



INVIVO STUDY OF AQUEOUS EXTRACT OF SNAKE FRUIT SEEDS IN FEMALE RATS WITH ANEMIA

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ABSTRACT

Anemia remains a global nutritional problem in children, pregnant women and female adolescents because of increasing its prevalence. This study aimed to analyze the effects of aqueous extract of snake fruit seed (AES) on body weight (BW), body length (BL), body fat percentage (BFP), and wet organ weight in female rats with anemia. This animal experiment used the pre-posttest control group design. A total of 30 female wistar rats with anemia were randomly divided into 5 groups: the negative and positive control groups were given a low iron diet with or without administration of 3.7 mg/200gBW iron supplements, and the treatment (AES1-3) groups were given a low iron diet+AES administration (25,50, and 100 mg/200gBW respectively) for 28 days. Data were analyzed using SPSS 26.0, and STATA MP 17. The average of BW, wet liver, and kidney weights in the AES2 group significantly increased, compared to other rat groups. The average of BFP in the AES2 group significantly decreased, compared to the control groups. The average of BL and wet spleen weight did not differ among rat groups. In conclusion, administration of 50 mg/200gBW AES increases BW, wet organ weights, and decreases BFP, but has no effect on BL.

Keywords: *Aqueous extract of snake fruit seeds, Anemia, Body weight, Body Length, Body Fat Percentage*

ABSTRAK

Anemia merupakan masalah gizi global pada anak-anak, ibu hamil, dan remaja putri karena prevalensinya terus meningkat. Penelitian ini bertujuan menganalisis pengaruh pemberian ekstrak air biji salak (AES) terhadap berat dan panjang badan, kadar lemak tubuh (BFP), dan berat organ basah pada tikus wistar betina dengan model anemia. Desain penelitian menggunakan true eksperiment with pre-posttest control group design. Sebanyak 30 ekor tikus wistar betina anemia dibagi secara acak menjadi 5 kelompok, yaitu kelompok kontrol negatif dan positif diberi diet rendah zat besi dengan atau tanpa pemberian suplemen zat besi 3,7 mg/200gBB, dan kelompok perlakuan (AES1-3) diberikan diet rendah zat besi+pemberian AES (masing-masing 25,50, dan 100mg/200gBB) selama 28 hari. Data dianalisis menggunakan SPSS 26.0, dan STATA MP 17. Rata-rata berat badan, berat basah hati dan ginjal pada kelompok AES2 meningkat signifikan dibandingkan kelompok tikus lainnya. Rata-rata BFP pada kelompok AES2 menurun secara signifikan dibandingkan kelompok kontrol. Rata-rata berat BL dan limpa basah tidak berbeda antar kelompok tikus. Kesimpulannya, pemberian AES 50 mg/200gBB dapat meningkatkan BB, berat organ basah, dan menurunkan BFP, namun tidak berpengaruh terhadap BL.

Kata Kunci: *Ekstrak air biji salak, Anemia, Berat Badan, Panjang Badan, Kadar Lemak Tubuh*

INTRODUCTION

Anemia remains a global nutritional problem over the world because of its increasing prevalence. Asian countries account for the highest anemia prevalence, from 46.3% in 2018 to 46.6% in 2019 for women of childbearing age (WHO, 2022). In addition, children, pregnant women, and adolescents are also vulnerable age groups for anemia. The common causes of anemia in women are inadequate iron intake, menstruation in women, gastrointestinal bleeding (Kumar et al. 2022), decreased iron absorption, and increased iron requirements during pregnancy, growth, and breastfeeding (Wibowo et al. 2021). Recently, obesity prevalence has increased significantly, which has become an important risk factor for anemia. Obesity induces a chronic and low level of systemic inflammation, which stimulates the production of many cytokines, such as Interleukin-6 (IL-6) and leptin. High IL-6 and leptin levels can upregulate hepcidin secretion, which inhibits iron absorption in the small intestine (Pande et al. 2019).

For a long-term period, anemia has negative impacts on growth and development. Pregnant women with anemia have a higher risk of premature birth, bleeding, and stunted children, compared to pregnant women without anemia (Kumar et al. 2022). As a result, anemia causes impaired cognitive abilities and linear growth defects (Soliman et al. 2014). The impact of anemia on adolescents is stunted growth and decreased enthusiasm for studying at school. Adolescent girls are more susceptible to anemia because adolescents are in a period of growth that requires more nutrients, including iron. In addition, adolescents are very concerned about their bodies thus they are concerned on food consumption, such as having a vegetarian diet (Wayan Dewi Tarini et al. 2020).

WHO recommended the administration of iron tablets for anemia treatment. The Indonesian government has implemented anemia treatment with iron tablets for female adolescents (12-18 years) that contain 60 mg iron and 400 µg folic acid for 52 weeks, and pregnant women minimal of 90 tablets when pregnancy. However, they feel some side effects such as nausea, vomiting, constipation, and unpleasant taste when consuming BAT cause non-compliance with BAT consumption (Jimenez et al. 2015). Therefore, we need other medicines to alleviate anemia in vulnerable age groups.

Snake fruit (*Salacca edulis* Reinw) is an exotic and native fruit from Indonesia (Arief and Asnawi 2021), which has become a priority commodity in Donokerto village, Turi district, Sleman regency, Yogyakarta. Around 25 – 30% of the snake fruit consists of seeds, which are no longer used. According to Susanti (2018), snake seed flour (SSF) contains 12.4 mg iron, 5.2 mg Zn, 12.6% protein, and 152.2 mg vitamin C. Giving 3.72g/BW SSF to an anemia rat model can increase Hb to normal after 4 weeks treatment, but the intervention needed a lot of SSF so it's not efficiency for people. Previous research showed that 0.175g/BW snake fruit seed extract with ethanol solvent could increase BW significantly ($p < 0.001$) for 2 weeks (Melati et al, 2019). However, this research has a side effect an increase in the number of leukocytes. Snake fruit seed extract using ethanol as a solvent contains saponins, flavonoids, quinones, monoterpenes or sequesterpes and polyphenolics (Warso, 2018). The polyphenol content in Pondoh snake fruit seeds (SFS) can act as an antioxidant (Musa et al., 2015).

This research provides an intervention involving snake fruit seed extract with water as a solvent in female rats with anemia models. Water is a simple compound that can dissolve iron; besides that, water is a cheap solvent (Plaza and Turner 2015). It is hoped that a higher nutritional content will have a more effective influence in treating anemia. This study aims to determine the effect of administering AES on BB, BL, BFP and wet organ weight in female anemia rat model.

METHOD

Materials

Fresh SFS were purchased from farmers in Donokerto village, Turi District, Sleman Regency, Yogyakarta. To make SSF, selected SFS were dried and grounded by using an existing method developed in our research group with IDS000005116 paten number. Female Wistar rats were purchased from CV. Dunia Kaca Kemuning village, Ngargoyoso district, Karanganyar Regency, Central Java (website: www.kemuning.co.id). BR-II comfeed feed was purchased from PT. Japfa Comfeed Indonesia Tbk in Sragen, Central Java, and AIN 93 Mineral mix free iron feeds provided by UNY laboratory Yogyakarta. Drinking water was provided by the integrated laboratory UNS.

Snake Fruit Seed Extraction

Hot water extraction of SSF referred to Denis et al (2019) and Sukmawati et al (2013) with slight modifications. Extraction of SSF was carried out at the Phytochemical Laboratory of Setia Budi University, Surakarta. Briefly, SSF was dissolved in 1:10 weight per volume (w/v) water and then heated at 65°C for 30 minutes. The extraction process was repeated 3 times until getting a thick solution. Collected filtrates were concentrated using a rotary vacuum evaporator at 60°C, 80 rpm, at 175 mbar. The thick extract obtained from 1kg of SSF is 87 g. Finally, aqueous extract of snake fruit seed (AES) was stored in a refrigerator at 4°C before use. Based on nutrition analysis, the AES contained 32.8 mg of iron, 10.3 mg zinc, magnesium 2764.2 mg of, vitamin C 495.5 mg, and tannin 0.62% per 100 gram (Febyawati et al. 2023).

Design, location, and time

This *in vivo* study used a pre-posttest control group design and was conducted at the integrated laboratory UNS from March to June 2023. The sample size was calculated using the degree of formula freedom (Charan and Kantharia 2013): $E = (nx 5) - 5$ ($E = 10 - 20$ range value; $n =$ sample number per group; $t =$ total number of treatment groups). The study consisted of 5 groups with 5 female

rats/group. To minimize dropout during the intervention, a 20% sample (1 rat) was added to each group (Noor et al 2022). Thirty Wistar female rats aged 6 weeks old and weighted 100 - 200 g, were used in this research study. Before the study began, the research protocol has been approved by the ethics committee of the Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Central Java Province, Indonesia, with number 59/UN27.06.11/KEP/EC/2023.

Development of Anemia Rat Model

Selected female rats were individually acclimatized in a plastic cage at 18-27°C and 80-90% humidity (Noor et al 2022), and 12 hours of light and dark cycles (Fitria 2014) for 7 days. During acclimatization, 10% of rat bw of comfeed BR-II was given and drinking water was provided ad libitum. The anemia rat model was generated by taking 1% bw venous blood. The next procedure was the replacement of comfeed BR-II to low iron diet AIN-93M mineral mix free Fe feed, which was given 10% bw every day. Female rats with anemia were characterized by blood hemoglobin levels <10 g/dL. Taking venous blood was repeated on days 7, 14, and 21 during anemia modeling.

The intervention of AES to anemia rat's model:

Once the female rats became anemia with <10 g/dL hemoglobin levels, all rats were randomly divided into 5 groups, negative control (NC), positive control (PC), treatment 1 (AES 1), treatment 2 (AES 2), and treatment 3 (AES 3). The NC group was only given a low-iron diet while the PC group was given a low-iron diet and 0.37 mg/200mgBW iron supplements. The AES 1-3 groups were given a low iron diet and 25, 50, and 100 mg/200 mgBW AES for 28 days. Drinking water was provided ad libitum for all rat groups.

Data Collection of Body Weight and Body Length

Body weight and body length data were measured using a digital scale (Joil, PT. Arta Joil Tappa, Tangerang Indonesia), and a metline (Onemed, PT. Jayamas Medica Industri, Sidoarjo Indonesia) before, during, and after the intervention. Body Fat Content is calculated using the body fat percentage formula via the TM index by Lee et al. 2011. $TM\ index = \frac{Body\ weight\ (g)}{Naso-anal\ length\ (cm)^{2.823}} \times 10^3$. $Body\ fat\ content = 0.581 \times TM\ index - 22.03$ (Lee et al. 2011).

Wet organ weight

Research to measure the effect of AES administration on female rats with anemia on wet organ weight was carried out in the last intervention, all rats were sacrificed based on an existing method (Noor et al 2022). The liver, spleen, and kidney organs were immediately taken and weighed using a digital scale. Then, those organs were washed using cold phosphate buffered saline (PBS) solution (Roche, Germany) and kept in the freezer.

Data analysis

All data were analyzed using SPSS (Statistical Program for Social Science) version 26 and STATA MP (Statistical and Data Multi Processor) version 17 and presented in mean \pm standard deviation. The averages of each group on BW, BL, and BFP were tested for normality based on Shapiro Wilk and homogeneity was analyzed using One way ANOVA in SPSS. The repeated measures ANOVA was used to compare mean BW, BL, and BFP, followed the Bonferroni post hoc test. Data of wet organ weight were analyzed using the Kruskal Wallis test, followed by the Man Whitney test. To evaluate mean differences of AES based on dose and duration, data were analyzed using Difference in Difference (DiD) Linear Regression. A significant value was set up $p < 0.05$.

RESULTS AND DISCUSSION

Body Weight, Body Length, and Body Fat Percentage Data of Female Rats with Anemia

Table 1 showed the mean of Body Weight (BW), Body Length (BL), and Body Fat Percentage (BFP) in female rats with anemia. In general, BW and BL data of control and treatment groups were almost

similar. The highest average of BW was in the PC (186.0 ± 5.13 g) and AES 2 group (183.3 ± 8.45 g), and the lowest average of BW was in the NC group (167.17 ± 7.5 g). Based on BL among rat groups, the NC group (18.67 ± 0.52 cm) was similar to the AES 3 group (18.83 ± 0.75 cm). Meanwhile, a similar BL was observed in PC, AES 1, and AES 2 rat groups (around 19 cm). However, the average of BFP varied among rat groups, and the highest average of BFP was in the AES 2 group (2.03 ± 1.11 g/cm³). The PC and AES 1 groups shared similar BFP (0.93 ± 0.40 vs. 0.78 ± 0.57 g/cm³). A similar pattern was observed in the NC (1.33 ± 0.61 g/cm³) and AES 3 (1.65 ± 1.10 g/cm³) groups.

Rat's group	BW (g)	BL (cm)	BFP (g/cm ³)
NC	167.2±7.5	18.7±0.5	1.3±0.6
PC	186.0±5.1a	19.5±0.6a	0.9±0.4
AES 1	179.0±12.4a	19.3±0.2a.b	0.8±0.2
AES 2	183.3±8.5a	19.4±0.2a.b	2.0±1.1a
AES 3	174.7±7.7b	18.8±0.3a.b	1.7±1.1
P	0.007*	0.429	0.080*

Table 1. Body Weight and Body Length Data of Female Rats with Anemia

*Statistically significant at 5% level by One Way ANOVA

The letter A designated mean differences between NC and other groups and the letter b showed mean differences between PC and other groups, which were analyzed using one-way ANOVA and followed by LSD post hoc test.

Effect of AES Intervention on Body Weight of Anemia Rats

Body weight is often used to test the effect/toxicity of a natural substance. Figure 1 showed significant differences in the PC, AES 1, and AES groups compared to the NC group on day 0. After 28 days of intervention, all groups were increase significantly on AES 2 compared NC, NC compared PC, and AES 3 compared PC ($p < 0.001$).

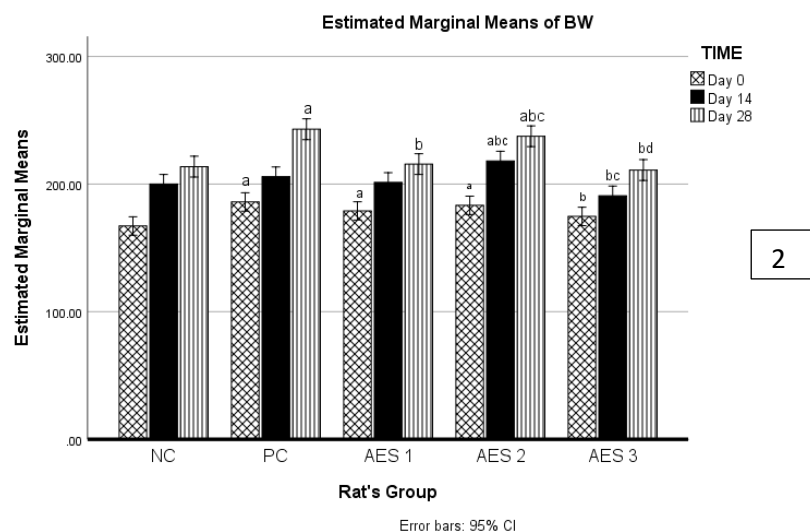


Figure 1. BW changes among rat groups with or without AES administration for 28 days, which were measured in day 0, 14, and 28 interventions. The letter a designated mean difference between NC and other groups, which were analyzed using one way ANOVA and followed by LSD post hoc test. The letter b showed mean differences between PC and other groups, which were analyzed using one-way ANOVA and followed by LSD post hoc test. The significant values were set up at $p < 0.05$.

AES administration increased the average of BW in female rats with anemia in dose dependent manner. After 14- and 28-days interventions, AES administration had different effects on BW among female rats with anemia (Figure 1 and Table 2). Compared to the NC (154.8 ± 8.4 g) group, the average of BW in PC (179.2 ± 8.9 g), AES 1 (161.3 ± 9.8 g), and AES 2 (181.2 ± 14.8 g) groups before intervention was significantly higher ($p < 0.024$). The average of BW in AES3 (154.2 ± 8.4 g) group did not differ from the NC group (154.8 ± 8.4 g), but significantly differed from the PC group ($p = 0.03$). At the 14th day intervention, the AES2 group (209.3 ± 11.9 g) had a significant higher of BW than the

NC (171.0±18.2 g), PC (192.2±19.2 g), and AES 1 (159.7±14.5 g, p<0.026) groups. The average of BW in AES3 (172.7±8.9 g) significantly decreased compared to the PC and AES1 groups (p=0.008).

Variables	BW			
	Coef	Std. err	P	R Square
Rat's group				0.799
PC	18.0	5.33	0.001*	
AES 1	12.3	5.33	0.024*	
AES 2	24.2	5.33	<0,001*	
AES 3	6.7	5.33	0.216	
Time				
Day 14	13.7	5.33	0.012*	
Day 28	44.5	5.33	<0,001*	
Rat's group and time				
PC (Day 14)	-1.1	7.55	0.878	
PC (Day 28)	-12.2	7.55	0.111	
AES 1 (Day 14)	-2.5	7.55	0.741	
AES 1 (Day 28)	-11.0	7.55	0.149	
AES 2 (Day 14)	-10.0	7.55	0.189	
AES 2 (Day 28)	-6.0	7.55	0.429	
AES 3 (Day 14)	-1.2	7.55	0.878	
AES 3 (Day 28)	-15.8	7.55	0.039*	

Table 2 . Further analysis of AES administration on BW based on rats' group and duration, compared to NC group.

*Statistically significant at 5% level

Source: processing results with the STATA 17 application

Table 2. Relationship between treatment effect and treatment time analyzed using linear regression in Stata. Coef shows the unstandardized beta coefficient as the value in the regression equation (+) shows an increase (-) shows a decrease. AES intervention in all groups over time significantly influenced changes in BW of anemia rats. The highest influence relationship is found in the BW variable at 79.9%.

On the 28th day of intervention (Figure 1), the average of BW in PC (182.0±21.7 g, p<0.001) and AES2 (222.3±10.1 g, p<0.001) groups significantly increased compared to the average of BW in NC group (182,0±21,7 g). By contrast, the AES1 (172,0±12.0 g) and AES3 (174.8±5.6 g) groups had a lower average of BW than the NC and PC groups, but it reached significantly only compared to the PC (p<0.001). In addition, the AES1 and AES3 groups had a lower average of BW compared to the AES2 group (p<0.001). Table 2 showed the effect of AES administration on rat's BW based on dose and duration. All rat groups (PC and AES1-2) had significantly increased BW, but increased BW in the AES3 group did not differ from the NC group. The highest increased BW was found in the AES2 (24.2 g) and PC (18.0 g) groups, followed by the AES1 group (12.3 g). According to the duration of AES administration, increased BW in all treatment groups in 14- and 28-days intervention was significantly higher than that off in the day before intervention (p<0.012). The highest increased BW occurred in the AES 3 group in the day 28 intervention and it reached significantly with p=0.039.

Effect of AES Intervention on the Body Length of Anemia Rats

Giving AES can increase the BL of rats for 28 days (p<0.01). All groups experienced significant improvement over time (p<0.002). On average, all BL increases occurred after days 0, 14, 28 (Figure 1). The BL in this study increased, thus supporting the role of AES in treating anemia.

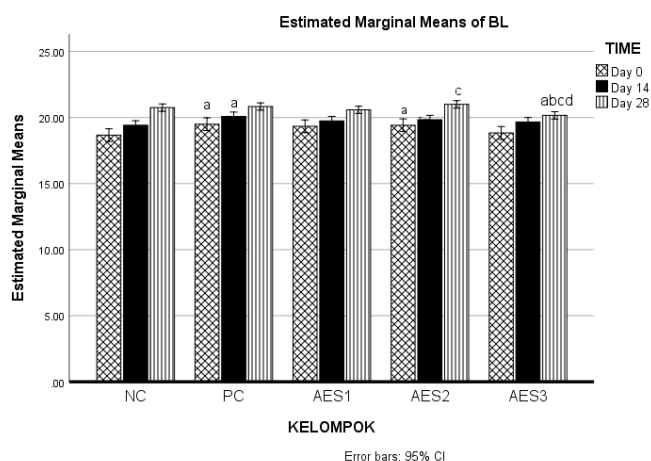


Figure 2. BL changes among rat groups with or without AES administration for 28 days, which were measured in day 0, 14, and 28 interventions. The letter a designated mean difference between NC and other groups, which were analyzed using one way ANOVA and followed by LSD post hoc test. The letter b showed mean differences between PC and other groups, which were analyzed using one-way ANOVA and followed by LSD post hoc test. The significant values were set up at $p < 0.05$.

AES administration did not affect the BL of female rats with anemia

From Figure 2 and Table 4, the average of BL increased in female rats with anemia at the 14- and 28-day interventions. On the day before intervention, the average of BL in PC (19.5 ± 0.5 cm, $p = 0.019$) and AES2 (19.4 ± 0.2 cm, $p = 0.032$) groups were significantly longer compared to the NC group (18.7 ± 0.5 cm). The average of BL among rat groups increased at the 14th-day intervention, but only the PC group (19.5 ± 0.6 cm) had a significantly longer of BL than the NC group (18.7 ± 0.5 cm, $p = 0.008$). On the 28th day of intervention, the increased BL had the same pattern as the increased BL at the 14 days of intervention. However, the increased BL in all treatment rat groups did not differ from the increased BL in the NC group. the AES2 (21 ± 0.0 cm) had a significantly longer average of BL compared to the AES1 (20.6 ± 0.5 cm, $p = 0.039$) and the AES3 group (20.2 ± 0.3 , $p < 0.001$). In addition, the average of BL in the AES3 group significantly decreased compared to the NC (20.8 ± 0.4 , $p = 0.005$), PC (20.8 ± 0.3 , $p = 0.002$) and AES1-2 groups.

Variable	BL			
	Coef	Std. err	P	R-squared
Rat's group				
PC	0.83	0.257	0.002*	0.7389
AES 1	0.67	0.257	0.012*	
AES 2	0.75	0.257	0.005*	
AES 3	0.17	0.257	0.519	
Time				
Day 14	0.75	0.257	0.648	
Day 28	2.08	0.257	0.043*	
Rat's group and time				
PC (Day 14)	-0.167	0.364	0.363	
PC (Day 28)	-0.75	0.364	0.043*	
AES 1 (Day 14)	-0.33	0.364	0.363	
AES 1 (Day 28)	-0.83	0.364	0.025*	
AES 2 (Day 14)	-0.33	0.364	0.363	
AES 2 (Day 28)	-0,5	0.364	0.174	
AES 3 (Day 14)	0.083	0.364	0.820	
AES 3 (Day 28)	-0.75	0.364	0.043*	

Table 3 . Further analysis of AES administration on BL based on rats group and duration, compared to NC group
*Statistically significant at a 5% level

Source: processing results with the STATA 17 application

Table 3. The relationship between treatment effect and treatment time was analyzed using linear regression in Stata. Coeff shows the unstandardized beta coefficient as the value in the regression equation (+) shows an increase (-) shows a decrease. AES intervention in all groups over time significantly influenced changes in BL in anemia rats, except in the AES 3 group was not significant. The effect of AES intervention for the PC, AES 1, and AES significantly decreased the BL for 28 days ($p < 0.05$).

Further analysis showed that AES administration did not influence increased BL in female rats with anemia (Table 3). Based on the duration of AES administration, the average BL increased significantly at the 28th-day intervention ($p = 0.043$) but did not increase significantly at the 14th-day intervention. In addition, the increased BL in the AES 2 group on the 28th day of intervention was not different from the NC group ($p = 0.174$). However, the average of BL in AES 1 ($p = 0.025$) and AES3 ($p = 0.043$) groups on the 28th day of intervention were significantly lower than that of the NC group.

Effect of AES Administration on BFP of Anemia Rats

BFP is the amount of fat content in the body. This study did not show any obese samples either before or after treatment.

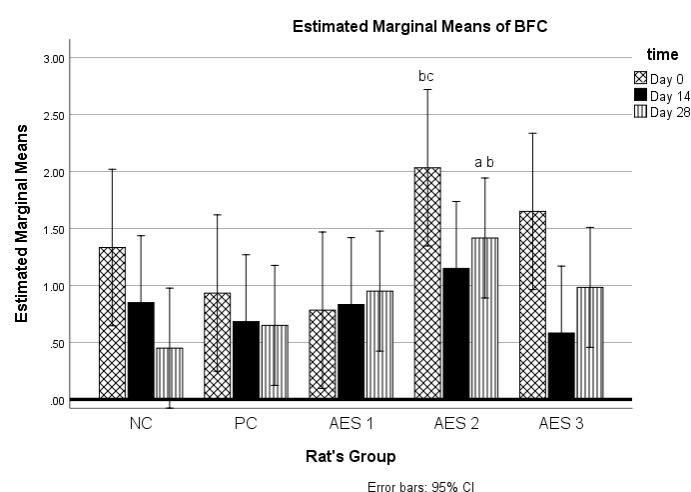


Figure 3. BFP changes among rat groups with or without AES administration for 28 days were measured in days 0, 14, and 28 interventions. The letter is a designated mean difference between NC and other groups. The letter b showed mean differences between PC and other groups, which were analyzed using one-way ANOVA and followed by LSD post hoc test. The significant values were set up at $p < 0.05$.

AES administration decreased the average of BFP in female rats with anemia in a dose dependent manner. After 14- and 28-day interventions, AES administration reduced the average of BFP among female rats with anemia (Figure 3 and Table 2), except in the AES1 group. On the day before the intervention, the average of BFP in the AES 2-3 groups ($2.1 \pm 1.9 \text{ g/cm}^3$) was higher than the average of BFP in the NC group ($1.3 \pm 1.1 \text{ g/cm}^3$), while lower averages of BFP were observed in the PC ($1.0 \pm 0.3 \text{ g/cm}^3$) and AES 1 ($0.8 \pm 0.6 \text{ g/cm}^3$, $p = 0.014$) groups. In addition, the average of BFP in the AES2 group was significantly higher than that of the PC ($p = 0.028$) and AES1 ($p = 0.014$) groups. On 14th day of the intervention, the average BFP was reduced among all rats group except in the AES 1 group ($0.8 \pm 0.5 \text{ g/cm}^3$). The reduced averages of BFP did not significantly differ. In contrast, the averages of BFP increased were found in the AES 1 group ($0.9 \pm 0.3 \text{ g/cm}^3$) on the 28th day of intervention. Meanwhile, reduced averages of BFP were found in the AES 2 ($1.4 \pm 1.0 \text{ g/cm}^3$), AES 3 ($1.0 \pm 0.5 \text{ g/cm}^3$), NC ($0.2 \pm 0.1 \text{ g/cm}^3$), and PC ($0.6 \pm 0.4 \text{ g/cm}^3$) groups. Overall, the BFP changes did not reach significantly. To further the effect of AES on the BFP of rats anemia model depends on dose and duration can be seen in Table 4.

Variable	BFP			
	Coef	Std. err	P	r-squared
Rat's group				
PC	-0.35	0.491	0.479	0.2703
AES 1	0.55	0.491	0.267	
AES 2	0.8	0.491	0.108	
AES 3	0.4	0.491	0.418	
Time				
Day 14	-0.63	0.491	0.201	
Day 28	-1.1	0.491	0.024*	
Rat's group and time				
PC (Day 14)	0.35	0.695	0.616	
PC (Day 28)	0.76	0.695	0.273	
AES 1 (Day 14)	0.7	0.695	0.317	
AES 1 (Day 28)	1.25	0.695	0.076	
AES 2 (Day 14)	0.34	0.695	0.633	
AES 2 (Day 28)	0.43	0.695	0.535	
AES 3 (Day 14)	-0.5	0.695	0.474	
AES 3 (Day 28)	0.4	0.695	0.567	

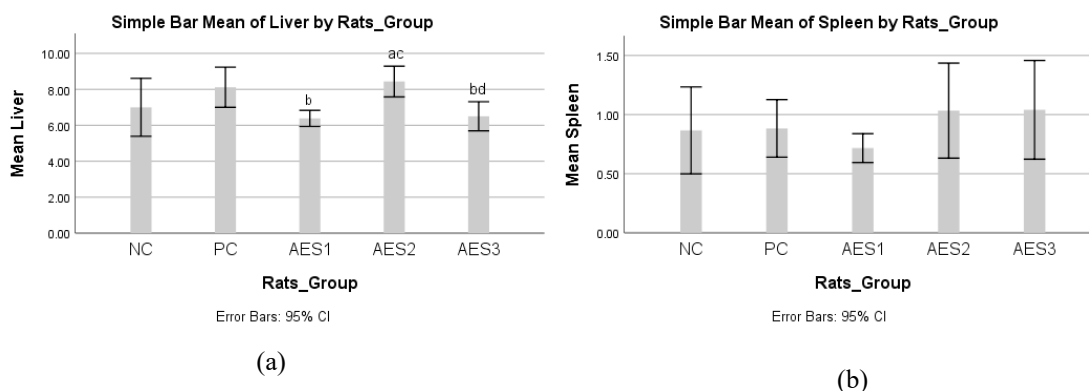
Table 4. Further analysis of AES administration on BFP based on rats group and duration, compared to NC group.
*Statistically significant at 5% level

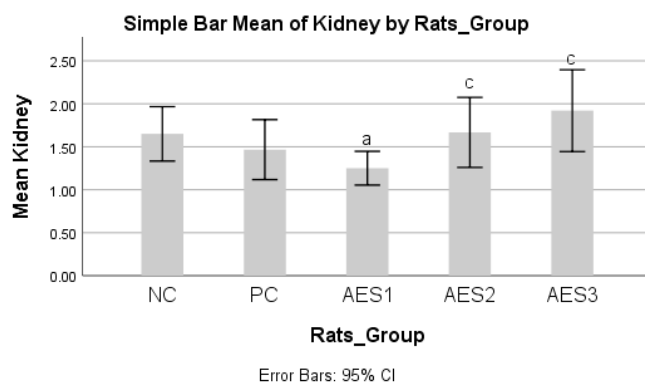
Source: processing results with the STATA 17 application

Table 4. The relationship between treatment effect and treatment time was analyzed using linear regression in Stata. Coeff shows the unstandardized beta coefficient as the value in the regression equation (+) shows an increase (-) shows a decrease. AES intervention in all groups over time not significantly changes for BFP, but the AES intervention during 28 days significantly decreased BFP of anemia rats (p=0.024).

Further analysis indicated that AES administration reduced the average of BFP at the day 28th intervention (p=0.024), but reduced averages of BFP in treatment groups did not differ from reduced average of BFP in the NC group, either at the day 14th or 28th day intervention.

AES administration had difference effects on wet liver, spleen, and kidney weights in female rats with anemia. Iron is one of most important trace elements that has vital roles in various biological processes including growth and development. Figure 4 indicated that AES administration increased wet liver, spleen, and kidney weight, but it did not depend on increased doses.





(c)

Figure 4(a) showed the wet organ weight of liver changes among rat groups with or without AES administration for 28 days, which were measured in the last interventions. Figure 4(b) Wet organ weight of spleen changes among rat groups with or without AES administration, which were measured in the last interventions. Figure 4(c) Wet organ weight of kidney changes among rat groups with or without AES administration, which were measured in the last interventions. The letter a designated mean difference between NC and other groups. The letter b showed mean differences between PC and other groups. The letter c showed differences in mean between AES1 and other groups. The letter d showed differences mean between AES 2 and AES 3, which were analyzed using Kruskal Wallis and followed by pairwise comparison post hoc test of Kruskal Wallis. The significant values were set up at $p < 0.05$.

At the end of the intervention, the average wet liver weight in the AES2 group (8.43 ± 0.81 g) was significantly higher compared to the average wet liver weight in the NC (7.00 ± 1.53 g, $p = 0.047$), AES1 (6.38 ± 0.42 g, $p = 0.033$) and AES 3 (6.50 ± 0.65 g, $p = 0.008$) groups. Meanwhile, the average wet liver weight in the AES1 (6.38 ± 0.42 g, $p = 0.014$) and AES3 ($p = 0.028$) groups were significantly lower compared to the average of wet liver weight in the PC group (8.11 ± 1.06 g). In contrast to the average of wet liver weight, higher averages of wet spleen weight were only found in AES 2 (1.03 ± 0.38 g) and AES 3 (1.04 ± 0.34 g) groups, but it did not reach significantly, compared to other groups (Figure 4b). In addition, the lowest wet spleen weight was found in the AES 1 group (0.72 ± 0.12 g). Interestingly, AES administration increased wet kidney weights depending on dose manner (Figure 4c). However, only the AES 3 group had a higher wet kidney weight average than the control groups (NC and PC) and treatment groups (AES1-2). The increased wet kidney weights of AES 2 and AES 3 (1.92 ± 0.38 g, $p = 0.002$) groups significantly differed from the AES 1 group but did not differ from NC and PC groups. In addition, a significant lower of wet kidney weight was found in the AES 1 group (1.25 ± 0.19 g), compared to the NC group (1.65 ± 0.30 g, $p = 0.026$). The average of wet kidney weight in the AES 3, and AES 2 (1.67 ± 0.39 g, $p = 0.034$) groups significantly higher compare the AES 1 group The average of kidney weight in AES1 significantly lower than the NC group.

DISCUSSION

In this study, we evaluated the effects of AES administration on BW, BL, BFP, and wet organ weight in female rats with anemia. The administration of 50 mg/200g BW AES for 28 days to female rats with anemia significantly increased the mean BW and significantly decreased the BFP percentage, but there was no effect for BL. Furthermore, the decreased BFP percentage of female rats with anemia was still higher than that of the control groups. Administration of 50 and 100 mg/200g BW AES also increased wet liver and spleen weights. These findings suggest that AES administration influences rat metabolism, increasing BW and wet organ weights.

Regarding the increased BW, our study indicated that increased AES doses were parallel to the increased BW. These findings are in line with another previous study conducted by Melati et al. (2019). Administration of 1.75 g/kg BW ethanolic extract of snake fruit seeds (SSF) for 4 weeks

increased BW significantly from 194.42 ± 2.82 g to 199.71 ± 3.03 g. However, the increase of BW in our study (28,1 g) is higher than that of Melati's study (5,29 g). The different effect of AES vs SSF is probably caused by their iron content, each 100 g of AES consists of 32.8 mg Fe, and SSF consists of 26.88 mg iron). Increased Fe absorption can stimulate the secretion of Ghrelin to regulate appetite and stimulate hunger (Ghrayeb et al. 2020). In addition, 100 g AES contains 41,10 g carbohydrate and 12,66 g protein, which increases carbohydrate, protein, and energy intake.

Another effect of Fe-induced ghrelin secretion is to stimulate the pituitary, releasing growth hormones involved in energy homeostasis. The ghrelin secretion positively correlated with growth hormone secretion (Melati et al. 2019). However, AES administration for 28 days has no effect on BL in female rats with anemia. Previous research has been carried out regarding differences in growth in Sprague-Dawley rats given low-Fe and high-Fe diets combined with copper. The BL of rats given high levels of Fe (8718 ppm) and Cu for 5 weeks increased higher than that of rats in the control group (Fe 93.7 ppm) (Ha et al. 2016). Iron deficiency in female rats can decrease their weight and growth by reduction of IGF-1 levels. This growth hormone is very important for iron metabolism and protoporphyrin synthesis. The IGF-1 secretion directly responds to growth hormone (GH) secretion, which plays an important role in growth and development (Soliman et al. 2014b). AES contains Fe and Zn, which can support growth in both rat and humans, but it takes longer administration to increase BL. AES has been shown to contain high levels of Fe. This can satisfy the daily Fe intake requirements (8-15 mg/day). The zinc content influences the increase in BL of female rats with anemia model in AES. Zinc is related to growth; previous research has shown that zinc deficiency (550 mg/kg) can affect the body length of puppies in pregnant female dogs for 2135 days (Yu et al. 2016). In this study, AES contained 10.3 mg of zinc, which can meet the daily zinc requirement of 8-9 mg/day for adolescents (Indonesian Ministry of Health 2019). Zinc, as an important element in cell metabolism, plays an important role in transcription factors and is involved in the activity of many enzymes and cellular functions (Jeng and Chen 2022). Magnesium in AES is 120% sufficient for daily needs (Zhan et al. 2014).

In this study, all female rats had normal nutrition status before AES administration. The BFP of female rats with anemia decreased at day 14 and increased again at day 28 intervention. The decrease in BFP in mice is in line with the decrease in BW body fat composition in male anemia rats, which was 6.38% (Moreno-Fernandez et al. 2019). In this research uses female rats, so it cannot be compared to compare the BFP. Fe deficiency impairs weight gain in the rats, with marked reductions in lean mass and body fat, indicating lower energy stores (Moreno-Fernandez et al. 2019). However, rats with the same BW do not necessarily have the same BFP. In this study, all rat diet was controlled with fat requirements according to daily requirements. Thus, BFP decreased along with increasing BW and BL of rats. The lack of iron intake causes changes the body composition on rats. Rats with anemia, shown decrease in cortisol secretion, which impact to decreased accumulation of body fat. Increased BFP might increase the risk of anaemia, this is due to the fact that ferritin is an acute-phase protein that is elevated by the low-grade inflammation that occurs when adipose tissues are enlarged. Obesity is associated as a risk factor for anemia (Pande et al. 2019). Reducing BFP in experimental animals can reduce chronic inflammation and serum hepcidin levels, thereby improving iron status because it increases iron absorption (Alshwaiyat et al. 2021).

Besides metabolism, iron is required for various physiological processes (Steinbicker and Muckenthaler 2013). The excessed iron levels will be stored in the body, such as the liver, muscles, bone marrow and spleen, to prevent iron toxic effects (Papanikolaou and Pantopoulos 2017). The liver weight in rats is generally 1-2% of total body weight ($\pm 10-15$ g) (Noor et al 2022). Administration of 50mg/200g BW AES increased significantly wet liver weight (8.43 ± 0.81 g) and kidney 1.67 ± 0.39 g but increased wet spleen weight (1.03 ± 0.38 g) did not differ significantly compared to the control female rats. By contrast, Sihombing et al. (2011) reported that wet liver

weight (4.39 ± 0.64 g), spleen (0.33 ± 0.03 g), and left kidney (0.52 ± 0.01 g) in normal female rats with 3 months old (Sihombing et al. 2011). Our findings indicate that the wet organ weights in female rats treated with 50 mg/200g BB AES are heavier than Sihombing's findings. These discrepancies are probably related to the different ages of female rats. We used female rats with anemia, aged 5 months old. Although AES administration positively affects BW and wet organ weights, we have some limitations during this study. At first, we did not determine other levels of micronutrients and IGF-1 required for rat body length. Secondly, the duration of AES administration is short so the BL remains unchanged. Finally, we also did not determine fat thickness and wet adipose weight, which are needed to verify BFP.

CONCLUSION

The administration of 50 mg/200g BW AES for 28 days to female rats with anemia significantly increased BW, wet liver, and kidney weights and decreased the BFP significantly. There was no effect for BL. Further research is needed to identify the levels of micronutrients and IGF-1 required for the body length. Besides that, need to determine fat thickness and wet adipose weight to confirm the effect of the intervention on reducing BFP. This study shows the potential for AES to be developed into a functional food for treating anemia.

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