

RESEARCH ARTICLE

Administration of Mung Bean Extract (*Phaseolus radiatus*) in Increasing Hb and Ferritin Level and Decreasing Malondyaldehyde (MDA) Level in Anaemic Rats

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ABSTRAK

LATAR BELAKANG: Kacang hijau, selain mengandung protein, karbohidrat, dan lemak, juga mengandung zat besi dan vitamin C yang terbukti dapat memperbaiki anemia pada wanita hamil. Kadar zat besi yang tinggi berpotensi meningkatkan produksi ROS melalui reaksi fenton. **TUJUAN:** untuk membuktikan bahwa pemberian ekstrak kacang hijau dapat meningkatkan kadar Hb dan ferritin, serta menurunkan kadar malondihaldehide (MDA) pada tikus wistar anemia.

METODE: Penelitian ini menggunakan rancangan *Post Test Only Control Group Design*. Sebanyak 25 ekor tikus galur wistar anemia dibagi menjadi 5 kelompok: kelompok normal (Nor-G); kelompok kontrol negative (Neg-G), diberi diet rendah Fe; dan kelompok perlakuan yang mendapatkan diet rendah Fe dan ekstrak kacang hijau dengan dosis 0,18g/200g/hari (GP-0,18), 0,36g/200g/hari (GP-0,36), dan 0,72g/200g/hari (GP-0,72). Diet rendah Fe dan ekstrak kacang hijau diberikan selama 14 hari. Kadar Hb diukur dengan metode Sahli, kadar ferritin digunakan Kit Immulite ferritin, dan MDA dengan TBA.

HASIL PENELITIAN: Kadar Hb dan ferritin pada kelompok yang mendapatkan ekstrak kacang hijau 0,18g (75.56), 0,36g (90.98) dan 0,72g (95.87) lebih tinggi bermakna, $p < 0.05$. Sedangkan kadar MDA pada kelompok yang mendapatkan kacang hijau 0,18g (4.646), 0,36g (3.396) dan 0,72g (1.92) lebih rendah bermakna, $p < 0.05$.

KESIMPULAN: Hasil penelitian ini menunjukkan bahwa pemberian ekstrak kacang hijau terbukti meningkatkan kadar Hb dan ferritin serta menurunkan kadar MDA pada tikus anemia.

Kata Kunci : Ekstrak Kacang Hijau, Kadar Ferritin dan Kadar MDA

ABSTRACT

BACKGROUND: Mung bean, not only contains protein, carbohydrates, and fats, but also contains iron and vitamin C which are proven to overcome anemia in pregnant woman. High iron levels are potential to increase ROS production through fenton reaction. **OBJECTIVE:** to prove the administration of mungbean can increase Hb and ferritin levels and decreasing malondihaldehide (MDA) in anaemic rats.

METHODS: This research uses *Post Test Only Control Group Design*. A total of 25 anaemic rats were divided into 5 groups: normal group (Nor-G); negative control group (Neg-G), were given low Fe diet; and treatment group with low Fe and mung bean extract at dose of 0,18g/200g/days (GP-0,18), 0,36g/200g/days (GP-0,36), dan 0,72g/200g/days (GP-0,72). Low Fe diet and mung bean extract were administered for 14 days. Hb levels were measured using Sahli method, ferritine level using Immulite Ferritine Kit, and MDA were measured using TBA.

RESULTS: Compare to Nor-G and Neg-G, levels of ferritin and Hb on groups with mung bean extract 0,18g (75.56), 0,36g (90.98) and 0,72g (95.87) were significantly higher ($p < 0.05$). While MDA levels on groups with mung bean 0,18g (4.646), 0,36g (3.396) and 0,72g (1.92) were significantly lower than on Nor-G and Neg-G groups ($p < 0.05$).

CONCLUSION: The administration of mung bean extract can increase Hb and ferritin, also lower MDA level on anaemic rats.

Keywords : Mung bean extract, Ferritin level and MDA level

INTRODUCTION

Anemia is a nutritional problem experienced by millions of people and still one of the major challenge for human health in developed or developing countries. The prevalence of anemia in developing countries is still very high, estimated 43%. While in developed countries, the prevalence of anemia is 9%. The group with higher risk of anemia are infants (47%), non-pregnant woman age 15-49 years old (30%), and pregnant women (42%)

(Sudikno, 2016). The prevalence of Anemia in Indonesia especially in women with reproductive age of 15–44th, according to the Riset Kesehatan Dasar/basic health research, in year 2013 was 35,3% (Risksedas, 2013). Anemia can cause maternal mortality during labor, low birth weight babies, prone to infections, abortions and premature births. In addition, anemic patients, especially chronic anemia, also cause a decrease in work capacity and productivity (AlDallal S, 2016). Therefore,

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efforts to decrease the prevalence of anemia become very important. Data showed that among the various causes of anemia, in addition to infection, nutritional factors, especially iron deficiency, also contributed to the incidence of anemia. (Agrawal S, et al. 2006).

Iron deficiency anemia caused by iron deficiency in the blood, which is characterized by incomplete hemoglobin (Hb) synthesis, the Hb levels become low and produce microcytic and hypochromic red blood cells (AlDallal S, 2016). Hemoglobin is a protein in the blood that serves as a means of transport of oxygen to all body tissues. If Hb level is decreasing, so does the body work capacity. There are some conditions that require Hb and iron sufficiency. Fetuses underwent increased tissue mass due to its growth, pregnant women and anemic patients with iron deficiency. Iron is an essential element needed to maintain the physicochemical process of the body (AlDallal S, 2016). The efforts to maintain the balance of physicochemical process in the body in order to remain physiological becomes very important. Iron absorption is mainly regulated in the intestine, and once iron absorbed, there is no mechanism of excretion either by the kidneys or the liver (Burke RM, et al. 2014; Andrews NC, 2008). For this reason, iron homeostasis in the body is strictly regulated. The body absorbs iron from food about 1-2 mg daily, which is offset by physiological processes such as menstruation and other bleeding within the body (Siah CW, et al. 2006; AlDallal S, 2016). Excessive iron levels can lead to increased production of hydroxyl radicals (OH•), a highly reactive oxygen species (ROS) and detrimental to the body through Fenton reaction (Barbusinski K, 2009). Increased OH• levels can cause cell membrane damage that contains unsaturated lipids due to lipid peroxidation. Peroxidation of erythrocyte membranes causes hemolysis resulting in a decrease hemoglobin levels.

Iron consumption in humans largely derived from heme iron (animal) and non-heme (plant). The heme iron is then broken down by the pancreatic enzyme and releases it into the intestinal lumen. The heme iron is absorbed by the enterocyte as metalloprotein via the divalent metal transporter 1 (DMT-1), then broken down by the heme oxygenase-1 enzyme to release the non-heme iron. Iron is then exported by ferroportin located in the basolateral enterocyte (Wimbley JTD, Graham D, 2011). Ferroportin can be bound by hepcidin, which prevents ferroportin from exporting iron so that the serum iron content decreases (Burke RM, et al. 2014). After absorption, the iron can then be stored in ferritin, which keeps the iron in a non-reactive state in the cell, or in transferrin, which also keeps the iron in a nonreactive

state in the circulatory fluid, but can be transferred into the cell (Burke RM, et al. 2014). Erythroblast through transferrin receptor binds to a complex of iron transferrin that endocytically incorporates iron into Hb. Iron absorption is maintained by erythropoiesis. The dynamic iron absorption process is mediated by hepcidin which regulates iron entry and prevents iron from escaping enterocytes and macrophages. Deposits of iron in the body, whether in cells or in serum, are mostly in the form of ferritin which is regulated through the process of iron absorption. Non-heme iron is absorbed in the form of ferro (Fe +2), reduced to iron ferric (Fe +3) by ascorbic acid in intestine, stomach acid, and intestinal reductase enzyme increases absorption of iron (Burke RM, et al. 2014; AlDallal S, 2016). Therefore, consuming heme iron along with ascorbic acid will improve the absorption of iron.

Mung beans are one food that can meet basic human needs because it contains macro and micro elements, vitamins, and amino acids. Various elements contained in mung beans include: Isoleucine 6.95%, Leucine 12.90%, Lysine 7.94%, Methionine 0.84%, Phenylalanine 7.07%, Threonine 4.50%, Valine 6.23%, and nonessential amino acids (Yusuf, 2014). Nutritional content in 100g of mung bean flour is 345 kcal energy, 4.5g protein, 83.5g carbohydrate, 1.0g fat, 50.0mg of calcium, 100mg phosphorus, and 1mg iron (Mahmud MK, et al. 2009; Yusuf, 2014). In addition to containing these nutritional elements, mung beans also contain isoflavones of 70.74 mg/100 grams of fresh mung beans (Iswandari, 2008). Furthermore, in two cups of mung beans consumed daily also contain vitamin C 80% of daily vitamin C requirement (75 mg). Results of the study conducted by Helty (2010) indicated that consuming mung 2 cups mung bean juice for 7 days in a row can increase the hemoglobin content of 1.12 g/dl and erythrocyte 0.5 million/ml in cancer patients undergoing chemotherapy (Helty, 2010). The study conducted by Maulina stated that the administration of mung beans with a dose of 18 gr/kg BW/day and 36 g/kgBW/day in rats showed an increase in hemoglobin levels of white rats respectively of 16, 50 /dL and 14.35 g/dL (Maulina N, 2011). Another study reported by Novidiyanto showed that the provision of mung bean sprouts could reduce Malondialdehyde (MDA) levels in high fat diet rats (Novidiyanto, 2016). The purpose of this study is to prove that administration of mung beans will increase levels of Hb and Ferritin, and lower levels of MDA in rats anemia.

METHODS

This study is an experimental study with Post

Table 1. Mean of Hb, Ferritin, and MDA Levels on each groups

| Variables | Groups | | | | | p (Anova) |
|---|-------------------------------|-------------------------------|---------------------------------|-------------------------------|--------------------------|--------------|
| | Nor-G N=5 Mean (±SD) | Neg-G N=5 Mean (±SD) | GP-0.18 N=5 Mean (±SD) | GP- 0.36 N=5 Mean (±SD) | GP-0,72 Mean (±SD) | |
| Hb level before Treatment (g/dl) | 12.85 (+0.023) | 6.80 (+.) | 6.73 (+ ..) | 6.73 (+ ..) | 6.59 (+ ..) | 0.000 |
| Hb level after treatment (g/dl) | 12.75 (+ ..) | 6.72 (+ ..) | 9.09 (+ ..) | 10.12 (+ ..) | 11.31 (+ ..) | 0.000 |
| ΔHb level before and after Treat (g/dl) | 0.12 | 0.08 | 2.37 | 3.39 | 4.72 | 0.000 |
| Ferritin level (µg/l) | 110,9 (±2,4) | 45,09 (±3,7) | 75,56 (±2,77) | 90,89 (±1,46) | 95,87 (±2,16) | 0,000 |
| MDA level (nmol/ml) | 1,206 (±0,96) | 9,07 (±0,12) | 4,646 (±0,48) | 3,396 (±0,23) | 1,982 (±0,23) | 0,000 |

Test Only Control Group Design. The number of samples in this study were 25 anemic wistar rats, divided into 5 groups randomly each consisting of 5 rats. The normal group (Nor-G), standard fed, without a low Fe diet ; negative control group (Neg-G), fed only with low Fe diet; and the treatment group who received a low Fe diet and mung bean extract respectively with a dose of 0. 18g/200 g BW/day (GP-018), 0. 36g/200 g BW/day (GP-036), and 0. 72 g/200 g BW/day (GP-072), dissolved in 3 ml of aquadest. The treatment is conducted for 14 days orally.

Mung Bean Extract

Mung bean extract was obtained by maceration method and using water as solvent. Dried mung beans were squashed, then each dose of 0. 18, 0. 36, and 0. 72 g/200 g BW/day dissolved into 3 ml of aquades. Each solution is then filtered using a coarse filter paper, followed by a fine filter paper. The filter results are an extract of mung beans.

Inducing Anemic Rats

Anemic rats is induced by giving a low iron diet in wistar rats for 14 days ad libitum.

Measurement of Hemoglobin, Ferritin, and MDA Levels

Hemoglobin levels of rats were measured by the Sahli method. Whereas ferritin level measured by Immulite Ferritin Kit, The MDA level was measured using a modified test method of tiobarbituric acid (TBA) with Spectrophotometer at 523 nm wavelength.

STATISTICAL ANALYSIS

All data were tested with Shaphiro Wilks and Levene's tests to determine whether the data distribution were normal and homogeneous. The result of statistical analysis showed that the data had normal and homogenous distribution of $p > 0.05$. Therefore to determine the difference of Hb, ferritin, and MDA levels among the parameters tested using parametric test by One Way Anova, followed by Post Hoc LSD test. The study was conducted, after obtaining approval from the Ethical Committee from Bioethics Commission of the Faculty of Medicine, Islamic University of Sultan Agung Semarang.

RESULTS

After oral administration of mung beans for 14 days in the three treatment groups, the results were shown in Table 1 below.

The highest Hb levels were in the normal group, followed by the GP-072, GP-036, GP-018, and the lowest level were in negative control group. Anova statistical analysis showed that there was a significant difference between groups, $p < 0.005$. The same pattern was found in difference Hb level between before and after treatment with mung bean extract. While on ferritin, the highest levels were in the normal group, followed by GP-0.72, GP-0.36, GP-0.18, and the lowest level found in the negative control group. On the contrary, the lowest MDA level was found in the normal group, followed by GP-0.72, GP-0.36, GP-0.18, and the highest level was in the negative control group. Statistical analysis of anova on ferritin and MDA levels in all groups showed a significant difference, $p < 0.005$.

Hb Levels Post Treatment

Post Hoc LSD analysis showed that Hb levels in the GP-0.18, GP-0.36, and GP-0.72 groups were significantly higher than the negative control group, $p < 0.05$. The increase of Hb levels in the treatment group (GP-0.18, GP-0.36, and GP-0.72) occurred in accordance with the increase in dose, so that the highest increase in Hb was at mung bean dose 0.72g. However, when compared with the normal group, the Hb level in GP-0.72 is still significantly lower, $p < 0.05$ (figure 1).

Differences (§)Hb Levels Before and After Treatment

Post Hoc LSD analysis showed that the difference of Hb levels before and after treatment in GP-0.72, GP-0.36, and GP-0.18 groups were significantly greater than the Neg-G and Nor-G groups, $p < 0.05$. The magnitude of the difference in the treatment group (GP-0.18, GP-0.36, and GP-0.72) occurred in accordance with the increase in dose (figure 1)

Ferritin Level

Post Hoc LSD analysis showed that ferritin levels in the GP-0.18, GP-0.36, and GP-0.72 groups were

significantly higher than the negative control group, $p < 0.05$. Increased levels of ferritin in the treatment group (GP-0.18, GP-0.36, and GP-0.72) occurred in accordance with the increased dose, resulting in increased ferritin levels. The highest level was in the dose of mung beans 0.72 g. Nevertheless, when compared with the normal group the ferritin levels at the 0.72 dose were still significantly lower, $p < 0.05$ (figure 1).

MDA Level

Post Hoc LSD analysis showed that the levels of ferritin in the group GP-0.18, GP-0.36, GP-0.72 significantly lower compared to the negative control group, $P < 0.05$. MDA levels decrement in the treatment group (GP-0.18, GP-0.36, and GP-0.72) occurred in accordance with an increase in dose, so the lowest levels of MDA is at a dose of 0.72 g mung beans. However, when compared with the normal group, MDA level of G-0.72 is still significantly higher, $p < 0.05$ (figure 1).

DISCUSSION

The results of this study indicated that a low iron diet in each group performed for 14 days on an ad-libitum is proven to lower hemoglobin levels up to

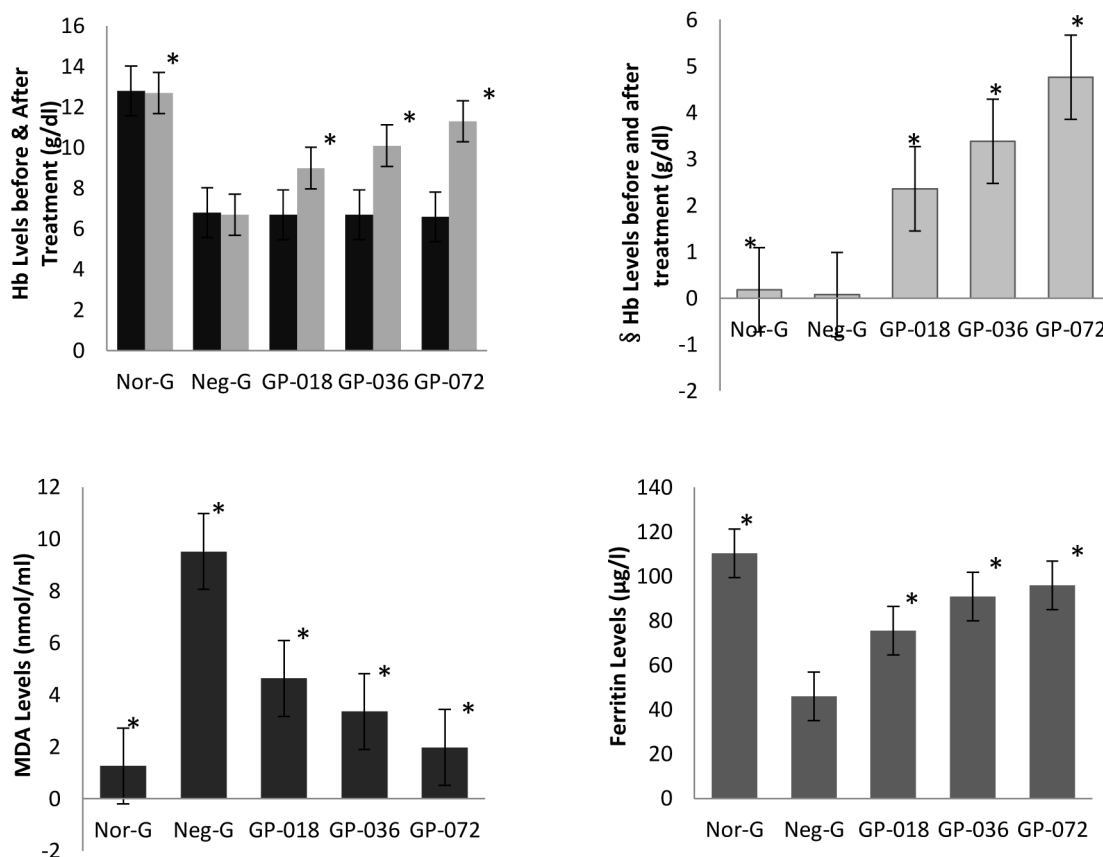


Figure 1. Level of Haemoglobin, §Hb before and after treatment, Ferritin, and MDA in each groups. * Significantly difference compared to that of Neg-G

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6.3- 6.88 g/dL, whereas hemoglobin levels in normal control rats was 12.70 - 13.17 g/dL. In groups receiving mung beans with doses of 0.18, 0.36, and 0.72g/200 g BW/day for 14 days showed significant increases in Hb levels in accordance with dose increases. The results of this study in accordance with studies conducted by Maulina which stated that the provision of mung beans with doses of 18 and 36 grams/kgBW/day increase Hb levels significantly (Maulina, 2015). Results of a study reported by Purushothaman, et al. also in line with the results of this study. Purushothaman, et al. (2008) reported that regular consumption of mung beans for one year could increase serum protein levels from 5.36 to 6.73 g/dL and serum iron levels from 16.6 to 46.7 µg/dL. Hb increase due to mung beans is caused by iron contained in mung beans. Data showed that mung beans contain iron, vitamin C and other nutrients that are useful for increasing iron absorption by the intestines, therefore mung bean consumption by infants and children are recommended (Ganesan K, 2017; Moriyama M, 2008). This finding is consistent with the theory that the absorption of iron in the intestine will be facilitated by ascorbic acid which reduces ferric iron (Fe +2) to iron ferrous (Fe + 3) (AlDallal S, 2016). Given that mung beans are foods that meet basic human needs, because they contain macro, micro, vitamin, and amino acids, it becomes very rational when mung beans are used to support food diversification in order to prevent anemia (Yusuf, 2014). Various amino acids, especially histidine, Phenylalanine, Leucine, alpha ketoglutarate, and glycine. The ketoglutarate alpha combined with the amino acid glycine forms a pyrrole compound. Four pyrrole compounds then combine to form protoporphyrin III. Protoporphyrin III reacts with Fe to form Heme. Four hemes combine with globin to form hemoglobin. In addition, folic acid and vitamin B12 are important for Hb formation, especially in the erythrocyte maturation phase (Ajioka RS, et al. 2006).

The results of this study also showed that ferritin levels in mung bean extract group with dose of 0.18 g/200 g BW/day, 0.36 g/200 g BW/day and 0.72g/200 g BW/day were higher than negative controls. Increased levels of ferritin in three doses of mung beans were in line with increment in dose. The higher the dose, the higher ferritin level increased. Similar results were reported by Purushothaman, which showed that the extract of mung beans also improved ferritin level from 3.56 to 5.94µg/dL and Hb from 7.54 to 8.29 g/dL (Purushothaman, et al. 2008). Among the three doses of mung beans extract, the most effective increase in ferritin levels was a dose of 0.72g/200 g BW/day.

Increased levels of ferritin as a result of the provision of mung beans associated with high level of serum iron. As described above that mung beans contain iron, folic acid, and vitamin C which facilitates the absorption of iron. As a consequence, the supply of iron into cells increases, as does ferritin synthesis. Various literatures showed that the synthesis of ferritin in a cell is determined by the supply of iron entering the cell directly, otherwise the synthesis of transferrin is inhibited. The results of this study are in accordance with the research reported by Purushothaman, which stated that the higher the iron inside the cell, then the higher the ferritin is synthesized (Purushothaman, et al. (2008). Given the iron circulating freely in serum is toxic, it must be kept by cells in the form of ferritin that is not toxic and can be transported to the place where it is needed. This is because free iron can act as a catalyst that converts Fenton re-action of H₂O₂ into free radicals OH• which is highly reactive and potentially caused oxidative stress (Barbusinski K, 2009).

Oxidative stress is a condition caused by an imbalance between oxidants and anti-oxidants which are marked by the rise in the levels of malondialdehyde (MDA). The study conducted by Patimah stated that anemia status influencing MDA levels before and after iron supplementation with a dose of 5.04 mg/200g of rat weight for 7 days of treatment (Patimah, 2009). Iron supplementation increase reactive oxygen species (ROS) and reactive nitrogen species (RNS), as well as lipid peroxidation and oxidative stress (Barbusinski K, 2009). Lipid peroxidation is the mechanism of cell membrane injury, caused by a reaction between OH• with unsaturated fatty acid membranes creating ROO. ROO react with unsaturated fatty acids to form MDA (Nasihun T, 2016). Referring to that fact, the MDA is used as a marker of oxidative stress on cells and tissues (Fisher, 2007). MDA levels in the negative control group had the highest value compared to the normal group and the group receiving a dose of mung beans 0. 18, 0. 36, and 0. 72 g/200 g BW/day. The administration of mung beans with a variety of doses can reduce MDA levels. Decreased levels of MDA are strongly suspected caused by decrement in lipid peroxidation due to increased absorption of iron-fortified vitamin C contained in mung bean extract. The statement indirectly based on the evidence that MDA levels in the normal group (without mung beans) has the lowest value. Decreased MDA levels due to mung beans, associated with phenolic acids and flavonoids contained in mung beans, act as natural antioxidants derived from plants (Ganesan K, 2017). Flavonoids function as primary antioxidants, chelators, and superoxide anion scavengers, and have

stronger antioxidant activity against peroxy radicals compared with vitamin E, vitamin C, and glutathione. Flavonoid and isoflavones contained in mung beans amounted to 70.74 mg/ 100g, containing hydroxyl groups which acts as a hydrogen donor that can capture free radicals (Zhan, 2007). Referring to these facts, the administration of mung bean extract, as an additional food to prevent especially iron deficiency anemia, and also to prevent oxidative stress is a rational choice.

CONCLUSION

Administration of mung beans extract with a dose of 0.18 g, 0.36 g, and 0.72 g/200 g BW/day can increase Hb and Ferritin levels, and decrease MDA levels in anemic wistar rats.

CONFLICT OF INTEREST

No conflict of interest to be reported in this study.

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