ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 9, Issue 1, 2016

ISSN - 0974-2441

Research Article

# LIPID PROFILE AND PLATELET AGGREGATION OF ETHANOLIC SEED EXTRACT OF AVOCADO (*PERSEA AMERICANA* MILL.) IN HYPERLIPIDEMIC MALE WISTAR RAT

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***Received: 29 October 2015, Revised and Accepted: 12 November 2015***

**ABSTRACT**

**Objective:** The objective of this research was to investigate the effect of ethanol seed extract of avocado (ESEA) (*Persea americana* Mill.) on lipid profile and platelet aggregation in hyperlipidemic male Wistar rat.

**Methods:** The avocado seed was extracted using 70% ethanol by the reflux method. The induction method was conducted by giving high cholesterol intake and oral administration of pure cholesterol, cholic acid, and propylthiouracil. Antiplatelet aggregation parameters were measured by observing of bleeding time (BT), coagulation time (CT), and antiplatelet aggregation activity using adenosine 5'-diphosphate (ADP) as aggregation inductor. Lipid profile such as total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL), as well as the parameters of platelet aggregation were measured before the induction, the end of induction and the end of therapy.

**Results:** ESEA 10 mg/kg bw showed decreasing in TC and LDL levels which was significantly different compare to control group ($p<0.05$) while ESEA 20 and 40 mg/kg bw gave decreasing in TG. All of doses of extract showed no increasing in HDL levels. In antiplatelet aggregation effect test, ESEA 10 mg/kg bw had the ability to extend BT. ESEA 20 and 40 mg/kg bw had the ability to extend the CT. All of doses of extract showed significantly different ($p<0.05$) on the measurement of aggregation activity by decreasing in plasma absorbance.

**Conclusion:** The result suggested lipid lowering and anti-aggregation potential of ESEA, which serves as a new potential herbal product and good for cardiovascular disease treatment because it can reduce both risk factors causing the disease.

**Keywords:** Cholesterol, Platelet aggregation, Avocado, *Persea americana* Mill., Seed.

## INTRODUCTION

Cardiovascular disease and coronary heart disease are the main causes of mortality which become global health issues in all of ethnic in the world. Some risk factors associated with the incidence of cardiovascular disease including hypercholesterol, dislipidemia, and hyperaggregation of platelets. The high level of cholesterol, particularly total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL) cholesterol are mainly responsible for the onset of coronary heart diseases [1]. Low-level circulation of high-density lipoprotein (HDL) particles is generally related with a higher incidence of atherosclerotic cardiovascular disease [2].

Hyperlipidemia and platelet aggregation are two interrelated circumstances where high levels of cholesterol in the blood lead to atherosclerosis. Atherosclerosis in the endothelium vascular related to stimulate platelet aggregation, coagulation or venous thrombosis, blood accumulation, blood flow interference, and venous inflammation that may lead to platelet adhesion and coagulation in the blood vessel [3].

The treatment of hypercholesterolemia and related cardiovascular diseases using medicinal plants has increased in recent years [4]. Related to antihyperlipidemia and anti-aggregation activity, phytochemical screening of avocado seed was investigated which showed the presence of saponin that allegedly responsible related with its general characteristic included cholesterol binding properties [5]. Saponin were highest in avocado seed than fruit and leaf [6]. While phenols in avocado could further indicate their ability to act as anti-inflammatory, anti-clotting, antioxidant, and immune enhancer [7]. The previous study revealed that phenols were highest in avocado seed than the other parts of the plant [6]. The phenolic compound had the ability to reduce plasma lipid level in the human body through the increasing in the regulation of LDL receptor expression, inhibition of hepatic lipid synthesis and lipoprotein secretion, and increase in cholesterol elimination through bile acids [8].

Therefore, the aim of this research was to investigate the effect of ethanol seed extract of avocado (*Persea americana* Mill.) on lipid profile and platelet aggregation in hyperlipidemic male Wistar rat and expected that it may have significant influence on lipid profile, and platelet aggregation were eventually able to reduce morbidity and mortality as risk of cardiovascular disease due to complication caused by these risk factors.

## METHODS

### Collection of sample

The seeds of avocado were collected from Lembang, Bandung. The plant was determined by Herbarium Bandungense, School of Life Science and Technology, Bandung Institute of Technology, Indonesia.

### *Animals*

Adult male Wistar rat 2-3 months old, weighing about 180-210 g, healthy with normal activity obtained from a animal laboratory, School of Pharmacy, Bandung Institute of Technology. The treatment was carried out according to ethics for the care and use of laboratory animals.

### Chemicals

Cholic acid and adenosine 5'-diphosphate (ADP) sodium salt were purchased from Sigma-Aldrich; pure cholesterol, propylthiouracil, and simvastatin were obtained from Dexa Medica; and aspirin was obtained from Bayer Pharmaceutical Industries, Indonesia. All other chemicals and reagents were of analytical grades.

**Preparation of plant extract**

The collected seeds were washed with tap water and dried in the oven with controlled temperature. Dried seeds were powdered using a mechanical and stored in air tight container. About 500 g powder was extracted with 70% ethanol using reflux apparatus. The extract was concentrated to dryness under reduced pressure and controlled temperature using a rotary evaporator. The dried extract (ethanolic seed extract of avocado [ESEA]) obtained was used in the present.

**Phytochemical screening**

The extract was qualitatively tested for the presence of secondary metabolite in extract.

**Experimental design**

Rats were randomly divided into 7 groups of 5 each. Initially, all rats were acclimatized by giving normal diet for 1 week. Furthermore, selected healthy mice were characterized by stable body weight and did not show strange behavior. Animals in Group I (normal control) were given normal diet, and animals in Group II (positive control/HCD control) were given 10% cholesterol diet, 0.2% cholic acid, and 12.5 mg/kg bw propylthiouracil [9]. Animals in Groups III-VII were given 10% high cholesterol diet (HCD), 0.2% cholic acid, and 12.5 mg/kg bw propylthiouracil for 2 weeks orally to induce hyperlipidemia and then for last 14 days of cholesterol treatment, Group III (10 mg/kg bw), Group IV (20 mg/kg bw), Group V (40 mg/kg bw) which served as a treated groups were fed with ESEA suspension. Animals of Groups VI and VII were fed with standard drug simvastatin (25 mg/kg bw) and aspirin (20 mg/kg bw), respectively. Extract and standard drug were suspended in a vehicle (CMC Na 0.5%). During the treatment period, administration of cholic acid and propylthiouracil were stopped but pure cholesterol still given.

**Estimation of serum lipid profile**

Serum of TC, TG, and HDL cholesterol were estimated using commercially available kits (Rajawali Nusindo, Indonesia), and LDL cholesterol was calculated using the Friedewald's equations [10]:

LDL = Total cholesterol − (HDL) − (Triglyceride/5)

**Antiplatelet aggregation test**

*Bleeding time (BT) method*

The tail of the rat was given 70% ethanol. A small cut was made in the tip of the tail with a scalpel. BT started when the first drop touched the tissue paper. It was checked until bleeding stopped. BT was calculated when the first drop until the blood stop flowing [11].

*Blood coagulation method*

The tail of the animal was given 70% ethanol. A small cut was made in the tip of the tail with a scalpel. A sample of blood was collected into a capillary tube. Capillary tube made broken, and formation of fibrin thread was observed. Coagulation time (CT) was calculated when the first drop until fibrin threads formed [12].

*Aggregation platelet activity*

Briefly, blood from animals was collected with sodium citrate (3.2%) solution as anticoagulant (blood:citrate=9:1) and centrifuged to obtain platelet rich plasma. Platelet-rich plasma was added 1 ml sodium chloride 0.9 % and shaken with vortex apparatus. Absorbance of this mixture was measured at wavelength 600 nm and read the initial absorbance. Furthermore, aggregation was induced by fix concentration of ADP and conducted at 37°C for 20 minutes then its absorbance was measured again at wavelength 600 nm and read the final absorbance. The average absorbance of aggregation platelet of each test group compared with control group [13].

**Statistical analysis**

Experimental results were expressed as mean±standard deviation. The result was analyzed statistically using Statistical Package for the Social Sciences 20. Analysis of variance with *post-hoc* least significant difference was used to analyze the data, and value of $p<0.05$ was set for statistical significance.

**RESULTS**

**Phytochemical screening**

Phytochemical screening of the ESEA showed the presence of alkaloids, phenols, flavonoid, saponin, steroid/triterpenoid, and tannin.

**Effect of ESEA on lipid profile**

*Effect of ESEA on TC*

Fig. 1 exposed that at the end of induction, all groups (except the normal group) had cholesterol level significantly different to baseline ($p<0.05$). The result showed that induction method for 2 weeks was able to increase TC significantly.

*Effect of ESEA on TG*

After 2 weeks of therapy, the groups with doses of 20 and 40 mg/kg bw showed significant reduction in TG level compared with control group ($p<0.05$) and no significant difference with simvastatin group (Fig. 2).

*Effect of ESEA on HDL*

At the end of therapy, HDL level of all test groups did not show significant difference to the control group. Neither the extracts nor simvastatin showed no significantly increasing in HDL level (Fig. 3).

*Effect of ESEA on LDL*

The data of Fig. 4 indicated that avocado seed extract 10 mg/kg bw, and simvastatin group had significant difference with the control group ($p<0.05$).

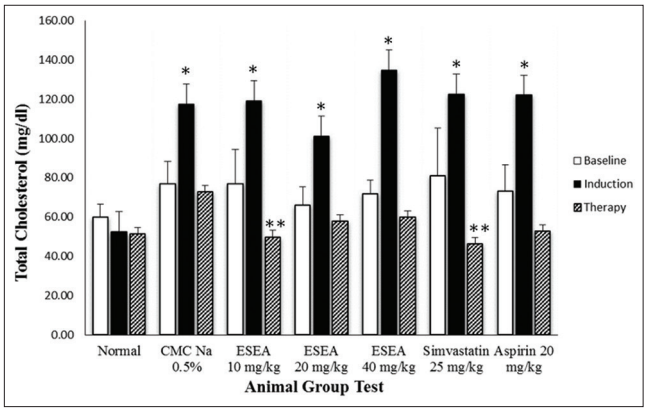


**Fig. 1: Effect of ethanol seed extract of avocado in total cholesterol level. *Significant compared to baseline p<0.05. **Significant compared to control group (CMC Na 0.5%) after treatment p<0.05**

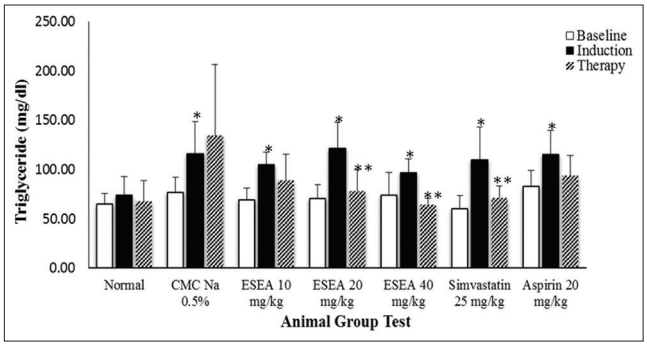


**Fig. 2: Effect of ethanol seed extract of avocado on triglyceride level. *Significant compared to baseline p<0.05. **Significant compared to control group (CMC Na 0.5%) after treatment p<0.05**

**Effect of ESEA on platelet aggregation**

*Bleeding time*

Extract 10 mg/kg bw and aspirin group showed increasing in BT, which was significant difference compared to control group (p <0.05) (Fig. 5).

*Coagulation time*

The extract with dose of 10 mg/kg bw which can prolong BT showed no significant difference compared to control group (Fig. 6).

**Antiplatelet aggregation activity**

The extract test group and aspirin group demonstrated significant decreasing in plasma absorption (p<0.05) compared to control group (Fig. 7).

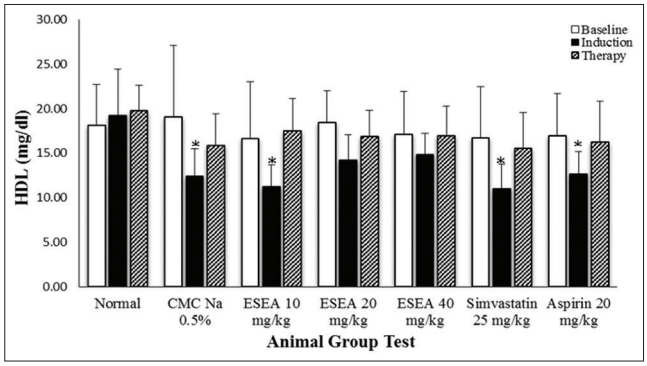


**Fig. 3: Effect of ethanol seed extract of avocado on high-density lipoprotein level. *Significant compared to baseline p<0.05**

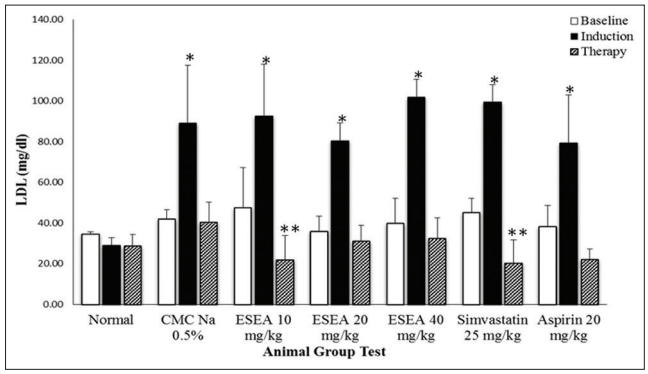


**Fig. 4: Effect of ethanol seed extract of avocado on low-density lipoprotein level. *Significant compared to baseline p<0.05. **significant compared to control group (CMC Na 0.5%) after treatment p<0.05**

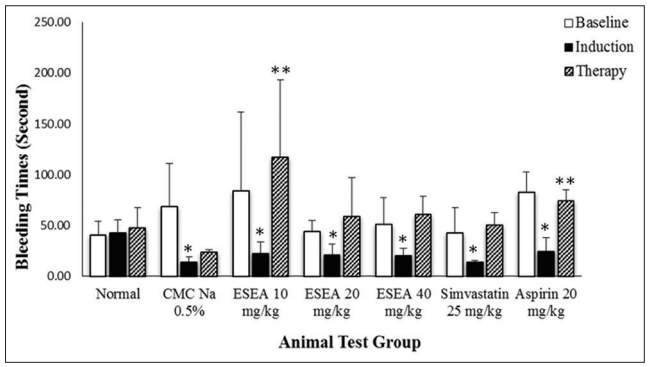


**Fig. 5: Effect of ethanol seed extract of avocado on bleeding time. *Significant compared to base line p<0.05. **Significant compared to control group (CMC Na 0.5%) after treatment p<0.05**

**DISCUSSION**

TC was measured before the induction, at the end of induction and the end of therapy. The level of TC at the end of the induction was significantly different compared to baseline (p<0.05). Animals were grouped into 7 groups: (1) Normal group, (2) positive control group (CMC Na 0.5%), (3) avocado seed extract dose of 10 mg/kg bw, (4) avocado seed extract dose of 20 mg/kg bw, (5) avocado seed extract dose of 40 mg/kg bw, (6) simvastatin group, and (7) aspirin group. The extracts were created in 3 variations to determine which one that could significantly affect the lipid profile and platelet aggregation. Measurement of cholesterol levels in the last week of induction intended as a parameter of success induction.

After induction was successful, the animal still given by high cholesterol feed for 2 weeks while administration of pure cholesterol, cholic acid, and propylthiouracil stopped. Induction termination of pure cholesterol, cholic acid, and propylthiouracil is based on the assumption that when people clinically detected hyperlipidemia then they will reduce the consumption of high cholesterol food gradually.

The result showed that induction method for 2 weeks was able to increase TC significantly (Fig. 1) where all groups (except the normal group) had cholesterol level significantly different to baseline (p<0.05) at the end of induction. Propylthiouracil was used to induce a hypothyroidism rat model that correlated with the hypothyroid rats had a fleshless body, significantly higher concentrations of TC and LDL cholesterol in the serum [14]. Cholic acid inhibits the conversion of cholesterol to bile acid [15,16] which may interfere with the action of pharmaceutical agents or nutrients that act in cholesterol metabolism by influencing the conversion of cholesterol to bile acids [16].

The group which was given by extract with dose of 10 mg/kg bw and simvastatin group showed decreasing in cholesterol level

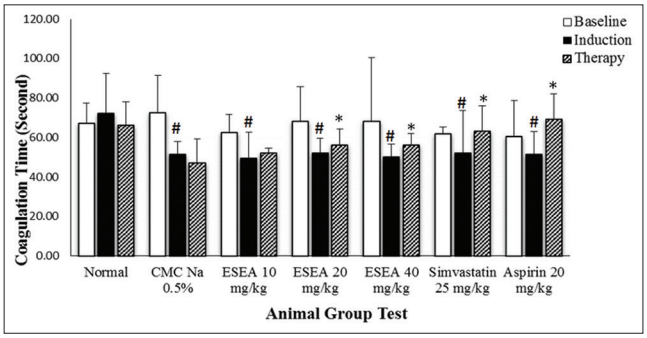


**Fig. 6: Effect of ethanol seed extract of avocado on coagulation time. #Significant compared to base line p<0.05. *Significant compared to control group (CMC Na 0.5%) after treatment p<0.05**

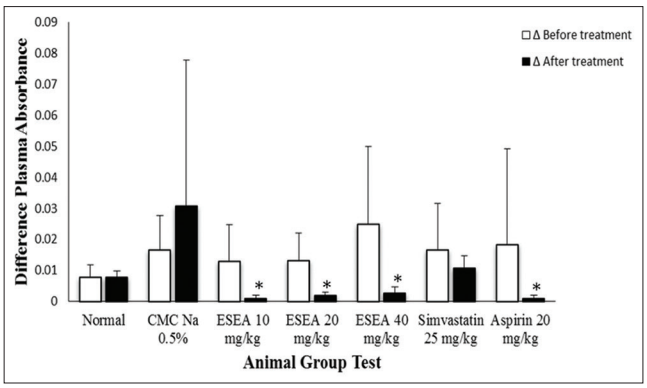


**Fig. 7: Difference of plasma absorbance before and after treatment. *Significant compared to control group (CMC Na 0.5%) after treatment p<0.05**

significantly ($p<0.05$) compared to control group, whereas there was no significant difference between extract 10 mg/kg bw compared to simvastatin. Avocado seed extract 10 mg/kg bw decreased cholesterol 58.12±15.78%, which was significantly different ($p<0.05$) compared to control group that decreased cholesterol 35.96±10.17%. Extract group dose of 10 mg/kg bw showed no significant difference ($p<0.05$) compared with simvastatin group that gave the largest cholesterol reduction 62.26±14.32%.

Phytochemical investigation of this plant showed the presence of alkaloid, phenols, flavonoid, saponin, steroid/triterpenoid, and tannin. It has been reported that flavonoid, saponin, and tannin play a role in hypolipidemic effect [17]. Saponin precipitate cholesterol, from micelles and interfere with the hepatic circulation of bile acid making it unavailable for intestinal absorption, this forces liver to produce more bile from cholesterol (serum) [1]. This may be lead to decrease serum cholesterol level.

Other components of lipoproteins are TG. High level of TG is one of the parameters of lipid metabolism abnormalities where is also an important risk factor for cardiovascular disease [18]. After 2 weeks of therapy, the groups with doses of 20 and 40 mg/kg bw showed significant reduction in TG level compared with control group ($p<0.05$) and no significant difference with simvastatin group. Synthetic drugs such as simvastatin, competitive inhibitor of HMG-CoA reductase enzyme is known has the ability to reduce blood TG level and slightly increase HDL level [1,19,20]. TG level of the control group was increased since this group was not given by therapy. The percentage of decreasing in TG level of the simvastatin group and extract 20 and 40 mg/kg bw showed decreasing in TG 25.25±14.05%; 27.10±13.62%; 32.45±11.81%, respectively, which were significantly different with the control group ($p<0.05$).

HDL was known as "good cholesterol" because it transports cholesterol that remaining in tissue to go back to the liver to be stored or for further synthesized into bile. At the end of therapy, HDL level of all test groups showed no significant difference to control group. Neither the extracts nor simvastatin showed no significant increasing in HDL level. The extract might be had no effect to increase HDL level while simvastatin still needs to be proved further related to its effect for HDL.

Several epidemiological studies in human demonstrated that increasing in LDL is atherogenic and showed a direct relationship between LDL level and incidence of coronary heart disease [21]. Level of LDL was obtained by calculation using the Friedewald formula:

LDL=Total cholesterol–HDL–(triglycerides/5).

The percentage of reducing in LDL of extract group with dose of 10 mg/kg bw and simvastatin group were 76.49±15.76% and 79.55±12.53%, respectively. This result indicated that avocado seed extract 10 mg/kg bw and simvastatin group had significant difference with the control group ($p<0.05$).

Hyperlipidemia-induced animals will have hyperaggregation, so it provides a shorter BT than before induction. It is known that high cholesterol level can increase the risk factor for cardiovascular disease such as cholesterol lead to increase platelet aggregation [22]. BT is one of the parameters of platelet aggregation test in this observation.

Extract 10 mg/kg bw and aspirin group showed increasing in BT, which was significant difference compared to control group ($p<0.05$) while between these two groups showed no significant difference. The extract 10 mg/kg bw increased BT 432±131% which was greater than the increasing in aspirin group 275±73%.

CT was observed based on the formation of fibrin threads. At the end of the induction demonstrated that the CT was significantly slower than CT before induction, and it can be concluded that the induction affects the CT of test animals.

The extract with dose of 10 mg/kg bw which can prolong BT, showed no significant difference compared to control group while the other test groups showed significantly different to control group ($p<0.05$). From these results, it could be seen that small dose did not have significant effect in CT.

The largest increasing in CT was shown by aspirin group 34.63±25.19%. The extract 10 mg/kg bw had no significant increasing in CT. This is probably due to the small dose of avocado seed extract had more influence in primary homeostasis than secondary homeostasis [12].

Antiplatelet aggregation activity was observed based on decreasing in plasma absorption using the Born method [13] were conducted on all of test groups. The antiplatelet aggregation activity of blood plasma was tested by ADP as aggregation inductor, and then plasma absorbance measured using spectrophotometer at wavelength 600 nm.

The extract test group and aspirin group demonstrated significant decreasing in plasma absorption ($p<0.05$) compared to control group, so it could indicate that the extract with various doses had antiplatelet aggregation activity which was induced by ADP while simvastatin group showed no significant difference compared to control group. The normal group did not show any differences between before and after treatment with the inhibition value 0.89%, meaning that there was no compound that inhibited the inductor to form aggregates. Avocado seed extract 10 mg/kg bw showed inhibition 99.51±14.91%, which was significantly different to control group ($p<0.05$), but did not have significant difference with aspirin group 95.06%. The extract 20 and 40 mg/kg bw had inhibition 62.76±44.13% and 87.81±5.05%, respectively, while simvastatin 45.39±25.85%.

The three of parameters testing of antiplatelet aggregation those are BT, CT, and decreasing in plasma absorption showed no extract group which have linear activity in three parameters. Extract 10 mg/kg bw showed significant activity on BT and decreasing in plasma absorption compared to control and had similar value with aspirin. While the extract with doses of 20 and 40 mg/kg bw showed significant activity in CT and decreasing in plasma absorbance.

## CONCLUSION

Avocado seed extract 10 mg/kg bw showed decreasing in TC level, LDL and had the ability to extend BT in antiplatelet aggregation effect test. The extract 20 and 40 mg/kg bw exposed decreasing in TG level and had the ability to extend the CT in antiplatelet aggregation effect test. All of tested doses had no activity to increase HDL level but had activity to inhibit aggregation of platelet by measuring plasma absorbance. The avocado seed extract had ability in lipid lowering and antiplatelet aggregation, which serves as a new potential herbal product and good for cardiovascular disease treatment because it can reduce both risk factors causing the disease.

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