase enzyme, works in glycolysis process. Its disruption cause disturbances in carbohydrate metabolism. This enzyme is affected by mercury metal ions binding.[12]

Generally, the absorption, distribution and accumulation of metals in organs and tissues is influenced by several factors. These include the characteristics and form of the metal, route, dose and duration of exposure, ability to bind ligands in cells, and species sensitivity. The hematopoietic system is one of the most vital organs for assessing toxicity. After oral administration, Hg, Cd and Pb undergo intestinal absorption and are transported through the blood. In the blood, they can be distributed via red blood cells and plasma proteins, especially albumin. According to Markowitz, M. (2000), there are several factors that can influence the toxicity of heavy metals.

Level and Length Exposure

It is known that heavy metals have bioaccumulative and biomagnification properties. Thus, the higher and longer the level of exposure to heavy metals, the higher the concentration of heavy metals in the bodies of organisms including humans and the toxic effects will be greater.

Chemichal Form

The chemical form of a heavy metal can affect the toxicity. For example, mercury in the form of $HgCl_2$ is more toxic than mercury (HgCl). This is because the divalent form is more soluble than the monovalent form. Besides, HgCl form is faster and easier absorbed so that the toxicity is higher. Inorganic mercury such as HgCl and $HgCl_2$ are known as kidney toxicants, while organic mercury such as methyl mercury is known to be toxic to the central nervous system.

Protein Complex

Some of heavy metal can bonded with protein because of the high afinity on sulfide group. Protein contains of amino acid that has S group such as methionine (met) and cysteine (cys), that easily binding with heavy metals. As for the example of heavy metal that form protein metal complex is the one that is formed by lead, bismuth, and mercury-selenium. Cadmium (Cd) and some water metal, such as copper (Cu) and Zinc (Zn) can bind with metalotioneine, a protein that has low molecule mass. Cd complex is not always toxic if compared with Cd^{2+}, but inside of renal tubular cells, Cd metalotioneine complex will release Cd^{2+} and resulted a toxic effect.

The administration of heavy metals in this study used safe doses according to SNI 2009 but was able to reduce Hb levels after 28 days of subacute administration, although the metals Hg, Cd and Pb+Hg+CD had no effect on Hb levels and body weight.

Conclusions

There was no effect of giving Pb metal; 0.01 mg/l = 0.006 mg/kgBW, Cd; 0.003 mg/l = 0.018 mg/kgBW, Hg; 0.001 mg/l = 0.0006 mg/kgBW and Pb+Cd+Hg; 0.006 mg/kgBB + 0.018 mg/kgBB + 0.0006 mg/kgBB on body weight of white mice. There was a significant decrease in Hb levels in the hemoglobin of white mice after administration of Pb metal; 0.01 mg/l = 0.006 mg/kgBB. Pb metal in water can influence changes in decreasing Hb levels during sub-acute administration.

Acknowledgements

This research was supported by Universitas Lambung Mangkurat, Banjarmasin, Indonesia through the Compulsory Research Lecturer Program (PDWM), based on grant agreement No 618/UN8/PG/2023. The funders were not involved in design of the study, collection and analysis of data, preparation of the manuscript, or decision to publish. The researchers would like to thank all participants involved in this research.

References

1. Herliwati, Rahman, M., Hidayat, A.S., Amri, U., Sumantri, I., 2022. The occurrences of heavy metals in water, sediment and wild srimps caught from barito estuary, South Kalimantan, Indonesia. *HAYATI J Biosci*, 29(5), pp. 643–647.
2. Rahmadani, Alawiyah, T., 2022. Deteksi logam berat merkuri (HG) pada air dan ikan pasca pertambangan emas di Sungai Barito Kabupaten Barito Utara. *J Surya Med*, 8(3), pp. 76–80.
3. Dinas Lingkungan Hidup Provinsi Kalimantan Selatan, 2021. Laporan kinerja Dinas Lingkungan Hidup Provinsi Kalimantan Selatan Tahun 2021. *Dinas Lingkungan Hidup Provinsi Kalimatan Selatan*.
4. Adhani R., Husaini. 2017. Logam berat sekitar manusia. *Lambung Mangkurat University Press*. Banjarmasin. pp.88–100.
5. Dwivedi K., 2020. Heavy metals toxicity and its effect on hematological parameters in albino rats. *International*

Journal of Creative Research Thoughts, 8(10), pp. 4126–4135.
6. Cimboláková, I., Kimáková, T., Pavolová, H., Bakalár, T., Kudelas, D., Seňová, A., 2020. Simulation of chronic intoxication in rats exposed to cadmium and mercury. *Appl Sci*, 10(22), pp. 1–14.
7. Hayet, S., Sujan, K.M., Mustari, A., Miah, M.A., 2021. Hematological and Biochemical Investigations in albino rat exposed to cadmium chloride. *Int J Adv Res Biol Sci*, 8(6), pp. 1–5.
8. Dinas Kesehatan Provinsi Kalimantan Selatan. 2021. Laporan kinerja Dinas Kesehatan Provinsi Kalimantan Selatan. *Dinas Kesehatan Provinsi Kalimantan Selatan*.
9. Badan Standardisasi Nasional. 2009. Batas maksimum cemaran logam berat dalam pangan. Badan Standardisasi Nasional, pp. 1–29.
10. Sani, A., Darma, A.,I., Imam, F.,A., Usman, B., U., 2021. Heavy metals mixture affects the blood and antioxidant defense system of mice. Journal of Hazardous Materials Advances. *Research Square*, pp.1–19.
11. Helwig, N.,E., Hong, S., Hsiao-wecksler, E.,T., 2016. Hypochromic anemias. In: Victor HA, Moss PAH, editors. Hoffbrand's essential haematology. 7th ed. *Hoboken*, pp. 29–40.
12. Pratidina EA, Suhartono E, Setiawan B. Impact Of Heavy Metals On Hexokinase Isoforms: An In Silico Study. Berkala Kedokteran. 2022;18(1):29

18783-55026-1-PB_1.pdf

ORIGINALITY REPORT

9%	2%	10%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Vianty Mutya Sari, Tonasih Tonasih, Siti Difta Rahmatika. "Supplementary Blood Tablets (Ttd) In Adolescent Women (Rematry) To Increase Hemoglobin (Hb) Levels", Jurnal Kebidanan Malahayati, 2022 Publication	2%
2	Sujatha, C. H., V. B. Pratheesh, and Yung-Tse Hung. "RIVER AND LAKE POLLUTION", Handbook of Environment and Waste Management Air and Water Pollution Control, 2012. Publication	2%
3	RK Apriyadi, T Winugroho, IDKK Widana, A Subiyanto. "Potential health disaster risks due to Ciliwung River Water quality in DKI Jakarta", IOP Conference Series: Earth and Environmental Science, 2021 Publication	2%
4	farfar.pharmacy.bg.ac.rs Internet Source	2%

5	Trivedi, Nitin G.. "A study on responses of Adhatoda vasica (L.) to heavy metal.", Proquest, 2015. Publication	2%

Exclude quotes	On	Exclude matches	< 2%
Exclude bibliography	On		