ANTI-DIABETIC EFFECT OF AGARWOOD LEAF EXTRACTS ON STREPTOZOTOCIN–INDUCED TYPE II DIABETIC ICR MICE

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ABSTRACT

Alternative treatments of insulin-independent diabetes mellitus have attracted many researchers over the years. The treatment should have a clearer mechanism of action and easier to administer. Asian traditional medicine contains a number of herbal extracts believed to combat the symptoms of diabetes mellitus. Thus, the purpose of this study was to investigate the potential of agarwood leaf extracts against type II diabetic ICR mice. A group of ICR mice of both sexes, aged 8 weeks were induced by a single intraperitoneal injection of 100 mg/kg body weight of streptozotocin (STZ). Mice (fasting) with blood glucose over 200 mg/dl were used in this experiment. Diabetic mice were divided into three groups and were forced fed with 50, 100 and 150 mg plant extract /kg body weight for 14 consecutive days respectivly. Two different types of solvents used in this study were distilled water and 95% methanol. Aqueous and methanolic extracts of *Aquilaria malaccensis* leaf exhibited a significant reduction of hyperglycemia in fasting diabetic mice at the lowest concentration (50 mg/kg b.w). Both extracts with different solvents reduced hyperglycemia within three days of administration and these were observed for all doses. For methanolic extract, low (50 mg/kg body weight) and moderate (100 mg/kg body weight) doses, reduced hyperglycemia in fasting diabetic mice compered with the highest dose (150 mg/kg body weight) after two hours of forced fed. In conclusion, these data suggest that both extracts of agarwood leaf possess potential anti-diabetic activity in both diabetic male and female mice. Hence, active constituents of agarwood leaf extracts which responsible for anti-diabetic activity requires further to be investigated and these findings contribute to potential diabetic treatment.

KESAN ANTI- DIBETIK EKSTRAK DAUN KARAS KE ATAS MENCIT ICR DIABETIK JENIS II ARUHAN- STREPTOZOTOCIN

ABSTRAK

Rawatan alternatif terhadap penyakit diabetes mellitus bebas insulin telah menarik perhatian ramai penyelidik sejak beberapa tahun dahulu. Mekanisma tindakbalas bagi rawatan ini perlulah jelas dan mudah untuk ditadbirkan. Perubatan traditional kalangan masyarakat Asia mengandungi beberapa ekstrak herba yang dipercayai dapat mengatasi gejala penyakit diabetic mellitus. Oleh itu tujuan kajian ini adalah untuk menyiasat potensi ekstrak daun pokok karas terhadap mencit ICR diabetic jenis II. Sekumpulan mencit ICR daripada kedua-dua jantina dan berumur lapan minggu telah disuntik dengan streptozotocin (STZ) secara intraperitoneal pada dos 100 mg/berat tubuh. Mencit (berpuasa) yang mempunyai aras glukos darah melebihi 200 mg/dl digunakan dalam eksperimen ini. Mencit-mencit diabetik ini dibahagikan kepada tiga kumpulan dan diberi makan secara paksa dos ekstrak tumbuhan 50, 100 dan 150 mg ekstrak tumbuhan / kg berat tubuh selama 14 hari berturut turut setiap satunya. Dua jenis pelarut yang berbeza digunakan dalam kajian ini iaitu air suling dan 95% metanol. Ekstrak larutan akues dan methanol daripada daun *Aquilaria malaccensis* menunjukkan penurunan yang signifikan terhadap hiperglisemia pada mencit diabetik yang berpuasa bagi kepekatan yang paling rendah (50 mg/kg berat tubuh). Kedua-dua ekstrak yang menggunakan pelarut yang berbeza menunjukkan pengurangan hiperglisemia dalam tiga hari selepas suntikan bagi kesemua dos. Untuk ekstrak metanol, dos yang rendah (50 mg/kg berat tubuh) dan sederhana (100 mg/kg berat tubuh) telah menurunkan paras hiperglisemia bagi mencit diabetik yang berpuasa berbanding dos yang tinggi (150 mg/kg berat tubuh) selepas dua jam diberimakan secara paksa. Kesimpulannya, hasil data kami mencadangkan kedua-dua ekstrak daun karas mempunyai potensi aktiviti anti-diabetik ke atas mencit jantan dan betina yang diabetik. Oleh itu, bahan-bahan aktif ekstrak daun karas yang bertanggungjawab untuk melawan diabetik ini memerlukan siasatan yang lebih lanjut dan penemuan ini dilihat mampu menyumbang kepada kaedah alternatif bagi rawatan penyakit diabetik.

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LIST OF ABBREVIATION

AQ	Aqueous Treatment
AChE	Acetylcholinesterase
BGL	Blood Glucose Level
BMI	Body Mass Index
DM	Diabetes Mellitus
DPN	Diabetic Peripheral Neuropathy
FFA	Free Fatty Acid
GLUT4	Glucose Transporter Type 4
HbA1c	Glycated Hemoglobin
I.p	Intraperitoneal
I.v	Intravenous
METH	Methanol Treatment
NHMS	National Health Morbidity Survey
OLETF	Otsuka Long-Evans Tokushima Fatty
ROS	Reactive Oxygen Species
STZ	Streptozotocin
TIIDM	Type II Diabetes Mellitus
TIDM	Type I Diabetes Mellitus
TZD	Thiazolidinedions
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Introduction

Diabetic Mellitus (DM) is a heterogeneous disease defined by high blood glucose levels and dyslipidemia. Diabetes is usually accompanied by a major metabolic deficiency which is the failure of peripheral tissues in the body to properly utilize glucose, thereby leading to chronic hyperglycemia (Rother, 2007). The percentage of diagnosis of this disease was recorded to be 2.22 per 1,000 of those < 20 years of age (Pettitt et al., 2014).

Extensively, the focus has been increased on diabetes and it is globally considered as a crucial public health concern due to its incidence and uncontrolled propagation rate since it is affecting more than 415 million adults worldwide (IDF, 2015). It is evident that people with diabetes disease are more vulnerable to disability than other healthy people without any history of diabetes disease (AIHW, 2013; Wong et al., 2014; Wong et al., 2016), as well as the limitation of core activity is more likely to occurs in diabetic people (AIHW, 2013). Thus, to the prevalence of diabetes, it is urgent to increase the awareness about, otherwise, more complications will probably result including disabilities and motility cases, Additionally, the medical costs will increase and more working days will be lost (Association, 2013). Type II diabetes mellitus (TIIDM) is known to reduce the life expectancy for those who are diagnosed of it, especially at a young age, which usually leads to early plunge and resulting in utmost costs due to the loss of wages and productivity (Pettitt et al., 2014). Therefore, type II diabetes disease is, in fact, one of the most serious public health epidemics that is causing an obvious financial burdensome. According to Association (2013), it was found that nearly US$245 billion in one way or another is ascribed to this chronic disease. Type II diabetes highly, include ethnic and conventional, basics; the incidence of TIIDM is evaluated to be 0.17 per 1000 for the Caucasian young generation, 1.06 per 1000 for African American young generation, 0.79 per 1000 for Hispanic young generation and 1.20 per 1000 for native American younger generation (Dabelea et al., 2014). The highest recurrent incidence of TIIDM can be observed at 22.3 per 1000 for the Pima Indians which represents 2.23% of those who were diagnosed with TIIDM at the age range between 10 and 14 years old (Reinehr, 2013). At present, TIIDM is reported to be the most common diagnosis between 20% and 50% of new diabetic

patients. The occurrence of the disease has apparently increased by 35 % among the age range of 10-19 years of the young population. (Dabelea et al., 2014). The chronic micro- and macro-vascular complications are promptly progressing in related to the incubation term of diabetes. Hence, the early diabetic onset in life is highly raising the risks of developing various types of complications (Pettitt et al., 2014).

It is worth mentioning, that the fragrant wood has been involved in many cultures worldwide, for example, the Japanese, Chinese and Arabian cultures. In addition to that fragrant wood is to some extent connected to sacred ceremonies, rituals, and history in different religions such as Islam, Christianity, Buddhism and Hinduism (Barden et al., 2000). Agarwood is the gummy heartwood of the Aquilaria tree, a genus belonging taxonomically to the Thymelaeaceae. Nineteen species have been reported up to date growing from India through Burma, Laos, Vietnam, and Cambodia to Malaysia, Sumatra, Borneo, the Philippines, and Papua–New Guinea, all of them producing agarwood. *Aquilaria malaccensis* is the main producer of agarwood in Malaysia. The species can be found abundantly in the Peninsular but not in the eastern part of Malaysia. Agarwood is usually produced from cultivated or wild *Aquilaria* trees by extrinsic factors such as microbial infection, physical injury and insect gnawing which induce wounding in the trees resulting in agarwood formation. It is well known that sundry years are wanted to form agarwood in the wounds of Aquilaria trees. Agarwood hold sedative, anti-emetic as well as carminative wares which make it an important trace in the traditional Chinese medication. Moreover, the essential oil of agarwood has an exceptional fragrance which makes it the most vital constituents in high-end perfumes, and it is usually used as incense

for religious ceremonies. Therefore, this chapter will discuss how this research will go to discuss type II DM by putting up this topic for discussion through the background of the study, problem statement, experimental design, objectives, research questions, hypothesis, limitation of the study, and significance of the study.

1.2 Background of the Study

Diabetes mellitus is the name that appears a wide range of defect groups which are well known by elevated plasma glucose. According to pediatric practice, type I diabetes is often diagnosed in about 96% of all diabetic children and it is identified by a certain insulin insufficiency which is caused by the autoimmune destruction of beta cells in the pancreas that are responsible for insulin production. Establishment of insulin treatment is of great concern and the failure to do so may lead to fatal cases in diabetic children. On the other hand, most adults with diabetes are usually diagnosed with type II diabetes which can be featured by a relative insulin secretory deficiency, and by targeting tissue opposition to the effect of insulin. Since about 1950s, a climactic increment in the propagation of type II diabetes in adult people has been recorded, and this tragic rise can be attributed to the increase spreads of obesity cases. Obesity is considered one of the most threatening issues in the current time and it is likely one of the main reasons that cause type II diabetes in children nowadays (Barrett, 2013).

The pathophysiology of diabetes mellitus is caused by a complex interaction between environmental parameters and genetic parameters. TIIDM is normally accompanied by a noteworthy deterioration in a pancreatic β-cell activity that occur in the setting of insulin resistance and hyperglycemia. At the time of adolescence, an increase in the augmentation in insulin resistance is usually detected.

An obvious increase in insulin resistance is commonly recognized during adulthood, and this is likely attributed to the aftereffects of increased secretion of sex steroids and growth hormone, which usually climax in a mid-adulthood and decline after adulthood (George & Copeland, 2013; Reinehr, 2013). Definite ethnic communities are more genetically vulnerable to insulin resistance than other groups such as Asian or Pacific Islanders, Native Americans, African Americans, and Latinos. For instance, it was found that African Americans between the age of 7 and 11 years old have higher levels of insulin in comparison to that of Caucasians of the same age range (Reinehr, 2013).

At present, metformin and insulin are the only permitted medicines for the remedy of TIIDM in the pediatric population (Copeland et al., 2013). For the first week, the primary dosage of metformin should not exceed 500 mg per day, thereafter the dosage can be increased by 500 mg per week or every other week in order to ease the associated gastrointestinal side effects, including abdominal discomfort and bloating loose stool (Copeland et al., 2013). It is imperative to introduce insulin in youths with HbA1c > 9%, in those who have a random plasma glucose level of 250 mg/dL, in those who are ketotic,

or in those for which their diagnosis of TIDM versus TIIDM is inconclusive (American Diabetes Association, 2016).

Traditional medicine has remained the most affordable and easily accessible source of treatment in the primary health care system of poor communities (Hosseinzadeh et al., 2015). In many developing countries; a large proportion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet health care needs. Although modern medicine may exist side-by-side with such traditional practice, herbal medicines have often maintained their popularity for historical and cultural reasons. Such products have become more widely available commercially, especially in developed countries (Humans et al., 2002). Herbal medicine is still the mainstay of about 75-80% of the whole population, mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and fewer side effects. However, the last few years have seen a major increase in their use in the developed world (Parekh et al., 2006).

Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Parekh et al., 2006). All plants containing active compounds are important. The secondary metabolites products existing in the plant are commonly the responsible elements of the advantageous medicinal effectiveness in the plant materials. These secondary compounds in plants are mainly secondary metabolites as for instance alkaloids, steroids, tannins, and phenol

compounds, which are produced and accumulated in precise parts or in all parts of the plant. In particular taxa such as family, genus, and species the secondary compounds of the plant are known to be more complex and distinct, while in wild species the secondary compounds are well recognized for their heterogeneity (Balandrin et al., 1985). The curative performance of plants is exceptional to a certain plant species or group which is compatible with the perception that the combination of secondary compounds in a certain plant is taxonomically distinguished (Wink, 1999). The secondary products of the plants may apply their action by resembling endogenous metabolites, ligands, hormones, signal transduction molecules of neurotransmitters and consequently have valuable therapeutic effects on humans because of the similarities in their potential target locations. Therefore, random screening of plants for active chemicals is as important as the screening of ethno botanically targeted species (Wagnor et al., 1989).

Agarwood (Known also as aloeswood or eaglewood) is typically recognized as condensed, heavy and scented gummy wood which is formed *Aquilaria Gonystylus* and *Gyrinops* trees (Hashim et al., 2016) (Figure 1.1). As reported by Chua (2008), the name ‘agarwood’ refers to resin-impregnated pieces of wood which at least have partially been scrapped from the non-impregnated woods.

Figure 1.1. Agarwood Trees (Liu et al., 2013)

For centuries, agarwood is defined as the finest natural incense and numerous communities have been using it to perform various religious rituals as well as cultural and medicinal purposes. Different names have been typically used to call agarwood, for instance in Malaysia and Indonesia it is called “gaharu”, and in Korea it is called “chim-hyuang”, while it is known as “chenxiang” or “chen hsiang” in China, in Thailand and Vietnam, agarwood is called “kritsana noi” and “bols d′agle” respectively, whereas in France and middle east it is recognized as “ca- lambour” and “oud” accordoongly (Burkill, 1935; Ng, 1997; Sidiyasa et al., 1986).

Moreover, agarwood, highly valuable resinous and fragrant heartwood, is used as incense for religious ceremonies, perfumes in the Arab world, ornamental materials, and medicinal components in oriental medicine (Kakino, Izuta, et al., 2010; Okudera & Ito, 2009). As stated by Lardos et al. (2011), agarwood is known to have many pharmacological

functions that include analgesic, anti-microbial, immunomodulatory, and wound healing properties. Agarwood is also used as a digestive in traditional medicine, and its medico-pharmacological analysis using model mice indicated the laxative effect (Kakino, Izuta, et al., 2010; Kakino, Tazawa, et al., 2010). Furthermore, the essential oil obtained from agarwood is also known to have anti-microbial properties (H. Chen et al., 2011).

Agarwood leaves are characterized by alternate, elliptic or lanceolate, 3-3.5 cm wide and 6-8 cm long with 12-16 pairs of veins(Sitepu, Santoso, & Turjaman, 2011). Many previous studies have shown the antihyperglycemic activity of agarwood leaf extracts including *Aquilaria Agallocha, Aquilaria SPP*. using different solvents and have indicated satisfactory results (Alam, Mujahid, Rahman, et al., 2015; Khalil et al., 2013; Pranakhon et al., 2011; Rajagopal et al., 2016; Shaari, 2013) (Figure 1.2).

Figure 1.2. Agarwood Leaf (S. Lee et al., 2013)

1.3 Problem Statement

Diabetes mellitus is a worldwide health issue due to the reported number of diabetic patients that is increasing on a daily basis. It is expected that the South-East Asia Region will have the highest diabetic patients in the world by 2025, with an estimated prevalence of 13.5% of the population which represents 145 million diabetic patients. In the year 2000, diabetes has resulted in 1404 deaths among women and 857 deaths among men (total of 2,261 death cases), as was reported by the Malaysian Burden of Disease and Injury Study (Letchuman et al., 2010). As stated by the National Health and Morbidity Surveys (NHMS

II), Type II diabetes is seen as no longer a disease of middle or old age were Type II diabetes was more apparent in people aged between 18 and 29 and also children below 10 years were reported to be inflicted by the disease.

The prevalence of type II diabetes in youth is increasing worldwide, coinciding with the rising obesity epidemic (Fagot-Campagna et al., 2000). In 2005, accounts for 50% of cases in some countries and ethnic groups (Pinhas-Hamiel & Zeitler, 2005). DM is mainly treated by injecting insulin or by orally administrating hypoglycaemic agents. Yet, several inconvenient side effects were observed from taking these agents, for instance, the liver intoxication developing hypoglycemia, the digestive system as well as weight gain (Aruna et al., 2014). Thus, the antidiabetic activity of various natural products has been the main interest of many researchers in recent time. The applications of plants as antihyperglycemic agents have been commonly reported since they were proved to be safe and possess lower side effects. The specified timings to monitor the blood glucose level in type II diabetic adolescents is in the morning, pre-meal, as well as a bedtime testing, should be done until glycemic control is achieved (Copeland et al., 2013). Insulin treatment is the launch of single- injection, long-acting basal insulin at bedtime (Huda et al., 2009). The insulin dose needs to be titrated to achieve a goal of fasting blood glucose level ranging between 70 and 130 mg/dL (Copeland et al., 2013). In case of failure to monitor the blood glucose level with this range, a rapid-acting insulin tablet prior to meals or twice-daily combination insulin is ordinarily added (George & Copeland, 2013).

The restricted approved medicinal options for type II diabetes are considered an obstacle for medical management of diabetic adolescents and this raises a serious challenging issue. It is worth mentioning that the only permitted oral diabetic medicine is Metformin, however, its efficiency in diabetic adolescents is limited. A group study conducted by "Diabetes in Adolescent and Youth (TODAY)" for 699 teenagers at the age range of (10-17) years, newly diagnosed with TIIDM, to look at the options of TIIDM treatments, observed that Metformin alone was merely capable to achieve glycemic control in half of the TODAY Participants (George & Copeland, 2013; Tryggestad & Willi, 2015).

Knowledge on the chemical constituents of plants is helpful in the discovery of therapeutic agent as well as new sources of economic materials such as oil and gums. The most important bioactive constituents of these plants are alkaloids, tannins, flavonoids and phenolic compounds (Satapathy et al., 2009). The *Aquilaria malaccensis* leaves extracted with ethanol were reported to exhibit hypoglycemic effects. Investigations on the hypoglycemic effect are commonly performed utilizing diabetic mice/rats. The blood glucose level was significantly decreased by the ethanoic extract and that can be attributed to the blood sugar reducing the effect of 2α-hydroxy ursolic acid (Mei et al., 2013). Additionally, the methanolic and aqueous extracts of *Aquilaria malaccensis* leaves have similar anti-hyperglycemic activity as insulin (Pranakhon et al., 2011). Furthermore, a rise in the blood glucose scale was noticed for *Aquilaria malaccensis* leaves extracted by ethyl acetate and ethanol-water, which can be ascribed to the increase in the levels of glucose transporter type 4 (GLUT4), a regulator of whole-body glucose homeostasis. Generally, the level of GLUT4 is increased more by the extracts than by pioglitazone (Pranakhon et

al., 2011). Some chemical constituents in ethanol extract of *Aquilaria sinensis* leave to act as antidiabetic agents inhibiting α-glucosidase activity and control the absorption of carbohydrate in the intestine which consequently resulting in a reduction in blood glucose level (Feng et al., 2011). These chemical compounds include iriflophenone 3,5-C-β-Ddiglucopyranoside, iriflophenone 2-O- 11 α-L-rhamnopyranoside, iriflophenone 3-C-β-D-glucoside, and mangiferin.

The potential of agarwood (*Aquilaria malaccensis*) leaves extract as a promising alternative for the antidiabetic drugs was investigated in this study. Two solvents, namely distilled water and methanol, were used to prepare the agarwood aqueous extract and agarwood methanolic extract, respectively. The potential of these extracts to treat type II DM, in animal models was examined by stimulating diabetic conditions to range using streptozotocin-induced diabetic male and female ICR mice.

1.4 Objectives

The main objective of the present study is to investigate the effect of agarwood leaf extract (*Aquilaria malaccensis)* on type II diabetes mellitus in streptozotocin-induced ICR mice: The specific objective of this study include,

1. To compare the blood glucose levels in diabetic mice treated with agarwood leaves aqueous extract with those treated with the methanolic extract.

2. To determine blood glucose levels in male diabetic mice compared to diabetic female mice after treatments with both aqueous and methanolic extracts.
3. To examine the effects of different concentrations of treatment at various intervals on fasting blood glucose levels in diabetic mice.

1.5 Research Questions

The research questions for this study are:

- Are there any differences in antidiabetic effect between *Aquilaria malaccensis* leave methanol extract with aqueous extract?
- Is there any gender-based difference in blood glucose levels between the treated diabetic mice?
- Is there any vital effect for exposure time and doses on the glucose levels of diabetic mice treated with agarwood extracts?

1.6 Hypothesis

The hypothesis for this study is as the followings:

- Agarwood leaf extract (*Aquilaria malaccensis*) has an antidiabetic effect on type II diabetes mellitus.
- The antidiabetic effect of *Aquilaria malaccensis* methanol extract differs from that of *Aquilaria malaccensis* aqueous extract.
- The antidiabetic effect of agarwood leaves extract is time- dependant.

1.7 Limitation of the Study

The source of *Aquilaria malaccensis* leaves was taken from Al-Hilmi Agrofarm in Slim river and the study was conducted at University Pendidikan Sultan Idris, Tanjung Malim, Perak under their environmental and laboratory conditions. The study investigated type II diabetes mellitus on ICR mice by induced STZ. Two solvents were used to extract agarwood (i.e. water, methanol). This research cannot be generalized to all sort of mice and areas worldwide.

1.8 The Significance of the Study

It has been demonstrated by the surge of the number of scientific publications in current years, mainly those investigating the pharmacological actions of agarwood, such as anti-depressant (Takemoto et al., 2008), anti-cancer (Dahham et al., 2016), anti-diabetic (Pranakhon et al., 2015), anti-oxidant (Tay et al., 2014), and anti-inflammatory (Rahman et al., 2012) activities of agarwood plant materials.

This study will lay down the ground for further pharmaceutical studies to develop new alternative products based on agarwood for treating diabetes rather than conventional drugs. These alternatives may reduce the use of conventional drugs and it may restrain their secondary effects. Furthermore, a low production cost is proposed for these alternatives, which promotes them for both pharmaceutical laboratories and consumers. It’s worth mentioning this study will investigate the antidiabetic effect of agarwood on both genders of ICR mice for the first time.