UNIVERSITI PUTRA MALAYSIA

NUTRITIONAL COMPOSITION OF *STROBILANTHES CRISPUS* JUICE AND ITS EFFECTS ON HYPERGLYCAEMIA, HYPERLIPIDEMIA, WOUND HEALING AND TOXICITY IN RATS

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By

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

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Chairperson : Professor Asmah Bt. Rahmat, PhD

Faculty : Medicine and Health Sciences

Strobilanthes crispus juice is reported to have good medicinal properties for treating diabetes mellitus and wound healing. The first part of this study evaluated the effect of *S. crispus* juice on hyperglycaemic, hyperlipidemic and antioxidant enzymes in normal and STZ-induced hyperglycaemic male and female rats at dosages of 140, 210 and 280 mg/kg of body weight (bw) for 30 days. Serum glucose, lipid profile (total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol), antioxidant enzymes (glutathione peroxidase and superoxide dismutase) were determined on day 0, day 15 and day 30. The result showed that significant decrease of serum glucose levels in male and female diabetic rats with treated glibenclamide and all groups treated with 140, 210 and 280 mg/kg bw of *S. crispus* juice on days 15 and 30 when compared to control group and baseline data (zero day). The highest reduction of glucose level was 80.9 % on day 30 in male diabetic group treated with 280 mg/kg bw *S. crispus* juice, followed by 78.9 % reduction in group treated with 210 mg/kg bw, and 67.4 % reduction in group treated with 140 mg/kg bw of *S. crispus* juice. In

female diabetic groups, reduction of glucose level was 78.2 %, 68.9 % and 68.6 % in groups treated with 140, 210 and 280 mg/kg bw of *S. crispus* juice respectively. Administration of *S. crispus* juice also reduced total cholesterol, triglyceride, LDL-cholesterol; increased HDL-cholesterol, the activity of glutathione peroxidase and superoxide dismutase in STZ-induced diabetic and normal rats.

Second part of this study was to determine the effect of *S. crispus* juice on wound healing and antioxidant enzymes (glutathione peroxidase and superoxide dismutase) in normal and diabetic rats. Three levels of dosage (70, 100 and 140 mg/kg of body weight) were orally and repeatedly administered for 7 days. Mid-dorsal linear incisions of 2 cm in length were made on each animal. The results showed that supplementation of *S. crispus* juice enhances wound closure in normal and diabetic rats. Glutathione peroxidase and superoxide dismutase were increased in diabetic group treated with *S. crispus* juice.

Third part of this study investigated the proximate composition, vitamin and mineral contents of *Strobilanthes crispus* juice. The proximate analysis showed that *S. crispus* juice contained high moisture (75.01 %), carbohydrate content (33.47 %) and dietary fibre (12.29 g/100g). The contents of vitamin A, C and E in *S. crispus* juice were found to be 2.32 mg/100g, 9.37 mg/100g and 5.89 mg/100g respectively. *S. crispus* juice was found high in minerals including, sodium (37.21 mg/100g), potassium (124.99 mg/100g), calcium (172.88 mg/100g), ferum (0.57 mg/100g), phosphorus (8.18 mg/100g), magnesium (27.86 mg/100g), copper (0.14 mg/100g) and zinc (1.49 mg/100g).

The fourth part of this study evaluated the acute toxicity of *S.crispus* juice. The LD_{50} was determined with four different dosages of *S. crispus* juice (700, 2100, 3500 and 4900 mg/kg of body weight) administered orally to normal female and male rats. The rats were treated with a single dose of juice and toxic effects were observed within 7 days. The results showed that no death or toxicity signs were observed in LD_{50} tested in normal rats. In the blood chemistry tests, no significant changes ($p<0.05$) were observed for most parameters (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatinine and albumin) tested in normal rats. The 30 days toxicity effect were determined by the repeated dosing study using normal and streptozotocin-induced diabetic rats of both sexes. Three level of dosage (140, 210 and 280 mg/kg of body weight) were orally and repeatedly administered for 30 days. The results showed that no significant changes in general behaviour, body weight and organ weight. No differences were noted between the test and control groups in haematological, macroscopic and histopathological findings. The administration of *S. crispus* juice to normal rats revealed insignificant change in liver and kidney functions, but significant reduction of aspartate aminotransferase (group female and male rats with administration 280 mg/kg b.w. of *S. crispus* juice), alanine aminotransferase (group female rats with 280 mg/kg b.w. of *S. crispus* juice) and alkaline phosphatase (group female rats with 210 mg/kg b.w. of *S. crispus* juice). In streptozotocin-induced diabetes, the rise in blood glucose is accompanied by an increase in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and creatinine. After the administration of diabetic rats with *S. crispus* juice for 30 days, there was a significant reduction in AST, ALT, ALP and creatinine. In conclusion, *S. crispus* juice has high nutritional

value and found non-toxic. It shows potential as an antidiabetic drink and additional nutraceutical supplement for wound healing for diabetic patients.

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

KOMPOSISI NUTRIEN JUS *STROBILANTHES CRISPUS* DAN KESAN KE ATAS HIPERGLISEMIA, HIPERLIPIDEMIA, PENYEMBUHAN LUKA DAN KETOKSIKAN PADA TIKUS

Oleh

NORFARIZAN HANOON BT NOOR AZMI

Jun 2009

Pengerusi : Profesor Asmah Bt. Rahmat, PhD

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Jus *Strobilanthes crispus* mempunyai potensi perubatan yang tinggi di dalam merawat diabetes mellitus dan penyembuhan luka. Pada peringkat pertama kajian ini menilai kesan jus *S. crispus* pada dos 140, 210 dan 280 mg/kg berat badan ke atas hiperglisemik, hiperlipidemik dan enzim antioksida bagi tikus jantan dan betina yang normal dan hiperglisemik diaruh streptozotocin selama 30 hari. Serum glukosa, profil lipid (jumlah kolesterol, trigliserida, HDL-kolesterol, LDL-kolesterol), enzim antioksida (glutathione peroksida dan superoxide dismutase) ditentukan pada hari 0, 15 dan 30. Keputusan menunjukkan penurunan yang signifikan bagi tahap serum glukosa pada tikus diabetik jantan dan betina yang dirawat dengan glibenclamide dan kesemua kumpulan yang dirawat dengan jus *S. crispus* 140, 210 dan 280 mg/kg berat badan pada hari ke 15 dan 30 dibandingkan dengan kumpulan kawalan dan data asas. Tahap penurunan glukosa yang tertinggi adalah sebanyak 80.9 % bagi kumpulan diabetik tikus jantan yang dirawat jus *S. crispus* pada hari ke 30 dengan dos 280

mg/kg berat badan, diikuti oleh penurunan sebanyak 78.9 % bagi kumpulan yang dirawat 210 mg/kg berat badan dan 67.4 % bagi kumpulan yang dirawat 140 mgl/kg berat badan. Bagi kumpulan diabetik betina, penurunan tahap glukosa adalah 78.2 %, 68.9 % dan 68.6 % pada kumpulan yang dirawat jus *S. crispus* 280, 210 dan 140 mg/kg b.w. Pemberian jus *S. crispus* juga dapat menurunkan jumlah kolesterol, trigliserida, LDL-kolesterol; meningkatkan HDL-kolesterol, aktiviti glutathione peroksidase dan superoxide dismutase pada tikus diabetik diaruh STZ dan tikus normal.

Pada peringkat kedua kajian ini adalah menentukan kesan jus *S. crispus* pada penyembuhan luka dan enzim antioksida (glutathione peroxidase dan superoxide dismutase) di dalam tikus normal dan diabetik. Tiga tahap dos (70, 105 dan 140 mg/kg berat badan) diberikan oral selama 7 hari. Luka sepanjang 2 cm dilakukan di tengah belakang setiap tikus. Keputusan menunjukkan pemberian jus *S. crispus* mengalakkan penyembuhan luka pada tikus normal dan diabetik. Enzim glutathione peroxidase dan superoxide dismutase menunjukkan peningkatan bagi kumpulan diabetik yang dirawat dengan jus *S. crispus*.

Pada peringkat ketiga kajian ini adalah menentukan komposisi proksimat, kandungan vitamin dan mineral bagi jus *S. crispus*. Analisis proksimat menunjukkan jus *S. crispus* mempunyai kandungan air (75.01 %), karbohidrat (33.47 %) dan gentian diet yang tinggi (12.29 g/100g). Kandungan vitamin A, C dan E yang diperolehi bagi jus *S. crispus* adalah 2.32 mg/100g, 9.37 mg/100g dan 5.89 mg/100g. Jus *S. crispus* mempunyai kandungan mineral yang tinggi iaitu sodium (37.21 mg/100g), potassium (124.99 mg/100g), kalsium (172.88 mg/100g), ferum (0.57 mg/100g), fosforus (8.18

mg/100g), magnesium (27.86 mg/100g), kuprum (0.14 mg/100g) dan zink (1.49 mg/100g).

Pada peringkat keempat kajian ini adalah menilai keselamatan jangka pendek (akut) bagi jus *S. crispus*. Ujian DM_{50} ditentukan dengan menggunakan empat dos jus *S. crispus* yang berlainan (700, 2100, 3500 dan 4900 mg/kg berat badan) yang diberikan secara oral (suapan) kepada tikus betina dan jantan yang normal. Tikus tersebut diberikan jus secara dos tunggal dan sebarang kesan toksik diperhatikan dalam masa 7 hari. Keputusan menunjukkan tiada kematian atau tanda ketoksikan melalui pemerhatian pada ujian DM_{50} pada tikus normal. Keputusan ujian kimia darah menunjukkan tiada perubahan yang signifikan yang dinilai ke atas kebanyakan parameter biokimia (aspartat aminotransferase, alanin aminotransferase, alkalin fosfatase, kreatinin dan albumin) yang diuji pada tikus normal. Pada 30 hari kesan toksikologi yang ditentukan oleh ujian subakut yang menggunakan tikus normal dan diabetik yang disuntik streptozotocin pada kedua-dua jantina. Tiga tahap dos (140, 210 dan 280 mg/kg berat badan) yang diberikan setiap hari selama 30 hari kepada tikus normal dan diabetik. Keputusan yang diperolehi menunjukkan tiada perubahan yang signifikan pada pemerhatian bagi kelakuan, berat badan dan berat organ. Tiada perbezaan didapati di antara kumpulan ujian dengan kawalan bagi keputusan hematologi, makroskopi dan histopatologi. Pemberian jus *S. crispus* kepada tikus normal didapati tiada perbezaan yang signifikan bagi fungsi hati dan ginjal kecuali penurunan yang signifikan bagi aspartat aminotransferase (bagi kumpulan tikus betina dan jantan yang diberikan jus *S. crispus* 280 mg/kg berat badan), alanin aminotransferase (kumpulan tikus betina dengan pemberian jus *S. crispus* 280 mg/kg berat badan), dan alkalin fosfatase (kumpulan tikus betina dengan pemberian jus *S.*

crispus 210 mg/kg berat badan). Pada tikus diabetik diaruh streptozotocin, peningkatan glukosa di dalam darah menyebabkan peningkatan serum aspartat aminotransferase (AST), alanin aminotransferase (ALT), alkalin fosfatase (ALP) dan kreatinin. Selepas rawatan ke atas tikus diabetik dengan jus *S. crispus* selama 30 hari menunjukkan penurunan yang signifikan bagi AST, ALT, ALP dan kreatinin. Kesimpulannya, jus *S. crispus* mempunyai nilai pemakanan yang tinggi yang berpotensi dan tidak toksik. Ia menunjukkan potensi sebagai minuman antidiabetik dan nutraseutikal yang dapat menyembuhkan luka pada pesakit diabetik.

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I certify that a Thesis Examination Committee has met on 25 June 2009 to conduct the final examination of Norfarizan Hanoon Bt Noor Azmi on her thesis entitled "Nutritional Composition of *Strobilanthes crispus* juice and its Effects on Hyperglycaemia, Hyperlipidemia, Wound Healing and Toxicity in Rats" 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.

Members of the Examination Committee were as follows:

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

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Date: 17 July 2009

DECLARATION

I declare that the thesis is 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.

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NORFARIZAN HANOON BT NOOR AZMI

Date: 1 July 2009

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ACUC	Animal Care and Use Committee
ADA	American Diabetes Association
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AOAC	Association of Official Analytical Chemists
g	gram
GPx	Glutathione peroxidase
HCl	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
H_2SO_4	Sulphuric acid
HDL	High Density Lipoprotein
IMR	Institute of Medical Research
KCl	Potassium chloride
K_2SO_4	Potassium sulphate
LD_{50}	Median lethal dose
LDL	Low Density Lipoprotein
µkat/L	Microkat per liter
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MES-TRIS	Morpholino ethansulfonic acid- TRIS-hydroxy-methyl aminomethane
pg	Pico gram
SCJ	*Strobilanthes crispus* juice

SOD	Superoxide dismutase
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background

Herbs refer to non-woody seed-producing plants that are annual, biennial or perennial and die at the end of each growing season. Several parts of the plant such as the flower, stem, seed or root are used for medical or aromatherapy qualities. In general, health care professionals consider herbs to be a crude drug, which is used to prevent diseases, treat infection or maintain a state of health (McCann, 2004). Herbs can be used as extracts, juices, tea, lozenges, inhalation, oils, salves, capsules and herbal baths.

Herbs have found its place in modern day biomedical medications. About one-fourth of all biomedical medications commonly prescribed today, contain at least an active ingredient derived from plants and the additional contents are chemically synthesized in the laboratory (McCann, 2004). Herbs are advertised as being able to improve overall health, cure illness and control diseases from diabetes to AIDS (Magee and Loiacono, 2004).

Herbal medicine are plant derived material or preparations with therapeutic or other human health benefit which contain either raw or processed ingredients from one or more plants. In some traditions material of inorganic or animal origin may also be present (WHO, 2005). Herbal medicine as the major remedy in traditional medical