Effect of wheat grass juice (*triticum aestivum* L.) against the erythrocytes and hemoglobin in male mice (*mus musculus* L.) anemia induced by sodium nitrite

E Yuniarti1*, L Hasanah1, L Advinda1 and P M Indika2

1Biology Department, Faculty of Mathematics and Science, Universitas Negeri Padang
2Health and Recreation Department Faculty of Sport Science Universitas Negeri Padang
Jl. Prof.Dr.Hamka Air Tawar Barat, Padang, West Sumatera, Indonesia

*elsayuniarti@gmail.com

Abstract. Anemia is one of the most common health problems found in Indonesia. Anemia is characterized by the low number of erythrocytes and hemoglobin in the blood caused by low levels of iron in the body. Wheat grass (*Triticum aestivum* L.) contain high existence of iron is supposed to be able to increase erythrocytes and Hb level in mice (*Mus musculus* L.). Completely Randomized Design was used in this experiment, consisting of 5 treatment (K1: without NaNO2 and WGJ (wheat grass juice), K2: given the NaNO2 without given the WGJ, P1: WGJ 25%, P2: WGJ 50%, and P3: WGJ 75%) and 5 replication. The parameters observed were the male mice erythrocytes and the Hb levels before and after being given the wheat grass juice. The data were anaylzed using ANOVA then continued DNMRT test with a 5% level. The results showed that giving wheat grass juice for 14 days can increased the erythrocytes and Hb levels in male mice. The most noticeable impact is the treatment with the highest dose i.e. P3 (WGJ 75%).

1. Introduction
The most common health problem in Indonesia is anemia. Anemia is a condition when level hemoglobin (Hb) in the blood less than normal, this can be caused that blood loss very fast or due to slow production blood cell in the body [1]. Anemia is a problem faced globally. WHO noted, globally in 2011, there were more than 273 million children aged 6 – 59 months suffering from anemia with 9,6 million of them being severe anemia, more than 496 million non – pregnant women aged 15 – 49 years suffering from anemia with 19,4 million of them are severe anemia, and 32,4 million pregnant women aged 15 – 49 years suffer from anemia with 800 thousand of them being severe anemia [18]. Approximately 50% of this figure is related to iron deficiency (children: 42%, not pregnant women 49% and pregnant women 50%) [19]. Based data in Indonesia there were 21,7% children ≥ 1 years, 28,1% children under 12 – 59 months and 37,1% pregnant women had anemia [20].

Anemia can be characterized by a low amount of erythrocytes in the blood. Erythrocytes in the blood function as carriers of hemoglobin and then transport oxygen from the lungs to the tissues. Red blood cells are able to concentrate hemoglobin in the cell fluid up to about 34 grams / dL of cells. If the formation of hemoglobin in the spinal cord is reduced, the percentage of hemoglobin in the cell is also reduced [1].
Lack of hemoglobin levels in the blood can be caused by various factors, but more than 50% of cases that occur are due to lack of iron (Fe) input. Hemoglobin levels that are too low due to iron deficiency are called iron deficiency anemia. Iron deficiency causes a lack of Hemoglobin (Hb) levels in the blood and can cause symptoms of lethargy, fatigue, forgetfulness, and can reduce endurance and result in infection with disease-causing viruses and bacteria [2].

Hemoglobin in the blood functions as an oxygen carrier by binding oxygen to oxyhemoglobin and then circulating throughout the body for metabolic needs. Besides oxygen, hemoglobin also carries carbon dioxide and with carbon monoxide forms carbon monoxihemoglobin (HbCO) bonds which also play a role in blood pH balance. When hemoglobin levels are low, the risk of developing anemia will be higher and blood will be difficult in binding oxygen [3].

One of the efforts made by a person to overcome anemia due to iron (Fe) deficiency is to consume wheat grass juice. Wheat grass (Triticum aestivum L.) is a plant from Gramineae family that can be consumed when the age of the plant reaches the seventh day since planting [4]. In addition to a short planting period, how to grow wheat grass is quite easy. Wheat grass can be planted directly on the ground or planted using hydroponic methods. Hydroponics method has many advantages, one of which is more awake hygiene because it is not exposed to the soil directly, so that the wheat grass will be cleaner because wheat grass will be consumed directly by juicing [5].

Wheat grass (Triticum sativum L.) which is used as juice contains lots of vitamins, minerals, enzymes and is also a food source that is rich in chlorophyll [6]. Chlorophyll on wheat grass has a structure that is almost the same as hemoglobin. Therefore, there will be a study on the effect of the administration of wheat grass juice (Triticum sativum) on hemoglobin levels and the number of erythrocytes of male mice (Mus musculus) in order to find out the benefits of wheat grass plants that are not well known by the Indonesian people, especially related to anemia.

2. Research Methods
The research was conducted at the Research Laboratory and Animal Division of the Department of Biology, FMIPA, Padang State University. This type of research is experimental, the research design is a Completely Randomized Design (CRD) with 5 treatments and 5 replications.

2.1. Tools and Materials
The equipment needed is a tray without holes and a hollow tray, sprayer, glass beaker, blender, filter, syringe that has been modified with gavage needles, mouse bottles, Sahli haemometer, suction pipette, stirring rod, drop pipette, static, surgical scissors, blood lancet, thoma pipette, Haemositometer, and microscope.

The materials used in this study were wheat grass (Triticum aestivum L.), fuel husk, sterile distilled water, Sodium Nitrite (NaNO2), 0.1% HCl solution, Hayem solution, 70% alcohol, xylol, betadin, gauze, and cotton.

2.2. Research Procedure

2.2.1. Animal Preparation Test. Mice are adapted for 7 days at room temperature in the laboratory before treatment. During adaptation, mice are fed with pellets and drinking water from PDAM. Mice were then divided into 5 treatment groups namely K1 (Control), K2 (Control Anemia), P1, P2, and P3. Each group consisted of 5 mice which were then treated.

2.2.2. Sample Group Distribution. The division of sample groups in this study are:

K1: not given wheat grass juice and not treated anemia (control)
K2: not given wheat grass juice after anemia treatment (anemia control)
P1: given 0.5 ml of wheat grass juice with a concentration of 25% after treatment of anemia
P2: given 0.5 ml of wheat grass juice with a concentration of 50% after treatment of anemia
P3: given 0.5 ml of wheat grass juice with a concentration of 75% after treatment of anemia
2.2.3. *Pathological Treatment (Anemia) in Mice.* Anemia treatment was carried out by giving Sodium Nitrite (NaNO₂) to mice. Based on previous research administration of Sodium Nitrite with the average LD₅₀ provision of Nitrite Sodium orally in mice was 250 mg / kg body weight. In this study, the weight of mice was 20 g, so the sodium nitrite level for each tail was 5 mg [7].

An effective anemia pathological treatment is LD₅₀ = ½ x 5 mg = 2.5 mg. So, the dose used in each tail is 2.5 mg dissolved in 1 mL of distilled water. Sodium Nitrite was given as much as 0.1 ml / 10 g BW / day, so that Sodium Nitrite was fed to mice as much as 0.2 ml.

2.2.4. *Planting of Wheat Grass.* Wheat grass used in this study came from wheat grass planted hydroponically. Before planting, wheat grass seeds are first soaked with water for ± 6 hours to accelerate germination. Then the seeds that have been soaked are rinsed with clean water, then the seeds are put into a basket and covered with plastic so that the roots grow quickly. The containers used for planting are two trays. The first tray is a tray without holes as a container to accommodate the excess hydroponic nutrition provided, and the second tray is a hollow tray whose bottom is covered with tissue. Perforated trays that have been covered with tissue and then sprinkled with roasted husks as planting media. After that, the container containing the husk is then planted with seeds of wheat grass that has germinated. Spray AB Mix solution with a sprayer to keep the seeds wet. Cover the tray with black plastic or cloth so that the wheat grass is not exposed to direct sunlight. This treatment aims to make the growth of wheat grass faster.

2.2.5. *Making Wheat Grass Juice.* Weigh 100 grams of wheat grass, then wash with clean water. Then the wheat grass is cut into small pieces and then blended. Next, the pulp is separated by filtering wheat grass using gauze. After filtering, obtained 100% concentration of wheat grass juice. This juice is then made dilution into three different concentrations, namely 25%, 50%, and 75%.

2.2.6. *Measurement Amount of Erythrocytes in Blood of Mice.* Calculation of the number of erythrocytes is done using a haemositometer. Sucked blood using an erythrocyte pipette to the 0.5 line mark. Then the diluent solution is sucked up to the sign line 101. 200 times the dilution occurs. The two ends of the pipette are then closed with the thumb and middle finger, the shuffle is done back and forth. Cleaned count booth and cover glass. Then it is removed and the droplets of 1-2 liquids that are in the erythrocyte pipette are removed, then the droplets are then attached to one side of the counting chamber that has been given a cover glass. The liquid in the erythrocyte pipette will flow to meet the counting chamber and then the count chamber is placed on a microscope.

The number of erythrocytes actually can be known by looking for factors to get the number of erythrocytes per uL of blood, namely 5 x 10 x 200 = 10,000. So to get the number of erythrocytes, namely: N x 10,000 [8]. Ket: N = number of erythrocytes from 5 counting chambers.

2.2.7. *Measurement of Hemoglobin Levels in the Blood of Mice.* Determination of hemoglobin levels is carried out using the Sahli method. The way it works inserting 0.1 N HCl solution into the sahli tube until the number 10 or underline. Then suck blood samples from the tail of mice that have been cut using a pipette to reach the line limit of 20 mm³ (0.02 cc). The blood sample is then put into a tube that has been filled with HCl solution and is waited for 3 minutes or until it turns blackish brown. Then add distilled water by drop while stirring using a stirring rod. Added again distilled water drops and stirred the mixture with the stirring rod until the color is the same as the standard color hemoglobinometer. The results of hemoglobin levels in the tube with units of g / dL are read on the hemoglobin tube [8].
2.3. Data Analysis
The data obtained were analyzed for variance or Analysis of Variants (ANOVA). From this analysis will be known whether or not the effect of treatment on the dependent variable, if $F_{\text{count}} > F_{\text{table}}$ then continued with further DNMRT (Duncan Multiple Range Test) with a significant difference of 5%.

3. Result And Discussion
The results of observation of the number of male mice erythrocytes before being treated (anemia condition) after being given wheat grass can be seen in the following diagram:

![Graph comparison of the average number of male mice erythrocytes in anemic condition with the number of erythrocytes after being given wheat grass juice.](image)

In this study, before the mice were treated with wheat grass juice, first the mice were made to become anemic by administering sodium nitrite orally for 14 days except in the control group (K1). The administration of Sodium Nitrite (NaNO2) can cause the formation of methemoglobin. Methemoglobin occurs due to nitrite oxidizing Fe2+ to Fe3+ (ferric ion) so that methemoglobin (metHb) is formed. If methemoglobin continues to form, then hemoglobin cannot do its job properly so it will cause anemia [9].

Based on the graph in Figure 1, the number of erythrocytes of male mice before and after treatment showed that the difference in the average number of erythrocytes in anemia (induced by NaNO2) with the average number of erythrocytes after being given the treatment of consecutive wheat grass juice from the highest to the lowest is P3 (75% concentration of wheat grass juice) which is $4.55 \times 10^6 / \text{mm}^3$, P1 (giving 25% wheat grass juice) which is $2.22 \times 10^6 / \text{mm}^3$, and P2 (giving 50% wheat grass juice) of $2.70 \times 10^6 / \text{mm}^3$. The increase in the number of erythrocytes in the control group was only $0.20 \times 10^6 / \text{mm}^3$ and in anemia control $1.94 \times 10^6 / \text{mm}^3$.

The number of red blood cells in normal mice is $6.86-11.7 \times 10^6 / \text{mm}^3$, if less than this amount the mice are said to be anemic. In this study, the average number of erythrocyte mice in the anemia control group was still normal, i.e. $7.49 \times 10^6 / \text{mm}^3$, but when compared with the average number of erythrocytes in the control group which was quite high, i.e. $10.26 \times 10^6 / \text{mm}^3$ then Sodium Nitrite proven to reduce the number of erythrocyte mice as much as $2.77 \times 10^6 / \text{mm}^3$ in the 14-day period [10].

The results of ANOVA analysis obtained are $F_{\text{count}} = 4.32$ and $F_{\text{table}} 0.05 = 2.9$. This means that $F_{\text{hit}} > F_{\text{table}}$ shows that there are significant differences so that the treatment of wheat grass juice treatment affects the number of red blood cells of male mice.

Furthermore, to find out whether there are differences in each treatment, further testing is done using the DNMRT test at a significance level of 5%.
Table 1. Summary data result of the test DNMRT erythrocyte number of male mice after given wheatgrass juice

| Action | Average Erythrocyte Number (10^6/mm^3) |
|--------|-------------------------------------|
| K2     | 10.46a                              |
| K1     | 9.43ab                              |
| P1     | 10.51bc                             |
| P2     | 11.38bc                             |
| P3     | 12.22c                              |

Indeks: The Number followed of alphabet same to show fact different of the test DNMRT 5% levels.
Action K1 (Control), K2 (Control Anemia with given NaNO2), P1 (Wheat grass juice 25%), P2 (Wheat grass juice 50%), P3 (Wheat grass juice 75%).

Research conducted by Sianturi et al (2012) on the effect of Dutch eggplant fruit on the number of anemic male erythrocytes also induced by Sodium Nitrite showed that in the anemia control group the mean number of erythrocytes was 11.58x10^6 / mm^3 and in the average blank control group the number of erythrocytes was 12.27x10^6 / mm^3. The average number of erythrocytes that are still in the normal range is affected by the presence of distilled water which affects the formation of the hormone erythropoietin. The production of erythropoietin in the body depends on tissue oxygen pressure and is modulated by a positive or negative feedback mechanism. At low oxygen pressures, production increases which results in increased production of erythrocytes in the bone marrow [13] 7, 13.

In this study, the average number of male mice erythrocytes after being given wheat grass juice for 14 days showed the highest yield in P3 treatment (75% wheat grass juice), which was 12.22x10^6 / mm^3 and the lowest was in P1 treatment (grass juice wheat 25%), which is 10.51x10^6 / mm^3. So that the average number of male mice erythrocytes after being given wheat grass juice was in the range between 10.51x10^6 - 12.22x10^6 / mm^3.

The increase in the number of erythrocytes in male mice after being given wheat grass juice was due to high Fe grass juice. According to table 1, the iron content in 100 grains of wheat is 126 mg. This amount of iron is high when compared to other vegetables, such as spinach (Amaranthushybridus) which iron content is only about 3.0 mg / 100 g of spinach. High levels of iron (Fe) in wheat grass can help the process of erythropoiesis by bone marrow [17]. The process of erythropoiesis in the bone marrow requires other precursors so that the process becomes effective, while the precursor one of them is iron [11].

The results of observation of the average Hemoglobin (Hb) level of male mice before being treated (anemia condition) after being given wheat grass can be seen in the following diagram:

Figure 2. Graph comparison of the average number of male mice anemic condition with the number of Hb after being given wheat grass juice
Based on the data obtained, the average Hb level in normal mice (K1) which were not given any treatment was 13.88 g / dL, while the Hb level of mice induced with Sodium Nitrite was 11.08 g / dL. This shows that Sodium Nitrite can reduce Hb levels by 2.8 g / dL.

The average level of hag increase in male mice after being given wheat grass juice for 14 days showed the highest yield in P3 treatment (giving 75% wheat grass juice), which was 13.64 g / dL with an increase of 2.24 g / dL from the previous one was only 11.40 g / dL. While the lowest average increase was in P2 treatment (25% wheat grass juice), which was from 12.32 g / dL to 13.44 g / dL with an increase of 1.12 g / dL. The average Hb level in the control group (without anemia treatment and without wheat grass juice) was 13.84 g / dL. When compared with the initial measurement of 13.88 g / dL, a decrease in hemoglobin levels of 0.04 g / dL was found. The decrease in Hb levels in this group of mice can be caused by the physiological conditions of the mice themselves and can also be influenced by environmental factors.

Furthermore, to find out whether the administration of wheat grass juice affects the hemoglobin levels of male anemia mice, one-way ANOVA variance analysis was performed. The results of ANOVA analysis obtained are F-count = 4.23 and F-table 0.05 = 2.9. This means that Fhit>Ftable indicates that there is a real difference, so the treatment of giving wheat grass juice has an effect on the hemoglobin levels of male mice. The increase in Hb levels is influenced by the presence of chlorophyll and Fe in the wheat grass juice. Chlorophyll is almost chemically identical to hemoglobin, the difference is only found in its central element, namely in hemoglobin in the form of Fe and in chlorophyll in the form of Mg. Similarities between the two are considered to be responsible for therapeutic effects that can help the process of hemoglobin synthesis [12].

In chlorophyll there is a four-ply ring molecule, one with the other associated with a methane group. In hemoglobin about 96% of the molecule is globulin and the rest is in the form of a heme molecule which is a compound of protoporphyrine compound with a central element in the form of Fe. Protoporphyrine also consists of four pyrole rings that are bound by methane groups. The presence of protoporphyrine in chlorophyll and Fe in wheat grass juice can make hemoglobin synthesis more efficient [14].

Most anemia occurs due to iron deficiency (iron deficiency). Iron itself has many important roles, one of which is to transport oxygen and carbon dioxide in the blood. Furthermore, hemoglobin is an important component of red blood cells. The number of red blood cells is affected by the hormone erythropoietin produced by the kidneys to stimulate the formation of red blood cells [15]. If iron from the body is insufficient, iron intake from food is needed. If iron intake from food is also not sufficient, anemia will occur, especially when considering red blood cells are only 120 days old [16].

Furthermore, to find out whether there are differences in each treatment, further testing is done using the DNMRT test at a significance level of 5%.

**Table 2. Summery data result of the test DNMRT Hb number of male mice after given wheat grass juice**

| Action | Average Hb Levels (g/dL) |
|--------|-------------------------|
| K2     | 12.16a                  |
| P1     | 12.48ab                 |
| P2     | 13.44bc                 |
| P3     | 13.64c                  |
| K1     | 13.84c                  |

*Indeks: The Number followed og alpabeth same to show fact different of the test DNMRT 5% levels. Action K1 (Control), K2 (Control Anemia with given NaNO2), P1 (Wheat grass juice 25%), P2 (Wheat grass juice 50%), P3 (Wheat grass juice 75%).*
Based on the results of the DNMRT test, it can be concluded that the most effective treatment for increasing Hb levels is treatment on P3 because it can increase Hb levels to 2.24 g / dL. The Hb level of the control group (without any treatment) was at a high average level because it was not treated with anemia. However, in the second measurement it was found that there was a decrease of 0.04 g / dL in the hemoglobin level. Presumably this condition is influenced by external factors such as temperature and the state of the mouse itself. Then in the anemia control group, the average initial hemoglobin level was 11.08 g / dL but in the second observation it was found that the Hb level of the mouse was 12.16 g / dL so that an increase of 1.08 g / dL occurred. It is suspected that this increase comes from food and drinking mice which also contain nutrients.

4. Conclusions and Suggestions

4.1. Conclusion
Based on the results of the study, it can be concluded that there is an effect of giving wheat grass juice to the hemoglobin level of male mice (Mus musculus L.) because F-count>F-table at a significance level of 5%. The treatment with the most significant effect after the administration of wheat grass juice was at a concentration of 75%.

4.2. Suggestion
Need to do research on the benefit of wheat grass juice on health like on pregnant, severe anemia and others condition.

References
[1] Guyton and Hall. 2011. Buku Teks Fisiologi kedokteran. Jakarta: EGC Penerbit Buku Kedokteran.
[2] Masrizal. 2007. Anemia Defisiensi Besi. Jurnal Kesehatan Masyarakat. 2(1): 140-145.
[3] Provan, D., Charles R. J. S., Trevor B., John L. 2004. Oxford Handbook of Clinical Haematology Second Edition. New York: Oxford University Press.
[4] Wigmore, A. 2011. The Wheatgrass Book. United States: Avery Publishing.
[5] Utama, H. S., Sani M. I., Arie I. 2006. Perancangan dan Implementasi Sistem Otomatisasi Pemeliharaan Tanaman Hydroponik. Jurnal Teknik Elektro. 8(1): 1-4.
[6] Bhikaji, P. K., Thakare M. P., Meshram D. S. 2015. The Effect of Wheatgrass Juice on Hemoglobin Level W.S.R. to Samanya-Vishesha Siddhanta. International Journal of Ayurved and Pharma Research. 3(7):66-70.
[7] Sianturi, S., Massita T., Emita S. 2013. Pengaruh Buah Terong Belanda (Solanum betaceum Cav.) Terhadap Jumlah Eritrosit dan Kadar Hemoglobin Mencit Jantan (Mus musculus L.). Anemia Strain DDW Melalui Induksi Natrium Nitrit (NaNO2). Jurnal Saintia Biologi. 1(2): 49-54.
[8] Kiswa, R. 2014. Hematologi dan Tranfusi. Jakarta: Erlangga.
[9] Suudah, E. N., Yusriana C. S., Dewi T. N. 2015. Uji Efektivitas Ketepatan Waktu Pemberian Kombinasi Natrium Tiosulfat dan Natrium Nitrit Sebagai Antidotum Ketoksikan Akut Kalium Sianida Pada Mencit (Mus musculus). Jurnal Permata Indonesia, 6(1): 21-28.
[10] Kusumawati, D. 2004. Bersahabatdengan Hewan Coba. Yogyakarta: UGM Press.
[11] Hofbrand, A. V., Pettit J. E., Moss P. A. H. 2005. Essential Haematology. Yogyakarta: Kanisius.
[12] Chauhan, M. 2014. A Pilot Study on Wheat Grass Juice For Its Phytochemical, Nutritional and Therapeutic Potential on Chronic Diseases. International Journal of Chemical Studies. 2(4):27-34.
[13] Sembiring, A., Massita T., Emita S. 2013. Pengaruh Ekstrak Segar Daun Rosela (Hibiscus sabdariffa L.) terhadap Jumlah Eritrosit dan Kadar Hemoglobin Mencit Jantan (Mus musculus L.) Anemia Strain DDW Melalui Induksi Natrium Nitrit (NaNO2). Jurnal Saintia
[14] Riswan, M. 2003. Anemia Defisiensi Besi Pada Wanita Hamil di Beberapa Praktek Bidan Swasta dalam Kota Madya Medan. *Jurnal Penelitian*. Sumatera Utara: Universitas Sumatera Utara.

[15] Muchtadi, D. 2008. *Pengantar Ilmu Gizi*. Bandung: Alfabet.

[16] Rasmaliah. 2004. Anemia Kurang Besi dalam Hubungannya dengan Infeksi Cacing pada Ibu Hamil. *Kajian Pustaka*. Sumatera Utara: Universitas Sumatera Utara.

[17] Rana S, Kamboj JK, Gandhi V. 2011. Living the natural way – Wheatgrass and Health. *Functional Food in Health and Disease*. 11: 444 – 456.

[18] World Health Organization. 2015. The Global Prevalence of Anemia in 2011. *WHO Rep.* 48.

[19] World Health Organization. 2017. *Global Prevalence of Anemia in 2011*. http://www.who.int/vmnis/prevalence/summary/anemia_data_status_t2/en/. Diakses tanggal 14 Oktober 2017.

[20] Badan Penelitian dan Pengembangan Kesehatan. 2013. *Riset Kesehatan Dasar 2013*. Diunduh dari http://www.depkes.go.id/. Diakses tanggal 14 Oktober 2017.