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Effect honje forest fruit juice administration on hemoglobin levels following mercury chloride treatment in mice

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ABSTRACT

Exposure to mercury chloride ($HgCl_2$) is known to induce oxidative stress, which negatively affects the hematological system, including a decrease in hemoglobin levels. This study aims to analyze the effect of honje fruit juice (Etlingera hemisphaerica), known for its high antioxidant content, on hemoglobin levels in mice (Mus musculus) exposed to mercury chloride. This study used an experimental method involving several groups of mice, namely group K1 (control), group K2 (given honje fruit juice), and group K3 (injected with mercury and given honje fruit juice). Hemoglobin levels were measured using a Sahli tube. The results showed that honje fruit juice significantly increased hemoglobin levels in mice exposed to mercury chloride compared to the control group. These findings indicate that the antioxidant content in honje fruit can help reduce the toxic effects of mercury chloride on the circulatory system. Therefore, honje fruit juice has the potential to be developed as a natural therapeutic agent in efforts to mitigate the toxic effects of mercury chloride.

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INTRODUCTION

The circulatory system functions to distribute oxygen, nutrients, hormones, and various other important substances evenly to all tissues and organs of the body so that they can function optimally (Aswan et al., 2022). Hemoglobin (Hb) is an important component of this system, a type of protein found in red blood cells that performs an important function in the process of binding and transporting oxygen from the lungs to all body tissues (Kim et al., 2013), and functions in the transport of carbon dioxide, a waste product of metabolism, out of body tissues back to the lungs to be extracted through the respiratory process. If there is a disturbance or

imbalance in hemoglobin levels in the body, it can cause various serious health problems, such as anemia, a disease in which the body lacks healthy red blood cells and oxygen (hypoxia) (Chen et al., 2020). Hypoxia hurts the functioning of vital organs and overall reduces the quality of life of the individual concerned (Tutik & Ningsih, 2019). One cause of Hb imbalance is exposure to heavy metals.

Exposure to heavy metals such as mercury (Hg) has long been known to have high toxicity potential to various biological systems in the body (Alik et al., 2022), including the hematopoietic system responsible for the production and maintenance of blood cells. One of the most commonly used forms of mercury compounds in toxicology research is $HgCl_2$ (mercury chloride), which is known to cause oxidative stress in the body (Mukaromah et al., 2020). An imbalance between the production of free radicals and the body's antioxidant system's ability to neutralize them causes this oxidative stress, resulting in damage to the structure of red blood cell membranes (Shandiutami et al., 2016), inhibiting the synthesis of hemoglobin, which is essential for oxygen transport, and triggering hemolysis or premature destruction of red blood cells. The results of various previous studies show that the administration of mercury chloride compounds to test animals, particularly mice (*Mus musculus*), causes a significant decrease in hemoglobin and erythrocyte levels in the blood, indicating a serious disturbance in hematological function (Shalan, 2022). In addition, an increase in leukocyte count was also observed as an immune response and compensation by the body to oxidative stress caused by mercury exposure, indicating the activation of the body's defense system against cellular damage (Armilda et al., 2022).

Heavy metal treatment can be carried out using several methods, either through chelation therapy or by using water and soil treatment methods. Chelation therapy uses drugs that bind heavy metals and help remove them from the body. Meanwhile, water and soil treatment can use various methods such as chemical precipitation, adsorption, ion exchange, and membrane technology. Effective materials for overcoming the negative effects of heavy metal exposure, such as mercury chloride researchers have begun to turn their attention to the use of natural materials known to have high antioxidant and antibacterial content as well as beneficial pharmacological effects (Sahidin et al., 2019). Natural materials can be used to overcome the negative impacts of heavy metals because they can neutralize free radicals and bind heavy metals naturally. Natural materials are relatively affordable, abundant, and have fewer side effects than chemical materials. One promising local plant that is currently the focus of research is honje (*Etlingera hemisphaerica*), which is a member of the Zingiberaceae family and has traditionally been used in various natural medicines (Andriani & Chaidir, 2017) and food consumption in a number of regions. This plant is known to contain various important bioactive compounds, including flavonoids, tannins, saponins, and polyphenols (Hikmah et al., 2022), each of which has been proven to have strong antioxidant activity and potential as an immunomodulator, a substance that can help regulate and strengthen the immune system. The presence of these compounds makes honje a highly potent natural candidate in warding off harmful free radicals produced by oxidative stress, as well as playing a role in protecting the integrity of cell structure and function from damage caused by exposure to heavy metals such as mercury chloride. Therefore, the use of honje in the field of health, particularly as a protective agent against mercury toxicity, is highly relevant for further investigation through scientific and experimental approaches (Susanti et al., 2024).

A number of previous studies have revealed that honje leaf extract has a significant effect on blood damage caused by exposure to mercury chloride in animal models, particularly mice. This effect is demonstrated by its ability to maintain erythrocyte (red blood cell) and leukocyte (white blood cell) counts within the normal range, even though the mice were exposed to this toxic substance. In addition to the leaves, honje fruit has also been the subject of research related to its pharmacologic potential, particularly in the context of its effect on blood lipid profiles. Several

studies have shown that honje fruit extract can significantly reduce triglyceride and total cholesterol levels in mice with hyperlipidemia, a metabolic disorder characterized by high blood fat levels. However, although the benefits of extracts from various parts of the honje plant have been widely reported, to date there has been very limited research specifically evaluating and documenting the effect of honje fruit juice administration on Hb levels in the blood of mice exposed to mercury chloride heavy metals. Therefore, further studies in this field are important to expand scientific understanding of the therapeutic potential of honje, particularly in the context of protection against hematological disorders induced by mercury chloride toxicity. The use of honje fruit juice as a therapeutic intervention has a number of advantages over leaf extract, including a more acceptable taste and the potential for different bioactive compounds. In addition, processing in the form of juice is more suitable for everyday use. Therefore, it is important to evaluate whether honje fruit juice has a protective effect against the decrease in Hb levels due to mercury chloride exposure in mice.

This study specifically aims to explore and analyze the effect of honje fruit juice (*Etlingera hemisphaerica*) administration on Hb levels in the blood of mice (*Mus musculus*) that have been exposed to the toxic compound mercury chloride, which is widely known as a dangerous heavy metal with damaging effects on the circulatory system. The honje fruit juice used in this study was Etlingera Fruit Syrup Drink (MSBE), which has a distribution permit number PIRT 2.13.3218.077.107-22. MSBE juice was used because it is practical and has undergone valid laboratory testing regarding the content of honje fruit juice. By utilizing animal models as an experimental approach that can represent biological responses to certain treatments (Franco, 2013), this study used mice as experimental animals (Mutiarahmi et al., 2021). This study is expected to produce valid scientific data that is methodologically accountable. The data will provide an initial overview of the potential of honje fruit juice as a natural therapeutic agent that functions to reduce or even counteract the toxic effects of mercury chloride on hematological parameters, particularly Hb levels. It is hoped that the results of this study will not only strengthen the scientific basis for the use of honje fruit in the fields of pharmacy and traditional medicine, but also open up opportunities for further development in the search for alternative treatments based on natural ingredients to support the health of the circulatory system threatened by exposure to heavy metals.

RESEARCH METHOD

Research Design

This type of research is quantitative research using a laboratory experiment method that aims to determine the effect of forest honje fruit juice on Hb level recovery in mice. In this study, the test was conducted once, after the experiment, by measuring the Hb level in mice at the end. This study was conducted for 10 days in February 2024. The subject of this study was the effect of honje forest fruit juice on the recovery of Hb levels in mice due to mercury chloride treatment. An observation table was created as a guide for experimenting. The following table and explanation describe the stages of the experiment to observe the Hb levels in mice injected with mercury chloride, which were then given honje juice to restore Hb levels in their blood. The results are shown in Table 1.

Population and Sample

The population in this study was male Swiss Webster mice imported from the Institute of Technology Bandung (ITB) that had passed health tests. The mice used were approximately 2-3 months old and weighed 25-30 grams. In this study, the mice were divided into three groups, namely group K1 (as control), K2 (injected with honje juice for 3 days), and K3 (gavage $HgCl_2$ + honje juice for 3 days). The research location was at SBIH Ruyani, Bengkulu City.

Table 1. Observation Table

Experimental animal group	N	Research Activity Day										
		0 (T)	1 (O)	2 (O)	3 (T)	4 (T)	5 (T)	6 (o)	7 (o)	8 (o)	9 (0)	10 (T)
K1: (Control)	3	No treatment	Rest	Rest	-	-	-	-	-	-	-	Final check using the Sahli device (Hemoglobin)
K2: (Honje juice 0.3mL/ 20 g body weight, 3 days)	3	No treatment	Rest	Rest	Gavage Honje juice 0.3mL / 20 g BW	Gavage Honje juice 0.3mL/ 20 g BW	Gavage Honje juice 0.3mL / 20 g BW	-	-	-	-	Final check using the Sahli device (Hemoglobin)
K3: ($HgCl_2$ 5 mg/kg BW + Honje juice 0.3mL/20 g BW, 3 days)	3	$HgCl_2$ injection 5 mg/kg BW	Rest	Rest	Gavage Honje juice 0.3mL / 20 g BW	Gavage Honje juice 0.3mL/ 20 g BW	Gavage Honje juice 0.3mL / 20 g BW	-	-	-	-	Final check using the Sahli device (Hemoglobin)

Description:
T = Treatment
O = Observation

Instruments

The tools used for research include: (1) Scales to measure the weight of mice and the weight of materials used, (2) 1 cc syringes used for intraperitoneal injections of mercury chloride to mice, (3) Gavage devices used to administer honje juice orally from the mouth to the stomach, (4) Mortar and pestle used to grind immunos, (5) Scissors for taking small volumes of blood, (6) Sahli tubes (Superior brand) for checking hemoglobin levels in mice. The materials used for the study included: (1) Honje juice (Hola juice No: PIRT 2.13.3218.077.107-22) (2) HCl solution used to dilute blood, (3) Distilled water, (4) Mercury chloride,(5) Alcohol, (6) Male Swiss Webster mice imported from the Institute of Technology Bandung (ITB) that had undergone health tests. The mice used were approximately 2 to 3 months old and weighed 25–30 grams. Upon arrival, the mice were acclimatized for one week to allow them to adapt to the new environment, while adhering to animal welfare ethics, including proper treatment of animals in accordance with the Five Freedoms principles.

Procedure

The first stage involved grouping the mice into three groups, namely K1 (control, no treatment), K2 (gavage with honje juice for 3 days), and K3 (injection of mercury chloride and gavage with honje juice for 3 days), with each group consisting of three mice. In the second stage, on day 0, mercury chloride was administered via intraperitoneal injection, due to the ability of the peritoneal cavity to rapidly absorb large amounts of the drug in group K3 at a dose of 5 mg/kg BW, while groups K1 and K2 were not injected with mercury chloride. The third stage on days 1 and 2 involved resting all groups/no treatment. The fourth stage on day 3 involved the K1 group as a control receiving no treatment, while the K2 and K3 groups were given honje juice for 3 days

via gavage at a dose of 0.3 mL/g BW. In the fifth stage, on day 10, Hb levels were measured using the Sahli method, which works by converting Hb in the blood into hematin acid using hydrochloric acid. A Sahli pipette was used to draw 20 mm^3 of blood, andthe tip of the pipette was cleaned with tissue. After that, the blood was placed in a Sahli tube containing 0.1 N HCl and left for three to five minutes to ensure that the Hb reacted with the HCl to form hematin acid. The blood was then stirred and distilled water was added drop by drop until the color matched the standard color. To read the scale, the surface of the solution is matched with a haemometer, which indicates the amount of hemoglobin in grams per 100 ml of blood.

Data Analysis

The data obtained from the research results were analyzed quantitatively. The data were analyzed by calculating the average Hb and standard deviation (SD) in the test animal group. The hypothesis test used in this study was the t-test. The research results will be described narratively to illustrate the effect of forest honje fruit juice on Hb level recovery in mice.

RESULTS

From the research conducted, the results are shown in Table 2. The results of the calculations with the mean and standard deviation with a sig value ($p<0.05$) for parameters including the increase in Hb levels in mice and the administration of honje fruit juice showed a significant difference between the control and the treatment. This means that the administration of honje fruit juice has an effect on increasing Hb levels in mice.

Table 2. Summary of Research Results

Test Animal Group	N	Average Hemoglobin ±SD (g/dL)
K1 (control)	3	11.9 ± 1.27
K2 ((Honje juice 0.3mL/ 20 g body weight, 3 days)	3	14.6 ± 2.88
K3 ($HgCl_2$ 5 mg/kg BW + Honje juice 0.3mL/20 g BW, 3 days)	3	11.8 ± 1.41

The results of Hb level measurements are presented in Table 2. The results for the average Hb level show significant variation between the test animals and the control group and the test animals in the treatment group. The average Hb diagram for the test animal group is presented in Figure 1.

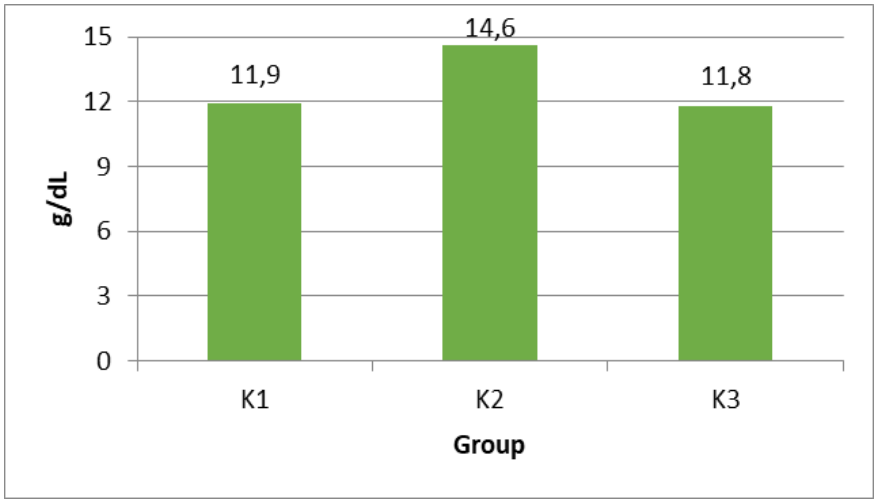

Figure 1. Difference in average Hb levels

In this study, using fresh forest honje fruit juice, can be seen in the picture below, in picture a is (a) photo of forest honje fruit, and in picture (b) is a photo of forest honje fruit juice.

(a) (b)

Figure 2. (a) Honje fruit, (b) Honje Juice

DISCUSSION

The results of Hb level measurements are presented in Table 2. The results for the average Hb level show significant variation between the test animals and the control group (11.9 ± 1.27) and the test animals in the treatment group. The test results show that the average hemoglobin level in the K2 treatment group increased (14.6 ± 2.88). Meanwhile, the average Hb level in the K3 treatment group (11.8 ± 1.41) showed a decrease in Hb levels in mice. This indicates that the administration of honje fruit juice and mercury chloride injections resulted in an increase and a decrease in hemoglobin levels in mice.

This study only observed the Hb levels of test animals after intraperitoneal injection of mercury chloride on day 0 and gavage of honje fruit juice on day 3, after the mice had rested. The results showed that the Hb levels of test animals were affected by honje fruit juice for 10 days. Figure 1 shows the percentage difference in average hemoglobin, indicating the effect of honje fruit juice on Hb due to the influence of mercury chloride. The effect of honje fruit juice was clearly seen in group K2, while in groups K1 and K3, honje fruit juice was shown to be able to restore Hb levels to/close to normal. (Etim et al., 2014) stated that Hb and erythrocytes are hematological components. Both components can be used to monitor the toxicity of a substance, especially those that affect blood and animal health. Erythrocytes or red blood cells function as hemoglobin carriers. During respiration, hemoglobin reacts with oxygen in the blood to form oxyhemoglobin (Opara et al., 2010). The results of a study conducted by (Benkovi**ć** et al., 2012) showed that the intensity of Hb levels in normal mice was 12.79 (g/dL). The results of research published by (Akin-Osanaiye et al., 2015) show that the Hb level in control mice is 12.80 (g/dL). These results indicate that the Hb level of the control group test animals is within the normal range. These results show that the test material used causes a difference in the mean between the control and treatment groups.

The effect of honje fruit juice administration can be assessed by comparing the Hb levels of each group injected with mercury chloride and honje juice with the control group that was not given honje fruit juice. Mercury chloride is known to inhibit erythropoiesis, which is the formation of red blood cells, by damaging erythrocytes or reducing their release into the bloodstream, which can ultimately trigger anemia. This anemia is closely related to iron metabolism disorders, which have an impact on the reduction of nutrient absorption in the intestines. When iron levels in the body decrease, hemoglobin production is also disrupted, causing a decrease in hemoglobin concentration in the blood, followed by a decrease in hematocrit levels. The effect of honje fruit

juice in increasing Hb levels as a result of this treatment is because honje fruit juice can help restore Hb levels through a combination of iron, vitamin C, antioxidants, and anti-inflammatory effects (Dilantika et al., 2024), as well as the antioxidant properties of vitamin C, which can neutralize free radicals in the body. To determine the effect of honje fruit juice on increasing Hb levels, it is necessary to compare it with the control group. The Hb levels of the control group showed a significant difference in the average increase in this treatment. Therefore, the treatment administered to the honje fruit juice group and the control group was effective in increasing Hb levels.

CONCLUSION

Based on the results of the study, it can be concluded that administering forest honje fruit juice (Etlingera hemisphaerica) can increase hemoglobin levels in mice (Mus musculus) exposed to mercury chloride ($HgCl_2$). This positive effect is thought to be closely related to the antioxidant compounds contained in forest honje fruit, which play a role in reducing oxidative stress and preventing damage to red blood cells due to mercury exposure. Thus, forest honje fruit juice can increase Hb levels in mouse blood due to $HgCl_2$ toxicity, making it a potential natural therapeutic agent in efforts to mitigate the effects of heavy metal toxicity on the hematological system.

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