Effect of *Triticum aestivum* L. Wheat Grass Juice Planted with Various Media on the Hemoglobin of Anemic Male Mice (*Mus musculus* L.)

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Abstract. Indonesia frequently experiences the health issue of anemia. This occurs when there are insufficient levels of hemoglobin in the blood, which is caused by a lack of iron, which is necessary for the synthesis of hemoglobin. Wheat grass is one of the iron-rich foods that can be consumed as an alternative to get the iron one needs (Triticum aestivum L.). Method: Factor A (planting material) and Factor B (control group) are the variables in this experimental study's factororial completely randomized design (treatment concentration). The samples consisted of forty male mice that were 8–10 weeks old and weighed 20–30 gr. Eight treatment groups, each with five male mice, were created. K1 received no wheat grass juice and was not treated for anemia; K2 received no wheat grass juice following the induction of NaNO2 (anemia treatment), After receiving treatment for anemia with a concentration of 50%, P1T was given wheat grass juice planted with soil. P2T: given wheat grass juice planted with soil with a concentration of 75% after anemic therapy, and P3T: given wheat grass juice planted with soil with a concentration of 100% after treating anemia. After receiving treatment for anemia, P1H was given wheat grass juice with a 50% concentration that was hydroponically grown. After receiving anemic therapy, P2H was given wheat grass juice with a 75 percent concentration that was hydroponically grown. After receiving treatment for anemia, P3H was given wheat grass juice with a 100% concentration that was hydroponically grown. Blood was drawn from the mice's tail tips before and after they received wheat grass juice for 14 days. Variance analysis (ANOVA) and DMRT follow-up with a level of 5% were used to analyze the data. Results: The study's findings demonstrated that if Fcount > Ftable, the planting medium had no impact on the hemoglobin levels of male anemic mice. Male mice with anemia whose Hb is affected by wheat grass juice at various concentrations where Fcount Ftable Conclusion: Wheat grass juice with a 100% concentration is the most obvious cure.

Keywords: Triticum aestivum · Hemoglobin · Anemia

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1 Introduction

Anemia is one of the health problems that often occurs in Indonesia. Anemia occurs when the hemoglobin level in the blood is less than normal due to excessive blood loss or because of red blood cell production too slow in the body [1]. The most common anemia is iron deficiency anemia. The Basic Health Research (Riskesdas) conducted in 2018 found that the prevalence of iron deficiency anemia in pregnant women around 48.9% its has increased from 37.1% conducted by Riskesdas in 2013, while the prevalence of anemia in boyteenagers is around 12% and in female teenagers around 23% [2].

Iron deficiency anemia results from a lack of iron that needed for the synthesis of hemoglobin. Iron is part of the Hemoglobin molecule, with reduced iron, the synthesis of hemoglobin will decrease and cause hemoglobin levels to drop. Hemoglobin is a very vital element for the human body, because low hemoglobin levels affect the ability to deliver O_2 which is needed by all body tissues [3].

The alternatives to fulfill the iron needed can be done by consuming iron-containing foods including wheat grass (*Triticum aestivum* L.). Wheat grass is a wheat plant that is harvested in vegetative development, aged 10 days after planting [4]. The iron content in wheat grass per 100 g is 126 mg [5].Wheat grass used as juice contains a lot of vitamins A, B, C E and K, minerals, enzymes and also rich in chlorophyll [6]. Chlorophyll in wheat grass has a forming structure that is almost the same as hemoglobin and allows the body to convert chlorophyll into hemoglobin [7].

Wheat grass can be planted directly on the ground or planted by hydroponic methods. Planting media is one of the main things that are considered in planting cultivation because the growing media play a role in storing nutrients and supporting plants [8]. Conventional plant cultivation uses soil planting media that have more complex nutrient content. The fuel husk planting media has advantages in terms of carrying good water and aeration. Fuel husk growing media requires special nutrients as a source of nutrients for plant growth and development, one of which is AB Mix. AB Mix nutrition contains essential nutrients consisting of nutrients A which contains macro nutrients and B nutrients that contain micro nutrients needed by plants to trigger growth.

The treatment of anemia in male mice is done by administering sodium nitrite. In this study using male mice with the reason that male mice did not change the estrus cycle, easily controlled and not supported by hormonal. Consequently, research on the impact of wheat grass juice was done (Triticum aestivum L.).

2 Methods

Experimental research is the type of study. The research used was Factorial Completely Randomized Design (RAL) method, held on February–June 2021 at the Biology Laboratory of FMIPA UNP.

48 male Mus musculus L. mice (Mus musculus) weighing 20–30 gr and being 8–10 weeks old made up the study's population. 40 male mice (Mus musculus L.) randomly chosen from the population served as the study's sample.

The tools used are a non-hole tray, perforated tray, sprayer and polybag, beaker glass, blender, sieve, gauze, modified syringe with gavage needle, mouse bottle, Sahli

haemometer, suction pipette, stirring rod, dropper, statif surgical scissors, and blood lancet. The ingredients used are wheat grass (Triticum aestivum L), nutrition AB Mix, fuel husk, humus soil, sterile aquades, and Sodium Nitrite.

In mice, anemia is treated by administering sodium nitrite (NaNO2). When given orally to mice, sodium nitrite has an average lethal dose (LD50) of.250.mg/kg body.weight. Since each mouse in this research weighed 20 gr, a sodium nitrite level was 5 mg/mouse. It is beneficial to treat pathological anemia with LD50 = 1/2 × 5 mg = 2.5 mg. Therefore, 2.5 mg of the dosage, each mouse receives, diluted in 1 mL of distilled water. The mice receive up to 0.2 ml of sodium nitrite daily, which is administered as 0.1 ml per 10 g of BB.

Test preparation Mice were housed at room temperature for seven days prior to treatment. Mice were given PDAM drinking water and pellet feed prior to adaption. The mice are then split into eight groups, with K1 receiving neither wheat grass juice nor anemia treatment (control), After receiving anemia treatment, K2 was not receive wheat grass juice (anemia control), P1T was receive 0.5 mL of wheat grass juice (contained at a 50% concentration), P2T was receive 0.5 mL of wheat grass juice (contained at a 75% concentration), P3T was receive 0.5 mL of wheat grass juice (contained at a 100% concentration), and P1H was given 0.5 mL of wheat grass juice (contained at a 50% concentration).

After soaking the wheat grass seeds in water for around 6 h, they are planted in soil medium. The seeds are soaked, emptied, and rinsed with clean water before being put into a polybag with soil and exposed to the light. When planting wheat grass with fuelhusk media, germination-stage seeds are placed in a basket and covered with plastic to encourage rapid root growth. Two trays are the planting container. The first tray is a tray with no holes that serves as a container for the additional hydroponic nourishment delivered, while the second tray is a hollow tray with a tissue-covered base. Use a perforated tray that has been tissue-wrapped and fuel husk-dusted as a planting medium.. Then, the germination of wheat grass seeds was sown in the container containing fuel husks. Apply AB. Spray the mixture onto the seeds to keep them moist. To avoid the wheat grass seeds struck direct sunlight, cover the tray with a piece of black fabric or plastic. The purpose of this therapy is to hasten the growth of wheat grass.

Wheat grass that had been thoroughly cleaned and weighed up to 100 g was used to make juice. Wheat grass should be chopped into small bits before being blended. Use gauze to filter the pulp and separate it.. After being filtered, wheat grass juice is obtained, and it has a 100% concentration. To separate the wheat grass juice into two different concentrations of 75% and 50%. Prepare the gavage for the procedure of administering 0.5 mL of wheat grass extract every two weeks to mice.

The Sahli method is used to measure hemoglobin levels; it involves inserting 0.1 N HCl solution into the Sahli tube to number 10 or underline. Use a pipette to suck blood samples from mice whose tails have been surgically removed, up to the 20 mm3 line (0.22 cc). Put the blood sample in an HCl solution-filled tube, and wait for 3 min, or until the color turns brownish brown. When the hue is the same as the typical hemoglobin color, add distilled water droplets and stir with a stirring rod. Use the unit of g/dL printed on the hemoglobin tube to read the results of the hemoglobin levels in the tube.

Table 1. Hemoglobin levels in anemic male mice were compared with those in male mice who had consumed wheat grass juice.

Group	Hb Levels (g/dL)		Sig(2-tailed)
	Pretest	postest	
K1	15, 14	15,30	0,001
K2	11,30	12,48	
P1T	10,96	13,08	
P2T	11,40	14,28	
P3T	12, 16	15,76	
P1H	11,56	13,00	
P2H	12,02	14,20	
P3H	12,08	15,56	

The data were assessed for variance or analysis of variations to establish whether or not the treatment had an impact on the dependent variable (ANOVA). The DMRT test should be run with a substantial difference of 5% if Fcount > Ftable.

3 Result and Discussion

The results of observations of paired T-test analysis (Paired Comparation) about Hemoglobin levels in male mice anemia condition with hemoglobin levels in male mice after being given wheat grass juice can be seen in Table 1.

*Information: sig.(2-tailed) < Sig. 5% shows a significant difference.*T (soil) H (hydroponic), KI (Normal Control), K2 (Anemia Control). P1(Wheat Grass Juice 50%), P2 (Wheat Grass Juice75%), P3 (Wheat Grass Juice 100%).

According to the paired t-test results in Table 1, the Sig. (2-tailed) < 0.05 indicates a significant difference between the Hb levels of male mice before and after receiving wheat grass juice.

Based on the average Hb levels of male mice under anemic conditions (induction of NaNO2), P3 (wheat grass juice with concentration 100 percent) is 3.6 g/dL, P2 (wheat grass juice with concentration 75 percent) is 2.88 g/dL, and P1 (wheat grass juice with concentration 75 percent) is 2.88 g/dL for soil planting media (wheat grass juice with concentrated 50 percent). ie 2.12 g/dL. For the fuel husk growing media using the hydroponic method, that is P3 (100% concentration) which is 3.42 g/dL, P2 (wheat grass juice with concentration 75%) which is 2.18 g/dL, P1 (wheat grass juice with concentration 50%) is 1.44 g/dL. The increase in Hb levels in the anemia control group was 1.18 g/dL and in the control group Hb levels increased by 0.16 g/dL.

Based on Table 2, the results of the ANOVA analysis revealed that the data after receiving wheat grass juice (Triticum aestivum L.) with the treatment of planting media (factor A) and the concentration of wheat grass juice (factor B) were found to show that the factor A Fcount 0.036 while Ftable is 2.62 at the level of 5%, this means that F_{count} <

Table 2. The impact of wheat grass juice is revealed by the ANOVA test (Triticum aestivum L.) planted with different media on hb levels of anemic male mice *Mus musculus* L.)

Factor	F_{count}	F_{table}
A (Plant Media)	0,036	2,62
B (Concentration of Wheat Grass Juice)	24,675	

Table 3. Summary data of DMRT test results Hemoglobin Levels of Male Mice at Different Concentrations After After being given Wheat Grass

Treatment	Average Hb levels (g/dL)
P1 (grass juice wheat concentration 50%)	12.9500^{a}
P2 (grass juice wheat concentration 75%)	14.2400^{ab}
P3 (grass juice wheat concentration 100%)	15.6600^{bc}

F_{table} shows that the wheat media planting factor does not affect the Hb level of anemic male mice so DMRT further testing is not carried out. While the factor B F_{count} is 24.675 while F_{table} is 2.62 at the level of 5%, this means that $F_{count} > F_{table}$ reveals that the Hb level of anemic male mice is affected by the concentration factor of wheat grass juice.

Next to find out whether there are differences in each concentration of wheat grass juice given, then a further test was performed using DMRT at a significant level of 5%.

Information: In a column whose values are followed by different superscript letters. Figures followed by identical letters show that there is no significant different in the DMRT test at the 5% level.

It is clear from Table 3 summary of the DMRT test results that there are significant differences between of treatments for anemic male mice in terms of the rise in hemoglobin levels, with the treatments that increase hemoglobin levels the most having the greatest impact on wheat grass juice at its purest form.

The presence of Fe content in wheat grass juice affects the growth in Hb levels in anemic male mice. In comparison to other plants like green spinach, which only contains around 8.1 mg of iron per 100 g, and red spinach, which only contains about 4.48 mg per 100 g [9], wheat grass has a high iron concentration of 126 mg per 100 g [5]. Around 7 mg of iron can be found in 100 g of moringa leaves. Wheat grass' high iron content can aid the bone marrow's erythropoiesis process. Other precursors are needed for the erythropoisis process in bone marrow to be effective, and iron is one among those precursors [3]. Red blood cells contain hemoglobin, which is crucial. The hormone erythropoitin, which is produced by the kidneys to promote the creation of red blood cells, has an impact on the quantity of red blood cells. Iron must come from food if the body doesn't get enough of it from its diet. Anemia will develop if it is lacking as well.

Between chlorophyll and hemoglobin have the same porphyrin structure, the difference between them is in the central atomic structure where chlorophyll is magnesium (Mg) and hemoglobin is iron (Fe) [10]. The structure of chlorophyll is similar

to hemoglobin and allows the body to convert chlorophyll into hemoglobin [7]. Most anemia occurs due to lack of iron intake. This iron deficiency anemia can be suffered by babies, children, even adults both men and women [3]. Hb levels in the blood are low due to iron deficiency, which can also induce lethargy, weariness, and forgetfulness symptoms. It can also lower the body's defenses, making it more susceptible to infection from harmful germs and viruses [11].

Chlorophyll functions as an antioxidant [12], Chlorophyll and its derivatives can lower serum, triglycerides, and constipation in addition to having antimutagenic and anticarcinogenic properties [13]. In addition to its general advantages for blood flow, digestion, and body cleansing, wheat grass has been demonstrated to have antioxidant and anti-cancer properties [14]. According to research on the clinical effectiveness of wheat grass pills for leukemia patients, wheat grass tablets with chlorophyll can also lower the amount of white blood cells [15]. Wheat grass juice can be used as an antioxidant supplement to reduce oxidative stress since it has a low rate of oxidative stress in chemotherapy patients [16].

Since wheat grass contains both enzymatic and non-enzymatic antioxidants and has higher antioxidant activity than other fruits and vegetables, it can be used as an antioxidant supplement in chronic conditions.. Enzymatic antioxidants such as peroxidase, superoxide dismutase, and cytochrome are present in wheat grass [17]. Wheat grass contains beta carotene, ascorbic acid, and chlorophyll as non-enzymatic antioxidants [18].

The results of the ANOVA test revealed that the planting media had no impact on the male mice's anemia's Hb level. Soil media has more micro pores than the macro pores, so the soil has the ability to bind water which is strong enough. Husk charcoal media is a mixture of charcoal and husk media. Husk charcoal media has advantages in terms of the ability to bring water and good aeration. Both soil media and medium of fuel husk have the same ability to absorb nutrients, one of which is nitrogen which is a nutrient that plays a role in the growth of the vegetative phase, especially the leaves and stems.

Availability of nutrients greatly influences plant growth and development. The husk charcoal planting media requires special nutrients as a source of nutrients for plant growth and development, the nutrients used are AB Mix. AB Mix Nutrition contains essential nutrients needed by plants. Fe, potassium nitrate, and calcium nitrate are all present in nutrition A. B comprises KH2PO4, Mono Ammonium Phosphate, Potassium Sulfate, Boric Acid, Ammonium Hepta Molybdat, or Molybdat Sodium in terms of nutrients [19].

The hydroponic method of feeding husk planting is preferable, however the planting media used to grow wheat grass has no effect on raising hemoglobin levels in anemic male mice. The hydroponic method of feeding husk planting is preferable, however the planting media used to grow wheat grass has no effect on raising hemoglobin levels in anemic male mice. There are several benefits to hydroponics, including not needing to prepare ground, sterile planting media, efficient use of water and fertilizers, the ability to protect plants from direct sunlight, and a higher level of hygiene due to the fact that plants are not directly exposed to soil [20], so wheat grass be consumed directly by juicing, it will be cleaner. In addition to the high iron content in wheat grass compared

to other green vegetables, the harvest period of wheat grass is faster and can be repeated over and over on the same plant so it is more efficient.

4 Conclusion

Based on the results of a research, it is conceivable that anemic male mice (Musmusculus L.) with hemoglobin levels after being fed wheat grass juice had substantial variations (Triticumaestivum L.). The hemaglobin level of anemic male mice's hemaglobin was unaffected by the juice of wheat grass (Triticumaestivum L.) grown on various conditions (Musmusculus L.). The hemoglobin level of anemic male mice (Musmusculus L.) was affected by a concentration of wheat grass juice (Triticumaestivum L.) planted with various media, with 100% wheat grass juice being the most effective treatment.

Suggestions in this study for people to try wheat grass juice (Triticum aestivum L.), which significantly improves body health.

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References

1. A.C. Guyton, J.E. Hall, Buku Teks Fisiologi Kedokteran, Jakarta: EGC Penerbit Buku Kedokteran, 2011.
2. Riskesdas, Badan Penelitian dan Pengembangan Kesehatan Kementrian Kesehatan RI, Riset Kesehatan Dasar, 2018.
3. A.V. Hoffbrand, J.E. Pettit, P.A.H. Moss, Essential Haematology, Yogyakarta, Kanisius, 2005.
4. L.R. Degraff, The Complete Guide To Growing And Using Wheatgrass, Everything You Need To Know Explained Simply Including Easy To make Recipes, Atlantic Publishing Grup, Inc., Florida, 2011.
5. S. Mathur, R. Mathur, G.K. Kohli, Therapeutic use of Wheat Grass Juice fot the Treatment of Anemia in Young Women of Ajmer City (Rajasthan, India). International Journal of Nutritional Sciences, 2(1), 2017, pp. 1–5.
6. A.K.W.R. Dutta, Wheatgrass a Perfect Food and Its Anti-Microbial Properties From The Different Solvent Extract.World Journal of Pharmacy and Pharmaceutical Sciences, 5(9), 2016, pp. 1818–1828.
7. P. Wakeham, The Medicinal and Pharmacological Screening Of Wheatgrass Juice (Triticum aestivum L.): An Investigation Into Chlorophyll Content And Antimicrobial Activity, Journal of Pharmacology, 6(2), 2013, 20–30.
8. A.D. Susila, K. Koesniawati, Pengaruh Volume dan Jenis Media Tanam pada Pertumbuhan dan Hasil Tanaman Selada (Lactuca sativa) dalam Teknologi Hidroponik Sistem Terapung, Buletin Agron., 32(3), 2004, pp. 16–21.
9. N. Nelma., Analisis Kadar Besi (Fe) Pada Bayam Merah (Iresine Herbstii Hook) dan Bayam Hijau (*Amaranthus tricolor*) yang Dikonsumsi Masyarakat, Jurnal Analis Kesehatan Poltekkes Kemenkes Medan, 2014.
10. M. Chauhan, A Pilot Study on WheatGrass Juice for its Phytochemical, Nutritional and Therapeutic Potential on Chronic Diseases.International Journal of Chemical Studies, 2(4), 2014, pp. 27–34.

11. M. Masrizal, Anemia Defisiensi Besi, Jurnal Kesehatan Masyarakat, 2(1), 2007, pp. 140–145.
12. C. Hsu, P. Chao, S. Hu, C. Yang, The Antioxidant and Free Radical Scavenging Activities of Chlorophylls and Pheophytins. Food and Nutrition Sciences, Vol. 4, 2013, pp. 1–8.
13. U. Lanfer-Marquez, R.M.C. Barros, P. Sinnecker, Antioxidant activity of chlorophylls and their derivatives, Food Research International., Vol. 38, 2005, pp. 885–891.
14. C. Payal, K. Davinder, S. Sunaina, K. Gurlaganjeet, S. Gagan, C. Amit, R.D. Dhawan, A Review On Pharmacognosy and Pharmacological Aspects, International Journal Of Phytopharmacology, 6(2), 2015, pp. 80–85.
15. M.J.K. Lansolanki, L. Bhaidpatel, Clinical Efficacy Evaluation of Wheat Grass Tablets as Supportive Treatment in Leukemia Patients, RJPBCS, 6(4), 2015, pp. 1451–1454.
16. S. Sharma, B.R. Shirvastav, A. Shirvastav, Effect of Wheat Grass Juice on Reduced Glutathione and Glutathione Related Enzymes in Lung CancerPatiensts During Chemotherapy, World Journal Of Pharmaceutical Research, 5(9), 2016, pp. 1608–1615.
17. S.B. Parit, V.V. Dawkar, S.R. Tanpure, S.R. Pai, A.D. Chougale, Nutritional Quality and Antioxidant Activity of Wheatgrass (Triticum aestivum) Unwrapby Proteome Profiling and DPPH and FRAP assays, Journal of Food Science, 2018, pp. 1–13.
18. A. Agrawal, E. Gupta, R. Chaturvedi, Determination of Minerals and Antioksidant Activities at Different Levels of Jointing Stage in Juice Wheat Gass-The Green Wonder. International Journal Of Pure & Applied Bioscience, 3(2), 2015, pp. 311–316.
19. Y. Sutiyoso, Meramu Pupuk Hidroponik, Jakarta, Penebar Swadaya, 2003.
20. F.D.S. Silvina, Penggunaan Berbagai Medium Tanam dan Konsentrasi Pupuk Organik Cair pada Pertumbuhan dan Produksi Mentimun Jepang (Cucumis sativus) secara Hidroponik, J. SAGU, 7(1), 2008, pp. 7–12.