Saudi Journal of Biological Sciences 27 (2020) 2025–2030

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

The Effects of stingless bee (*Tetragonula biroi*) honey on streptozotocin-induced diabetes mellitus in rats<!– Effects of stingless bee (*Tetragonula biroi*) honey on streptozotocin-induced diabetes mellitus in rats

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ARTICLE INFO

Article history:
Received 18 September 2019
Revised 12 November 2019
Accepted 26 November 2019
Available online 2 December 2019

Keywords:
Diabetes mellitus
Stingless bee honey
STZ-induced diabetic rats
Tetragonula biroi
T. laeviceps

ABSTRACT

Diabetes mellitus (DM) is a metabolic disease characterised by chronic hyperglycaemia with impaired carbohydrate, fat and protein metabolism caused by defects in insulin secretion or action. Based on our previous research, stingless bee honey (SLBH) from *Tetragonula biroi* and *T. laeviceps* can inhibit alpha-glucosidase activities. Therefore, the aim of the present study was to determine the effects of daily oral administration of SLBH on body weight (BW) and fasting blood glucose (FBG) levels of male rats with streptozotocin (STZ)-induced DM. Thirty-six male Sprague Dawley rats were divided into six groups of six rats each. One group of normal non-diabetic rats served as a positive control. The diabetic groups were intraperitoneally (i.p.) injected with STZ (50 mg/kg BW) for induction of DM and divided into five equal subgroups of six animals each: an untreated group as a negative control; a group treated with 0.6 mg/kg BW of glibenclamide as a positive control and three SLBN treatment groups that had daily oral administration of 0.5, 1.0 or 2.0 g/kg BW, respectively, for 35 days. The results showed that SLBH significantly reduced loss of BW in diabetic rats. FBG levels in diabetic rat blood, collected from the tail, were measured using Accu-Chek test strips. The FBG levels in diabetic rats that have oral administered intake with glibenclamide and SLBH were stable. There were no changes in serum FBG levels in SLBH-treated diabetic rats for 35 days. Pancreatic histopathology results from all groups showed no abnormalities or tissue damage in either diabetic or non-diabetic rats. The results of this study show that administration of SLBH reduced BW loss or improved BW of rats with STZ-induced DM. Meanwhile, the reduction in loss of BW that occurred in diabetic rats after 35 days of SLBH administration was the result of reduced formation of fats and proteins, which are broken down into energy. Further research is needed to determine the antidiabetic effects of honey from other stingless honeybee species.

1. Introduction

Diabetes mellitus (DM) is a metabolic disease having various aetiologies that is characterised by chronic hyperglycaemia with impaired carbohydrate, fat and protein metabolism caused by defects in insulin secretion or insulin action, or both, which result in long-term damage, dysfunction and failure of various organs.

DM is a global public health concern, having affected an estimated 285 million people worldwide in 2010. This is expected to increase to 439 million by 2030, with the majority of new cases

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2019.11.039
1319-562X/

expected to emerge in developing countries (Shaw et al., 2010). Although various types of antidiabetic agents are currently available, DM remains a leading cause of morbidity and mortality worldwide (Kokil et al., 2010; Roglic and Unwin, 2010).

Honey is typically consumed directly and has many uses in traditional medicine and functional food (Meo et al., 2017; Sahlan et al., 2019). For example, honey has been used to promote wound healing (Jull et al., 2008), has antimicrobial activities (Lusby et al., 2005), regulates food absorption in the intestine (Nemoseck et al., 2011), has anti-inflammatory and analgesic activities (Alzubier and Okechukwu, 2011), antibacterial and antioxidant activities (Sahlan et al., 2019), reduce cell proliferation (Shafira et al., 2019) and enhancement of overall sperm quality (Fakhrildin and Alsaadi, 2014).

Honey is produced by both honey bees and stingless bees (Apidae: Meliponinae) is comprised of stingless bees that form colonies that are much smaller than those of the European honey bee (*Apis mellifera*) (Arias et al., 2006). By comparison the honey produced by stinging bees such as *A. mellifera*, stingless bee honey (SLBH) has higher water content and is generally more acidic than honey produced by stinging bees (Vit et al., 2004).

Based on our previous research, stingless bee honey (SLBH) from *Tetragonula biroi* and *T. laeviceps* can inhibit alpha-glucosidase activities (Rahmawati et al., 2019). There are several types of SLBH that have different effects on various conditions because of the distinctive compositions. However, it is unclear whether SLBH from *T. biroi* conveys an antidiabetic effect. Therefore, the aim of the present study was to determine the effects of SLBH from *T. biroi* on streptozotocin (STZ)-induced DM in male rats.

2. Materials and methods

2.1. Animal

Male Sprague Dawley rats with a minimum body weight (BW) of 200 g were housed at 4–5 animals per cage and acclimatised for 14 days before experimentation. Each rat was fed as much as 15 g of standard rat chow per day under a 12-h light/dark cycle with access to water ad libitum. All animal protocols were approved by the Animal Ethics Commission of the Centre for Tropical Biopharmaca Studies, IPB University (Bogor Agricultural University), Bogor, West Java, Indonesia (No. 0007/2018 KEH TROP BRC).

2.2. Stingless bee honey (SLBH)

SLBH of *T. biroi* was collected from North Luwu District, South Sulawesi Province, Indonesia. The honey was stored in a closed container at room temperature.

2.3. Induction of DM

Rats were in fasting condition for about 16 h before treatment. DM was induced by intraperitoneal (i.p.) injection of STZ dissolved in 0.1 M citrate buffer (pH 4.5) at 45–50 mg/kg BW. At 48 h after induction of DM, fasting blood glucose (FBG) levels and BW were measured to assess the overall condition of the rats.

2.4. FBG levels and BW

FBG levels and BW were monitored every week. BW was measured using a digital scale and FBG levels were measured in blood collected from the tail vein after overnight fasting using Accu-Chek test strips (Roche Diabetes Care, Inc., Indianapolis, IN, USA). A FBG concentration of $\geq$216 mg/dL was considered a diabetic state.

2.5. Study design

Thirty-six male Sprague Dawley rats were divided into six groups of six rats each. The control group consisted of normal non-diabetic rats. For induction of DM, rats in the diabetic groups were injected i.p. with STZ at a concentration of 50 mg/kg BW. The diabetic rats were divided into the following five subgroups: untreated negative control group; positive control group consisting of rats treated with glibenclamide at 0.6 mg/kg BW and three diabetic groups of rats that received SLBH at doses of 0.5, 1.0 or 2.0 g/kg BW (groups D + 1, D + 2 and D + 3, respectively). SLBH and glibenclamide were given orally for 35 days. The SLBH dose administered in this study was based on that of previous studies involving the administration of SLBH to rats with STZ-induced DM (Aziz et al., 2017).

2.6. Histological preparation

All rats were sacrificed by sedation with a combination of ketamine and xylazine (80 and 10 mg/kg BW, respectively) and exsanguination. Afterwards, the pancreas was immediately removed and stored in formalin.

For histological analysis, tissue samples were dehydrated in ascending concentrations of alcohol (70%, 80%, 90%, 95% and 100%) for 1 h each, then rinsed with xylol solution three times for 1 h each. Afterwards, the samples were embedded in paraffin and the paraffin block was cut into sections of a thickness of 5 μm using a microtome. The samples were then mounted on a glass slide and stained with hematoxylin and eosin (H&E).

2.7. H&E staining

The embedded tissue samples were deparaffinised by rinsing with xylol solution (×3, 10 min each) and were rehydrated by immersing in decreasing concentrations of alcohol solution (100%, 95%, 90%, 80% and 70%; 5 min each) and rinsed with distilled water for 10 min. Afterwards, the tissue preparations were immersed in hematoxylin dye solution for approximately 2 min and rinsed again with running tap water, while being observed under a light microscope to determine the colour intensity. Following immersion in eosin solution for 2 min, the tissue samples were dehydrated using an ascending concentration of alcohol, rinsed again with xylol solution and mounted on a glass slide under a cover glass using Entellan® mounting medium (EMD Millipore Corporation, Billerica, MA, USA).

2.8. Statistical analysis

Data were analysed for normal distributions using the Shapiro–Wilk test. The Levene test was performed to assess the homogeneity of data. Data were compared using the Mann–Whitney *U* test, the Kruskal–Wallis rank test and one-way analysis of variance. A probability (p) value of >0.05 was considered statistically significant. Data are presented as the mean ± standard error of the mean (SEM).

3. Results

3.1. Changes in BW

Changes in BW among the groups are shown in Fig. 1. The BW of the non-diabetic rats increased after 35 days of treatment, while

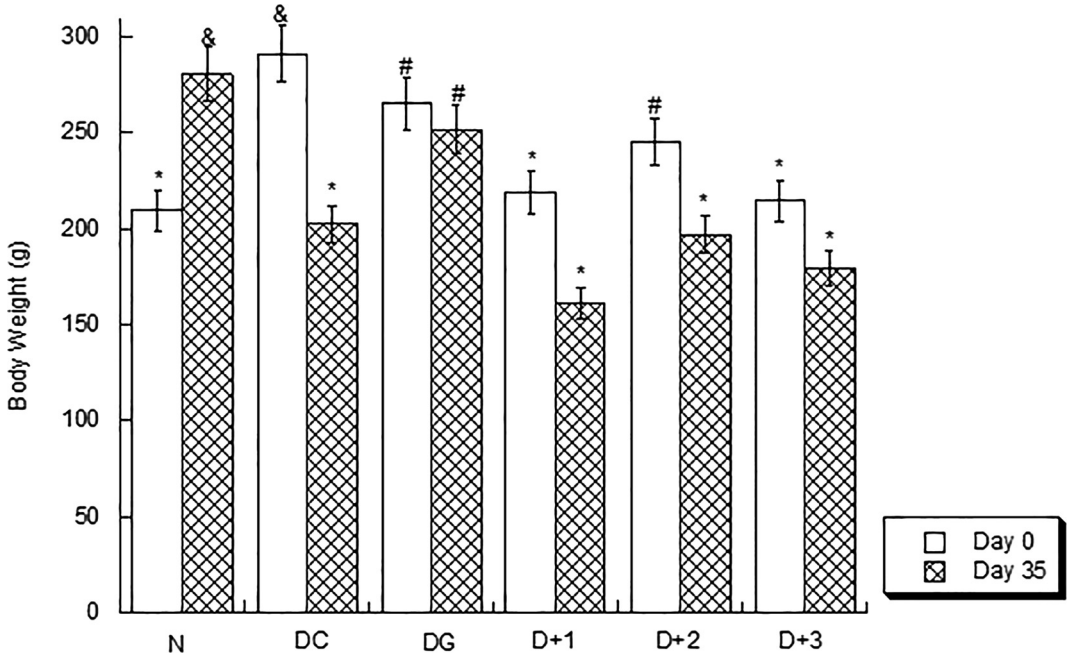

Fig. 1. Bar chart showing BW at days 0 and 35. Data were obtained from four rats in each group and are presented as the mean ± standard error of the mean (SEM). Bars with different symbols indicate significance at $p < .05$. Abbreviations: N, Group 1 (Normal, nondiabetic control); DC, Group 2 (Diabetic control); DG, Group 3 (Diabetic, treated with 0.6 mg/kg BW glibenclamide); D + 1, Group 4 (Diabetic, treated with 0.5 g/kg BW SLBH); D + 2, Group 5 (Diabetic, treated with 1 g/kg BW SLBH); D + 3, Group 6 (Diabetic, treated with 2 g/kg BW SLBH).

those of the diabetic rats decreased. The greatest reduction in BW occurred in the untreated diabetic rats (negative control group). Diabetic rats treated with glibenclamide (positive control group) experienced lower BW loss compared to other diabetic rats.

Overall, SLBH reduced BW loss in diabetic rats. Diabetic rats treated with SLBH at 0.5 g/kg BW experienced lower reductions in BW compared to the untreated diabetic rats. The diabetic rats treated with SLBH at 2 g/kg BW experienced the lowest reduction in BW among the three groups of diabetic rats. The reduction in BW that occurred in this group was close to that of the glibenclamide-treatment group.

3.2. Changes of FBG levels

All diabetic rats developed DM, as is evidenced by increased FGB levels. FBG levels were higher in diabetic rats compare to the non-diabetic rats. As shown in Fig. 2, the FBG levels of non-diabetic rats increased slightly every week, but were fluctuated in diabetic rats. On post-treatment day 7, the FBG levels of the diabetic rats decreased but increased again on day 14. In diabetic rats treated with glibenclamide, as well as those treated with SLBH at 1.0 g/kg BW, FBG levels were quite stable with no significant alter-

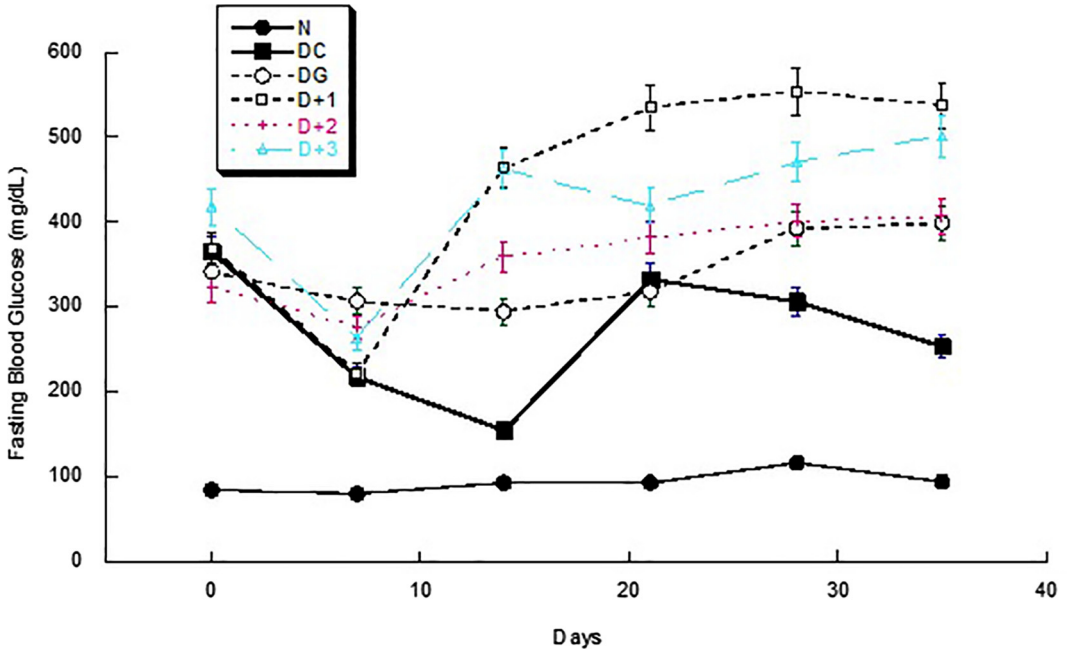

Fig. 2. Chart showing changes in glucose levels at day 0 to day 35. Data were obtained from four rats in each group and are presented as the mean ± standard error of the mean (SEM).

ation. There was no change after 35 days in the FBG serum levels in SLBH-treated diabetic rats as well.

3.3. Histopathology

The pancreatic histopathology results showed no abnormalities or organ damage in either the diabetic or non-diabetic rats (Fig. 3). There was no significant difference between the pancreatic histopathology of the untreated and treated diabetic rats.

4. Discussion

The results of the present study showed that SLBH from *T. biroi* had antidiabetic effects in rats with STZ-induced DM. STZ triggers DM in almost all species with either a single injection or with multiple low-dose injections (Junod et al., 1967). STZ-induced DM is the most widely used model of type II DM in research, as this type is common in humans (Bastai, 2005).

Honey has many biological, biochemical and physiological activities in animals and humans (Rao et al., 2016), and has not been shown in the present study to convey any good and beneficial effects regarding the management of DM. These afore-mentioned beneficial effects are in the form of better hyperglycaemic control, limiting the incidences of metabolic disturbances and preventing complications of DM (Rao et al., 2016). Honey (not specified from stingless bee species) is known to reduce FBG levels, increase fasting C-peptide levels and lower the glycaemic index (GI) (Bobiş et al., 2018). In fact, consumption of moderate amounts of honey (not specified from stingless honey bee species) has been reported to reduce blood glucose levels in rat models of both type I and II diabetes (Meo et al., 2017).

Fructose is the main carbohydrate in most types of honey (Fasanmade and Alabi, 2008). Carbohydrates with a low GI will trigger a slight increase in blood sugar level, as compared to those carbohydrates with a high GI. Fructose has a GI of 19, while that of sucrose is 68. Hence, fructose will theoretically trigger a lower rise

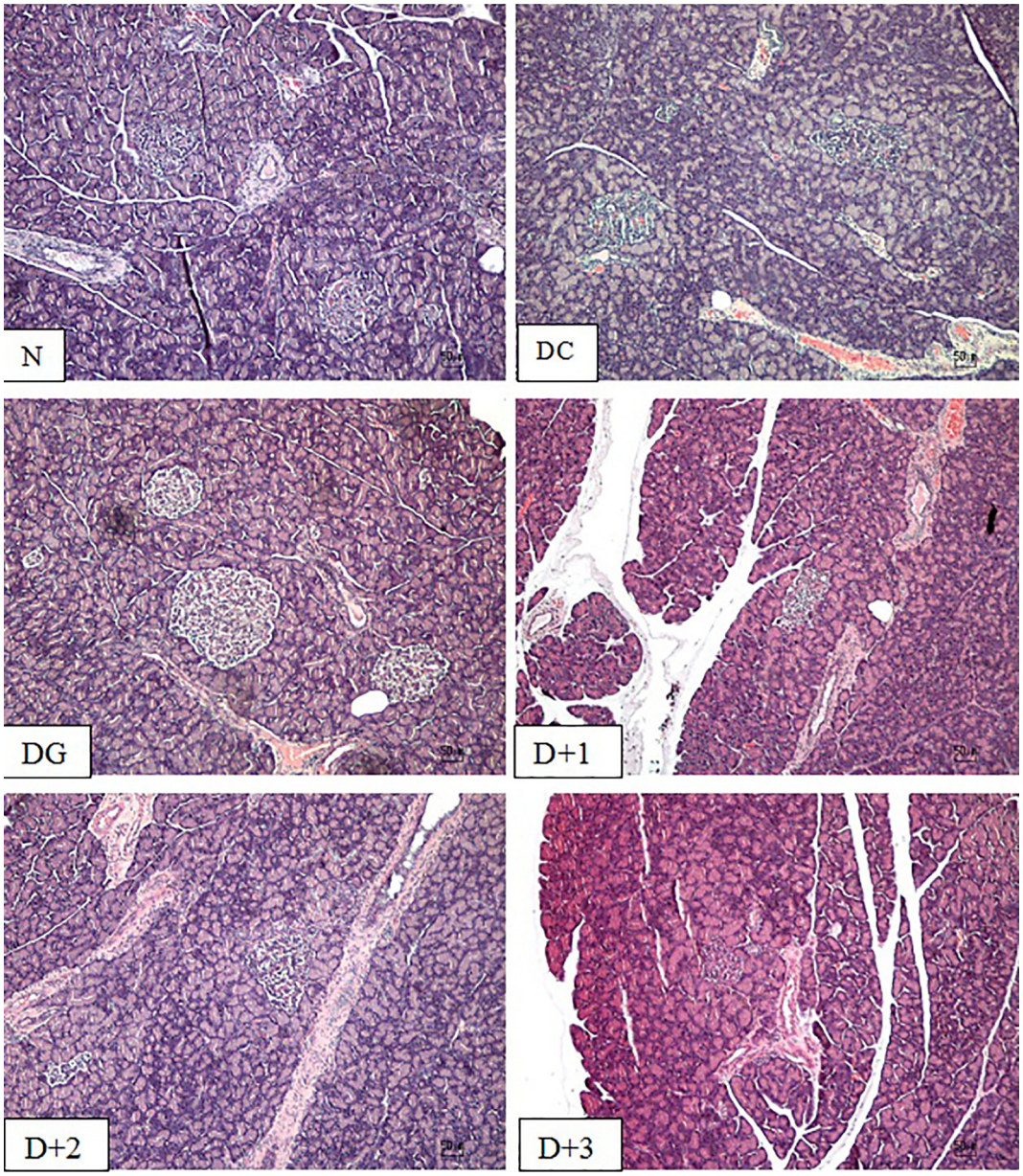

Fig. 3. Representative histological images showing the appearance of islets of Langerhans in different groups. Abbreviations: D + 1, D + 2 and D + 3, SLBH treatment groups (0.5, 1.0 or 2.0 g/kg BW, respectively); NC, negative control; PC, positive control.

in blood sugar than sucrose (Elliott et al., 2002). Honey, as compared to dextrose, was also shown to decrease glucose and insulin levels in normal subjects. Therefore, administration of honey can avoid the occurrence of hyperglycaemia and hyperinsulinemia, by comparison to other sources of carbohydrates (Foster-Powell et al., 2002).

Previous studies have shown that fructose tends to reduce blood sugar in animal models of diabetes (Al-Waili, 2004; Kwon et al., 2008), by triggering a reduction in sugar absorption in the intestine (Erejuwa et al., 2012) and reducing food intake (Kellett et al., 2008; Thibault, 1994). Small amounts of fructose can increase hepatic glucose uptake through a novel mechanism of glucokinase activation, promote glycogen rapid accumulation storage in the liver, increase triglyceride levels and also showed weight gain (Meirelles et al., 2011). The lack of glycogen storage in the liver triggers the release of stress hormones that can impair glucose metabolism, resulting in insulin resistance (Watford, 2002).

Data were analysed for normal distributions using the Shapiro–Wilk test, that the most powerful normality test, compared than the other test such Kolmogorov-smirnov, lilliefors and Andersondarling tests (Granato et al., 2014). In animal models of alloxan- and STZ-induced DM, administration of honey was shown to improve and reduce BW, respectively (Meo et al., 2017; Kwon et al., 2008). In the present study, administration of SLBH to non-diabetic rats had no effect on BW, demonstrating that SLBH does not trigger the formation of fat, which is generally the main cause of BW gain. Meanwhile, the reduction in BW loss in diabetic rats that occurred after 35 days of SLBH administration was the result of reduction in the formation of fats and proteins, which can be broken down into energy (Aziz et al., 2017). The combination of SLBH and glibenclamide, or metformin, was shown to increase BW in treated diabetic rats, by comparison with untreated diabetic rats (Wang et al., 2015).

Besides containing sugar (fructose and glucose) and water, SLBH also contains organic acids, phenolic compounds, proteins, amino acids, enzymes, vitamins and minerals (Erejuwa et al., 2011). The efficacy of honey is dependent on the content of phenolic compounds (Habib et al., 2014). Honey is a naturally produced substance that contains many phenolic compounds (Erejuwa et al., 2011). Flavonoids are generally the most common form of phenolic compounds produce by plants (Habib et al., 2014). Flavonoids in honey prevent oxidation from low-density lipoproteins and increase the amounts of high-density lipoprotein, (Wang and Li, 2011), while SLBH has been shown to reverse these activities in a rat model of DM (Aziz et al., 2017). Moreover, SLBH was found to reduce serum glucose, insulin and fructosamine levels in diabetic rats (Wang et al., 2015).

SLBH also contains many minerals, including potassium, calcium, sodium, magnesium and manganese (Biluca, 2016). Honey increases serum levels of zinc and copper, which are important for insulin and glucose metabolism (Fuhrman and Aviram, 2001), and improves glucose and lipid metabolism by increasing adiponectin levels and/or by reducing oxidative stress-mediated lipid peroxidation in a rat model of DM (Khalil et al., 2010). Meanwhile, administration of SLBH to normal rats had no effect on serum insulin levels, but resulted in higher levels of serum insulin in diabetic rats, by comparison with untreated diabetic rats (Aziz et al., 2017).

5. Conclusion

The results of the present study showed that the administration of SLBH reduced BW loss or improved BW of rats with STZ-induced DM. However, there were no changes in serum FBG levels in glibenclamide or SLBH-treated diabetic rats. Meanwhile, the reduction in BW loss in diabetic rats that occurred after 35 days of SLBH administration was the result of reduced production of fats and proteins, which can be broken down into energy. It can be concluded from this that honey from stingless bees can potentially reduce BW loss in DM patients. However, further studies are needed to determine the antidiabetic effects of honey from other stingless honeybee species.

Acknowledgements

This research is funded by Universitas Indonesia, Republic of Indonesia, under Grant of Hibah Q1Q2 (Grant No. NKB-0314/UN2.R3.1/HKP.05.00/2019), and the World Class Professor Programme Scheme – A (Grant No. T/41/D2.3/KK.04.05/2019) and World Class University (WCU) Program managed by Institute Teknologi Bandung, from the Ministry for Research, Technology and Higher Education (Republic of Indonesia) for partially financial support.

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