ANTI-DIABETIC AND ANTI-LETHARGIC EFFECTS OF *Stichopus horrens* EXTRACT IN TYPE 2 DIABETES-INDUCED SPRAGUE DAWLEY MALE RATS

ENG VIXUAN

SCHOOL OF HEALTH SCIENCES

UNIVERSITI SAINS MALAYSIA

2025

ANTI-DIABETIC AND ANTI-LETHARGIC EFFECTS OF *Stichopus horrens* EXTRACT IN TYPE 2 DIABETES-INDUCED SPRAGUE DAWLEY MALE RATS

by

ENG VIXUAN

Dissertation submitted in partial fulfilment

of the requirements for the degree

of Bachelor of Health Sciences (Honours)

(Biomedicine)

January 2025

CERTIFICATE

This is to certify that the dissertation entitled "Anti-diabetic and Anti-lethargic Effects of *Stichopus horrens* Extract in Type 2 Diabetes-Induced Sprague Dawley Male Rats" is a genuine record of research work done by Ms ENG VIXUAN during the period from August 2024 to January 2025 under my supervision. I have read this dissertation and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality as a dissertation to be submitted in partial fulfilment for the degree of Bachelor of Health Science (Honours) (Biomedicine).

Main Supervisor,

AP Dr Sabreena Safuan
Lecturer
School of Health Sciences
Universiti Sains Malaysia
Health Campus
16150 Kubang Kerian
Kelantan, Malaysia

Date: 26.02.2025

Co-Supervisor,

Dr Musfirah Zulkarnain
Lecturer
School of Industrial Technology
Universiti Sains Malaysia
Main Campus
11700 Gelugor
Pulau Pinang, Malaysia

Date: 26.02.2025

DECLARATION

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

……..

(ENG VIXUAN)

Date: 26.02.2025

ACKNOWLEDGEMENT

This dissertation would not have been possible without the support, help, dedication and guidance of several individuals who have greatly contributed in the midst of preparation and completion of the study.

First and foremost, I would like to express my heartful appreciation to my main supervisor, AP Dr Sabreena binti Safuan and my co-supervisor, Dr Musfirah binti Zulkarnain for their unwavering help, guidance and feedback throughout my entire research. Their expertise has shaped me into a better researcher in understanding the flow and direction of this study. My sincere gratitude goes to my postgraduate seniors, Ms. Bibi, Ms. Fatihah Ahmad, Ms. Azmina and Mr. Muhammad Hamizan for their constant help in laboratory work and data analysis. Furthermore, I am also grateful to the staff in Cell culture, and Biomedicine laboratories (School of Health Sciences) and Animal Research and Service Centre (USM Health Campus) for generously allowing me to execute my work in their laboratories. Special thanks go to my friends, Koo Joanne, Muhammad Aiman bin Raymond, Muhammad Alzam bin Nafiz, and the rest of my classmates who sincerely encouraged me throughout my study. In addition, I would like to thank my parents and siblings for paving me with love and support for these 6 months. Thank you all for being an integral part of this academic endeavour.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LISTS OF SYMBOLS AND ABBREVATIONS

°C	Degree Celsius
%	Percentage
ADP	Adenosine diphosphate
AGE	Advanced glycation end product
ANOVA	Analysis of variance
ARASC	Animal Research and Service Centre
ATP	Adenosine triphosphate
cAMP	Cyclic adenosine monophosphate
CER	Ceramide
DAG	Diacylglycerol
DM	Diabetes mellitus
ER	Endoplasmic reticulum
ETC	Electron Transport Chain
FuCS	Fucosylated chondroitin sulfate
GLUT2	Glucose transporter 2
GLUT4	Glucose transporter 4
GPx-1	Glutathione-peroxidase I
HbA1c	Hemoglobin A1c

HFD	High fat diet
HUVEC	Human umbilical vein endothelial cells
HPP	High pressure processing
IAAP	Islet amyloid polypeptides
IR	Insulin receptor
IRS	Insulin receptor susbstrate
mg/kg	Milligram per kilogram
mg/mL	Miligram per millimetre
MODY	Maturity-onset diabetes of the young
NA	Nicotinamide
NPD	Normal pellet diet
P13K	Phosphatidylinositol 3-kinase
PCG 1α	Peroxisome proliferator-activated receptor γ co-activator 1α
ROS	Reactive oxygen species
SD	Sprague Dawley
SOD2	Superoxide dismutase 2
STZ	Streptozotocin
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
UPR	Unfolded protein response

KESAN ANTI-DIABETIK DAN ANTI-LESU *Stichopus horrens* EKSTRAK DALAM DIABETIK JENIS 2 TIKUS JANTAN SPRAGUE DAWLEY

ABSTRAK

Penyakit kencing manis dianggap sebagai gangguan metabolik kronik yang disebabkan oleh kekurangan penghasilan insulin oleh pankreas atau jejasan tindak balas sel badan terhadap insulin. Pelbagai ubat seperti metformin memberi kesan terapeutik untuk merawat penyakit ini tetapi kesan sampingan yang dihasilkan olehnya mendorong penyelidik untuk meneroka rawatan alternatif lain terutamanya produk semula jadi. Kajian ini bertujuan untuk mengkaji potensi gamat yang berbeza dari segi kaedah pengekstrakan dari *Stichopus horrens* (gamat) dalam mengurangkan tahap glukosa, meminimumkan keletihan, dan memulihkan struktur dan fungsi organ tikus diabetik jenis 2 (T2DM). Dua kaedah pengekstrakan telah digunakan dalam kajian ini, iaitu hidrolisis, dan pemprosesan tekanan tinggi. Ekstrak pertama gamat melalui pemprosesan hidrolisis sahaja manakala ekstrak kedua melalui pemprosesan tekanan tinggi sahaja. Ekstrak ketiga gamat melalui pemprosesan hidrolisis dan pemprosesan tekanan tinggi. Dalam kajian ini, HUVEC sel telah didedahkan kepada tiga *Stichopus horrens* dinding badan sampel serta satu kawalan positif iaitu metformin dengan kepekatan 6.25 hingga 4000 μg/mL dalam MTT assay untuk mendapatkan dos permulaan rawatan dalam kajian haiwan. Kajian haiwan ini melibatkan tikus jantan Sprague Dawley (SD) yang mengaplikasi induksi diet tinggi lemak selama lima minggu, 110 mg/kg suntikan nikotinamida, diikuti dengan 55 mg/kg streptozotocin (STZ) dalam mencipta model tikus T2DM. Tikus dibahagi secara rawak kepada enam kumpulan iaitu kawalan normal (n=3), kawalan kencing manis (n=3),

kumpulan rawatan metformin (n=3), kumpulan rawatan ekstrak gamat (hidrolisis sahaja) (n=3), kumpulan rawatan ekstrak gamat (pemprosesan tekanan tinggi sahaja) (n=3), dan kumpulan rawatan ekstrak gamat (pemprosesan tekanan tinggi dan hidrolisis) (n=4). Penemuan melalui kajian ini telah menunjukkan trend penurunan dalam proliferasi sel selari dengan peningkatan kepekatan gamat dan mempunyai nilai IC_{50} sebanyak 3.771 ± 0.152 μg/mL dalam ekstrak gamat (hidrolisis sahaja), 3.783 ± 0.063 μg/mL dalam ekstrak gamat (pemprosesan tekanan tinggi sahaja), dan 4.006 ± 0.054 μg/mL dalam ekstrak gamat (pemprosesan tekanan tinggi dan hidrolisis) manakala metformin menunjukkan peningkatan trend sel proliferasi dan mempunyai nilai EC_{50} sebanyak 4.931 ± 4.044 μg/mL. Oleh itu, dos permulaan bagi semua rawatan untuk kajian haiwan ialah 150 mg/kg. Penemuan dalam kajian ini menunjukkan trend penurunan glukosa dalam darah selepas empat minggu rawatan dengan sampel gamat sebanyak 150 mg/kg serta peningkatan toleransi senaman dalam kumpulan ekstrak gamat (pemprosesan tekanan tinggi dan hidrolisis) sebanyak 150 mg/kg. Di samping itu, sampel gamat menunjukkan sedikit pemulihan dalam histologi hati, buah pinggang, dan pankreas. Tempoh rawatan gamat yang singkat terhadap tikus kencing manis membuktikan bahawa gamat mungkin merupakan salah satu produk yang berkesan pada masa hadapan.

ANTI-DIABETIC AND ANTI-LETHARGIC EFFECTS OF *Stichopus horrens* EXTRACT IN TYPE 2 DIABETES-INDUCED SPRAGUE DAWLEY MALE RATS

ABSTRACT

Diabetes mellitus is a chronic metabolic disorder, that is attributed to either insufficient insulin production by the pancreas or impaired response of body cells to insulin. Present medication like metformin has brought significant therapeutic effects in treating this disease but the side effects prompt the researchers to explore other alternative treatments especially natural products. This study aimed to investigate the potential of different sea cucumber samples of *Stichopus horrens*, differentiated by extraction methods, in reducing fasting blood glucose level, minimising fatigue condition, and restoring structure and function in organs of diabetic type 2-induced rats. Two extraction methods were utilised in this study, which are hydrolysis and high-pressure processing (HPP). The first extract of sea cucumber was subjected to hydrolysis only, while the second extract was exposed to HPP only. The last extract of sea cucumber was subjected to both hydrolysis and HPP. In the *in-vitro* assay, human umbilical vein endothelial cells (HUVECs) were exposed to three *Stichopus horrens* body wall samples, and a positive control, metformin with concentrations ranging from 6.25 to 4000 μg/mL in MTT assay to obtain starting dosage of treatments for animal study. The *in-vivo* study involved Sprague Dawley (SD) rats, which included the induction of high fat diet for five weeks, 110 mg/kg of nicotinamide injection, followed by 55 mg/kg of streptozotocin (STZ) into creating Type 2 diabetes mellitus (T2DM) rat model. Rats were randomly grouped into six groups; normal control (n=3), diabetic control (n=3), metformin-treated group (n=3), sea cucumber extract (hydrolysis only) (n=3), sea cucumber extract (HPP only) (n=3), and sea cucumber extract (HPP and hydrolysis) (n=4). The findings displayed a decreasing trend in cell viability with increasing sea cucumber concentration with IC_{50} values of 3.771 ± 0.152 μg/mL in sea cucumber extract (hydrolysis only), 3.783 ± 0.063 μg/mL in sea cucumber extract (HPP only), and 4.006 ± 0.054 μg/mL in sea cucumber extract (HPP and hydrolysis) while metformin depicted an increasing trend of cell viability with EC_{50} value of 4.931 ± 4.044

μg/mL. As a result, the starting dosage for animal study pertaining to different treatments is 150 mg/kg each. The findings depicted decreasing trend of blood glucose level after four weeks of treatments with 150 mg/kg sea cucumber samples and increased exercise tolerance in sea cucumber extract (HPP and hydrolysis) group with 150 mg/kg. Apart from that, sea cucumbers exhibited mild restoration in histological changes of liver, kidney, and pancreas. The short treatment duration of sea cucumber on diabetic rats might prove that sea cucumber is a promising candidate for the development of novel product in the future.

CHAPTER ONE: INTRODUCTION

1.1 Research Background

Characterised by chronic hyperglycaemia, diabetes mellitus is defined as a metabolic disorder that occurs due to defective insulin secretion by the beta cells of islet of Langerhans or/and impaired insulin action that is specifically known as insulin resistance (Goyal *et al.*, 2023). Insulin is a polypeptide hormone that notably plays a role in glucose metabolism, homeostasis, and cell growth (Rahman *et al*., 2021).

Worldwide, 460 million diabetic adults (9.3%) were confirmed by the International Diabetes Federation in 2019, in which, the number is expected to rise to 10.2% by 2030 and 10.9% by 2045 even with the current treatment of Type 2 diabetes (Akhtar *et al.*, 2022). From year 2000 to 2019, the mortality rates of diabetic individuals increased at about 13% especially in low and middle-income countries (Antar *et al.*, 2023). The World Health Organisation (2024) stated that among 1.6 million deaths in 2021, 47% of them were due to traditional complications like stroke, coronary heart disease, retinopathy, and peripheral neuropathy, that were associated with diabetes.

Diabetes mellitus is classified into several categories, which are Type 1 diabetes, Type 2 diabetes, gestational diabetes, maturity-onset diabetes of the young (MODY), steroid-induced diabetes, and neonatal diabetes. Among these, type 1 diabetes, and type 2 diabetes are the main subtypes with significantly higher prevalence of T2DM (Goyal *et al.*, 2023). In T2DM, the impairment in insulin sensitivity (insulin resistance) is due to the tolerance made against insulin hormones, causing it to be ineffective despite having sufficient amount. In response to the insulin resistance, pancreas will secrete more insulin to maintain blood glucose homeostasis in the body. Therefore, circulating insulin will

subsequently increase in individuals with T2DM until the body could not keep up with the high rise of glucose in the bloodstream, and eventually progresses to T2DM (Freeman & Pennings, 2022). This occurs because Type 2 diabetic patients have a low disposition index, in which it is defined as the curvilinear relationship between insulin sensitivity and insulin secretion. The low disposition index makes the body unable to make more insulin to combat insulin resistance. Unlike Type 1 diabetes that is caused by genetic factors, T2DM occurs mainly because of inappropriate lifestyle and environmental factors such as chemicals and toxin. Thus, the progression of diabetes mellitus might be associated with oxidative stress, beta-cells dysfunction, inflammation, and tissue insulin resistance. This complex mechanism will disturb glucose homeostasis and consequently lead to the worsening of diabetes mellitus (Antar *et al.*, 2023).

In addition, the linkage of fatigue to T2DM is greater than other medical disorders, that might encompass lifestyle, physiological or psychological factors. Fatigue is defined as a common physiological state of mental and physical strength deprivation, that is characterised by temporary decline in the working ability of the body (Wang *et al*., 2021). Physiologically, fatigue in diabetes is mainly due to the alteration in the blood glucose level with chronic hyperglycaemia, causing several symptoms and disease complications to arise (Fritschi & Quinn, 2010). A study by Van Der Does *et al*. (1996) proved that there is a correlation between fatigue and glycated haemoglobin (haemoglobin A1c) which is a form of haemoglobin linked to sugar. High HbA1c indicates that there is a high number of haemoglobin coated with sugar in the bloodstream which suggests high possibility of T2DM, which is often associated with fatigue. Furthermore, the pathogenesis of fatigue is contributed by substrate depletion, high level of hydrogen ions, presence of inorganic phosphate and potassium. In a diabetic individual, low insulin secretion may shift the

energy substrate from carbohydrates to fat, leading to low glycogen stores. This will lead to low ADP phosphorylation rate and ATP synthesis, proceeding to fatigue in individuals (Kalra & Sahay, 2018).

Recently, there are numerous findings proving the benefits of natural products as replacement to available modern treatments such as jungle garlic and *Etlingera elatior* with minimal side effects. A high commercial value natural product, sea cucumber body wall, has been long used as folk medicine among Asia and Middle East countries because of its extensive therapeutic properties, provided by rich nutrients and bioactive substances (Fagbohun *et al*., 2023). In terms of nutrients, the body wall of sea cucumber offers a great profile of valuable components such as Vitamin A, Vitamin B1, Vitamin B2, Vitamin B3, and minerals. Furthermore, it provides good biological and pharmacological action as an anti-diabetic, anti-lethargic, anti-inflammatory, anti-thrombotic, and anti-tumor agent because of selective bioactive substances extracted from the body wall. These include proteins (collagen, and peptides), polysaccharides, saponins, phenolics, flavonoids, and N-acetylglucosamine compounds. High nutritional value of sea cucumber has encouraged the exploitation of multiple sea cucumber species for modern treatment, and pharmaceutical uses. *Stichopus horrens* or specifically known as durian sea cucumber, is a sea cucumber species that is widely used. This species of sea cucumber is mainly distributed in the Indo-Pacific, South-Pacific, and Hawaii regions and can be found in shallow tropical waters. The body wall composition of *S. horrens* consists of mainly proteins, followed by collagen, carbohydrate, polysaccharide, and glycosaminoaglycan content (Umam *et al*., 2024). From the different extraction methods discussed such as enzymatic hydrolysis, boiling using hot water, and UV-assisted

autolysis hydrolysate, enzymatic hydrolysis method liberated the highest content of sulfated polysaccharide, and minerals.

High pressure processing (HPP), also known as high-hydrostatic pressure processing (HHP) is a non-thermal processing method that subjects pressures of about 400 to 600 MPa at mild temperature of less than 45 °C to inactivate microorganisms in food products (Koutsoumanis *et al.*, 2022). Upon its usage as a pre-treatment on sea cucumbers, it is proved by Hossain *et al*. (2022) that HPP exhibited higher yield extraction of phenolics, and flavonoids from *Cucumaria frondosa* as compared to untreated sea cucumber samples. Moreover, HPP can modify the structure of phenolics to increase intestinal absorption in humans and avoid thermal degradation to the chemical structures.

Enzymatic hydrolysis incorporates the use of commercial enzymes in obtaining peptides, and polysaccharides as the enzymes will cleave the peptide bonds in the protein, releasing encrypted peptides or polysaccharides under optimized temperature, time, and enzyme concentration (Cruz *et al.*, 2021). This method has proved to be promising as it can fully utilise the nutritional value of the sea cucumber with not only extraction of the major component of sea cucumber, which is protein, but also other bioactive substances like saponin and polysaccharides. Thus, combination methods of enzymatic hydrolysis and HPP can increase the yield of most of the bioactive substances from the sea cucumber body wall.

1.2 Problem Statement

Diabetes mellitus, especially Type 2 diabetes, accounts for one of the leading mortality rates in Malaysia but is treatable by practicing good diet habit, exercising, weight managing, and taking medications. As stated by the International Diabetes Federation, Malaysia has the highest rate of diabetes complications in Western Pacific region, where it was recorded that the total number of adults diagnosed with diabetes was 4,431,500 out of 22,130,900 adult population, withholding 20% prevalence in 2021 (International Diabetes Federation, 2024). With more than 95% of diabetic individuals diagnosed with Type 2 diabetes, the increase in prevalence from 11.2% in 2011 to 18.3% in 2019 was noted. Despite numerous drugs such as thiazolidinediones, and alpha glucosidase inhibitors available on the market, it is concerning as of the high prevalence and death rate worldwide. This global issue has encouraged the study of a natural product, sea cucumber, due to its well-known health benefits and broad biological effects like anti-diabetic, anti-lethargic, anti-inflammation, and antioxidant properties with this study focused on anti-diabetic and anti-lethargic effects on experimental animals. This study is conducted due to lack of study on this sea cucumber species, *Stichopus horrens* in diabetic and fatigue animal model. In addition, the extent of anti-diabetic and anti-lethargic effect differences brought by different processing methods of sea cucumber body wall for maximal extraction of bioactive substances is studied. Theoretically, extracts exposed to two different processing methods (hydrolysis and HPP) are expected to exert the highest effects as compared to extracts exposed to only one processing method due to higher concentration of bioactive compound yielded. Therefore, this study will examine anti-diabetic and anti-lethargic effect of different sea cucumber processing samples on Type 2 diabetes-induced SD rats.

1.3 Objectives

1.3.1 General Objective

To investigate the anti-diabetic and anti-lethargic effects of *Stichopus horrens* extract in Type 2 diabetes-induced Sprague-Dawley (SD) male rats.

1.3.2 Specific Objectives

1. To determine the fasting blood glucose level of diabetic-induced SD rats upon treatment with *Stichopus horrens* extract (hydrolysis only, HPP only, and HPP and hydrolysis).
2. To determine the anti-lethargic effect of *Stichopus horrens* extract (hydrolysis only, HPP only, and HPP and hydrolysis) in diabetic-induced SD rats using open field maze test.
3. To observe the histopathological changes of liver, kidney, and pancreas in diabetic-induced SD rats upon treatment with *Stichopus horrens* extract (hydrolysis only, HPP only, and HPP and hydrolysis).

1.4 Hypothesis

1. *Stichopus horrens* extract (HPP and hydrolysis) will reduce fasting blood glucose in diabetic-induced SD rats.
2. *Stichopus horrens* extract (HPP and hydrolysis) will reduce lethargy of diabetic-induced SD rats in open field maze test.
3. *Stichopus horrens* extract (HPP and hydrolysis) will restore histopathological changes in liver, kidney, and pancreas of diabetic-induced SD rats.

1.5 Rationale of the Study

This study is significant to come up with a good natural product candidate in reducing blood glucose level and lethargic in T2DM individuals as one of the alternative treatments in addition to current medication. This specific species of sea cucumber, *S. horrens* had been chosen in this study because it has a high value in Malaysia, easy to obtain and make into nutritional product. Furthermore, as there are many other sea cucumber species that have been demonstrated as medicinal value by other researchers, comparison can be made between different sea cucumber species to determine which sea cucumber species can display the highest anti-diabetic and anti-lethargic effect with low side effects.

CHAPTER TWO: REVIEW OF LITERATURE

2.1 Diabetes mellitus

Diabetes mellitus is defined as a group of metabolic diseases that is characterised by high blood glucose level. It is initiated with insufficient production of insulin or/and diminished response of tissue to insulin that subsequently leads to the defect in insulin action. Patients with DM often present symptoms like weight loss, polyuria, polydipsia, and impairment in vision but the worsening complications of DM may lead to potential kidney failure (nephropathy), loss of vision (retinopathy), and increased risk of amputation of body parts. Furthermore, it is mentioned by American Diabetes Association (2013) that patients of DM may develop risk to atherosclerotic cardiovascular, hypertension, and cerebrovascular disease.

In normal condition, blood glucose level will rise after food intake, causing the secretion of insulin to reduce the glucose level back to the baseline. The circulating insulin will then trigger the glucose to travel to the adipocytes and skeletal muscle for further glucose uptake via tyrosine kinase receptor pathway. This pathway is initiated when hormones bind to cell-surface insulin receptors (IRs), facilitating an activation in the receptor that subsequently causes tyrosine phosphorylation of insulin receptor substrate (IRS). This phosphorylated IRS will activate phosphatidylinositol 3-kinase (PI3K), resulting in phosphorylation of serine/threonine-protein kinase (Akt). When Akt is activated, it will facilitate the translocation of intracellular glucose transporter 4 (GLUT4) or glucose transporter 2 (GLUT2) to the plasma membrane to enhance glucose uptake. Thus, this P13K/Akt signaling pathway will generate glycogen synthesis, glucose uptake, and glucose output. However, this signaling pathway is inhibited in insulin resistance state, thus, most research study focus on the activation of P13K/Akt signaling pathway in

Type 2 diabetic individuals (Wang *et al.*, 2020). This explains the metabolic abnormalities caused by diabetes mellitus to organs like skeletal muscle, adipose tissue, liver, and kidney when the cells are unable to use glucose as the main energy source due to lack of insulin.

2.1.1 Types of diabetes mellitus

DM is generally divided into several types which are type 1 diabetes, type 2 diabetes, and gestational diabetes. Accounting for only about five to ten percent of diabetic patients, Type 1 diabetes or insulin-dependent diabetes is categorised based on the etiology which is immune-mediated diabetes, and idiopathic diabetes. Immune-mediated diabetes is described as cellular-mediated destruction of the pancreatic beta cells that depict absolute deficiency in insulin to counter high glucose level. The destruction is evidenced by the presence of markers such as insulin autoantibodies, islet cell autoantibodies, and autoantibodies to tyrosine phosphatase IA-2 and IA-2β (American Diabetes Association, 2013). Moreover, this type of diabetes has a strong association with HLA-DR/DQ alleles and is also related to environmental factors. T1DM is more common in children than adults and these individuals need lifelong exogenous insulin replacement with no other treatment to reverse the condition (Antar *et al.*, 2023).

Type 2 diabetes or non-insulin dependent diabetes, accounting for 90% of diabetes cases, is due to the impaired insulin sensitivity and/or defective insulin secretion. Patients with T2DM are normally associated with obesity and this is linked to the extent of insulin resistance in an individual. Unlike T1DM, T2DM is asymptomatic in early stage as the symptoms are not severe enough for patients to detect any change in the body as only hyperglycaemia is present without ketoacidosis. Continuous hyperglycaemia from

reduced insulin levels will cause target organ damage (TOD) by enhancing the risk of microvascular disease, and atherosclerotic macrovascular disease. Unlike individuals diagnosed with T1DM, they might not be dependent on insulin for life since they can be treated by multiple medications available on the market, and changes in lifestyle practice (Antar *et al.*, 2023).

Gestational diabetes is a pregnancy-related hyperglycaemia occurring in the mother but may induce high risk of diabetes in newborn or fetus when they reach adulthood. It is characterised when the pregnant mother initially does not have diabetes prior to the pregnancy but depicts hyperglycaemia during pregnancy. Normally, this will be resolved once the fetus is born but mothers with gestational diabetes do have the risk of developing pre-eclampsia, and gestational hypertension. The pathogenesis of gestational diabetes is unclear, but it might be due to complex interaction of several factors like genetic, metabolic, and environmental factors (Antar *et al.*, 2023).

2.1.2 Prevalence of diabetes mellitus

According to Hossain *et al*. (2024), 240 million individuals are estimated to be having undiagnosed diabetes, occupying half of the adults worldwide. The anticipation of diabetes prevalence made by the International Diabetes Federation (2024) in 2030 and 2045 is 643 and 783 million respectively as it is assumed that approximately 537 million individuals were living with diabetes in 2021. India, China, USA, Japan, Russia, Brazil, Pakistan, Bangladesh, Italy and Indonesia occupy the top 10 nations with highest diabetes prevalence around the world (International Diabetes Federation, 2024). In addition, in 2021, global prevalence of DM outbreaks North Africa and Middle East with 39.4% and Qatar with 76.1%. The substantial rise in the prevalence of T2DM from 2000 to 2021 is

probably due to the high rise of economic growth, and rapid urbanization that drives the increase in obesity cases, which poses as a risk factor for DM. Moreover, most of the diabetes prevalence comes from low and middle-income countries as compared to high-income countries. As reported by Ruben & Khairul Hafidz Alkhair Khairul Amin (2023), known diabetic individuals in Malaysia had decreased from 1,999,450 in 2019 to 870,771 in 2023.

2.1.3 Metabolic profile on development of Type 2 Diabetes in human

In humans, the progression of T2DM transitions from healthy state, prediabetes, and eventually to diabetic state. In healthy individuals, excess glucose will be accommodated by liver and muscle tissue in the form of glycogen until it fully occupies the storage. This will then initiate *de novo* lipogenesis in the liver and adipose tissue to remove excess glucose from the bloodstream. However, the conversion to prediabetic state initiates insulin resistance, hyperinsulinaemia, and dyslipidaemia that affects several organs like liver, skeletal muscle, and adipose tissue. When excess glucose is directed to adipose tissue store, it results in the expansion of adipose tissue. The dysfunctional adipose tissue will cause accumulation of fat in non-adipose tissue like muscle, liver, and beta cells, leading to hepatic insulin resistance with reduced glycogen synthesis and increased gluconeogenesis. This contributes to hyperglycaemia, and the progression of prediabetes to frank diabetes takes place due to genetic susceptibility. In frank diabetes, it is characterised by the significant loss of functional beta cell mass. It is defined as the product of physical beta cell mass and its function in suppressing insulin secretion and glucose-stimulated pulsatile insulin release. Therefore, in this state, one may experience

loss of insulin secretion, increased circulating pro-insulin-to-insulin ratio, and loss of pulsatile insulin oscillation (Skovsø, 2014).

2.2 Pathophysiology of diabetes mellitus

Since diabetes is a complex metabolic disease that results from the malfunctioning of the feedback loops between secretion and action of the glucose-lowering hormone, insulin, pathophysiology of the disease should be understood that further involves the beta cells dysfunction, mitochondrial dysfunction, and insulin resistance in several organs like liver, skeletal muscle, and adipose tissue that play part in the pathogenesis of T2DM.

2.2.1 Beta cell dysfunction

Beta cells are cells that take control over the secretion of insulin which initially are synthesized as pre-proinsulin. As pre-proinsulin, it will undergo a conformation change to proinsulin with the help of some proteins in the endoplasmic reticulum (ER) so that it can be packaged by Golgi apparatus in secretory vesicles and cleaved into insulin and C-peptide. After maturation, insulin will be stored in granules until the trigger of insulin is activated, which is mainly from the high blood glucose level. In this case, beta cells will take in glucose via GLUT2 for glucose catabolism. This will increase the intracellular ATP/ADP ratio, causing ATP-dependent potassium channels to be closed. When potassium channels are closed, membrane depolarisation will take place, leading to the opening of calcium channels and entry of calcium ion into the cells. The increase of calcium ions in the cells will trigger exocytosis.

The dysfunction of beta cells has been proven to be associated with a complex network of environmental and molecular pathways interaction, especially in excessive nutritional states like obesity. Upon obesity, excess free fatty acid and glucose in the body will favor the increase in ER stress, leading to chronic inflammation and insulin resistance (IR) in several organs by the activation of apoptotic unfolded protein response (UPR) pathway. This UPR pathway is activated by several mechanisms like inhibition of sarcoplasmic and endoplasmic reticulum calcium ion ATPase (SERCA) that facilitates mobilisation of calcium ion in ER and direct impairment in the ER homeostasis. Furthermore, when excess glucose is present, proinsulin biosynthesis and islet amyloid polypeptides (IAAP) will rise, causing misfolded insulin and IAAP to be increased in beta cells and favor ROS production (Galicia-Garcia *et al*., 2020).

2.2.2 Mitochondrial dysfunction

Several evidence had proved that T2DM development also arises from mitochondrial dysfunction due to several factors like defective mitochondrial biogenesis, oxidative stress, aging, and genetic mutations that affect the integrity of mitochondria. For instance, a study by Mootha *et al*. (2003) found that patients with T2DM had downregulation of oxidative metabolism genes that are regulated by peroxisome proliferator-activated receptor γ co-activator 1α (PCG 1α) and reduced phosphocreatine re-synthesis rate that point to impairment in mitochondrial function. PCG 1α is generally involved in regulating the expression of genes in mitochondrial biogenesis, acting as transcription coactivator. While mitochondria's main function is to synthesise ATP in response to metabolic demand, it also takes part in other roles like ROS clearance, stress response, production of metabolites, and maintenance of ion homeostasis. Therefore, severe accumulation of ROS in the mitochondria, especially in the complex I and III of ETC due to increase in

the electron supply to the mitochondria ETC, will lead to the generation of hydrogen peroxide. The ROS generated will damage the DNA, and lipid membrane, subjecting it to mitophagic processes to eliminate the dysfunctional mitochondria. As a result, it will reduce substrate utilisation and enhance accumulation of lipid intermediates like ceramide (CER) and diacylglycerol (DAG). This lipid intermediate will interrupt the insulin signaling pathway, in which CER will inhibit protein kinase AKT while DAG will increase serine/ threonine phosphorylation of IRS-1, causing the downstream propagation of insulin signaling pathway (Galicia-Garcia *et al*., 2020).

2.2.3 Insulin resistance

Due to the linkage between T2DM and insulin resistance, where they are often present together, insulin resistance is defined as the impaired response of insulin-responsive cells to circulating insulin, in which it is a state where they are not influenced by the increase or decrease in the blood glucose level. Therefore, this insulin-deficient condition can be categorised into three types, which are insulin antagonists in plasma, diminished insulin response, and reduced insulin secretion by the beta cells of pancreas. Additionally, during fasting state, insulin response is controlled by several hormones such as glucagon, catecholamines, and glucocorticoids, with each having respective functions. For instance, glucagon will perform glycogenolysis while catecholamines will promote lipolysis and glycogenolysis. Glucocorticoids will take part in gluconeogenesis, lipolysis, and lastly muscle catabolism. However, in conditions where these are excessively produced, it may kick off the start of insulin resistance in several organs like skeletal muscle, liver, and adipose tissue, leading to development of systemic IR and causing T2DM (Galicia-Garcia *et al*., 2020).

An individual skeletal muscle cell comprises of highly structured myofibers, in which, in each myofiber, there are lots of myofibrils within it, that skeletal muscle is almost half the body weight weight. In fed state, about 70% of glucose will be directed to the skeletal muscle for uptake by GLUT4, while the rest will be used by the liver. This proves that skeletal muscle holds the largest portion of the glucose uptake in the body. Therefore, during the progression of T2DM, the high glucose level will stimulate the pancreas to make more insulin, leading to hyperinsulinaemia, and increased uptake by the skeletal muscle. This is explained when there is evidence of the decrease in muscle volume in individuals with T2DM, that may affect the glucose metabolism in the body (Rahman *et al*., 2021). Thus, if there is any mutation that reduces expression of GLUT4, insulin receptor or upstream and downstream signaling pathway, glucose uptake into muscle will be greatly impaired (Galicia-Garcia *et al*., 2020).

Liver is the major site of insulin action for glycaemic control, like synthesizing glucose into glycogen for storage, converting excess glucose into fatty acids and triglyceride, and upregulating glucose utilisation, glycolysis, and glycogenesis. At the same time, insulin functions to downregulate glucose production, gluconeogenesis, and glycogenolysis by the glucose uptake into adipose tissue and skeletal muscle. In healthy individuals, the reduction in glucose takes place by the P13K/phosphorylation of the Akt/IRS-1 pathway to enhance glycogen synthesis, and at the same time, inhibit gluconeogenesis. However, in T2DM individuals, the P13K/Akt signaling pathway is inhibited by insulin resistance mechanism, that other studies have confirmed the importance of activating P13K/Akt signaling pathway as one of the strategies to improve progression of T2DM (Rahman *et al.*, 2021). IR state allows impairment in glycogen

synthesis and promotes an increase in the lipogenesis and proinflammatory CRP (Galicia-Garcia *et al*., 2020).

Adipose tissue takes part in the production of several biologically active compounds that regulate metabolic homeostasis at systemic level. Moreover, adipose tissue is linked to insulin as it helps to stimulate triglyceride synthesis and suppress triglyceride hydrolysis. In fed state, glucose uptake into adipocytes will take place via GLUT4, allowing glycerol-3-phosphate to be produced for the formation of triacylglycerol together with fatty acids. Thus, if there is an impaired response of adipose tissue to the insulin, it may cause impaired suppression of lipolysis and increase free fatty acid release into the plasma. This condition is caused by defective AKT activation that has affected the translocation of GLUT4 to the plasma membrane (Galicia-Garcia *et al*., 2020).

2.3 Effect of environmental toxin and chemicals in diabetes mellitus

In T2DM, the long-term exposure to environmental toxins and chemicals is found to be one of the risk factors to DM (Bonini & Sargis, 2018). Environmental exposure may induce high oxidative stress to the body, compromising the viability of beta cells in the pancreas. In some research, it was found that oxidative stress can induce changes in the way target cells react to insulin and sense them. For example, ROS can induce pro-oxidative reactions, modifying insulin receptors and its substrate in signaling pathway. As oxidative stress is defined as the overwhelming production of reactive oxygen species (ROS), ROS differs in terms of chemical structure, in which some are superoxide radical that act as either oxidant or reductant and some are hydrogen peroxide, and peroxynitrite that act as oxidants. The action of ROS will bring a disequilibrium between oxidation and antioxidation system, that may consequently cause oxidative damage to the tissue and

cells in the body. If oxidative stress acts on beta cells of the islet of Langerhans, it may affect the function and amount produced (Antar *et al.*, 2023). For instance, there are several environmental toxins like arsenic that have been proven to contribute to the accumulation of ROS, progressing to T2DM.

Due to its ability as mitochondrial electron transport (ETC) disruptor, arsenic (As) is known to be a potent inducer of oxidative stress, allowing ROS to be significantly produced in the mitochondria. It acts by the mechanism of dampening the expression of sirtuin-3, a mitochondrial deacetylase that helps to activate some ETC complexes and superoxide dismutase activity of SOD2. In the case of an increase in mitochondrial ROS, cellular metabolism will execute a switch from oxidative phosphorylation to glycolysis, resulting in the increase in reactive carbonyls like acetoacetate and methylglyoxal that has been linked to the T2DM development. However, to counter the detrimental effect of As, dietary selenium is often used which involves a selenoprotein, known as glutathione-peroxidase I (GPx-1). By consuming one molecule of glutathione, GPx-1 can reduce one molecule of hydrogen peroxide, allowing harmful ROS to be reduced to water (Bonini & Sargis, 2018).

2.4 Complications of Type 2 diabetes mellitus

T2DM is known as a multisystem disease that has strong correlation with complications in several organs like aorta, and kidney as portrayed in Figure 2.1, leading to an increase in the mortality rate of diabetic individuals or posing a long-term health risk. Poorly controlled blood glucose level for long term duration might inevitably bring damaging effects to some organs.

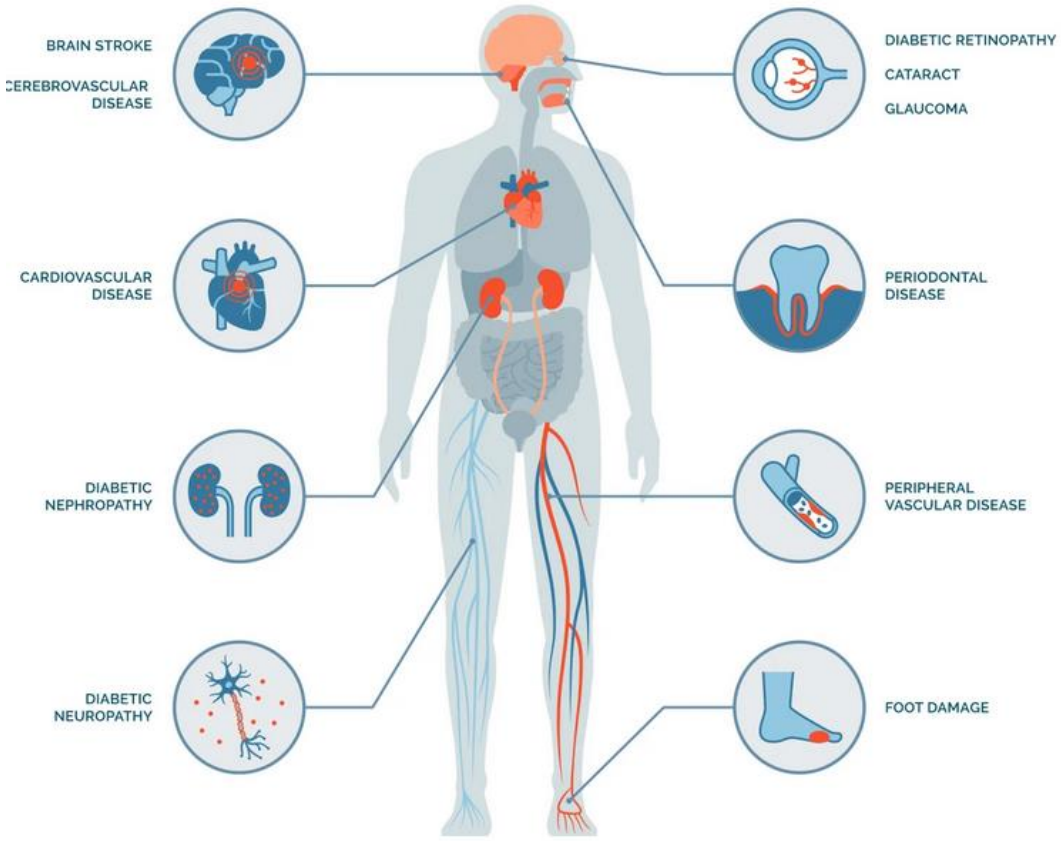

Figure 2.1: Effects of diabetes on human organs

Source: Choksi *et al*. (2022)

2.4.1 Aorta

Cardiovascular risks like heart disease and stroke are one of the most life-threatening complications that may result in the development of atherosclerosis in endothelium, subsequently leading to severe coronary artery disease, and peripheral vascular disease. Pertaining to endothelium, it functions to regulate the structure and tone of the vascular system by balancing the release of endothelial-derived contracting and relaxing factors. In this way, appropriate contraction and relaxation of the vascular wall can be performed. However, this balancing property of endothelium is much affected and altered in T2DM

conditions due to endothelial dysfunction, oxidative stress, and inflammation that takes place. In hyperglycaemic state, vascular endothelial cells are susceptible to developing into intracellular hyperglycaemia because glucose can diffuse through the plasma membrane. This is explained when the excess glucose is metabolised via polyol pathway to convert it to sorbitol and fructose by an enzyme known as aldose reductase. This enzyme will stimulate the activation of aldose reductase secondary metabolic pathway, oxidize NADPH to NADP+ and reduce NAD+ to NADH. This consistent stimulation will cause the depletion of NADPH and conversely, increase the NADH/NAD+ ratio. This change will accelerate glycolysis and speed up de novo synthesis of DAG, causing protein kinase C (PKC) to be activated. When PKC is activated, nitric oxide (NO) will be reduced. These will eventually cause vascular permeability and increase the contractility of vascular wall. In addition to that, if aldose enzyme is significantly produced, this will initiate more protein glycosylation that may yield Amadori products and AGE. Since AGE is highly associated with ROS production, oxidative stress production will be increased, causing vascular lesion (Galicia-Garcia *et al.*, 2020).

2.4.2 Kidney

Diabetic-induced hyperglycaemia may also result in renal complications with hemodynamic, inflammatory, fibrosis, and oxidative stress as major determinants of this pathological process. As for hemodynamics, hyperglycaemia will lead to an increase in osmolarity within the capillaries of glomerulus that promotes high glomerular pressure and dilation of afferent arterioles. When afferent arterioles dilate too often, hyperfiltration might occur, altering the vasoactive system within the kidneys. Several vasoactive systems like renin-angiotensin-aldosterone system (RAAS), polyol, protein kinase C (PKC), and AGE dependent pathways are highly activated. The constant activation of

RAAS is due to the increase in angiotensin II hormone that will further stimulate vasoconstriction of efferent arterioles. The dual combination of vasodilation in afferent arterioles and vasoconstriction in efferent arterioles leads to increased glomerular filtration rate and pressure, causing glomerular and tubular injury. Some key features of renal complications induced by diabetes are glomerulosclerosis, thickening of glomerular basement membrane, mesangial expansion, and progressive decline in renal function due to the morphological and functional changes in the glomerulus and kidney tubules (Jha *et al.*, 2024).

2.5 Treatment of Type 2 diabetes mellitus

To slow down the progression and development of T2DM and its related complications, treatment algorithms have been designed with a combination of pharmacological treatment and lifestyle changes to achieve great glycaemic control. American Diabetes Association (ADA), and European Association for the Study of Diabetes (EASD) suggested that initial intervention of T2DM should start from lifestyle changes for patients that have minor and acute DM. Those with severe diabetes complications, drug therapy should be advised based on the specific requirements for each patient. For lifestyle changes, physical activity and diet are the most important determinants of energy balance in achieving desired blood pressure, blood glucose, lipid, and body weight. Overweight individuals are mostly associated with insulin resistance as calories intake per day is most likely very high, worsening the condition for diabetes. By reducing body weight, insulin sensitivity could likely be improved.

In terms of pharmacological treatments, there are several oral and injectable hypoglycemic drugs available for the treatment of T2DM, but metformin of biguanide

drug, an oral agent still acts as first-line T2DM treatment. Metformin is approved by the U.S. Food and Drug Administration (FDA) in 1994 for T2DM treatment that can reduce fasting blood glucose by 20%. It can be taken together with glinides, sulfonylureas, alpha glucosidase inhibitors, and thiazolidinediones. Metformin has the potential to induce weight loss and is unlikely to cause hypoglycaemia. It acts to inhibit gluconeogenesis by activating AMP-activated protein-kinase (AMPK), an enzyme that helps to maintain homeostasis of cell energy or inhibiting glucagon-induced cAMP production by blocking adenylyl cyclase. However, metformin may produce side effects like abdominal discomfort, diarrhea, and nausea in mild cases and lactic acidosis in severe cases. Although the cases of lactic acidosis resulting from metformin side effect are low, it depicts a high fatality rate. On the other hand, glinides like repaglinide, and nateglinide, and sulfonylureas serve the same function, which is to stimulate the beta cells for insulin production by regulating ATP-sensitive potassium channels in membrane of beta cells.

Another oral treatment is thiazolidinediones that act to increase insulin sensitivity by acting on organs like liver, skeletal muscle, and adipose tissue for high glucose utilisation and decreased glucose production. Thiazolidinediones are similar to metformin in terms of efficacy level as monotherapy, but metformin is more preferred due to lower cost and minimal adverse effects. Dipeptidyl peptidase-4 inhibitors help to increase incretin agents (GLP1 and GIP) that are secreted by intestine cells as to increase production of insulin and inhibit glucagon secretion. It can be used as monotherapy or dual therapy with other oral agents like metformin, and thiazolidinediones.

One of the approved injectable agents is insulin that can be used to treat patients with all types of diabetes. The insulin induced in T2DM patients is not an imitation of

endogenous insulin, but it acts like an analogue such as lispro, detemir, glargine, glulisine, and aspart (Marín-Peñalver *et al*., 2016).

2.6 Fatigue

Fatigue, which is a common symptom of physiological and psychological condition, occurs normally after a mental or physical exertion to a point that it has brought stress, exhaustion, and tiredness to the body (Wang *et al*., 2021). Fatigue is generally divided into two types, which are acute and chronic fatigue, in which the occurrence of acute fatigue is temporary, and can be alleviated but chronic fatigue is normally associated with prolonged fatigue even after rest and this occurs when the body is in a disease state. In conjunction with the processes and systems involved in fatigue mechanism, it has been categorised into peripheral and central fatigue. Peripheral fatigue is associated with skeletal muscle or cardiac muscle, with the decrease in muscle tension. This results from the reduction in glycogen store, and also alteration in the muscle fiber from physical inactivity or aging. On the other hand, central fatigue has been associated with disruption to the nervous system that is defined as decreased motivation to carry out tasks or physical activity. Therefore, central fatigue differs from peripheral fatigue because motor weakness might not be detected clinically as in peripheral fatigue. In addition, central fatigue is usually related to neurological disorders like multiple sclerosis, but peripheral fatigue is mostly associated with diabetes mellitus, that has been described as purely physiological condition (Fritschi & Quinn, 2010).

2.7 Sea cucumber

As one of the nutrient-rich marine invertebrates, sea cucumbers have long been used as traditional medicine worldwide especially Asian countries like Japan, Korea, Malaysia, Indonesia, and China due to their broad therapeutic values. Functional purpose of sea cucumber on human health has been researched and validated through scientific literature that has exhibited properties of wound healing, anti-thrombotic, antioxidant, anti-hyperglycemic, anti-microbial, and anti-coagulant. These are due to the presence of bioactive compounds extracted from the sea cucumber including saponins (triterpene glycosides), phenolics (flavonoids and phenolic acids), polysaccharides, and proteins. Apart from that, sea cucumber also provides high nutritional value because of low lipid and high protein content such as lysine, arginine, and tryptophan. Additionally, gelatin extracted from sea cucumber is much more valuable than others due to the amino acid composition with essential amino acids that have excellent function in immune regulation. However, different sea cucumber species will withhold divergent nutraceutical, medicinal, and pharmacological activities due to its diversity (Fagbohun *et al*., 2023).

In contemporary market, there are various sea cucumber products originating from different body parts like liquid extract from whole sea cucumber, dry tablets from the body wall, and extract from the skin of sea cucumber but among these, body wall of the sea cucumber can be considered as the most marketable product. Therefore, those non-marketable body parts like gonads, intestine, and respiratory tract will be discarded as waste (Hossain *et al*., 2022).

Stichopus horrens or referred to durian sea cucumber is a variable whitish to grey marine invertebrate with brown irregular dots covering the body wall surface that belongs to class Holothuroidea. It is characterised by large and irregular papillae, smooth integument, and big tubercles, giving an external appearance of irregular and soft invertebrate, resembling the skin of durian fruit. Furthermore, this sea cucumber species reaches its sexual maturity at about 16 to 18 cm long, and it undergoes asexual reproduction by binary fission. In Malaysia, this species is well known for its body fluid back then as a health tonic, which is believed to have healing effects (Tan, 2008). The illustration of *Stichopus horrens* is shown in Figure 2.2.

Figure 2.2: *Stichopus horrens*

Source: Tan (2018)

2.7.1 Saponin

Constituting secondary metabolites of sea cucumbers, saponins act as a basis of chemical defense, in which it is known to elucidate various biological properties like anti-obesity, anti-bacterial, anti-fungal, anti-tumor, and immune modulatory activities. In terms of chemical structure, saponins are composed of a polar linear or branched saccharide chain that is attached to a non-polar aglycone by glycosidic bond. The diversity in carbon skeleton of aglycone regions has divided saponin into steroidal glycosides and triterpenoidal glycosides (Fagbohun *et al.*, 2023). However, triterpene glycosides are dominantly identified in sea cucumbers like *S. horrens*. Since saponin