PHYTOCHEMICAL SCREENING AND ANTIHYPERGLYCAEMIC ACTIVITIES OF *Cordyceps sinensis* AND ITS BASED PRODUCT (ESULIN)

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by

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LIST OF SYMBOLS AND ABBREVIATIONS

ALP	Alkaline phosphatase
ALT	Alkaline aminotransferase
ALX	Alloxan
ANOVA	Analysis of variance
Anti-GAD	Anti-glutamic acid decaboxylate
ARASC	Animal Research and Service Centre
AST	Aspartate aminotransferase
BMI	Body mass index
BUN	Blood urea nitrogen
CS	*Cordyceps sinensis*
DALYs	Disability Adjusted Life Years
DM	Diabetes mellitus
DNS	Dinitrosalicylic acid
FBG	Fasting blood glucose
FFA	Free fatty acid
FPG	Fasting plasma glucose
GC-MS	Gas Chromatography Mass Spectrometry
GDM	Gestational diabetes mellitus
H_2SO_4	Sulphuric acid
HbA1c	Glycated haemoglobin
HCL	Hydrochloric acid
HDL	High density lipoprotein
IC_{50}	Half maximal inhibitory concentration

ICA	Islet cell antibody
IDF	International Diabetes Federation
IDDM	Insulin-dependent diabetes mellitus
im	Intramuscular
ip	Intraperitoneal
iv	Intravenous
K^+ATP	Sodium-potassium adenosine triphosphatase
LDL	Low density lipoprotein
LOD	Limit of detection
Na_2CO_3	Sodium carbonate
NAD	Nicotinamide-adenine dinucleotide
ND	Not detected
NHMS	National Health Morbidity Survey
NIDDM	Non-insulin dependent diabetes mellitus
OGTT	Oral glucose tolerance test
PPARγ	Peroxisome proliferator-activated receptor-gamma
Rpm	Resource prospector mission
SEM	Standard error mean
STZ	Streptozotocin
TMC	Traditional Chinese Medicines
TTM	Traditional Tibetan Medicines
WHO	World Health Organization
VLDL	Very low density lipoprotein
g	Gram
g/day	Gram per day

Kg	Kilogram
L	Litre
ml	Millilitre
mg/dL	Miligram per decilitre
mg/kg/day	Milligram per kilogram per day
mg/ml	Milligram per millilitre
mmol/L	Milimol per litre
U/day	Unit per day

PENYARINGAN FITOKIMIA DAN AKTIVITI ANTIHIPERGLISEMIK *Cordyceps sinensis* DAN PRODUK ASASNYA (ESULIN).

ABSTRAK

Diabetes mellitus merupakan satu gangguan metabolik umum dengan komplikasi vascular mikro dan makro yang mengakibatkan morbiditi dan kematian yang ketara. Kajian yang diterangkan di dalam tesis ini adalah satu usaha untuk menilai potensi antihiperglisemik ekstrak akues *Cordyceps sinensis* (*C.sinensis*) dan produk asasnya, Esulin. Tiga esperimen utama telah dijalankan untuk mencapai objektif umum. Esperimen pertama ialah mengenal pasti kelas fitokimia *C.sinensis* dan Esulin untuk menyediakan nilai terapeutik herba ini sebagai asas untuk penyelidikan selanjutnya. Penyaringan fitokimia untuk kedua-dua ekstrak telah dijalankan dengan menggunakan prosedur standard bagi pengesanan alkaloid, tanin, saponin, terpenoid dan flavonoid. Keputusan yang diperolehi secara positif menunjukkan kehadiran empat sebatian bioaktif utama iaitu alkaloid, saponin, tanin, dan flavonoid dalam kedua-dua *C.sinensis* dan Esulin. Selain itu, terpenoid hanya hadir di dalam Esulin. Bahagian kedua kajian ini adalah untuk menentukan kesan rencatan ekstrak akues *C.sinensis* dan Esulin terhadap aktiviti enzim alfa amilase dan alfa glukosidase. Kedua-dua ekstrak dan juga acarbose (ubat standard sebagai kawalan positif) dilarutkan dalam air suling dan disediakan pada lima kepekatan yang berbeza iaitu 0.125, 0.25, 0.5, 1 dan 2 mg/ml. Setiap analisis dilakukan tiga kali dan purata digunakan untuk mengira peratusan enzim perencatan. Dalam analisis *in vitro* ini, ekstrak akueus Esulin mempunyai aktiviti rencatan alfa amilase dan alfa glucosidase yang baik dengan nilai IC_{50} yang rendah dan menunjukkan perencatan peratusan tertinggi pada kepekatan yang diuji (2 mg/ml) berbanding ekstrak akueus *C.sinensis*. Kajian ketiga yang dijalankan ialah penilaian *in vivo* terhadap kesan

C.sinensis dan Esulin pada tikus diabetis aruhan streptozotocin. Tikus diberikan secara oral ekstrak akueus *C.sinensis* dan Esulin (75, 150, 300 mg/hari masing-masing), metformin (150 mg/hari) dan Humulin R (5 U/hari) sekali sehari selama 28 hari. Keputusan yang diperolehi daripada kajian ini menunjukkan bahawa ekstrak *C.sinensis* dan Esulin menurunkan paras glukosa darah, fruktosamin, jumlah kolesterol dan trigliserida tikus diabetis secara ketara ($p < 0.05$). Pemberian kedua-dua ekstrak herba juga menyebabkan peningkatan ketara serum insulin dan kolesterol lipoprotein berketumpatan tinggi (HDL) apabila dibandingkan dengan tikus kawalan diabetis. Pada tikus aruhan diabetik, peningkatan glukosa darah disertai peningkatan tahap bilirubin, fosfat alkali (ALP), alanin amino transferes (ALT), aspartat aminotransferes (AST), urea dan kreatinin. Walaubagaimanapun, selepas rawatan dengan kedua-dua ekstrak bagi tempoh empat minggu, aktiviti serum bilirubin, ALP, urea dan kreatinin menunjukkan penurunan yang ketara. Penemuan ini adalah setanding dengan tikus diabetis yang menerima ubat standard, metformin dan Humulin R. Kesimpulannya, ekstrak akueus *C.sinenis* dan Esulin menunjukkan kesan antihiperglisemia dan hypolipidemia pada tikus diabetis dengan menurunkan paras glukosa darah, fruktosamin, kolesterol dan trigliserida dan pada masa yang sama dapat meningkatkan paras insulin dan kolesterol HDL. Kajian ini juga mencadangkan salah satu mekanisma potensi antidiabetis kedua-dua ekstrak ialah melalui perencatan alfa amilase dan alfa glukosidase. Walaubagaimanapun, mengambil kira semua data, kajian ini menunjukkan ekstrak akueus Esulin bekerja lebih baik berbanding *C.sinensis* dalam mengurus dan mengawal diabetes. Oleh itu, produk herba ini berpotensi menjadi calon untuk penyelidikan pembangunan ubatan pada masa hadapan terutama dalam rawatan diabetes mellitus.

PHYTOCHEMICAL SCREENING AND ANTIHYPERGLYCAEMIC ACTIVITIES OF *Cordyceps sinensis* AND ITS BASED PRODUCT (ESULIN).

ABSTRACT

Diabetes mellitus is one of the common metabolic disorders with micro and macro vascular complications that result in significant morbidity and mortality. The present study described in the thesis is an effort to evaluate the antihyperglycaemic potentials of the aqueous extracts of *Cordyceps sinensis* (*C.sinensis*) and its based preparation namely Esulin. Three main experiments were conducted to achieve the general objective. The first experiment identified the phytochemical classes of *C.sinensis* and Esulin in order to provide the therapeutic value of these herbs as a basis for further research. Phytochemicals screening for both extracts were carried out using the standard procedures for alkaloid, tannin, saponin, terpenoid and flavonoid detection. Results acquired positively showed the presence of four main bioactive compounds i.e. alkaloids, saponins, tannins, and flavonoids in both *C.sinensis* and Esulin. Additionally, terpenoids were only present in Esulin. The second part of this study was to determine the inhibitory effects of the *C.sinensis* and Esulin aqueous extracts on the activities of alpha amylase and alpha glucosidase enzymes. Both extracts as well as acarbose (standard drug as positive control) were dissolved in distilled water and were prepared at five different concentrations of 0.125, 0.25, 0.5, 1 and 2 mg/ml. Each analysis was performed three times and the mean was used to calculate the percentage of enzymes inhibition. In this *in vitro* analysis, the aqueous extract of Esulin had good alpha amylase and alpha glucosidase inhibitory activity with the lowest IC_{50} value and showed the highest percentage inhibition at the concentration tested (2 mg/ml) compared to *C.sinensis* extract. The third study conducted was the *in vivo* assessment on the effects of *C.sinensis* and

Esulin on streptozotocin-induced diabetic rats. The rats were administered orally with the aqueous extracts of *C.sinensis* and Esulin (75, 150, 300 mg/day each), metformin (150 mg/day) and Humulin R (5 U/day) once daily for 28 days. Results obtained from this part of study indicated that *C.sinensis* and Esulin extracts significantly ($p < 0.05$) reduced the fasting blood glucose, serum fructosamine, total cholesterol and triglycerides levels of diabetic rats. Administration of both herbal extracts also caused significant increase in the serum insulin and high density lipoprotein (HDL)-cholesterol levels when compared to diabetic control rats. In diabetic-induced rats, the rose of blood glucose was accompanied by an increased in serum bilirubin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea and creatinine levels. However, following treatment with both extracts for a period of four weeks, activities of serum bilirubin, ALP, urea and creatinine exhibited significant reduction. These findings were comparable with those receiving standard drugs, metformin and Humulin R. In conclusion, *C.sinensis* and Esulin aqueous extracts displayed antihyperglycaemic and hypolipidemic effects in diabetic rats by lowering blood glucose, serum fructosamine, cholesterol and triglyceride and at the same time can increase the levels of serum insulin and HDL-cholesterol. This study also suggests that one of the mechanisms for antidiabetic potential of both extracts is by the inhibition of alpha amylase and alpha glucosidase enzymes. However, taking all data together, this study revealed that the Esulin aqueous extract worked better compared to *C.sinensis* extract in managing and controlling diabetes. Thus, this herbal product could become the next potential candidate for drug development research, particularly in the treatment of diabetes mellitus.

CHAPTER 1

INTRODUCTION

1.1 Background

Diabetes mellitus (DM) is a serious and chronic endocrine metabolic disorder characterised by persistent hyperglycaemia. It is caused by inadequate insulin either in its production or its function (American Diabetes Association (ADA), 2014; Kumar and Clark, 2002). Insulin deficiency leads to chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism (Beverley and Eschwege, 2003; Manikandan *et al.*, 2013). It has a significant impact on health, quality of life and life expectancy of patients even though it is regarded as a common and controllable disease (Kirkman *et al.*, 2012). As a global burden, DM is recognised as one of the five major causes of morbidity in the world (Ahmed *et al.*, 2010a) that leads to severe health complications such as neuropathy, retinopathy, nephropathy, cardiovascular complication and ulceration (Fowler, 2008; Soumya and Srilatha, 2011).

The epidemic of diabetes has enormously affects more than 382 million individuals throughout the world (Ramachandran *et al.*, 2014; Sugathan *et al.*, 2013). In year 2013, global estimates further growth of this disease for almost 50% with the greatest increase in the developing countries of Asia, South America and Africa (Zimmet *et al.*, 2001). According to Diabetes Atlas of the same year (2013), the current highest number of people with diabetes are reported in the European and Western Pacific regions with 53 and 138 million, respectively (Chan*et al.*, 2014).

However, by the year 2025, the South East Asia region is expected to have the greatest number of diabetes with the estimated prevalence of 13.5% or in number is 145 million people (Letchuman *et al.*, 2010).

In Malaysia, diabetes progressively gained momentum over the decades. According to the World Health Organization (WHO), Malaysia is expected to have a total number of 2.48 million diabetic people in 2030 as compared to 0.94 million in year 2000 (Subramanian *et al.*, 2008). The fact that, this statistic was worrying because among the top ten chronic diseases in the country, DM was ranked sixth for man and fifth for women according to the Disability Adjusted Life Years (DALYs) (Letchuman *et al.*, 2010). With such scenario, DM was ranked at sixth place in the top 20 causes of death in Malaysia (Yusoff *et al.*, 2013). WHO (2014) reported that this disease caused 4,750 or 3.74% of total deaths in the country, where Malaysia was ranked 95th worldwide. This statistic showed that diabetes ranks higher than hypertension and lung cancer (WHO, 2014).

The increasing prevalence of diabetes is not only regarded as a recent medical challenge but has contributed for a rapid increase in the cost of health care management of the particular disease and its chain complications over the last decade. The International Diabetes Federation (IDF) estimated around US$ 213 billion and as high as US$ 396 billion will be spent on medical cost for this disease by year 2025 (Ali and Jusoff, 2009). It is calculated that there will be almost 50% increase in direct health care costs from year 2003 to 2025 (Ibrahim *et al.*, 2010). In Malaysia, it was reported that the direct outpatient cost of diabetic patient per year was RM 802.15 for health clinic without specialist and RM 1127.00 for health clinic

with specialist (Ibrahim *et al*., 2010). Furthermore, it was revealed that the total costs of diabetic foot were RM 47373.70 per year. This charge only reflects the cost for managing an acute infection. However, the expenditure is estimated to escalate for outpatient follow up, physiotherapy and indirect costs of emotional suffering and reduced productivity (Lam *et al.*, 2014).

Despite the escalation of medical expenditure, there is still lacking of satisfactory and effective therapies for diabetes up to the moment (Ahmed *et al.*, 2010a; Ghosh and Suryawanshi, 2001). Although insulin therapy and other non-insulin medications can control many aspects of DM, there are numerous drawbacks include hypoglycaemia, gastrointestinal disturbances, weight gain, nausea, oedema, as well as kidney and liver complications which are commonly experienced among diabetic patients (Piedrola *et al.*, 2001). Hence, there is an urgent need to use multiple medications with acceptable tolerability and safety profiles. This aims to reduce the risk of serious adverse events including kidney and liver diseases, blindness, vascular and neurological problems that can lead to amputation and increased mortality (Cade, 2008).

For centuries there are growing interest in the use of medicinal plants as an alternative management for various chronic diseases including DM. Ethnobotanical reports have stated that, approximately 800 plants are being utilised as traditional remedies for the diabetes treatment, but many of them do not have scientific explanation (Aguilara *et al.*, 1998; Pushparaj *et al.*, 2000). To the present day, the medicinal values of various plant extracts have also been continuously studied by many diabetic research scientists worldwide (Daisy and Eliza, 2007; Lodha *et al.*,

2010; Noor *et al.*, 2008). From that, plenty of traditional plant preparations particularly the compounds with both hypoglycaemic and antioxidative properties have been selected for the development of antidiabetic agents (Day, 1998).

Among various medications and alternative medicines, several herbs have been known to cure and control diabetes. *Cordyceps sinensis* (*C.sinensis*) is one of the most valued herbs in the traditional Chinese medicine, which has a broad application in diabetes treatment. *C.sinensis* is classified as an Ascomycetes fungus and has been reported to possess various pharmacological actions, such as anti-inflammatory (Yu *et al.*, 2004), antitumor (Nakamura *et al.*, 1999), hypolipidemic (Koh *et al.*, 2003), anti-aging (Ji *et al.*, 2009) and hypoglycaemic (Kiho *et al.*, 2000). The popularity of *C.sinensis* is manifested by the emergence of many commercial products in the local and international market including Esulin. The product of Esulin also contains small amounts of other herbs and it has been formulated to be a potential candidate for the management of DM which is increasing rapidly in Malaysia.

1.2 Scope of the study

In this present study, *C.sinensis* was evaluated for its phytochemical analysis and antidiabetic activities both *in vitro* and *in vivo*. This herb was chosen due to its popularity as been widely utilised as antidiabetic agent and for treating various ailments. Although there are several previous experiments of the antidiabetic activities of *C.sinensis* (Fatma El Zahraa *et al.*, 2012; Kiho *et al.*, 2000; Lo *et al.*, 2006; Zhang *et al.*, 2006), but this current work is different in various aspects. This study focused on evaluation of the effectiveness between *C.sinensis* compared to its

based product, Esulin particularly on their *in vitro* and *in vivo* antidiabetic activities and fairly important was the analysis of their phytochemical classes.

Therefore, this present study was divided into three phases as listed below:-

a) Study I:- Screening of phytochemicals of the aqueous extracts of *C.sinensis* and its based product, Esulin.

   This experiment was conducted to determine the classes of secondary plant metabolites that present in both aqueous extracts of *C.sinensis* and Esulin.

b) Study II:- *In vitro* antidiabetic activity of the aqueous extracts of *C.sinensis* and its based product, Esulin.

   This *in vitro* antidiabetic study was investigated using alpha amylase and alpha glucosidase inhibition assays.

c) Study III:- *In vivo* antidiabetic activity of the aqueous extracts of *C.sinensis* and its based product, Esulin.

   This last phase of experiment was the confirmatory *in vivo* antidiabetic studies of both extracts that were eventually conducted in streptozotocin induced diabetic rats.

This set of study was aimed to generate a stronger pharmacological rationale towards understanding the efficacy of these extracts as potential antihyperglycaemic candidates.

1.3 Objectives of the study

1.3.1 General objective

The general objective of this study was to evaluate the potential antihyperglycaemic activities of the aqueous extracts of *C.sinensis* and its based formulation (Esulin) on management of diabetes.

1.3.2 Specific objectives

The specific objectives of this study are listed as follow:-

1. To identify the classes of secondary plant metabolites present in the aqueous extracts of *C.sinensis* and Esulin from the phytochemical screening analysis by means of Study I.

2. To assess the *in vitro* alpha amylase and alpha glucosidase inhibiting activities of the aqueous extracts of *C.sinesis* and Esulin by means of Study II.

3. To investigate the effects of aqueous extracts of *C.sinensis* and Esulin on the management of diabetes in streptozotocin (STZ) induced diabetic rats by means of Study III.

1.4 Importance of study

As the prevalence of DM is increasing, more research on alternative treatments based on natural resources need to be further investigated. However, one of the major problems with herbal medicine is lacking definite and complete information about the composition of extracts and there is little biological knowledge on the action of diabetes treatment.

With regards to the present study, scientific information on the types of a phytochemical group of *C.sinensis* and its based product, Esulin are not widely available. Thus, the identification of the constituents classes performed in this study is crucial to assist in the analysis of the therapeutic efficacy of these herbs.

Apart from this, the limited scientific literature pertaining to the *in vitro* antidiabetic activities of *C.sinensis* and Esulin enhance the urgency of conducting this present research. This *in vitro* investigation is fairly important to rule out the antidiabetic effects of both herbs on particular digestive enzymes. The encouraging findings of the enzymes inhibition assays pave way for the confirmatory *in vivo* study.

As in most efficacy studies, the *in vivo* experiment is necessary to be conducted in a complete body-system model in order to relatively substantiate the *in vitro* findings. Hence, the antidiabetic activities of both *C.sinensis* and its based product were finally investigated in experimental diabetic rats prior to the confirmation of the antidiabetic potential of these aforementioned herbs.

The information gathered from this study could be utilised as an initial scientific platform for the scientific community in particular, as well as for the public in general. This study could initiate other comprehensive research in order to develop a safe antidiabetic agent. Further, this research can also indirectly enlighten consumers about the potential efficacy of *C.sinensis* and Esulin. This will help in ensuring better understanding among the public and expanding new knowledge regarding these herbal formulations.

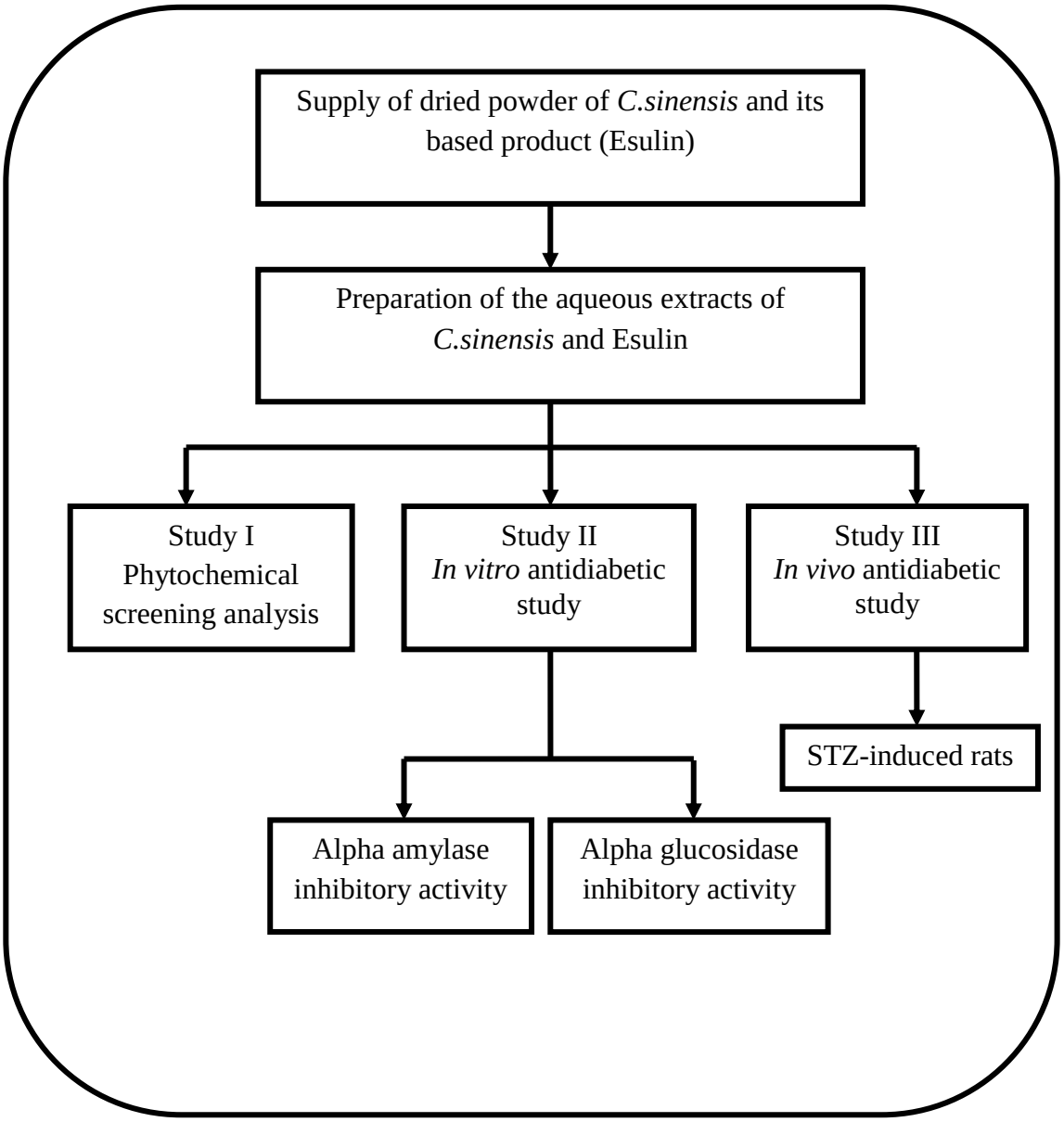

Figure 1.1 Experimental design of the overall study. The experiment comprised of screening of phytochemicals, the *in vitro* and *in vivo* studies of *C.sinensis* and its based product, Esulin.

CHAPTER 2

LITERATURE REVIEW

2.1 Diabetes mellitus

The phrase ‘diabetes mellitus’ is originally derived from the Greek words dia (= through) and bainen (= to go). The word mellitus (honey sweet) was added by Thomas Willis, a physician from Britain in 1675 after rediscovering the sweetness of urine and blood of patients. It was only in 1776 that Dobson from Britain firstly confirmed the presence of excess sugar in urine and blood as a cause of their sweetness (Ahmed, 2002).

WHO reported that diabetes will be the seventh leading cause of death in 2030 (Sugathan *et al.*, 2013). The escalation trend in the number of patients with diabetes due to many factors such as population growth, aging urbanization, increasing the prevalence of obesity and physical inactivity (Wild *et al.*, 2004). In fact, 90% of diabetic individuals have type 2 (non-insulin dependent) DM. Within this category, no more than 10% can be accounted for other rare forms of diabetes that are directly inherited (Pozzili and Di Mario, 2001).

In our country, the earliest diabetes study was carried out in 1960 (Pillay and Lim, 1960) and in 1966 (West and Kalbfleisch, 1966). The first National Health and Morbidity Survey (NHMS) in Malaysia was carried out in 1986, where prevalence of diabetes among adults age 35 years old and above was found about 6.3% (NHMS, 1986). Within ten years later, in the NHMS II, the figure had increased to 8.3% which was among adults of age 30 years old and above as shown in Figure 2.1

(Letchuman *et al.*, 2010). This shocking rise had encouraged the Ministry of Health Malaysia to initiate numerous national healthy lifestyle campaigns. Thus, a national steering committee was set up to improve the screening and management of DM among residents in primary and secondary care clinics.

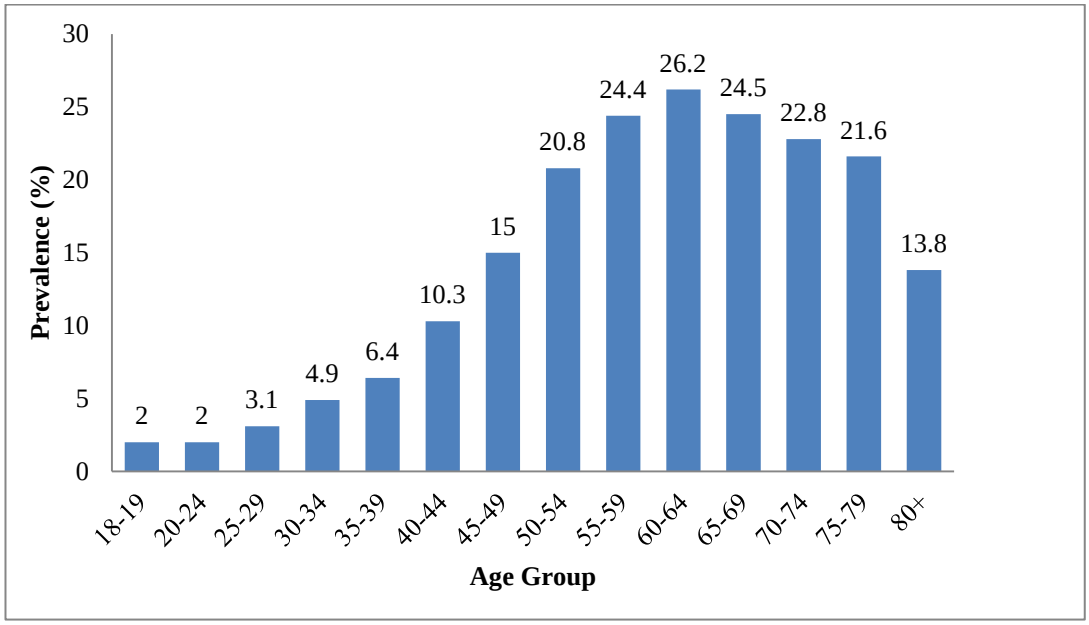

Figure 2.1 Prevalence of diabetes mellitus by age group in Malaysia

Source from Letchuman *et al*., (2010).

2.1.1 Major classifications of diabetes mellitus

The WHO (1980) classified diabetes based on the clinical characteristic, where there are two common types of diabetes, which are insulin-dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM). The WHO classification also recognises malnutrition related DM, which is other type of DM associated with specific conditions. Further is gestational diabetes which is diabetes diagnosed during pregnancy. There is a comparison of two major types of DM (type 1 and type 2) as shown in Table 2.1.

Table 2.1 Comparison between type 1 and type 2 diabetes mellitus

Features	**Type 1**	**Type 2**
Usual age of onset	Childhood/ young adulthood	Middle age or later
Body build	Normal	Overweight
Plasma insulin	Absent	Normal/high
Complications	Ketoacidosis	Hyperosmolar coma
Response to insulin	Normal	Reduced
Response to oral antidiabetic	Unresponsive	Responsive

Source from Alberti and Zimmet (1998).

2.1.1.1 Type 1 diabetes mellitus

Type 1 DM represents around 5-10% of all cases of diabetes, effecting approximately around 20 million people worldwide (Maahs *et al.*, 2010). Type 1 diabetes also known as juvenile diabetes or IDDM and it is usually diagnosed in childhood and adolescence. Type 1 is caused by an absolute deficiency of insulin, usually as the result of a T-cell mediated autoimmune destruction of the β-cells of the pancreas. Insulin is a hormone that is required to allow sugar (glucose) to enter the cell and produce energy. The two main forms of clinical type 1 DM are type 1a which is thought to be due to immunological destruction of pancreatic β-cells, which is resulting in insulin deficiency (Foulis *et al.*, 1991). Type 1a is characterized by the presence of islet cell antibody (ICA), anti-glutamic acid decaboxylate (anti-GAD), insulin antibodies that identify the autoimmune process with β-cell destruction (Epstein *et al.*, 1994). Another one istype 1b (idiopathic), on the other hand, is the condition where there is no evidence of autoimmunity. There is no known etiological basis for type 1b DM, some of patients have permanent insulinopaenia and tend to be ketoacidosis, but have no evidence of autoimmunity (McLarty *et al.*, 1990).

Various factors may contribute to type 1 DM, including genetics and environment. Although the genetic susceptibility to type 1 DM is inherited, only 12% to 15% of type 1 diabetes occurs in families. This indicates that genetic factors do not account entirely for the development of type 1 DM. Thus, environment plays a primary factor that triggers type 1 diabetes patients through viral infections, nutritional factors, low birth weight and parental age (Akerblom *et al.*, 2002).

2.1.1.2 Type 2 diabetes mellitus

Type 2 DM or NIDDM is the most common type of diabetes, affecting almost 5% to 7% of the world's population (Amos *et al.*, 1997; King *et al.*, 1998).The prevalence of diabetes is increasing rapidly. The WHO has predicted that by year 2030 the number of adults with diabetes worldwide will be more than doubled from 171 million in 2000 to 366 million (Wild *et al.*, 2004). The incidence of type 2 DM increases with age and it is common in individuals over the age of 40. However in some specific ethnic such as African-American, Asian-Pacific and American Indians show that type 2 diabetes is increasing among children and it is common than type 1 DM (Cockram, 2000). It is often related with obesity (BMI $\geq$25 kg/m2), decreased physical activity and genetic predisposition (Zimmet *et al.*, 1990). In general, the rate of type 2 DM is higher in urban populations than rural communities (King *et al.*, 1998) because it is associated with population, whose lifestyle has changed from traditional patterns to a modern (Bloomgarden, 1996).

Type 2 DM is a heterogeneous disorder that results from an interaction between a genetic predisposition and environmental factors, that leads to the combination of insulin deficiency and insulin resistance (DeFronzo *et al.*, 1997). Decreased insulin secretion and decreased insulin sensitivity are both involved in the onset of type 2 DM but the proportion of their involvement differs according to the patient. Pancreatic β-cell function is retained to a certain degree and insulin injections are rarely required for survival. Though, complications such as infection can lead to ketoacidosis temporarily but can arise with stress associated with another illness such as infection (Abate and Chandalia, 2003).

In fact, regular physical activity, healthy diet, maintaining a normal body weight and avoiding tobacco use are important components in the management of type 2 DM when blood glucose level cannot be controlled (Sugathan *et al.*, 2013). Patients are advised to use oral hypoglycaemic agents or insulin as treatments of DM. Based on United States National Institutes of Health, exercise and diet are the essential strategies to reduce body weight, improve glycaemic index and also reduce risk of cardiovascular complication, which show approximately 70% to 80% of mortality among those with type 2 DM. It has been shown that weight reduction and increment in daily energy expenditures will improve glucose tolerance and decrease insulin resistance (Staffers *et al.*, 1997). Based on epidemiological prospective study, regular physical activity performed several times a week has been associated with a decreased incidence of the disease in long term for both men and women at different age groups (Ross *et al.*, 2000).

2.1.1.3 Gestational diabetes mellitus

Gestational diabetes mellitus (GDM) is a carbohydrate intolerance associated with hyperglycaemia of variable severity with the onset or first recognition of glucose intolerance during pregnancy, which usually occur during the second or third trimester (ADA, 2003). In early pregnancy, fasting and postprandial glucose concentrations are normally lower than women, who are not pregnant. Individual with high risk of GDM includes older woman, woman with previous history of glucose intolerance, woman with history of babies larger for gestational age, woman from certain ethnic groups of higher risk for type 2 DM and any pregnant woman who has any elevation of fasting or casual blood glucose levels (WHO, 1999b). It is established that GDM can be harmful to both foetus and mother. It will increase the

risk of intrauterine foetal death during the last four to eight weeks of gestation and also causes other complications, including congenital abnormalities (Comess *et al.*, 1969). It occurs in about 4% of all pregnancies and patients with GDM have a 30% to 50% chance of developing type 2 DM later in life (Salim, 2005).

2.1.1.4 Other specific types of diabetes mellitus

Other specific types are small minority of cases estimated at about 2%, arise as the consequence of some other well-defined disease or predisposing factors such as pancreatitis or steroid excess (ADA, 2001). This category includes variety types of diabetes that is secondary to other specific conditions or associated with particular diseases or syndromes with a distinct aetiology. The categories and the causes of the other types of diabetes are shown in Table 2.2.

Table 2.2 Other specific types of diabetes mellitus

Types	**Diseases/syndromes**
1) Genetic defects of β-cell function	Chromosome 20,HNF4α (MODY1) Chromosome 7,glucokinase (MODY2) Chromosome 12,HNF1α (MODY3) Chromosome 13,IPF1 (MODY4) Chromosome 17,HNF3β (MODY5) Mitochondrial DNA, A3243G mutation
2) Genetic defects in insulin action	Type A insulin resistance Leprechaunism Rabson-Mendenhall syndrome Lipoatrophic diabetes
3) Others genetic syndromes sometimes associated with diabetes	Down syndrome Friedreich ataxia Huntington disease Klinefelter syndrome Laurence-Moon-Biedl syndrome Myotonic dystrophy Porphyria Prader-Willi syndrome Turner syndrome Wolfram syndrome
4) Uncommon forms of immune mediated diabetes	Insulin autoimmune syndrome (antibodies to insulin) Anti insulin receptor antibodies Stiffman syndrome
5) Diseases of the exocrine pancreas	Fibrocalculous pancreatopathy Pancreatitis Trauma/pancretectomy Neoplasia Cystic fibrosis Hemochromatosis Wolcott-Rallison syndrome
6) Endocrinopathies	Crushing syndrome Acromegaly Pheochromocytoma Glucogonoma Hyperthyroidism Somatostatinoma

7) Drug or chemical induced	Nicotinic acid Glucocorticoids Thyroid hormone α-adrenergic agonist β-adrenergic agonist Thiazides Phenytoin Pentamidine Pyriminil (Vacor) Interferon-α
8) Infections	Congenital rubella Cytomegalovirus

Sources from WHO (1999b).

2.1.2 Diagnosis of diabetes mellitus

Diagnosis of DM is the process of confirming that subject conforms to the disease concept. DM is diagnosed on the basis of the WHO recommendations from year 1999, which is incorporating both fasting plasma glucose and 2 h plasma glucose levels after 75 g oral glucose tolerance test (OGTT) into a practicable diagnostic classification (WHO, 1999a) (Table 2.3). If the fasting plasma glucose level is in the diagnostic range for diabetes, an OGTT is not required for diagnosis. A confirmatory test should be performed because a diagnosis of diabetes carries considerable and lifelong consequences for the patients as intraindividual variation or incomplete fasting may result in invalid diagnosis (Alberti and Zimmet, 1998).

Table 2.3 The 1999 World Health Organization diagnostic criteria for diabetes

	Glucose concentration (mmol/L)
Diabetes mellitus (DM)	Fasting ≥ 7.0 or 2h post glucose load ≥ 11.1
Impaired glucose tolerance	Fasting (if measured) < 7.0 and 2h post glucose load ≥ 7.8 and< 11.1
Impaired fasting glucose	Fasting ≥ 6.1 and < 7.0 and 2h post glucose load (if measured) < 7.8

Glucose load = 75 g glucose orally

Source from WHO (1999a).

2.1.3 Symptoms of diabetes mellitus

Symptoms are similar for both type 1 and type 2 DM but they are different in their intensity. Symptoms develop more rapidly and typical in type 1 DM. These include polydipsia, polyphagia, polyurea, weight loss, fatigue, cramps, blurred vision, constipation and candidiasis (Kumar and Clark, 2002). Although symptoms of type 2 DM are similar but insidious in onset. Most cases are diagnosed because of complications or incidentally. Type 2 DM carries high risk for large vessel atherosclerosis, which is commonly associated with hypertension, hyperlipidaemia and obesity (Saely *et al.*, 2004; Svensson *et al.*, 2004; Zimmet *et al.*, 1990). In early stages of the disease, the symptoms may be deficient but unfortunately the damage to organs occurs even in the absence of symptoms. For this reason, it is important for the community who may be at risk for diabetes to get their blood sugar level checked regularly. Those at risk include people age 40 and above, overweight or obese, anyone with parent or sibling who has diabetes and woman who has diabetes during pregnancy or a baby weighing more than 4 kg at birth (Alberti and Zimmet, 1998).

2.1.4 Complications associated with diabetes mellitus

DM is a long term disorder associated with a number of clinical problems that causing ill and mortality. Complications of DM can be divided into two categories, which are microvascular and macrovascular diseases. The diseases affect the small blood vessels in the retina, kidney, and peripheral nerves which appear to be most directly related to the duration and severity of the raised blood glucose (hyperglycaemia). Microvascular complications of DM may result in blindness, chronic renal failure and nerve damage to the feet contributing to the formation foot

ulceration and amputation. Macrovascular diseases on the other hand occur when large blood vessels in heart, brain and peripheral circulation are affected. Therefore, people with DM have higher rates of getting stroke, heart disease and peripheral vascular disease compared to normal people of the same age and sex (Kannel and McGee, 1979).

One of the most important complications of DM is it can affect the eye through the development of retinopathy. This common complication cause blindness and responsible for 10,000 new cases of blindness every year in the United States (Fong *et al.*, 2004). The prevalence of diabetic retinopathy in Malaysia has been reported progressively rise and much higher than the figures reported from U.K Australia and Japan (Tajunisah *et al*., 2011). The main risk of developing diabetic retinopathy and other complications of DM depends on both duration and severity of the patient with hyperglycaemia. According to literature, the severity of hyperglycaemia and uncontrolled hypertension are the crucial factors for the development of diabetic retinopathy in patients with type 2 DM. It is begins to develop as early as seven years prior to the diagnosis of diabetes in these patients (Fong *et al.*, 2004). Most patients with type 1 diabetes on the other hand develop evidences of retinopathy within 20 years of diagnosis (Keenan *et al.*, 2007).

Another complication of DM is diabetic nephropathy, which is the most common cause of established renal failure by damaging small blood vessels in kidney. This difficulty is one of the leading causes of morbidity and mortality in diabetic patient. The initial stage of diabetic nephropathy is microalbuminuria, which is defined as albumin excretion of 30-299 mg/24 hours higher than normal albumin

excretion (<20 mg/24 hours) (Fowler, 2008). Usually, diabetic patients with microalbuminuria will develop proteinuria and overt diabetic nephropathy, as this progression occurs in both types 1 and 2 DM. It affects about 30% of people with type 1 DM, typically appearing about 10 years after diagnosis. In those with type 2 diabetes, it tends to be appearing after a shorter time scale (Thomas, 2003). Like other microvascular complications of DM, patients should be treated to the lowest safe glucose level and normalization of blood pressure must be obtained to prevent or control diabetic nephropathy (Adler *et al.*, 2003; Gross *et al.*, 2005).

Diabetes also results in neuropathy which is a damage of the peripheral nervous system or the network of nerves. According to the ADA (2007), diabetic neuropathy is a symptom and/or signs of peripheral nerve dysfunction in the patient with diabetes after the exclusion of other causes. Neuropathy affects around 20% to 50% of patients with type 2 DM and out of this more than 75% of amputations occur after developing foot ulceration (Boulton *et al.*, 2005). Diabetic neuropathy can be divided into several different forms, including acute reversible and other persistent neuropathies, such as symmetrical, focal or multifocal and autonomic neuropathies. From that, symmetrical neuropathy also known as peripheral neuropathy is damage to the axon tips that begin in the longest nerves and has become the most common form among diabetic patients. In this condition, patients lose their sense to light touch, vibration and temperature and it leads to numbness (Fowler, 2008). However, there is no specific treatment of diabetic neuropathy, although many drugs are available to treat these symptoms.