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A PRELIMINARY STUDY ON THE EFFECTS OF STINGLESS BEE HONEY (SBH) ON FASTING BLOOD GLUCOSE IN STREPTOZOTOCIN (STZ)-INDUCED DIABETIC RAT MODELS

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ABSTRACT

Diabetes is a metabolic disorder characterized by hyperglycemia due to defects in insulin secretion, insulin action, or both. SBH has good antihyperglycemic potential and has traditionally been used as an alternative treatment for diabetes mellitus (DM). Since the role of SBH in glucose control is still unclear in animal and human studies, the present study was designed to evaluate the antihyperglycemic effects of SBH in streptozotocin-induced diabetic rat models. Fifteen Sprague-Dawley rats (200-250 g) were equally divided into five groups, the first group being a normal (non-diabetic) rat and the other four groups being diabetic. The normal and untreated diabetic groups received normal saline while the other diabetic groups were treated with SBH (2 g/kg body weight), metformin (MET /250 mg/kg body weight) and SBH + MET respectively. The treatment was given within 12 days. Fasting blood glucose (FBG) was measured at baseline and every two weeks thereafter. On days 7 and 12, SBH significantly lowered FBG, comparable to the normal group (p<0.05). In the group treated with MET and the combination of SBH-MET, FBG improved only on the 12th day of treatment (p<0.05). The results show that a single SBH treatment is effective in lowering blood glucose levels. Thus, SBH could be of great value in the treatment of diabetes in humans.

Key words: Antihyperglycemic, diabetes mellitus, fasting blood glucose, Stingless bee honey, STZ-induced diabetic rats

INTRODUCTION

Worldwide, there has been an increase in the incidence of diabetes mellitus (DM) in recent years. In all countries, including Malaysia, DM is a serious public health problem. It is a category of metabolic disorders characterized by hyperglycemia due to defects in insulin secretion, insulin action or both (Dineshkumar *et al*., 2010; Martin *et al*., 2014). DM is a global public health problem, affecting an estimated 285 million people worldwide in 2010 (Sahlan *et al*., 2020). It is predicted that the overall number of people with diabetes will explode to 439 million in 2030, with the majority of new cases in developed countries expected to appear (Nain *et al*., 2012; Sahlan *et al*., 2020). The most prevalent form

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of diabetes is type 2 diabetes mellitus (T2DM) which is also known as noninsulin-dependent diabetes mellitus (NIDDM), responsible for 90% of the disease prevalence (Mahmoud *et al*., 2012). It is considered a complex disease caused by insulin resistance associated with impaired function of β-cells as the body's cells cannot utilize the glucose properly (Aizzat *et al*., 2010; Gonzalez *et al*., 2011). According to estimates, 8 billion people around the world will be suffering from type 2 diabetes by 2025 (Dong *et al*., 2014). This condition leads to DM remains as a leading cause of morbidity and mortality worldwide, although different forms of antidiabetic agents are currently available (Naskar *et al*., 2011; Park *et al*., 2020; Takım, 2021). Since DM needs a lifelong treatment and the economic burden of patients should be taken into accounts, a search for alternative treatment is highly demanded.

Honey has been known for its therapeutic values since a long time ago. It has been studied against various ailments in animal and human models and was denoted as a novel antioxidant agent as it contains a variety of phytochemicals with high flavonoids and phenolic content (Rao *et al*., 2016; Mohamad *et al*., 2019). Numerous medicinal benefits such as antimicrobial, antioxidant, anti-inflammatory, anticancer, antihyperlipidemic, antidiabetic, and cardio-protective properties have been recorded to be demonstrated by honey (Shadan *et al*., 2018). However, the composition of honey depends primarily on its floral source, seasonal, and environmental factors (Abu Bakar *et al*., 2017; Nweze *et al*., 2017). Therefore, different varieties of honey may exhibit different health-promoting properties.

The *Trigona* sp. of stingless bee, known as Kelulut, is a stingless bee species found in Malaysia. It has been determined to have a medicinal value and is widely used as it is considered to yield a better performance than other honey bees honey (Rao *et al*., 2016; Mohamad *et al*., 2019). Stingless bees are a broad monophyletic class of highly eusocial bees widely found throughout the globe in abundance in warm humid forests. They belong to the Apidae family, the Apinae subfamily, and the Meliponini tribe (Shamsudin *et al*., 2019). The most abundant species found in the southern part of Malaysia is *Heterotrigona itama* (Akhir *et al*., 2017; Shamsudin *et al*., 2019; Abdul Malik *et al*., 2020). The biological properties that make it ideal as a medicine are antibacterial, anti-inflammatory, healing effects of wound and sunburn, antioxidant activity and antimicrobial activity (Nweze *et al*., 2017). Other findings have proven that this natural product does not only exhibit antioxidant properties but also can act as an anti-inflammatory, antidiabetic, antimicrobial agent and among of the best wound healers available in nature (Zulkhairi Amin *et al*., 2019). Since the role of SBH in controlling glucose levels is still controversial in animal and human studies, this study aimed to determine the anti-hyperglycemic effects of SBH on fasting blood glucose levels in STZ-induced diabetic rat models.

MATERIALS AND METHODS

Chemicals and reagents

Chemicals and reagents used in this research include streptozotocin (Sigma-Aldrich), used to induce diabetic and metformin, act as a standard drug.

The Na-citrate buffer solution (0.1 M) used in this experiment was prepared as follows: 50 mL of distilled water was prepared in a suitable container. Na-citrate buffer was prepared by dissolving 0.54 g of Citric Acid and 0.71 g of Sodium Citrate into the solution, followed by adjusting the pH to 4.5 using HCL or NaOH.

Preparation of Stingless Bee Honey (SBH)

Fresh stingless bee honey from *Heterotrigona itama* species was extracted from three stingless bee hives in Kelantan state on 2 March 2020. All samples were protected from direct sunlight and stored at 4°C. Prior to dilution, the SBH was allowed to be at room temperature and freshly prepared with normal saline (2.0 g/kg body weight) each time it was administered. The dose (2.0 g/kg body weight) was chosen based on previous study (Mustafa *et al*., 2019; Sahlan *et al*., 2020). MET (250 mg/kg body weight) was dissolved in distilled water just before oral administration on each day.

Animals

The experimental animals in this study were eight to ten weeks-old male Sprague-Dawley rats, at a range weight of 200-250 g. They were purchased from Animal Research and Service Centre (ARASC), Universiti Sains Malaysia, Health Campus, Kubang Kerian. All rats were housed in plastic cages and maintained under standard laboratory conditions (21 ± 2°C) with alternating cycles of 12 h light and dark. They were allowed free access to normal standard rat pellet diet (10%/kg BW) supplied by ARASC USM and water *ad libitum*. The rats were acclimatized to laboratory conditions for one week before commencement of the experiment. All procedures performed in this study were in accordance with the Institutional Guidelines for the Care and Use of Animals for Scientific Purposes and were approved by the Institutional Animal Care and Use Committee USM, Kubang Kerian (USM/IACUC/ 2019/ (120) (1020)).

Induction of diabetes

After an overnight fasting period, blood was drawn using the tail- prick technique. URight blood glucometers were used to measure fasting blood glucose in rats. First, the test strip was inserted into the groove of the URight blood glucometer. Then, about 0.05 to 0.01 mL of blood was collected from the tail vein of the rats. The collected venous blood was then transferred to the detection area of the test strip. This procedure was performed at baseline, day 7 of STZ injection, and day 13 before the animals were euthanized (Feng *et al*., 2018).

Experimental design

In the experiment, a total of fifteen rats were used. They were equally divided into five groups and were treated orally, once daily with normal saline, SBH, MET and a combination of SBH and MET for 12 consecutive days, 7 days after STZ injection (see Table 1).

Both SBH and MET were administered to the rats by oral gavage once daily until the end of the study. All groups of rats were maintained in standard environmental conditions and fed with a standard diet and water. Body weight, food and water intake were measured every two days during this study. Day 7 of STZ induction is designated as day 1 of treatment administration. The evaluation of fasting blood glucose was performed on day 13 after the initiation of treatment.

1.1 Determination of Fasting Blood Glucose Level

After an overnight fasting period, blood was drawn using the tail prick technique. URight blood glucometer was used to measure fasting blood glucose in rats. First, the test strip was inserted into the groove of the URight glucometer. Then, approximately 0.05 to 0.01 mL of blood was collected from the tail vein of the rats. The collected venous blood was then transferred to the detection area of the test strip. This process was performed at baseline, day 7 of STZ injection, and day 13 before the animals were euthanized (Feng *et al*., 2018).

1.2 Statistical analysis

All data in this study are expressed as mean ± standard errors of mean (SEM). One-way analysis of variance (ANOVA) was used for multiple comparisons among groups followed by Tukey's HSD. All analyses were carried out by SPSS, version 21 (Chicago, II, USA). Results were considered significant at $p<0.05$.

RESULTS AND DISCUSSIONS

Table 2 shows the effects of SBH, MET and the combination of SBH and MET on the fasting blood glucose levels in normal rats and STZ-induced diabetic rats. The data was collected at the baseline and biweekly thereafter. At the beginning of the study, during the pre-injection of STZ, there was no significant difference between each group of rats ($F = 1.083$, $p>0.05$). After STZ was administered, the data indicated that FBG level was significantly increased in the STZ-lesioned rats compared with the normal rats ($p<0.05$, n=3). Interestingly, the elevated blood glucose in diabetic rats was inhibited after receiving SBH after 7 days of treatment, exhibiting a statistically significant amelioration relative to the diabetic untreated ($p<0.05$, n=3), refer Table 3. Meanwhile, the blood glucose-lowering level in the MET-treated group and SBH-MET group were slightly lower than that in the SBH-treated group ($p<0.05$, n=3) but also have a significant difference as compared to the diabetic untreated rat

Table 1. Rat treatment groups

Group	Treatment
1	Normal rats + Normal saline
2	Diabetic untreated + Normal saline
3	Diabetic + SBH (2 g/kg BW)
4	Diabetic + MET (250 mg/kg BW)
5	Diabetic + SBH (2 g/kg BW) + MET (250 mg/kg BW)

Table 2. The effects of SBH, MET and the combination of SBH and MET on the fasting blood glucose levels in normal and diabetic group rats

Group (n=3)	Treatment	Fasting blood glucose (mmol/L)		
		Week of STZ injection		Euthanized
		Pre-injection	Day 7	Day 13
I	Normal	4.13 + 0.35	4.03 + 0.38	4.03 + 0.32
II	Diabetic untreated	3.83 + 0.23	6.25 + 0.55	28.65 + 1.76
III	SBH (2g/kg)	3.87 + 0.34	18.27 + 2.64	6.10 + 1.88
IV	MET (250mg/kg)	4.60 + 0.24	23.93 + 1.65	4.20 + 0.23
V	SBH (2g/kg)+ MET (250 mg/kg)	4.30 + 0.29	27.10 + 2.85	5.40 + 1.04

Values are expressed as mean + SEM (n = 3). Fasting blood glucose (FBG) of all samples is expressed in mmol/L.

Table 3. The effects of SBH, MET and the combination of SBH and MET on blood glucose levels in normal and diabetic group rats

Group (n=3)	Treatment	Blood glucose (mmol/L)		
		Week of STZ injection	Week of treatment	
		Day 3	Day 7	Day 12
I	Normal	5.10 + 0.21	5.10 + 0.31	4.77 + 0.18
II	Diabetic untreated	27.67 + 0.95	30.05 + 1.76	28.20 + 0.80
III	SBH (2g/kg)	20.27 + 4.32	15.90 + 5.45	20.37 + 7.25
IV	MET (250mg/kg)	a23.33 + 0.93	27.80 + 1.45	22.87 + 2.36
V	SBH (2g/kg)+ MET (250 mg/kg)	16.00 + 2.21	23.17 + 2.03	18.83 + 5.59

Values are expressed as mean + SEM (n = 3). Blood glucose of all samples is expressed in mmol/L.

group. After 12 days of treatment, all the diabetic rats that received SBH, MET or their combination had significantly reduced the fasting blood glucose concentrations compared with diabetic untreated group rats ($p<0.05$).

Honey has recently been recognised in several studies as an emerging novel antidiabetic agent. It could have the potential to act as a multi-targeted agent in patients with DM through its antioxidant, antimicrobial, immune modulator and anti-inflammatory effects. It is also known to decrease levels of FBG, increase levels of fasting C-peptides and decrease the glycaemic index (GI) (Sahlan *et al*., 2020).

The composition of SBH differs from other species due to some physiochemical parameters (Yaacob *et al*., 2018). Alongside water and sugars are the major contents of SBH, it also known to be rich in vitamins, amino acids and minerals with almost 200 different compounds were reported (Erturk *et al*., 2019). In most forms of honey, fructose is the key carbohydrate (Abu Bakar *et al*., 2017). Compared to carbohydrates with a high GI, carbohydrates with a low GI can cause a small increase in blood sugar levels. Potentially, fructose would trigger a lower risen of blood sugar as compared to sucrose as it has a lower GI than sucrose. A study from (Krishnasree & Mary Ukkuru, 2017) stated that SBH could be reported as the best honey for treating diabetics as the GI content was lower than other honey being analysed.

The most common group of polyphenols that exist in honey are flavonoids and phenolic acids (Ewnetu, Lemma, and Birhane 2013). Honey is known for its antioxidant activity. Prior research has shown that the total antioxidant activity of honey is primarily provided by its phenolic composition rather than other components (Ewnetu *et al*., 2013). Thus, SBH is believed to possess a stronger antioxidant activity as it has a higher phenolic content (Ewnetu *et al*., 2013; Kek *et al*., 2014). Therefore, SBH administration, compared to other sources of carbohydrates, can prevent the occurrence of hyperglycaemia and hyperinsulinemia.

When streptozotocin is injected into rats during the neonatal phase, it resembles human type 2 DM with regard to insulin secretory response abnormalities (Dewanjee *et al*., 2011). Mild hyperglycemia occurs between 2 and 3 months of life in this model, along with partial insulin deficiency. In the current study, the blood glucose level increased progressively after induction with STZ. This mechanism partially destroyed the beta cell's function and lead to an increase in the generation of reactive oxidative stress (ROS). Pancreatic β-cells regulate the blood glucose level in an animal's body, so any changes in this mechanism can lead to DM.

SBH could impede this mechanism as it has antioxidative properties. Administration of SBH to diabetic rats resulted in glucose reduction after daily treatment for 12 days. Within the first week of treatment, there was a significant decrease of FBG in STZ-induced diabetic rat models when be orally treated with SBH (2.0 g/kg body weight). This suggests that antioxidant properties from flavonoid and phenolic compounds that are present in SBH might be responsible for the observed antidiabetic effect of this honey. Our result was in line with a study from (Aziz *et al*., 2017; Sahlan *et al*., 2020), which reported that SBH reduced the FBG level in diabetic rat models.

To the best of our knowledge, this is the first study that reports the benefits of SBH in reducing FBG levels of type 2 diabetic rat models. However, the mechanism of SBH in suppressing other DM complications was unknown. Thus, these research gaps should be bridged in future research.

CONCLUSIONS

DM is a common chronic disease that can cause multiple complications if the glucose level is uncontrolled. Our present study demonstrated that

decreased FBG levels occur in diabetic rats treated with SBH at the early stage of observation. Thus, it can be said that SBH provides an antihyperglycemic effect to diabetic induced rats and it serves as a potential countermeasure of diabetic treatment. However, further studies are needed in human subjects to determine if these results can be appropriately extrapolated to human diabetes. This may result in the better and more efficient management of DM and its related complications.

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