UNIVERSITI PUTRA MALAYSIA

ANTI-OBESITY AND ANTI-OXIDATIVE EFFECTS OF METHANOLIC Albizia myriophylla (L. BENTH) BARK EXTRACT IN OBESE MICE

AZMAH SA'AT

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By

AZMAH SA'AT

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

July 2017

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

ANTI-OBESITY AND ANTI-OXIDATIVE EFFECTS OF METHANOLIC *Albizia myriophylla* (L. BENTH) BARK EXTRACT IN OBESE MICE

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July 2017

Chairman : Goh Yong Meng, DVM, PhD
Faculty : Veterinary Medicine

Globally and in Malaysia, obesity causes early morbidity and mortality and is costly to treat.The antioxidant activities in herbal plants have been suggested as one of the mechanisms working against obesity. Hence, this study examined the antioxidant activity of *Albizia myriophylla* bark or known locally as tebu gajah. Its effect on anti-obesity via *in vivo* test in high-fat diet-induced obesity in mice consisting of the normal control group (NC), the high-fat diet control group (HFDC), the high fat diet group treated with *Albizia myriophylla* methanol extract at 10 mg/kg, 20 mg/kg and 30 mg/kg for 5 weeks. Antioxidant activity of the methanol extract and its derived fractions namely hexane, chloroform, ethyl acetate, butanol and a residual aqueous fraction of the bark of ABZ was assessed. In (1,1-diphenyl-2-picrylhidrazyl) radical scavenging test, (2-2'-azinobis 3-ethyl-6-sulfonic acid) radical scavenging test and reducing activity on ferrous iron test, the total antioxidant capacity was found to be varied in different fractions. The IC_{50} calculated value of the three assays showed that the methanolic extract of ABZ bark had the lowest IC_{50} value for each assay, compared to the other extracts signifying highest antioxidant activity and was selected for use in the anti-obesity study. The body weight of mice, adipose cellularity study, and levels of cholesterol, triglyceride, low-density lipoprotein (LDL), the malondialdehyde levels in the muscles of the obese mice, the serum aspartate transaminase (AST) and serum alanine transaminase (ALT) were analysed. Results showed significant weight loss with significant reduction in the number and size of adipose cells in the mice treated with 20 mg/kg and 30 mg/kg of methanolic extract of ABZ bark.Inaddition, there was a significant reduction of blood cholesterol, triglyceride and LDL following treatment with 20 mg/kg and 30 mg/kg of methanolic extract of ABZ bark, compared to HFDC. Furthermore, the significant reduction of AST, ALT and malondialdehyde in the 20 mg/kg and 30 mg/kg treated group, compared with HFDC, suggest the safety of methanolic extract of ABZ bark in a mice model. The regulatory effects of methanolic extract of ABZ bark on genes including hormone sensitive lipase (HSL), peroxisome proliferator-activated receptors α and γ (PPAR α and PPAR γ), sterol regulatory element-binding protein-2 (SREBP2) and Stearoyl-Coenzyme A-Desaturase (SCD)

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gene expression and adipocytokines including tumor necrosis factor-α (TNFα), interleukin-6 (IL-6), adiponectin and leptin involved in lipid metabolism were also studied. The obese mice treated with the 30 mg/kg of methanolic extract of ABZ showed significant up-regulated of PPARα and significant down-regulated activity of PPARγ, SREBP2, and SCD gene expression when compared to the HFDC group. In addition, the mice treated with 20mg/kg and 30 mg/kg of ABZ methanolic extract showed a significant reduction of TNFα and IL-6 and significant increase of adiponectin and leptincompared to the HFDC. In conclusion, the antioxidant properties of 20mg/kg to 30 mg/kg methanolic extract of ABZ bark is able to avert obesity by regulation of the genes and adipocytokines involved in lipid metabolism.

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AKTIVITI ANTI-OBESITI DAN ANTIOKSIDAN EKSTRAK METANOL KULIT *Albizia myriophylla* (L. BENTH) PADA TIKUS OBES

Oleh

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Di peringkat global dan di Malaysia, obesiti merupakan antara penyebab morbiditi dan kematian di peringkat awal berserta kos rawatan yang mahal. Aktiviti antioksidan dalam tumbuhan herba telah dicadangkan sebagai salah satu daripada mekanisma untuk mengatasi masalah obesiti.Oleh itu, kajian ini mengkaji aktiviti antioksidan *Albizia myriophylla* (ABZ) yang lebih dikenali sebagai tebu gajah di Malaysia. Kesannya kepada anti-kegemukan melalui ujian *in vivo* dalam obesiti yang dirangsang melalui diet lemak tinggi di kalangan tikus yang terdiri dari kumpulan kawalan normal (NC), kumpulan diet lemak tinggi (HFDC), kumpulan diet lemak tinggi yang di rawat dengan ekstraksi metanol kulit ABZ pada aras 10 mg/kg, 20 mg/kg dan 30 mg/kg turut dikaji selama 5 minggu.Aktiviti antioksidan ekstraksi metanol dan pecahan ekstrak yang diperolehi daripadanya seperti hexana, klorofom, etil asetat, butanol dan sisa pecahan akueus daripada kulit ABZ telah dilakukan dengan menggunakan analisis kimia secara *in vitro*. Dalam ujian memerangkap radikal DPPH (1,1-diphenyl-2-piccrylhidrazyl), ujian memerangkap radikal ABTS (2-2'-azinobis 3-ethyl-benzothiazoline-6-sulfonic acid) dan ujian pengurangan aktiviti terhadap ion ferus (FRAP), jumlah keupayaan antioxidan didapati berbeza-beza dalam fraksi yang berlainan. Nilai IC_{50} daripada tiga kajian ini menunjukkan bahawa ekstraksi metanolik kulit ABZ menunjukkan nilai IC_{50} yang terendah untuk ketiga-tiga ujian, dibandingkan dengan ekstraksi yang lain, serta menunjukkan aktiviti antioksidan yang tertinggi.Dapatan-dapatan di atas menunjukkan bahawa ekstrak metanolik kulit ABZ adalah sumber antioxidan semulajadi yang berpotensi dan telah dipilih untuk digunakan dalam kajian anti-obesiti.Penilaian berat badan tikus, kajian sellulariti adipos, dan kadar ketinggian kolestrol, trigliserida, lipoprotin berketumpatan rendah (LDL), aras malondialdehyde dalam otot tikus gemuk, serum aspartate transaminase (AST) dan serum alanine transaminase (ALT) turut dikaji.Hasilnya telahmenunjukkan kehilangan berat yang signifikan berserta penurunan ketara dalam bilangan dan saiz sel-sel adipos pada tikus-tikus yang telah diberikan 20 mg/kg dan 30 mg/kg ekstraksi metanolik kulit ABZ.Tambahan pula, berikutan rawatan 20 mg/kg dan 30 mg/kg ekstrak metanol dahan ABZ, terdapat pengurangan kolesterol, TG dan LDL yang

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ketara dilihat dalam kumpulan-kumpulan ini. Di smping itu, pengurangan AST, ALT dan malondialdehyde yang ketara dalam kumpulan 20 mg/kg dan 30 mg/kg dibandingkan dengan kumpulan HFDC, menggambarkan ekstraksi metanolik kulit ABZ adalah selamat digunakan dalam model tikus.Kesan pengawalaturan ekstrak metanol ABZ pada gen yang melibatkan lipase hormon sensitif (HSL), peroksisom reseptor proliferator-diaktifkan α dan γ (α PPAR dan PPAR γ), sterol peraturan unsur mengikat protein-2 (SREBP2) dan stearoyl-Koenzim A-desaturase (SCD) gendan adipositokin termasuk tumor nekrosis faktor-α (TNFα), interleukin-6 (IL-6), adiponectin dan leptin yang terlibat dalam metabolisma lemak juga telah dikaji. Tikus gemuk yang dirawat dengan 30 mg/kg ekstrak metanol ABZ menunjukkan aktiviti yang ketara kepada kenaikan kadar aktiviti PPARα dan penurunan kadar aktiviti PPARγ, SREBP2, dan SCD ekspresi gen apabila dibandingkan dengan kumpulan HFDC. Di samping itu, kumpulan yang dirawat dengan 20 mg/kg dan 30 mg/kg menunjukkan pengurangan ketara TNFα dan IL-6 serta menunjukkan peningkatan ketara adiponektin dan leptin berbanding dengan kumpulan HFDC. Kesimpulannya, sifat-sifat antioksidan 20 mg/kg kepada 30 mg/kg ekstrak metanol kulit ABZ mampu mengelakkan kegemukan dengan pengawalaturan gen dan adipositokin yang terlibat dalam metabolisma lemak.

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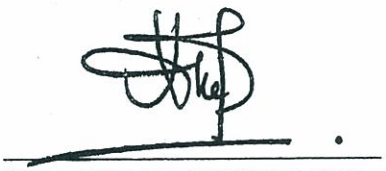

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TABLE OF CONTENTS

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LIST OF TABLES

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LIST OF FIGURES

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LIST OF ABBREVIATIONS

ABTS	2-2'-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid)
ABZ	*Albizia myriophylla*
ADP	Adenosine diphosphate kinase
AKT	Protein kinase B
ALT	Alanine Transaminase
AMPK	Adenosine monophosphate kinase
AST	Aspartate transaminase
BAT	Brown adipose tissue
BMI	Body mass index
CAT	Catalase
CT	Cycle Threshold
CVD	Cardiovascular Disease
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1 picrylhydrazyl
ELISA	Enzyme-linked immunosorbent assay
FRAP	ferric reducing antioxidant power
GP	Glutathione peroxidase
H&E	Haematoxylin and eosin
HDL	High-Density Lipoprotein
HFD	High-fat diet
HFDABZ1	High-fat diet mice treated with ABZ 10 mg/kg
HFDABZ2	High-fat diet mice treated with ABZ 20 mg/kg
HFDABZ3	High-fat diet mice treated with ABZ 30 mg/kg
HFDC	High-fat diet control

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HKG	House keeping gene
HMG-CoA-R	Hydroxy-methylglutaryl-Coenzyme A reductase
HRP	Horseradish peroxidase enzyme
HSL	Hormone-sensitive lipase
IC	Inhibition concentration
IL-6	Interleukin-6
LDL	Low-Density Lipoprotein
MDA	Malondialdehyde
mRNA	Messenger ribonucleic acid
mTOR	Mechanism target of Rapamycin
MUFA	Monounsaturated fatty acid
NAFLD	Non-alcoholic fatty liver disease
NC	Normal control
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
pAMPK	Phosphorylation of Adenosine monohosphate-activated protein kinase
PCR	Polymerase chain reaction
PPARα	Peroxisome proliferator-activated receptor alpha
PPARγ	Peroxisome proliferator-activated receptor gamma
PUFA	Polyunsaturated fatty acid
qPCR	Quantitative reverse transcription PCR
ROS	Reactive oxygen species
RT-PCR	Reverse transcription polymerase chain reaction
SCD	Stearoyl-Coenzyme A-Desaturase
SFA	Saturated fatty acid
SOD	Sodium dismutase

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SREBP	Sterol Regulatory Element-Binding Protein
T2DM	Type 2 diabetes mellitus
TG	Triglyceride
TMB	3,3,5,5'-tetramethylbenzidine
TNF-α	Tumor necrosis factor alpha
TSFA	Total saturated fatty acid
VLDL	Very low-density lipoprotein
WAT	White adipose tissue

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CHAPTER 1

INTRODUCTION

Obesity is fast becoming a major health concern throughout the world (Hurt *et al.,* 2010). The predisposing factors of obesity have multi-factorial facets involving diet, sedentary lifestyle, dysregulation of the genes and adipocytokines involved in the lipid metabolism (Valavanis *et al.,* 2010). The health burden from obesity is largely driven by an increased risk of type 2 diabetes, cardiovascular diseases, and several forms of cancer such as colon and breast cancer (Renehan *et al.,* 2008). The medical costs of obesity represent the monetary value of health-care resources devoted to managing obesity-related disorders, including the costs incurred by excess use of ambulatory care, hospitalisation, drugs, radiological or laboratory tests, and long term care (Guh *et al.,* 2009).Therefore, obesity proves to be a challenge in the prevention and treatment of the foreseeable conditions stated above.

Obesity is closely related to increasing inflammatory environment and enhanced oxidative stress conditions in the cells (Savini *et al.,* 2013). Obesity consisting of adipose cells accumulation is generally known as a crucial energy supplier and storage and was recently acknowledged as an important endocrine tissue which is actively involved in lipid metabolism (Wang *et al.,* 2014). Undeniably, the endocrine function of the adipose tissue plays a major role in the energy balance with the involvement of the adipocyte derivatives involving the pro-inflammatory and anti-inflammatory adipocytokines (Wang and Huang, 2015). When the generation and release of pro-inflammatory adipocytokines such as TNFα and Il-6 exceed the production of anti-inflammatory adipocytokines such as adiponectin and leptin, systemic inflammation and obesity associated metabolic disorders occurs (Johnson *et al.,* 2012). Hence, the development of obesity is related to the chronic low-grade inflammatory condition (Wang *et al.,* 2014).

Dietary consumption of nutrients rich with anti-inflammatory bioactive components from plants which are high with antioxidant activities such as omega-3 fatty acids and polyphenols, has demonstrated anti-inflammatory activity with reduced inflammation in breast cancer tissue and obesity (Kalupahana *et al.* 2011; Siriwardhana *et al.,* 2013; Savini *et al.,* 2013; Khan and Mukhtar, 2013; Jeong et al., 2016). A review article by Esfahani *et al.* (2011), stated that consumption of mixed fruits and herbal supplements, considerably reduces the oxidative stress activity and elevates the antioxidant levels in the systemic circulation. This evidence supports the dietary guidelines of Malaysians which emphasize the importance of a diet rich in high fiber diet consisting of fruits and vegetables, to prevent the development of obesity (Tee, 2011).

Several researches stated that polyphenols consumption prevents development of obesity via numerous probable mechanism which includes reducing adipogenesis via suppressing the growth and differentiation of adipocytes by inhibiting the PPARγ and SREBP2 gene expression (Mayoral *et al.,* 2015; Moseti *et al.,* 2016), reducing the

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inflammatory activity and attenuating the oxidative stress condition via stimulation of the PPARα gene expression which reduces the anti-inflammatory precursors namely the TNFα and IL-6, and increases the anti-inflammatory adipocytokines which are the adiponectin and leptin (Jung and Choi, 2014; Jia *et al.,* 2016) and also by reduction of the cholesterol synthesis viathe suppression of SREBP2 gene (Taylor *et al.,* 2011). In addition, the down regulation of SCD gene will help in stimulating the insulin sensitivity action, thus producing effective energy utilization (Karahashi *et al.,* 2013).

Among the most common pharmacological drugs concurrently used as an anti-obesity agent in the clinical settings are orlistat, which inhibits the gastric and pancreatic lipases thus, reducing the lipid absorption from the gut and sibutramine, which acts as an oral anorexiant (Kang and Park, 2012). The high rate of side effects such as myocardial ischemia specifically associated with sibutramine, has been the reason for the drug to be withdrawn from the market in several countries (Kang and Park, 2012). This side effect seen in the current synthetic anti-obesity drug stresses the necessity of finding a suitable replacement of alternative with a natural or herbal product.

1.1 Significance of Study

Natural or herbal products have shown great promise as novel drug leads (Cragg and Newman, 2013). Numerous herbal products have been used as an anti-obesity agent. For example, astaxanthin, a dietary carotenoid reduces lipid accumulation in mice through the inhibition of PPARγ and activation of PPARα (Jia *et al.,* 2016). Consequently, for a more efficient treatment and control of obesity or weight gain and to satisfy unmet medical needs, there is a necessity to develop new anti-obesity agents that specifically up-regulate the PPARα expression and down-regulate the PPARγ and SREBP2 expression. To explore this possibility, in this study, the researcher has used the ABZ bark and its various fractions extracted through maceration process, and tested its antioxidant activities and gene transcription studies.

In Peninsular Malaysia, ABZ or tebu gajah aqueous bark extracts was used as a tonic drink which was mixed with other herbal supplementation. It was taken as an alternative medication for the treatment of diabetes. Following this, a study by Saat *et al.* (2012) has proven that the ABZ aqueous bark extract had significant hypoglycemic activity in streptozotocin nicotinamide induced diabetic rat. As diabetes is closely associated with obesity, with similar pathogenesis, ABZ bark extract may have a potential anti-obesity effect, as well, via indirectly from the antioxidant or free radical scavenging activity. A patent in the United States had used ABZ bark to prevent obesity but not as an anti-obesity agent, as the bitter after taste of saponin thwarts over eating (Patent Application 20040146468).

In the present study, the effect of high fat diet on the liver of the obese mice was also evaluated. The levels of liver enzymes which are AST and ALT, along with the hematoxylin and eosin staining of the hepatocytes of the mice were done. The safety of consumption of the selected doses of ABZ bark in mice was investigated through

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evaluation of the obese mice liver. The histology of the kidney was not done due to financial constraint.

Thus, the significance of this study is in the hope that the anti-obesity action of *Albizia myriophylla* bark extract will provide an alternative and play a significant role in body weight management.

1.2 Hypothesis

The antioxidant activity of *A. myriophylla* will regulate the adipogenesis pathway by controlling the adipocytokines and genes involved in the lipid metabolism, thus leading to a reduction in the number and size of adipocyte cells, eventually controlling obesity and is safe in mice model.

1.3 General Objective

The main objective of this study was to investigate the potential anti-obesity activity of *A. myriophylla* bark extract in lipid metabolism and gene regulation of obesity-induced mice.

1.4 Specific Aims

1. To determine the polyphenol composition and content in ABZ bark extract and its antioxidant activity.
2. To analyse the body composition, adipocyte cellularity and blood serum parameters in obesity-induced mice given ABZ bark extract.
3. To evaluate the mRNA expression patterns of different genes in the liver.
4. To evaluate the effect of ABZ bark extract in obesity-induced mice liver.

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