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Biochemical Evaluation of Hypoglycaemic Effect of Stingless Bee Pollen in High Fat Diet Fed-Low Dose STZ Induced Experimental Type 2 Diabetes in Rats

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Abstract

The present study was aimed to evaluate the hypoglycaemic properties of stingless bee pollen in HFD fed-low dose STZ induced experimental type 2 diabetes in rats. The rats were divided into four groups of six animals each. Group I control group. Group II fed HFD induced STZ (35mg/kg. b.w. ip). Group III were induced with diabetes and treated stingless bee pollen (100 mg/kg b.w/rat/day). Group VI was diabetic rats treated with metformin (50mg/kg.b.w.). Body weight, food and fluid consumption were recorded periodically. At the end of the experimental period, the animals were starved overnight and OGTT was performed. After 30 days of experimental period, the animals were overnight fasted, anesthetized and scarified by cervical decapitation. Blood was collected with and without anticoagulants for the separation of plasma and serum, respectively. The biochemical analysis performed includes fasting blood glucose, HbA1c, plasma insulin, C-peptide and urine sugar. Insulin resistance, β-cell function and insulin sensitivity were also manipulated from HOMA-IR, HOMA β-cell function and QUICK-I index. The results of the present study clearly indicate that the stingless bee pollen significantly decreased the levels of fasting blood glucose and HbA1c. Stingless bee pollen improves the plasma insulin and C-peptide levels. From the arithmetic data, stingless bee pollen reduces the insulin resistance and enhances β-cell function and insulin sensitivity. The antidiabetic efficacy was more pronounced in rats treated with stingless bee pollen. The results obtained clearly evidenced that the stingless bee pollen significant antidiabetic properties used in the present study.

Keywords

Type 2 diabetes mellitus, High fat diet, stingless bee pollen, Antidiabetic properties

Introduction

Type 2 diabetes mellitus (T2DM) or noninsulin dependent diabetes mellitus (NIDDM) is a chronic progressive disease characterized by chronic fasting hyperglycaemia and resulting in defective insulin action [1]. T2DM can cause severe damage to body systems such as kidneys, eyes and the heart, as well as

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the vascular system more generally. Individuals with T2DM does not produce enough insulin (insulin deficiency), or cell body that are not able to use insulin properly (insulin resistance). Insulin is a hormone produced by the β-cells in the pancreas to control blood sugar levels [2]. Insulin resistance is related to genetic factors, obesity, sedentary lifestyle and aging. Consumption of high fat food and physical inactivity are important predictors of obesity and T2DM [3].

In obese individuals, response is inadequate to overcome insulin insensitivity particularly contributing to increased production of glucose by the liver. The metabolism of carbohydrate, fat, and protein are disturbed as the disease progresses. This condition lead to "prediabetes", where the glucose levels are high in the T2DM glucose range [4]. Hyperglycaemia results when the cell β fail to compensate insulin resistance with excess insulin output. The progressive decline of the β cell function and mass over time with hyperglycaemia marks the development of T2DM [5].

Nature is an inexhaustible source of products and bioactive molecules that can be used as alternative treatments, stingless bee pollen one of them. Stingless bee pollen is another bee product that is extremely rich in bioactive molecule [6]. Stingless Bee pollen come from mixture of flower pollen, nectar and bee secretions. It can be collected by beekeepers without damage to the beehive. This natural product, that has been gaining prominence, is recognized to be valuable apitherapeutic product with potential for medical and nutritional application [7].

As the previous studies, flavonoids in Stingless Bee pollen have an antioxidants activity and are thought to be the compound that is able to lower serum glucose level through the inhibition of oxidative stress [8]. In addition, the antioxidants activity of stingless bee pollen can improve insulin receptor signaling in insulin resistant conditions, therefore the insulin sensitivity can be increased [9].

Accordingly, stingless bee pollen used in the present study was from kelulut bees (Trigona sp). Kelulut bees is small bees which has not sting at their tails. Kelulut bees are found in the forests of East Kalimantan. The advantages of bee kelulut is produces more bee pollen than other type bee [10].

Since, Type 2 Diabetes Mellitus is a multifactorial and multisystemic metabolic disorder, the current trend in diabetes is towards nature product from bee product that is extremely rich in bioactive molecule. Accordingly, the study objective is to examine biochemical evaluation of hypoglycaemic effect of stingless bee pollen in high fat diet fed-low dose STZ induced experimental type 2 diabetes in rats.

Materials and Methods

Chemicals

Streptozotocin were procured from Sigma Aldrich, stored at 2-4°C and protected from light. All other chemicals used were purchased from standard commercial suppliers and were of analytical grade quality.

Animals

Male rats of Wistar strain weighing about 160-180 g were procured from Gadjah Mada University, Yogyakarta, Indonesia. The rats were housed in spacious polypropylene cages lined with husk. The experimental rats were maintained in a controlled environment (12:12±1h light/dark cycle; temperature 22°C ± 3°C; relative humidity 55%). Animal were acclimatized to standard husbandry conditions for one week to eliminate the effect of stress prior to initiation of the experiments. The rats were fed with commercial pellet rat chow and free access to water ad libitum [11].

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Induction of T2DM in Experimental Rats

The rats were allocated into two dietary regimens by feeding either normal pellet diet (NPD) or high fat diet (HFD) for 2 weeks or dietary manipulation. The composition of HFD is powdered NPD-365g/kg, Lard-310 g/kg, Casein-250g/kg, Cholesterol-10 g/kg, vitamin and mineral mix-60 g/kg. DL-methionine-3kg/kg, Yeast powder-1g/kg, NaCl-1g/kg. After 2 weeks of HFD, the group II, group III and group IV rats were injected with a single dose of STZ (35 mg/kg b.w/rat), while the group I rats fed with fed with NPD was injected with 0,5 ml of freshly prepared cold citrate buffer (pH 4.5) in a same volume intraperitoneally. After one week of STZ injection, rats with fasting blood glucose levels > 120 mg/dL were considered as diabetic and chosen for further studies [12]. The animals were divided into four group, each comprising of a minimum of six rats as follows.

Experimental Animal Design

The animals were divided into four groups, comprising a minimum of six animals in each group as follows: Group I = Normal control rat group fed with normal pellet diet (NPD) will be treated with water for 30 days (negative control), Group II = HFD+ STZ (i.p.; 35mg/kg b.w.) -induced diabetic rats will be treated with water for 30 days (positive control), Group III = HFD+ STZ (i.p.; 35mg/kg b.w.) -induced diabetic rats will be treated orally with bee pollen (100 mg/kg b.w/rat/day) for 30 days, Group IV = HFD+ STZ (i.p.; 35mg/kg b.w.) -induced diabetic rats will be treated orally with metformin (50 mg/ kg b.w/rat/day; in aqueous solution orally) for 30 days. At the end of 30 days experimental period, rats were fasted overnight, anaesthetized, using ketamine (80 mg/kg b.w./rat, i.p.) and sacrificed by cervical decapitation. Blood was collected with and without anticoagulant for plasma and serum separation respectively.

Oral Glucose Tolerance Test (OGTT)

Overnight fasted rats of all groups were subjected to oral glucose tolerance test on the last week of the experimental period. The blood glucose levels were monitored at 0, 30, 60, 90 and 120 min using One Touch glucometer (Life scan, Johnson and Johnson Company) after oral administration of 2 g/kg b.w. glucose as aqueous solution [13].

Determination of Biochemistry Parameters

The fasting blood level was determined by glucose oxidase diagnostic enzyme kit and the level of plasma insulin and C-peptide were assayed using rat ELISA kit. The presence of urines sugar was detected using urine strips.

Assessments of Insulin Resistance and β Cell Function

Insulin resistance was assessed by QUICKI (Quantitative Insulin Check Index) and HOMA-IR. HOMA-β score was calculated using blood glucose and plasma insulin concentrations according to the following formula: HOMA-IR= [Blood glucose (mg/dl) x Insulin (μU/ml)]/ 405, HOMA-β= [360 x Insulin (μU/ml)]/ Blood glucose-63%.

Ethical Statement

The experiments were designed and conducted in strict accordance with the current ethical norms approved by Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine Gadjah Mada University, Yogyakarta, Indonesian No: KE/FK/325/EC/2023.

Statistical Analysis

The results were expressed as mean ± S.E.M of six rats per group and statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS program followed by LSD.

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Results

Figure 1 shows the effect of oral administration of stingless bee pollen in body weight gain of experimental groups of rats. The raise in body weight was far less in diabetic control rats as compared to diabetic rats treated with stingless bee pollen. However, diabetic rats treated with Stingless Bee Pollen showed a significant increase in body weight gain when compared to the diabetic rats which have showed a marginal increase in body weight and the efficacy of stingless bee pollen was comparable with metformin, a standard drug widely used for the treatment of T2DM.

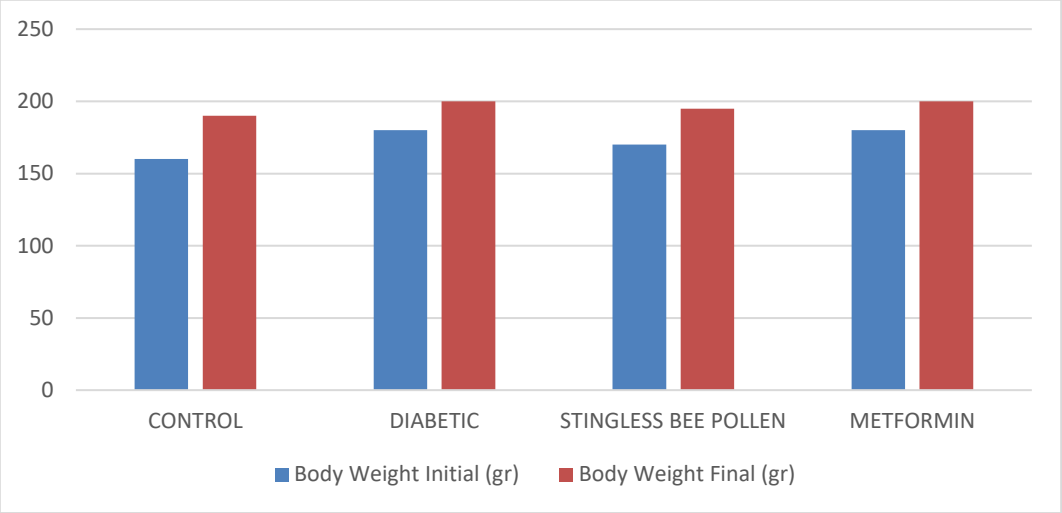

Figure 1: Effect of stingless bee pollen on body weight gain in experimental groups of rats
Values are given as mean ± SEM for groups of six rats in each. One-way ANOVA followed by post hoc test LSD. Statistical significance was compared within the groups as follows:
[a]compared with control
[b]compared with diabetic rats

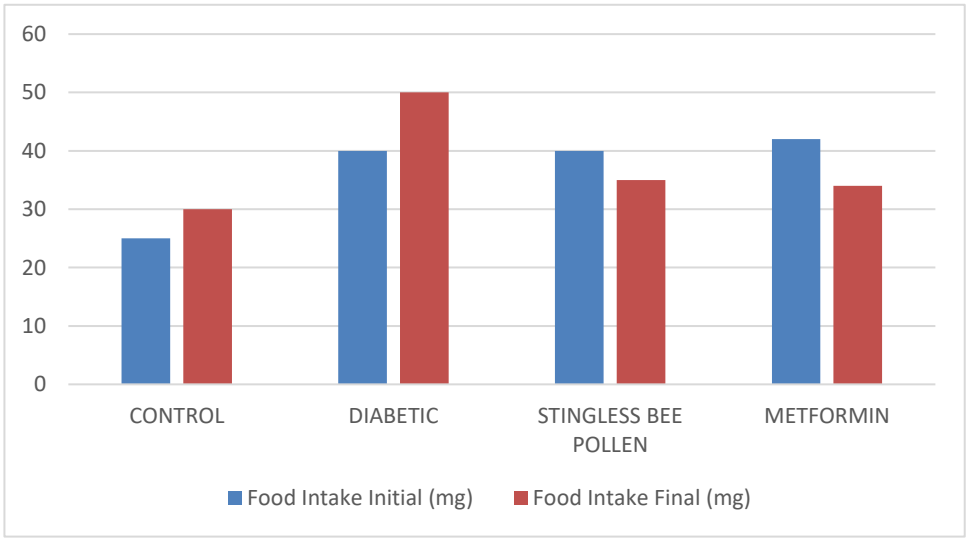

Figure 2: The levels of food consumption in control and experimental groups
Values are given as mean ± SEM for groups of six rats in each. One-way ANOVA followed by post hoc test LSD. Statistical significance was compared within the groups as follows:
[a]compared with control
[b]compared with diabetic rats

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The levels of food (Figure 2) and fluid (Figure 3) consumption were significantly increased in diabetic groups of rats when compared with control groups of rats. The increased crave of food and fluid utilization was normalized after oral treatment of stingless bee pollen which was comparable with metformin.

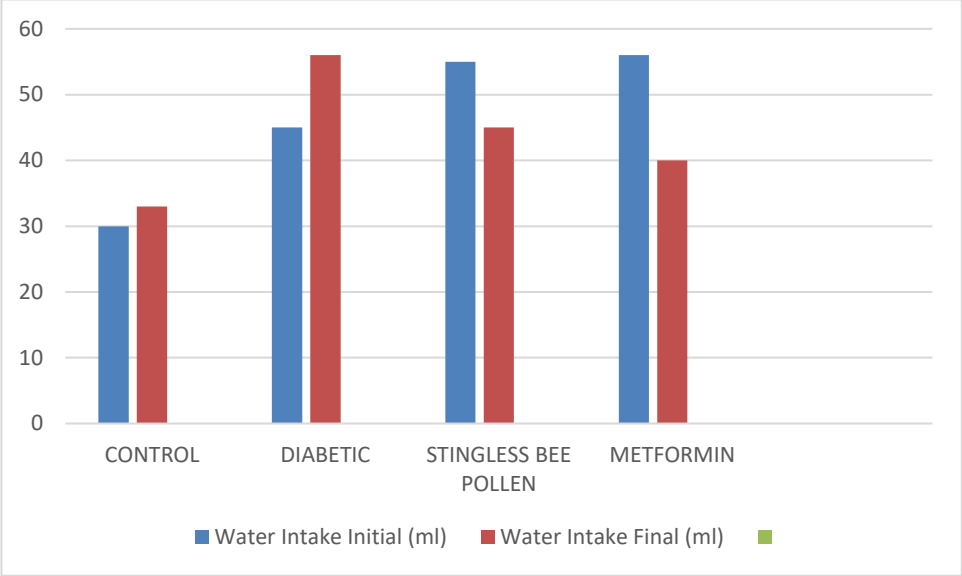

Figure 3: The levels of fluid intake in control and experimental groups of rats
Values are given as mean ± SEM for groups of six rats in each. One-way ANOVA followed by post hoc test LSD. Statistical significance was compared within the groups as follows:
[a]compared with control
[b]compared with diabetic rats

Figure 4 depicts the levels of blood glucose in control and experimental groups of rats after receiving an oral glucose load (2g/kg. b.w.). In control group of rats, the blood glucose level reached the maximum peak at 60 min after an oral glucose load and it was progressively declined to near normal level at 120 min representing the maintenance of normal glucose homeostasis. Conversely, the blood glucose levels in HFD-STZ induced diabetic rats reached the maximum peak at 60 min and remained unsubsidized over the next 60 min. However, the diabetic rats treated with stingless bee pollen showed a significant decrease in blood glucose levels at fasting, 30 and 60min time interval when compared with the diabetic control group of rats. In addition, the blood glucose levels returned to near basal level at 120 min after the oral glucose load in treated groups of rats. However, diabetic rats treated with stingless bee pollen showed a statistically significant improvement in glucose homeostasis which was comparable with metformin.

The levels of fasting blood glucose, glycosylated hemoglobin (HbA1c), plasma insulin, c-peptide and urine sugar in control and experimental groups of rats are depicted in table 1. The levels of fasting blood glucose and HbA1c% were found to be significantly elevated in diabetic group of rats when compared with control rats. Likewise, the levels of plasma insulin and C-peptide were moderately decreased in HFD-STZ induced diabetic rats. Urine sugar was observed in the diabetic group of rats. Oral administration of stingless bee pollen as well as metformin to experimental groups of rats showed improved levels of altered biochemical parameters.

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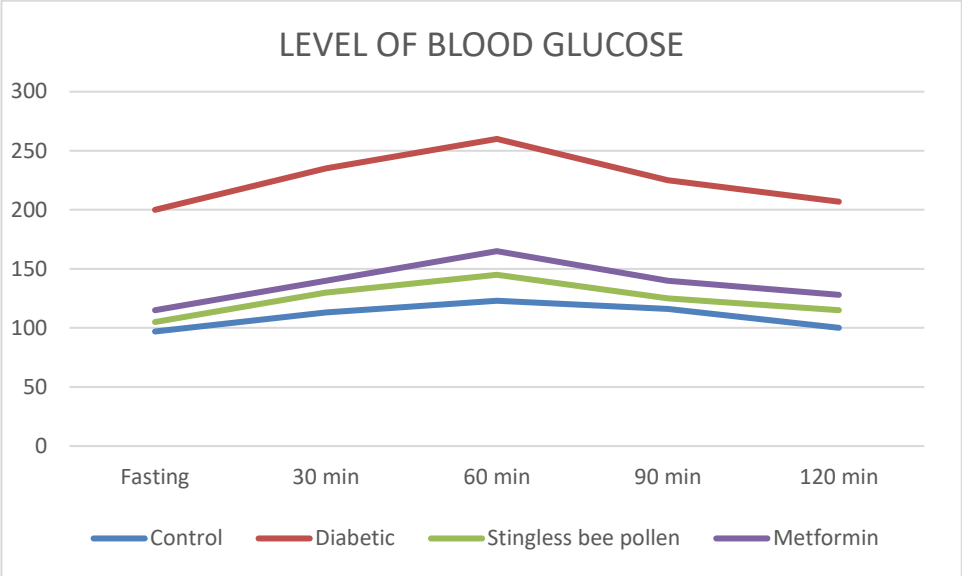

Figure 4: The levels of blood glucose in control and experimental groups of rats after receiving an oral glucose load

Values are given as mean ± SEM for groups of six rats in each. One-way ANOVA followed by post hoc test LSD. Statistical significance was compared within the groups as follows:
[a]compared with control
[b]compared with diabetic rats

Tabel 1: Effect of Stingless Bee pollen on fasting blood glucose, HbA1C, Plasma Insulin, C-Peptide and urine sugar in experimental of Rats

Groups	Fasting Blood Glucose (mg/dl)	HbA1C (% Hemoglobin)	Plasma insulin (µU/ml)	C-Peptide (pmol/mL)	Urine Sugar
Control	85.33±3.35	5.87±0.16	13.87±0.17	0.27±0.023	Nil
Diabetic	304.21±8.32[a]	10.70±0.38[a]	9.25±0.13[a]	0.11±0.011[a]	+++
Stingless Bee Pollen	110.20±3.02[a]	5.80±0.46[b]	13.02±0.63[b]	0.23±0.012[b]	Nil
Metformin	103.72±4.11[b]	5.76±0.19[b]	14.41±0.70[b]	0.21±0.011[b]	Nil

Results are expressed as mean ± SEM [n = 6]. One-way ANOVA followed by post hoc test LSD was done. The results were [a]compared to control rats and [b] compared to diabetic rats.

The altered levels of HOMA-IR and HOMA-β indices (Figure 5 & 6) were reverted to near normal in diabetic rats treated with stingless bee pollen as well as metformin. Figure 7 depicts the quantitative insulin check index (QUICKI). Diabetic rats showed reduced insulin sensitivity which indicates the extent of insulin resistance. Stingless bee pollen as well as metformin restored the insulin sensitivity.

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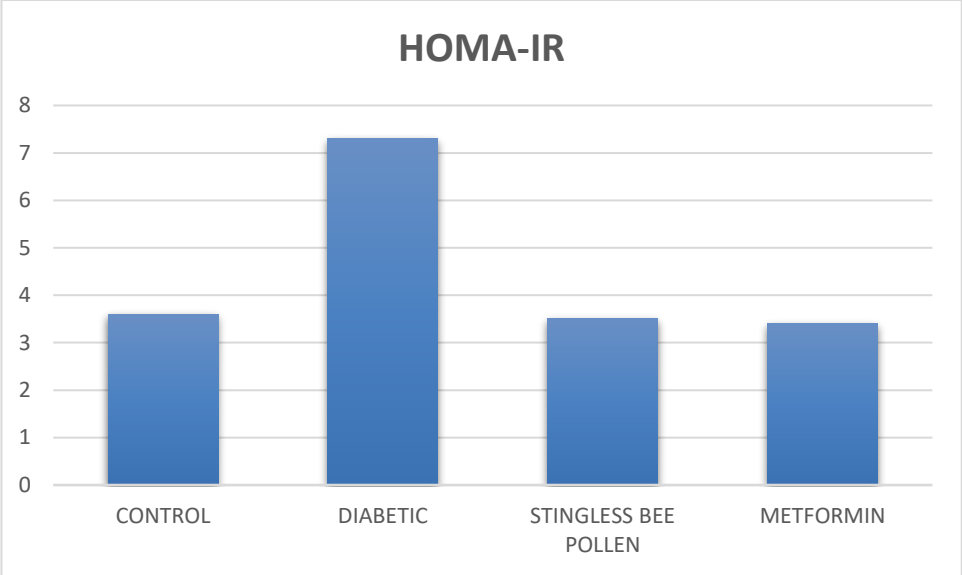

Figure 5: Effect of stingless bee pollen on HOMA-IR

Values are given as mean ± SEM for groups of six rats in each. One-way ANOVA followed by post hoc test LSD. Statistical significance was compared within the groups as follows

[a]compared with control

[b]compared with diabetic rats

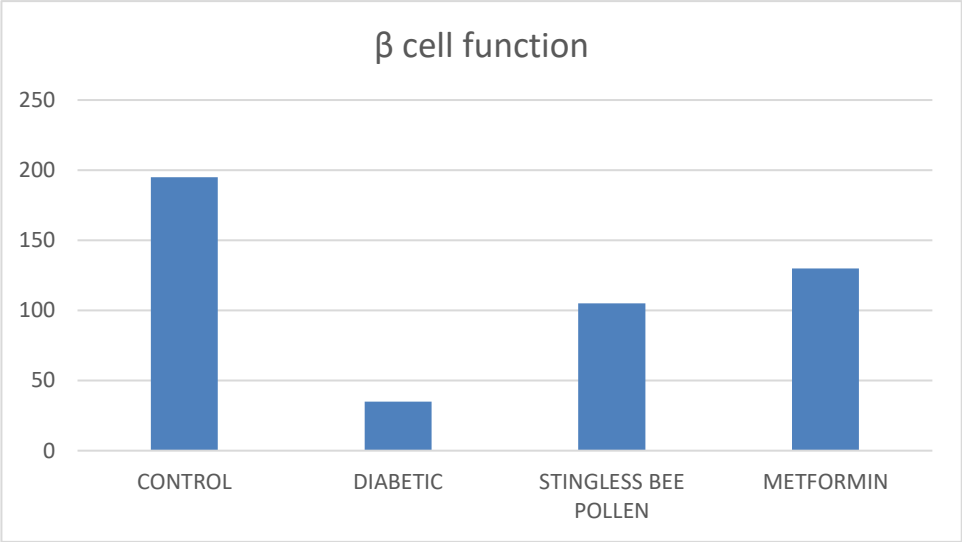

Figure 6: Levels of b-cell function in control and experimental of rats

Values are given as mean ± SEM for groups of six rats in each. One-way ANOVA followed by post hoc test LSD. Statistical significance was compared within the groups as follows

[a]compared with control

[b]compared with diabetic rats

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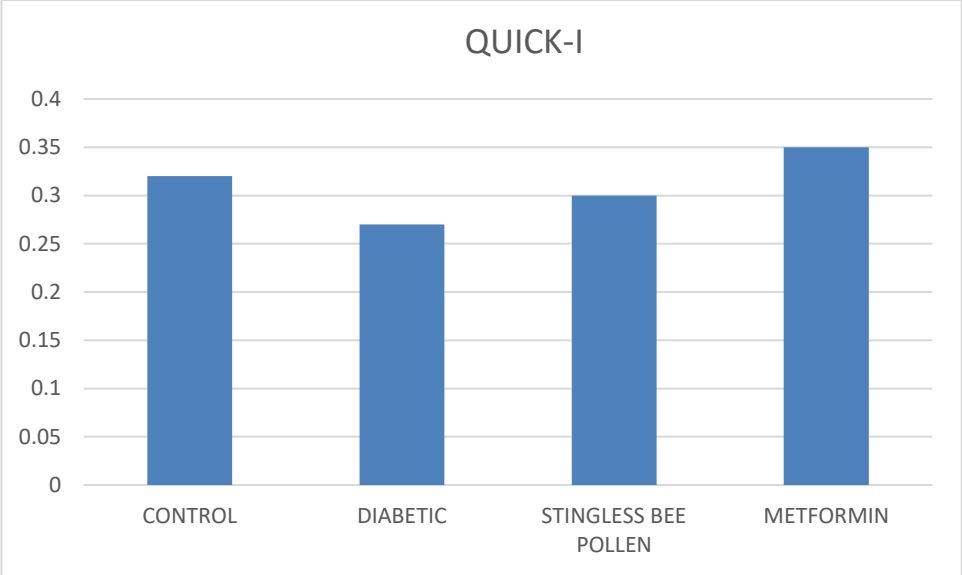

Figure 7: Effect of stingless bee pollen on QUICK-I index in experimental groups of rats

Values are given as mean ± SEM for groups of six rats in each. One-way ANOVA followed by post hoc test LSD. Statistical significance was compared within the groups as follows

[a]compared with control

[b]compared with diabetic rats

Discussion

To combat the increasing prevalence of T2DM, there is an urgent need for more effective treatments as most of the currently available drugs for the treatment of T2DM elicit undesirable side effects after prolonged use. It is a fact that the type 2 diabetic patients are often treated with combinatorial drugs due to its multifactorial and multisystemic nature. The animal model used in the present study involves a combination of a diet high in fat to bring about insulin resistance, hyperinsulinemia and/or impaired glucose tolerance followed by treatment with a low dose of STZ, a specific β cell toxin which results in reduction in functional β cell mass [13]. The pathological condition induced by the stressors namely the high fat diet and low dose STZ closely resembles the clinical features of T2DM, though on a shorter time scale than found in the human condition [14].

The transition from a metabolically healthy state to an obese and subsequent state involves a vicious cycle comprising of hyperinsulinemia, insulin resistance, dyslipidemia and dysfunctional adipose tissue, ectopic fat accumulation in both liver as well as skeletal muscle and failure of insulin producing β cell function [15]. The composition and duration of high fat diet feeding timeframe greatly influence the onset of T2DM in experimental rats. The most commonly used approach is to feed rats with a diet high in fat but normal levels of carbohydrates rather than a diet high in carbohydrates to produce a 'high energy' feeding regimen [16].

STZ is a nitrosourea analogue, preferentially taken up by pancreatic β cells via GLUT2 glucose transporter and causes DNA alkylation followed by the activation of poly ADP ribosylation leading to depletion of cytosolic concentration of NAD+ and ATP. Enhanced ATP dephosphorylation after STZ treatment provides substrate for xanthine oxidase resulting in the generation of superoxide radicals [17].

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Further, nitric oxide (NO) moiety is liberated from STZ leading to irreversible destruction of β cells by necrosis [18]. It was observed that STZ administration at first abolished the β cell response to glucose. Temporary return to responsiveness then appears which is followed by its permanent loss and β cells are damaged [19]. Though, STZ is widely used to induce both type 1 and type 2 diabetes in animal models, the STZ dose will greatly affect the β cell mass remaining in the rats. Likewise, variation in the amount of β cell mass left in both type 1 and type 2 diabetes do exist in human [20].

However, the loss of β cell mass in the pathogenesis of type 1 diabetes occurs mainly as a result of an autoimmune reaction, which is not the case in T2DM. The dose of STZ itself obviously has a significant impact on the phenotype of HFD-fed rats. STZ induces robust not absolute β cell ablation in a manner that depends on the dose, number of doses, time interval between the doses, route of administration, fed/fasted state upon STZ administration and the rat strain/vendor. However, several reports are available in the literature that a single low dose of STZ (<45mg/kg) induced experimental diabetes as a suitable model of experimental type 2 diabetes to screen the efficacy of lead molecules [21].

The observed weight loss in the untreated group of diabetic rats may be due to excessive degradation of tissue structural proteins known to contribute to body weight [21]. Though, all the experimental groups of rats were continued to feed with a high fat diet, the untreated diabetic rats have shown a marginal increase in body weight gain when compared to other experimental groups of rats indicating the alteration in the major metabolic pathways due to decreased insulin sensitivity coupled with insufficiency [22].

The positive effect of stingless bee pollen and metformin in maintaining normal homeostasis was demonstrated by the significant improvement in body weight gain of rats in other treatment groups. However, diabetic rats treated with stingless bee pollen showed a relatively better improvement in body weight gain and the efficacy of stingless bee pollen was comparable with metformin [23].

Increased food and fluid consumption was observed in diabetic group of rats. This indicates the polyphagia and polydipsic condition. The classic triad of diabetic symptoms are polyphagia, polydipsia and polyuria [24]. An increase in renal loss of glucose is accompanied by an excessive loss of water and electrolytes. Excessive loss of water leads to the activation of thirst mechanism, termed as polydipsia. An increased thirst may be due to hyperglycemia which raises the osmolarity of blood and makes it more concentrated. Glucosuria and increased tissue protein catabolism leads to crave excess amount of glucose for energy production, an increase in appetite and food intake called as polyphagia [25].

The food and fluid intake of diabetic groups of rats treated with individual as well as stingless bee pollen were significantly reduced after 30 days of treatment. From the data, it is clearly manifested that the stingless bee pollen as well as metformin considerably reduced the pathophysiological symptoms through its significant hypoglycemic properties [23]. Like, the effect on body weight gain, the diabetic rats treated with stingless bee pollen significantly improved the polyphagia and polydipsic condition than the experimental rats treated with metformin [26].

Oral glucose tolerance test (OGTT) has been the mainstay for diagnosing diabetes for decades. It more efficiently detects prediabetics as well as patients with impaired glucose tolerance [27]. OGTT has the utility for evaluating insulin sensitivity and β-cell function during glucose administration via a physiological route [27, 28]. OGTT is commonly used to evaluate the disease progression, outcome of treatments and to assess the physiological and pathophysiological conditions of diabetes mellitus. Oral glucose tolerance test provides more physiological conditions for the estimation of β-cell function than does a test based on intravenous glucose administration. OGTT provides significant and valuable information for predicting the ensuing incidence of type 2 diabetes [28].

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The routinely used imperative marker for estimating the degree of protein glycation in diabetes includes glycated hemoglobin levels. Extensive studies on HbA1c bring out the importance of HbA1c as a non-manipulatable and reliable biochemical parameter in assessing metabolic control as compared to one-point blood glucose estimation [29] . HbA1c is likely to be a more physiological assessment of glucose intolerance than the artificial conditions of the OGTT and hence it should be the preferred diagnostic test. During persistent hyperglycemia, glucose irreversibly binds to the N-terminal valine of the β chain of hemoglobin [30].

The process of glycation at other positions such as lysine on the β-chain or at sites on the α-chain may be imperative at higher levels of glycation. The percent glycation of hemoglobin also depends on the average age of the erythrocytes in the sample and the percent of HbA1c is higher in older cells [31]. HbA1c levels represent average glycemia over the entire 120-day life span of the red blood cell. HbA1c measurement may also be used as a tool to stratify the risk of the patient developing microvascular complications because there was an exponential rise in the rate of secondary complications with increasing HbA1c values. Each 1% reduction in glycated hemoglobin was associated with a significant reduction in diabetes related mortalities [31, 32] .

HbA1c signifies the mean glucose levels maintained over the previous 6-8 weeks. Normoglycemia was achieved in diabetic groups of rats treated with individual as well as stingless bee pollen possibly through their ability to maintain glucose homeostasis [33]. However, the diabetic rats treated with stingless bee pollen showed a significant decrease in the levels of fasting blood glucose as well as HbA1c and increased levels of plasma insulin next to diabetic rats treated with stingless bee pollen and the efficacy of stingless bee pollen was comparable with metformin evidencing that the stingless bee pollen possesses significant insulin stimulatory and insulin sensitivity properties [33, 34]. Patients with most depressed β-cell function had a higher HbA1c and higher baseline glucose, but lower baseline insulin and C-peptide[35].

The balance between the utilization and production of glucose is maintained by the hormone, insulin. It stimulates glucose uptake, utilization and storage while suppressing hepatic glucose production, thus reducing plasma glucose levels. Insulin inhibits the production and release of glucose by the liver, due to the blockage of gluconeogenesis and glycogenolysis [36]. C-peptide, a bioactive peptide plays a crucial role in the biosynthesis of and has a half life of 30 min. Subsequent to the cleavage from the proinsulin molecule; C-peptide is released into the blood stream as equimolar concentrations to insulin [37]. The serum levels of insulin and C-peptide greatly increase in the early stage of T2DM because of insulin resistance. Although T2DM is a state of insulin resistance to relative insulin insufficiency, it may progress to a late-stage insulin and C-peptide-deficient state due to pancreatic β-cell demise [37, 38].

Physiological concentrations of C-peptide activates extracellular signal regulated kinases, phosphatidyl inositol 3-kinase, protein kinase C, elevates intracellular calcium, and stimulates peroxisome proliferator activated receptor- γ [39]. The observed increased in the levels of C-peptide in diabetic rats treated with stingless bee pollen as well as metformin may be due to the stimulation of molecular factors that produce c-peptide from the pancreatic β-cells, thereby ameliorate the secondary complications of diabetes [39, 40].

The homeostasis model assessment of insulin resistance (HOMA-IR) developed by Matthews et al. (1985) have been extensively used for the determination of insulin resistance [41] . Insulin resistance is the primary metabolic disorder associated with obesity and appears to be the primary mediator of metabolic syndromes. Likewise, QUICKI is also an empirically derived mathematical conversion of fasting blood glucose and plasma insulin concentrations. However, it provides a reliable, reproducible and accurate index of insulin sensitivity with a better optimistic prognostic power [41, 42].

HOMA- β cell function is effectively used to measure the pancreatic β-cell function. Evidences suggested that hyperglycemia causes additional functional impairments in insulin release that go beyond the actual β-cell deficit [43]. The majority of genes associated with type 2 diabetes have been linked to the β-cell dysfunction and impairments in β-cell mass. If β-cell mass is reduced by 50%, the secretory burden for the remaining β-cells increases by 100%, thereby leading to chronic β-cell stress [43, 44]. The data obtained through the above three arithmetical indices suggest that the diabetic rats treated with stingless bee pollen significantly improved the β-cell function and insulin sensitivity indicating its synergistic efficacy in ameliorating chronic hyperglycemia.

Conclusion

In conclusion, the results of the present study clearly evidenced that the stingless bee pollen possess significant antidiabetic properties which are evidenced from the decreased levels of fasting blood glucose, glycosylated hemoglobin and increased levels of plasma insulin and C-peptide. The arithmetic indicators also evidenced the antidiabetic efficacy of stingless bee pollen. Thus, stingless bee pollen may be considered as a safe and effective drug for the successful treatment of type 2 diabetes. Detailed studies are in progress to evaluate the effect of stingless bee pollen on lipid and protein metabolism as well as on oxidative stress in experimental type 2 diabetes.

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References

1. Maqbool M, Naeem A, Aamer S. Diabetes Mellitus and Its Various Management Strategies in Practice. Indo Am J Pharm Sci. 2018;5(8):8163-+.
2. Dendup T, Feng X, Clingan S, Astell-Burt T. Environmental risk factors for developing type 2 diabetes mellitus: A systematic review. Int J Environ Res Public Health. 2018;15(1).
3. Nolan CJ, Damm P, Prentki M. Type 2 diabetes across generations: From pathophysiology to prevention and management. Lancet [Internet]. 2011;378(9786):169–81. Available from: http://dx.doi.org/10.1016/S0140-6736(11)60614-4
4. Cole JB, Florez JC. Genetics of diabetes mellitus and diabetes complications. Nat Rev Nephrol [Internet]. 2020;16(7):377–90. Available from: http://dx.doi.org/10.1038/s41581-020-0278-5
5. Zaccardi F, Webb DR, Yates T, Davies MJ. Pathophysiology of type 1 and type 2 diabetes mellitus: A 90-year perspective. Postgrad Med J. 2016;92(1084):63–9.
6. M C B Nascimento A, E Luz Jr G. Bee pollen properties: uses and potential pharmacological applications-a review. J Anal Pharm Res. 2018;7(5):513–5.
7. Setyawan AB, Satria AP, Arung ET, Paramita S. Effect of Bee Pollen Kelulut Bees on HBA1C in Type 2 Diabetes Mellitus Patients. Malaysian J Fundam Appl Sci. 2023;19(1):56–60.
8. Ibrahim N, Mohd Niza NFS, Mohd Rodi MM, Zakaria AJ, Ismail Z, Mohd KS. Chemical and Biological Analyses of Malaysian Stingless Bee Propolis Extracts. Malaysian J Anal Sci. 2016;20(2):413–22.
9. Kocot J, Kiełczykowska M, Luchowska-Kocot D, Kurzepa J, Musik I. Antioxidant potential of propolis, bee pollen, and royal jelly: Possible medical application. Oxid Med Cell Longev. 2018;2018.
10. Magdaleni SDAR. Secondary Metabolite Compounds of Bee Pollen the Geniotrigona Incise Sakagami & Inoue. from Samarinda East Borneo. Int J Sci Res [Internet]. 2018;7(2):556–60. Available from: https://www.ijsr.net/archive/v7i2/ART201710012.pdf
11. Furman BL. Streptozotocin-Induced Diabetic Models in Mice and Rats. Curr Protoc Pharmacol. 2015;70(1):5.47.1-5.47.20.
12. Omotayo EO, Gurtu S, Sulaiman SA, Wahab MSA, Sirajudeen KNS, Salleh MSM. Hypoglycemic and

antioxidant effects of honey supplementation in streptozotocin-induced diabetic rats. Int J Vitam Nutr Res. 2010;80(1):74–82.

13. Yingying Z, Lianger D, Lu H, Zhenxing S, Jilin D, Yang Y. Effects of oat β-glucan , oat resistant starch , and the whole oat flour on insulin resistance , inflammation , and gut microbiota in high-fat-diet-induced type 2 diabetic rats. 2020;69(April).
14. Erejuwa OO, Sulaiman SA, Wahab MS, Sirajudeen KNS, Salleh MSMD, Gurtu S. Action protectrice anti-oxydante du miel malésien Tualang sur le pancréas de rats normaux ou diabétiques induits par la streptozotocine. Ann Endocrinol (Paris) [Internet]. 2010;71(4):291–6. Available from: http://dx.doi.org/10.1016/j.ando.2010.03.003
15. Janoutov J. Optimal Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) Cut-O ff s : A Cross-Sectional Study in the Czech Population. 2019;
16. Denisow B, Denisow-Pietrzyk M. Biological and therapeutic properties of bee pollen: a review. J Sci Food Agric. 2016;96(13):4303–9.
17. Yahfoufi N, Alsadi N, Jambi M, Matar C. The immunomodulatory and anti-inflammatory role of polyphenols. Nutrients. 2018;10(11):1–23.
18. Badrulhisham NSR, Ab Hamid SNP, Ismail MAH, Yong YK, Muhamad Zakuan N, Harith HH, et al. Harvested locations influence the total phenolic content, antioxidant levels, cytotoxic, and anti-inflammatory activities of stingless bee honey. J Asia Pac Entomol [Internet]. 2020;23(4):950–6. Available from: https://doi.org/10.1016/j.aspen.2020.07.015
19. Cheng CW, Villani V, Buono R, Wei M, Kumar S, Yilmaz OH, et al. Fasting-Mimicking Diet Promotes Ngn3-Driven β-Cell Regeneration to Reverse Diabetes. Cell [Internet]. 2017;168(5):775-788.e12. Available from: http://dx.doi.org/10.1016/j.cell.2017.01.040
20. Lytrivi M, Castell AL, Poitout V, Cnop M. Recent Insights Into Mechanisms of β-Cell Lipo- and Glucolipotoxicity in Type 2 Diabetes. J Mol Biol [Internet]. 2020;432(5):1514–34. Available from: https://doi.org/10.1016/j.jmb.2019.09.016
21. Sihota P, Yadav RN, Poleboina S, Mehandia V, Bhadada SK, Tikoo K, et al. Development of HFD-Fed/Low-Dose STZ-Treated Female Sprague-Dawley Rat Model to Investigate Diabetic Bone Fragility at Different Organization Levels. JBMR Plus. 2020;4(10).
22. Sohrabipour S, Sharifi MR, Talebi A, Soltani N. GABA dramatically improves glucose tolerance in streptozotocin-induced diabetic rats fed with high-fat diet. Eur J Pharmacol [Internet]. 2018;826:75–84. Available from: https://doi.org/10.1016/j.ejphar.2018.01.047
23. Setyawan AB, Mahadeva Rao US, Sairazi NSM. Therapeutic Potential of Stingless bee Pollen: A Review. Res J Pharm Technol. 2023;16(5):2549–56.
24. Honesta K, Edna M, Benjamin K, Lucy M. Clinical Presentation and Factors Associated with Diabetic Ketoacidosis at the Onset of Type-1 Diabetes Mellitus in Children and Adolescent at Muhimbili National Hospital, Tanzania: A Cross Section Study. Int J Diabetes Clin Res. 2020;7(3):1–8.
25. Fauziyah Y, Sunarti S, Hanoum IF, Wahyuningsih MSH. Ethanol Extract of Tithonia diversifolia (Hemsley) A Gray Standardized Ameliorates Hyperglycemia, Polyphagia, and Weight Loss in Diabetic Rats. Molekul. 2018;13(1):72.
26. Khalifa SAM, Elashal MH, Yosri N, Du M, Musharraf SG, Nahar L, et al. Bee pollen: Current status and therapeutic potential. Nutrients. 2021;13(6):1–15.
27. Kuo FY, Cheng KC, Li Y, Cheng JT. Oral glucose tolerance test in diabetes, the old method revisited. World J Diabetes. 2021;12(6):786–93.
28. Jagannathan R, Neves JS, Dorcely B, Chung ST, Tamura K, Rhee M, et al. The oral glucose tolerance test: 100 years later. Diabetes, Metab Syndr Obes. 2020;13:3787–805.
29. Gummesson A, Nyman E, Knutsson M, Karpefors M. Effect of weight reduction on glycated haemoglobin in weight loss trials in patients with type 2 diabetes. Diabetes, Obes Metab. 2017;19(9):1295–305.
30. Carlsson H, Rappaport SM, Törnqvist M. Protein adductomics: Methodologies for untargeted

screening of adducts to serum albumin and hemoglobin in human blood samples. High-Throughput. 2019;8(1).

31. Rossello X, Raposeiras-Roubin S, Oliva B, Sánchez-Cabo F, García-Ruíz JM, Caimari F, et al. Glycated Hemoglobin and Subclinical Atherosclerosis in People Without Diabetes. J Am Coll Cardiol. 2021;77(22):2777–91.
32. Mitsios JP, Ekinci EI, Mitsios GP, Churilov L, Thijs V. Relationship between glycated hemoglobin and stroke risk: A systematic review and meta-analysis. J Am Heart Assoc. 2018;7(11).
33. Syafrizal, Ramadhan R, Kusuma IW, Egra S, Shimizu K, Kanzaki M, et al. Diversity and honey properties of stingless bees from meliponiculture in east and north kalimantan, indonesia. Biodiversitas. 2020;21(10):4623–30.
34. Brodkiewicza Y, Marcinkevicius K, Reynoso M, Salomon V, Maldonado L, Vera N. Studies of the Biological and Therapeutic Effects of. J Drug Deliv Ther. 2018;8(5):382–92.
35. Babaya N, Noso S, Hiromine Y, Taketomo Y, Niwano F, Yoshida S, et al. Relationship of continuous glucose monitoring-related metrics with HbA1c and residual β-cell function in Japanese patients with type 1 diabetes. Sci Rep [Internet]. 2021;11(1):1–9. Available from: https://doi.org/10.1038/s41598-021-83599-x
36. Hatting M, Tavares CDJ, Sharabi K, Rines AK, Puigserver P. Insulin regulation of gluconeogenesis. Ann N Y Acad Sci. 2018;1411(1):21–35.
37. Gar C, Rottenkolber M, Prehn C, Adamski J, Seissler J, Lechner A. Serum and plasma amino acids as markers of prediabetes, insulin resistance, and incident diabetes. Crit Rev Clin Lab Sci [Internet]. 2018;55(1):21–32. Available from: https://doi.org/10.1080/10408363.2017.1414143
38. Yaribeygi H, Maleki M, Sathyapalan T, Sahebkar A. The effect of C-peptide on diabetic nephropathy: A review of molecular mechanisms. Life Sci [Internet]. 2019;237:116950. Available from: https://doi.org/10.1016/j.lfs.2019.116950
39. Luppi P, Drain P. C-peptide antioxidant adaptive pathways in β cells and diabetes. J Intern Med. 2017;281(1):7–24.
40. Landreh M, Jörnvall H. Biological activity versus physiological function of proinsulin C-peptide. Cell Mol Life Sci [Internet]. 2021;78(3):1131–8. Available from: https://doi.org/10.1007/s00018-020-03636-2
41. de Fátima Haueisen Sander Diniz M, Beleigoli AMR, Schmidt MI, Duncan BB, Ribeiro ALP, Vidigal PG, et al. Homeostasis model assessment of insulin resistance (HOMA-IR) and metabolic syndrome at baseline of a multicentric Brazilian cohort: ELSA-Brasil study. Cad Saude Publica. 2020;36(8).
42. Park SY, Gautier JF, Chon S. Assessment of insulin secretion and insulin resistance in human. Diabetes Metab J. 2021;45(5):641–54.
43. Wu S, Gao L, Cipriani A, Huang Y, Yang Z, Yang J, et al. The effects of incretin-based therapies on β-cell function and insulin resistance in type 2 diabetes: A systematic review and network meta-analysis combining 360 trials. Diabetes, Obes Metab. 2019;21(4):975–83.
44. Eguchi N, Vaziri ND, Dafoe DC, Ichii H. The role of oxidative stress in pancreatic β cell dysfunction in diabetes. Int J Mol Sci. 2021;22(4):1–18.