ANTI-DIABETIC ACTIVITY OF *PSYCHOTRIA MALAYANA* JACK LEAF AQUEOUS EXTRACT IN INDUCED TYPE 1 DIABETIC ADULT ZEBRAFISH

BY

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ABSTRACT

Type 1 diabetes is a perpetual and profound disease impacting people at all ages. It is diagnosed with the loss of insulin production because of dynamic demolition of the β-cells in the pancreas. One bottleneck of the drug discovery on type 1 diabetes is the expensive and long testing period of the established *in vivo* assays. *Psychotria malayana* has been reported traditionally to treat diabetes. Thus, the aim of this study were to develop a type 1 diabetic adult zebrafish model through chemical induction, and to evaluate the anti-diabetic activity of *Psychotria malayana* Jack leaf extract on the developed type 1 diabetic adult zebrafish. Different chemical inducers, streptozotocin and alloxan, with different doses were evaluated in elevating the blood glucose level of zebrafish. Two positive controls (glibenclamide and insulin) were tested for the confirmation of the developed model. Finally, the anti-diabetic activity of a traditional medicinal plant, *P. malayana* aqueous extract was evaluated using the developed model. LC-MS based fingerprinting of the zebrafish and the histological examinations were applied to confirm the ability of this plant to treat type 1 diabetes. The LC-MS data set was pre-processed and then statistically calculated using a multivariate data analysis, partial least square-discriminant analysis (PLS-DA). The result of this study indicated that a single intraperitoneal injection of alloxan with the dose of 300 mg/kg was the optimal dose to elevate the fasting blood glucose level of the zebrafish. The blood glucose level (>150 mg/dL) was significantly higher ($P<0.05$) than that of the healthy zebrafish (70-80 mg/dL), and can be maintained for 7 days. This model was able to test the anti-diabetic activity of *P. malayana* leaf extract. The plant extract with the dose of 1, 2, and 3 g/kg significantly reduced the blood glucose level of the diabetic zebrafish from (123.50±10.89) to (75.67±17.82, 90.83±7.86, and 73.50±7.66, respectively) ($P< 0.05$). Furthermore, LC-MS based fingerprinting exhibited that all doses of the plant extract were able to shift the serum metabolite profile of the treated zebrafish toward the healthy group alongside PLS-DA component 2. While PLS-DA component 1 indicated that the plant extract with the dose of 3 g/kg was superior compared to other doses in shifting the metabolite profile toward the healthy zebrafish. Finally, the histopathological examination showed that this plant extract could not repair the enormous reduction of the Langerhans cells in the pancreas of the diabetic zebrafish. In addition, this extract did not affect the liver structure of the healthy zebrafish. Hence, the mode of action of this plant extract in lowering the blood glucose level should not be through the recovery of the pancreas, but through other mechanisms which is recommended to be examined in future research.

خلاصة البحث

يعتبر مرض السكري من الأمراض الشائعة التي تصيب كبار وصغار السن. يشخص مرض السكري بارتفاع سكر الدم نتيجة لفقدان الخلايا البائية في البنكرياس وظيفتها في إنتاج الانسولين. تعتبر حيوانات التجارب المخبرية من أحسن الطرق لدراسة الخصائص المرضية لكل الأمراض التي تصيب الانسان. بسبب الفوائد التي تقدمها zebrafish تم اختيارها لتكون نموذج مخبري لدراسة آثار الأدوية. توجد حتى الآن طريقتين فقط لجعل zebrafish تصاب بمرض السكري، إما بوضعها في محلول يحتوي على الغلوكوز أو alloxan أو بحقنها بـSTZ . بالنسبة للطريقة الأولى تبقي كمية مسبب مرض السكري مجهولة لأنه لكل سمكة وزن معين وبالتالي يجب أن تكون كمية مسبب المرض واحدة ومدروسة جيدا. أما بالنسبة لـ STZ فهو يعتبر حساس للضوء وتعتبر نصف حياته قصيرة جدا (حوالي 20 دقيقة فقط) بالإضافة إلى كلفته المادية. وبالتالي فإن محلول STZ يحتاج الكثير من الجهد لحقنه للسمك خصوصا أنه يتم حقنه في ظروف معتمة. في هذه الدراسة تم اكتشاف أن الجرعة 300 ملغ لكل كيلوغرام من وزن السمكة من alloxan هي الجرعة المناسبة لتحدث مرض السكري في السمك، تم الأخذ بعين الاعتبار نسبة السكر في دم السمك الصائم ودرجة الضرر المحدث في الخلايا البائية في البنكرياس كمؤشرات على مرض السكري نوع 1. كما تم التوصل إلى أن حقنة واحدة من alloxan بالجرعة المذكورة سابقا تحدث مرض السكري لمدة أطول من جرعة STZ، بالإضافة إلى أنه تم اكتشاف أن نبات P. malayana له قدرة علاجية ضد مرض السكري في كل الجرعات المعطاة للسمك. غير أن تحاليل LC-MS بينت أن التوصيف الأيضي لدم السمك المعطي الجرعة 3 غ لكل كيلوغرام كان أفضل من الجرعتين السابقتين 1 غ لكل كيلوغرام من وزن السمكة و2 غ لكل كيلوغرام من وزن السمكة حيث أرجع التوصيف الأيضي أقرب إلى السمك السليم.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master in Pharmaceutical Science (Pharmacology)

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LIST OF ABBREVIATIONS

ADP	Adenosine diphosphate
AGES	Advanced Glycation End Products
AKT	Protein kinase B
APCI	Atmospheric pressure chemical ionization
APPI	Atmospheric pressure photoionization
ATP	Adenosine triphosphate
CNS	Central nervous system
DME	Diabetic macular edema
ESI	Electrospray ionization
FAB	Fast atom bombardment
GC-MS	Gaze chromatography- mass spectroscopy
GLP-1	Glucose transporter 1
HLA	Human leukocyte antigen
IFN-Ɣ	Interferon gamma
IL-1β	Interleukin 1 beta
IRS	Insulin receptor substrate
K_{ATP} channel	ATP-dependent potassium channel
LC-MS	Liquid chromatography–mass spectrometry
LC-QTOF MS	Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometer
MHC	Major histocompatibility complex
NAD+	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NF-ƘB	Transcription factor nuclear factor

NMR	Nuclear Magnetic Resonance
NO	Nitric oxide
NPDR	Non-proliferative diabetic retinopathy
PCA	Principal component analysis
PDK-1	PIP3-dependent protein kinase
PDR	Proliferated diabetic retinopathy
PIP2	phosphatidylinositol-4,5-Kinase
PNS	Peripheral nervous system
STZ	Streptozotocin
TCA	tricarboxylic acid cycle
TGF-α	Transforming growth factor
TNF-α	Tumour necrosis factor alpha
TOF	Time of fly detector
UPLC-MS	Ultra-performance liquid chromatography
VEGF	Vascular endothelia growth factor
IL-6	Interleukin 6
IL-1β	Interleukin 1β
APOH	Apolipoprotein H
SREBF1	Sterol regulatory element-binding protein 1
PPARα/γ	Peroxisome proliferator-activated receptor gamma
NR1H3	Nuclear receptor 1H3
LEP	Leptin

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Diabetes is a metabolic disease diagnosed by high blood glucose levels. The high blood glucose level causes numerous serious complications such as cardiovascular disease and retinopathy. Based on the Malaysian National Health Morbidity Survey (Letchuman et al., 2010), around 24,000 individuals diagnosed with type 1 diabetes. In addition, Diabetes Atlas estimates that this number will increase to 145 million individuals by 2025 (Jan Mohamed et al., 2015).

In order to understand the system biology and the underlying complex disorder as well as discovering novel drugs, a valid disease animal model is required. The analogy of the physiological mechanism and the heredity between certain animal and human is a brilliant idea through the use of animal model in understanding the concept of human diseases (Hau, 2008). The human body is a complex system containing different organs working in different functions. The interaction between the tested drugs and the organisms could not simply be explained through *in vitro* studies. Consequently, the use of animal model is important to understand drug interaction within different organs in the body system. The animal models allow studying the mechanism of absorption, distribution, metabolism, excretion, and toxicity (Swerdlow, Braff, & Geyer, 2000).

Zebrafish has been used effectively on drugs screening (Hill, Teraoka, Heideman, & Peterson, 2005). The advantages of utilizing zebrafish as an animal model are: lessen the maintenance cost, shorten period of testing, easy to control the

experimental conditions, and the most imperative preferred standpoint is the similar hereditary of zebrafish to human (around 70%) (Howe et al., 2013). The small size of zebrafish (3-5 cm) allowed them to be kept in small tank (Lieschke & Currie, 2007). Zebrafish as a vertebrate model shows a significant similarity to the human physiology system. The same carbohydrates regulated genes of mammalian have been detected in zebrafish. Moreover, the pancreas of zebrafish has the same functions as the mammalian counterpart for glucose homeostasis. It is producing and secreting insulin, glucagon, somatostatin and digestive enzymes such as amylase. Despites its advantages, the work with zebrafish requires handling skills in extracting the organs for histology purpose (Biemar et al., 2001).

There are certain products have shown promising results as hyperglycemia drugs through a screening using *in vivo* tests. However, some of these potential drugs lost the anti-diabetic activity when testing through the mammalian animal models due to the pharmacokinetics issue and/or toxicity. In this stage, the zebrafish model is worth to be developed as an alternative screening tool prior to testing on mammalian model (Tabassum, Tai, Jung, & Williams, 2015).

Psychotria malayana Jack is widespread plants in tropical and subtropical countries. It is known locally by different names such as ‘altoko’, ’dumamai’, and ’kadpaayan’. Due to its curative activities, the species of Psychotria herb is used for treating different medical issues such as diabetes. Chemical studies of Psychotria species showed the presence of enormous alkaloid compounds in the aerial parts of the plant (Carvalho Junior et al., 2017). It has been traditionally used to treat gastrointestinal disease, stomachache and infections of the female reproductive system in certain countries such as India, Indonesia, and Brazil. Several studies on antioxidant, antinflammatory and antimicrobial properties of this plant have been

reported (Currais et al., 2014; Formagio et al., 2014; Tran et al., 2017). However, the scientific proof of this plant on diabetes treatment is still lacking.

Several groups of phytoconstituents have been identified in this plant. In general, all *Psychotria sp* species tend to have alkaloid together with other groups of compounds. It was reported containing different type of compounds such as: pentacyclic triterpenes (ursolic and oleanolic acid); steroids (24-methylene-cycloartanol, stigmasterol and β-sitosterol); glycosylated steroids (3-O-β-D-glucosyl-β-sitosterol and 3-O-β-D-glucosyl-stigmasterol); polyunsaturated triterpene (squalene); the esters of glycerol (1-palmitoylglycerol and triacylglycerol); and indole alkaloids (N,N-dimethyltryptamine and N-methyltryptamine) (Soares et al., 2017). While an alkaloid hodgkinsine was reported to be present in *P. malayana* (Hadi, Rahmawati, Asnawati, Ersalena, & Azwari, 2014).

In this study, the antidiabetic activity of *P. malayana* leaf was evaluated on the induced type 1 diabetes zabrafish followed by the analysis of the serum utilizing LC-MS based fingerprinting. Metabolomics approach was applied in the fingerprinting by calculating the LC-MS dataset through multivariate data analysis. Metabolomics is an holistic approach emphasizing the analysis of all metabolites in a sample (Guasch-Ferré et al., 2016). Metabolites are small molecules (less than 1 kDa), defined as an intermediate metabolism product. The metabolites can be detected in the blood, serum and urine. Metabolomics have been used in an extensive variety of research fields, e.g. toxicology, pharmacology, microbial biotechnology, and plant biotechnology (C˘ uperlovic´-Culf, Barnett, Culf, & Chute, 2010).

1.2 PROBLEM STATEMENT

Taking its advantages into consideration, zebrafish has been chosen to be an ideal animal model for different diseases including diabetes. Nevertheless, the condition to induce adult zebrafish to become type 1 diabetes was varied depend on unpredictable factors. Thus, the optimized condition to induce type 1 diabetes on zebrafish should be developed prior to testing the anti-diabetic activity of the potential samples.

The available approaches to treat diabetes disease include diet control, insulin injection, and intake of oral diabetic medications. However, the oral medications have been confirmed to be inadequate and cause several side effects, whilst insulin injection only solves the problem temporarily (Snyder & Berns, 2004). Moreover, hypertrophy as a result of many insulin injections on the body area is another complication of insulin therapy (Gkaliagkousi, Shah, & Ferro, 2007). This condition leads to the necessity of finding new drugs or treatment with minimum side effects.

Natural products are the most suitable sources due to less side effects compared to synthetic drugs and are considered as a potential database in drug discovery (Karimi, Majlesi, & Rafieian-Kopaei, 2015). One of the prospective medicinal plants is *P. malayana* which is used traditionally in the treatment of diabetes. However, the scientific proof of this plant for diabetes treatment is still lacking. Hence, a proper scientific experiment was designed in this study to proof its anti-diabetic activity.

1.3 RESEARCH OBJECTIVES

The objectives of this study are per the following:

1- Development of the type 1 diabetic model using adult zebrafish model by:

a) Determination of the optimum dose to increase the fasting blood glucose level using alloxan and streptozotocin.

b) Determination of glucose lowering activity using positive controls glibenclamide and insulin.

2- Evaluation of the anti-diabetic activity of *Psychotria malayana* leaf aqueous extract on the developed type 1 diabetic adult zebrafish by:

a) Determination of serum metabolites profile using LC-MS fingerprinting.

b) Determination of its effects on the liver and pancreas using histology examination.

1.4 RESEARCH HYPOTHESIS

1- Zebrafish can be used as an alternative model for type 1 diabetes using chemical inducers.

2- *Psychotria malayana* leaf is effective in treating the induced type 1 diabetes zebrafish.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

Diabetes mellitus is a group of diseases characterized by high blood glucose levels as a result of low insulin production, or a defect in insulin metabolism (American Diabetes Association, 2010). High levels of blood glucose can induce micro vascular damage and cause ischemic heart disease, stroke, and peripheral vascular disease, as well as other serious complications such as retinopathy, nephropathy, and neuropathy (Lazzarini, Gurr, Rogers, Schox, & Bergin, 2012). Type 1 diabetes is diagnosed with high fasting blood glucose or random blood sugar test and glycated hemoglobin (HbA1c) test (Atkinson, Eisenbarth, & Michels, 2014). Several pathogeneses are involved in the development of type 1 diabetes. Among them is the destruction of β-cells in the islets of Langerhans, which are responsible for insulin production. Due to the insulin reduction, the metabolic abnormalities of carbohydrates, fats, and proteins occur in the cells pathways (Atkinson et al., 2014).

2.2 INSULIN PRODUCTION

Insulin is a peptide hormone that is synthesised and secreted from β-cells in the pancreas (Sonksen & Sonksen, 2000). The regulation of insulin gene expression is influenced by blood glucose levels; however, it can be activated by other hormones, such as the Glucagon-Like Peptide-1 (GLP-1) (Kjems, Holst, Vølund, & Madsbad, 2003). The primary form of insulin is known as preproinsulin, a 98 amino acid long peptide, which is cleaved in the rough endoplasmic reticulum to become proinsulin by