UNIVERSITI PUTRA MALAYSIA

INSULINOTROPIC PROPERTIES OF SEVERAL MALAYSIAN HERBS AND GANODERMA SPECIES

MOHD SAUFI BASTAMI.

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MOHD SAUFI BASTAMI

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Chairman : Muhajir Hamid, PhD

Faculty : Biotechnology and Biomolecular Sciences

Diabetes Mellitus incident is currently one of the major and common diseases in Malaysia and increasing every year. To date, no herbs or drugs are able to completely cure the disease. In this study, insulinotropic properties of Malaysian herbs were investigated using pancreatic β cells BRIN-BD11. Three out of 13 herbs samples studied were belongs to fungi (*Ganoderma sp*). The studied showed seven samples are able to induce insulin secretion over 200 % by BRIN-BD11 cell lines. Based on traditional practitioner reports, *Gynura procumbens* was choosed for further investigation on its anti-hyperglycemic properties. Glucose tolerance test were carried on normal and streptozotocin-induced diabetic rats showed at 1000 mg/kg/ b.w *Gynura procumbens* methanolic extract was able to reduce blood glucose level in rats. Toxicity towards BRIN-BD11 cells was analyzed using MTT assay and alkaline comet assay. Toxicity levels at IC_{50} was 300 ug/ml using MTT assay. Furthermore, Alkaline Comet Assay has proved this toxicity is not due to DNA damage in BRIN-BD11 cells. Acute toxicity was carried on ICR strain mice for 14 days. *Gynura procumbens* did not show any toxicity at concentration 7 g/kg b.w after administered orally. These results suggest *Gynura procumbens* and other herbs contain active constituents and have potentials for diabetes treatment.

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

CIRI-CIRI INSULINOTROPIK BEBERAPA HERBA MALAYSIA DAN SPESIES GANODERMA

Oleh

MOHD SAUFI BASTAMI

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Pengerusi : Muhajir Hamid, PhD

Fakulti : Bioteknologi dan Sains Biomolekul

Kes penyakit Diabetis Mellitus atau Kencing Manis kini menjadi penyakit utama dihadapi oleh kebanyakkan pesakit manakala bilangannya semakin meningkat setiap tahun di Malaysia. Sehingga kini masih belum ada ubat atau herba yang dapat memulihkan daripada penyakit ini sepenuhnya. Dalam kajian ini, ciri-ciri insulinotropik dari tumbuhan herba Malaysia di jalankan bagi melihat kebolehan tumbuhan herba merangsang penghasilan insulin oleh sel selanjar model sel β pankreas BRIN-BD11. Sejumlah tiga dari 13 sampel yang di kaji terdiri dari golongan kulat (*Ganoderma sp*). Kajian mendapati tujuh daripada sample kajian berupaya merangsang penghasilan insulin lebih dari 200 % oleh sel BRIN-BD11. *Gynura procumbens* dipilih berdasarkan laporan pengamal perubatann tradisional untuk ujian lanjut ciri-ciri anti hyperglycemia. Seterusnya, kajian toleransi glukosa ekstrak methanol *Gynura procumbens* terhadap tikus normal dan tikus diabetik yang di aruh oleh streptozotocin mendapati 1000 mg/kg b.w ekstrak berupaya menurunkan paras glukosa darah. Kajian toksisiti terhadap sel BRIN-BD11 mengunakan asai MTT dan Asai Komet Beralkali dijalankan. Asai MTT memberikan keputusan paras toksisiti *Gynura procumbens* pada IC_{50} adalah 300 ug/ml. Walaubagaimanapun, Asai Komet Beralkali membuktikan toksisiti ini berlaku bukan

disebabkan oleh pemusnahan DNA di dalam sel BRIN-BD11. Tahap toksisiti akut dijalankan kepada mencit dari strain ICR untuk tempoh 14 hari. *Gynura procumbens* didapati tidak memberi kesan toksik pada kepekatan 7 g/kg b.w selepas ekstrak di masukkan secara oral. Keputusan ini menunjukkan *Gynura procumbens* dan tumbuhan herba yang lain mengandungi komponen aktif dan berpotensi dalam rawatan kencing manis.

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LIST OF ABBREVIATIONS

b.w	Body weight
CO_2	Carbon dioxide
cm^2	Centimeter square
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic Acid
^oC	Degree Celsius
EDTA	Ethylenediamine tetraacetic acid
ELISA	Enzyme-Linked Immunosorbent Assay
EtBr	Ethidium Bromide
FBS	Feotal bovine serum
g	Gram
IC_{50}	Concentration of 50% inhibitory
KRBB	Krebs Ringer bicarbonate buffer
LMA	Low melting agarose
M	Molar
mA	mili Ampere
ml	Milliliter
mM	milimolar
MTT	3-[4,5-dimetiltiazol-2-yl]-2,5-dimetiltetrazolium bromide
N	Normality
NaCl	Sodium Chloride
NMA	Normal melting agarose
nm	Nanometer
OGTT	Oral glucose tolerance test
PBS	Phosphate buffer saline
RPMI 1640	Roswell Park Memorial Institute 1640 Medium
W	Watt

v/v	Volume /volume
ul	Microliter
mg	Miligram
w/v	Weight / volume
mmol	Milimole

CHAPTER 1

INTRODUCTION

Herbal and natural products have been used for centuries throughout the world in every culture especially on disease treatments by traditional method. Traditional medicines are medicine is made from natural resources whether plant, fauna or mineral. It can be found throughout the world either in the water or land. Malaysia is known for its diversity which are claimed to possess medicinal value. The Malaysians also practice traditional and herbal remedies as an alternative choice for disease treatment. Traditional medicines are still a source of disease treatment of the world population. It has been shown by Phillipson and Wright that 80 % of the world populations depends on traditional medicine (Rahman *et al.,* 1999). In two separate surveys recently conducted in Australia and United States respectively that almost 48.5 % and 34 % of respondent have used at least one form of unconventional therapy including herbal medicine (Grover *et al.,* 2001). Furthermore, many of the current commercial drugs are derived from traditional plants based. In the United States, approximately 25 % of prescription contains active ingredients derived from plants. However the proportion in others countries are varies, such as in Germany, 40 % of drugs are based on plant materials (Lee, 1999). Well known drug for headache, aspirin, was originally created from two herbs, - white willow bark and meadowsweet (Lee, 1999). Traditional herbs were also generating an economic value to some countries in the world on medicinal aspect. About 30 % of the worldwide sales of drugs are based on natural product or herbs. Developing new potential drugs

might cost millions dollars. Pharmaceutical companies have to spend US$ 350 million dollars to develop new drugs (Grabley & Thiericke, 1999).

Malaysian herbal market is valued at about US$ 20 billion and growing at a rate of 20 % annually. Much of it is imported either from Indonesia, China or India (Kadir & Lope Pihie, 2001). Malaysian herbal research and development are focusing into few herbs discoveries such as anti-malarial, cytotoxicity, anticancer agents from *Goniothalamus* and male aphrodisiacs agent from the root extract of *Eurycoma lingofolia* (Kadir & Shaari, 2000). There are many more potential herbs which were used by traditional practitioner which are undiscovered.

Noor and Ashcroft (1989) described that over the past years; scientific and medical knowledge on the role of plant-derived product in the treatment of diabetes mellitus has advanced and created an exciting new area of research which could provide valuable information for the development of alternative drugs. With rich of natural resources, almost 6800 species of seed plants and 600 seedless plants, Malaysia may able to develop drugs towards many diseases.

Most studies on remedies usage are not really understood. Scientific studies have been carried out on several plants which believed to possess anti-diabetic properties. *Andrographis paniculata*, the king of bitter is used since immemorial times as Chinese and Ayuverdic medicine mainly for liver disease and dysentery was combined with *Orthosiphon spicatus* on diabetes treatment (Akowuah *et al.*, 2004). In India,

hypoglycaemic activity of *Momordica charantia* or bitter gourd, known as '*peria katak*' in Malaysia, was studied since 1981 by Virdi *et al.*, (2003). Meanwhile, *Gynura procumbens*, found in various part of Southeast Asia, has been used for the treatment of eruptive fevers, rash and kidney disease (Zhang & Tan, 2000). Other potential herbs reported to have anti hyperglycaemic activity are *Alium cepa*, *Allium sativum*, *Murraya koeinigii*, *Viscum album*, *Sumbucus nigra*, *Tinospora crispa* and *Morinda citrifolia* (Grover *et al.,* 2002). Most of these plants are easily found in Malaysia.

Development for potential drugs on diabetes treatments are not limited to plants, but also able to be produced from Ganoderma. *Ganoderma lucidum* which known as '*Ling Zhi*' in China or '*reishi*' in Japan was studied by Tomoda *et al.,* (1986), is reported to have hypoglycaemic activity. It is also deemed as an elixir of life that could augment good health and well-being. However, hypoglycaemic activity and medicinal value of *Ganoderma tropicum* and *Ganoderma tsugae* are unknown.

About 1200 species of Malaysian plants have been reported to have medicinal value. Phytochemical screening, which aimed at scoring the presence of different types of chemical compounds such as alkaloids, flavonoids, saponins, terpenoids, eugenols, polyphenols, sterols, tannins, aloin, plysacharides, diterpenes and glycosides have been carried out (Kadir and Shaari, 2000; Grover *et al.,* 2002). These phytochemical compounds have been reported to have anti-tumor, anti-oxidant also male and women aprodiasiac properties (Kadir and Shaari, 2000). It is also believed to have potential on

enhancing insulin secretion from pancreatic β cells since some Malaysian herbs were used by traditional practioner on diabetes treatment.

The main objectives of the experiment are to study insulinothropic properties of some Malaysian Herbs including some species of Ganoderma. Plants that have potential on diabetes treatment as reported by traditional practioner will be used for insulinotrophic acting using rat pancreatic β cell lines. Measurement was carried on insulin secreting cell lines (BRIN-BD11 cell lines) using insulin ELISA methods. Various insulin concentrations are released by BRIN- BD11 cell lines affect from its stimulator. Plants and Ganoderma samples was extracted and tested towards BRIN-BD11 cells on their ability in enhancing insulin secretion and determine the cytotoxicity level and IC_{50} value by [3-(4, 5-dimethylthiazol-2-yl)-2, 5-dimethyltetrazolium bromide (MTT) assay.

The second objective is to study the anti-hyperglycemic properties of *Gynura procumbens.* A further study of *Gynura procumbens* methanolic extract on hypoglycaemic activities and its toxicity was carried as *Gynura procumbens* was reported by traditional practitioner to be safe taken as edible and useful in controlling diabetes. Study were carried to determine the maximum insulin levels secreted by BRIN-BD11 cell lines and *in vivo* study of OGTT (oral glucose tolerance test) on normal and induced diabetic rats by *Gynura procumbens* methanolic extract. Toxicity level of *Gynura procumbens* methanolic extract towards BRIN-BD11 cell lines and ICR strain mice will be evaluated. The genotoxicity towards BRIN-BD11 also were studied by Comet Assay and AOPI assay.

CHAPTER 2

LITERATURE REVIEW

2.1 Diabetes Mellitus

Diabetes mellitus (DM) is a complex disorder of carbohydrate, fat and protein metabolism. It is primarily a result of a defect in secretion or action of insulin, the hormone that facilitates and control the use of glucose in the cells. Due to the deficiency of insulin, diabetic patients have an impaired tolerance to glucose that leads to numbers of short term and long term complications. Early diagnosis of diabetes is determined by blood glucose level. Glucose levels before meal are generally run between 4-7 mmol/l or 60-100 mg/dl in normal people (Williams & Porte, 1974; Dagget, 1981). Diabetes not only limited to human being but also occurred on canine especially on type 1 diabetes, insulin dependent diabetis *mellitus* (IDDM) (Davison *et al.,* 2002).

Diabetes Mellitus is the commonest endocrine disorder that affects more than 100 million people worldwide (6 % of the population) and projected to grow over 220 millions within 40-50 years (Proietto *et al.,* 1999). In next 10 years, diabetes may affect about five times more people than it does now (Bailey, 2000; Grover *et al.,* 2002). Diabetes derived from Greek for *siphon*, which refers to the copious excretion of water that characterizes the disease, meanwhile *mellitus* came from Latin word for honey, characterized by the high sugar content in urine.

In Malaysia, diabetes mellitus is increasing towards serious level. The Ministry of Health has recorded 657,988 people in Malaysia suffering from diabetes in 2002 increasing from 525,858 in year 2001 (Abdullah, 2003). The number of diabetics estimated to be increased to 0.8 million cases in 2025. From the reports of admissions to government hospitals in Peninsular Malaysia, diabetes mellitus had increased from 5024 cases in 1979 to 17808 cases in year 1990, and in year 2020 about 144 600 Malaysians will suffering diabetes (Amos *et al.,* 1997).

2.1.1 Types of Diabetes

Diabetes can be divided into three distinct types, in which subtypes have been identified.

2.1.2 Type 1: Insulin –Dependent Diabetes Mellitus (IDDM)

Type 1 or insulin-dependant diabetes mellitus (IDDM) occurs in approximately 10 % of all diabetics' patients in western world. The symptoms are hyperglycaemia due to secondary insulin deficiency, occurring as a result of autoimmune destruction of pancreatic endocrine β cells (Perfetti & Ahmad, 2000). Type 1 commonly occurs on childhood (Docherty, 2001). This type of diabetes accounts for 3 % of all diabetes worldwide in 1997, meanwhile type 2 is by far the most common type (Proietto *et al.,* 1999).

2.1.3 Type 2: Non-Insulin Dependent Diabetes Mellitus (NIDDM)

Non-insulin dependent diabetes mellitus occurs when insulin is no longer reacting as metabolic hormone in reducing blood sugar level (insulin resistant) or insufficient ability to secrete insulin (Proietto *et al.,* 1999; Perfetti & Ahmad, 2000). It is reported that type 2 diabetes has strong genetic tendency (Proietto *et al.,* 1999). Combination of insulin resistant and insufficient insulin released has accounting 90-95% of diabetes in developed countries (Husen *et al.,* 2004). About 90 % of diabetics in western world are categorized by type 2 rather than type 1. Type 2 diabetic shows direct contact with obesity. Hartz *et al.*, 1983 and Kissebah *et al.,*1984, reports that a close relationship between upper body obesity with type 2 diabetes by increased free fatty acid oxidation could impair glucose oxidation thus leading to insulin resistance (Proietto *et al.,* 1999). The risk of diabetes seems to be increased ten times in women with both severe obesity and high waist to hips ratio.

2.1.4 Other Types of Diabetes

Other types of diabetes including entities secondary to or associated with certain other conditions or syndromes. Diabetes may be secondary to pancreatic disease removal of pancreatic tissues, endocrine disease such as acromegaly, Cushing's syndrome, glucagonoma, somatostatinoma, or the administration of certain drugs, hormones or chemicals that causing hyperglycaemia (Hamid, 1999).