UNIVERSITI PUTRA MALAYSIA

EFFECTS OF TAMARILLO (Cyphomandra betacea (Cav.) Sendtn.) ON BODY WEIGHT, BIOCHEMISTRY, ANTIOXIDANT ENZYME, INFLAMMATORY BIOMARKERS, LIVER AND KIDNEY IN HIGH FAT DIET-INDUCED OBESE RATS.

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By

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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November 2015

Chairman: Professor Asmah Rahmat, PhD
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Obesity has reached an epedimic levels in Malaysia, where the prevalence of overweight and obese adult increased from 29.1% and 14.0% in 2006 to 29.40% and 15.10% in 2011 respectively. This study aims to investigate the beneficial effects of *Cyphomandra betacea* on body weight, biochemistry, antioxidant enzyme, inflammatory biomarkers, and liver and kidney in adult male Sprague-Dawley rats fed with high fat diet. Rats were fed on either normal chow or high fat diet for 10 weeks for obesity induction and subsequently received tamarillo at low dose (150 mg kg^{-1}) (TLDG), medium dose (200 mg kg^{-1}) (TMDG), high dose (300 mg kg^{-1}) (THDG) or distilled water via oral gavages for another 7 weeks for treatment phase. In this present study, *C. betacea* treated group showed lower bodyweight and BMI as compared to control positive group.THDG showed lowest bodyweight and BMI followed by TMDG and TLDG. Interestingly, treatment of obese rats with *C. betacea* led to significant decrement of cholesterol ($p<0.05$) and significant increment of HDL-c ($p<0.05$). Positive reduction also can be seen in the triglyceride, LDL-C and also blood glucose. Additionally, there was a positive improvement of superoxide dismutase (SOD) and glutathione peroxidase (GPX) activity along with a significant increase ($P<0.05$) of total antioxidant status (TAS) in *C. betacea* treated rats. Further, rats treated with *C. betacea* show significantly lower in TNF- α and IL-6 ($p<0.05$). As the liver section of *C. betacea* treated group were observed, the liver appeared normal with radiating hepatocytes and displayed less fat vacuoles as the dosage increase accordingly. Meanwhile, the kidney histology section of *C. betacea* treated group showed normal glomerulus, proximal convoluted tubule and distal convoluted tubule. Overall, this study demonstrates the potential use of *Cyphomandra betacea* for weight maintenance and complimentary therapy to supress some obesity complication sign.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains.

KESAN TAMARILLO (*Cyphomandra betacea* (Cav.) Sendtn.) TERHADAP BERAT BADAN, BIOKIMIA, ENZIM ANTIOKSIDAN, PENANDA BIOLOGI RADANG, DAN HATI SERTA GINJAL TERHADAP TIKUS TERARUH OBESITI- DIET TINGGI LEMAK

Oleh

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Obesiti telah mencapai tahap epidemik di Malaysia, statistik menunjukkan berat badan berlebihan dan obesiti telah meningkat dari 29.1% dan 14.0% pada tahun 2006 kepada 29.40% dan 15.10% pada tahun 2011. Kajian ini bertujuan untuk menyiasat kesan-kesan positif *Cyphomandra betacea* terhadap berat badan, biokimia, enzim antioksidan, penanda biologi radang, serta hati dan ginjal dalam tikus jantan dewasa Sprague Dawley yang diberi makan dengan diet tinggi lemak. Tikus diberi makan sama ada diet chow normal atau diet tinggi lemak selama 10 minggu untuk induksi obesiti dan seterusnya mendapat *C. betacea* pada dos yang rendah (150 mg kg^{-1}) , dos sederhana (200 mg kg^{-1}), dos yang tinggi (300 mg kg^{-1}) atau air suling melalui gavages oral selama 7 minggu untuk fasa rawatan. Di dalam kajian ini, kumpulan rawatan *C. betacea* telah menunjukkan berat badan dan BMI yang rendah berbanding dengan kumpulan kawalan rawatan positif. THDG menunjukkan berat badan dan BMI yang paling rendah diikuti oleh TMDG dan TLDG. Menariknya, terdapat pengurangan signifikan aras kolesterol ($p<0.05$) dan kenaikan signifikan aras HDL-c ($p<0.05$) hasil daripada penggunan rawatan *C. betacea*. Terdapat juga penurunan positif dalam trigliserida, LDL-C dan juga glukosa darah dalam kumpulan rawatan *C. betacea*. Terdapat peningkatan positif bagi aktiviti superoxide dismutase (SOD) dan glutathione peroxidase (GPX) serta peningkatan signifikan ($p<0.05$) bagi aktiviti status antioksida (TAS) dalam tikus yang dirawat *C. betacea.* Tikus yang dirawat *C. betacea* juga menunjukkan pengurangan aras TNF-α dan IL-6 yang signifikan ($p<0.05$). Bahagian histologi hati kumpulan rawatan *C. betacea* menampilkan lobul hati yang normal berserta dengan hepatosit dan pengurangan vakules lemak selari dengan peningkatan dos *C. betacea*. Sementara itu, bahagian histologi ginjal menunjukkan bahagian glomerulus, tubulus proksimal dan distal berbelit yang normal. Secara keseluruhan, kajian ini menunujukkan bahawa *C. betacea* mempunyai potensi dalam mengekalkan berat badan dan bertindak sebagai terapi komplementari untuk mengurangkan beberapa tanda komplikasi obesiti.

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Respectfully yours with sincere gratitude,

Noor Atiqah Aizan Abd. Kadir
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This thesis was submitted to the Senate of University Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid
ADA	American Dietician Association
AOA	Antioxidant Activity
ARP	Anti Radical Power
BMI	Body Mass Index
BW	Bodyweight
CAT	Catalase
CDC	Centre of Disease Control
CN	Control Negative
CP	Control Positive
CRP	C-reactive Protein
CVD	Cardiovascular Disease
DM	Diabetes Mellitus
DPPH	2,2-diphenyl-1-picrylhydrazyl
FDA	Food and Drug Administration
FFA	Free Fatty Acids
FV	Fruit and Vegetable
FW	Fresh Weight
g	Gram
GAE	Gallic Acid Equivalent
GB	Gastric Bypass
gDM	Gram Per Dried Mass
GI	Glycemic Index
GLUT4	Glucose Transporter 4
GPX	Glutathione Peroxide
HDL	High Density Lipoprotein
IC_{50}	Inhibitory Concentration
IFGA	Impaired Fasting Glucose
IGF	Insulin-like Growth Factor
IGFBP	Insulin-like Growth Factor Binding Protein
IGT	Impaired Glucose Tolerance
IL-6	Interleukin 6
LCD	Low Calorie Diets
LDL	Low Density Lipoprotein
LPa	Lipoprotein a
mg	Milligram
NEFA	Nonesterified Fatty Acids
NHANES	Nutrition and Health Examination Survey
NHLBI	Nation Institutes of Health
ORAC	Oxygen Radical Absorbance Capacity
PC-1	Prohormone Convertase 1
PPAR	Peroxisome Proliferator Activator
PPAR-g	Proliferator Activated Receptor-g
RAAS	Renin-angiotensin-aldosterone System
ROS	Reactive Oxygen Species
RP	Reducing Power
RS	Reactive Species
SOD	Superoxide Dismutase

TC	Total Cholesterol
TEAC	Trolox Equivalent Antioxidant Capacity
TG	Triglyceride
THDG	Tamarillo High Dosage Group
TLDG	Tamarillo Low Dosage Group
TMDG	Tamarillo Medium Dosage Group
TNF-α	Tumor Necrosis Alpha
U.S	United States
USDA	United States Department of Agriculture
VGB	Vertical Banded Gastroplasty
VLCDs	Very Low Calorie Diets
VLDL	Very Low Density Lipoprotein
WHO	World Health Organisation
WHR	Waist Hip Ratio
µM TE	Micro Molar Trolox Equivalent
µmol	Micro Mol

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Based on Malaysian National Health and Morbidity Survey, the prevalence of overweight and obese adult increased from 29.1% and 14.0% in 2006 to 29.40% and 15.10% in 2011 respectively. Obesity was defined standardly by chronic disease consisting of increase in body fat stores. BMI, calculated as weight in kilograms divided by height in metres squared, is a simple estimate of total body fat content in adults. In general, BMI < 18.5 kg m^{-2} is considered underweight, meanwhile 18.5-22.9 kg m^{-2} is normal, 23.00-24.9 kg m^{-2} is overweight and 25 kg m^{-2} or greater is obese (World Health Organization, 2014).

Obesity is significantly associated with potentially life-threatening co-morbidities. Bagchi and colleagues reported that obesity is associated with most of the component of metabolic syndrome, the leading cause of type 2 diabetes (Bagchi and Preuss, 2007). Meanwhile, in was reported that as the BMI increases, there is a curvilinear growth in excess mortality. This excess mortality rises more rapidly when the BMI is above 30 kg/m^2. BMI over 40 kg/m^2 is associated with a further increase in overall risk and for the risk of sudden death (Bray, 2004).

Inflammatory cytokines which are largely produced in the adipose tissue are believed to play a danger role in obese person and leading cause in insulin resistance. Interestingly, blood concentrations of these cytokines are lowered following weight loss. The main cytokines responsible of chronic inflammation are tumor necrosis factor-α (TNFα), interleukin-6 (IL-6), and the inflammasome-activated IL-1β (Rodriguez-Hernandez *et al*., 2013).

Prolonged obesity condition may lead into antioxidant enzyme depletion, such as superoxide dismutase (SOD), and catalase (CAT) (Amirkhizi *et al*., 2007). In obese person, the activity of SOD and glutathione peroxidise (GP_X) is significantly lower compared with that in healthy persons therefore, continuously showed implications for the development of obesity-related health problems (Ozata, *et al*., 2002).

Fruits and vegetable are placed at second level of the pyramid. According to Malaysian Food Pyramid recommendation, the number of servings recommended for this group is five servings per day. Eating fruits and vegetables can ensure the adequate supply of micronutrient, dietary fibres, and phytochemicals which can help to maintain the body in healthy state (Norimah *et al.*, 2008). Thus, fruits and vegetable can help to prevent and lower risk for certain disease. The WHO Technical Report Series 916 recommends intake of 400 g to 500 g fresh fruits and vegetables a day to improve overall health and reduce the risk of certain noncommunicable disease (World Health Organization, 2003).

Cyphomandra betacea is locally known as "Buah Cinta," "Moginiwang," or "Tamarillo" among local people in Sabah, Malaysia. Whereas, in Peninsular Malaysia this fruit is commonly known as "Pokok Tomato" or "Tamarillo". *C. betacea* can grow naturally in the higher-humidity and low-temperature area. The ripe fruit of *C. betacea* is usually eaten raw by local community (Ali Hassan and Abu Bakar, 2013). It is an egg-shaped bright red fruit with yellow-orange flesh and black seeds that are surrounded by purple gelatine. The red colour is due to pigments called anthocyanins and the yellow-orange colour is due to carotenoids. This fruits are available in both red and yellow varieties. However, the red varieties are more popular and more common. (Lister *et al*., 2005).

Cyphomandra betacea demonstrated antioxidant properties and contain phytochemicals such as beta-carotene, anthocyanins, phenolic acids and large amounts of ascorbic acids. (Lister *et al*., 2005; Vasco *et al*., 2008; Vasco *et al*., 2009; Ghosal and Mandal, 2012; Prakash *et al*., 2012). *C. betacea* have shown to be very useful plant and it is expectable that the interest of this kind of plant will arise in the forthcoming years. *C. betacea* remains unexplored except for its antioxidant profile and to the best of our knowledge, this is the first study to evaluate the protective effects of *Cyphomandra betacea* on body weight, biochemistry profile, antioxidant enzyme, inflammatory biomarkers, liver and kidney histology in high fat diet- induced obese rats and therefore, it may play a certain role in assisting people in the management of some obesity complication sign.

1.2 Objective

1.2.1 General Objective

To study the effects of tamarillo (*Cyphomandra betacea*) on body weight, biochemistry profile, antioxidant enzyme, inflammatory biomarkers, liver and kidney histology in high fat diet- induced obese rats.

1.2.2 Specific Objective

1. To determine and to compare the body weight, biochemistry profile (Blood Glucose, Total Cholesterol, Triglycerides, HDL-C and LDL-C), antioxidant enzyme (SOD,GP_X & TAS), inflammatory biomarkers (TNF-α and IL-6) and liver and kidney histology between control negative, control positive and *C. betacea* treated groups after 10 weeks post-obesity induction and 7 weeks post-treatment.

2. To determine and to compare the effects of low (150 mg/kg), medium (200 mg/kg) and high (300 mg/kg) dosage of *C. betacea* on body weight, biochemistry profile (Blood Glucose, Total Cholesterol, Triglycerides, HDL-C and LDL-C), antioxidant enzyme (SOD, GP_X & TAS), inflammatory biomarkers (TNF-α and IL-6) and liver and kidney histology in *C. betacea* treated group after 7 weeks post-treatment.

1.3 Null Hypothesis

H_{O1}: There are no significant differences in mean of:

a) Body weight
b) Biochemistry profile (Blood Glucose, Total Cholesterol, Triglycerides, HDL-C and LDL-C)
c) Antioxidant enzyme (SOD,GP_X & TAS)
d) Inflammatory biomarkers (TNF-α and IL-6)
e) Liver and kidney histology

between control negative, control positive and *C. betacea* treated groups after after 10 weeks post-obesity induction and 7 weeks post-treatment.

H_{O2}: There are no significant differences in mean of:

a) Body weight
b) Biochemistry profile (Blood Glucose, Total Cholesterol, Triglycerides, HDL-C and LDL-C)
c) Antioxidant enzyme (SOD,GP_X & TAS)
d) Inflammatory biomarkers (TNF-α and IL-6)
e) Liver and kidney histology

between low, medium and high dosage of *C. betacea* in *C. betacea* treated group after 7 weeks post-treatment.

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