EVALUATION OF ANTI-DIABETIC ACTIVITY OF *Etlingera elatior* FLOWER (BUNGA KANTAN) AQUEOUS EXTRACT IN RAT WITH TYPE 2 DIABETES MELLITUS INDUCED BY HIGH-FAT DIET AND STREPTOZOTOCIN

NOR ASIAH BINTI MUHAMAD NOR

UNIVERSITI SAINS MALAYSIA

2022

EVALUATION OF ANTI-DIABETIC ACTIVITY OF *Etlingera elatior* FLOWER (BUNGA KANTAN) AQUEOUS EXTRACT IN RAT WITH TYPE 2 DIABETES MELLITUS INDUCED BY HIGH-FAT DIET AND STREPTOZOTOCIN

by

NOR ASIAH BINTI MUHAMAD NOR

Thesis submitted in fulfilment of the requirements

for the degree of

Doctor of Philosophy

MARCH 2022

ACKNOWLEDGEMENTS

In the name of ALLAH, The Most Gracious and The Most Merciful.

First and foremost, praise and thanks to ALLAH, The Almighty for His blessing and giving me the strength to successfully complete my research project. I would like to express my deep and sincere gratitude to my research supervisor, Associate Professor Dr. Wan Amir Nizam bin Wan Ahmad, for giving me the opportunity to do research and providing valuable guidance throughout this project. His dedication, supervision, and patience over the years have greatly motivated me. His timely advice and scientific approaches have helped me to a very great extent to accomplish this project. It was a great pleasure and honour to work and study under his guidance.

Special thanks are dedicated to my co-supervisors, Dr. Liza Noordin and Dr. Nor Hidayah Abu Bakar for their continuous support, opinions, and contributions towards the completion of this project. Their expertise and encouragement were appreciated. I also would like to acknowledge all staff from the Animal Research and Service Centre (ARASC), Central Research Laboratory (CRL), Scanning Electron Microscope Laboratory, and Biomedicine Laboratory, USM for their kind help and co-operation during my study period. Next, I am extremely grateful to my parents and family for their love, support, and prayers. To my husband, son and daughters, thank you very much for being understanding, supportive, and always be with me wherever I am. Last but not least, my special thanks go to my friends, Nurul Syahida, Nur Afifah, Norlida, Nur Hamizah, Marhasiyah, and Bibi for their unconditional support.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF EQUATIONS

LIST OF ACRONYMS, ABBREVIATIONS AND SYMBOLS

ADA	American Diabetes Association
AGE	Advance glycation end-product
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of variances
AOPPs	Advance oxidation protein products
ARASC	Animal Research and Service Centre
AST	Aspartate aminotransferase
BHT	Butylated hydroxytoluene
BP	Blood pressure
BUN	Blood urea nitrogen
CAT	Catalase
CTGF	Connective tissue growth factor
DKD	Diabetic kidney disease
DM	Diabetes mellitus
DN	Diabetic nephropathy
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
DPX	Distyrene plasticiser xylene
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
EEAE	*Etlingera elatior* flower aqueous extract
ELISA	Enzyme-linked immunosorbent assay
ESRD	End-stage renal disease
FBG	Fasting blood glucose
FRAP	Ferric reducing antioxidant power

FRIM	Forest Research Institute Malaysia
GA	Gallic acid
GAE	Gallic acid equivalent
GBM	Glomerular basement membrane
GDM	Gestational diabetes mellitus
GPX	Glutathione peroxidase
HbA1c	Glycated haemoglobin
HFD	High-fat diet
H&E	Haematoxylin & Eosin
H_2O_2	Hydrogen peroxide
HCL	Hydrochloride acid
HDL	High-density lipoprotein
HPLC	High-performance liquid chromatography
HRP	Horseradish peroxidase
IC_{50}	Half inhibitory concentration
IGT	Impaired glucose tolerance
IFG	Impaired fasting glucose
IL-1	Interleukin-1
IL-6	Interleukin-6
IP	Intraperitoneal
LDL	Low-density lipoprotein
LPO	Lipid peroxidation
MDA	Malondialdehyde
mg GAE/g	mg gallic acid equivalents per 1 g of plant material
mg QE/g	mg quercetin equivalents per 1 g of plant material
mM FE /mg	mM Ferrous equivalents per 1 mg of the dry extract
MT	Masson’s trichrome

NaCl	Sodium chloride
NCD	Non-communicable disease
NHMS	National Health and Morbidity Surveys
NOx	Nitrogen oxides
O_2	Superoxide anion radical
OD	Optical density
OECD	Organization for Economic Co-operation and Development
OGTT	Oral glucose tolerance test
OHA	Oral hypoglycaemic agent
Ox-LDL	Oxidised-low density lipoprotein
OH	Hydroxyl radical
PAS	Periodic acid Schiff
PKC	Protein kinase C
ppm	Parts per million
QE	Quercetin equivalent
R^2	Regression coefficient
Rf values	Retention factor
ROS	Reactive oxygen species
SBP	Systolic blood pressure
SD	Sprague-Dawley
SD	Standard deviation
SEM	Scanning electron microscope
SGPT/ ALT	Serum glutamic pyruvic transaminase/Alanine transaminase
SOD	Superoxide dismutase
STZ	Streptozotocin
TAC	Total anthocyanin content

TAOC	Total antioxidant capacity
TBA	Thiobarbituric acid
TFC	Total flavonoid content
TGF-β	Transforming growth factor-beta
TLC	Thin-layer chromatography
TNF-α	Tumour necrosis factor-alpha
TPC	Total phenolic content
TPTZ	2,4,6-Tris (2-pyridyl)-s-triazine
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
T2DR	Type 2 diabetes mellitus rat
UAE	Ultrasound-assisted extraction
UV	Ultraviolet
UV-Vis	Ultraviolet-visible
WHO	World Health Organisation
X	Distance of solvent travelled from its origin
Y	Distance of spot travelled from its origin
α	Alpha (Refers to type1 error probability)
β	Beta (Refers to power of study)
δ	Delta (Refers to the difference in means for selected parameter)
σ	Sigma (Refers to within group standard deviation)

PENILAIAN AKTIVITI ANTI-DIABETIK EKSTRAK AKUES BUNGA *Etlingera elatior* (BUNGA KANTAN) TERHADAP MODEL TIKUS DIABETIS MELLITUS JENIS 2 DENGAN ARUHAN DIET TINGGI LEMAK DAN STREPTOZOTOCIN

ABSTRAK

Diabetis mellitus (DM) adalah masalah kesihatan awam sejagat yang membawa kepada pelbagai komplikasi, termasuk kegagalan ginjal. Pada masa ini, penggunaan tumbuhan perubatan dalam pengurusan penyakit meningkat secara mendadak kerana kesan kesihatannya yang bermanfaat. Secara tradisional, bunga *Etlingera elatior* (bunga kantan) telah digunakan sebagai makanan tambahan untuk pengurusan diabetis. Walau bagaimanapun, sangat sedikit bukti saintifik mengenai keberkesanan tumbuhan ini dalam DM. Kajian ini bertujuan untuk menentukan kesan anti-diabetik ekstrak bunga *E. elatior* (EEAE) terhadap model tikus DM jenis-2 (T2DR). Aktiviti antioksidan *in vitro* dan anti-diabetik EEAE ditentukan melalui perencatan enzim pencernaan karbohidrat. Model T2DR telah dihasilkan menggunakan kombinasi makanan diet-tinggi lemak (HFD) dan streptozotocin (STZ). Empat puluh sembilan ekor tikus Sprague-Dawley (SD) dibahagikan sama rata kepada tujuh kumpulan; normal, obes, diabetis-tidak terawat, metformin-terawat, dan EEAE-terawat pada 500, 1000, dan 2000 mg/kg. Semua rawatan diberikan secara oral selama 6 minggu. Paras glukosa puasa dalam darah (FBG) dan berat badan diukur setiap minggu, sementara tekanan darah sistolik (SBP) setiap dua minggu. Pada akhir kajian, paras parameter biokimia dan penanda antioksida ditentukan. Histologi hati, ginjal, pankreas, dan aorta dinilai melalui pewarna haematoxylin dan eosin (H&E). Struktur

tisu ginjal seterusnya dinilai menggunakan analisis mikroskopi elektron (SEM) dan pewarnaan khas; “periodic acid Schiff” (PAS) dan “Masson’s trichrome” (MT). Selanjutnya, paras interleukin-6 (IL-6), “transforming growth factor-beta” (TGF-β), dan “connective tissue growth factor” (CTGF) dalam tisu ginjal dikaji. Terdapat aktiviti antioksida *in vitro*, dan perencatan *in vitro* terhadap enzim α-amilase dan α-glukosidase yang ketara. Dalam T2DR EEAE-terawat, FBG dan SBP berkurang dengan ketara berbanding kumpulan lain. Berbanding dengan T2DR-tidak terawat, EEAE memulihkan profil lipid, fungsi ginjal dan hati, dan meningkatkan paras penanda antioksida (“superoxide dismutase”/SOD, “catalase”/CAT dan “glutathione”/GSH). Tambahan pula, terdapat penambahbaikan pada histologi hati, ginjal, dan pankreas EEAE-terawat pada dos rendah dan pertengahan. EEAE juga menurunkan paras IL-6, TGF-β, dan CTGF dalam tisu ginjal. Sebagai kesimpulan, EEAE mempunyai aktiviti anti-diabetik, yang boleh dikaitkan dengan sifat antioksidan dan penurunan proses keradangan.

EVALUATION OF ANTI-DIABETIC ACTIVITY OF *Etlingera elatior* FLOWER (BUNGA KANTAN) AQUEOUS EXTRACT IN RAT WITH TYPE 2 DIABETES MELLITUS INDUCED BY HIGH FAT DIET AND STREPTOZOTOCIN

ABSTRACT

Diabetes mellitus (DM) is a global public health concern that leads to multiple complications, including renal failure. Nowadays, the use of medicinal plants in disease management is increasing exponentially due to their beneficial health effects. Traditionally, the flower of *Etlingera elatior* (bunga kantan) has been used as a supplement for diabetic management. However, very little scientific evidence on the efficacy of this plant in DM. This study aims to determine the anti-diabetic effect of *E. elatior* flower aqueous extract (EEAE) and improvement in renal damage on the type 2 DM rat (T2DR) model. The *in vitro* antioxidant, and *in vitro* anti-diabetic activity of EEAE were determined by inhibition of carbohydrate digestive enzymes. The T2DR model was achieved using a combination of high-fat diet (HFD) feeding and streptozotocin (STZ). Forty-nine male Sprague-Dawley (SD) rats were equally divided into seven groups; normal, obese, untreated-DM, metformin-treated, and EEAE-treated at 500, 1000, and 2000 mg/kg. All treatments were orally administered for 6 weeks. The fasting blood glucose (FBG) and body weight were measured weekly, while the systolic blood pressure (SBP) was fortnightly. At the end of the study, levels of biochemical parameters and antioxidant biomarkers were determined. The histology of the liver, kidney, pancreas, and aorta was assessed using haematoxylin and eosin (H&E) stain. The kidney tissue was further evaluated by

scanning electron microscopy (SEM) analysis and special staining; periodic acid Schiff (PAS) and Masson's trichrome (MT). Furthermore, the levels of interleukin-6 (IL-6), transforming growth factor-beta (TGF-β), and connective tissue growth factor (CTGF) in kidney tissue were elucidated. There were significant *in vitro* antioxidant activities, and *in vitro* inhibition on α-amylase and α-glucosidase enzymes. In EEAE-treated T2DR, FBG and SBP were significantly lower compared to other groups. When compared to untreated-T2DR, EEAE improved lipid profile, renal and liver function tests, and significantly increased antioxidant biomarkers levels (superoxide dismutase/SOD, catalase/CAT, and glutathione/GSH). In addition, there was an improvement in liver, kidney, and pancreatic histology of EEAE-treated at low and medium doses. EEAE also decreased the level of IL-6, TGF-β, and CTGF in kidney tissue. In conclusion, *E. elatior* has anti-diabetic activity, which can be attributed to its antioxidant properties and reduction of the inflammatory process.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Diabetes mellitus (DM) is a chronic disease associated with the elevation of blood glucose levels due to insulin secretion and action defects. It is commonly characterised by hyperglycaemia, which contributes to various complications that are responsible for morbidity, disability, and premature death in young adults (Canto et al., 2019). The global prevalence of diabetes is increased greatly from 2017 to 2019 with sedentary lifestyles, excess body weight, and physical inactivity being the primary consequences (International Diabetes Federation, 2019). This situation has impacted productivity and had a negative effect on the country's socio-economic growth.

DM is categorised into type-1 (T1DM) and type-2 (T2DM) based on the inability of the pancreas to produce insulin or insulin resistance. It is manifests as weight loss, polyuria, polyphagia, and polydipsia (World Health Organization, 2016). Obesity is the leading cause of T2DM, contributing to elevated triglyceride levels, hypertension, and insulin resistance (Gheibi et al., 2017). Uncontrolled diabetes can lead to retinopathy, nephropathy, neuropathy, atherosclerosis, and coronary heart disease as its complications. Diabetes causes a change in organs and tissues, with the pancreas being the disease's primary target organ (Elkotby et al., 2018). According to experimental and clinical data, DM affects the liver, blood vessels, kidneys, retina, and nerves (Kocaman & Kulolu, 2020; Mauricio et al., 2020). Chronic kidney disease (CKD) is one of the common complications of DM, which affect 30 to 40 per cent of

diabetic patients in urban areas (Mestry et al., 2017). Diabetes-related kidney disease or diabetic nephropathy (DN) is the leading cause of the end-stage renal disease (ESRD) in T2DM patients worldwide (Saran et al., 2019; Jitraknatee et al., 2020). The levels of blood urea nitrogen (BUN) and creatinine rise when the kidneys fail, as does microalbuminuria, which has been used as a marker for DN. Morever, excessive mesangial matrix aggregation, thickening of the tubular and glomerular basement membranes, and tubular fibrosis are manifestations of DN on renal cellular elements (Cohen & Viswanathan, 2012; Pourghasem et al., 2015).

The pathogenesis of diabetes and its complications is linked to oxidative stress (Dos Santos et al., 2019). Oxidative stress occurs when there are too many free radicals and the body's antioxidant defences aren't working properly. Hyperglycaemia produces reactive oxygen species (ROS), which cause extensive damage to body cells (Ullah et al., 2014), including the pancreas, liver, and kidneys (Asmat et al., 2016). Inflammation, cytokine release, and apoptosis are stress-related signalling pathways that are activated due to stressors (Yaribeygi et al., 2020). Additionally, antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are diminished in diabetic patients, causing oxidative stress to become worse (Oguntibeju, 2019).

Medicinal plants have become more popular in disease control in recent years due to their numerous health benefits. The medicinal plant contains phytochemicals and antioxidants that can slow or stop the oxidation process by stabilising free radicals (Atta et al., 2017). It is also less expensive and relatively safer than synthetic prescription medications, which usually have more side effects. In the plant kingdom,

the Zingiberaceae is one of the largest families. It is an important family that provides man with a wide range of useful items in the categories of food, medicine, essential oils, and aesthetics (Jaafar et al., 2007). *Etlingera elatior* belongs to the ginger family that is widely cultivated across Southeast Asia, and among Malaysians, it is known as "Kantan" (Habsah et al., 2005[a]; Lachumy et al., 2010). It has been used as a seasoning, a fresh vegetable, and an essential ingredient in Malaysian cuisine (Jackie et al., 2011). Interestingly, this plant has traditionally been utilised as an alternative medicine source for diabetes and hypertension (Jeevani et al., 2011).

The phytochemistry and pharmacological activity of this plant have been studied extensively using various methods. *E. elatior* had a wide range of antioxidant activity due to a high concentration of secondary metabolites (Chan et al., 2011; Ghasemzadeh et al., 2015; Ramasamy et al., 2016). Antimicrobial, antioxidant, anticancer, anti-diabetic, anti-inflammation, anti-ageing, and potential wound healing properties have also been demonstrated (Lachumy et al., 2010; Hueh Zan et al., 2011; Srey et al., 2014; Ghasemzadeh et al., 2015). The plant's safety also has been established by various studies (Lachumy et al., 2010; Sungthong & Srichaikul, 2018). Furthermore, no acute or sub-acute toxicity associated with *E. elatior* flower extract has been identified, indicating that the extract is safe for both male and female rats (Afifah, 2016; Afifah et al., 2019).

A T2DM rat (T2DR) model has been developed using a combination of a high-fat diet (HFD) to induce obesity and a low dose of streptozotocin (STZ) to cause minimal pancreatic β-cell dysfunction. This model is based on Fang et al. (2019), who found that obese animals develop insulin resistance and have metabolic and disease

progression timelines similar to human T2DM. Furthermore, the model developed a stable and long-lasting hyperglycaemia, making it a valuable research tool in diabetes study (Guo et al., 2018).

In this study, the efficacy of *E. elatior* flower aqueous extract (EEAE) on T2DR was compared to metformin, an oral hyperglycaemic agent (OHA). Blood was drawn at the end of the study period (6 weeks) for biochemical analysis and the determination of oxidative stress biomarkers. Haematoxylin and eosin (H&E) staining is used to examine diabetes-related organ changes histopathologically. To identify precise changes in the tissue following EEAE treatment, a special staining analysis of renal tissue was performed. Inflammatory marker, interleukin-6 (IL-6), and fibrotic markers, transforming growth factor-beta (TGF-β), and connective tissue growth factor (CTGF) were also measured in kidney tissue.

1.2 Problem statement

Diabetes is becoming more prevalent. Diabetes affects 463 million people worldwide, estimated to reach over 700 million by 2045 (International Diabetes Federation, 2019). In Malaysia, the prevalence of diabetes is on the rise, with 11.6 per cent in 2006, 15.2 per cent in 2011, 17.5 per cent in 2015, and 18.3 per cent in 2016 (Institute for Public Health, 2020). Although anti-diabetic drugs are commercially available, they have adverse effects. In addition, there is currently no adequate and effective cure for the disease. As a result, scientists are focusing their efforts in developing an anti-diabetic medicine from natural resources with reasonable glycaemic control, fewer side effects, and lowers diabetic complications.

The prevention of DM is now associated with the regular intake of vegetables and fruits rich in natural antioxidant. Since it is readily available, affordable, and believed to have fewer side effects, dietary herbs and medicinal plants are gaining more attention in diabetes management. Locals in Malaysia have historically used the flower of *E. elatior* for food flavouring and medicinal purposes. This is based on the belief that consuming raw inflorescence regularly will help with diabetes (Jeevani, 2011). However, data on the effectiveness of *E. elatior* flower in the treatment of diabetes is limited. Efforts to evaluate the anti-hyperglycaemic properties of crude *E. elatior* aqueous flower extract (EEAE) in *in vitro* and *in vivo* systems have yet to be undertaken.

1.3 The rationale of the study

This study aimed to gather evidence on the *in vivo* efficacy of *E. elatior* flower on the T2DR model. Even though this plant is frequently used for treating numerous illnesses, little is known about its anti-diabetic effect. Therefore, this study corroborates the anti-diabetic activity of *E. elatior* flower aqueous extract in both *in vitro* and *in vivo* experimental models.

As a result, the findings of this study will help to determine the extract's anti-diabetic activity *in vitro* and secondary metabolites that possibly contribute to this activity. Furthermore, the results are used in basic science research to determine the possible effect of *E. elatior* flower as an anti-diabetic agent in an *in vivo* model and the safety of using this plant as alternative medicine.

1.4 The objective of the study

1.4.1 General objective

To evaluate the effect of *E. elatior* flower aqueous extract (EEAE) as an anti-diabetic agent on the T2DR model.

1.4.2 Specific objectives

i) To determine the *in vitro* antioxidant and *in vitro* anti-diabetic activity of EEAE.

ii) To determine the effects of EEAE on fasting blood glucose level, blood pressure, body weight, food and water intake of T2DR.

iii) To determine the effect of EEAE on biochemical parameters (renal function test, liver function test, lipid profile) related to DM in T2DR.

iv) To evaluate the effects of EEAE on the oxidative stress biomarkers in T2DR (SOD, MDA, CAT, GSH, and TAOC).

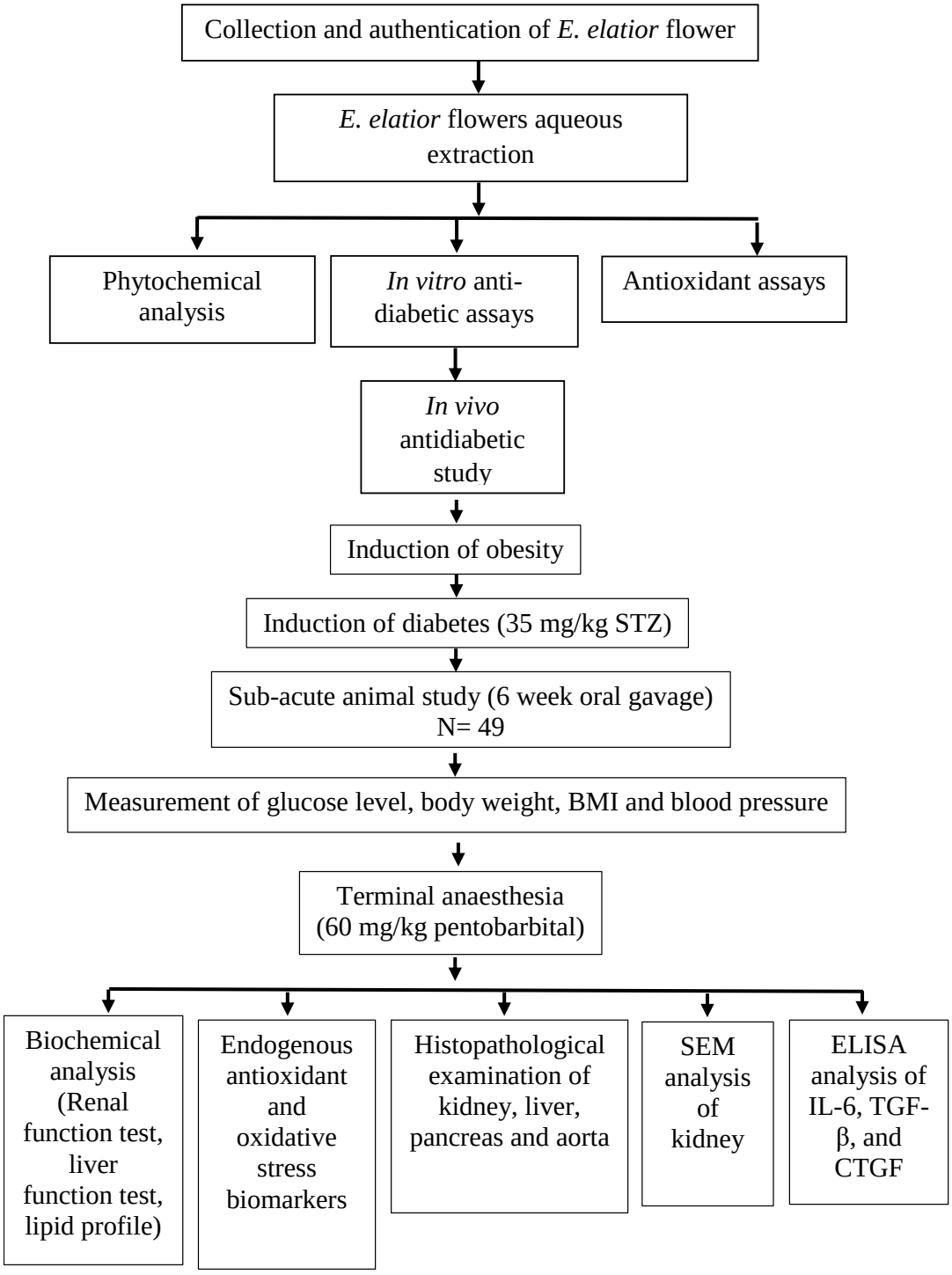
v) To evaluate the histopathological and ultrastructural improvements of DM-target organs following EEAE treatment.

vi) To evaluate the effect of EEAE on the expression of inflammatory (IL-6) and fibrotic markers (TGF-β, and CTGF) in kidney tissue.

1.5 Hypothesis

The aqueous extract of *E. elatior* flowers (EEAE) will improve diabetic rats' blood glucose levels, body weight, lipid profile, renal function, and liver function. In addition, consuming EEAE reduces oxidative damage by increasing endogenous antioxidant enzymes and decreasing oxidative stress markers. The histology changes in the liver, pancreas, and aorta are restored when hyperglycaemia is improved. Next, diabetic kidney complications will be improved in terms of histopathology, ultrastructure, and levels of IL-6, TGF-β, and CTGF.

1.6 Study design



HFD: High fat diet; STZ: Streptozotocin; BMI: Body mass index; IL-6: Interleukin 6; TGF-β: Transforming growth factor beta; CTGF: Connective tissue growth factor; SEM: Scanning electron microscopy.

Figure 1.1 General flow chart of the study.

CHAPTER 2

LITERATURE REVIEW

2.1 Diabetes Mellitus (DM)

2.1.1 Overview of the disease

Diabetes mellitus (DM) is a chronic metabolic disease characterised by an increase in blood glucose levels caused by an inability of the pancreas to produce enough insulin or utilise it efficiently (International Diabetes Federation, 2017). Insulin is an important hormone produced by pancreatic beta cells. Its primary function is to transport glucose from the bloodstream into the body's cells, which are then converted into energy. Under normal conditions, the pancreas secretes digestive enzymes along with insulin and glucagon for glucose homeostasis in the body. Insulin lowers blood glucose levels by allowing glucose to reach body cells. In low blood glucose levels, the pancreas releases glucagon and stimulates glucose release from the liver (Röder et al., 2016). After meals, glucose and amino acids are absorbed directly into the bloodstream. Increased blood glucose levels signal the pancreatic beta cells to secrete insulin that allows glucose to enter body cells, particularly muscle and liver. The liver stops producing glucose (gluconeogenesis) at high insulin levels and stores it as glycogen. As blood glucose levels reach their peak, the pancreas decreases insulin production until both blood glucose and insulin are minimal.

Lack of insulin or failure of body cells to respond to insulin contributes to blood glucose accumulation, known as hyperglycaemia. Type-1 diabetes mellitus (T1DM) is

caused by an autoimmune mechanism that destroys the pancreatic islet cells. In the meantime, type-2 diabetes mellitus (T2DM) results from insulin resistance due to lack of compensatory insulin secretion, which is more common in obese people. It affects insulin-sensitive tissue such as liver, muscle, and adipose tissue which leads to insulin resistance and pancreatic beta-cell dysfunction (Zatterale et al., 2020). Uncontrolled diabetes leads to various organs damage, leading to disabling and life-threatening health complications seen as the consequences of macro and microvascular damage (Mauricio et al., 2020). The mechanisms involved in disease progression include glycosylation of proteins due to the formation of the advanced glycation end products (AGEs), the production of superoxide, the activation of protein kinase C (PKC), acceleration of hexosamine and polyol pathways leading to accumulation of sorbitol, hypertension, and dyslipidaemia (Ighodaro et al., 2018[b]).

2.1.2 Types of DM

Diabetes was previously classified into three types: type-1, type-2, and gestational diabetes. Prediabetes is a condition where blood sugar levels are higher than normal but below the defined threshold of diabetes, and is considered a precursor of T2DM. Prediabetes refers to impaired glucose tolerance (IGT) or impaired fasting glucose (IFG), which causes a high risk of developing T2DM (Bansal, 2015). Early treatment, a healthy lifestyle, and ideal body weight may return blood sugar levels to a normal range for some people with pre-diabetes and lower the risk of developing T2DM. Historically, the distinctions of T1DM and T2DM were based on age at onset, degree of β-cell loss, level of resistance to insulin, the presence of autoantibodies associated with diabetes and insulin therapy requirements. However, as the prevalence of obesity

at a young age rises, the phenotypes of T1DM and T2DM has become less distinctive (American Diabetes Association, 2021). Traditional assumptions that T2DM exclusively affects adults and T1DM only affects children are no longer valid, as both disorders affect people of all ages. Moreover, the advances in molecular genetics and increase knowledge in pathophysiology have allowed clinicians to identify growing numbers of diabetes subtypes.

2.1.2 (a) Type 1 diabetes mellitus (T1DM)

Type 1 diabetes mellitus (T1DM) represents only 10% of all diabetes cases worldwide. T1DM can occur at any age, but it is more common in children and adolescents. In T1DM, glucose accumulates in the blood, and the body cells become starved of energy. In this condition, people with T1DM require daily insulin injections to maintain glucose levels within an acceptable range (Pathak et al., 2019).

T1DM is the result of a combination of genetic and environmental influences. Most commonly, it is caused by an autoimmune disorder in which the body's immune system attackx the insulin-producing cells of the pancreas. As a result, the β-cells of the pancreas do not produce enough or any insulin. According to Paschou et al. (2018), the major histocompatibility complex (MHC) region, also known as HLA (human leucocyte antigen), is one of the main genes predisposing to T1DM. Polymorphic alleles of the HLA complex are responsible for 40 to 50 per cent of the genetic risk of developing T1DM. Whereas, the insulin gene (Ins-VNTR) polymorphisms and cytotoxic T lymphocyte-associated antigen-4 gene (CTLA-4) account for 15 per cent of the genetic predisposition.

In comparison to the genetic factors, the environmental factors that influence the development of T1DM are less well understood, but contact with certain microorganisms is emerging as an important factor. In some cases, viral infections, toxins, and dietary factors can trigger an autoimmune response (International Diabetes Federation, 2019). Infections that affect immune regulation include rubella, coxsackie virus B and enteroviruses (Ilonen et al., 2019). There is also evidence that nutritional factors such as cow's milk and cereals are important, but the exact effect of these factors is unknown.

2.1.2 (b) Type 2 diabetes mellitus (T2DM)

T2DM is the most common form of diabetes, representing more than 90% of all diabetes cases (Zhuo et al., 2018). It is most common in older adults, but has recently become more common in children and adolescents due to obesity. T2DM is characterised by chronic hyperglycaemia due to a lack of body cell response to insulin, known as insulin resistance. Insulin resistance is thought to develop through the accumulation of fat in the liver and muscle tissue. It also accumulates in the pancreas, and causes impaired β-cell function, inflammation of the islet cells, and ultimately β-cell death (Skyler et al., 2017). As shown in Figure 2.1, insulin resistance contributes to increased glucose production in the liver and decreases glucose uptake in muscle and adipose tissue (Freeman & Pennings, 2020). Both insulin resistance and β-cell dysfunction occur in the early pathogenesis of T2DM, with interactions between genetic factors and lifestyle being the most important determinants (Galaviz et al., 2018; Zheng et al., 2018). Although the causes of T2DM are not fully understood, overweight, obesity, physical inactivity, prediabetes, increasing age, ethnicity, and

family history are strongly associated with the pathogenesis of T2DM (Institute for Public Health, 2020).

T2DM is a complex metabolic disorders characterised by hyperglycaemia due to insulin resistance as well as hyperlipidaemia due to obesity. In this condition, hyperglycaemia induces oxidative stress and enhance ROS production, while hyperlipidaemia contributes to the release of inflammatory cytokines (Teodaro et al., 2019). Over time, hyperglycaemia induces toxic effects, leading to a variety of complications including nephropathy, retinopathy, neuropathy, stroke and coronary heart disease. The pathophysiology that links T2DM and its complications is complex and multifactorial. In fact, the precise mechanism is still unknown, but the consequences of oxidative stress and inflammation have been identified as a major contributors to the progression of T2DM and its complications (Santilli et al., 2015).

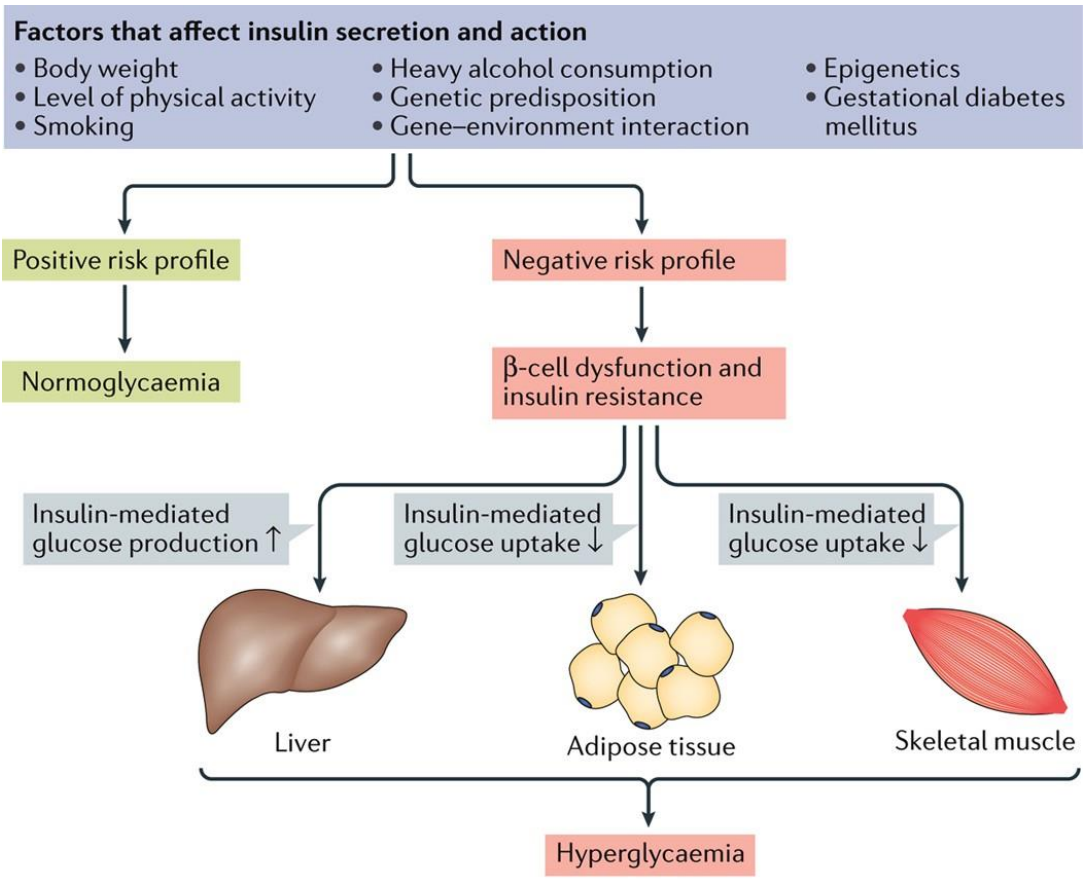


Figure 2.1 Pathophysiology of hyperglycaemia in type-2 diabetes mellitus (Zheng et al., 2018).

2.1.2 (c) Gestational diabetes mellitus (GDM)

Gestational diabetes mellitus (GDM) is defined as abnormal carbohydrate intolerance that is recognised during pregnancy. GDM is a diabetogenic state characterised by hyperinsulinemia and insulin resistance due to progressive changes in maternal metabolism. Pregnant women with hyperglycaemia are at higher risk of developing GDM in subsequent pregnancies (International Diabetes Federation, 2019). Approximately 7% of all pregnancies are complicated by GDM worldwide, while the prevalence of GDM in Asia is 11.5% of all pregnancies (Lee et al., 2018). Most patients with GDM may not have any symptoms, but features such as polyuria and lethargy that are often associated with diabetes may be related to pregnancy. The clinical importance of GDM is associated with a high risk of developing congenital malformations and macrosomia or abnormal size of the foetus at their gestational age (Nurain et al., 2019). Increased risk of pre-eclampsia, polyhydramnios, and increased caesarean section also contributed to maternal morbidity in GDM. The patient also is at risk of developing hypertension, hyperlipidaemia, and T2DM in the future (Mumtaz, 2000).

2.1.2 (d) Other types of DM

A recent WHO report listed a new type of diabetes known as specific types of diabetes, including monogenic and 'secondary diabetes' that occurs due to other causes. Monogenic diabetes results from an alteration of a single gene, including neonatal DM and maturity-onset diabetes of the young (MODY). Monogenic diabetes is uncommon, accounting for 1.5 to 2% of all cases. Meanwhile, secondary diabetes refers to diabetes caused by other factors such as disease of the exocrine pancreas, endocrine disorders, drugs, chemicals, and infections that induced the incidence of diabetes (International Diabetes Federation, 2019; World Health Organization, 2019). The details classification of DM is shown in Table 2.1.

Table 2.1 Types of diabetes (World Health Organization, 2019).

Types of diabetes	Descriptions
T1DM	β-cell destruction (mostly immune-mediated) and absolute insulin deficiency; onset most common in childhood and early adulthood
T2DM	Most common type, various degrees of β-cell dysfunction and insulin resistance; commonly associated with overweight and obesity
Other specific types of diabetes	Monogenic diabetes - Monogenic defects of β-cell function and monogenic defects in insulin action
	Diseases of the exocrine pancreas - Various conditions that affect the pancreas can result in hyperglycaemia (trauma, tumour, inflammation, etc.)
	Endocrine disorders - Occurs in diseases with excess secretion of hormones that are insulin antagonists
	Drug or chemical-induced - Some medicines and chemicals impair insulin secretion or action, some can destroy β-cells
	Infection related-diabetes - Some viruses have been associated with direct β-cell destruction
	Uncommon specific forms of immune-mediated diabetes - Associated with rare immune-mediated diseases
	Other genetic syndromes sometimes associated with diabetes
Gestational DM	Hyperglycaemia below diagnostic thresholds for diabetes in pregnancy
****Diagnostic criteria for diabetes:** fasting blood glucose ≥ 7.0 mmol/L or 2-hour post-load blood glucose ≥ 11.1 mmol/L or HbA1c ≥ 6.5% ****Diagnostic criteria for gestational diabetes:** fasting blood glucose 5.1–6.9 mmol/L or 1-hour post-load blood glucose ≥ 10.0 mmol/L or 2-hour post-load blood glucose 8.5–11.0 mmol/L	

2.1.3 The prevalence of DM

DM has become one of the common diseases globally, reaching epidemic proportions in the last decade. Diabetes affects and disables people at their most productive age, and shorten the life expectancy of the elderly. The disease contributes to considerable damages in organs or systems internally and increases the risk of premature death and permanent disability (World Health Organization, 2016). Now it becomes a major cause of morbidity and mortality affecting the youth and middle age people. Recently, diabetes has been identified among the top 10 causes of death globally (Zheng et al., 2018).

The International Diabetes Federation (IDF) had reported in 2017 that 425 million people aged 20 to 79 years old were diabetic (International Diabetes Federation, 2017). In 2019, the global diabetes prevalence was 9.3% (463 million people), estimated to increase to 10.2% (578 million) by 2030 and to 10.9% (700 million) by 2045, with 50.1% of them remaining undiagnosed (International Diabetes Federation, 2019). It was accounted that rapid urbanisation, unhealthy diet, and a sedentary lifestyle have resulted in the upward trend of DM cases (Saeedi et al., 2019). Diabetes has become a serious global health concern, and its expenditure continues to increase and lead to significant social, financial, and health system implications (Ogurtsova et al., 2017).

In Malaysia, the incidence of DM was dramatically increased in adults aged 18 years and above. The National Health and Morbidity Survey (NHMS) had reported that the number of diabetes cases was 11.2% in 2011, increasing to 13.4% in 2015 and

expanding up to 18.3% in 2019 (Figure 2.2) (Institute for Public Health, 2020). The highest prevalence was observed in the middle age groups revealed that the risk of getting DM increases significantly among young Malaysian adults (Institute for Public Health, 2015). According to the National Diabetes Registry report, 99.3% of all registered patients were diagnosed with T2DM. There were 897,421 active diabetes patients in 2019, and the majority of the patients were female and Malay (Figure 2.3) (Ministry of Health Malaysia, 2020). Based on audited patients, 80.4% had hypertension, and 74.3% had dyslipidaemia. As for the complications, 14.6% of patients had been diagnosed with nephropathy, 10.6% had retinopathy, and 5.9% had ischaemic heart disease.

Surprisingly, a vast percentage of all cases was classified as undiagnosed diabetes, where the patient was unaware of the disease. Undiagnosed diabetes has become substantial public health implications as they remain untreated and at high risk to develop serious complications. Obesity, age, ethnicity, educational level, and hypertension are the key related risk factors for undiagnosed DM in Malaysia (Ismail et al., 2018). In NHMS 2019 reports, an increasing trend of overweight and obesity in the Malaysian population indicated that the prevalence was increased by 1.5 and 3 times within less than two decades, respectively (Tee & Yap, 2017). Consequently, early screening is essential to enable timely intervention and prevention of the disease.

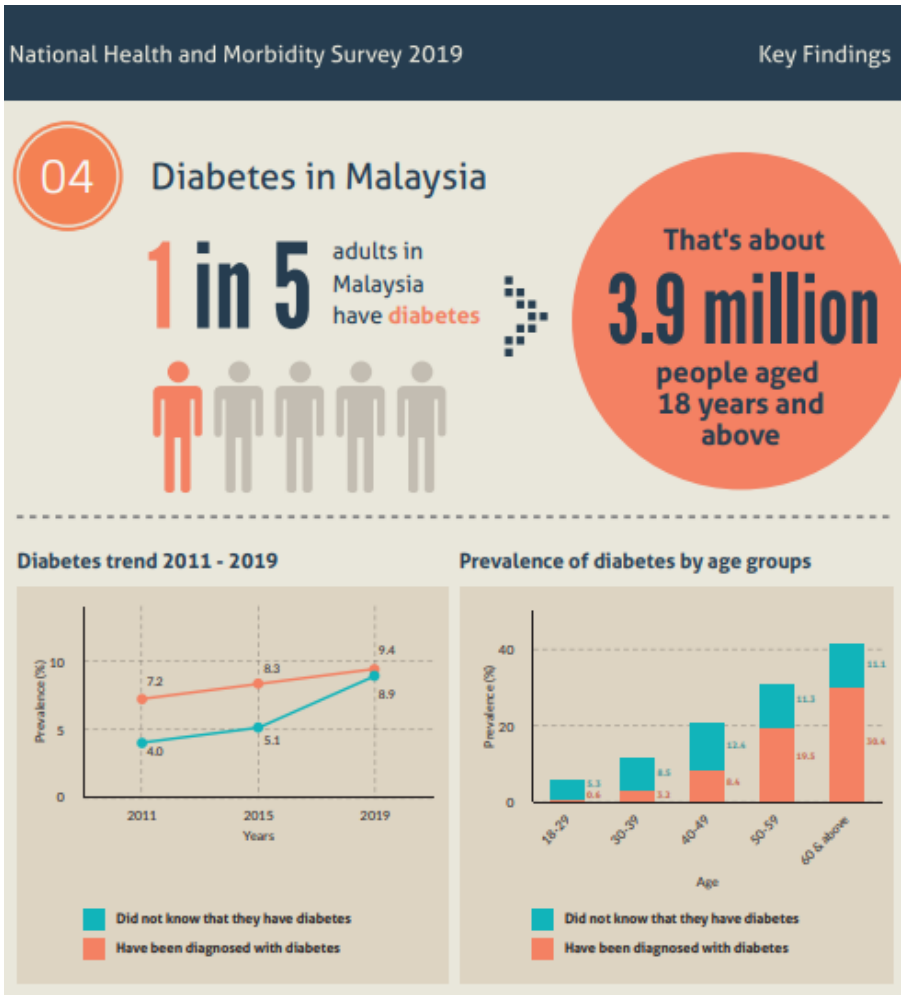


Figure 2.2 Prevalence of diabetes in Malaysia from 2011 to 2019 (National Health and Morbidity Survey, Institute for Public Health, 2020).

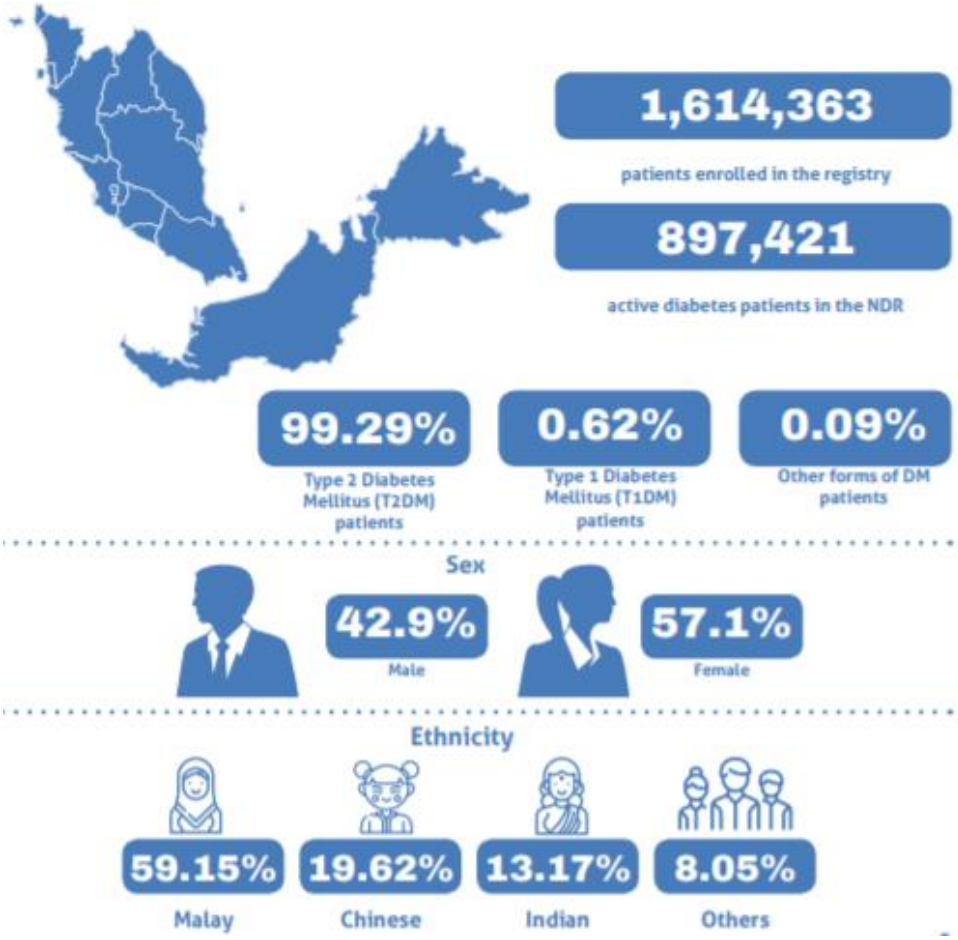


Figure 2.3 Populations of diabetes patients in Malaysia from National Diabetes Registry Report, 2013-2019 (Ministry of Health Malaysia, 2020).

2.1.4 The diagnosis of DM

Diabetes can be diagnosed based on blood glucose criteria; either fasting blood glucose (FBG) or 2-hour blood glucose (2-h BG) after the 75 g oral glucose tolerance test (OGTT) or glycated haemoglobin (HbA1c) criteria (International Diabetes Federation, 2017; American Diabetes Association, 2020) (Table 2.2). FBG test is the preferred method of screening for diabetes. The FBG measures blood sugar levels after fasting for at least 8 hours. Normal FBG is less than 5.6 mmol/L, while FBG is greater than 5.6 mmol/L and less than 6.9 mmol/L implies that the person has impaired fasting glucose. A diagnosis of diabetes is made when FBG is greater than 6.9 mmol/L.

In OGTT, diabetes is diagnosed when 2-hour blood glucose is greater than or equal to 11.1 mmol /L. Gestational diabetes is diagnosed based on OGTT with 75 g of oral glucose during the 24^{th} and 28^{th} weeks of gestation (International Diabetes Federation, 2019). Further, random blood glucose in non-fasting also can be used to diagnose diabetes. This test can be applied to someone having severe symptoms of diabetes. Diabetes is diagnosed when blood glucose is higher than or equal to 11.1 mmol/L.

The HbA1c measures average blood sugar for the past 2 to 3 months. This test offers several advantages compared to FBG and OGTT, including greater convenience and excellent pre-analytical stability. However, higher costs, limited availability of HbA1c tests in some regions, and an incomplete correlation between HbA1c and average glucose could be the limitation of this test. Diabetes is diagnosed when the level of HbA1c is greater than or equal to 6.5%.

Table 2.2 Criteria for the diagnosis of DM (adapted from American Diabetes Association, 2021).

Diagnosis	Normal	Pre-diabetes	Diabetes
Fasting blood glucose (FBG)*	< 5.6 mmol/L	5.6 mmol/L to 6.9 mmol/L	≥ 7.0 mmol/L
2-hour blood glucose**	< 7.8 mmol/L	7.8 mmol/L to 11 mmol/L	≥ 11.1 mmol/L
Random blood glucose	-	-	> 11.1 mmol/L
Glycated haemoglobin ($HbA1_C$)	< 5.7%	5.7% to 6.4%	≥ 6.5%

*Fasting is defined as no caloric intake for at least 8 h.
**The test should be performed, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water (WHO).

2.1.5 The symptoms of DM

Diabetes usually presented with the symptoms illustrated in Figure 2.4. The classic symptoms of diabetes, such as polyuria (frequent urination), polydipsia (excessive thirst), and polyphagia (excessive hunger) are most frequently occur in T1DM and severe T2DM. Polydipsia occurs due to high blood glucose that raises the osmolarity of blood. The condition encourages polyuria due to excess fluid intake and glucose-induced urination. Unexplained weight loss, fatigue, restlessness, and body aches are signs of T1DM and undetected diabetes, especially in T2DM (Ramachandran, 2014). The weight loss occurs when the body cells lack glucose to use as energy due to the insufficient insulin hormone. Thus, the body starts to burn fat and muscle to gain energy, which results in a reduction of overall body weight.

The symptoms of T1DM might be comparable to those of T2DM, including increased thirst, frequent urination, fatigue, slow-healing wounds, persistent infections, and numbness in hands and feet. However, the presentation of T2DM is much less dramatic, and the condition may be wholly symptomless, and the onset is usually impossible to determine (International Diabetes Federation, 2019). There is often a long pre-detection period, and a half (50.1%) of global diabetes cases in 2019 were unaware of their conditions as they may remain asymptomatic for many years (Saeedi et al., 2019). Thus, it may be first diagnosed when a complication occurs due to hyperglycaemia, such as foot ulcers, vision changes, renal failure, or infection.