COMMUNICATIONS IN SCIENCE AND TECHNOLOGY
Homepage: cst.kipmi.or.id

Klanceng Honey (*Tetragonula laeviceps*): Its effect on fasting blood glucose, lipid and hematological profiles, and pancreatic histopathology of diabetic rats

Agussalim[a], Nafiatul Umami[a], Nurliyani[b], Wan Iryani Wan Ismail[c], Dewi Masyithoh[d], Ustadi[e], Ali Agus[a,*]

[a]*Department of Animal Nutrition and Feed Science, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia*
[b]*Department of Animal Products Technology, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia*
[c]*Cell Signaling and Biotechnology Research Group, Universiti Malaysia Terengganu, Terengganu 21030, Malaysia*
[d]*Faculty of Animal Husbandry, Universitas Islam Malang, Malang 65144, Indonesia*
[e]*PT. Kembang Joyo Sriwijaya, Malang 65152, Indonesia*

Article history:
Received: 15 August 2024 / Received in revised form: 10 November 2024 / Accepted: 7 Desember 2024

Abstract

This study aims to assess the daily ingestion of Klanceng Honey (KH) on fasting blood glucose (FBG), high-density lipoprotein (HDL), total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL), and pancreatic histopathology. Thirty rats were involved and divided into six groups: G1 = untreated normal rats, G2 = untreated diabetic rats (DR), G3 = DR treated with 0.9 mg/kg BW glibenclamide, G4, G5, and G6 = DR treated with 1, 2, and 3 g/kg BW KH, respectively for 28 days. The FBG level of G1 was found lower than that of diabetic rat groups (G2–G6) ($p<0.01$). The TC level of G1 group on 14th and 28th days was lower than that of G2–G6 groups ($p<0.01$). The TG levels in all rat groups showed no significant difference at 0–28 days. On 14th day, the LDL level of diabetic groups were higher than the one in G1 group ($p<0.01$). Meanwhile, on 14th and 28th, the HDL levels of G3–G6 groups, were higher than the one in G1 group ($p<0.01$). The pancreatic histopathology of all rat groups showed no abnormalities of the Langerhans islets. The daily ingestion of KH prevented FBG, TC, TG, and LDL levels from rising, increased the HDL levels, and protected the pancreatic glands against damage. *T. laeviceps* honey can potentially be used as a functional food for therapy of DM patients.

Keywords: Diabetes mellitus; fasting blood glucose; insulin; stingless bee honey; pancreas gland

1. Introduction

Diabetes mellitus (DM) is a metabolic disease characterized by the high chronic levels of blood glucose (hyperglycemia) and disorders of protein and lipids metabolisms, which are determined by abnormal insulin secretion or action [1,2]. This disease occurs when the fasting plasma glucose is at ≥126 mg/dL [1]. One of the impacts of DM on humans is the development of blindness, caused by the damage in the retinal blood vessels or diabetic retinopathy [3].

Honey is known not only as a traditional functional food but also as medicine for human health improvement. It is consumed fresh by humans due to its rich in nutrients and contains sugars (mainly glucose, sucrose, fructose, and trehalulose), proteins (enzymes and amino acids), organic acids, phenolic compounds (flavonoids and phenolic acid), minerals, vitamins, and volatile compounds [4]. Honey can be produced by both honeybees and stingless bees around the world. In Indonesia, the stingless bee is known as Klanceng bee, with *Tetragonula laeviceps* as one of the most domesticated [5,6]. Klanceng bee mostly creates a nest in bamboo, sugar palm stalks, tree's trunk, and in the ground to produce honey, pot-pollen, and propolis [7–25].

The physicochemical properties of Klanceng honey (KH) from *T. laeviceps* in Indonesia (North Lombok, Magelang, Purworejo, Klaten, Gunungkidul, and Sleman) have been studied. These properties include protein (at least 17 amino acids), minerals (Ca, Mg, Cu, Na, Fe, K, Mn, Al, and Zn), vitamin C, sugars (glucose, fructose, sucrose, and reducing sugar), phenolics, flavonoids, antioxidant activity DPPH, and at least 6 organic acids [7–11,18,20,21,25]. The honey physicochemical properties from several species of KH from different countries (Malaysia, Australia, Brazil, Venezuela, Thailand, Costa Rica, Ecuador, and Tanzania) include sugars (fructose, glucose, sucrose, and trehalulose), flavonoid,

* Corresponding author.
Email: aliagus@ugm.ac.id
https://doi.org/10.21924/cst.9.2.2024.1503

phenolic, antioxidant activity, minerals, and amino acids have been studied [26–46]. KH physicochemical properties are more strongly determined by the origins and plant flowers as a feed source [8,25,37,47] and species [47].

Nutritional honey might be used as a functional food for diabetic patients' therapy. The honey produced by honeybees have been previously studied in diabetics in-vitro and preclinical and clinical research that overall revealed the decreasing the blood glucose, low density lipoprotein (LDL), very low-density lipoprotein (VLDL), total cholesterol (TC), triglyceride (TG), glycosylated hemoglobin (HbA1c), reduced body weight (BW) loss, increasing high density lipoprotein (HDL), and pancreas gland protected from damage [2,48–59]. However, inconsistent results also were found on honey produced by honeybee in which it had no impact on the change of hyperglycemia and hyperlipidemia [60,61].

KH contains trehalulose sugar, which is known more slowly release to blood compared to sucrose, to have low insulinemic index, low GI, high antioxidant activity, and to be acariogenic [39,43]. The information about the use of KH on preclinical and clinical diabetic studies, so far seems to be limited. A preclinical investigation by Aziz et al. [62], showed that the ingestion of KH from *Geniotrigona thoracica* can prevent a rise in fasting blood glucose (FBG), TC, LDL, and TG, but with the increasing levels of HDL and serum insulin rose. Sahlan et al. [63] observed that the oral treatment of KH from *T. biroi* for 35 days resulted in the stable FBG levels, reduced BW loss, and absence of abnormalities in the pancreatic gland of diabetic rat groups. In a clinical study, Rashid et al. [64] reported that the daily ingestion of 30 g Kelulut honey for 30 days showed no effect on FBG, TC, HDL, LDL, TG, blood pressure, and body mass index of patients with impaired fasting glucose.

KH from *T. laeviceps* used in our study has lower in sugars content (sucrose at 2.56–4.49% w/v, fructose at 7.79–22.92% w/w, glucose at 11.49–22.78% w/w, and reducing sugar at 44.07–60.14%) [11], rich in antioxidant (47.3–90.5%), phenolic (0.65–2.30% GAE/100 g), and flavonoid (0.21–1.00 mg QE/g) [8,10]. The preclinical studies on diabetic rats using KH from Gunungkidul, Indonesia has never been previously studied. This study, in turn, aims to assess the daily ingestion of KH on the BW, FBG, TC, TG, LDL, HDL, hematological profiles, and pancreatic histopathology of diabetic rats.

2. Materials and Methods

2.1. Animals, feed, and ethical clearance

Male rats (Wistar) aged 2–3 months with BWs 153–234 g were adapted for 14 days in individual cages (20 × 12 × 15 cm in size) at 21ºC and 70% humidity with 12 hours in a controlled light and dark cycles. This study used the American Institute of Nutrition-93 Maintenance (AIN-93M) [65] standard feed of 15 g/rat/day and ad libitum drinking water. The protocol for the study has received the approval of the Ethical Commission from the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Indonesia (0079/EC-FKH/Eks./2019).

2.2. DM induction

The DM induction, with minor modifications referred to previous method that has been reported by Sahlan et al. [63]. After 14 hours of fasting, rats were induced with intraperitoneal with the aid of injections using streptozotocin (STZ) of 40 mg/kg of BW, which was dissolved in 0.1 M citrate buffer with pH of 4.4. After three days of DM induction, blood was collected and centrifuged at 10,000 rpm within 5 min. Afterward, serum was collected to analyze the FBG levels. The FBG levels were measured by means of a GOD-PAP enzymatic photometric method using a reagent DiaSys kit based upon the protocols from the manufacturer (DiaSys Diagnostic Systems GmbH, Germany) to assess the condition of all rats. DM was induced when FBG ≥126 mg/dL [1]. Every week, the rats BWs were assessed using a digital scale.

2.3. Study design

A total of 30 rats were divided into six groups (G1–G6), each of which consisted of five rats. The study was designed as follows: G1 = untreated normal rats (positive control), G2 = untreated diabetic rats (negative control), G3 = diabetic rats treated with 0.9 mg/kg BW glibenclamide (positive control), G4–G6 = diabetic rats treated with 1, 2, and 3 g/kg BW KH, respectively. We here used the dosages of KH in the study based on previous study as reported by Sahlan et al. [63] for stingless bee honey in diabetic rats based on the daily human consumption for honey, i.e. 90–100 g/day converted to the rats consumption of 1–3 g/kg BW [8,54,66]. All treatments were administrated for 28 days. Fresh KH (*T. laeviceps*) from Nglipar Gunungkidul farm was harvested the day before the treatment and stored at 5ºC. Fresh honey and glibenclamide were administrated to diabetic rats by oral gavage at 8 am before being fed with AIN-91M.

2.4. FBG level

Blood was collected thrice from all rat groups, that is, on day 0 (before treatment), day 14 (14 days after being treated with KH), and day 28 (28 days after being treated with KH) for the analyses of FBG, lipid profiles, and hematological profiles. All rats were fasted for 14 hours, and blood was collected from the orbital sinus using a microhematocrit. The blood was centrifuged at 10,000 rpm for 5 minutes and serum was collected for the analyses of FBG, TC, TG, LDL, and HDL. Meanwhile, Eppendorf tubes containing the anticoagulant ethylenediamine tetra-acetic acid were used for the hematological profile analysis.

The FBG levels were measured using a glucose oxidase (GOD-PAP) enzymatic photometric method by means of a reagent DiaSys kit in accordance to the protocols of the manufacturer (DiaSys Diagnostic Systems GmbH, Germany). Meanwhile, the samples were prepared with the stock solutions. The blank (10 µl aquadest + 1,000 µl reagent), standard 100 mg (10 µl aquadest + 1,000 µl reagent), samples (10 µl serum + 1,000 µl reagent), and solutions were mixed using a vortex. Furthermore, the solutions were incubated at 20–25ºC for 20 minutes in a dark condition. The FBG level

was measured using Microlab 300 at 540 nm and calculated by:
FBG level (mg/dL) = (Δ samples/Δ standard) × standard concentration/cal (mg/dL).

2.5. Blood lipid profiles

The serum was used in lipid profiles analyses (TC, TG, HDL, and LDL) using an enzymatic photometric method. A reagent DiaSys kit was used in accordance with the manufacture's protocols (DiaSys Diagnostic Systems GmbH, Germany). The CHOD-PAP kit (DiaSys Diagnostic Systems GmbH, Germany) was used for TC analysis, glycerol-3-phosphate-oxidase TG kit, DyaSis) for TG, HDL precipitant for HDL, and LDL precipitant for LDL.

2.5.1. TC level

For TC level analysis, the serum was prepared by preparing stock solutions which were blank (10 µl aquadest + 1,000 µl reagent), 100 mg/dL standard (10 µl standard + 1,000 µl reagent), and sample (10 µl serum + 1,000 µl reagent). Furthermore, the solutions were mixed and incubated for 20 min at 20–25ºC. The TC level was measured by means of a Microlab 300 at 500 nm and calculated by:
TC level (mg/dL) = (Δ samples/Δ standard) × standard concentration/cal (mg/dL)

2.5.2. TG level

For TG level analysis, the serum was prepared by preparing stock solutions which were blank (10 µl aquadest + 1,000 µl reagent), 200 mg/dL standard (10 µl standard + 1,000 µl reagent), and sample (10 µl serum + 1,000 µl reagent). Furthermore, the solutions were mixed and incubated for 30 min at 20–25ºC. The TG level was measured by means of a Microlab 300 at 500 nm and calculated by:
TG level (mg/dL) = (Δ samples/Δ standard) × standard concentration/cal (mg/dL).

2.5.3. HDL level

For precipitation, the serum was prepared as follows: 100 µl serum and 250 µl HDL precipitant solution were added in tubes, mixed, and incubated at 20–25ºC for 10 min. Furthermore, the solution was centrifuged at 12,000 rpm for 2 min and the supernatant was used to determine the HDL level. Afterward, stock solutions were made using the blank (100 µl aquadest + 1,000 µl reagent), 200 mg/dL standard (100 µl standard + 1,000 µl reagent), and sample (100 µl serum + 1,000 µl reagent). The solutions were mixed and incubated for 10 min at 20–25ºC. The HDL level was measured by means of a Microlab 300 at 500 nm and calculated by:
HDL level (mg/dL) = (Δ samples/Δ standard) × standard concentration/cal (mg/dL).

2.5.4. LDL level

For precipitation, the serum was prepared as follows: 50 µl serum and 500 µl LDL precipitant solution were added in tubes, mixed, and incubated for 15 min at 20–25ºC. Then, the solution was centrifuged at 12,000 rpm for 2 min and the supernatant was used to determine the LDL level. The stock solutions were prepared using the blank (100 µl aquadest + 100 µl standard + 1,000 µl reagent), standard (10 µl standard + 9 g/L NaCl + 1,000 µl reagent), and sample (100 µl supernatant + 1,000 µl reagent). The solutions were mixed and incubated for 10 min at 20–25ºC. The LDL level was measured by means of a Microlab 300 at 500 nm and calculated by:
LDL precipitant level (mg/dL) = (Δ samples/Δ standard) × standard concentration/cal (mg/dL)
LDL level (mg/dL) = cholesterol level–LDL precipitant level.

2.6. Hematological profiles

Hematological profiles were measured using an automated hematology analyzer (Sysmex model KX-21, Japan). The blood samples were homogenized and placed under an aspiration probe and the start switch was pressed. Meanwhile, the hematological profiles included hemoglobin (HGB), white blood cell (WBC), mean corpuscular volume (MCV), hematocrit (HCT), red blood cell (RBC), platelet (PLT), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), neutrophil (NEUT), lymphocytes (LYM), red cell distribution width (RDW), mean platelet volume (MPV), and platelet large cell ratio (P-LCR).

2.7. Deparaffinization process

All rats were injected intraperitoneally with 10% ketamine at a dose of 0.3 ml/rat, and they were sacrificed after 5 min. Afterward, the rats' abdomen was opened to remove the pancreatic glands, which were placed in tubes containing 10% neutral buffered formalin. The pancreas were dehydrated for 1 h each using different concentrations of alcohol (50%, 60%, 70%, 80%, 90%, and 100%). It was followed by rinsing thrice using a xylol solution for 1 h. Furthermore, the pancreas were embedded in paraffin and cut using a microtome into 5 µm of the thickness. Finally, the samples were stained by hematoxylin and eosin on glass slides [63].

2.8. Hematoxylin eosin staining

The deparaffinized embedded tissues were rinsed thrice with a xylol solution for 10 min each, rehydrated using alcohol solutions (100%, 95%, 90%, 80%, and 70% for 5 min on each) and then rinsed using an aquadest for 10 min. Furthermore, the preparate tissues were drowned in 75 ml of hematoxylin for 3–5 min and rinsed thrice by aquadest for 1 min each. Next, the tissues were immersed in eosin solution for 1–2 min, rehydrated using alcohol solutions (100%, 95%, 90%, 80% and 70% for 5 min on each), and rinsed again using a xylol solution for 3 min on each. Finally, the tissues were mounted on glass slides and the islet of Langerhans were imaged by means of an Optilab microscope camera [63].

2.9. Statistical analysis

The data of BW, FBG, TC, TG, LDL, HDL, and hematological profiles were analyzed by covariate analysis followed by post-hoc Tukey's HSD test (SAS studio version

3.8). The pancreatic histopathology was performed a descriptive analysis. All data were presented as the mean ± standard error of the mean (SEM).

3. Results and Discussion

3.1. Rats BW

The results showed that the BWs and the BWs increment of the G1 group were higher than those of diabetic rat groups (G2–G6) ($p<0.01$) (Table 1). The BWs of the diabetic rat groups (G2–G6) on day 7 days decreased and increased again on day 14–28 days, but the G1 group steadily increased. In the G1–G6 groups, the BW showed the increments of 52.57, 10.74, 26.94, 41.80, 11.35, and 24.79 g/rat, respectively, compared to day 0 (Table 1). Based on the increments, the data revealed that the G2 and G5 groups were lower than that of the G1, G3, G4, and G6 groups, respectively (Table 1). Overall, the administration of KH on diabetic rats ameliorated the BWs.

Table 1. The BW of normal and diabetic rat groups treated with KH and glibenclamide for 28 days

Observation times	Body weight (g/7 days)						*p* value
	G1	G2	G3	G4	G5	G6	
0 day	260.64±2.67[b]	195.78±3.11[a]	181.19±9.61[a]	168.84±3.90[a]	184.98±6.77[a]	198.13±7.63[a]	<0.001
7 days	275.43±3.77[b]	181.81±4.61[a]	177.93±9.45[a]	167.49±7.78[a]	175.31±7.73[a]	195.19±8.41[a]	<0.001
14 days	289.08±6.54[b]	190.30±7.05[a]	187.42±9.38[a]	182.74±10.16[a]	181.20±7.18[a]	202.57±10.93[a]	<0.001
21 days	303.01±9.32[b]	200.62±8.73[a]	199.60±9.73[a]	199.63±11.72[a]	190.58±9.66[a]	217.11±11.38[a]	<0.001
28 days	313.21±12.41[b]	206.52±9.56[a]	208.13±9.95[a]	210.64 ±12.22[a]	196.32±9.39[a]	222.91±12.36[a]	<0.001

[ab]Superscripts with different letters in the same row indicate significance at $p<0.01$. Abbreviations: G1 = untreated normal rats, G2 = untreated diabetic rats, G3 = diabetic rats treated with 0.9 mg/kg BW glibenclamide, G4, G5, and G6 = diabetic rats treated with 1, 2, and 3 g/kg BW KH, respectively.

3.2. FBG level

The G1 group presented a lower FBG level compared to diabetic rat groups (G2–G6) ($p<0.01$). However, no difference ($p>0.05$) was observed among the diabetic rat groups (G2–G6) (Fig. 1). The FBG level of G1 group decreased on day 0–28 but the one in G2 group increased in the same period. Furthermore, the FBG levels of G5 and G6 groups increased on day 14 but stabilized on day 28 after given with the treatment of KH. Interestingly, the FBG level of G4 group was found lower on day 14–28 after given treatment compared to G3 group. Interestingly, FBG level in the G1 and G5 group decreased (Fig. 1). These results indicated that the KH treatment prevented the raise of FBG level.

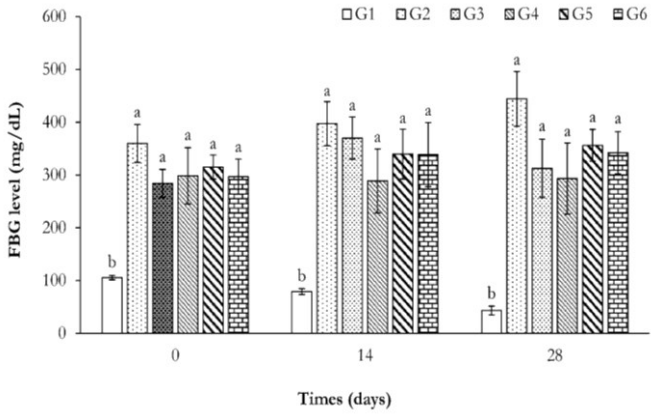

Fig. 1. The FBG levels of the normal and diabetic rat groups at day 0, 14, and 28 after given the treatment with KH and glibenclamide. Different letters (a and b) on the same day indicates significant at $p<0.01$

3.3. Blood lipid profiles

The TC level at day 0 did not differ ($p>0.05$) between G1 group (normal rats) and G2–G6 groups (diabetic rats). The G1 group exhibited a lower TC level on day 14 and 28 compared to G2–G6 groups ($p<0.01$) (Fig. 2A). Interestingly, 28 days after the treatment with 1 g/kg BW KH, the TC level of G4 group showed no difference with the one in the G1 group and was lower than that of G3 group. On day 28, the TC level in the G1, G4, and G6 groups showed a decrease by 12.48, 10.42, and 7.34 mg/dL, respectively compared to day 0 (Fig. 2A). These results indicated that KH can decrease the TC level of diabetic rats.

The TG levels in the G1 group (normal rats) and diabetic rat groups (G2–G6 groups) showed no significant difference on day 0, 14, and 28. The TG levels increased on day 14 for G3–G6 groups but decreased after 28 days when compared to day 0 (Fig. 2B). These results indicated that KH can prevent the TG level of diabetic rats from rising.

The LDL level on day 0 did not differ among rat groups (G1–G6). Moreover, after 14 days the treatment with KH and glibenclamide, the LDL level of diabetic groups (G2–G6) were found higher than that of G1 groups ($p<0.01$) (Fig. 2C). However, no difference at the LDL levels were observed on day 28 after given the treatment with KH and glibenclamide (Fig. 2C). These results indicated that the ingestion of KH by the diabetic rats prevented the rise in LDL levels.

The HDL level in the normal rats (G1 group) and diabetic rats (G2–G6 groups) showed no significance on day 0. However, on 14 and 28 days after the treatment with KH for G3–G6 groups, the HDL levels were found higher than that of the G1 group ($p<0.01$) (Fig. 2D). These findings revealed that the KH treatment can increase the HDL levels of diabetic rats.

3.4. Hematological profiles

For all rat groups (G1–G6), the hematological profiles on day 0 showed significant difference in terms of RDW, PDW, MPV, and P-LCR ($p<0.01$) but not in terms of RBC, WBC, HCT, HGB, MCH, MCV, MCHC, LYM, NEUT, and PLT

(Table 2). Furthermore, 14 days after the treatment with KH and glibenclamide, a significant difference was observed in RBC, MCV, and MCH ($p<0.05$) of all rat groups, but not in WBC, HGB, HCT, PLT, MCHC, NEUT, LYM, PDW, RDW, P-LCR, and MPV (Table 3). After 28 days of treatment with KH and glibenclamide significant differences were observed in RBC, HGB, HCT, MCV, and PDW ($p<0.05$) but had no effect on WBC, LYM, MCH, MCHC, NEUT, PLT, MPV, and RDW (Table 4).

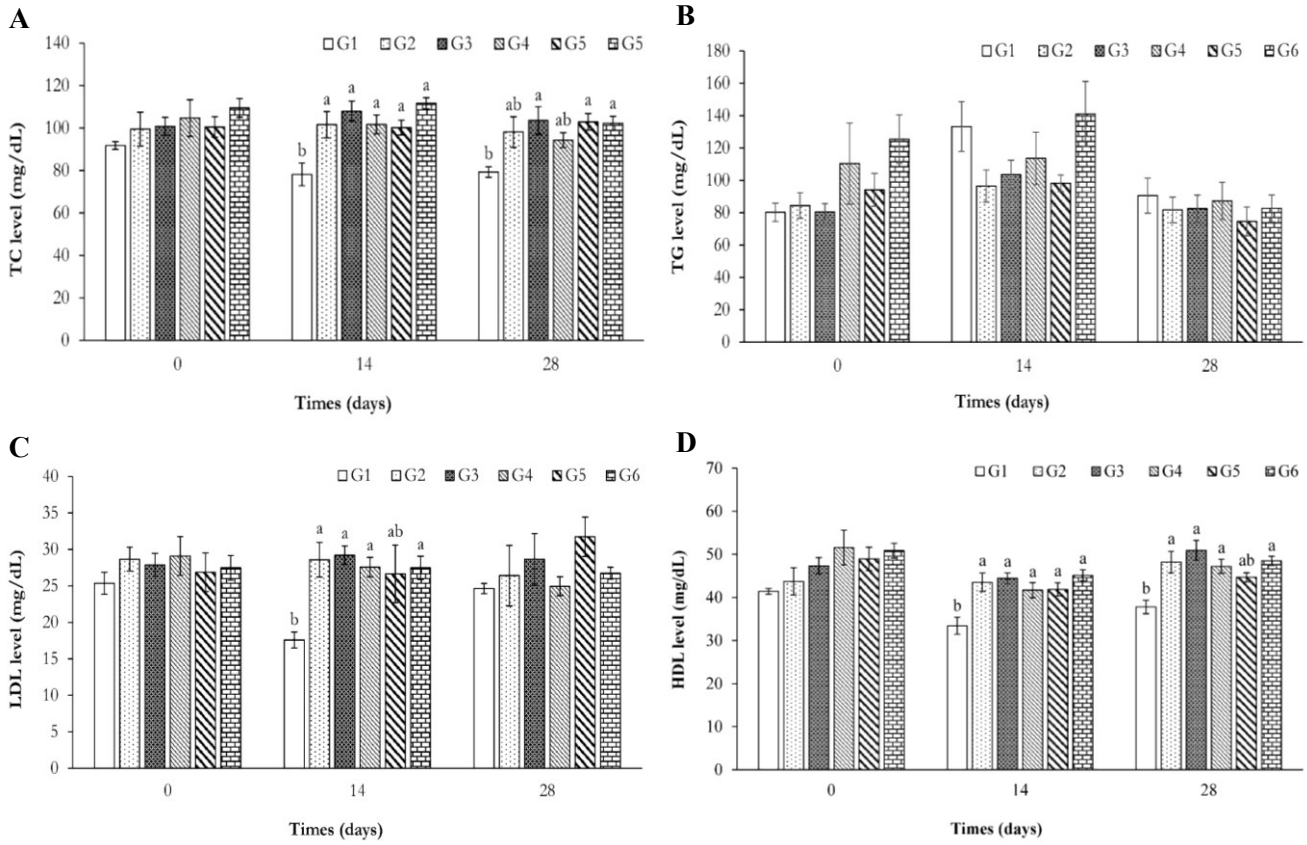

Fig. 2. The TC (A), TG (B), LDL (C), and HDL (D) levels of the normal and diabetic rat groups on day 0, 14, and 28 after given the treatment with KH and glibenclamide. Different letters (a and b) on the same day indicates significant at $p<0.01$. Abbreviations: G1 = untreated normal rats, G2 = untreated diabetic rats, G3 = diabetic rats treated with 0.9 mg/kg BW glibenclamide, G4, G5, and G6 = diabetic rats treated with 1, 2, and 3 g/kg BW KH, respectively

Table 2. Hematological profiles of normal and diabetic rat groups before the treatment with KH and glibenclamide on day 0

Parameters	G1	G2	G3	G4	G5	G6	*p* value
WBC (10^3/µL)	8.08±0.66	10.26±0.69	10.52±0.99	11.60±2.46	14.16±1.13	10.14±0.98	0.070
RBC (10^6/µL)	8.25±0.87	7.97±0.52	7.18±0.19	7.91±0.49	8.20±0.30	8.14±0.63	0.754
HGB (g/dL)	17.20±2.16	16.38±1.35	14.56±0.45	16.10±1.10	16.70±0.62	16.30±1.04	0.765
HCT (%)	51.08±5.03	49.06±3.10	45.12±1.06	48.72±2.90	50.90±1.75	50.28±3.68	0.787
MCV (fL)	62.10±0.53	61.64±0.41	62.86±0.30	61.64±0.26	62.12±0.51	61.90±0.68	0.485
MCH (pg)	20.70±0.64	20.50±0.40	20.28±0.39	20.34±0.31	20.38±0.33	20.22±1.14	0.995
MCHC (g/dL)	33.36±1.16	33.22±0.68	32.28±0.62	33.00±0.55	32.84±0.74	32.74±1.85	0.928
PLT (10^3/µL)	764.80±38.75	932.00±90.95	978.00±19.14	849.20±77.63	958.20±35.46	887.00±31.64	0.109
LYM (%)	74.18±2.47	82.82±1.67	79.42±5.04	82.20±2.23	73.36±7.48	80.66±2.41	0.479
NEUT (%)	25.82±2.47	17.18±1.67	20.58±5.04	17.80±2.23	26.64±7.83	19.34±2.41	0.479
LYM (10^3/µL)	6.04±0.57	8.52±0.65	8.22±0.51	9.38±1.72	10.26±1.19	8.10±0.67	0.102
NEUT (10^3/µL)	2.04±0.23	1.74±0.19	2.30±0.79	2.22±0.77	3.90±1.44	2.04±0.42	0.435
RDW (fL)	31.68±0.61^{b}	30.52±0.32^{ab}	29.40±0.34^{a}	30.6±0.30^{ab}	30.40±0.38^{ab}	29.68±0.44^{a}	0.010
PDW (fL)	8.26±0.08^{b}	9.04±0.15^{a}	9.24±0.09^{a}	9.22±0.07^{a}	9.22±0.19^{a}	8.98±0.25^{a}	<0.001
MPV (fL)	6.86±0.08^{b}	7.30±0.06^{a}	7.52±0.10^{a}	7.40±0.00^{a}	7.42±0.15^{a}	7.22±0.10^{ab}	<0.001
P-LCR (%)	7.18±0.36^{a}	9.28±0.33^{ab}	10.46±0.54^{b}	10.12±0.36^{b}	10.00±0.79^{b}	9.12±0.48^{ab}	<0.001

^{ab}Superscripts with different letters in the same row indicate significant at $p<0.01$

Table 3. Hematological profiles of normal and diabetic rat groups after the treatment with KH and glibenclamide for 14 days

Parameters	G1	G2	G3	G4	G5	G6	*p* value
WBC (10^3/µL)	5.52±0.50	6.12±0.86	6.38±0.97	5.86±0.52	5.90±0.46	5.62±1.05	0.970
RBC (10^6/µL)	7.38±0.12^{b}	8.00±0.19^{ab}	7.75±0.13^{ab}	8.04±0.11^{ab}	8.13±0.08^{a}	8.05±0.29^{ab}	0.038
HGB (g/dL)	14.76±0.27	15.36±0.37	14.76±0.20	14.96±0.13	15.12±0.18	15.20±0.42	0.600
HCT (%)	47.58±0.76	49.46±0.84	48.14±0.53	49.26±0.78	49.88±0.35	49.80±0.71	0.426
MCV (fL)	64.46±0.56^{b}	61.86±0.69^{a}	62.14±0.50^{a}	61.24±0.40^{a}	61.40±0.24^{a}	61.86±0.27^{a}	0.001
MCH (pg)	20.00±0.16^{b}	19.18±0.24^{ab}	19.06±0.23^{a}	18.60±0.11^{a}	18.62±0.26^{a}	18.90±0.14^{a}	<0.001
MCHC (g/dL)	31.04±0.25	31.04±0.33	30.68±0.31	30.38±0.22	30.32±0.32	30.54±0.26	0.328
PLT (10^3/µL)	925.20±34.32	800.20±52.60	877.60±61.98	807.80±64.25	863.80±48.50	830.60±12.95	0.389
LYM (%)	82.20±2.20	84.40±3.18	81.08±0.80	85.18±2.52	81.34±2.31	85.12±2.27	0.652
NEUT (%)	17.80±2.20	15.60±3.18	18.92±0.80	14.82±2.52	18.66±2.31	14.88±2.27	0.652
LYM (10^3/µL)	4.52±0.36	4.92±0.73	5.18±0.82	4.96±0.43	4.76±0.27	4.86±1.00	0.989
NEUT (10^3/µL)	1.00±0.20	0.88±0.20	1.20±0.15	0.90±0.16	1.14±0.22	0.76±0.07	0.488
RDW (fL)	35.20±1.05	33.14±0.47	32.66±0.57	34.86±0.86	34.92±0.97	33.06 ±0.36	0.084
PDW (fL)	8.32±0.22	8.32±0.27	8.38±0.16	8.96±0.29	8.44±0.22	8.20±0.28	0.335
MPV (fL)	7.08±0.12	7.02±0.17	7.14±0.14	7.08±0.29	7.04±0.14	7.02±0.15	0.996
P-LCR (%)	7.54±0.49	7.28±0.69	7.82±0.72	8.64±1.00	7.24±0.73	7.18±0.86	0.753

^{ab}Superscripts with different letters in the same row indicate significant at p<0.01

Table 4. Hematological profiles of normal and diabetic rat groups after the treatment with KH and glibenclamide for 28 days

Parameters	G1	G2	G3	G4	G5	G6	*p* value
WBC (10^3/µL)	6.10 ± 0.56	5.70 ± 0.51	6.70 ± 0.90	7.52 ± 0.50	4.84 ± 0.32	5.56 ± 0.71	0.071
RBC (10^6/µL)	7.50 ± 0.14^{a}	8.70 ± 0.49^{b}	8.02 ± 0.15^{ab}	8.44 ± 0.11^{ab}	8.19 ± 0.07^{ab}	8.12 ± 0.13^{ab}	0.026
HGB (g/dL)	15.26 ± 0.24^{a}	16.06 ± 0.30^{ab}	16.20 ± 0.21^{b}	16.44 ± 0.14^{b}	16.12 ± 0.13^{ab}	16.24 ± 0.15^{b}	0.009
HCT (%)	49.98 ± 0.70^{b}	57.00 ± 3.04^{a}	52.50 ± 0.97^{ab}	54.96 ± 0.35^{ab}	53.20 ± 0.71^{ab}	52.74 ± 0.55^{ab}	0.006
MCV (fL)	66.68 ± 0.60^{a}	65.58 ± 0.38^{b}	65.50 ± 0.39^{ab}	65.12 ± 0.53^{ab}	64.96 ± 0.39^{ab}	65.02 ± 0.49^{ab}	0.033
MCH (pg)	20.34 ± 0.13	18.74 ± 1.21	20.22 ± 0.24	19.48 ± 0.10	19.68 ± 0.23	20.02 ± 0.28	0.136
MCHC (g/dL)	30.52 ± 0.14	28.54 ± 1.73	30.88 ± 0.32	29.92 ± 0.21	30.34 ± 0.41	30.78 ± 0.27	0.319
PLT (10^3/µL)	962.20 ± 51.86	908.00 ± 51.22	956.20 ± 30.93	876.20 ± 36.10	945.00 ± 83.98	894.40 ± 17.70	0.287
LYM (%)	88.58 ± 0.77	83.12 ± 2.14	84.62 ± 2.71	87.40 ± 2.63	81.32 ± 4.51	89.46 ± 0.72	0.766
NEUT (%)	11.42 ± 0.77	16.88 ± 2.14	15.38 ± 2.71	12.60 ± 2.63	18.68 ± 4.51	10.54 ± 0.72	0.209
LYM (10^3/µL)	5.42 ± 0.54	4.74 ± 0.48	5.68 ± 0.82	6.54 ± 0.32	3.96 ± 0.40	4.96 ± 0.62	0.209
NEUT (10^3/µL)	0.68 ± 0.04	0.96 ± 0.12	1.02 ± 0.22	0.98 ± 0.26	0.88 ± 0.21	0.60 ± 0.10	0.057
RDW (fL)	34.52 ± 0.46	39.52 ± 0.65	39.28 ± 0.71	40.70 ± 0.82	39.70 ± 1.01	36.86 ± 1.15	0.451
PDW (fL)	9.26 ± 0.45^{a}	9.30 ± 0.48^{bc}	9.02 ± 0.30^{bc}	9.76 ± 0.30^{c}	8.62 ± 0.25^{bc}	8.96 ± 0.38^{ab}	0.000
MPV (fL)	7.66 ± 0.27	7.54 ± 0.23	7.48 ± 0.19	7.96 ± 0.17	7.22 ± 0.17	7.54 ± 0.21	0.399
P-LCR (%)	10.44 ± 1.66	10.16 ± 1.51	9.60 ± 1.15	12.16 ± 0.92	7.84 ± 0.81	9.80 ± 1.18	0.286

^{ab}Superscripts with different letters in the same row indicate significant at p<0.01

3.5. Pancreatic histopathology

The pancreatic histopathology of all rat groups showed no abnormalities of the islets of Langerhans (Fig. 3). The G1 group (positive control) exhibited the normal islets of Langerhans islets with normally distributed β-cells (Fig. 3G1). The G2 group (untreated diabetic rats, negative control) suffered from the serious losses of β-cells after inducement by STZ (Fig. 3G2). Furthermore, the diabetic rat groups treated with glibenclamide (positive control, Fig. 3 G3) and KH (Fig. 3; G4–G6) had normally distributed β-cells although few losses were observed. In addition, the Langerhans islets from KH (Fig. 3 G4–G6) were larger compared to untreated diabetic rat (Fig. 3 G2, negative control). These results indicated that ingestion KH could prevented the damage to pancreatic gland and may indicate the recovery of β-cells.

3.6. Discussion

DM is a metabolic disease marked by the high chronic levels of blood glucose (hyperglycemia) and caused by insulin deficiency or disorder in insulin action, affecting the impairment of the metabolism of proteins and lipids, making leading blood glucose levels higher [1,2]. It is a nutritional

functional food to improve human and animal models' health when used in clinical and pre-clinical studies, respectively. Honey contains sugars, proteins (enzymes and amino acids), flavonoid compounds (phenolic acid and flavonoids), vitamins, minerals, organic acids, and aromatic substances [4]. KH (*T. laeviceps*) from Indonesia has been studied for its physicochemical properties; it is characterized by low sugar contents (fructose at 4.82–22.92% w/w, glucose at 11.36–22.78% w/w, sucrose at 2.56–6.74% w/v, and reducing sugars at 40.90–60.14%) [11,21], high amounts of phenolics (0.65–2.30% GAE/100 g), flavonoids (0.21–1.00 mg QE/g), and antioxidants (47.3–90.5%) [8,10].

Furthermore, a previous study revealed that 17 amino acids such as proline, lysine, arginine, phenylalanine, histidine, leucine, isoleucine, methionine, tyrosine, valine, aspartic acid, serine, glutamic acids, glycine, threonine, alanine, and cysteine [18], and minerals such as Na, Ca, Mg, Cu, K, Zn, Fe, Al, and Mn can be found in the honey [20].

The findings of the study indicated that the ingestion of KH ameliorated several diabetic rat parameters that were induced with STZ. The diabetic rat groups (G4–G6 groups) that ingested KH showed an ameliorated and stable increment in the BW for 28 days. The findings are consistent with an earlier studied by Sahlan et al. [63] stating that KH (*T. biroi*) ingestion of 0.5, 1, and 2 g/kg BW could reduce BW loss in diabetic rat groups induced with STZ. Additionally, according to Aziz et al. [62], the consumption of 1 and 2 g/kg BW KH (*Geniotrigona thoracica*) can prevent the BW decrease in diabetic rats induced by nicotinamide and STZ. Gholami et al. [67] reported that jujube honey ingestion of 1 and 2 g/kg BW could increase the diabetic rats' BW induced with STZ. Erejuwa [2] stated, that the consumption of moderate fructose is associated with no change in BW however, the excessive doses of fructose may have an impact on weight gain.

Our study showed the fluctuating FBG levels in diabetic rat groups treated with KH (G4–G6 groups). However, the FBG level in G4 group was stable and did not increase in contrast to the one in the untreated diabetic rats (G2 group) (Fig. 1). FBG level was determined by the ingestion of carbohydrates such as fructose and glucose and was related to glycemic index (GI). Low and high GI indicate low and high blood glucose levels, respectively [2,60,61]. Honey contains fructose, isomaltose, palatinose, and α-cyclodextrin which possibly modulate its hypoglycemic effect or antidiabetic activity. These effects may be related to gastric emptying with prolonged time, food intake, and decreased intestinal absorption rate and, gut microbiota modulation, increased activity of glycogen synthase and glucokinase, and reduced peripheral glucose. Furthermore, honey fructose is an essential stimulator of insulin secretion from isolated pancreas and leads to triglyceridemic bypassing of the phosphofructokinase regulatory step in glycolysis [52].

KH (*T. laeviceps*) used in our study is known to have lower sugar contents, such as those of glucose, sucrose, fructose, and reducing sugars [11,21]. Furthermore, the KH from *T. hockingsi, T. carbonaria, G. thoracica, Heterotrigona itama,* and *T. angustula* contains trehalulose as the main sugar. Trehalulose sugar is known to release in blood in which it is considerably slower than that of sucrose [39,43]. Meanwhile, Zulkifli et al. [68] stated that regular trehalulose consumption is able to ameliorate insulin by regulating glucose metabolism to reduce diabetes risk. Thus, the pancreatic gland may be repaired by KH to insulin secretion to the metabolism of carbohydrates that later can make blood glucose stable and being prevented from rising. The FBG levels in our study (Fig. 1) were found similar to those observed in earlier study by Sahlan et al. [63] and Aziz et al. [62] showing that KH (*T. biroi* and *G. thoracica*, respectively) administrated on diabetic rats could cause fluctuation at the FBG levels.

Our study observed that the lipid profiles, consisting of TC, TG, and LDL fluctuated. However, the HDL in the diabetic rat groups increased after given a treatment with KH and glibenclamide compared to the untreated normal rats (G1 group) (Fig. 2). After 28 days, the TG level under the treatment with KH tended to decrease when compared to day 0 and 14 (Fig. 2B). The KH ingestion by diabetic rat groups could prevent the increase at TC, TG and LDL levels. However, the HDL level was also stably higher. This result is found in line with the report by Erejuwa et al. [69], showing that Nigerian honey (1.0 or 2.0 g/kg BW) ingestion by diabetic rats induced by alloxan substantially increased the HDL level and decreased hyperglycemia, TG, VLDL, and non-HDL cholesterol. By contrast, the consumption of 3.0 g/kg BW Nigerian honey only considerably reduced TC and VLDL.

Furthermore, from the use of honey in the treatment of diabetes, Erejuwa [2] discovered that it had a hypoglycemic impact on diabetic rats induced by alloxan and STZ. Honey ingestion raised the levels of serum insulin and HDL in STZ-induced diabetic rats and decreased those of fructosamine, LDL, VLDL, TC, TG, and hepatic dysfunction. In addition, Aziz et al. reported that KH (*G. thoracica*) ingestion by diabetic male rats induced by STZ and nicotinamide could prevent the increase at TG, TC, and LDL levels, but the levels of HDL and serum insulin increased [62]. Alghamdi et al. reported that honey extract of 2 mg/kg BW was decreased the FBG level in mouse [70]. The daily ingestion of 100 g honey for patients with type 1 DM revealed lower GI and peak incremental index [66], and increased C-peptide level compared to the daily ingestion of sugars (glucose and sucrose) [66]. Furthermore, Asaduzzaman et al. reported that the honey administration of 1 g/kg BW for 3 weeks in diabetic rats decreased TC, TG, LDL, VLDL, and increased HDL [71].

The study on honey flavonoids in type-1 diabetic rats revealed ameliorated dyslipidemia (impaired lipid metabolism) and decreasing blood glucose levels. Furthermore, flavonoids inhibited the activities of α-amylase- and α-glucosidase- and might be helpful in the management of postprandial hyperglycemia [52]. KH (*T. laeviceps* and *T. biroi*) can inhibit α-glucosidase; KH (*T. laeviceps*) extracted by methanol exhibited a higher inhibition compared to other honeys [72]. Additionally, according to Meo et al. [61], honey flavonoids can prevent LDL oxidation and increase the HDL level. KH contains minerals, Cu and Zn [20]. Honey increased the levels of Zn and Cu in serum, which are required in the metabolisms of glucose and insulin [73]. Moreover, honey minerals can ameliorate lipid and glucose metabolisms by raising adiponectin levels and decreasing the oxidative stress brought on by lipid peroxidation in diabetic rats [2,74].

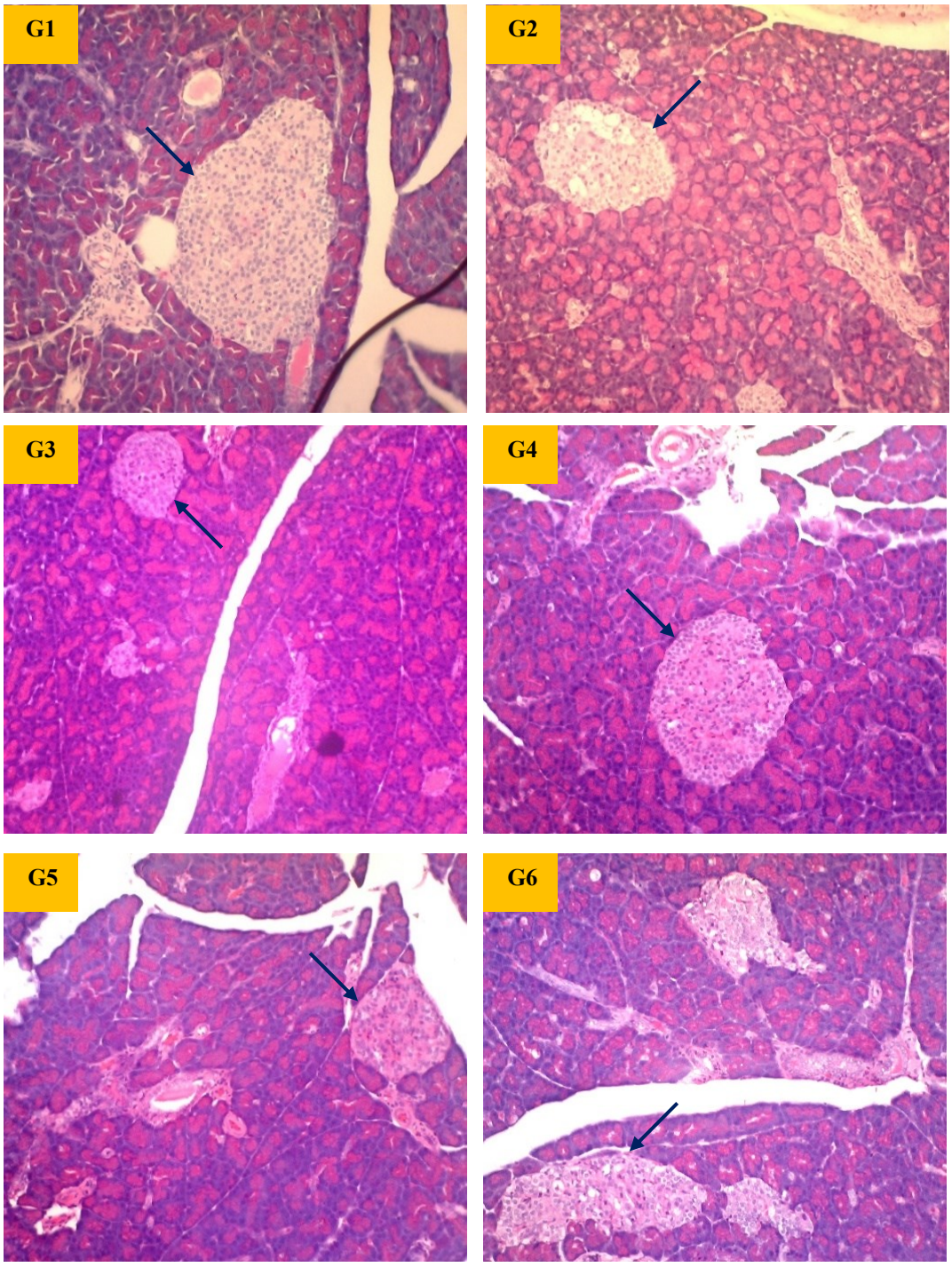

Fig. 3. Islets of Langerhans of the normal and diabetic rat groups after given the treatment with KH and glibenclamide. Abbreviations: G1 = untreated normal rats, G2 = untreated diabetic rats, G3 = diabetic rats treated with 0.9 mg/kg BW glibenclamide, G4, G5, and G6 = diabetic rats treated with 1, 2, and 3 g/kg BW KH, respectively

Compared to those of the untreated normal rats (G1 group), the hematological profiles of the diabetic rat groups on day 14 after given the treatment with KH and glibenclamide showed an increasing RBC as well as decreasing MCV and MCH levels (Table 3). However, on day 28 after given the treatment with KH, the RBC and HGB levels increased (Table 4). High RBC and HGB levels are required by the diabetic rat groups for normal oxygen transport and nutrients from the bloodstream to all organs. This finding is consistent with an earlier study by Agussalim et al. [8], reporting that malnourished rats showed increasing HGB and lymphocyte levels after the treatment with KH (1.8 g/kg BW) for 7 weeks.

Based on pancreatic histopathology, the islets of Langerhans in untreated normal rats and diabetic rat groups treated with KH and glibenclamide showed no damage. However, the size of islets Langerhans in diabetic which is treated with KH was bigger than that of untreated diabetic rats with honey (Fig. 3 G2, control negative). These results indicated that the ingestion of KH can ameliorate pancreatic glands from damage. During the DM, pancreatic damage

gradually diminishes their ability to produce and secretion of insulin. The administration of honey from *G. thoracica* stingless bee showed as ability to produce near normal insulin secreted by the pancreas in diabetic rats supported by the near normal of the pancreatic islets with the size bigger than untreated diabetic [62].

KH used in our study is rich in phenolic and flavonoid, which are the excellent sources of antioxidants to inhibit lipid oxidation and eliminate reactive oxygen species [2,4,8,60,61,75,76]. Ofor et al. explained that the pancreas condition was expressed by the antioxidant capacity of honey. The higher antioxidant of honey can protect the pancreas gland from glucotoxicity of β-cells mediated by oxidative stress. Therefore, the use of honey in type 2 diabetes mellitus can increase insulin secretion and protect the pancreas from damage as well as reactive oxygen species (ROS). Several studies revealed that ROS could bring an impact on the impaired function of pancreatic β-cells such as inhibiting biosynthesis and secretion of insulin and increase β-cell apoptosis [77].

Erejuwa [2] explained that honey ingestion increased the serum insulin level of diabetic rats induced by STZ. These findings revealed that the consumption of honey can stimulate remnant pancreatic β-cells to produce insulin. The administration of honey can protect pancreas from damage and oxidative stress, increase the number and size of islet cells, and reduce the damage of Langerhans islets. In addition, honey has a ability to stimulate β-cells (health, diseased, and remnant) to increase the secretions of insulin and C-peptide beneficial for deficiency insulin patients. According to Sahlan et al. [63], the ingestion of KH (*T. biroi*) diabetic rat groups showed no abnormalities of the pancreatic glands. Furthermore, Aziz et al. [62] reported that the ingestion of KH (*G. thoracica*) can protect the pancreatic gland from inflammation and oxidative stress via a mechanism that maintains the near-normal insulin secretion.

4. Conclusion

The ingestion of *T. laeviceps* has increased the BW of diabetic rats induced by STZ. *T. laeviceps* honey also prevented the raise of the FBG, TC, TG, and LDL levels. Interestingly, the honey increased the HDL levels, protected the pancreas gland from damage and showed no abnormalities. *T. laeviceps* honey can potentially be used as a functional food for therapy DM patients. Therefore, advanced study on the clinical administration of KH for long periods are deemed necessary.

Acknowledgements

The authors would like to thank the Directorate of Research, Reputation Improvement Team World Class University-Quality Assurance Office of Universitas Gadjah Mada, Indonesia for funding through Post-Doctoral Program 2022 (grant no. 10922/UN1.P.II/Dit-Lit/PT.01.02/2022). We also to thank the Publishers and Publications Board, Universitas Gadjah Mada for language editing service.

References

1. IDF, *International Diabetic Federation (IDF) diabetic atlas 10th edition*. International Diabetes Federation. www.diabetesatlas.org, 2021.
2. O. O. Erejuwa, Honey: *Profile and features: Aplications to diabetes*. In: R. R. Watson and V. R. Preedy, editors. *Bioactive food as dietary intervensions for diabetes*. New York: Academic Press, 2019, pp. 461–494.
3. R. A. Aras, T. Lestari, H. A. Nugroho and I. Ardiyanto, *Segmentation of retinal blood vessels for detection of diabetic retinopathy: A Review*. Commun. Sci. Technol. 1 (2016), 33–41.
4. P. M. Da Silva, C. Gauche, L. V. Gonzaga, A. C. O. Costa and R. Fett, *Honey: Chemical composition, stability and authenticity*. Food Chem. 196 (2016) 309–323.
5. R. D. Rachmawati, A. Agus, N. Umami, Agussalim and H. Purwanto, *Diversity, distribution, and nest characteristics of stingless bees (Hymenoptera: Meliponini) in Baluran National Park, East Java, Indonesia*. Biodiversitas 23 (2022) 3890–3901.
6. S. Kahono, P. Chantawannakul and M. S. Engel, *Social bees and the current status of beekeeping in Indonesia*. In: P. Chantawannakul, G. Williams and P. Neumann, editors. *Asian beekeeping in the 21st century*. Singapore: Springer, 2018, pp. 287–306.
7. Agussalim, A. Sabir, M. Sahlan and A. Agus, *Evaluation of stingless bee honey quality (Tetragonula laeviceps) based on their physicochemical from different origins*. Biodiversitas 24 (2023) 2134–2142.
8. Agussalim, N. Umami, Nurliyani and A. Agus, *Stingless bee honey (Tetragonula laeviceps): Chemical composition and their potential roles as an immunomodulator in malnourished rats*. Saudi J. Biol. Sci. 29 (2022) 103404.
9. Agussalim, A. Agus, Nurliyani, N. Umami and I. G. S. Budisatria, *Physicochemical properties of honey produced by the indonesian stingless bee*: *Tetragonula laeviceps*. IOP Conf. Ser. Earth Environ. Sci. 387 (2019) 012084.
10. A. Agus, Agussalim, Nurliyani, N. Umami and I. G. S. Budisatria, *Evaluation of antioxidant activity, phenolic, flavonoid and vitamin c content of several honeys produced by the indonesian stingless bee*: *Tetragonula laeviceps*. Livest. Res. Rural Dev. 31 (2019) 152.
11. Agussalim, A. Agus, Nurliyani and N. Umami, *The sugar content profile of honey produced by the indonesian stingless bee*, *Tetragonula laeviceps*, *from different regions*. Livest. Res. Rural Dev. 31 (2019) 91.
12. L. K. Dewi, M. Sahlan, D. K. Pratami, A. Agus, Agussalim and A. Sabir, *Identifying propolis compounds potential to be Covid-19 therapies by targeting SARS-CoV-2 Main Protease*. Int. J. Appl. Pharm. 13 (2021) 103–110.
13. Erwan, Habiburrohman, I. K. G. Wiryawan, M. Muhsinin, B. Supeno and Agussalim, *Comparison of productivity from three stingless bees: Tetragonula sapiens, T. Clypearis and T. biroi managed under same feed sources for meliponiculture*. Biodiversitas 24 (2023) 2988–2994.
14. M. N. Pratama, A. Agus, N. Umami, Agussalim and H. Purwanto, *Morphometric and molecular identification, domestication, and potentials of stingless bees (Apidae: Meliponini) in Mount Halimun Salak National Park, West Java, Indonesia*. Biodiversitas 24 (2023) 6107–6118.
15. D. K. Pratami, T. Indrawati, I. Istikomah, S. Farida, P. Pujianto and M.

Sahlan, *Antifungal activity of microcapsule propolis from Tetragonula spp. to Candida Albicans*. Commun. Sci. Technol. (2020) 16–21.

16. B. Supeno, Erwan and Agussalim, *The production of honey and pot-pollen from stingless bee Tetragonula clypearis and their contribution to increase the farmers income in West Lombok*, *Indonesia*. Livest. Res. Rural Dev. 34 (2022) 42.
17. Erwan, M. Astuti, Syamsuhaidi, M. Muhsinin and Agussalim, *The effect of different beehives on the activity of foragers, honey pots number and honey production from stingless bee Tetragonula* sp. Livest. Res. Rural Dev. 32 (2020) 158.
18. Agussalim, N. Umami, Nurliyani and A. Agus, *The physicochemical composition of honey from indonesian stingless bee (Tetragonula laeviceps)*. Biodiversitas 22 (2021) 3257–3263.
19. Erwan, Suhardin, Syamsuhaidi, D. K. Purnamasari, M. Muhsinin and Agussalim, *Propolis mixture production and foragers daily activity of stingless bee Tetragonula sp. in bamboo and box hives.* Livest. Res. Rural Dev. 33 (2021) 82.
20. A. Sabir, A. Agus, M. Sahlan and Agussalim, *The minerals content of honey from stingless bee Tetragonula laeviceps from different regions in Indonesia*. Livest. Res. Rural Dev. 33 (2021) 22.
21. A. Agus, Agussalim, M. Sahlan and A. Sabir, A. *Honey sugars profile of stingless bee Tetragonula laeviceps (Hymenoptera: Meliponinae).* Biodiversitas 22 (2021) 5205–5210.
22. B. Supeno, Erwan and Agussalim, *Enhances production of coffee (Coffea robusta): The role of pollinator, forages potency, and honey production from Tetragonula sp. (Meliponinae) in Central Lombok, Indonesia*. Biodiversitas 22 (2021) 4687–4693.
23. Agussalim, Nurliyani, N. Umami and A. Agus, *The Honey and propolis production from indonesian stingless bee: Tetragonula laeviceps*. Livest. Res. Rural Dev. 32 (2020) 121.
24. J. Thipraksa, P. Michu, A. Kongthong and P. Chaijak, *Exploring the impact of co-fermentation Saccharomyces cerevisiae and Lactobacillus Sp. on stingless bee-honey cider fermentation*. Commun. Sci. Technol. 8 (2023) 93–99.
25. Agussalim and A. Agus, *Production of honey, pot-pollen and propolis production from indonesian stingless bee Tetragonula laeviceps and the physicochemical properties of honey: A review*. Livest. Res. Rural Dev. 34 (2022) 66.
26. M. A. I. Al-Hatamleh, J. C. Boer, K. L. Wilson, M. Plebanski, R. Mohamud and M. Z. Mustafa, *Antioxidant-based medicinal properties of stingless bee products: Recent progress and future directions.* Biomolecules 10 (2020) 923.
27. S. Ávila, P. S. Hornung, G. L. Teixeira, L. N. Malunga, F. B. Apea-Bah, M. R. Beux, et al., *Bioactive compounds and biological properties of Brazilian stingless bee honey have a strong relationship with the pollen floral origin*. Food Res. Int. 123 (2019) 1–10.
28. H. O. Mokaya, K. Nkoba, R. M. Ndunda and N. J. Vereecken, *Characterization of honeys produced by sympatric species of Afrotropical stingless bees (Hymenoptera, Meliponini).* Food Chem. 366 (2022) 130597.
29. F. I. N. Moreira, L. L. de Medeiros, L. M. de Carvalho, L. S. Olegario, M. de Sousa Galvão, S. A. M. da Franca, et al., *Quality of Brazilian stingless bee honeys: Cephalotrigona capitata/Mombucão and Melipona scutellaris Latrelle/Uruçu*. Food Chem. 404 (2023) 134306.
30. L. C. Ng, S. B. A. Razak, Rudiyanto, S. P. Tan and F. T. Ahmad, *Low heating effects on the total microbial activity and physico-chemical properties of stingless bee (Heterotrigona itama) honey*. Malaysian Appl. Biol. 52 (2023) 43–52.
31. Z. Ngaini, H. Hussain, E. S. Kelabo, R. Wahi and S. Farooq, *Chemical profiling, biological properties and environmental contaminants of stingless bee honey and propolis*. J. Apic. Res. 62 (2023) 131–147.
32. M. Popova, D. Gerginova, B. Trusheva, S. Simova, A. N. Tamfu, O. Ceylan, et al., *A preliminary study of chemical profiles of honey, cerumen, and propolis of the African stingless bee Meliponula ferruginea.* Foods 10 (2021) 997.
33. S. Shamsudin, J. Selamat, M. Sanny, S. B. Abd. Razak, N. N. Jambari, Z. Mian, et al., *Influence of origins and bee species on physicochemical, antioxidant properties and botanical discrimination of stingless bee honey*. Int. J. Food Prop. 22 (2019) 238–263.
34. S. N. Sharin, M. S. A. Sani, M. A. Jaafar, M. H. Yuswan, N. K. Kassim, Y. N. Manaf, et al., *Discrimination of Malaysian stingless bee honey from different entomological origins based on physicochemical properties and volatile compound profiles using chemometrics and machine learning*. Food Chem. 346 (2021) 128654.
35. E. C. A. Souza, C. Menezes and A. *Flach, Stingless bee honey (Hymenoptera, Apidae, Meliponini): A review of quality control, chemical profile, and biological potential.* Apidologie 52 (2021) 113–132.
36. E. Umaña, G. Zamora, I. Aguilar, M. L. Arias, R. Pérez, L. A. Sánchez, et al., *Physicochemical differentiation of stingless bee honeys from Costa Rica*. J. Apic. Res. 62 (2023) 873–882.
37. I. Villacrés-Granda, D. Coello, A. Proaño, I. Ballesteros, D. W. Roubik, G. Jijón, et al., *Honey Quality parameters, chemical composition and antimicrobial activity in twelve Ecuadorian stingless bees (Apidae: Apinae: Meliponini) tested against multiresistant human pathogens*. LWT - Food Sci. Technol. 140 (2021) 110737.
38. F. C. Biluca, F. Braghini, L. V. Gonzaga, A. C. O. Costa and R. Fett, *Physicochemical profiles, minerals and bioactive compounds of stingless bee honey (Meliponinae)*. J. Food Compos. Anal. 50 (2016) 61–69.
39. N. Zawawi, J. Zhang, N. L. Hungerford, H. S. A. Yates, D. C. Webber, M. Farrell, et al., *Unique physicochemical properties and rare reducing sugar trehalulose mandate new international regulation for stingless bee honey*. Food Chem. 373 (2022) 131566.
40. F. Braghini, F. C. Biluca, M. Schulz, L. V. Gonzaga, A. C. O. Costa and R. Fett, *Stingless bee honey: A precious but unregulated product - reality and expectations*. Food Rev. Int. 38 (2022) 683–712.
41. B. Chuttong, Y. Chanbang, K. Sringarm and M. Burgett, *Physicochemical profiles of stingless bee (Apidae: Meliponini) honey from South East Asia (Thailand)*. Food Chem. 192 (2016) 149–155.
42. A. C. dos Santos, F. C. Biluca, F. Braghini, L. V. Gonzaga, A. C. O. Costa and R. Fett, *Phenolic composition and biological activities of stingless bee honey: An overview based on its aglycone and glycoside compounds*. Food Res. Int. 147 (2021) 110553.
43. M. T. Fletcher, N. L. Hungerford, D. Webber, M. Carpinelli de Jesus, J.

Zhang, I. S. J. Stone, et al., *Stingless bee honey, a novel source of trehalulose: A biologically active disaccharide with health benefits*. Sci. Rep. 10 (2020) 1–8.

44. A. Gela, Z. A. Hora, D. Kebebe and A. Gebresilassie, *Physico-chemical characteristics of honey produced by stingless bees (Meliponula Beccarii) from West Showa Zone of Oromia Region, Ethiopia*. Heliyon 7 (2021) e05875.
45. V. A. Isidorov, J. Maslowiecka, N. Pellizzer, D. Miranda and S. Bakier, *Chemical composition of volatile components in the honey of some species of stingless bees.* Food Control 146 (2023) 109545.
46. N. F. Ismail, M. Maulidiani, S. Omar, M. F. Zulkifli, M. N. F. Mohd Radzi, N. Ismail, et al., *Classification of stingless bee honey based on species, dehumidification process and geographical origins using physicochemical and ATR-FTIR chemometric approach*. J. Food Compos. Anal. 104 (2021) 104126.
47. A. Nordin, N. Q. A. V Sainik, S. R. Chowdhury, A. Saim and R. B. H. Idrus. *Physicochemical properties of stingless bee honey from around the globe: A comprehensive review*. J. Food Compos. Anal. 73 (2018) 91–102.
48. A. K. Hassan, D. A. El-kotby, M. M. Tawfik, R. E. Badr and I. M. Bahgat, *Antidiabetic effect of the egyptian honey bee (Apis mellifera) venom in alloxan-induced diabetic rats*. J. Basic Appl. Zool. 80 (2019) 58.
49. D. Cianciosi, T. Y. Forbes-Hernández, S. Afrin, M. Gasparrini, P. Reboredo-Rodriguez, P. P. Manna, et al., *Phenolic compounds in honey and their associated health benefits: A review*. Molecules 23 (2018) 2322.
50. O. O. Erejuwa, S. A. Sulaiman and M. S. Ab Wahab, *Oligosaccharides might contribute to the antidiabetic effect of honey: A review of the literature*. Molecules 17 (2012) 248–266.
51. M. Zamanian and F. Azizi-Soleiman, *Honey and glycemic control: A systematic review*. PharmaNutrition 11 (2020) 100180.
52. R. Sharma, N. Martins, A. Chaudhary, N. Garg, V. Sharma, K. Kuca, et al., *Adjunct use of honey in diabetes mellitus: A consensus or conundrum?* Trends Food Sci. Technol. 106 (2020) 254–274.
53. V. R. Pasupuleti, C. S. Arigela, S. H. Gan, S. K. N. Salam, K. T. Krishnan, N. A. Rahman, et al., *A review on oxidative stress, diabetic complications, and the roles of honey polyphenols*. Oxid. Med. Cell. Longev. (2020) 8878172.
54. M. A. Abdulrhman. *Honey as a sole treatment of type 2 diabetes mellitus*. Endocrinol. Metab. Syndr. 5 (2016) 232.
55. S. B. Khedekar, B. Ravishankar and P. K. Prajapati, *Anti-diabetic activity of dried extract of Tionspora cordifolia (Guduchi ghana) and honey in streptozotacin induced diabetic rats*. Int. J. Green Pharm. 9 (2015) S31–S38.
56. M. Moein, S. Moein, F. Farmani, S. Rozbehan and Z. Sabahi, *Examination the antioxidant potentials and antidiabetic properties of phenolic extracts of some Iranian honeys*. J. Nephropharmacology 11 (2022) e06.
57. O. O. Erejuwa, S. A. Sulaiman and M. S. Ab Wahab, *Honey: A novel antioxidant*. Molecules 17 (2012) 4400–4423.
58. D. A. M. Kamal, S. F. Ibrahim, H. Kamal, M. I. A. M. Kashim and M. H. Mokhtar, *Physicochemical and medicinal properties of tualang, gelam and kelulut honeys: A comprehensive review*. Nutrients 13 (2021) 197.
59. S. Rana, M. Mishra, D. Yadav, S. K. Subramani, C. Katare and G. B. K. S. Prasad, *Medicinal uses of honey: A review on its benefits to human health*. Prog. Nutr. 20 (2018) 5–14.
60. S. A. Meo, M. J. Ansari, K. Sattar, H. U. Chaudhary, W. Hajjar and S. Alasiri, *Honey and diabetes mellitus: Obstacles and challenges–road to be repaired*. Saudi J. Biol. Sci. 24 (2017) 1030–1033.
61. S. A. Meo, S. A. Al-Asiri, A. L. Mahesar and M. J. Ansari, *Role of honey in modern medicine*. Saudi J. Biol. Sci. 24 (2017) 975–978.
62. M. S. A. Aziz, N. Giribabu, P. V. Rao and N. Salleh, *Pancreatoprotective effects of Geniotrigona thoracica stingless bee honey in streptozotocin-nicotinamide-induced male diabetic rats*. Biomed. Pharmacother. 89 (2017) 135–145.
63. M. Sahlan, O. Rahmawati, D. K. Pratami, R. Raffiudin, R. R. Mukti and H. Hermasyah, *The effects of stingless bee (Tetragonula biroi) honey on streptozotocin-induced diabetes mellitus in rats*. Saudi J. Biol. Sci. 27 (2020) 2025–2030.
64. M. R. Rashid, K. N. Nor Aripin, F. B. Syed Mohideen, N. Baharom, K. Omar, N. M. S. Md Taujuddin, et al., *The effect of kelulut honey on fasting blood glucose and metabolic parameters in patients with impaired fasting glucose.* J. Nutr. Metab. (2019) 3176018.
65. P. G. Reeves, F. H. Nielsen and G. C. Fahey, *AIN-93 Purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet.* J. Nutr. 123 (1993) 1939–1951.
66. M. Abdulrhman, M. El Hefnawy, R. Ali, I. Abdel Hamid, A. Abou El-Goud and D. Refai, *Effects of honey, sucrose and glucose on blood glucose and c-peptide in patients with type 1 diabetes mellitus*. Complement. Ther. Clin. Pract. 19 (2013) 15–19.
67. M. Gholami, M. Hemmati, A. Taheri-Ghahfarokhi, R. Hoshyar and M. Moossavi, *Expression of glucokinase, glucose 6-Phosphatase, and stress protein in streptozotocin-induced diabetic rats treated with natural honey*. Int. J. Diabetes Dev. Ctries. 36 (2016) 125–131.
68. M. F. Zulkifli, M. Sivakumar, M. Maulidiani and W. I. Wan Ismail, *Bibliometric Approach to trehalulose research trends for its potential health benefits*. Food Biosci. 53 (2023) 102677.
69. O. O. Erejuwa, N. N. Nwobodo, J. L. Akpan, U. A. Okorie, C. T. Ezeonu, B. C. Ezeokpo, et al., *Nigerian honey ameliorates hyperglycemia and dyslipidemia in alloxan-induced diabetic rats*. Nutrients 8 (2016) 1–14.
70. A. H. Alghamdi, I. M. Shatla, S. Shreed, A. H. Khirelsied and M. F. El-Refaei, *Bee honey extract attenuates hyperglycemia in Induced type 1 diabetes: Impact of antioxidant and angiogenesis activities on diabetic severity in vivo*. Appl. Sci. 13 (2023) 8045.
71. M. Asaduzzaman, R. Sohanur, S. Munira, R. Muedu, M. Hasan, M. Siddique, et al., *Effects of honey supplementation on hepatic and cardiovascular disease (CVD) marker in streptozotocin-induced diabetic rats*. J. Diabetes Metab. 6 (2015) 1000592.
72. O. Rahmawati, D. K. Pratami, R. Raffiudin and M. Sahlan, *Alpha-glucosidase inhibitory activity of stingless see honey from Tetragonula biroi and Tetragonula laeviceps*. AIP Conf. Proc. 2092 (2019) 030001.
73. B. Fuhrman and M. Aviram, *Flavonoids protect LDL from oxidation and*

attenuate atherosclerosis. Curr. Opin. Lipidol. 12 (2001) 41–48.

74. M. I. Khalil, S. A. Sulaiman and L. Boukraa, *Antioxidant Properties of Honey and Its Role in Preventing Health Disorder*. Open Nutraceuticals J. 3 (2010) 6–16.
75. R. Mărgăoan, E. Topal, R. Balkanska, B. Yücel, T. Oravecz, M. Cornea-Cipcigan, et al., *Monofloral honeys as a potential source of natural antioxidants, minerals and medicine*. Antioxidants 10 (2021) 1023.
76. H. Scepankova, J. A. Saraiva and L. M. Estevinho, *Honey health benefits and uses in medicine.* In: J. M. Alvarez-Suarez, editor. *Bee products - chemical and biological properties.* Switzerland: Springer. (2017) pp. 83–96.
77. C. C. Ofor, O. O. Erejuwa, G. C. Akuodor, D. O. Aja, A. U. Mba and E. N. Shu, *The role of honey in the treatment of type 2 diabetes mellitus: A Review of literature*. Int. J. Basic Clin. Pharmacol. 12 (2022) 120–124.