UNIVERSITI PUTRA MALAYSIA

HISTOPATHOLOGICAL EXAMINATION ON PANCREATIC TISSUES OF STREPTOZOTOCIN - INDUCED DIABETIC SPRAGUE DAWLEY RATS TREATED WITH PURPLE SWEET POTATO (IPOMOEA BATATAS) LEAF EXTRACT

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ABSTRACT

Histopathological Examination on Pancreatic Tissues of Streptozotocin - Induced Diabetic Sprague Dawley Rats Treated with Purple Sweet Potato (*Ipomoea batatas*) Leaf Extract

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Introduction: Type 2 diabetes mellitus (T2DM) is a metabolic disorder that occurs due to the impairment of insulin to regulate the normal blood glucose level in the body. A few conventional medicines used in treating T2DM have been reported to develop unfavourable side effects on health. Hence, scientists keep investigating the potential of plant-based therapeutic agents to reduce the prevalence of T2DM among people. Purple sweet potato leaves have been identified as a functional food having a variety of bioactive chemicals shown to promote health. On the other hand, previously published studies have reported excessive oxidative stress in patients with T2DM can induce inflammation in the organs, resulting in premature cell senescence. **Objective:** The purpose of this study is to determine the effect of *Ipomoea batatas* leaf ethanolic extract (IBEE) on the diameter of Islets of Langerhans and the number of pancreatic β cells in treated rats. **Methodology:** A semi-quantitative histopathological assessment of the pancreas of treated rats was performed. The organs underwent a series of histopathological processes including tissue fixation, tissue processing, tissue embedding, tissue sectioning, slide staining with haematoxylin-eosin stain, microscopic examination and histopathological scoring. The data were analysed using one-way ANOVA, followed by Tukey's post hoc test for multiple comparisons. **Result:** The results showed that oral administration of IBEE to streptozotocin-induced diabetic rats could improve the inflammation of the pancreas compared with those of untreated diabetic rats. **Discussion:** Plant ethanolic extracts have been suggested to contain an abundance of polyphenolic compounds which pharmacological effects include the anti-oxidation and anti-inflammation. Various phytochemical studies have reported that purple sweet potato leaf contain high content of polyphenolic compounds. The impressive protective effects of IBEE could be due to the phenolic and flavonoid compounds present in IBEE which possibly help scavenge the reactive oxygen species and regenerate the injured β cells. **Conclusion:** Findings from the study showed that IBEE has the potential to be developed as an alternative therapeutic agent to treat T2DM.

Keywords: Type 2 Diabetes Mellitus, *Ipomoea batatas*, streptozotocin, histopathological changes

ABSTRAK

Pemeriksaan Histopatologi Tisu Pankreas Pada Tikus Sprague Dawley Diabetik Teraruh Streptozotocin Dirawat Dengan Ekstrak Daun Keledek Ungu (*Ipomoea batatas*)

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Pengenalan: Diabetes mellitus jenis 2 (T2DM) adalah gangguan metabolik yang berlaku akibat gangguan insulin untuk mengawal paras glukosa darah normal dalam badan. Beberapa ubat konvensional yang digunakan dalam merawat T2DM telah dilaporkan menghasilkan kesan sampingan yang tidak baik terhadap kesihatan. Oleh itu, saintis terus menyiasat potensi agen terapeutik berasaskan tumbuhan untuk mengurangkan kelaziman T2DM. Daun keledek ungu telah dikenal pasti sebagai makanan yang mempunyai pelbagai bahan kimia bioaktif yang menggalakkan kesihatan. Di samping itu, kajian yang diterbitkan sebelum ini telah melaporkan tekanan oksidatif yang berlebihan pada pesakit T2DM boleh menyebabkan keradangan pada organ, mengakibatkan penuaan sel pramatang. **Objektif:** Tujuan kajian ini adalah untuk menentukan kesan ekstrak etanol daun *Ipomoea batatas* (IBEE) terhadap diameter Kelompok Langerhans dan bilangan sel β pankreas dalam tikus yang dirawat. **Metodologi:** Penilaian histopatologi separuh kuantitatif pada pankreas tikus yang dirawat telah dilakukan. Organ-organ tersebut menjalani beberapa siri proses histopatologi termasuk penetapan tisu, pemprosesan tisu, pembenaman tisu, keratan tisu, pewarnaan slaid dengan pewarnaan haematoxylin-eosin, pemeriksaan mikroskopik dan pemarkahan histopatologi. Data dianalisis menggunakan ANOVA sehala, diikuti dengan ujian post hoc Tukey untuk pelbagai perbandingan. **Keputusan:** Keputusan menunjukkan bahawa pemberian oral IBEE kepada tikus diabetes yang disebabkan oleh streptozotocin boleh mengurangkan keradangan pankreas berbanding tikus diabetes yang tidak dirawat. **Perbincangan:** Ekstrak etanol tumbuhan telah dicadangkan mengandungi banyak sebatian polifenol yang yang mempunyai kesan farmakologi termasuk anti-pengoksidaan dan anti-keradangan. Pelbagai kajian fitokimia telah melaporkan bahawa daun keledek ungu mengandungi kandungan sebatian polifenol yang tinggi. Kesan perlindungan IBEE yang baik mungkin disebabkan oleh sebatian fenolik dan flavonoid yang terdapat dalam IBEE yang membantu menghilangkan spesies oksigen reaktif dan menjana semula sel β pancreas yang cedera. **Kesimpulan:** Dapatan kajian menunjukkan IBEE berpotensi untuk dibangunkan sebagai agen terapi alternatif untuk merawat T2DM.

Kata kunci: Diabetes Jenis 2, *Ipomoea batatas*, streptozotocin, perubahan histopatologi

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LIST OF ABBREVIATIONS

T2DM	Type 2 diabetes mellitus
IBEE	*Ipomoea batatas* leaf ethanolic extract
PSPL	Purple-fleshed sweet potato leaves
β	Beta
GLUT4	Glucose transporter type 4
IST	Insulin signal transduction
MRC	Mitochondrial respiratory chains
NADPH	Nicotinamide adenine dinucleotide phosphate
KATP	ATP-sensitive potassium channels
DAMPs	Damage-associated molecular patterns
PRRs	Pattern recognition receptors
TLRs	Toll-like receptors
NF-κB	Nuclear factor kappa B
IκB	Inhibitor of NF-κB
TNF-α	Tumour necrosis factor-alpha
ROS	Reactive oxygen species
RNS	Reactive oxygen species
MAPK	Mitogen-Activated Protein Kinase
JAK-STAT	Janus kinase-signal transducer and activator of transcription
Nrf2	Nuclear factor erythroid 2–related factor 2
COX-2	Cyclooxygenase-2
iNOS	Inducible nitric oxide synthase
%	Percent

°C	Degree Celsius
S.E.M	Standard error mean
BW	Body weight
AMPK	Adenosine Monophosphate-Activated Protein Kinase

CHAPTER 1

INTRODUCTION

1.1 Type 2 Diabetes Mellitus

Type 2 diabetes mellitus (T2DM) is a metabolic disorder that occurs due to high blood glucose levels, resulting from insulin resistance and impairment of insulin secretion. T2DM is the most common type of diabetes suffered by people worldwide. Statistics recorded that T2DM accounts for more than 90% of all diabetes cases. T2DM is most typically found in people over 45 years old (Goyal & Jialal, 2021). Nonetheless, due to increased overweight and obesity, physical inactivity, and unhealthy diets, T2DM is increasingly found in younger people, adolescents, and even children (Zheng et al., 2018). In 2020, about 900 000 people in Malaysia were diagnosed with diabetes, with T2DM accounting for 99.33 percent of the cases (Chandran & Zakariah, 2021).

Persistent hyperglycemia in untreated diabetes mellitus can lead to various acute and chronic complications. Hypertension among T2DM patients has recorded the highest prevalence at 80.00% in 2020, followed by dyslipidemia (75.72%) and erectile dysfunction (15.50%). Besides that, patients with T2DM also suffer from nephropathy (14.38%), retinopathy (11.52%), ischaemic heart disease (5.64%), cerebrovascular disease (1.88%), diabetic foot ulcers (1.24%) and amputations (0.73%) (Chandran & Zakariah, 2021). It has been discovered that oxidative stress contributes to the aetiology of diabetic complications. Hyperglycemia-induced oxidative stress is believed to raise pro-inflammatory protein levels with infiltrating

macrophages secreting inflammatory cytokines, resulting in local and systemic inflammation.

1.2 *Ipomoea batatas* as a Potential Therapeutic Agent for T2DM

Sweet potato (*Ipomoea batatas*), locally known as ubi keledek, is a dicotyledonous plant originating from the Convolvulaceae family. It is a popular tropical and subtropical staple food with nutritional benefits, as indicated by its increased production and consumption (Alam et al., 2016). The sweet potato has been recognised as a functional food containing several bioactive compounds shown to promote health (Amagloh et al., 2021). Some people in many parts of the globe consume sweet potato as a traditional medicine to treat various diseases such as asthma, diarrhea, nausea, fever and stomach distress (Meira et al., 2012).

Makori et al. (2020) discovered that the sweet potato leaf is more potent than other parts of plant components in scavenging free radicals. Research on the functional constituents of sweet potato leaves shows that high levels of polyphenols, carotenoids and flavonoids contribute to its health advantages (Nguyen et al., 2021). Furthermore, Ji et al. (2015) reported that purple-fleshed sweet potato leaves (PSPL) had much more anthocyanin than yellow, red, and green sweet potato leaves. Hence, it is essential to investigate the antidiabetic property of PSPL in T2DM.

1.3 Problem Statement

T2DM is a complex metabolic disorder that affects humans, affecting several organs in the body. Hence, it is crucial to reduce the prevalence of diabetes among people to prevent comorbidities and complications. However, the management of diabetes is challenging and its treatments are frequently accompanied by side effects that can harm people's health (Rafiu & Luka, 2018). As a result, scientists are increasingly looking for natural compounds that have antidiabetic properties with low adverse effects (Luo et al, 2021).

1.4 Justification

Ipomoea batatas leaf contains significant antioxidant, anti-inflammatory and immunomodulatory properties, making this plant a viable candidate for antidiabetic treatment (Ayeleso et al., 2016). Furthermore, 130 known metabolites have been found in the leaf and storage root of *Ipomoea batatas* using a simple extraction method (Drapal et al., 2019).

1.5 Objective

1.5.1 General Objective

To evaluate the effects of *Ipomoea batatas* leaf ethanolic extract (IBEE) on the pancreas of streptozotocin-induced diabetes in Sprague Dawley rats by histopathological analysis.

1.5.2 Specific Objective

1. To determine the effect of IBEE on the diameter of Islets of Langerhans in streptozotocin-induced diabetic Sprague Dawley rats.

2. To determine the effect of IBEE on the number of pancreatic β cells in streptozotocin-induced diabetic Sprague Dawley rats.

1.7 Hypothesis

Ipomoea batatas leaf ethanolic extract exhibit larger diameter of Islets of Langerhans and higher number of pancreatic β cells compared to the untreated rats (depending on the doses of IBEE administered).

CHAPTER 2

LITERATURE REVIEW

2.1 Pancreas: An Endocrine Gland

The pancreas is a long, flat organ that resides in the loop of the duodenum in the belly, posterior to the stomach. It weighs around 100g and measures about 14-23 cm in length (Longnecker, 2021). The Islets of Langerhans are a group of cells in the pancreas responsible for producing and releasing hormones that control glucose levels. It consists of five different types of cells: alpha cells, beta cells, delta cells, gamma cells, and epsilon cells which release glucagon, insulin, somatostatin, pancreatic polypeptide, and ghrelin respectively. Pancreatic islet cells perform critical functions in appetite regulation and glucose homeostasis (Xavier, 2021).

The essential hormones involved in glucose homeostasis are insulin and glucagon. The beta (β) cells detect an increase in blood glucose concentration after eating a high-carbohydrate meal and release insulin directly into the bloodstream. Other factors such as fatty acids, amino acids, and hormones can also stimulate insulin release (Mann et al., 2020). Insulin binds to its receptor, causing glucose transporter type 4 (GLUT4) to move from inside the cytoplasm of the target cell to the plasma membrane. GLUT4 functions as a channel protein that allows glucose to enter the cell quickly and be utilised for metabolism or converted into glycogen or fat for storage. This mechanism is known as the insulin response, and it is necessary for lowering and stabilising blood glucose levels (Taylor & Knight, 2021).

2.2 Pathogenesis of T2DM

2.2.1 Insulin Resistance

Insulin resistance is defined as a loss or reduction in the responsiveness of target tissues to insulin (Maciejczyk et al., 2018). Insulin resistance occurs due to the malfunction of insulin receptors, insulin receptor substrates, and the insulin receptor's binding activity (Holloway et al., 2014). As a result, insulin transmission is reduced in insulin resistance patients. Oxidative stress has been shown to impede normal insulin signal transduction (IST) at several levels. Oxidative stress can potentially affect IST by downregulating proteins necessary for optimal IST function, lowering insulin sensitivity and leading to insulin resistance (Balbaa et al., 2017).

2.2.2 Impairment in Insulin Secretion

Normal glucose homeostasis requires a healthy and functional mass of pancreatic β cells, and diabetes is associated with variable degrees of β cell dysfunction (Esser et al., 2020). As a result, insulin synthesis from β cell is deregulated and diminishes, causing postprandial glucose to rise over the normal level (White et al., 2016). Finally, a defective steady-state and basal insulin secretion occurs, resulting in complete β cell failure (Yaribeygi et al., 2020).

The main contributors of free radicals in pancreatic β cells are mitochondrial respiratory chains (MRC) and NADPH (nicotinamide adenine dinucleotide phosphate) oxidase activity with superoxide anion being the primary free radical species generated (Hurrle & Hsu, 2017). Chronic hyperglycemia induces the formation of free radicals in the pancreas via numerous biochemical mechanisms, including an increase in cytosolic calcium and protein kinase activation (Newsholme et al., 2009). Since β cell has a limited antioxidant defence system capability, oxidative stress in β cell is

common in diabetes mellitus and contributes significantly to their function loss (Gerber & Rutter, 2017).

Oxidative stress disrupts proinsulin vesicle inclusion into the plasma membrane and lowers their exocytosis in response to glucose in the circulation. Free radical species have a deleterious impact on metabolic pathways in β cells and inhibit nuclear transcription factors involved in insulin gene expression. Besides that, oxidative stress induces mitochondrial failure in the β cell and causes apoptosis in pancreatic cells. These mechanisms lead to β cell dysfunction and reduce/inhibit insulin secretion by lowering the blood glucose level in the body (Yaribeygi et al., 2020).

2.3 *Ipomoea batatas*

2.3.1 Background History

Sweet potato (*Ipomoea batatas*) belongs to the Convolvulaceae family and is closely related to morning glory (*Ipomoea indica*) and kangkung (*Ipomoea aquatica*) (Tan, 2015). It originated in Central America and is now extensively cultivated and consumed worldwide. Sweet potatoes are grown primarily for their tubers (Ayeleso et al, 2016). On the other hand, the leaves are occasionally used as a substitute for other green vegetables (Franková et al., 2022). With yearly production around 90 million tonnes, it is one of the world's most important food crops, contributed mainly by Asian countries, particularly China (Leite, 2020). Figure 1 shows the total production of sweet potato from the year 2010 to 2020 across the countries.

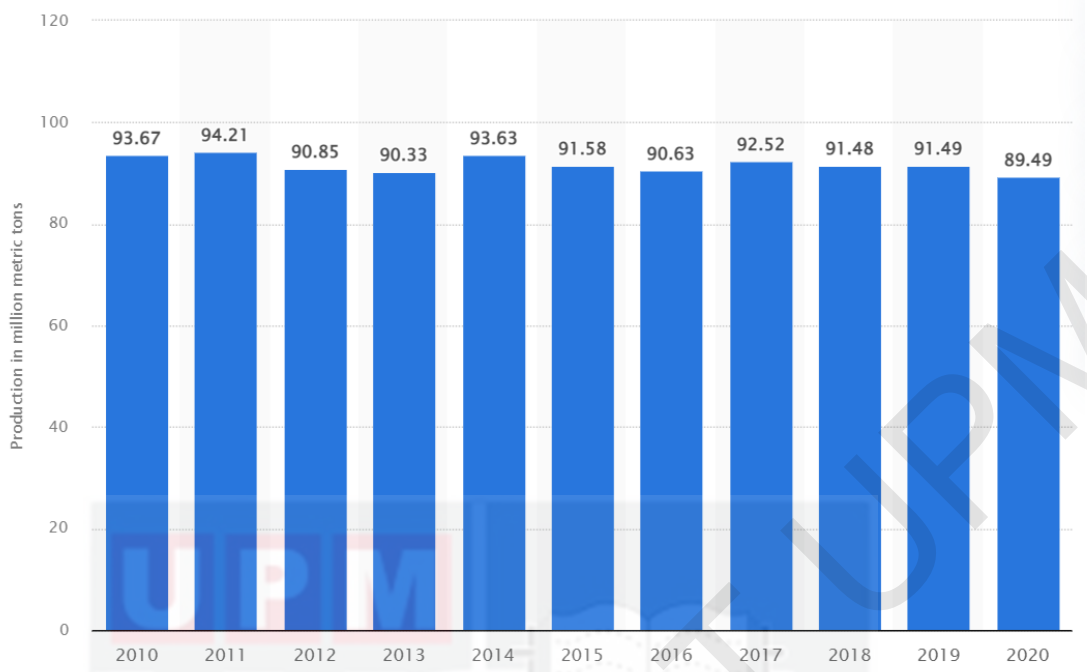

Figure 1 Statistic depicts the total production of sweet potato globally from 2010 – 2020 (Statista, 2022).

Besides that, sweet potato is also a significant food crop in tropical and subtropical locations, providing nutritional benefits to rural and urban populations (Alam et al., 2016). Sweet potato is the sixth most significant food crop after rice, wheat, potatoes, maise, and cassava (Prathiksha & Ramachandra Naik, 2019). According to Teow et al. (2007), the consumption of sweet potatoes can help to reduce and prevent the occurrence of cardiovascular diseases and functions as anticarcinogen. Sweet potatoes are a vital crop in many regions of the world, with over 100 nations producing them (Alam, 2021). Figure 2 depicts the widespread sweet potato cultivation across the globe in 2018.

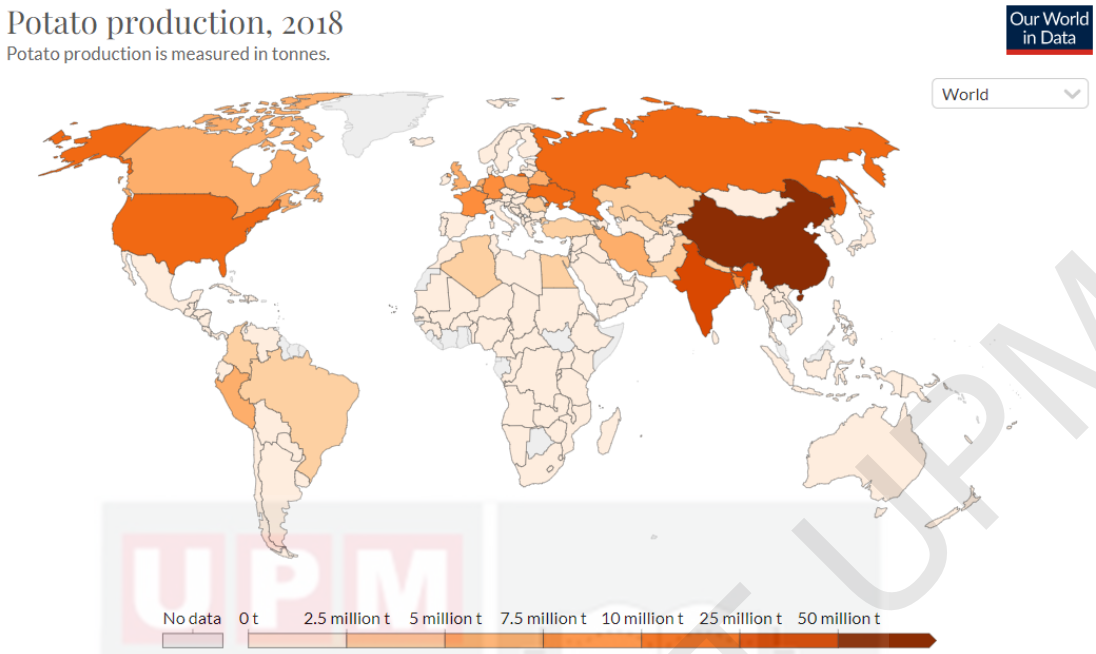

Figure 2 A global map presenting the production of sweet potatoes across the world (Ritchie & Roser, 2020).

2.3.2 Botanical Information

Ipomoea batatas are propagated vegetatively as an annual plant from stem cuttings or storage roots (Huaman, 1999). According to Van de Fliert and Braun (1999), the growth cycle of sweet potato occurs in three phases (establishment, intermediate, and store root bulking phase) during a period of 90 to 120 days, from crop establishment to storage root harvest. Sweet potatoes have a substantially greater yearly production than many other green vegetables since they can be harvested many times (Alam, 2021). Because of its short production cycle, this crop may be produced in the tropics and the temperate zone throughout the summer. Sweet potatoes can thrive at a wide variety of altitudes, from sea level to 2,500 metres in the tropics (Tan, 2015). Sweet potatoes generate the most and highest quality roots in well-drained, sandy, or silt loam soil.

For optimal growth and root development, it requires both warm days and nights (Verma, 2014). It is quite sensitive to alkaline and saline environments, impacting its development. The optimum soil pH for sweet potatoes ranges between 5.6 and 6.6 (Brandenberger et al., 2022)). According to Alam (2021), the crop is more resistant to illnesses, pests, and excessive moisture than many other tropical green vegetables. Sweet potatoes come in various shapes, including round, fusiform, globular, and ovate with smooth, rough, and ridged surfaces. The flesh can be white, yellow, reddish, orange, or purple, while the skin can range from white to yellow, orange, purple, red, or brown (Alam et al., 2016). Figure 3 illustrates various shapes, flesh and skin of sweet potato tubers available. According to Rosnani et al. (2017), its weight ranges from 150 to 250 g.

Figure 3 Sweet potato varieties with varying flesh and skin colour (van Eijck, 2021).

2.3.3 Local-based Sweet Potato Production

Sweet potato is the second most important tuber crop in Malaysia after cassava (Hanim et al., 2014). Sweet potato is widely planted in Perak, Selangor, Kelantan, and Terengganu, where approximately 41 244 metric tonnes of sweet potatoes were produced in 2017 (Zulkifli et al., 2021). It is used in the food sector as an ingredient in bakery items such as bread, biscuits, cakes, drinks, and noodles (Soison et al., 2015). In addition, it is utilised as a stabiliser, thickener, and tissue reinforcing agent in food to enhance the texture and quality (Mu et al., 2017).

Purple sweet potato is an uncommon crop among Malaysians due to a scarcity of commercial food products. Purple sweet potato is often used in only a few Malaysian dishes and traditional snacks (Muhammad et al., 2021). On the other hand, Purple-fleshed sweet potatoes are more popular among researchers due to their high nutritional and functional properties, which results in beneficial antioxidants and other biological activities (Maddipatla at al., 2017). As a result, the scientific study strives to improve understanding of the nutritional qualities of purple-fleshed sweet potatoes and increase their usefulness, particularly in managing T2DM.

2.4 Purple-fleshed Sweet Potato Leaf

2.4.1 Pharmacological Properties

Other than tubers, the other edible parts of sweet potatoes such as the leaf, stem and stalk were discovered to contain valuable components in terms of nutrient, bioactive and non-nutrient compounds beneficial for humans (Alam, 2021). Bioactive compounds, known as phytochemicals, found in plant-based foods are essential in providing human health benefits beyond basic nutrition, notably decreasing the risk and likelihood of chronic diseases (Jimenez-Garcia et al., 2018). Sweet potato leaves

are high in antioxidants such as polyphenols, flavonoids, anthocyanins, and caffeic acid derivatives. These bioactive components can reduce oxidative stress to varied degrees, which is reported to be the most important and common risk factor for chronic diseases (Johnson & Pace, 2010).

It has been proposed that polyphenols are the potent antioxidant compounds that provide health benefits upon consuming fruit and vegetables (Pandey & Rizvi, 2000). PSPL has the highest total phenolic content compared to other commercial vegetables, including sweet potato tubers and potato roots (Kurata et al., 2019; Makori et al., 2020). The polyphenolic contents in the leaves were reported to exhibit various physiological functions such as antimutagenic, anticancer, antimicrobial, antidiabetic, antioxidant and anti-inflammatory activity in *in vitro* and *in vivo* study, which is essential for human health (Alam, 2021).

Furthermore, phytochemical analysis by using high performance liquid chromatography (HPLC) revealed that the polar metabolites such as β-carotene, anthocyanins, as well as some phenolic acids and flavonoids were found to be present in relatively higher concentrations in purple sweet potato compared to the other colour-fleshed sweet potato (Park et al., 2016). Anthocyanin, the purple pigment present in purple sweet potatoes is effective as an antioxidant because it can react with free radicals in the body by reducing the ability of free radicals to cause harm and damage to the body. The pigment anthocyanin in purple sweet potato has higher stability than other sources, such as anthocyanin in elderberries, blueberries, red cabbage and red corn (Dwiyanti el al., 2021).

Besides that, intake of PSPL contributed to substantial improvements in proliferative responses of peripheral blood mononuclear cells and natural killer cell cytotoxic activities. More specifically, it can increase lymphocyte proliferation,

interleukin-2 and interleukin-4 levels. People that consume PSPL in their diets exhibit relatively higher cytotoxic natural killer cell activity, indicating that dietary polyphenols can deactivate or reduce free radicals in the body, decrease lipid peroxidation, and improve immunity to destroy free radicals (Johnson & Pace, 2010).

2.5 Sprague Dawley Rat as an Animal Model

The rat has been preferred for investigating neurobiological processes and providing neuropsychological models for human behavioural problems for almost a century. Rats are widely used as the primary rodent model for research due to their bigger size, which facilitates surgical procedures and other types of testing (Cowley et al., 2004). In addition, rats and humans share roughly 95% of the same DNA, so they are more or less prone to the same illnesses and respond to therapies in the same way. Experimental research employs a variety of rat strains, both inbred and outbred. Sprague Dawley rats, an outbred albino breed of rats that exhibits calmness and ease in handling, are generally recognised and commonly used research models in numerous studies such as reproduction and development, toxicology, safety and efficacy testing, nutrition, behaviour, and pharmacology studies (Delwatta et al., 2018).

2.6 Inflammation

Under normal circumstances, inflammation protects tissues from both endogenous and exogenous harm. Inflammation can be caused by various stimuli, including microbial and viral infections, autoimmune illnesses, exposure to allergens or toxic substances, and even metabolic problems. Inflammation can be classified into two stages which are acute and chronic inflammation. Acute inflammation lasts only

a few days and is typically helpful to the host since it aids in the restoration of normal homeostasis, such as by digesting invading germs. On the other hand, chronic inflammation is defined as inflammation that lasts for a long time. Chronic inflammation is harmful to the body since it can create inappropriate physiological reactions, raise the risk of cellular damage, and eventually contribute to the development of chronic disorders.

2.6.1 Vascular Response

When there is an injury in the tissue, the small blood vessels in the injured area constrict for a brief period. This process is known as vasoconstriction. Next, vasodilation of blood vessels occurs to increase the blood flow into the injury site. Consequently, the walls of the blood vessels become more permeable and allow exudate to enter the tissues from the circulatory system. The exudate contains various substances including clotting factors which help to prevent or limit infectious agents from spreading throughout the body. Besides that, antibodies also aid in destroying the pathogen that invades the body. The blood flow becomes progressively sluggish when fluid and other substances escape the blood vessels. The white blood cells subsequently adhere to the blood vessel wall, which is the initial stage in their emigration into the extravascular space of the tissue (Britannica, 2020).

2.6.2 Cellular Response

The most crucial element of inflammation is the increase of white blood cells at the injury site. Neutrophils are the primary type of white blood cells involved in acute inflammation. These cells can be obtained in adequate amounts from the blood circulation when tissue injury is mild. The bone marrow will create more neutrophils

if the damage is severe. Neutrophils must escape through the blood vessel wall and actively migrate from the blood vessel to the site of tissue damage by the chemotaxis mechanism. After days or weeks, macrophages, a biological hallmark of chronic inflammation, become increasingly abundant at the injury site. The presence of macrophages indicates that persistent inflammation is present at the cellular level (Britannica, 2020).

2.6.3 Inflammatory Mediators

Inflammation is a tightly controlled process mediated by immune signalling molecules known as cytokines. In the first phase, pathogen-associated molecular patterns (PAMPs) are employed to identify infection signals or damaged tissues (Patel & Patel, 2015). These PAMPs are specific to the molecules that the pathogens produce. Meanwhile, damage-associated molecular patterns (DAMPs) are endogenous danger compounds generated by injured or dying cells that stimulate the innate immune system via their interaction with pattern recognition receptors (PRRs) (Roh & Sohn, 2018).

When the innate immune system successfully recognises DAMPs, either transmembrane Toll-like receptors (TLRs) or inflammasomes will activate particular immunological signalling mechanisms, ultimately activating nuclear factor kappa B (NF-κB). Activation of NF-κB will result in the dissociation of NF-κB from inhibitor of NF-κB (IκB) and it further translocates to the nucleus to up-regulate the transcription process by binding to target genes. Following the transcription and translation of the genes, pro-inflammatory cytokines such as interleukin-1, interleukin-6 and tumour necrosis factor-alpha (TNF-α) will be expressed (Ashley et al., 2012).

These pro-inflammatory cytokines then attract immune cells like monocytes and neutrophils to the site of damage, where they produce reactive oxygen species (ROS) and reactive nitrogen species (RNS) that destroy macromolecules like proteins and DNA. Under normal circumstances, restoration limits the recruitment of additional neutrophils for wound healing and restores the equilibrium of the tissues. Meanwhile, a persistent inflammatory response induces tissue injury in chronic inflammation, increasing the risk of cellular damage (Saha et al., 2020).

On the other hand, inflammatory cells such as macrophages, mast cells, lymphocytes and monocytes will produce inflammatory mediators including chemokines, cytokines, and prostaglandins to attract more inflammatory cells to the site of damage. As a result, the tissue will experience respiratory bursts and increased oxidative stress. These attract more macrophages and directly stimulate the NF-κB, Mitogen-Activated Protein Kinase (MAPK), and Janus kinase signal transducer and activator of transcription (JAK-STAT) signalling pathways involved with the inflammatory response. Transcription factor activation, such as NF-κB and nuclear factor erythroid 2–related factor 2 (Nrf2) are critical components of the inflammatory signalling cascade and oxidative stress responses (Kaulmann & Bohn, 2014).

2.7 Recent Pharmacological Findings of *Ipomoea batatas*

Previous studies have reported that polyphenols in the PSPL can help treat T2DM. Dietary polyphenols have long been recognised for their potent antioxidant capabilities and protective effects against diseases (Shahwan et al., 2022). Polyphenols can be divided into three groups: phenolic acids, flavonoids, and non-flavonoids (Rambaran, 2020). Phenolic acids and flavonoids are primarily responsible for the health-promoting biological activity in sweet potato leaves (Wang et al., 2016).

Several polyphenols can modulate the primary processes of glucose homeostasis and carbohydrate metabolism, such as glycolysis, gluconeogenesis, and glycogenesis, which are all compromised in diabetes (Shahwan et al., 2022).

Flavonoids are the main bioactive substance isolated from *Ipomoea batatas* leaves (Mbayei-Nwaoha & Emejulu, 2013). Flavonoids possess a powerful antioxidant that aids in the treatment of diabetes (Sarian et al., 2017). According to Soares et al. (2017), an increase in intracellular calcium concentration triggers a number of cellular pathways that increase the synthesis of insulin. Flavonoids may act as insulin secretagogues and enhance peripheral glucose absorption by influencing the pleiotropic pathways of insulin signalling. Additionally, catechin and quercetin are involved in calcium regulation via changes in calcium fluxes. Quercetin can also lessen insulin resistance while increasing peripheral tissue's ability to absorb glucose (Rafiu & Luka, 2018).

Besides that, anthocyanin, the purple pigment present in purple sweet potatoes, contains antioxidant and anti-inflammatory qualities that are helpful in *in vivo* and *in vitro* models of chronic diseases (Blesso, 2019). According to many investigations, anthocyanins have demonstrated promising outcomes in blocking the majority of the inflammatory signalling cascade and minimising inflammation-mediated tissue damage. On the other hand, anthocyanins work as antioxidants because they can directly trap free radicals, hence reducing ROS production in afflicted cells (Alam et al., 2021).

Meanwhile, *Ipomoea batatas* leaf extract contains a high concentration of phenolic components such as caffeic acid and its derivatives (Niwa et al., 2011). Caffeic acid and its derivatives also have a considerable inhibitory effect on cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) synthesis

(Choi et al., 2018). Caffeic acid is a potent inhibitor of mitogenic activity, which promotes T cell proliferation, lymphokine synthesis, and NF-κB activation (Sidoryk et al., 2018). Activation of NF-κB can stimulate the transcription of numerous genes and eventually control inflammation.

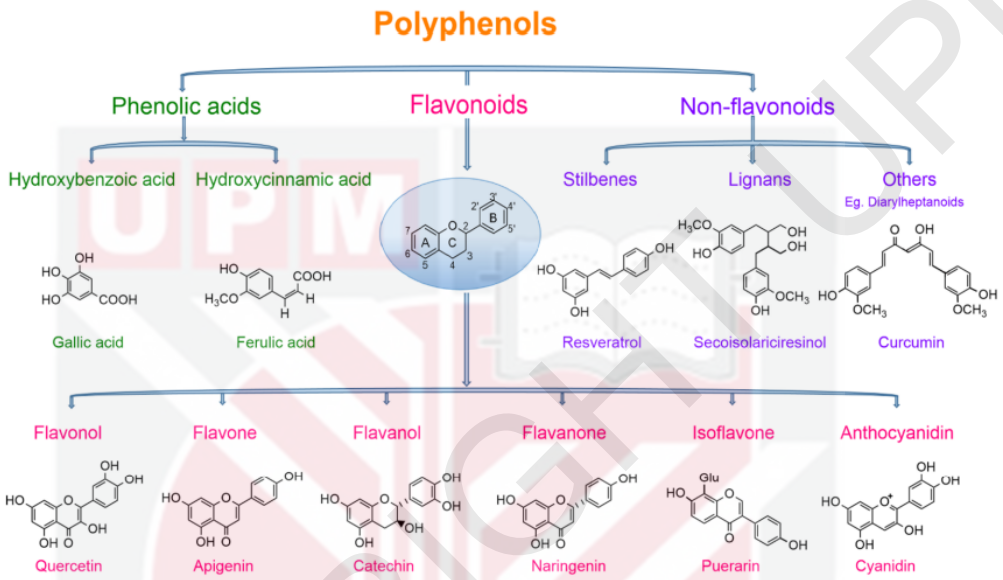

Figure 4 Classification of main polyphenols (Rambaran, 2020).

CHAPTER 3

METHODOLOGY

3.1 Materials

3.1.1 Reagents and Instruments

The reagents used in the histopathological procedure of the pancreas were formalin, alcohol, xylene, hematoxylin and eosin staining. The instruments used were the cold plate Leica HI1130, oven Memmert ULM400, Leica TP1020 automatic benchtop tissue processor, Leica RM2255 fully automated rotary microtome, feather microtome blade high profile, microscope frosted glass slide, mounting bath Leica HI1220, Leica ST5010 autostainer XL, Leica EG1160 tissue embedding station, fume hood, coverslip, Sigma-Aldrich DPX mountant and Dino-Eye microscope.

3.1.2 Specimens

The pancreas of Sprague Dawley rats was obtained from a previous study conducted by a postgraduate student. The Sprague Dawley rats had been induced with streptozotocin and treated with different dosages of IBEE and positive control groups. There were six groups of Sprague Dawley rats, consisting of five rats in each group. Table 1 depicts the distribution of the groups.

Table 1 The groups and treatments of the Sprague Dawley rats

Group	Treatment
1	Non-diabetic rats treated with distilled water
2	Diabetic rats treated with distilled water
3	Diabetic rats treated with oral 200 mg/kg body weight of IBEE
4	Diabetic rats treated with oral 400 mg/kg body weight of IBEE
5	Diabetic rats treated with oral 30 mg/kg body weight of statin
6	Diabetic rats treated with oral 25 mg/kg body weight of gliclazide

3.2 Methods

3.2.1 Tissue Fixation

The pancreas of the rats was immersed in 10% formalin solution for 24-48 hours. Formalin solution acts as a fixative solution to preserve and maintain the cell morphology of the pancreas (Hani et al., 2015). The ideal specimen-to-formalin volume ratio should be at least 1:10 (example: 1 cm^3 of specimen per 10ml of formalin). The containers of the pancreas filled with formalin were labelled correctly to prevent misidentification.

3.2.2 Tissue Processing

After fixation, the specimens underwent a series of tissue processing to remove the water from the tissues and solidify them to facilitate the thin microscopic sections (Alturkistani et al., 2015). The specimens were transferred into tissue cassettes and subsequently the tissue cassettes were placed in the specimen baskets for processing.

Fixed tissues required extra grossing to fit correctly inside the cassette before processing them. The tissues were trimmed not to exceed 2.5 × 2.0 × 0.4 cm in size (Knoblaugh et al., 2012). Leica TP1020 automatic benchtop tissue processor was used to control the temperature and processing time of the procedure. The specimens were immersed in increasing concentrations of alcohol which were 80% of ethanol one time, 95% of ethanol two times, followed by 100% of ethanol three times. Each immersion required 1 hour to complete. This step is called dehydration which aims to remove the water and formalin from the tissues of the pancreas. After that, the specimens were immersed in xylene three times to remove the alcohol. Same as alcohol, each immersion with xylene required 1 hour to complete. Finally, the specimens were immersed in paraffin wax two times, where each immersion required 2 hours to complete.

3.2.3 Tissue Embedding

After the specimens were infiltrated with paraffin wax, the specimens were removed from the automatic tissue processing machine. Before use, solid paraffin wax was placed in a paraffin container and melted at 60 °C. The warm melted paraffin wax was filled into the tissue embedding mould according to tissue size. The specimens were carefully placed into each mould containing the melted paraffin wax. Next, a cassette was placed on top of the mould followed by additional paraffin wax and the mould was transferred to the cold plate Leica HI1130 until the paraffin wax became completely frozen. Finally, the blocks and the cassettes were removed from the mould and kept in the freezer before performing the trimming procedure.

3.2.4 Tissue Sectioning

Leica RM2255 fully automated rotary microtome was used to cut the specimens into 4 μm thickness. After that, the tissue ribbons were carefully placed in the mounting bath Leica HI1220 at 40 °C to remove the wrinkles. The tissue ribbons were allowed to float on the water's surface and were scooped up onto the slides. The slides were then dried in an oven Memmert ULM400 at 37 °C for a few hours to melt the excess paraffin wax.

3.2.5 Slide Staining

The slides were immersed in xylene two times for 5 minutes each to remove the paraffin wax. After deparaffinisation, the slides were rinsed in decreasing concentrations of alcohol starting from 100% ethanol, 95% ethanol and 80% ethanol. The slides were immersed two times for each concentration for 5 minutes each. After rehydration, the slides were washed in running tap water. Next, the slides were stained with hematoxylin for 15-20 minutes, followed by washing the slides in running tap water. The slides were then dipped in 1% acid alcohol for a few seconds to decolourise the pink colour of hematoxylin to bluish colour. The slides were washed again and subsequently stained with eosin for 5-10 minutes. The slides were washed in running tap water to remove the excess stain. After that, the slides were immersed in increasing concentrations of alcohol from 80% ethanol, 95% ethanol and 100%. The slides were immersed two times for each concentration for 2 minutes each. Following dehydration, the slides were transferred to xylene two times for 2 minutes each to remove the alcohol. The slides were then mounted with mounting media. Sigma-Aldrich DPX

mountant was applied 2-3 drops on each slide, and coverslips were placed on the slides. The slides were dried overnight at room temperature in the fume hood.

3.2.6 Microscopic Examination

The slides were examined using Dino-Eye microscope under 100X and 400X magnification to observe the morphology of pancreatic β cells in the pancreatic tissue. The diameter of the Islets of Langerhans were measured and the number of pancreatic β cells were counted.

3.2.7 Statistical Analysis

The statistical analysis was performed using IBM SPSS. The values were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to analyse the effect of IBEE in comparison with the series of dosages and corresponding control on the diameter of Islets of Langerhans and the number of pancreatic β cells in streptozotocin-induced diabetic Sprague Dawley rats. Lastly, all the data were interpreted as mean ± standard error mean (S.E.M). Values with $p < 0.05$ were considered statistically significant.

CHAPTER 4

RESULTS

4.1 Histology of Pancreas

The histologic examination of pancreas tissue samples was recorded. Figure 5 shows the morphology of the pancreas tissue samples under 400X magnification. From the picture, it can be observed that the islets of Langerhans of the non-diabetic group were nearly a round shape with a large diameter and the β cells were distributed regularly with appropriate structure. As compared to the diabetic group, the islets of Langerhans were severely atrophied and degenerated, exhibited irregular shape, loose arrangement and disordered structure. Interestingly, the regeneration of β cells shown by diabetic rats administered with 400mg/kg body weight (BW) of IBEE were comparable with groups treated with gliclazide and statin. Diabetes rats treated with 400mg/kg BW of IBEE, gliclazide and statin seemed to have a pancreas with an apparently normal structure when compared to the untreated diabetic group.

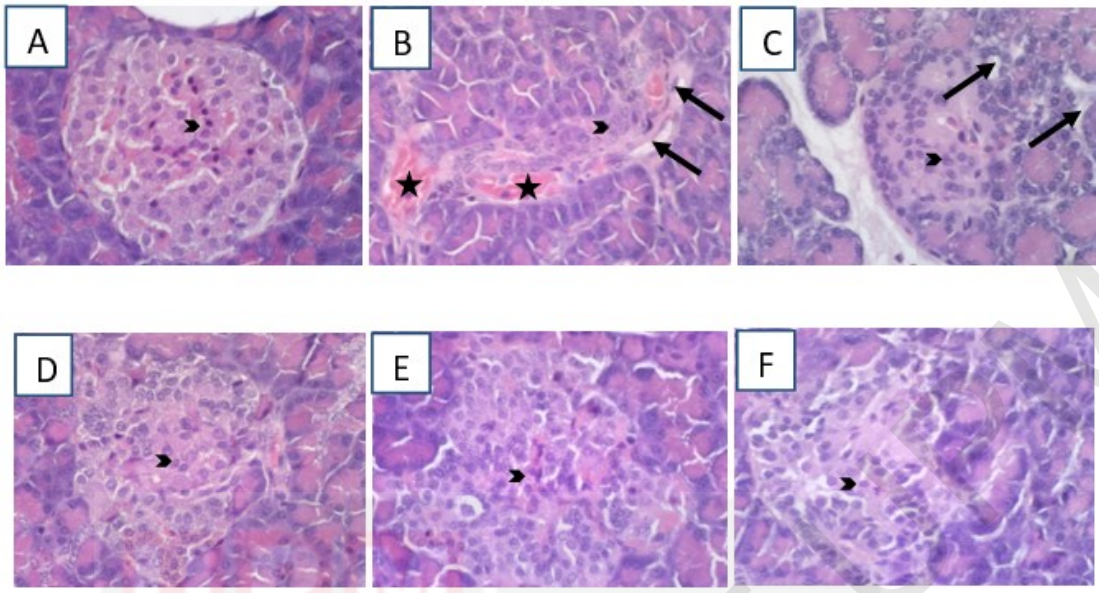

Figure 5 Histologic examination of pancreas tissue samples. Hematoxylin and eosin (H&E) staining, original magnification X 400. **A.** Non-diabetic group; **B.** Diabetic group; **C.** Diabetic treated (IBEE 200mg/kg) group; **D.** Diabetic treated (IBEE 400mg/kg) group; **E.** Diabetic treated (statin 30mg/kg) group; **F.** Diabetic treated (gliclazide 25mg/kg) group. arrowhead: β cell; arrow: vacuolation; asterisk: β cell necrosis

4.2 Effect of IBEE on the Diameter of the Islets of Langerhans and the Number of Pancreatic β cells in Streptozotocin-Induced Diabetic Sprague Dawley Rats

The results presented in Table 2 revealed the diameter of the Islets of Langerhans and the number of pancreatic β cells of the non-diabetic, diabetic and treated rats. It was recorded that the non-diabetic group exhibited the largest diameter of Islets of Langerhans compared to the other groups, while the diabetic group exhibited the smallest diameter of Islets of Langerhans. When the rats were given the treatment of 200 mg/kg BW of IBEE, the diameter was increased. The diameter continued to increase when 400mg/kg BW of IBEE were treated to the rats. Treatment with statin and gliclazide further increased the diameter of Islets of Langerhans. From the result obtained, the non-diabetic group did not differ significantly (p-value > 0.05) between the rats treated with 400 mg/kg BW of IBEE and positive control groups. Meanwhile, the non-diabetic group showed a significant difference (p-value < 0.05) between the diabetic group and the diabetic group treated with 200 mg/kg BW of IBEE.

As for the number of pancreatic β cells, the data showed a similar pattern to that of the diameter of the Islets of Langerhans. The only difference was that the diabetic group treated with 400mg/kg BW exhibited a higher number of pancreatic β cells compared to diabetic group treated with gliclazide. From the results obtained, we can also say that the non-diabetic group did not differ significantly (p-value > 0.05) between the rat treated with 400 mg/kg BW of IBEE and the positive control group. Meanwhile, the non-diabetic group showed a significant difference (p-value < 0.05) between the diabetic group and the diabetic group treated with 200 mg/kg BW of IBEE.

Table 2 The effect of IBEE on the diameter of the Islets of Langerhans and the number of pancreatic β cells in streptozotocin-induced diabetic Sprague Dawley rats.

Group	Diameter of Islets of Langerhans (mm)	Number of pancreatic β cells
Non-diabetic	0.217 ± 0.021^{a}	134.33 ± 15.02^{a}
Diabetic	0.078 ± 0.013^{b}	28.67 ± 7.88^{b}
Diabetic + IBEE (200 mg/kg)	0.130 ± 0.014^{bc}	65.67 ± 12.68^{bc}
Diabetic + IBEE (400 mg/kg)	0.152 ± 0.014^{ac}	99.67 ± 2.67^{ac}
Diabetic + statin (30 mg/kg)	0.175 ± 0.006^{ac}	110.67 ± 20.19^{ac}
Diabetic + gliclazide (25 mg/kg)	0.156 ± 0.008^{ac}	91.33 ± 11.84^{ac}

All values are expressed as mean ± standard error of mean; n = 5. Different letters indicate significant differences between groups ($P < 0.05$).

CHAPTER 5

DISCUSSION

Streptozotocin is a genotoxic methylating agent produced by Streptomyces achromogenes, is often used to cause hyperglycemia in experimental animal models by inducing pancreatic islet β cell cytotoxicity (Luo et al., 2019). Previous research has shown that streptozotocin can increase the overproduction of reactive oxygen species and cause oxidative damage, which can lead to cell death and decreased insulin biosynthesis. Streptozotocin causes deterioration of tissues such as the liver, kidneys, and pancreas (Ly et al., 2019). Oxidative stress, inflammation, and apoptosis have all been linked to pancreatic islet degeneration after administration of streptozotocin (Roh et al., 2016).

Oxidative stress has a crucial role in the development and progression of diabetes and its associated consequences. Diabetes mellitus promotes oxidative stress, which might be due to increased free radical generation or decreased antioxidant defences (Pashapoor et al., 2019). Oxidative stress arises when the production of free radicals surpasses the antioxidant capability of a cell. In the pancreas, glucose auto-oxidation and protein glycosylation may generate free radicals that harm β cells (Nna et al., 2018).

Since oxidative stress due to the production of free radicals is believed to be the primary cause of hyperglycemia, antioxidants have been reported to be useful in the treatment of diabetes. Epidemiological research and clinical trials have established an inverse correlation between the incidence of illnesses such as diabetes, cardiovascular disease, inflammation, cancer, and age-related problems when fruits and vegetables are consumed. The beneficial health benefits of phytochemicals found

in vegetables and fruits are attributable to their antioxidative characteristics (Taheri Rouhi et al., 2017)

In other words, oxidative stress may be averted by consuming antioxidants that delay and limit the oxidation of vulnerable cellular substrates. Fresh fruits and vegetables have been proposed to improve human health due to their high phytochemical content and other beneficial micronutrients. Additionally, a number of *in vitro* studies have shown that polyphenols may enhance the ability of peripheral tissues to absorb glucose, which would lower blood sugar levels (Taheri Rouhi et al., 2017)

Nowadays, the scientific community is paying close attention to the medicinal properties of functional foods for the treatment of T2DM. PSPL is used in traditional medicine to treat a variety of illnesses due to its bioactive components (Lee at al., 2015). According to Nguyen et al. (2021), sweet potato leaf was reported to possess anticancer activity, anti-inflammatory activity, hepatoprotection, antimicrobial activity and antidiabetic activity. The biological effects of sweet potato leaf contribute to its classification as a healthy fruit due to its high concentration of phytochemicals that scavenge a broad range of free radicals.

Sweet potato leaves contain more polyphenols than other regularly eaten vegetables such as spinach, kale, amaranth, eggplant, cabbage, cauliflower, green peas and lettuce (Kurata et al., 2019; Makori et al., 2020). Wang et al. (2016) stated that sweet potato leaves have polyphenol concentrations that were 7-9 times higher than grape seeds. In this study, we aim to investigate the effect of IBEE on the pancreas of streptozotocin-induced diabetes in Sprague Dawley rats by histopathological analysis. Plant ethanolic extracts have been suggested to contain an abundance of polyphenolic

compounds which pharmacological effects include anti-oxidation and anti-inflammation

Extraction is the first step in separating and purifying bioactive chemicals from biomass sources for subsequent use. The best solvent for extraction should be chosen to optimise the amount of a target component and biological activity (Nguyen et al., 2021). Ethanol has been employed as a solvent for extraction in this study since ethanol has a reputation as a reliable solvent for the extraction of polyphenols and is safe for human consumption (Do et al., 2014). Besides that, the ethanol solvent also served as a self-preservative, reducing the likelihood of bacterial and mould development inside the plant extract (Abubakar & Haque, 2020).

The histological findings of *I. batatas* leaf ethanolic extract (IBEE)-treated pancreas in streptozotocin-induced diabetic rats demonstrated that IBEE therapy can restore pancreatic function by increasing pancreatic β cells diameter and number.

Based on these findings, we established that the hypoglycemic effects of IBEE may be attributable to their ability to mitigate oxidative stress. The results were consistent with the findings of Ly et al. (2019), who discovered that plant leaf polyphenols could enhance pancreatic β cell regeneration by increasing the area of the Islet of Langerhans and the number of β cells. This suggests that polyphenols have a preventive and recuperative impact on pancreatic islets.

Contrarily, reduced β cell function under oxidative stress and impaired peripheral tissue responsiveness to insulin result in hyperglycemia and hyperinsulinemia in conjunction with persistent low-grade inflammation (Manawi et al., 2021). B cell dysfunction develops when β-cells become depleted as a result of their efforts to compensate for an increased demand for insulin (Wickramasinghe et al,

2021). It was observed from the experiment that diabetic rats treated with distilled water exhibited vacuolation in the pancreas, β cell necrosis, reduced diameter in the islet of Langerhans and a lower number of pancreatic β cells. These results were in agreement with prior research, which found a considerable decrease in the islet diameter, area, number, and volume in streptozotocin-induced rats (Adeyemi et al, 2010: Ramadan et al, 2017)

Sweet potato leaves contain various antioxidants that help the body defend itself against oxidative and free radical reactions, resulting in a decrease in low-density lipoprotein oxidation and DNA damage in human lymphocytes (Nguyen et al., 2021). PSPL diets had greater cytotoxic natural killer cell activity, indicating that dietary polyphenols may deactivate free radicals and promote improved immunity (Johnson & Pace, 2010). Due to the high polyphenol content of the leaves, PSPL may influence several immunological processes, including the release of cytokines and natural killer cells, which can boost the proliferative responsiveness of peripheral blood mononuclear cells (Nguyen et al., 2021).

On the other hand, *Ipomoea batatas* extracts had hypoglycemic characteristics that helped to restore the hyperglycaemia caused by streptozotocin (Rafiu & Luka, 2018). In particular, polyphenols like flavonoids and tannins strongly suppress α-amylase and α-glucosidase, two glycosidase enzymes that are essential for carbohydrate digestion and regulate postprandial glycemic levels, without side effects such as diarrhoea and other intestinal problems caused by current medications (Zeng et al., 2019).

Several polyphenols such as epigallocatechin-3-gallate, resveratrol, and quercetin increased glucose uptake in muscles and adipocytes by translocating glucose

transporter type 4 (GLUT4) to the plasma membrane through the Adenosine Monophosphate-Activated Protein Kinase (AMPK) pathway (Shahwan et al., 2022). Quercetin is also recognised to have antidiabetic potential through pancreatic cell proliferation, which is believed to suppress apoptosis in β cells during the progression of diabetes. Meanwhile, phenolic compounds help to delay the deterioration of pancreatic islets and the onset of T2DM by lessening the load of persistent stimulation of β cells and strain on β cells (Anjani et al., 2018)

According to Ji et al. (2015), PSPL had a much greater anthocyanin concentration than yellow, red and green sweet potato leaf. Anthocyanins have anti-inflammatory effects by promoting cell membrane strengthening and reducing capillary permeability and fragility (Grebla-Al-Zaben et al, 2021). In addition, the PSPL extracts suppress neuroinflammatory reactions by preventing the synthesis of pro-inflammatory mediators such as TNF-α, COX-2, nitric oxide and iNOS. The significant antioxidant effects of PSPL extract were thought to be connected to its anti-neuroinflammatory properties (Kang et al., 2014)

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The diameter of the islets of Langerhans and the number of pancreatic cells in rats were measured in different concentrations of IBEE and compared to the corresponding controls, gliclazide and statin. Ethanolic extract of *Ipomoea batatas* leaf at 400mg/kg BW exhibited significant improvements in the diameter of the Islet of Langerhans and regeneration of β cells of the pancreas in streptozotocin-induced diabetic rats. Findings from the study showed that PSPL has the potential to be developed as an alternative therapeutic agent to treat T2DM.

6.2 Recommendation

In this study, two dosages of IBEE were given to investigate the effect of IBEE on diabetic rats, which are 200mg/kg BW and 400mg/kg BW. From the results obtained, it is highly recommended that a dosage of more than 400mg/kg BW of IBEE be given to understand how it will affect the histopathology analysis of the pancreas, either resulting in further regeneration of the pancreas or the degradation of the pancreas. It is also suggested that a future study be conducted using the same procedure but with a bigger sample size (n = 5) than this study in order to increase the significance and confidence if any changes in parameters occur. Last but not least, extensive toxicological studies should be carried out to determine the safety of PSPL's bioactive ingredients. This will increase scientific interest in exploring the medicinal potential of PSPL beyond its significance as a food source.

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