IN VITRO ASSESSMENT OF ANTIDIABETIC PROPERTIES OF HERBS AND SPICES EXTRACTS IN ADIPOCYTES.

BY

NURANIZA BINTI AZAHARI

A thesis submitted in fulfilment of the requirement for the degree of Master of Health Sciences (Nutrition Sciences)

Kulliyyah of Allied Health Sciences
International Islamic University
Malaysia

MARCH 2013

ABSTRACT

The prevalence and suffering of diabetes and obesity has been increasing among the various communities of the world including Malaysia. The cost of treatment is also rising. Therefore, it is important to explore non pharmacological regime that are non-invasive and with less health risks and cost burden to the patients, healthcare professionals and nations. Medicinal plants have been used for the treatment and prevention of diabetes since ancient time. Therefore, nine common traditional antidiabetic herbs namely *Andrographis paniculata* (Hempedu bumi), *Lagerstroemia speciosa* (Banaba/bungur), *Orthosiphon stamineus* (Cat whisker), *Peronema canescens* (Sungkai), *Momordica charantia* (Bitter gourd/bitter melon), *Tinospora crispa* (Patawali), *Pithecellobium jiringa* (Jering) and spices namely *Syzygium polyanthum* (Bay leaf) and *Cinnamomum zeylanicum* (Cinnamon), were screened for their antidiabetic properties in *in vitro* model. Water extracts of these herbs and spices were prepared and evaluated for their effects on cell proliferation, adipogenesis, adipolysis, glucose uptake and glucose oxidase assay in 3T3-L1 preadipocytes. The study was then continued with quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) for selected herbs and spices extracts for the genes; adipogenesis-regulator (*Pparγ* mRNA*),* insulin-sensitive glucose transporter 4 (*Glut4* mRNA) and gene associated with obesity and insulin resistance (*Adiponectin* mRNA). The results of aforementioned extracts promoted cell proliferation at a concentration of 0.25mg/ml which showed a maximum viability after 48 hours of treatment. Insulin and *A. paniculata* extract significantly ($p<0.05$) induced adipocyte differentiation, inhibited lipolysis and stimulated glucose uptake/oxidase in adipocytes. This activity was accompanied by significant ($p<0.05$) up-regulation of *Pparγ*, *Glut4* and *Adiponectin* transcriptional levels. This finding reveals that *A. paniculata* extract have similar effect to that of insulin activity. Whilst the study on *C. zeylanicum* and *O. stamineus* extracts have similar activity for adipogenesis significantly ($P<0.01$), stimulated glucose uptake and reduced adipolysis activity. Thus, it is suggested that these extracts might have insulin-mimicking effects which could be potentially used as preventive agents for diabetes. In contrast to insulin, water extracts of *L. speciosa* did not induced adipocyte differentiation, significantly ($P<0.01$) decreased *Pparγ* mRNA, exhibited adipolysis activity and stimulated glucose uptake/oxidase due to significant ($P<0.05$) up-regulation of *Glut4* transcriptional levels in adipocytes. This combination suggested that *L. speciosa* extract may be useful for the treatment of hyperglycemia and obesity in type 2 diabetes. It is well known fact that an appropriate balance between the adipogenesis, adipolysis and glucose uptake/oxidase in diabetes is of primary importance. The present study provides some important clues both on the biochemical and transcriptional aspects of the effect induced by the herbs and spices screened. The present study suggests that these herbs and spices possess antidiabetic properties as well as can be used for the associated metabolic dysfunctions.

ملخص البحث

إن الانتشار والمعاناة من الداء السكري والسمنة تزداد في الكثير من المجتمعات ومن ضمنها ماليزيا. تكلفة العلاج أيضاً ترتفع. لذلك من الضروري اكتشاف أنظمة دوائية لااجتياحية بأقل أخطار صحية وتكون محتملة التكلفة من قبل المريض، الاختصاصيين الصحيين وكذلك للأمم. استخدمت النباتات الطبية للعلاج والوقاية من الداء السكري منذ أقدم العصور. لهذا السبب تمت دراسة تسعة نباتات لاكتشاف خواصها المضادة للسكري في نوذج في الزجاج وهذه النباتات هي *Andrographis paniculata* (Hempedu bumi)، *Lagerstroemia speciosa* (Banaba/bungur)، *Orthosiphon stamineus* (Cat whisker)، *Peronema canescens* (Sungkai)، *Momordica charantia* (Bitter gourd/bitter melon)، *Tinospora crispa* (Patawali)، *Pithecellobium jiringa* (Jering) وأنواع وهي *Syzygium polyanthum* (Bay leaf) و *Cinnamomum zeylanicum* (Cinnamon). تم تحضير الخلاصات المائية للنباتات والبهارات المذكورة وتقييم تأثيرها على تكاثر الخلايا، تكوين الدهون، تحلل الدهون، قبض الغلوكوز ومعايرة الأكسيداز للغلوكوز في خلايا 3T3-L1 preadipocytes. كما تمت متابعة الدراسة بإجراء الـــ quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) لخلاصات نباتات وبهارات منتقاة للجينات التالية: adipogenesis-regulator (*Ppary* mRNA), insulin-sensitive glucose transporter 4 (*Glut4* mRNA) والجين المتعلق بالسمنة والمقاومة للإنسولين (*Adiponectin* mRNA). نتائج الخلاصات المذكورة حرضت تكاثر الخلايا بتركيز 0.25mg/ml والذي أظهر حيوية قصوى بعد 48 ساعة من العلاج. الإنسولين وخلاصة ال ـــ*A. Paniculata* حرضت تمايز الخلايا الدهنية على نحو هام ($p<0.05$)، ثبطت تحلل الدهون وحرضت على قبض وأكسدة الغلوكوز في الخلايا الدهنية. هذه الفعالية كانت مترافقة بزيادة التنظيم على نحو هام ($p<0.05$) للمستويات النسخية لـــ*Ppary*, *Glut4* و *Adiponectin*. تظهر هذه النتائج ان خلاصة الـــ*A. Paniculata* لها تأثير مشابه لفعالية الإنسولين. بينما الدراسة على *C. Zeylanicum* و *O. Stamineus* كان لها فعالية مشابهة لتكوين الدهون على نحو هام ($P<0.01$)، القبض المحرض للغلوكوز ونقصان تحلل الدهون. وبذلك من المقترح أن هذه الخلاصات قد يكون لها تأثيرات محاكية للإنسولين يمكن استخدامها كعوامل وقائية من الداء السكري. على خلاف الإنسولين، الخلاصة المائية للـــ*L. Speciosa* لم تحرض على تمايز الخلايا الدهنية، على نحو هام ($P<0.01$) أنقصت *Ppary* mRNA، أظهرت فعالية تحلل الدهون و حرضت على قبض؟أكسدة

الغلوكوز نتيجة زيادة التنظيم على نحو هام ($P<0.05$) للمستويات النسخية لـ*GLUT4* في الخلايا الدهنية. هذه المشاركة تقترح أن خلاصة الـ*L. Speciosa* يمكن أن تكون مفيدة لعلاج ارتفاع السكر والسمنة في الداء السكري من النمط الثاني. من الحقائق المعروفة جيداً أن التوازن المناسب بين تكون الدهون، تحلل الدهون وقبض/أكسدة الغلوكوز في الداء السكري له أهمية خاصة. تقدم هذه الدراسة بعض المفاتيح الهامة على المستويات الكيميائية الحيوية والنسخية للتأثيرات المحرضة بالأعشاب والبهارات التي تمت دراستها. وكذلك تقترح أن هذه الأعشاب والبهارات تملك خواص مضادة للداء السكري إضافة إلى أنه يمكن استخدامها في الخلل الوظيفي الاستقلابي المترافق.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Health Sciences (Nutrition Sciences).

……

Muhammad Muzaffar Ali Khan Khattak
Supervisor

……

Muhammad Taher
Co-supervisor

……

Solachuddin Arief Jauhari Ichwan
Co-supervisor

I certify that I have read this thesis and in that my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Health Sciences (Nutrition Sciences).

……

Norafiza Binti Zainuddin
Internal Examiner

……

Hamid Jan Bin Jan Mohamed
External Examiner

This thesis was submitted to the Department of Dietetics and Nutrition Sciences and is accepted as fulfilment of the requirement for the degree of Master of Health Sciences (Nutrition Sciences).

……

Wan Azdie Bin Mohd Abu Bakar
Head, Department of Nutrition Sciences

This thesis was submitted to the Kulliyyah of Allied Health Sciences and is accepted as fulfilment of the requirement for the degree of Master of Health Sciences (Nutrition Sciences).

……

Nik Mazlan Bin Nik Mamat
Dean, Kulliyyah of Allied Health Sciences

DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Nuraniza Binti Azahari

Signature …….. Date ……..

INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA

DECLARATION OF COPYRIGHT AND AFFIRMATION OF FAIR USE OF UNPUBLISHED RESEARCH

IN VITRO ASSESSMENT OF ANTIDIABETIC PROPERTIES OF HERBS AND SPICES EXTRACTS IN ADIPOCYTES

Affirmed by Nuraniza Binti Azahari.

…… ……

Signature Date

Dedicated to my beloved husband; Muhammad Hafrizan Bin Hassan, his loving contribution has no boundary and kept me going at difficult times. To my loving parents; Azahari Bin Mahasan and Che Hamidah Binti Abdullah, parents in law and siblings, who had provided me with spiritual and emotional support throughout this long journey.

ACKNOWLEDGEMENTS

Bismillahirrahmanirrahim,

In the Name of Allah S.W.T, the Most Gracious, the Most Merciful, who has given me the strength and ability to complete my research. With God's willing, this work can finally be completed. All perfect praise belongs to Allah S.W.T, Lord of the Universe.

I am grateful to express my utmost appreciation and special thanks to all of my supervisors; Assistant Professor Dr. Muhammad Muzaffar Ali Khan Khattak, Associate Professor Dr. Muhammad Taher and Assistant Professor Dr. Solachuddin Arief Jauhari Ichwan for their guidance, encouragement and supervision throughout the course of this research. Their patient and hard work will always be admired.

I am also thankful to International Islamic University Malaysia (IIUM) for funding this research. I am thankful for Department of Nutrition Sciences, Kulliyyah of Allied Health Sciences and Department of Pharmaceutical Technology, Kulliyyah of Pharmacy, International Islamic University Malaysia for giving me the opportunity to conduct and complete my research studies here.

My extended thanks go to my husband, Muhammad Hafrizan Bin Hassan for his love and patience during my study. I would also like to convey my deepest thanks to my parents, brothers and sisters for their invaluable moral supports.

Last but not least, thank you to Sis Wastuti, all of my friends and everyone who was involved and helped me in completion of this thesis.

Nuraniza Binti Azahari

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

µg	Microgram
µL	Microliter
µm	Micrometer
µM	Micromolar
ANOVA	Analysis Of Variance
C/EBP	C/AAT Enhancer Binding Protein
$CaCl_2$	Calcium chloride
cAMP	Cyclic adenosine monophosphate
cDNA	complementary DNA
DEPC	Diethyl pyrocarbonate
DEX	Dexamethasone
DM	Diabetes Mellitus
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic Acid
DNA	Deoxyribonucleic Acid
dNTP	deoxynucleoside Triphosphate
e.g.	(exempli gratia); for example
ELISA	Enzyme-Linked Immunosorbent Assay
et al.	(et alia); and others
FBS	Foetal bovine serum
FFA	Free Fatty Acid
Fig	Figure
g	Gram
g	Gravity
GDM	Gestational Diabetes Mellitus
GLUT-4	Glucose transporter-4
h	hour
H2O	Water
H_2SO_4	Sulfuric acid
HEPES	(N-[2-Hydroxyethyl]piperazine-N'- [2-ethanesulfonic acid])
HKG	Housekeeping Gene
HSL	Hormone sensitive lipase
IBMX	3-isobutyl-1-methylxanthine
IDDM	Insulin-Dependent Diabetes Mellitus
IDDM	Insulin-Dependent Diabetes Mellitus
IGF-1	Insulin-like growth factor 1
KCl	Potassium chloride
KRPH	Krebs Ringer Phosphate Hepes
L	Liter
M	Molar
MgCl2	Magnesium Chloride
MgSO4	Magnesium sulphate
MIX	Methylisobutylxanthine
mL	Mililiter

mM	Millimolar
mRNA	messenger Ribonucleic Acid
MTT	3- (4, 5-Dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide
N	Normality
Na_2HPO_4	Disodium hydrogen phosphate
Na_3VO_4	sodium orthovanadate
NaCl	Natrium Chloride
NaOH	Sodium Hydroxide
NHMS III	National Health Morbidity Survey III
NIDDM	Non-Insulin-Dependent Diabetes Mellitus
NIDDM	Non-Insulin-Dependent Diabetes Mellitus
N.T.	Not tested
nm	Nanometer
OD	Optical Density
PBS	Phosphate buffered saline
PBSA	Phosphate buffered saline A
PCR	Polymerase Chain Reaction
PenStrep	Penicillin streptomycin
pH	ATP
PKA	Protein Kinase A
PPARγ	Peroxisome proliferator-activated receptor gamma
qRT-PCR	Real Time Reverse Transcription quantitative PCR
rpm	Revolutions per minute
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
S.D	Standard deviation
TNF α	Tumor necrosis factor alpha
UV	Ultraviolet
WHO	World Health Organization

LIST OF SYMBOLS

<	Less than
>	More than
–	Similar to control
%	Percent
*	Statistical significance denotation
±	Approximation
↑	Increase activity
↓	Decrease activity
®	Registered trademark
°C	Degree Celsius
X	Times
α	Alpha
β	Beta
γ	Gamma

LIST OF APPENDICES

CHAPTER ONE

INTRODUCTION

1.1 PREVALENCE OF DIABETES MELLITUS (DM)

Currently, many countries face greater increase in the number of people suffering from DM. Diabetes is a serious condition of pancreatic beta cells dysfunction resulting in deregulation of blood glucose which leads to a serious problem of the individual in particular and of the society in general. Its rapid increase in the global prevalence is of greater concern to the countries and global communities. According to Shaw, Sicree, and Zimmet (2010), it is estimated that the world prevalence of diabetes among adults aged 20 - 79 years were affected 285 million people worldwide in 2010 and will increase to 439 million by year 2030. Within these 20 years, there will be 69% increase in numbers of adults with diabetes in developing countries and there will be 20% increase in the developed countries. The World Health Organization (WHO) estimated that 30 million people suffered from diabetes in year 1985 and the number increased to more than 171 million in year 2000. Subsequently, the incidence is expected to be 366 million by year 2030 and among them, large increase will occur in developing countries, especially among people aged between 45 - 64 years (WHO, 2002; Wild et al., 2004). Other estimates had been produced by the International Diabetes Federation (IDF) which showed that in year 2011, 366 million people suffered from diabetes, and it was expected to rise to 552 million by 2030 (Whiting et al., 2011) .

The prevalence of diabetes reported for Malaysia in National Health and Morbidity Survey (NHMS) III (2006) was 11.7% compared to 6.3% and 8.3% in

NHMS I (1986) and NHMS II (1996) respectively. The incidence is expected to increase to 13.3% in year 2030. In National Health and Morbidity Survey (NHMS) III (2006), Indians had the highest prevalence of diabetes which is 19.9% followed by Malays 11.9% and Chinese 11.4% (Letchuman et al., 2010).

The increase in DM prevalence is projected to occur because of obesity and sedentary lifestyle. Obesity and type 2 DM are generally considered being multifactorial and polygenic diseases associated with an increased risk of mortality and morbidity. According to World Health Report 2002, approximately 58% of diabetes globally can be attributed to body mass index (BMI) above 21 kg/m^2. The prevalence of obesity in Malaysia has increased from 4.4% in 1996 to 14.0% in 2006 with highest prevalence of 19.3% seen among adults aged between 45 - 49 years old. Based on the Malaysian National Health and Morbidity Survey (NHMS), the number of overweight and obese adult males increased from 20.1% and 4.0% respectively, in 1996 to 29.7% and 10.0% respectively, in 2006. The prevalence was found to be higher among adult females from 7.6% in 1996 and increased to 17.4% in 2006 (Mohamud et al., 2011).

1.2 ETIOLOGY OF DIABETES MELLITUS

Diabetes mellitus is a metabolic disorder of multiple etiologies characterized by elevated plasma glucose concentrations resulting from defects in insulin secretion, insulin action or both that can lead to metabolic abnormalities in carbohydrates, proteins and lipids (World Health Consultation, 1999). This situation can also cause body tissues in particular, the muscle, adipose tissues and liver fail in the uptake and utilizing of glucose from the blood circulation due to lack or abnormality in insulin

molecule. Thus, resulting in elevated blood glucose concentration known as hyperglycaemia. If blood glucose levels remain higher over a longer period of time, this can result in a damage of important organs in the body such as nerves, kidneys, eyes, blood vessels and heart. Complications to these organs can lead to death (Brownlee, 2001; Hirsch, 1995; Weiss & Sumpio, 2006). Diabetes may be asymptomatic or may be associated with symptoms like thirst, polydipsia, polyuria, sudden weight loss or may progress to ketoacidosis and coma, depending on the severity of the metabolic abnormality. There are three main types of diabetes mellitus which are type 1, type 2 and gestational diabetes mellitus.

Type 1 diabetes is also known as insulin-dependent diabetes mellitus (IDDM), immune-mediated or juvenile-onset diabetes. This disease can affect people at any age, but it is often present in children or young adult. It is caused by an auto-immune reaction where the body's defence system attacks their own cell, which is insulin-producing cells (ß-cells in the Islets of Langerhans) pancreas. This reaction can cause people with type 1 diabetes to produce very little or no insulin (Williamson et al., 1996).

Type 2 diabetes is also known as non-insulin dependent diabetes mellitus (NIDDM) or adult-onset diabetes. It accounts for at least 90% of all cases of diabetes which sometimes associated with people who are obese or overweight. Type 2 diabetes can cause insulin resistance and lead to elevated blood glucose levels. This disease is caused by a relative deficiency or diminished effectiveness of insulin. Treatment for type 2 diabetes are oral hypoglycaemic drugs, sugar restricted diet and complex carbohydrate preparation which delay the absorption of glucose into the gut (Williamson et al., 1996).

Gestational diabetes mellitus (GDM) is caused by high blood glucose levels during pregnancy. International Diabetes Federation (2011) reported that GDM usually develops in 1 of 25 pregnancies worldwide and usually disappears after delivery. But, some of the individuals with GDM and their children are at increased risk of developing type 2 diabetes in their later life.

1.3 RISK FACTORS

Risk factors associated with diabetes mellitus can be categorized into modifiable and non-modifiable factors. Modifiable risk factors are including diet, obesity, physical inactivity and westernization in lifestyle. However, age, ethnicity, family history and history of gestational diabetes mellitus are the main non-modifiable determinants of diabetes prevalence (Colagiuri et al., 2006; Libman and Arslanian, 2007).

1.4 MANAGEMENT OF DIABETES MELLITUS

Currently, available therapies to manage diabetes mellitus are dietary modification, exercise, modern drugs including insulin and oral administration of hypoglycaemic agents such as metformin, sulfonylureas and acarbose etc. Insulin plays a key role in glucose homeostasis and counter regulatory hormone like glucagon, which raises serum glucose. According to Bailey (1999), the standard approach begins with healthy lifestyle which includes diet control and exercise, particularly designed to facilitate weight loss in obese people. In general, insulin therapy has been considered to be the last therapeutic option when diet control, exercise and oral hypoglycaemic agent/therapies or combination of two different classes of oral drugs have failed. Sometimes, insulin therapy is also supplemented with an oral agent to further improve glycaemic control (Figure 1.1). However, there are still challenges for the medical

system to find out treatment and management for diabetes without any side effects. This leads to an increasing research to improve commercially available drugs. Apart from this, traditionally plants are also used to improve control over hyperglycaemia worldwide (Koski, 2006; Pari and Saravanan, 2004).

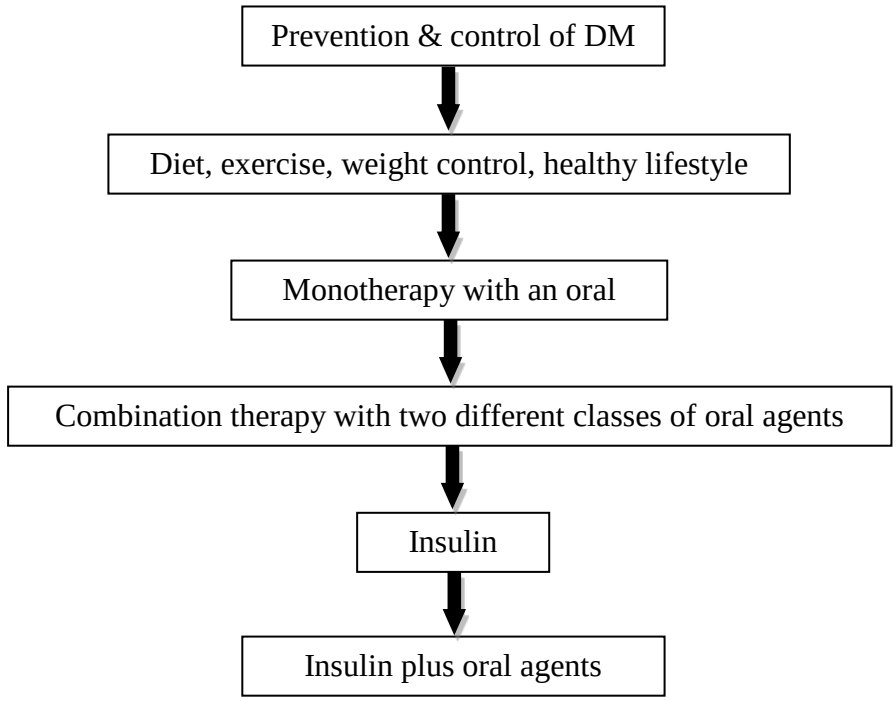

Figure 1.1: Typical treatment paradigm for type 2 diabetes mellitus. When the patient exhibits inadequate glycaemic control, he/she is moved to the next treatment level. Patients with severe complications may be "jumped" immediately to insulin therapy. Modified from Bailey, (1999)

1.4.1 Mechanism of Action of Conventional Oral Hypoglycaemic Drugs

Oral hypoglycaemic agents control blood glucose level through variety of mechanisms/ actions (Figure 1.2). Metformin is a biguanide agent that lowers the hepatic glucose production as well as reduces insulin resistance. Sulphonylurea acts as insulin secretagogue by increasing insulin secretion. Thiazolidinediones promote