
UNIVERSITI PUTRA MALAYSIA

# EFFECTS OF Anacardium occidentale L. LEAF EXTRACT ON SERUM GLUCOSE LEVELS AND INTESTINAL GLUCOSE ABSORPTION IN RATS

MARY KHOO GAIK HONG

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By

MARY KHOO GAIK HONG

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

February 2004

# DEDICATION

To my family

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

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**Chairman:** **Associate Professor Hamdan Hj. Mohd. Noor, Ph.D.**

**Faculty:** **Medicine and Health Sciences**

The objective of this study was to verify the potential hypoglycaemic property of *Anacardium occidentale* L. vein and leaf aqueous extract in type 2 diabetic rat model. The rats were treated with the extracts (5, 50, and 500 mg/kg), which were incorporated into the drinking water and given daily for 6 weeks. Oral glucose tolerance test (OGTT) with 1.5 g/kg of glucose challenge was then performed to monitor the serum glucose level. An additional dose of their respective treatment was given again orally during OGTT to enhance the effect. The results did not show any improvement in the glucose tolerance even after 6 weeks of treatment, except for the positive control—glybenclamide. However, the serum glucose level rate of increase after oral loading with glucose was suppressed in the group treated with 500 mg/kg of the leaf extract. Glucose uptake into the blood was delayed by 46.81%, 33.02%, and 42.01% after 1, 3, and 6 weeks of treatment compared to before treatment. The same but milder effect was also seen in the 500 mg/kg of the vein-treated group.

The extract may have affected glucose absorption in the gastrointestinal tract. Therefore, another study was conducted to determine whether the extract has any significant effect on intestinal glucose absorption. *In situ* absorption technique according to Doluisio *et al.* (1969) was carried out. Kreb-Ringer phosphate buffer (KRPB) containing 7.5 mM glucose with or without the addition of *A. occidentale* L. leaf extract (1, 5, 10, and 15 mg/ml) was then introduced into the lumen. Luminal samples were collected at various time intervals for glucose determination by GOD-PAP-method. 0.5 mM phlorizin was used as the positive control. The results showed that 10 mg/ml of the leaf extract inhibited glucose absorption in the jejunal segment of the experimental rats. Luminal glucose concentration was significantly higher at 5, 10, and 20 minutes than the control (95.32% vs. 68.91%, 79.71% vs. 45.97%, and 49.38% vs. 20.76% respectively), indicating less glucose was being absorbed. Nonetheless, the effect of 10 mg/ml could not beat the inhibition portrayed by phlorizin.

At the same time, the leaf extract was also found to be able to interact with the standard blood glucose measurement [GOD-PAP-method], causing the measured value to be lower than the actual concentration. GOD-PAP-method is based on a simple oxidation reaction while the leaf extract was found to have high reducing power. The high reducing power of the leaf extract was believed to have counteracted the normal process of GOG-PAP-method, causing the values to be under-read. Nevertheless, the advantage of an extract with high reducing power is one could also be a good antioxidant.

Thus, this study suggests that the aqueous leaf extract of *A. occidentale* L. might contain chemicals for treating diabetes mellitus by inhibiting intestinal glucose absorption. It may also exhibit antioxidant activity in biological system because of its high reducing power. This will be a good supporting therapy for diabetes as antioxidant has beneficial effects on glycaemic control and may help to ameliorate diabetes complications.

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

# KESAN EKSTRAK DAUN *Anacardium occidentale* L. KE ATAS ARAS GLUKOSA SERUM DARAH DAN PENYERAPAN GLUKOSA PADA USUS TIKUS

Oleh

**MARY KHOO GAIK HONG**

**Februari 2004**

**Pengerusi:** **Professor Madya Hamdan Hj. Mohd. Noor, Ph.D.**

**Fakulti:** **Perubatan dan Sains Kesihatan**

Kajian ini dijalankan untuk menentukan keberkesanan ekstrak akues urat daun dan daun *Anacardium occidentale* L. dalam menurunkan kandungan aras glukosa serum darah pada model tikus diabetes jenis 2. Rawatan ekstrak urat daun dan daun *A. occidentale* L. (5, 50, dan 500 mg/kg) dicampurkan dalam air minuman dan diberikan setiap hari kepada tikus diabetik selama enam minggu. Ujian toleransi glukosa secara oral dengan "cabaran glukosa" sebanyak 1.5 g/kg dijalankan untuk menentukan kesan ekstrak terhadap kandungan glukosa serum darah. Pada masa yang sama, dos tambahan berdasarkan rawatan masing-masing diberikan secara oral untuk menambahkan kesan ekstrak tersebut. Malangnya, tiada sebarang dos rawatan yang berupaya merendahkan aras glukosa serum darah tikus diabetik walaupun setelah dirawat selama enam minggu, kecuali kumpulan kawalan positif iaitu glibenclamide. Walau bagaimanapun, terdapat perencatan pada kadar peningkatan aras glukosa serum darah setelah diberi "cabaran glukosa" secara oral pada kumpulan yang dirawat dengan 500 mg/kg ekstrak daun. Kadar peningkatan glukosa menurun sebanyak 46.81%, 33.02%, and 42.01% selepas dirawat selama 1, 3, dan 6

minggu berbanding dengan sebelum rawatan. Kesan yang sama turut diperhatikan pada kumpulan yang dirawat dengan 500 mg/kg ekstrak urat daun tetapi kesannya adalah lebih lemah.

Ini mungkin disebabkan ekstrak akues tersebut telah mengganggu penyerapan glukosa pada salur gastrousus. Oleh itu, satu lagi kajian telah dijalankan untuk menentukan sama ada ekstrak daun itu mempunyai kesan yang bererti terhadap kadar penyerapan glukosa pada usus. Teknik penyerapan *in situ* berdasarkan kaedah yang dikemukakan oleh Doluisio *et al.* (1969) telah dilakukan. Larutan penimbal fosfat Kreb-Ringer (KRPB) yang mengandungi 7.5 mM glukosa yang ditambah dengan ekstrak daun (1, 5, 10, dan 15 mg/ml) atau tanpa ekstrak dimasukkan ke dalam lumen dan disampel pada masa-masa tertentu. Sampel diuji dengan kaedah GOD-PAP untuk menentukan kandungan glukosa pada lumen. 0.5 mM phlorizin digunakan sebagai kawalan positif. Keputusan menunjukkan bahawa ekstrak daun pada kepekatan 10 mg/ml berupaya merencatkan penyerapan glukosa pada bahagian jejunum. Kandungan glukosa dalam lumen adalah lebih tinggi secara bererti pada minit ke-5, 10, dan 20 berbanding dengan kawalan (95.32% vs. 68.91%, 79.71% vs. 45.97%, and 49.38% vs. 20.76% masing-masing). Ini menunjukkan kurang glukosa yang diserap. Walau bagaimanapun, kesan ekstrak pada kepekatan 10 mg/ml tidak dapat menandingi kesan yang ditunjukkan oleh phlorizin.

Pada masa yang sama, ekstrak daun juga didapati berupaya bertindak balas dengan kaedah GOD-PAP yang digunakan untuk mengukur kepekatan glukosa darah. Ini menyebabkan nilai bacaan adalah lebih rendah daripada yang sebenarnya. Prinsip kaedah GOD-PAP adalah berdasarkan pada tindak balas pengoksidaan. Ekstrak

daun pula didapati mempunyai kuasa penurunan yang tinggi. Kuasa penurunan ekstrak daun ini disyaki telah menghalang tindak balas asal GOD-PAP yang menyebabkan bacaannya menjadi lebih rendah daripada yang sebenarnya. Walaupun begitu, sesuatu ekstrak yang mempunyai kuasa penurunan yang tinggi juga berkemungkinan merupakan bahan antioksida yang baik.

Oleh itu, keputusan kajian ini menunjukkan bahawa ekstrak akues daun *A. occidentale* L. mungkin mengandungi bahan kimia untuk merawat diabetes mellitus dengan merencatkan kadar penyerapan glukosa pada usus. Ekstrak daun juga mungkin mempunyai kesan antioksida pada sistem biologi kerana kuasa penurunannya yang tinggi. Kesan antioksida ini boleh dijadikan rawatan tambahan untuk diabetes kerana bahan antioksida adalah baik dalam mengawal nilai glisemia dan komplikasi diabetes.

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| | |
|---|---|
| % | percent |
| ADP | adenosine diphosphate |
| ATP | adenosine triphosphate |
| $Ca^{2+}$ | calsium ion |
| cAMP | adenosine 3', 5'-cyclic monophosphate |
| cc | cubic centimetre |
| cm | centimetre |
| dL | decilitre |
| DNA | deoxyribonucleic acid |
| g | gramme |
| $H^+$ | hydrogen ion |
| $HBA_{1c}$ | glycosylated heamoglobin A1c |
| HDL | high-density lipoprotein |
| HLA | human leukocyte antigen |
| i.p. | intraperitoneal |
| $K^+$ | kalium ion |
| kg | kilogramme |
| L | litre |
| LDL | low-density lipoprotein |
| mg | milligramme |
| mg% | milligramme percent (equivalent to mg/100 ml) |
| ml | millilitre |
| mM | millimolar |
| mmol | millimole |
| n | number of replicates |
| $Na^+$ | natrium ion |
| $Na^+$-$K^+$ ATPase | sodium-potassium pump |
| NADP | nicotinamide adenine dinucleotide phosphate |
| NADPH | nicotinamide adenine dinucleotide phosphate (reduced) |
| ng | nanogramme |
| nm | nanometre |
| P | probability of an event due to chance alone |
| p.o. | *per os* |
| pH | $-\log_{10}[H^+]$ |
| rpm | revs per minute |
| S.E.M. | standard error of mean |
| VLDL | very low-density lipoprotein |
| vs. | versus |
| wk | week |
| $\alpha$ | alpha |
| $\beta$ | beta |
| $\gamma$ | gamma |
| $\mu$l | microlitre |
| °C | degrees Celcius |
| $\approx$ | approximately |

# CHAPTER I

# INTRODUCTION

## Diabetes Mellitus—Disease of the 21st Century

Diabetes mellitus rank sixth in 1999 as the leading cause of death in the United States (U.S.)—after heart disease, cancer, stroke, chronic respiratory disease, and unintentional injuries (Hoyert *et al.*, 2001). Diabetes has been described since antiquity. It has been with us for over 3500 years and it is still with us today, without any ultimate cure. Every day, thousands of diabetes sufferers die of this disease and its complications. Death by diabetes was estimated to account to 810,000 in the year 2000 (in WHO regions), 1.5% of the total deaths (World Health Organization, 2001).

Diabetes is not contagious, yet it is probably the world's fastest growing metabolic disease. It usually leads to other complications such as long-term damage, dysfunction, and failure of various organs e.g. blindness, renal failure, heart disease, high blood pressure, neuropathy, gangrene, infection, and foot disease that frequently leads to amputation (Montgomery, 1975; Kloppel *et al.*, 1998; World Health Organization, 1998a). In U.S., the mortality rate of diabetes had increased 3.3% causing it to jump from the seventh rank in 1998 to the sixth in 1999 as the leading cause of death (Hoyert *et al.*, 2001).

In the developing countries, life expectancy may be halved, where the prevalence is increasing and adequate treatment is often unavailable (King *et al.*, 1993). The prevalence of diabetes in adults worldwide was estimated to be 4% (135 million) in 1995. This figure is expected to project to 5.4% (300 million) by the year 2025. Although prevalence is higher in the developed countries than in the developing countries (6% and 3.3% in 1995 respectively) and will remain so in 2025 (7.6% and 4.9% respectively), but the proportional increase is higher in the developing countries. There will be only 42% increase (from 51 to 72 million) in the developed countries but a 170% rise (from 84 to 228 million) is expected in the developing countries. Hence, more than 75% people with diabetes will reside in the developing countries in 2025 compared to only 62% in 1995 (King *et al.*, 1998).

Diabetes is not in the list of the top ten principal causes of deaths in the government hospitals of Malaysia (Ministry of Health, Malaysia, 1999a & 2000). Nevertheless, it is still a problem faced by many Malaysians. In Peninsular Malaysia alone, the prevalence was reported to increase approximately 2.5% within 10 years—from 6.3% in 1986 to 8.8% in 1996. The prevalence for the whole of Malaysia in 1996 (inclusive of Sabah and Sarawak) was reported to be 8.3%. By estimating the total population of Malaysia to be approximately 21 million in 1996, Malaysia was homed to over 1.7 million sufferers. With longevity and further industrialisation, the number of people affected with diabetes may double by the year 2010 (Rugayah *et al.*, 1999).

Furthermore, the number of admission to government hospitals in Peninsular Malaysia for diabetes increased from 19,503 cases in 1990 to 25,125 cases in 1997,

which is about 30% increment over the span of 7 years. Mortality due to diabetes also increased from 231 deaths in 1990 to 386 death in 1997, an increment of approximately 70% (Ministry of Health, Malaysia, 1999b).

Diabetes is recognised worldwide as an important community health problem because it has significant impact on health, quality of life, and life expectancy of individuals, as well as on the health-care system. The economic burden of this disease is enormous. As a major non-communicable disease, diabetes mellitus alone claimed on the average around 8% of total health budgets in developed countries (World Health Organization, 1998b).

Economic analyses performed in the 1980s suggested that the total (direct and indirect) economic costs associated with diabetes in the U.S. were between $13.7 and $20.4 billion in the 1980s-era dollars. After adjusting to the 1990 dollars using the Consumer Price Index-U Inflater, the values were estimated between $17.2 and $28.2 billion (Javitt & Chiang, 1995). A more recent study by Ray *et al.* (1993) estimated the total cost of diabetes in 1992 to be $91.8 billion ($45.2 billion for direct costs and $46.4 billion for indirect costs)—four times of the previous estimates, even after adjusting for inflation (cited in Javitt & Chiang, 1995). In another study, the total direct medical care costs for people confirmed diabetes was $85.7 billion or 11.9% of U.S. health care expenditures in 1992 (Rubin *et al.*, 1994). Meanwhile, American Diabetes Association (1998) estimated that $44.1 billion was attributed for direct medical expenditures and $54.1 billion for indirect costs, totalling up to approximately $98 billion for 1997. The full burden and exact cost of this disease is not easy to measure, as there are many hidden costs associated such as

loss of human lives and abilities that transcends numerical measurement. Whatever the real cost may be, one thing for sure is that the economic burden of diabetes mellitus is immense.

## Therapy and Management of Diabetes

Diagnosing diabetes was never a problem. According to ancient Hindu writings dated 1500 B.C., ants and insects were attracted to the urine of some people associated with a mysterious emaciating disease (Gordon, 1959; MacCracken & Hoel, 1997). But for thousand of years, no one knew how to live with it, let alone correct diabetes. All they understood was the victims had an intense thirst, enormous urine output, and wasting away of the body that slowly expired. Children with the disease died quickly and older folks struggled with devastating complications.

Aretaeus of Cappadocia (in the second century A.D.) was one of the firsts to provide a fairly complete clinical description of diabetes. He was the man who coined the term diabetes which means "going through" or "siphon", the literal translation which reflects the early understanding of this disease that drained patients off more fluid than they could consume (Morton, 1970; Sonksen, 1984). Later, in the 17$^{th}$ century, the Latin word for honey—"mellitus" was appended to diabetes for its link with sweet urine, which was observed at a much earlier time (Sonksen, 1984). This disease was also mentioned by other ancient physicians such as Sushrut, Charak, Celsus, Galen, Demetius of Apamea, Caelius Aurelianus, Paul of Aegina, Aetius, Rhazea, Avicenna, and Haly Abbas (Gordon, 1959; Goldfine & Youngren, 1998).

In the early days, treatment for diabetes ranged from herbs to "cataplasms to the hypochondrium over the kidneys" and even venesection (Gordon, 1959; MacCracken & Hoel, 1997). Emetics, purgatives, astringents, and refrigerant remedies were also prescribed. Engaging in exercise was encouraged, preferably on horseback to "employ moderate friction" and alleviate excess urination (Gordon, 1959). For more than two millennia, diabetes was thought to be a disease of the kidney and bladder. Therefore, remedies prescribed were mostly for combating polyuria. According to Traditional Chinese Medicine, the Chinese believed that improper diet such as indulgence in alcohol and intemperance in sexual life was the main cause of this disease (Chen, 1994). Hence, avoidance of sex and wine were also prescribed. There were also those who practised bleeding, blistering, doping with opium, and overfeeding to compensate the loss of fluid and weight (Bliss, 1982 cited in MacCracken & Hoel, 1997). Some tried strict meat diet and others tried a diet high in fat, which did more harms than good. Frederick Allen's starvation approach, although seems to be radical and cruel had the best result and allowed a number of young people to survive and became the first insulin users (Bliss, 1982 cited in MacCracken & Hoel, 1997).

The important milestone in understanding this disease occurred in the 19$^{th}$ century, with the discovery of islets of Langerhans by Paul Langerhans in 1869 and the observation by Minkowski and von Mering that removing the pancreas leads to diabetes. With further research by Banting, Best, Macleod, Collip, and many others, we have come to understand the cause of diabetes and the link between this disease with insulin (Morton, 1970; Goldfine & Youngren, 1998). Today, we know that diabetes means just one thing—a high blood sugar level.

Appropriate dietary planning and regular physical exercise is the initial therapy for type 2 diabetes patients. According to Bell & Ovalle (2000), 5-10% reduction in body weight will lower insulin resistance. Decrease in calorie intake without significant weight loss will likewise decrease insulin resistance, as well as a low fat diet. Walking at least 40 minutes, four times weekly, is enough to enhanced insulin sensitivity. 6% reduction in the chance of developing diabetes has been shown with every 5 miles per week walk. And an even greater reduction in those at higher risk, i.e. with family history of diabetes and those that are obese.

When the response is insufficient, the next step is appropriate pharmacotherapy, with the aim of a safe glycaemic control. Current pharmacological agent offers a wide range of selection to reduce hyperglycaemia. Generally, they can be categorised into five groups with different mode of action, namely biguanides, sulfonylureas, thiazolidinediones, alpha-glucosidase inhibitors, and meglitinides, used either alone or in combination. When oral therapy too failed, patients usually switched to insulin injection, with or without oral agent to improve insulin action (Leiter, 1999; Rajan, 1999; Gilmet, 1999; Cara, 1999; Bell & Ovalle, 2000; Edelman & Henry, 2001). With the advance in medical science, pancreatic beta cells transplant especially for type 1 diabetes sufferers is also possible (Lacy, 1995; Davalli *et al.*, 2000; Stevens *et al.*, 2001).

However, there had been alarming reports on the adverse effects of these modern treatments. In the treatment of type 1 diabetes, pancreas transplant patients may suffer spontaneous hypoglycaemia (Battezzati *et al.*, 1998). While those with insulin therapy should be aware of the possibilities of increase amount and distribution of