UNIVERSITI PUTRA MALAYSIA

EFFECTS OF *STROBZLANTHES CRISPUS* CRUDE AND TEA EXTRACTS IN STREPTOZOTOCIN-INDUCED HYPERGLYCEMIC RATS

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

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UPM

To my beloved mom and dad, for their outstanding support and patience
To my lovely siblings; Adik, Ina and Ain
To diabetics and their families, clinicians and researchers
Who are at war fighting this disease

fadzelly

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

EFFECTS OF *STROBILANTHES CRISPUS* CRUDE AND TEA EXTRACTS IN STERPTOZOTOCIN-INDUCED HYPERGLYCEMIC RATS.

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September 2005

Chairman: **Associate Profesor Asmah Rahmat, PhD**

Faculty: **Medicine and Health Sciences**

Strobilanthes crispus leaf has been used ethnomedically to treat diabetes mellitus and related disorders in Asia. The first part of this study is to develop a tea from leaves of *S. crispus* and investigate its antioxidant properties *in vitro*. Fermented and unfermented teas from young and old leaves of *S. crispus* were developed according to *Camellia sinensis* and *Camellia theifera* preparations for black and green tea, respectively. Three methods were used to determine the antioxidant activities i.e 1) β-carotene bleaching method 2) DPPH free radical scavenging assay 3) Ferric reducing/antioxidant power (FRAP) assay. The total phenolic content was also estimated using Folin-Ciocalteu method. The result showed that unfermented *S. crispus* tea displayed a higher antioxidant activity compared to fermented *S. crispus* tea. Tea developed from old or matured leaves possessed higher antioxidant activity compared to young leaves. However, commercial green (Sencha, UK) and black (Boh, Malaysia) tea that were developed from leaves of *C. sinensis* exhibited higher antioxidant activity among all

teas tested. The second part of this study was aimed at determining the effect of *S. crispus* crude extract on STZ-induced hyperglycemic rats. *S. crispus* (young and old leaves) were extracted with distilled water and given to normal and hyperglycemic rats at concentrations of 2.5, 5.0 and 7.5% for 21 days. Plasma glucose, lipid profile (total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol), total antioxidant status and serum potassium and magnesium contents were determined on baseline (day 0), day 7 and day 21. The results showed that *S. crispus* crude extract at concentrations of 2.5, 5.0 and 7.5% from old or matured leaves reduced glucose level significantly in hyperglycemic rats ($p<0.05$). Third part of this study evaluated the effect of *S. crispus* fermented and unfermented tea in STZ-induced hyperglycemic rats at a concentration of 2% for 21 days. Plasma glucose, lipid profile (total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol), total antioxidant status and serum potassium and magnesium contents were determined during baseline (day 0), day 7 and day 21. The results showed that both fermented and unfermented *S. crispus* tea reduced glucose level in hyperglycemic rats ($p<0.05$). Fermented and unfermented *S. crispus* tea also improved antioxidant status and lipid profile in hyperglycemic rats by lowering the total cholesterol, triglyceride, and LDL-cholesterol. Total antioxidant status and HDL-cholesterol also increased in hyperglycemic rats treated with fermented or unfermented tea *S. crispus*. Both fermented or unfermented *S. crispus* tea failed to prevent the reduction of serum magnesium in hyperglycemic rats.

PERPUSTAKAAN SULTAN ABDUL SAMAD
UNIVERSITI PUTRA MALAYSIA

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

KESAN EKSTRAK KASAR DAN EKSTRAK TEH DARI DAUN *STROBILANTHES CRISPUS* PADA TIKUS YANG DIARUH HIPERGLISEMIA MENGGUNAKAN STREPTOZOTOCIN

Oleh

MOHD FADZELLY ABU BAKAR

September 2005

Pengerusi: **Profesor Madya Asmah Rahmat, PhD.**

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Strobilanthes crispus telah digunakan terutamanya di Asia dalam perubatan tradisional untuk merawat diabetes mellitus dan penyakit yang berkaitan. Bahagian pertama kajian ini adalah untuk membuat teh dari daun *S. crispus* dan mengkaji ciri-ciri antioksidan dalam teh tersebut. Teh yang difermentasi dan yang tidak difermentasi dibentuk menggunakan kaedah membuah teh masing-masing dari daun *C. sinensis* dan *C. theifera.* Tiga kaedah digunakan untuk menentukan aktiviti antioksidan iaitu 1) Kaedah pelunturan β-karoten 2) Kaedah penghapusan radikal bebas DPPH 3) Kaedah penurunan ferik/kuasa antioksidan (FRAP). Kandungan fenolik keseluruhan juga ditentukan menggunakan kaedah Folin-Ciocalteu. Keputusannya menunjukkan bahawa teh *S. crispus* yang tidak difermentasi menunjukkan paras aktiviti antioksidan yang lebih tinggi dari yang difermentasi. Teh yang dibentuk dari daun tua menunjukkan aktiviti antioksidan yang lebih tinggi berbanding dengan daun muda. Walaubagaimanapun, teh hijau (Sencha, UK) dan teh hitam (Boh, Malaysia) yang

dibuat dari daun teh (*C. sinensis*) menunjukkan tahap aktiviti antioksidan yang paling tinggi dalam semua teh yang dikaji. Bahagian kedua kajian ini adalah untuk menentukan kesan ekstrak kasar pada tikus yang diaruh hiperglisemia menggunakan STZ. Ekstrak kasar daun *S. crispus* (daun muda dan tua) disediakan dengan menggunakan air suling dan diberi pada tikus normal and tikus hiperglisemia pada kepekatan 2.5%, 5.0% dan 7.5% selama 21 hari. Paras glukosa, profil lipid (kolesterol keseluruhan, trigliserida, HDL-kolesterol, LDL-kolesterol) plasma, status antioksidan keseluruhan dan kalium serta magnesium ditentukan pada hari 0, 7 dan 21. Keputusannya menunjukkan ekstrak kasar daun tua *S. crispus* pada kepekatan 2.5%, 5.0% dan 7.5% menurunkan paras glukosa secara signifikan pada tikus yang diaruh hiperglisemia ($p<0.05$). Bahagian ketiga kajian ini adalah untuk menilai kesan pengambilan teh *S. crispus* (difermentasi atau tidak difermentasi) pada tikus normal and tikus hiperglisemia pada kepekatan 2.0% selama 21 hari. Paras glukosa, profil lipid (kolesterol keseluruhan, trigliserida, HDL-kolesterol, LDL-kolesterol) plasma, status antioksidan keseluruhan dan kalium serta magnesium ditentukan pada hari 0, 7 dan 21. Keputusannya menunjukkan bahawa ke dua-dua teh *S. crispus* yang difermentasi atau tidak difermentasi menurunkan secara berkesan paras glukosa plasma pada tikus yang diaruh hiperglisemia ($p<0.05$). Kedua-dua teh *S. crispus* yang difermentasi atau yang tidak difermentasi juga mampu membaiki paras antioksidan keseluruhan dan juga membaiki profil lipid dalam tikus yang diaruh hiperglisemia dengan menurunkan paras kolesterol keseluruhan, trigliserida dan LDL-kolesterol. Status antioksidan keseluruhan dan HDL-kolesterol juga meningkat pada tikus hiperglisemia yang dirawat dengan teh *S. crispus* yang difermentasi atau yang tidak difermentasi. Walaubagaimanapun, ke dua-

dua teh tersebut gagal menghalang penurunan magnesium serum dalam tikus hiperglisemia.

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AAS = atomic absorption spectrometer

FRAP = ferric reducing ability of plasma or ferric reducing / antioxidant power

HDL-cholesterol = high density lipoprotein cholesterol

LDL-cholesterol = low density lipoprotein cholesterol

VLDL-cholesterol = very low density lipoprotein cholesterol

Min = minute

Hr = hour

Temp = temperature

TBA = thiobarbituric acid

TCA = trichloroacetic acid

DPPH = 1,1-diphenyl-2-picrylhydrazyl

HSDA = N-(2-hydroxy-3-sulfopropyl)-3, 5- dimethoxyaniline

STZ = streptozotocin

GOD = glucose oxidase

POD = peroxidase

TPTZ = 2,4,6-tripyridyl-s-triazine

CHAPTER I

INTRODUCTION

1.1 Background

Prevalence of diabetes in adults worldwide was estimated to be 4.0% in 1995 and this figure was estimated to rise to 5.4% by the year 2025. The prevalence is higher in developed than in developing countries. The number of adults with diabetes in the world will rise from 135 million in 1995 to 300 million in the year 2025. The major part of this numerical increase will occur in developing countries including Malaysia. There will be a 42% increase, from 51 to 72 million, in the developed countries and a 170% increase, from 84 to 228 million, in developing countries. Thus, by the year 2025, more than 75% of people with diabetes will reside in developing countries, as compared with 62% in 1995 (King *et al.*, 1998).

Diabetes is a costly disease and is associated with major long-term implications, not only for the health and well being of the affected individuals, but also for the government. WHO (2002) reported that the direct health costs of treating diabetic patients range from 2.5% to 15% of annual budget, depending on local diabetes prevalence and effectiveness of the treatment available. WHO estimated that there are over 171 million people worldwide who are afflicted with diabetes mellitus (WHO, 2004). These complications contribute to the enormous cost, both economic and

personal, that are associated with this disease. Generally, symptoms of diabetes complications develope years after this disease has occurred (Foster, 1991).

In Malaysia, the high demand for herbs has caused some herbs to be imported from other countries in high quantities. Based on estimated by the Director of Pusat Sumber Genetik Tumbuhan, Institut Biosains, UPM, Dr. Mohd Saad found that value of imported medicinal plants in Malaysia has increased from RM 167 million (1990) to RM 401 million (1997). Meanwhile, the value of medicinal plants exports have also increased from RM 17 million in 1990 to 58 million in 1997. In *Berita Minggu Newspaper* (1998), he also said that research from their institute found that 80% of the world's population still depend on traditional medicine, including herb. World Health Organization (WHO), as reported in the *Berita Minggu Newspaper* estimated that approximately 75 to 95% of world population still depend on traditional medicine for health care.

Malaysia is a country blessed with many kinds of herbs or plants which is frequently used in traditional medicine. In Malaysia, over 15 000 species of higher plants were found and about 1200 of these plant species have been reported to have potential pharmaceutical value some of which are being used as herbal medicine (Soepadmo, 1991). Furthermore, throughout the development of human culture, the use of natural products (especially from medicinal plants) has had magical-religious significance and different points of view regarding the concepts of health and disease existed within each culture (Rates, 2001)

One of the herbs that have great potential and is believed to have health-giving properties is "*pecah beling*" or *Strobilanthes crispus* (Figure 1.1). It is commonly known as "*daun pecah beling*" in Jakarta or "*enyoh kilo*", "*kecibeling*" or "*kejibeling*" in Java (Sunarto, 1977). It is also locally known as "*pecah kaca*" or "*jin batu*".

Figure 1.1 : *Strobilanthes crispus* ZII 109 (L) Bremek or *Saricocalix crispus* ZII 109 (L) Bremek (Acanthaceae)

Traditionally, the leaves of pecah beling are boiled with water and the filtrates used in traditional medicine in Malaysia and Indonesia as antidiabetic, diuretic, antilytic and laxative agents. This plant has many cystoliths of calcium carbonate and an infusion is mildly alkaline (Perry & Metzger, 1980). A recent study indicated that the water extract of *S. crispus* contained compounds that inhibits the proliferation of retrovirus; an agent in viral disease such as acquired immune deficiency syndrome (AIDS) and adult T-cell Leukemia (Kusumoto *et al.,* 1992). This plant also possesses antimicrobial properties (Soediro *et al.*, 1983), high antioxidant activity (Ismail *et al.*, 2000), anticancer properties (Endrini, 2003) and antihepatocarcinogenesis (Jaksa *et al.*, 2004)

1.2 Problem statement

Until today, there is no treatment that can completely cure diabetes mellitus. Presently, insulin is used to treat diabetes mellitus type 1. On the other hand, the pharmacological agents currently used for the treatment of type 2 diabetes include sulphonylureas, biguanide, thiazolidinedione and acarbose. These agents however have restricted usage due to several undesirable side effects and failure to significantly alter the course of diabetic complications. Current insulin regimens (in type 1 diabetes mellitus) are problematic in maintaining physiological blood glucose profile (Groop *et al.*, 1985). Hypoglycemic agent such as glibenclamide can cause acidosis and impair cardiac function (Legtenberg *et al.*, 2002) and is not effective in long-term treatment (Gerich *et al.*, 1985). In addition, hypoglycemic drug such as sulphonylurea, leads to a higher risk