ORIGINAL ARTICLE

Reduction in Transaminase Enzymes Action after Application of Leaves Extract of IR Bagendit Paddy on Liver Cell of Lead Exposed Rat

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ABSTRACT

Introduction: When entering the body, plumbum metabolized in the liver may turn to free radicals causing hepatocyte necrosis and increasing Serum Glutamic Oxaloacetic Transaminase (SGOT)and Serum Glutamic Pyruvic Transaminase (SGPT). Rice leaf water extract containing metallothionein protein functions to bind plumbum. This study aims to determine the effect of water extract of IR Bagendite rice leaf on the SGOT and SGPT levels in plumbum-induced Wistar rats. **Methods:** This study used a negative control group and a positive control which was givenplumbum acetate 60 mg/kg/BW per day. A group of rats with a preventive extract of IR Bagendite rice leaf water 0.2; 0.4, 0.8 ml per day and each treatment were induced with plumbum 60 mg/kg/body weight per day. The study was conducted for 60 days. The enzymatic colourimetric method was used to measure the SGOT and SGPT and analyzed using the One Way ANOVA and Bonferroni 5% test. **Results:** The results showed the mean SGOT of negative control was lower than those of the positive. Treatment 1 (99.7) and 2 (98.7) were lower than the positive control (100.2), while treatment 3 (118.7) was higher than the positive control. There was a significant difference between positive control and treatment 1 (p=0.00) and 2 (p=0.01). **Conclusion:** Infusion of rice leaf water of Blora location can prevent liver function disorders at doses of 0.2 ; 0.4 at SGOT and 0.2 at SGPT.

Keywords: IR Bagendit, SGOT SGPT, Plumbum

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INTRODUCTION

Lead is a heavy metal with the symbol Pb (from the Latin plumbum). Pb is toxic to cells, organs, and body tissues (1,2,3). Exposure to plumbum is still commonly found in residential areas as a result of its use in various industries such as battery, nail paint, cosmetics and others (4). Plumbum enters the body through inhalation, skin, and digestion(5). Plumbum that enters the body will be metabolized in the liver and if exposed continuously will be toxic and excreted together with bile and blood. A series of metabolic processes in the liver can cause damage to the liver due to the loss of function and structure of liver cells (6). Liver function can be indicated by changes in the levels of Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT)(7).

SGOT and SGPT are transaminase enzymes produced by liver cells and are used to measure liver function disorders. Many factors can cause damage to liver cells, including exposure to heavy metals such as Pb. Continuous exposure to Pb will accumulate in the liver and can become free radicals (8). Free radicals are molecules that contain electrons and are unpaired so that they induce a chain reaction of unsaturated fats in the cell membrane layer that will cause hepatocyte cell necrosis (9). The number of hepatocyte cell necrosis causes impaired liver function which can be observed due to an increase in SGOT and SGPT.

So far, the chelating agents are used to treat Pb toxicity. Some chelating agents include dimercaprol (BAL), 2,3-dimercaptosuccinic acid (DMSA), and d-penicillamine (DPCN) (10,11,12). Due to the complexity of parenteral administration, toxic effects, and a susceptibility to exacerbate the neurotoxicity of particular metals, therapeutic usage of EDTA (ethylenediamine tetraacetate) and BAL (2,3-dimercaptopropanol) is now restricted (13). A chelating agent is needed to reduce or prevent heavy metal exposure (14,15). One of the chelating materials is metallothionein(16,17) protein. This protein is rich

in sulfhydryl groups which can bind covalently to heavy metals (18). Metallothionein is a small cytosolic (25-82 aa, 2.5-8.0 KDa), cysteine-rich protein, which plays a role in chelating heavy metals with high affinity located in the thiol group of cysteine (Cys) (18-23 Cys contained in the conserved gene region). The presence of metallothionein can be detected using the ELISA method (19).

The results of previous studies showed that various plants such as rice, corn, beans, soybeans contain a lot of metallothionein protein and the most abundant is in the leaves of IR Bagendite type of rice (20). Isolation, Identification Similarity and Qualitative Expression of Metallothionein Gene in various rice leaf varieties, namely IR Bagendite, Inpari, IR 34, IR 35, Umbu, Umbuk, and Sticky Rice showed that the quantification and purity of DNA and RNA were mostly found in IR Bagendite rice leaves. The results of insilico analysis of metallothionein gene sequences in rice leaves based on the NCBI database show that the metallothionein gene is located on chromosome 3 of Rice plant (Oryza sativa) which functions for stress-inducible protein in drought conditions, soil and water conditions containing metals, so the presence of metallothionein can be used as a biomarker of heavy metal exposure (21).

IR Bagendite rice is widely planted in various areas of Central Java, Indonesia including Boja, Blora, Batang, and Weleri. In the preliminary study, it was found that the highest metallothionein content was IR Bagendite at Blora location amounting to 380,636 ng/L, Weleri 252,189 ng/L, Batang 121,746 ng/L, and Boja 28.836 ng/L. In this study, an infusion of rice leaf water from IR Bagedite in Blora location was tested as a preventive measure to prevent Pb-induced liver function disorders in Rattus norvegicus. IR Bagediterice leaves in the Blora location were chosen because they have the highest metallothionein protein content.

MATERIALS AND METHODS

Research Design
This research was experimental with a randomized post-test only control group design. The Integrated Research and Testing Laboratory (LPPT) at the University of Muhammadiyah Semarang kept and intervened with experimental animals. The length of time for raising the animalsfrom the selection period to the treatment period took place within 8 weeks.

The number of samples was calculated by using the formula: BS = (t − 1) (r − 1)≥15. The number of rats used was 6 for each group (1 negative control group, 1 positive control group, and 3 treatment groups) so the total number of samples used in this study was 30 Rattus norvegicus. All rats were selected which were male and 15 weeks old.

Infusion Making Process
Rice leaves of various varieties are cleaned and washed with running water before being chopped into small pieces. As much as 100 g was put into pan A and added 1 litre of distilled water, then closed. Plan B (as a water bath) is added with enough water so that the upper pan (A) is partially submerged. It is heated for 15 minutes, starting with pan A reaching a temperature of 90oC while stirring occasionally. The infusion is spread while hot through the flannel. The supernatant is an infusion. The obtained infusion was examined for metallothionein protein using the ELISA method.

Intervention in experimental animals and data analysis Water extract of rice leaves at Blora location was given to treatment groups 1(P1), 2(P2), and 3(P3) with graded doses of 0.2, 0.4, 0.8 ml/day via a probe, while the negative and positive controls were not given IR Bagendite rice leaf extract. The positive control group and all treatment groups were given Pb exposure at a level of 60 mg/kg of body weight(BW)/day for 8 weeks.

On the last day of week 8, blood was drawn from the control and treatment groups through the retro-orbital plexus to check the levels of SGOT and SGPT using the Enzymatic colourimetric method at an accredited Unimus chemistry laboratory. Liver function tests using SGOT and SGPT parameters were distinguished from the negative control group, positive control group, and treatment group 1, 2, and 3 using the one way ANOVA test and Bonferroni test.

Ethical Clearance
The study obtained ethical clearance from the ethics committee of FKM UNIMUS Semarang with No. 503/ KEPK-FKM/UNIMUS/2021. The head of the Integrated Research and Testing Laboratory (LPPT), University of Muhammadiyah Semarang, is notified of the ethical clearance results and the research was accepted.

RESULTS

Description of experimental animal characteristics
Measurement of body weight (BW) was carried out at the beginning and end of the treatment with the results of the average weight listed in table I. Based on table I, the average initial body weight was highest in the 1st treatment group and the lowest in the 3rd treatment group. The control group had the greatest final mean

Table I: Average Body Weight

Group	Average Weight		
	Before (gram)	After (gram)	difference
C	183.6	208.5	24.9
T1	191.7	202.1	10.4
T2	162.4	189.3	26.9
T3	154.8	179.4	24.6

Note: C= Control, T=Treatment

weight, while the third treatment group had the lowest. The highest weight gain occurred in the 2nd treatment group.

Liver function

SGOT level
SGOT levels in the negative control group were the lowest on average when compared to other groups. SGOT levels increased in the positive control group and experienced a downward trend in treatment groups 1 and 2, while treatment group 3 experienced an increase above the positive control group. The average per group in detail can be seen in the Table II.

Table II: Average SGOT Levels in Control and Treatment Groups

Group	Level of SGOT (U/L)	
	Average	
C -	81.6 ±2.64	P=0.00*
C +	100.2± 4.96	
T1	99.7 ±2.95	
T2	98.7 ±2.86	
T3	118.7 ± 1.31	

*ANOVA

Based on the normality test using Shapiro-Wilk, the p-value of 0.47 was obtained, which means that the data was normally distributed, so the ANOVA test was carried out to determine the differences between groups. The results of the ANOVA test obtained p-value of 0.00, which means that there were significant differences in SGOT levels between groups. To determine the differences between groups, the Bonferroni test was carried out. Table III shows the summary of the Bonferroni test results. The results of the Bonferroni test showed that the levels of SGOT in the negative control group were significantly different from those in the positive control group (p=0.00). The positive control group was significantly different from all groups with p-value of less than 0.05 (p-value < 0.05).

SGPT Level
SGPT levels in the negative control group were lower than in the positive control group. The positive control group was higher than treatment groups 1 and 2 but lower than treatment group 3. The highest SGPT levels occurred in treatment group 3. The average of SGPT

Table III: Bonferroni Test Results for Each Control and Treatment Group

Group		Mean Defference	Sig	95% Confidence Interval	
				Lower Bound	Upper Bound
C -	C +	18.5	0.00	4.48	32.58
	T1	0.5	1.00	-13.51	14.58
	T2	1.4	1.00	-14.11	17.01
	T3	-18.5	0.02	-34.11	-2.98
C +	C -	-18.5	0.00	-32.58	-4.48
	T1	-18.0	0.00	-31.39	-4.60
	T2	-17.1	0.01	-32.06	-2.10
	T3	--37.1	0.00	-52.06	-22.10

levels for each group can be seen in the Table IV. The results of the normality test using Shapiro-Wilk obtained a p-value of 0.97 which means that the data is normally distributed, so the ANOVA test was carried out to determine the differences between groups.

Table IV: Average of SGPT levels in Each Control and Treatment Group

Group	Amount of SGPT (U/L)	
	Average (%)	
C -	55.6 ±1.35	p=0.01*
C +	58.2 ±2.55	
T1	50.0 ± 2.03	
T2	56.5 ± 0.86	
T3	59.75± 1.31	

*ANOVA

The results of the ANOVA test obtained a p-value of 0.01, which means that there are significant differences between groups. To determine the differences between groups, the Bonferroni test was carried out. Table V shows a recapitulation of the results of the Bonferroni test. Based on the results of the Bonferroni test, only 1 group was significant, namely the positive control group against the treatment group 1 with a p-value of 0.03.

Table V: Bonferroni Test Results for Each Control and Treatment Group

Group		Mean Defference	Sig	95% Confidence Interval	
				Lower Bound	Upper Bound
C -	C +	-2.5	1.00	-10.40	5.33
	T1	5.6	0.27	-1.83	13.17
	T2	-0.8	1.00	-9.22	7.55
	T3	-4.0	1.00	-12.47	4.30
C +	C -	2.5	1.00	-5.33	10.40
	T1	8.2	0.03	0.32	16.07
	T2	1.7	1.00	-7.02	10.42
	T3	-1.5	1.00	-10.27	7.17

DISCUSSION

The average bodyweight of the rats complied with the inclusion criteria and was quite homogeneous. All rats in the control group and the treatment groups until the end of the treatment experienced an increase in body weight, and the highest increase was found in the 2nd treatment (T2) with a delta value of 26.5. In the control and treatment groups, the weight gain was quite varied, the influencing factors were the average initial body weight and the amount of Pb exposure. Giving Pb can affect the metabolism of nutrients so that there is an impact on weight loss.

The results of the study on SGOT levels showed that the treatment groups 1 and 2 treated with IR bagendite leaf infusion 0.2 and 0.4 ml/day, SGOT level decreased significantly when compared to the positive control. This proves that the administration of IR Bagendite leaf infusion can prevent liver function disorders in Pb-induced rats. In treatment 3 also significantly different but the levels were higher than the positive control group, the dose may be too high so that it is feared to be a toxic dose.

SGPT levels in treatment groups 1 and 2 were decreased when compared to the positive control group, but only treatment group 1 experienced a significant decrease (p=0.03) when compared to the positive control group. Treatment group 3 has the highest mean although it is not significant when compared to the control group, it is necessary to take into account toxic doses. Based on the results of this study, the administration of IR Bagenditerice leaf water infusion in the Blora location was able to significantly prevent liver function disorders, especially at doses of 0.2 to 0.4 ml/day for SGOT and 0.2ml/day for SGPT, with Pb exposure 60 mg/kg bw per day and the metallothionein content in the infusion was 380,636 ng/L.

SGOT and SGPT are transaminase enzymes produced by liver cells and are used to measure liver function disorders(22). The liver is an organ that is very susceptible to the influence of chemicals and is the main target organ for the toxic effects of chemicals (toxicants) including Pb (5). In general, the effect of plumbum on the hepatobiliary system is to catalyze the peroxidation of unsaturated fatty acids, reduce nitrogen oxides and increase hydroxyl radicals. Pb exposure can also increase Kupffer cells and hepatocytes significantly characterized by low levels of lipopolysaccharide and increased proteolytic activity (23).

Plumbum (Pb) is a heavy metal and can cause cell injury so that it can cause degeneration and necrosis of liver cells. The presence of cytoplasmic swelling, which can be induced by cell damage, might be interpreted as a feature of cell degeneration. Cell necrosis can occur directly or can lead to cell degeneration (reversible injury). The microscopic picture of necrosis is pyknosis, karyorexis, and karyolysis. The location of necrosis is observed in threeregions, namely focal, zonal, and submassive necrosis (24,8,25)

Plumbum can cause histopathological and biochemical changes in the liver of rat and cause disturbances in the balance of oxidants and antioxidants that cause an increase in oxidative stress, an increase in the percentage of abnormal livers, inducing lipid proxinase which can damage tissues and cell membranes (26) resulting in changes in cell structure and function further impacting on the increase in SGOT and SGPT (27).

IR Bagenditerice leaf water infusion has been investigated and proven to contain metallothionein protein rich in sulfhydryl groups which can bind covalently to heavy metals including Pb. Metallothionein protein perhaps binds with Pb and eventually enters the detoxification process to reduce or prevent Pb induction/exposure inside the body.

CONCLUSION

Giving IR Bagenditerice leaf water infusion of Blora location can prevent liver function disorders in Pbexposed Rattus norvegicus. Doses of 0.2 and 0.4 ml of rice leaf water infusion were significantly able to prevent the increase in SGOT while the dose of 0.2 ml of rice leaf water infusion was able to significantly prevent the increase of SGPT.However, increasing the dose ofpaddy leaves extract did not significantly reduce the activityof the transaminase enzymes.

ACKNOWLEDGMENT

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