HYPOGLYCEMIC AND ANTIOXIDATIVE EFFECTS OF *ANACARDIUM OCCIDENTALE* LINN. IN DIABETIC RATS

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MASTER OF SCIENCE
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DEDICATION

Especially dedicated to……

Almighty God,

My dearest daddy, Ling Kuok Miew and mummy, Tang Swei Ting

My brother, Han Lee and sister, Eileen

My late younger sister, Pyleen

My beloved husband, Tang Tung Kwong

Brothers and sister in Christ ……

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

HYPOGLYCEMIC AND ANTIOXIDATIVE EFFECTS OF *ANACARDIUM OCCIDENTALE* LINN. IN DIABETIC RATS

By

LETTY LING

April 2006

Chairman : Zulkhairi Haji Amom, PhD

Faculty : Medicine and Health Sciences

Diabetes mellitus is found to be associated with oxidative damage which coexists with a reduction in the antioxidant status. The Malay folklore medicine in Malaysia believes that by consuming the decoction of the vein and leaves (vascular bundle) of *A. occidentale* L. (cashew-nut), it is able to lower blood glucose level of diabetic patients. The objective of this study was to verify the potential hypoglycemic and antioxidative effects of *Anacardium occidentale* L. leaves aqueous extract (AOE) in Type 2 diabetes rat model. Freeze-dried AOE of various doses (50 mg/kg, 250 mg/kg, 500 mg/kg and 1000 mg/kg body weight) were administered to streptozotocin induced Type 2 diabetic rats. The rats were force-fed with the extracts once daily for six weeks. Oral glucose tolerance test (OGTT) with 1.5 g/kg body weight of glucose challenge was then conducted to monitor the serum glucose level. Blood was collected through cardiac puncture to examine the levels of lipid peroxidation and the enzymatic activities in the experimental rats. The result showed an improvement in the glucose tolerance after six weeks of treatment significantly as compared to the diabetes control ($P<0.05$). After treatment, the rats treated with AOE at all doses

have lower fasting glucose levels compared to the pre-treatment week. It was also noticed that all the doses of the leaves extract managed to delay the rise of glucose level in oral glucose tolerance curve. Thus, *A. occidentale* leaves aqueous extract had suppression effect on the increase of serum glucose levels in oral glucose load.

The AOE at all doses exhibited low lipid peroxidation product indicated by serum malondialdehyde (MDA) levels after six weeks of extract administration as compared to the pre-treatment week. The reduced MDA levels of group treated with AOE 250mg/kg, AOE 500 mg/kg and AOE 1000mg/kg are comparable to the MDA levels as obtained by the normal control groups after six weeks of extract treatment.

Serum catalase activities were found to be significantly elevated in diabetic groups treated with AOE as compared to the diabetic control groups whereas in the normal groups, the serum catalase activities in blood were much higher than the diabetic groups. Plasma superoxide dismutase (SOD) activities of AOE treated were found to be higher than the diabetes control after three weeks of administration. The SOD activities were higher than the normal groups significantly ($P<0.05$). However, there were reductions on SOD activities at Week 6. Diabetes treated groups (AOE 50 mg/kg, AOE 250 mg/kg and AOE 500 mg/kg) showed an increased of plasma gluthathione peroxidase (GPx) activities at Week 3 as compared to the pre-treatment groups.

Histological study of the pancreas showed an extensive damage of the islets of Langerhans and reduced dimensions of islet in the diabetic-induced rats. There were significant increase in the area, perimeter and diameter of pancreatic islets in both

glybenclamide and AOE treated rats. The diabetic rats treated with AOE 250 mg/kg have the highest increase in area, perimeter and diameter of the islet of Langerhans and have no significant difference compared to the normal control rats. This may suggest that AOE 250 mg/kg could improve and protect the islet Langerhans cells from oxidative degeneration resembling the normal rats.

The results of this study indicate that *A. occidentale* L. leaves might possess hypoglycemic activity. The alterations of the enzymatic antioxidant activities in the experimental animals provides evidence that the preventive effects of *A. occidentale* L. may be due to inhibition of lipid peroxidation by its antioxidant properties. Thus, *A. occidentale* L. possesses antioxidant properties which counteract the oxidative damage in diabetic subjects.

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

KESAN HIPOGLISEMIK DAN ANTIOKSIDATIF *ANACARDIUM OCCIDENTALE* LINN. PADA TIKUS DIABETIK

Oleh

LETTY LING

April 2006

Pengerusi : Zulkhairi Haji Amom, PhD

Fakulti : Perubatan dan Sains Kesihatan

Penyakit diabetes mellitus telah dikatakan berhubung kait dengan kemusnahan oksidatif yang diakibatkan oleh pengurangan status antioksidan. Masyarakat Melayu di Malaysia mempercayai perubatan traditional di mana penggunaan daun *A. occidentale* L.(gajus) berupaya menurunkan kandungan aras glukosa darah pada pesakit diabetes. Kajian ini bertujuan untuk menentukan keberkesanan ekstrak akuas daun *A. occidentale* L. (AOE) dalam menurunkan kandungan aras glukosa serum darah dan kesan antioksidannya pada model tikus teraruh diabetes jenis 2. Ekstrak daun berakuas yang telah disejukbekukan telah diberikan dos (50 mg/kg, 250 mg/kg, 500 mg/kg dan 1000 mg/kg berat badan) kepada tikus teraruh diabetes jenis 2 melalui suntikan streptozotosin. Tikus telah diberikan AOE melalui oral setiap hari sekali, selama enam minggu. Ujian toleransi glukosa secara oral (OGTT) dengan "cabaran glukosa" sebanyak 1.5 g/kg berat badan telah dijalankan untuk menentukan kesan ekstrak terhadap kandungan glukosa serum darah. Darah juga diambil melalui tusukan jantung untuk menguji aras oksidasi lipid dan aktiviti enzim antioksidan

pada tikus kajian. Keputusan daripada kajian ini telah menunjukkan dos rawatan AOE berjaya menurunkan aras glukosa darah tikus diabetik selepas enam minggu tempoh rawatan berbanding dengan kumpulan kawalan diabetes melalui ujian toleransi glukosa. Kumpulan tikus yang diberikan rawatan AOE pada semua dos menunjukkan aras glukosa serum darah yang lebih rendah sebelum diberikan "cabaran glukosa" berbanding dengan tempoh sebelum rawatan diberikan. Kajian ini mendapati bahawa AOE pada semua dos berupaya merencatkan peningkatan aras glukosa serum darah setelah diberikan "cabaran glukosa" dalam Ujian toleransi glukosa. Ekstrak akuas daun *A. occidentale* ini dicadangkan telah menunjukkan penindasan terhadap peningkatan glukosa dalam Ujian toleransi glukosa.

Tikus yang diberikan AOE pelbagai dos telah menunjukkan kandungan oksidasi lipid, iaitu aras malondialdehid (MDA) yang lebih rendah berbanding dengan sebelum rawatan diberikan selepas enam minggu tempoh rawatan. Kandungan MDA yang diperolehi daripada kumpulan rawatan AOE 250 mg/kg, AOE 500 mg/kg, dan AOE 1000 mg/kg selepas enam minggu rawatan dijalankan adalah setara seperti yang diperolehi daripada kumpulan kawalan normal.

Aktiviti katalase pada tikus diabetik yang diberikan rawatan AOE telah meningkat berbanding dengan kumpulan kawalan diabetik. Manakala, aktiviti katalase pada kumpulan normal adalah sentiasa lebih tinggi daripada kumpulan diabetik. Tikus yang diberikan rawatan AOE juga menunjukkan aktiviti superoksida dismutas (SOD) yang lebih tinggi berbanding dengan kumpulan kawalan diabetik selepas tiga minggu rawatan dijalankan dan lebih tinggi daripada kumpulan normal secara ketara ($P<0.05$). Kumpulan diberikan dos rawatan AOE 50 mg/kg, AOE 250 mg/kg and

AOE 500 mg/kg telah menunjukkan peningkatan aktiviti glutathion peroksides (GPx) pada minggu ke-3 berbanding dengan minggu sebelum diberikan rawatan.

Pemerhatian histologi pada pankreas yang dikaji telah menunjukkan kemusnahan pada pepulau Langerhans dan dimensi pepulau Langerhans telah berkurangan pada tikus diabetes yang disuntik streptozotosin (STZ). Luas permukaan, perimeter dan panjang diameter pada pepulau Langerhans telah menunjukkan peningkatan yang ketara pada kumpulan tikus yang diberikan rawatan glibenklamida dan AOE. Tikus yang diberikan rawatan AOE 250 mg/kg telah menunjukkan peningkatan luas permukaan, perimeter dan panjang diameter pepulau Langerhans yang tertinggi antara tikus yang diberikan dos rawatan yang lain ($P<0.05$). Peningkatan luas permukaan, perimeter dan panjang diameter pada tikus yang diberikan rawatan AOE 250 mg/kg tiada perbezaan ketara berbanding dengan tikus kawalan. Ini menunjukkan dos AOE 250 mg/kg mungkin mampu memperbaiki dan melindungi sel pada pepulau Langerhans seperti yang ditunjukkan pada tikus kumpulan kawalan.

Kesimpulannya, kajian ini telah mendapati ekstrak akuas daun *A. occidentale* L. mungkin mempunyai aktiviti hipoglisemik dan antioksidan. Kesan antioksidannya boleh dilihat melalui perubahan aktiviti antioksidan yang diperolehi pada haiwan dalam kajian ini. Perencatan oksidasi lipid pada haiwan eksperimen berkemungkinan disebabkan oleh aktiviti antioksidan yang bertindak semasa berlakunya kemusnahan oksidatif di dalam subjek diabetik.

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With all of my love, thank you very much. May God bless each one of you abundantly.

Amen.

Trust in the Lord with all your heart and lean not on your own understanding;

In all your ways acknowledge him, and he will make your paths straight.

Proverbs 3: 5-6

THANK YOU

I certify that an Examination Committee has met on 12 April 2006 to conduct the final examination of Letty Ling on her Master of Science thesis entitled "Hypoglycemic and Antioxidative Effects of *Anacardium occidentale* Linn. in Diabetic Rats" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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

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DECLARATION

I hereby 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 or concurrently submitted for any other degree at UPM or other institutions.

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LETTY LING

Date:

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