Effectiveness of Taro Leaf Extract in Increasing Hematocrit Levels and Mean Corpuscular Volume (MCV) in Wistar Rats Induced by $NaNO_2$

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Abstract. Anemia is a condition of the reduced number of red blood cells or erythrocytes resulting in decreased ability to bind oxygen in the body as indicated by reduced hemoglobin levels, hematocrit, and Mean Corpuscular Volume (MCV). Ethanol taro leaf extract contains phytochemicals that can potentially increase hematocrit and MCV levels. This study aims to provide scientific information on the effect of taro leaf extract on hematocrit and MCV levels in male Wistar rats induced by NaNO2. This study used a qualitative true experimental test with a pre-post randomized control group trial design using 30 male Wistar rats and divided into 5 negative control groups (K1), positive control (K2), and taro leaf extract treatment with a dose of 100mg/kgBW (P1), 200mg/kgBW (P2), and 400mg/kgBW (P3). Statistical data processing was analyzed using the Wilcoxon test to assess the differences between groups before and after taro leaf extract treatment. It did not provide a statistically significant effect but experienced changes in the mean hematocrit levels and MCV count in the post-test group of taro leaf extract treatment with one-way ANOVA test ($p > 0.05$).

Keywords: Anemia, Hematocrit, MCV, taro leaf extract, NaNO2

1 INTRODUCTION

The problem of anemia is still a health problem that needs attention in Indonesia. Anemia can be seen from the decrease in hemoglobin levels and the number of red blood cells.[1] The cause of anemia can be caused by a lack of protein, minerals, and vitamins as well as infectious diseases. The cause of anemia is 50-80% due to iron deficiency. Prevention and treatment of anemia in addition to taking blood-boosting tablets, can also be done by consuming foods that have high nutritional content, one of which is taro leaves. Consuming foods that have high nutritional content has the potential to increase hemoglobin levels, hematocrit, and the number of red blood cells. Around ten percent of the global population consumes taro. Taro plants are rich in important nutrients including carbohydrates, proteins, and fats, as well as minerals and

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vitamins that can be used to make various medicinal products.[2] Taro can be consumed from the leaves, stalks to the stems.

As a source of food and medicinal herbs, taro plants are known for their many uses. Saponins, tannins, alkaloids, steroids, and terpenoids are all found in taro stems and leaves.[3] Taro leaf extract contains compounds that can increase blood hemoglobin levels. These compounds include vitamin A, vitamin C, flavonoids, phosphorus, and many more. Similar to vitamin C which helps the production of hemoglobin, vitamin C also facilitates the absorption of iron from food, which is needed for further processing into red blood cells.[4] The reason for giving NaNO2 to experimental mice is because nitrate is a chemical that is not excreted by the body, so it can accumulate and cause health problem.[5] $NaNO_2$ induction for 20 days can cause hematological and rheological disorders.[6] The mechanism of $NaNO_2$ works by inhibiting the distribution of O_2 which causes the formation of methemoglobin. The reaction between NO from $NaNO_2$ and hemoglobin will form nitrosohemoglobin which causes hemoglobin levels to decrease so that the ability of erythrocytes is inadequate in carrying out its function to carry O_2 throughout the body.[7] The mechanism of $NaNO_2$ which is a free radical will cause hypoxia in the organs of mice. The free radicals formed can affect the erythrocytic membrane which can disrupt the hematopoiesis process and damage to erythrocytes. The damage to erythrocytes that occurs is the lysis of erythrocytes which causes a decrease in hemoglobin levels.[8,9] This study aims to analyze the effectiveness of taro leaf extract administration in increasing hematocrit levels and Mean Corpuscular Volume in Wistar rats induced by $NaNO_2$. Anemia diagnosis examination can be diagnosed by erythrocyte index (Mean Corpuscular Volume and hematocrit).

2 METHODS

2.1 Research Design

This study is a qualitative true experimental test using a pre-post randomized control group trial design method. The sample in this study was 30 male Wistar rats (Rattus norvegicus) weighing 150-250 grams and aged 6-8 weeks. Used ats aged 7 and 10 weeks because the average value of the erythrocyte index at that age tends to be lower than the literature but is within the normal range. The rats were placed in separate cages for each group. The rats were divided into 5 groups, namely the negative control group (K-), the positive group (K+), and the treatment groups P1, P2, and P3 consisting of 5 rats for each group. The test rats were acclimatized for 7 days to reduce the effects of stress in the new environment and were fed ad libitum so that the rats' metabolic processes were not disturbed. During the adaptation period, some rats died during the research process so only 27 rats were analyzed for blood samples. Division of test animal groups: Negative control (K-) Ad libitum; Positive control (K+) Ad libitum + iron tablets 4.5mg/kgBW; Dose 1 (P1) Taro leaf extract, 100mg/kgBW for 21 days; Dose 2 (P2) Taro leaf extract, 200mg/kgBW for 21 days; Dose 3 (P3) Taro leaf extract, 400mg/kgBW for 21 days.

2.2 Induction of $NaNO_2$ in Rat

Administration of $NaNO_2$-induced rats to cause anemia with a dose used for each mouse is ± 12.5 mg NaNO2/mouse dissolved in 1 ml of aquadest. The sample used was 30 mice divided into 3 groups, namely the negative control group (K1) without treatment, the positive control group (K2) which was treated with anemia and given $NaNO_2$ for 1 day without diet extract, and the treatment group (K2) treated with anemia which was given NaNO2 for 1 day as much as 2 ml/mouse given using a probe. On the 9th day, blood sampling of the experimental mice was carried out using the retroorbital plexus method which was punctured through the eye vein with microhematocrit and inserted into an Eppendorf tube up to 0.5 µl or 1-5 ml, then the mouse blood was incubated using an automated hematology analyzer to check the hematocrit levels and the number of MCVs analyzed at the Semarang City Animal Health Laboratory, Srondol.

2.3 Data Collection and Data Analysis

The maintenance and treatment of test animals were carried out in the Animal Physiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Semarang State University (UNNES), while the biology laboratory at the same institution was tasked with determining and producing ethanol extract of taro leaves. Ethical approval was obtained from the Health Research Ethics Commission, Faculty of Public Health, Muhammadiyah University of Semarang with No.526 / KEPK-FKM / UNIMUS / 202. The data were tested for normality using the Shapiro-Wilk test, and the homogeneity of data variance using the Levene test, because the number of samples in this study was less than 50. The results of the Wilcoxon test showed statistical significance before and after the study. testing differences between treatment groups. To determine the effective dose between the treatment group and the control group, the data obtained from this study were statistically analyzed using advanced methods. The data were in the form of average blood cell volume and hematocrit levels of experimental mice. The tests used were one way ANOVA and post hoc test with a confidence level of 95%.

3 RESULT AND DISCUSSION

The production of taro leaf powder (Colocasia esculenta L. Schott) from the drying results can produce a yield of 15.4% from the initial material weight of 3000 grams and the resulting powder weight of 462 grams. Continued with the yield of taro leaf powder simplicia which obtained a yield of 11.64% resulting from the weight of taro leaf extract of 52.4 grams from the initial material weight of 450 grams. This maceration method is in line with previous studies to obtain leaf extract yields and can be used as antioxidants and medicines.10 The extraction method in this study was carried out by maceration using 96% ethanol solvent and obtained leaf extract that was green-reddish black.

At the beginning of the study, the sample size of the experimental mice was 30 male white mice (Rattus novergicus). During the study, only 27 mice were taken and blood samples were analyzed, there were 2 mice that died in group P1 and group P2, and mice that experienced a decrease in body weight of ⩾10% of their initial weight in group P3. Based on the results of direct observations in the animal physiology laboratory, the experimental mice experienced a weak condition and did not move much after being given sodium nitrite for 1 x 24 hours. The experimental mice were no longer weak and moved a lot after being given taro leaf extract treatment for 21 days.

Table 1. Data on changes in body weight

Group	Body Weight Pre-test (g)	Body Weight Post-test (g)	Body Weight Delta
K1	152,0±15,54	162,8±15,35	10,8±2,17
K2	147,2±14,92	154,8±11,03	7,6±4,39
P1	151,0±14,50	159,2±13,66	8,2±2,28
P2	152,0±7,23	160,4±7,30	7,8 ±1,64
P3	142,4±10,45	150,4±7,56	8±4,06

Description:
K1 = Negative control group, without taro leaf extract administration
K2 = Positive control group, ad libitum + 4.5 mg/kgBW iron tablets
P1 = Treatment group with taro leaf extract 100mg/kgBW
P2 = Treatment group with taro leaf extract 200mg/kgBW
P3 = Treatment group with taro leaf extract 400mg/kgBW

Based on Table 1, it is known that the difference in body weight between the Pre-Test and Post-Test experimental mice, it can be analyzed using the paired samples t-test followed by the ANOVA test to be more significant. The results showed that body weight increased without the administration of taro leaf extract. The control group (K1) experienced a higher body weight (10.8 ± 2.17 gr) than the treatment group. These results follow what was found in the literature.[5] With 23 g of carbohydrates, 1.9 g of protein, and 0.2 g of fat per 100 g of taro, weight gain can help the healing process of anemia.[2]

According to Widodo et al., anemia is characterized by a decrease in hemoglobin levels below 11 g/dl, hematocrit, and the number of red blood cells (erythrocyte count).[11] Figure 1 shows the effect of taro leaf extract on hematocrit levels.

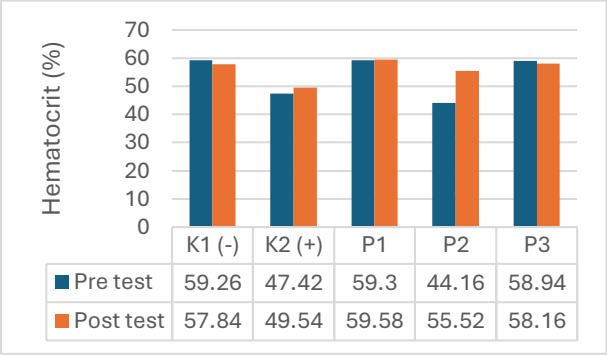

Fig. 1. Changes in Mean Hematocrit Levels of Rats

Table 2. Analysis of hematocrit data before and after intervention

	Pre-test (%)	Post-test (%)	Delta	*p**
K1	59.26±4.24	57.84±4.71	-1.42±7.474	0.693
K2	47.42±17.88	49.54±6.64	2.12±16.19	0.500
P1	59.30±3.82	59.58±7.13	0.28±8.29	0.943
P2	44.16±17.03	55.52±6.02	11.36±19.18	0.256
P3	58.94±3.28	58.16±2.63	-0.78±5.75	0.777
*p***	0.058	0.080	0.700	

Description:
*p** = Paired t-test
*pp*** = Anova test

According to Douglas and Wardrop, the range of normal hematocrit values in rats is between 37.6%-51%.[12] The average hematocrit values of groups P1 (0.28±8.29) and P2 (11.36±19.18) increased more than P3 (-0.78±5.75). This shows that giving taro leaf extract at a dose of 100mg/kgBW and 200mg/kgBW is effective in increasing hematocrit values. According to Mc Donald (1973), the lower the concentration of nutrients, the greater the absorption of food by the rat's body. It is known that the delta hematocrit levels of male rats were highest in the pre-post P2 group with a dose of taro leaf extract of 200mg/kgBW, namely 11.36±19.18 and the lowest average hematocrit levels were in the P3 treatment group with a dose of 400mg/kgBW, namely -0.78±5.75 (Table 2). If the hematocrit levels before and after the paired t test, the asymp value is 0.914 (2-tailed) (p> 0.05). And continued by conducting a one-way ANOVA test to determine the significant hematocrit levels (p> 0.05). These levels are 0.058 for the pre-test, 0.080 for the post-test, and 0.700 for the combined group. There is no significant difference in the effect of taro leaf extract on hematocrit levels with the first, second, and third treatment groups of mice induced by NaNO2 and the resulting hematocrit levels are still within the normal range. This study is in line with Aulia et.al When compared with the hematocrit levels of normal mice, all doses including the control

group and all treatment groups are still within the normal range, so that the administration of taro leaf extract does not affect its normal hematocrit levels.[13]

The average hematocrit level of post-test K2 (49.54%) is within the normal range according to Douglas and Wardrop (2010). The average of mice in the negative control group (K1), P1, P2, and P3 showed hematocrit levels above the normal range (Table 2). This is because the mice in each group have different metabolisms. Treatment of group P3 was not significant in reducing hematocrit levels but was reduce better than the positive control group K1 and K2.

The administration of taro leaf extract contains flavonoids, tannins, alkaloids, saponins, and phenols.[14] Phenols, tannins, saponins, steroids, quinine, triterpenoids, glycosides, and alkaloids are phytochemicals found in taro leaf extract that provide antibacterial properties.[15] To determine whether the effect of the treatment group is additive or subtractive, this is done to compare the hematocrit level with the positive control group. In groups P1, P2, and P3, the hematocrit levels were higher than K2, indicating that the administration of taro leaf extract can decrease hematocrit levels approaching the normal hematocrit range. The hematocrit value of P3 mice was even higher than K2, indicating that the administration of taro leaf extract can increase hematocrit as effectively as consuming iron tablets. The hematocrit levels of the treatment group after administration of taro leaf extract were higher than those of the positive control group (49.54 ± 6.64%).

Table 3. Analysis of MCV data before and after intervention

	Pre-test (µL)	Post-test (µL)	Delta (µL)	*p**
K1	90.72±2.8	87.28±1.65	-3.44±2.45	0.034
K2	91.32±3.37	87.86±3.21	-3.46±5.51	0.233
P1	88.32±1.98	85.52±1.37	-2.80±1.29	0.008
P2	86.16±2.46	83.10±2.41	-3.18±3.72	0.035
*p***	0.038	0.015	0.998	

Description:
*p** = Paired t-test
*pp*** = Anova test

Fig. 2. Changes in the Mean MCV Number of Rats

The observation results (Table 3) showed a significant difference in the average number of MCV in each treatment group. MCV or erythrocyte volume is an index to determine the size of red blood cells.[16] The research test of Laksmindra Fitria suggest calculating the erythrocyte index of hematological profile data, namely MCV, to get good and complete results.17 The normal value of Mean Corpuscular Volume in mice ranges from 48.9-57.9 fL.18 There was a significant effect of giving taro leaf extract on the number of MCV in the treatment group ($p = 0.015$). In the treatment group, giving taro leaf extract P1 ($p = 0.008$) and P3 ($p = 0.035$) significantly reduced MCV levels in mice compared to the negative control group ($p = 0.233$) without giving the extract. Meanwhile, the P2 treatment group ($p=0.128$) was not significant in reducing MCV levels when compared to P1 and P2 treatments, but better than the positive control group ($p=0.233$).

Based on Figure 2, it can be seen that the change in the number of MCV before the administration of taro leaf extract in mice after being induced by $NANO_2$ had the highest change in the number of MCV in the K2(+) control group, which was 91.32±3.37 fL with a delta of -3.46±5.51 fL (administration of ad libitum and iron tablets 4.5 mg/kgBW), while the lowest number of MCV was in the P1 treatment with a change in MCV of -2.80±1.29 fL (treatment of taro leaf extract with a dose of 100mg/kgBW).

If the number of MCV before and after the paired t-test, an asymp value of 0.000 (2-tailed) was obtained ($p <0.05$). And continued by conducting a one-way ANOVA test to determine the significant hematocrit level ($p>0.05$). The level is 0.038 for the pre-test, 0.015 for the post-test, and 0.998 for the combined group (Table 3). There is no significant difference in the effect of taro leaf extract on the number of MCV with the control group and the NaNO2-induced treatment group so that the number of MCV produced is still within the normal range.

In the post-test, the groups with MCV values close to normal were P1, P2, and P3. In groups K1 and K2, the MCV values ranged from 87 µL, while the group given taro leaf extract had an MCV value of 83-85 µL. The results of the research observations (Fig 2) showed a difference between the first, second, and third treatments with each dose of taro leaf extract given, namely P1 (100 mg/kgBW), P2 (200 mg/kgBW), P3 (400 mg/kgBW). The group with the highest average MCV was the positive control group (K2) of 87.86 ± 3.21 fL with ad libitum administration and additional iron tablets of 4.5 mg/kgBW. The average hematocrit level after being given taro leaf extract in mice was the lowest in the P2 treatment group of 83.10 ± 2.41 fL with a dose of 200 mg/kgBW. After 21 days of treatment with taro leaf extract, the MCV count of the test mice was found to be higher than that normally seen in the wild. This study contradicts the findings of Khairunnisa et al (2022), which showed that mice given Andaliman extract had reduced MCV counts. Iron deficiency anemia, thalassemia, and secondary anemia can be characterized by MCV values that are below the normal range.[19]

Flavonoids in taro leaves can increase erythropoiesis (the process of forming erythrocytes) in the spinal cord so that it will increase erythrocytes. The change in hematocrit and MCV levels towards normal levels after giving taro leaves is due to the

content of phytochemical compounds found in taro leaves. Hematocrit is a percentage of the total volume of erythrocytes in the blood whose value is expressed as a percentage (%) so that changes in hematocrit levels in the treatment of giving taro leaves are influenced by the number and size of erythrocytes. The number of erythrocytes is positively correlated with the hematocrit value.[20]

Changes in the number of erythrocytes that occur are caused by compounds contained in taro leaves, namely iron (Fe), vitamin C, protein, and other antioxidant compounds. These compounds are important precursors in the formation of hemoglobin and erythrocytes (red blood cells). 21 MCV (Mean Corpuscular Volume) is the average volume of erythrocytes. The size of the MCV value is influenced by the number of erythrocytes and hematocrit in the blood. A decrease in hematocrit will affect a decrease in MCV.

4 CONCLUSION

Oral administration of taro leaf extract to the post-test group of male rats (Rattus norvegicus) induced by sodium nitrite did not have a statistically significant effect but experienced changes in the mean hematocrit and MCV levels of the rats.

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