CHAPTER V
DISCUSSION

The aim of study was observing the effect of EVOO on serum ICAM-1 and eNOS levels of HFD rats. The HFD male wistar rats used in the study was developed for atherosclerotic animal model. EVOO is the highest quality with low acidity and the least processed. Additionally, many beneficial effect of EVOO had been reported. This study was done to further explore the mechanism used by EVOO in protecting vascular endothelial cells in HFD individuals. EVOO effect on sICAM-1 and eNOS levels were observed in rats receiving a combination of EVOO and HFD for 2 months period. Endothelial NOS that is commonly expressed in endothelial cells, has many favorable effects including maintaining vascular dilatation, influencing blood pressure, vasoprotective and anti-atherosclerotic. Lower plasma concentrations of sICAM-1 was used as indicator for a treatment preventing an atherosclerosis development and progression.

This study showed that the EVOO can reduce levels of ICAM-1 of wistar rats that have been fed by HFD. Therefore, the optimum dose administration of EVOO which may reduce levels of ICAM-1, is 1ml. Although, it was not statistically significant. CAM-1 concentrations in sera were not changed significantly over the entire course of the HFD period. The serum ICAM-1 levels of negative controls receiving normal diet were not different than those of positive controls receiving HFD. The HFD may not be sufficient or may not have been fed for a sufficient duration to induce changes in ICAM-1 concentrations. The previous study which used baboons as the model of animal showed that high
cholesterol of high fat on VCAM – ICAM-1 did not significant change during period of 7 weeks.\textsuperscript{47}

Our study showed that the eNOS levels of positive control consuming HFD only were significantly lower than those of receiving normal diet. The eNOS concentrations were significantly reduced by consuming high cholesterol high fat diet for period of 7 weeks.\textsuperscript{47} This findings partly supported by a study done in rats obtaining moderately high-fat diet which showed reduced eNOS expression in the liver, heart and kidney medulla.\textsuperscript{48} HFD rats used in this study were confirmed as atherosclerosis animal model indicated by the present of foam cells on the aortic wall.\textsuperscript{44} The reduced circulating eNOS levels observed in HFD rats possibly involves in foam cell formation because the present of eNOS is able to prevent the foam cell formation.\textsuperscript{49} Foam cell formation, occur after macrophages taking up OxLDL.\textsuperscript{50,51} Interestingly, Ox-LDL has ability in inhibiting eNOS expression/activity.\textsuperscript{52} Several other factors might explain the reduced eNOS production in HFD rats. TNF-\(\alpha\) might be one of factor contributed in the decreasing eNOS levels. Study using similar HFD rats showed a significant increase number of cells expressing TNF-\(\alpha\).\textsuperscript{53} TNF-\(\alpha\) reduced eNOS mRNA levels of cultured human coronary artery endothelial cells.\textsuperscript{54} Shear stress might involve in eNOS production by blood vessels. Atherosclerosis was aggravated by decline shear stress which was associated with reduced eNOS production of vascular wall.\textsuperscript{55} HFD mice model for atherosclerosis showed reduced myocardial-VEGF expression and eNOS production.\textsuperscript{56} Interestingly, the eNOS production had a positive correlation with VEGF but negatively with LOX-1 expression.\textsuperscript{57}
Additionally, LOX-1 also reduce eNOS activity and reduce eNOS bioavailability.\textsuperscript{58} LOX-1 expression was upregulated by oxLDL.\textsuperscript{59,60} Ox-LDL promoted the expression of LOX-1, but reduced eNOS expression at the same time.\textsuperscript{61} This might explain the association between eNOS and LOX-1 expression mention above. It will strengthen our study when we are able to know what factors involve in decreasing circulating eNOS levels in these HFD rats.

The eNOS levels of HFD rats receiving EVOO in dose of 3ml/kg BW/day were significantly higher than positive controls consuming HFD only. EVOO was given in the same time period of HFD. This therefore, suggested that EVOO consumed concomitantly with HFD, was able increase eNOS levels of HFD rats. Anti-inflammatory compound of EVOO might contribute in the elevation of eNOS levels. Effect of olive oil is related to high polyphenols contents, strong antioxidant. The polyphenols are functional against free radical induced-tissue damage (scavenger activity), reduce oxidative stress, inhibit endothelia adhesion molecules, increase eNOS expression as well as greater increase concentration NO, improve endothelia function by controlling vasodilatations, improve lipid profile by increasing HDL and prevent the oxidation of LDL.\textsuperscript{62,63} The intake of the polyphenol-rich breakfast was associated with an improvement in endothelial function, as well as a greater increase in concentrations of NO. That the consumption of a meal based on virgin olive oil with a high phenolic compound content improves endothelium-dependent micro vascular vasodilatation during the first 4 h of the postprandial period in patients with hypercholesterolemia. This
improvement is associated with a decrease in oxidative stress and an increase of the final products of nitric oxide.\textsuperscript{13}

5.1 Limitation of the study

This study has not measured yet the levels of ox-LDL and TNF-\(\alpha\) which will give influences to the level of ICAM-1.