CHAPTER I
INTRODUCTION

1.1. Background

Broadly speaking, fat is required as the element of the diet because of its beneficial effect, but the excessive consumption of fat may cause serious problems for the health. Saturated fat intake is associated with heart disease. Increasing saturated fat intake increases total blood cholesterol and LDL cholesterol. Trans fat intake is associated with an increased risk of heart attack, heart disease and atherosclerosis.\textsuperscript{1,2}

Atherosclerosis (also known as arteriosclerotic vascular disease or ASVD) is a condition in which an artery wall thickens as a result of the accumulation of fatty materials such as cholesterol and triglyceride. It is a syndrome affecting arterial blood vessels, a chronic inflammatory response in the walls of arteries, caused largely by the accumulation of macrophage white blood cells and promoted by low-density lipoproteins (LDL, plasma proteins that carry cholesterol and triglycerides) without adequate removal of fats and cholesterol from the macrophages by functional high-density lipoproteins (HDL). It is commonly referred to as a hardening or furring of the arteries. It is caused by the formation of multiple plaques within the arteries. Thus atherosclerosis is a disease in which plaque builds up inside the arteries. Arteries are blood vessels that carry oxygen-rich blood to the heart and other parts of the body.\textsuperscript{2}
Olive oil is a fat obtained from the olive. Olive oil is the main source of unsaturated fatty acids in the Mediterranean region, a nutritional regimen gaining ever-increasing renown for its beneficial effects on inflammation. The Mediterranean diet is considered to be a protective factor in the primary and secondary prevention of coronary heart disease (CHD), and against oxidative stress associated processes. Olive oil and its phenolic compounds improved myocardial oxidative stress in standard-fed conditions. The most important components in olive oil are the fatty acids. Fatty acids are simple structures made up of long chains of various numbers of carbon atoms. There are only a few types of fatty acids in olive oil, but the proportions of each strongly influence the characteristics and nutritive value of the oil.\textsuperscript{3,4}

Endothelium is a specialized layer of cells that forms the inner lining of all blood vessels. Endothelial cells produce nitric oxide and other substances that regulate blood pressure, maintain balance between pro-thrombotic (blood clotting) and anti-thrombotic mechanisms, and act as a selective barrier between the blood and surrounding tissues. The functions of the endothelium are crucial; endothelial dysfunction is an early event in atherosclerotic plaque development and cardiovascular disease. Nitric oxide is a principal factor involved in the anti-atherosclerotic properties of the endothelium.\textsuperscript{5}

Atherosclerosis is associated with reduced endothelial NO production, the observation of deficient NO-mediated vasorelaxation in hypercholesterolemia suggests that loss of NO bioactivity is an early feature of atherosclerosis. Several studies had been conducted addressing the beneficial effect of olive oil to enhance
the expression of endothelial nitric oxide. An important feature of a healthy endothelium is an adequate output of nitric oxide (NO), which is produced by the enzyme endothelial nitric oxide synthase (eNOS). The MUFA of olive oil seems to be an important determinant of beneficial vascular effects of olive oil. In addition, the triterpene olanolic acid and possibly polyphenols may positively modulate eNOS, and another pathway seems involved.\textsuperscript{5,6}

Another inflammatory modulator which involve is cell adhesion molecules (CAMs). ICAM-1 plays a role in the development of arterial plaque. In atherosclerosis patients, increased endothelial cell ICAM-1 expression was observed within the neovasculature of coronary plaques, but not in endothelial cells directly overlying the plaque. Plaque is made up of fat, cholesterol, calcium, and other substances found in the blood. Over time, plaque hardens and narrows the arteries, reducing blood flow to the organs (such as the heart) and other parts of the body. This can lead to serious problems, including heart attack, stroke, or even death. The prominent role of ICAM-1 during inflammation and its ability to govern endothelial cell migration, act as a common denominator for many endothelial cell functions observed during angiogenic and inflammatory processes. The study demonstrated that its activity is undergoing through a nitric oxide-dependent pathway. Intercellular adhesion molecule-1 (ICAM-1) regulates endothelial cell motility through a nitric oxide-dependent pathway.\textsuperscript{7}

There is a gradual decreased of CVD mortality as olive oil intake increased. The current study is needed to find out the effect of administration of extra virgin olive oil (EVOO) which is high quality of olive oil in different
dosages to experimental animal. The doses used are 1ml, 2ml, 3ml/kg/day and after taking these doses for 60 days will cause dietary restriction on lipid profile and myocardial antioxidant defences (Faine L. A et al, 2004). So far, there is still no experimental study which is conducted addressing the effect of different dosage of olive oil to the serum level of eNOS and the expression of ICAM1 in endothelial cells.

1.2. RESEARCH QUESTION

Based on explanation in the background of the study, the main research question could be as following:

Does administration of EVOO have effect in reducing ICAM-1 and increasing eNOS in high fat diet wistar rats?

The general research question mentioned above is divided into specific research questions as follow:

a. Is administration of EVOO can increase the level of eNOS serum of wistar rats which are given high fat diet?

b. Is administration of EVOO can reduce the level of ICAM-1 serum of wistar rats which are given high fat diet?

1.3. RESEARCH OBJECTIVES

1.3.1 General objectives

To prove the administration of EVOO has effect on reducing ICAM-1 and increasing eNOS in high fat diet wistar rats.
1.3.2. Specific objectives

a. To analyse eNOS serum level after administration of 1ml, 2ml and 3ml/kg/day for 60 days EVOO of wistar rats given high fat diet.

b. To analyse ICAM-1 serum level after administration of 1ml, 2ml, and 3ml/Kg/day for 60 days EVOO of wistar rats given high fat diet.

1.4. Research Benefits

1.4.1. Benefit for science

The results of this study may give information about the possibility of benefit of EVOO on the endothelial cells activation by reducing production ICAM-1 level and improve eNOS.

1.4.2. Benefit for health care services

The results of this study give more information whether EVOO can be used as a supplement for inhibiting the atherosclerosis in HFD individuals.

1.4.3. Benefit for research

The results of this study give more ideas to the next studies about the benefit of EVOO for atherosclerosis.

1.5. Research originality

Based on literature tracing on National Library of Health of United State of America and data base Research and Development Unit of Indonesia Health Department, study of benefit of EVOO for endothelial protection on wistar rats
Given high fat diet has not conducted yet. There were several studies related to protection of endothelial function as follow:

**Table 1.** Previous report related to study on protective effect olive oil on high fat diet.

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<th>Method</th>
<th>Results</th>
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<td>1</td>
<td>Acute Effects of High-Fat Meals Enriched With Walnuts or Olive Oil on Postprandial Endothelial Function.</td>
<td>By randomizing in a crossover design 12 healthy subjects and 12 patients with hypercholesterolemia to 2 high-fat meal sequences to which 25 g olive oil or 40 g walnuts had been added. Both test meals contained 80 g fat and 35% saturated fatty acids, and consumption of each meal was separated by 1 week.</td>
<td>Adding walnuts to a high-fat meal acutely improves flow-mediated dilation (FMD) independently of changes in oxidation, inflammation, or asymmetric dimethylarginine (ADMA). Both walnuts and olive oil preserve the protective phenotype of endothelial.</td>
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<td>2</td>
<td>Effects of olive oil and its minor phenolic constituents on obesity-induced cardiac metabolic changes.</td>
<td>Male Wistar rats were divided into two groups (n = 24/group): receiving standard-chow and receiving hypercaloric-chow. After 60 days the groups were divided into four subgroups (n = 6/group) which received standard-chow and saline; standard-chow and olive-oil; standard-chow pleuropein; standard-chow and cafeic-acid; hypercaloric-chow and saline; hypercaloric-chow and olive-oil; hypercaloric-chow and cafeic acid</td>
<td>The study demonstrated that olive-oil, oleuropein and cafeic-acid enhanced fat-oxidation and optimized cardiac energy metabolism in obesity conditions. Olive oil and its phenolic compounds improved myocardial oxidative stress (myocardial beta-hydroxyacyl coenzyme-A dehydrogenase) in standard-fed conditions.</td>
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<td>3</td>
<td>Dietary enrichment of apolipoprotein E-deficient mice with extra virgin olive oil in combination with seal oil inhibits atherogenesis. Eilertsen K-E, Marhre H K, Cludts K, Olsen J O, Hoylaerts M F.</td>
<td>In total, 62 (30 females and 32 males ApoE mice (B6.129P2 Apoe) were for 12 weeks fed a lipid rich diet containing 19.5% fat and 1.25% cholesterol without any supplement, with 1% (wt/wt) mixture of EVOO and seal oil (EVO/n-3), or 1% corn oil, respectively.</td>
<td>Dietary supplementation of a marine/olive oil combination inhibits atherosclerotic lesion formation in the female apoE/- mice by antithrombotic, antihypertriglyceridemic, and antioxidant effects.</td>
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<td>4</td>
<td>Efficacy of Olive Oil Diet on the Histopathological Changes induced in Hypercholesterolemic Rat. Al-Rawi M, Ali A.</td>
<td>Forty male Sprague-Dawley rats (150-200g) maintained under a 12 h photoperiod (08.00-20.00) at an ambient temperature of 22ºC, were fed the appropriate rat chow until 12 h prior to the experiment, when food was withdrawn. Four different groups of 10 animals each, were studied: normolipemic diet, atherogenic diet, normolipemic diet with 10% olive oil, atherogenic diet with 10% olive oil. Treatment were given for 16 weeks.</td>
<td>Co-administration of olive oil+mixture of (cholesterol 4% + Cholic acid 1% + thiouracil 0.5%) lessen the most histopathological changes in aorta and liver as compared to animals treated with the mixture of (cholesterol 4% + Cholic acid 1% + thiouracil 0.5%) alone. This indicated that olive oil showed improvement in the structure of the aorta and liver of rat.</td>
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<td>5</td>
<td>Phenolic Content of Virgin Olive Oil Improves Ischemic Reactive Hyperemia in Hypercholesterolemic Patients</td>
<td>Twenty-one hypercholesterolemic volunteers received two breakfasts, using a randomized sequential crossover design. Both arms received the same</td>
<td>A meal containing high-phenolic virgin olive oil improves ischemic reactive hyperemia during the postprandial state. This phenomenon might be mediated via</td>
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<td>Ruano J, Lopez-Miranda J, Fuentes F, et al.\textsuperscript{13}</td>
<td>olive oil, but one had its phenolic acid content reduced from 400 to 80 ppm. Ischemic reactive hyperemia (IRH) was measured with a laser-Doppler procedure at baseline and 2 h and 4 h after oil intake. Postprandial plasma concentrations of lipid fractions, lipoperoxides (LPO), 8-epi prostaglandin-F2, and nitrates/nitrites (NO) were obtained at baseline and after 2 h of the fat meal.</td>
<td>reduction in oxidative stress and the increase of nitric oxide metabolites.</td>
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Current study is different with several previous study that mentioned above. The differences are:

- Using of EVOO for protection of endothelial, while previous study used walnut and another treatment.
- The outcome measured of current study are eNOS and ICAM-1 serum level, while previous studies measured the level of ADMA, myocardial oxidative stress marker (myocardial beta-hydroxyacyl coenzyme-A dehydrogenase), and atherosclerotic lesion using pathologic anatomy examination.