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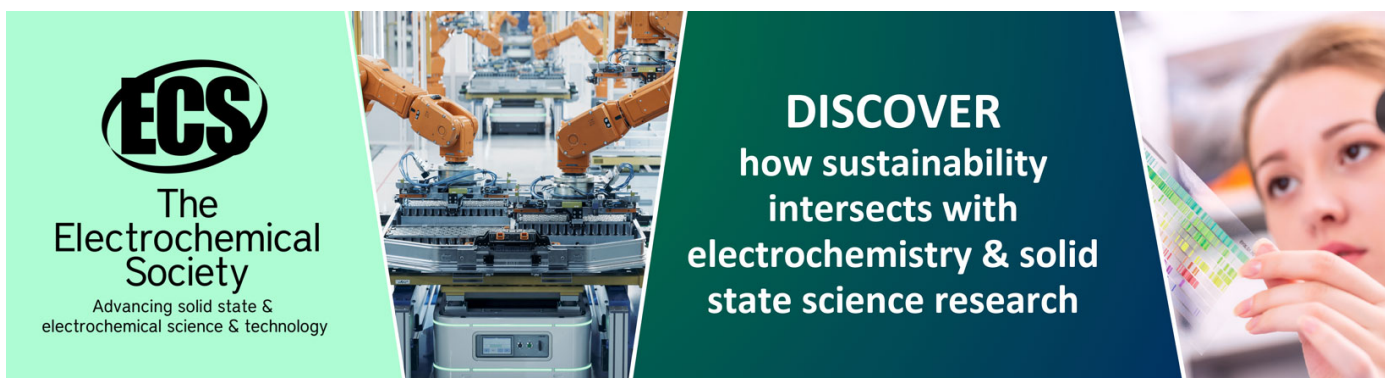
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# The Effect of Snake Fruit (*Salacca edulis Reinw.*) Seed Extract On Body Weight In Female Rats Model with Iron Deficiency Anemia

D Melati<sup>1,2</sup>, D G Tamtomo<sup>2,3</sup> and D Indarto<sup>2,4,5\*</sup>

<sup>1</sup>Human Nutrition, Postgraduate Program of Nutrition Sciences, Universitas Sebelas Maret, Surakarta

<sup>2</sup>Postgraduate Program of Nutrition Sciences, Universitas Sebelas Maret, Surakarta

<sup>3</sup>Department of Anatomy, Faculty of Medicine, Universitas Sebelas Maret, Surakarta

<sup>4</sup>Department of Physiology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta

<sup>5</sup>Biomedical Laboratory, Faculty of Medicine, Universitas Sebelas Maret, Surakarta

\*Corresponding author's email: dono@staff.edu.ac.id

**Abstract.** Anemia is a global nutritional problem in developing countries. Oral iron supplementation is recommended for anemia treatment in pregnant woman, and adolescent girl. However, this supplementation frequently has some side effects. In a previous study, snake fruit seeds naturally contain high level of iron, which is potentially used for alternative anemia supplementation. This study aimed to investigate the effect of snake fruit seed extract (SSE) on body weight (BW) in female rat model with anemia. The study was a pre-posttests control group design, 14 female rats strain Wistar were selected based on inclusion criteria. After 10 days modelling of anemia, all rats were randomly divided to 2 groups: control and treatment groups. For the remaining days, all rats received low iron feed, but added 1.75 g/kg BW/day SSE in the T group 14 days treatment. Body weight was measured before and after treatment, and all data were analyzed using independent and paired T-tests with  $p < 0.05$ . Before treatment, the average of BW in the C group is lower than T group ( $p = 0.005$ ). After treatment, the average of BW in T and C groups were statistically differed from BW before treatment ( $p < 0.001$ ). In addition, a greater number of BW was found in the T group, compared with the C group ( $p < 0.001$ ). The administration of 0.175 g/100 g BW/day SSE can increase BW in female rats model with anemia.

**Keywords :** Anemia, Iron Deficiency Anemia, Snake Fruit Seed Extract, Body Weight

## 1. Introduction

Anemia, which is characterized by reduction of hemoglobin levels below normal, becomes a global health problem from 1990 to 2013 [5]. In 2013, 1.93 billion people around the world are estimated to suffer anemia and 59.5% among them have iron deficiency anemia (IDA) [6,1]. In addition, the prevalence of anemia in Indonesian population who aged over 1 year is 21.7% [7]. Pregnant women is



one of the most vulnerable age groups and the prevalence of anemia increases from 37.1% in 2013 to 48.9% in 2018 [8].

Iron deficiency anemia is mainly found in children and childbearing women including adolescent girls and pregnant women [9,10]. The cause of IDA in those age groups can be low nutritional intake, decreased iron absorption or increased iron loss throughout the human body [11], which result in some IDA symptoms such as fatigue, weakness, low concentration and low productivity. Therefore, the IDA symptoms will cause several serious health problems like premature birth, low birth weight, miscarriage and pregnancy mortality [12].

Furthermore, recent studies have exhibited that decreased appetite, delayed growth and low weight gain were reported in rats model with IDA is known can affect growth. Rat with IDA will shown limitations in growth as expressed by low weight gain [13,14]. Based on Moreno-Fernandez et al (2019) study, rats with IDA can increase some metabolic hormones such as Thyroid-stimulating hormone (TSH), Glucose-dependent Insulinotropic Polypeptide (GIP), glucagon, insulin, cortisol and adrenocorticotrophic hormone (ACTH) and decrease ghrelin, which plays an important role in regulation of metabolism and appetite. The changes of those hormones lead to decrease of muscle mass, body fat and energy expenditure.

Iron supplementation is recommended by WHO for anemia treatment in public setting because of simple, cheap and easy [2,16]. In rats with IDA, iron supplementation improves BW for 5 weeks of treatment [13]. However, several unwanted effects such as nausea, vomiting, constipation, diarrhea and abdominal pain are reported in patients who received iron supplementation [2,17]. Therefore, herbal medicine becomes an alternative treatment for IDA. Some natural compounds have intensively been studied around the world. Snake fruit seeds are industrial waste of home industries that process snake fruits to produce chips, pie and snack bar. From data of laboratory analysis, 100 g of snake fruit seed flour (SSF) contains 26.88 mg iron, 4.56 mg zinc, 3.71 % w/w protein and 0.88 mg vitamin C [18]. Rats model with IDA which was given 37.2 g/kg BW/day SSF for 2 weeks shows a significant higher hemoglobin levels ( $14.0 \pm 0.4$  g) and BW ( $213.0 \pm 3.3$  g), compared to the control group ( $8.6 \pm 0.1$ g) ( $p < 0.001$ ); ( $203.5 \pm 4.2$  g) ( $p < 0.001$ ) [3]. However, the amount of SSF is too much if given to patients with IDA. Extraction of SSF is the best way to increase iron concentration to overcome that problem [19]. We have successfully generated seed extract (SSE) that contains 211 mg iron/100 g extract, 7.87 times higher than iron content in SSF [20]. Therefore, the aim of this study was to investigate the effect of SSE on BW in rats with IDA.

## 2. Methods

### 2.1. Extraction of Snake Fruit Seeds

Snake fruit seeds were collected from 3 home industries at Turi district, Sleman Regency, Yogyakarta. Snake seed extract (SSE) used a modification of extraction process, was done by Nursucihta et al (2014). Briefly, simplicia of snake fruit seeds was extracted using the maceration method with ethanol solvent [21]. Collected extracts of snake fruit seeds were dried using rotary vacuum evaporator for 60 minutes in Food Engineering Laboratory, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta. For the next drying process, SSE was placed into oven for 12 hours in Physiology Laboratory, Faculty of Medicine, Universitas Sebelas Maret, Surakarta.

### 2.2. Animals Model of Anemia Care

This research study used female rats, which were obtained from Nutrition Laboratory Center for Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta. These rats were placed in to a plastic cage with a wire cover and adaptation was done by controlling the temperature (12-hour light and 12-hour dark), providing unlimited access of water and controlling standard food [22]. The standard food was an AIN-93M and provided by the Nutrition Laboratory Center. The protocol of this study has been approved by the Ethical Committee of Medical Research, Faculty of Medicine, Universitas Sebelas Maret.

Rat model of anemia was adopted from Susanti et al (2018), which iron in AIN-93M standard food was removed. The amount of the standard food was given 10-15 g/day or 10% of BW rat/day for 10 days [23].

### 2.3. Experiment Design

This research study was an experimental laboratory with pre-posttests control group design. We used 14 female rats, which were Wistar species, aged 8-10 weeks old, had  $\pm 200$  g body weight, and were healthy condition. Selected female rats were randomly divided into 2 : control (C) and treatment (T) groups. The C group was given low iron feed, while the T group was given low-iron feed and 1.75 g/kg BW/day SSE for 14 days. The SSE dose was based on the calculation of daily iron requirement, performed by Susanti (2018) [3]. The SSE was dissolved in mineral water and given in the morning via intragastric administration using a 3 ml gastric bulb. The body weight of rats in both groups was periodically monitored every week using a digital Camry weight scale, to ensure that all rats were healthy.

### 2.4. Data Analysis

Data of body weight before and after treatment were presented as mean  $\pm$  standard deviation (SD). We used independent and paired T-tests to analyze body weight since those data were normal and homogenous. Because the average of body weight in C and T groups were significantly difference, multiple regression test was used and the p value was set up  $<0.05$ .

## 3. Results and Discussion

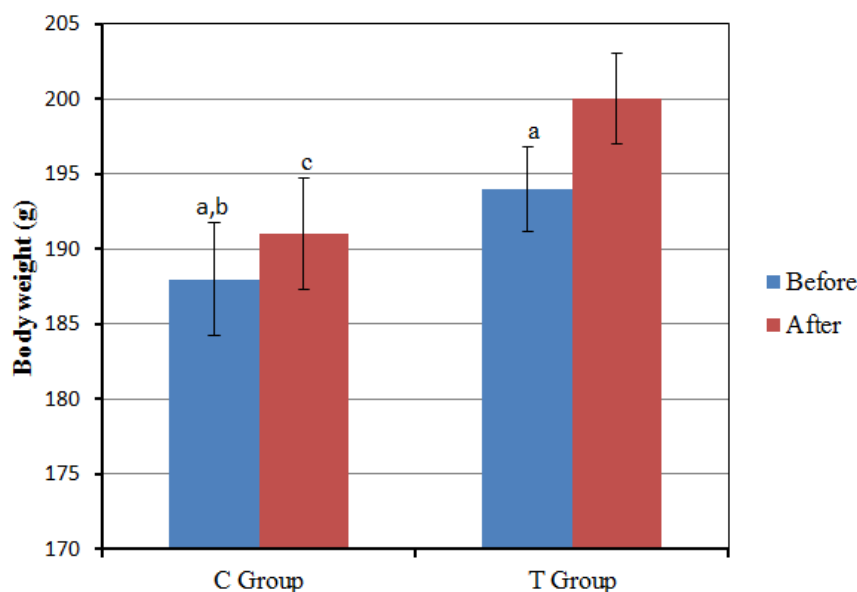
After 10 days treatment with low iron feeding, all rats showed IDA signs such as pale in the skin, claws, ears, tails and eyes, rough and sparse fur, and inactive or lethargic. The average of hemoglobin (Hb) levels in the C group was  $9.4 \pm 0.6$  g/dL lower than that of T group ( $9.9 \pm 0.7$  g/dL) with  $p=0.206$ . These results indicate that low iron feed is able to reduce Hb levels to become iron deficiency anemia. This finding indicates that there is no difference between C and T groups. Table 1 shows the results of multiple linear regression among treatment group and BW before treatment to delta BW.

**Table 1.** The Result of Linear Multiple Regression Analysis Among Treatment Group and BW Before Treatment to  $\Delta$  Body weight.

| Variable            | B Coefficients | <i>p</i> | R     | R square | Sig   |
|---------------------|----------------|----------|-------|----------|-------|
| Treatment group     | 3.067          | 0.01     |       |          |       |
| BW before treatment | -0.011         | 0.892    |       |          |       |
| (constant)          | 1.270          |          | 0.884 | 0.781    | 0.000 |

The results of multiple regression analysis show that BW before treatment was not significantly effected to weight gain, with p value was 0.892. The weight gain of female rats were effected with administration of 1.75 g/kg BW/day SSE. Based on the value of R square indicated that BW before treatment and treatment group influence amount 78% of the weight gain. Whereas other variables were not examined in this study.

From Figure 1, BW of all rats was evaluated before and after treatment with SSE. The C group had  $188.28 \pm 3.72$  g the average of BW while the average of rats BW in the T group was higher  $194.42 \pm 2.82$  g. A significant difference was observed in the average of rats BW with  $p = 0.005$ .



**Figure 1.** Body Weight Changes in Female Rats with IDA Treated with 1.75 g/kg BW/day SSE for 2 weeks. The C group (negative control) was only given low iron feed, while the T group (treatment) was given low-iron feed and 1.75 g /kg BW/day SSE. Each bar represented mean  $\pm$  SD in both group. Independent and paired T-tests were used to analyze differences between C and T groups. a indicated significant differences between before and after treatments within groups ( $p < 0.001$ ). b and c indicated significant differences of before treatment and after treatment between C and T groups respectively ( $p = 0.005$  and  $p < 0.001$ ).

After treatment, the average rats BW increased by  $2.28 \pm 0.48$  g in the C group and  $5.28 \pm 1.11$  g in the T group. The increased BW in both group reached significantly with  $p < 0.001$ , compared to the average of BW before treatment. Rats in the C group had  $190.57 \pm 3.69$  g the average of BW, significantly lower than the average of BW in the T group ( $199.71 \pm 3.03$  g;  $p < 0.001$ ).

After the administration of SSE no abnormal clinical signs or macroscopic findings were observed. Female rats that receive SSE survived during the study period, the behaviour was normal, and the condition of their skin and fur, eyes, nose, oral cavity and abdomen were normal.

IDA rats model without iron supplementation, seen in the C group had low metabolism, which results in growth disorders like BW. Iron deficiency in the IDA rats model can cause reduction of enzyme activities and oxygen supply to all cells in the rats tissues. As a result, cellular energy will decrease and disturb all metabolisms in the rats body [16,15]. The results of this study show that 1.75 g /kg BW/day administration of SSE increases rats BW for 14 days. It is not surprised that iron administration derived from fruit and plant extracts can increase BW. Cui et al (2018) use polysaccharide-iron III extracted from *Enteromorpha prolifera* for treatment of anemic rats. Higher average of BW was observed in rats treated with polysaccharide-iron III, compared to the negative control group [14]. The same results of increased BW are also found in anemic rats given cocoa and mango extracts [24].

The decrease of body growth and weight loss on IDA can be considered with several mechanism. Tang et al (2014) study found that rats with IDA would be loss of appetite and shown apathy, this was due to decrease on ghrelin hormone. Ghrelin hormone will decrease if low iron stores in the body such as on IDA patient [25,26,27]. Ghrelin is a hormone secreted in the stomach and duodenum, and stimulates the central and peripheral nervous system pathways. Ghrelin functions is regulate appetite

by stimulates hunger. The ghrelin plasma will increase before eating and decrease after eating [28]. Decreasing ghrelin levels from normal limits can cause cachexia, increase corticosterone secretion and induce catabolism [29,30].

The lack of iron intake causes changes in body composition on rats. Rats with IDA, shown decrease in cortisol secretion, which impact in decreased accumulation of body fat and increase energy expenditure. A decrease in the cortisol hormone causes an increase in lipolysis, plasma triglyceride and cholesterol, thus affecting the body fat composition in rats with anemia [31,32].

Other studies found IDA impaired the decrease in thyroid hormone. Thyroid hormones cause central nervous system changes, which play a role in the thermoregulatory response. Low thermoregulation responses cause a decrease in basal metabolic rate (BMR) and BW [33]. Increased TSH levels in IDA patient cause a compensatory mechanism by increasing T3 and T4 which relates in decreased bone formation and increased resorption [34]. Delayed skeletal development causes low weight gain [15].

After administration of Fe therapy in IDA patients, Hb levels returned to the normal level [35]. The normal Hb levels are associated with O<sub>2</sub> consumption, CO<sub>2</sub> production and energy expenditure are higher in IDA patients [15]. Increased appetite also was found in IDA patients [36] so that there was an increase in energy, protein and carbohydrate intake [26]. Administration of Fe therapy for IDA has been shown to improve physiological function and metabolism of IDA patients so that normal growth and weight gain can be achieved.

#### 4. Conclusion

Administration of 1.75 g /kg BW/day SSE for 2 weeks increases body weight in IDA female rats model, higher than the control group. Further investigation is needed to find out the mechanism of SSE increasing body weight.

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