

The Influence of Wedge Sea Hare (*Dolabella auricularia*) Extract and Papaya Juice on Hemoglobin (Hb) and Ferritin Levels of Mice Strain (Balb / C) with Anemia

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Abstract

The aim was to see the effect of wedge sea hare extract and papaya juice, on Hemoglobin and Ferritin levels of Mice Strain (BALB / c).

Method, True Design Experiment with Control Group, has been used. Mice were divided into 5 groups, each group 5 tails, reserves 5 tails. Intervention of mice was given a solution of collaborative compounds (a mixture of sea rabbit extract with papaya juice) for 21 days, with the distribution of the following doses: Group A, Control (-), Group B, Control (+), Fe tablets 0.65 mg / kg BW, Group C, dose treatment = 100 mg / kg BW of mice / day, Group D, dose treatment = 150 mg / kg BW of mice / day, Group E, dose treatment = 250 mg / kg BW mice / day. Blood is taken before and after treatment 21 days. Data Analysis using a Paired t-test and ANOVA statistical tests.

The results, statistical tests showed a significant difference in hemoglobin levels that were significant in the three treatments, between before and after treatment, with $P < 0.05$. This means that the administration of wedge sea hare extract and papaya juice can increase Hemoglobin levels in anemia mice, where there is a tendency to increase the average hemoglobin level along with the large dose of treatment given. Besides, it is also due to the presence of primer metabolic substances in papaya juice, such as vitamin C, Beta carotene, Calcium, Potassium, and Phosphorus.

Conclusion, there is a different increase in hemoglobin levels in the three forms of treatment between before and after treatment. This means that there is a significant effect of giving collaborative compounds to Hemoglobin levels in mice.

Keywords : *Wedge sea hare, Hemoglobin, Ferritin, Wedge Sea Hare extract, Papaya Juice.*

Introduction

The Basic Health Research in 2007 showed that the national prevalence of anemia (in urban areas) was 12.8%, of which 70.1% were IDA. This number increased to 21.7% in 2013,¹⁴. This showed that the biggest proportion of anemia is IDA. IDA, in general, may not be known and felt by children or parents, but its effect causes a decrease in aerobic endurance which will have a decreased physical ability to disrupt the child's growth and development itself,²⁰.

Wedge sea hare is a food source of animal protein that is not widely known by the public. Recent research has found that wedge sea hare is very rich in amino acids such as aspartic acid, glutamate, and glycine, moreover, the amino acid arginine which is an essential amino acid for growing children,^{8,17}. Providing food sources of vitamin C and β -Carotene greatly influences the mechanism of action of Fe in forming hemoglobin,²¹.

Hb is an element that contains Fe Ferro (Fe 2+) as the main element of Hb. This Hb is present in erythrocytes, where each Hb is bound to one protein (globin) and four molecules of heme. Hb levels are often used as

indicators of anemia,⁶. Ferritin is an intact form of Fe deposits. Serum ferritin is an excellent indicator of iron reserves unless there are symptoms of inflammation and malignancy,². The objective of this research was knowing the effect of giving wedge sea hare extract, papaya juice, on Hb and Ferritin levels in Mice Strains (BALB / c) with anemia.

Materials and Method

Pre-clinical study research design, True Experiments with Control Group Design. Mice divided into 5 groups, each group of 5 animals, reserve 5 animals. The total number of samples was 30. The following groups of procedures form the treatment:

Group A = Control (-) given aquades

Group B = Control (+), Fe tablets 0.65 mg / kg BW / day,¹⁵

Group C = Treatment I, Collaborative fluid of wedge sea hares, papaya juice, dose = 100 mg / kg BW of mice / head / day = 0.75 mg / day

Group D = Treatment II Dose = 150 mg / kg BW = 2.25 mg / day

Group E = Treatment III Dose = 250 mg / kg BW / mice / day = 3.25 mg / day,

Experimental Animals (Mice strain BALB / c):

Mice weighing 25-35 grams/head, aged 8-12 weeks, healthy and free of pathogens, obtained from mice experimental animal farmers in the city of Kendari. Implementation of this research trial as a basis using the “Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicines” according to WHO standards.

Adaptation of Experimental Animals

Mice before being treated were acclimatized for 1 week. This process was carried out indoors with sufficient air circulation, the room temperature was maintained at standard conditions, ie 28 ± 2 °C, and humidity of 50 ± 10 % with a room lamp that has a cycle of 12 hours on and 12 hours off.

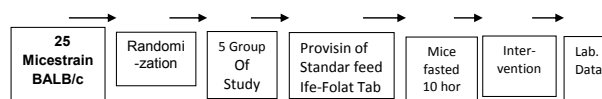
Sample size

The sample size was calculated using a modification of the Federer formula: 45 each test group consisted of

6 mice,¹¹.

Federer’s formula:	3n	≥ 18
(t-1) (n-1)	≥ 15	n
(4-1)(n-1)	≥ 15	≥ 6
3 (n-1)	≥ 15	Information :
3n-3	≥ 15	t = number of test groups
		n = sample size per group

The ideal sample size according to the Federer formula above was 6 or more mice. Thus, the total number of mice in all 5 test groups was 25.



Research design: *The pretest and posttest control group design*

The Making of wedge sea hare extract and papaya Juice

1. The making process of wedge sea hare extract

Wedge sea hare is gastropods living in shallow areas where there are algae. Wedge sea hares were taken from the coast of Toronipa, Soropia District, Konawe Regency. Wedge sea extract before being made, first rinsed 5-7 times to reduce salt levels, then dried without direct sunlight.

2. Making Papaya Juice

Papaya was taken from the Mandonga Mall market, Kendari City. Both papaya juice and wedge sea hare extracts were made in the Biopharmaca laboratory, Faculty of Pharmacy, Haluoleo University, Kendari. Thickening papaya was washed, peeled, cut into 10 x 7 cm sizes and blended until crushed, then filtered.

3. Making Combined Extracts (Collaborative Fluids)

After wedge sea hare and papaya extract were obtained, then preparations were made. The extract was then made into 4 comparisons between wedge sea hare and papaya juice, as followed :

1. 1.5 grams: 40 grams

- 2. 2.0 grams: 30 grams
- 3. 2.5 grams: 20 grams
- 4.3.0 grams: 10 grams.

Then each of these comparisons was dissolved in Na-CMC until the volume became 100 mL.

Intervention Implementation (Provision of wedge sea hare extract and papaya juice)

1. Pathological Treatment of Anemia

The pathological treatment was through the administration of NaNO₂ with a dose used in each mice that is 3.75 mg dissolved in 1 ml of distilled water. The dose given was 0.3 ml/mice/ day,²⁰. This was done for all groups of mice.

2. Treatment (wedge sea hare extract and papaya juice)

Feeding mice must be careful because they must meet their nutritional needs and fluids. The need for food for mice ranges from 2-4 grams/day of dry matter or approximately 20% of body weight, while the water needs are only 3 ml/day. Collaborative fluids in this study were given orally using a gavage needle through a sonde⁵.

3. Initial and final blood sampling/examination:

Taking too much blood in small animals, causing hypovolemic shock, stress and even death. Blood sampling that was not according to the rules, caused anemia in experimental animals.

Results

a. Homogeneity Test

Table 1: Description of Homogeneity Test from the results of initial blood tests of mice (before treatment).

Variables	Number of samples	Mean Hb level (mg / dl)	Standard Deviation
Control (+)	5	5.88	3,7
Control (-)	5	3.90	0.88
Treatment-1	5	4.70	0.15
Treatment-2	5	4.60	0.34
Treatment-3	5	4.80	2.02

P-Value = 0,421

ANOVA statistical test showed a significance level $\alpha > 0.05$, H₀ was accepted, there was no difference in mean Hb levels between groups.

b. Hemoglobin levels before and after the intervention

Table 2: Mean Hb levels before and after treatment

Group	Hb Level	
	Before	After
Control (-)	7,42 + 3,39	8,30 + 2,22
Control (+)	4,00 + 0,83	11,76 + 2,67
Treatment-1	5,90 + 3,16	13,10 + 1,45
Treatment -2	6,00 + 3,30	13,06 + 1,39
Treatment -3	6,22 + 5,02	14,40 + 1,68

Table 2, there was a mean increase in Hb levels before and after treatment. In the treatment group, both treatments one, two and three increased Hb levels, compared with negative controls (P <0.05). The mean increased in the three treatments was higher than the increase in positive controls (given iron folate capsules).

Table 3: Mean difference in hemoglobin levels before and after treatment

Group	Mean difference in Hb level	
	Difference	Standard Deviation
Control (-)	0,88	+ 3,25
Control (+)	7,76	+ 1,37
Treatment-1	7,20	+ 1,39
Treatment -2	7,06	+ 1,49
Treatment -3	8,18	+ 1,68

Table 3 showed the difference in Hb levels before and after treatment, where treatment groups one and treatment two had a difference in Hb levels that almost resembled positive control (iron-folat etablets) of 7.76 gr/dL, while in treatment three, instead, it exceeds positive control, which is 8.18 gr /dL.

4. Ferritin Levels

The results of the laboratory examination of Ferritin levels in this study could not be displayed as Hb levels, because the results of the examination showed un iformity among all groups, namely with a value < 0.500 µg/L.

Inferential Analysis

Paired t-test

Table 4: Paired t-test statistical test results:

Group	Mean	t – test	Sig (2-tailed)
Control (-)	-4,02	-1,74	0,155
Control (+)	-7,76	-6,45	0,003
Treatment-1	-7,20	-3,65	0,022
Treatment-2	-7,66	-12,38	0,000
Treatment-3	-8,66	-7,78	0,001

Table 4 showed the mean difference between the treatment group and the control group, both in treatments one, two and three ($P < 0.05$).

Discussion

Wedge sea hares are gastropods that are incorporated into the genus *Dolabella*, living in shallow areas where there are algae. Stated that wedgesea hares favor *Cladophora* green algae, *Cymodocearotunseagrass* data, and *Sargassumcristaefolium* brown algae species,²². The characteristics of this animal have a pair of tentacles found in the dorsal part. The ventral part of the wedge sea hare has a shell that covers the purple ink glands. Purple ink glands in wedge sea hares are used to protect against danger.

The compound in Rabbit Sea Extract

Phytochemical examination results prove that rabbit sea hare extract in this study contained several secondary metabolic active compounds, such as Saponin Flavonoids and Tannins. Research by Uffelle S.A. reported that active phytochemical compounds, namely flavonoids and tannins were very involved in hematological processes,²⁰.

Compounds in Papaya juice

In the Indonesian Food Composition Table-(2018), fresh papaya contains several primary metabolic nutrients such as vitamin C, Beta carotene, Thiamin, and the minerals Ferum, Calcium, and Potassium. A study explained that Fe absorption is strongly influenced by things such as the level of Fe deficiency, the level of body Fe availability, gastric acidity, the availability of vitamin C, the presence of Phosphate and Fitat,¹⁹. Hoffbrand & Moss explained that for the formation of hemoglobin, iron is transported by transferrin proteins to erythroblasts in the bone marrow, bound to globin proteins, assisted by vitamin B6 which is a coenzyme and vitamin C which plays a role in reducing ferric iron (Fe^{3+}) to ferrous (Fe^{2+}) in the small intestine,⁷. This reduction process causes iron to be easily absorbed for hemoglobin synthesis,¹⁵.

The collaborative compound of hemoglobin formation

This was likely the effect of the collaboration compound in three doses which can increase the Hb level, which exceeds the effect of the iron folate capsule, ie at a dose of three (250 mg/kg BW). The protein of wedge sea hare did not differ much in the research of Abdullah et al, which stated that the protein content of *Anadara antiquate* is 12.89%,¹. The protein content of wedge sea hare is greater than *Pleuroploca trapezium* 10.348%,⁴. The high protein content of wedge sea hare showed that wedge sea hare can be used as an alternative food source of beneficial animal protein.

Hemoglobin level difference

Table 2 showed that there was an increase in the average Hb level in mice in the treatment group, both one, two and three treatments, especially when compared to negative controls ($P < 0.05$). The mean increase in the three treatments was higher than the increase in positive controls (given iron folate). This proved that the treatment of wedge sea hare extract and papaya juice (collaboration fluid) influences the formation of Hb levels. Purwaningsih S reported that these wedge sea hare, besides containing several nutrients as primary metabolic sources such as amino acids, fatty acids, and minerals, also contained many secondary metabolic substances such as Flavonoids, Saponins, and Tannins, through the results of phytochemical tests,¹¹.

Foods that contain iron and protein are needed for the formation of hemoglobin, including consuming food sources of animal protein from the sea such as fish, shellfish and wedge sea hare. Consumption of wedge sea hare is highly recommended because in addition to being a source of animal protein is also a source of minerals such as Fe, Cu, and Zn. Purwaningsih S stated that wedge sea hares contained 12.49 + 0.34% protein, mineral 23.02 + 3.23%, fat 0.87 + 0.55%, where the amino acid value of wedgesea hare is very good from other sea animals,^{13,19}

Results of statistical tests Table (5-4) in which there were significant differences in mean Hb levels in the three treatments, $P < 0.05$. This means that the administration of collaborative fluids can increase hemoglobin levels in anemic mice, there is a tendency to increase the mean hemoglobin level along with the large dose of treatment given. The formation of Hb will run smoothly if a sufficient number of triggers is available, the absorption rate of Fe,³. The availability of Fe in the body will greatly affect the formation rate of Hb and Erythrocytes. Hb is a protein with a molecular weight of 64,450 is a red pigment, which functions to carry globular oxygen, where each molecule contains 5% heme-containing iron and 95% globular polypeptides. This pigment is a chromogen which has 4 metal pyrrole groups¹⁶.

Conclusion

Giving several doses (100 mg / kg body weight, 150 mg / kg body weight, and 250 mg / kg body weight) of wedge sea hare extract on hemoglobin levels in mice (BALB / c) that have anemia showed an increase in the mean level of hemoglobin along with the magnitude the dose given. This is because the collaboration of many secondary metabolic phytochemical compounds from wedge sea hares that work together with the primary metabolic compounds of papaya extract can synthesize the formation of heme which can stimulate hemoglobin levels.

Ethical Clearance- Taken from faculty ethical committee

Source of Funding- Self

Conflict of Interest –Nil

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