ORIGINAL ARTICLE

IR-Bagendit Leaves Water Extract as Preventing Agent in Hematopoiesis, Degeneration and Necrosis in Kidney Tubulus of Lead-Exposed Rats

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ABSTRACT

Background: Lead exposure causes anemia indicated from hemoglobin, hematocrit and erythrocytes count. Lead accumulation in kidney causes cells degeneration, necrosis in tubulus and function disorder.

Aim: To analyze the effect of IR-Bagendit rice leaves water extract in preventing hematopoiesis and cell degeneration and necrosis in lead-exposed rat's kidney.

Methods: There were three treatment groups (0.2 ml/day, 0.4 ml/day, and 0.8 ml/day IR-Bagendit water extract) and control with no extract treatment in lead-exposed rats. After 100 days of treatment, the average hemoglobin, hematocrit, and erythrocyte counts were higher than control group.

Results: The results of extract treatment in lead-exposed rats were higher compared to the control group, so does in hematocrit levels and the number of erythrocytes. The leukocytes number was relatively same between control and treatment group. The highest hemoglobin and hematocrit levels were in the 0.4 ml treatment group (9.95 gr% and 11.88%), the highest average no of erythrocytes was in 0.2 ml extract (3.74 million/mm3) treatment group

Conclusion: 0.4 ml IR-Bagendit extract was the optimal dose as preventive agent in lead-exposed rats.

Keywords: IR-Bagendit, lead (Pb), hematopoiesis, cell degeneration, necrosis

INTRODUCTION

Lead (Pb) is a toxic metal in human that cause anemia[1] and disorder in detoxification organ such as liver and kidney, especially in children, pregnant women, and industrial workers[2]. Lead is infiltrated into the body via respiratory, skin, and digestion and then will be accumulated[3]. The use of lead in fuel had been reduced unfortunately lead pollution is still high obtained from paints, batteries, jewelries, cosmetics, kid toys and gasoline industries [(4, 5)]. In the other hand, phosphate fertilizers, pesticides, herbicides in agricultural product and environment contamination in natural health products are increasing the source of lead poisoning in food[6,7].

Lead can disturb heme synthesis by inhibit ferochelatase and δ-aminolevulinate synthase (ALAS)[8,9]. Lead exposure will decrease the activity of glucose-6 phosphate dehydrogenase (G-6PD) enzyme, which will shorten the erythrocyte lifetime. This shortening induced by the cell membrane damage, since lead is a pro-oxidant that will cause oxidative stress[10,11], which can be measured through hematocrit (Ht). Interleukin 3(IL-3) has an important role in the process of hematopoiesis especially in triggering the proliferation of various hematopoietic cells, cell development activities, differentiation and cell apoptosis[12].

Lead accumulation occurs in soft tissues such as the kidneys and liver. In the kidney, the proximal tubular epithelium will degenerate, hyperplate and cariomegaly. Cell degeneration is an alteration in cell morphology due to an injury, which can be reversible and irreversible. If pathological stimulation is too severe, irreversible injury will be occurred and cells will dead or necrosis[13]. Degeneration and necrosis of renal tubular cells due to exposure of lead is have an impact on expression of erythropoietin hormones (EPO), urea levels and creatinine levels[14].

Preventive efforts to prevent lead toxicity become more severe should be developed. Metal binder or chelating agent can be used to bind toxic metals such as lead, so it can be removed from the body. EDTA and 2,3-Dimercaprol is chelating agent for curative procedure, unfortunately it is harmful[15].

Research on chelating agent using natural ingredients had been carried out by Santosa et al[16] that used water extract of IR-Bagendit rice leaves (*Oryza sativa L.*). IR-Bagendit rice leaves has the highest metallothionein protein compared to the other plants such as corn, soybeans, and beans. Metallothionein can bind lead ion through sulfidril groups[17]. Metallothionein is synthesized in the liver and gastrointestinal wall through absorption of various natural ingredients or micronutrient such zinc, it had been proved to improve biosynthesis of heme, hematopoiesis, degeneration and necrosis of epithelial tubular renal, and basophilic stipplings[18,19].

METHODS

Experimental Animals and Treatment: Twenty eight male *Rattusnovergicus*, aged 15 weeks old, 180-220g in weight, divided into 3 treatment groups and 1 control group. Animal keeping and treatment was conducted in Laboratory Integrated Research and Testing (LPPT) Universitas Gadjah Mada, Yogyakarta for 100 days. During treatment period, *R. norvegicus* was given standardized pellet feed (*ad libitum*), lead exposure using 0.5 gr/kg body weight/day [(20)] and treated using water extract of IR-Bagendit rice leaves were 0.2 ml/day, 0.4 ml/day, and 0.8 ml/day in each treatment group respectively, given through oral gavages and the control group was not treated with the extract. Randomized post test only control-group design was used to determine the number and randomized the samples. Ethical clearance was acquired from Faculty of Medical UNISSULA register No. 209/VI/2017/Komisi Bioetik.

Measurement of Hemoglobin (Hb), Hematocrit (Ht), Erythrocyte, Interleukin 3 (IL3), EPO, Degenerative Cells and Necrosis, Ureum and Creatinine: The

samples used to calculate Hb, Ht levels and number of erythrocytes was using whole blood with EDTA as anticoagulant. Blood serum specimens were collected for measuring the levels of IL3, EPO, ureum, and creatinine. Histological examination using Hematoxyline-Eosin stain was carried out to count the number of degenerate and necrosis cells in renal tubular tissue. The normal, degenerated, and necrotic cell of renal tubules were calculated as percentages. Hb, Ht and erythrocyte levels were measured using a hematology analyzer machine. IL3 and EPO were measured using ELISA method. The urea level was measuring was conducted using the Urease GLDH enzymatic UV test. Jaffe method was used to measure the creatinine. These measurements of blood and tissue parameters were conducted at the Clinical Pathology Laboratory and Cyto-Histotechnology Laboratory in Universitas Muhammadiyah Semarang (UNIMUS).

Data analysis: The collected data was described and analyzed the optimum concentration of water extract of IR bagendit rice leaves using a statistical Kruskal Wallis test, followed by Bonferroni.

RESULTS

The results of extract treatment in lead-exposed rats were higher compared to the control group, so does in hematocrit levels and the number of erythrocytes. The leukocytes number was relatively same between control and treatment group. The highest hemoglobin and hematocrit levels were in the 0.4 ml treatment group (9.95 gr% and 11.88%), the highest average number of erythrocytes was in 0.2 ml extract (3.74 million/mm3) treatment group (Table 1.).

The treatment in lead-exposed rats was found that the normal renal tubule cells count in the treatment group was higher than the control group. The highest normal cells count was in the 0.8 ml extract treatment (44.00±9.61) and the lowest was in the control group (19.42±4.50). Result in degeneration and necrotic cells number were contrast compared to normal cells, the control group result was higher than treatment group. The highest cell degeneration in the control group was 24.57±7.29 and the lowest was in the 0.8mL IR-bagendit extract treatment (16.40±4.56). The highest of necrotic cells was in the control group (56.00±5.59) and the lowest was in the 0.8mL IR-bagendit treatment group (39.60±5.94) (Table 2).

The highest level of ureum was in the 0.4 ml extract treatment group (55.62±19.17) and the lowest was in the control group (36.00±12.12). The highest creatinine level was in the 0.8 ml extract treatment group (0.76±0.08) and the lowest was in the control group (0.50 ± 0.060). The highest IL3 level was in the control group (93.69±52.75), and the lowest was in the 0.4 ml extract treatment group (73.34±47.60). The average EPO was quite varies between group, 0.4ml extract treatment group was the highest (85.77±45.56), and it was above the average of the control group (73.04±29.38) (Table 4.).

The trend levels of urea, creatinine, IL3 and EPO were shown in Figure 4. Ureum and creatinine levels from control group to the 0.2 ml and 0.4 ml extract treatment were increased and slightly decreased for the 0.8 ml of extract treatment group. The average EPO levels decreased in the control group and increased in the 0.4 ml extract treatment and then decreased in 0.8 ml extract treatment. The average level of IL3, in the control group was slightly decreased compared to 0.4 ml extract treatment group and slightly increased in 0.8 ml extract treatment group.

Table 4: The measurement results of ureum, creatinine,IL3, and EPO level based on water extract of IR-Bagendit rice leaves treatments in rats exposed to lead

Variabel	Perlakuan				P value
	Control group	0,2 ml IR-Bag. extract	0,4 ml IR-Bag. extract	0,8 ml IR-Bag. extract	
Ureum	36.00 ± 12.12	39.88 ± 9.92	55.62 ± 19.17	41.94 ± 17.39	0.92
Creatinin	0.50 ± 0.06	0.68 ± 0.14	0.72 ± 0.07	0,76 ± 0.08	0.01
IL 3	93.69 ± 52.75	80.73 ± 31.80	73.34 ± 47.60	84.30 ± 61.80	0.93
EPO	73.04 ± 29.38	66.64 ± 48.49	85.77 ± 45.56	58.37 ± 44.30	0.77

Table 1. The result of Hb, Ht, and blood cells in treatments and control.

Variables	Treatment				P value
	Control group	0,2 ml IR-Bag. extract	0,4 ml IR-Bag. extract	0,8 ml IR-Bag. extract	
Hemoglobin (gr%)	9.54 ± 134	9.68 ± 0.87	9.95 ± 0.83	9.80 ± 0.86	0.89
Hematocrit (%)	10.87 ± 0.70	11.00 ± 0.06	11.82 ± 0.88	11.82 ± 1.13	0.91
Erythrocyte count (mm^3)	3.36 ± 0.71	3.74 ± 0.30	3.47 ± 058	3.44 ± 0.26	0.76
Leukocyte count (mm^3)	10.6 ± 2.22	10.56 ±2.87	9.61 ± 2.64	9.64 ± 3.57	0.87

Table 2: The measurement results of normal, degenerated, and necrotic cells.

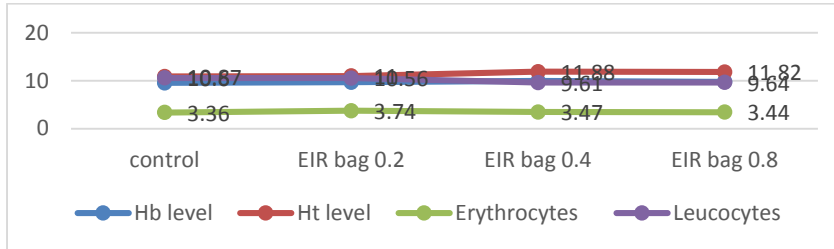| Variabel	Treatments				P value
	Control	0,2 ml IR-Bag. extract	0,4 ml IR-Bag. extract	0,8 ml IR-Bag. extract	
Normal	19.42 ± 4.50	34.33 ± 14.09	35.50 ± 9.46	44.00 ± 9.61	0.00
Degenerated	24.57 ± 7.29	21.00 ± 7.29	21.85± 7.33	16.40 ± 4.56	0.16
Necrotic	56.00 ± 5.59	44.66 ± 8.84	43.00 ± 4.20	39.60 ± 5.94	0.00

Fig. 1. Hb, Ht, and blood cells in treatments and control.

Table 3. Tukey test results on normal and necrotic cells comparison

Variabel	P value
Normal cells	
Control- 0,2 ml IR-Bag. extract	0.03
Control- 0,4 ml IR-Bag. extract	0.03
Control- 0,8 ml IR-Bag. extract	0.00
Nekrosis	
Control- 0,2 ml IR-Bag. extract	0.03
Control- 0,4 ml IR-Bag. extract	0.00
Control- 0,2 ml IR-Bag. extract	0.00

Fig. 2: Graphical results of normal, degenerated, and necrotic cells in water extract of IR-Bagendit rice leaves treatments in rats exposed to lead
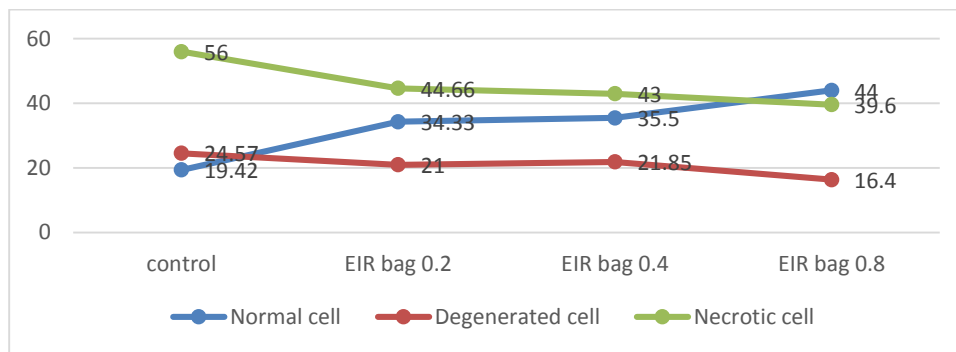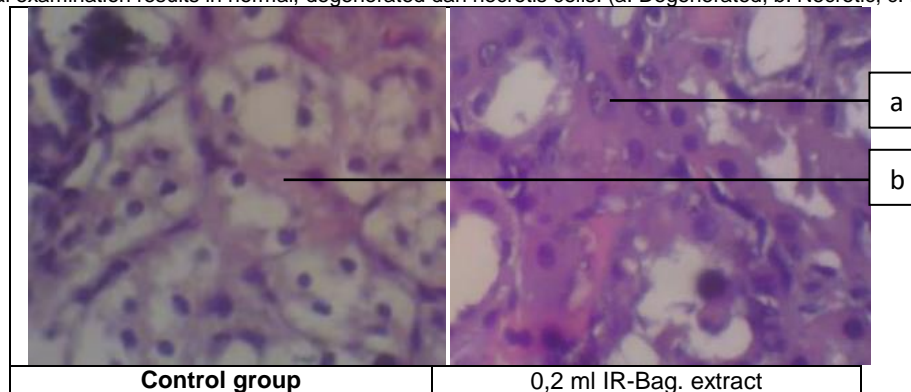

Fig. 3: Histological examination results in normal, degenerated dan necrotis cells. (a. Degenerated; b. Necrotic; c. Normal cells)

| Control group | 0,2 ml IR-Bag. extract |

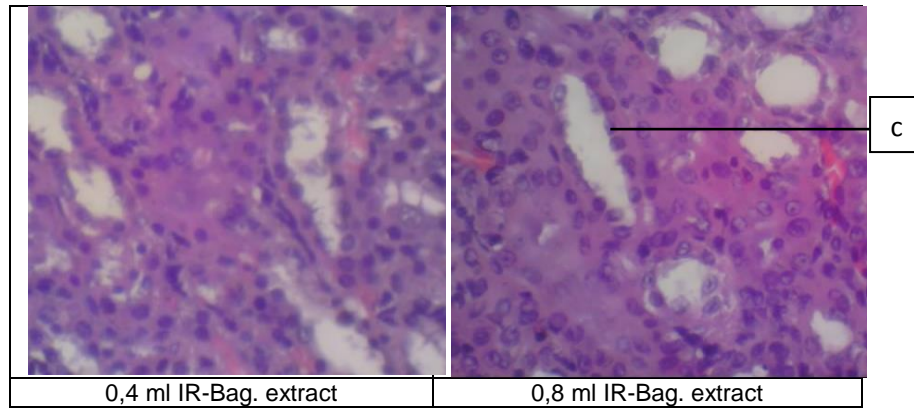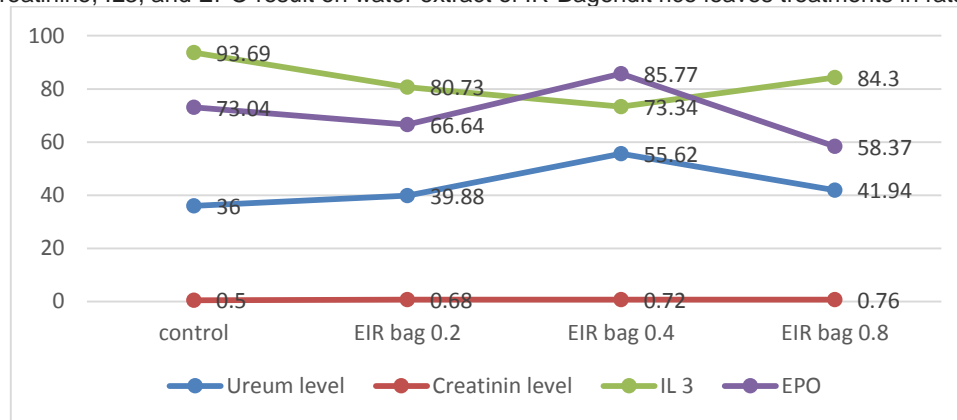| 0,4 ml IR-Bag. extract | 0,8 ml IR-Bag. extract |

Figure 2. shows the trend of the increasing average normal cells in renal tubules in the 0.2 ml; 0.4 ml; 0.8 ml extract treatment groups. Whereas cell degeneration and renal tubular necrosis were decreasing in the treatment group than control group in lead exposed rats (Fig. 3.).

Fig. 4: Ureum, creatinine, IL3, and EPO result on water extract of IR-Bagendit rice leaves treatments in rats exposed to lead.

DISCUSSION

In this study, in the treatment group, the average Hb, Ht levels, and erythrocyte counts were relatively increased and the number of leukocytes was relatively decreased. The lowest Hb, Ht, and total erythrocyte levels were found in the control group and the optimum result mean was in the 0.4 ml extract.

The results of this study were strengthened by the previous study[20], which was concluding that the exposure to 0.5 g/kg/BW in rats was proved to increase the blood profile in heme, Hb, Ht, and erythrocyte levels after 10 weeks. Lead inhibits pyrimidine-5'-nucleotidase and converts other nucleotide functions[21]. Lead is able to interfere the heme synthesis, thereby changing the concentration of enzymes in heme synthesis or its derivatives. Lead poisoning can cause an increase in anemia cases which can be seen from Hb, Ht levels and the number of erythrocytes, and reticulocytes[22].

The results of this study illustrate that Hb, Ht, levels and the number of erythrocytes was improved after the water extract of IR-bagendit rice leaves treatments in *R. norvegicus* exposed to lead, and are very likely applied to prevent lead-induced anemia.

Erythrocyte life time was shortened of less than 120 days in lead-exposed individuals so that the number of erythrocytes decreases through the mechanism of erythrocyte destruction, and it will accelerates erythrocytes spleen sequestration through phosphatidylserine (PS) and then increases the erythrophagocytosis[23]. Erythrocyte membrane damage due to exposure to lead is a cause of short erythrocyte lifetime[11,24]. Membrane permeability has a very important role in maintaining the integrity of erythrocyte cells[25] and even a low exposure to Pb (NO_3) can cause membrane damage which begins with hemolysis in erythrocyte cells[26].

The mechanism of lead toxicity is also from free radical such as reactive oxygen species (ROS) which reacts with cellular macromolecules (DNA, proteins, lipids)[27]. Increased lead accumulation that occurs in erythrocyte membranes can trigger the pro-oxidants that cause oxidative stress which are sensitive to the amount and erythrocyte volume[23,28]. Lead can also cause deficiency of the G-6PD enzyme (glucose-6 phosphate dehydrogenase) which can inhibit the maturation of erythrocytes in the bone marrow[10,11].

In this study the number of erythrocytes were increased in the treatment group compared to the control group. IR bagendite rice leaves extract contains the highest levels of metallothionein protein compared to the other rice leaves variety [16]. Metallothionein protein as a factor for binding to Pb exposure so that it can improve erythropoiesis activity due to exposure to lead[9].

In this study, cell degeneration and necrosis in the treatment group was relatively lower than the control group, while the normal cells were higher than the control group. Statistically, there were significant differences between the control group with treatment groups 1,2 and 3 in normal cells and necrosis cells. This proves that metallothionein protein found in IR bagendit rice leaves extract can inhibit the damage in the kidney tubule cells. In the control group without the extract, renal tubular cells were unable to receive pathological stimuli and physiological stress, and the manifestations of these stimuli were cell injury. If cells are able to adapt to stimulation, reversible injury will occur so that the cell only degenerates and allows it to return to normal[31]. Degenerated cells are characterized by enlarged and paled epithelial tubular cells and clear vacuoles in the cytoplasm which cause the tubular lumen to become narrower. If pathological stimuli and physiological stress were too high, cell injury is irreversible so the cell becomes necrotic[30] which is characterized by eosinophilic cytoplasm, nucleus of karyolysis, a mass of red amorphous in the tubular lumen[31]. The leads will inhibit the sulphydryl-dependent enzymes and slowly injures proximal tubular cells[32,33], causing chronic interstitial nephritis with fibrosis, exacerbated by hypertension[34,35].

The levels of urea and EPO in the treatment of 0.4 ml rice leaves extract were higher than the control group. EPO is a hormone produced by the kidneys and functions for the erythropoiesis process[36,37,38,39]. When associated with the results of Hb, Ht levels, and the number of erythrocytes, the increase in EPO has a direct corelation. In accordance to Foley RN *et al.* (2009)[40] which use the EPO to increase hemoglobin levels in patients with kidney failure who do hemodialysis[40,41,42,43]. Urea levels are biomarkers of kidney function. In this study the levels of urea and creatinine in the treatment group remained higher than the controls[44,45,46]. The improvement of renal tubular cells as evidenced by the decrease in necrosis cells in the treatment group has not been able to reduce urea levels. The large number of tubular cell necrosis describes impaired renal function with manifestations of increased urea levels. Chronic renal failure showed an increase in urea and creatinine levels[45,46,47,48,49]

Further research is needed to prove it clinically by considering the optimal dose of IR bagendit rice leaves extract and the gene encoding for metallothionein protein in rice leaves.

CONCLUSION

Concentration of 0.4 ml water extract of IR-bagendit leaves optimally prevents hematopoiesis disorder, cell degeneration, necrosis and renal dysfunction in lead-exposed rats.

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