

The administration of sea hare gonad and moringa leaf formula increases body weight hemoglobin in female Wistar rats

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Abstract

Sea hare gonad and moringa leaf contain essential nutrients for growth and tissue formation and have physiological effects on the body. The research was to study the effects of sea hare gonad

flour and moringa leaf flour formula on body weight and Hemoglobin (Hb) levels in female Wistar anemic rats. This research was conducted in a laboratory using a randomized control pretest-posttest design. The study sample consisted of 30 female Wistar rats (*Rattus norvegicus*) aged 5-7 months and weighing 100-150 g, all of which were anemic. The rats were divided into five treatment groups: K+ received 1.1 mg/week of iron supplementation, K- received no treatment, F1 received 3 g of sea hare gonad formula, F2 received 3 g of sea hare gonad formula and 1 g of moringa leaf flour, and F3 received 3 g of sea hare gonad formula and 2 g of moringa leaf flour. The intervention lasted for 24 days, and measurements of body weight and Hb levels were taken before and after the intervention. The change in body weight before and after the intervention in each group was as follows: K- = 51.5 ± 17.1 g, K+ = 41.2 ± 2.6 g, F1 = 14.3 ± 5.7 g, F2 = 30.4 ± 8.6 g, and F3 = 55.1 ± 16.2 g. There was a significant difference in the increase in body weight among the groups ($p=0.002$). Hb levels also increased after the intervention, with changes as follows: K- = 2.56 ± 0.95 g, K+ = 9.10 ± 1.87 g, F1 = 7.10 ± 1.2 g, F2 = 8.68 ± 0.22 g, and F3 = 8.98 ± 2.7 g. There was a significant difference in the increase in Hb levels among the intervention groups ($p=0.000$). The administration of sea hare gonad formula and moringa leaf flour can increase body weight and Hb levels in female anemic rats. This finding suggests the potential use of these ingredients as beneficial nutritional supplements to improve nutritional status in individuals with anemia.

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Key words: gonad, moringa leaf, body weight, hemoglobin.

Contributions: WR, conceptualization, data curation, formal analysis, methodology, validation, visualization, writing – original draft, review & editing; ND, conceptualization, investigation, methodology, validation, and writing – original draft, review & editing; SW, conceptualization, methodology, formal analysis, validation, and writing; HR, methodology, visualization, writing – review & editing; SR, resources, investigation, and writing – review & editing. All the authors have read and approved the final version of the manuscript and agreed to be held accountable for all aspects of the work.

Conflict of interest: the authors declare no potential conflict of interest.

Funding: none.

Ethics approval and consent to participate: the research has received ethical approval from the Health Research Ethics Commission, Faculty of Medicine, Hasanuddin University, based on ethical certificate 383/UN 4.6.4.5.31/PP36/2023. During the research, the researcher pays attention to the ethical principles of respect for animal rights, beneficence and non-maleficence.

Patient consent for publication: this research uses non-human subjects.

Availability of data and materials: all data generated or analyzed during this study are included in this published article.

Acknowledgments: we would like to thank Poltekkes Kemenkes Kendari for preparing this research fund and UHO FK Animal Laboratory partners, FK-UNHAS Ethics Commission for their valuable insights and contributions to this study.

Received: 12 October 2023.

Accepted: 26 June 2024.

Early access: 8 August 2024.

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Healthcare in Low-resource Settings 2024; 12:11952

doi:10.4081/hs.2024.11952

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Introduction

Low Hemoglobin (Hb) levels are a significant global health issue, particularly in developing countries.¹ Anemia, often caused by iron deficiency, can impair physiological functions and reduce quality of life. Various efforts have been made to address this issue, one of which is the use of natural supplements.² Recent research highlights the potential of natural ingredients such as sea urchin gonad (*Deadema setosum*)³ and moringa leaves (*Moringa oleifera*) in increasing Hb levels due to their rich nutritional content.⁴ Sea urchin gonads are known to contain proteins, vitamins, and minerals essential for blood health. Recent studies report that sea urchin gonads have a positive effect on Hb levels in test animals.⁵ On the other hand, moringa leaves are a high source of iron and other vitamins and minerals. Previous studies have shown that moringa leaves effectively increase Hb levels in anemic rats.⁶ The primary issue in this study is how to increase Hb levels in anemic female Wistar rats. Nutritional deficiencies like iron deficiency can impair physiological functions. A common solution that has been widely applied is the administration of synthetic iron supplements. However, the use of these supplements often comes with side effects such as gastrointestinal irritation.

As an alternative, the use of natural supplements rich in iron and other nutrients is the focus of this study. A flour formula combining sea urchin gonads and moringa leaves is expected to be an effective supplement to increase Hb levels without significant side effects. A study by Setiawan *et al.* (2019) showed that moringa leaf extract could increase Hb levels in anemic rats.⁷

Sea urchin gonads, although less commonly used in traditional medicine, are known to have high nutritional content, including proteins, omega-3 fatty acids, and other essential minerals that support blood health. Consumption of sea urchin gonads can increase Hb levels in experimental rats.⁸ Several studies have proven that moringa leaves can increase Hb levels in anemic subjects.⁹⁻¹¹ However, few studies have explored the effects of combining moringa leaves with sea urchin gonads. Existing studies focus more on the use of each ingredient separately. Therefore, there is a gap in the literature examining the effectiveness of these combined ingredients as a Hb-boosting supplement.

Female Wistar rats were chosen for this study due to their well-documented physiology and hormonal cycles, which are relevant to understanding anemia, particularly in females. Their consistent response to experimental conditions and manageable size make them ideal for controlled laboratory studies. The scientific objective is to assess the efficacy of a sea urchin gonad and moringa leaf flour formula in raising Hb levels, which is crucial for predicting its potential in human treatments. This relevance to human biology lies in the similarity of iron metabolism and erythropoiesis processes between Wistar rats and humans, offering insights before human trials.

This study aims to evaluate the effectiveness of a flour formula combining sea urchin gonads and moringa leaves in increasing Hb levels in female Wistar rats. The novelty of this research lies in the use of the combination of these two natural ingredients, which has not been extensively explored in previous scientific literature. The results of this study are expected to provide an effective natural supplement alternative to combat anemia with minimal or no side effects. The scope of the research includes a comparative analysis between the control group and the groups given the flour formula, with Hb levels as the main parameter.

Materials and Methods

Type and research design

This research is an experimental study using female *Rattus norvegicus* rats with a pretest-posttest randomized control design. The research groups were randomly divided into five groups using simple random sampling. The treatment and intervention for each group were carried out for 24 days. One day before and after the intervention (day 25), body weight and Hb levels were measured. Hb levels were also measured on day 12.

Sample

The research sample used female *Rattus norvegicus* rats aged 5-7 months, weighing 100-150 g, and healthy (not disabled and exhibiting normal activity). The sample size was determined using Cohen's table, considering research ethics and effect size (0.8), resulting in a minimum sample size of 5 rats/group. A total of 25 rats were divided into 5 treatment groups, with each group consisting of 5 rats:

Positive control (K+) received Fe equivalent to 1.1 mg Fe/week.

Negative control (K-) did not receive any nutritional support other than normal feed.

Formula 1 (F1) received 3 g of sea urchin gonad flour.

Formula 2 (F2) received 3 g of sea urchin gonad flour + 1 g of moringa leaf flour.

Formula 3 (F3) received 3 g of sea urchin gonad flour + 2 g of moringa leaf flour.

Sample selection for each group was done using simple random sampling by numbering the rats from 1-25, writing the numbers on paper strips, rolling them up, placing them in a container, and mixing them several times. Five rolled papers were drawn at each stage, and the sequence of numbers drawn represented the sample group. This process was repeated five times, resulting in 25 rats divided into 5 groups.

Sample treatment

Pre-treatment

Before treatment, all samples (Wistar rats) were weighed and adapted for one week. The adaptation process used standard cages, food, and drink. The cages used were conventional plastic cages with wire covers, measuring 100x80x20 cm. Five Wistar rats were allocated to each cage. The cages were cleaned daily before feeding. Room temperature and humidity were monitored and maintained between 20-25°C and 40-60%, respectively. The room was equipped with ventilation and lighting to stimulate the day-night cycle. Pellet feed (Ad2 brand) was given twice daily (morning and evening), 15 g/day. Water was provided *ad libitum* using 70-80 mL plastic bottles, cleaned daily. The laboratory was specially designed for animal research, with minimal external activity, and managed by two lab technicians and a supervisor. Interaction with humans and other animals was minimized to avoid stress on the samples. Treatments considered stress and pain from procedures like weighing and blood sampling, performed by skilled and experienced technicians. Sample health was continuously monitored.

Initial treatment (pre-test)

Initial treatment was done to induce anemia in the rats using aluminum sulfate for 7 days. On day 8, initial weight and Hb levels were measured. The rats were kept in the same cages as during adaptation.

Intervention implementation

During the intervention process, the rats were placed in the same cage as in the previous process. The intervention was carried out for 24 (twenty-four) days through feeding according to the group. Feeding was done using a sonde 4 times a day, namely the positive control group (K+) was given Fe equivalent to 1.1 mg Fe/week, the negative control group (K-) received no nutritional support other than normal feed, formula group 1 (F1) was given 3 g of gonad flour, formula group (F2) was given 3 g of gonad flour + 1 g of moringa flour, and formula group (F3) was treated with 3 g of gonad flour + 2 g of moringa flour. On day 12, blood was taken and on day 25, body weight was measured and blood was taken for Hb examination.

Materials and Methods

The formula was made from sea urchin gonad flour obtained from the coastal area of Soropia, Southeast Sulawesi. The formula was made using 1080 g of gonad flour and 120 g of moringa leaf flour for F1, 240 g for F2, and 360 g for F3. Fresh gonads were removed from the shell, steamed for 5 minutes, dried using an electric oven at <100°C for 5 hours, ground with a warning blender,

and sieved with a 100 mesh. Moringa leaves were cleaned, air-dried for <5 hours, dried using an electric oven, ground with a warning blender, and sieved with a 100 mesh. The nutritional content of the intervention materials was as follows: Formula F1 2.87% moisture, 6.2% ash, 50.2% protein, 47% carbohydrates, 26.8% fat, 17.1 mg/100 g iron, and 0.73 mg/100 g zinc; Formula F2 4.1% moisture, 6.7% ash, 50.9% protein, 43.6% carbohydrates, 22% fat, 17.5 mg/100 g iron, and 0.6 mg/100 g zinc; Formula F3 5.6% moisture, 7.4% ash, 48.3% protein, 49.3% carbohydrates, 20.3% fat, 19 mg/100 g iron, and 0.53 mg/100 g zinc.

Data collection

Data collected in this study included Hb levels and body weight measured three times: at the beginning, middle, and end of the intervention. Hb levels were measured using the cyanmethemoglobin (Drabkin's) method. Blood samples for Hb measurement were taken from the rats' ears and analyzed in the animal research laboratory of the Faculty of Medicine, Haluoleo University. Body weight was measured using a digital scale with a 0.1-gram scale.

The procedure for checking Hb levels using the Cyanmethemoglobin (Drabkin's) method is as follows:^{7,12} i) ensure that the Drabkin solution is ready and in good condition - Drabkin's solution is generally clear in color; ii) draw blood from the patient using an Ethylenediaminetetraacetic Acid (EDTA) tube to prevent coagulation; iii) pipette 20 μ L (0.02 mL) of blood into a test tube containing 5 mL of Drabkin's solution - ensure complete mixing by gently swirling the tube; iv) leave the blood sample and Drabkin's solution mixture for 5-10 minutes at room temperature - during this time, the Hb in the blood will react with the components in the Drabkin solution to form stable cyanmethemoglobin; v) switch on the spectrophotometer and set the wavelength at 540 nm, calibrate the spectrophotometer using a blank containing Drabkin's solution without a blood sample, measure the absorbance of the sample solution that has been mixed with Drabkin's solution; vi) calculate the Hb concentration using the formula: Haemoglobin Concentration (g/dL) = (Sample Absorbance : Standard Absorbance) \times Standard Concentration (g/dL).

Table 1. Body weight (g) of rats between, before, and after the intervention.

Group	n	Before (mean \pm SD)	After (mean \pm SD)	Sig*	Weight change (mean \pm SD)
K+	4	117.4 \pm 3.7	168.9 \pm 5.6	0.002	-41.9 \pm 15.2
K-	4	148.4 \pm 5.8	106.4 \pm 11.2	0.009	51.5 \pm 17.1
F1	4	125.6 \pm 4.5	139.9 \pm 8.1	0.012	14.3 \pm 5.7
F2	4	129.1 \pm 2.2	159.4 \pm 9.9	0.016	30.4 \pm 8.6
F3	4	141.4 \pm 3.9	196.5 \pm 19.3	0.006	55.1 \pm 16.2
Sig**	0	0		0.002	

*Two-sample paired *t*-test; **One-way ANOVA test; SD, Standard Deviation.

Table 2. Hemoglobin level (g/dL) of rats between, before, and after intervention.

Group	n	Before (mean \pm SD)	After (mean \pm SD)	Sig*	Weight change (mean \pm SD)
K-	4	6.4 \pm 1.4	3.8 \pm 0.54	0.004	-2.56 \pm 0.95
K+	4	5.6 \pm 1.1	14.7 \pm 0.78	0.002	9.10 \pm 1.87
F1	4	6.5 \pm 1.0	13.26 \pm 0.28	0.000	7.10 \pm 1.2
F2	4	5.5 \pm 0.6	14.17 \pm 0.51	0.000	8.68 \pm 0.22
F3	4	5.9 \pm 1.0	14.85 \pm 2.2	0.007	8.98 \pm 2.7
Sig**	0.628	0.000		0.000	

*Two-sample paired *t*-test; **One-way ANOVA test; SD, Standard Deviation.

Data processing and analysis

Measurement and examination data were inputted and processed through the Statistical Package for Social Sciences (SPSS) for Window. Data on body weight and Hb levels are presented as numerical data so they are presented using mean and standard deviation values. Data analysis was performed using statistical tests, namely the two-sample paired *t*-test to assess the effect of the intervention on body weight and Hb levels between before and after the intervention in each group. One-way ANOVA test was used to analyze differences in changes in baseline, final, and inter-group Hb levels, as well as baseline weight, final weight, and inter-group changes. Data analysis used 5% alpha ($\alpha=0.05$).

Ethical recommendations

All stages of this research will be carried out after obtaining ethical recommendations from the Health Research Ethics Commission (KEPK) Faculty of Medicine, Hasanuddin University number: 383/UN 4.6.4.5.31/PP36/2023.

Results

Body weight

Table 1 shows a significant increase in body weight of rats between before and after the intervention, especially in each group, namely K+ ($p=0.002$), K- ($p=0.009$), F1 ($p=0.012$), F2 ($p=0.016$) and F3 ($p=0.006$). ANOVA test results showed there was a difference in the increase in body weight between groups ($p=0.000$). The highest increase in rat body weight was obtained by group F3, which was 55.1 g.

Hemoglobin level

Table 2 shows that each treatment group had relatively similar initial Hb levels ($p=0.628$). The Hb level of rats increased significantly between before and after the intervention in each treatment group, both group K- ($p=0.004$), group K+ ($p=0.002$), group F1

($p=0.000$), group F2 ($p=0.000$) and group F3 ($p=0.007$). The results of the ANOVA test showed that there were differences in the increase in body weight between groups ($p=0.002$). The highest increase in rat body weight was obtained by group F3, which was 10.0 g. Figure 1 shows that each group had relatively similar initial Hb levels. Each group Experienced an increase in Hb levels during the intervention, except for group K- which continued to experience a decrease in Hb levels during the intervention. The highest final Hb levels were obtained by group F3 (14.85 g/dL), group K+ (16.65 g/dL), group F2 (14.17 g/dL) and F1 (13.26 g/dL).

Discussion

We found that the administration of the sea urchin gonad and moringa leaf flour formula can increase the body weight of anemic rats, particularly in group F3, which received 3 g of gonad flour and 2 g of moringa leaf flour ($p=0.006$). The average weight gain in the F3 group reached 10.0 ± 2.9 g. The weight gain in the F3 group was significantly higher compared to the control group and other treatment groups ($p=0.000$).

This finding supports previous research using supplementary food products with either gonad flour, moringa leaf flour, or a combination of both. The results of this study reinforce previous findings, both those using supplementary food products with a mixture of sea urchin gonad flour or moringa leaf flour, and a combination of both. The intervention in the form of Bagea Gonad supplementary food can increase the body weight of Bajo children.¹³ Interestingly, the weight gain from the Bagea Gonad intervention group was higher compared to those consuming biscuits from the government program. Previous studies found that the intervention with moringa-enriched gonad cookies as supplementary food could increase the body weight of malnourished children. The weight gain in the gonad intervention group was higher compared to the government program biscuits and moringa biscuits intervention groups.^{14,15}

The *setosum* gonad extract can enhance immune function, as indicated by increased Immunoglobulin M (IgM) antibody production.¹⁶ This made the F3 group healthier and experienced less illness compared to other groups, leading to better growth. Gonads contain essential nutrients such as amino acids and essential fatty acids. The highest fatty acids content includes palmitic acid and omega-9.¹⁷ Amino acids and essential fatty acids play a crucial role in growth and tissue maintenance, facilitating the formation of new tissues and the maintenance of existing tissues.¹⁸ This condition resulted in higher body weight growth in the F3 group compared to other groups.

The use of moringa leaves can increase Hb levels. For instance, consuming 2100 mg/day of moringa leaf powder for 30 days increased Hb levels by 1.76 ± 0.8 g/dL in adolescents.¹⁹ Different research reports have also shown that the administration of moringa leaf extract alone increased the average Hb level by 1.19 g/dL. Similarly, the administration of a mixed formula of moringa leaf powder capsules and sweet orange juice in post-menstrual adolescents increased the average Hb level from 11.27 ± 1.14 to 12.72 ± 1.35 .²⁰ Moringa leaves contain high levels of iron and vitamin C. Each 100 g of the formula contains 17.5-19 mg of iron. Adding moringa leaves to this formula potentially increases vitamin C levels. Vitamin C consumption can enhance iron absorption and bioavailability.^{21,22} Several previous studies have shown similar results. The administration of moringa leaf extract can increase the Hb level of adolescent girls aged 17-24 years.²⁰ The results of research on high school students 12-18 years old with mild anemia in Takalar, South Sulawesi showed an increase in Hb levels of 1.1 g/dL.²³ The results of moringa leaf extract intervention are more visible in anemic targets. Yulina's 2022 study on high school adolescent girls assessed the effect of giving moringa leaf extract twice a day for 14 days on increasing the Hb levels of anemic schoolgirls. The results showed an increase in Hb levels by 12.9 g/dL. Moringa leaf intervention can help meet the nutritional needs of adolescent girls. Moringa leaves are rich in iron, necessary for Hb formation, and are also a source of vitamin C, which is essential for enhancing iron absorption in the body.^{24,25} By incorporating vitamin C, the absorption of iron in the body can be improved, thus helping to increase Hb levels.²⁶ Adding gonad flour to the moringa leaf flour formula can potentially increase iron absorption and bioavailability as reported.¹²

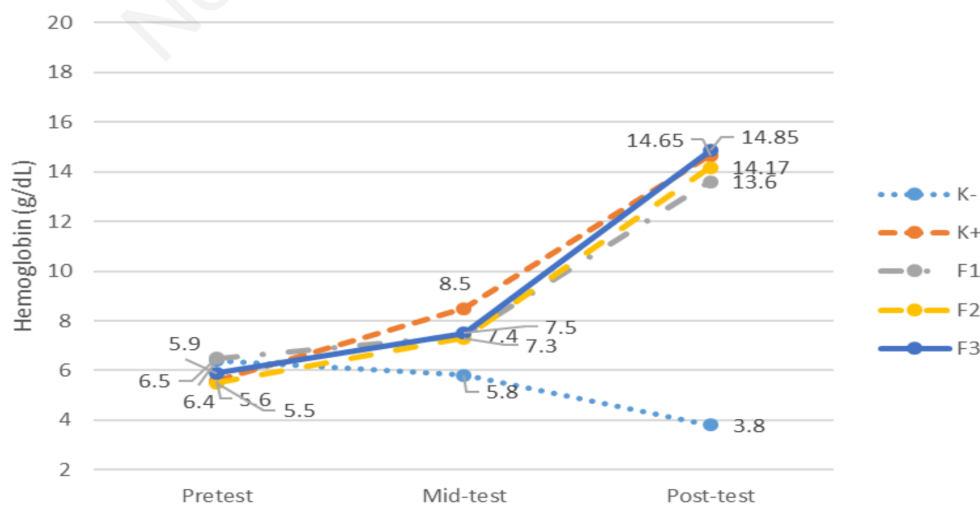


Figure 1. Mean Hemoglobin levels in rats during the intervention.

We found that the gonad and moringa leaf flour formula intervention was as effective as the positive control group (K+). This means that administering the gonad and moringa leaf formula could be an alternative for preventing anemia in adolescent girls, especially in preventing anemia. Utilizing local food sources to address nutritional problems will enhance the sustainability of nutritional programs and community self-reliance.

Although this study was conducted on rats, the results show significant potential for implementation in humans, especially in populations vulnerable to anemia and malnutrition. Supplements based on the combination of gonad and moringa leaf flour can be an effective and natural alternative to increase Hb levels and body weight in children and adults with nutritional deficiencies.

The study has several limitations. Firstly, it was conducted on a small sample size of rats, which may not fully represent the variability seen in larger populations. Secondly, the study duration was relatively short, limiting the observation of long-term effects and potential side effects of the supplement. Additionally, the study was performed on animals, and results may not directly translate to humans without further clinical trials. The specific nutritional environment and controlled conditions in the laboratory also may not accurately reflect real-world scenarios.

Conclusions

This study concludes that administering the F3 formula, a combination of sea urchin gonad (*Deadema setosum*) and moringa leaves (*Moringa oleifera*), for 24 days significantly increased body weight and Hb levels in female Wistar rats. With its rich nutritional content, this combination shows higher effectiveness compared to formulas containing only one type of ingredient. Therefore, further research on humans is recommended to confirm the health benefits of this combination and to develop widely usable supplements for addressing anemia and malnutrition.

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