Biosaintifika 9 (3) (2017) 417-422

Biosaintifika
Journal of Biology & Biology Education

http://journal.unnes.ac.id/nju/index.php/biosaintifika

Effect of *Carica papaya* Leaf Juice on Hematology of Mice (*Mus musculus*) with Anemia

✉Afreni Hamidah, Evita Anggereini, Nurjanah

DOI: 10.15294/biosaintifika.v9i3.11427

Study Program of Biology, Faculty of Teacher Training and Education, Universitas Jambi, Indonesia

History Article

Received 10 September 2017
Approved 28 November 2017
Published 31 December 2017

Keywords

Anemia; Hematologics disease; Induction Sodium Nitrite; Papaya Leaf Juice

Abstract

Anemia is defined as a decrease in haemoglobin concentration and the number of erythrocytes. One source of iron in vegetable is the papaya leaf. This study aimed to determine the number of erythrocytes and hemoglobin levels after the application of *Carica papaya* leaf on mice through the sodium nitrite induction. The research design used was Randomized Design Complete by using five treatments with five repetitions on each treatment. The data measured in this study was number of erythrocytes and hemoglobin levels of rats before being given sodium nitrite, after being given sodium nitrite and after being given papaya leaf juice. The data obtained were analyzed statistically by using variance analysis (ANOVA), and the data that significantly different was then analyzed by Duncan Multiple Range Test (DMRT). The results showed that papaya leaf juice significantly affected the increase of erythrocyte number and hemoglobin level. The concentration that gives the best effect in increasing the number of erythrocytes is 50% papaya leaf juice concentration and 25% papaya leaf juice concentration which gives the best effect in increasing the hemoglobin content. Further, the findings can be used as baseline information for further scientific investigation for using papaya leaf as an alternative medicine in curing anemia disease and analyzing phytochemical, pharmaceutical and other biological activities on other plant or vegetable and to conducted further research by using female mice and pregnant female mice.

How to Cite

Hamidah, A., Anggereini, E., & Nurjanah. (2017). Effect of *Carica papaya* Leaf Juice on Hematology of Mice (*Mus musculus*) with Anemia. *Biosaintifika: Journal of Biology & Biology Education*, 9(3), 417-422.

✉ Correspondence Author:
Jl. Lintas Jambi - Muara Bulian Km. 15, Mendalo Darat, Jambi 36122
E-mail: afreni3103@gmail.com

p-ISSN 2085-191X
e-ISSN 2338-7610

INTRODUCTION

Indonesia still has four major nutritional problems: less calorie protein, less vitamin A, less iodine, and lack of iron disorders called nutritional anemia. Among the four nutritional problems one of which until now has not shown the bright spot of the success, that countermeasure is the problem of iron deficiency (Kiswari, 2014).

Lately, the pattern of human life tends towards unhealthy patterns. Lack of rest time (sleep), irregular eating patterns and unnoticed food intake can cause disruption to health such as anemia.This disease is caused by the disturbances in the circulatory system, where conditions in this anemia disease the production of erythrocytes decreased causing the lack of oxygen supply throughout the body. According to data obtained from the World Health Organization (WHO) (2002), anemia is one of the problems that contributes to the increase in maternal mortality (MMR) and Infant Mortality (IMR) in Indonesia. In Indonesia, about 20% of women, 50% of pregnant women, and 3% of men are iron deficient. Further data obtained from one of the hospitals in Jambi (RS. Dr. Bratanata) that shows that patients with anemia in 2015 reached 145 people.

The less iron levels in the body will aggravate the anemia suffered. Iron that comes from animal is the most easily absorbed by the body. However, not everyone can consume food derived from the animal because of the allergies or even the economic condition. Indonesia is a developing with predominantly middle and low in economic level. Food sources containing vegetable iron that have been widely known one of them is papaya which also has many benefits as a natural medicine (Arisandi & Andriani, 2008). *Carica papaya* is one of the plant species with antimalarial activity in Kupang. Root, stem, and leaf contents alkaloids, karpain, caricaxantine, violaxantine, papain, saponins, flavonoids, polyphenol (Ihwan *et al.,* 2016). The same phytochemical analysis of the three plant extracts detected saponins, tannins, cardiac glycosides, flavonoids and alkaloids in the three extracts. This study therefore, shows that *Mangifera indica* and T. occidentalis extracts have antianaemic potential (Ogbe *et al.,* 2010).Thus, methanolic leaf extracts of *Carica papaya* L. possible in vitro antisickling and membrane-stabilizing activities. Plant extract reduced hemolysis and protected erythrocyte membrane integrity under osmotic stress conditions (Imaga *et al.,* 2009).

This plant has many benefits, easy to find and relatively inexpensive. In Jambi Province, papaya have a high production rate of 161,820 trees in 2014 and with different numbers of production in each district. In Muaro Jambi Regency, in 2014 the number of papaya plants was 22,745 trees (Department of Agriculture of Crops and Food of Jambi Province, 2015). According to Milind and Gurditta (2011) papaya are included in the family of Caricaceae. It has been widely used in traditional medicine. Papaya leaves contain alkaloids, carpaine, pseudocarpaine, vitamins C, vitamin E, and corpocid. Papaya leaves also contain minerals such as potassium, calcium, magnesium, copper, iron, zinc, and manganese.

Sodium nitrite is one of the additives in food that works as a preservative, especially in meat product. It can inhibit the growth of *Clostridium botulinum* toxin's production in meat. The use of prolonged sodium nitrite can cause methemoglobinemia (Endreswari, 2000). Sodium nitrite works by the distribution of inhibition mechanism. Sodium nitrite will lead to the formation of methemoglobin, is the process by which Fe^{2+} in hemoglobin is oxidized to Fe^{3+} (Suudah, *et al.,* 2015). According to Ambarwati (2012), nitrite is a compound in the form of crystals or powder crystals that are odorless, white and soluble in water. The hemoglobin (Hb) decreased after $NaNO_2$ induction treatment group for 18 days. In contrast, mean of, MDA, ferritin level, and schistocytes percentage's in mice which were induced with $NaNO_2$ induction increased. Adduction of Katuk leaves chlorophyll and Cu-chlorophyllin proven to increase Hb level in mice although it has been induced by $NaNO_2$ (Suparmi *et al.,* 2016).

The aim of this research was to analyze the effect of papaya leaf juice on the hematological condition of mice (*Mus musculus* Linn.) with anemia through the induction of sodium nitrite ($NaNO_2$). The hematological condition analyzed including the number of erythrocytes and hemoglobin level. This study also determine the concentration of papaya leaf which can give the best hematological effect. The benefits of this research are:1. As experience and increase science for researchers in conducting basic research, 2. Can provide information for researchers and the public about the effect of giving papaya leaf to the hematological condition of male mice (*Mus musculus* Linn.) Anemia, and 3. To enrich the material of Animal Physiology on Biology Education Study Program.

METHODS

The method used in this study was an experiment using Randomized Design Complete (RDC) with five groups consisting of control (control with distilled water and control with the administration of $NaNO_2$ and fero fumarate) and treatment (treated with $NaNO_2$ and the administration of papaya leaf juice with different percentages: 25%, 50% and 75%) with five repetitions on each group

Sample of Research

The samples used in this study were 25 mice (*Mus musculus* Linn,) male DDW strain which was about 3 months old with an average weight between 20-30 g totaled 25 tail obtained from Biology Laboratory, Department of MIPA, Faculty of Science and Technology of Jambi University. The sampling technique used in this research was simple random sampling. Papaya used in this study was the California Varieties and obtained from community plantations of RT 03, Dusun IV, Kasang Pudak Village, Sub District Kumpe Ulu, District Muaro Jambi.

Preparation of experimental animals

Before the study was started, first prepared animal cages measuring 60 cm^2/animal, made of a wire-shaped box (Kusumawati, 2004). The cage was equipped with sawdust as a bedding which is always replaced every two times a week. Additionally, cage drinking tools also made of plastic bottles were accompanied by a glass or metal pipe so that mice can drink through the pipe (Smith & Mangkoewidjojo, 1988). Then the experimental animals were acclimatized for one week in a cage with room temperature 20-24ºC, and fed in the form of pellets as much as 4-8 g/day and drink as much as 5-8 ml/day (Hedrich, 2014).

Papaya Leaf Juice

As much as 1 kg young papaya leaves from the stem at the nodes 3 and 4, fresh green and clean were collected, then cut into small pieces. Then blended and added 1000 ml of distilled water and then squeezed and filtered using filter paper. A pure papaya leaf juice was considered as a concentration of 100%. Then the papaya leaf juice was diluted into several concentrations for the treatment of 75%, 50% and 25%.

Sample Group

After the acclimatization process for one week, the mice were grouped into five groups randomly. Each group contained of five mice.

Group I: Control, given distilled water for 14 days after anemia.
Group II: Comparative control, given ferrous fumarate for 14 days after anemia.
Group III: Given papaya leaves with concentration of 25% for 14 days after anemia.
Group IV: Given papaya leaf juice withconcentration of 50% for 14 days after anemia.
Group V: Given papaya leaf juice with concentration of 75% for 14 days after anemia.

The treatment of anemia was through the administration of Sodium Nitrite ($NaNO_2$). Based on previous research conducted by Sianturi *et al.* (2013), the average LD50 requirement of oral Sodium Nitrite in mice was 250 mg $NaNO_2$/ kg BW. Thus, the dose used on each rat was 2.5 mg.

Determination of Comparative Dosage of Fero Fumarate

For comparison, anemia drug used in the form of iron tablets contains 325 mg of ferrous fumarate. Each 325 mg ferrous fumarine tablet contains 250 mg of iron element. The dose conversion for humans weighing 70 kg in mice weighing 20 g is 0.0026. Then the dose for mice is: = 250 mg x 0.0026 = 0.65 mg/20 g mice = 0.65/20 g of BW.

Calculation of Red Blood Cells and Hemoglobin Levels

Blood collection was done through the tail of the mice and sequenced by hand to the tip of the tail for blood to flow and accommodated into Eppendorf tube filled with EDTA anticoagulation (Kusumawati, 2014).The calculation of the amount of red blood cell was performed using a haemocytometer device according to Kiswari (2014) by blood-sucking using erythrocyte pipettes up to the 0.5 mark line. The actual number of erythrocytes can be done by calculating the dilution in the erythrocyte pipette is 200 times. Factors to get a number of erythrocytes per uL of blood to be: 5 x 10 x 200 = 10,000

So, to get number of erythrocytes are N x 10,000 (N: number of erythrocytes from 5 chambers count). Determination of hemoglobin level was done by using Sahli method according to Kiswari (2014). Read the results of hemoglobin levels on the tube with the unit g/ml shown on the hemoglobin tube.

Pathological Treatment (Anemia)

The pathological treatment (anemia) was carried out by administering sodium nitrite ($NaNO_2$) for 14 days orally to the experimental

animals (Endreswari, 2000). According to Wolfensohn and Lloyd (1998) the volume of giving to oral mice is 20 ml $NaNO_2$/kg, so in mice weighing 20 grams of 0.4 ml $NaNO_2$/mice/day.

Treatment of Papaya Leaf Juice

Treatment was given for 14 days after the mice was already suffering from anemia, as much as 0.4 ml $NaNO_2$/20 g/day. The treatment was done orally with papaya leaf juice concentration of 25 %, 50%, and 75%.

Data collection and Analysis

The data collection in this research was conducted before the administration of papaya leaf juice, after the treatment of anemia and after the administration of papaya leaf juice. After the treatment of anemia and papaya leaf juice is measure a number of erythrocytes by using hemocytometer and measure of hemogobin level by using Hb tool Sahli. The data obtained then were analyzed statistically with analysis of variance (ANOVA). The data with significant effect then were proceed with Duncan Multiple Range Test (DMRT) test at 5% real level (Gaspersz,1994: 92).

RESULTS AND DISCUSSION

Erythrocytes Count

The average number of erythrocytes increased after papaya leaf juice, with the highest increase erythrocytes occurred in the treatment of papaya leaf juice 75%. The results of the analysis of variance shows significant effect of papaya leaf juice on the increase of erythrocytes counts

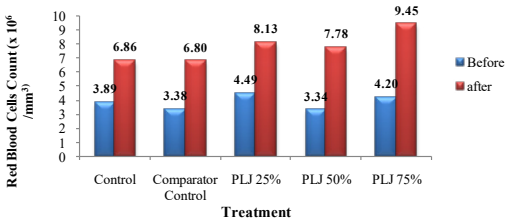

Figure 1. Diagram of the Average Number of erythrocytes Count of Male mice Before and After Treatment

The administration of papaya leaves had a significant effect on increasing the number of mices' erythrocytes (5%). The number of erythrocytes in control treatment was not significantly different from the comparison control, the treatment of papaya leaf juice 25% and 50%, but significantly different with the treatment with papaya leaf juice 75%. There was an increase in the number of erythrocytes after being treated with papaya leaf juice.

The highest increase of the number of erythrocyte count was found in treatment with papaya leaf juice 75% ($5.25x10^6$ /mm^3), followed by 50% (4.44×10^6 /mm^3) and 25% (3.63×10^6/mm^3). While the average increase in the number of erythrocytes is lowest in the control treatment that is equal to 2.97×10^6 /mm^3. The mice were induced by sodium nitrite orally for 14 days, resulted in the decreasing number erythrocytes. This is in accordance with Kusumawati (2004) which stated that the number of erythrocytes of mice is 6,86-11,7 x 10^6 /mm^3. If it less than this amount, it is said to be anemia. The cause of decrease in the number of erythrocytes is because of the distruption in iron absorption process so that the mice have iron deficiency anemia.

According to Suudah, *et al.* (2015), sodium nitrite causes the formation of methemoglobin. Natrium nitrite will oxidize a portion of hemoglobin so that ferric ions will be formed in the bloodstream. Yuningsih (2007) added that if nitrite ions are absorbed in the blood and when it comes in contact with erythrocytes, nitrite oxidizes Fe2 + in hemoglobin to Fe3 + to form methemoglobin (metHB).

Based on the results of analysis of variance, the treatment of papaya leaf 75% significantly different with the control, comparative control and treatment by using papaya leaf 25%. This is presumably because the content of Fe and vitamin C is high enough in papaya leaf. Results of research by Sianturi, *et al.* (2013), states that iron and vitamin C can increase the amount of erythrocytes of male mice anemia. Kiswari (2014) added that iron in the human body with high concentrations is present in erythrocytes as a part of the hemoglobin that serves to transport oxygen from the lungs to the cells that need it to perform the metabolism of glucose, fat, and protein into energy (ATP).

The results of Sembiring, *et al.* (2013) stated that iron play a role in the formation and maturation of erythrocytes in the process vitamin C serves as a trigger of the iron. Vitamin C can reduce Fe^{3+} to Fe^{2+} in the gut making it easy to absorb iron. Kiswari (2014) explains that the conversion of iron in the form of ferric to ferrous occurs in the stomach with the help of HCl. In patients with iron deficiency anemia the production of stomach acid decreases so it is difficult to convert iron in the form of ferri (Fe^{3+}) to ferro (Fe^{2+}). The presence of vitamin C SH (sulfidril) and amino acid sulfur can increase iron absorption because it can reduce iron in the form of ferric to ferro. Vitamin C can increase iron absorption

from food through the formation of ferro ascorbate complexes.

The number of eryhtrocyte of control group was not significantly different compared with the papaya leaf juice 25% and the papaya leaf juice 50%, but significantly different with the papaya leaf juice treatment 75%. This indicates that the treatment of papaya leaf juice is more effective than comparative control treatment with synthesis drug administration. The presence of vitamin C content in papaya leaves is greater than the content of vitamin C in synthesis drug, causing more iron absorption process. The highest increase of eryhtrocyte count in papaya leaf juice treatment was found in papaya leaf juice 75%, but statistically, the result of treatment using papaya leaf juice 75% was not significantly different with the papaya leaf 50%. This shows that the treatment of papaya leaf 50% gives the best effect is with a lower concentration of papaya leaf juice 75%, papaya leaf juice 50% can give the same effect.

Hemoglobin Level

Papaya leaf juice give a significant effect on the increase of hemoglobin level of mice at 5% level. The control treatment was significantly different with papaya leaf juice 75%, papaya leaf juice 25% and 50%, but not significantly different with control comparator. Comparative controls were significantly different from those of papaya leaf 75%, but were not significantly different from control, papaya leaf 25% and papaya leaf 50%.

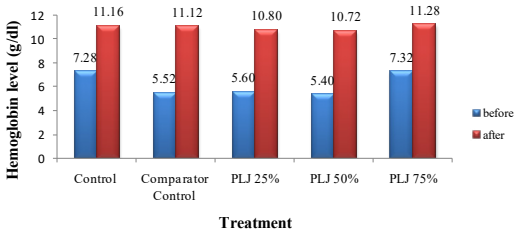

Figure 2. Diagram of the Average Hemoglobin Levels of Male Mice Before and After the Treatment

Each treatment of papaya leaves showed a significant difference to the control group (only distilled water). Increased hemoglobin levels are thought to be due to papaya leaf content Fe and vitamin C which can increase hemoglobin levels of mice. Rumimper *et al.* (2014) stated that iron and vitamin C minerals can increase Wistar rat hemoglobin levels. Sembiring, *et al.* (2013) added that the mineral content of Fe and Vitamin C can increase the hemoglobin rate of male mice anemia. The presence of vitamin C can increase the absorption of iron fourfold that is used in the process of formation of eryhtrocytes and hemoglobin. Vitamin C helps in the process of iron absorption so that synthesis of hemoglobin increases. Mehta and Hoffbrand (2006) suggest that vitamin C can increase iron absorption and is an important substance needed for the production of hemoglobin in normal erythrocytes.

Increased hemoglobin levels allegedly caused other than due to the presence of iron and vitamin C content, is also caused by the presence of Vitamin A in the papaya leaf. Results of research by Sianturi, *et al.* (2013), stated that vitamin A affects the formation of hemoglobin. This is because vitamin A and iron are very good for maintaining the health of epithelial tissue including endothelium in blood vessels. An adequate vitamin A will increase the value of hemoglobin along with the increase in vitamin A.

Treatment with the papaya leaf juice is more effective than comparative control treatment with synthesis drug administration. This is because vitamin C content in papaya leaf is higher than the content of vitamin C in synthesis drugs and its play a role in the process of iron absorption that can increase the synthesis of hemoglobin. The highest increase of hemoglobin was in the treatment with papaya leaf juice 50%. However statistically, but after analyzed statistically the papaya leaf juice 50% was not significantly different from the papaya leaf juice 25% and 75%. So, it can be determined that the treatment of papaya leaf juice 25% is the most effective because it can give the same effect in lower concentration.

CONCLUSION

The administration of papaya leaf juice significantly effect the hematological condition of mice with anemia. The most effective concentration of papaya leaf juice in increasing the number of erythrocyte is 50% and in improving the hemoglobin content is 25%.

Further, the findings can be used as baseline information for further scientific investigation for using papaya leaf as an alternative medicine in curing anemia disease and analyzing phytochemical, pharmaceutical and other biological activities on other plant or vegetable. It is expected to conducted further research by using female mice and pregnant female mice.

REFERENCES

Ambarwati, R. (2012). Effect Of Sodium Nitrite ($NaNO_2$) To Erithrocyte And Hemoglobin

Profile In White Rat (*Rattus norvegicus*). *Folia Medica Indonesiana,* 8(1), 1-5.

Arisandi, Y., & Andriani, Y. (2008). *Khasiat Tanaman Obat Edisi V*. Jakarta: Pustaka Buku Murah.

Endreswari, S. (2000). Penelitian Toksisitas Akut Natrium Nitrit Pada Hewan Uji Tikus. *Media Penelitian dan Pengembangan Kesehatan*, 10(2),12-15.

Gaspersz. (1994). *Metode Perancangan Percobaan.* Bandung: C.V. Armico.

Hedrich, H. (2014). *The Laboratory Mouse*. USA: Elsevier Science's.

Ihwan, I., & Koda, S. H. A. (2017). Antimalarial Herbal Plants in Kupang, Indonesia. *Biosaintifika: Journal of Biology & Biology Education*, 9(1), 95-104.

Imaga, N. O. A., Gbenle, G. O., Okochi , V. I., Akanbi, S. O., Edeoghon, S. O., Oigbochie, V., Kehinde, M. O., & Bamiro, S. B. (2009). Antisickling property of *Carica papaya* leaf extract. *African Journal of Biochemistry Research*, 3(4), 102-106.

Kiswari, R. (2014). *Hematologi dan Transfusi.* Jakarta: Erlangga.

Kusumawati, D. (2004). *Bersahabat dengan Hewan Uji Coba*. Yogyakarta: UGM Press.

Mehta, A., & Hoffbrand, V. (2006). *Hematology at A Glance*. Jakarta: Erlangga.

Milind, P., & Gurditta. (2011). Basketfull Benefits of Papaya. *International Research Journal of Pharmacy*, 2(7), 6-12.

Ogbe, R. J., Adoga, G. I., & Abu, A. H. (2010). Antianaemic potentials of some plant extracts on phenyl hydrazine-induced anaemia in rabbits. *Journal of Medicinal Plants Research*, 4(8), 680-684.

Rumimper, E. A., Posangi, J., & Wuisan, J. (2014). Uji Efek Perasan Daun Bayam Merah (*Amaranthus tricolor*) Terhadap Kadar Hemoglobin Pada Tikus Wistar (*Rattus norvegicus*). *Jurnal e-Biomedik (eBM)*, 2(2), 1-3.

Sembiring, A., Tanjung, M., & Sabri, E. (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 ($NaNO_2$). *Saintia Biologi*, 1(2), 60-65.

Sianturi, S., Tanjung, M., & Sabri, E. (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 ($NaNO_2$). *Saintia Biologi*, 1(2), 49-54.

Smith, J. B., & Mangkoewidjojo, S. (1988). *Pemeliharaan, Pembiakan, dan Penggunaan Hewan Percobaan di daerah Tropis.* Jakarta: Universitas Indonesia.

Suparmi., Sampurna., Nur A. C. S., Alvenia, M. E., Galuh, D. U., Iqrommatul, L. B., & Heavin, R. S. (2016). Anti-anemia Effect of Chlorophyll from Katuk (*Sauropus androgynus*) Leaves on Female Mice Induced Sodium Nitrite. *Pharmacognosy Journal,* 8(4), 375-379.

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.

Wolfensohn, S., & Lloyd, M. (1998). *Handbook of Laboratory Animal Mnagement and Welfare second edition*. USA: Blackwell Science.

Yuningsih. (2007). Keracuanan Nitrat-Nitrit Pada Ternak Ruminansia dan Upaya Pencegahannya. *Jurnal Litbang Pertanian*, 26(4), 153-159.