UNIVERSITI PUTRA MALAYSIA

EFFECT OF "JIN BATU" *(STROBILANTHES CRISPUS)* LEAF EXTRACT ON LIPOLYSIS IN HIGH-FAT DIET-INDUCED OBESE RATS

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By

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April 2007

Chairman: **Professor Maznah Ismail, PhD**

Faculty : **Medicine and Health Sciences**

The aim of the present study was to assess the effects of Jin Batu (*Strobilanthes crispus)* leaves extract (1% w/w) on diet-induced obese rats and to evaluate its potential development for the treatment of obesity. *S. crispus* leaves were extracted in chloroform-methanol (3:5), and the extracts were given in drinking water of obese rats. This experiment was divided into 2 phases. In the first phase, Sprague-Dawley rats (n=42) aged 4 months old (mature age) were given semi-purified high-fat (HF) diet for 14 weeks to induce obesity. In the second phase, Obese rats were treated for another 14 weeks with 1% (w/w) chloroform-methanol extract of *S. crispus* leaves.

Results obtained in the first phase were as follows. After 14 weeks of HF diet, 18 diet-induced rats were identified as obese based on the body weight gain. The obese rats had significantly ($p < 0.05$) higher energy intake, adipose tissue and liver weights and plasma leptin levels compared to Normal rats. Obese rats also had higher feed efficiency and lower adipose tissue lipolysis and lipoprotein

lipase (LPL) gene expression than Normal rats. A severe case of fatty liver (non-alcoholic fatty liver disease) with the development of hepatic steatosis was also noted in Obese rats. No significant difference was found in mean food intake, HDL, LDL, cholesterol, triglyceride, glucose, glycerol and insulin levels between Normal and Obese rats.

In the second phase, Obese rats from HF diet treatment were divided into two groups; Obese and Obese + Treatment (OT). The use of Normal rats as positive control in the first phase was continued in the second phase. Only OT group was given *S. crispus* extract (1% w/w) in drinking water. High-Fat diet (during first phase) was switched to normal rat chow diet for all groups to imitate diet modification as a method of therapy in human. After 14 weeks of treatment with *S. crispus* extract, OT group was found to have lower mean body weight than control Obese group. The mean body weight gain and feed efficiency was also lowest in OT group when compared to Normal and Obese groups. However, for both results the difference was not significant. The anti-obesity effect of OT group was further confirmed with significantly ($p < 0.05$) lower adipose tissue and liver weight and leptin level compared to Obese group. Lower glucose level and hepatic steatosis combined with high lipolysis rate and LPL mRNA expressed in adipose tissue was also noted in OT group.

In conclusion, this study indicates that 1% (w/w) *S. crispus* extract has some anti-obesity effect on obese rats especially in decreasing adipose tissue weight, lowering leptin level and hepatic steatosis.

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

KESAN EKSTRAK DAUN "JIN BATU" (*STROBILANTHES CRISPUS*) KE ATAS LIPOLISIS PADA TIKUS OBES ARUHAN DIET TINGGI LEMAK

Oleh

NORHASNIDA BINTI ZAWAWI

April 2007

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Kajian ini dijalankan bertujuan untuk menilai kesan ekstrak (1 % w/w) daun Jin Batu (*Strobilanthes crispus)* terhadap tikus obes yang teraruh melalui diet tinggi lemak. Daun *S. crispus* telah diekstrak dalam larutan kloroform-metanol (3:5) dan kemudiannya dimasukkan ke dalam air minuman tikus obes. Kajian ini telah dibahagikan kepada 2 fasa. Dalam fasa pertama, tikus Sprague-Dawley (n=42) berumur 4 bulan (umur matang) telah diberikan diet berlemak tinggi (semi-tulen) selama 14 minggu untuk menjadikannya obes. Dalam fasa kedua, tikus obes telah diberikan 1% (w/w) ekstrak kloroform-metanol daun *S. crispus* selama 14 minggu.

Setelah 14 minggu diberikan diet berlemak tinggi, 18 ekor tikus telah diidentifikasikan sebagai obes berdasarkan pertambahan berat badannya. Didapati tikus obes tersebut mempunyai jumlah pengambilan tenaga dari makanan yang lebih tinggi secara signifikan ($p < 0.05$) berbanding tikus normal. Di samping itu,

berat tisu adipos, berat hati dan kandungan leptin dalam plasma darah tikus obes turut didapati lebih tinggi dan signifikan ($p < 0.05$) berbanding tikus normal. Kadar pengekspresan gen lipoprotein lipase (LPL) turut didapati lebih tinggi dalam tisu adipos tikus obes berbanding tikus normal walaupun ianya tidak signifikan. Kes hati berlemak yang kronik turut diperhatikan dalam tikus obes. Variabel lain yang dikaji seperti jumlah pengambilan makanan, profil darah (aras HDL, LDL, kolesterol dan trigliserida), aras glukosa, aras gliserol dan insulin plasma didapati tidak jauh berbeza dalam kedua-dua kumpulan obes dan normal.

Dalam eksperimen fasa kedua, tikus obes dari eksperimen fasa pertama telah diambil dan dibahagikan kepada dua kumpulan yang diberi nama kumpulan Obese (sebagai kawalan) dan kumpulan Obese + Treatment (OT). Penggunaan tikus Normal sebagai kawalan positif dalam fasa pertama juga diteruskan dalam fasa kedua. Hanya kumpulan OT yang diberi rawatan ekstrak daun *S. crispus* melalui air minuman. Dalam semua kumpulan, diet berlemak tinggi yang diberikan sebelum ini (fasa pertama) telah ditukar kepada diet makanan tikus normal. Setelah 14 minggu ekstrak *S. crispus* diberikan kepada kumpulan OT, kumpulan OT didapati mempunyai purata berat badan akhir yang lebih rendah berbanding kumpulan Obese. Purata pertambahan berat badan dan efisiensi pengambilan makanan kumpulan OT pula adalah yang paling rendah berbanding kumpulan lain. Kesan anti-obesiti dalam kumpulan OT dibuktikan melalui berat tisu adipos, berat hati dan paras leptin dalam plasma darah yang lebih rendah dengan signifikan ($p < 0.05$) berbanding kumpulan Obese. Selain itu, aras glukosa yang rendah, pengurangan lemak dalam hati, kadar lipolisis yang tinggi dalam

adipos tisu dan jumlah pengekspresan gen yang tinggi dalam tisu adipos berbanding kumpulan Obese turut diperhatikan dalam kumpulan OT walaupun ianya tidak signifikan.

Secara kesimpulannya, kajian ini menunjukkan bahawa ekstrak 1% (w/w) *S.crispus* mempunyai kesan anti-obesiti terhadap tikus obes terutamanya dalam mengurangkan berat tisu adipos, aras leptin dan lemak dalam hati.

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I certify that an Examination Committee met on 13/4/2007 to conduct the final examination of Norhasnida Zawawi on her Master of Science thesis entitled "Effect of "Jin Batu" (*Strobilanthes crispus*) Leaf Extract on Lipolysis in High-Fat Diet-Induced Obese Rats" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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NORHASNIDA BINTI ZAWAWI

Date:

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LIST OF ABBREVIATIONS

BMI	Body mass Index
CART	Cocaine-amphetamine-regulated transcript
cDNA	Complimentary deoxyribonucleic acid
CM	Condensed milk
CNS	Central nervous system
CT	Crossing point or threshold value
DEPC	Diethyl pyrocarbonate
DIO	Diet-induced obese
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked Immunosorbent Assay
FFA	Free fatty acids
H & E	Hematoxylin and eosin
HDL	High-density lipoprotein
HF	High-fat
HSL	Hormone-sensitive lipase
LDL	Low-density lipoprotein
LED	Low-energy diet
LPL	Lipoprotein lipase
M-MLV	Mouse-Moloney murine leukemia virus
mRNA	Messenger ribonucleic acid
NAFL	Non-alcoholic fatty liver

NCBI	National Center for Biotechnology Information
NEFA	Non-esterified fatty acids
OR	Obese-resistant
OT	Obese + treatment
PCR	Polymerase chain reaction
POMC	Proopiomelanocortin
PPAR	Peroxisome proliferators-activated receptor gamma
RT-PCR	Real-time polymerase chain reaction
SD	Sprague Dawley
TAG	Triacylglycerol
TBE	Tris-borate
TG	Triglycerides
TLC	Thin layer chromatography
TMB	Tetra methyl benzidine
TNF	Tumor necrosis factor
UCP	Uncoupling protein
UV	Ultra violet
VLDL	Very-low-density lipoprotein
WAT	White adipose tissue
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

International Diabetes Institute (2000) defined obesity as a condition in which there is an excess of body fat while Björntorp (1998) specifically defined obesity as an increased mass of total adipose tissue triglycerides. The operational definitions of obesity and overweight however are based on body mass index (BMI), which is closely correlated with body fatness. Body Mass Index is defined as weight per height squared (kg/m^2) (International Diabetes Institute, 2000). Excessive body mass for stature, and more specifically an excessive body fat content, is a condition of concern because it is in and of itself socially and physically debilitating and it represents a risk factor for increased morbidity and mortalility rate. It is now clear that a high body mass for height or a high body fat level and upper body obesity plus weight gain in adult life are associated with the risk of developing several chronic diseases (Bray *et al.*, 1998).

In Malaysia, more than 6 million or about 20-28 percent of Malaysians are obese and are at risk of developing serious diseases (Utusan Malaysia, 2005). World Food and Agriculture Organization (FAO) had stated that the obesity problem arises as Malaysians consumed high calorie food daily especially food with high fat content (Utusan Malaysia, 2005). The Malaysia National Health Morbidity Survey (1996) revealed that in adults, 20.7% were overweight and 5.8% obese (0.3% of whom had BMI values of >40.0 kg m^{-2}); the prevalence of obesity was

clearly greater in women than in men. In women, obesity rates were higher in Indian and Malay women than in Chinese women, while in men the Chinese recorded the highest obesity prevalence followed by the Malay and Indians (Lim *et al.*, 2000).

The remedies proposed over the years for losing body weight, or more precisely body fat, are exhaustive and have essentially concentrated on traditional methods aimed at manipulating food intake and/or increasing physical activity. Many dietary regimens have been proposed with various permutations on the three major energy-providing components of the diet (Dwyer, 1980), and although they may work for a few individuals, the fact that overweight and obesity continue to rise is evidence that they do not work for the vast majority who wish to lose or maintain their lost weight. Increasing physical activity increases energy expenditure and provides other health benefits; however, when one calculates the amount of exercise that must be performed to lose a few grams of fat it must appear a daunting task for someone who has 20 to 50 kg of excess fat to lose (Garrow, 1995). The observations that most weight-reducing programs have a low rate of success indicate that both diet and exercise have limited efficiency (Acheson & Tappy, 2004). Failing, or in addition to, dietary management and physical exercise, an individual can also use pharmaceutical aids to help control body weight. However, the numbers of drugs that are presently approved for the treatment of obesity are relatively few (Bray, 2000).

In the past a variety of drugs that influence appetite, energy expenditure, or both were used; however, all have been found to cause serious side effects and are considered inappropriate for the pharmacological treatment of obesity (Acheson & Tappy, 2004). At the present time Sibutramine, and Orlistat are in use. Sibutramine, a beta-phanethylamine, is the only centrally acting anti-obesity compound approved for use in most countries. Cardiovascular side effects include an increase in systolic and diastolic blood pressure, and an increase in heart rate, tachycardia and palpitations and vasodilatation (Halford, 2006). Weight loss produced by sibutramine has a number of beneficial effects on key risk factors for non-communicable diseases. However, perhaps the most critical issue is cardiovascular function. Sibutramine has been shown to increase heart rate (3–7 beats per minute) and blood pressure. These adrenergic side effects are a particular concern for patients with hypertension (Halford, 2006). Orlistat (tetrahydrolipstatin) is a semi synthetic hydrogenated derivative of a naturally occurring lipase inhibitor produced by *Streptomyces toxytricini* (Weibel *et al.*, 1987; Hochuli *et al.*, 1987). This compound can block up to one-third of ingested fat from being digested. This undigested fat is then eliminated in the bowel movements. Side effects may include oily stools or spotting, increased number of bowel movements, bowel movement urgency, poor bowel movement control, or gas with discharge (Harp, 1998). Weight loss of 10% to 15% can decrease health risks. However, failure to lose weight or failure to improve comorbid conditions indicates either non-compliance with the drug or that the patient is not responding to the drug. Either setting requires reevaluation of therapy and addition of other medications or other modalities of treatment (Bray, 2000).

Recently, there has been a marked increase in the use of preparations based on medicinal plants in the developed countries, more so as these are generally regarded as being free from the side effects associated with synthetic drugs (Chada & Singh, 1991). Health care products, natural cosmetic, and health foods are becoming increasingly popular. The observed efficacy of many traditional medicines is another important reason for the revival of interest in medicinal plants (Chada & Singh, 1991). A study of the Malaysian plants has yielded a large harvest of natural products (Goh *et al.*, 1995). Several international agencies are now taking an interest in the promotion of the use and research on medicinal plants in the developing countries (Chada & Singh, 1991). The World Health Organization (WHO) supports such research programed (Hedberg, 1987). In the declaration of WHO on primary health care for all by the year 2000, medicinal plants have been assigned an important role (Chada & Singh, 1991).

Since the early years, the Malays have been using plant products such as the leaves, roots, stems, flowers and fruit as ingredients in the preparation of traditional medicine. Traditional medicine has been used since immemorial time and until today, it remains a popular method of treatment. In the tropics, a total of 6,000 floral species have been reported to possess medicinal values (Muhamad & Mustafa, 1994; Goh *et al.*, 1995). From this, a total of 1,230 have been recorded in Malaysia as plants used in traditional medicine. Scientific studies have proven that several medicinal plants used in Malay traditional medicine indeed do contain organic compounds which produce therapeutic effects, in other words, possess medicinal values. Malaysia, a developing country, is rich in natural resources. We

should not waste these resources by leaving them to grow wild only to be destroyed, without utilizing them on a larger commercial basis (Muhamad & Mustafa, 1994). Judging from the large amount of useful substances already obtained from plants, and in view of the fact that only 7% of those now known to science have been properly investigated, research in this area offers a wide scope for the future (Hedberg, 1987).

Strobilanthes crispus plant is a native plant to countries from Madagascar to Indonesia, and is commonly known as daun picah beling in Indonesia and enyoh kelo, kecibeling or kejibeling in Jawa (Sunarto, 1977). This bush-like plant can be found on riverbank or abandoned field while some Javanese use this plant as fence. From a chemical investigation done by Ahmed Faress (1999), it was found that the active compound of *S. crispus* is a phenolic acid named verbascoside. Phenolic compounds have been shown to reduce plasma cholesterol and triglycerides (Thompson, 1993).

Other than that, high level of total ash (21.6%) found in the dried plant leaves were the result of high content of minerals such as potassium (10,900 mg/100 g sample), followed by calcium (5,185 mg/100 g sample) (Maznah *et al.*, 2000). There were not many studies done to investigate the potential of *S. crispus* leaves as medicinal plant. This research will look into the possible role of *S. crispus* leaves as anti-obesity agent because of its high mineral contents. More recently, it has been proposed that dietary calcium regulates energy metabolism and lipolysis