The Antiplatelet Aggregation Effect of Extract and Ethyl Acetate Fraction of Velvet Bean Seed (*Mucuna pruriens* L.) in Dyslipidemic Rat

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**ABSTRACT**

Cardiovascular disease (CVD) is the first cause of death in the world, CVD has complex and multifactorial process including atherogenic lipoprotein, oxidized low density lipoprotein (LDL), endothelial dysfunction, plaque stability, vascular inflammation, thrombotic and fibrinolytic disorder, exercises and genetic factor. Inhibiting the platelet aggregation is one of the CVD prevention. Velvet bean seed (*Mucuna pruriens* L.) can be found abundantly in Indonesia, but has not been used as herbal medicine. Ethanol extract and ethyl acetate of velvet bean seed contain high flavonoids and antioxidants properties which is expected could inhibit platelet aggregation. The objectives of the research were to determine the activity of ethanol extract and ethyl acetate fraction of velvet bean seed towards clotting and bleeding time in dyslipidemic rats. This research used completely randomized design in dyslipidemic rats which were given by ethanol extract of velvet bean seed at the concentrations of 50, 100 and 200 mg/kg BW/day and ethyl acetate fraction of velvet bean seed at the concentrations of 15, 30 and 60 mg/kg BW/day and 42.2 mg/kg BW/day aspirin for ten days. Clotting and bleeding time were measured at days 0, 10, and 20. Data were analyzed using One way analysis of variance and continued with Duncan’s post Hoc test with 95 % level of significancy. The results showed that administration of 60 mg/kg BW/day ethyl acetate fraction of velvet bean seed and at the concentrations of 100 and 200 mg/kg BW/day ethanol extract of velvet bean seed, prolong the clotting time at day 10, ethyl acetate fraction at the concentration of 60 mg/kg BW/day, 200 mg/kg BW/day ethanol extract of velvet bean seed prolong bleeding time at day 10.

**Keywords**: antiplatelet, flavonoid, platelet aggregation, *Mucuna pruriens* L., cardiovascular disease, dyslipidemic

**INTRODUCTION**

Cardiovascular disease (CVD) is the first cause of death in the world; around 19.8 % from total death in 1993 and become 24.4 % in 1998. An estimated 17.1 million people died from CVD in 2004, representing 29% of all global deaths. Of these deaths, an estimated 7.2 million were due to CVD and 5.7 million were due to stroke (AHRQ, 2003; WHO, 2009). Incidence of CVD is increasing in developed countries, the incidences are estimated to be three to four times higher in developing countries. Globally, CVD is estimated 31 % of the worldwide mortality. Each year 17.2 million people die of CVD, 80 % of them in the developing world and emerging economies. By 2030, almost 23.6 million people will die from CVD, mainly from heart disease and stroke. These are projected to remain the single leading causes of death. The largest percentage increase will occur in the Eastern Mediterranean Region. The highest increase in number of death will occur in the South-East Asia Region (WHO, 2009). Based on the data of Medical Department and Yayasan Penyakit Jantung (Cardiac Disease Foundation) showed that CVD is the leading cause of death in Indonesia. Based on World Health Organization (WHO) data, around 12 million people died of heart attack in the world. According to the family health survey (survei kesehatan rumah tangga) in 1992, CVD causes around 16 % of death and become 26.4 % in 2001 (Irawan, 2007).

Atherosclerosis is the main cause of CVD with complex and multifactorial process involving genetic and environment factors. The risk factors of CVD are atherogenic lipoprotein,
oxidized low-density lipoprotein (LDL), endothelial dysfunction, plaque instability, vascular inflammation, thrombotic and fibrinolytic disorder, exercises and genetic factor (Wijaya, 1998; Halliwel and Gutteridge, 1999). Atherosclerosis is an inflammatory disease and high plasma cholesterol concentrations, in particular those of LDL cholesterol, is one of the principal risk factor of atherosclerosis. If LDL particles become trapped in an artery, they can undergo progressive oxidation and be internalized by macrophages by means of the scavenger receptors on the surfaces of these cells (Ross, 1999). The internalization leads to the formation of lipid peroxides and facilitates the accumulation of cholesterol esters. Oxidized LDL may also be involved in atherogenesis by inducing smooth muscle cell proliferation and smooth muscle foam cell generation (Holvoet et al., 1998). Subendothelial accumulation of foam cells plays a key role in the initiation of atherosclerosis. These foam cells may be generated by the uptake of oxidized LDL and/or malondialdehyde (MDA)-modified LDL (Holvoet et al., 1998; Ross, 1999). Numerous in vitro studies using a variety of oxidation methods and measurements have shown that polyphenolics from red wine, green tea and chocolate can inhibit LDL oxidation (Anderson et al., 2001).

Platelet aggregation is an important factor in thrombosis with uncontrollable clotted blood (Wu et al., 2007). Formation of platelet aggregates in an atherosclerotic vessel can cause total blockade of blood flow, leading to myocardial infarction and thromboembolic diseases. Atherosclerosis promotes increased adhesiveness in the endothelial wall for platelets, and a lost of anticoagulant properties, which contribute to atherosclerotic plaque formation. Platelets adhesion is initiated when platelets contact with exposed connective tissue of injured tissues. Adhesion stimulates platelets to secrete a variety of factors, including fibrinogen and von Willebrand factor, which facilitates aggregation and platelet plaque on the vessel wall. Activated platelets also secrete thromboxane A2 that further stimulates platelet aggregation, and the activated immune cells also secrete platelet activating factor (PAF) which stimulate platelet aggregation (Boik, 1996; Geraldo, 2010). The activated platelets change shape, put out pseudopodia, discharge their granules, and stick to other platelets thus initiating the process of platelets aggregation (Resh and Ernst, 1995; Klepser and Klepser, 1999; El-Sabban, 2009). The rupture of plaques is the most important mechanism for the progression of vascular disease because this rupture exposes thrombogenic subendothelial matrix of protein and collagen, triggering a cascade of platelet activity (Geraldo, 2010).

Flavonoids are component of a wide variety of edible plants, fruits, and vegetables and of beverage appears to have antioxidative properties toward LDL, lipid peroxidation (Fuhrman et al., 1995; Sesso et al., 2003). Flavonoid from *Garcinia cambogia* inhibit atherosclerosis, endothelial damaged, leucocyte activation, platelet adhesion and platelet aggregation (Koshy et al., 2001). According to the previous research by Widowati and Retnaningsih (2007) antioxidant activity in ethanol extract and ethyl acetate fraction of velvet bean seed (*Mucuna pruriens L.*) compared with α-tocopherol, ascorbic acid and butylated hydroxytoluene (BHT), ethanol extract and ethyl acetate fraction of velvet bean seed contain higher flavonoid compared to the others fraction (hexane fraction, butanol and water fraction) (Widowati et al., 2007; Widowati et al., 2010), thus this compound can be possibly used as antiaggregation platelet agent.

Many drugs as antiplatelet agents for treating CVD, however some have several collateral effects and resistance in long term therapy, such as the known clinical aspirin resistance (Geraldo, 2010). Thus, the purpose of this research is to develop new antiplatelets agents with low collateral effects, from ethanol extract and ethyl acetate fraction of velvet bean seed.

**MATERIALS AND METHODS**

**Extraction and Fractionation**

The velvet bean seed (*Mucuna pruriens L.*) was collected from Sukoharjo District, Central Java, Indonesia, during April, 2007. Five kilograms of velvet bean seeds were milled and soaked in distilled ethanol (EtOH) and extracted by maceration technique; evaporated filtrate and yielded 516.845 g (10.335 %) ethanol extract of velvet been seed. The ethanol extract (133.889 g) was partitioned with solvent mixture of 75% n-hexane and 25 % water, produced 30.466 g (22.71 %) hexane fraction and the water filtrate residue was partitioned with solvent mixture of 95 % ethyl acetate and 5 % water, produced 0.498 g (0.37 %) ethyl acetate fraction and the water filtrate residue was partitioned with solvent mixture of 90 % n-buthanol and 10 % water, produced 3.578 g (2.67 %) ethyl acetate fraction. Finally, the water filtrate residue was evaporated and produced 4.358 g (3.26 %) water fraction. In this study, we used ethanol extract and ethyl acetate fraction of velvet bean seed, since the previous in vitro study showed that ethanol extract and ethyl acetate fraction of velvet bean seed had the highest antioxidant activity.

**Animals and Diets**

Thirty adult male Wistar rats obtained from School of Life Science, Bandung Institute of Technology, were housed in standard cages provided with food and water ad libitum. Rats were adapted for 7 days until the body weight were 175-200 g. The rats were feed by high fat diet (3000 g 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 rats which feed with high fat diet showed the average percentage
of crude fat was 20.78% compared to the standard diet rats which was only 7.37%.

In spite of high fat diet, each rat was also given with 1 mL/day fructose liquid 60% (120 g/200 mL aquadest). The high fat diet and fructose liquid were given for 2 weeks until the body weight achieved 200 – 250 g, but the high fat diet was still given continually for 20 days. The ethanol extract and ethyl acetate fraction of velvet bean seed were given only for 10 days.

Rats were divided into 9 groups (3 rats) with different treatments. The first group, as negative control, was given standard diet. The second group, as positive control, was given high fat diet. The third, fourth, and fifth groups were treated with high fat diet plus ethyl acetate fraction 15 mg/kg BW, 30 mg/kg BW and 60 mg/kg BW daily. The sixth, seventh and eighth groups were treated with high fat diet plus ethanol extract 50 mg/kg BW, 100 mg/kg BW and 200 mg/kg BW daily. Group ninth was treated with high fat diet and aspirin 42.2 mg/kg BW daily.

Sample Preparation for The Lipid Test and MDA

After the treatments, 1.5 mL blood from orbital vein was collected in the tube contained heparin. It was centrifuged at 3,000 rpm for 10 minutes and the plasma was used for measuring the total cholesterol, LDL-cholesterol, high-density lipoprotein (HDL-cholesterol), triglyceride, and MDA level.

Total plasma cholesterol and triglyceride were measured according to the instruction manuals by the diagnostic kits from Abbott Clinical Chemistry (Abbott Clinical Chemistry, 2006), HDL-cholesterol, LDL-cholesterol were measured according to the instruction manuals of the diagnostic kits from Daiichi Pure Chemicals Co., Ltd (Daiichi Pure Chemical, 2008).

After feeding high fat diet and fructose for 2 weeks, the lipid profile of negative control group (standard diet) was as follow: total cholesterol 58.33 mg/dL, LDL 8.00 mg/dL, HDL 33.00 mg/dL, triglyceride 63.67 mg/dL and the dyslipidemic rats had total cholesterol 70.67 mg/dL, LDL 14.33 mg/dL, HDL 24.33 mg/dL, and triglyceride 112.33 mg/dL. Thus, feeding with high fat nutrient induced dyslipidemic rats.

Clotting Time Assay

The rat’s tail was injured, blood collected with capillary tube for 30 seconds. Capillary tube was broken every 15 seconds until the fibrin filament appeared at the broken capillary tube as clotting time. Clotting time was measured at days 0, 10 and 20.

Bleeding Time Assay

The rat’s tail was injured, blood absorbed with filter paper for 15 seconds. The interval time between the first dropping blood until the flowing blood stopped was calculated as bleeding time. Bleeding time was measured at days 0, 10 and 20.

Statistical Analysis

To verify the statistical significance of all parameters, the data were calculated the values of means and standard deviation (M ± SD) and 95% confidence interval (CI) of means. This research used completely randomized design. To compare several groups, analysis of variance (ANOVA) was used. P-values of less than 0.05 were considered as statistically significant. Furthermore to know the best treatment, Duncan’s post-Hoc test at 95% confidence interval was used. Statistical analysis was done using SPSS 16.0 version.

RESULTS AND DISCUSSION

Based on the statistical analysis, at day 10 showed that HDL, LDL and MDA significantly different among treatment, high fat diet significantly increased the LDL-cholesterol, decreased the HDL-cholesterol and decreased MDA compared to standard diet (Table 1). Extract and ethyl acetate fraction of

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LDL level (mg/dL)</th>
<th>HDL level (mg/dL)</th>
<th>MDA level (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>8.00±1.00 ab</td>
<td>33.00±2.67 c</td>
<td>5.52±0.67 ab</td>
</tr>
<tr>
<td>Positive control</td>
<td>14.33±1.53 cd</td>
<td>24.33±1.53 a</td>
<td>11.77±1.13 c</td>
</tr>
<tr>
<td>Ethyl acetate fraction 15 mg/kg BW/day</td>
<td>10.00±2.00 ab</td>
<td>25.33±3.22 ab</td>
<td>5.62±0.18 ab</td>
</tr>
<tr>
<td>Ethyl acetate fraction 30 mg/kg BW/day</td>
<td>8.33±2.52 ab</td>
<td>26.67±2.3 abc</td>
<td>4.06±0.55 ab</td>
</tr>
<tr>
<td>Ethyl acetate fraction 60 mg/kg BW/day</td>
<td>7.33±1.53 a</td>
<td>29.67±1.15 abc</td>
<td>3.39±0.22 a</td>
</tr>
<tr>
<td>Ethanol extract 50 mg/kg BW/day</td>
<td>11.33±2.52 bc</td>
<td>26.33±3.51 abc</td>
<td>6.03±2.35 b</td>
</tr>
<tr>
<td>Ethanol extract 100 mg/kg BW/day</td>
<td>10.33±1.53 ab</td>
<td>28.67±5.51 abc</td>
<td>5.54±1.35 ab</td>
</tr>
<tr>
<td>Ethanol extract 200 mg/kg BW/day</td>
<td>11.67±3.22 bc</td>
<td>31.67±3.51 bc</td>
<td>5.14±1.86 ab</td>
</tr>
<tr>
<td>Aspirin 42.2 mg/kg BW/day</td>
<td>15.33±1.53 d</td>
<td>26.33±3.51 abc</td>
<td>9.60±1.61 c</td>
</tr>
</tbody>
</table>

Results are expressed means ± SD, Duncan post Hoc test are shown by little letter, different letter at the same column are significantly different among treatment groups at confidence interval 95%.

Table 1. HDL, LDL and MDA level in dyslipidemic rats treated with ethanol extract and ethyl acetate fraction of velvet bean seed for 10 days.
velvet bean seed decreased the LDL level and ethanol extract of velvet bean seed 200 mg/kg BW/day increased HDL level in dyslipidemic rats. Extract and ethyl acetate fraction of velvet bean decreased the MDA level in dyslipidemic rats.

After treatment with various doses of ethanol extract, ethyl acetate fraction of velvet bean seed, and aspirin 42.2 mg/kg BW/day for 10 days, the effect on clotting time was shown in Table 2. Based on the statistical analysis, at day 0, clotting time showed no significant difference among groups because at day 0 rats had not been given with ethanol extract, ethyl acetate and aspirin yet. At day 10, clotting time showed significant difference among groups because rats had been given ethanol extract and ethyl acetate fraction. At day 20, clotting time showed no significant difference among groups because rats had been given ethanol extract and ethyl acetate fraction had no effect on clotting time after the treatment had been stopped. To determine the difference of the clotting time (days 0, 10 and 20) among treatment groups, we analyzed using Duncan’s post Hoc test (Table 2), which showed that on day 0, no significant difference in bleeding time among treatment groups. At day 10, the bleeding time showed that ethyl acetate group (60 mg/kg BW/day) was significant difference compared to positive control, even showed a better result compared to group treated with aspirin as anticoagulant. At day 20, the shortest bleeding time was only in positive control (dyslipidemic rats), negative control, rats given ethyl acetate fraction 60 mg/kg BW/day and rats given with aspirin were significant difference compared to positive control. Although aspirin and ethyl acetate fraction had been stopped at day 10, it means that aspirin and ethyl acetate fraction could prolong the bleeding time until day 20.

Based on statistical analysis, at day 0, the bleeding time showed no significant difference among treatment groups. While, at day 10, bleeding time showed significant difference among treatment groups, since rats had been given ethanol extract and ethyl acetate fraction. At day 20, bleeding time showed significant difference among treatment groups although the ethanol extract, ethyl acetate and aspirin had been stopped at day 10, indicated that ethanol extract and ethyl acetate still influenced the bleeding time. To determine the difference of the bleeding time (0, 10 and 20 days) among treatment groups, we analysed using Duncan’s post Hoc test (Table 3), which showed that on day 0, no significant difference in bleeding time among treatment groups. At day 10, the bleeding time showed that ethyl acetate group (60 mg/kg BW/day) was significant difference compared to positive control, even showed a better result compared to group treated with aspirin as anticoagulant. At day 20, the shortest bleeding time was only in positive control (dyslipidemic rats), negative control, rats given ethyl acetate fraction 60 mg/kg BW/day and rats given with aspirin were significant difference compared to positive control. Although aspirin and ethyl acetate fraction had been stopped at day 10, it means that aspirin and ethyl acetate fraction could prolong the bleeding time until day 20.

Based on the clotting time data (Table 2) at day 10 indicated that dyslipidemic rats were given with extract and ethyl acetate prolonged clotting time compared to positive control. The bleeding time (Table 3) at days 10 and 20 in dyslipidemic rats were shorter than negative control. At day 10 showed that the dyslipidemic rats which were given ethanol extract and ethyl acetate fraction prolonged the bleeding time. This research was verified by previous study that hyperlipidemic in rats exhibit significant increase in ADP or collagen-induced platelet aggregation and cholesterol/phospholipid molar ratio in platelets. The increase in cholesterol/phospholipid ratio was responsible for hyperaggregation of platelet in animals (Nobukata, 1999). This data was verified with previous research that high cholesterol consumption can induce dyslipidemic and endothelial damaged, also effect thrombocyte adhesion in collagen furthermore activate thrombocyte (Aprami, 1993;...
Wijaya, 1998). Active thrombocyte release ADP and thromboxane A2 and initiate aggregation of thrombocyte and vasoconstriction. Thrombocyte aggregation is important factor in thrombosome formation in uncontrol blood clotting (Wu et al., 2007). The high LDL and low HDL level in dyslipidemic rats (Table 3) were shorter clotting and bleeding time compared to negative control, this research was verified that elevating levels of LDL and low levels of HDL are widely used for predicting risk to atherosclerosis (Sevanian et al., 1998).

Velvet bean seed ethanol extract and ethyl acetate fraction contain high antioxidant and flavonoids (Widowati and Retnaningsih, 2007; Widowati et al., 2007, Widowati et al., 2010), were capable to prolong the clotting and bleeding time at day 10 in dyslipidemic rats (Table 3) were shorter clotting and bleeding time compared to negative control, this research was verified that elevating levels of LDL and low levels of HDL are widely used for predicting risk to atherosclerosis (Sevanian et al., 1998).

Based on the data (Table 1) displayed that velvet bean seed ethanol extract and ethyl acetate fraction had high antioxidant activity (Widowati and Retnaningsih, 2007), and were capable to reduce lipid peroxidation and decreased MDA, LDL level compared to dyslipidemic rats which were not given extract and ethyl acetate fraction as antioxidant and flavonoid sources. Antioxidant activity of polyphenols were shown to possess many biological properties including the inhibition of platelet aggregation, vasorelaxing activity, modulation of lipid metabolism, and inhibition of LDL oxidation (Agli et al., 2004).

In Table 2, the LDL in dyslipidemic rats 14.33 mg/dL was higher than negative control (8.00 mg/dL) and will highly influenced the oxidative damage (MDA 11.77 mmol/L), due to the high LDL level accelerating atherogenicity (Parthasarathy et al., 1999). The oxidation of LDL was led by free radical-mediated chain reaction, yielding phosphatidylcholine hydroperoxide (PCOOH) cholesteryl ester hydroperoxide (CEOOH) as the primary oxidations product (Noguchi et al., 1998). It means that high LDL level increases oxidized LDL (ox-LDL) and MDA level (Table 2) and play role in atherosclerosis. LDL level increases amounts of thiobarbituric acid reactive materials (often attributed to increase amounts of MDA, 4-hydroxynonenal, and related carbonyl compounds), lipid peroxides, cholesterol oxides, decrease amounts of specific phospholipids, showed increase lysophospholipids and diminish antioxidants level (Sevanian et al., 1998). The evidence indicate that oxidative modification of LDL may play a causative role in atherosclerosis (Sevanian et al., 1998). Oxidized LDL is cytotoxic to endothelial cells, and nLDL is not toxic (Sevanian et al., 1998; Halliwel and Gutteridge, 1999). The previous research indicated that atherogenicity was due to oxidative damage to LDL cholesterol. Modified or oxidized LDL (ox-LDL) has been shown to accelerate several steps in atherosclerosis including endothelial damage, monocyte/macrophage recruitment, increased uptake of LDL by foam cells (Hannekens, 1999). The oxidation of LDL is important in atherosclerosis, it is likely to occur in the subendothelial intima rather than the plasma (Parthasarathy, 1999). In Table 1, showed that dyslipidemic rats, high LDL concentration increased ox-LDL. The MDA level in dyslipidemic rat was higher 11.77 mmol/L compared to normal rat was 5.52 mmol/L, furthermore the bleeding time at 10 day of dyslipidemic rats were shorter than those in normal rats. This research result supports previous research, that oxLDL could accelerate atherosclerosis stages including endothelial damage, monocyte/macrophage recruitment, increased uptake of LDL by foam cells, alteration in vascular tone (Hannekens, 1999; Parthasarathy et al., 1999). The oxidative modified LDL is taken up by macrophage, which results in its unregu-
lated uptake and eventual formation of foam cells as the early stages in cardiovascular disease (Noguchi et al., 1998). Atherosclerosis is a systemic inflammatory disease characterized by the accumulation of monocytes/macrophages and lymphocytes in the intima of large arteries. Rupture or erosion of the advanced lesion initiates platelet activation and aggregation on the surface of the disrupted atherosclerotic plaque (Massberg et al., 2002). The term of endothelial dysfunction has been used to refer several pathological conditions, including altered anticoagulant and anti-inflammatory properties of the endothelium, impaired modulation of vascular growth, and dysregulation of vascular remodeling (Cai and Harrison, 2000), damaging the platelet activity, leukocyte adhesion, and thrombosis and is intimately involved in the development of atherosclerosis (Heitzer et al., 2001). Increased production of oxygen-derived free radicals such as the superoxide anion has been linked to impaired endothelial vasomotor function (Heitzer et al., 2001). Ox-LDL is the key event in endothelial injury and dysfunction. LDL in the subendothelial space, undergoes progressive oxidation and activates the expression of macrophage chemotactic protein 1 (MCP-1) and macrophage colony stimulating factor (M-SF) in the endothelium. MCP-1 and M-CSF promote the entry and maturation of monocytes to macrophages, which further ox-LDL. Ox-LDL is specifically recognized by the scavenger receptor of macrophages and once internalized, formation of foam cells. Ox-LDL and modified LDL induce endothelial, associated with changes of the adhesiveness to leukocytes or platelet and to wall permeability (Virgilli and Scaccini, 2001).

Extract and ethyl acetate fraction of velvet bean could prolong the clotting and bleeding time, and was verified with the previous research that using apple juice as flavonoids source (total flavonoids 1.36 ± 0.03 g/100 mL equivalent catechin and total anthocyanin 3.05 ± 0.85 mg/100 g) in cholesterolemic rabbit, significantly decreased fibrinogen and factor VII levels compared to high-cholesterol group (Setorki et al., 2001). Based on the data (Table 2,3) displayed velvet bean seed extract and ethyl acetate fraction could prolong the clotting and bleeding time due to contained high flavonoid (Widowati et al., 2007). The previous research showed that ethyl acetate fraction of velvet bean seed 500 µg/mL exhibited antiaggregation platelet against adenosin diphosphate (ADP) inducer by in vitro test (Widowati and Ratnawati, 2009). Flavonoids are subgroup of polyphenol, capable of: 1). inhibiting atherosclerosis, endothelial disruption, leukocyte activity, adhesion, aggregation platelet, decrease LDL level in normal and hypercholesterolemic rats, also inhibit in vitro LDL oxidation; 2). decreasing atherogenesis, inhibit hyperlipidemia, inhibit triglyceride accumulation in blood and liver, decrease phospholipid and fatty acids in rat’s tissue (Koshy et al., 2001; Hidgon, 2005). Giving 10 mL and 5 mL apple juice in cholesterolemic rabbits, the atherosclerotic thickness of right and left coronary arteries was 0.8 – 1.4 and 0.92 –1.92, respectively and the plaque degree for both was grade 1. High cholesterol groups, right and left coronary arteries, the atherosclerotic thickness of right and left coronary arteries was 3.47 ± 0.37 and 3.28 ± 0.26, respectively and the plaque degree in both was grade 3. Giving apple juices as flavonoid sources decreased the plaque degrees (Setorki et al., 2009). Atherosclerotic changes were absent in normal diet group, whereas in the intimal surface of the coronary arteries from high-cholesterol diet group were seen many fat-laden macrophages. The cytoplasm of the macrophages filled with lipid droplets (foam cell) as the result of lipid digestion by the macrophage. Apple juice as flavonoids source may be useful in preventing hypercholesterolemic atherosclerosis and lowering the related risk of coronary artery disease (Setorki et al., 2009).

Flavonoids play role as an antiinflammation, antioxidant, antiallergic, hepatoprotective, antithrombotic, neuroprotective and anticarcinogenic (Ganapaty et al., 2007). Antiaggregation platelet activity of flavonoid is through inhibition of thromboxane \( \text{A}_2 \) mechanism. The inhibited thromboxane \( \text{A}_2 \) stimulates adenyl cyclase further increase cAMP and the increasing cAMP will reduce ion Ca (calcium) concentration in thrombocyte, furthermore inhibit aggregation and adhesion (Hoffbrand and Pettit, 1987), prolong clotting and bleeding time.

Flavonoids protect against atherosclerosis, through reducing the susceptibility of LDL to oxidation (Benito et al., 2002), and vasodilator properties observed in vitro (Benito et al., 2002)

The research results showed that giving velvet bean seed extract 200 mg/kg BW/day increased HDL level (31.67 mg/dL) compared to positive control (24.33 mg/dL) (Table 2). This results were verified with previous study that HDL concentration inhibited atherosclerosis progression and advance cardiovascular disease (Sevanian et al., 1998; Santoso and Setiawan, 2005).

Based on Table 2 showed that aspirin prolonged clotting time in dyslipidemic rats and prolonged bleeding time in dyslipidemic rats (Table 3), aspirin is antiplatelet agent, medication that blocks the formation of blood clots by preventing the clumping of platelets. Aspirin as anti-thrombotic compounds is through the inhibition of platelet cyclooxygenase-1 (COX-1) by irreversible acetylation of a specific serine moiety, thereby blocking the formation of thromboxane \( \text{A}_2 \) (TXA2) for the life time of the platelets (McKee et al., 2002; Ohmori et al., 2006). Table 1 showed that aspirin has no antioxidant effect, can not decrease the MDA level, has no anticholesterol activity, and can not decrease the LDL level in dyslipidemic rats.
CONCLUSIONS

Ethanol extract and ethyl acetate fraction of velvet bean seed exhibited antiplatelet aggregation in dyslipidemic rats, treated with ethanol extract 100 mg/kg BW/day, 200 mg/kg BW/day and ethyl acetate fraction 60 mg/kg BW/day prolonged the clotting time at day 10, ethanol extract 200 mg/kg BW/day and ethyl acetate fraction 60 mg/kg BW/day prolonged the bleeding time at day 10.

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