



UNIVERSITI PUTRA MALAYSIA

**THE POTENTIAL OF *Momordica charantia*, *Morinda citrifolia* and
Centella asiatica EXTRACTS AS ANTI-OBESITY AGENTS**

By NAJLA GOODA SAHIB

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NAJLA GOODA SAHIB

**Thesis submitted to the School of Graduate studies, Universiti Putra Malaysia, in
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THE POTENTIAL OF *Momordica charantia*, *Morinda citrifolia* and *Centella asiatica* EXTRACTS AS ANTI-OBESITY AGENTS.

By

NAJLA GOODA SAHIB

October 2009

Chairman: Dr Azizah Abdul Hamid, PhD

Faculty: Food Science and Technology

The effect of *Momordica charantia* Extract (MCE), *Centella asiatica* Extract (CAE) and *Morinda citrifolia* Fruit Extract (MCFE) on lipoprotein lipase (LPL) activity, pancreatic lipase activity, proliferation and differentiation of 3T3-L1 preadipocytes were investigated to evaluate their potential application for obesity treatment. Dried plant samples were extracted in 99.5% ethanol. Extracts were analyzed for total phenolic compounds and flavonoids utilizing Folin Ciocalteu assay and HPLC respectively. The anti oxidative potential of all extracts were also evaluated using the FRAP and DPPH assays. The inhibitory effects of the extracts on lipoprotein lipase (LPL) and pancreatic



lipase (PL) were determined *in vitro*. The extent of cell proliferation was determined by cell viability assay and the degree of differentiation was measured by Oil Red O staining assay. Results of the study showed that all extracts tested consisted of excellent concentration of phenolics compounds, and catechin and epicatechin was predominantly present in all three extracts. All extracts also showed good anti oxidative potential in scavenging free radicals. At a concentration of 1 mg/mL, the extracts inhibited LPL activity differently, with MCFE showing the highest inhibitory effect (21.4±2.3%), followed by CAE (16.3±1.1 %) and MCE (10.6 ±0.6%). At a concentration of 0.02mg/ml, all three extracts inhibited PL, with CAE showing the highest inhibition (31.1%), followed by MCFE (29.7%) and lastly MCE (21.5 %), with no significance difference. MCE showed the highest inhibitory effect on both cell proliferation and differentiation of preadipocytes. After 72 hours, MCE was found to be most toxic with an LC₅₀ of 1.6mg/L, followed by CAE with an LC₅₀ of 2.4 mg/mL and MCFE had the least cytotoxic effect with LC₅₀ of 4.5 mg/mL. Results after 48 hours of plating showed that MCE exhibited the highest inhibitory effect on differentiation in a dose dependent manner. The highest inhibition, 74.7 ±5.41% of control, occurred at concentration of 0.5 mg/mL. Data from the study indicate that MCE exert potential anti obesity effects by inhibiting proliferation and differentiation of preadipocytes. MCFE can also be used as a therapeutic treatment for obesity due to its relatively high anti LPL and PL activity and low cytotoxic effect.

Key words: Obesity, *Momordica charantia*, *Morinda citrifolia*, *Centella asiatica*, 3T3-L1 cells, cell differentiation, Lipoprotein lipase, flavonoids, catechin and phenolic compounds.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah master sains

**EKSTRAK *Momordica charantia*, *Morinda citrifolia* dan *Centella asiatica*
SEBAGAI AGEN ANTI OBESITI**

Oleh

NAJLA GOODA SAHIB

Oktober 2009

Pengerusi: Azizah Abdul Hamid, PhD

Fakulti: Sains dan Teknologi Makanan

Kesan ekstrak *Momordica charantia* (MCE), ekstrak *Centella asiatica* (CAE) dan buah *Morinda citrifolia* (MCFE) ke atas aktiviti lipase lipoprotein (LPL), aktiviti lipase pancreatic, pengandaan dan perbezaan 3T3-L1 preadipocytes telah dikaji untuk menilai potensi untuk rawatan kegemukan .Sampel tumbuhan yang telah dikeringkan telah diekstrak dalam 99.5 % etanol. Jumlah komponen fenolik/ flavonoids telah dianalisis menggunakan Kaedah Folin Ciocalteau dan HPLC. Keupayaan anti- pengoksidaan semua ekstrak juga dinilai menggunakan kaedah FRAP dan DPPH. Kesan pencegahan semua ekstrak keatas LPL dan PL ditentukan secara in vitro. Tahap pengandaan sel telah

ditentukan oleh kaedah MTT dan darjah perbezaan diukur oleh assey Oil Red O staining. Keputusan daripada kajian menunjukkan semua ekstrak yang diuji mengandungi kepekatan komponen fenolik yang baik dan katekin dan epikatekin adalah paling dominan di antara kesemua ekstrak. Kesemua juga menunjukkan keupayaan antioksidan yang tinggi terhadap pemerangkapan radikal bebas. Pada kepekatan 1mg/mL, ekstrak merencat aktiviti LPL secara berbeza dengan MCFE menunjukkan kesan perencatan paling tinggi ($21.4 \pm 2.3\%$), diikuti CAE ($16.3 \pm 1.1\%$) dan MCE ($10.6 \pm 0.6\%$). Pada kepekatan 0.25 mg/mL CAE, MCFE and MCE menunjukkan pencegahan aktiviti lipase pankreatik tinggi pada masing masing (31.1%), (29.7%), (21.5%). MCE menunjukkan kesan perencatan paling keatas pengandaan sel dan pembezaan preadiposites. Selepas 72 jam, MCE didapati lebih toksik dengan LC_{50} 1.6 mg/mL diikuti oleh CAE dengan nilai LC_{50} dan 2.4 mg/mL dan MCFE mempunyai kesan sitotoksik paling rendah dengan nilai LC_{50} 4.5 mg/mL. Keputusan selepas 48 jam menunjukkan MCE mempunyai kesan perencatan paling tinggi Perencatan paling tinggi 74.7 ± 5.41 daripada kawalan berlaku pada kepekatan 0.5 mg/mL. Data daripada kajian menunjukkan MCE boleh memberi kesan anti kegemukan dengan mencegah pengandaan dan perbezaan preadiposite dan MCFE juga anti LPL dan anti PL yang tinggi.

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I certify that an Examination Committee has met on 27th October 2009 to conduct the final examination of **Najla Gooda Sahib** on her degree entitled ” **The potential of *Momordica charantia*, *Morinda citrifolia* and *Centella asiatica* extracts as antiobesity agents** ” in accordance with the Universities and Universities Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia (P.U(A) 106) 15 March 1998. The Committee recommends that the student awarded the Master of Science.

Members of the Examination Committee were as Follows:

Azizah Osman, PhD

Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Suhaila Mohamed, PhD

Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Internal Examiner)

Asmah Rahmat, PhD

Professor
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Internal Examiner)

Laily Din, PhD

Professor Dato’
Centre For Graduate Management
Universiti Kebangsaan Malaysia
Malaysia
(External Examiner)

BUJANG KIM HUAT, PhD

Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date : 24 December 2009



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

Azizah Abdul Hamid, PhD

Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Nazamid Saari , PhD

Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

Faridah Abas, PhD

Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

HASANAH MOHD. GHAZALI, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 14 January 2010



DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

NAJLA GOODA SAHIB

Date : 7 December 2009



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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
APOC-II	Apolipoprotein C II
BHA	Butylated Hydroxyanisole
BMI	Basic Metabolic Index
BSA	Bovine Serum Albumin
CA	<i>Centella asiatica</i>
CAE	<i>Centella asiatica</i> Extract
C/EBP- α	CCAAT/enhancer binding protein
DEX	Dexamethasone
DMEM	Dulbecco's Modified Eagle's Medium
DMRT	Duncan's Multiple Range Test
DPPH	Dinitrophenyl hydrazine
EGCG	Epillogalocatechin Gallate
FDA	Food and Drug Administration
FFA	Free Fatty Acids
FRAP	Ferric Reducing Antioxidant Potential
GA	Gallic Acid
GSE	Grape Seed Extract
HCl	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
IBMX	Isobutylmethylxanthine

IDL	Intermediate Density Lipoprotein
LDL	Low Density Lipoprotein
LFO	Licorice Flavonoids Oil
LPL	Lipoprotein Lipase
MCE	<i>Momordica charantia</i> Extract
MCFE	<i>Morinda citrifolia</i> Fruit Extract
mRNA	messenger Ribose Nucleic Acid
NHMS	National Health and Morbidity Survey
PL	Pancreatic Lipase
PPAR- γ	Peroxisome Proliferator Activated Receptor
SPSS	Statistical Package for Social Sciences
TG	Triglycerides
TPC	Total Phenolic Compounds
TPTZ	Tris (2-pyridyl)-5 triazine
VLDL	Very Low Density Lipoprotein
WAT	White Adipose Tissue
WHO	World Health Organization

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CHAPTER 1

1 INTRODUCTION

Obesity is a rapidly growing epidemic worldwide, presenting an increase in the risk of morbidity and mortality in many countries across the world (World Health Organization, 2000). It is believed that today, more than 1.1 billions people are overweight worldwide and 312 million are classified as obese (Hossain et al., 2007). Complications associated with obesity, such as hypertension, hyperlipidemia, diabetes mellitus, cardiovascular disease, cancer, metabolic disorders are forcing researchers to come up with long term solutions for weight management and control (Ferraro et al., 2002; Mukherjee 2003). Unfortunately, Malaysia has not been spared by the escalating problem of obesity. The Third National Health and Morbidity Survey (NHMS III, 2001/2002) revealed that 23 % of adults were overweight and 14 % were in the obese category (Fatimah et al., 2005). Obesity has also been defined as an increased adipose tissue mass which is the result of an enlargement in fat cells and/or an increase in their number (Couillard et al., 2000). The main cause of obesity is believed to be an imbalance between energy input and energy expenditure and weight loss requires a sustained negative energy balance: energy output must exceed energy intake (Stunkard, 1996).

Although reduction of caloric intake by diet and increased level of physical activity are very well known approaches to lose weight, the needs for drugs and other supplements are fast gaining acceptance. Pancreatic lipase (PL) is one of the exocrine enzymes of



pancreatic juice that is essential for the digestion of dietary fats in the intestinal lumen. Pancreatic lipase fulfills an important function in dietary fats absorption by hydrolyzing triglycerides into diglycerides and subsequently into monoglycerides and free fatty acids (Winkler et al., 1990). As hydrolysis of dietary triacylglycerol is essential for subsequent absorption by enterocytes, inhibition of pancreatic lipase will function by reducing the availability of dietary fats calories preventing the assimilation of fats and thus mimic the effect of reduced food intake (Mukherjee, 2003).

Lipoprotein Lipase (LPL) is a non covalent homodimeric protein produced mainly by the adipose, heart and muscle tissues (Brunzell et al., 1989). LPL is the enzyme that hydrolyses triglycerides (TG) component of circulating lipoprotein, hence ensuring that fatty acids are delivered to the tissues. LPL activity in the adipose tissue and muscle will determine to what extent ingested lipids are stored or utilized, hence the amount of fat deposited in the adipose tissue. Due to this action, LPL is closely related to obesity. It was also observed that LPL activity seems relatively high in obese rats (Gamarallage et al., (2003)

Lu and co workers (2006) reviewed that fat tissues are regulated by the number and capacity of adipocytes. An increase in the proliferation and differentiation of preadipocytes may promote excess fat accumulation in the tissues. A regulation of the proliferation and differentiation of preadipocytes could mean control over fat deposition in the tissues. Moreover since the conversion from undifferentiated fibroblast-like preadipocytes into mature adipocytes, constitutes the adipocyte life cycle (Rayalam et al.,



2008), the inhibition of adipocyte differentiation can be used as a control for obesity (Okano et al., 1997).

The condition of obesity has also been linked with poor antioxidant status (Ozata et al., 2002). People with higher Body Mass Index (BMI) have been shown to have high oxidative stress in relation to the content of antioxidative compounds and minerals in the blood (Reitman et al., 2002).

Tropical countries like Malaysia are very rich in herbs and plants and much research has to be done to make maximum use of their benefits. Research in this area is very crucial as the use of medication and pharmaceuticals in the management of obesity is highly controversial and does not address a long term solution. *Momordica charantia*, (MC) Bitter melon; cucurbitacea family, locally known as “peria katak” is a vegetable indigenous to subtropical and tropical regions of South America and Asia. Fruits and seeds of this plant have been used since the dawn of time for treatment of diabetes in Southeast Asian countries (Platel and Srinivasan, 1997). *Centella asiatica* (CA) or “pegaga” is one of the local herbs that is claimed to possess various physiological effects (Zainol et al., 2003). *Centella asiatica* have been known to be used for wound healing, memory improvement, treating mental fatigue (Goh et al., 1995), allergies and cancer, especially leukemia (Kan, 1986). *Morinda citrifolia* L. (MCL) (Rubiaceae), locally known as mengkudu, has been used in folk remedies for over 2000 years. All parts of the plants, which include fruits, leaves, bark and roots have been shown to contain active compounds that have high medicinal values. Medical applications have been reported for

diabetes, antiseptic, hypertension, anticancer and antibiotic properties (Dixon et al., 1999). These plants are also known to be used as slimming aids by various communities in Malaysia.

Hence the objectives of this study were:

1. To determine the total phenolic content, flavonoids content and antioxidant activity of extracts of *Momordica charantia*, *Morinda citrifolia* Fruit and *Centella asiatica*.
2. To determine the effects of extracts on Pancreatic lipase and Lipoprotein lipase activity.
3. To determine the effects of extracts on the proliferation and differentiation of 3T3-L1 preadipocytes.

CHAPTER 2

2. LITERATURE REVIEW

2.1 Obesity and Causes of Obesity

Obesity is a rapidly growing, worldwide epidemic and increases the risk of morbidity, in many countries across the world (World Health Organization, 2000). The most common definition of obesity is the BMI, calculated as body weight in kilogram divided by the square of height in meters. Being overweight is defined as a BMI of 25.0 – 29.9 kgM⁻² and a BMI exceeding 30 kg M⁻² is considered as obese. An extreme obesity is defined as a BMI of greater than 40 kg M⁻²(Thompson et al., 2007). Today, more than 65 percent of adults in the United States are overweight or obese (National Institutes of Health, 2006). Although the problem of Obesity in Malaysia is not as severe as it is in the United States, it has been a source of worry lately for the Malaysian Government. Based on the Third National Health and Morbidity Survey (NHMS III, 2001/2002) that was carried out nationwide, the prevalence of overweight and obesity among adults aged 18 years were 23 % and 14 % respectively (Fatimah et al., 2005). Obesity is a major source of concern as it is now characterized as a chronic disease (Stunkard, 1999). A chronic disease is defined as a disruption of bodily functions that develops slowly, and persists for an extended period, often for the life of the affected individual (Butler, 1991). As described by Mukherjee (2003), not only obesity is a threat to the general health of the public but is also a risk factor for clinical disorders such as hypertension, hyperlipidemia, diabetes

