The Relationship Between Systolic Blood Pressure and LDL Cholesterol Male Sprague Dawley Rats Given High Fat Diet and Mung Bean Sprouts (*Phaseolus radiatus* L.)

Novian Swasono Hadi¹, Dwi Lestari², Arta Farmawati³, Ahmad Ghozali⁴, and Lily Arsanti Lestari⁵

¹Department of Nutrition, Politeknik Kesehatan Kemenkes Gorontalo, Jl Taman Pendidikan, Gorontalo, 96113, Indonesia
²School of Public Health Graduate Programme, Faculty of Medicine, Universitas Gadjah Mada, Jl Farmako, Sekip Utara, Yogyakarta, 55281, Indonesia
³Department of Pathology Anatomy, Faculty of Medicine, Universitas Gadjah Mada, Jl Farmako, Sekip Utara, Yogyakarta, 55281, Indonesia
⁴Department of Health Nutrition, Faculty of Medicine, Universitas Gadjah Mada, Jl Farmako, Sekip Utara, Yogyakarta, 55281, Indonesia

Abstract

Lifestyle changes with high fat food consumption are one of the factors the risks of cardiovascular diseases like coronary heart disease and atherosclerosis. The formation of atherosclerotic plaque in the walls of blood vessels initiated by the absorption of LDL through the blood to the endothelial cells that cause LDL is oxidized by the Reactive Oxygen Species, which initiated in the pathogenesis of human hypertension. Mung bean sprouts contain antioxidant compounds can be used as a functional food in the treatment of cardiovascular disease and hypertension. Male Sprague-Dawley was 24 at 8 wk and it is divided into four groups. The first group was given the standard diet, the second group was given a high-fat diet, the third group was given a high-fat diet, and mung bean sprouts 1 mL · 200 gBW⁻¹, the fourth group was given a high-fat feed and supplements of vitamin E dose of 23 IU. Blood pressure and LDL concentration were measured after the treatment. Systolic Blood pressure and levels of LDL cholesterol who were given mung bean sprouts and vitamin E has no statistical difference with group of normal. Levels of blood pressure and LDL cholesterol have a positive correlation. Mung Bean sprouts give the effect of a decrease in blood pressure and impeded elevated levels of LDL male Sprague Dawley rats.

Keywords: Blood pressure, High fat diet, LDL cholesterol, Mung bean sprouts, *Sprague Dawley* rats.
1. Introduction

Peoples lifestyle to consuming food high fats into one of the causes of cardiovascular diseases like atherosclerosis and of coronary heart disease. The risk atherosclerosis would increase if preceded dyslipidemia [1]. Dyslipidemia is an abnormality of metabolism lipoproteins, either that or lack of excessive. The state of being might be risen from total cholesterol levels, low density levels lipoproteins (LDL), and the levels of triglyceride and reducing levels of high density lipoproteins (HDL) in blood [2]. Dyslipidemia can improve lipoproteins LDL levels and lower the levels HDL that is the risk of cardiovascular diseases [3].

Increased concentrations of LDL lead to the liver cannot metabolize maximally. The concentration of LDL containing polyunsaturated fatty acids in the lipid and cholesterol ester, causing lipid peroxidation process, thus easily oxidized LDL [4]. Increased free radicals in dyslipidemia affect the increase in lipid peroxidation products so that the body experiences oxidative stress [5]. Atherosclerosis causes the coronary arteries to become inelastic and narrow so that the resistance to blood flow in the arteries increases. High blood pressure caused by increased systolic pressure caused blood vessels elastic not accompanied by the increase in diastolic blood pressure due to narrowing of the arteries [6].

A healthy diet and balanced as well as foods that contain lots of antioxidants is one effective way to prevent dyslipidemia. Antioxidant properties can neutralize free radicals cause dyslipidemia and cardiovascular disease [7]. Mung bean sprouts are known to have vitamin E content is high enough so that potential as a source of antioxidants that come from food (exogenous antioxidants) [8]. Vitamin E has a preventive effect on cardiovascular disease because vitamin E can protect polyunsaturated fatty acids against oxidative damage in the cell membrane [4].

2. Materials and Methods

2.1. Diet and intervention

Standard diet given in this experiment was AIN-93 M and a high-fat diet modified AIN-93 M using tallow (Table 1)

The Sprouts were used in this study are sprouts of mung bean species *Phaseolus radiatus* (L.) with germination time of 48 h. Sprouts were smoothed using a blender. After that, homogenized using a homogenizer. The dose of 1 mL sprouts intervention
TABLE 1: The composition of the diet.

| Component         | Modification of High |       |       |
|-------------------|----------------------|-------|-------|
|                   | Standard Diet*       | Fat Diet** |
| Corn Oil          | 620.692              | 440.66 |
| Casein            | 140                  | 140    |
| Sucrose           | 100                  | 100    |
| Soybean Oil       | 40                   | 40     |
| Fiber             | 50                   | 50     |
| Mineral Mix AIN 93| 35                   | 35     |
| Vitamin Mix AIN 93| 10                   | 10     |
| Tallow            | 0                    | 180    |
| L-sistin          | 1.8                  | 1.8    |
| colin Bitartat    | 2.5                  | 2.5    |
| TBGQ              | 0.008                | 0.04   |
| Total (g)         | 1 000                | 1 000  |

based on the content of vitamin E and adjusted to the optimal dose as an antioxidant in humans. For comparison, the use of vitamin E supplements was adjusted to the optimal dose of vitamin E supplements that recommended in humans. Commercial vitamin E supplements are used as controls.

2.2. Animal experiment

Amount of 24 male rats *Sprague Dawley* were aged 8 wk. In this study, experimental animals male *Sprague Dawley* rats were divided into four groups randomly. The first group was given the standard diet, the second group was given a high-fat diet, the third group was given a high-fat diet, and intervention in the form of green bean sprouts 1 mL · 200 g BW⁻¹, and group IV was given a high-fat diet and supplements of vitamin E doses of 23 IU. In this study, test animals first adapted using the standard feed for 3 d in individual cages with temperature and optimal lighting. The study lasted 28 d (4 wk).

2.3. Systolic blood pressure and ldl cholesterol analysis

Blood pressure measurement is performed twice during the study by using tail-cuff detection. Analysis of blood LDL levels also performed twice during the study. The first measurement is done at the beginning of the experiment after undergoing acclimatization mice in the laboratory for 3 d; the second measurement was done at the end of the intervention. Blood serum analysis was done by the end of the intervention.
Blood pressure measurement using the tool sphygmomanometer, with such a device can be seen with systolic blood pressure in Sprague Dawley rats [9]. LDL cholesterol analysis using mathematical calculations with the formula LDL = total cholesterol - (HDL + triglycerides/5) [10].

2.4. Ethical clearance

This study has been approved and granted by the Research Ethics Committee of Faculty of Medicine, Universitas Gadah Mada with the letter number KE/FK485/EC dated May 8th, 2015.

2.5. Statistical analysis

The data were analyzed using ANOVA with posthoc Bonferroni test if there are significant differences. Pearson correlation was used to test the relationship between triglycerides and MDA liver also HDL and MDA liver. Significance level used was 95 %. Stata program used in this statistical analysis.

3. Result

3.1. Correlation between LDL cholesterol and systolic blood pressure

Levels of blood pressure and serum LDL levels were significantly different in each group. Blood pressure who were given the mung bean sprouts and vitamin E is not different meaning the blood pressure in the normal group. Levels of LDL in the group given the mung bean sprouts were not significantly different when compared to the group given vitamin E (Table 2).

Analysis of the correlation between serum levels of LDL and liver tissue MDA showed a positive correlation. If the serum LDL levels increase, it will be followed by an increase in blood pressure. Has a regression formula and correlation coefficient Pearson correlation $y = 1.0317x - 61.02$ and $r = 0.6541$.

Increased levels of LDL in the group with the provision of a high-fat diet was higher than the control group and the other treatment groups. Increased levels of fats in the blood may occur due to increased synthesis or decreased degradation that can be caused by genetic abnormalities or other abnormalities such as high consumption of...
## Table 2: Systolic Blood Pressure and LDL Cholesterol.

| Group             | Variable                      | Systolic blood pressure (mmHg) | DL Cholesterol (mg · dL⁻¹) |
|-------------------|-------------------------------|--------------------------------|----------------------------|
| Normal            |                               | 93 ± 2.68a                     | 30.58 ± 3.30a              |
| Control           |                               | 129.83 ± 2.31b                 | 73.31 ± 4.89b              |
| Mung bean sprouts |                               | 96.83 ± 4.35a                  | 42.03 ± 1.81c              |
| Vitamin E         |                               | 95.66 ± 1.63a                  | 38.48 ± 1.84c              |

Data in mean±SD

Different letter in the same column shows that there has been a significant, \( P < 0.05 \)

Fat [2]. Hyperlipidemia can also be caused by the consumption of high-fat foods, such as cow brain, seafood, egg yolk and others [6]. Diets high in cholesterol can make the atherosclerotic plaques in experimental animals, lipid principal in atheroma (plaque) is cholesterol and cholesterol ester derived from plasma, epidemiological analyzes of large-scale demonstrated significant correlation between cholesterol plasma total or LDL levels and severity of atherosclerosis [11]. Research conducted Onggang [12], the provision of high-fat diet (lard and egg yolk) in mice.

Figure 1: Relationship between systolic blood pressure and LDL cholesterol male Sprague Dawley rats given high fat diet and vitamin.

Free fatty acid esterification into triacylglycerol could experience both in the liver, adipose tissue, and muscle. Excessive fat intake resulting in the accumulation of triacylglycerol in the muscles, one of which is the heart muscle. Triacylglycerol accumulation in the liver, transported by VLDL into the blood stream, and then headed to the peripheral tissues or muscles that have the enzyme lipoprotein lipase so that more fat accumulation in heart muscle cells. This condition is called fatty heart muscle [11]. The hyperlipidemic
condition can cause high blood pressure which affects cardiac dysfunction. In the early stages, the heart will perform an adaptation mechanism by adding a period of cell protein to maintain blood pressure, it is known as swelling of the heart (cardiac hypertrophy) [13].

Research shows that the function of endothelium affected by Reactive Oxygen Species that can inactivate Nitric Oxide (NO) degradation. NO degradation would cause vasomotor dysfunction, smooth muscle proliferation, and the expression of inflammatory genes that can disrupt endothelial-dependent vasodilation, all these are mechanisms that initiate the development of atherosclerosis, hypertension, and coronary heart disease [14]. Increased blood pressure in humans and experimental animals is associated with increased vascular peripheral resistance, which is caused by decreased levels of Nitric Oxide for an increase in superoxide [15].

Vitamin E is a fat-soluble antioxidant that can prevent lipid peroxidation. Vitamin E as a chain-breaking antioxidant can break free radical formation that can stop the oxidative process and prevent the formation of MDA [16]. Previous research associated with supplementation of vitamin E shows that the administration of vitamin E and a combination of capsaicin and vitamin E can reduce LDL and increase HDL in hypercholesterolemic mice [17].

Reduction on LDL in the group treated with the administration of the sprouts, because of the antioxidant compounds contained in sprouts, such as flavonoids and phenolic compounds. Flavonoids are antioxidants that can capture free radicals. Flavonoids can stop the early stages of the reaction to liberate the hydrogen atoms of the hydroxyl groups which then binds to a free radical. With this bond, it will stabilize peroxide radicals that make the activation energy is reduced and will further impede and inhibit oxidation of LDL cholesterol [18].

The decrease in blood pressure in the group with the provision of sprouts is caused by the flavonoids and polyphenols which are derivatives are compounds found only in plants. These compounds have a strong antioxidant effect, increases the ability of platelets to release nitric oxide and inhibit the formation of thrombus. Increase in NO will lead to vasodilation of blood vessels which ultimately will cause a decrease in blood pressure [19]. Research Maslachah [20] mention vitamin E has potential as a chain-breaking antioxidant in the membrane that can prevent cell damage by inhibiting lipid peroxidation and free radical formation.

In addition to vitamin E, mung bean sprouts are also known to contain vitamin C [21]. Vitamin C found in mung bean sprouts also has a role as a non-specific electron donor (reducing agents). Vitamin C donates electrons as part of the hydrogen atom [22]. Epidemiological studies prove that there is a correlation between vitamin C intake with
the risk of heart disease, the status of vitamin C were associated with high levels of HDL cholesterol and low blood pressure [23]. Increased in blood pressure systole in the given of high fat diet higher than group who were given a high fat diet and mung bean sprout and also on group who were given high fat diet and vitamin E, but there is no difference effect a decrease in blood pressure between the provision of mung bean sprouts and vitamin E [24]. Research conducted Hadi et al. [25], a dose of mung bean sprout 0.67 g is optimal doses in preventing a rise in blood pressure and prevent alterations histopathology Sprague Dawley male rats.

4. Conclusions

Blood pressure and LDL cholesterol levels were given the green bean sprouts, and vitamin E did not have significant different to the blood pressure in the normal group. Levels of blood pressure and LDL cholesterol have a positive correlation. Mung bean sprouts can give the effect of a decrease in blood pressure and inhibits the increase in blood LDL levels Sprague Dawley rats.

Acknowledgments

This study was part of a master’s thesis from Public Health Graduate Program the Faculty of Medicine, Universitas Gadjah Mada in 2015.

References

[1] Wijaya A. Parameter biokimiawi untuk sindrom koroner akut (dalam) forum diagnisticum. [Biochemical parameters for acute coronary syndromes (in) diagnographic forums]. Prodia Educational Services. 2000:1–15. [in Bahasa Indonesia]. http://prodia.co.id/id

[2] Adam LB. Guidelines for adolescent nutrition service hyperlipidemia center of leadership, education, and training and child nutrition. Division of Epydemiology and Community Health, School of Public Health. University of Minnesota. 2006; 109-124 http://www.epi.umn.edu/let/pubs/adol_book.shtm

[3] Carey VJ, Bishop L, Laranjo N, Harshfield BJ, Kwiat C, Sacks FM. Contribution of high plasma triglicerides and low high-density lipoprotein cholesterol to residual risk of coronary heart disease after establishment of low density lipoprotein cholestrol control. American Journal of Cardiology 2010;106:757–763. https://www.ncbi.nlm.
nih.gov/pubmed/20816113

[4] Muchtadi D. Pangan dan kesehatan jantung. [Food and heart health]. Alfabeta. Bandung; 2013. p. 90–97. [in Bahasa Indonesia]. http://www.cvalfabeta.com/0412-detail-pangan_dan_kesehatan_jantung.html

[5] Yang R, Shi Y, Hao G, Li W, Le G. Increasing oxidative stress with progressive hyperlipidemia in human: Relation between malondialdehyde and atherogenic index. J. Clin Biochem. Nutr. 2008;43:154–115. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2581765/

[6] Price SA, Wilson LM. Patofisiologi, konsep klinis proses-proses penyakit Ed. 6, Vol. 1. [Pathophysiology: Clinical concepts of disease processes 6th Ed]. EGC. Jakarta; 2006. p. 355–363. [in Bahasa Indonesia]. https://www.belbuk.com/patofisiologi-vol-1-2-edisi-6-p-3612.html

[7] Lautan J. Radikal bebas pada eritrosit dan leukosit. [Radicals in erythrocytes and leukocytes]. Cermin Dunia Kedokteran, 1997;116–130. [in Bahasa Indonesia]. http://www.cdkjournal.com/

[8] Anggrahini S. Pengaruh lama pengecambahan terhadap kandungan α-tokoferol dan senyawa proksimat kecambah kacang hijau (phaseolus radiatus l). [Effect of germinating time on the α-tocopherol and proximate content of mung bean sprout (Phaseolus radiatus l)]. Agritech 2007; 27(4):152–157 https://journal.ugm.ac.id/agritech/article/view/9850

[9] Matos SL, Paula H, Pedrosa ML, Santos RC, Oliveira EL, Junior DAC, Silva ME. Dietary models for inducing hypercholesterolemia in rats. Braz. Arch. Biol. Technol. 2005;48(2):203–209. http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-89132005000200006

[10] Friedwald WT, Levy RI, Fredrikson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultra-centrifuge. Clin Chem, 1972;8:499–502. https://www.ncbi.nlm.nih.gov/pubmed/4337382

[11] Murray RK, Mayes PA, Granner DK, Rodwell VW. Harper’s biochemistry 25th Ed. [translated]. Hartono A. Biokimia Harper Edisi ke-25. Penerbit Buku Kedokteran EGC. Jakarta; 2003. p. 254–262. [in Bahasa Indonesia] http://kin.perpusnas.go.id/DisplayData.aspx?pld=86582&pRegionCode=UNTAR&pClientId=650

[12] Onggang FS. Pencegahan dislipidemia dengan pemberian rosela (Hibiscus Sabdariffa). [Prevention of dyslipidemia by administration of roselle (Hibiscus Sabdariffa). [Thesis]. Fakultas Kedokteran. UGM. Yogyakarta; 2009. p. 65–70. [in Bahasa Indonesia]. http://etd.repository.ugm.ac.id/index.php?mod=penelitian_detail&sub=PenelitianDetail&act=view&typ=html&buku_id=42547&obyek_id=4
[13] Hascheck WM, Wallig MA, Rousseaux C. Fundamental of toxicologic pathology 2nd Ed. Mc Millian Publishing Sollutions; 2010. p. 333–334. https://www.elsevier.com/books/fundamentals-of-toxicologic-pathology/haschek/978-0-12-370469-6

[14] Taniyama Y, Griendling K. Reactive oxygen species in the vasculature. Molecular and cellular mechanisms. J. Hypertension 2003;42:1075–1081 https://www.ncbi.nlm.nih.gov/pubmed/14581295

[15] Bohr DF, McIntyre M, Dominiczae AE. Endothelial function in hypertension: The role of superoxida anion. Hypertension, 1999;34:539–545. https://www.ncbi.nlm.nih.gov/pubmed/10523323

[16] Schafer FQ, Kelley EE, Buettner GR. Oxidative stress and antioxidant intervention. In: Critical reviews of oxidative stress and aging: Advances in basic science, diagnostics and intervention. Cutler RG, RodriguezH (Eds). World Scientific. New Jersey; 2003. p. 849–869. https://www.worldscientific.com/doi/abs/10.1142/9789812775733_0049

[17] Tamba DA. Efek kombinasi capsaicin dan vitamin E terhadap perubahan kadar hdl dan ld1 pada tikus hiperkolesterolemia. [The effect of combination of capsaicin and vitamin E on changes in HDL and LDL levels in hypercholesterolemic rats]. [Thesis]. [unpublished]. Fakultas Kedokteran. UGM. Yogyakarta; 2013. p. 68–70. [in Bahasa Indonesia].

[18] Adeneye AA, Olagunju JA. Preliminary hypoglycemic and hypolipidemic activities of the aqueous seed extract of carica papaya linn. In Wistar Rats. Biology and Medicine 2009;1(1):1–10. https://pdfs.semanticscholar.org/73e4/d5418836ccf0f505b055b694e4fdd2dd81f.pdf

[19] Freedman JE, Keaney JF. Vitamin E inhibition of platelet aggregation is independent of antioxidant activity. J Nutr., 2001;131:3748–3778. https://www.ncbi.nlm.nih.gov/pubmed/11160564

[20] Maslachah L, Sugihartuti R, Kurniasanti R. Hambatan produksi reactive oxygen species radikal superoksida oleh antioksidan vitamin e pada tikus putih yang menerima stressor ranjatan listrik. [The inhibition of vitamin e antioxidant to superoxide radical reactive oxygen species production on the white rat (rattus norvegicus) stressed by an electric shock]. Media Kedokteran Hewan 2008; 24:18–24. [in Bahasa Indonesia] http://www.journal.unair.ac.id/filerPDF/04-Lilik%20Farm. pdf

[21] Orozco RF, Frias J, Zielinski H, Piskula MK, Kozlowska H, Valverde CV. Kinetic study of the antioxidant compounds and antioxidant capacity during germination of Vigna cv. emmerald, Glycine max cv.jutro and Glycine max cv.merit. Food chemistry 2008;111:622–630. http://agris.fao.org/agris-search/search.do?recordID=US201300913906
[22] Murray RK, Granner DK, Mayes PA, Rodwell VW. Harpers illustrated biochemistry, 26th Ed. McGraw-Hill, Boston; 2000. 95–185. https://archive.org/details/HarpersIllustratedBiochemistry26thEd2003

[23] Saputra MA. Pengaruh jus jambu biji merah (Psidium Guajawa L.) terhadap kejadian aterosklerosis pada tikus putih (Rattus Norwegicus) yang diberi diet tinggi lemak. [The influence of red guava juice (Psidium Guajava L.) to the incidence of atherosclerosis in white rats (Rattus Nor-wegicus) given a high-fat diet]. [Undergraduate Thesis]. [Unpublished]. Fakultas Kedoteran. UGM. Yogyakarta; 2007. p. 71–72. [in Bahasa Indonesia].

[24] Hadi NS, Farmawaty A, Ghozali A. Pengaruh pemberian kecambah kacang hijau (Phaseolus radiata (L)) terhadap tekanan darah sistole dan histopatologi aorta tikus Sprague Dawley jantan yang diberi diet tinggi lemak. [The influence of mung bean sprout (Phaseolus radiata (L)) on systole bloodpressure and histopathology aorta of Sprague Dawley male rat was given a high-fat diet]. [Thesis]. Fakultas Kedokteran. UGM. Yogyakarta; 2015. p. 51. [in Bahasa Indonesia]. http://etd.repository.ugm.ac.id/index.php?mod=penelitian_detail&sub=PenelitianDetail&act=view&typ=html&buku_id=91771&obyek_id=4

[25] Hadi NS, Farmawaty A, Ghozali A. Pencegahan hipertensi dan penebalan dinding aorta dengan pemberian kecambah kacang hijau (Phaseolus radiatus(L)) pada tikus putih Sprague Dawley. [The prevention of hypertension and thick walls aorta using mung bean sprouts (Phaseolus radiatus (L)) of Sprague-Dawley rats]. Jurnal Gizi Klinik Indonesia 2016;12(3):116–122. [in Bahasa Indonesia]. https://jurnal.ugm.ac.id/jgki/article/view/22454