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Effect of Pomelo (Citrus grandis) Ethanolic Extract on Atherosclerotic Plaque Formation

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Abstract—Cardiovascular disease is a major health problem in developed and developing countries and still be the first rank causing death in the world, including Indonesia. Most of cardiovascular disease caused by atherosclerosis. Macrophage apoptosis can reduce the size of atherogenic lesions and the progression of atherosclerotic plaque formation. Proapopstosis effects on macrophages owned by flavonoids. This study aims to know the potential of flavonoids found in Citrus grandis extract which works by suppressing the phosphorylation of AKT Ser473 so that the formation of foam cells (foam call) can be minimized. The research sample is 38 male Wistar rats weighing 90-150 grams. Samples were divided into 5 groups. All groups except normal control group were induced atherosclerosis by utilizing the shear stress mechanism at the branch of the abdominal aorta. Shear stress created by injecting adrenaline i.v. followed by hyperlipidemic diet (egg yolk) for 3 weeks. Then, during the next two weeks, the positive control group treated with simvastatin, while the treatment group were treated with extracts of C. grandis (ECG) known to contains flavonoids. Macroscopic showed that ECG could reduce atherosclerotic lesions. of citrus grandis. The sample data captured in the form of mouse blood and abdominal aortic tissues. Based on the results of the TLC screening, Ethanolic Extrxct Meanwhile, the group given simvastatin as a positive control also showed fewer atherosclerotic lesions. ECG proven to lower cholesterol and triglyceride levels of rats that had been induced atherosclerosis. Award ECG doses of 500 and 1000 mg/kg showed a significant decrease in cholesterol ($p < 0.01$) compared to the negative control group. ECG is proven to reduce atherosclerotic lesions, so that ECG can be used as an adjuvant for simvastatin to achieve maximal therapeutic effect against atherosclerosis.

Keywords—atherosclerosis, Citrus grandis, simvastatin, hyperlipidemic.

INTRODUCTION

Cardiovascular disease is a major health problem in developed and developing countries and still be the first rank causing death in the world. There are 7.4 million people globally die from ischemic heart disease in 2012[1]. Indonesia as a developing contry also shown a same condition, the incidence of cardiovascular disease keep increasing.

Atherosclerosis is an inflammatory disorder which underlied cardiovascular disease. Fatty streaks in atherosclerosis is caused by inflammatory lesion mediated by macrophage and T lymphocytes. The macrophage can be found in every phase of atherosclerosis and has important role.

Progressive atherosclerosis may lead to sustained fatal heart diseases such as myocardial infarction and ischemic heart disease[2]. To avoid the progression of atherosclerosis, it is necessary to do the prevention.indeed, the macrophage apoptosis can reduce the size of atherogenic lesions and can reduce the progression of atherosclerotic plaque formation [3]. The utilization of natural compounds from plants to reduce the progression of atherosclerosis is a prospective opportunities.

Citrus grandis (pomelo) or in Indonesia known as Jeruk Bali contains various flavonoids. Flavonoids has anti-inflammatory effects, antiplatelet, antitumor and proapoptosis effect. Proapoptosis effect may reducing atherosclerotic lesions by suppressing the phosphorylation of AKT Ser473 so, the formation of foam cells can be minimized[4]. Accordingly, Citrus grandis is potential in preventing the progression of atherosclerotic lesion formation.

METHODS

a. Plants

Pomelo (Citrus maxima or Citrus grandis) obtained from Pasuruan, Indonesia, in March 2016. Citrus grandis is Magnoliopsida family. This plant has determined by experts from the Botanical Laboratory of Sciences and Mathematics Faculty, Universitas Jember, Indonesia. Ethanol extract of Citrus grandis are obtained through the extraction process. Citrus grandis specimens deposited at the herbarium of the Faculty of Sciences and Mathematics Universitas Jember.

b. Animals

Male Wistar rats (12 weeks old) were purchased from Malang, Indonesia. The weights of rats at the beginning of the study ranged from 90-150 g. Rats were randomly divided into five groups, each group containing 7-8 rats.

c. Research Design

Before being treated, all rats were adapted for 2 weeks (adaptation phase). The rats were housed in an environmentally controlled room, Physiological Laboratory of Medical Faculty Universitas Jember. The next stage is the induction of atherosclerosis for 3 weeks, with a variety of treatments as follows: 1. Normal Control. Rats were injected with NaCl 0.9% just on the first day, then were fed a standard diet. 2. Negative Control. Rats were injected with adrenalin 0.06mg / KgBW i.v. on the first day of induction period, then were fed a standard diet. 3. Positive Control, Treatment 1, and Treatment 2. Rats were injected with adrenalin 0.06mg / KgBW i.v. on the first day of induction period, then were fed a standard diet and high-fat diet (egg yolk). Prior to extract citrus grandis, rat's blood-lipid-profile (cholesterol and triglycerides) was measured as a mid-test to determined whether the hyperlipidemic condition successfully induced. The last phase is the extract of Citrus grandis for the treatment group 1 (dose of 500mg / KgBW) and treatment group 2 (dose of 1000mg / KgBW). While the negative control group treated with simvastatin 1.53mg / KgBW P.O.

d. Extraction method

Pomelo (Citrus grandis) were immediately peeled after purchased. The tissue removed was the pericarp region (peel), which includes the epicarp and mesocarp. The peels were dried then crushed using a blender. A total of 602.65 grams of citrus grandis' peels powder was extracted by maceration process using ethanol 70% (ratio of powder and ethanol 70% was 1:3). The solution was filtered using filter paper to obtain the filtrate. Then, this filtrate was evaporated using Vacuum Rotatory Evaporator to obtain 103 grams of concentrated extract. The concentrated extract was given to rats.

e. Measurements of Plasma Lipid Concentration

The concentrations of triglyceride (TG) and cholesterol in the rats' plasma were enzymatically measured with a Glycerol-3-Phosphate-Oxidase (GPO)

kit for TG and CHOD-PAP kit for cholesterol using a spectrophotometer at 546 nm wavelength.

f. Histopathological Studies

Portions of abdominal aortic tissue were fixed in 10% formalin. The washed tissue was dehydrated in descending grades of isopropanol and finally cleared in xylene. The tissue was then embedded in molten paraffin wax. Sections were cut to 5 µm thickness, and stained with hematoxylin and eosin. The sections were then viewed under a light microscope (DM RXE, Leica, Germany and Olympus CX21LED, US) for histopathological changes.

g. Statistical Analysis

Analysis is conducted normality test and variant test. If the data distribution is normal (P> 0.05) then use a hypothesis test one way ANOVA followed by Post Hoc Tuckey test. However, if the data distribution is not normal (p <0.05) then use the Kruskal Wallis test followed by Mann Whitney test to determine whether there were significant differences in each group. It is significant if the p value is <0.05.

RESULTS

a. Phytochemical screening results Extract Citrus grandis

Results of Thin Layer Chromatography (TLC) screening shows that ECG contains flavonoids. Examination using stationary phase: silica gel 60 F254, the mobile phase of butanol: acetic acid: water (8: 2: 10), and ammonia vapor. Seen on a UV light with a wavelength of 546 nm.

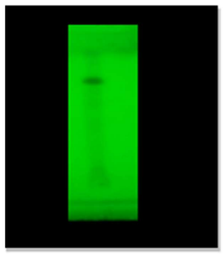


Fig 1. Thin Layer Chromatography of ECG

b. Extract Citrus grandis (ECG) Effects in abdominal aorta macroscopic

Group given ECG showed atherosclerotic lesions less than the negative group (Fig 2). Meanwhile, the group given simvastatin as a positive control also showed fewer atherosclerotic lesions.

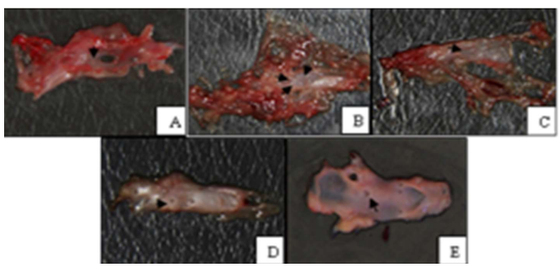
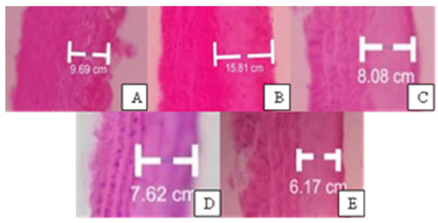


Fig 2. Macroscopic view of abdominal aorta

c. Extract Citrus grandis (ECG) effects in abdominal aorta histopathology

ECG shown to decrease abdominal aortic intima thickness of rats that had been previously induced atherosclerosis compared to the negative control group. Intima thickness also decreased in the positive control group treated with simvastatin (Fig 3).



	A	B	C	D	E
Adrenalin + egg yolk	-	+	+	+	+
ECG 500	-	-	-	+	-
ECG 1000	-	-	-	-	+
Simvastatin	-	-	+	-	-

Fig 3. Histopathology of all group

d. Extract Citrus grandis (ECG) effects in Cholesterol Levels

ECG proven to lower cholesterol of rats that had been induced atherosclerosis. Award ECG doses of 500 and 1000 mg/kg showed a significant decrease in cholesterol (p <0.01) compared to the negative control group. Meanwhile, the two groups showed no significant difference when compared to the positive control group.

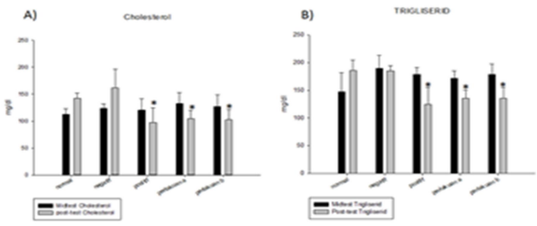


Fig 4. Cholesterol and triglyceride levels.

Inducing effects of atherosclerosis using epinephrine and egg yolk diet on cholesterol levels total male Wistar rats at day 22, the effect of ECG on cholesterol levels (A) and triglycerides (B) on day 37.

e. E. Extract Citrus grandis (ECG) effects in triglyceride Levels

ECG was also proven to reduce triglyceride levels of rats that had been induced atherosclerosis before. Award ECG doses of 500 and 1000 mg / kg showed a significant decrease in cholesterol (p <0.01) compared to the negative control group. Meanwhile, the two groups showed no significant difference when compared to the positive control group.

DISCUSSION

Two main treatment of atherosclerosis is a change of lifestyle and pharmacologic therapy. Pharmacological treatment is primarily aimed to reduce levels of LDL-cholesterol and triglycerides. Statins are a group of drugs that can be used for the treatment of atherosclerosis. This drug works by blocking the action of HMG Co-A reductase, which catalyzes the conversion of HMG Co-A into mevalonic acid in the early stages of the synthesis of cholesterol. Simvastatin has been widely used as an anti-atherosclerosis drug, but as a chemicals drug, statins are not free from side effects such as gastrointestinal disorders and myopathy. Even the FDA (Food and Drug Administration) has revised the label for simvastatin-related myopathy. Therefore, the potential for atherosclerosis therapy being sought and developed, including herbal and natural theraphy. Herbal treatment has a great chance considering Indonesia is known as a mega center of biological diversity (biodiversity) 2nd largest in the world.

This study shows that the ECG proven to reduce the thickness of the intima of the abdominal aorta on

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histopathologic examination. This study used the abdominal aorta branching part to make preparations of renal histopathology. This area was selected because even though the entire aorta is exposed to the effects of atherosclerosis, but the atherosclerotic lesions are formed only in some special parts that undergo endothelial shear stress. The blood flow will cause shear stress in the vessel wall which affect cell physiology. Shear stress is influenced by the direction of flow and blood viscosity. The area of aortic arch or aortic branching susceptible to high shear stress. Meanwhile atherosclerotic lesions appear mainly in areas with high shear stress. In the abdominal aorta, branching at the infrarenal area will experience higher shear stress due to the direction of blood flow that comes from above (Figure 3). High shear stress will cause injury to the endothelium and accumulation of foam cell. Indicators of atherosclerosis can be determined by measuring the thickness of the aortic intima. The result showed that the induction of adrenaline and egg yolks provide an overview intima is thicker than normal controls. While ECG is able to decrease the thickness of the intima is similar to simvastatin. This shows potency of ECG as an agent antiaterosclerotic.

This study shows that ECG proven to reduce blood lipid profile such as cholesterol and triglycerides. In this study, cholesterol and triglyceride levels have been selected as a representative of rat blood lipid levels as both are major lipid components which contained in fatty foods. Normal cholesterol levels in Wistar rats was 110.85 mg/dl (Lilian, 2010). On the negative group there was an increase in total cholesterol levels average to 161.60 mg/dl (Figure 4.2). ECG with variation dose in the group A treatment (500mg/kg) and B (1000mg/KgBW) can lower total cholesterol levels on average respectively 104.80 mg/dl and 102.63 mg/dl. Meanwhile, normal triglyceride levels of male Wistar rats was 69.63 mg / dL. The Increasing of triglyceride levels in the negative control group is 184.77 mg / dl. Award simvastatin lowers triglyceride levels were significantly ($p < 0.01$), although still above the normal value is 124.95 mg / dl. ECG also proven to decrease triglyceride levels ($p < 0.01$) menjadi135,83 mg/dl and 135.35 mg/dl in the group A treatment and group B treatment respectively. This indicates that ECG has a similar potency to simvastatin in lowering blood lipid profile. Variations dosage in the treatment group 1 and 2 but not significant effect on the reduction in cholesterol and triglycerides. This is possible because the dosage range used too narrowly. Further research is needed to determine the effective dose and lethal dose ECG.

ECG effects on the improvement of atherosclerotic lesions and the levels of lipid profile is due to the flavonoids content. Flavonoids have antioxidant effects that can reduce blood cholesterol by inhibiting the oxidation of LDL. The mechanism of how flavonoids can inhibit the oxidation of LDL is not fully known, but the possibility of flavonoids may protect the alpha-tochopherol in LDL to oxidation by inhibiting the formation and release of free radicals. Another effect of flavonoids is as agents that inhibit anti-atherogenic lipoprotein synthesis in the gut, as well as anti-inflammatory that can prevent the development of atherosclerotic lesions and the occurrence of coronary heart disease due to aterosklerosis. Moreover, thick skin on grapefruit contains pectin most compared with other citrus fruits. Every 15 grams of pectin can lower cholesterol levels 10 percent. Cholesterol reduction on providing ECG, accompanied by high-cholesterol diet supports previous studies conducted by Maria Ema Lestari Lamanepa in a similar field. From these data we can conclude that ECG was able demonstrate improvement histopathology induced rat aortic atherosclerosis. Moreover ECG also reduce levels of triglycerides and cholesterol in the blood

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REFERENCES

[1] World Health Organization. The 10 leading causes of death in the world, 2000 and 2012. 2014. http://www.who. int/ mediacentre /factsheets/ fs310/en/ [23 Agustus 2015].

[2] Isselbacher, Braunwald, Wilson, Martin, Fauci, dan Kasper. Harrison Prinsip-Prinsip Ilmu Penyakit Dalam Edisi 13 Vol 3 2014. Jakarta: EGC.

[3] Babaev, V.R, Chew J.D, Ding L. Macrophage EP4 deficiency increases apoptosis and suppresses early aterosclerosis. Cell Metabolism 2008, 8(6): 492–501

[4] Ministry of Health Indonesia, 2015. Situasi Penyakit Kanker. Jakarta: Pusat data dan informasi Kementrian Kesehatan RI.

[5] Zeng, P., Liu, B., Wang, Q., Fan, Q., Diao, J., Tang, J., Fu, X., dan Sun, X. Apigenin Attenuates Aterogenesis through Inducing Macrophage Apoptosis via Inhibition of AKT Ser473 Phosphorylation and Downregulation of Plasminogen Activator Inhibitor-2. Hindawi 2015: 1-12.