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Effectiveness of Dried Juwet Cereal Bar (Syzygium cumini L.) as an Antianemic Intervention in Lead-Induced Female White Rats: A Preclinical Study

Ni Luh Putu Anggie Pramesthi Putri[1], Ni Komang Puja Pertiwi[1], Anak Agung Cahaya Dayita Nariswari[1], Shiendy Ayu Puspita[1], Ni Putu Ayu Widya Anggreni[1], Ketut Agus Adrianta[2]

[1] Bachelor of Pharmacy, Faculty of Pharmacy, Universitas Mahasaraswati Denpasar, Bali, Indonesia
[2] Departemen of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Mahasaraswati Denpasar, Bali, Indonesia

*Correspondence: agusaick@unmas.ac.id

ABSTRACT

In contemporary times, lifestyle changes and increased air pollution from vehicle emissions have impacted health, particularly through chronic lead exposure. Lead disrupts iron absorption and hemoglobin synthesis, leading to anemia—a condition marked by insufficient red blood cells or hemoglobin. Anemia affects 29.9% of women of reproductive age globally, with notably high rates in Indonesia among pregnant women and adolescent girls. Tradition al iron supplements often cause side effects, highlighting the need for alternative non-pharmacological solutions. *Syzygium cumini* L., which is rich in iron, vitamin C, and anthocyanins, offers a promising alternative. This study evaluates the effectiveness of Dried Juwet Cereal Bar in treating anemia using a quantitative experimental approach. Twenty-eight female rats were divided into four groups: negative control, positive control, normal, and treatment, receiving Dried Juwet Cereal Bar containing 5.9% juwet extract. Hemoglobin, hematocrit, and erythrocyte levels were measured using a hematologic analyzer, with statistical analyses performed using paired sample T-tests, Kruskal-Wallis tests, and Mann-Whitney tests. The juwet extract, prepared with a 96% ethanol-water mixture, retained significant bioactive compounds. Phytochemical screening revealed alkaloids, flavonoids, triterpenoids, saponins, and anthocyanins. The extract showed high antioxidant activity (IC_{50} of 215.6887 ppm) and a substantial vitamin C content (18,586.7 mg/100 g). Results indicated that Dried Juwet Cereal Bar significantly increased hemoglobin, hematocrit, and erythrocyte levels after administering dried juwet cereal bars in anemic female white rats with a p-value<0,05, suggesting their potential as an effective non-pharmacological treatment for anemia. This approach supports local agriculture and provides a nutritious, practical, and sustainable product reflecting Indonesian tropical fruit diversity.

Keywords: Anemia, Lead, Dried Juwet, Antianemic

INTRODUCTION

In the modern era, people's lifestyles have changed, including unbalanced dietary habits and increased air pollution from motor vehicles, which have led to various health issues, one of which is anemia. Exposure to heavy metals like lead (Pb) from air pollution can decrease the body's iron reserves and inhibit hemoglobin synthesis, resulting in anemia (Sinatra et al., 2020). Anemia is characterized by a deficiency in red blood cells or hemoglobin concentration, which leads to reduced hematocrit and red blood cell levels (Krisnanda, 2020; Paramastri et al., 2021).

According to WHO data from 2019, the global prevalence of anemia in women of reproductive age reached 29.9%, while in Indonesia, the prevalence of anemia among pregnant women was 48.9% based on 2018 Riskesdas data (Kementerian Kesehatan RI, 2023). This high prevalence indicates significant anemia issues among pregnant women and adolescent girls, which, if not properly managed, can persist into adulthood and increase risks such as maternal and infant mortality, premature birth, and developmental delays (Susanti, 2022).

Iron supplements are commonly used to treat anemia, but side effects such as nausea, vomiting, and constipation

often reduce patient compliance (Kementerian Kesehatan RI, 2019). Therefore, non-pharmacological alternatives, such as consuming iron-rich fruits like dried juwet (*Syzygium cumini* L.), offer a promising solution. Juwet fruit contains anthocyanins, iron, and vitamin C, which have potential anti-anemic effects by increasing hemoglobin levels and aiding in blood purification (Azkiyah et al., 2021; Hapsari & Setyawan, 2023; Hidayah et al., 2023).

METHODS

This research uses a quantitative experimental method with a pre-posttest control group design. Twenty-eight female rats were divided into four groups: negative control, positive control, normal, and treatment groups given Dried Juwet Cereal Bar containing 5.9% juwet extract. Hemoglobin, hematocrit, and erythrocyte levels were measured using a hematologic analyzer by testing blood samples taken from the orbital vein. The animal testing followed Ethical Clearance (EC) No. DP.04.02/F.XXXII.25/0694/2024. Statistical analysis employed the Paired Sample T-Test to test the differences between groups before and after the Dried Juwet Cereal Bar treatment, the Kruskal-Wallis test to examine the effect of the Dried Juwet Cereal Bar administration, followed by the Mann-Whitney test. Samples of juwet fruit (*Syzygium cumini* L.) obtained from Duren Jaya Village, East Bekasi District, Bekasi Regency, West Java Province. The plant was determined at the MIPA Laboratory Unit, IAIN Syekh Nurjati Cirebon, with determination number 46/In.08/LB.1.1/PP.009/5/2024.

Tools and Materials

The tools used in this study include an oven (Memmert), digital scale (Acis), analytical balance (Pioneer), food grinder (Ossel), blender (Philips), UV-Vis spectrophotometer, rotary evaporator (Buchi), hotplate (Thermo Scientific), hematologic analyzer (Ayto), furnace (Thermo Scientific), centrifuge, desiccator (Shuniu), AAS (Shimadzu), water bath, Buchner funnel and vacuum. The materials used include 96% ethanol, distilled water, chloroform, lead(II) acetate, 1cc and 3cc syringes, 3 mL EDTA tubes, juwet fruit *(Syzygium cumini* L.), capillary tubes, female white rats, rat feed, filter paper, magnesium powder, concentrated HCl, 0.1 N HCl, NaOH, acetic anhydride, concentrated H_2SO_4, $FeCl_3$, KCl-HCl buffer (1M, pH 1), NaOAc buffer (1M, pH 4.5), methanol, DPPH, ascorbic acid, alcohol, Kjedhal tablets (0.5g), boric acid, lead acetate, filter paper, honey, rice crispy, milk powder, butter, sugar, and vanilla extract.

Preparation Of Dried Juwet And Juwet Fruit Extract

A total of 2 kg of juwet fruit (*Syzygium cumini* L.) is weighed and dried using an oven at 50 °C for a specified duration (Parfiyanti et al., 2016). This method is chosen to facilitate the control of the heat used, ensuring that the active compounds in the juwet fruit are preserved. Next, the dried juwet fruit is ground using a blender and extracted through maceration for 3 x 24 hours with a solvent mixture of 96% ethanol and water in a ratio of 1:2:2. The solvent is removed using a rotary evaporator and further evaporated using a water bath to obtain a thick juwet extract (Putri et al., 2024).

The extract standardization is carried out using two parameters: specific and non-specific. The specific parameters include organoleptic properties (form, smell, taste, and color), water-soluble extract, ethanol-soluble extract, and phytochemical content. The non-specific parameters include drying loss and moisture content, ash content, and acid-insoluble ash content (Mangalu et al., 2022).

Formulation and Preparation of Dried Juwet Cereal Bar

Melt the butter in a small saucepan over low heat. Add honey and granulated sugar, heating until the mixture is completely melted (mixture A). In a large bowl, combine rice crispy, powdered milk, and dried juwet thoroughly (mixture B). Quickly pour mixture A into mixture B and incorporate the juwet extract, which has been mixed with vanilla extract, stirring until well combined. Transfer the mixture into a mold and press down with a spatula to compact. Refrigerate until the cereal bars are set and firm.

Table 1
Dried Juwet Cereal Bar Formulation

Ingridients	Amount % (w/v)
Juwet Extract	5,9
Dried Juwet	9,09
Butter	9,09
Sugar	27,2
Milk Powder	4,54
Natural Honey	18,18
Vanilla Extract	1 ml
Rice crispy	Ad 22 grams

Effectiveness Analysis of Dried Juwet Cereal Bar

The effectiveness test for anti-anemia was conducted following Ethical Clearance (EC) No. DP.04.02/F.XXXII.25/0694/2024. Female rats were divided into four groups, each consisting of 7 rats: negative control (I), positive control (II), treatment (III), and normal (IV). The rats were acclimated for 7 days. Groups I, II, and III were induced with a lead acetate solution at a dose of 15 mg/200 g body weight orally for 21 days. After 21 days, blood samples were collected from each rat (pretest). Following the pretest, the rats were treated as follows: Group I received a cereal bar solution with no active ingredients; Group II received an iron supplement syrup; Group III received a Dried Juwet Cereal Bar solution; and Group IV received a normal diet. Each group was treated for 2 weeks before undergoing a posttest evaluation (Ifada et al., 2018).

Hemoglobin, Hematocrit, and Erythrocyte Level Analysis in Female Rats

For both the pretest and posttest, blood was collected from the orbital vein and stored in 1 mL EDTA tubes. The samples were placed on a blood roller mixer and subsequently analyzed using a hematology analyzer (Ifada et al., 2018).

Total Anthocyanin Analysis in Juwet Fruit Extract

Total anthocyanin content was determined using the differential pH spectrophotometry method, which measures absorbance changes between pH 1.0 and 4.5 (Zahroh & Agustini, 2021). A 0,02904 g concentrated extract was dissolved in 5 mL of HCl and methanol (1:9) in a centrifuge tube. One milliliter of the extract solution was added to a cuvette, and absorbance was measured at 510 nm and 700 nm using a UV-Vis spectrophotometer after adding buffer solutions at pH 1.0 and 4.5.

Vitamin C Content Analysis in Juwet Extract and Dried Juwet Cereal Bar

Vitamin C content was analyzed using UV-Vis spectrophotometry (Abriyani et al., 2023). Dissolve 0.1 g of ascorbic acid in 100 mL of distilled water to prepare a 100 ppm solution. From this, prepare additional solutions at concentrations of 800 ppm, 400 ppm, 200 ppm, 100 ppm, 80 ppm, 60 ppm, 40 ppm, 20 ppm, and 10 ppm. Pipette 0.3 mL of the standard solution, add 3 mL of reagent, vortex, and incubate for 90 minutes, then cool and measure absorbance at 695 nm. For sample analysis, weigh 1 g of the sample, dilute with distilled water to 5 mL, pipette 0.3 mL of the sample solution, add 3 mL of reagent, vortex, and incubate for 90 minutes, then cool and measure absorbance at 695 nm (Sudiarta et al., 2021).

Iron Content Analysis in Dried Juwet Cereal Bar

Weigh 5–10 g of the solid sample and place it in a beaker glass. Add 10 mL of H_2SO_4 and 10 mL of HNO_3. Heat the solution slowly until it darkens, avoiding excessive foaming. Add 1-2 mL of HNO_3 and continue heating until the solution darkens further. Continue adding HNO_3 and heating for 5–10 minutes until the solution is no longer dark (all substances are oxidized), then cool. Add 10 mL of distilled water (the solution will turn light yellow if it contains Fe), heat until boiling, then cool. After digestion, analyze the sample using atomic absorption spectroscopy (AAS) to determine the iron content (Faqihuddin & Ubaydillah, 2021).

Antioxidant Activity Analysis Using the DPPH Method in Juwet Extract and Dried Juwet Cereal Bar

A 0.1 g sample of Dried Juwet Cereal Bar was dissolved in 5 mL of methanol and homogenized by centrifugation at 3000 rpm for 15 minutes. The supernatant was filtered, and the filtrate was diluted to a final volume of 5 mL (0.1 g / 5 mL = 20 mg/mL). From this solution, 0.5 mL was pipetted into a reaction tube. To each reaction tube, 3.5 mL of 0.1 mM DPPH solution (0.0039 g DPPH in 100 mL methanol, 99.9%) was added. As a positive control and for comparison, vitamin C solutions with concentrations of 10, 20, 30, 40, and 50 ppm were prepared. Each concentration was pipetted into 0.5 mL and mixed with 3.5 mL of a 0.1 mM DPPH solution in reaction tubes. Both the samples and the comparison solutions were incubated at 37 °C for 30 minutes, and then absorbance was measured at 517 nm. The IC_{50} values were calculated using a regression equation.

Regression Equation: $y = ax + b$

Concentration (% weight) =

$$= \frac{\text{Concentration}(mg/mL) \text{ x Dilution Factor x } 100}{\text{Sample Concentration } (mg/mL)}$$

Concentration (% weight) = g/100g x 10000 = mg/L GAEAC

Proximat Analysis Of Dried Juwet Cereal Bar

Analysis of Moisture Content

Porcelain dishes were heated in an oven for approximately 2 hours, then placed in a desiccator to cool. The weight of the empty dish was measured until a constant weight was achieved. A sample of 2–3 g was placed in the porcelain dish, which had a known constant weight. The dish was then dried in an oven at 105°C for 4–5 hours, cooled, and placed in a desiccator for 15 minutes. Finally, the dry sample in the porcelain dish was weighed until a constant weight was obtained (weight differences between consecutive measurements were less than 0.2 mg) (Huriawati et al., 2016). The moisture content was determined using the following formula:

Moisture Content (%w) = $\frac{W1-W2}{W1} x100\%$

Description:

W1 = Weight of the sample before drying

W2 = Weight of the sample after drying

Analysis of Ash Content

Porcelain crucibles were heated in a furnace at 600 °C for approximately 2 hours, then cooled in a desiccator for 15 minutes and weighed until a constant weight was achieved. A 2-3 g sample was then placed in the crucible and incinerated in the furnace at 600 °C for 4-5 hours, then placed in a desiccator for 15 minutes. The crucible containing the ash was weighed until a constant weight was achieved (Sunartaty & Yulia, 2017). Ash content was calculated using the following formula:

Ash Weight (g)= Weight of the porcelain dish with ash – Weight of the empty porcelain dish

Ash Content (%) = $\frac{\text{Ash Weight } (g)}{\text{Sample Weight } (g)} x\ 100$

Analysis of Protein Content

The protein content was determined using the Kjeldahl method. A 0.1 g sample was placed in a digestion flask and mixed with 0.5 g Kjeldahl tablets, 5 mL of concentrated H_2SO_4, and boiling stones. The sample was then digested using a block digestor at 420 °C until the solution became clear, then cooled. The base tank of the automatic distiller was filled with a NaOH-$Na_2S_2O_3$ solution, while a 4% H_3BO_3 solution was placed in the acid tank. The clear digestion solution was then attached to the automatic distiller. A 250-mL Erlenmeyer flask was prepared to collect the distillate and was also attached to the distiller. The distillate collected in the Erlenmeyer flask was titrated with a 0.1 N HCl solution. Before titration, 3 drops of phenolphthalein indicator were added. The endpoint of the titration was indicated by a color change to pink in the Erlenmeyer flask. The volume of 0.1 N HCl used to reach the endpoint was recorded. For the blank test, 1 mL of distilled water was used as a substitute for the initial test sample (Rosaini et al., 2015). Protein content was calculated using the following formula:

$$\%N=\frac{\text{Vol HCL titrated (sampel}-\text{blank) x Cons HCl (0,1)x14,007}}{Sample\ Weight\ (mg)}x100\%$$

Protein Content = % N x Conversion Factor (6,25)

Analysis of Fat Content

A sample weighing 2–5 grams, from which water has been removed, is wrapped in filter paper. The filter paper containing the sample is then wrapped again with another sheet of filter paper and placed in the extraction tube, while hexane solvent is added to the boiling flask up to one-third of its volume. After all parts of the Soxhlet apparatus (i.e., the boiling flask, extraction tube, and condenser tube) are correctly assembled, the fat extraction process is run for 4 hours. Subsequently, the fat in the boiling flask is poured into a porcelain dish and dried in an oven at 80°C for 12 hours. The next step involves placing the porcelain dish containing the sample fat in a desiccator for 15 minutes and weighing it until its weight is constant (Pargiyanti, 2019). The fat content in the sample is determined using the following calculation:

$$\%\ \text{Fat} = \frac{A-B}{C}\ x\ 100\%$$

Description:

A = Weight of flask with sample fat from the desiccator

B = Weight of the empty flask

C = Weight of the sample (g)

Analysis of Carbohydrate Content

The total carbohydrate content is determined using the by-difference method. The carbohydrate content is calculated as 100% minus the sum of the moisture, ash, protein, and fat content (Kole et al., 2020).

RESULT AND DISCUSSION

Preparation of Juwet Extract

The extraction of juwet extract utilized the maceration method due to its simple processing and equipment, avoiding heating to prevent degradation of active substances from temperature effects and heat-sensitive compounds. Maceration is advantageous for isolating natural compounds and marine materials as soaking samples breaks down cell walls due to pressure differences inside and outside cells, allowing secondary metabolites in the cytoplasm to dissolve in organic solvents, ensuring complete compound extraction (Makalunsenge et al., 2022; Wendersteyt et al., 2021). Maceration was conducted for 3x24 hours with two re-macerations or solvent replacements to thoroughly extract compounds from the sample. Ethanol 96% mixed with water served as the solvent of choice due to its universal, polar nature, ease of access, applicability in various extraction methods, and safety for extracts intended for medicinal and food purposes. Ethanol 96% was selected for its selectivity, non-toxicity, excellent absorption, and high search capability, thereby searching for non-polar, semi-polar, and polar compounds (Fauziyah et al., 2022; Wendersteyt et al., 2021).

Table 2

Result of Standardization Analysis of Spesific and Non-Specific Parameters

Specific Parameters			
Organoleptic			
Color	Aroma	Taste	Form
Dark Purple	Distinctive aroma of juwet fruit	Sour	Thick Extract
Water Soluble Extract			
Replication 1	Replication 2	Replication 3	Average
108,69%	31,91%	57,45%	66,016% ≥ 24%; meets criteria
Ethanol Soluble Extract			
17,25%	47,69%	49%	37,98% ≥ 11%; meets criteria
Phytochemical Screening			
Alkaloids	Positive	Triterpenoids	Positive
Flavonoids	Positive	Anthocyanins	Positive
Saponins	Positive		
Non-Spesifik Parameters			
Water Content/Drying Loss			
Replication 1	Replication 2	Replication 3	Average
1,20%	1,42%	2,61%	1,743% < 10%; meets criteria
Ash Content			
3,94%	3,54%	3,64%	3,706% < 16,6%; meets criteria
Acis Insoluble Ash Content			
3,32%	2,93%	3,02%	3,09% < 4,5%; meets criteria
Total Anthocyanin	**Vitamin C Content**		**IC_{50}**
12,615 mg/100 grams	18.586,7 mg/100 grams		215,6887 ppm

juwet fruit, with a distinctive aroma and slightly acidic taste, and a thick texture. Determination of these organoleptic parameters aimed to provide an initial,

Standardization of the extract has been carried out in accordance with the requirements set by MMI (Indonesian Pharmacopoeia) Volumes I-IV as outlined in (Zahroh & Agustini, 2021).

Spesific Parameters

Organoleptic dan Phytochemical Screening

In the organoleptic examination, juwet extract was obtained in a dark purple color, characteristic of the objective, and simple assessment of the extract using sensory perception (Mangalu et al., 2022). In the organoleptic examination, juwet extract was obtained in

a dark purple color, characteristic of the juwet fruit, with a distinctive aroma and slightly acidic taste, and a thick texture. Determination of these organoleptic parameters aimed to provide an initial, objective, and simple assessment of the extract using sensory perception (Andrian et al., 2018).

Water-Soluble and Ethanol-Soluble Extract Testing

Soluble substance levels were tested to determine the purity of the extract by quantifying the minimum chemical content of the extract soluble in a specific solvent (Andasari et al., 2021). The test results for the water-soluble extract showed significant variation between replications, averaging 66.016%. Despite the variation, all replications met the requirements set by MMI (Indonesian Pharmacopoeia), i.e., ≥ 24% (Zahroh & Agustini, 2021). This indicates that juwet extract contains a considerable amount of water-soluble components essential for body absorption. The ethanol-soluble extract yielded an average of 37.98%, with all replications meeting the MMI (Indonesian Pharmacopoeia) requirement of ≥ 11% (Zahroh & Agustini, 2021). This suggests that juwet extract also contains components soluble in ethanol, indicating the presence of important bioactive compounds (Situmorang & Ricky, 2022).

NON-SPESIFIC PARAMETERS

Moisture Content or Drying Shrinkage Test

The moisture content or drying shrinkage test results for three replications showed an average of 1.743%. All replication results met the stipulated requirement, i.e., less than 10% (Zahroh & Agustini, 2021). This low moisture content indicates good stability of the juwet extract with minimal risk of microbial growth, suitable for long-term storage (Leviana & Paramita, 2017).

Ash Content Test

The determination of ash content aimed to quantify the amount of inorganic or mineral substances remaining after the combustion process (Fauziyah et al., 2022). Test results indicated an average ash content of 3.706%. The low ash content suggests that the extract contains appropriate mineral content without excess, crucial for product quality. According to applicable standards, this content should not exceed 16.6% (Ulfah et al., 2020).

Acid-Insoluble Ash Test

The acid-insoluble ash content test aimed to identify the amount of ash remaining after impurities

originating from external materials such as sand or soil (Fatimawali et al., 2020). Test results for acid-insoluble ash in juwet extract yielded an average of 3.09%, with all replication results meeting the MMI (Indonesian Pharmacopoeia) requirement of less than 4.5%. This indicates that the majority of minerals present in the extract are easily absorbed and non-contaminant.

Total Anthocyanin Test

Total anthocyanin content was calculated using differential pH spectrophotometry based on the difference in absorbance between pH 1.0 and 4.5. Anthocyanins tend to change color, reversible with pH changes, allowing for quantitative measurement of anthocyanin content in the sample (Zahroh & Agustini, 2021). Measurement results showed a total anthocyanin content of 12.615 mg per 100 grams of juwet extract. Anthocyanins act as antioxidants by donating hydrogen atoms to DPPH, resulting in color changes from purple to yellow (Zahroh & Agustini, 2021).

Antioxidant Test

IC_{50} is the concentration required to inhibit 50% of free radical activity, indicating significant antioxidant activity in juwet extract (Miksusanti et al., 2012). IC_{50} (Inhibition Concentration 50) is a parameter used in the DPPH method to measure the antioxidant activity of a compound. Antioxidant activity was measured by observing the decrease in color intensity or absorbance of the DPPH solution. Anthocyanins, as antioxidants, donate hydrogen atoms to DPPH, causing the solution to change color from purple to yellow (Elvi Trinovani et al., 2022). This color change causes a change in absorbance at the maximum DPPH wavelength using UV-Vis spectrophotometry, allowing the determination of the free radical scavenging activity known as the inhibitory concentration (IC_{50}) value (Pramiastuti et al., 2021). The antioxidant activity of juwet fruit extract yielded an IC_{50} value of 215.6887 ppm.

Vitamin C Content Test

Vitamin C content in the antioxidant activity measurement of juwet fruit extract was used as a comparison. Juwet extract contained a very high vitamin C content of 18,586.7 mg per 100 grams. Vitamin C is known for its strong antioxidant properties and is important for skin health, immune system function, and iron absorption (Rozi et al., 2023).

Organoleptic Test

The taste, color, aroma, shape, and texture of the product were evaluated through organoleptic testing (also known as sensory evaluation) (Arziyah et al., 2022). The resulting color of the cereal bar was purple-yellow. The yellow and purple color of the cereal bar was derived from rice crispy and juwet extract containing anthocyanins, imparting a dark purple color (Hajrin et al., 2021). The aroma produced by the cereal bar was caramel. The characteristic caramel aroma is produced by caramelization reactions due to heat during cooking (Laga et al., 2019).

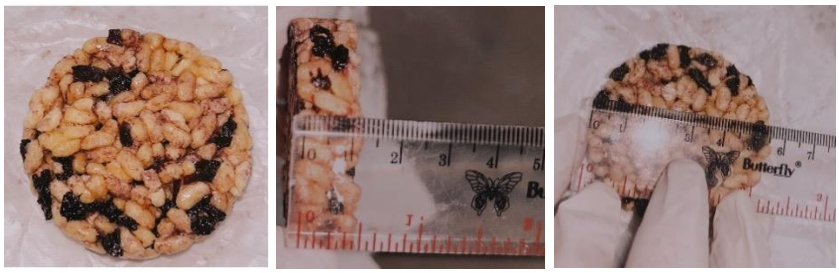

Figure 1. Measurement and Shape of Dried Juwet Cereal Bar from The Top Dried Juwet Cereal Bar with a diameter of 6 cm and a thickness of 1.2 cm weighs 22 grams. Dried Juwet Cereal Bar contains 5.9% juwet extract and was administered in solution form with additives formulated for experimental animals.

The caramelization reaction is caused by the interaction of sugar at high temperatures (80 °C), which is above the melting point. This reaction occurs when sugar is heated in acid, base, or dry heating conditions (Adna Ridhani & Aini, 2021). The taste of this cereal bar was sweet and slightly acidic. The sweet and acidic taste of the cereal bar is due to the composition of cereal bar ingredients such as sugar, honey, powdered milk, and the distinctive sour taste of juwet fruit. This cereal bar was made in a round shape with a firm texture.

Table 3
Nutrient Analysis Results of Dried Juwet Cereal Bar

Organoleptic				
Color	**Aroma**	**Taste**	**Shape**	**Texture**
Yellowish Purple	Caramel	Sweet-Sour	Round	Crispy
Iron Content Analysis	**Vitamin C Content Analysis**		**IC_{50}**	
2,411 ppm	2.408,4 mg/100 grams		1962,5581 ppm	
Proximate Analysis			**Requirements**	
Moisture Content	7,9837% bb		Max. 11,3%	
Ash Content	1,25657% bb		Max. 1,72%	
Protein Content	6,3364% bb		Min. 9,38%	
Fat Content	4,1513% bb		Max. 10,9%	
Total Carbohydrate Content	80,2721% bb		Min. 66,7%	
Caloric value	383,7954 Kcal/100 grams		Min. 403 Kcal/100 grams	

Iron and Vitamin C Content Test

The iron content of the cereal bar was quantitatively tested. The purpose of the quantitative analysis of the cereal bar was to analyze the concentration of iron or Fe in the sample using Atomic Absorption Spectrophotometer (AAS) (Nurhasanah, 2023). The iron content contained in the cereal bar was 2.411 ppm. Detection and determination of vitamin C content in a sample can be analyzed using UV-Vis Spectrophotometry (Abriyani et al., 2023). The vitamin C content contained in Dried Juwet Cereal Bar in this study was 2,408.4 mg/100 grams.

Antioxidant Test

Antioxidant activity is observed from the IC_{50} value. A low IC_{50} value indicates greater antioxidant activity (Widnyani et al., 2023). The IC_{50} value found in the cereal bar was 1962.5581 ppm. The antioxidant activity in this cereal bar is still considered weak. A compound is considered a very strong antioxidant if its IC_{50} value is less than 50 ppm, strong if the IC_{50} value is between 50-100 ppm, moderate if the IC_{50} value is between 100-150 ppm, and weak if the IC_{50} value is between 150-200 ppm. If the IC_{50} of a substance is above 500 ppm, it is considered less active or very weak, but still has potential as an antioxidant (Pratiwi et al., 2023).

Proximate Test

The National Standardization Body does not yet have an official guideline on the standard nutritional composition for snack bar products that could be used as a reference in this study (Mawarno & Putri, 2022). The proximate analysis information in this study refers to the Nutri-Grain Fruit and Nut Bar product (U.S. DEPARTMENT OF AGRICULTURE, 2018). Overall, the proximate analysis of cereal bar products has not fully met USDA quality standards, especially in terms of protein and calorie content. The protein content in these cereal bars still does not meet the standard because the composition of cereal bar ingredients contains little protein. The calorie content in cereal bars also does not yet meet the standard. The caloric value of a cereal bar is influenced by the carbohydrate, fat, and protein content. In this study, the main nutrients are carbohydrates and fats (Choiriyah et al., 2020).

Result of Average Hemoglobin, Hematocrit, and Erythrocyte Levels Pre and Post Anti-Anemic Therapy

Table 4
Normality Test Results

Parameters			Shapiro-Wilk		
			Statistic	df	Sig.
Positive	Hemoglobin	Pre	0.827	5	0.132
		Post	0.955	5	0.772
	Hematocrit	Pre	0.934	5	0.623
		Post	0.927	5	0.579
	Erythrocytes	Pre	0.987	5	0.970
		Post	0.961	5	0.818
Negative	Hemoglobin	Pre	0.932	5	0.613
		Post	0.960	5	0.806
	Hematocrit	Pre	0.920	5	0.532
		Post	0.897	5	0.391
	Erythrocytes	Pre	0.870	5	0.268
		Post	0.920	5	0.533
Treatment	Hemoglobin	Pre	0.839	5	0.163
		Post	0.922	5	0.544
	Hematocrit	Pre	0.983	5	0.950
		Post	0.996	5	0.997
	Erythrocytes	Pre	0.928	5	0.582
		Post	0.970	5	0.875
Normal	Hemoglobin	Pre	0.918	5	0.515
		Post	0.896	5	0.387
	Hematocrit	Pre	0.858	5	0.220
		Post	0.983	5	0.949
	Erythrocytes	Pre	0.849	5	0.191
		Post	0.969	5	0.872

Table 4 shows that all parameters have a p-value > 0.05, indicating that the data are normally distributed, so the statistical test used is the paired sample t-test.

Table 5
Result of Average Pre and Post Therapy Antianemic Hemoglobin, Hematocrit and Erythrocyte Levels

Parameters	Treatment Group	Sig.
Pre - Post Hemoglobin	Positive	0.008*
	Negative	0.777
	Treatment	0.040*
	Normal	0.439
Pre - Post Hematokrit	Positive	0.005*
	Negative	0.197
	Treatment	0.001*
	Normal	0.356
Pre - Post Eritrosit	Positive	0.030*
	Negative	0.142
	Treatment	0.001*
	Normal	0.362

*Significant difference

The anti-anemic activity testing was conducted in 4 treatment groups: positive control group, negative control group, treatment group, and normal group (Ifada et al., 2018). The positive control group was given Sangobion Vita-Tonic at a dose of 0.2 ml/200 grams/body weight of rats, the negative control group was given cereal bars without juwet extract,the treatment group was given Dried Juwet Cereal Bar at a dose of 5.9%, and the normal group was only given feed and water. The results of anti-anemic activity tests in each group were observed based on hemoglobin, hematocrit, and erythrocyte levels (Table 5). Table 5 shows that the levels of hemoglobin, hematocrit, and erythrocytes in the positive group given Sangobion Vita-Tonik and the treatment group given Dried Juwet Cereal Bar have p-values < 0.05, indicating a significant difference between pretest and posttest in the test groups.

This indicates that the administration of Dried Juwet Cereal Bar for 14 days was able to increase hemoglobin, hematocrit, and erythrocyte levels due to its iron content reaching 2,411 ppm per 2 grams of Dried Juwet Cereal Bar. Iron in Dried Juwet Cereal Bar is in the form of ferric iron (Fe^{3+}) or non-heme iron which is difficult to absorb (Bloor et al., 2021). In the stomach, Fe^{3+} can only be

absorbed if it has been reduced by gastric acid into Fe^{2+}. Therefore, the presence of vitamin C in Dried Juwet Cereal Bar can help increase iron absorption by chelating with ferric iron (Fe^{3+}) at the acidic pH of the stomach while remaining soluble in the alkaline pH of the duodenum, the first part of the small intestine. Iron becomes a precursor in hemoglobin formation, thus increasing hemoglobin levels, the number of erythrocytes, and the oxygen binding capacity of erythrocytes. The availability of stable oxygen results in perfect erythropoiesis, thereby producing perfect erythrocytes. Additionally, Dried Juwet Cereal Bar has a protein content of 6.3364% wb that can assist in hematopoietic growth processes and play a crucial role in managing blood cell proliferation and differentiation (Pradawahyuningtyas et al., 2020).

Statistical Analysis Results of Differences Between Pre and Post Levels of Hemoglobin, Hematocrit, and Erythrocytes in Experimental Animal Groups

Table 6
Shapiro-Wilk Normality Test Results

Test Groups		Sig.
Positive	Hemoglobin	0.871
	Hematocrit	0.508
	Erythrocytes	0.361
Negative	Hemoglobin	0.931
	Hematocrit	0.275
	Erythrocytes	0.979
Treatment	Hemoglobin	0.254
	Hematocrit	0.531
	Erythrocytes	0.936
Normal	Hemoglobin	0.873
	Hematocrit	0.353
	Erythrocytes	0.413

Table 7
Variance Test Results

Parameters	Sig.
Hemoglobin	0.030
Hematocrit	0.231*
Erythrocytes	0.070*

*Homogeneous data

Table 6 shows that all parameters have a p-value > 0.05, indicating that the data are normally distributed. However, Table 7 shows that hemoglobin has a p-value < 0.05, indicating that the data are not homogeneous, so the statistical test used is the Kruskal-Wallis test followed by the Mann-Whitney test.

Table 8
Kruskal-Wallis Test Results

Hemoglobin	Sig. 0.007
Hematocrit	Sig. 0.001
Erythrocytes	Sig. 0.002

Table 8 shows that hemoglobin, hematocrit, and erythrocytes in the four groups of experimental animals have p-values < 0.05, indicating significant differences, so the Mann-Whitney Test is conducted.

Table 9
Difference in Mean Post-Pre Differences in Test Groups

Groups	Comparison	Sig.		
		Hemoglobin	Hematocrit	Erythrocytes
Positive	Negative	0.016*	0.016*	0.047*
	Treatment	0.249	0.009*	0.009*
	Normal	0.036*	0.009*	0.047*
Negative	Positive	0.016*	0.016*	0.047*
	Treatment	0.009*	0.009*	0.009*
	Normal	0.465	0.142	0.117
Treatment	Positive	0.249	0.009*	0.009*
	Negative	0.009*	0.009*	0.009*
	Normal	0.021*	0.009*	0.009*
Normal	Positive	0.036*	0.009*	0.047*
	Negative	0.465	0.142	0.117
	Treatment	0.021*	0.009*	0.009*

*Significant difference

Table 9 shows that between the treatment group and the positive group, as well as the treatment group and the negative group, there are p-values < 0.05, indicating significant differences.

This indicates that juwet extract can increase hemoglobin, hematocrit, and erythrocyte levels. In terms of hemoglobin levels, the treatment group shows no

significant difference compared to the positive control group. However, in terms of hematocrit and erythrocyte levels, the treatment group shows a significant difference compared to the positive control group. Table 10 shows that the treatment group has higher hematocrit and erythrocyte levels compared to the positive control group. This indicates that Dried Juwet Cereal Bar has activity comparable to Sangobion Vita-Tonic.

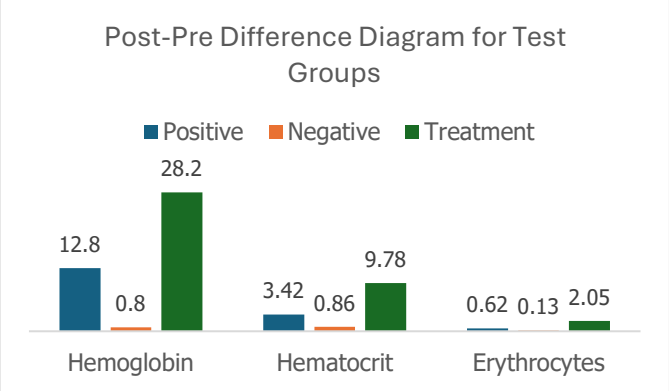

Figure 2. Post-Pre Difference Diagram for Test Groups

Table 10
Difference In Increase of Hemoglobin, Hematocrit, and Erythrocytes in Test Groups

Group	Comparison	Parameters	Increase
Treatment	Positive	Hemoglobin	2,203 times greater
		Hematocrit	2,859 times greater
		Erythrocytes	3,306 times greater
	Negative	Hemoglobin	35,25 times greater
		Hematocrit	11,372 times greater
		Erythrocytes	15,769 times greater

Table 10 shows a significant increase between the treatment group given Dried Juwet Cereal Bar and the positive group given Sangobion Vita-Tonik in hematocrit and erythrocyte levels, which are 2.859 and 3.306 times higher, respectively. Another significant increase is seen in the treatment group compared to the negative group given cereal bar without juwet extract in hemoglobin, hematocrit, and erythrocyte levels, which are 35.25, 11.372, and 15.769 times higher, respectively.

CONCLUSION AND SUGGESTIONS

This research aims to determine the effectiveness of Dried Juwet Cereal Bar (*Syzygium cumini* L.) as an anti-anemic agent in female white rats (*Rattus novergicus*). The main results of the study indicate that Dried Juwet Cereal Bar have effective anti-anemic activity in increasing hemoglobin, hematocrit, and erythrocyte levels in female rats induced with lead acetate. These findings support juwet fruit as a potential alternative for treating anemia caused by lead exposure. The study also demonstrates that Dried Juwet Cereal Bar have the potential to be an anti-anemic agent that meets good physical quality standards, is safe and effective, and can minimize the side effects of chemical drugs. This natural-based formulation can also be developed into a market-ready product, potentially attracting consumers seeking natural products. However, this research requires toxicity testing to ensure long-term consumption safety, stability testing to evaluate product shelf life during storage, and clinical trials to assess effects on humans directly. Additionally, bioavailability tests are needed to determine how well the substance is absorbed and utilized by the body.

In this article, the authors suggest conducting clinical trials on human subjects to ensure the safety and effectiveness of Dried Juwet Cereal Bar in preventing anemia in humans. These clinical trials are crucial for analyzing the health benefits of the cereal bar product, especially in increasing hemoglobin levels and preventing iron deficiency in the human body.

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