EVALUATION ON HYPOGLYCAEMIC PROPERTIES OF AQUEOUS *Etlingera elatior* FLOWER EXTRACT ON DIABETIC-INDUCED RATS: A PILOT STUDY

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by

LIM BEE HUI

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CERTIFICATE

This is to certify that the dissertation entitle "Evaluation on hypoglycaemic properties of aqueous *Etlingera elatior* flower extract on diabetic-induced rats: a pilot study" is file record of research work done by Ms Lim Bee Hui during the period from February 2020 to July 2020 under my supervision.

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated and duly acknowledged. I also declare that it has not been previously or concurrently submitted as a whole for any other masters at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research, and promotional purposes.

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Lim Bee Hui

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

%	Percent
°C	Degree Celsius
±	More or less
>	Greater than
<	Less than
≥	Greater than or equal to
≤	Less than or equal to
g	Grams
iu	International Unit
mg	Milligrams
mg/dl	Milligram/decilitre
mg/kg	Milligram/kilogram
mmHg	Millimetre mercury
mL	Millilitre
mmol/L	Millimoles per litre
µL	Microlitre
µm	Micrometre
µmmol/L	Micromoles per litre

LIST OF ABBREVIATIONS

ABCC8	ATP Binding Cassette Subfamily C Member 8
ADA	American Diabetes Association
AEEFE	Aqueous Extract *Etlingera elatior* Flower Extract
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ATP	Adenosine Triphosphate
BB	Biobreeding
BMI	Body Mass Index
BP	Blood Pressure
BUN	Blood Urea Nitrogen
CAD	Coronary Artery Disease
CAPN10	Calpain 10
CDC	Centers for Disease Control and Prevention
DM	Diabetes Mellitus
DPP-4	Dipeptidyl-Peptidase-4
FBG	Fasting Blood Glucose
FDA	Food and Drug Association
GCGR	Glucose Receptor
GCK	Glucokinase

GDM	Gestational Diabetes Mellitus
GKRP	Glucokinase Regulatory Protein
GK	Goto-Kakizaki
GLP-1	Glucagon-Like Peptide 1
GLUT2	Glucose Transporter 2
GSH	Gluthathione
H&E	Hematoxylin-Eosin
HCC	Hepatocellular Carcinoma
HDL	High Density Lipoprotein
HFD	High-Fat Diet
hIAPP	Human islet amyloid polypeptide
HLA	Human Leukocyte antigen
HNF4a	Hepatocyte nuclear factor 4*a*
IDF	International Diabetes Federation
IP	Intraperitoneal
IPH	Institute for Public Health
LDL	Low Density Lipoprotein
LFT	Liver Function Test
MCO	Movement Control Order

MODY2	Maturity-Onset Diabetes of the Young 2
NAFLD	Non-Alcoholic Fatty Liver Disease
NASH	Non-Alcoholic Steatohepatitis
NHMS	National Health and Morbidity Survey
NOD	Non-Obese Diabetic
NZO	New Zealand Obese
OHA	Oral Hypoglycaemic Agent
OLETF	Otsuka Long-Evans Tokushima Fat
PAS	Periodic Acid-Schiff
PGB	Post-Prandial Blood Glucose
PKP	Perintah Kawalan Pergerakan
PPSK	Pusat Pengajian Sains Kesihatan
PSS	Power Sample Size
RFT	Renal Function Test
ROS	Reactive Oxygen Species
SBP	Systolic Blood Pressure
SD	Sprague Dawley
SEM	Scanning Electron Microscope
SGLT2	Sodium Glucose Cotransporter 2
SOD	Superoxide Dismutase

STZ	Streptozotocin
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
TCF7L2	Transcription Factor 7-Like 2
TC	Total Cholesterol
TG	Triglycerides
TNF	Tumour Necrosis Factor
UPMS	Unit Pengurusan Makmal Sains
USM	Universiti Sains Malaysia
WHO	World Health Organization
ZDF	Zucker Diabetic Fatty

Penilaian Sifat Hipoglisemik Ekstrak Akueus Bunga *Etlingera elatior* pada Tikus yang Diabetes: Kajian Rintis

ABSTRAK

Diabetes mellitus (DM) merupakan gangguan metabolik yang bercirikan hiperglisemia yang berterusan, di mana jumlah glukosa terlebih beredar dalam darah. Di Malaysia, *Etlingera elatior* telah digunakan secara tradisional untuk mengurangkan kadar glukosa darah. Kajian rintis ini bertujuan untuk menilai sifat hipoglisemik ekstrak akueus bunga *E. elatior* (AEEFE) pada tikus Sprague Dawley (SD) diabetik yang diaruh dengan diet lemak-tinggi buatan-sendiri (HFD) dan streptozotocin (STZ). Kajian rintis ini terbahagi kepada dua fasa: induksi kegemukan (keadaan prediabetik) menggunakan HFD buatan sendiri dan induksi diabetes mellitus Jenis 2 (T2DM) menggunakan 30 mg/kg STZ. Oleh sebab Perintah Kawalan Pergerakan (PKP) yang dikuatkuasakan oleh kerajaan Malaysia terhadap kes pandemik Covid-19, hanya keputusan daripada bahagian pertama telah dianalisis. Indeks Jisim Badan (BMI) tikus SD mencapai kategori kegemukan setelah 4 minggu pemakanan HFD walaupun terdapat penurunan pengambilan makanan. Kadar glukosa darah dan tekanan darah sistolik (SBP) kumpulan eksperimen juga meningkat. Walaupun fasa kedua tidak dapat dilaksanakan kerana PKP, keputusan yang dijangkakan masih dilapor berdasarkan data dan maklumat daripada artikel yang telah diterbitkan. Kesan AEEFE dalam pemeriksaan biokimia dan histopatologi dijangkakan berkesan dalam pemulihan jika dibandingkan dengan kumpulan diabetes tidak terawat. Sebatian bioaktif yang terdapat dalam AEEFE seperti antosianin dan flavonoid dijangkakan dapat mengurangkan komplikasi daripada hiperglisemia dan meningkatkan kepekaan insulin. Oleh itu, semua hasil dapatan yang dijangkakan dalam kesan AEEFE, bunga *E. elatior* dijangka mempunyai potensi hipoglisemik dalam rawatan T2DM.

Evaluation on Hypoglycaemic Properties of Aqueous *Etlingera elatior* Flower Extract on Diabetic-Induced Rats: A Pilot Study

ABSTRACT

Diabetes mellitus (DM) is a metabolic disorder characterised by persistent hyperglycaemia, in which an excessive amount of glucose circulates in the blood. In Malaysia, *Etlingera elatior* has been used traditionally for reducing blood glucose levels. This pilot study was designed to evaluate the hypoglycaemic properties of aqueous *E. elatior* flower extract (AEEFE) on a self-made high-fat diet (HFD) and streptozotocin (STZ)-induced diabetic Sprague Dawley (SD) rats. There were 2 phases in the pilot study: induction of obesity (prediabetic state) using self-made HFD and induction of Type 2 diabetes mellitus (T2DM) using 30 mg/kg STZ. Due to the Movement Control Order (MCO) imposed by the Malaysian government because of the pandemic Covid-19 outbreak, only the results from the first part of the study had been analysed. The Body Mass Index (BMI) of the Sprague Dawley (SD) rats reached the obesity category after 4 weeks of HFD feeding despite there was a reduction of food intake. The blood glucose level and systolic blood pressure (SBP) of the experimental group were also increased. Although the second phase of the study cannot be continued due to MCO, the expected results were still being reported based on the data and information from the previously published articles. The effects of AEEFE in the biochemical and histopathological examinations were expected to exhibit improved results when compared to the untreated diabetic group. The bioactive compounds present in the AEEFE such as anthocyanin and flavonoid were expected to minimise the complications from hyperglycaemia and increase insulin sensitivity. Therefore, from all the evidence collected in the expected effects on AEEFE, *E. elatior* flower is expected to have hypoglycaemic potential in treating T2DM.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Diabetes mellitus (DM) is a disorder characterised by persistent hyperglycaemia caused by insulin defect, insulin secretion or both (Ozougwu et al., 2013; ADA, 2020). There are three types of diabetes, Type 1 diabetes mellitus (T1DM), Type 2 diabetes mellitus (T2DM) and gestational diabetes. T2DM is the most common form of diabetes due to obesity, older age, and family history of the disease as risk factors (ADA, 2020). This disease has a very serious health effect and results in secondary complications, including cardiovascular, ocular, neurological and renal abnormalities that cause morbidity, disability and premature death in young adults.

DM is a group of metabolic disorders that are characterized by hyperglycaemia. Dysfunction in carbohydrate, lipid and protein metabolism and insulin signalling defect leads to hyperglycaemia (ADA, 2020). This condition is associated with oxidative stress, which increases the formation of reactive oxygen species (ROS) and reduces antioxidant levels. Under oxidative stress, a highly toxic product is released, resulting in oxidative damage to the pancreas, liver and kidneys (Sharma et al., 2011).

Nowadays, diabetes management is now focusing on the usage of medicinal plant because of their lower side effects compared to synthetic pharmaceutical drugs, which are more costly. In Malaysia, the flowers of *E. elatior* (bunga kantan) are traditionally used by the locals for food and medicinal purposes. They believed that daily intake of raw inflorescence could reduce diabetes (Wijekoon et al., 2011). However, the effects of *E. elatior* flower extract on diabetes have not been scientifically well studied.

The aim of this pilot study was therefore to evaluate the effects of anthocyanin-rich aqueous flower extract *E. elatior* as a potential antidiabetic agent and its mechanism in the T2DM rat model.

The aqueous *E. elatior* flower extract (AEEFE) was prepared in this study using a sonication process at the School of Health Sciences, Universiti Sains Malaysia (USM) Kelantan. Sprague Dawley (SD) rats will be induced by streptozotocin (STZ) to become diabetic. The efficacy of the extract will be assessed against metformin, an oral hypoglycaemic agent (OHA) in subacute animal studies. Blood will be collected within and at the end of the animal study; subject to biochemical investigation and assessment of oxidative stress biomarkers. The rat organs will be subject to histopathological examination.

1.2 Problem statement

In Malaysia, *E. elatior* inflorescences are traditionally used by the locals for food flavouring and medicinal purposes. They believed that daily intake of raw inflorescence could reduce diabetes (Wijekoon et al., 2011). As of today, no such effort has been made to understand the mechanism of action of the crude AEEFE associated with the reduction of hyperglycaemic properties in *in vivo* systems and histopathological changes in organs. This study is therefore proposed.

1.3 Rational of study

Previous study has shown that the potential of *E. elatior* flower to reduce blood glucose level in T2DM rat model (Nor et al., 2019). As of this date, it remains to be assessed whether the flower has an effect on the reduction or prevention of an end-organ complication due to diabetes. In addition, the relationship between the *E. elatior* flower and the antioxidant properties also has an effect on diabetes that has yet to be clarified. This study is therefore proposed to evaluate the effects of AEEFE as a potential antidiabetic agent in the T2DM rat model.

1.4 Study objectives

1.4.1 General objective

To evaluate the effects of anthocyanin-rich AEEFE in the T2DM rat model as a potential hypoglycaemic agent.

1.4.2 Specific objectives

1. To elucidate the effects of self-made HFD on the food intake, BMI, blood glucose, and blood pressure in the SD rats.
2. To evaluate the effects of oral AEEFE on lowering blood glucose levels in diabetic-induced rats.
3. To assess the effects of oral AEEFE on the lipid profile, liver and renal function tests in diabetic-induced rats.
4. To determine the relationship between oral AEEFE and oxidative stress biomarkers in diabetic-induced rats.
5. To assess histopathological changes in liver, kidney and pancreas in diabetic-induced rats.

1.5 Hypothesis

The hypothesis of the study are as follows：

The AEEFE may has the hypoglycaemic properties on diabetic rat.

Specifically,

1. The oral AEEFE significantly lowers the blood glucose in diabetic-induced rats
2. The oral AEEFE significantly improves on lipid profile, liver and renal function tests in diabetic-induced rat.
3. The antioxidant biomarkers significantly improved with oral AEEFE.
4. There is significant improvement of liver, kidney and pancreas structures in diabetic-induced rat.

1.6 Significance of the Study

Diabetes mellitus is a serious public health problem that has raised concerns around the world. Diabetes management is now focusing on the usage of medicinal plants because of their readily available, affordable and lower side effects compared to synthetic pharmaceutical drugs (Nasri, 2013; Nor et al., 2019). Results of this subacute analysis are therefore intended to clarify the possible antidiabetic effect of the *E. elatior* medicinal plant as a potential treatment for T2DM.

CHAPTER 2

LITERATURE REVIEW

2.1 Diabetes mellitus in general

Diabetes mellitus (DM) is a group of metabolic and physiological disorders characterised by elevated blood glucose level or hyperglycaemia resulting from either impairment in the insulin secretion, resistance to insulin action or inadequate response to the insulin secretion in pancreas (ADA, 2020; CDC, 2020; WHO, 2020). Chronic hyperglycaemia leads to prolonged damage, dysfunction, and failure of various body systems particularly the blood vessels, eyes, heart, kidneys, and nerves (Asmat et al., 2016; Tan et al., 2019).

2.2 Epidemiology

International Diabetes Federation (IDF) stated there are approximately 463 million adults aged 20-79 years were suffering from diabetes in 2019; and expected that this will rise to 700 million by 2045. Meanwhile, diabetes prevalence has been increasing rapidly in low- and middle-income countries. Approximately 1 in 5 of those over 65 years of age with diabetes and 1 in 2 (232 million) with diabetes worldwide have not been diagnosed. Diabetes is one of the top 10 causes of death in adults and is estimated to cause 4.2 million deaths worldwide in 2019. Global health expenditure on diabetes was estimated to be at least USD 760 billion in 2019, which was 10% of total adult spending. More than 1.1 million children and adolescents live with Type 1 diabetes mellitus (T1DM), while approximately 374 million people are at increased risk of developing Type 2 diabetes mellitus (T2DM). In addition, more than 20 million live births, which accounts for approximately 1 in 6 live births, are affected by diabetes during pregnancy.

In Malaysia, there is an alarming increase in the diabetes prevalence as reported by the National Health and Morbidity Survey (NHMS). The overall diabetes prevalence was 18.3% in 2019, affecting 3.6 million individuals, with approximately 9.4% known diabetes and 8.9% raised blood glucose among unknown diabetes (IPH, 2020). A study in Malaysia found the highest prevalence of T2DM among Indians, followed by Malays and Chinese (Hussein et al., 2015). This growing chronic disease has obviously affected the nation productivity particularly would have negative impacts on the socio-economic development in Malaysia.

2.3 Types of diabetes mellitus

The main categories of diabetes are type 1, type 2 and gestational diabetes mellitus.

2.3.1 Type 1 diabetes mellitus

Type 1 diabetes mellitus (T1DM) is generally considered as an autoimmune disorder and it represents only around 10% of the diabetes cases globally (Katsarou et al., 2017; Paschou et al., 2018). Autoimmune disorders occur when the immune system attacks the body's own tissues and organs. The T-cells respond to the autoantigens and damages the pancreatic insulin-producing β-cells. Damage to these cells impairs insulin production and leads to the signs and symptoms of T1DM. Generally, insulin controls how much glucose is passed from the blood into cells for conversion to energy. Lack of insulin results in the inability to use glucose for energy or to control the amount of sugar in the blood.

Scientists believe that the majority of T1DM is influenced by hereditary genes, namely the human leukocyte antigen (HLA) types (Noble et al., 2010; Noble and Valdes, 2011; Richardson et al., 2016). HLA complex polymorphic alleles, which located on chromosome 6, are responsible for 40-50% of the genetic risk of T1DM pathogenesis. In addition, some studies have shown that T1DM can also be triggered by the environmental factors including low vitamin D levels, lower exposure to ultraviolet rays, and viral infections (Coppieters et al., 2012; Craig et al., 2013; Beeck and Eizirik, 2016).

There is no cure for T1DM and the disease is progressing towards the complete destruction of β-cells of the pancreas. T1DM may occur at any age; but it usually develops at an early age, most of which begins in adolescence (Simmons, 2015; Stenberg, 2015; Katsarou et al., 2017).

People with T1DM need lifelong treatments of daily insulin injections to manage their condition, which still leaves them at risk of long-term complications. The injected insulin is made up for the insulin that is not produced by the body. Most people with T1DM require two to four injections per day (Kahanovitz et al., 2017; Katsarou et al., 2017).

2.3.2 Type 2 diabetes mellitus

Type 2 diabetes mellitus (T2DM) is a progressive health problem and accounts for around 90% of all diabetes cases worldwide (Toplak et al., 2016; WHO, 2020). Formerly known as non-insulin-dependent, or adult-onset, T2DM happens as a result of dysregulation of carbohydrate, lipid and protein metabolism, and results from hyperglycaemia, impaired of insulin resistance, insulin secretion, or a combination of both (Lin and Sun, 2010; Olokoba et al., 2012).

T2DM is characterised by insulin insensitivity (Asghar and Sheikh, 2017; Czech, 2017; Gaballah et al., 2017). Unlike people with T1DM, people with T2DM are making insulin. However, the body is becoming resistant to the normal effects of insulin, or is gradually losing the capacity to produce enough insulin in the pancreas. This condition makes the body unable to utilise the insulin effectively to maintain the glucose homeostasis over a long period of time.

T2DM is most commonly seen in persons older than 45 years of age (Ginter, 2012; Chaudhury et al., 2017). However, it is increasingly seen in children, adolescents, and younger adults due to rising levels of alcohol consumption, cigarette smoking, family history, hypertension, obesity, overweight, physical inactivity, poor diets and sedentary lifestyle.

Although there is yet no cure for the T2DM, it can be prevented through diet control, lifestyle modification, weight control. In addition, there are many medicines available in the market that have been approved by Food and Drug Association (FDA) to improve blood glucose level control (Olokoba et al., 2012; Marín-Peñalver et al., 2016).

2.3.3 Gestational diabetes mellitus

Gestational diabetes mellitus (GDM) is defined as hyperglycaemia with onset and first recognition between 24 to 28 weeks of gestation of a woman (Baynest, 2015; Kc et al., 2015). Placental production of diabetogenic hormones such as human placental lactogen in late pregnancy, leading to progressive insulin resistance; when adaptation β-cell function during pregnancy fails to compensate for maternal insulin resistance, it may lead to gestational diabetes. In addition, some researchers believe that gestational diabetes is caused by the hormonal changes of pregnancy combined with genetic and lifestyle factors. Gaining too much weight during pregnancy can also be a factor. The condition caused by GDM is typically temporary. It usually resolves after childbirth, but it carries pre-, peri-, and postnatal risks of adverse outcomes in the mother and the offspring.

As reported by IDF, about 7-10% of all pregnancies worldwide suffered from GDM. The vast majority of cases of hyperglycaemia during pregnancy have occurred in low- and middle-income countries, where access to maternal care is often limited (Behboudi-Gandevani et al., 2019).

Gestational diabetes is diagnosed by prenatal screening, rather than through reported symptoms. Although GDM may occur anytime during pregnancy, it generally affects pregnant women during the second and third trimesters, and at delivery (Kc et al., 2015; Mukerji et al., 2020).

Women with gestational diabetes are at subsequent high risk of developing T2DM, particularly three to six years after delivery (Reece, 2010; Joham et al., 2014; Zhu et al., 2019). In the meantime, exposure to hyperglycaemia in the womb predisposes offspring to a lifelong risk of becoming overweight or obese, due to the development of T2DM.

It is important for pregnant women with diabetes or GDM to carefully control and monitor their blood glucose levels in order to reduce the risk of adverse pregnancy outcomes with the support of their healthcare provider (Kc et al., 2015; Shen et al., 2019). GDM can usually be controlled through eating healthy foods and regular exercise (CDC, 2020). However, sometimes a woman with GDM must also take insulin to keep blood glucose under control. Table 2 summarised the comparison between T1DM, T2DM, and GDM.

Table 2.1: Comparison of T1DM, T2DM, and GDM (Adapted from Kommoju and Reddy, 2011; Heianza and Qi, 2017; Katsarou et al., 2017; Tan et al., 2019; CDC, 2020)

Features	Type 1 Diabetes Mellitus (T1DM)	Type 2 Diabetes Mellitus (T2DM)	Gestational Diabetes Mellitus (GDM)
Clinical	• Young onset: <20 years • Normal weight • Markedly decreased blood insulin	• Older onset: >30 years, obese • Increase blood insulin	• First detected in pregnancy, overweight
Prevalence	• 5% of all cases	• 90% of all cases	• 7-10% of all pregnancies
Risk factors	• Genetic • Environmental	• Age • Family history • Genetic • Obesity	• Family history • Obesity
Pathogenesis	• Autoimmune destruction of pancreatic β-cells	• Adipose tissue and liver • β-cells destruction • Insulin deficiency • Insulin resistance in skeletal muscle	• Insulin resistance • Placental hormones
Treatment	• Insulin for lifetime	• Exercise • Healthy lifestyle • Insulin • Oral medicines	• Healthy diet and lifestyle • Insulin • Oral medicines • Regular exercise

2.4 Signs and symptoms

The signs and symptoms of diabetes are caused by the increasing blood glucose level or hyperglycaemia. The classic symptoms of diabetes including polyuria (frequent urination), polydipsia (excessive thirst), polyphagia (extreme hunger), unusual weight loss, increased fatigue, irritability, blurry vision, pain or numbness of hands and legs (Kooti et al., 2016; Katsarou et al., 2017; Pawar et al., 2017).

Mild symptoms such as unexplained weight loss and easy fatigue may remain unnoticed at an early stage of diabetes (Ford et al., 2010; Bansal, 2015). People do not consider this to be serious problems because, unlike many other diseases, the consequences of hyperglycaemia are not immediately manifested. People are not aware that damage began many years until the symptoms become noticeable. This is unfortunate because recognition of early symptoms can act as warning signs for people to immediately control the disease and prevent vascular complications. Moreover, prediabetic conditions, which are disregarded by many, may lead to a chronic progression of the disease.

2.5 Diagnosis

Some symptoms of diabetes may have remained unnoticed for many years. Hence, there are several ways to test for diabetes in laboratory. Each way usually needs to be repeated on a second day to confirm diagnosis of diabetes. Table 2.2 summarise the measurements, diagnostic value, and characteristics of the measurements in diabetes diagnosis.

Table 2.2: The measurements, diagnosis value, and characteristic in diabetes diagnosis (Adapted from Inzucchi, 2012; ADA, 2020)

Measurement	Diagnosis values			Characteristics
	Normal	**Prediabetes**	**Diabetes**	
Glycated haemoglobin (HbA1C)	< 5.7%	5.7 – 6.4%	≥ 6.5%	Testing should be performed in a laboratory using a method that is National Glycohemoglobin Standardization Program (NGSP) certified and standardised to the Diabetes Control and Complications Trial (DCCT) assay. The test reflects average blood glucose levels over past 2- to 3 months.
Fasting plasma glucose (FPG)	< 100 mg/dl	100 mg/dl – 125 mg/dl	≥ 126 mg/dl (7.0 mmol/L)	Fasting is defined as no caloric intake for at least 8 hours.
Oral Glucose Tolerance Test (OGTT)- 2 hours plasma glucose	< 140 mg/dl	140 mg/dl – 199 mg/dl	≥ 200 mg/dl (11.1 mmol/L)	The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.
Random plasma glucose	80 – 140mg/dl (4.4 – 7.8 mmol/L)	140 – 200mg/dl (7.8 – 11.1 mmol/L)	≥ 200mg/dl (11.1 mmol/L)	Random is defined as any time of the day, regardless of the interval since the last meal.

2.6 Diabetes risk factors

2.6.1 Genetic factors

Genetic factors play important roles in the pathogenesis of diabetes. The human leukocyte antigen (HLA) complex accounts for the major genetic susceptibility to autoimmune disorder of T1DM (Noble and Valdes, 2011; Stenberg, 2015; Richardson et al., 2016). Polymorphism in HLA is strongly associated with the genetic susceptibility to T1DM. Some variants of the HLA-DQA1, HLA-DQB1, and HLA-DRB1 genes increase the risk of developing T1DM. These genes provide instructions on how to make proteins that play a critical role in the immune system.

Numerous mutations have been shown to reliably contribute to the risk in T2DM. Genes associated with T2DM risk including ATP binding cassette subfamily C member 8 (*ABCC8*), calpain 10 (*CAPN10*), glucose transporter 2 (*GLUT2*), glucagon receptor (*GCGR*) and transcription factor 7-like 2 (*TCF7L2*). These genes control production of glucose, insulin production and regulation, and glucose level homeostasis in the body (Hansen, 2002; Kommoju and Reddy, 2011; J. Song et al., 2017).

GDM is thought to have strong heritability. Compared to pregnant women with normal glucose tolerance, GDM women have a significantly greater parental history of T2DM (Joham et al., 2014; Mukerji et al., 2020). Besides that, maturity-onset diabetes of the young 2 (MODY2) is an autosomal dominant form of MODY due to mutations in glucokinase (GCK) (Shaat et al., 2006; Gjesing et al., 2017; Rosik et al., 2020). GCK mutations cause pancreatic glucose sensing defects and result in lower insulin levels during pregnancy. Apart from that, many studies have shown that the genes that play role in the occurrence of T2DM, exhibit strong connection to GDM risk. The genes such as *TCF7L2*, hepatocyte nuclear factor 4α (*HNF4a*), and glucokinase regulatory protein (GKRP) may increase the susceptibility to GDM (Shaat et al., 2006; Freathy et al., 2010; Zhang et al., 2013; Gjesing et al., 2017).

2.6.2 Environmental factors

Environmental factors such as unhealthy diet, sedentary lifestyle, stress, and viral infection, are generally believed to account for the rapid global increase in diabetes prevalence and incidence in recent decades (Chan and Woo, 2010; Heianza and Qi, 2017). Sedentary lifestyles and HFD consumption are generally behavioural factors conducive to the high prevalence of T2DM. Accumulation of fat from surplus calories and physical inactivity in the liver, muscles, and pancreas contributes to β-cell dysfunction and insulin resistance (Al-Goblan et al., 2014; Heianza and Qi, 2017). Long term of the unhealthy dietary and lifestyle promotes obesity or overweight, which increase the chance of developing T2DM.

On the other hand, viral infection has been shown to trigger T1DM. Enteroviruses are a group of viruses that usually cause mild illnesses, like the common cold. Certain enterovirus strains, such as the coxsackievirus, enterovirus-D68, poliovirus, rotavirus, mumps virus have been specifically linked to T1DM especially in children (Coppieters et al., 2012; Craig et al., 2013; Beeck and Eizirik, 2016). Children infected with enteroviruses may have no signs and symptoms of diabetes. In fact, they may exhibit symptoms of common cold like abdominal pain, diarrhoea, eye inflammation, fevers, sore throat, vomiting, skin rash, and sharp pains in the rib muscle that mask the real disease to be left untreated.

2.7 Diabetes complications

2.7.1 Diabetic nephropathy

Diabetic nephropathy is a serious kidney related complication that commonly present in patients suffering from both T1DM and T2DM. Over many years, poorly controlled diabetes can cause damage to blood vessels in the kidneys that delicate filtering system. This can lead to kidney damage and cause high blood pressure. High blood pressure can cause further kidney damage by increasing the pressure in the delicate filtering system of the kidneys. Diabetic nephropathy may gradually decrease kidney function and cause kidney failure over time (Teoh et al., 2010; Haneda et al., 2015; Pourghasem et al., 2015).

Three major histologic changes occur in the glomeruli of people with diabetic nephropathy. First, mesangial expansion is directly induced by hyperglycaemia, possibly through increased matrix production or glycation of matrix proteins (Tervaert et al., 2010; Nguyen and Goldschmeding, 2019). Second, thickening of the glomerular basement membrane (GBM) occurs and the kidney function decreases as the injury worsens (John, 2016; Kriz et al., 2017). Third, glomerular sclerosis is caused by intraglomerular hypertension induced by dilatation of the afferent renal artery or from ischemic injury induced by hyaline narrowing of the vessels supplying the glomeruli (Kovesdy et al., 2017; Trevisan and Dodesini, 2017). Histological changes of the kidney lead to renal function failure and eventually become the major factors of dialysis and transplantation.

2.7.2 Non-alcoholic Fatty Liver Disease (NAFLD)

Diabetes is closely associated with the rising prevalence and severity of non-alcoholic fatty liver disease (NAFLD) (Dietrich and Hellerbrand, 2014; Divella et al., 2019; Polyzos et al., 2019). This NAFLD refers to a wide spectrum of liver diseases ranging from the most common, fatty liver or steatosis, to advanced diseases such as non-alcoholic steatohepatitis (NASH), NASH-related cirrhosis, and hepatocellular carcinoma (HCC). NAFLD has a prevalence of 70% among T2DM patients. Particularly, the risk factors in T2DM such as overweight or obesity and insulin resistance have been strongly linked with NAFLD.

Fatty liver or steatosis, the hallmark of NAFLD, occurs when accumulation of fat in the liver. While NASH is the liver inflammation caused by fat in the liver. On the other hand, cirrhosis is an irreversible and advanced scarring of the liver as a result of chronic inflammation of the liver (Dietrich and Hellerbrand, 2014; Polyzos et al., 2019).

All stages of NAFLD are now believed to be due to insulin resistance, a condition closely associated with obesity. Both BMI and obesity correlate with the degree of liver damage (Sharma et al., 2011; Dietrich and Hellerbrand, 2014). In fact, the greater the BMI, the greater the damage to the liver.

2.7.3 Other complications

There are many other complications associated with DM namely diabetic cardiomyopathy, diabetic retinopathy, and diabetic neuropathy (Jia et al., 2018; Yang et al., 2019; Preguiça et al., 2020).

Diabetic cardiomyopathy is defined as the existence of abnormal cardiac morphology and performance of the myocardium in the absence of other confounding factors such as coronary artery disease (CAD), hypertension, and significant valvular disease (Marcinkiewicz et al., 2017; Jia et al., 2018). Both T1DM and T2DM patients are at risk of cardiomyopathy that leads to heart failure.

Diabetic retinopathy can also develop in anyone who suffering from T1DM or T2DM. At first, diabetic retinopathy may cause no symptoms or only mild vision problems. However, it can eventually affect significant vision on a global scale (Duh et al., 2017; Liew et al., 2017; Wang and Lo, 2018).

Diabetic neuropathy is another serious and common complication of T1DM and T2DM (Tesfaye, 2006; Said, 2013; Vinik et al., 2013; Mojto et al., 2019). It is a type of nerve damage caused by long-term hyperglycaemia. The condition usually develops slowly, sometimes over the course of several decades (Vinik and Erbas, 2013; Freeman, 2014).

2.8 Diabetes management

2.8.1 Non-pharmacology

The role of non-pharmacological treatment in diabetes is well known for the prevention or reduction of diabetes. Exercise not only reduces hyperglycaemia, but also reduces insulin resistance by reducing obesity (Balducci et al., 2014; Kameswaran et al., 2014). Therapeutic diet also is helpful in management of diabetes.

Kameswaran et al. (2014) reported that non-pharmacological interventions aimed at preventing T2DM including diet and physical activity. Lifestyle interventions appears to be at least as effective as drug treatment. Exercise is perceived to be beneficial for glycaemic control and weight loss in patient with T2DM. Physical activity not only improves blood glucose control in T2DM patients, but also reduces body weight and blood pressure. It improves the adverse lipid profile by reducing total cholesterol and low-density lipoprotein (LDL) cholesterol and increasing high-density lipoprotein (HDL) cholesterol.

Nutritional interventions are important in achieving optimal blood glucose level control in patients with T2DM (Hansen, 2002; Kameswaran et al., 2014). Most patients with T2DM are overweight or obese and, therefore, weight loss due to dietary restriction of energy helps in diabetes management (Raveendran et al., 2018). Tuomilehto (2009) has shown that lifestyle management is highly effective in the prevention and early management of T2DM. This evidence of lifestyle modification in diabetes prevention is stronger than in most other multifactorial diseases.

Obesity, physical inactivity, and unbalanced diet are well known to be the major risk factors for diabetes (Lin and Sun, 2010; Ng et al., 2010). In people genetically predisposed to the disease, the likehood of developing T2DM diabetes is high once they are exposed to unhealthy lifestyles.

2.8.2 Pharmacological

Pharmacologic approaches must be based on the patient characteristics, the level of glucose control and cost considerations. A combination of different oral agents may be useful for controlling hyperglycaemia before insulin therapy becomes necessary. Table 2.3 summarises the class, characteristics, and generic, and brand names of anti-diabetic drugs.

Table 2.3: The class, characteristics, and generic name, and brand name of anti-diabetic drugs (Adapted from Surya et al., 2014; Marín-Peñalver et al., 2016)

Class	Characteristic	Generic name	Brand name
Alpha-glucosidase inhibitors	Slow the breakdown of sugars and starchy foods	Acarbose Miglitol	Precose Glyset
Biguanides	Reduce the amount of glucose liver	Metformin	Glucophage
Dipeptidyl-peptidase-4 (DPP-4) inhibitors	Improve blood glucose level without making it drop too low	Alogliptine, Linagliptine, Saxagliptine, Sitagliptine	Tradjenta Onglyza Januvia
Glucagon-like peptides 1 (GLP-1) agonist	Change the way body produces insulin	Exenatide Liraglutide Dulaglutide	Trulicity Byetta Victoza
Meglitinides	Stimulate the pancreas to release more insulin	Nateglinide repaglinide	Starlix Prandin
Sodium glucose cotransporter 2 (SGLT2) inhibitors	Release more glucose into the urine	Canagliflozin Dapagliflozin Empagliflozin	Invokana Farxiga
Sulfonylureas	Stimulate the pancreas to release more insulin	Glipizide Glimepiride Glyburide	DiaBeta, Glynase Glucotrol Amaryl
Thiazolidinediones	Help insulin work better	Pioglitazone Rosiglitazone	Actos Avandia

2.9 *Etlingera elatior* (Bunga Kantan)

2.9.1 General description

Etlingera elatior or torch ginger is one of the most popular plants belongs to genus *Etlingera* in the ginger family known as Zingiberaceae. The genus *Etlingera* brings many beneficial products including dyes, foods, fragrance, essential oils, male aesthetics, medicine, and spices (Jaafar et al., 2007).

In Malaysia, *E. elatior*, also well known as Bunga kantan, is traditionally used by local folks for food flavouring and medicinal purposes. Young shoots and buds of *E. elatior* are consumed raw as fresh vegetables or *ulam* (Ghasemzadeh et al., 2015). Besides that, its flower (Figure 2.1) is commonly used as condiment for many iconic Malaysian dishes, for example asam laksa, nyonya laksa, asam pedas, nasi kerabu, and nasi ulam.

Figure 2.1: The flower of *E. elatior*

Traditionally, the local folks believe that daily intake of raw inflorescence of *E. elatior* can reduce diabetes and hypertension (Jeevani et al., 2011). The *E. elatior* flower methanol extract is used as traditional remedy for stomach aches or headache and applied externally to relieve itching (Lachumy et al., 2010). Apart from that, the decoction of its leaves is traditionally used in wound healing, while the fruits are used to treat earache (Chan et al., 2011; Jackie et al., 2011). Consumption of *E. elatior* inflorescence along with bitter leaves (*Vernonia amygdalina*) is thought to relieve flatulence in postpartum women (Mai et al., 2009; Wijekoon et al., 2011). In addition, the mixture of *E. elatior* leaves with other aromatic herbs are used for bathing to remove body odour especially among women (Chan et al., 2011).

2.9.2 Phytochemistry

In recent years, the phytochemical in *E. elatior* has received increasing interest due to their importance for biological properties. Phytochemical screening of *E. elatior* inflorescence has been reported to be rich in biochemical compounds such as anthocyanin, flavonoid, phenolic, saponins, tannins, and terpenoids contents (Lachumy et al., 2010; Wijekoon et al., 2011). In *E. elatior* leaves, chlorogenic acid, flavonoids of kaempferol 3-glucuronide, quercetin 3-glucuronide, quercetin 3-glucoside, and quercetin 3-rhamnoside have been reported. Meanwhile, diarylheptanoids, labdane, diterpenoids and steroids have been isolated from *E. elatior* rhizomes (Habsah et al., 2000; Ghasemzadeh et al., 2015). Its inflorescence has also been reported to contain a significant amount of carbohydrate, crude protein, fibre, leucine, lysine, unsaturated fatty acid (Jeevani et al., 2011; Juwita et al., 2018).

2.9.3 Potential therapeutic values

Traditionally, it is believed that the daily intake of raw inflorescence can reduce diabetes and hypertension diseases. Some studies have proven that *E. elatior* rhizomes have significantly exhibited anti α-glucosidase and anti α-amylase, antioxidant, anti-inflammatory properties (Ud-Daula et al., 2016; Juwita et al., 2018; Anzian et al., 2020) . In addition, various studies have shown that the inflorescence of *E. elatior* has many other biological properties such as anti-microbial, anti-cancer, anti-proliferative, cytotoxic activity and antioxidant (Chan et al., 2011; Ghasemzadeh et al., 2015). Researches conducted by Lachumy et al. (2010) and Susanti et al. (2013) proved that flower extract of *E. elatior* exhibit antibacterial activities against *Bacillus cereus* and *Staphylococcus aureus*, and antifungal activity against *Candida albican* and *Cryptococcus neoformans.* Their studies claimed that Gram positive bacteria were more susceptible toward the *E. elatior* flower extract compared to that of Gram negative bacteria. Apart from that, the extract of *E. elatior* has also showed promising cytotoxic and anti-tumour promoting activity (Ghasemzadeh et al., 2015; Juwita et al., 2018).