Original Research Article

High intake of green tea decreased hemoglobin and hematocrit levels in *Rattus novergicus* strain wistar albino

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ABSTRACT

Background: Anemia is one of four nutritional problems in Indonesia with a prevalence of 21.7%, the type of anemia is iron deficiency anemia. One of the causes of iron by tannins present in green tea. The purpose of this study is to determine the effect of green tea on hemoglobin and hematocrit amount in *Rattus novergicus* strain wistar albino.

Methods: This research used experiment with post-test only control group design. Twenty four rats were divided into four groups: the control group and the treatment group were given a different dose of 5.6g in a 1.8ml/200gr solvent, 8.4g in a 1.8ml/200g solvent, 11.2g in 1.8ml/200gr solvent for a month. Blood is taken through 2 ml orbital sinus using capillary pipette. hemoglobin examination using spectrophotometer and hematocrit checking using microhematocrit tool. The study was conducted in pharmaceutical laboratories and biochemical laboratories. Data analysis using one way ANOVA and Kruskal Wallis with degree of significance $p<0.05$.

Results: The results of the study were found the mean differences amount of hemoglobin and hematocrit. The mean of control group in hemoglobin content was 16.39gr%, in treatment group were P1: 13.64gr%, P2: 11.19gr% and P3: 10.49 gr%, while in hematocrit control group was 44.83%, in treatment group were P1: 44.83%, P2: 39.17% and P3: 37.17%. Result of the test using one way ANOVA, which found the effect of green tea on hemoglobin (p value = 0.000) and result of the test using Kruskal-Wallis, which found the effect of green tea on hematocrit (p value = 0.017).

Conclusions: Based on the results of this study concluded that there is influence of green tea on the decrease of hemoglobin and hematocrit amount in *Rattus novergicus* strain wistar albino. It is suggested to pay more attention to dose of green tea with normal limit of 8.4gr.

Keywords: Green Tea, Hematocrit, Rat (*Rattus novergicus*), Level of hemoglobin

INTRODUCTION

Tea is the highest drink consumption in the world. According to research by archaeologists, the Chinese and Indian communities have been consuming tea leaves soaked in boiling water since about 5,000 years ago. In 2011, Indonesia becomes the 8th largest tea producing country in the world.[1]

Based on Euromonitor (2015), the growth of tea drinks has increased 14% annually. In Indonesia, about 90% of tea farmers cultivate their crops into green tea which then develops into jasmine tea, while the remaining (10%) becomes black tea. Green tea is traditionally used as a treatment. It is specifically consumed by the people of Asia and the middle east.[1]

The content of catechins in green tea is good for the health of the body. Benefits of green tea include lowering and maintaining weight, improving brain performance, stroke antidote, lowering cholesterol and preventing cancer.[1] The negative effect of green tea is the occurrence

of inhibit iron absorption derived from non-heme iron of the body caused by tannin substance.[2]

Scollary GR, states tea content which well-known responsible for the bitter taste of tea is called tannin. It is a class of the polyphenol compound-a compound with phenol groups in its chemical structure found in plants, so it is often referred as plant polyphenols. One of the negative things is the deficiency of Iron/Fe (Ferum) elements in the body cause anemia. Tannin can indeed reduce or inhibit the absorption of Fe, leading to impaired Fe metabolism which can cause macrocytic anemia.[3]

Green tea contains a mixture of polyphenol compounds in the form of tannin which is one of the most powerful inhibiting factors in the absorption of iron by binding the iron. Consume tea while eating in the case of low iron in the body will cause the deficiency of Fe.[4]

When Iron in the blood plasma is in a low state, the iron released from iron stores to be brought by transferin to plasma and the whole body, then it transported to the mitochondria as a place of heme synthesis. In erythrocytes, low hemoglobin and hematocrit can lead to anemia, one of the causes is the presence of flow interruption of iron to erythoblast.[5]

World Health Organization (WHO) states that anemia occurs in the community as amount 40-88% in 2013.[6] There are four nutritional problems in Indonesia, one of them is anemia.[6] Basic health research in 2013 states, anemia sufferers in Indonesia has reached 21.7%. At the age of 65-74 reaches 34.2% and age >75 years reaches 46.0%. The highest proportion of anemia in terms of sex is women.[7]

Based on health profile of west Sumatera in 2011, the number of the prevalence of anemia is 24.63%.[8] According to West Sumatera Provincial Health Office, based on Nutrition Status Monitoring (PSG) survey conducted in 2015, the highest proportion of anemia is pregnant women as 43.1%. Moreover, data from Padang Health Office in 2012 shows that the prevalence of anemia reaches 24.5%.[9]

Anemia occurs because the red blood cell protein and red blood cell volume and erythrocytes decrease.[10] It can be seen from normal hemoglobin amount is 12g%, mild anemia occurs if the hemoglobin amount is 10 to 12 g%, moderate anemia occurs if the hemoglobin amount is 8 to 10gr% and its weight is less than 8%.[11]

Anemia can be prevented by consuming foods that help the absorption of iron. It can be getting from animal-based foods such as chicken and other meat and plant-based foods such as green vegetables, fruits contain vitamin C. it also can be prevented by providing iron supplements to the body.[10]

The iron obtained from food consists of heme and non-heme iron. Heme iron is found in animal food and non-heme iron is found in plant food. However, tannin in tea only affects the absorption of nonheme iron.[12] Anemia is said to be severe if the hemoglobin amount is less than 7g/dl. Anemia is caused by dominant relationship between biological, ecological, social and political factors that occur in the community.[13]

Based on research conducted by Lubis M et al, the result showed that there was no effect of green tea on the reduction of hemoglobin and hematocrit amount in wistar.[14] Different finding obtained by Bait Y, which found there was an influence of green tea in decreasing hemoglobin amount in sprague dawley.[15]

The volume of erythrocytes in blood circulation may have an interruption as a result of anemia, so the occurrence of anemia decrease hematocrit and haemoglobin values.[16]

Based on the background of problems above, the researchers are interested in doing research on the effect of green tea on hemoglobin and hematocrit amount in *Rattus norvegicus* strain wistar albino.

METHODS

The type of this research was experimental research by using post-test only control group design that was conducted in Pharmacology laboratory of pharmaceutical faculty of Andalas University for maintenance and blood taking. Examination of hemoglobin amount by spectrophotometer method and hematocrit amount by microhematocrit method were carried out at Biochemistry Laboratory of Andalas University. This research was conducted in October until November 2017.

The population of this research was male strains Wistar Albino obtained from the animal maintenance unit of Andalas University, by consideration that the male strain wistar albino was trial mammals or often called as animal laboratory because it is often used in biology research. The sample used was strain male wistar albino with the criteria that the population was 5-6 years and the average weight was 150-250grams while the exclusion criteria was that the male strains Wistar Albino which were not eating, then the drop-out criteria was the male which faced the decline in physical conditions and dead while research was being conducted. Time of feeding and drinking was every day as libitum in the form of pellets. Animal trials stable used was 60 cm x 40 cm x 20cm as a place to maintain the rats, consisting of plastic edges with a water container, wire as a lid, drinking water bottle, feeding and powdering as a cage base. The cage should be easy to be grabbed and reattached. The cage should be a bit of a rock, the animals were not easy to release and clearly visible outside.

Operational definitions included green tea with a measuring instrument using a scale, measuring results with a dose based on weight, and a measuring scale with a ratio, while on examination of hemoglobin levels measuring instrument with a spectrophotometer, the measurement results were clarified in grams% and the measuring scale in the ratio, then on hematocrit examination, this measurement was the percentage of red blood cells in the blood after the specimen was centrifuged with a measuring instrument using a microhematocrit, the results of the measurements were clarified in % and the measuring scale with a ratio.

In the implementation procedure, the control group rats were placed in a cage without being given treatment, while the treatment group of rats were divided into three groups, namely group 1 (P1), group 2 (P2) and group 3 (P3) where each group was given a daily dose. Green was given by inserting spuisonde into the mouth to the stomach and injecting it directly into the stomach of the rat. The data obtained were analyzed by using one way ANOVA and Kruskal-Wallis test with a significance level of p >0.05.

RESULTS

Data of hemoglobin amount were analyzed by using one way ANOVA and post hoc test boferoni (Table 1).

Table 1: The mean of hemoglobin of negative control group and treatment group on *Rattus norvegicus* wistar strain albino (n = 24).

Subject of groups	Hemoglobin level (gram %) Mean±SD	P value
Negative control	16.39±1.62	0.000
Treatment 1	13.64±1.84	
Treatment 2	11.19±1.28	
Treatment 3	10.49±0.63	

The Table 1 shows there is a difference hemoglobin level between the control group and the treatment group. It can be seen that the hemoglobin level of treatment groups are lower than control group, and every treatment groups show different Hb levels. To see the difference of hemoglobin level between control and treatment group in details, it is necessary to have further testing with the post hoc test bonferoni.

Based on the Table 2, it can be concluded that there is a significant effect of hemoglobin level between negative control group and P1, P2 and P3 groups. The result shows that those P groups have p value <0.05.

The Table 3 the changes mean of hematocrit level between control group and treatment group. The decrease of hematocrit level can be seen from each treatment group. To see the difference of hematocrit level between control and treatment group in details, it is necessary to have further testing with Man-Whitney U test.

Table 2: The result of post hoc test bonferroni on hemoglobin levels in each research group.

Group	Hemoglobin levels			
	Negative control	P1	P2	P3
Negative control	-	0.019*	0.000*	0.000*
P1	0.019*	-	0.044*	0.006*
P2	0.000*	0.044*	-	1.000
P3	0.000*	0.006*	1.000	-

Table 3: The mean of hematocrit level of negative control group and treatment group on *Rattus norvegicus* strain wistar albino (n = 24).

Subject of groups	Hemoglobin level (gram %) Mean±SD	P value
Negative control	44.83±5.77	0.017
Treatment 1	44.67±5.92	
Treatment 2	39.17±2.48	
Treatment 3	37.17±2.13	

Table 4: The result of Mann-Whitney U Test on hematocrit level in each research group.

Group	Hematocrit levels			
	Negative control	P1	P2	P3
Negative control	-	0.81	0.02*	0.01*
P1	0.81	-	0.14	0.02*
P2	0.02*	0.14	-	0.16
P3	0.01*	0.02*	0.16	-

From the Table 4, it can be concluded that there is significant effect of hematocrit level between KN group and P2 and P3 group and P3 and P1 group because of p value <0.05.

DISCUSSION

The effect of green tea on hemoglobin amount in Ratus norvegicus strain wistar albino

Based on the analysis result, it is found that green tea affects hemoglobin levels of control group and treatment groups with different doses. From those groups, group three has the highest decreased hemoglobin level with dose 11.2/200grBB. Decreased levels of hemoglobin are due to absorption disorders, caused by tannins substance in green tea.[4]

Tanin is a polyphenol compound that forms an inorganic complex compound in the body.[4] Tannin in tea also has negative effects.[7] Bitter taste in tea occurs due to the colloids in water, so high consumption of tannin can result protein absorption.[17] Tannins turns into toxic if it exceeds the consumption limit. Tannins can precipitate proteins that inhibit the absorption of iron and reduce the bioavailability of iron.[18] When tannins enter the body orally, it will follow the absorcit path like other nutrients. It will be dissolved in small intestine. Tanin is a polyphenol compound that forms an inorganic complex compounds in the body. Iron minerals will be bound due to the presence of tannin.[17]

Iron in ferritin will be easily removed if the iron in the blood plasma is low so that transferin will bring iron throughout the body which needs the iron. In heme synthesis precisely in mitochondria, iron is also released.[5] Iron that continuously used in ferritin will reduce iron deposits.[11] if there is too little iron, there will be transferrin disruption in bringing iron to erythoblast, the worst impact is the occurrence of hemoglobin and hematocrit deficiency in erythrocytes resulting an anemia.[5]

The same result is also shown in a study conducted by Bait Y, which giving green tea to sprague dawley rats for 16 days. Based on the evaluation result after giving green tea on the day 16, there was a decrease level of hemoglobin in treatment group (p <0.05). It means that green tea affects the hemoglobin level on wistar rats.[15] In contrast, a study conducted by Lubis et al, different result. By giving green tea for 30 days in wistar rats, there was no decreased hemoglobin and hematocrit levels in treatment group (p >0.05) when hemoglobin and hematocric amount were measured on day 31.[14]

The effects of decreased hemoglobin and hematocrit may cause anemia. This situation can be seen when a person feels weakness, headache and loss of concentration.[19] Changing the habit not to drink tea while eating, avoiding too much drink tea, consuming varied foods such as animal and vegetable food are several ways to prevent anemia.[20]

The effect of green tea on hematocrit amount in Rattus norvegicus strain wistar albino

Based on the analysis results, it is found that green tea influences hematocrit levels of control and treatment groups with different doses. Among those three treatment groups, group three has the highest decreased hematocrit levels with dose 11.2/200grBB.

Green tea contains a mixture of polyphenol compounds in the form of tannins.[4] Tannin is one of the most powerful inhibiting factors in iron absorption process. It is a kind of polyphenols that form an inorganic complex compound that inhibit iron absorption by binding the iron.[4] Tannin can be harmful if proteins and minerals

bound to tannins cannot be utilized for the body. It is about 64% of iron cannot be absorbed by erittrosite because of the presence of tannin so that the erythrocytes will be disrupted and cause anemia.[19]

Increasing iron-binding capacity by tannin causes a reduction in iron stores resulting iron deficiency in the body. This condition is also characterized by decreasing transferin saturation and reducing bone marrow hemosiderin.[17] Iron deficiency can cause anemia in which decreases the quantity of red blood cells in circulation. As a result, it also reduced hematocrit and hemoglobin level in the blood.[16] According to a study by Bait Y, it was found that green tea affected the reduction of hematocrit on wistar rats.[15] This is different from the research conducted by Lubis et al, which found that green tea did not affect the hematocrit level in wistar rats.[14]

Dosage and brewing time affect the amount of tannin in a cup of tea. The upper limit of green tea consumption is 5 cups per day.[4] Consuming too much green tea will affect the absorption of iron and causes iron deficiency.[3] The amount of substances dissolved in water is closely related to the brewing time. Brewing green tea for 8 minutes had the highest tannin level 83,503 ppm.[21] The brewing process is done by using extraction method with water solvent, which the optimum temperature is at 85^0C and the brewing time is 5 min.[22]

The effects of decreased hemoglobin and hematocrit level may cause anemia.[5] iron deficiency caused by anemia is characterized by the reduction of iron serum, transferin saturation, ferritin serum due to low iron stores in the body.[11] therefore, the hemoglobin and hemotocrit level falls.16 In this study, the researchers did not conduct pre-test on hemoglobin and hemotocrit level in *Rattus norvegicus* strain wistar albino.

CONCLUSION

There is an influence of green tea on hemoglobin and hematocrit amount in Rattus Norvegicus Strain Wistar Albino.

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