THE BIOCHEMICAL EFFECTS AND HISTOLOGICAL FEATURES OF ORAL ADMINISTRATION OF STINGLESS BEE HONEY (*Heterotrigona itama*) ON DIABETIC SPRAGUE-DAWLEY WOUND RATS MODEL

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by

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LIST OF SYMBOLS

g	Gram
%	Percentage
µL	Microliter
µm	Micrometer
µmol/l	Micromole per liter
°C	Degree Celsius
cm	Centimeter
g/kg	Gram per kilogram
g/L	Gram per liter
M	Molar
mg	Milligrams
mg/dl	Milligrams per deciliter
mg/kg	Milligrams per kilogram
mg/mL	Milligrams per milliliter
min	Minute
ml	Milliliter
mm	Millimeter
mmol/l	Millimole per liter
nm	Nanometer
pg	Pictograms
pg/mL	Picograms per milliliter
U/L	Units per liter

LIST OF ABBREVIATIONS

Ab	Antibody
ALP	Alkaline phosphatase
ALT	Alanine transaminase
ANOVA	Analysis of variance
ARASC	Animal Research and Service Centre
AST	Aspartate transaminase
BG	Blood glucose
BUN	Blood urea nitrogen
BW	Body weight
Ca^{2+}	Calcium
CAT	Catalase
Cu	Copper
DFU	Diabetic foot ulcer
dH_2O	Distilled water
DM	Diabetes mellitus
DN	Diabetic nephropathy
DPX	Dibutylphthalate polystene xylene
ELISA	Enzyme-Linked Immunosorbent Assay
ESRD	End-stage renal disease
EW	Excisional wound
FBG	Fasting blood glucose
Fe	Iron
FFA	Free fatty acid
GBM	Glomerular basement membrane
GFR	Glomerular filtration rate
GI	Glycemic index
GLUT2	Glucose transporter
GR	Glutathione reductase
H&E	Haematoxylin and eosin
HCL	Hydrochloric acid

HDL-C	High-density lipoprotein cholesterol
HFD	High fat diet
HFHSD	High fat high sucrose diet
HRP	Horseradish peroxidase
HSC	Hepatic stellate cell
IACUC	International Animal Care and Use Committee
IDF	International Diabetes Federation (IDF)
IL-10	Interleukin-10
IL-6	Interleukin-6
IOL	Islets of Langerhans
IP	Intraperitoneal
IPS	Institut Pengajian Siswazah
IR	Insulin resistance
IV	Intravenous
IW	Incisional wound
K^+	Potassium
LCMS	Liquid chromatography-mass spectrometry
LDL-C	Low-density lipoprotein cholesterol
LFT	Liver function test
MDA	Malondialdehyde
MET	Metformin
Mg^{2+}	Magnesium
Mn	Manganese
Na^+	Sodium
NA	Nicotinamide
NAD	Nicotinamide dinucleotide
NAFLD	Non-alcoholic fatty liver disease
NaOH	Sodium hydroxide
NASH	Non-alcoholic steatohepatitis
ND	Non-diabetic
OD	Optical density
P	Phosphorus
PAS	Periodic-Acid Schiff

RFT	Renal function test
ROS	Reactive oxygen species
SBH	Stingless bee honey
SBME	SBH + MET
SD	*Sprague-Dawley*
SEM	Standard error of mean
SOD	Superoxide dismutase
SOP	Standard operating procedure
STZ	Streptozotocin
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TC	Total cholesterol
TG	Triglyceride
TNF	Tumor necrosis factor
UNT	Untreated
USM	Universiti Sains Malaysia
Zn	Zinc

LIST OF APPENDICES

KESAN BIOKIMIA DAN CIRI HISTOLOGI KE ATAS ORAL ADMINISTRASI MADU LEBAH TANPA SENGAT (*Heterotrigona itama*) TERHADAP MODEL LUKA TIKUS SPRAGUE-DAWLEY YANG MENGHIDAP DIABETES

ABSTRAK

Diabetes mellitus (DM) telah menjadi isu kesihatan awam yang utama dan ianya semakin berkembang. Kadar morbiditi dan kadar kematian DM telah meningkat disebabkan oleh komplikasinya, termasuk kerosakan pankreas, nefropati diabetik (DN), dan kerosakan hepatoselular serta penyembuhan luka yang teruk. Menurut pelbagai kajian, pengambilan madu berkait sebaliknya dengan risiko menghidap diabetes. Walau bagaimanapun, ia masih tidak diberi penekanan oleh pengamal perubatan kerana terdapat perbahasan tentang kawalan glisemik dalam individu yang mengambil madu. Oleh itu, kajian semasa dijalankan untuk mengkaji kesan oral administrasi madu lebah tanpa sengat (SBH) terhadap pengurusan DM untuk menekankan kepentingan SBH dalam rawatan diabetes. Enam puluh ekor tikus jantan *Sprague-Dawley* (SD) telah diinduksi dengan suntikan intraperitoneal streptozotocin (STZ) (50 mg/kg) untuk menjadi DM. Mereka dibahagikan kepada lima kumpulan utama berdasarkan rawatan yang diberikan. Tikus bukan diabetes dan tikus diabetes yang tidak dirawat diberikan air garam biasa. Sementara, dos SBH 2.0 g/kg, metformin (MET) 250 mg/kg dan gabungan kedua-dua SBH dan MET (SBME) telah diberikan melalui administrasi oral untuk menentukan kesannya pada tikus diabetes dan juga untuk menilai perkembangan dalam penyembuhan luka berbanding tikus bukan diabetes. Bagi setiap kumpulan utama, Luka eksisi (luka terbuka) dan luka insisi (luka

tertutup), n=6 untuk setiap kumpulan, dicipta untuk menyerupai luka pada pesakit diabetes. Dos sebanyak 2.0 g/kg SBH diberikan selama 12 hari berturut-turut menunjukkan aktiviti antidiabetik dengan menurunkan glukosa darah puasa (FBG) dengan ketara dalam model tikus diabetes. Tambahan pula, SBH mengurangkan penanda tekanan oksidatif (MDA), penanda pro-radang (IL-6), beserta dengan meningkatkan tahap IL-10. Paras serum C-peptida, insulin dan profil lipid tidak mempunyai perbezaan yang ketara dalam semua kumpulan tikus di EW dan IW. Sel pankreas tikus diabetes yang dirawat SBH menunjukkan penjanaan semula vakuola islets selepas diwarnai dengan haematoxylin & eosin (H&E) dan periodic acid-Schiff (PAS). Paras serum kreatinin dan urea menunjukkan perbezaan yang tidak ketara antara semua kumpulan tetapi buah pinggang tikus kumpulan SBH menunjukkan lapisan nipis membrane glomeruli dan kurang pada perubahan hidropik berbanding kumpulan lain (EW dan IW). Walaupun pengurangan dalam serum ALT, AST dan ALP tidak ketara, tetapi secara histologi, rawatan dengan SBH memperbaiki sel-sel hepatik dan mengurangkan pembentukan vakuol lemak dan dilatasi darah dalam hati tikus diabetes berbanding kumpulan lain. SBH juga mempercepatkan penutupan luka dengan ketara dan memberikan peratusan penguncupan luka yang lebih tinggi pada tikus DM. Kesimpulannya, pemberian SBH (2.0 g/kg) selama 12 hari telah menunjukkan peningkatan yang ketara dalam kawalan glisemik (FBG), memberikan tahap IL-6 dan MDA yang rendah serta tahap IL-10 yang lebih tinggi berbanding kumpulan lain yang dirawat, (EW dan IW). Ia juga meningkatkan ciri histologi pankreas, buah pinggang dan hati serta menggalakkan penyembuhan luka tikus diabetes.

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ABSTRACT

Diabetes mellitus (DM) has emerged into a major and growing public health issue. The morbidity and mortality rate of DM has increased due to its complications, including pancreatic damage, diabetic nephropathy (DN), and hepatocellular damage as well as impaired wound healing. According to various research, honey consumption is inversely related to the risk of developing diabetes. However, it still not be emphasize by the medical practitioner, due to debate on glycemic control in individual with honey supplementation. Therefore, the current study was carried out to examine the effects of oral administration of stingless bee honey (SBH) on the management of DM in order to emphasize the significance of SBH in the treatment of diabetic. Sixty male *Sprague-Dawley* (SD) rats were induced with intraperitoneal injection of streptozotocin (STZ) (50 mg/kg) to become DM. They were divided into five main groups according to treatment given. Non-diabetic rats (ND) and diabetic untreated rats (UNT) were orally gavage with normal saline. While, the SBH dosage of 2.0 g/kg, metformin (MET) 250 mg/kg and the combination of both SBH and MET (SBME) were administered through oral gavage to determine their effects on diabetic rats and to assess the wound healing progress compared to ND rats as controlled. For each main group, excisional wound (open wound) and incisional wound (close wound), n=6 for each wound, were created to mimic wound in diabetic patient. The dose of 2.0 g/kg SBH for 12 consecutive days exhibited antidiabetic activity by significantly lowering fasting blood glucose (FBG) in diabetic rat models. Furthermore, SBH give a lower level of oxidative stress marker (MDA), pro-inflammatory marker (IL-6), coupled together with higher level of IL-10 in EW and IW groups. The level of serum C-peptide, insulin and lipid profile were not significantly difference in all group of rats in EW and IW. Pancreatic cells of SBH-treated diabetic rats showed regeneration of islets after stained with haematoxylin & eosin (H&E) and periodic acid-Schiff (PAS). The serum creatinine and urea level showed insignificant different among groups but the rat kidney of SBH group showed thin layer of glomeruli basement membrane and less hydropic changes as compared to other groups (EW and IW). Although the reduction in serum ALT, AST and ALP was not significant but histologically, treatment with SBH improved the hepatic cells and reduced the formation of fatty vacuoles and dilatations of blood sinusoids in the liver of diabetic rats as compared to other groups. SBH also significantly accelerated the wound closure and give a higher wound contraction percentage in DM rats. In conclusion, administration of SBH (2.0 g/kg) for 12 days has shown significant improvement in glycemic controlled (FBG), give low level of IL-6 and MDA as well as higher level of IL-10 as compared to other treated groups (EW and IW). It also improve the histological features of pancreas, kidney and liver as well as promote wound healing of diabetic rats.

CHAPTER 1

INTRODUCTION

1.1 Background

Diabetes mellitus (DM) is currently one of the leading metabolic disease and a major public health concern in Malaysia (Al-Awar et al., 2016). Globally, there has been a rise in the incidence of diabetes from the past three decades due to lifestyle modifications (Balamash et al., 2018). In 2019, the International Diabetes Federation (IDF) estimated about 463 million people had diabetes worldwide and it is predicted that by 2040, the number would rise to 700 million (Saeedi et al., 2019). With 4.2 million fatalities reported in 2019, type 2 diabetes mellitus (T2DM) is actually one of the top causes of illness and premature mortality worldwide (Galicia-garcia et al., 2020). Both developed nations and developing regions are primarily impacted by the disease (Pasupuleti et al., 2020).

DM is characterized by persistent hyperglycemia and inadequate production or action of insulin produced by the pancreas inside the body (Damasceno et al., 2014). If left untreated, sustained hyperglycemia causes abnormalities of the metabolism of carbohydrates, proteins, and lipids, which can result in dysfunction of various organs and leading to diabetic complications include, pancreatic damage, diabetic nephropathy (DN), hepatocellular damage and impaired wound healing in diabetic patients (Li et al., 2014; Mestry et al., 2017). Elevations in plasma glucose level and free fatty acids (FFA) contribute to the increasing of reactive oxygen species (ROS) levels that will activate inflammation signalling pathways and cause insulin resistance (IR) (Damasceno et al., 2014). Additionally, it might cause oxidative stress, which might influence the activity of the insulin (Ma et al., 2018).

Oxidative stress is mediated mainly by hyperglycemia-induced generation of free radicals (Johansen et al., 2005). Any change in the ratio of pro-oxidants to antioxidants, favoring the latter, can be referred as oxidative stress. This imbalance can be caused by a variety of circumstances, including ageing, the effects and toxicity of drugs, inflammation, and/or addiction (Ullah et al., 2016). The oxidative stress play a vital role in developing vascular complications particularly in T2DM. Understand the pathophysiology of diabetic and its progression to vascular complication will help to determine the best treatment for diabetic and these will reduce the morbidity and mortality rate in diabetic patients (Ali et.al, 2020).

Regulating blood glucose (BG) levels is crucial for reducing the risk of diabetic complications and enhancing the health of diabetic patients (Yang and Kang, 2018). Up until recently, conventional therapies have only attempted to manage BG levels and have been unsuccessful in controlling diabetic complications (Du et al., 2016). Various number of oral diabetic drugs available in reducing diabetes-associated hyperglycemia. However, those drugs have numerous limitations as they give another side effect towards patients, such as, hypoglycemia, diarrhea, vomiting and others (Dong et al., 2014; Yang & Kang, 2018). In addition, the high expenses of the treatments also led to a financial stress, particularly for those diabetic patients living in developing nations (Ali et al., 2020). Considering these matters, a search for an alternative treatment is highly demanded. Researchers from all over the world have carried out further investigation in order to find an alternative medicine that can act as an anti-hyperglycemic agent. Honey, as a natural substance, is believed to help in lowering the BG levels of diabetic patients as well as reduce diabetic complications (Biluca et al., 2016).

Honey is defined as a natural sweet substance produced by honeybees, from the nectars of plant flowers and honeydew (Sohaimy et al., 2015). It possesses a valuable therapeutic property due to the presence of approximately 200 compounds such as vitamins, enzymes, amino acids, and minerals with the major content of water and sugars (Małgorzata et al., 2018). Honey has been studied against various ailments in animal and human models and it is denoted as a novel antioxidant agent (Ahmed et al., 2018).

Numerous studies have shown that the presence of phenolic compounds and flavonoids in the honey enhances its antioxidant properties, which are crucial for reducing oxidative stress (Rao et al., 2016). Besides, it is also reported that honey is among the best wound healers available in the nature and can be one of the antidiabetic agents. The presence of the polyphenols such as quercetin, catechin and kaempferol in honey exhibit antidiabetic properties and successfully inhibit the α-glucosidase enzymes, which involved in the elevation of BG (Zulkhairi Amin et al., 2018). The composition of honey depends primarily on its floral source, seasonal and environmental factors (Erejuwa et al., 2016; Alvarez-suarez et al., 2014; Zakaria et al., 2016). Therefore, different varieties of honey may exhibit different health promoting properties.

Stingless bee honey (SBH) is a precious bee product of the stingless bee. It is reported to contain rich phytochemical contents and has been widely used traditionally and in well-established meliponary across time and place. The composition of SBH is differ from other species due to some physicochemical parameters. The distinctive feature of this honey is that it has distinctive colour, taste and viscosity and is stored naturally in the pot (cerumen) which contains anti-inflammatory agent such as interleukin-10 (IL-10) that inhibits the synthesis of pro-inflammatory cytokine such as interleukin-6 (IL-6) (Biluca et al., 2016; Ramadan et al., 2017).

Besides its anti-inflammatory and antioxidant properties, SBH also grow as a research of interest for wound healing promotion (Biluca et al., 2016; Nweze et al., 2017). It is proved that SBH are more valuable, and it has been used for a long time to treat various diseases (Yaacob et al., 2018). Besides, the nature of stingless bee that does not sting and easy to be manipulated and cultivated by the beekeepers is another special properties of SBH. Some studies has been carried out to evaluate the effects of SBH on DM (Da Silva et al., 2015; Ramli et al., 2019; Sahlan et al., 2020; Zulkifli et al., 2018). A study investigated the effects of SBH (*Tetragonula biroi*) on streptozotocin (STZ)-induced DM in rats by Sahlan et al., (2020), reported that SBH was significantly reduced the BG levels of diabetic rats. Similarly, a study of Zulkifli et al., (2018) stated that, supplementation of SBH from *Heterotrigona itama* showed a promising effect of SBH in controlling the BG levels and enhanced the C-peptide and insulin action of diabetic rats, suggesting its potential role against diabetes.

Besides its antidiabetic property, SBH could also be useful in reducing several diabetic complications due to its antioxidant property. As SBH comprises various compounds that act as antioxidants, it is believed that the synergistic effect of a wide range of these compounds including phenolic acids, flavonoids, enzymes, organic acids and other minor compounds, contribute to its antioxidant activity (Shamsudin et al., 2019). A study has proved that the used of honey caused malondialdehyde (MDA) levels to decrease noticeably (Hassan et al., 2019). This suggests a reduction of oxidative stress occurred in diabetic rats as shown by the reduction of MDA levels (Da Silva et al., 2015; Ramli et al., 2019).

SBH also help in improving the instability of lipid profile. Another study from Erejuwa et al., (2016) showed that administration of SBH significantly reduced the BG levels, serum triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein (VLDL) while give a high level of high-density lipoprotein cholesterol (HDL-C) in diabetic rats. Inducing diabetes with the injection of STZ also caused a toxic effect on other organs, including the kidney (Kitada, 2016). It led to the alterations in renal architecture, which exhibit hyperglycemia-induced glomerular lesions, such as thickening of glomerular basement membrane (GBM), mesangial matrix expansion, oxidative stress and inflammation. When the renal is damaged or not functioning properly, the serum urea and creatinine levels will rise. Interestingly, a study by Hamad et al., (2015) reported that, honey consumption in diabetic rats significantly reduced both urea and creatinine levels suggesting the improvement in renal function, which associated with better renal morphology preservation. It can be concluded that, honey is effective in preventing the renal damage in diabetic rats.

In addition, DM also led to hepatocellular damage in diabetic rats. It caused a high level of liver enzymes such as alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP), which act as an indicator of liver function (Mustafa, 2018). This is clear from the findings by El-Haskoury et al., (2019), comparing untreated diabetic rats with normal control rats, clearly showed a high ALT, AST and ALP level. However, honey administration reduced the higher levels of ALT, AST and ALP in diabetic rats, and this might be as a result of hepatoprotective effects of honey constituents (Elkotby et al., 2018).

Wound healing is impaired in diabetes and diabetic foot ulcers (DFUs) are most common wound associated with diabetic patients and affected about 15% of diabetic patients (Okonkwo & Dipietro, 2017; Patel et al., 2019). It can be classified as both microvascular and macrovascular complication of diabetic (Ullah et al., 2016). Decrease in ability to metabolize glucose in diabetic patients, because of hyperglycemic condition, lead to complicate the wound healing process. Diabetes is believed to delay the process of healing as it impairs each stages of wound healing including haemostasis, inflammation, proliferation and remodelling phase. Honey has been well established in wound healing treatment. A study by Nordin et al., (2018) have proved that a low dose of SBH possess a mesmerizing effect on the human dermal fibroblast viability and proliferation. This result concluded that SBH could potentially improve and promote the wound healing process.

Therefore, the present study is carried out to determine the biochemical effects and histological features of oral administration of SBH on diabetic *Sprague-Dawley* (SD) wound rats model. The test for biochemical analysis including the fasting blood glucose (FBG) levels, lipid profile (TC, TG, HDL-C and LDL-C), renal function test (urea, creatinine, sodium (Na^{+}), potassium (K^{+}), calcium (Ca^{2+}) and magnesium (Mg^{2+})), liver function test (albumin, total protein, ALT, AST and ALP), C-peptide, insulin, oxidative stress marker (MDA) and inflammatory markers (IL-6 and IL-10) levels as well as histopathological analysis of pancreas, kidney and liver architecture of diabetic rats were included in the study.

1.2 Problem statement

In Malaysia, DM is currently one of the most prevalent metabolic diseases and a significant public health issues. The chronic hyperglycemia influenced the development of diabetic complications including pancreatic damage, DN, hepatocellular damage and impaired wound healing as well as exaggerate IR. Various number of anti-diabetic drugs available in reducing diabetes-associated hyperglycemia. However, most of the hypoglycemic agents used as conventional therapies are reported to have certain side effects including diarrhea, vomiting, hypoglycemia and others (Dong et al., 2014; Yang & Kang, 2018).

Despite of treatment given, DM still become a major concern as it influenced the development of diabetic complications. Currently, there are a lot of study that revealed the therapeutic properties of different types of honey in terms of antidiabetic, antioxidant, antibacterial, anti-inflammatory and wound healing activities. However, it still not be emphasize by the medical practitioner, probably due to the debate on the glycemic control in individual with honey supplementation and the role of honey in diabetic complication.

Hence, to expand the field of knowledge in this area, recent study is conducted to determine the biochemical effects and histological features of oral administration of SBH from *Heterotrigona itama* in diabetic wound SD rats model. Besides, this study also comprises on the effects of oral administration of SBH in treating the wound that was created on the rat dorsum, which is excisional wound and incisional wound, instead of being topically applied onto the wound site. Unadulterated SBH was chosen as it can easily cultivated by beekeepers and more confidence to get the pure honey as they are reared commercially in Malaysia. In addition, stingless bee also have an important socioeconomic role as they can pollinate diverse species of cultivable plants, which is not restricted to only natural flora (Lavinas et al., 2018). The appropriate dose of unadulterated SBH able to control the glucose level and prevent diabetic complications.

1.3 Significant of the study

Establishment of oral administration of SBH in STZ-induced diabetic rats model in regulating the glycemia and manage its complications would provide scientific evidence for its use in the management of diabetes. These findings could pave the way for further studies that may help to produce medicinal products that derived from this natural substance to be used in clinical field of application.

1.4 Objectives

1.4.1 General objective:

To investigate the effects of oral administration of SBH in diabetic wound rats model on glycemic control, biochemical parameters and histological features of pancreas, kidney, and liver.

1.4.2 Specific objectives:

(1) To determine the level of fasting blood glucose (FBG), lipid profiles (TC, TG, HDL-C and LDL-C), renal function test (RFT), liver function test (LFT), C-peptide and insulin by an oral administration of SBH in STZ-induced diabetic wound rats model.

(2) To assess the effects of oral administration of SBH on oxidative stress marker (MDA), pro-inflammatory marker (IL-6), and anti-inflammatory marker (IL-10) in STZ-induced diabetic wound rats model.

(3) To assess the effects of oral administration of SBH on histopathological architecture in pancreas, kidney and liver in STZ-induced diabetic wound rats model.

(4) To determine the effects of oral administration of SBH on the macroscopic observation of the wound and measurement of wound contraction percentage in STZ-induced diabetic wound rats model.

CHAPTER 2

LITERATURE REVIEW

2.1 Honey

2.1.1 Introduction

Honey is well-known for its wide-spectrum medical therapeutics agents in treating physiological dysfunctions (Talebi et al., 2020). It is produced from secretions of some plants, honeydew or nectar of flowers by honeybees (*Apis melifera;* Family: Apidae*)*. Honey is a complex food product and considered as the only natural sweetener that can be used without processing (Pauliuc & Dranca, 2020). Traditional medical practitioner worldwide had used raw honey for centuries, in treating numerous medical treatments, such as treatments of leg ulcers in Ghana, eye diseases in India, measles in Nigeria and cough and sore throat in Bangladesh (El-Soud, 2012). Since ancient times, it was also used as a traditional medicine in several therapeutics activity like treating DM, wound healing, tissues regeneration and alleviating gastrointestinal disorders. The therapeutic potential of honey also have increasingly documented in the modern science literature (Rahman et al., 2014).

Honey is produced all over the world and the global production of honey is approximately reached 1.20 million tons annually (Meo et al., 2017). It is stated that this global production of honey increased by 10% from approximately 1.4 million tons to 1.5 million tons between 2005-2010 (Rahman et al., 2014). There are about 320 different varieties of honey originating from various floral sources (Sohaimy et al., 2015). Honey may be assigned by the name of geographical or topographical region where it is produced. It is also named according to the botanical or plant source if it comes completely or predominantly from that specific source. As for an example, Acacia honey and Gelam honey were named according to the plant that providing nectars as a source of honey while for Tualang honey and SBH refers to the types of bees that produced honey (Shahira et al., 2021). Since there are variety of plants and bee species that produce honey, there are no equivalent quality of honey to one another.

In general, honey is economically produced by two types of bees which are *Apis* sp. (honeybee) and *Melipona* sp. (stingless bee) (Ranneh et al., 2019). Apis, the only one genus of clan Apini, are the genuine and social honeybees that set up a permanent colony. For honeybee, there are not many types of species exist; *Apis mellifera, Apis cerana, Apis florea,* and *Apis dorsata* (Shahira et al., 2021). For stingless bees, it can be classified into many genera as these bees are the largest group of eusocial bees on earth (Lavinas et al., 2018). There are about more than 600 species can be found and they spread throughout the tropical and subtropical areas of the globe including Southeast Asia, South America, Central America, south of North America, Africa and in Northern Oceania (Hrncir et al., 2016).

Despite of various research proved the benefits of honey as antidiabetic, the issue of adulterated honey to be use in the management of DM become worst. According to reports, 80% of the honey marketed in Malaysia is adulterated (Yong et al., 2022). These adulterated honeys, sometimes referred to as "diabetic honey" in Malaysia, are harmful to health, particularly for people with or without diabetes. SBH can cost up to US $100 per kilogram, which is in contrast with floral honey, is often marketed for US $20-40 per kilogram. Owing to this, dishonest food fraudster have turned to adulterating SBH in order to generate quick profits. Glucose syrup, fructose syrup and floral honey are the most often utilized adulterants in SBH (Tosun, 2013). Besides, vinegar has also been added to less expensive floral honey to resemble the sour flavor of SBH, and this floral honey then be sold as fake SBH or used to adulterate SBH to increase the volume with the less expensive adulterant (Mail et al., 2019).

2.1.2 Chemical constituents in honey and their benefits

Honey contains macronutrients and micronutrients, which depends on various factors, such as bee type, floral sources and environmental processing factors. In general, the major constituents of honey (75%) represented by fructose and glucose (monosaccharides) with low amounts of disaccharides (sucrose) and polysaccharides. Enzymes, amino acids and minerals can be found in honey as minor constituents (Alqarni et al., 2014). Honey is rich in flavonoids and phenolic acids, which act as natural antioxidants (Al-hatamleh et al., 2020; Khalil et al., 2010). Besides, honey also contains about 180 types of different chemical composition including free amino acids, sugars, enzymes, proteins, vitamins, essential minerals and other various phytochemicals (Carter et al., 2016; Pauliuc & Dranca, 2020). These constituents can be added by bees or they come directly from nectar during ripening process.

The constituents present in different variety of ratio and give rise to different in color, taste, viscosity and also affects the therapeutic benefits of each honey (Ranneh et al., 2021). The synergistic effects of these constituents provide an antioxidant properties of honey although little is known about the mechanism involved by each of the constituents (Chua et al., 2013). Therefore, the composition of honey can be analyze to determine its botanical and geographical origin (Islam et al., 2016). Due to its chemical constituents, honey has been discovered to have a substantial impact on human nutrition, healing and prevention of illness (Alqarni et al., 2014). Table 2.1 showed the chemical constituents that present in several types of honey.

Table 2.1 Chemical constituents that present in several types of honey

Category	Constituents	References
	Macronutrients	
Sugars	(Monosaccharides: fructose, glucose, (disaccharides: maltose, turanose, amltulose, sucrose), (trisaccharides: panose, ketose, isomaltose, cellobiose, isopanose	(Loh et al., 2011; Ranneh et al., 2021)
Amino acids	Proline, alanine, phenylalanine, tyrosine, glutamic acid, isoleucine, leucine, glutamine, histidine, glysine, threonine, arginine, valine, methionine, cysteine, tryptophan, lysine, serine	(Sakač et al., 2019)
Enzymes	Diastase (α-amylases, ß-amylases), glucose oxidase, invertase, catalase, superoxide dismutase	(De-melo et al., 2017)
Organic acids	Gluconic acid, aspartic acid, citric, acetic, formic, fumaric, galacturonic, malonic, acetoglutaric, gluconic, glutamic, butyric, glutaric, isocitric, lactic, malic, methylmalonic, quinic, succinic, tartaric, oxalic.	(Mato et al., 2003; Sakač et al., 2019)
Flavonoids	Apigenin, catechin, chrysin, galangin, hispidulin, kaempferol, luteolin, myricetin, pinobanksin, pinocembrin, quercetin, taxifolin, isorhamnetin	(Chettri & Kumari, 2020)
Phenolic acids	Caffeic acid, chlorogenic acid, cinnamic acid, ellagic acid, ferulic acid, gallic acid, sinapic acid, syringic acid, vanillic acid, kaempferol, quercetin	(Chettri & Kumari, 2020)
	Micronutrients	
Minerals	Potassium (K^+), calcium (Ca), magnesium (Mg), zinc (Zn), phosphorus (P), manganese (Mn), iron (Fe), copper (Cu) and sodium (Na^+)	(Abu Bakar et al., 2017; Biluca et al., 2016)

2.2 Stingless bees

2.2.1 Taxonomy, morphology and distribution

Stingless bees, as their common name implies, are distinguished from *Apis mellifera* bees by not having a sting (Esa et al., 2022). They build their hives horizontally, travel shorter distances while looking for food, and create honeypots rather than honeycombs to store honey (Ávila et al., 2018). Stingless bees are vast of monophyletic class of highly eusocial bees, which belong to the family Apidae and tribe Meliponini (Akhir et al., 2017).

Taxonomically, the stingless bee species are divided into two main genera: *Trigona*, which comprises the largest group that can be found throughout the tropics, and *Melipona*, which only includes the genus *Melipona* and exclusively found in the Neotropics (Vijayakumar and Jeyaraaj, 2014). There are three characteristics of stingless bees that distinguish them apart from other types of bees; diminished wing venation, a weakened or absent sting (Figure 2.1) and the presence of a penicillum on the hind tibia (Figure 2.2) (Engel & Rasmussen, 2017). Even though their sting has shrunk in size, they are still capable of biting if their nest is disturbed (Azizi et al., 2020).

Figure 2.1 Reduction of wing venation and sting in stingless bee

Figure 2.2 Presence of penicillum on the hind tibia

Stingless bees are one of the natural type of bees, that exist in almost every continent including Eastern and Southern Asia, Latin America, the mainland of Australia and Africa, which consists approximately 500 species within the genus worldwide (Abd Jalil et al., 2017). Malaysia is one of the honey production countries as the living conditions are suitable for bees. The rainforest topical climate of Malaysia allows honeybees to have an optimal warm temperature and high humidity throughout the year with an average temperature at 27℃ and rainfall at 250 centimeters (cm) (Zakaria et al., 2020). Figure 2.3 shows the distribution of stingless bees' subgenera throughout the world (Combey, 2010).

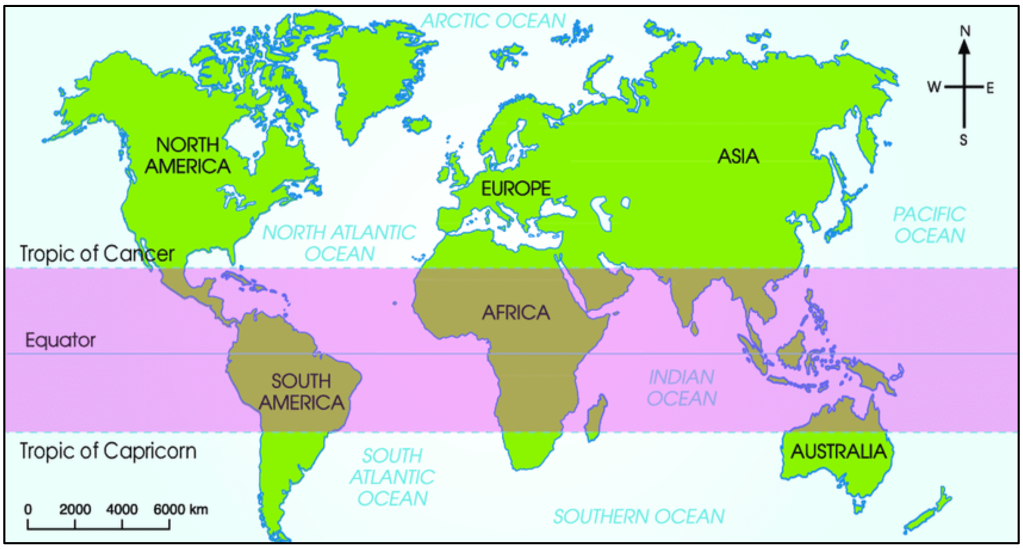

Figure 2.3 The distribution of subgenera of stingless bees in the world

Among the stingless bee species, *Heterotrigona itama*, *Geniotrigona thoracica*, *Lepidotrigona terminate* and *Tetragonula laeviceps* are reared commercially in Malaysia (Zulkifli et al., 2018). Honey produced by these stingless bees is known with various names such as *Kelulut* honey (in Malaysia), Meliponine honey, SBH pot honey and also sugar bag honey (in Australia) (Ranneh et al., 2019; Zulkhairi Amin et al., 2018).

2.3 Stingless bee honey

SBH is considered as one of food substances that can be used as natural remedies. It is produced from the nectar of flowers or another part of the tree by the stingless bees from the family of *Meliponini*, which made up of various types of genera; *Melipona, Trigona and Heterotrigona* (Ali et al., 2020). SBH is rich in phytochemical contents and it has been reported to have an antidiabetic, anti-inflammatory, antioxidant, antimicrobial, anticancer properties and also can boost immune system (Abd Jalil et al., 2017; Akhir et al., 2017). Environmental conditions of plants determined the variety of physical and chemical properties of SBH being harvested.

SBH is kept in pots or brood cells made of cerumen, a waxy substance made of beeswax, resin and mandibular secretion that maintains a sterile environment in the hive (Anderson et al., 2011). The transformation of nectar to become honey is an impressively complex process. In cerumen pots, nectar undergoes three transformations as shown in Figure 2.4. Large amount of water are drained from nectar in cerumen pots or brood cells during the first transformation process. Then, process that involves microbial fermentation take place, and the final transformation process involves worker bees secrete enzymes that chemically hydrolyze the nectars sucrose into fructose and glucose (Chettri & Kumari, 2020).

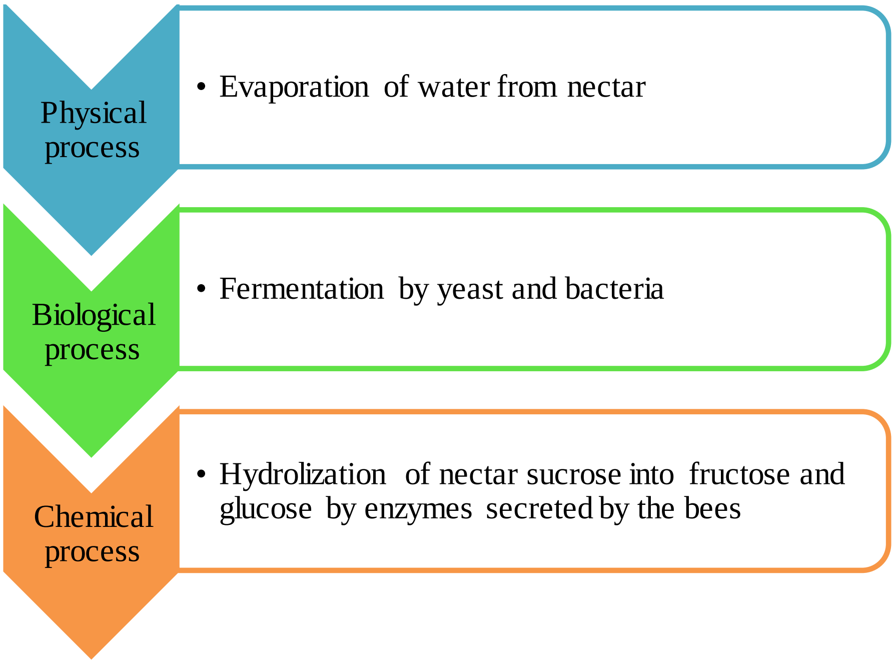

Figure 2.4 Transformation process of nectar into honey

SBH contains approximately 200 substances (Zulkhairi Amin et al., 2019). The distinctive sweetness and acidic flavor of SBH are well known (Abd Jalil et al., 2017; Biluca et al., 2016). It has a distinctive color, aroma, viscosity and taste and also has more fluidic texture and a slower crystallization process as compared to other types of honey such as Manuka, Gelam and Tualang honey (Zulkifli et al., 2018). Besides, it is also reported that SBH has high water content and acidity due to the presence of organic acids and minerals, when compared to honey produced by stinging bees (Sahlan et al., 2020). Ranneh et al., (2019) also stated that SBH has been found to be rich in polyphenols as compared to other types of honey. The composition of SBH is complex and varies depending on the geographical and botanical origin of nectar or honeydew. Thus, these affects in the varieties quality of honey produced, which then influenced their bio-medicinal properties.

2.3.1 Nutritional and chemical constituents of stingless bee honey

2.3.1(a) Moisture content

Moisture content is an important criteria in determining the quality of honey as it influences a number of its properties including viscosity, specific weight, flavor, maturity and crystallization (Alfredo et al., 2015). Different factors may affect the moisture content in honey including botanical and geographical origin of nectar, degree of maturation, season of harvesting, processing and storage conditions by beekeepers during harvesting period (De-melo et al., 2017). Honey with low moisture content are very difficult to be handled and processed due to its high viscosity (Shahira et al., 2021).

SBH has been reported to contain higher moisture content as compared to *Apis mellifera* honey (Sahlan et al., 2020). A study by Lim et al., (2019) reported that the moisture content of six SBH being tested was ranged from 27 to 31%. The result obtained was coincide with the specified range by Malaysian Standards (MS 2683:2017), which stated that the moisture content of unprocessed Malaysian SBH should not be more than 35%. It can be concluded that, although SBH have a high moisture content than that of *Apis mellifera* honey, it is still within a standard range as proposed by Malaysian SBH standard.

2.3.1(b) Sugar content

Fructose and glucose are said to be responsible for most of physical and nutritional characteristics of honey as they are the predominant sugars present in SBH (Rahman et al., 2014). According to studies, the ratio of fructose and glucose influence the crystallization process of SBH and affect its flavor (Escuredo et al., 2014; Johann et al., 2020). Besides, the glycemic index (GI) of fructose is reported to be low as compared to GI of sucrose (19 and 60; respectively) (Bantle, 2009). Compared to *Apis mellifera* honey, SBH has been reported to have less sugar content, which is less sweet and has higher moisture content (Islam et al., 2016; Shamsudin et al., 2019).

Besides fructose and glucose, a new novel disaccharide trehalulose was also found to be a major component of SBH from Malaysia, Australia and Brazil (Fletcher et al., 2020). It is a naturally occurring isomer of sucrose, but releases monosaccharides into the bloodstream at a significantly slower rate than sucrose does. As a result, this disaccharide is very advantageous as it has a low GI as well as a low insulinemic index. Trehalulose is also known as a potent antioxidant, which contributed to the beneficial health properties of SBH. Hence, SBH is suitable to be used as an antidiabetic agent.

2.3.1(c) Proteins and amino acids

The amino acids profile could be characterized by its botanical and geographical origins (Sakač et al., 2019). Pollen is believed as the main source of protein, but other animal and vegetal sources such as, fluid and nectar secretions of the salivary glands of honeybees, also can be the sources of protein and amino acids in honey (Ranneh et al., 2021). Based on the study by Shamsudin et., (2019), phenylalanine was found to be the most abundance amino acids found in SBH. However, different botanical origin and location may influenced the type and concentration of amino acids present in different type of SBH (Sun et al., 2017).

2.3.1(d) Organic acids

The major organic acid that can be found in all SBH samples is gluconic acid, a product of glucose oxidation by glucose oxidase, with the mean content ranging from 0.07 to 1.48 g/kg of honey (Shamsudin et al., 2019). The study reported that, honey produced by *Hetrotrigona itama* showed higher amounts of gluconic acid content as compared to those from *Geniotrigona thoracica*. Besides, other organic acids such as formic, acetic, citric, oxalic, lactic, malic, maleic and succinic acids also been detected but in such minor amount of them (Mato et al., 2003). The presence of these organic acids contributed to the acidic condition of honey (pH value between 3.2 and 4.5). Thus, the organic acids profile can act as useful biomarker to identify the originality of honey.

2.3.1(e) Polyphenolic compounds

Polyphenolic characterization is suitable in determining the differences of the floral origin of honey (Alvarez-suarez et al., 2014). Polyphenols in honey play a vital role in prevention and management of variety of illness which has been reported in previous studies (Talebi et al., 2020). These compounds possess panoramic advantageous indications to contain antioxidant effects, anti-inflammatory, antidiabetic and other numerous protecting activities as well as helps in scavenging free radicals (Chan et al., 2017).

Flavonoids and phenolic acids are the most abundant categories of phenolic compounds that can be found in SBH. When compared to honey made by *Apis mellifera,* SBH contains higher level of flavonoids and antioxidants. This is in accordance with a study by Shamsudin et al., (2019), which reported that the total phenolic content of SBH from *Heterotrigona itama* was higher than that of *Apis mellifera* honey. Another study by Alvarez-suarez et al., (2018) also reported that SBH from *Melipona beecheii* honey had better antioxidant capacity values as well as higher concentrations of phenolic compounds, flavonoids, carotenoids, ascorbic acid, proteins and free amino acids as compared to honey from *Apis mellifera*. Another type of SBH, Australian *Trigona carbonaria* honey was also examined, and it can be concluded that it has a stronger antioxidant activity than that of European floral honey; even its capacity to scavenge free radicals was on par with samples of European honey (Oddo et al., 2008). As SBH increased the antioxidant activity higher than *Apis mellifera* honey, it is expected that SBH is the best honey to be used in treating diseases related to oxidative stress including diabetic wound and other complications (Souza et al., 2006).

2.3.1(f) Minerals

Minerals are essential in regulation of metabolic pathways in the living body. The chemical elements in honey can be divided into macro elements and micro elements or trace elements (Islam et al., 2016). The composition of these elements vary in different types of honey depending on the botanical and geographical origins of honey. The value of different minerals in honey is dependent on the soil composition as the minerals are transported into plants through the roots and shifted to the nectar before producing honey (Shahira et al., 2021). Although their present in honey as a minor constituents, they play a vital role in determining the quality of honey.

SBH has been reported to have a higher mineral content as compared to *Apis mellifera* (Kek et al., 2018). The variations in mineral concentration in SBH may be due to the materials that bees collect while feeding on plants, to the irregularities in beekeeping and harvesting practices, as well as the fact that different types of SBH derive from various botanical sources (Chan et al., 2017; Shamsudin et al., 2019). Potassium (K^+) is believed as the most abundance element in SBH, followed by calcium (Ca^{2+}) and magnesium (Mg^{2+}) (Abu Bakar et al., 2017; Biluca et al., 2016). These mineral are important in promoting insulin function (Kumar et al., 2019).

2.3.2 Beneficial therapeutic effects of stingless bee honey

It has been suggested that honey can be used as a transporting vehicle for plant medicinal properties as many of them can be transported into honey. It has been used as remedies centuries ago and still being include in both traditional and modern medical practices. Most of the biological properties of honey are attributed to the presence of their polyphenols, such as flavonoids. SBH possess several beneficial effects under pathological conditions, rendering it a promising functional food with antidiabetic, antioxidant, anti-inflammatory and wound healing properties (Ranneh et al., 2019). Figure 2.5 showed the beneficial therapeutics effects of SBH consumptions and their protection against diseases.

Figure 2.5 Beneficial therapeutic effects of SBH consumptions

2.3.2(a) SBH and antidiabetic properties

DM is a chronic, widespread illness that affects people and its prevalence has been increasing steadily all over the world (Okonkwo & Dipietro, 2017). Among the therapeutic property of SBH is antidiabetic. α-amylase and α-glucosidase enzyme inhibition assays were used to examine the antidiabetic effects of honey because these enzymes are essential for the breakdown of complex sugar molecules into simple sugar in the brush border epithelium of the intestine. Trigona honey was shown to have the highest percentage of inhibition against α-amylase and α-glucosidase enzymes as the present of polyphenols, such as quercetin and luteolin in honey helps in treating diabetes by lowering the amount of BG level (Aziz et al., 2017; Krishnasree & Mary Ukkuru, 2017).

Erejuwa et al., (2016), stated that daily intake of 1.0 and 2.0 g/kg BW of Nigerian honey for three weeks give a better effect on FBG, improvement of lipid profiles and other metabolic parameters in diabetic rats induced with alloxan. In addition, based on previous study, SBH administration to diabetic male rats also give a positive effects in preventing the risen in FBG, TC, TG and also lowering the LDL-cholesterol levels (Aziz et al., 2017). The study also reported that SBH could account for its antidiabetic properties as it has a great potential in protecting the pancreas against damage and dysfunction.

2.3.2(b) Oxidative stress and antioxidant properties of SBH

Oxidative stress is one of the occurrence that happened under diabetic condition (Waer & Helmy, 2012). It generally refers to an overproduction of ROS which can assault various tissue damages in patients with diabetic as it play an important role in the pathogenesis of DM and diabetic complications (Carolo et al., 2018; Kaur et al., 2016;