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Anticholesterol Activity of Velvet Bean (*Mucuna pruriens* L.) towards Hypercholesterolemic Rats

(Aktiviti Antikolestrol daripada Kacang Velvet (*Mucuna pruriens* L.) terhadap Tikus Hiperkolestromik)

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ABSTRACT

*Hypercholesterolemia is the main risk factor of atherosclerosis which is directly related to coronary heart disease. Velvet bean (*Mucuna pruriens *L.) is predicted to have potential anticholesterolemia since a previous study showed that this plant had high antioxidant activity. The purpose of this research was to determine the anticholesterol activity of crude extract and ethyl acetate fraction of velvet bean compared to simvastatin and vitamin E in decreasing the plasma level of total cholesterol, LDL and triglyceride and increasing the HDL level on hypercholesterolemic rats. Thirty male Wistar rats were divided into ten groups. Group I was fed with standard diet as negative control group and all the other groups were fed with high-fat diet and were given fructose solution. High-fat diet and fructose solution increased the level of total cholesterol, LDL-cholesterol and triglyceride and decreased the level of HDL-cholesterol significantly compared to the negative control group. The treatment groups were given 3 various doses of crude extract (50, 100, 200 mg/kgBW) and ethyl acetate fraction (15, 30, 60 mg/kgBW) of velvet bean, simvastatin 2.7 mg/kgBW and vitamin E 60 mg/kgBW daily for ten days. The data were analyzed by one way Analysis of Variance (ANOVA) and Duncan's Post Hoc Test. The best results were shown by 15 mg/kgBW of ethyl acetate fraction in decreasing total cholesterol and 60 mg/kgBW of ethyl-acetate fraction for the LDL-cholesterol, 200 mg/kgBW of crude extract in increasing HDL-cholesterol and 50 and 200 mg/kgBW of crude extract and 60 mg of ethyl acetate fraction in lowering the triglyceride.*

Keywords: Anticholesterol; hypercholesterolemia; Mucuna pruriens *L.; velvet bean*

ABSTRAK

*Hiperkolesterolemia adalah factor utama aterosklerosis yang berkaitan dengan penyakit jantung koronari. Kacang Velvet (*Mucuna pruriens *L.) diramalkan mempunyai potensi antikolestrolemia kerana kajian sebelumnya menunjukkan bahawa tanaman ini mempunyai aktiviti antioksida yang tinggi. Tujuan kajian ini adalah untuk menentukan aktiviti antikolestrol ekstrak kasar dan bahagian etil asetat daripada kacang velvet dibandingkan dengan simvastatin dan vitamin E dalam menurunkan jumlah tahap kolesterol dalam plasma, LDL dan trigliserida serta meningkatkan kadar HDL pada tikus hiperkolesterolemia. Tiga puluh tikus jantan Wistar dibahagikan kepada sepuluh kumpulan. Kumpulan I diberikan diet standard sebagai kumpulan kawalan negatif dan semua kumpulan yang lain diberikan diet tinggi lemak dan larutan fruktosa. Diet tinggi lemak dan larutan fruktosa dapat meningkatkan tahap jumlah kolesterol, kolestrol LDL dan trigliserida serta menurunkan kadar kolestrol HDL secara signifikan jika dibandingkan dengan kumpulan kawalan negatif. Kumpulan perlakuan diberikan 3 dos pelbagai ekstrak kasar (50, 100, 200 mg/kgBW) dan bahagian etil asetat (15, 30, 60 mg/kgBW) daripada kacang velvet, simvastatin 2.7 mg/kgBW dan vitamin E 60 mg/kgBW setiap hari selama sepuluh hari. Data dianalisis dengan Analisis Varian satu hala (ANOVA) dan Kajian Duncan Post Hoc. Keputusan yang terbaik ditunjukkan oleh bahagian etil asetat dengan 15 mg/kgBW menurunkan jumlah kolestrol dan 60 mg/kgBW bahagian etil asetat kolestrol LDL, 200 mg/kgBW ekstrak kasar dalam meningkatkan kolestrol HDL dan 50 dan 200 mg/kgBW ekstrak kasar dan 60 mg bahagian etil asetat dapat menurunkan trigliserida.*

Kata kunci: Antikolesterol; hiperkolesteralemia; Mucuna pruriens *L.; kacang velvet*

INTRODUCTION

The blood lipids (total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides) have been shown to be related to the development of coronary heart disease (CHD), since these risk factors play an important role in determining atherogenesis and the subsequent pace of atherosclerosis. Evidence from clinicopathological and epidemiological studies overwhelmingly confirms that hyperlipidemia is the primary prerequisite for atherosclerosis manifested in premature cardiovascular disability and death. Hyperlipidemia is caused by a diet high in fat, especially saturated fat and cholesterol. The International Atherosclerosis Project, found that the degree of atherosclerosis was directly proportional to the prevalence of CHD and stroke, and that lipid levels were directly related to plaque damage. The higher the serum

cholesterol, the greater the plaque build up (Temple & Burkitt 1994).

With the etiological preeminence of hyperlipidemia in CHD, various drugs have been utilized to lower the blood lipids, such as clofibrate, niacin, cholestyramine, statin and gemfibrozil. Although these drugs were successful in reducing serum cholesterol levels, they produced unpleasant and distressing side effects (Temple & Burkitt 1994).

Nowadays, natural medicine is constantly expanding, especially in Indonesia which has various kinds of herbal medicine. Velvet bean (*Mucuna pruriens* L) is one of the tropical legumes which was empirically used to improve the cholesterol profile. The previous study which examined the antioxidant activity of crude extract and fractions of velvet bean *in vitro,* shows that crude extract and ethyl acetate fraction has high antioxidant activity and contains high flavonoid (Widowati et al. 2007). *In vitro* studies demonstrated that flavonoids are potent free radical scavengers. Isoflavones are flavonoid compounds found in a variety of legumes. Approximately half of the ingested flavonoids are absorbed into the bloodstream through the gastrointestinal tract lining and half are metabolized to other compounds by gastrointestinal microflora. However, this varies depending on the flavonoid (Boik 1996; Koshy et al. 2001).

Free radicals are chemical species that have a single unpaired electron in an outer orbit. Free radicals in the presence of oxygen may cause peroxidation of lipids within plasma and organellar membranes. Antioxidants could block the initiation of free radicals and terminate radical damage and inhibit lipid peroxidation which was the factors of atherosclerosis. Natural antioxidants are widely used because they are safe and cause less adverse reactions (Boik 1996; Kumar 2005).

Since the clinical efficacies of velvet bean in lowering the lipids profile is unknown, it is important to study the anticholesterol activity of hexan, ethyl acetate, butanol and water fractions of velvet bean compared to simvastatin in decreasing the plasma level of cholesterol total, LDL and triglyceride and increasing the HDL level on hypercholesterolemic rats.

Materials and Methods

EXTRACTION AND FRACTIONATION

The velvet bean seed (*Mucuna pruriens* L.) was collected from Sukoharjo district, Center Java, Indonesia, in April 2007. Five kilograms of *Mucuna pruriens* L. seeds were soaked in EtOH and evaporated and the result was 10.335% crude extract (516.845 g). The ethanol crude extract was partitioned with *n*-hexane and 25% water, the result was 22.71%. The residue was partitioned with ethyl acetate and 5% water and the result was 0.37%. The residue was then partitioned with n-buthanol and 10% water and the result was 2.67%. Finally the residue of water filtrate was evaporated and the result of water fraction was 3.26%. This experiment used crude extract and ethyl acetate fraction of velvet bean, because the *in vitro* experiment previously showed that crude extract and ethyl acetate had the highest antioxidant activity.

ANIMALS AND DIETS

Thirty adult male Wistar rats obtained from Biology School, Bandung Technology of Institute were housed in standard cages and provided with food and water *ad libitum*. The rats were adapted for 7 days until the body weight were 175-200 g. The hypercholesterolemic rats were induced with high fat diet (3000 g of standard diet mixed with 250 g duck egg yolk, 500 g palm oil, 1250 g wheat flour, 500 g lamb fat and hot water). The difference between the standard diet and high fat diet is shown in Table 1.

TABLE 1. The nutrient of standard and high fat diet

Item	High fat diet	Standard diet
Water (%)	13.61	8.61
Ash (%)	5.19	5.96
Crude protein (%)	16.05	23.34
Crude fiber (%)	11.64	10.12
Crude fat (%)	20.78	7.37
Carbohydrate (%)	46.34	53.21

In spite of high fat diet, the rats were given fructose liquid 60% (120 g/200 ml aquadest) 1 mL/rat/day. The high fat diet and fructose liquid were given for 2 weeks until the body weight were 200 – 250 g.

The rats were devided into 10 groups (n=3) for different treatments. The first group of animals was given standard diet (negative control). The second group of rats was given high fat diet (positive control). The third, fourth and fifth groups of rats were treated with high fat diet plus ethyl acetate fraction 15 mg/kgBW, 30 mg/kgBW and 60 mg/kgBW daily. The sixth, seventh and eighth groups of rats were treated with high fat diet plus crude extract 50 mg/kgBW, 100 mg/kgBW and 200 mg/kgBW daily. Group nine and ten were treated with high fat diet plus simvastatin 2.7 mg/kgBW and vitamin E 60 mg/kgBW daily. All the treatments were given for 10 days.

SAMPLE PREPARATION FOR LIPID PROFILE TEST

After the treatments, 1.5 mL blood from the orbital vein were collected in tubes contanining EDTA. The samples were centrifuged at 3000 rpm for 10 min and the plasma were used for measuring the total cholesterol, LDL-cholesterol, HDL-cholesterol and triglyceride level.

The total plasma cholesterol and triglyceride were measured according to the instruction manual accompanying the diagnostic kits from Abbott Laboratories (Abbott Laboratories, 2006). High density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol were measured according to the instruction manuals accompanying the diagnostic kits from Daiichi Pure Chemicals Co., Ltd.

STATISTICAL ANALYSIS

Mean and standard deviation (M ± SD) were calculated and 95% confidence interval (CI) of means was used. To compare between groups, analysis of variance (ANOVA) were calculated. P-values of less than 0.05 were considered as statistically significant. Furthermore, to know the best treatment, Duncan's post-Hoc test 95% confidence interval was used. Statistical analysis was carried out using SPSS 16.0 program.

RESULTS AND DISCUSSION

A high fat diet and fructose liquid significantly increased the cholesterol total, LDL-cholesterol and triglyceride and decreased the HDL-cholesterol compared to basal diet.

After treatment with various dose of crude extract, various dose of ethyl acetate fractions of velvet bean, 60 mg/kgBW of vitamin E and 2.7 mg/kgBW simvastatin for 10 days, the effect on total cholesterol is as shown in Figure 1. ANOVA showed that the treated groups were significantly different (p = 0.031) and Duncan post-Hoc test showed that 15 mg/kgBW/day of ethyl acetate fraction of velvet bean gave the best result in decreasing the cholesterol total.This is better compared to simvastatin (patent anticholesterol medicine) and vitamin E. Ethyl acetate 30 and 60 mg/kgBW/day have similar activity with simvastatin.

The effect of treatment towards LDL-cholesterol is shown in Figure 2. The statistical analysis showed that there was significant difference (p = 0.018) and 60 mg/

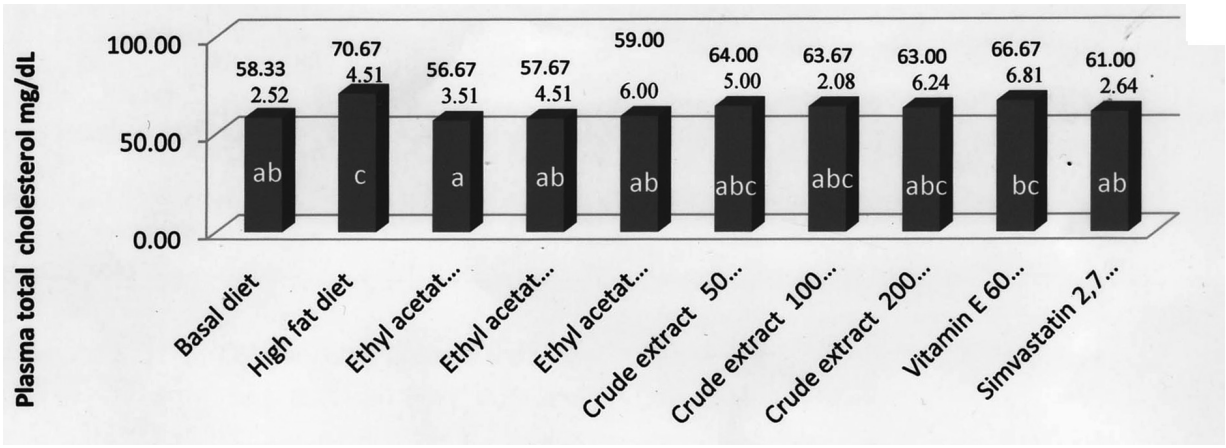

FIGURE 1. The plasma total cholesterol in hypercholesterolemic rat treated with crude extract and ethyl acetate fraction of velvet bean. Result are expressed as mean ± SD of three animals per group, Duncan's post-Hoc tests are showed by letter a, b, c are significantly different among treatment groups at confidence interval 95%

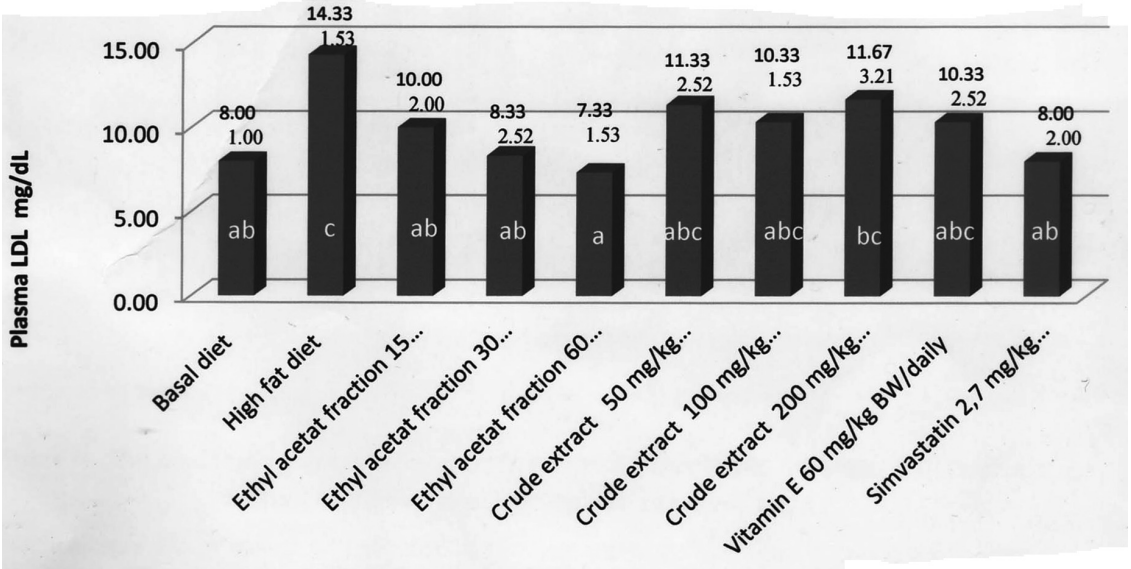

FIGURE 2. The plasma LDL-cholesterol level in hypercholesterolemic rat treated with crude extract and ethyl acetate fraction of velvet bean. Result are expressed as mean ± SD of three animals per group, Duncan's post-Hoc tests are showed by letter a, b, c are significantly different among treatment group at confidence interval 95%

kgBW/day of ethyl acetate fraction of velvet bean gave the best result in decreasing the LDL-cholesterol compared to simvastatin and vitamin E. Ethyl acetate fraction at concentration of 15 and 30 mg/kgBW/day showed similar activity to simvastatin. Crude extract at concentration of 50 and 100 mg/kgBW/day decreased LDL-cholesterol more effectively compared to vitamin E.

The effects of the treatments on HDL-cholesterol is shown in Figure 3. There were significant differences with $p \leq 0.073$ and 200 mg/kgBW/day crude extract of velvet bean increased the HDL-cholesterol better than simvastatin and other treatments. Ethyl acetate fractions at concentration of 60 mg/kgBW/day, crude extract at concentration 100 mg/kgBW/day, vitamin E and simvastatin showed similar activity in increasing the HDL-cholesterol level.

Figure 4 shows the effect of treatments on triglyceride concentration. Statistical analysis with ANOVA showed a highly significant ($p = 0.000$) difference and Duncan's post-Hoc test showed that ethyl acetate fraction 60 mg/kgBW/day, crude extract 50 and 200 mg/kgBW/day of velvet bean, vitamin E and simvastatin had similar activity in decreasing the triglyceride concentration.

The seeds of velvet bean are high in protein, carbohydrates, lipids, fiber and minerals. They are also rich in novel alkaloids, saponins, and sterols. The seeds of all *Mucuna* species contain a high concentration of L-dopa; velvet bean seeds contain 7-10% L-dopa. Experimental

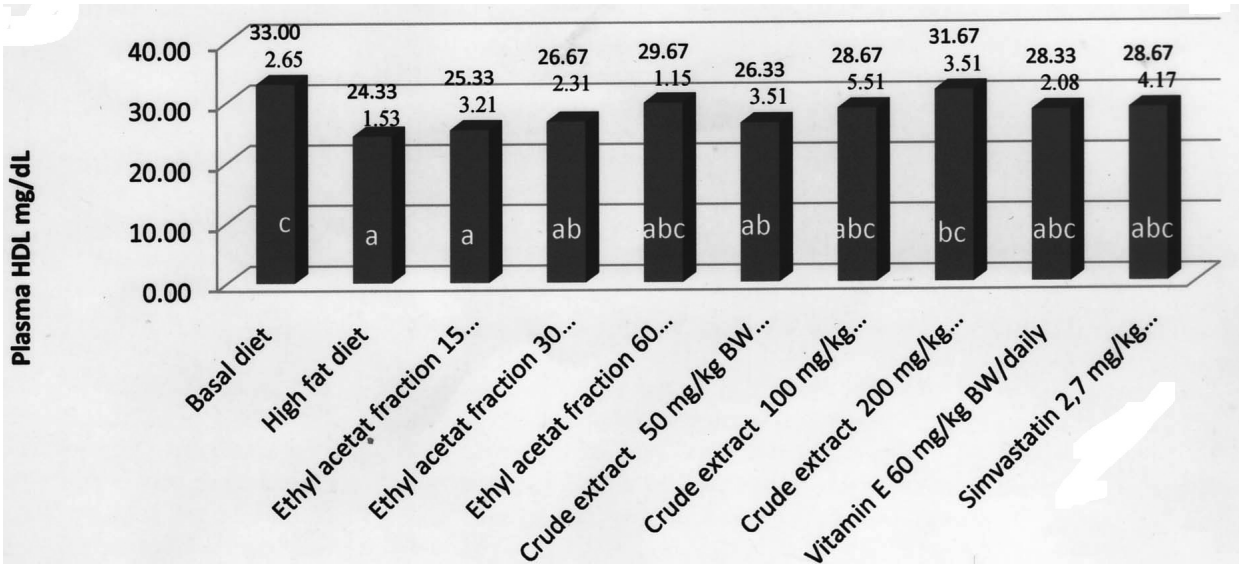

FIGURE 3. The HDL-cholesterol level in hypercholesterolemic rat treated with crude extract and ethyl acetate fraction of velvet bean. Result are expressed as mean ± SD of three animals per group, Duncan post-Hoc tests are showed by letter a, b, c are significantly different among treatment group at confidence interval 95%

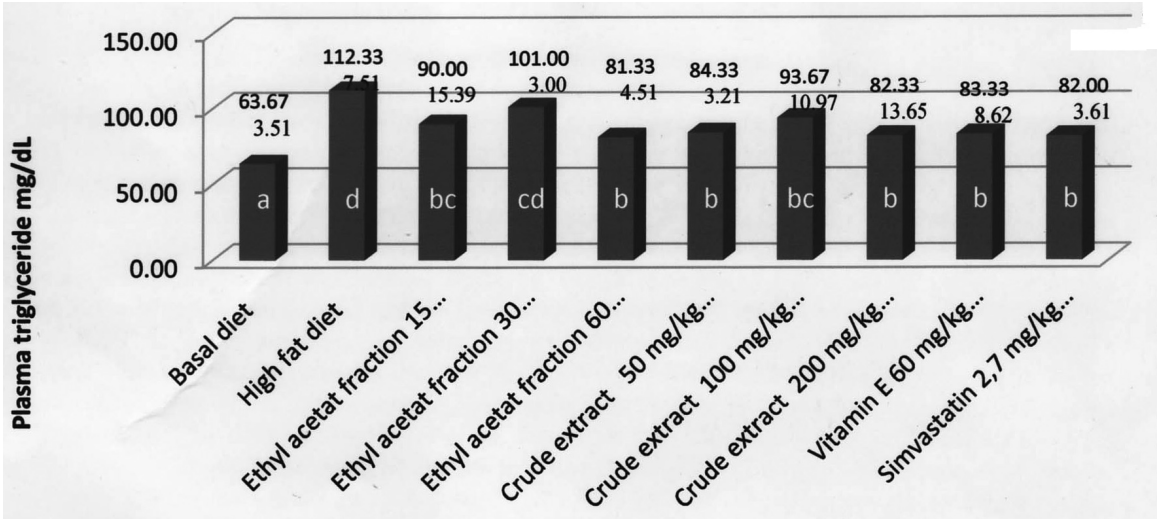

FIGURE 4. The plasma triglyceride level on hypercholesterolemia rat treated with crude extract and ethyl acetate fraction of velvet bean. Result are expressed as mean ± SD of three animals per group. Duncan's post-Hoc tests are showed by letter a, b, c are significantly different among treatment group at confidence interval 95%

model showed that although a neurotransmitter pre-cursor, L-Dopa also helps in the reduction of cholesterol and blood sugar levels (Herbal powers 2007).

Phytosterols and saponins are common constituents of legumes. Plant sterols are structurally similar to cholesterol, and oral administration of phytosterols has been shown to bind cholesterol and reduce its absorption. Saponins are plant compounds that have surfactant properties and like phytoestrogens, bind to cholesterol and bile acids. Because of their surfactant properties, herbs that contain saponins may help to increase the absorption of plant constituents that are not water-soluble (Boik 1996).

In vitro studies with alcohol extract of *Mucuna pruriens* showed that *Mucuna pruriens* significantly inhibited $FeSO_4$ induced lipid peroxidation (Mahfouz & Kummerow 2000). It also inhibited the specific chemical reactions induced by superoxides and hydroxyl radicals. The alcohol extract of the seeds of *Mucuna pruriens* has an anti-lipid peroxidation property, which is mediated through the removal of superoxides and hydroxyl radicals. In another study a decoction of the leaf and the seed of *Mucuna pruriens* reduced total cholesterol in rats (Gorinstein et al. 1998).

The anticholesterolemia effect of ethyl acetate and crude extract of velvet bean could be due to its high content of flavonoid and high antioxidant activity. Isoflavones could protect the circulating and membrane lipids by sparing endogenous antioxidant (Tsai & Huang 1999). Flavonoid as an antioxidants either blocks the initiation of free radical formation or inactivate (e.g. scavenge) free radicals and terminates radical damage (Kumar et al. 2005). Free radicals in the presence of oxygen may cause peroxidation of lipids. The lipid-free radical interactions yield peroxides, which are unstable and reactive which can result in extensive membrane, organellar and cellular damage. Free radical could be captured by a scavenger, such as flavonoid, a potent free radical scavengers, even greater than vitamin E (Boik 1996). For example, superoxide is unstable and decays (dismutates) spontaneously into oxygen and hydrogen peroxide in the presence of water. However, several nonenzymatic and enzymatic systems contribute to inactivation of free radical reactions. A series of enzymes act as free-radical scavenging systems and break down hydrogen peroxide and superoxide anion. Superoxide dismutase (SOD), which is found in many cell types, can convert superoxide to hydrogen peroxide. Flavonoid as an antioxidant in the ethyl acetate fraction of velvet bean could increase SOD activity in hypercholesterolemic rats.

Crude extract and ethyl acetate fraction of velvet bean significantly decreased the total cholesterol, LDL-cholesterol and triglyceride and increased the HDL-cholesterol in hypercholesterolemic rats.

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