THE EVALUATION OF *Etlingera elatior* FLOWER (BUNGA KANTAN) AQUEOUS EXTRACT (EEAE) EFFECTS ON COGNITIVE IMPAIRMENT IMPROVEMENT IN DIABETIC RATS (PILOT STUDY)

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by

TOH ZENY

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DECLARATION

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

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(Toh Zeny)

Date: 1/3/2025

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LIST OF SYMBOLS AND ABBREVIATIONS

AGEs	Advanced Glycation End Products
AMPK	AMP-Activated Protein Kinase
ANOVA	Analysis of Variance
APP	Amyloid Precursor Protein
ARASC	Animal Research and Service Centre
ATP	Adenosine Triphosphate
Aβ	β-amyloid glycogen synthase kinase-3β (GSK-3β)
BBB	Blood Brain Barrier
BMI	Body Mass Index
CA	*Cornu Ammonis*
CAT	Catalase
CNS	Central Nervous System
DG	Dentate Gyrus
DM	Diabetes Mellitus
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DPX	Dibutylphthalate Polystyrene Xylene
EEAE	*Etlingera elatior* Flower Aqueous Extract
FBG	Fasting Blood Glucose
FRAP	Ferric Reducing Antioxidant Power
GLUT1	Glucose Transporter 1
GLUT2	Glucose Transporter 2
GPx	Glutathione Peroxidase
GSH	Glutathione

GSK-3β	Glycogen Synthase Kinase-3 Beta
H&E	Haematoxylin and Eosin
HbA1c	Glycated Haemoglobin
HPLC	High-Performance Liquid Chromatography
IC_{50}	Half-maximal Inhibitory Concentration
IDE	Insulin-degrading Enzymes
IDF	International Diabetes Federation
IGF-1	Insulin-like Growth Factor 1
IL-1β	Interleukin-1 Beta
IL-2	Interleukin-2
IL-6	Interleukin-6
MDA	Malondialdehyde
MWM	Morris Water Maze
NHMS	National Health and Morbidity Survey
NO	Nitric Oxide
NVC	Neurovascular Coupling
NVU	Neurovascular Units
PARP	Poly (ADP-Ribose) Polymerase
PPSK	School of Health Sciences
RAGEs	Receptor of Advanced Glycation End Products
RIN	Rat Insulinoma Cell Line
ROS	Reactive Oxygen Species
SD	Sprague-Dawley
SEM	Standard Error of the Mean
SOD	Superoxide Dismutase

T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
T-AOC	Total Antioxidant Capacity
TNF-α	Tumour Necrosis Factor-Alpha
UAE	Ultrasonic-Assisted Extraction
UPMS	Unit Pengurusan Makmal Sains

PENILAIAN KESAN EKSTRAK AKUEUS BUNGA *Etlingera elatior* (BUNGA KANTAN) (EEAE) TERHADAP PENAMBAHBAIKAN GANGGUAN KOGNITIF DALAM TIKUS DIABETIK (KAJIAN RINTIS)

ABSTRAK

Diabetes mellitus (DM) merupakan isu kesihatan global yang memberi kesan kepada individu di semua negara, tanpa mengira jantina dan umur. DM meningkatkan risiko komplikasi multi-organ, termasuk gangguan kognitif yang dikaitkan dengan hiperglisemia, tekanan oksidatif, rintangan insulin, disfungsi neurovaskular, dan neuroinflamasi. Walaupun drug antidiabetik farmaseutikal berkesan dalam mengawal darah glukosa, ia sering disertai pelbagai kesan sampingan. Oleh itu, penggunaan tumbuhan ubatan yang mengandungi sebatian bioaktif untuk menangani gangguan kognitif akibat diabetes semakin menjadi tumpuan kerana mempunyai kesan sampingan yang minimum dan lebih menjimatkan kos. Kajian rintis ini bertujuan untuk menilai kesan ekstrak akueus bunga *Etlingera elatior* (EEAE) terhadap gangguan kognitif pada tikus diabetik. Sembilan ekor tikus jantan Sprague-Dawley dibahagikan secara rawak kepada tiga kumpulan (n=3/kumpulan): (1) kumpulan kawalan normal, (2) kumpulan kawalan diabetes, dan (3) kumpulan rawatan diabetes yang menerima 1000 mg/kg EEAE secara oral selama 6 minggu. Ujian DPPH digunakan untuk mengukur aktiviti antioksidan EEAE dan menunjukkan bahawa EEAE mempunyai potensi antioksidan sederhana, dengan sedikit penambahbaikan dalam paras darah glukosa puasa (FBG) dan indeks jisim badan (BMI) berbanding tikus diabetes. Selain itu, EEAE menunjukkan penambahbaikan

dalam semua parameter berkaitan pembelajaran dan ingatan spatial dalam ujian Morris Water Maze (MWM). Pemeriksaan histologi tisu otak menggunakan pewarnaan hematoksilin dan eosin (H&E) menunjukkan pengurangan degenerasi neuron dan mikroglia. Penemuan ini mencadangkan bahawa kesan antidiabetik, antioksidan, anti-radang, dan perlindungan saraf disebabkan oleh antosianin, iaitu sebatian bioaktif dalam EEAE. Oleh itu, EEAE berpotensi sebagai produk terapeutik semula jadi bagi memperbaiki gangguan kognitif akibat diabetes.

THE EVALUATION OF *Etlingera elatior* FLOWER (BUNGA KANTAN) AQUEOUS EXTRACT (EEAE) EFFECTS ON COGNITIVE IMPAIRMENT IMPROVEMENT IN DIABETIC RATS (PILOT STUDY)

ABSTRACT

Diabetes mellitus (DM) is a global health concern that affects individuals across all countries, sexes, and age groups. It increases the risk of developing multi-organ complications, including cognitive impairment, which is often linked to hyperglycaemia, oxidative stress, insulin resistance, neurovascular dysfunction, and neuroinflammation. Although the pharmaceutical antidiabetic drugs are effective in controlling blood glucose levels, they are often associated with various side effects. Hence, the trend of using medicinal plants that contain bioactive compounds to improve diabetes-induced cognitive impairment is increasing due to their minimal side effects and cost-effectiveness. This pilot study aims to evaluate the effects of *Etlingera elatior* flower aqueous extract (EEAE) on improving cognitive impairment in diabetic rats. Nine Sprague-Dawley male rats were randomly separated into three groups (n=3/group): (1) normal control group, (2) diabetic control group, and (3) diabetic treatment group receiving 1000 mg/kg EEAE orally for 6 weeks. The DPPH assay was used to measure the antioxidant activity of EEAE, which showed that EEAE exhibited moderate antioxidant potential. Furthermore, EEAE treatment showed slight improvements in fasting blood glucose (FBG) levels and body mass index (BMI) compared to the diabetic control group. Additionally, it improved all parameters related to spatial learning and memory in the Morris Water Maze (MWM) test.

Histological examination of brain tissue using haematoxylin and eosin (H&E) staining revealed reduced neuronal degeneration with fewer microglia in the EEAE-treated group. These findings suggest that the antidiabetic, antioxidant, anti-inflammatory, and neuroprotective effects could be due to the anthocyanins, a bioactive compound in EEAE. Therefore, it can potentially be developed as a natural therapeutic product in improving diabetes-induced cognitive impairment.

CHAPTER 1: INTRODUCTION

1.1 Background of Study

Diabetes mellitus (DM) is a chronic metabolic disease characterised by elevated blood glucose levels due to insufficient insulin secretion, insulin resistance or a combination of both, affecting the metabolism of carbohydrates, lipids and proteins (Antar *et al.*, 2023). It is primarily classified into type 1 DM (T1DM), type 2 DM (T2DM), gestational DM and other specific types of DM. T1DM results from the autoimmune destruction of pancreatic β-cells leading to insulin deficiency. In contrast, T2DM is often associated with insulin resistance, where the body does not respond well to insulin, which is not an autoimmune condition (Elsayed *et al.*, 2022). The factors that lead to T2DM include obesity, sedentary lifestyles, environmental stress and unhealthy dietary patterns. Besides that, gestational diabetes is usually defined as pregnancy-related hyperglycaemia that brings health problems to the mother and foetus. It will increase the risk of newborns developing diabetes during adulthood if their mothers are diagnosed with gestational diabetes during pregnancy. Furthermore, other specific types of diabetes can arise from the disease of the exocrine pancreas, endocrinopathies, infection or the use of certain drugs and chemicals (Antar *et al.*, 2023).

Diabetes can affect individuals of any age group, gender and geographic location. As a result, it has become one of the leading causes of mortality and morbidity worldwide and its prevalence is expected to rise significantly in the coming decades. Additionally, the prevalence of diabetes in low- and middle-income countries is much higher when compared to high-income countries. IDF Diabetes Atlas (2021) reported that 1 in 10 adults (20-79 years), approximately 537 million, were living with diabetes in 2021 compared to 108 million in 1980 and predicted to rise to 783 million by 2045 globally.

Besides that, diabetes caused around 6.7 million deaths in 2021. According to the National Health and Morbidity Survey (NHMS) 2023, the prevalence of diabetes among adults in Malaysia was 15.6 %, showing an increasing trend from 2011 to 2023 (Institute for Public Health, 2024).

In addition, patients with diabetes may experience signs and symptoms of frequent urination (polyuria), increased appetite, increased thirst (polydipsia), weight loss, extreme fatigue, blurred vision and slow wound healing. In diabetic patients, hyperglycaemia will lead to the excessive production of reactive oxygen species (ROS) with decreasing antioxidant protection resulting in oxidative stress that affects multiple organ systems. Hence, diabetes can lead to various complications such as neuropathy, nephropathy, retinopathy, cardiovascular diseases and cognitive impairment which may include problems with memory, attention, language, judgment and making decisions (Aderinto *et al.*, 2023). Besides that, individuals with diabetes have a higher risk of developing neurodegenerative diseases such as Alzheimer's disease and dementia. Epidemiological studies showed that 63.8% of diabetic patients experience cognitive impairment when compared to only 10.8% of non-diabetics (Varghese *et al.*, 2022). The key factors that mediate the impact of diabetes on cognitive impairment include insulin resistance, neuroinflammation, neurovascular dysfunction, hyperglycaemia and oxidative stress (Jin *et al.*, 2022; Aderinto *et al.*, 2023). All these factors collectively affect the hippocampus which is crucial for memory and learning. Thus, treatments are needed to manage diabetes-induced cognitive impairment.

However, antidiabetic drugs such as sulphonylureas, insulin, meglitinides and amylin analogues may cause side effects that lead to health problems, including hypoglycaemia, allergy, kidney complications and weight gain. Hence, medicinal plants with blood sugar-lowering, anti-inflammatory and antioxidant properties are explored to

improve diabetes-induced cognitive impairment because they have fewer side effects. Among these, *Etlingera elatior*, known as torch ginger and bunga kantan which belong to the family of Zingiberaceae, are investigated. It is a tropical and subtropical plant native to Southeast Asia, including Malaysia, Thailand and Indonesia. Besides that, it is also well-known for culinary, spices, medicines, fragrances, dyes as well as essential oils. Moreover, *E. elatior* holds significant potential as an alternative treatment for diabetes and its complications because it contains bioactive compounds such as anthocyanins, which help reduce oxidative stress and inflammation while enhancing glucose metabolism. Besides that, previous studies showed that *E. elatior* has various biological properties including antimicrobial, antioxidant, anticancer, antidiabetic, anti-ageing, and anti-inflammation that bring health benefits to society (Nor *et al.*, 2019; Nor *et al.*, 2020; Noordin *et al.*, 2022).

After the treatment of *E. elatior* flower aqueous extract (EEAE), the Morris Water Maze (MWM) test, a hippocampus-dependent behavioural task, was conducted to assess spatial learning and memory in rats (Frame *et al.*, 2019; Njan *et al.*, 2020). Throughout the repeated trial sessions, distal cues around the water tank guided the rats in locating the submerged hidden platform below the water surface, thereby facilitating spatial learning. Then, the hidden platform is removed during the probe trial to assess the reference memory by measuring the time spent by the rats in the target area (platform's previous location). As a result, the MWM test is an effective tool in this study to evaluate the effect of EEAE on improving cognitive impairment in diabetic rats.

1.2 Problem Statement

Diabetes mellitus (DM) is associated with various systemic complications, including cognitive impairment. According to Hossain, Al-Mamun & Islam (2024), diabetes prevalence continues to increase globally and is predicted to rise to 643 million by 2030. Besides that, the prevalence of cognitive impairment among diabetic patients (63.8%) was significantly higher when compared to only 10.8% of non-diabetic individuals (Varghese *et al.*, 2022). Although antidiabetic pharmaceutical drugs have aided in managing diabetes and its complications, including cognitive impairment, many individuals are still concerned about the undesirable side effects associated with long-term administration, such as severe hypoglycaemia, renal failure, increased oedema and lactic acidosis. Hence, there is growing interest in exploring the need for alternative treatments using medicinal plants with antidiabetic, anti-inflammatory and antioxidant properties as potential neuroprotective agents with fewer side effects. Previous studies have indicated that *E. elatior* flower has the potential for blood sugar-lowering and high antioxidant activity. However, the effects of *E. elatior* flower on cognitive impairment in diabetic models have not been fully studied. Therefore, this pilot study aims to investigate the effect of EEAE on improving cognitive impairment in diabetic rats.

1.3 Research Questions

1. Does EEAE exhibit *in vitro* antioxidant properties?
2. Does EEAE improve cognitive function in diabetic rats?
3. What structural changes in the brain of diabetic rats are observed after treatment with EEAE?

1.4 Objectives

1.4.1 General Objective

To evaluate the effects of *Etlingera elatior* flower aqueous extract (EEAE) on cognitive function in diabetic rats.

1.4.2 Specific Objectives

1. To evaluate the *in vitro* antioxidant properties of EEAE.
2. To determine whether EEAE improves cognitive function in diabetic rats.
3. To evaluate the structural changes to the brain in diabetic rats by EEAE.

1.5 Hypothesis

1.5.1 Null Hypothesis

1. EEAE does not exhibit significant *in vitro* antioxidant properties.
2. EEAE does not significantly improve cognitive function in diabetic rats.
3. EEAE does not cause significant structural changes to the brain in diabetic rats.

1.5.2 Alternative Hypothesis

1. EEAE exhibits significant *in vitro* antioxidant properties.
2. EEAE significantly improves cognitive function in diabetic rats.
3. EEAE causes significant improvements in structural changes to the brain in diabetic rats.

1.6 Significance of Study

Diabetes mellitus (DM) is a significant risk factor for cognitive impairment. While antidiabetic pharmaceutical drugs effectively control blood glucose levels, they are often associated with adverse side effects, and there is limited study on their effects in improving cognitive impairment caused by diabetes. Hence, it highlights the need for natural products containing antidiabetic, anti-inflammatory and antioxidant properties because they are safer for long-term administration. This study is significant in filling a gap in the current understanding of natural treatments by investigating the ability of EEAE to manage diabetes-induced cognitive impairment. It helps to advance our understanding of diabetes-induced cognitive impairment by integrating traditional medicinal knowledge with modern scientific research. Furthermore, the findings of the study could significantly contribute to the development of alternative treatments to reduce reliance on conventional treatments with undesirable side effects. It could also have broader implications for plant-based interventions in future research, thereby improving the overall management of diabetes-induced cognitive impairment effectively and reducing the burden of disease globally.

CHAPTER 2: LITERATURE REVIEW

2.1 Diabetes-induced Cognitive Impairment

Diabetes mellitus (DM) has evolved in recent years from a medical disorder mainly linked to metabolic abnormalities to a complicated illness with possible consequences that go well beyond glucose regulation, including significant effects on cognitive function. Cognitive impairment is a complex and growing health issue in diabetic patients. Previous studies have proven a substantial correlation between DM and cognitive impairment, as well as the fact that DM has been identified as a risk factor for the development of mild cognitive impairment into dementia and Alzheimer's disease (Yuan & Wang, 2017; Aderinto *et al.*, 2023). This recognition highlights the importance of understanding the pathophysiology of diabetes-induced cognitive impairment, which affects memory, learning, language, attention, information processing speed, and executive functions, including planning, problem-solving, decision-making, and effective communication (Aderinto *et al.*, 2023). This condition represents a significant challenge to the overall quality of life, especially for older people. The contributing factors that result in diabetes-induced cognitive impairment involve hyperglycaemia, oxidative stress, insulin resistance, neurovascular dysfunction, and neuroinflammation (Jin *et al.*, 2022; Aderinto *et al.*, 2023; Yosef *et al.*, 2023). All these factors affect the structure and function of the hippocampus, a region responsible for memory and learning. Figure 2.1 shows the pathway of diabetes-induced cognitive impairment.

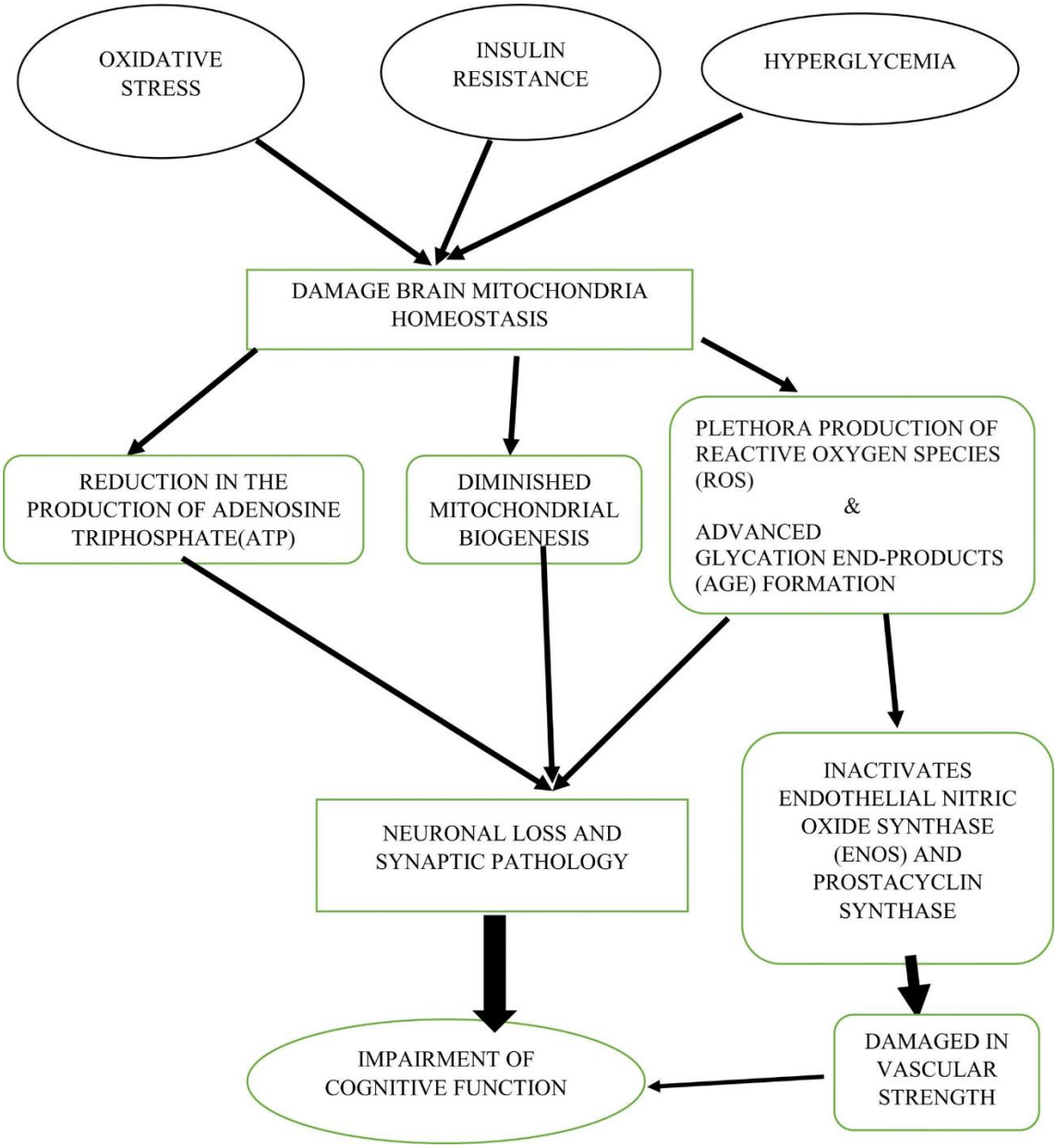

Figure 2. 1: The overall pathway of diabetes-induced cognitive impairment (Aderinto *et al.*, 2023).

2.1.1 Hyperglycaemia and Oxidative Stress

Prolonged hyperglycaemia in diabetes is the primary drive of cognitive impairment that triggers a series of detrimental reactions, including increased production of reactive oxygen species (ROS), particularly hydrogen peroxide, superoxide anion, and hydroxyl radicals (Aderinto *et al.*, 2023). Under normal conditions, antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) play an important role in neutralising these ROS. However, high blood glucose levels increase the production of ROS, resulting in the depletion of antioxidant mechanisms. All these ROS will further increase the activation of the polyol pathway, protein kinase C pathway, hexosamine pathway, and advanced glycation end products (AGEs) formation (Oguntibeju, 2019; Caturano *et al.*, 2023).

Apart from that, prolonged hyperglycaemia impairs the function of endothelial cells lining blood vessels via activation of the polyol pathway, resulting in the reduction of nicotinamide adenine dinucleotide phosphate, endothelial nitric oxide synthase activity, and nitric oxide (NO) production (Aderinto *et al.*, 2023). Loss of NO impairs the ability of blood vessels to dilate, which in turn causes atherosclerosis, thrombus formation, and cerebral infarction, followed by cognitive impairment (Abo hagar *et al.*, 2018). Besides that, the formation of AGEs happens when the excess glucose reacts non-enzymatically with the free amino groups of protein, forming Schiff bases that undergo rearrangement to form Amadori products that subsequently contribute to the AGEs formation (Twarda-clapa *et al.*, 2022; Aderinto *et al.*, 2023). The interaction of AGEs with the receptor of AGEs (RAGEs) causes oxidative stress and inflammation (Aderinto *et al.*, 2023).

Generally, all these processes shown in Figure 2.2 will induce oxidative stress. In this condition, the production of ROS, including free radicals and other highly reactive molecules, exceeds the capacity of the body's antioxidant defence system to neutralise

them (Twarda-clapa *et al.*, 2022; Caturano *et al.*, 2023). The brain is susceptible to oxidative stress because it contains high lipid content, high oxygen consumption rate, and low antioxidant enzymes (Yosef *et al.*, 2023). Therefore, ROS causes damage to cellular structure, including mitochondria, DNA, and proteins, resulting in neuronal apoptosis and synapse loss followed by cognitive dysfunction. According to Luo *et al.* (2022), diabetes diminishes mitochondrial dynamics, disrupts mitophagy, and causes proteostasis dysfunction, resulting in brain mitochondrial damage that causes the reduction in adenosine triphosphate (ATP), which develops into brain cell apoptosis (Luo *et al.*, 2022).

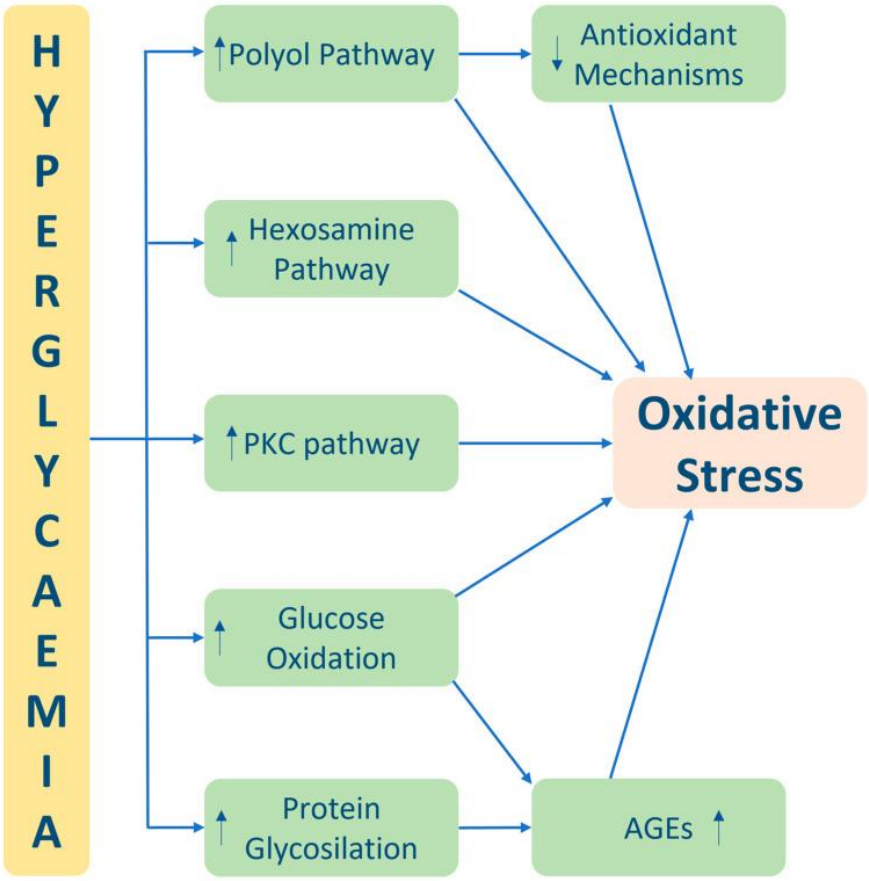

Figure 2. 2: The pathophysiological mechanism of hyperglycaemia-induced oxidative stress (Caturano *et al.*, 2023).

2.1.2 Insulin Resistance

In addition, previous studies have proven that insulin receptors are widely distributed in brain regions, including the hippocampus and frontal cortex, which are important for insulin cognitive effects by allowing insulin and insulin-like growth factor 1 (IGF-1) to regulate metabolism and growth (Beishon *et al.*, 2019). Insulin is essential for neurotransmission, neuroprotection, and the management of central glucose metabolism (Duarte, Moreira & Oliveira, 2012). When insulin signalling is impaired, it affects these critical processes, leading to impaired neuronal function, weakened synaptic connections, and reduced glucose uptake, which causes energy deficits in the brain.

In diabetes, amyloid processing and accumulation are negatively affected by insulin resistance and hyperinsulinemia. Insulin resistance impairs the clearance of β-amyloid (Aβ) due to the decreased level of insulin-degrading enzymes (IDE), thus promoting the accumulation of β-amyloid (Aβ), which is a product of β- and γ-secretase action on the amyloid precursor protein (APP) (Aderinto *et al.*, 2023; Yosef *et al.*, 2023). Then, it increases glycogen synthase kinase-3β (GSK-3β) activity, triggering tau hyperphosphorylation and fibrillary tangle formation, which cause neuronal dysfunction. Besides that, insulin resistance weakens the permeability of the blood-brain barrier (BBB) that regulates the microenvironment of the brain, thereby limiting the brain insulin to enter it (Qiu *et al.*, 2014). Hence, these processes impair cerebrovascular function and cognition. Furthermore, chronic low-grade inflammation that contributes to neuroinflammation is also associated with insulin resistance (Aderinto *et al.*, 2023).

2.1.3 Neurovascular Dysfunction

Diabetes-induced cognitive impairment is significantly associated with microvascular and macrovascular complications. Microvascular complications encompass diabetic nephropathy, retinopathy, and neuropathy, which may impair cognitive function (Ryan *et al.*, 2016; Aderinto *et al.*, 2023). As the brain and kidney have comparable anatomical and functional characteristics of the vasculature system, nephropathy that causes albuminuria and kidney dysfunction is at a higher risk of developing cognitive impairment (Jin *et al.*, 2022; Xie *et al.*, 2022).

Furthermore, the retina shares a similar microvascular network with the brain, thus retinal damage reflects potential small-vessel disease in the brain (Jin *et al.*, 2022). When the retinal arteriolar diameters become narrower, the cognitive processing becomes slower, suggesting that retinal microvasculature damage may contribute to the progression from mild cognitive impairment to Alzheimer's disease. Decreased mental efficiency and executive function are caused by the narrower retinal arterioles, cerebral microbleeds, and wider venules related to the brain's white matter atrophy (Jin *et al.*, 2022). Generally, the transport of nutrients to nerve tissue is impacted by structural changes in the microvasculature, such as capillary reduction and arteriovenous shortcuts (Forte *et al.*, 2019). This makes the brain more vulnerable to oxygen deprivation, which may result in cognitive impairment.

Apart from that, macrovascular complications contribute to cognitive impairment primarily through cerebrovascular and cardiovascular diseases that impair brain function via cerebral hypoperfusion, ischaemia, neuroinflammation, and structural brain damage (Hu *et al.*, 2023). The integrity of neurovascular units (NVU), including neurons, astrocytes, interneurons, endothelial cells, blood-brain barrier (BBB), myocytes, pericytes, microglia, and extracellular matrix components, is compromised in diabetes

(Muoio, Persson & Sendeski, 2014; Jin *et al.*, 2022). Previous studies have mentioned that diabetes may compromise BBB integrity and impair astrocytic gap junctional communication (McConnell *et al.*, 2019; Hajal *et al.*, 2022). Damage to any compound of NVU may impair neurovascular coupling (NVC), resulting in a mismatch between blood flow and neuronal activity followed by cognitive impairment.

2.1.4 Neuroinflammation

Neuroinflammation plays an important role in diabetes-induced cognitive impairment characterised by the activation of immune responses. Diabetes may exhibit the breakdown of immune tolerance, resulting in the development of autoreactive T CD 8+ cells and CD 20+ B cells, contributing to inflammation by infiltrating peri-islet regions (Willcox *et al.*, 2009; Yosef *et al.*, 2023). Furthermore, islet inflammation also results from the production of ROS and cytokines by macrophages, such as interleukin-1 beta (IL-1β) and tumour necrosis factor-alpha (TNF-α) (Yosef *et al.*, 2023). Besides that, adipose tissues produce pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 that contribute to insulin resistance and chronic low-grade inflammatory states (Caturano *et al.*, 2023). Under normal conditions, adipose tissues release leptin, which enhances insulin sensitivity, and adipokines, which have anti-inflammatory properties. However, diabetes leads to an increase in leptin, which causes leptin resistance and reduces its effectiveness. Besides that, the formation of adipokines is also suppressed.

Next, the activation of microglia by IL-2, IL-6, and TNF-α leads to the primary immune defence mechanism in the central nervous system (CNS) associated with modifying the gene expression of the pro-inflammatory cytokines (Yosef *et al.*, 2023). Hence, this process amplifies the inflammatory response by attracting the white blood cells to the CNS, resulting in more glial damage and neuronal apoptosis (Grigsby *et al.*,

2014). It increases the oxidative stress that promotes the production of Aβ and further activates nuclear factor kappa B (NF-kB), which increases the production of pro-inflammatory cytokines. All these inflammatory processes will affect cerebral vasoregulation and are associated with decreased cerebral vasoreactivity and vasodilation, thus developing cognitive impairment (Chung *et al.*, 2015).

2.2 Animal Models for Diabetic Study

Most medical research, including diabetic studies, requires laboratory animals such as rats and mice because they have around 95 % DNA similar to humans. Thus, they develop diseases and respond to treatments in ways that are quite similar to human physiological and pathological processes (Delwatta *et al.*, 2018). Furthermore, animal models play an important role in studying the mechanism of diabetes and its complications, as well as evaluating new antidiabetic agents and interventions for future clinical applications.

Among these, male Sprague-Dawley (SD) rats, an outbred strain of albino laboratory rats belonging to *Rattus norvegicus*, are often chosen as diabetes models due to their well-established phenotype and genetic variability with extensive baseline data (Patterson *et al.*, 2015; Delwatta *et al.*, 2018; Fajarwati *et al.*, 2023). Additionally, their physiological and immunological similarities to humans make them valuable for providing insights into biomedical research. Apart from that, SD rats are calm and docile, making them easier to handle. As SD rats show greater resilience to stress-induced behavioural depression, they are suitable for behavioural tasks, including chronic restraint stress, chronic unpredictable stress, or genetic manipulation (Wang *et al.*, 2017).

Most of the diabetic models involve using male rats because they are more sensitive to islet-cell toxins such as STZ (Kim *et al.*, 2020; Furman, 2021). After the

injection of STZ, male rats had lower β-cell mass associated with a higher glucagon/insulin ratio compared to female rats, resulting in higher fasting blood glucose levels (Kim *et al.*, 2020; Ghasemi & Jeddi, 2023). In female rats they had a higher resistance to STZ toxicity than male rats due to the presence of the protective effects of oestrogen that enhance β-cell function, insulin production, and glucose homeostasis, thus they require a higher dosage of STZ to reach the same level of hyperglycaemia as male rats. Besides that, female rats are not recommended due to their highly fluctuating hormonal conditions, including oestrogen and progesterone during oestrous cycles, leading to variable responses and may affect the results (Husein, Indarto & Wasita, 2024). Therefore, the results are inconsistent because each rat has a different oestrous cycle phase during the measurement day. Furthermore, the survival rates of female rats were lower than those of male rats (Vital, Larrieta & Hiriart, 2006). Consequently, male SD rats act as a reliable model for studying biological systems, diseases, behaviour, and treatments.

2.3 Streptozotocin

Several methods can be used to develop diabetes in animal models, including dietary or nutritional induction, genetic manipulation (spontaneous and transgenic/knockout diabetic animals), surgical intervention (pancreatectomy), and diabetogenic agents such as streptozotocin (STZ) and alloxan (Fajarwati *et al.*, 2023). Since alloxan has a narrow diabetogenic dose, even a slight overdose can result in general toxicity primarily affecting the kidney; thus, alloxan is the least favourable (Wszola *et al.*, 2021).

Streptozotocin, (also known as 2-deoxy-2-(3-(methyl-3-nitrosoureido)-D glucopyranose) derived from the culture of *Streptomyces achromogenes*, is a highly selective, rapid and irreversible pancreatic islet β-cell-cytotoxic agent that causes

hypoinsulinaemia and hyperglycaemia (Patterson *et al.*, 2015; Ghasemi & Jeddi, 2023). This selective toxicity happens because pancreatic β-cells express a high glucose transporter 2 (GLUT2) level that acts as the predominant glucose transporter in facilitating the movement of STZ across the plasma membrane. Previous studies on RIN (rat insulinoma cell line) cells have proved that the cytotoxic effects of STZ are associated with the glucose transport capabilities of the cells (Ghasemi & Jeddi, 2023). RIN cells that express GLUT1 instead of GLUT2 show higher resistance to STZ. However, they become more susceptible to STZ when GLUT2 is introduced into these cells, indicating that GLUT2 plays an important role in facilitating the uptake of STZ (Wszola *et al.*, 2021; Ghasemi & Jeddi, 2023). As a result, STZ is selectively accumulated in pancreatic β-cells via GLUT2 due to its chemical structural similarity to glucose, enabling GLUT2 to transport it into the cells (Patterson *et al.*, 2015; Wszola *et al.*, 2021).

After entering the cells, STZ causes DNA damage by transferring the methyl group to the DNA molecule, resulting in DNA fragmentation. Then, it leads to the overstimulation of poly (ADP-ribose) polymerase (PARP) to repair DNA, causing the depletion of NAD^+ and ATP (Lenzen, 2008; Wszola *et al.*, 2021). It is associated with dephosphorylation, which increases the substrate for xanthine oxidase, resulting in the generation of hydrogen peroxide and hydroxyl radicals, thereby inducing oxidative stress (Wszola *et al.*, 2021). Additionally, the nitrosourea group of STZ increases nitric oxide production, exacerbating oxidative stress and disrupting mitochondrial function. As a result, all these mechanisms accelerate the apoptotic and necrotic β-cell death, resulting in the inhibition of insulin biosynthesis and secretion, which in turn leads to hyperglycaemia (Lenzen, 2008; Wszola *et al.*, 2021; Ghasemi & Jeddi, 2023).

To conclude, the STZ molecule contains two functional parts that contribute to its diabetogenic action, including the glucopyranosyl group, which facilitates its uptake into

pancreatic β-cells via glucose transporter 2 (GLUT2), and the nitrosourea group, which is responsible for the destruction of pancreatic β-cells (Ghasemi & Jeddi, 2023). Previous studies have mentioned that T1DM rat models can be established using two methods, including a single high dose of STZ (55 mg/kg) administered intraperitoneally or multiple low doses of STZ (20 mg/kg) administered intraperitoneally daily for five consecutive days (Nor *et al.*, 2019; Ghasemi & Jeddi, 2023). Also, T2DM rat models are developed by administering 35 mg/kg of STZ intraperitoneally after two weeks of a high-fat diet (58% of total kcal from fat) (Ghasemi & Jeddi, 2023). Figure 2.3 summarises the mechanism of STZ in causing the insulin-producing β-cells impairment, resulting in diabetic development.

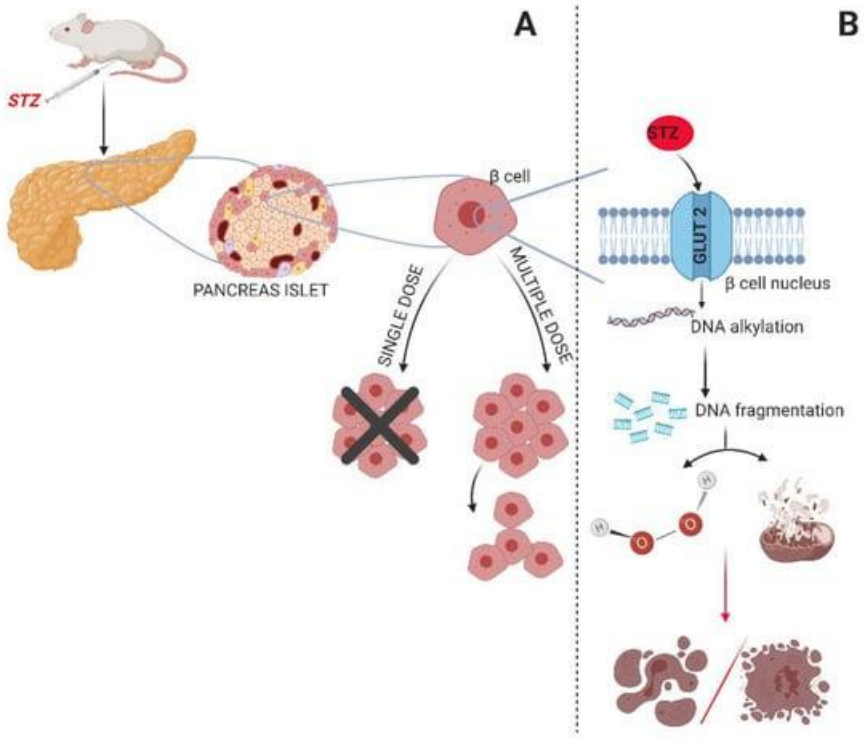

Figure 2. 3: Mechanism of action of single or multiple doses of STZ damages the β cells **(A)**. Mechanism of action of STZ in the β cells nucleus **(B)** (Wszola *et al.*, 2021).

2.4 The Need for Alternative Treatments Compared to Pharmaceutical Antidiabetic Drugs

The management of DM primarily involves pharmacological treatment based on the specific type of diabetes in order to improve glycaemic control and prevent the development of diabetes complications. T1DM requires lifelong insulin therapy, while T2DM can be managed through a combination of lifestyle modification, pharmaceutical antidiabetic drugs, and in advanced stages, insulin therapy (Blahova *et al.*, 2021; Thota & Akbar, 2023). Despite effectively controlling blood glucose levels, these treatments have side effects that can affect health-related quality of life.

Insulin can be categorised based on the duration of action, such as rapid-acting, short-acting, intermediate-acting, and long-acting. Furthermore, the administration routes of insulin include subcutaneous, intravenous, inhalation, and intramuscular (Thota & Akbar, 2023). The most common side effect of insulin therapy is hypoglycaemia, which can lead to irritability, confusion, and tachycardia, and may progress to severe conditions such as loss of consciousness, seizure, coma, or death (Chamberlain *et al.*, 2017; Thota & Akbar, 2023). Furthermore, short- and intermediate-acting human insulin are associated with a higher risk of severe hypoglycaemia, while rapid- and long-acting insulin are associated with fewer hypoglycaemic events (Chamberlain *et al.*, 2017). The other side effects also involve weight gain, hypokalaemia, and peripheral hyperinsulinemia.

The available therapy for T2DM is pharmaceutical antidiabetic drugs such as biguanides, thiazolidinediones, sulphonylureas, meglitinides, alpha-glucosidase inhibitors, glucagon-like peptide-1 receptor agonists, dipeptidyl peptidase-4 inhibitors, and sodium-glucose cotransporter two inhibitors (Blahova *et al.*, 2021). Patients with T2DM should start treatment with metformin monotherapy at the time of diagnosis unless

there are contraindications. If the HbA1c target is not achieved after 3 months of monotherapy, dual combination therapy consisting of metformin with another type of antidiabetic drug should be started. If the target is still not achieved, triple therapy that involves metformin with two additional antidiabetic drugs should be considered. However, combination injection therapy that includes metformin and insulin should be initiated if there is still no improvement after three months of triple therapy. Previous studies showed that long-term administration of metformin might lead to vitamin B_{12} deficiency and gastrointestinal side effects (Chamberlain *et al.*, 2017). The other pharmaceutical antidiabetic drugs may also cause bloating, diarrhoea, and weight gain (Nor *et al.*, 2019). In some severe cases, they will increase the risk of heart failure, bladder cancer, bone fracture, and kidney complications (Blahova *et al.*, 2021).

Since prolonged administration of pharmaceutical antidiabetic drugs is associated with various side effects, there is growing interest in natural therapeutic products that contain bioactive compounds in improving glycaemic control and cognitive impairment with fewer side effects. Besides that, natural therapeutic products not only can be used in pre-diabetic and early stages of diabetes but also the advanced stages (Chamberlain *et al.*, 2017). Therefore, research is needed on medicinal plants that have the potential to manage diabetes and its complications to investigate their efficacy, safety, and mechanisms.

2.5 Phytochemical and Pharmacological Properties of *Etlingera elatior*

Etlingera elatior, commonly known as torch ginger, is a medicinal and culinary plant that can be found in South Asia. It has been traditionally utilised in medical practice due to its rich phytochemicals and bioactive compounds that contribute to numerous pharmacological activities, including antimicrobial, antioxidant, anti-inflammatory, antihyperglycemic, antihyperuricaemic, anti-ageing, antilarval, wound healing, and skin whitening (Juwita, Puspitasari & Levita, 2018). The phytochemical analysis of *E. elatior* flower aqueous extract (EEAE) from the previous study showed that it contained phenolic, flavonoid, coumarin, tannin, and quinone (Nor *et al.*, 2019).

Generally, the unopened *E. elatior* flowers were specifically chosen for extraction due to their highest level of total phenols content, total flavonoids content, ferric-reducing assay power, and scavenging activity when compared to mature open flowers (Binti Anzian *et al.*, 2017). Previous studies have mentioned that EEAE has higher antioxidant activity than ethanolic extract of *E. elatior* flowers as determined by two different methods, including 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant potential (FRAP) assay (Ghasemzadeh *et al.*, 2015). Studies by Nor *et al.* (2020) and Noordin *et al.* (2022) have shown that EEAE exhibits a significant total phenolic content [39.06 ± 1.59 mg gallic acid equivalent per gram extract (mg GAE/g)], total flavonoid content [39.00 ± 2.42 mg quercetin equivalent per gram extract (mg QE/g)], and total anthocyanin content (54.26 ± 5.34 mg/L), which contribute to its potent antioxidant and antidiabetic activity, bringing beneficial effects in managing diabetes mellitus (DM) and its complications.

By using the high-performance liquid chromatography (HPLC) method, EEAE shows the presence of a high level of cyanidin-3-O-glycosides, which is a plant-derived secondary metabolite classified under the flavonoid family and specifically within the

anthocyanin subgroup (Cásedas *et al.*, 2019). In addition, anthocyanins play a significant role in facilitating anti-inflammatory, antimicrobial, anticarcinogenic, antioxidant, and antidiabetic (Noordin *et al.*, 2022). Besides that, six primary anthocyanin compounds have been recognised for their antidiabetic properties, including cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin (Oliveira *et al.*, 2020). It shows antidiabetic effects by regulating the blood glucose levels through various mechanisms; including decreasing oxidative stress, reducing inflammation, preserving β-cell function, stimulating insulin secretion, enhancing insulin sensitivity, promoting glycolysis, suppressing gluconeogenesis, inhibiting sugar digestion by suppressing α-amylase and α-glucosidase activity as well as activating AMP-activated protein kinase (AMPK) (Oliveira *et al.*, 2020). Furthermore, anthocyanin can cross the blood-brain barrier, thus it also plays an important role in neuroprotection.

Nor *et al.* (2020) and Noordin *et al.* (2022) shown that there was a significant reduction of fasting blood glucose levels in diabetic rats treated with EEAE. Besides that, the antioxidant properties of EEAE facilitate the reduction of malondialdehyde (MDA), an oxidative stress marker, indicating the improvement of lipid peroxidation. This enhancement is associated with the improvement of antioxidant markers, including superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and total antioxidant capacity (T-AOC) (Noordin *et al.*, 2022). Additionally, the antioxidant properties act as a free radical scavenger in repairing pancreatic β-cells and maintaining their performance in stimulating insulin secretion to prevent the development of diabetes complications by mitigating oxidative stress caused by hyperglycaemia (Nor *et al.*, 2019). In short, the antidiabetic activity is related to the presence of bioactive compounds in EEAE with high antioxidant and anti-inflammatory properties.

2.6 Roles of Hippocampus in Cognitive Function

The hippocampus is crucial for forming and reconstructing relational memories by integrating multiple inputs to form associations between the elements of scenes and events, thereby facilitating the transfer from short-term memories into long-term memories (Wible, 2013). Other than learning and memory, it also plays an important role in spatial navigation, emotional behaviour, and regulation of hypothalamic functions (Anand & Dhikav, 2012). It is a paired functional system with an S-shaped structure located medially in the temporal lobe, forming part of the limbic system (Idunkova, Lacinova & Dubiel-Hoppanova, 2023). It can be divided into head (expanded part), body, and tail (thin curved part).

Furthermore, it is comprised of the dentate gyrus (DG), subiculum, and *cornu ammonis* (CA) fields, which can be divided into three subregions, including CA1, CA2, and CA3, based on the size, density, and branching patterns of pyramidal cell axons and dendrites (Wible, 2013; Idunkova, Lacinova & Dubiel-Hoppanova, 2023). However, the classification of CA4 remains debated because some studies reclassified CA4 as the hilus area that separates CA3 and DG rather than considering it a separate CA region. The subiculum is located in the dorsal part of the parahippocampal gyrus, which includes the entorhinal and perirhinal areas, thereby facilitating the connection between the hippocampus and the entorhinal cortex (Wible, 2013). Figure 2.4 shows the structural anatomy of the hippocampus in the coronal plane.

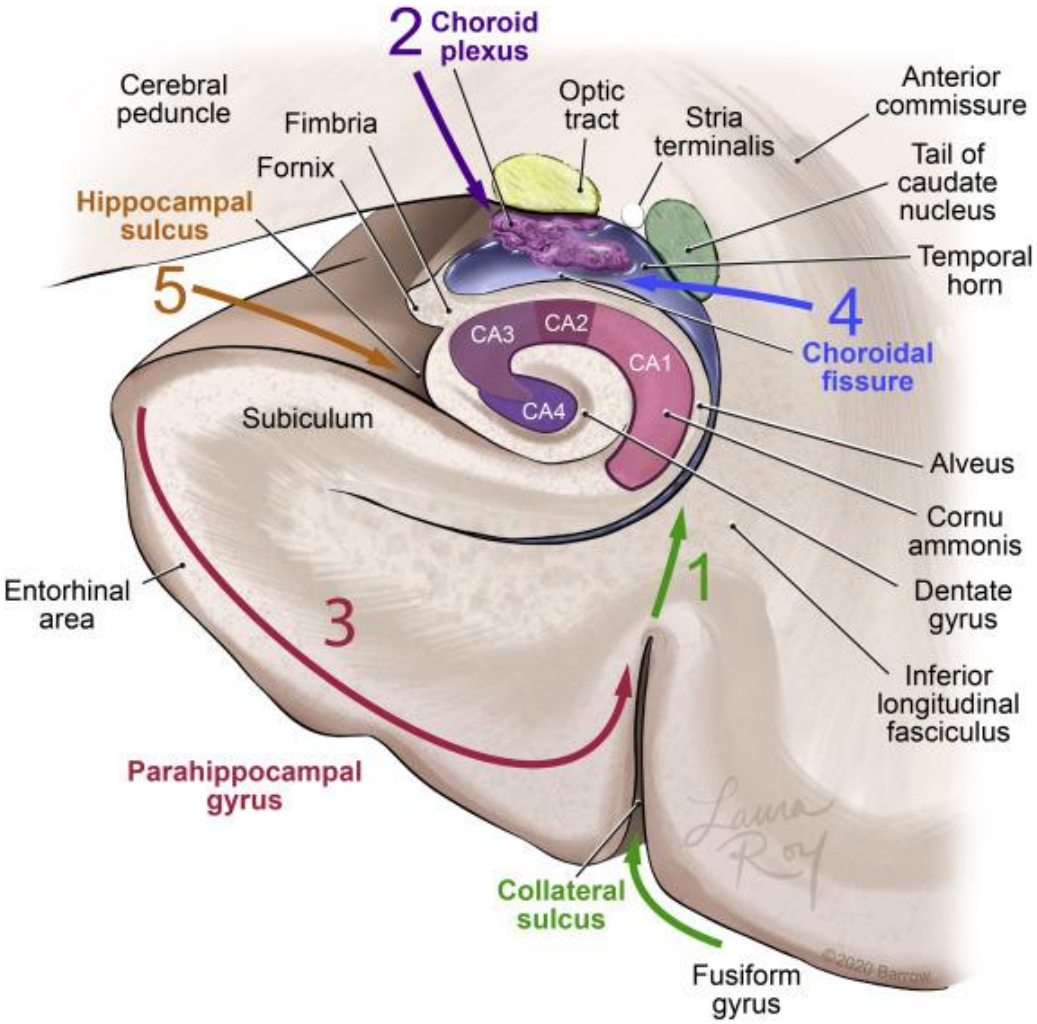

Figure 2. 4: Structural anatomy of the hippocampus in the coronal plane (Przybylowski *et al.*, 2021).

Generally, the entorhinal cortex serves as the bridge between the hippocampus and the cerebral cortex by giving major input through superficial layers to the hippocampus and receiving output through deeper layers (Coutureau & Di Scala, 2009; Anand & Dhikav, 2012). The perforant path arises from different layers of the entorhinal cortex and connects it to the hippocampal formation, facilitating the information flow. Axons in the perforant path primarily originate from layers II and III of the entorhinal cortex, with minimal input from deeper layers IV and V. While axons from layers II and IV target the granule cells of the dentate gyrus and the pyramidal cells of CA3, those from

layers III and IV project to the pyramidal cells of CA1 and the subiculum (Anand & Dhikav, 2012). The overview of the basic pathway of the hippocampus is shown in Figure 2.5.

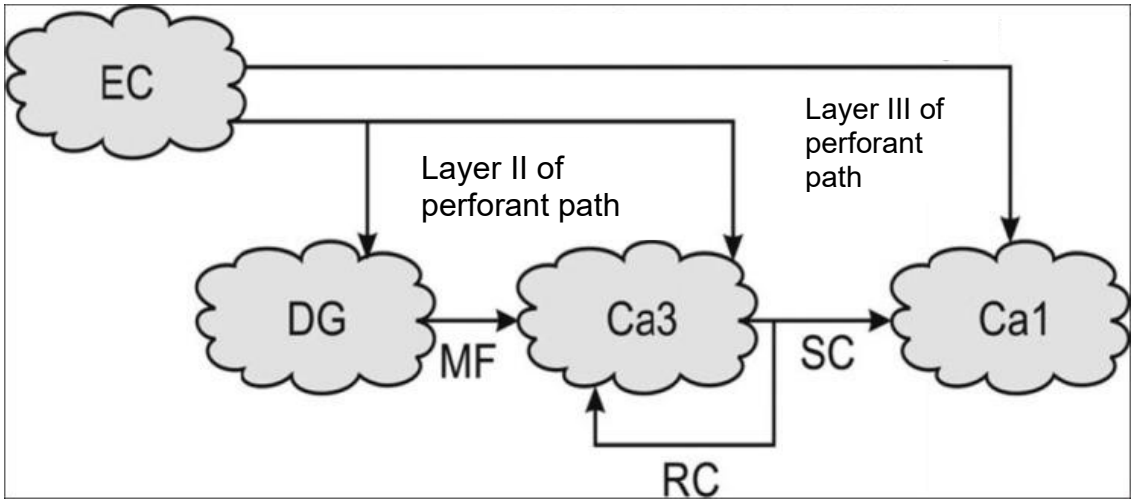

Figure 2. 5: Basic pathway of the hippocampus (Anand & Dhikav, 2012).

For learning and memory loops, it can provide inputs through two pathways, including the polysynaptic and direct intra-hippocampal pathways. In the polysynaptic pathway, the entorhinal cortex (layer II of the perforant path) sends projections from the parietal, temporal, and occipital areas to the dentate gyrus, which is important for pattern recognition and memory encoding (Kerr *et al.*, 2007; Anand & Dhikav, 2012). Then, projections move to CA3 through the mossy fibres for information processing (Anand & Dhikav, 2012). After that, the fibres, known as Schaffer collateral, move from CA3 to CA1, which is crucial for memory formation. Next, they move to the subiculum, alveus, fimbria, fornix, mammillothalamic tract, anterior thalamus, posterior cingulate, and retrosplenial cortex (Anand & Dhikav, 2012). For the direct intra-hippocampal pathway, the entorhinal cortex (layer III of the perforant path) provides input from the temporal