THERAPEUTIC POTENTIAL OF ANTHOCYANIN-RICH ROSELLE EXTRACT AS ANTI-OBESITY AGENT

By

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DECLARATION

I hereby declare that this dissertation is the result of my own investigation, except where otherwise stated and duty acknowledged. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at Universiti Sains Malaysia or other institution. I grant Universiti Sains Malaysia the right use the dissertation for teaching, research and promoting purpose.

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Date:

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LIST OF SYMBOLS AND ABBREVIATIONS

%	= percentage
°C	= degree celcius
cm	= centimeter
g	= gram
kg	= kilogram
mg/kg	= milligram per kilogram
mm	= millimeter
μL	= microliter
mL	= milliliter
i.p.	= intraperitoneal
CVD	= cardiovascular disease
WHR	= waist-to-hip ratio
BMI	= Body mass index
H_2O_2	= hydrogen peroxide
O_2^-	= superoxide
OH^-	= hydroxyl
CAT	= catalase
SOD	= superoxide dismutase
GPx	= glutathione peroxidase
GR	= glutathione reductase
GSH	= glutathione
TBARS	= thiobarbituric acid reactive substances
DPPH	= 2,2-diphenyl-1-picrylhydrazyl
EDTA	= ethylenediaminetetraacetic acid
AST	= aspartate aminotransferase
ALT	= alanine aminotransferase
NAFLD	= non-alcoholic fatty liver disease
NASH	= non-alcoholic steatosis hepatitis
MDA	= malondialdehyde

TBAR	= thiobarbiturate
ROS	= reactive oxygen species
HMDS	= hexamethyldisilazane
PBS	= phosphate buffered saline
SEM	= scanning electron microscope
H&E	= hematoxylin and eosin
ELISA	= enzyme-linked immunosorbent assay
OD	= optical density
XO	= xanthine oxidase
WR	= working reagent
HFD	= high fat diet
SD	= Sprague-Dawley
ARASC	= Animal Research and Service Centre
NHMS	= National Health and Morbidity Survey
WHO	= World Health Organization

ABSTRAK

Obesiti adalah penyakit kronik yang berasal dari pelbagai faktor dan ditakrifkan sebagai peningkatan pengumpulan lemak di dalam tisu adipos. Selain berperanan dalam penyimpanan trigliserida, ia juga bertanggungjawab mengeluarkan adipokin. Terdapat kaitan di antara adipokin dengan tekanan oksidatif yang berkaitan dengan obesiti. Roselle atau nama saintifiknya *Hibiscus sabdariffa* Linn tergolong di dalam kalangan keluarga Malvaceae yang ditanam di seluruh dunia dan digunakan sebagai minuman, ejen perisa, dan ubatan herba. Antosianin adalah salah satu daripada fitokimia di dalam roselle yang bertindak sebagai antioksidan. Roselle telah dituntut secara tradisional sebagai anti-obesiti melalui ciri-ciri antioksidannya. Tujuan kajian ini adalah untuk mengkaji kesan antosianin di dalam ekstrak roselle berperanan sebagai anti-obesiti. Parameter yang dikaji merangkumi indeks jisim badan (BMI), histopatologi hati dan aorta dan penilaian tekanan oksidatif. Dua puluh lapan ekor (n=28) tikus Sprague-Dawley telah dibahagikan kepada 5 kumpulan. Kumpulan 1 ialah tikus normal, sebagai kawalan negative, manakala 4 kumpulan yang lain diberi makanan komersial diet-tinggi lemak (HFD) selama 6 minggu untuk menghasilkan tikus obes. Kumpulan 2 ialah tikus-obes tanpa rawatan yang ditetapkan sebagai kawalan positif, Kumpulan 3 tikus-obes yang dirawat dengan 300 mg / kg Roselle akueus + 1% TFA ekstrak, kumpulan 4 tikus-obes yang dirawat dengan 300 mg / kg Roselle etanol + 1% TFA dan kumpulan 5 tikus-obes yang dirawat dengan 20 mg / kg vitamin C. Tempoh kajian yang dijalankan adalah selama 3 minggu. BMI telah diukur setiap minggu. Pada akhir kajian, semua tikus dieutanasia. Paras penanda tekanan oksidatif (superoxide dismutase / SOD dan glutathione reductase / GR) telah dinilai. Manakala, hati dan salur darah 'thoracic descending aorta' digunakan untuk kajian histopatologi menggunakan pewarnaaan hematoxylin dan eosin (H&E) dan juga mikroskop imbasan elektron (SEM). Keputusan rawatan selama tiga minggu menunjukkan BMI dalam semua kumpulan yang dirawat berkurang dengan signifikan. Roselle akueus + 1% TFA menunjukkan perubahan peningkatan histopatologi di dalam hati dan aorta toraks berbanding rawatan lain. Walau bagaimanapun, tiada perubahan dalam penanda tekanan antioksidan dalam semua kumpulan kajian. Keputusan menunjukkan roselle akueus + 1% TFA ialah rawatan yang berpotensi untuk mengurangkan obesiti.

Kata kunci: Roselle, obesity, BMI, aorta, hati, SOD, GR

ABSTRACT

Obesity is a chronic disease of multifactorial origin and can be defined as an increase accumulation of fat in adipose tissue. Besides its roles in triglycerides storage, adipose tissue also responsible for secretion of certain types of adipokines. There is an association between adipokines with oxidative stress in relation to obesity. Roselle or scientifically known as *Hibiscus sabdariffa* Linn belongs to Malvaceae family which cultivating worldwide and consumed as beverages, flavoring agent, and herbal medicine. Anthocyanin is one of the phytochemicals present in roselle which act as an antioxidant. Roselle has been claimed traditionally to reduce obesity through its antioxidant properties. The aim of this study was to investigate the effect anthocyanin-rich roselle extracts on obesity. The parameters studied include body mass index (BMI), histopathological of liver and aorta and oxidative stress assessment. Twenty-eight (n=28) Sprague-Dawley rats were divided into 5 groups. Group 1 served as negative control, normal rat. The remaining 4 groups fed with commercial High-Fat diet (HFD) for 6 weeks to induce obesity in the rat. Group 2 designated as positive control obese-rat without treatment, Group 3 obese-rat, treated with 300 mg/kg Roselle aqueous + 1% TFA extract, Group 4 obese-rat treated with 300 mg/kg Roselle ethanol + 1% TFA and Group 5 obese-rat treated with 20 mg/kg vitamin C. The intervention period was 3 weeks. BMI was measured weekly. Rats were euthanized at the end of the study. The serum levels of oxidative stress markers (superoxide dismutase/SOD and glutathione reductase/GR) were assessed. While the liver and descending thoracic aorta were subjected for histopathological studies using hematoxylin and eosin staining (H&E stain) and scanning electron microscope (SEM). Three-weeks of treatment significantly reduced BMI in all treated group. Roselle aqueous + 1% TFA significantly improved histopathological changes in liver and thoracic aorta as compared to other treatments. However, there were no changes in antioxidant markers in all study groups. These results demonstrated roselle aqueous + 1% TFA is a potential treatment for obesity.

Keywords: Roselle, obese, BMI, aorta, liver, SOD, GR

CHAPTER 1

INTRODUCTION

1.1 Background of study

The prevalence of obesity has increased worldwide. Obesity becomes a major contributor to the chronic diseases and disability that affects virtually all ages and socioeconomic groups. According to World Health Organization (WHO) in 2015, at least 2.8 million people dying each year as a result of being overweight or obese. In 2014, more than 1.9 billion adults, 18 years and older, were overweight; of these over 600 million of adults were obese that contributes about 13% of the world's adult populations. The increase in obesity prevalence has been observed in Malaysia. A survey from WHO in 2010 showed Malaysia was in the sixth rank in Asia with the highest adult obesity rate. The Malaysian National Health and Morbidity Survey 2015 (NHMS) reported about 30.0% (56 million) are pre-obese while 30.6% (3.3 million) are obese. The 2006 Malaysian National Health and Morbidity Survey (NHMS) databases from 1996 to 2006 showed alarming statistics with the percentages of females was higher than males; 7.6% to 17.4 and 4.0% to 10.0% respectively (Wan Mohamud *et al.*, 2011).

Obesity is defined as a condition of abnormal or excessive fat accumulation in adipose tissue that is detrimental to human health. The Body Mass Index (BMI) is commonly used to indicate overweight and obesity in adults (Gill, 2006). It is calculated as body weight in kilograms divided by the square of height in meters (BMI = kg/m^2). In human, BMI greater than or equal to 25 kg/m^2 is overweight while BMI greater than or equal to 30 kg/m^2 is considered obesity (Gill, 2006; WHO, 2015). Figure 1.1 shows BMI classification. BMI has limitations because it does not distinguish between lean mass and fat; thus, it may overestimate body fat in well-trained body builders and underestimate body fat in older persons. Moreover, BMI does not identify fat distribution. Waist circumference (WC) or waist-to-hip ratio (WHR) is useful indicators of visceral fat distribution. WC equal to or more than 80 cm in women or 94 cm in men, and WHR above 0.90 for males and 0.85 for females are associated with high risk for cardiovascular disease (CVD) (Savini *et al.*, 2013).

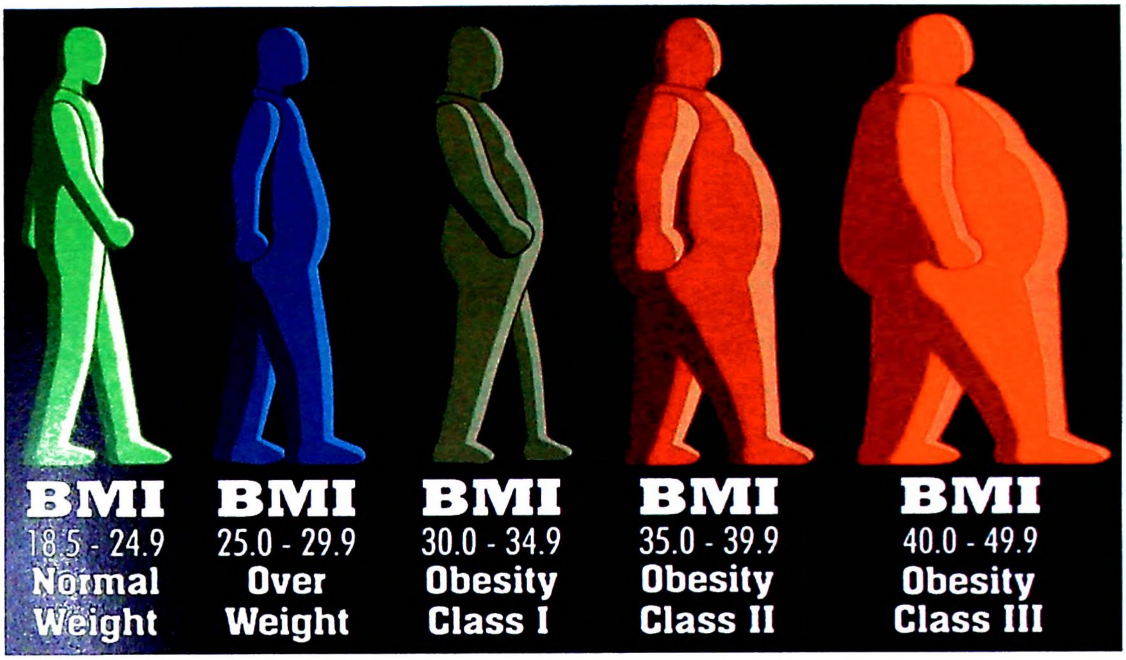

Figure 1.1: BMI classification adapted from World Health Organization, 2015 stated five categories of BMI consist of normal weight, overweight and classes of obesity

Obesity is characterized by excessive storage of adipose tissue. Besides increased secretion of adipokines, an overproduction of reactive oxygen species (ROS) has also been demonstrated (Marseglia *et al.*, 2015) in obesity. In addition, adipocytes have been identified as a source of pro-inflammatory cytokines including TNF-α, IL-1, and IL-6; which are potent stimulators for the production of ROS (Marseglia *et al.*, 2015). Increased oxidative stress caused damage to cellular structures and reduces antioxidant production, thus leads to the development of obesity-related complications. The depletion of antioxidant sources, including superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT), vitamin A, vitamin E, vitamin C, and β-carotene have been demonstrated in obese people (Esposito *et al.*, 2006).

A complex interaction between the environment, genetic predisposition and human behavior including unhealthy diets and physical inactivity has been linked to obesity. Obesity is associated with an increased risk of morbidity and mortality as well as reduced life expectancy. Several diseases have been attributed to obesity such as diabetes mellitus, cardiovascular disease, fatty liver disease and cancer (Marseglia *et al.*, 2015). Various efforts are now concentrated on many herbal plant extracts because of their potential to induce antioxidant effects (Vashi & Devarajan, 2011).

In Malaysia, one of the plants commonly used in the treatment of obesity is Roselle *(Hibiscus sabdariffa Linn)* due to its properties as antioxidant and anti-obesity (Pooja and Priscilla, 2009). Pharmacotherapy, in addition to diet and exercise, has been demonstrated to facilitate a weight loss. However, as compared to conventional medicine, natural products have some advantages including lower cost, fewer side effects and well tolerated by the patient. Judicious use of this plant can promote the development of a range of functional food such as health supplement in treating hypercholesterolemia and diseases associated with it at a nominal cost.

Obesity harms virtually every aspect of health, from shortening life and contributing to chronic diseases. As obesity is a global health problem, the study of the disease has undergone considerable development. However, the treatment of obesity remains largely ineffective. Furthermore, the anti-obesity potential of natural products and its mechanism of action have not been widely studied. In the present study, we are postulating that Roselle is able to combat oxidative stress and prevent subsequent development of obesity-related diseases.

1.2 Objectives

1.2.1 General objective

To investigate the effects of anthocyanin-rich roselle extract on obesity and obesity-related complications

1.2.2 Specific objectives

1. To evaluate the effect of commercial high fat diet (HFD) inducing obesity in rats model
2. To evaluate the effects of anthocyanin-rich roselle extract on body mass index (BMI) in obese rats
3. To evaluate the effects of anthocyanin-rich roselle on histopathological changes of liver and thoracic aorta in obese rat
4. To determine the effects of anthocyanin-rich extract on oxidative stress markers in obese rats

1.3 Scope of the research

This study was conducted to evaluate the effects of anthocyanin rich roselle extract in obese-rat model. Rats were induced with commercial high-fat diet (HFD) to become obese. The body mass index (BMI) was measured throughout the period of study. The blood was withdrawn by cardiac puncture technique for measurement of oxidative stress markers; superoxide dismutase (SOD) and glutathione reductase (GR). In addition, the liver and thoracic descending aorta were isolated to study the histological changes associated with obesity using Hematoxylin and Eosin staining and scanning electron microscope (SEM). The experiments were done in a clean environment and using appropriate equipment in ARASC and Biomedical Laboratories, School of Health Sciences, USM.

1.4 Research hypothesis

Roselle anthocyanin is able to reduce the body weight and other complication risks associated with obesity in obese rat model

1.5 Significant of study

This study may discover roselle as a potential anti-obesity agent. Roselle may be used as an alternative medicine in place of conventional medicine in the treatment of obesity as it is cheaper and has minimal side effects. The results obtained may identify oxidative stress as a factor that involved in the pathogenesis of obesity and its complications. The study may also give some guidance for further research that will be performed in human in the future.

CHAPTER 2

LITERATURE REVIEW

2.1 Obesity and its associated complications

Obesity has become an important public health problem with its prevalence continues to increase globally. The problem seems to affect not only in affluent countries but also in developing countries. BMI is a tool to determine obesity that has been described earlier in Chapter 1. Obesity that refers to an excess amount of body fat causes a wide range of health problems.

Previous study showed there are few factors that contribute to obesity such as poor eating habits and lack of exercise. Fundamentally, obesity is the result of excessive energy consumption compared with the energy expended. Increase portion of high calorie foods, sugar-sweetened drinks and consumption of processed foods, such as meat, contribute to overweight and obesity (Fernández-Sánchez *et al.*, 2011). Lack of exercise or physical activities leads to obesity. Evidence suggests that being physically active is one of the most important steps to maintaining health because its contribute between 25% and 50% of total daily energy expenditure (Bouchard *et al.*, 20070) and can thus be important factors for weight control.

Obesity increases the risk of developing a variety of pathological conditions, including insulin resistance, type 2 diabetes, dyslipidemia, hypertension and non-alcoholic fatty liver disease (NAFLD). Accumulating evidence suggests that chronic inflammation in adipose tissue may play a critical role in the development of obesity-related metabolic dysfunction (Jung & Choi, 2014).

2.2 Current management of obesity

Endocrine Society (2015) release new guidelines on the treatment of obesity by diet, exercise, and behavioral modification are cornerstones of therapy for obesity that approaches for body mass index (BMI) of 25 kg/m^2 or higher (Apovian *et al.,* 2015). However, diet and lifestyle changes resultant weight loss is often small. For more effective weight loss, individuals have shown to benefit from anti-obesity medications. Recent anti-obese medications affect biological mechanisms that suppress appetite and absorb nutrients to regulate body weight.

Numerous anti-obesity agents have been used for weight loss in general populations as well as in individuals with diabetes. These drugs act through a variety of mechanisms, including increased appetite suppression (sibutramine and phentermine), increased energy expenditure (ephedrine and caffeine) and decreased food absorption from the gastrointestinal tract (orlistat). Metformin and fluoxetine are approved for safe use in weight loss, but only fluoxetine is not approved for that purpose due to their toxic side effects. Generally, these anti-obesity drugs may be available over-the-counter or by prescription (Khan *et al.,* 2012).

Despite these drugs showed reduction of weight loss, these drugs give an adverse effect when consumed for long term. Therefore, the study on anti-obesity from natural product is highly needed. The advantages of using natural plants compared to conventional medicine are lower cost. Research, testing, and marketing add considerably to the cost of prescription medicines. Next, natural plant showed reduced risk of side effects. Most natural medicines are well tolerated by the patient, with fewer unintended consequences than pharmaceutical drugs. Besides have fewer side effects, and may be safer to use over time. Judicious use of this plant can promote the development of range of functional food such as health supplement in treating obesity and diseases associated with it at a nominal cost.

2.3 Obesity and oxidative stress

Adipocytes produce a variety of biologically active molecules known as adipokines, including plasminogen activator inhibitor–1 (PAI-1), TNF-α, resistin, leptin, and adiponectin (Furukawa *et al.*, 2004). Dysregulated production of these adipokines participates in the pathogenesis of obesity-associated metabolic syndrome due to increased production of fat. In addition, interleukin 8 (IL-8), interleukin 10 (IL-10), interferon gamma (IFN-γ) and inducible protein 10 (IP-10 or CXCL10) also associated with excessive body weight (Sharabiani *et al.*, 2011).

Abnormal production of these adipokines will induce production of reactive oxygen species (ROS), generating oxidative stress (OS) and consequently lead to obesity (Fernández-Sánchez *et al.*, 2011). Figure 2.1 summarize roles of adipokines in obesity.

Adipose tissue in obese state secrete of inflammatory adipokines with increase releasing of free fatty acids. The free fatty acids and pro-inflammatory adipokines get to metabolic tissues, including skeletal muscle and liver, and modify inflammatory responses as well as glucose and lipid metabolism, thereby contributing to metabolic syndrome. In addition, obesity induces a phenotypic switch in adipose tissue from anti-inflammatory (M2) to pro-inflammatory (M1) macrophages. On the other hand, the adipose production of insulin-sensitizing adipokines with anti-inflammatory properties, such as adiponectin, is decreased in obese state (Jung & Choi, 2014).

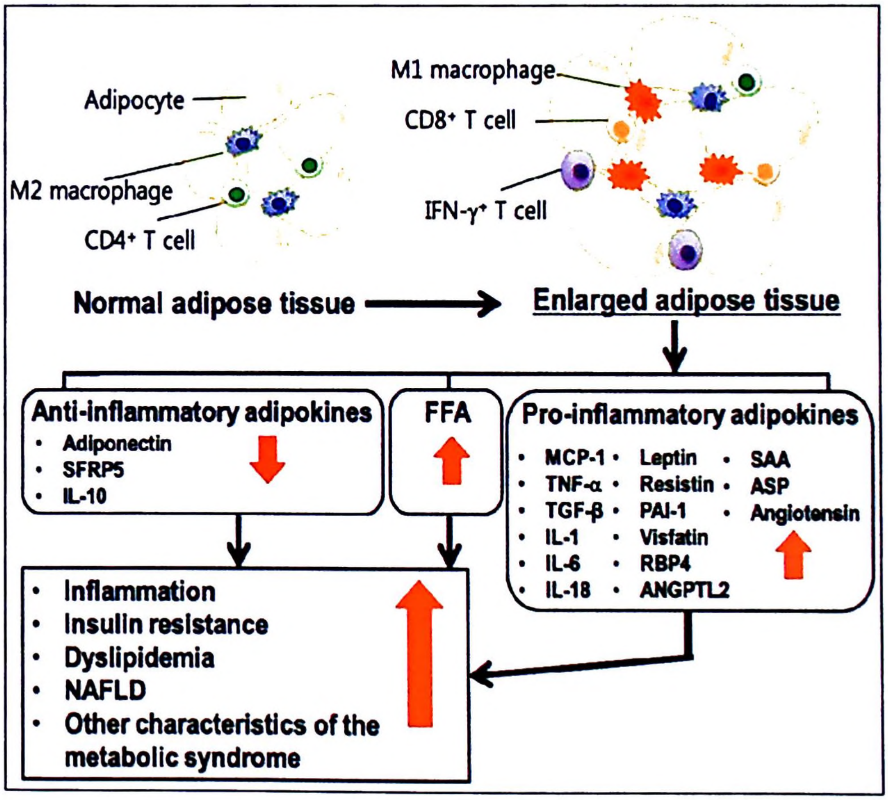

Figure 2.1: Secretion of adipokines in obesity cause phenotypic switch from anti-inflammatory to pro-inflammatory adapted from Jung & Choi, 2014.

Few studies showed oxidative stress also play an important role in the pathogenesis of non-alcoholic fatty liver disease (NAFLD) or non-alcoholic steatohepatitis (NASH) in animal and human studies due to increased lipolysis and increased delivery of fatty acids from adipose tissue to liver (Madan *et al.,* 2006; Machado *et al,* 2008). NAFLD is a progressive disease, initially manifested by accumulation of lipid droplets in the liver (hepatic steatosis). Following steatosis, inflammation may lead to non-alcoholic steatohepatitis (NASH). NASH may progress to fibrosis, cirrhosis and eventually liver failure or hepato-carcinogenesis. Historically NAFLD was linked to high caloric intake, physical inactivity, genetics, and certain medications' side effects. Emerging studies have demonstrated that other factors may play a role in the genesis and development of NAFLD or may act as a 'second hit' in the progression of hepatic steatosis to steatohepatitis (Ahmed *et al.,* 2010; Dowman *et al.*, 2010).

The 'two hit' theory is the likely explanation for the pathogenesis of NAFLD. Fat accumulation in the liver is the first 'hit'; and most likely occurs because of an imbalance between triglyceride accumulation, breakdown and export from the liver. The presence of fat in the liver makes the hepatocyte more vulnerable to further damage and injury. Insulin resistance, inflammation, excess alcohol and obesity are the causes of NAFLD mediating the second 'hit'. At the cellular level, factors involved in the second 'hit' are thought to be oxidative stress, subsequent lipid peroxidation and an inflammatory response (Ahmed *et al.,* 2010).

Currently there are no specific, sensitive biochemical markers that identify the different conditions of NAFLD (Ahmed, 2007). However, fatty liver is associated with elevated serum ALT and GGT concentrations and these liver enzymes are generally considered as surrogate markers of parenchymal cell and bile duct canaliculi dysfunction. In addition, the increase in liver enzymes is often mild and is usually restricted to one or both of ALT and AST. One studied showed patient with NASH have significantly ($P>0.05$) higher levels of plasma glucose, AST, ALT, GGT, alkaline phosphatase and triglycerides than the non-NASH patient. It can conclude these biochemical markers are often more pronounced in the presence of a metabolic disorder caused by liver disease than just by obesity, since these are factors closely related to the pathophysiology of NASH (Leghi *et al.*, 2015).

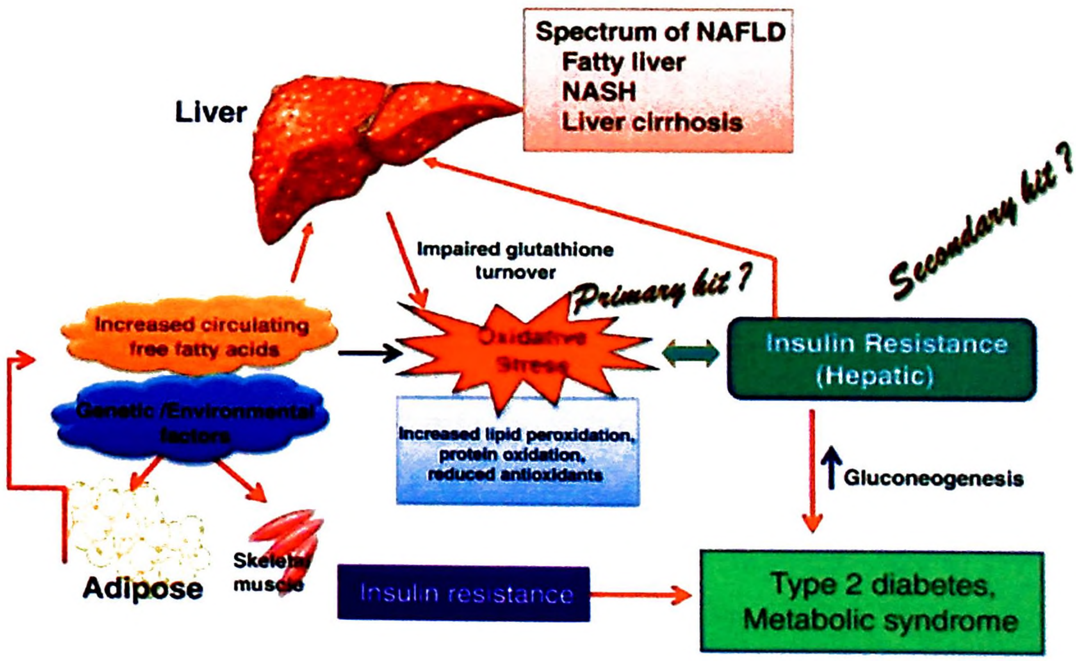

Figure 2.2: Illustration showing plausible interactions of oxidative stress. The 'two' hits theory for pathogenesis of NAFLD adapted from Narasimhan *et al.*, 2010

Reactive oxygen species (ROS) are highly reactive transient chemical species formed in all tissues during normal aerobic cellular metabolism which can be both harmful and beneficial in biological system depending the environment (Lopaczynski and Zeisel 2001; Glade 2003). Beneficial effects of ROS involve in defense against infectious agent and in cellular signaling systems. In contrast, at higher concentration ROS can potentially to initiate damage to various intracellular components including proteins, nucleic acids and polyunsaturated fatty acids in cell membranes and plasma lipoprotein; this damage is often referred as 'oxidative stress' (Sirag *et al.*, 2014). There are several types of ROS include superoxide anion radicals (O_2), singlet oxygen, nitric acid radical, hydrogen peroxide (H_2O_2), hydroxyl radicals (OH) and various lipid peroxides (Favier,2003; Chandra *et al.*, 2012).

As a result from this process, it have received a lot of attention especially in experimental or clinical medicine and biology because of their role in the aetiology of various chronic and degenerative diseases, including aging, coronary heart disease, inflammation, stroke, diabetes mellitus, cancer, rheumatic and neurodegenerative desorders (Luximon-Ramma *et al.*, 2002; Dasgupta & De, 2007; Halliwell, 2012). The current hypothesis suggests oxidative stress as an underlying mechanism by obesity induces tissue damage or provokes several human diseases (Ma *et al.*, 2011).

Therefore, in order to eliminate oxidative stress leading to obesity, it is very essential to keep ROS at a correct level. The harmful effects of ROS can be balanced by the action of antioxidants, some of which are enzymes present in the body (Halliwell, 1996). Antioxidant is a compound capable of stabilizing or deactivating free radicals. Human have evolved highly complex antioxidant systems (enzymic and nonenzymic), which work synergistically and in combination with each other to protect the cells and organ systems against free radical damage (Rahman, 2007). The most efficient enzymatic antioxidants involve glutathione peroxidase (GSHx), catalase (CAT) and superoxide dismutase (SOD) (Mates *et al.,* 1999). While for non-enzymatic antioxidants include Vitamin E and C, thiol antioxidants (glutathione, thioredoxin and lipoic acid), melatonin, carotenoids, natural flavonoids, and other compounds (McCall and Frei 1999). Furthermore there are also antioxidant from natural source such as fruits, vegetables and spices in boosting antioxidant status, enhancing better health and preventing many diseases (Oboh and Rocha, 2007). Thus, supplement with antioxidant from plants is believed to reduce the risk of complications related obesity and oxidative stress (Wang *et al.*, 2000; Suboh *et al.*, 2004).

2.4 Lipid metabolism

With fat gain, lipid deposition can impair tissue and organ function in two possible ways. First, the size of fat pads around main organs may increase substantially by modifying organ function either by simple physical compression or because fat cells of organs secrete various locally acting substances. Second, the lipid accumulation can occur in non-adipose cells and may lead to cell dysfunction or cell death known as lipotoxicity (Unger, 2001; Montani *et al.*, 2004). In addition, Montani *et al*, 2004 suggested that the development of obesity leads not only to increased fat depots in classical adipose tissue locations but also to significant lipid deposits within and around other tissues and organs known as ectopic fat storage.

Lipid metabolism balanced is necessary to maintain homeostasis. When the balance is lost, obesity or hyperlipidemia develops, leading to a variety of serious diseases, including atherosclerosis, hypertension, diabetes and functional depression of certain organs (Birari & Bhutani, 2007). Previous study showed pancreatic lipase involved in lipid metabolic pathways is being identified and characterized (Shi & Burn, 2004). Lipases are enzymes that digest fats, including triacylglycerol and phospholipids. The human lipases include the pre-duodenal (lingual and gastric) and the extra-duodenal (pancreatic, hepatic, lipoprotein and the endothelial) lipases (Mukherjee, 2003).

Pancreatic lipase (PL) synthesized and secreted by the pancreas. PL is responsible for the hydrolysis of 50–70% of total dietary fats. Of the various lipases, PL is the principle lipolytic enzyme accounting for the hydrolysis of dietary fats to their respective fatty acids and monoglycerides (MGs). The MGs and free fatty acids, released by lipid hydrolysis, form mixed micelles with bile salts, cholesterol and lysophosphatidic acid and are absorbed into enterocytes where resynthesis of triglycerides (TGs) takes place. TGs are stored in adipocytes as their main energy source (Mukherjee, 2003; Shi & Burn, 2004; Birari & Bhutani, 2007). Figure 2.3 showed physiological role of enzymes lipase in lipid absorption.

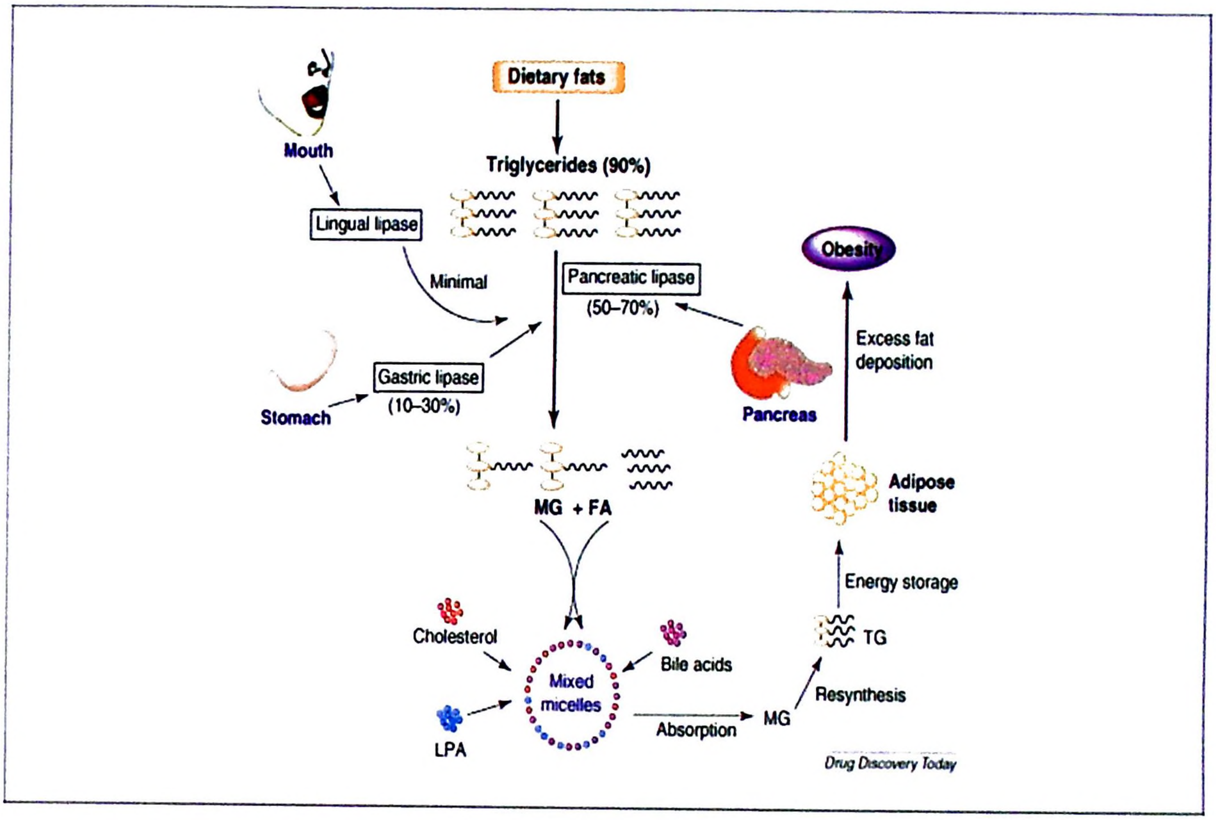

Figure 2.3: **Physiological role of enzymes lipase in lipid absorption**. In obesity individual, there are excessive of fat accumulation in adipose tissue. Adapted from Birari & Bhutani (2007)

2.5 Roselle (*Hibiscus sabdariffa* Linn)

Hibiscus is one of the most common flower plants worldwide. There are more than 300 species of hibiscus around the world. One of them is roselle or its scientific name is *Hibiscus* sabdariffa Linn or Roselle that belongs to the *Malvaceae* family is known as *Asam Susur* or *Asam Paya* in East Coast of Malaysia. In addition, other country called with Rozelle, Sorrel, Red sorrel, Jamaican sorrel, Sour-sour, Queensland jelly plant, Jelly okra, Lemon bush and Florida cranberry. While in North Africa and Near Easy, roselle is called *Karkada* or *carcada*. In India, it is called as Gongura, Lalambari, Patwa (Hindi), Lal-mista, Chukar (Bengali), Lal-ambadi (Marathi), Yerra gogu (Telugu), Pulichchai kerai (Kannada), Polechi, Pulichchai (Malayalan) and Chukiar (Assam) (Gautam, 2004). It is a tropical plant native to India and Malaysia. However, it is also grows widely in the tropics and subtropics regions such as Caribbean, Central America, Africa, Brazil Australia, Hawaii, Florida and Philippines (Morton, 1987; Ross, 2003).

Roselle is an annual, erect, bushy, herbaceous sub-shrub that grows to 8 ft. (2.4 m) in height and has deep penetrating taproot (Figure 1.2). The stems are smooth, cylindrical, typically dark green to red colored stalk. This roselle plant is containing of leaves, flower, calyces, capsule and seeds that contains different phytochemical composition and usage. The leaves are arranged alternately with variable in shape, 7.5-12.5cm long, green with reddish veins and have long or short petioles. Leaves of young seedling and upper leaves of older plants are simple; which contains three to five lobe and the margins are toothed (Figure 1.3). Flowers borne singly in leaf axils, yellow or buff with rose or maroon eye, and turn pink as they wither at the end of the day (Figure 1.4) (Mahadevan *et al.*, 2009).

The calyx consists of 5 large sepals with a collar (epicalyx) of 8-12 slim, pointed bracteole around the base and fully enclose the fruits (Figure 1.5 and figure 1.6). The fruits are velvety capsule, green color for immature and have 5 valves (each valve containing 3-4 seeds) (Figure 1.7). It will turn brown in color and split open when mature and dry. Seeds are kidney-shaped, light brown and covered with minute, stout and stellate hairs (Figure 1.8) (Mahadevan *et al.*, 2009).

Roselle is hardy herbaceous shrub, one of plant which is easy to grow in most well drained soils and requires about 4-8 months growth with night-time temperatures with a minimum of 20 °C, 13 hours of sunlight and a monthly rainfall ranging from 5–1000 mm ?(130–250 mm) during the first few months to prevent premature flowering. Rain or high humidity during the harvest time and drying process can downgrade the quality of the calyces and reduce the yield (Ecocrop, 2007). According to Plotto (2004), about 1.5 kg of fruits is produces by a single plant. The quality of hibiscus is determined by seed stock, local growing conditions, time of harvest, post-harvest handling and mainly the drying step. Usually, propagation is done by seeds or by rooting short cuttings. The edibles fleshy calyces are collected after 15-20 days of flowering (Mahadevan *et al.*, 2009).

Various studies have been done on different parts of roselle plant that use in daily meals and also for medical purpose. In daily meals, fresh or dried calyces are used in the preparation of herbal drinks, hot and cold beverages, fermented drinks, wine, jam, jellied confectionaries, ice cream, pudding and cake (Tsai *et al.*, 2002). Besides, red color and unique flavor of calyces also can be used as food coloring agent (Tsai and Ou, 1996; Ali *et al.*, 2005). In Sudan, the leaves are eaten green or dried, cooked with onion and ground nuts. While in Malaysia, the cooked leaves are eaten as vegetables (Mat Isa *et al.*, 1985; Emmy, 2006). In Africa, roselle seeds are roasted and ground into powdered and used for soups and sauces (Duke, 1983)

In medical application, calyces of roselle extract are claimed to have mild diuretic and purgative effects, and serve home remedies for cancer. In addition, juice from the calyces is proved to have high content of antioxidants compounds including anthocyanin and vitamin C that enhance health status (Mohamed *et al*, 2002). In China, roselle extract is used to treat hypertension, pyrexia and liver damage, and also in ayurvedic medicine (Odigie et al., 2003). Evidence suggests that sepal extracts give effective treatment against leukemia due to its high content of polyphenols, particularly protocatechuic acid (Tseng *et al.*, 2000). Duke (1983) proved the seeds also can be used as medicine. In Burma, it is used for debility, while in Taiwan as diuretic, laxative and tonic. Furthermore, roselle also has certain therapeutic properties such as soothing colds, clearing a blocked nose, clearing mucous, as an astringent, promote kidney function and aids in digestion (Morton, 1987).

The roselle plant is used in this study because of its potential as anti-obesity. The anthocyanin level in roselle has been found the most abundant especially in calyces, which exhibits hypocholesterolemic, antioxidative, hepatoprotective and cardioprotective (Jonadet *et al.*, 1990; Wang *et al.*, 2000). Thus, the present study was designed to determine the effects of anthocyanin roselle extract in obese-rat models. The previous study showed the aqueous extract of red and green *Hibiscus Sabdariffa* calyces caused significant decreases in the LDL and cholesterol levels while no significant effect was observed on HDL-cholesterol and triglycerides levels (Olatunji *et al.*, 2005).

Antioxidant activity of the Roselle extract correlated strongly to its anthocyanin content (Tsai *et al.,* 2002). According to Wong *et al.* (2002), roselle is an electron donor that can react with free radicals and convert them into more stable products and terminate radical chain reactions. The plant has been shown to have a very low degree of toxicity with no adverse effects rather than a commercial drug, which makes it safe for human consumption.

Roselle (*Hibiscus sabdariffa* L.) plant:

Figure 2.4: Roselle plant from Royalty Free Stock Photos (2015)

Figure 2.5: Roselle leaves from Green Tropical (2012)

Figure 2.6: Roselle flower from Flickrver (2014)

Figure 2.7: Roselle fruit from Free Stock Photo (2015)

Figure 2.8: Roselle calyces from On the Green Side of Life (2013)

Figure 2.9: **Roselle capsule containing seeds** from On the Green Side of Life (2013)

Figure 2.10: **Roselle seeds from Nyonya (2013)**

2.5.1 Phytochemistry of Roselle

Roselle (*Hibiscus sabdariffa* Linn) possesses various nutritional and medicinal values from its flowers, calyces and seeds (Ali *et al.*, 2005; Vangalapati *et al.*, 2014). This plant is reported to have important sources of vitamins, minerals, dietary fibers and bioactive compounds, such as organic acid and polyphenols (Jafarian *et al.*, 2014). Petals of this plant also contain alkaloids, anthocyanins, flavonoids, saponins and tannins (Obouayeba *et al.* 2014). Anthocyanins showed the major compounds, followed by phenols and flavonoids, which all of them possess antioxidant activities (Lin *et al.*, 2007; Obouayeba *et al.*, 2014).

The analysis using High Performance Liquid Chromatography (HPLC) method showed a petal contains two phenolic acid, 16 flavonoids and four types of anthocyanin (Obouayeba *et al.*, 2014). Two phenolic acids are chlorogenic acid and protocatechuic acid. While 16 types of flavonoid are glossypetrin, sabdaretin, gossypetin, luteolin, gossytrin, hibiscetin, rutin, hibiscetrin, myricetin, eugenol, nicotiflorine, quercitrin, quercetin, kaempferrol, astragalin and cyanoside. The four types of anthocyanin are cyanidin 3-O-glucoside, cyanidin 3-O-sambubioside, delphinidin 3-O-glucoside and delphinidin 3-O-sambubioside (Hirunpanich *et al.*, 2005; Obouayeba *et al.*, 2014) (Figure 2.11). Cyanidin 3-O-sambubioside and delphinidin 3-O-sambubioside are the major anthocyanins abundantly present in the calyx (Jafarian *et al.*, 2014) (Figure 2.12).

The calyces of roselle also contain chemical compound such as pectin, crude proteins and minerals like ascorbic acid, aluminum, calcium citrate, iron, manganese, phosphorus, potassium and sodium (Vangalapati *et al.*, 2014). There are many factors contribute to different compounds in roselle plant depend on genetically differences, environmental, ecology, harvest condition (Da-Costa-Rocha *et al.*, 2014). The color of sepals used either dark red, light red and green colored also one of the factors (Vangalapati *et al.*, 2014).

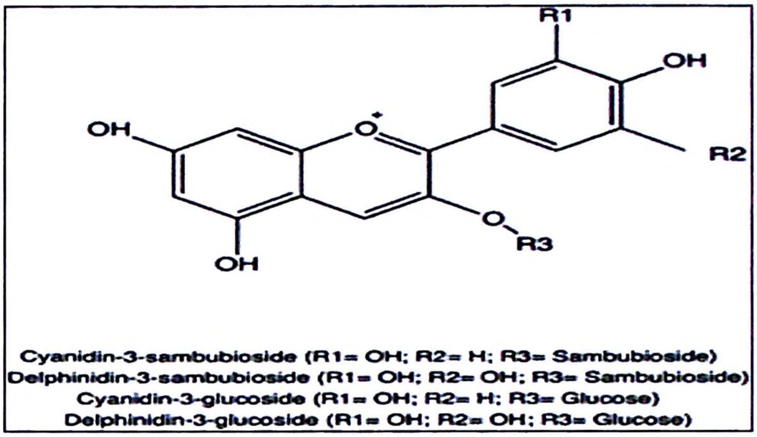

Figure 2.11: **Chemical structure of main Anthocyanins** from Da-Costa-Rocha *et al.* (2014)

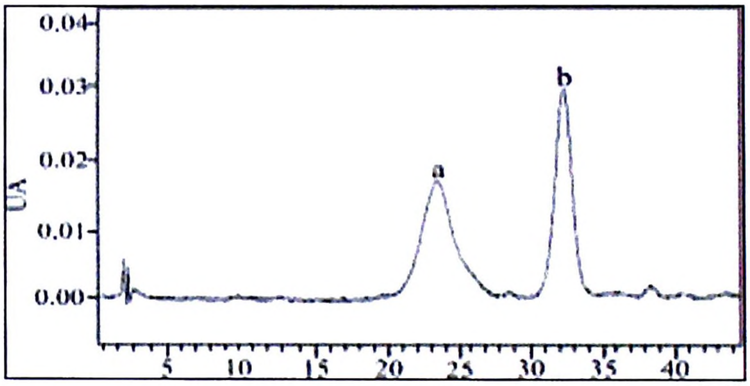

Figure 2.12: **HPLC chromatogram of Roselle calyces extract** (a): delphinidin-3-O-sambubioside, (b): cyanidin-3-O-sambobioside. Conditions: C18 column, 1mL/min, 520 nm from Jafarian *et al.* 2014.