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Article

Effectiveness of Red Guava Leaf Ethanol Extract (*Psidium guajava L.*) on Increasing Hemoglobin Levels in Female White Rats (*Rattus novergicus*)

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ABSTRACT

Hemoglobin is the protein found in red blood cells that has the task of delivering oxygen to the lungs. When the hemoglobin level degrades, it can cause symptoms of anemia. Guava fruit is often used by people in traditional medicine to increase hemoglobin levels. The people only use the fruit because it contains vitamins, minerals, fiber and phytochemical compounds that the body needs. However, these ingredients also found in guava leaves have the potential to increase hemoglobin levels and prevent anemia which is easily available and economical. The purpose of this study is to identify the effects of extracted from guava leaves (Psidium guajava L.) on anemic hemoglobin levels of female wistar strains. The study used a laboratory experimental design with pretest and post-test group design, purposive sampling technique. The sample in this study was 30 female wistar strains divided into 5 groups, namely negative control without treatment, positive control was given blood increasing tablets to rats with anemia, and treatment groups I, II, III were given guava leaf extract at doses of 100 mg/KgBb, 250 mg/KgBb and 500 mg/KgBb to rats with anemia. Increased hemoglobin levels of guava leaves (Psidium guajava L.) respectively 13.56 gr/dl, 15.56 gr/dl dl, and 16.85 gr/dl. The most influential guava leaf extract is treatment group 3 with a dose of 500mg/kgBB and in post hoc analysis using LSD shows a significant difference between guava leaf extract at a dose of 100 mg/KgBb, 250 mg/KgBb and 500 mg/KgBb (p-value = 0.0000) in increasing hemoglobin levels.

INTRODUCTION

Hemoglobin is a protein found in red blood cells that is responsible for delivering oxygen to the lungs [1]. If hemoglobin levels decrease, it can cause symptoms of anemia, namely 5L (weak, tired, lethargic, tired, careless), pale face, blurred vision, headaches, easily infected with diseases, and pale nails. There are several risk factors for anemia including nutritional deficiencies, genetic disorders of hemoglobin, infections (e.g. hookworm), and various other conditions such as menstrual conditions, gastrointestinal diseases, frequent blood donations, and chronic diseases) that cause blood loss or destruction of red blood cells [2]. The effects of decreased hemoglobin levels include decreased concentration of learning, decreased physical fitness, and impaired growth so that height and weight do not reach normal.

WHO estimates that in 2019 30% of women aged 15–49 years, 37% of pregnant women, and 40% of children 6–59 months of age were affected by anaemia, with the WHO African Region and South-East Asia Region being most affected [3]. The threshold value of hemoglobin (Hb) levels in adult females is 12.0 gr/dl and males 13.0 gr/dl, while the normal value of rat Hb ranges from 11.6-16.1 gr/dl [4].

Anemia can be treated in two ways, namely pharmacological and non-pharmacological therapy. Pharmacologically, supplements (TTD), folic acid and blood transfusions can be given. Non-pharmacological therapy can also be done by giving foods rich in Vit C, Vit B and Vit A such as spinach, carrots, guava and others fruit which can help the absorption process of iron in the body [5].

According to Prawirohardjo, the prevention of anemia in adolescents can be given enough sulfas ferrosus 1 tablet a day. However, side effects that will arise such as nausea, difficulty defecating, and black stools because the iron contained is very high, namely 200 mg or 60 mg of elemental iron and 0.25 mg of folic acid, this is what causes people to tend to be non-compliant with taking iron tablets [16]. Meanwhile, blood transfusion is done if the Hb level is <7 gr/dl [6].

In addition, Indonesia is known as one of the countries with the largest biodiversity in the world. This diversity can be seen from the many plants that can be processed into herbal medicines. The benefit of using traditional medicine is that the raw materials are easily available and cheap. One example of a plant that can be utilized is the guava plant (Psidium guajava L.). This plant is most known as antidiarrheal, antibacterial, antiviral, antidiabetic and antioxidant. Guava leaves (*Psidium guajava* Linn.) are known to help raise blood hemoglobin levels. They contain compounds such as iron, copper, and phosphorus, which contribute to this increase.

In this study, researchers wanted to see the benefits of *guava* leaves (*Psidium guajava* L.) by direct observation on experimental animals in the form of white rats (*Rattus novergicus*) related to anemia treatment using herbal plants in the form of guava leaves

METHODS

This type of research a *Quasi Experimental*. The research was conducted from January to March 2023 at the Pharmacology Laboratory, Faculty of Medicine, University of North Sumatra. This study is a *Quasi Experimental* with a *pre-test and post-test group* design that uses female white rats (*Rattus novergicus)* as many as 25 samples divided into 5 groups. Coupled with a reserve of 10% (f = 0.1) of the total sample so that 1 rat is obtained as a reserve. So the total sample needed is 30 rats which are divided into 5 groups, which means 1 group consists of 5 rats and 1 rat as a reserve. Inclusion criteria are rats in healthy condition, no wounds, rats aged 2-3 months, weighing 200-250 grBB, Hb rats < 11.6 gr / dl and exclusion criteria for deformed rats, looking sick and dead during the study. The sample is calculated using the Federer formula as follows :

$$(n-1)(t-1) \geq 15$$
$$(n-1)(5-1) \geq 15$$
$$(n-1).(4) \geq 15$$
$$4n-4 \geq 15$$
$$4n \geq 15+4$$
$$4n \geq 19$$
$$n \geq \frac{19}{4} = 4{,}75 \approx 5$$

The tools used in this practicum are cages and drinking containers, measuring cups, analytical scales, oral probes, digital scales, rotary evaporators, maceration bottles, masks, gloves, syringes, injection needles, Hb Check (easytouch GCHB) and HB strips. The materials used in this practicum are rats, guava leaf extract, 96% ethanol, Aquades, and Nacl.

Before being used as experimental animals, all rats were kept for approximately one week for environmental adjustment and health control. Experimental animals were placed in cages with an area of 150 cm and a cage height of 20 cm that had been cleaned and placed in a maintenance room free from noise and then given standard feed ad libitum.

After all the five groups were adapted for 7 days. On the 8th day, 3 ml of blood was taken from the lateral vein of the rat's tail to reduce hemoglobin levels in rats [7]. On the 9th day, hemoglobin levels were measured before treatment using *easytouch GcHb.* On the 10th day, the treatment began to be given and lasted for 7 days according to the group. Then on the next day, the hemoglobin level in rats was measured again after treatment.

Health Research Ethics Commission, Faculty of Medicine, Malikussaleh University has granted this study ethical feasibility, with number: 033/KEPK/FKUNIMAL-RSUCM/2022. The

samples used are guava leaves and have been terminated at the Herbarium Medanes Laboratory (MEDA), University of North Sumatra.

Hemoglobin level data were tested using Microsoft Excel 2019 and SPSS Version 26, 2019. The mean difference test data used the LSD (*Least Significant Difference*) test, and the increase in hemoglobin levels used the median test

RESULTS

The average percentage increase in hemoglobin levels after blood collection in rats (*pretest*) and hemoglobin levels after treatment with extracts (*post-test*) is shown in Table I. Rats that have been treated for 7 days show an increase in hemoglobin levels in group K (+), group 3, group 2, and group 1, respectively.

Table 1. *Percentage increase in hemoglobin level before and after treatment.*

Group	***N***	***Haemoglobin level before treatment (g/dL)***	***Haemoglobin level after treatment (g/dL)***	***Average increase***
Negative control	6	10.9	10.75	
Positive control	6	10.5	16.75	58%
Group I	6	10.7	13.56	27%
Group II	6	10.8	15.56	44,4%
Group III	6	10.7	16.85	56,3%

Based on table 1, the percentage increase in haemoglobin levels after treatment shows an increase in hemoglobin levels in the positive control group (blood increasing tablets), group I (dose of 100 mg/KgBb), group II (dose of 250 mg/KgBb), and group III (dose of 500 mg/KgBb) when compared to the negative control group (distilled water).

The results of the effect of increasing post-test haemoglobin levels compared to blood increasing tablets can be seen by comparing the average percentage increase of each treatment dose with the average percentage of positive control, namely sangobion. The goal is to determine the effectiveness of the treatment dose compared to the effectiveness of the positive control, namely Sangobion.

Table 2. *LSD test on post-test hemoglobin levels*

Group	***Negative control***	***Positive control***	***P1***	***P2***	***P3***
Negative control	-	0,000*	0,000*	0,000*	0,000*
Positive control	0,000*	-	0,000*	0,000*	0,222
Group I	0,000*	0,000*	-	0,000*	0,000*
Group II	0,000*	0,000*	0,000*	-	0,000*
Group III	0,000*	0,222	0,000*	0,000*	-

*There is a significant difference (p<0.05)

Based on the results of the Post-hoc LSD further test, it shows that the significance value of the difference in means between groups is 0.000 (α <0.05), so it can be explained that there is a significant difference in the mean Hb Post-Test levels between groups, except in group 3 with control (+).

DISCUSSION

The study, the measurement of *pretest* hemoglobin levels was carried out to determine the decrease in hemoglobin levels after taking 3 ml of blood in each group. The decrease shows a state of anemia seen from descriptive data. Taking 3 ml of blood in the lateral vein of rats can reduce hemoglobin levels in each group without risking the safety of the test animals [6,7].

The average hemoglobin level after being given *guava* leaf extract (*Psidium guajava L.*) dose of 100 mg/KgBb has an average hemoglobin level of 13.56 gr/dl (27%), dose of 250 mg/KgBb has an average hemoglobin level of 15.56 (44.4%), and dose of 500 mg/KgBb has an average hemoglobin level of 16.85 (56.3%). This shows that among the three treatment groups the closest to the positive control is the 500 mg/KgBb dose group. In line with research conducted by Lina Winarti and Wantiyah, the higher the dose, the higher the effect given. This increase can occur due to the flavonoid content in guava leaves [6,8].

In line with FP's research. Hardimarta et al. mentioned that quercetin contained in flavonoid compounds has been shown to be associated with antioxidant effects. Flavonoids are lipophilic so that they are able to bind to the erythrocyte cell membrane and function as a protector against free radicals. Flavonoids present in the bloodstream stimulate the kidneys' plasma globulin cells to produce the hormone erythropoietin. Once released into the blood, erythropoietin promotes red blood cell production by the bone marrow. In response, the bone marrow's primitive stem cells continuously generate new hemocytoblasts. These hemocytoblasts initially develop into basophilic erythroblasts, which then begin the process of hemoglobin synthesis [17]. The results of Unigwe and Nwakpu showed that flavonoid antioxidant activity can also increase hemoglobin levels [9].

In addition to flavonoids, guava leaves also contain iron. Based on biochemical and mineral analysis in previous research conducted by Lintu Thomas, Anitha et al. showed that guava leaves have an iron content of 13.50 mg/100 grams, which is higher than guava fruit, which is 0.26 mg/100 grams [10]. Another study by Angela Ditauli Lubis by giving a dose of *guava* leaf extract (*Psidium guajava L.*) as much as 500 mg / kgBB [6]. The study showed an increase in hemoglobin levels after giving guava leaf extract. This is because one of the hemoglobin-forming content is

higher in leaves than in fruit. This happens because of the photosynthesis process in the leaves involving iron (Fe) as an electron carrier in the bright phase of photosynthesis so that iron (Fe) is deposited more in the leaves. When the body experiences iron deficiency, it will reduce the rate of formation and concentration of hemoglobin in the blood circulation [11]. This is because iron is an important microelement in protein synthesis. Iron is a vital trace element that is needed by the body for the formation of hemoglobin, myoglobin, and various enzymes .

When iron enters the body it is in the form of ferric (Fe^{3+}) and then enters the stomach. In the stomach, iron will be converted into ferrous (Fe^{2+}) with the help of stomach acid and vitamin C. Ferrous iron will enter the small intestine and be absorbed in the proximal part. After absorption, iron will bind to apotransferrin and enter the mucosal cells. Then the iron will separate into three parts, some of which will still bind to apotransferrin and form serum transferrin, some will bind to apoferitin and form ferritin, and some will bind to serum transferrin. Iron that binds to serum transferrin will be distributed throughout the body, especially the liver, spleen, and bone marrow. Iron that enters the bone marrow will bind to erythrocytes and porphyrins to form *heme* compounds. *Heme* will bind to globulin and form hemoglobin. Hemoglobin functions to bind and carry oxygen throughout the body [11].

Another chemical compound contained in guava leaf ethanol extract is vitamin C. This compound serves to accelerate the absorption of iron in the stomach. Vitamin C can help reduce ferric iron (Fe^{3+}) to ferrous (Fe^{2+}) in the small intestine so that it is easily absorbed by the body, the reduction process will be greater if the pH in the stomach is more acidic. Vitamin C can increase the pH in the stomach so that it can increase the absorption process of iron by up to 30%. The vitamin C content in guava leaves is higher than citrus fruits, in 100 grams of guava leaves it contains 103 mg of vitamin C, while in 100 grams of citrus fruits it contains 50-70 mg of vitamin C.

Vitamin C has a very important role in iron absorption, especially from *nonheme* iron, which is found in many plant foods. Vitamin C is a substance that helps increase the absorption and metabolism mechanism of iron in the body [12,13]. However, if the amount of vitamin C is insufficient or consuming vitamin C with insufficient iron consumption, the function of vitamin C in the body in iron absorption cannot function optimally, which will result in a decrease in hemoglobin levels[14].

in line with research by Nova lusiana, et al related to the increase in hemoglobin levels more found in extra Guava leaves as much as 45.79% while Banyan leaf extract can increase by 40.36%. So that guava leaf extract is an extract with the highest increase in Hb levels compared to banyan leaf extract [15].

CONCLUSION

Based on the research that has been done, there are differences in the effectiveness of guava leaf ethanol extract (Psidium guajava L.) with various doses on increasing hemoglobin levels in female white rats (Rattus norvergicus). Guava leaf extract (Psidium guajava L.) with doses 100 mg kgBb, 250 mg/kgBb and 500 mg/kgBb is effective in increasing hemoglobin levels in female white rats.

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