UNIVERSITI PUTRA MALAYSIA

GLUCOSE UPTAKE MECHANISM OF MUSCLE CELLS AND ADIPOCYTES STIMULATED BY *SCOPARIA DULCIS* LINN EXTRACT

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By
BEH JOO EE

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfillment of the requirements of the Degree of Master of Science

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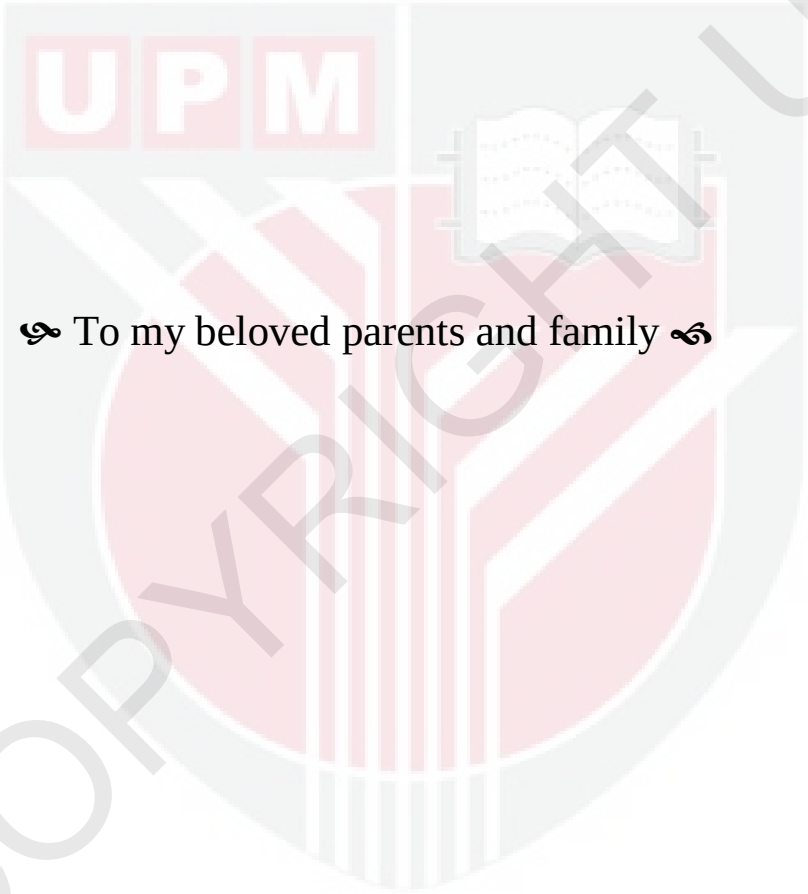
Dedication



ঌ To my beloved parents and family ঌ

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of requirement for the degree of Master of Science

GLUCOSE UPTAKE MECHANISM OF MUSCLE CELLS AND ADIPOCYTES STIMULATED BY *SCOPARIA DULCIS* LINN EXTRACT

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April 2010

Chairman: Associate Professor Muhajir Hamid, PhD

Faculty: Faculty of Biotechnology and Biomolecular Sciences

Diabetes mellitus is a metabolism disease which is mainly caused by glucose uptake disorder and decrease of body peripheral cells insulin sensitivity. The aim of the study is to investigate the effect of *Scoparia dulcis* Linn extracts on glucose uptake mechanism of L6 myotubes and 3T3-F442a adipocytes. In this study, the major problems that need to clarify are cytotoxicity of herbal extracts, complexity of the extracts and cellular protein fractionation.

Cytotoxicity studies showed that the water extract of *S. dulcis* L. leaves showed less toxicity effect on L6 myotubes and 3T3-F442a adipocytes compared with other extracts, which were from petroleum ether, ether acetate and ethanol. Among the extracts, the leaves water extract showed the maximum cell viability from the Trypan blue exclusion test, the highest inhibitory concentration 50 (IC_{50}) from the MTT assay and the lowest tail moment from the Comet assay.

The 2-deoxy-D-[^{3}H] glucose uptake assay showed that the leaf water extract significantly enhanced glucose uptake activity on L6 myotubes and 3T3-F442a adipocytes. The result confirmed that the maximum glucose uptake activity due to direct stimulation of TLC-separated fraction 7 (SDF7) of the crude extract on these cells. Four active compounds were successfully identified from SDF7 fraction using C18 reverse phase of HPLC, Mass Spectrometry and NMR assessments.

Further studies were carried out to investigate the stimulation effects of glucose uptake mechanism by SDF7 on L6 myotubes and 3T3-F442a adipocytes separately using immunoblotting assay. This study emphasized the allocation and expression of the important downstream effectors of insulin signaling pathway concomitant with the plasma membrane Glut 4 translocation in the SDF7-treated L6 myotubes and 3T3-F442a adipocytes. So, these peripheral cells were divided into four cellular fractions which are plasma membrane fraction, cytosolic fraction, high density fraction and low density fraction using an ultracentrifugation with the different methods approach.

For L6 myotubes, the results showed that SDF7 was able to stimulate the expression of IRS-1, PI 3-KINASE, PKB/Akt 2 and TC 10 on the plasma membrane fraction of these cells and triggered the translocation of Glut 4 from the intracellular pool to the plasma membrane of L6 myotubes through an immunoblotting study. Furthermore, the expression of Glut 4 protein on the L6 myotubes plasma membrane was confirmed by an immunofluorescence assay. Glycogen was accumulated in L6 myotubes after being

stimulated by of SDF7 as demonstrated by the DNS colorimetric assay and PAS staining assay.

An immunoblotting assay demonstrated that the translocation of Glut 4 was accompanied by the expressions of IRS-1, PI 3-Kinase, PKC and TC 10 that traffick from the intracellular pool to 3T3-F442a adipocytes' plasma membrane when treated with SDF7. Further study was performed to evaluate the amount of Glut 4 on plasma membrane by using an immunofluorescence quantification assay. The results showed that there were a significant increased of Glut 4 on SDF7-treated 3T3-F442a adipocytes membrane in time-dependent and concentration-dependent mode. In addition, the level of adiponectin was increased whereas the level of leptin and TNF-α were decreased on 3T3-F442a adipocytes in response to the SDF7 treatment as evaluated by an ELISA assay. Adiponectin and PPAR-γ mRNA level were being significantly up-regulated in the SDF7-treated 3T3-F442a adipocytes using the Quantigen 2.0 mRNA assay.

As the conclusion, *Scoparia dulcis* Linn has a potential to be categorized as a hypoglycemic medicinal plant based on its good glucose transport activity and Glut 4 translocation on muscle cells and adipocytes.

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

KESAN RANGSANGAN EKSTRAK *SCOPARIA DULCIS* KE ATAS MEKANISMA PENGAMBILAN GLUKOSA OLEH SEL-SEL OTOT DAN ADIPOS

Oleh

BEH JOO EE

April 2011

Pengerusi: Profesor Madya Muhajir Hamid, PhD

Fakulti : Fakulti Bioteknologi dan Sains Biomolekul

Penyakit kencing manis merupakan sesuatu penyakit metabolik yang disebabkan oleh gangguan pengambilan glukosa dan kekurangan sensitiviti terhadap insulin pada sel-sel persisian di badan manusia. Tujuan pengajian ini adalah untuk menyelidik kesan-kesan *Scoparia dulcis* Linn ekstrak terhadap mekanisma pengambilan glukosa daripada sel-sel otot L6 dan sel-sel adipos 3T3-F442a. Dalam kajian tersebut, masalah-masalah utama yang dihadapi seperti kesan sitotoksik herba ekstrak terhadap sel-sel yang diuji, kerumitan herba ekstrak dan protein fraksinasi perlu ditangani dan dijelaskan dengan segera.

Kajian ini menguji kesan masa dan ruang khusus kepada mekanisma pengambilan glukosa yang dirangsangkan oleh ekstrak mentah *Scoparia dulcis* Linn. (Salam Baik) pada sel otot L6 dan sel adipos 3T3-F442a. Kajian sitotoksik jelas menunjukkan bahawa ekstrak air *S. dulcis* L. menyebabkan ketoksikan yang kurang berbanding dengan ekstrak-

ekstrak daripada pelarut-pelarut yang lain seperti petroleum eter, eter asetat dan etanol pada sel-sel otot dan sel-sel adipos. Di kalangan ekstrak tersebut, bahagian daun daripada ekstrak air *S. dulcis* L. menunjukkan maksima kebolehidupan sel dengan ujian pengecualian Trypan biru, kepekatan perencatan 50 (tindakan keracunan yang menyebabkan jumlah daripada 50 peratusan sel-sel dibunuh) yang paling tinggi pada ujian MTT dan ekor masa yang terendah pada ujian Komet berbanding dengan ekstrak yang lain.

Kajian lanjutan telah dilakukan ke atas asai pengambilan 2-deoxy-D-[^{3}H] glukosa pada ekstrak air bahagian daun daripada *Scoparia dulcis* telah menunjukkan aktiviti pengambilan glukosa yang ketara pada kedua-dua sel otot L6 dan adipos 3T3-F442a. Keputusan telah membuktikan bahawa aktiviti pengambilan glukosa yang maksima adalah disebabkan oleh rangsangan *Scoparia dulcis* L. fraksi 7 (SDF7) daripada teknik pemisahan TLC-penyiapan pada sel-sel tersebut. Selain daripada ini, kajian ini berjaya mengenalpasti empat sebatian aktif daripada SDF7 dengan menggunakan frasa terbalik C18 HPLC, spektrometri jisim dan penilaian NMR.

Seterusnya, kajian lanjut telah menjuruskan kepada kesan stimulasi daripada mekanisma pengambilan glukosa oleh SDF7 pada sel otot L6 myotubes dan sel adipos 3T3-F442a secara berasingan. Kajian ini buat kali pertama menitikberatkan lokasi dan penzahiran unsur-unsur pengesanan daripada hiliran pengisyaratan insulin serta mengaktifkan pengangkutan Glut 4 dalam sel otot L6 dan sel adipos 3T3-F442a yang telah diberikan rawatan SDF7 pada waktu tertentu. Oleh itu, sel-sel tersebut telah dibahagikan kepada

empat bahagian selular, antaranya adalah bahagian membrane sel, bahagian sitosol, bahagian ketumpatan tinggi dan bahagian ketumpatan rendah dengan menggunakan pelbagai jenis teknik pemisahan dalam pengemparan ultra.

Untuk sel otot L6, keputusan telah menunjukkan bahawa fraksi SDF7 mampu memberangsangkan penampilan IRS-1, PI 3-kinase, PKB / Akt 2 dan TC 10 pada bahagian sel membran dan mencetuskan pengangkutan Glut 4 yang bermakna dari kawasan intraselular ke sel membran sel otot L6 melalui ujikaji immunoblot. Selain itu, penzahiran protein Glut 4 pada sel membran daripada sel-sel otot L6 sekali lagi disahkan dengan menggunakan asai imuno-pendarfluo. Asai pengukuran warna DNS dan pewarnaan PAS telah menunjukkan bahawa terdapat pengumpulan glikogen yang nyata di sel-sel otot L6 selepas dirawati oleh SDF7.

Asai imunoblot menunjukkan bahawa pengangkutan Glut 4 adalah diseringi oleh penzahiran IRS-1, PI 3-kinase, PKC dan TC 10 yang telah diangkutkan dari kawasan intraselular ke sel membran pada sel-sel adipos 3T3-F442a. Kajian yang lebih lanjut telah dilakukan untuk menilai jumlah Glut 4 pada sel plasma dengan menggunakan asai imuno-pendarfluo. Keputusan-keputusan tersebut memaparkan bahawa terdapat peningkatan bilangan Glut 4 yang bermakna pada sel membran daripada sel-sel adipos 3T3-F442a yang dirawati oleh SDF7. Tambahan pula, terdapat peningkatan paras perembesan adiponektin and pengurangan paras perembesan leptin dan TNF-α yang nyata dalam sel-sel adipos 3T3-F442a yang dirangsangkan oleh SDF7 melalui asai ELISA. Paras mRNA adiponektin dan PPAR-γ telah ditingkatkan secara ketara setelah

memberikan perawatan SDF7 kepada sel-sel adipos 3T3-F442a yang diuji dengan menggunakan asai 2.0 mRNA Quantigen.

Kesimpulannya, *Scoparia dulcis* Linn berpotensi dikelaskan sebagai tumbuhan perubatan hipoglisemia yang baik dengan kesan pengambilan glukosa dan kesan-kesan biologikal lain terhadap sel-sel otot and adipos yang telah dihuraikan dalam kajian tersebut.

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I want to give a special appreciation to my parents and brother for their endless supports and encouragements in this project.

APPROVAL

I certify that an Examination Committee has met on 25 April 2011 to conduct the final examination of Beh Joo Ee on his Master of Science thesis entitled "Glucose Uptake Mechanism of Muscle Cells and Adipocytes that Stimulated by *Scoparia dulcis* Linn Extract" accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follow:

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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 follow:

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Date:

DECLARATION

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

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BEH JOO EE

Date: 25 April 2011

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