

RESEARCH ARTICLE

IR Bagendit Paddy Leaves Extract Improves Liver Cell Morphology and Reduces The Activity of Transaminase Enzymes After Lead Exposure in Rat

Budi Santosa¹, Henna Ria Sunoko², Andri Sukeksi¹, Siti Thomas Zulaikhah^{3,*}

¹Departement of Medical Laboratory Science, Faculty of Nursing and Health Sciences, Universitas Muhammadiyah Semarang, Jl. Kedungmundu Raya No.18, Semarang 50273, Indonesia

²Departement of Pharmacy, Faculty of Medicine, Universitas Diponegoro, Jl. Prof. Soedarto, Semarang 50275, Indonesia

³Departement of Public Health, Faculty of Medicine, Universitas Islam Sultan Agung, Jl. Kaligawe Raya KM. 4, Semarang 50112, Indonesia

*Corresponding author. E-mail: sitiethomas@unissula.ac.id

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Abstract

BACKGROUND: Lead (Pb) was known as one of systemic toxic agent. In the body, lead may be deactivated by the metallothioneins. Paddy leaves contain metallothioneins, sugars and pythosterols, and studies have shown the pharmacological activity of rice leaves on the protective effect of lead-induced rats against kidney function. The aim of this study was to evaluate the role of IR Bagendit paddy leaves extract as hepatoprotective agent.

METHODS: Twenty-eight rats were divided into four groups: one control and three treatment groups. Control and treatment groups were exposed to lead of 0.5 g/kg body weight (BW)/day and then the treatment groups were administered with paddy leaves extract of 0.2; 0.4; and 0.8 g/kg BW/day per oral for 8 weeks. On the last day of the 8th week, body weight was measured and the numbers of normal, degenerative and necrotic liver cells were examined with hematoxylin-eosin staining. Serum glutamic

oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were measured as liver function parameter. Difference of variables between control and treatment groups were examined by Friedman test.

RESULTS: There was no association in different BW between groups. The normal liver cells are higher in treatment than control group ($p<0.001$) and necrotic liver cells are lower in treatment than control group ($p\leq 0.001$). There was no association in degenerative liver cells between groups ($p=0.153$). The activity of transaminase enzymes are lower in treatment than control group ($p<0.001$).

CONCLUSION: IR Bagendit Paddy leaves extract reveals hepatoprotective activity by improving liver cells morphology and reducing the activity of transaminase enzymes after lead exposure.

KEYWORDS: paddy leaves extract, liver normal cell, necrotic cell, transaminase enzymes

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Introduction

Lead (Pb) has been used in more than 900 industries, including mining, smelting, refining, battery manufacturing and so on.(1) Lead exposures have serious consequences for the health. In the high levels of exposure, lead could induce brain and central nervous system damage, coma, convulsions and even death. Chronic lead poisoning may

be left with mental retardation and behavioural disorders in children.

Liver is one of the most complex and largest metabolism organs in the body. It involves in each metabolism of the food substances, most drugs and toxicants.(2) Lead is one of liver damage inducing toxicants. The mechanism of liver injury can be separated as biochemical and immune mechanism. The biochemical mechanism involves various chemical agents those

are metabolized in the liver via phase 1 and phase 2 metabolisms. The metabolite products may alter the intracellular homeostasis, mitochondria or other organelles. The immune mechanism involves cell cooperation and is mediated with complement, cytokines or nitrogen oxide (NOx). Lead may involve directly in normal biochemical process in hepatobiliary system.(3,4)

High concentration of lead is toxic to the cells. Lead will act as prooxidant and bind to organic molecules. In the cell, lead ions bind to the cellular proteins and convert the protein structure into the inactive conformation.(5,6) Intraperitoneal administration of 20 mg/kg body weight (BW) lead acetate in rats resulted histopathologic and biochemical changes in liver, prooxidant and antioxidant balance disorders, oxidative stress, lipid peroxidase.(7)

Paddy leaves water extract contains sucrose, oxalate, β -sitosterol, stigmasterol and metallothionein.(8,9) Previous studies have shown the pharmacological activity of rice leaves on the protective effect of lead-induced rats against kidney function (10) and blood toxicity (11). Metallothionein is a protein/polypeptide with small molecular mass (4-8 kDa), containing cysteine amino acid (Cys), but having no aromatic amino acid or histidine. The classification of metallothionein is based on the composition of amino acid, number and division of Cys-amino in sequence, and the similarity of phylogenetic sequence and relationship.(12) Metallothionein is a protein rich of sulfidryl groups and may bind covalently with lead.(13-16) Previous study showed that paddy leaves, especially Bagendit IR has the highest metallothionein content.(8) In this study, the effects of paddy leaf extract on liver cell morphology and liver function after lead exposure in rats will be evaluated.

Methods

Determination and Extraction of IR Bagendit Paddy Leaves

Fresh Paddy leaves were cleaned and washed with distilled water. The leaves were dried at the room temperature, pulverized with mortar and sieved at 0.1 mm diameter size. One-hundred g pulverized leaves were put in the bottle and distilled water was added up to 1 kg as total volume. The mixture was boiled and filtered through a filter paper.(14) The extract was kept and used for animal study.

Animal Model

Healthy white *Rattus norvegicus*, 180-220 g, aged 15 weeks in condition, were acclimatized for 7 days, housed in cages,

and maintained under standard laboratory conditions with dark and light cycles (12/12 hours) according to the ethical standards. Standard feed and drink were given *ad libitum*. No vitamin was added. Ethical permission for this study was obtained from the Ethics Commission of Medical Faculty of Universitas Sultan Agung Islamic, Semarang (No. 209/VI/2017/Komisi Bioetik).

Animal Treatment and Sample Collection

An experimental research with randomized post-test only control-group design was conducted. The sample size was calculated with the formula $BS=(t-1)(r-1)\geq 15$.

Twenty-eight rats in this study were divided into 4 groups, which were 1 control and 3 treatment groups. The control group was given lead acetate 0.5 g/kg BW, food and drink. Meanwhile, the treatment groups were given 0.5 g/kg lead acetate BW and 0.2 mL (T1 group), 0.4 mL (T2 group) and 0.8 mL (T3 group) IR bagendite paddy leaves extract. Lead exposure and paddy leaves extract administration were administered to both control and treatment groups for 8 weeks. After that all rats were sacrificed. The liver and heart were immediately dissected, weighed, transferred into the tubes with Bouin solution and paraffinized. Blood was drawn from orbital vein. Serum was separated by centrifugation at 5,000 rpm for 10 minutes and kept in frozen storage.

Hematoxyline-Eosin (HE) Staining

Paraffinized tissues were sectioned and placed on the glass slides. Then the tissue slides were deparaffinized, rehydrated, dipped in hematoxylin, eosin, and washed with water. Then the slides were dehydrated, mounted with cover glass and observed under a 40x-magnified light-microscope. Manual observation was carried out by 2 operators. Histological examinations of the liver were carried out in 5 microscopic fields for each slide. Histopathological changes were observed in the form of congestion, inflammatory cell infiltration, and necrosis.

Scoring and Identification of Liver Cell

The cell numbers were the number for liver cell that were counted per every 100 cells for each groups. Normal pattern liver lobules were observed with the central vein in the middle and liver cell cords radiating towards the periphery. The areas containing the hepatic artery, portal vein and bile duct were surrounded by connective tissue. Sinusoids were observed between the liver cell cords, and the polygonal-shaped hepatocytes mostly contained a single oval-shaped euchromatic nucleus.

Degenerative liver cell were observed focal parenchymal necrosis together with evidence of regeneration and many hypertrophic, often multinucleated liver cells. The hypertrophic liver cells were often observed to contain peculiar inclusions of irregular size and shape, located predominantly at the periphery of the cytoplasm. Necrotic liver cell were identified using the criteria as increased eosinophilia, cell swelling and lysis, loss of architecture, karyolysis and karyorrhexis.

Measurements of Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT)

SGOT and SGPT measurements were conducted based on SGOT kit (Catalog No. 2601 99 83 021, Diasys Diagnostic System GmbH, Holzheim, Germany) and SGPT kit (Catalog No. 2701 99 83 021, Diasys Diagnostic System GmbH) from the manufacturer. The principle of SGPT measurement was based on the changes L-alanine and 2-Oxoglutarate to L-Glutamate and pyruvate. The pyruvate formed reacted with nicotinamide adenine dinucleotide (NADH) to D-lactate and NAD^+ . The NAD^+ formation was measured using kinetic method. Meanwhile, the principle of SGOT measurement was based on the changes L-aspartate and 2-oxoglutarate to L-glutamate and oxalacetate. The oxalacetate formed reacted with NADH to D-lactate and NAD^+ . The NAD^+ formation is measured using kinetic method as well.

Statistical Analysis

Data distribution was tested with kolmogorov-smirnov test. Normal data was expressed as mean \pm SD and abnormal data was expressed as median with min-max values. The differences between multiple groups were calculated by Friedman test. The differences between 2 groups were calculated by Mann Whitney test. The statistical calculations were performed using SPSS version 16.0 (IBM Corporation, New York, USA).

Results

Rats' BW

The rats' BW measurement was conducted at the baseline and the end of the study. Based on the results of BW measurements before and after treatment, BW difference data of T1 and T3 groups were lower than the control group (Table 1).

Liver Cells Morphology

In the control group, there were many necrotic and degenerative cells but few normal cells. In contrast with T1, T2 and T3 groups, there were more normal cells than degenerative or necrotic cells (Figure 1).

The average and *p*-value of liver normal, degenerative, and tubular necrotic cells were shown in Table 2. Based on Table 2, number of normal cells was higher in the treatment groups than the control group and the highest number was found in T3 group. Number of necrotic cells were lower than in the treatment groups and the lowest number was found in the T3 group.

Friedman test results showed that there was a strong positive relationship ($p < 0.001$) in the number of normal cells and paddy leaves extract dose, suggesting that higher dosage of IR Bagendit paddy leaves extract resulted higher number of liver normal cells. In contrast, the number of necrotic cells are lower depending on the dose of paddy leaves extract ($p < 0.001$). There was no relationship between number of degenerative cells and paddy leaves extract dose.

Liver Functions

SGOT levels of all treatment groups were lower than the one of control group ($p < 0.001$) but there was no association between the treatment groups (Table 3). There was no significant difference between the treatment groups.

SGPT levels of all treatment groups were lower than control group ($p < 0.001$) but there was no association

Table 1. The difference of body weight before and after treatment.

Group	Body Weight (g)			
	Before	After	Difference	<i>p</i> -value*
Control	180.2 (169.9 - 206.7)	215.8 (70.6 - 256.2)	24.6 (-1.6 - 51.1)	0.012
T1	197.4 (154.1 - 214.4)	208.9 (192.1 - 220.7)	9.0 (-5.7 - 58.9)	
T2	161.5 (144.2 - 181.3)	191.2 (160.5 - 240.6)	27.3 (-1.0 - 59.3)	
T3	150.1 (135.9 - 179.4)	173.9 (150.3 - 210.0)	15.6 (-16.1 - 74.1)	

*The differences between multiple groups were calculated by Friedman test.

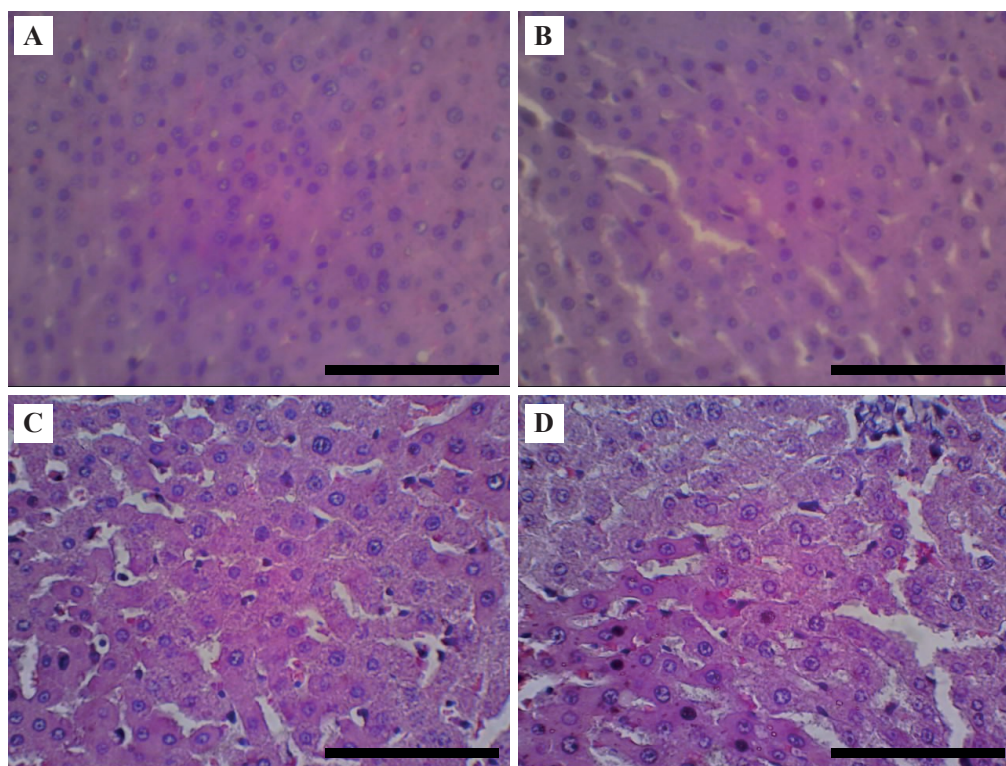


Figure 1. Liver cells morphology between control and treatment groups. A: Control group; B: Treatment T1 group; C: Treatment T2 group; D: Treatment T3 group. Black bar: 100 μm.

between the treatment groups (Table 4). The average of SGPT levels in treatment groups was around 35 μ/L, while the average of SGPT levels in control group was 40 μ/L.

Discussion

There was an association of BW difference between control and treatment groups in this study. The treatment groups had lower BW difference than the control group. A previous study reported that lead-exposed rats had BW reduction.(17,18) Several factors, such as lead dose, period of exposure, nutrition, could affect these findings.

Liver is a vital organ that has many functions. Many chemicals could induce liver dysfunction. These chemical agents came from food, occupation or environment.(19) Lead will induce liver damage. As prooxidant, lead affect hepatobiliary system, oxidize unsaturated fatty acids, reduce nitrogen oxide and increase hydroxyl radicals.(20)

The histopathological studies are the evidence of efficacy of drug as hepatoprotective agent. Simultaneous treatment IR Bagendit paddy leaves extract improves the liver cells morphology. Dose of paddy leaves extract was positively associated with the number of normal cells and negatively associated with the number of degenerative cells. Dose of paddy leaves extract was not associated with the

Table 2. Liver cells morphology quantification.

Group	Normal Cells		Degenerative Cells		Necrotic Cells	
	Average	<i>p</i> -value	Average	<i>p</i> -value	Average	<i>p</i> -value
Control	23.71±2.69		21.42±2.43		54.85±3.18	
T1	37.33±5.78	<0.001	16.00±4.09	0.153	46.66±3.50	<0.001
T2	36.57±4.42		20.42±5.85		43.00±2.30	
T3	46.00±10.27		18.80±4.08		35.20±9.83	

*The differences between multiple groups were calculated by Friedman test.

Table 3. Average of SGOT levels in control and treatment groups.

Group	SGOT Level	
	Average (μ /L)	<i>p</i> -value
Control	79.57 \pm 24.60	
T1	62.50 \pm 33.15	<0.001
T2	64.00 \pm 26.28	
T3	60.40 \pm 20.79	

*The differences between multiple groups were calculated by Friedman test.

number of necrotic cells. Phytosterols and metallothioneins are in group of active agent in paddy leaves extract.(8,9) These compounds have antioxidant activity.(21-23) Metallothioneins also have activity as metals chelating agent.(24,25) Metallothioneins can bind lead in digestive tract and in the systemic circulation.(24,26) This condition will prevent the formation of free radicals. Reduction of free radicals will prevent cells damage. In this paddy leaves extract, degenerative cells may return to normal cell.(27)

Liver transaminases can be used to monitor liver damage. SGPT and SGOT are the most commonly used biomarkers of liver damage. SGPT is involved in the transfer of an amino group from alanine and presents in the cytoplasm. SGPT can be found in various tissues, mostly in liver. SGPT is a good biomarker of hepatocellular injury. SGOT is involved in the transfer of an amino group from aspartate. More than 80% of SGOT is present in the mitochondria and the remaining 20% of SGOT is present in the cytoplasm. In case of mitochondrial damage, SGOT activity increases more than SGPT.(25) In this study, SGOT and SGPT level are higher in control group than the treatment group (p <0.001). However, increasing the dose of paddy leaves extract did not significantly reduce the activity of the transaminase enzymes.

Table 4. Average of SGPT levels in control and treatment groups.

Group	SGPT Level	
	Average (μ /L)	<i>p</i> -value
Control	40.28 \pm 9.46	
T1	35 \pm 14.56	<0.001
T2	34.71 \pm 12.4	
T3	34.8 \pm 11.12	

*The differences between multiple groups were calculated by Friedman test.

Conclusion

IR Bagendit Paddy leaves extract reveals hepatoprotective activity by improving liver cells morphology and reducing the activity of transaminase enzymes after lead exposure.

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