UNIVERSITI PUTRA MALAYSIA

EVALUATION OF ANTIHYPERGLYCEMIC ACTIVITY OF FICUS DELTOIDEA JACK AND ELUCIDATION OF ITS ANTIDIABETIC MECHANISMS USING IN VITRO MODEL

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FBSB 2012 24

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DOCTOR OF PHILOSOPHY
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2012

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

February 2012

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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Faculty: Biotechnology and Biomolecular Sciences

Diabetes mellitus is a metabolic disease characterized by persistent hyperglycemia. It is the first leading causes of death in developed country and has been an epidemic in many developing countries including Malaysia. This disease remains as a major global health problems even though many antidiabetic drugs are available. This could possibly be due to the limitations of these drugs such as adverse effects and poor clinical efficacy. Therefore, searching for new antidiabetic drugs should be continued. *Ficus deltoidea* or locally known as Mas cotek is one of the common medicinal plant used in Malaysia. It has been traditionally claimed to possess antidiabetic activity. However, the scientific studies to confirm its efficacy and its possible mode of actions are still lacking.

This study was carried out to authenticate the antidiabetic property of *F. deltoidea* through *in vivo* antihyperglycemic evaluation and to elucidate its possible antihyperglycemic mechanisms using *in vitro* model. *In vivo* antihyperglycemic evaluation was performed in adult male Sprague Dawley rats. Two modes of treatment were applied; acute and sub-chronic. Antihyperglycemic evaluation following acute treatment was carried out in normal and streptozotocin-induced diabetic rats at three different prandial states; fasting, postprandial and post-glucose loaded state. Antihyperglycemic evaluation following sub-chronic treatment (15-days) was carried out in streptozotocin-induced diabetic rats. *In vitro* antihyperglycemic mechanisms evaluation of *F. deltoidea* was carried out to elucidate the potential of the plant to stimulate insulin secretion from pancreatic β-cells, to enhance glucose uptake by adipocytes, muscle and liver cells, to augment adiponectin secretion from adipocytes cells and to delay glucose absorption from small intestine by inhibiting α-glucosidase (sucrase) activity. The viability of cells that were used in the *in vitro* evaluation of antihyperglycemic mechanisms in the presence of *F. deltoidea* extracts was determined using MTT assay.

The results had shown that acute treatment of hot aqueous and ethanolic extracts of *F. deltoidea* reduced fasting and postprandial hyperglycemia and improved glucose tolerance activity in normal and streptozotocin-induced diabetic rats. Furthermore, following 15-days treatment, hot aqueous extract reduced fasting hyperglycemia and stimulated insulin secretion in streptozotocin-induced diabetic rats. Both extracts did not reduced blood glucose level below the normal range. Antihyperglycemic

mechanisms elucidation had revealed that hot aqueous, ethanolic and methanolic extracts of *F. deltoidea* have significantly stimulated insulin secretion from pancreatic β-cells. Among all extracts, the hot aqueous stimulated the insulin secretion the most and was further evaluated for determination of insulin secreting mechanisms. The result revealed that the insulin secretory action of hot aqueous extract involved K^+_{ATP} channel-dependent and K^+_{ATP}- channel independent pathway. The extract also has the ability to induce the uses of intracellular Ca^{2+} to trigger insulin release.

Hot aqueous, ethanolic and methanolic extracts of *F. deltoidea* significantly enhanced basal and insulin-mediated glucose uptake into adipocytes, muscles and liver cells. The extracts showed either insulin-mimetic or insulin-sensitizing activity or combination of both activities during enhancing glucose uptake into these cells. The ethanolic extract exhibited the highest potential of glucose uptake activity followed by methanolic and hot aqueous extract. Evaluation for adiponectin secretion activity found that the hot aqueous and methanolic extracts of *F. deltoidea* significantly augmented basal and insulin-mediated adiponectin secretion from adipocytes cells. Hot aqueous extract exhibited higher adiponectin secreting activity as compared to methanolic extract. Meanwhile, ethanolic extract did not showed any effect on adiponectin secretion activity.

α-Glucosidase inhibition study showed that hot aqueous, ethanolic and methanolic extracts of *F. deltoidea* significantly inhibited *in vitro* and *in vivo* rat intestine α-glucosidase (sucrase) activity. All extract possess mixed-type inhibition mechanism

against this enzyme activity. The *in vivo* study had shown that these extracts ameliorate postprandial hyperglycemia following sucrose administration in normal and diabetic rats. Among all extracts, methanolic possesses the most potent rat intestine sucrase inhibitory activity. The viability study showed that methanolic extract at higher concentrations (500 and 1000 μg/ml) exhibited cytotoxic effect against BRIN BD11 cells and ethanolic at the same concentration possess cytotoxic effect against BRIN BD11 and L6 myotubes. Therefore, insulin secreting and glucose uptake activities possess by both extracts could not be taken into consideration.

In conclusion, this study had shown that *F. deltoidea* has the ability to reduce hyperglycemia following acute and sub-chronic treatment. The suggested mechanisms by which *F. deltoidea* reduce hyperglycemia are stimulation of insulin secretion from pancreatic β-cells, enhancement of glucose uptake into adipocytes, muscle and liver cells, augmentation of adiponectin secretion from adipocytes cell and inhibition of α-glucosidase activity in small intestine. This dual pancreatic and extrapancreatic action of *F. deltoidea* together with its ability to maintain normal glycemia illustrate the enormous potential of this plant to be developed as new oral antidiabetic agent. Furthermore, adiponectin secreting and insulin-sensitizing activities exhibited by *F. deltoidea* indicated that this plant has the ability to ameliorate insulin resistance and may potentially be beneficial for the treatment of insulin-resistance related-Type 2 diabetes mellitus.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PENILAIAN AKTIVITI ANTIHIPERGLISEMIK *FICUS DELTOIDEA* JACK DAN PENJELASAN MEKANISMA ANTIDIABETIKNYA MENGGUNAKAN MODEL *IN VITRO*

Oleh

ZAINAH ADAM

Februari 2012

Pengerusi: Muhajir Hamid, PhD

Fakulti: Fakulti Bioteknologi dan Sains Biomolekul

Diabetis melitus merupakan penyakit metabolik yang dicirikan oleh hiperglisemia berterusan. Ia merupakan penyebab kematian yang utama di negara maju dan telah menjadi wabak di kebanyakan negara membangun termasuk Malaysia. Diabetis melitus masih menjadi masalah kesihatan global yang utama walaupun terdapat banyak drug antidiabetik, Ini mungkin disebabkan oleh batasan-batasan pada drug antidiabetik konvensional seperti kesan buruk dan keberkesanan klinikal yang lemah. Maka dengan itu, pencarian drug antidiabetik baru harus diteruskan. *Ficus deltoidea* atau nama tempatannya Mas cotek merupakan salah satu tumbuhan ubatan yang lazim digunakan di Malaysia. Ia telah dipercayai secara tradisional mempunyai aktiviti antidiabetik.

Namun begitu, kajian-kajian saintifik untuk mengesahkan keberkesanannya serta mekanisma tindakan yang mungkin masih kurang.

Kajian ini telah dijalankan untuk mengesahkan sifat antidiabetik *F. deltoidea* melalui penilaian antihiperglisemik secara *in vivo* dan dan untuk mencari penjelasan mekanisma antihiperglisemik yang mungkin menggunakan model *in vitro*. Kajian antihiperglisemik *in vivo* telah dilakukan di dalam tikus Sprague Dawley jantan. Dua bentuk rawatan telah diaplikasikan; akut dan sub-kronik. Kajian antihiperglisemik berikutan rawatan akut telah dijalankan di dalam tikus normal dan diabetik aruhan-streptozotocin pada tiga keadaan prandial yang berbeza; puasa, selepas makan dan selepas diberi glukosa. Kajian antihiperglisemik berikutan rawatan sub-kronik (15-hari) telah dijalankan di dalam tikus diabetik aruhan-streptozotocin. Kajian mekanisma antihiperglisemik *F. deltoidea* telah dijalankan secara *in vitro* bagi menilai potensi pokok ini untuk merangsang perembesan insulin daripada sel-β pankreatik, untuk meningkatkan kadar pengambilan glukosa oleh sel adipos, otot dan hati, untuk meningkatkan kadar perembesan adiponektin oleh sel adipos dan untuk melambatkan penyerapan glukosa daripada usus kecil dengan merencat aktiviti α-glukosidase (sukrase). Keupayaan untuk hidup bagi sel-sel yang digunakan dalam kajian mekanisma antihiperglisemik dengan kehadiran ekstrak *F. deltoidea* ditentukan menggunakan asai MTT.

Keputusan kajian menunjukkan bahawa rawatan secara akut ekstrak akuas panas dan etanolik *F. deltoidea* telah menurunkan hiperglisemia puasa dan selepas makan serta memperbaiki aktiviti toleransi glukosa di dalam tikus normal dan diabetik aruhan-

streptozotocin. Tambahan pula, berikutan 15-hari rawatan, ekstrak akuas panas menurunkan hiperglisemia puasa dan merangsang rembesan insulin di dalam tikus diabetik aruhan-streptozotocin. Kedua-dua ekstrak tidak menurunkan paras glukosa darah di bawah julat normal. Kajian tentang mekanisma-mekanisma antihiperglisemik *F. deltoidea* menunjukkan bahawa ekstrak akuas panas, etanolik dan metanolik merangsang rembesan insulin dari sel-β secara signifikan. Di antara semua ekstrak, akuas panas menunjukkan potensi yang terbaik dalam merangsang rembesan insulin. Tindakan perembesan insulin oleh ekstrak akuas panas telah melibatkan laluan ketergantungan-K^+_{ATP} dan ketidaktergantungan-K^+_{ATP}. Ekstrak ini juga mempunyai kebolehan untuk merangsang penggunaan Ca^{2+} dalaman untuk mencetuskan perembesan insulin.

Ekstrak akuas panas, etanolik dan metanolik *F. deltoidea* ini secara signifikannya menambahkan pengambilan glukosa basal dan diperantarakan-insulin ke dalam sel-sel adipos, otot dan hati. Kesemua ekstrak menunjukkan sama ada aktiviti meniru-kesan insulin atau menambah sensitiviti-insulin atau kombinasi kedua-dua aktiviti semasa menambahkan pengambilan glukosa ke dalam sel-sel ini. Ekstrak etanolik mempamerkan potensi aktiviti pengambilan glukosa yang tertinggi diikuti oleh ekstrak metanolik dan akuas panas. Penilaian aktiviti merembes adiponektin menemukan bahawa ekstrak akuas panas dan metanolik *F. deltoidea* secara signifikannya meningkatkan aktiviti perembesan adiponektin basal dan diperantarakan-insulin. Ekstrak akuas panas mempamerkan aktiviti perembesan adiponektin yang tinggi jika

dibandingkan dengan ekstrak metanolik. Sementara itu, ekstrak etanolik tidak menunjukkan apa-apa kesan terhadap aktiviti perembesan adiponektin.

Kajian tentang perencatan α-glukosidase menunjukkan bahawa ekstrak akuas panas, etanolik dan metanolik *F. deltoidea* secara signifikannya merencat aktiviti α-glukosidase (sukrase) usus tikus secara *in vitro* dan *in vivo*. Kesemua ekstrak mempamerkan mekanisma perencatan jenis-campuran. Kajian *in vivo* menunjukkan bahawa kesemua ekstrak menurunkan hiperglisemia selepas makan berikutan pengambilan sukrosa pada tikus normal dan diabetik. Di antara kesemua ekstrak, metanolik memiliki sifat perencatan paling kuat terhadap enzim sukrase usus tikus. Kajian keupayaan hidup menunjukkan bahawa ekstrak metanolik pada kepekatan tinggi (500 and 1000 μg/ml) mempamerkan kesan sitotoksik terhadap sel BRIN BD11 dan ekstrak etanolik pada kepekatan yang sama mempunyai kesan sitotoksik terhadap sel BRIN BD11 dan L6 myotubes. Maka dengan itu, aktiviti perembasan insulin dan pengambilan glukosa oleh kedua-dua ekstrak tidak boleh diambil kira.

Kesimpulannya, kajian ini menunjukkan bahawa *F. deltoidea* mempunyai keupayaan untuk menurunkan hiperglisemia berikutan rawatan akut dan sub-kronik. Mekanisma yang dicadangkan bagaimana *F. deltoidea* menurunkan hiperglisemia adalah perangsangan perembesan insulin daripada sel- β pankreatik, peningkatan pengambilan glukosa oleh sel-sel adipos, otot dan hati, penambahan perembesan adiponektin daripada sel adipos dan perencatan aktiviti α-glukosidase di dalam usus kecil. Tindakan pankreatik dan extra-pankreatik *F. deltoidea* ini berserta keupayaannya untuk

mengekalkan glisemia normal membuktikan bahawa pokok ini berpotensi ini untuk dibangunkan sebagai agen antidiabetik oral yang baru. Tambahan pula, sifat perembes adiponektin dan menambah sensitiviti-insulin yang dipunyai oleh *F. deltoidea* menunjukkan yang tumbuhan ini berupaya untuk menurunkan kerintangan insulin dan sesuai digunakan dalam rawatan diabetis mellitus Jenis 2 yang berkaitan dengan kerintangan terhadap insulin.

ACKNOWLEDGEMENTS

First and foremost, all thanks to Allah the Al Mighty, for giving me the strength to complete the study. I would like to express my deepest gratitude and appreciation to my main supervisor, Associate professor Dr Muhajir Hamid and my co-supervisors, Professor Dr Amin Ismail and Dr Shafii Khamis for the great guidance, advices, assistance, encouragement and motivation throughout this study.

I would like to express my gratitude to Malaysian Nuclear Agency for providing grant (project no: MINT-R&D-05-009) to support this study and for granting me scholarship and study leave to pursue my postgraduate degree. My gratitude also goes to Dr Shamsul Khamis for identification of plant material. I wish to extend my appreciation to everyone, although not individually named here, who had contributed either directly or indirectly towards the completion of this project. Last but not least, I would like to thanks my beloved husband, daughter and parents for their sacrifice and continuous love and being the pillars of my strength.

I certify that a Thesis Examination Committee has met on 3 February 2012 to conduct the final examination of Zainah bt Adam on her Doctor of Philosophy thesis entitle "Evaluation of Antihyperglycemic activity of *Ficus deltoidea* and its Antidiabetic Mechanism Elucidation using *in vitro* Model" in accordance with the Universities and University Colleges Act 1971 and the constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The committee recommends that the student be awarded the Doctor of Philosophy degree.

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

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DECLARATION

I 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, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

ZAINAH BT ADAM

Date: 3 February 2012

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