UNIVERSITI PUTRA MALAYSIA

ANTI-OBESITY EFFECT OF CROCIN AND ETHANOLIC EXTRACT OF SAFFRON (Crocus sativus L.) IN RATS FED WITH HIGH-FAT DIET

MARYAM MASHMOUL

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By

MARYAM MASHMOUL

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATION

To my beloved daughter,
ELENA

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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July 2017

Chair: Associate Professor Azrina Azlan, PhD
Faculty: Medicine and Health Sciences

Obesity is one the most common nutritional disorder in the world which can increase the risk of numerous medical problems. Saffron, a source of bioactive compounds, recently has attracted interest for many health benefits. The aim of this study was to evaluate anti-obesity effects of saffron ethanolic extract and its most bioactive compound; crocin in animal model. Obesity was induced by feeding high-fat diet to male *Sprague Dawly* rats for 12 weeks. Then the animals were divided into seven groups in which three of them served as controls; normal diet, high-fat diet, high-fat diet plus orlistat and the other four groups were treated with saffron extract and crocin at low and high doses (40 and 80 mg/kg/day) in their food for eight weeks. Changes in anthropometrical parameters and food intake were recorded. At the end of study blood samples were collected by cardiac puncture for biochemical analysis. The visceral fat pad were dissected and weighed and the liver, pancreas and kidney samples were removed to stain with hematoxylin & eosin (H&E) for histopathological examination. Results showed that crocin (80 mg/kg) significantly decreased the rate of body weight gain (0.09±0.01), total visceral fat pad (7.19±0.22) and weight ratio of epididymal fat to body (1.72±0.80) ($p<0.05$) compared with high-fat group while saffron extract (80 mg/kg) displayed a significant ($p<0.01$) reduction on food intake in experimental rats. Furthermore, crocin (80 mg/kg) significantly reduced plasma levels of triacylglyceride (TG) (1.86±0.29 mM/L) and total cholesterol (TC) (1.07±0.08 mM/L) compared with high-fat control group ($p<0.05$). Treatment with 80 mg/kg of saffron extract ($p< 0.001$) and crocin ($p<0.05$) on the obese rats significantly decreased fasting blood glucose level rather than orlistat. The plasma insulin and TNF-α levels reduced significantly while catalase and adiponectin levels were increased markedly in groups treated with saffron extract and crocin at the high dose (80 mg/kg). Moreover, a significant increment in ghrelin ($p<0.05$) and decrement in leptin ($p<0.05$) were observed in crocin (80 mg/kg) treated group. Saffron extract and crocin dose dependently alleviated the level of liver enzymes in diet induced obese-rat model compared to high-fat diet fed group. Moreover semi-quantitative histological assessment of saffron extract and crocin supplementation on fatty liver tissue indicated that hepatic steatosis and ballooning sig-

nificantly decreased by high dose of saffron extract ($p<0.01$) and crocin ($p<0.05$) treatments. In term of none-alcoholic fatty liver disease (NAFLD) activity score (NAS), saffron extracts dose dependently ($p<0.01$) and crocin 80 mg/kg ($p<0.05$) ameliorated the NAS value. Histopathological examination of kidney and pancreatic tissues of obese rats treated with crocin and saffron extract indicated that saffron extract at both doses exhibited similar protective effect against renal injuries in term of vascular congestion and interstitial inflammation induced by high-fat diet. High dose (80 mg/kg) of the saffron extract supplementation showed significant protective effect in reducing pancreatic adipose tissue infiltration ($p<0.05$). Overall anthropometrical, biochemical and histopathological outcomes suggest that saffron extract and crocin supplementations at the level of tested concentrations have anti-obesity property through enhancement of glucose and lipid metabolism, anti-inflammatory and regulatory effects of adipocytokines and protective effects against fatty liver and untoward impact of fatty diet in kidney and pancreatic tissues of diet-induced obese rats which are encouraging.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

KESAN ANTI-OBESITI CROCIN DAN EKSTRAK ETANOL SAFFRON (*Crocus sativus* L.) KE ATAS TIKUS YANG DIBERI DIET TINGGI LEMAK

Oleh

MARYAM MASHMOUL

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Pengerusi: Prof. Madya Azrina Azlan, PhD
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Obesiti merupakan masalah pemakanan yang biasa pada masa kini dan ia boleh meningkatkan risiko untuk menghadapi masalah kesihatan yang lain. Crocin, merupakan sumber sebatian bio-aktif dan ia telah mendapat perhatian meluas kerana khasiat dan kebaikannya terhadap kesihatan manusia. Tujuan kajian ini adalah untuk menilai kesan anti-obesiti ekstrak etanol safron dan crocin di dalam model haiwan. Obesiti telah didorong dengan memberi diet tinggi lemak kepada tikus jantan *Sprague Dawly* selama 12 minggu. Haiwan tersebut kemudian dibahagikan kepada tujuh kumpulan, di mana tiga daripada kumpulan tersebut dijadikan sebagai kumpulan kawalan; diet biasa, diet tinggi lemak, diet tinggi lemak dengan orlistat dan empat kumpulan lagi diberi ekstrak safron dan crocin (40 dan 80 mg/kg/hari) dalam makanan mereka selama lapan minggu. Perubahan antropometrik and parameter pemakanan telah direkodkan. Pada akhir kajian, sampel darah telah dikumpul dengan cara tusukan jantung untuk tujuan analisis biokimia. Pad lemak viseral telah dibedah siasat dan ditimbang dan sampel hati telah dikeluarkan untuk diwarnakan dengan hematoxylin & eosin (H & E) untuk pemeriksaan histopatologi. Crocin (80 mg / kg) menunjukkan penurunan yang signifikan bagi kadar peningkatan berat badan, jumlah pad lemak dan nisbah berat lemak epidermis kepada badan ($p<0.05$) manakala ekstrak safron (80 mg/kg) mengurangkan pengambilan makanan di kalangan tikus eksperimen secara signifikan ($p <0.01$). Tambahan pula, crocin (80 mg/kg) mengurangkan paras trigliserida (TG) dan jumlah kolesterol (TC) ($p <0.05$) plasma secara signifikan. Rawatan dengan 80 mg/kg ekstrak safron ($p<0.001$) dan crocin ($p<0.05$) pada tikus obes telah menurunkan paras glukosa darah secara signifkan berbanding dengan orlistat. Paras insulin plasma dan TNF-α dikurangkan secara signifikan manakala paras katalase dan adiponektin telah meningkat dengan ketara dalam kumpulan yang dirawat dengan ekstrak safron dan crocin pada dos yang tinggi. Seiringan itu, peningkatan ghrelin ($p <0.05$) dan penurunan leptin ($p <0.05$) yang signifikan telah diperhatikan dalam kumpulan yang dirawat dengan crocin (80 mg/kg). Tambahan pula, ekstrak safron dan crocin mengurangkan paras enzim hati dan steatosis hepatik secara dos

bersandar dalam model tikus obes yang disebabkan oleh diet berbanding dengan kumpulan yang diberi diet tinggi lemak. Kajian ini menunjukkan bahawa ekstrak safron mungkin menjadi prospek yang berpotensi untuk rawatan obesiti dan implikasinya terhadap aktiviti antioksidan yang tinggi, kesan anti-inflamasi dan kesan mengawal adipositokin. Hasil penemuan daripada kajian histopatilogi juga mencadangkan bahawa ekstrak safron dan suplemen crocin mempunyai kesan perlindungan terhadap hati berlemak dan kesan yang tidak diingini daripada diet tinggi lemak di buah pinggang dan tisu pankreas di kalangan tikus obes yang disebabkan oleh diet berlemak.

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I certify that a Thesis Examination Committee has met on 28 July 2017 to conduct the final examination of Maryam Mashmoul on her thesis entitled "Anti-Obesity Effect of Crocin and Ethanolic Extract of Saffron (*Crocus sativus* L.) in Rats Fed with High-Fat Diet" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AC	Abdominal Circumference
AI	Atherogenic Index
ALB	Albumin
ALFD	Alcoholic Fatty Liver Disease
ALP	Alkaline Phosphatase
ALT	Amino Alanine Transferase
AST	Aspartate Amino Transferase.
BHT	Butylated Hydroxytoluene
BMI	Body Mass Index
CAT	Catalase
CCK	Cholecystokinin
CKD	Chronic Kidney Disease
CVD	Cardiovascular Disease
DE	Dried Extract
EI	Energy Intake
FDA	Food and Drug Administration
FE	Feed Efficiency
FFA	Free Fatty Acid
FPG	Fasting Plasma Glucose
GLP	Glucagon-Like Peptide
GPX	Glutathione Peroxidase
H&E	Hematoxylin and Eosin
HDL	High-Density Lipoprotein
HFD	High Fat Diet
HPLC	High Performance Liquid Chromatography
IGT	Impaired Glucose Tolerance
LDL	Low-Density Lipoprotein
NAFLD	Non-Alcoholic Fatty Liver Disease
NAS	Nonalcoholic Fatty Liver Disease Activity Score
NASH	Nonalcoholic Steatohepatitis
ND	Normal Diet
ROS	Reactive Oxygen Species
SD	Sprague Dawley
SNS	Sympathetic Nervous System
SOD	Superoxide Dismutase
T2DM	Type2 Diabetes Mellitus
TBARS	Thio-Barbituric Acid Reactive Species
TC	Total Cholesterol
TG	Triglyceride
TNF	Tumour Necrosis Factor
VLDL	Very Low-Density Lipoprotein
WC	Waist Circumference
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background

Obesity or excess body weight is the most common nutritional and public health problem in the world. Obese people are at increased risk for many serious diseases and health conditions such as high blood pressure, dyslipidemia, cardiovascular disease, type 2 diabetes, stroke and certain sorts of cancer (WHO, 2015). The primary treatment regarding obesity involves dieting and physical activity. Although dietary plans may produce weight-loss temporary, however maintaining this status is usually challenging (Tate, Jeffery, Sherwood, & Wing, 2007). The complex pathogenesis underlying obesity status implies the necessity of various intervention strategies to address this challenge (WHO, 2013).

Herbal dietary supplements for weight loss and obesity management are one of the most common alternative medicine therapies (Barnes, Powell-Griner, McFann, & Nahin, 2004). As the alternative treatment of obesity and its related complications, there are a number of natural products which includes medicinal plants either in the form of pure compounds or as extracts having different anti-obesity outcomes on metabolism and fat oxidation in the body (Hasani-Ranjbar, Jouyandeh, & Abdollahi, 2013).

Saffron is the dried stigma of the blossoms of *Crocus sativus L.* (*Iridaceae* family) plant that has been commonly used as a natural remedy from the ancient times (Christodoulou, 2015). Saffron is one of the most precious spices in the world as it is an extremely labor-intensive crop. The significant level of saffron bioactive compounds; i.e. Carotenoids (Winterhalter & Straubinger, 2000; Abdullaev, 2006; Negbi, 2003) and polyphenols (Karimi, Oskoueian, Hendra, & Jaafar, 2010); caused it an interesting topic to study for potential health benefits. Several scientific reports indicated that the most health properties of saffron have been attributed mainly to crocin, a carotenoid substance with high antioxidant activity, which provides the particular golden red color of saffron (Caballero-Ortega, Pereda-Miranda, & Abdullaev, 2007; Chen et al., 2008; G Gutheil, Reed, Ray, Anant, & Dhar, 2012; Giaccio, 2004). Although bulk quantities of saffron are expensive, high concentration of the bioactive contents and small treatment doses are enough that affordability may be within the reach of most and makes it still a cost-effective natural product. Moreover, crocin as a water-soluble carotenoid also can be found and extracted from the fruits of gardenia (Gardenia Jasmin ides Ellis) (Pham, Cormier, Farnworth, Tong, & Van Calsteren, 2000) which is an inexpensive plant.

Although high-antioxidant characteristic of saffron and its most bioactive component crocin, along with the some other therapeutic properties of saffron such as lowering

cholesterol and triglyceride levels (He et al., 2007; Shirali, Bathaie, & Nakhjavani, 2012), modulating insulin and blood glucose uptake (Shirali et al., 2012; Mohajeri, Mousavi, & Doustar, 2009; Xi et al., 2007) as well as suppressing inflammatory adipocytokines (Xi, 2007) has been detected in different diseases-health model of studies earlier, however there was a lack of human or animal study to examine the efficacy of saffron as a dietary supplement in individuals with obesity for body weight loss and potentiality to suppress obesity's complications as well.

1.2 Problem statements

The prevalence of obesity has expanded to epidemic proportion and is commonly a key contributor towards the worldwide burden of chronic disease and unhealthy issues. Obesity increases the risk for morbidity coming from non-communicable disease. The medical, psychological, and economical burden regarding the obesity has gotten considerable inferences in developed and developing communities recently (WHO, 2015).

The excessive worldwide prevalence of overweight and obesity, suggesting that diet and exercise alone are not adequate for millions of people trying to lose weight and manage their body weight (Hainer, 2012). Even though two different kinds of obesity-treatment drugs, namely orlistat (*Xenical*) which decreases intestinal malabsorption of fat over the inhibition of pancreatic lipase activity and drugs which act as an anorectic, or appetite curbing chemical such as sibutramine (*Reductil*) are already available on the market, however both kinds of drugs have disturbing side-effects such as hypertension, headache, dry mouth, constipation, insomnia and etc. (Arterburn, Crane, & Veenstra, 2004).

At this time, for the reason of dissatisfaction with even high costs and possibly dangerous side-effects of available anti-obesity drugs, scientific research toward the investigation of potential natural compounds for managing body weight status is extremely encouraging. A number of natural products, including plant crude extracts as well as isolated bioactive compounds such as polyphenols and carotenoids, can induce body weight-loss and prevent obesity (Mohamed, Ibrahim, Elkhayat, & Dine, 2014; Sergent, Vanderstraeten, Winand, Beguin, & Schneider, 2012; Yun, 2010). To date, despite the existence of numerous studies on potential anti-obesity products (Rodgers, 2012; Johansson, 2014; Fu, 2016), still there is an extensive demand to study new safe, natural and effective anti-obesity products.

1.3 Significance of study

The proposed study will serve as a first animal study to investigate the anti-obesity effect of crocin and saffron ethanolic extract compared with orlistat in animal model. In this study, the efficacy of saffron supplementation in both forms of crude ethanolic extract and a pure bio-active compound (crocin) in comparison with orlistat (an FDA drug approved for obesity management) has been assessed. Data from this research

project will be used in future studies to evaluate the beneficial impacts of saffron on prevention and control of obesity and the related comorbidities in the human body.

1.4 General objective:

To evaluate anti-obesity properties of crude ethanolic extract and a pure bio-active compound of saffron (*crocin*) in rats fed a high-fat obesity-induced diet.

1.5 Specific objectives:

i. To determine the changes of anthropometrical parameters and food intake in control and treatment groups subjected to high fat diet-induced obese rats
ii. To determine the level of obesity associated biomarkers, including: fasting plasma glucose, insulin, lipid profile, leptin, ghrelin, adiponectin, serotonin, TNF-α and catalase as well as liver function enzymes (ALT, AST, ALP and ALB) of all experimental rats
iii. To examine the histopathological changes of fatty liver, kidney and pancreatic tissues following 8 weeks of crocin and saffron extract supplementation in animals
iv. To compare the anti-obesity effectiveness of saffron extract and crocin at the level of testing concentration among treatment groups

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