

Conference Paper

Effect Supplementation of Mung Bean Sprouts (*Phaseolus radiatus* L.) and Vitamin E in Rats Fed High Fat Diet

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Abstract

The high fat diet consumed in daily will increase total cholesterol and oxidative stress levels, a risk factor of cardiovascular disease. Mung bean sprouts as a functional food and vitamin E is an antioxidant component which acts to inhibit lipid peroxidation process. The objective of this study is to determine the effect of supplementation of mung bean sprouts and vitamin E on total cholesterol and MDA plasma level in rats fed high-fat diet. Male rats *Sprague Dawley* ($n = 24$) were randomly divided into four groups (6 in each group). The groups were fed on a normal diet (Group I), high fat diet (HFD) (Group II), HFD supplemented with 1 mL · g BW⁻¹ mung bean sprout (Group III), and HFD supplemented with 23 IU Vitamin E (Group IV). After 28 d, total cholesterol and MDA plasma level study were performed. The level of total cholesterol and plasma MDA in rats which were given mung bean sprouts and vitamin E was significantly lower than the rats which were given high fat diet only ($P < 0.05$), but higher than the normal diet. There was a very strong correlation ($r = 0.87$; $P < 0.05$) between levels of total cholesterol and plasma MDA level. Administration of mung bean sprouts and vitamin E in a high fat diet significantly to prevent oxidative stress, as indicated by total cholesterol dan MDA plasma lower than high fat diet only.

Keywords: High fat diet, Mung bean sprout, Plasma MDA, Total cholesterol, Vitamin E.

1. Introduction

Hyperlipidemia is a major cause of atherosclerosis and atherosclerosis-associated conditions, such as coronary heart disease (CHD) [1]. The prevalence of CHD in Indonesia based on Riskesdas (Indonesia health survey) 2013 is 1.5 %. In 2008, out of the 17 300 000 cardiovascular deaths, heart attacks were responsible for 7.300 000 deaths and strokes were responsible for 6 200 000 deaths [2]. Hyperlipidemia is a major cause

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of increased atherogenic risk; lifestyle changes (sedentary behavior and diets high in calories, saturated fat, and cholesterol) [3].

Hyperlipidemia characteristic is high levels of total cholesterol, low-density lipoprotein cholesterol (LDL) and very low density lipoprotein cholesterol (VLDL), with decreased levels of high density lipoprotein cholesterol (HDL). High levels of LDL cholesterol containing polyunsaturated fatty acids (PUFA) in lipid and cholesterol ester, leading easily oxidized lipids, known as lipid peroxidation process [4]. Lipid peroxidation process by free radical oxidation. In addition, free radical oxidation can affect the blood plasma and which can lead to liver damage. Malondialdehyde (MDA) is an aldehyde compound formed from lipid peroxidation products in polyunsaturated fatty acids [5].

Vegetables and fruit are rich sources of a variety of nutrients, including vitamins, trace minerals, and dietary fiber, and many other classes of biologically active compounds. One of the popular vegetables in Indonesia is mung bean sprouts. In Indonesia, Mung bean sprouts as functional food are known to contain dietary fiber and vitamin E as antioxidants component acts to inhibit lipid peroxidation process. Mung bean sprouts are often processed into a variety of dishes or as fresh vegetables are often eaten raw. The seeds and sprouts of mung beans are also widely used as a fresh salad, vegetable or common food in India, Bangladesh, South East Asia, and western countries [6].

Mung bean sprouts contain much nutritional value, including protein, dietary fiber, and phytochemicals, antioxidants bioactive compounds. Antioxidant compounds found in mung bean sprouts, such as vitamin E, C, and polyphenols. Vitamin E is a nonenzymatic endogenous fat-soluble vitamin with high anti-oxidant potency. Vitamin E is important for protecting cell membranes and LDL cholesterol [7] Vitamin E may reduce free radicals and break the chain reaction of lipid peroxidation [8]. The purpose of this study was to determine the effect supplementation of mung bean sprouts and vitamin E on total cholesterol and plasma MDA level in high fat diet-fed rats.

2. Materials and Methods

The research is an experimental research design with the post-test only with the control group. Research conducted at the Centre for the Study of Food and Nutrition, University of Gadjah Mada (UGM PSPG) Yogyakarta, Indonesia. All aspects of the in vivo experiment were conducted according to the guidelines provided by The Ethical Committee of Medical Research of Faculty of Medicine, Gadjah Mada University (No. KE/FK/485/EC).

2.1. Mung bean sprout

Mung bean seed was soaked in water for 8 h. Then, the seeds were washed with distilled water. Germination time for seeds was chosen to get the sprouts of full freshness. Mung bean sprouts are sprouts obtained from the process of germination green beans for 48 h, then crushed using a blender and uniform size by using a homogenizer. Germination processes were carried out in darkness.

2.2. Vitamin E

Vitamin E is a vitamin E supplement commercial. The dose of supplemental vitamin E used was 23 IU, derived from Centre for the Study of Food and Nutrition, University of Gadjah Mada (PSPG UGM). This dose was based on the recommended dietary allowance issued by NIH [9].

2.3. Animal

Sprague Dawley rats, 8 wk male ($n = 24$, body weight 147 g to 222 g) were supplied by the Faculty of Animal Husbandry Institute of Bogor Agricultural (Bogor, Indonesia) and maintained under controlled temperature (± 25 °C) and lighting conditions (12 h light:12 dark photoperiods). All rats were housed individually. The rats were fed on standard rat diet for 10 d for adaptation. Rat diet and water were given ad libitum.

2.4. Diet

The standard diet used in this study are AIN-93M [10]. High fat diet is standard AIN 93M supplemented with tallow. Diet composition and group supplements in the study were presented in Table 1.

2.5. Steps

Rats were divided into four groups ($n = 6$ in each group): group I, II, III and IV. After 10 d of adaptation, all group rats received treatment based on dietary composition in Table 1. Group I was given standard diet. Group II was given HFD. Group III was given HFD and 1 mL · g WB⁻¹ mung bean sprouts. Group IV was given HFD, and 23 IU vitamin E. Rats of group III and group IV were the mung bean sprouts and vitamin E through oral sonde.

TABLE 1: Diet composition and group supplements in the study.

| Component | Group I (Standard diet) | Group II (HFD) | Group III (HFD + MBS) | Group IV (HFD + Vit E) |
|--|----------------------------|----------------|--------------------------|---------------------------|
| Cornstarch (g) | 620.692 | 620.692 | 620.692 | 620.692 |
| Casein (kg) | 140 | 140 | 140 | 140 |
| Sucrose (g) | 100 | 100 | 100 | 100 |
| Soybean oil (g) | 40 | 40 | 40 | 40 |
| Fiber (g) | 50 | 50 | 50 | 50 |
| Mineral mix (g) | 35 | 35 | 35 | 35 |
| Vitamin mix (g) | 10 | 10 | 10 | 10 |
| L-cystine (g) | 1.8 | 1.8 | 1.8 | 1.8 |
| Choline bitartrate (g) | 2.5 | 2.5 | 2.5 | 2.5 |
| TBHQ (mg) | 0.008 | 0.008 | 0.008 | 0.008 |
| Tallow (g) | 0 | 180 | 180 | 180 |
| Mung beans sprouts (mL · gWB ⁻¹) | 0 | 0 | 1 | 0 |
| Vitamin E (IU) | 0 | 0 | 0 | 23 |

This research carried out for 28 d. Parameters such total cholesterol and MDA plasma were measured after 28 d intervention.

2.6. Total cholesterol analysis

Blood sampling was done via the orbital plexus of rats. The total cholesterol level was measured by using principle enzymatic hydrolysis and oxidation methods. Serum containing lipoproteins reacted cholesterol reagent — cholesterol esters in lipoprotein broken down by enzyme cholesterol esterase into cholesterol and fatty acids. Cholesterol then undergoes oxidation with the enzyme cholesterol oxidase as a catalyst to produce compounds peroxide reacted together phenol, and 4-amino antipyrine produce imine quinone compounds are red and can be measured with a spectrophotometer at a wavelength (λ) of 500 nm.

2.7. Plasma MDA analysis

The level of plasma MDA conducted at the end of the study. Blood samples were collected from plexus orbitalis and centrifuged at 4 000 rpm (1 RPM = 1/60 Hz), 15 min. The plasma MDA level was measured by TBARS (thiobarbituric acid reactive substances) method. This method depends on the formation of MDA as an end product of lipid peroxidation, which reacts with thiobarbituric acid producing thiobarbituric acid reactive substance (TBARS), a pink chromogen, which can be measured spectrophotometrically

at 532 nm, an MDA standard was used to construct a standard curve against which readings of the samples were plotted.

2.8. Statistical analysis

All data were expressed as mean \pm standard deviation (mean \pm SD). The data obtained are then tested for normality and variance. The data total cholesterol is not normally distributed, the non-parametric statistical test Kruskal Wallis and Mann-Whitney test. The data plasma MDA is normally distributed, the parametric statistical test one-way ANOVA and post hoc Bonferroni. The average difference in total cholesterol and plasma MDA level at $P < 0.05$ was considered significant. To determine the correlation of total cholesterol and plasma MDA level is used, Spearman.

3. Results

3.1. Total cholesterol analysis

The level of total cholesterol of the ND group was significantly lower than the three other groups ($P < 0.05$). The level of total cholesterol of the high fat diet group (group II) was significantly higher than other groups ($P < 0.05$). No significant difference of total cholesterol level between group III and vitamin E group (group IV) ($P > 0.05$), but the level of total cholesterol significantly lower than the level of the high fat diet group (group II) (Table 2).

TABLE 2: Effect supplementation of Mung Bean Sprout and Vitamin E on Plasma MDA Level.

| Group | Plasma MDA (nmol · mL ⁻¹) |
|----------------------------|---------------------------------------|
| Group I (Standar diet) | 1.20 \pm 0.11 ^a |
| Group I (HFD) | 3.99 \pm 0.19 ^c |
| Group III (HFD + MBS) | 1.74 \pm 0.08 ^b |
| Group IV (HFD + Vitamin E) | 1.53 \pm 0.07 ^b |

The results are presented in mean \pm SD

a, b, and c The notation is different in the same column indicating significant different ($P < 0.05$)

3.2. Plasma MDA analysis

The level of plasma MDA of the high fat diet group (group II) was significantly higher than other groups ($P < 0.05$). The level of plasma MDA of the group I (standar diet) was

significantly lower than the three other group ($P < 0.05$). No significant difference of plasma MDA level between high fat diet-mung bean sprout 1 mL/g WB (group III) and vitamin E group (group IV) ($P > 0.05$), but significantly lower than the level of plasma MDA of group II (Table 3).

TABLE 3: Effect supplementation of Mung Bean Sprout and Vitamin E on Total Cholesterol Level.

| Group | Total Cholesterol Levels (nmol · mL ⁻¹) |
|----------------------------|---|
| Group I (Standard diet) | 92.12 ± 1.62 ^a |
| Group I (HFD) | 182.68 ± 3.99 ^c |
| Group III (HFD + MBS) | 143.61 ± 2.74 ^b |
| Group IV (HFD + Vitamin E) | 143.13 ± 1.68 ^b |

The results are presented in mean ± SD
^{a, b, and c} The notation is different in the same column indicating different significant $P < 0.05$

3.3. Correlations between total cholesterol and plasma MDA levels

There were correlations between total cholesterol and plasma MDA levels in all subjects. The significant positive correlation between plasma MDA levels and total cholesterol was observed ($r = 0.87, P < 0.05$). Thus, the total cholesterol was high in groups with increased lipid peroxidation (plasma MDA) level (Fig 1).

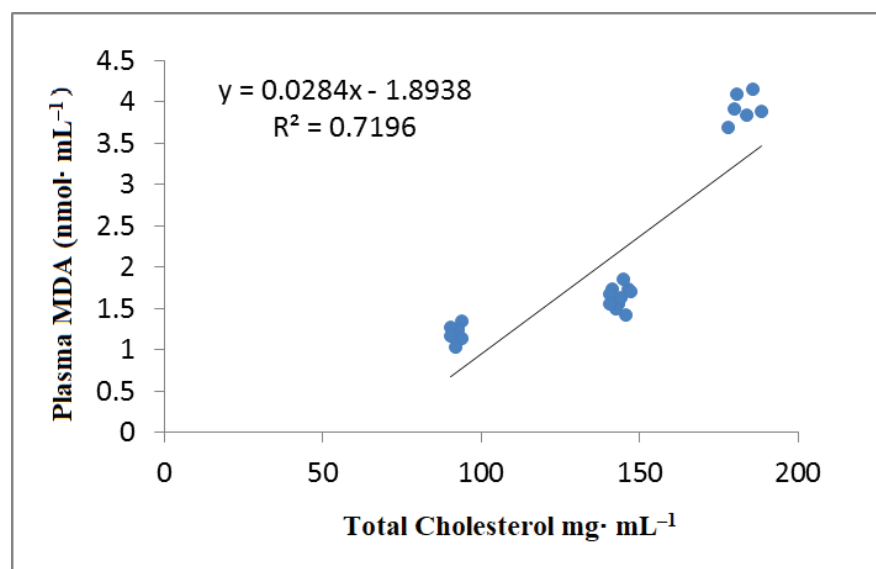


Figure 1: Correlation of total cholesterol and plasm MDA in all subjects.

4. Discussion

The results showed that total cholesterol levels in rats were higher than without supplementation of mung bean sprout (Table 2). Consumption of a high fat diet can increase total cholesterol levels in the blood [9]. Phytosterol content of mung bean sprouts to provide a positive effect of lowering total cholesterol levels in the blood. The content of phytosterol in mung bean sprout is 15 mg in 100 g of edible portion mung bean sprout [12]. Phytosterol can lower blood cholesterol levels and can prevent the absorption and biosynthesis of cholesterol [13].

Beside to the content of phytosterols, dietary fiber in mung bean sprouts also to affect the total cholesterol levels in the blood. The mung bean sprouts containing dietary fiber and its resistant to starch are well-known of decreased total cholesterol level. The mechanism of decreased total cholesterol levels by dietary fiber soluble is prevention of bile salt (BS) re-absorption from the small intestine leading to an excess fecal BS excretion; reduced glycemic response leading to lower insulin stimulation of hepatic cholesterol synthesis; and physiological effects of fermentation products of SDF, mainly propionate [14].

The results showed that a high fat diet could to changes in plasma MDA in rats. MDA plasma levels rats fed a high fat diet (group II) is higher than the plasma levels of MDA in rats of other groups (Table 3). The high levels of plasma MDA indicate that rats experience oxidative stress. Oxidative stress can occur due to an imbalance between antioxidants and free radical compounds in the body [15]. The high fat diet of this study contains poly unsaturated fatty acids (PUFAs). Previous studies have suggested that PUFA is easily oxidized to produce free radical compounds. The toxic aldehydic degradation products, by-product of PUFA metabolism, could result in significant damage to cells present in the arterial walls and may be related to atherosclerotic lesions [4].

Supplementation of mung bean sprouts (group III) made a significant difference in plasma MDA (Table 3). Supplementation of mung bean sprouts in rat high fat diet causes lower plasma MDA levels than without mung bean sprout ($P < 0.05$). The functional content of mung bean sprouts to provide a beneficial effect on plasma MDA levels. Mung bean sprouts are known to contain flavonoids, isoflavones and ascorbic acid [12]. These compounds can inhibit the occurrence of oxidative compounds in low density lipoprotein (LDL), to improve the immune system in the body [16]. Volatin antioxidant compounds capable of inhibiting the formation of plasma malondialdehyde [17].

Level of total cholesterol rats consuming a high-fat diet and vitamin E is lower than the rats fed a high-fat diet without supplementation of vitamin E (Table 2). Vitamin E is a

lipid soluble chain-breaking antioxidant that protects LDL particles from oxidative attack. Vitamin E as an antioxidant has the effect of lowering lipid oxidation products, thus contributing to the pathology of atherosclerosis. Vitamin E supplementation of a high fat diet may protect very low density lipoprotein (VLDL) from alteration and consequently decrease its atherogenic effect [11].

Supplementation of vitamin E in rats fed a high-fat diet had an effect on plasma MDA levels (Table 3). Plasma MDA levels in rat fed a high fat diet, and vitamin E (group IV) were lower than plasma MDA levels given a high fat diet without vitamin E supplementation (group II and III). The mechanism of vitamin E as an antioxidant compound is to give the H atom at a radical reaction chain [18]. α -tocopherol is the most abundant lipid soluble antioxidant in vivo and acts as an important inhibitor of lipid peroxidation in membrane systems [19]. α -tocopherol containing the -CH group will donate the hydrogen atom (H) to Reactive Oxygen Species (ROS). Vitamin E in the lipid phase (membrane) captures peroxy radicals in the lipid peroxidation process and protects polyunsaturated fatty acids (PUFA-H). Vitamin E reacts with the peroxy radical of polyunsaturated fatty acids (PUFA-OO*) in cell membrane phospholipids to form a slightly reactive radical vitamin E (a tocopheryl radical) that breaks the propagation chains of a radical chain reaction [20].

In Fig. 1, it seems that changes in total cholesterol level followed by changes in plasma MDA levels in each treatment group, both groups of rat were given the mung bean sprouts or vitamin E. Correlation total cholesterol level and plasma MDA levels of rat in the study had a correlation with category is very strong ($r = 0.87$) and significantly ($P < 0.05$).

The total cholesterol level was high in groups with increased lipid peroxidation (plasma MDA) level. Plasma MDA levels and total cholesterol were increased due to the lower antioxidant status in the body, can increase the susceptibility of LDL to oxidation in the circulation. As a lipid peroxidation process leading to increased atherogenicity of LDL, it follows that antioxidant status should have a major impact not only on the rate of LDL oxidation but perhaps on development of atherosclerosis [21].

Triglycerides are the main constituent of the component chylomicron and VLDL. Transport of triglycerides from the small intestine in the form of chylomicrons and the liver in the form of VLDL. The concentration of VLDL, which are rich in cholesterol and triacylglycerol by the liver and is converted to intermediate density lipoprotein (IDL). After entering the bloodstream stream, VLDL begins to lose its triacylglycerol content as it is hydrolyzed by lipoprotein lipase (LPL) into fatty acids and glycerol. After some triglycerides are hydrolyzed by LPL, VLDL becomes IDL. IDL concentrations are

absorbed by the liver directly through LDL receptors (LDLR) or can be converted to LDL [20].

High LDL levels can cause very easily oxidized LDL [4]. As the LDL level increases, the availability of oxidized substrate is also increased so that the likelihood of the oxidation process increases. The higher levels of total cholesterol, the greater the rat plasma MDA levels in each treatment [15]. MDA plasma levels rats fed a high fat diet were higher than mice fed a standard diet. In addition to plasma MDA levels, total cholesterol levels of rats fed a high fat diet was higher than rats fed a standard diet [11].

5. Conclusions

In conclusion, the supplementation of mung bean sprout and vitamin E effect on total cholesterol and plasma MDA of rats given HFD. Although supplementation of vitamin E on a high fat diet on total cholesterol and plasma levels of MDA are lower than supplementation of mung bean sprouts, but did not different significantly. This study proves that the consumption of green bean sprouts as a functional food is good for health, and can prevent oxidative stress caused by the consumption of high fat diet on the human body.

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