International Conference on Biology Education, Natural Science, and Technology
Vol. 1 No. 1 (2023)

Number of Erythrocytes of White Rats (*Rattus norvegicus*) Sonded with a Combination of Porang Tuber and Moringa Leaf Extract

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KEYWORDS:
Glucomannan
Moringa leaves
Number of erythrocytes
Obest rat
Porang tubers

ABSTRACT

Overcoming obesity which is increasing in the pandemic era. Obesity or overweight is an abnormality in the amount of body weight that results in excessive accumulation of body fat and can pose a risk of other diseases. This also affects the supply of the number of erythrocytes that enter the body. This study aims to determine the number of erythrocytes of obese white rats (*Rattus norvegicus*) that were sounded with a combination of pouring tuber glucomannan (*Amorphophallus mulleri* Blume) and moringa leaf extract. This research method used a true experimental which was prepared using a Completely Randomized Design (CRD) in the form of a Posttest Only Control Design. The data obtained were analyzed using One Way ANOVA SPSS version 20 followed by the DMRT test. The results of this study indicated that the combination of pouring tuber glucomannan and moringa leaf extract affected increasing the number of erythrocytes in obese white rats. Administration of pouring tuber glucomannan and moringa leaf extract increased the number of erythrocytes, with the order of increasing P7 (60:40), P5 (80:120), and P3 (100:100). With these results, it can be concluded that the combination of pouring tuber with moringa leaf extract is effective in increasing the number of erythrocytes in obese people.

1. INTRODUCTION

Since the COVID-19 pandemic era, the government has issued a Work From Home (WFH) policy that requires all activities to be done from home or online. With this policy, it changes the way of life and also the environmental conditions of the community, thus bringing some emerging problems. One of them is an increasing number of cases of obesity in the community. This is due to the presence of restrictions on leaving the house, which indirectly causes the time to play gadgets as well as the habit of snacking snacks to increase. According to (Mutia et al., 2022), based on the results of the analysis of the relationship between dietary patterns and the incidence of adolescent obesity during the covid-19 pandemic, 11 (23.9%) obese students with frequent diets were obtained, while non-obesity students with regular diets was 35 (76.1%) students. There is a link between dietary patterns and the incidence of adolescent obesity during the Covid-19 pandemic. A diet where a person consumes foods that tend to contain high energy, carbohydrates and fat and low fiber can lead to obesity.

Obesity is an abnormal amount of body weight caused by excess body fat. Obesity is also associated with increased inflammation and abnormal body metabolism, thereby increasing the risk of insulin resistance, type 2 diabetes, stroke, and cardiovascular disease (Septiyanti & Seniwati, 2020). Based on the national data of obesity in Indonesia, the number of centrally obese

people aged >15 years of age increased in 2018, which is 31.0% compared to 2013. In contrast, the national prevalence of obesity in the proportion of adults aged >18 in 2018 ranged from 21.8% to 14.8% in 2013 (Depkes, 2018). There is a need for a solution to the growing problem of obesity. The prevalence of central obesity among the population in Indonesia was 31%. The prevalence of central obesity in adolescents continues to increase every year. There was an increase in cases of 2.9% from 2007-2010. Then, 2010-2013 increased by 4.9%, and 2013-2018 by 4.4%. DKI Jakarta province ranks second in the province with 42% of the population aged ≥15 years suffering from central obesity.5 The prevalence of obesities tends to be higher in teenage girls compared to teenage boys, which is 1.5% in teenaged girls and 1.3% in teenager boys (Zaki et al., 2022).

Obesity is influenced by several factors, such as excessive food intake, a lack of physical activity, technological advances, and genetic factors (Praditasari & Sumarmik, 2018). There are also treatments for obesity. Obesity is usually dealt with through diet. Some people have done this way to lose their weight. Diet is part of the key to weight loss success because it affects how many calories enter the body. A high-fiber diet that recommends the consumption of foods high in fiber becomes one of the diets recommended for obese people. Through the implementation of this high-fiber diet, one is expected to be able to modify the arrangement and portion of daily foods by increasing the high-fiber content more than usual or exceeding the recommended daily intake of fiber. This diet aims to provide food intake according to the nutritional needs of the body and is high in fiber so that it can trigger the intestinal peristaltic movement so that the process of urination becomes normal. A high-fiber diet includes a diet that is good for health, easy to do and affordable. This is because foods that are high in fiber are safer for the human body, and most have a purchase price that is not too high. Therefore, high-fiber diets can be applied by all groups of society (Zaki et al., 2022).

There is also an alternative, natural way to deal with obesity is by using natural ingredients such as leaf extracts and grain plants. Glucomannan is a polysaccharide compound of 67% D-manose and 33% D-glucose, with properties between cellulose and galactomanan, which crystallize forming fine fibrous structures. The main content of the porridge plant is glucomannan, that is found in the part of the bulbs. Glucomannan is a hydrocoloid that has the ability to thicken and form a gel so it is widely used in various industries such as food, chemistry, biotechnology and pharmaceuticals (Wardani et al., 2021). In the field of health, glucomannan lowers cholesterol and is used to prevent disease by lowering the blood sugar response. Glucomannan can be used as a functional nutrient-provider food ingredient that gives a sense of satiety because it contains one of the active ingredients contained in the foam and can also be used by dissolving it in water. Glucomannan also plays a role in decreasing and inhibiting blood lipids, preventing cancer, dealing with constipation, and reducing obesity. (Alamsyah, 2019).

Amorphophallus muelleri Blume is one of the most promising export commodities for Japan, China, Vietnam, Australia, and other countries (Pertanian Kementerian, 2020). Indonesia still exports pork in the raw form of chipped pork or crude pork flour. Grapefruit is widely used as a cosmetic, glue, and supplement. Proper processing of light can be profitable in its sale and use because light cannot be consumed directly. The use of pork as a food material in Indonesia is controlled by the high content of calcium oxalate, which is 0.19% (Wahyuni et al., 2020). This is due to the content of oxalate crystals, which can cause an allergic reaction or irritation in the mouth and damage the organs in the body. Based on research (Saputro et al., 2014) the level of

glucomannan obtained after the purification process to remove larger oxalate crystals ranged from 36.69% to 64.22% compared to the pre-purification flour of 28.76%.

In this study, not only is glucomannan important in dealing with obesity, but there is also a role for leafy vegetables. Kelor is a type of multiguna plant, and almost all parts of the kelor plant can be used as antimicrobial material. The parts of the clay plant that have been proven to be antimicrobial substances include leaves, seeds, oils, flowers, roots, and skins (Luluk Sutji Marhaeni, 2021). The leaves are known to contain alkaloids, saponins, phitosterols, tanins, polyphenols, phenols, and flavonoids that act as antioxidants (Saputra et al., 2020). The leaves are also known to contain 120mg of vitamin C per 100g. The role of flavonoids and alkaloids as hypoglycemic agents works through intra- and extra-pancreatic mechanisms, playing a role in the repair and regeneration of pancreatic cells and stimulating the release of the hormone insulin. Arkanoid stimulates the sympathetic nerve and increases insulin secretion. Giving decoctions of leaves can lower blood sugar levels in diabetics. (Lakshita, 2017).

Overweight and obesity also increase the risk of anemia due to the occurrence of fat storage. Fat accumulation in overweight or obese people causes inflammatory reactions and increased cytokines (IL-6), thus stimulating increased hepsidine and decreasing iron absorption in the intestines. In addition, the accumulation of fat in the liver can trigger the formation of lipid peroxide, which damages the membranes of red blood cells and disrupts the synthesis of hemoglobin. The accumulation of fat in the liver can trigger the formation of lipid peroxide,e which will ultimately affect the iron metabolism process so that free radicals occur. This causes the synthesis of Hb tob noto run perfectly. In the final stage, hemoglobin decreases in number and erythrocytes decrease,e sot anemia can occur. The accumulation of fat in the liver can trigger the formation of lipid peroxide,e which will ultimately affect the iron metabolism process so that free radicals occur. This causes the synthesis of Hb tob noto run perfectly. In the final stage, hemoglobin decreases in number and erythrocytes decrease,e sot anemia can occur (Pasalina et al., 2019). A person is called anemic when their red blood cells are lower than the reference value. The reference value in this study for men is 4.50–6.50 x106µl and for women is 3.80–4.80 x 106µl. The results of the erythrocyte examination are important to know. If a person has an abnormality in the number of erythrocytes, then the results of the erythrocyte examination should be reliable. (Haiti et al., 2021).

White rats (*Rattus norvegicus*) are a trial animal that is often used in obesity reduction research because they have more agility compared to other trial animals, are easy to handle, can be obtained in large quantities without being too expensive, and have valid repeat results. (Wistar et al., 2019). White rats (*Rattus norvegicus*) are often used as food tests and food deficiency tests on all types of animals, including humans (Rejeki et al., 2018).

Based on reference searches, research on the number of white rats' erythrocytes fed a combination of porang tuber glucomannan and moringa leaf extract, researchers saw the effect of high protein and fat on the process of erythropoiesis which affected the number of erythrocytes. In addition, researchers also looked at the effect of porang glucomannan and porang leaf extract on reducing obesity. The purpose of this study was to determine the effect of the glucomannan content of porang tubers and moringa leaf extract on the increase in the number of erythrocytes in obese rats.

2. MATERIALS AND METHODS

2.1. The Location of The Study

The research was conducted from September 2022 to May 2023, from the selection of titles to the drawing of conclusions. The study was conducted at the university's Eleven March Animal Experiment Laboratory as a place of maintenance, treatment, and blood sampling. The research was also conducted in the Biology Education Laboratory of the Keguruan Faculty and Education Sciences of the Muhammadiyah Surakarta University as a place of examination and analysis of the number of esritrocytes under a microscope.

2.2. The Materials and Tools Used

The tools used in this research include: (1) A set of hemositometers consists of two boxes that include a leucocyte pipette and an erythrocyte pipette, a counting chamber, and a sucker. (2) 1 cup of petri (3) Glass and deck glass for 4 sets (4) Olympus CX 23 binocular microscope (5) Counter-count to two. (6) 1 tissue, and (7) The blood tubes contain the anticoagulant EDTA.

The materials used include: (1) blood samples of white Wistar rats (Rattus norvegicus) with obesity from 4 treatment samples; (2) Reagen Hayem, and (3) The Aquades.

2.3.Determination of The Sample

The sample is taken at the UNS Animal Test Laboratory by preparing the microhematocrit and divided into two, while the mice are held properly, then the microhematocrit is scratched on the medial canthus of the eye under the eyeball towards the foramen opticus. The blood is stored in the blood tubes that already contain the anticoagulant EDTA. Before taking blood, the mice are first fasted overnight and then blood is taken from the retro-orbitalis sinus. Blood sampling is carried out slowly so that it does not hurt the animal and is guarded so that no clots occur. In the microtube, add a 0.1% heparin anticoagulant solution and warm it slowly to prevent licking and clotting. Given that hematological measurements are cellular measures, the presence of clots can affect the outcome, as well as cause collapse in the measuring machine (Rosidah, 2020).

2.4. Measurement Methods

The method used in this study is (1). Experimental method with a post-only control design. (2). Documentation method: this method helps in taking pictures of research used in documenting tools, materials, research processes, and research results. (3). The library method, the technique of collecting data by library, is carried out by searching for references in books, national and international journals, existing scientific works, and scripts and studying the same scope. This method is used to support the creation of library reviews and speeches.

2.5.Experimental Design

The study used a true experimental method composed of a complete random plan (RAL) of a posttest-only control design using 4 groups of treatments and each treatment having 2 repetitions. The details are as follows:

1). Treatment 1 (Control): Control group with normal feeding (P1).

2). Treatment 3: high-fat dietary feed treatment group + glucomannan and moringa leaf extract 100 mg/kg BBB (P3)

3). Treatment 5: high-fat dietary feed treatment group + glucomannan and moringa leaf extract, 80 mg/kg BBB: 120 mg/kg BBB (P5)

4). Treatment 7: high-fat dietary feed treatment group + glucomannan and kelor moringa extract (60 mg/kg BBB): 40 mg/kg BBB (P7)

2.6.Stages of Research Activities

The stages of activity in this study include: (1) level of maintenance; (2) level of treatment; and (3) Stage Examination of the number of erythrocytes by installing the aspirator pipette on the thoma pipette, preparing the hayem reagent to avoid contamination, pouring the hayem reactor on the glass beaker, and before pressing the blood, first homogenize the blood. Blood in the pipette to the limit of 0.5 ml. Clean the pipette with tissue. The pipette uses the same pipette, trimming the hemoglobin reagent to the limit of 101, removing the aspirator from the pipet, and homogenizing the pipette by stretching for about 1 minute. Execute a blood test using a binocular microscope by placing a blood sample on the deck glass and the counting chamber. Test and calculate the number of erythrocytes.

2.7. Data Calculation and Analysis

The result of the next examination of the number of erythrocytes will be calculated using the following formula:

Jumlah Eritrosit : $\frac{n}{v}\ x\ p$

Where (n) is the total cell counted, (v) is volume (p x l x t) x number of boxes, and (p) is a dilution of the sample on the thoma haemositometer pipette.

Then the results of the study were analyzed statistically using the one-way analysis of variance (ANOVA) in SPSS version 20, followed by Duncan's multiple range test (DMRT) to find out the rates between the treatment groups.

3. RESULTS AND DISCUSSION

The results of the analysis of the number of erythrocytes of fat white rats dissected with a combination of porang tubers and moringa leaf extract showed that there was a difference in the dose of the treatment group with the number of normal white rats, namely the first treatment group was positive in the control group (P1) with normal feeding and showed the number of erythrocytes 7.16 million /l. In the P3 treatment group, the dose of a mixture of porang tuber glucomannan and moringa leaf extract with a ratio of 100:100 mg/kgBB showed an erythrocyte count of 6.20 million/L. Then in the P5 treatment group with a combined dose of porang tuber glucomannan and moringa leaf extract 80:120 mg/kgBB, the red blood cell count was 5.05 million/L. And in the P7 treatment group with a dose of 60:40 mg/kg BB, the number of erythrocytes was 4.55 million/L.

In the number of erythrocytes, based on the statistical tests in the treatment group, there was a distinct and significant increase that can be seen in the following table:

Table 1: Results of statistical tests on measuring the number of erythrocytes

ANOVA

Jumlah Eritrosit

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8.269	3	2.756	220502.333	.000
Within Groups	.000	4	.000		
Total	8.269	7			

Description: There were significant differences in the number of erythrocytes between the doses of the treatment groups (P 0.05).

Based on the results of the Anova test, it can be seen that the significance value of 0.000 is less than 0.05 (P 0.05), so the different treatment is real or has a significant influence on the blood erythrocyte number. Because the treatment is different, further tests are carried out using the Duncan test, with the following results:

Table 2. Results of the Uji DMRT (*Duncan Multiple Range Test*)

Jumlah Eritrosit
Duncan

Kelompok Perlakuan	N	Subset for alpha = 0.05			
		1	2	3	4
P7	2	4.5500			
P5	2		5.0500		
P3	2			6.2000	
P1	2				7.1650
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

The Duncan test results above can be concluded as follows:
1. P1 treatment is different from P7, P5, and P3 treatment, and vice versa.
2. P7 treatment is different from P1, P5, and P3 treatment, and vice versa.
3. P5 treatment is different from P1, P7, and P3 treatment, and vice versa.
4. P3 treatment is different from P1, P7, and P5 treatment, and vice versa.

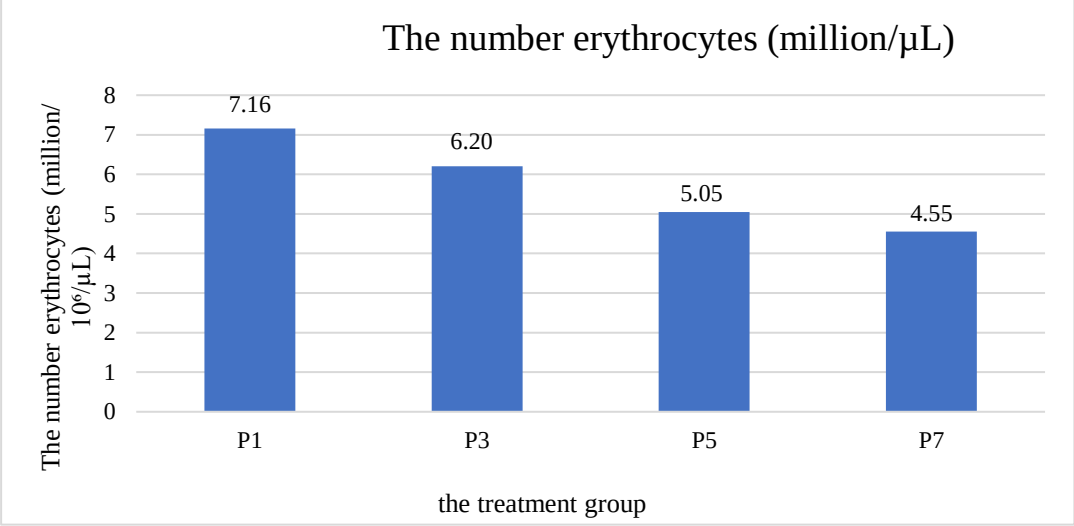
Table 3. The Duncan test results above can be made in the form of a notation, which is as follows:

Treatment	Average	Noted
P7	4.55	a
P5	5.05	b
P3	6.20	c
P1	7.16	d

Letters (a) indicate the lowest to (d) the highest.

Treatment with the same notation is not really different; on the contrary, treatment with different notations is really different. Based on the above table, it can be concluded that treatments

are really different from each other because they have different notations. The normal total number of erythrocytes in white rats ranges between 7 While the total erythrocytes in this study were in treatment group 1, a positive control group (P1) with normal feeding showed a number of erythrocyts of 7.16 million/L, which correlated with the letter "a," which indicates having the highest result among all treatment groups. While the treatment group P3 doses of a mixture of glucomannan porang tuber and moringa leaf extract with a ratio of 100:100 mg/kgBB showed a number of erythrocytes of 6.20 million/L, the letter "b" was connoted, indicating that the result was a moderate number. Then, in the P5 treatment group, a combination dose of glucomannan porang tuber and moringa leaf extract of 80:120 mg/kgBB showed a number of red blood cells of 5.05 million/L with the connotation "c," which showed results with small amounts. And in the treatment group P7 with a dose of 60:40 mg/kgBB, the number of erythrocytes was 4.55 million/L, associated with the letter "d," referring to the lowest outcome among the other treatment groups.



Description: P1 (control), P3 (100:100), P5 (80:120), and P7 (60:40)

Figure 1: A graph of the average number of erythrocytes in obese white mice that have been treated with a combination of glucomannan and leaf extract.

The average total range of erythrocytes resulting from this study is (3.16–5.10) x 10^6/µL. At P1 (normal), that is, 7.16 x 10^6/µL, erythrocytes are within the normal range of the number of white rats erythrocyts based on the (Dewi et al., 2022) the normal number of erythrocytes in white rats ranges around between (7-11)x10^6/µL. This is due to the treatment one, given normal feeding and does not contain high fat, so the number of erythrocytes is still within the normal range. At P3 (100:100) 6.20x10^6/µL, this number decreases from the normal range of white-mouse erythrocytes ranging from (7-11) x10^6 /µL. This is due to the dose (100:100) of glucomannan porang tuber and moringa leaf is not high enough to make the normal number of red blood cells in obese rats. However, in these three treatments, the number of erythrocytes is higher than in the treatments P5 (80:120) 5.05x10^6/µL and P7 (60:40) 4.55x10^6/µL. According to (Sundayani et al., 2016),the number of erythrocytes is influenced by gender, age, body condition, daily variation, and stress conditions. The number of erythrocytes is also due to the size of the blood cell itself. An abnormal number of erythrocytes in mice was caused by mice who had not reached the age of 3 months, weighed below the standard weight (250 g), and were at the time of blood collection.

The results of statistical tests showed that the administration of a combination glucomannan porang tuber and (*Amorphophallus muelleri* Blume) and moringa leaf extract (*Moringa oleifera*) with different dosage levels had a real effect (P 0.05) on the decrease and increase in the number of white rats erythrocytes. The lack of precursors such as iron and amino acids that help the erythrocyte formation process causes a decrease in the number of erythrocytes. This condition can be caused by a disruption in the absorption of decreased nutritional values in the feed given, which will affect the organs that play a role in the production of blood cells. Other factors that affect the difference in the number of erythrocytes are age, nutrition, blood volume, species, height, location, season, time of sampling, and type of anticoagulant (Zuraidawati et al., 2019).

The effect of the administration of the combination glucomannan porang tuber and (*Amorphophallus muelleri* Blume) and moringa leaf extract (*Moringa oleifera*) on the total number of erythrocytes through the Duncan trial can be known that the treatment of P1, P3, P5, and P7 had a significant difference in the number of the erythrocyte, but when seen a decrease in the amount of obese mice erytrocyte from the normal number of mice, the most observed in the treatment group P7 (60 mg/kgBB: 40 mg / kgBB) with the erytrocyte number of $4.55x10^{6}/\mu L$. Glucomannan porang tuber helps in increasing the number of red blood cells against obese people as found in the study (Alamsyah, 2019), which states that the fiber of the gallbladder will inhibit the process of recycling and bile salts will be secreted through the feces, so that only a small amount of bile salt is returned to the liver. This will stimulate the liver to form new bile salts and will take cholesterol from the blood as a bile salt-forming material. The more bile salts are formed, the less cholesterol circulating in the blood.

The average obese person has a low iron intake. When a good-quality diet is not balanced, such as with high-energy foods, low iron intake, and absorption enhancers, it can support the onset of anemia. 20% of obese subjects had a low iron status, and 94% had an average low-quality diet score of 52.04 5.2 (Nurramadhani et al., 2019). In the research conducted by (Suzana et al., 2017), Moringa extract contains 27.33% protein by Kjeldahl's method. The total iron content in moringa extract is an average of 14.67 mg/100g. The extract containing vitamin C is 759.05 mg/100 g with HPLC (*High Performance Liquid Chromatography*). The analysis of the nutrient levels in the capsules used in the study was based on the amount of iron in 100 g of the extract. If one capsule containing 700 mg of iron extract contains 0.103 mg of iron per capsule, vitamin C is 5.313 mg/cappule and the protein content is 39.043 mg in one capsule.

Therefore, the administration of glucomannan porang tuber and the moringa leaf extract is expected to increase the number of erythrocytes in patients with hypercholesterolemia, so that it can be concluded through Table 1 that the use of the combination of glucomannan porang tuber and the moringa leaf extract can increase the amount of the erythrocyte, with an increased sequence of P7 (60:40), P5 (80:120), and P3 (100:100).

4. CONCLUSIONS

The addition of a combination of porang tuber glucomannan (Amorphophallus muelleri Blume) and moringa leaf extract (Moringa oleifera) had an effect on increasing the number of erythrocytes in white rats suffering from obesity but did not affect the decrease in the number of erythrocytes. The combination of porang tuber glucomannan and Moringa leaf extract with a dose of P3 (100 mg/kgBW : 100 mg/kgBW) was able to increase the number of erythrocytes in obese

white rats. This research can be continued to calculate the differential erythrocytes and see the effect of giving a combination of porang tuber glucomannan and Moringa leaf extract on antibodies.

5. ACKNOWLEDGMENTS

The author thanks the Biology Education Study Program for providing funding for the incobest article.

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