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Canavalia ensiformis Protein Extract Effect Toward Serum Lipid Profile of Hypercholesterolemic Sprague Dawley Rat

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Abstract

Riskesdas 2013 showed the prevalence of abnormal total cholesterol levels in Indonesia is 35.9%, low HDL cholesterol is 22.9%, high LDL cholesterol is 15.9%, and high triglyceride is 11.9%. This study aimed to prove the effect of jack bean protein extract toward serum lipid profile of a hypercholesterolemic rat. This was a true experimental study conducted in September 2016 with post-only control group design. Twenty four male Sprague Dawley rats were randomly classified into 4 different groups; control negative group (C-), control positive group (C+), first experimental group (X1) which was given jack bean extract 4g/200g of body weight of the rat per day, and second experimental group (X2) which was given jack bean extracts 6g/200g of body weight of the rat per day. Treatment for 2 weeks showed a significant effect. Total cholesterol, LDL cholesterol, and triglycerides levels of groups with protein jack bean extract administration were lower than the positive control group ($p < 0.05$). Increasing doses of jack bean extracts was linear with serum lipid profile improvement of Sprague Dawley Rat. These result indicated the positive effect of jack bean protein extract toward serum lipid profile of hypercholesterolemic Sprague Dawley Rat.

Introduction

Cardiovascular disease is the largest cause of death worldwide until present. The data from WHO in 2012 showed 17.5 million of deaths globally is caused by cardiovascular disease, which is dominated by coronary artery disease (7.4 million) and stroke (6.7 million). World heart federation (2008) showed more than 75% of deaths due to cardiovascular disease occurred in developing countries and Southeast Asia Region becomes the region with the third largest deaths after Europe and West Pacific. The death rate of man, which is 363-443 per 100,000 population, is higher than the death rate of woman, which is 181-261 per 100,000 population. One of the risk factor of these cardiovascular diseases is dyslipidemia.

Dyslipidemia is the abnormalities of lipid metabolism in the blood. This condition is marked by the increase of total cholesterol level, triglyceride levels, or both. Other sign of dyslipidemia is the decrease of HDL cholesterol level, while other cholesterol level and triglyceride level are increasing. According to National Cholesterol Education Program (NCEP) Adult Treatment Program III (ATP-III), the level of Low Density Lipoproteins (LDL) is the primary target of the management of dyslipidemia because 60-70% of total cholesterol level in the blood are contained in the LDL cholesterol (National Cholesterol Education Program, 2012). Moreover, Wiklund

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et al., (2013) showed that triglyceride level is also important as an indicator of hyperlipidemia and as a target of its management because increasing triglyceride level is a sign of lipid abnormalities in the blood, atherogenic dyslipidemia, and metabolic syndrome. High Density Lipoproteins (HDL) cholesterol has an independent role in the development of cardiovascular disease. Several sources showed that HDL cholesterol had a protective effect on this disease. The high level of HDL cholesterol indicates the compensation of the body on re-transport of cholesterol to the liver, because of the high level of lipid in extra hepatic tissue. The total cholesterol level also plays important role as a good general indicator of dyslipidemia by knowing the level of cholesterol other than LDL and HDL cholesterol (Nelson, 2013; Fakhrzadeh & Malazy, 2012). In addition to drug consumption, Bazzano *et al.,* (2011) showed that consumption of legumes has become a considered effort in preventing and treating dyslipidemia.

Legumes are still poorly known and are not optimally utilized. One of these legumes is Jack Bean. The benefit of Jack Bean in medical field, especially its effect on hypercholesterolemia, has been shown in several studies. However, its popularity and utility are lesser than other conventional legume, such as soya bean (Subagio, 2016). One of the component of legumes, including Jack Bean, that has an effect on lowering cholesterol level, is protein. Study on alloxan-induced rats (Nimenibo-Uadia, 2003) showed that there was a significant decrease of cholesterol and triglyceride level after administration of Jack Bean extract compared to control group.

Kayashima *et al.,* (2005) showed that administration of 0.34% Concanavalin A, which is one of protein fraction of Jack Bean, was proven to exert a positive effect. The treatment group had both lower serum cholesterol and triglyceride levels than control group.

According to recent studies, the mechanism of decreasing serum cholesterol and triglyceride levels by the protein occurred by an increase in the transcription of LDL receptor (Cho, *et al.,* 2007). Other mechanism was through absorption of peptides of the liver, which modulates cholesterol homeostasis, stimulating production and excretion of bile acid through feces. Therefore, cholesterol in the liver and the heart was reduced. The secretion of cholesterol in the liver can also be reduced. In addition, reduction of triglyceride level was due to the decrease of fatty acids synthesis in the liver (Rigamonti *et al.,* 2010; Spielmann *et al.,* 2008).

The protein of Jack Bean can be extracted with extraction and precipitation methods using NaOH and HCl. These methods need only several easily obtained tools and materials. Furthermore, the product of its extract has been applied for food processing, such as tofu, cake, and so on (Garba & Kaur, 2014).

The Sprague Dawley Rats was used as subjects because of its higher similarity of physiology characteristic with human than rabbit or other omnivore. It is also docile, easy to adapt, resistant to treatment, not able to vomit, and easy to feed. Therefore, bias can be minimal (Segupta, 2013).

Study about Jack Bean consumption and its effect on reducing lipid level in the blood are already performed. However, the study of hypercholesterolemic effect of protein from the Jack bean in the form of an extract is limited. Therefore, we conducted this study to prove the effect of Jack Bean (*Canavalia ensiformis*) protein extract toward serum lipid profile of Sprague Dawley Rat, which was fed hyper-cholesterol food.

Method

Current study was a true experiment study with post-test only design using control group. It was carried out in Food and Nutrition Study Center of Universitas Gadjah Mada, Yogyakarta, on September 2016. Twenty four Sprague Dawley Rats were randomly divided into 4 groups. The first group was just given standard food during the study (C-). The second group was given additional hyper cholesterol food as positive control group (C+). The third group was given hyper cholesterol food and 4g/200g of body weight of Jack Bean protein extract (X1). The fourth group was given hyper cholesterol food and 6g/200g of body weight of Jack Bean protein extract (X2). The duration of the study was 5 weeks. The first week was the adaptation period, whereas the second and the third week were the hypercholesterolemic

induction of C+, X1, and X2 groups. The last two weeks were the period of intervention for X1 and X2 groups, and continuation of the hyper cholesterol diet for C+, X1, and X2 groups. The hyper cholesterol diet consisted of 0.1% cholic acid, 10% pig oil, and 5% boiled quail egg yolks, which was mixed with ADII standard food . Ethical clearance was obtained from Ethical Committee of Medical Faculty, Diponegoro University, Semarang, with number 865/EC/FK-RSDK/VIII/2016.

Jack Bean were obtained from Temanggung Regency, Central Java, Indonesia. The beans were sorted, washed, and detoxified using Ajeigbe et al., (2012) method. Jack Bean protein extract was produced using extraction and precipitation method with 0.1 N NaOH and 0.1 M HCl (Bazzano et al., 2011).

Proximate test was performed according to SNI 01-2891-1992. The weight of the protein was measured using Kjeldahl method. The lipid level, ash content level, water level, and crude fiber were measured using soxhletasi method, dry ashing method, thermogravimetry, and acid-base hydrolysis, respectively. Carbohydrate level was obtained by reducing the total weight by protein, lipid, ash, and water weight (Musfiroh *et al.,* 2007; Sayangbati *et al.,* 2013).

The given dosage and the duration of treatment were referred to previous study. Pig nuts protein, soya bean, and pea bean dosage were 233g/kg of feed, 20g/100g of feed, and 20g/100g of feed, respectively. If they were converted into daily dose of feed, which was 10% per 200g body weight (BW) of rat (20g), the dose of pig nuts, soya bean, and lupine nuts became 4.7g/200g BW, 4g/200g BW, and 4g/200g BW, respectively. Therefore, we administered 4g/BW Jack Bean protein. The duration of treatment in current study was adjusted according to previous study, which was 14 days. Protein intervention for 14 days in previous study was proven to produce significant difference in lipid level between treatment and control group (Rigamonti *et al.,* 2010).

The blood was drawn at retro orbital plexus as much as 2ml. The serum was kept for 15 minutes and was centrifuged for 20 minutes at 300rpm. Total cholesterol level was measured on the 21st and 35th day, while other lipid profile were only measured on the 35th day. Cholesterol level on the 21st day was obtained to evaluate the fulfillment of hypercholesterolemic condition (≥180 mg/dl of total cholesterol). Total cholesterol (CH) and HDL cholesterol (HDL-C) were analyzed using cholesterol oxidase-amino antipyrine (CHOD-PAP) method, while LDL cholesterol (LDL-C) was analyzed using precipitation method (Sa'adah *et al.,* 2017). Triglyceride (TG) level was measured using enzymatic-colorimetric method (GPO-PAP).

The data were presented in average ± standard deviation. The difference of average of total cholesterol data and other lipid profile were analyzed using one-way analysis of variance (ANOVA) with post hoc LSD test. The result was considered significant if the $p<0.05$.

Result and Discussion

Jack Bean or *Canavalia ensiformis* is a tropical legume with white color and long shape. It originated from South America and is widely planted in South Asia, Southeast Asia, Africa, and Latin America. Currently, it is widely planted in several region of Indonesia (Lenkala, 2014; Sridhar & Seena, 2006; Wahyuningsih & Saddewisasi, 2013). Unlike Mung Bean, which is widely used in Indonesia as Tempe, Jack Bean is only utilized as animal feed and its younger form is processed as vegetable (Winarni & Dharmawan, 2016; Sridhar & Seena, 2006; Fagbenro *et al.,* 2004).

The composition of nutrients in Jack Bean extract was measured to understand the percentage of protein and other substance, which are assumed to have role in lipid level reduction. Jack Bean extract was produced to increase the percentage of protein proportion in 100g of material. Jack Bean protein extract in this study had 44.5% of protein and 12.53% of crude fiber (Table 1). It was different with the previous study, which protein isolates had 60.88-73.23% of protein level. Protein extraction in other study (Ugwuona & Suwaba, 2013) produced similar nutrients proportion with current study, which was 49.47% of protein and 18.29% of crude fiber. Higher fiber proportion in Jack Bean protein extract was better in supporting its role in the mechanism of reducing blood lipid, compared to the protein in the form of isolates.

Table 1. Proximate Analysis Result of Jack Bean Extract

Contents	Content (%)
Ash	7.13
Water	11.38
Crude fiber	12.53
Carbohydrate	21.76
Protein	44.5
Fat	3.34

Source: Primary Data

Total cholesterol level before the administration of Jack Bean protein extract was assumed to be homogenous. There was no significant difference between average of total cholesterol in C+, X1, and X2 group on the 14th day with $p>0.05$. Table 2 showed the difference of lipid profile level between each groups of the study.

The administration of Jack Bean protein extract for two weeks was capable of reducing total cholesterol level, even during the ongoing consumption of hyper cholesterol feed. The two groups of protein extract treatment (X1 and X2) had reduction of total cholesterol level, while C+ group had increased total cholesterol level (post Hoc test result, $p<0.05$). The difference of total cholesterol level between group X1 and X2 was not significant. However, X2 group had greater reduction than X1 group.

The result of current study was reciprocal with a study by Nimenibo-Uaida (2003), which stated that the administration of Jack Bean extract in diabetic rats can reduce the plasma cholesterol level from 2.98±0.05 mmol/L to 1.80±0.03 mmol/L and reduce triglyceride level form 1.83±0.01 mmol/L to 0.76±0.05 mmol/L after ten days of treatment.

Kayashima *et al.,* (2005) also showed that Concanavalin A (one of the protein within the Jack Bean) had a positive effect on serum cholesterol level. The cholesterol levels before and after the treatment were 3.10±0.34 mmol/L and 0.592±0.057 mmol/L, respectively. Cholesterol and triglyceride levels on the liver were also reduced (33.0±2.1 μmol/g and 60.0±9.8 μmol/g), while HDL cholesterol level were higher than control group (0.845±0.110 mmol/L). Total cholesterol level, triglyceride, HDL cholesterol in the serum, and cholesterol level in the liver of control group were 5.42±0.40 mmol/L, 0.803±0.098 mmol/L, 0.802±0.059 mmol/L, 52.3±3.7 μmol/g and 69.8±9.0 μmol/g, respectively.

The reduction of cholesterol after administration of Jack Bean protein extract can be affected by its protein and crude fiber contents. Jack Bean protein can increase the expression of cholesterol-7α-hydroxylase gene in the liver and subsequently increase the amount of secreted bile acids in the feces. This mechanism prevent secretion of cholesterol through VLDL (Cho *et al.,* 2007; Spielmann et al., 2008). On the other hand, Concavalin A (lectin) had different mechanism in reducing cholesterol level. Lectin Concavalin A affects lipid absorption in gastro intestinal tract without affecting protein and glucose absorption. Concavalin A binds to glycolipid in gastro intestinal tract and prevents the activity of brush border enzyme in enterocyte, thereby limiting cholesterol absorption (Kayashima *et al.,* 2005).

In the case of other beans, protein was also proven to be able to improve blood lipid profile. The administration of 233g/kg protein isolates of pig nuts for 2 weeks in hypercholesterolemic rat was proven to be able to significantly reduce total cholesterol level and LDL+VLDL cholesterol level from 3.48 mmol/L to 2.44 mmol/L and from 2.54 mmol/L to 1.61

Table 2. Lipid Profile of Rats after Administration of Jack Bean Protein Extract

Group	ΔCH(mg / dL)	LDL-C (mg / dL)	TG (mg / dL)	HDL-C (mg / dL)
C-	0.70 ± 1.76^{a}	29.62 ± 9.31^{a}	79.75 ± 2.83^{a}	63.13 ± 3.03^{a}
C+	25.18 ± 2.59^{b}	107.44 ± 1.09^{b}	142.24 ± 3.02^{b}	19.85 ± 1.39^{b}
X1	-48.30 ± 2.03^{c}	54.83 ± 5.75^{c}	114.80 ± 2.88^{c}	45.69 ± 1.95^{c}
X2	-69.62 ± 2.17 ^{c}	36.08 ± 1.24^{c}	91.93 ± 2.07^{d}	48.37 ± 9.82^{c}

$^{a-c}$ Letter on the same column indicate the difference in average.

Source: Primary Data

mmol/L, respectively. However, there was no significant difference on triglyceride and HDL cholesterol levels. The administration of 20% protein isolates of soya bean without isoflavon for two weeks was proven to be more effective than casein in reducing cholesterol level of rats. Other study by Rigamonti *et al.,* (2010) also proved that the administration of protein isolates from pea bean for 14 and 28 days were capable of significantly reducing cholesterol level and increasing HDL cholesterol level.

Cellulose polysaccharide and hemicellulose in the crude fiber of Jack Bean protein extract were known to have hypercholesterolemic effect (Sridhar & Seena, 2006). These compound bind to lipid along with bile salt within the intestinal tract and increase its excretion through feces. Furthermore, the fiber can alter the bile salt pool from cholic acid to chenodeoxycholic acid, which can inhibit the activity of *3-hydroxy 3-methylglutaryl* (HMG) *CoA Reductase*. These inhibition resulted in the reduction of cholesterol synthesis. Moreover, fibers will synthesize its degradation product, such as propionate or short chain fatty acid, in order to inhibit the synthesis of fatty acid. Meanwhile, the result of other study showed that a water soluble fiber was more effective than non-water soluble fiber in reducing blood cholesterol level (Gropper *et al.,* 2009).

The administration of Jack Bean protein extract also affected LDL cholesterol level. Treatment group with administration of Jack Bean protein extract (X1 and X2) had a lower LDL cholesterol level than C+ group. The increase dose of the extract did not provide significant difference toward LDL cholesterol level and total cholesterol level. However, cholesterol level in the group with the administration of 6g of protein extract was higher than the group with the administration of 4g of protein extract.

The protein within the legumes can increase transcription of LDL receptor in the liver and other extrahepatic tissue. The amount of LDL receptor increased that the LDL cholesterol in the blood stream were tied more frequently in the liver and extrahepatic tissue. Consequently, LDL clearance in the circulation would increase (Spielmann *et al.,* 2008). The fibers were also capable of reducing LDL cholesterol. Mechanism of reduction of total cholesterol level was mediated by LDL cholesterol reduction. Consumption of fiber was proven to increase the amount of receptor of LDL by as much as 26% higher than placebo (Shrestha *et al.,* 2007). Fiber intake reduced cholesterol by increasing the bile acid excretion and triggering bile acid synthesis. The low level of liver cholesterol triggers its intake from the circulation, including the increase of LDL receptor. Therefore, cholesterol on the LDL can be immediately taken (Bloedon *et al.,* 2004).

The level of HDL cholesterol between groups of treatment was significantly different. The level of HDL cholesterol on X1 and X2 group were normal, which were above 40 mg/dL. This result proved that the administration of Jack Bean protein extract could also affect the HDL cholesterol level. Higher HDL cholesterol in treatment group compared to control group could be the mechanism of the decrease of non-HDL cholesterol. Mechanism of re-transportation by HDL is known to reduce the cholesterol level in extra hepatic tissues. The HDL delivers the cholesterol to the liver to be excreted (Kayashima *et al*, 2005).

The intake of fiber is also known to be related by re-transportation mechanism by HDL. The relation is on the cholesteryl ester transfer protein (CETP), which had a function in transferring cholesteryl ester from HDL to triglyceride particle. Fiber intake can reduce the activity of CETP. The inhibition of CETP will change the route of RCT. It will be delivered more often through HDL than VLDL. Therefore, the HDL cholesterol will be increased (Bazzano, 2011; Tall, 2010).

Current study revealed that both treatment groups had a lower serum triglyceride level than control group. The absorption of the lipid is inhibited by the protein of Jack Bean in the gastrointestinal tract. Other mechanism related to this result is the capability of the protein in reducing fatty acid synthesis due to reduction of mRNA SREBP-1c concentration and its target gene, which is FAS, as the key enzyme of the lipid synthesis in the liver. Therefore, production and secretion of very low density lipoprotein (VLDL) can be reduced. VLDL protein is a lipoprotein that contains high level of triglyceride. The reduction of its secretion

Table 3. Correlation Test between Weight Gain and the Difference in Cholesterol Level

Group	ΔBW(g)	ΔCH(mg / dL)	Pearson Test
C-	15.5±1.05	0.70 ± 1.76[a]	0.000
C+	24.33±1.21	25.18 ± 2.59[b]	
X1	13.66±1.03	-48.30 ± 2.03[c]	
X2	11.33±1.03	-69.62 ± 2.17 [c]	

Source: Primary Data

in the liver will also reduce triglyceride level, when VLDL circulates on the bloodstream (Rigamonti *et al.,* 2010; Spielmann *et al.,* 2008).

Body weight was not always related to cholesterol level (Wang *et al.,* 2010; Zamani *et al.,* 2012). However, obesity is related to high cholesterol level (Listiyana *et al.,* 2013). The average gain in body weight in the current study on the 21st day to 35th day between each groups were not significantly different (p=0.804). However, correlation test between average weight gain and the difference in total cholesterol level showed significant result (p=0.000), indicating that during intervention, weight gain of the rats was related with the change of total cholesterol level. Protein intake is known to increase satiety, to help in weight reduction, and to lower the level of triglyceride (Santesso, 2012).

Conclusions

The administration of Jack Bean protein extract can improve the blood lipid profile of hypercholesterolemic Sprague Dawley Rats compared to the control group. Increasing dose of Jack Bean protein extract did not provide significant effect except on serum triglyceride level. Further study about the effect of Jack Bean protein extract in longer duration is needed.

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