Abstract

Leucaena leucocephala (Lam.) deWit (Leguminosae) also known as 'Petai Belalang' is a fast growing shrub or tree found in tropical and subtropical areas. Its fruit has been consumed as food by people in Malaysia, Thailand, Phillipines, Indonesia and Mexico. In Indonesia and Mexico the fruits have been used to treat diabetes. The aim of this study was to assess *in vivo* antioxidant and anti-diabetic potential of *L. leucocephala.* Preliminary screening was done using *in vitro* antioxidant (data not shown), complemented by food nutrition analyses. The anti-diabetic *in vitro* study was conducted using adipocyte cellular model and RT-PCR method, and the *in vivo* efficacy of the extract was assessed using OGTT method as well as oral feeding in Streptozocin-induced diabetic rats. The finding exhibited that *L. leucocephala* dry fruit water extract posessed high phenolic content and rich in vitamin B_2, calcium and magnesium. In preliminary adipocyte cellular study, *L. leucocephala* up-regulated GLUT-4 gene expression twofold which implied the potential to facilitate glucose uptake. In OGTT, *L. leucocephala* fruit showed significant reduction in glucose level at the 60^{th} minute ($p < 0.05$) in normal rats and exhibited glucose lowering effect comparable to Glibenclamide (no significant difference at the 150^{th} minute) in diabetic rat. Thus, *L. leucocephala* posess hypoglycemic activity *in vitro* and *in vivo* which was further investigated in chronic study (30 days). *L. leucocephala* dry fruit water extract were partially purified to separate bioactive compounds using solvent extraction (hexane and ethyl acetate) and the bioactivity was confirmed using sub-chronic study (7 days) in Streptozocin-induced diabetic rats. Both chronic and sub-chronic study showed that *L. leucocephala* water extract prevented worsening of hyperglycemia and its partially purified extract exerted hypoglycemic effects ($p < 0.001$), respectively. Both studies also showed stimulation in secretion of insulin possibly by exerting protection against pancreatic damage. In

chronic study (30 days), *L. leucocephala* dry fruit water extract restored body weight and stimulated adiponectin levels. Lipid and protein damages in the brain and kidney were significantly reduced in diabetic rats treated with *L. leucocephala* extract. In *L. leucocephala* water-hexane partially purified extract treated rats, a high total non-enzymic antioxidant level and high glutathione peroxidase activity were observed. This extract was subjected to further identification of bioactives compounds using HPLC and LC-MS. The results revealed that the active compound is a polar compound with a molecular weight of 217.0937 g/mol and molecular formula of $C_7H_{13}N_4O_4$. However the exact structure could not be identified as it required more extensive investigation and is beyond the scope of this study. In conclusion, *Leucaena leucocephala* has a potential to be developed as an anti-diabetic agent.

Abstrak

Leucaena leucocephala (Lam.) deWit (Leguminosae) atau telah dikenali sebagai Petai Belalang adalah tumbuhan renek atau pokok yang mempunyai tumbesaran yang cepat dan ditemui di kawasan-kawasan tropika dan sub-tropika. Buahnya dimakan sebagai makanan oleh orang di Malaysia, Thailand, Filipina, Indonesia and Mexico. Di Indonesia dan Mexico buah tersebut telah digunakan untuk merawat penyakit diabetes. Tujuan kajian ini adalah untuk menilai potensi antioksidan dan anti-diabetik *L. leucocephala.* Saringan awal telah dilakukan menggunakan ujian antioksida *in vitro* (data tidak ditunjukkan), dilengkapkan dengan analisis nutrisi pemakanan. Kajian anti-diabetik *in vitro* telah dijalankan menggunakan model sel adipos dan kaedah RT-PCR, dan keberkesanan ekstrak secara *in vivo* telah dinilai menggunakan kaedah OGTT serta secara oral ke atas Streptozocin-induksi tikus diabetik. Hasil telah menunjukkan bahawa ekstrak air buah kering *L. leucocephala* memiliki kandungan fenol yang tinggi dan kaya dengan vitamin B_2, kalsium dan magnesium. Pada kajian awal sel adipos, *L. leucocephala* telah meningkatkan ekspresi gen GLUT-4 sebanyak dua kali ganda yang menunjukkan potensi untuk memudahkan pengambilan glukosa. Pada kajian OGTT, buah *L. leucocephala* telah menunjukkan penurunan signifikan dalam tahap glukosa pada minit ke 60 ($p < 0.05$) dalam tikus normal dan mempamerkan kesan penurunan glukosa setanding dengan Glibenclamide (tidak menunjukkan perbezaan signifikan pada minit ke 150) dalam tikus diabetik. Dengan ini, *L. leucocephala* memiliki aktiviti hipoglisemik secara *'in vitro'* dan '*in vivo'* yang mana penyelidikan telah dilanjutkan dalam kajian kronik (30 hari). Ekstrak air buah kering *L. leucocephala* telah disepara tulenkan untuk mengasingkan kandungan bioaktif menggunakan pengekstrakan pelarut (heksana dan etil asetat) dan bioaktiviti telah disahkan menggunakan kajian separa kronik (7 hari) keatas Streptozocin-induksi tikus diabetik. Kedua-dua kajian kronik dan

sub-kronik telah menunjukkan bahawa ekstrak air buah kering *L. leucocephala* telah mencegah pemburukan hiperglisemia dan ekstrak separa tulenannya telah menghasilkan kesan hipoglisemia ($p < 0.001$). Kedua-dua kajian juga telah menunjukkan stimulasi terhadap perembesan insulin yang mungkin diberikan oleh perlindungan daripada kerosakan pankreas. Dalam kajian sub-kronik (30 hari), ekstrak air buah kering *L. leucocephala* telah memelihara berat badan dan merangsang paras adiponektin. Kerosakan lipid dan protein di dalam otak dan buah pinggang ketara berkurangan dalam tikus-tikus diabetik yang telah dirawat dengan ekstrak *L. leucocephala.* Dalam kumpulan tikus yang telah dirawat dengan ekstrak separa penulenan air-heksana *L. leucocephala* telah menunjukkan jumlah antioksidan bukan enzim dan aktiviti glutatione peroksida yang tinggi. Ekstrak ini telah dipilih untuk identifikasi komposisi-komposisi bioaktif menggunakan HPLC dan LC-MS. Keputusan mendedahkan bahawa komposisi bioaktif tersebut adalah sebatian polar dengan berat jisim molekul 217.0937 g/mol dan formula molekul $C_7H_{13}N_4O_4$. Walaubagaimanapun, struktur sebenar tidak dapat dikenalpasti kerana ia memerlukan penyelidikan yang lebih luas dan ia di luar skop kajian ini. Kesimpulannya, *Leucaena leucocephala* mempunyai potensi untuk dibangunkan sebagai agen anti-diabetik.

Acknowledgements

I am very grateful to the Mighty Lord for giving me an opportunity in this life to learn and understand more about science. To my dearest parents, siblings and loved ones; Mohd. Ridzwan thank you so much for your continuous support and care at all times.

I wish to express my greatest gratitude to my main supervisor, Professor Umah Rani Kuppusamy for her tireless, patience, invaluable guidance and believe in me. Also, special thanks goes to my co-supervisor Professor Mahmood Ameen Abdulla, the workaholic and helpful person who helped and guided me tirelessly to complete all the *in vivo* experiments successfully. I wish to express my deepest appreciation and gratitute to both my supervisors for making my dream come true.

This works would not be completed without the supports of my postgraduate mates Dr. Chai Jen Wai, Dr. Chandra, Ms. Dahlia, Dr. Lee Ai Li, Mrs. Lim Siang Ling, Mrs. Nazratun Nafizah and Ms. Bahiyah, Mrs. Siti Zainab and all my tutor-mates.They all are such a wonderful friends and thanks God that i have them around me.

My gratitude also goes to the senior staff in Molecular Medicine Department, Ms. Kim and all support staff, Clinical Diagnostic Laboratory (CDL) and animal house staff especially Mrs. Hidaitul Alaina for expert technical support. My warmest thanks also goes to Ms. Suzainor and Dr. Dharmani for sharing and exchanging ideas.

This study was supported by University Malaya under research fund PPP PS124 / 2008C.

TABLE OF CONTENTS

Content **Page**

Content **Page**

Content **Page**

Content **Page**

Content **Page**

Content **Page**

Content **Page**

Content **Page**

Content **Page**

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS AND ABBREVIATIONS

Symbol/Abbreviations	Description
+	Plus
–	Minus
-	Negative
±	Plus Minus
=	Equal
<	Less
≥	Equal or more
%	Percent
ºC	Degree Celcius
α	Alpha
β	Beta
δ	Gamma
ΔC_T	Delta Ct
$\Delta\Delta C_T$	Delta-delta-Ct
$2^{-}\Delta\Delta CT$	Log scale, base 2.Delta-delta-Ct
CT	Cycle threshold
µ	Micro
µl	Microlitre
µm	Micrometer
µg/ml	Microgram per millilitre
µM	Micromolar
µg/ml	Microgram per millilitre
IV	Four
xg	Times gravity
3T3-L1	Original Cell line derived from 3T3-Swiss albino rats
A	Absorbance
AACC 32-10	American Association of Cereal Chemists Method 32-10
Acrp30	Adipocyte Complement Related Protein of 30 kD
ACTH	Adrenocorticotropic hormone
ADD1	Adipocytes Determination and Differentiation Factor 1
AdipoR2	Adiponectin receptor
ADP	Adenosine diphosphate
apM1	Adipose most abundant gene transcript 1
AGE	Advanced Glycated Endproduct
AKT	Protein Kinase B
ANOVA	Analysis of Variance
AOAC	Association of Official Agricultural Chemists
AOPP	Advanced Oxidation Protein Product
AOX	Antioxidant
Aq	Aqueous
ATP	Adenosine triphospate
BHA	Butylated hydroxyanisole
BSA	Bovine Serum Albumin
$C_8H_{15}N_3O_7$	2-deoxy-2-(3-(methyl-3-nitrosoureido)-D glucopyranose
CAT	Catalase
CAM	Complimentary and Alternative Medicine

Symbol/Abbreviations	**Description**
CBBG	Coomassie brilliant blue G-250
CDL	Clinical Diagnostic Laboratory
cDNA	Complimentary DNA
c GMP	Cyclic Guanosine Monophosphate
CVs	Coefficient of Variances
D	Diabetic rats
Da	Dalton
DAG	Diacylglycerol
DM	Diabetes Mellitus
DM1	Diabetes Mellitus type 1
DMEM I	Dulbecco's Modified Eagle's Medium I
DMEM II	Dulbecco's Modified Eagle's Medium II
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
DPX	Distyrene, plasticizer,xylene
EAR	Estimated Average Requirement
ELISA	Enzyme Linked Immunosorbent Assay
eNOS	Endothelial Nitric Oxide Synthase
EPR	Electron Paramgnetic Resonance
Et	Ethyl acetate
FAS	Fatty Acid Synthase
FBG	Fasting Blood Glucose
FBS	Fetal Bovine Serum
Fe(II)	Ferrous ion
$FeCL_3.6H_2O$	Ferric chloride
FRAP	Ferric Reducing Antioxidant Power
$FeSO_4.7H2O$	Ferrous sulfate heptahydrate
g	Gram
g/kg	Gram per kilogram
g/100g	Gram per hundred gram
g/day	Gram per day
G	Glibenclamide
GAEs	Gallic Acid Equivalents
GC	Gas Chromatography
G 1.25 mg/kg	Glibenclamide at dose of 1.25 milligram per kilogram
G6Pase	Glucose-6-Phosphatase
GlcNAc	N-acetylglucosamine
GLUT-2	Glucose Transporter 2
GLUT-4	Glucose Transporter 4
GPx	Glutathione Peroxidase
GSH	Glutathione (reduced)
GSSG	Glutathione Disulphide (oxidized)
GSSG-R	Glutathione Reductase
H_2O_2	Hydrogen Peroxide
Hb	Haemoglobin
HDL	High Density Lipoprotein
He	Hexane
His	Histidine
HM	Hydrophobic Motif

Symbol/Abbreviations	Description
HOCl	Hypochlorous Acid
HPLC	High Performance Liquid Chromatography
HRP	Horse Reddish Peroxidase
HSL	Hormone Sensitive Lipase
H + E	Heamatoxylin and Eosin
ICP-OES	Inductively coupled plasma atomic emission spectroscopy
ID	Identify
IDDM	Insulin Dependent Diabetes Mellitus
IgG	Immunoglobulin G
IL-6	Interleukin 6
IRS-1	Insulin Receptor Substrate-1
K	Pottassium
K ATP	ATP-sensitive potassium
KK/Ay	KK mice with Ay alelle congenic strains
LD_{50}	Median Lethal Dose
LDL	Low Density Lipoprotein
LIPE	Lipase
LL	*Leucaena leucocephala*
LY294002	2-morpholin-4-yl-8-phenylchromen-4-one
Lys	Lysine
M	Molar
MCP-1	Monocyte chemotactic protein-1
MDA	Malodialdehyde
mg/100g	Milligram per hundred gram
mg kg^{-1}	Milligram per kilogram
mRNA	Messenger Ribonucleic Acid
ml	Millilitre
mg/dL	Milligram per decilitre
mg/mL	Milligram per mililitre
mM	Millimolar
mins	Minutes
min/mg	Minute per milligram
ml/kg	Mililitre per kilogram
mmol/L	Milimolar per Litre
ng/ml	Nanogram per mililitre
nmol	Nanomolar
N	Normal rats
Na_2CO_3	Disodium carbonate
NAD^+	Nicotinamide Adenine Dinucleotide
$NADP^+$	Nicotinamide Adenine Dinucleotide Phosphate
NADPH	Nicotinamide Adenine Dinucleotide Phosphate(reduced)
NIDDM	Non- Insulin Dependent Diabetes Mellitus
nm	Nanometer
$O_2^{\bullet-}$	Superoxide anion species
•OH	Hydroxyl radical
ONOO-	Peroxynitrite
NO	Nitric oxide
NOS	Nitric Oxide Synthase

Symbol/Abbreviations	Description
O-GlcNAc	O-linked N-acetylglucosamine
O-GlcNAcase	Enzyme of O-linked N-acetylglucosamine
OGTT	Oral Glucose Tolerance Test
P	Probability
p110	Catalytic subunit
p85	Regulatory subunit
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PDK 1	Phosphoinositide-dependent kinase-1
PDE3B	Phosphodiesterases
PEPCK	Phosphoenolpyruvate carboxykinase
pH	Power of Hydrogen
PH	Pleckstrin Homology
Phe	Phenylalanine
PKB	Protein Kinase B
PKC	Protein Kinase C
PPAR ∝	Peroxisome Proliferator-Activated Receptor Gamma
PUFA	Polyunsaturated Fatty Acids
PI3K	Phosphatidylinositol 3-kinases
PIP	Porcine Insulin Precursor
PtdIns(4,5)	Phosphatidylinositol 4,5-bisphosphate
PtdIns(3,4,5) P_3	Phosphatidylinositol 3,4,5-trisphosphate
PtdIns(4,5) P_2	Phosphatidylserine
qRT-PCR	Quantitative Real-Time PCR
r	Correlation coefficient
®	Registered trademark
R^2	Coefficient of determination
RBC	Red Blood Cells
RDA	Recommended Daily Allowance
RDV	Recommended Daily Value
Rn	*Rattus norvegicus*
RNA	Ribonucleic acid
RNS	Reactive Nitrogen Species
RO•	Alkoxy
ROO•	Peroxy radicals
ROOH	Organic hydroperoxide
ROS	Reactive Oxygen Species
rpm	Revolutions per minute
RPMI	Roswell Park Memorial Institute
rRNA	Ribosomal Ribonucleic Acid
RT	Reverse transcription
RT-PCR	Reverse transcription Polymerase Chain Reaction
SD	Standard Deviation
sdH_2O	Sterile distilled water
SEM	Standard Error Mean
sec	Second
SLC2A4	Gene encoded GLUT 4
SOD	Superoxide Dismutase
SPSS	Statistical Package for Social Sciences

Symbol/Abbreviations	**Description**
SREBP	Sterol Regulatory Element Binding Proteins
STZ	Streptozotocin
TBA	Thiobarbituric Acid
TBARS	Thiobarbituric Reactive Substances
TCA	Trichloroacetic Acid
TG	Triacylglycerol
TGFβ	Transforming Growth Factor Beta
™	Trade mark
TNF	Tumor Necrosis Factor
TPTZ	Tripyridyltriazine @ 2,4,6-tripyridyl-s-triazine
Tyr	Tyrosine
UDP	Uridine diphosphate
UMMC	University Malaya Medical Centre
UK	United Kingdom
USA	United State America
USRDA	Universal Standard Recommended Daily Allowance
VEGF	Vascular Endothelial Growth Factor
Vers	Version
VIC/MGB	Probe labeled with VIC™ dye – MGB
v/v	Volume per Volume
WHO	World Health Organization
w/v	Weight per Volume
X	Times
XOD	Xanthine Oxidase