CHAPTER I

INTRODUCTION

1.1. Background

Diet is one of the main environmental factors that contribute to high fat diet (HFD). Human studies have shown that increased fat intake is associated with body weight gain which can lead to high serum of fat diet and other related metabolic diseases.[1] Fasting or feeding a high fat diet abolished lipogenesis (fat formation) in adipose tissue and reduced glucose oxidation markedly. Lipogenesis increased to the highest levels on a high-carbohydrate, fat-free diet. HFD is the second killer in America it causes 300,000 deaths a year. Several more highly publicized social evils are comparatively small problems. A highdiet in saturated fat can dramatically raise the level of cholesterol, increasing the risk of heart disease. The AHA recommends limiting total fat intake to between 25 to 35 percent of total calories with only 7 percent coming by way of saturated fats. [2,3]

In obesity case, there is a chronic systemic inflammation with increased of proinflammatory cytokines in the circulation. TNF- α in adipose tissue is the first cytokine observed in obese mice. Inflammation is considered to play an important role in the pathogenesis of insulin resistance in obese individuals. Foam Cells are fat-laden immune cells of the type macrophage.They are an indication of plaque-build up, or atherosclerosis, which is commonlyassociated with increased risk of heart attack and stroke. Foam cells are formed

when the body sends macrophages to the location of a fatty deposit on the blood vessel walls. The macrophage surrounds the fatty material in an attempt todestroy it. The cell becomes filled with lipids (fats). The lipids surrounded by the macrophage give it a "foamy" appearance. Obese individuals with insulin resistance showed elevated levels of TNF-α that may have a causal relationship.[4,5]

Cytokines play a dual role in atherosclerosis. Proinflammatory and Th1-related cytokines promote the development and progression of the disease, whereas anti-inflammatory and regulatory T cell–related cytokines exert clear antiatherogenic activities. Cytokines are low-molecular-weight protein mediators that usually act at short range between neighboring cells in lymphoid organs or inflamed tissues, cytokines when they are administered to humans , they produce inflammation, fever and in some cases death. They are involved in many physiological processes and are especially important for regulating inflammatory and immune (innate and adaptive) responses. All cells involved in atherosclerosis are capable of producing and responding to cytokines. At a more advanced stage of the disease, proinflammatory cytokines destabilize atherosclerotic plaques by promoting cell apoptosis and matrix degradation. Macrophage apoptosis results in the formation of cell debris, which contributes to the enlargement of the lipid core.[6,7]

High-cholesterol diet plays a important role for the occurrence of chronic inflammation, including atherosclerosis. This diet will induce inflammatory reaction, and the release of chronic inflammatory cytokines including, TNF-α

and IL-1.[8] TNF α are the most important proinflammatory cytokines to be studied, due to their effect to stimulate inflammation. Thereby, their effect should be suppressed by giving anti-inflammatory agents.

The commonly held belief that high fat and cholesterol consumptioncauses atherosclerosis has been questioned, yet there is still known to be a strong link between lipids and atherosclerosis based on experimental and clinical relationships between hypercholesterolaemia and atheroma. Because fat and cholesterol are the substances of which plaque consists, they are both considered to be contributors to the cause of atherosclerosis. This however remains unverified. Inflammation is considered to be a cause of atherosclerosis rather than fat and cholesterol. [9,10]

One of the functional foods that could be expected to lowering metabolic disease is olive oil. EVOO is a type of oil very different from other oils, As how to obtain and composition, EVOO is one of the food that have a functional content of mono unsaturated fatty acids (MUFA), which partiallylarge oleic acid present in form and contains a lot of antioxidants (such as tyrosol,hydroxytyrosol) and oleuropein which known as antidiabetic andantioxidant.[11]

Olive oil is the main source of fat, which has anti-inflammatory compounds including at minimum nine categories of polyphenols and more than twenty well researched anti-inflammatory nutrients. Research has documented a wide variety of anti-inflammatory mechanisms used by EVOO polyphenols to lower the risk of inflammatory problems. These anti-inflammatory benefits of

EVOO do not depend on large levels of intake. As little as 1-2 tablespoons of EVOO per day have been shown to be associated with great anti-inflammatory benefits.[12,13] So far, there is no experimental study which evaluate the effect of EVOO in different doses for reducing the level of pro inflammatory cytokine (TNF α) and presence of foam cell. This study will determine the effects of EVOO for 60 days of treatments period on the foam cell and TNF α as inflammatory markers in rat undergo high fat diet.

1.2.Research Questions

Whether EVOO have effectiveness to reduce level of proinflammatory cytokine (TNF-α) and fat laden cells (foam cells) in HFD *Wistar rats*?

1.3.Objectives of the study

1.3.1. General Objectives

Based on the research question mention above, the general purpose of this study is to investigate the effectiveness of EVOO on the level of proinflammatory cytokine (TNF-α) and fat laden cells (foam cells) in Wistar rats induced by HFD.

1.3.2. Specific Objectives

a. To proof the levels of TNF α among high fat diet rats that receive EVOO in doses are lower than those which do not receive EVOO.
b. To find the effectiveness of EVOO in reducing TNF-α.
c. To proof reducing the presence of foam cells among high fat diet rats that receive EVOO in doses than those which do not receive EVOO.
d. To find the effectiveness of EVOO in reducing the presence of foam cells.

1.4.Study benefit

1.4.1. Benefit for science

This study is provide scientific evidence of the important role of EVOO to reduce proinflammatory cytokine and fat laden cells in high fat diet individual.

1.4.2. Benefit for research

The results of this study may give information for developing further research whether EVOO can be used as prevention for reducing the possibility of inflammation due to high fat diet.

1.5.Originality of the study

Based on searching on research publication on Pubmed National Library of Medicine National Institute of Health USA and other websites related with health, the study which analyze the effect of EVOO on foam cells and TNF α has not conducted yet in rat with high fat diet. There were several study related to current study as listed below.

Table (1) Originality of the study

NO	Title, Auther, Journal	Method	Results
1.	Chronic effects of a high-fat diet enriched with virgin olive oil and a low-fat diet enriched with *a*-linolenic acid on postprandial endothelial function in healthy men . Fuentes1, J. et al. 2008; 100. 159–65.[14]	Twenty male medical students Age :from 18 to 30 years three of different diets for 4-week Western diet, rich in saturated fat, a Mediterranean diet, rich in MUFA , a low-fat diet enriched in ALA.	Plasma concentrations of lipid parameters, nitrates and nitrites (NOx) were examined . The endothelium-dependent vasodilatory response was greater after the ingestion of the MUFA-rich diet than after the SFA or ALA low-fat diets. The bioavailability of NOx was higher following the MUFA diet than after the SFA and ALA low-fat diets.
2.	Defining high-fat-diet rat models: metabolic and molecular effects of different fat types. Buettner R,	Male Wistar rats , age: six-week-old, high-fat diet based on lard, olive oil, coconut fat or fish oil ,Weight gain and food intake . After 12 weeks, an	detected classical HF effects only in diets based on lard and olive oil .PUFA did not induce insulin resistance. Diets based on LC-SFA and MUFA induced hepatic

NO	Title, Auther, Journal	Method	Results
	Parhofer K G, et al. 2006; 36, 485–501.[15]	insulin tolerance test was undertaken.	steatosis with SREBP1c activation.
3.	Differential Effects of High-Carbohydrate and High-Fat Diet Composition on Metabolic Control and Insulin Resistance in Normal Rats. Jorge L. Ble-Castillo, María A. et al. 2012; *9*, 1663-76.[16]	Male Wistar rats . age : 7 weeks and 180–200 g High-saturated fat diet containing lard and the high-unsaturated fat diet containing olive oil. High-digestible carbohydrate diet containing digestible corn starch and the high-resistant starch diet with native banana starch .	HFDs and HCDs reduced weight gain in comparison with CD. Calorie intake was similar in both HFDs and CD,HUFD exhibited the most beneficial effects on glycemic control meanwhile HRSD induced the highest reduction on lipid content and did not modify insulin sensitivity.
4.	Comparison of Serum Cholesterol Fractions Levels in albino rats on monounsaturated (Olive Oil) fat diet. Abro A. K., Bukhari M. H. 2009.[17]	Albino Rats ,age :32 weeks, with equal number of males and females. experimental diets for 20 weeks. added with olive oil serum LDL-c	serum HDL-c without atherogenic diet at week 0 and 32 . serum HDL-c with atherogenic diet at week 0 and 32 . serum LDL-c without atherogenic diet at week 0 and 32 . serum cholesterol at week 0 and 32 . serum HDL-c at week 0 and 32 . The difference is statistically very highly signi-ficant .

The current study is different with several previous studies that listed above. In current study, research subjects are: first, effect of different doses of EVOO on the level of proinflammatory cytokine in Wistar rats induced by high fat diet. Second, the effect of EVOO in reducing the level of the current study variables TNFα and foam cells. The current study also use high fat diet and treatment in the same time.