

Hemoglobin Levels of Wistar Rats After Saluang Fish (*Rasbora Spp*) Powder Administration

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ABSTRACT

It is estimated that 40% of children aged between 6-59 months experience anemia worldwide. In Indonesia prevalence of anemia in children under five reaches 38.5%, and 26.8% in children ages 5-14 years. Food first consumed by baby tends to support the absorption substance iron that causes Iron Deficiency Anemia (IDA). Saluang fish powder is a material food proven locally and contains high in protein, calcium, and substances iron. Research objectives : This study was to determine hemoglobin levels in Wistar Rats after being given a dose of Saluang fish powder. The method used is true experimental with post-test only design in Wistar Rats for 16 weeks in the Laboratory of Pharmacology, Universitas Muhammadiyah Palangkaraya. Control group divided into positive control group and negative control group. Whereas treatment group divided into of three groups which each given dose differently, low dose (0,36 g/ 200 g BW), moderate dose (0,54 g/ 200 gr BW), high dose (0,72 g/ 200 g BW) for 2 times a day *intraperitoneal* and standar feed *ad libitum* . The results show the average levels of hemoglobin group negative control was 19.617 mg/dL, and the group control positive 20.28 mg/dL. The average level of hemoglobin in the group in each treatment is that given dose light (19.65 grams/ dL), dose moderate (18.96 mg/dL), and dose high (20.33 mg/dL). Statistical test results show there is no difference in average levels of hemoglobin in a low dose group (p-value=0.63), moderate dose group (p-value=0.77), and high dose group (p-value=0.189) with hemoglobin levels in the negative control group. However, there is a difference in average levels of hemoglobin in the treatment group(p-value=0.000) with average levels of hemoglobin in the positive control group.

Keywords: Anemia, Hemoglobin, Saluang Fish Powder, Wistar Rats

INTRODUCTION

Anemia is a condition where the hemoglobin concentration is lower than normal it is estimated that 40% of children aged between 6-59 months experience anemia worldwide (WHO, 2023). In Indonesia prevalence of Anemia in children under five reaches 38.5%, and 26.8% in children ages 5-14 years (Anonim, 2019). Iron Deficiency Anemia (IDA) reduces oxygen transport capacity, energy metabolism, and immune function and affects cognitive function and motor development (Gillespie, 1998; Nasution, E, 2004; Miranda et al 2014). IDA in children is caused by

several factors: malnutrition, inadequate diet, infection, inflammation, chronic disease, genetic and obstetric conditions, and red blood cell disorders (Gupta PM, et al. 2016). Therefore, providing good weaning food must include a variety of foods to ensure adequate nutrition, especially micronutrients. Children who do not receive weaning food according to WHO recommendations have a risk (OR=1.214) of illness such as anemia (Nurhalina, N. et al, 2020).

The food consumed by babies tends to support iron absorption. One of the national strategies to overcome nutritional problems among children under five is the development of local food ingredients to increase family food security through cheap and affordable food. Developed local ingredients such as Saluang Fish (*Rasbora spp*) are an alternative for residents around Central Kalimantan to meet children's nutritional needs. Fish is a source of animal protein besides meat, milk and eggs, which is rich in essential vitamins and minerals such as long chain fatty acids, omega-3 (DHA) and omega-6 which play a role in growth and health (Dewi, PFA, et al., 2018).

Saluang fish (*Rasbora spp*) is a local fish found in the freshwater of Kalimantan and Sumatra. The nutritional content per 100 grams of saluang fish is 18 grams of protein (Lestari RM, et al., 2016; Yunanto A, Et al 2020) and iron is 2.17 grams (Tumongka, S, et al., 2022). Besides, saluang fish also contains omega-6 in the form of linoleic acid (1013%) and arachidonic acid (1.27%) as well as higher levels of lysine, nitrogen 580 mg/g (Hesty, H., 2020) and calcium of 1.6 calcium per grams (Yunanto A, Et al 2020). However, fish consumption among children in Indonesia is still low, around 26.1% (Nurhalina, N. et al, 2020). One of the factors behind the low consumption of fish in children is due to the lack of mother's knowledge (Dewi, PFA, et al., 2018), the fishy smell of fish and the sharp bones of the fish so that children are often rejected (Ari, Y. 2014).

Therefore, we developed saluang fish powder which is processed with other food ingredients so as to increase children's appetite for consuming fish. We explored the possible role of the nutritional content of Saluang fish in increasing hemoglobin levels to prevent anemia. The results of previous research that mixed saluang fish powder with food ingredients were reported to have the same effectiveness as iron supplementation in reducing the prevalence of iron deficiency anemia in school children (Miranda et al 2014). Saluang fish powder can be used as an alternative to iron supplements, especially because iron supplementation has gastrointestinal side effects such as stomach ache, nausea and diarrhea (Aurora, WID. 2021).

Wistar rats are often used as experimental animals because they are mammals that have complete organs, nutritional needs, biochemical metabolism, reproductive, respiratory, circulatory and excretory systems. Rats also have a number of advantages in their ability to reproduce, maintainance, and larger in size so they are easy to research (Lestari RM, et al., 2016; Smith, JB, et

al. 1988). Therefore, we developed Saluang fish powder which contains protein, iron and calcium which has the ability to increase hemoglobin levels. The aim of the research was to determine the hemoglobin levels of the Wistar rats after administering saluang fish powder.

METHODOLOGY

This research is an experimental study with a post-test only control group design for two months carried out at the Animal house of Muhammadiyah University, Palangkaraya.

1. Saluang Fish Powder Production

The selected saluang fish is cleaned and weighed, then steamed for ± 15 minutes. After the fish is drained, the fish flesh is separated from the bones and the antioxidant BHT 0.02% is added and then stirred well. Shred the fish meat, then weigh it and then dry it at 50 o C for ± 9 hours. After the shredded fish is dry, grind the fish until it becomes powder, then sift it and weigh it.

2. Try Animal Care

This study was approved by the Ethical Committee of Medical Research, Medical Faculty, Palangkaraya University. 2-week-old Sprague-Dawley rats weighing 42 ± 6 grams were placed in the animal cage at Palangkaraya Muhammadiyah University with controlled temperature (24 ± 1 °C, humidity $50 \pm 10\%$). After adaptation for 7 days, 30 rats were randomly selected and divided into five groups, consisting of a positive control group, a negative control group, and 3 treatment groups with different doses, each group consisting of 6 rats.

The positive control group was the group that given 50% standard feed + distilled water per day. Meanwhile, in the negative control group, rats were given standard food + distilled water per day. The treatment group was divided into three, each group was given 50% standard feed + Saluang fish powder in different doses, low dose (0.36 g/ 200 g BW per day), medium dose (0.54 g/g BW per day), high dose (0.72 g/ 200 g BW per day). Saluang fish powder was dissolved in distilled water and immediately given to rats parenterally. Oral administration is carried out once every day for 16 weeks. During this period, body weight was measured weekly, and food intake was recorded daily.

3. Hematology Test

At the end of the experimental period, rats fasted for 8 hours before anesthetized with kloroform. Blood sample collected from the retro orbital sinus vessel. hemoglobin were measured using Point-of-care testing (POCT) for hemoglobin (Hb) using digital hemoglobinometers from *easytouch*.

4. Statistical test

Data was analyzed using SPSS tools. Data analysis was carried out using the ANOVA test to determine the difference between the average hemoglobin levels of the treatment group and the average hemoglobin levels of the control group.

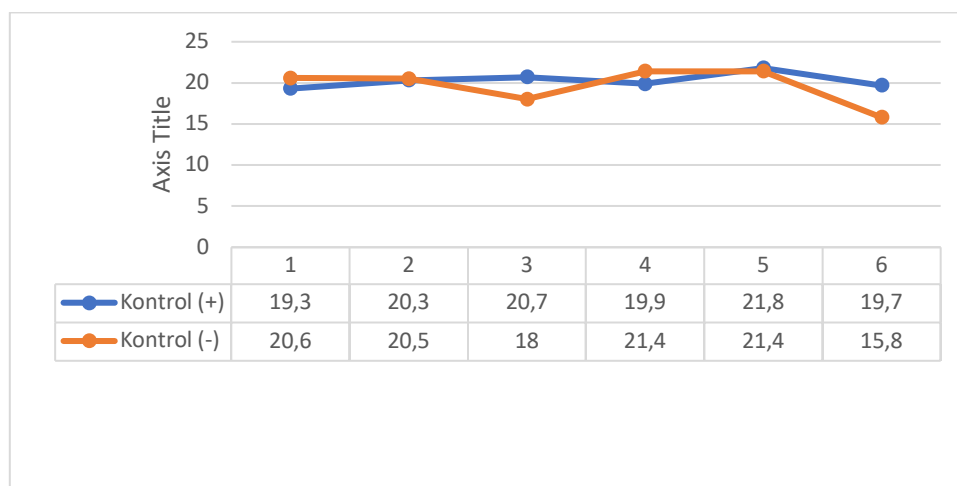
RESULTS AND DISCUSSION

1. Hemoglobin Levels

Table 1 shows that the average level of hemoglobin in the negative control group was 19.617 mg/dL, with the highest level was 21.4 mg/dL. Meanwhile, in the positive control group, the average level of hemoglobin was 20.28 mg/dL, with the highest rate was 21.8 mg/dL.

Table 1 Hemoglobin Levels in the Group Control

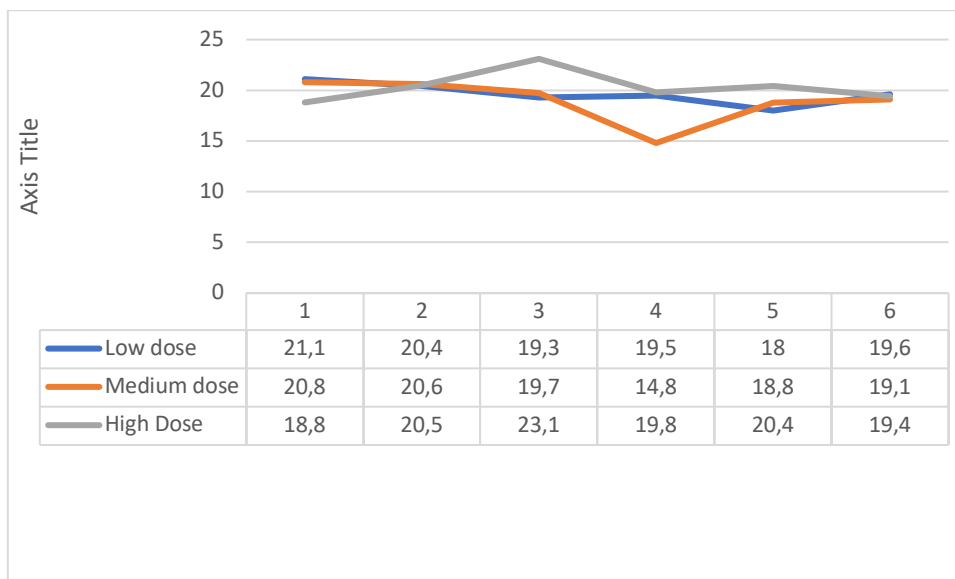
	Control negative	Control Positive
Mean	19,617	20,283
Median	20,550	20,100
SD	22,489	0.8864
Min	15.8	19.3
Max	21.4	21.8



Graph 1. Hemoglobin levels in the negative control and positive control groups

Table 2 Hemoglobin Levels in the group Treatment

	Low Dose (0.36 g/ 200 g BW per day)	Medium Dose (0.54 g/ 200 g BW per day)	High Dose (0.72 g/ 200 g BW per day)
Mean	19,650	18,967	20,333
Median	19,550	19,400	20,100
SD	10,521	21,897	14,962
Min	18.0	14.8	18.8
Max	21.1	20.8	23.1



Graph 2. Hemoglobin Level in the positive control and negative control groups

Table 2 shows the average level of hemoglobin in the treatment group. Low dose group was 19.65 grams/dL with highest level was 21.1 mg/ dL. The average levels of hemoglobin in the medium dose group was 18.96 mg/ dL with highest level was 20.8 mg/ dL. Meanwhile in high dose group, has average levels of hemoglobin 20.33 mg/ dL, with the highest level was 23.1 mg/dL.

2. The difference between hemoglobin level in the treatment and control group

Table 3. Differences in Hemoglobin Levels between group treatment and negative control group

	Mean Square	df	F	p-value
Low Dose	1,103	4	0.980	0.63
Moderate Dose	3,993	4	0.499	0.77
High Dose	2,753	4	15,296	0.189

Table 3 shows There is no difference in average levels of hemoglobin between the treatment group; low dose (p-value= 0.63), medium dose (p-value=0.77), high dose (p-value=0.189)) with the negative control group.

Table 4. Differences in hemoglobin Levels between group treatment and positive control group

	Mean Square	df	F	p-value
Low Dose	5,535	5	0.0	0.00
Moderate Dose	23,973	5	0.0	0.00
High Dose	11,193	5	0.0	0.00

Table 4 shows that There is a difference in average levels of hemoglobin group treatment (p-value=0.000) with average levels of hemoglobin in the positive control group.

The statistical test results showed that there was no difference in hemoglobin levels average between the treatment group and the negative control group. Meanwhile, when compared with the positive control group, the results of statistical tests show that there are differences. Thus, the hemoglobin levels average in the group given 50% standard feed had different hemoglobin levels average when compared to the hemoglobin levels in the group only given standard feed. This is because various types of ingredients in standard feed and saluang fish powder can increase hemoglobin levels. In this study, administration of saluang fish powder and standard feed showed an increase in hemoglobin levels so that it was effective in preventing anemia in Wistar Rats. Thus, providing food with high protein, calcium and iron content can increase hemoglobin levels. The results of this study are also relevant to the finding that combining calcium and iron supplementation is reported to have the same effectiveness as single iron supplementation in reducing the prevalence of iron deficiency anemia in children⁵.

Protein functions to produce amino acids, and broken down later becomes muscle protein reduced to become the substance iron. Substance iron is very important in the production functioning hemoglobin in the delivery of oxygen from the lungs to the network body, inside transportation electrons in cells, and inside synthesis enzyme necessary iron to use production of oxygen For energy mobile(Gillespie S. 1998; Dallman PR. Et al. 1980; Storz JF. 2018). Children tend to own safe substances low in iron because use the substance iron for the growth and expansion of blood volume (Dallman PR. Et al. 1980). The peak prevalence of lack of substance iron in children happens at the time growth ends brain (6-24 months) when ability motor and cognitive start forming. Children with anemia deficiency iron risk experience disturbed psychomotor compared to kids who don't experience

anemia(Gupta PM. Et al., 2016; Consortia, 1995; Miller JL, et al. 2013). Babies anemia because deficiency iron have a risk disturbance development of mental and motoric in a long (Consortia, 1995). Habit consumption containing foods high in protein increases the rate of hemoglobin(Consortia, 1995; Harahap, LAH. 2023). Meanwhile, children aged school (2-4 years), show exists enhancement rate of hemoglobin after supplementation substance iron, which is given in a way consistent(Miranda et al 2014, Miller JL. 2023; Mohammed SH, et al. 2019; Tumer J. et al. 2023).

Apart from containing protein and substances like iron, Saluang fish powder also contains tall calcium (Lestari RM. Et al., 2016; Tumongka A et al., 2020). The study has previously shown that giving calcium give influences the positive rate of hemoglobin (Babitt JL. And Lin HY. 2012). However different with some studies that calcium lowers the rate of hemoglobin Because the effect can hinder the absorption substance iron(Diana R, et al. 2019; Pratiwi R. et al. 2018; Toaha A. et al. 2015). Calcium can hinder the absorption substance iron good given in calcium salt form or dairy products (Lonnerdal B. 2010). Calcium is a substance nutrition includes factor inhibitors or absorption inhibitors substance iron so that the amount of ferritin will also reduced which has the effect of decreasing the substance iron that will used For the synthesis of hemoglobin and replace damaged hemoglobin, so substance rate hemoglobin will be be crease (Rieny EG. Et al., 2021).

Human studies where the intake of calcium in the period a long time does not show a change in size hematology or indicators of substance status iron. Interaction between calcium and substances iron possible is incident luminal, which affects absorption of iron in the membrane apical or inhibition during transportation of iron in circulation, which shows exists role of Ferroportin (FPN) and hephaestin as exporter serosal. In research, this giving saluang fish powder and feed standard showing enhancement rate hemoglobin so that effectively prevent anemia in white rats.Thus giving food with tall combined calcium with other foods that contain substance nutrition can increase the rate of hemoglobin. The research results are also relevant with findings that supplement combined calcium and substances iron reported the same effectiveness as supplementation substance iron single in reducing the prevalence of anemia deficiency iron in children's schools⁵.

CONCLUSION

The results study showed that there was no difference in hemoglobin levels average between the treatment group and the negative control group. Meanwhile, when compared with the positive control group, the results of statistical tests show that there are differences. However, a limitation in this study is that we did not measure hemoglobin levels before administering saluang fish powder. It

is possible that the research results were influenced by other things such as body weight, diet, and calcium levels that were not measured in the study. Therefore, further research is needed by comparing the hemoglobin levels of subjects before and after given saluang fish powder and subjects given iron supplementation and/or calcium supplementation.

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CONFLICT INTEREST

The authors declare no conflict of interest

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