UNIVERSITI PUTRA MALAYSIA

HYPOGLYCEMIC AND ANTIOXIDATIVE EFFECTS OF *EUGENIA AROMATICA* AND *ARCHIDENDRONE JIRINGA* ON DIABETIC RATS

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By

RADHIAH BINTI SHUKRI

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

June 2006

To my husband and family…….

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

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June 2006

Chairman : Professor Suhaila binti Mohamed, PhD

Faculty : Food Science and Technology

The study conducted for 15 weeks involved 56 Sprague Dawley male rats aged three weeks that were divided into seven groups. Two control groups were normal rats and induced-diabetic rats given a basal diet, four other groups were 2 normal rat groups and 2 induced-diabetic rat groups either supplemented with a basal diet containing 5% of cloves (*Eugenia aromatica*) or jering (*Archidendrone jiringa*) respectively. The basal diet that contained Glibenclamide (3mg/kg body weight) was supplemented to the remaining one group of diabetic rats. Body weight and feed consumption were monitored weekly and daily respectively. During a 3 weeks interval, blood samples were drawn via cardiac puncture for the purpose of biochemical analysis that consisted of glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD) activities; malondialdehyde (MDA) level and levels of urea, creatinine, alanine aminotransferase (AST) and aspartate aminotransferase (AST). Somatic index and histological changes of liver, heart, lung, eye, brain, kidney and pancreas of the experimental rats were also evaluated.

The results observed showed a slight lowering of blood glucose level of 6.1±0.4 and 6.2±0.5 mmol/l for cloves and jering supplemented STZ-diabetic rats respectively. The body weight of the diabetic rats supplemented with the herbs was also improved with R^2 value of 0.9924 and 0.9068 for jering and cloves, respectively. Weak anti-oxidative property in blood and organs was revealed with the supplementation of the herbs but was more effective in cloves. While evidence of toxicity of jering was shown mainly in the liver, kidney and heart of normal and diabetic rats, cloves was seen to be toxic to the pancreas of diabetic rats through histology. Jering-supplemented normal and diabetic groups had high cardiosomatic index with 0.51±0.11 (NJ) and 0.49±0.04 (DJ), necrotic hepatocytes and Kupffer cells of 50.5±5.0 (NJ) and 71.2±5.2 (DJ), respectively.

However, toxicity effect of these herbs towards certain organs at the 5% dose given suggests that a dose-response effect need to be further studied.

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

KESAN HIPOGLISEMIK DAN CIRI-CIRI ANTIOKSIDAN OLEH *EUGENIA AROMATICA* DAN *ARCHIDENDRONE JIRINGA* TERHADAP TIKUS KENCING MANIS

Oleh

RADHIAH BINTI SHUKRI

Jun 2006

Pengerusi : Profesor Suhaila binti Mohamed, PhD

Fakulti : Sains dan Teknologi Makanan

Kajian yang telah dijalankan selama 15 minggu melibatkan 56 ekor tikus Sprague Dawley jantan yang berumur 3 minggu yang dibahagikan kepada 7 kumpulan. Sementara 2 kumpulan kawalan tikus normal dan tikus kencing manis yang diaruh hanya diberikan diet normal, 4 lagi kumpulan yang terdiri daripada 2 kumpulan tikus normal dan 2 kumpulan tikus kencing manis diberikan sama ada diet yang mengandungi 5% cengkih (*Eugenia aromatica*) atau 5% jering (*Archidendrone jiringa*). Diet yang mengandungi Glibenclamide (3mg/kg berat badan) diberikan kepada satu lagi kumpulan tikus kencing manis. Berat badan diselia seminggu sekali manakala jumlah makanan diselia setiap hari. Setiap 3 minggu, sampel darah diambil melalui jantung untuk dianalisa secara biokimia iaitu aktiviti glutation peroksidase (GSH-Px), katalase (CAT) and superoksid dismutase (SOD); paras malondialdehid (MDA) dan paras urea, kreatinin, alanin aminotransferase (AST) dan aspartat aminotransferase (AST). Indeks

somatik dan perubahan histologi bagi hati, jantung, paru-paru, mata, otak, buah pinggang dan pankreas tikus eksperimen turut dinilai.

Keputusan yang diperolehi menunjukkan penurunan yang tidak ketara paras glukos dalam darah sebanyak 6.1±0.4 dan 6.2±0.5 mmol/l masing-masing bagi tikus kencing manis STZ yang diberikan cengkih dan jering. Berat badan tikus-tikus kencing manis yang diberikan herba juga menunjukkan pencapaian yang baik dengan nilai R^2 0.9924 dan 0.9068 for masing-masing bagi jering dan cengkih. Penstabilan ciri-ciri tekanan oksidatif yang tidak ketara dalam darah dan organ-organ juga ditunjukkan dengan pemberian herba tetapi cengkih lebih menunjukkan kesan yang baik. Ketoksikan jering dapat dikesan terutamanya dalam hati, buah pinggang dan jantung tikus normal dan tikus kencing manis sementara cengkih menunjukkan kesan toksik pada pankreas tikus kencing manis melalui histologi. Kumpulan tikus normal dan kencing manis yang diberikan jering mempunyai kardiosomatik indeks yang tinggi dengan nilai 0.51±0.11 (NJ) dan 0.49±0.04 (DJ) serta hepatosit nekrotik dan sel Kupffer yang tinggi dengan nilai 50.5±5.0 (NJ) dan 71.2±5.2 (DJ).

Kesan toksik 5% herba ini terhadap organ-organ tertentu menunjukkan bahawa kajian lanjut berkenaan dengan respon kepada dos yang berbeza perlu dijalankan.

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I certify that an Examination Committee has met on 30 Jun 2006 to conduct the final examination of Radhiah binti Shukri on her Master of Science thesis entitled "Hypoglycemic and Antioxidative Effects of *Eugenia aromatica* and *Archidendrone jiringa* on 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:

Azizah binti Abdul Hamid, PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Asmah binti Rahmat, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Maznah binti Ismail, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Jamaluddin bin Mohamed, PhD
Professor
Faculty of Health Sciences
Universiti Kebangsaan Malaysia
(External Examiner)

_____
HASANAH MOHD. GHAZALI, PhD
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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:

Suhaila binti Mohamed, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Noordin bin Mohamed Mustapha, PhD
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Nazimah binti Sheikh Abdul Hamid, PhD
Senior Lecturer
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

_____
AINI IDERIS, PhD
Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 14 DECEMBER 2006

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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RADHIAH BINTI SHUKRI

Date:

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3-DG	3-deoxyglucosones
AGEs	Advanced glycation end product
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
C	Cloves
CAT	Catalase
CML	N-carboxymethyl-lysine
D	Diabetic control
DC	Diabetic rats treated with cloves
DJ	Diabetic rats treated with jering
DG	Diabetic rats treated with Glibenclamide
DM	Diabetes mellitus
DR	Diabetic retinopathy
EDTA	Ethylene diamine tetra-acetic acid
g	Gram
G	Glibenclamide
GBM	Glomerular basement membrane
GFR	Glomerular filtration rate
GSH	Glutathione
GSH-Px	Glutathione peroxidase
GSSG	Oxidized glutathione
H_2O_2	Hydrogen peroxides

HCL	Hydrochloric acid
IDDM	Insulin-dependent diabetes mellitus
IU	International Unit
J	Jering
KCl	Potassium chloride
MDA	Malondialdehyde
mg	Miligram
MGO	Methylglyoxal
Ml	Mililiter
N	Normal control
NC	Normal rats supplemented with cloves
NJ	Normal rats supplemented with jering
NASH	Non-alcoholic steatohepatitis
NIDDM	Nitric oxide
NO	Non-insulin-dependent diabetes mellitus
O_2	Oxygen
PKC	Protein kinase C
RBC	Red blood cells
ROS	Reactive oxygen species
SD	Standard deviation
SOD	Superoxide dismutase
STZ	Streptozotocin
TBA	Thiobarbituric acid

TBARS	Thiobarbituric acid reactive substances
TGF- β	Growth factor β

CHAPTER 1

INTRODUCTION

For the most part of this century, health concerns in the field of human nutrition that have been centered around deficiency disorders of macro and micronutrients with emphasis on the role of essential nutrients in health and disease. In recent years, various dietary constituents have been found to provide protections against any disease. Any significant role by dietary intervention is encouraging and emerging as an acceptable approach for controlling the diabetes mellitus incidence worldwide (Dasgupta et al., 2004). Currently there are over 150 million diabetics worldwide and this number is likely to increase to 300 million or more by the year 2025 due to increase in sedentary lifestyle, consumption of energy rich diet, and obesity (King *et al*., 1998). Prevalence of diabetes mellitus among Malaysians was 10.5% in 1996 and is dangerously increasing to 15% in 2003 (Mafauzy, 2005).

Despite remarkable progress in the management of diabetes mellitus by synthetic drugs, there has been a renewed interest in indigenous anti-diabetic agents, especially the medicinal plants attributed with therapeutic virtues (Grover *et al*., 2003). High prevalence and long-term complications of diabetes mellitus (Palumbo, 2001) have prompted a search for new oral hypoglycemic agents from such antidiabetic plants (Grover *et al.*, 2003). The ethno-botanical information reports about 800 plants that may possess anti-diabetic potential. Many plant extracts and plant products have been shown to have significant antioxidant activity as well as having hypoglycemic properties (Sekar