



Blood Profile of *Rattus Nurvegicus* Exposed to $HgCl_2$ and Received Combination of IR Bagendit Rice Leaves-Infused Water and Young Coconut Water

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Abstract: Mercury chloride ($HgCl_2$) is widely used in industries; in fact, this chemical substance is deadly for health and causes various health problems, such as liver damage, kidney damage, and hematopoietic disorders, especially in erythropoiesis. This study aims to determine the blood profile in *Rattus nurvegicus* exposed to $HgCl_2$ by combining IR Bagendit rice leaves-infused water and young coconut water. This study employed an experimental method to examine the positive control group exposed to $HgCl_2$ and the negative control group receiving a placebo. Meanwhile, the treatment groups received a combination of IR Bagendit rice leaves-infused water in stratified compositions and young coconut water. The hematologic profile was examined using a hematologic analyzer, while the reticulocyte count was examined using wet and dry methods. The mean value of each group was tested using ANOVA. This study has successively obtained average levels of Hb (C+ 12,0;C- 14,2;T1 12,5;T2 12,9;T3 12,8), the erythrocyte count (C+ 6,52;C- 7,80;T1 1,44;T2 1,07;T3 7,32), levels of Ht (C+ 35,5;C- 42,9;T1 37,8;T2 37,9;T3 38,2), MCV (C+ 73,7;C- 79,9;T1 80,4;T2 78,7;T3 79,6), MCH (C+ 27,3;C- 29,9;T1 29,5;T2 28,5;T3 29,1), and MCHC (C+ 31,1;C- 34,2;T1 31,9;T2 33,3;T3 32,1), and the reticulocyte count (C+ 2,0;C- 1,2;T1 1,3;T2 1,1;T3 1,3). The significant differences in the groups are found in levels of Hb, Ht, MCV, and MCHC (p-value 0,00; 0,00; 0,03; 0,01). This study concludes that combining IR Bagendit rice leaves-infused water and coconut water could prevent blood profile exposure to $HgCl_2$. Suggestions for further research to increase the intervention time and measure oxygen levels.

Keywords: Blood profile; IR bagendit rice leaves; young coconut water

INTRODUCTION

Various forms of Mercury (Hg), consisting of metallic element mercury (Hg element), mercury salt (Hg+dan Hg²⁺), and organic mercury (methylmercury and dimethylmercury), are massively used in multiple daily products, such as industrial products (paint preservatives, etc.), health products (medicine, hospitals, laboratories), and agricultural products (fungicides)(Iqbal & Asmat, 2012; Sakamoto et al., 2018). Mercury in liquid forms is applicable for thermometers that could damage the brain and lungs when exposed to mercury vapours (Azevedo et al., 2012). Mercury chloride ($HgCl_2$) is an example of inorganic mercury or organic salt, while methylmercury is an example of organic mercury.

Mercury exposure could disrupt the transport process through the cell membrane because mercury is a heavy metal with a high affinity with sulfur, damages the cell membrane, and disrupts the enzymes' proper functions. Moreover, organic Hg inhibits sulfhydryl enzyme activities (Jan et al., 2015; Ajsuvakova et al., 2020). Hg covalent bonds with sulfur cause the biological effect. If the sulfur is in the form of

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sulfhydryl, divalent mercury will replace hydrogen atoms to form mercaptides. Severely acute toxicity, caused by inorganic and ionic mercury (mercuric chloride), could endanger various organs, such as the liver, kidneys, and hematopoietic systems because continuous exposure to mercury could cause oxidative stress in cells or tissues and the formation of the reactive oxygen species (ROS) (Crespo-López et al., 2019).

Barriers to the hematopoietic system due to mercury exposure are related to liver and kidney damage. Liver and kidney damages impair the production of the erythropoietin hormone, which serves to erythropoiesis. The blood profile consists of haemoglobin (Hb), erythrocyte cell count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCV), and mean corpuscular haemoglobin concentration (MCHC). Reticulocyte cell count is a key haematological marker to measure hematopoietic system disorders exposed to mercury, especially in erythropoiesis.

The current treatment of inorganic mercury toxicity uses chelating agents, such as 2,3-dimercaptosuccinic acid (DMSA), dimercaprol (BAL), or d-penicillamine (DPCN) (Bjørklund G, Mutter J, 2017; Aaseth et al., 2015). The chelating ingredients are therapeutic and have a non-optimal success rate. Therefore, it is necessary to develop natural materials with chelating and antioxidant properties to prevent mercury toxicity. The natural ingredients that potentially serve as chelating agents in this study are IR Bagendit rice leaves and coconut water as antioxidants.

IR Bagendit rice leaves-infused water is rich in the metallothionein protein (Santosa et al., 2018; Santosa et al., 2020) and has many sulfhydryl groups that function in the HG detoxication mechanism. A large number of sulfhydryl groups in the metallothionein protein affects the protein's ability to bind Hg. Sulfhydryl residues of Cys could bind one metal ion to 2 or 3 sulfhydryl residues (SH)(Ruttkey-nedecky et al., 2013). The binding coordination of each metal ion of the Cys forms a tetrahedral structure of tetrathionate (Nezhad RM, Shahpiri A, 2013). A previous study has proven that metallothionein genes in chromosome 3 function as a protein induced by environmental stresses, such as the presence of metallic contaminants (Santosa et al., 2020)

Coconut water drink is found in tropical countries, such as Indonesia. Moreover, coconut water has been commonly consumed as a thirst-quenching drink. Several studies have proven that coconut water contains many antioxidants, vitamins, minerals, amino acids, and enzymes. Therefore, consuming coconut water could reduce oxidative stress and increase antioxidants due to the decreasing MDA (Li et al., 2018)

Based on the explanation mentioned earlier, it is interesting to investigate the ability of IR Bagendit rice leaves-infused water combined with coconut water to improve the hematopoietic system, especially in the erythropoiesis of *Rattus norvegicus* exposed to $HgCl_2$. The novelty of this research is that coconut water contains many antioxidants, and IR Bagendit rice leaves contain a lot of metallothionein protein as a chelating agent. Antioxidants combined with chelating agents how strong can neutralize $HgCl_2$ exposure, especially in the erythropoiesis process.

MATERIALS AND METHODS

Research Design

This study employed the randomized post-test-only control-group design and examined *Rattus norvegicus* in the Integrated Research and Testing Laboratory (LPPT) of Universitas Muhammadiyah Semarang, Indonesia. The research sample

was selected using the formula: $BS = (t - 1) (r - 1) \geq 15$. The selected rats had been acclimatized for a week before the intervention. This study employed one negative control group, one positive control group, and three treatment groups; each group consisted of six rats. Thus, the total number of samples was 30 male *Rattus norvegicus* aged three weeks.

Process of Making Infusion

Since IR Bagendit rice leaves-infused water from Blora contains a high level of metallothionein, this study employed it. Before being chopped, the rice leaves had been cleaned and washed under running water. Then, 100 g of chopped leaves were put into a pot and added with one litre of aquades. Afterwards, the pot was closed. Pot B (as a water bath) was added with enough water until the top of pot A was partially submerged. The pots were heated for 15 minutes until the temperature inside pot A reached 90°C while stirring occasionally. The infusion was sprayed while it was hot using the flannel. The process of producing the infusion is described as figure 1. Young coconut water was taken from a location around Semarang and given in a single form, as much as 4 ml/100 gr BW, after the infusion of rice leaves IR Bagendit.

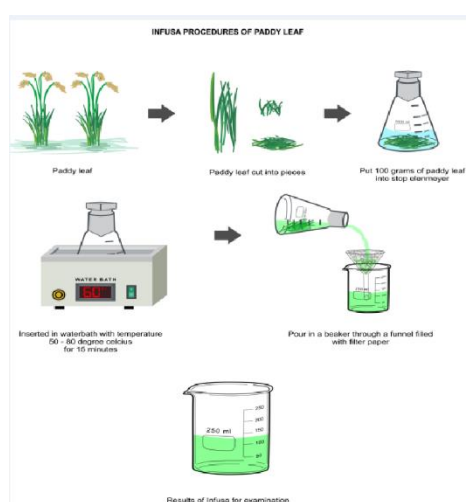


Figure 1. Production of IR Bagendit Rice Leaves-Infused Water. (Santosa, 2021)

Intervention on Research Animals

The negative and positive control groups received different treatments; the negative control group received a placebo in aquades; the positive control group received 20 mg/kg BW of $HgCl_2$ per day. They were made in the form of stock solution with a concentration per ml of 20 mg $HgCl$, administered via a probe every day, 1 ml. Afterwards, 0.2 ml, 0.4 ml, and 0.8 ml of IR Bagendit rice leaves-infused waters from Blora combined with 4 ml/100 gr BW of coconut water were given to treatment groups 1 (T1), 2 (T2), and 3 (T3), respectively. Moreover, all treatment groups were exposed to 20 mg/kg of mercury chloride daily. On day 15, the blood of the control and treatment groups was taken using the retro-orbital plexus. The blood was examined using a haematology analyzer device to reveal the haemoglobin (HB) rate, erythrocyte count, haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) rates. The number of reticulocytes was checked using the Brilliant Creacyl Blue (BCB) painting.

Blood Profile Examination

The blood profile examination covers Hb levels, the erythrocyte count, and Ht, MCV, MCH, and MCHC rates. This study employed the Hematology Analyzer Mindray BC-2800. The reticulocyte count was counted using the wet method with BCB reagent

in methanol and the dry method with BCB reagent in NaCl. The reticulocyte count was obtained using the formula calculation: the reticulocyte cell count per 1000 erythrocytes was multiplied by 100%. Blood profiles were examined in the Clinical Pathology Laboratory of Universitas Muhammadiyah Semarang; the laboratory is licensed with ISO 9000-2015.

Statistical Analysis

Data on blood profiles were described based on each group's average, SD, minimal, and maximal scores. Meanwhile, the data normality test (p -value:Hb=0,08; erythrocyte=0,28;Ht=0,10; MCV=0,08; MCH=0,29; MCHC=0,85; reticulocyte=0,12) was conducted using the Shapiro Wilk to determine the differences between the groups. Since the data had a normal distribution, the statistical analysis employed the ANOVA test. Meanwhile, the statistical test employed the SPSS.

Ethical Clearance

This study received ethical clearance from the Bioethics Commission for Medical/Health Research of the Faculty of Medicine of Universitas Islam Sultan Agung Semarang following the Declaration of Helsinki no.203/VII/2020/Komisi Bioetik.

RESULTS AND DISCUSSION

The results of the blood profile investigation show the average Haemoglobin levels, erythrocyte counts, levels of Haematocrit, MCV, MCH, and MCHC, and reticulocyte counts. These results are presented in the following table.

Table 1. Average Values of MCV, MCH, and MCHC

Group	MCV (fl)		MCH (pg)		MCHC (%)	
	Average	p -value	Average	p -value	Average	p -value
C+	73.7 ± 7.06		27.3 ± 44.66		31.1 ± 1.0	
C-	79.9 ± 2.16		29.9 ± 0.87		34.2 ± 1.1	
T1	80.4 ± 1.48	0.03*	29.5 ± 2.22	0.54	31.9 ± 0.38	0.01*
T2	78.7 ± 0.48		28.5 ± 1.86		33.3 ± 0.93	
T3	79.6±0.85		29.1 ± 1.75		32.1 ± 0.87	

The ANOVA test calculated the differences between multiple groups. C+ = Positive control, C- = Negative control, T1 = Treatment 1 T2 = Treatment 2, T3 = Treatment 3. Significant p -values are marked with*

Table 1 shows that the MCV values of all treatment groups are higher than that of the positive control group. Meanwhile, the highest value is found in treatment 1 (80.4 g/dl). Moreover, the MCH and MCHC have similar trends, but MCH has an insignificant difference (0.54). Finally, MCH and MCGC have significant differences (p -values of 0.03 and 0.01).

Table 2 shows that the highest Hb level (14.2 g/dl) is found in the negative control. However, the level decreases in treatments 2, 3, and 1. Finally, the lowest Hb level is found in the positive control. The lowest mean of erythrocyte count is found in the positive control group, while the highest is in the negative control group. All treatment groups have higher average erythrocyte count values than the positive control group. The same pattern also occurs in Ht levels. Moreover, this study has found that the positive control group has the highest average score of reticulocyte counts. Haemoglobin and Haematocrit levels are significantly different (p -values = 0.00). Finally, the erythrocyte and reticulocyte counts are not significantly different (p -values = 0.14 and 0.06).

Table 2. Means of Hb Levels, Erythrocyte Counts, Ht levels, and Reticulocyte Counts

	Hb (g/dl)		Erythrocyte Counts (mm ³ blood)		Ht Level (%)		Reticulocyte Counts (%)	
	Average	p-value	Average	p-value	Average	p-value	Average	p-value
C+	12.0 ± 0.33		6.52 ± 0.89		35.5 ± 1.16		2.0 ± 0.32	
C-	14.2 ± 0.86		7.80 ± 0.54		42.9 ± 3.14		1.2 ± 0.64	
T1	12.5 ± 0.66	0.00*	7.44 ± 0.54	0.14	37.8 ± 3.26	0.00*	1.3 ± 0.2	0.06
T2	12.9 ± 0.49		7.01 ± 0.85		37.9 ± 1.46		1.1 ± 0.4	
T3	12.8 ± 0.33		7.32 ± 0.55		38.2 ± 1.11		1.3 ± 0.5	

*The ANOVA test calculated the differences between multiple groups. C+ = Positive control, C- = Negative control, T1 = Treatment 1 T2 = Treatment 2, T3 = Treatment 3. *Significant p-values are marked with*

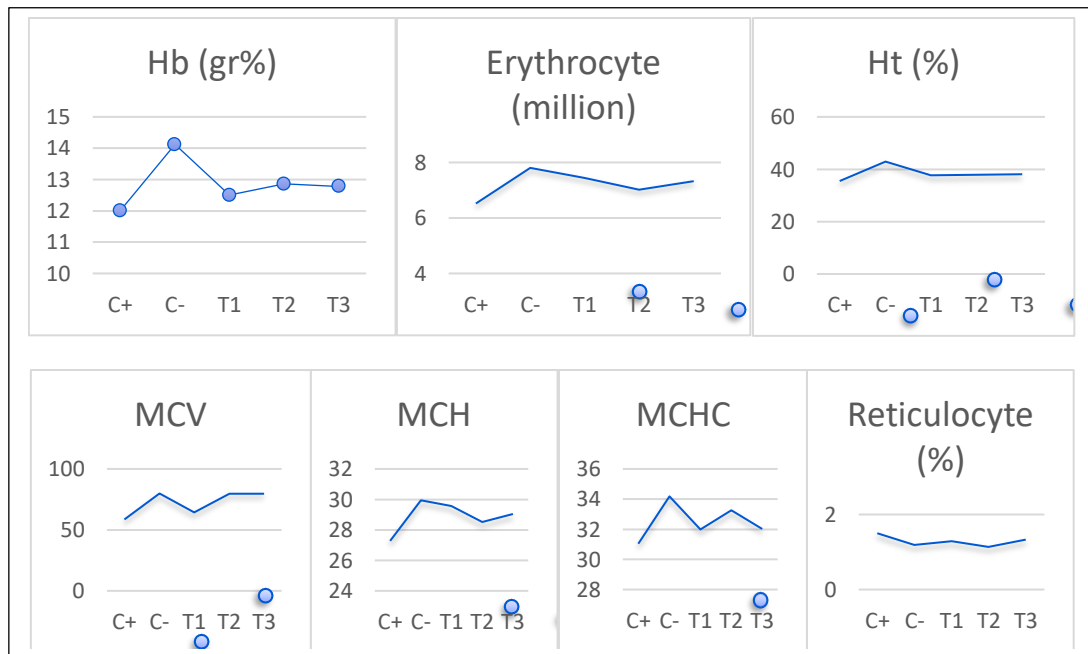


Figure 2. Means of Haemoglobin, Erythrocyte Count, Levels of Haematocrit, MCV, MCH, and MCHC, and Reticulocyte Count in Each Treatment Group

This study has discovered nearly identical patterns, except in the reticulocyte count. The positive control group has lower Hb levels, erythrocyte counts, and levels of HT, MCV, MCH, and MCHC than those of n the negative control group. Meanwhile, the treatment groups have slightly decreased values, but their values are still higher than the positive control group's. Meanwhile, the positive control group has the highest reticulocyte count among the negative control group and all treatment groups. The

statistical test has revealed significant differences in the Hb level, Ht level, MCV, and MCHC. According to figure 2, the erythrocyte count, MCH, and reticulocyte count are not significantly different.

These results prove that combining IR Bagendit rice leaves-infused water and young coconut water could improve hematopoiesis, Hb levels, Ht levels, MCV, and MCHC. IR Bagendit rice leaves contain a lot of metallothionein protein, which functions as a chelating agent that could bind Hg. Meanwhile, coconut water contains antioxidants that could capture free radicals from Hg to prevent damaged erythrocyte membranes (Gonzalez et al., 2012). The erythrocyte count and reticulocyte count are not significantly different because the erythrocyte cells have not been damaged before the half-life of the cell; such a condition also affects the reticulocyte cells. The reticulocyte cell count will increase if the erythropoiesis increases (Piva et al., 2015). In this study, the erythropoiesis has not increased because Hg exposure lasts only 15 days, and the erythrocyte count is still within normal limits.

Coconut water is a drink rich in nutrients, such as antioxidants, vitamins, minerals, amino acids, and enzymes, and a source of L-arginine and vitamin C (Li et al., 2018). Consuming coconut water could reduce oxidative stress and increase antioxidants (Zulaikhah, 2019). Meanwhile, IR Bagendit rice leaves-infused water contains a lot of metallothionein protein, which is rich in sulfhydryl (SH) groups. Sulfhydryl groups in metallothionein protein will compete with sulfhydryl in membrane proteins to bind mercury; consequently, no disruption occurs in the membrane integrity. The blood profile results conclude that the combination of coconut water and extract water of IR Bagendit rice leaves could significantly prevent hematopoiesis system disorders, especially erythropoiesis (Santosa et al., 2022). Compared to previous studies, this study has the novelty of combining an antioxidant-containing ingredient with a chelating agent. Previous studies, on average, only used a single ingredient. The implication of the results of this study is the use of natural materials that are cheap and easily available for health purposes. The limitation of this study is that the antioxidant content of various young coconut types is unknown.

CONCLUSION

The combination IR Bagendit rice leaves-infused water and coconut water could significantly increase the levels of Haemoglobin, Haematocrit, MCV, and MCHC in rats exposed to Haemoglobin. This research is very useful in using natural ingredients that are cheap and easily available for health purposes. Further suggestions from this study are extended intervention time and known antioxidant content of various types of young coconuts.

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CONFLICT OF INTEREST

The author has no conflict of interest.

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