



The Effect of Administration of Ethanol Extract of Talas Kimpul Stems ((*Xanthosoma Sagittifolium* (L) Schott)) on Female Wistar Rats on Hemoglobin Level, Erythrocyte Level and Birth Weight of Little Wistar Rats



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Abstract

Lack of nutritional intake of pregnant women is at risk of anemia and giving birth to low birth weight (LBW) babies. LBW is at risk of stunting. In 2018 the proportion of pregnant women experiencing anemia was 48.9%, stunting was 30.8%. This study aimed to determine the effect of giving ethanol extract of Kimpul taro stems ((*Xanthosoma Sagittifolium* (L.) Schott)) to female Wistar rats on hemoglobin levels, erythrocyte level, and birth weight of little rats. The Randomized Pretest Posttest Control Group Design. The population of this research is female Wistar rats aged 12-13 weeks, healthy, weight 170-180 g. Sampling technique: simple random sampling. The sample size of 25 was divided into five groups, namely Negative Control (KN), Positive Control (KP), Treatment 1 (P1), Treatment 2 (P2), and Treatment 3 (P3). The intervention duration is 4 weeks, using an oral sonde. Data analysis includes descriptive, bivariate, and multivariate, using computer assistance. The results: the average group pretest, hemoglobin levels 12.44-13.38 g/dL; erythrocyte levels 6.22 – 7.18 10^6 /uL. The average post-test group, Hb levels 12.56–14.45g/dL; erythrocyte levels 6.93 – 8.31 10^6 /uL; None of the KN, KP, and P1 groups were pregnant; The birth weight of children in the P2 group was 3.97 g, the P3 group was 4.06 g. The results of the pretest Anova test Hb levels p 0.498, erythrocyte levels 0.58; posttest Hb levels p0.014, erythrocyte levels 0.034. Conclusion: the administration of ethanol extract from Talas Kimpul stems affects Hb levels, erythrocyte levels, and birth weight of rat pups. Recommendation: research needs to be continued to determine ferritin levels.

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1 Introduction

The fetus gets nutrition from its mother through uteroplacental circulation. The transport of nutrients and oxygen is carried out by red blood cells, especially hemoglobin (Hb). Oxygen is bound to Hb, because O₂ is not easily soluble in plasma, Hb also plays a role in the transport of CO₂, CO, NO, and H⁺ gases. Iron is absorbed from the small intestine, immediately binds to beta globulin, transferrin and is transported into the blood. The total amount of iron in the human body is an average of 4 grams, 65% of which is in the form of hemoglobin and is stored in the liver about 15-30% bound to ferritin. When the amount of iron in the plasma decreases, ferritin will be excreted easily (Sherwood, 2010).

Pregnant women are responsible for nutritional intake, for their bodies and the fetus they contain. If the mother lacks nutritional intake, the fetus will also experience the same thing. One of the effects of lack of nutritional intake that most often occurs is iron deficiency anemia. Anemic pregnant women have Hb levels below normal (Director et al., 2010).

The results of the Indonesian Basic Health Research (Riskesdas) in 2018 found that the proportion of pregnant women with anemia was 48.9%, this figure is higher than the results of the same research in 2013, which was 37.1% (Agency for Health Research and Development of the Ministry of Health, 2018). The target of the National Medium-Term Development Plan (RPJMN) in 2024 is to reduce the MMR to 183 per 100,000 KH, and the IMR to fall to 16 per 1,000 KH (Bappenas, 2019).

Fetuses conceived by anemic pregnant women are at risk of experiencing growth disorders so they have the potential to experience low birth weight and stunting. The results of Riskeddas 2018 found the proportion of LBW in Indonesia was 6.2%, Bali <6% and the proportion of PB less than 48 cm was 22.7%, Bali Province <10%. Stunting is one of the health problems faced by the Unitary State of the Republic of Indonesia. The proportion of stunting in toddlers from the 2018 Riskesdas results is 30.8%. The target for reducing stunting in 2024 is 19% (Bappenas, 2019).

Talas Kimpul (*Xanthosoma Sagittifolium* (L.) Schott) or Belitung taro (in Bali called taro) is a type of taro plant that can grow throughout the year, this taro is well known and used by people in Indonesia. Taro Kimpul midrib contains various nutrients that the body needs, including carbohydrates, protein, calcium, iron, vitamin C, fiber (Eyasu et al., 2019; de Souza Araújo et al., 2019).

Research on kimpul taro midrib extract harvested from Buleleng Regency, Bali Province contains nutrients, anti-nutrients, and phytochemicals. Nutrient content includes 18.18% water by wet weight (bb); Ash 2.75% bb; Calories 326.83% bw; protein 0.873% bw; fat 2.11% bw; carbohydrates 76.09% bw; Fe 0.43 mg/kg; beta carotene 15.19 mg/100g; vitamin C 7.69 mg/100g (Budiani & Armini, 2021).

Research result on giving talas leaf (*Colocasia esculenta*) to young mice, improves hematological parameters (Ufelle et al., 2018), and is useful as a rabbit wound medicine (Wijaya et al., 2014). Research on the benefits of Talas kimpul stems (*Xanthosoma Sagittifolium* (L.) Schott) related to reproductive health has not been found, so it needs to be investigated. This study aimed to determine the effect of the administration ethanol extract of Talas kimpul stems (*Xanthosoma Sagittifolium* (L.) Schott) to female Wistar rats on ferritin levels, hemoglobin levels, and birth weight of rats (De Bernabé et al., 2004; Luyckx & Brenner, 2005).

2 Materials and Methods

The design of this research is The Randomized Pretest Posttest Control Group Design. The research was conducted at Udayana University. Preparation of extracts and phytochemical tests in the food biotechnology laboratory. Research on experimental animals was carried out at the Maintenance and Breeding of Experimental Animals at Biomice and Rats. Laboratory examinations (Erythrocyte and Hemoglobin levels) were carried out at the Mantra Medika Denpasar laboratory. The study was carried out from April to October 2022.

Population and sample

The population of this study was female wistar rats aged 15-16 weeks. Inclusion criteria: 1 week pregnant, healthy, not having eating and drinking disorders, weight 170-180 g. The sample size was 25 individuals, which were divided into 5 groups, each with 5 individuals. Sampling technique; This research is simple random sampling.

Making Talas Kimpul Stems extract

The skin of the kimpul taro is cleaned, then steamed, then dried in the oven for 24 hours at 50°C. The dried fronds were sifted and made into flour, then macerated with 96% ethanol for 24 hours. Furthermore, evaporation is carried out to obtain a liquid extract.

Tests on mice

- 1) Acclimatization is carried out for 7 days.
- 2) Cyclophosphamide 50 mg/kg BW, single dose. Dosage determination is based on research (Ketaren, 2016)
- 3) Pretest: examination of Hb and ferritin levels 6 days after administration of cyclophosphamide.
Blood sample was taken from the orbital sinus 1 mL
- 4) Rats mated
- 5) Treatment for 4 weeks (28 days): KN was given 3 ml of distilled water; KP was given Fe 3.78 mg/kg BW rats; P1: Taro kimpul leaf extract with Fe content of 3.78 mg/kg BW (450 mg). P2: administration of kimpul leaf extract with Fe content of 7.56 mg/kg BW (950 mg). P3: Taro kimpul leaf extract was given with Fe 3.78 mg/kg BW (450 mg) + FE 3.78 mg/kg BW. The dose of FE is based on the standard of physiological antenatal care, which is 60 mg per day (WHO, 2016).
- 6) Laboratory examination
At the end of the treatment, hemoglobin levels, serum ferritin levels, and birth weight of rats were examined. Hemoglobin levels were checked using the cyanmethemoglobin method. Serum ferritin levels were checked by the ELIZA method using the Elabscience Rat FE (Ferritin) kit, cat Number: E-EL-R3018.
- 7) Weighing the baby mice
Weighing weight using ACIS Multi-Purpose Digital Scale.

Data analysis

Data analysis included descriptive, bivariate (paired t-test), and multivariate (Anova and Post Hoc). Computer-assisted analysis, SPSS program.

3 Results and Discussions

3.1 Result

Characteristics

Table 1
Body Weight and Leukocyte Levels Parent

Characteristic	Pretest	Posttest	Comparison	
	Mean \pm SD	Mean \pm SD	Mean difference	p
Body Weight parent (gram)				
KN	179,80 \pm 1,48	135,80 \pm 8,38	39,00	0,000
KP	180,00 \pm 1,58	135,80 \pm 18,44	44,20	0,007
P1	180,00 \pm 1,41	153,40 \pm 6,025	26,60	0,001
P2	180,00 \pm 1,27	153,33 \pm 1,97	26,67	0,000
P3	179,80 \pm 0,84	158,60 \pm 1,67	21,20	0,000
Comparison	0,998	0,004		
Leukocyte level ($10^3/uL$)				
KN	2,70 \pm 0,35	7,32 \pm 1,12	-4,62	0,000
KP	1,68 \pm 0,35	7,52 \pm 0,58	-5,84	0,000
P1	4,50 \pm 4,14	6,74 \pm 0,99	-2,24	0,241
P2	2,13 \pm 1,17	8,00 \pm 2,58	-5,87	0,002
P3	2,94 \pm 0,42	8,22 \pm 1,78	-5,28	0,002
Comparison	0,211	0,633		

Note: KN (control negative) is given 0,3 ml of distilled water; KP (control positive) was given Fe 3,78 mg/kg BW rats; P1 (Perlakuan 1): giving Talas kimpul stems extract with Fe content of 3.78 mg/kg BW (450 mg). P2 (perlakuan2) 2: giving Talas kimpul stems extract with Fe content of 7.56 mg/kg BW (950 mg). P3 (Perlakuan3): giving Talas kimpul stems extract with Fe content of 3.78 mg/kg BW (450 mg) + FE 3.78 mg/kg BW.

Table 1 shows the average body weight of the brood stock, erythrocyte levels, and leukocyte levels before being given treatment did not differ in the five groups. Means were significantly different for all groups after being given treatment on parent weight and red blood cell (RBC) levels. Differences before and after the parent BB treatment occurred in all groups. There was a decrease in the average weight of 21.2 – 44.2 g. the most decrease in the KP group, the lowest in the P3 group. Increased levels of erythrocytes in P2 and P3 groups. Leukocyte levels increased in the KN, KP, P2, and P3 groups

Table 2
Hemoglobin and Erythrocyte Levels Before and After Intervention

Groups	Pretest	Posttest	Comperison	
	Mean \pm SD (g/dL)	Mean \pm SD (g/dL)	Mean difference (Posttest – Pretest)	p
Hemoglobin level				
KN	12,62 \pm 1,07	12,56 \pm 0,97	-0,06	0,697
KP	12,46 \pm 0,66	12,78 \pm 1,23	0,32	0,457
P1	13,38 \pm 1,72	14,06 \pm 0,97	0,68	0,481
P2	13,33 \pm 1,40	14,45 \pm 0,86	1,12	0,010
P3	12,44 \pm 0,15	13,60 \pm 0,51	1,16	0,011
Comparison	0,498	0,014		
Erythrocyte level ($10^6/uL$)				
KN	6,40 \pm 0,42	7,04 \pm 0,65	0,64	0,056
KP	6,22 \pm 0,39	6,93 \pm 0,71	0,72	0,071
P1	7,18 \pm 1,02	7,47 \pm 0,29	0,29	0,573

P2	7,11 ± 0,67	8,31 ± 1,27	1,21	0,018
P3	6,36 ± 0,15	7,08 ± 0,12	0,722	0,000
Comparison	0,58	0,034		

Note: KN (control negative) is given 0,3 ml of distilled water; KP (control positive) was given Fe 3,78 mg/kg BW rats; P1 (Perlakuan 1): giving Talas kimpul stems extract with Fe content of 3.78 mg/kg BW (450 mg). P2 (perlakuan2) 2: giving Talas kimpul stems extract with Fe content of 7.56 mg/kg BW (950 mg). P3 (Perlakuan3): giving Talas kimpul stems extract with Fe content of 3.78 mg/kg BW (450 mg) + FE 3.78 mg/kg BW

Table 2 provides information that the Hb and erythrocyte levels before the intervention were not different in all groups ($p > 0.05$). After the intervention, the mean Hb and erythrocyte levels were found to increase among the five groups ($p < 0.05$). Decreased Hb levels in the KN group. Significant increases in Hb and erythrocyte levels occurred in groups P2 and P3. The results of the analysis show that H_0 is rejected. The provision of kimpul taro leaf extract affected Hb levels, namely there was an increase in Hb levels in the treatment group.

Table 3
Birth weight of little rats

Group	Average Number of Children Born	Average BB Born ± SD
KN	0	0
KP	0	0
P1	0	0
P2	2	3,97
P3	2	4,06

Note: KN (control negative) is given 0,3 ml of distilled water; KP (control positive) was given Fe 3,78 mg/kg BW rats; P1 (Perlakuan 1): giving Talas kimpul stems extract with Fe content of 3.78 mg/kg BW (450 mg). P2 (perlakuan2) 2: giving Talas kimpul stems extract with Fe content of 7.56 mg/kg BW (950 mg). P3 (Perlakuan3): giving Talas kimpul stems extract with Fe content of 3.78 mg/kg BW (450 mg) + FE 3.78 mg/kg BW

Table 3 provides information that female wistar rats that are pregnant and give birth are groups P2 and P3. The other Wistar rats were infertile. The results of the data analysis showed that there was an effect of giving ethanol extract of kimpul taro leaves on the fertilization of rats and children's body weight. The mean birth weight of rat pups in the P3 group was greater than that in the P2 group (Schober et al., 2019; Marais et al., 2008).

3.2 Discussion

Talas Kimpul or Kladi is well known by the public. The part of Kladi that is consumed so far is the tuber. Kladi stems and old leaves are used for animal feed, such as pigs. Young stems (fronds) or lompong can be consumed by humans, for vegetables. However, not many people take advantage of it (Zinatullina et al., 2021). There are two types of macronutrients and micronutrients. The results of this study found that 100 grams of wet-weight of kimpul taro fronds contained 0.87 g of protein; 2.11 g fat; 76.09 g carbohydrates; 2.75 g Ash, 326.84 kcal, 15.19 mg beta-carotene; 0.428 mg/kg, and 7.690.03 mg of vitamin C. All these nutrients are needed by the body to build the body (organogenesis), and body development including regulating body physiology (Dulińska et al., 2006; John et al., 2001).

Hemoglobin consists of heme and globin. Heme contains an iron component, while globin is a protein that is broken down into amino acids. Iron is delivered to tissues by circulating transferrin, a transporter that captures iron released into the plasma primarily from intestinal enterocytes or reticuloendothelial macrophages (John et al., 2005; Cappellini & Motta, 2015). The binding of iron-charged transferrin to the cell surface transferrin receptor (TfR) 1 results in endocytosis and absorption of the metallic charge. Internalized iron is transported to the mitochondria for the synthesis of heme or iron-sulfur groups, which are an integral part of several metalloproteins, and excess iron is stored and detoxified in cytosolic ferritin. Ferritin levels

along with hemosiderin reflect body iron stores. Stored iron in an insoluble form, mainly in the liver, spleen, and bone marrow. Most of the iron is bound to iron-binding proteins. Hemosiderin is an iron storage complex that is difficult to release iron for the body's needs. In fit conditions, serum ferritin concentration is positively correlated with total body iron stores (Abbaspour et al., 2014).

The content of protein and iron in the ethanol extract of the kimpul midrib given daily can increase the hemoglobin levels of rats. Pregnant women need an intake of micronutrients, such as folic acid, and vitamin C. Non-adherence causes anemia (Nadiyah et al., 2021). The results of this study are in line with the description (Moorthy et al., 2020), that children who are given Fe every day have Hb levels that are high higher than intermittent administration and reduces the risk of anemia. Children aged 12-24 months need 8 mg of iron daily. Parenting patterns, the mother's education, and family income also influence (Nurjannah et al., 2021).

Macronutrients are mainly distinguished in carbohydrates, proteins and lipids. Micronutrients are dietary components that do not make a significant contribution to caloric intake but are important for health and vital function, even if required in smaller amounts, including vitamins (both fat-soluble and water-soluble) and minerals (Savarino et al., 2021). Protein is a source of energy. The central role of protein in the maintenance of energy balance has considerable support. Several studies have shown that rodents fed a protein-deficient diet or animals experiencing protein stress (eg, pregnancy) spontaneously choose a high-protein diet under preferred feeding conditions (Carreiro et al., 2016). Carbohydrates are one source of calories, which are produced by staple foods that can provide a feeling of fullness. The regulation of nutrient intake begins with the recognition that the body has a limited capacity for carbohydrate storage but requires a constant supply of glucose for the central nervous system (CNS). The lateral (LH) and ventromedial (VMH) hypothalamus contain glucoreceptors that are sensitive to changes in circulating carbohydrate concentrations (Olivier et al., 1998; Anand et al., 1999).

4 Conclusion

Based on the results of the study, the following conclusions can be drawn

- 1) The average Hb level before the intervention ranged from 12.44 - 13.38 g/dL, while after the intervention it ranged from 12.56 - 14.45 g/dL. There is an increase in Hb levels after the intervention
- 2) The average erythrocyte levels before the intervention ranged from 6.22 to 7.18 10^6 /ul, while after the intervention it ranged from 6.93 to 8.31 10^6 /ul. There is an increase in erythrocyte levels after the intervention
- 3) Almost all rats are not pregnant and give birth. Rats that were pregnant and gave birth were in groups P2 and P3.
- 4) There is an effect of giving ethanol extract of kimpul taro leaves on Hb levels, erythrocytes, and birth weight of rat pups.

Recommendation

The researcher continued the research to find ferritin levels before and after the intervention and analyzed the effect of giving ethanol extract of kimpul taro leaves on ferritin levels.

Ethical Considerate

It has been approved by Poltekkes Kemenkes Denpasar Ethics Committee with The Letter Number LB.02.03/EA/KEPK/ 0594 /2022

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