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Antidiabetic Properties of Stingless Bee Pollen in High-Fat Diet FED-Low Dose STZ Induced Experimental T2DM Rats

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Abstract

Diabetes mellitus (DM) is a multisystem metabolic disease because of either insulin insufficiency or ineffectiveness. Individuals DM continuously measure their blood glucose levels during fasting and after eating to manage their condition effectively. Despite the availability of several drugs, there is no treatment proven to be perfect due to adverse side impacts as well as reduced long-term efficacy. Moreover, the excessive formation of free radicals has been linked to diabetes progression, development, as well as complications. Therefore, this study analyzes the antidiabetic properties of stingless bee pollen scientifically. This natural product was fed a high-fat diet and low streptozotocin doses in experimental rats with T2DM. An oral glucose tolerance test aimed investigate stingless bee pollen effect on glucose homeostasis. An insulin toleration test was executed to obtain stingless pollen influence on insulin sensitivity. This study measures Insulin resistance with HOMA-IR. Oral administration of stingless bee pollen for 30 days raised glucose homeostasis in T2DM rats, as shown by the OGTT, ITT, and HOMA-IR results. In addition, this treatment significantly improved changes in glycated hemoglobin, fasting blood glucose, protein, uric acid, urea, as well as creatinine levels. The activity changes of ALP, AST, and ALT liver enzyme markers in T2DM rats were nearly normalized after given with stingless bee pollen. The activity of carbohydrate metabolism enzymes includes pyruvate kinase, glucokinase, glucose-6-phosphatase, glucose-6-phosphate dehydrogenase, fructose-1,6-bisphosphatase and lactate dehydrogenase in T2DM rats' liver tissue. Conclusion this change was significantly restored to near-normal levels after stingless bee pollen treatment.

Keywords
Diabetes Mellitus, High Fat Diet, Metformin, Stingless Bee Pollen

Introduction

Type 2 diabetes, also defined as T2DM or non-insulin-dependent DM, is a chronic and progressive condition indicated by elevated blood sugar levels and impaired insulin function [1]. T2DM can lead to severe complications affecting vital body systems, such as the kidneys, eyes, heart, and overall vascular system. In individuals with T2DM, there is either insufficient insulin production (insulin deficiency) or ineffective utilization of insulin by cells (insulin resistance). Insulin, created in the pancreas by the beta cells, is

important in regulating blood glucose levels (Dendup et al., 2018). Insulin resistance, related to genetic predisposition, obesity, physical inactivity, and aging, contributes to the T2DM development. A high-fat diet consumption and leading a sedentary lifestyle are significant predictors of obesity and T2DM [2].

In the case of obese individuals, the body's response to insulin insensitivity is insufficient, particularly regarding the liver's glucose production. As the disease progresses, the metabolism of carbohydrates, fats, and proteins is disrupted. This condition leads to "pre-diabetes," where blood sugar levels are above the T2DM blood sugar range [3]. Hyperglycaemia occurs when beta cells cannot keep up with insulin resistance by perspiring excess insulin. Over time, hyperglycaemia leads to progressive declines in β-cell function and quality, marking T2DM development [4].

In the search for alternative treatments, nature provides an abundant source of bioactive molecules, including stingless bee pollen. Stingless bee pollen, a rich yield from bees, is composed of a combination of pollen, nectar, and bee secretions. Beekeepers are able to collect it without causing harm to the hive. This increasingly popular natural product, known as Stingless Bee Pollen (SBP), is considered a beneficial apitherapy product that potentially has nutritional and medical benefits [5].

Figure 1: Stingless Bee Products (Honey, Bee Pollen, Propolis)

As mentioned in previous studies, flavonoids present in stingless bee pollen possess antioxidant properties and are believed to have the ability to reduce blood glucose levels by inhibiting oxidative stress [6]. Additionally, the stingless bee pollen antioxidant activity may enhance insulin receptor signalling in diseases characterized by insulin resistance, potentially improving insulin sensitivity [7].

In light of these findings, the stingless bee pollen utilized in this research was obtained from the Kelulut bee (Trigona sp), which is known for its small size and lack of stingers on its tails. These bees are discovered in the East Kalimantan forests. The advantage of using the Kelulut bee is its ability to produce a higher quantity of bee pollen [8]. Considering that T2DM is a complex metabolic disorder affecting multiple systems, the current trend in diabetes research is to explore natural products derived from bee products, which are known for their rich content of bioactive molecules. Accordingly, the study objective is to examine the impact of stingless bee pollen on T2DM rats induced by a high-fat diet as well as low-dose streptozotocin (STZ).

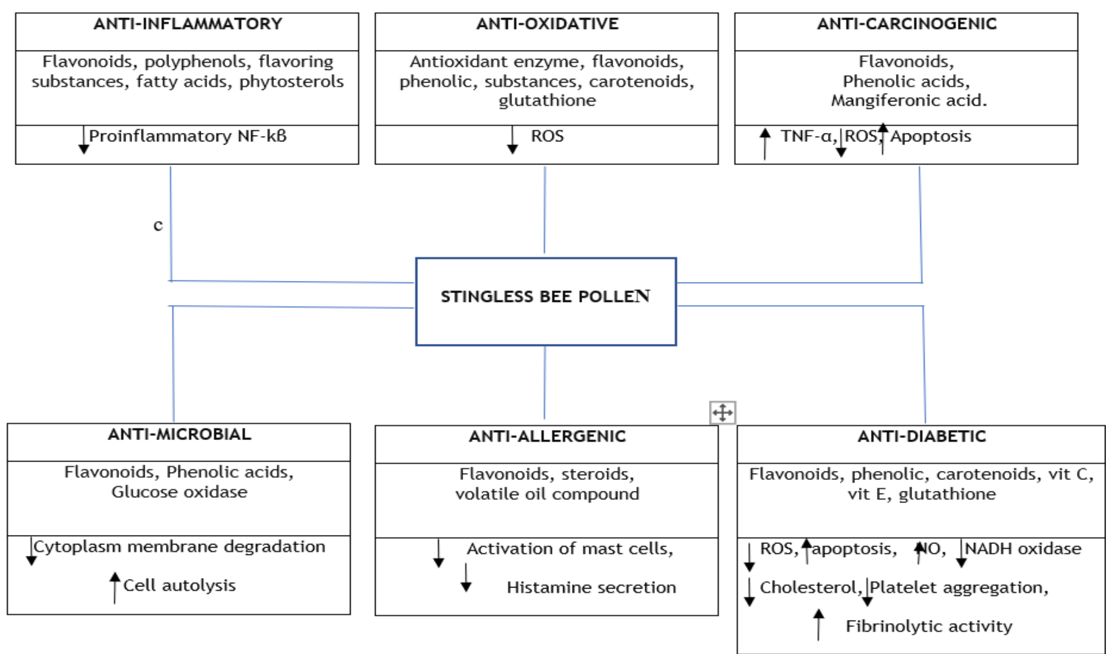

Figure 2: Stingless Bee pollen's primary and secondary metabolites have a complementary and alternative public medicine by which the pollen compounds act. Abbreviations: NF-kβ, nuclear factor kappa-light-chain-enhancer of activated B cells; ROS, reactive oxygen species; TNF-α, tumors necrosis factor alpha; ALA, α-linolenic acid; ↓, decrease/inhibition; ↑, increase/activation

Materials and Methods

Chemicals

Streptozotocin were bought from Sigma Aldrich and kept in a dark environment at a temperature ranging from 2-4°C. All of the different chemicals utilized were obtained from retail suppliers and have analytical grades.

Animal

Male Wistar strain rats were acquired from Gadjah Mada University, Yogyakarta, Indonesia with a weight of around 160-180 g. The rats were lodged in ample polypropylene cages equipped with bedding. The study kept the rats in controlled conditions, including a 12:12 ± 1-hour light-dark cycle, maintained at 22°C ± 3°C, as well as the relative humidity of 55%. To minimize the effects of stress, the rats were acclimated to the standard housing conditions within a week before the commencement of the experiment. The feed given was commercially available pelleted rat food and had unrestricted access to water during the study time [9].

HFD Streptozotocin-Induced T2DM

Rats were assigned to the 2 diets by receiving an HFD or NPD control within two weeks. The HFD arrangement is powdered NPD - 365 g/kg, lard - 310 g/kg, casein - 250 g/kg, cholesterol - 10 g/kg, and vitamin as well as mineral mixture - 60 g/kg. DL-methionine - 3kg/kg, yeast - 1g/kg, sodium chloride - 1g/kg. After 2 HFD weeks, rats in groups II, III, and IV were given an unmarried STZ dose injection (35 mg/kg body weight/rat), while group I fed NPD were infiltrated with 0.5 mg/kg body weight/rat ml of freshly prepared. The similar cold citrate buffer (pH 4.5) volume was injected intraperitoneally. After the STZ injection in one week, rats which have fasting blood glucose >120 mg/dl were classified as T2DM as well as selected for deep analysis (Omotayo et al., 2010). Animals were split into four categories, apiece including at least 6 rats, as follows.

Experimental Animal Design

The four groups consisted of six rats respectively. Furthermore, the group I categorized as the normal control, where provided a normal pellet diet (NPD), as well as treated with water for a month (acting as the

negative control). Group II consisted of rats with induced T2DM by administering a high-fat diet (HFD) along with the injection of streptozotocin (STZ) (35 mg/kg bw). These rats were also treated with water for a month (acting as the positive control). In Group III, T2DM rats induced by HFD and STZ injection were given stingless bee pollen (100 mg/kg bw/rat/day) for a month. Group IV included T2DM rats induced by HFD and STZ injection, and they were administered oral metformin (50 mg/kg bw/rat/day) dissolved in an aqueous solution for a month. The rats underwent an overnight fasting period during the final experimental phase, anesthetized with ketamine (80 mg/kg body weight/rat, intraperitoneally), as well as euthanized by cervical decapitation. Moreover, blood samples were gathered to separate plasma and serum, with and without anticoagulants, severally.

Oral Glucose Tolerance Test (OGTT)
During the final week, an OGTT was executed on all overnight fasted rats' groups. Blood glucose levels were monitored with a one-button blood glucose meter (Life Scan, Johnson and Johnson Company) at 0 to 120 minutes behind oral administration at 2 g/kg body weight. Dextrose in water [10].

Homeostasis Model Determination of Insulin Assessment
Since a measurement of insulin or blood glucose cannot accurately detect insulin abnormalities, insulin resistance was assessed by the HOMA-IR, as follows [11]: HOMA-IR = fasting insulin (μU/L) x Fasting blood glucose (nmol/L)/ 22.5

Biochemical Parameters
Values for fasting blood glucose, blood urea, glycated haemoglobin, uric acid, serum creatinine, and plasma protein were calculated [12]. Plasma insulin levels were determined using an ELISA kit for rat insulin determination (Linco Research, St. Charles, MO, USA). The glucose in the urine was tested utilizing a dipstick (Diastix). Check the activities of ALT, ALP, and AST pathological marker enzymes in serum [12].

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
Effects are described as an average ± normal error of 6 rats per class, and statistical importance was assessed by ANOVA using the SPSS and LSD program. Values were assessed as statistically influential if $p<0.05$.

Results and Discussion
In T2DM rats induced with low-dose STZ and fed HFD, a high-fat diet combination induced hyperinsulinemia, insulin resistance, as well as glucose tolerance, then are given by β-cell toxin STZ treatment, significantly reduced functional β-cell mass. At the same time, these two stressors are said to mimic the T2DM pathology in a shorter time than humans [13]. Furthermore, the influence of oral stingless bee pollen administration toward the OGTT of rats in both groups is shown in Figure 3. Intraperitoneal insulin exposure is shown in Figure 4. T2DM rats treated with stingless bee pollen and metformin had significantly lower blood sugar levels.

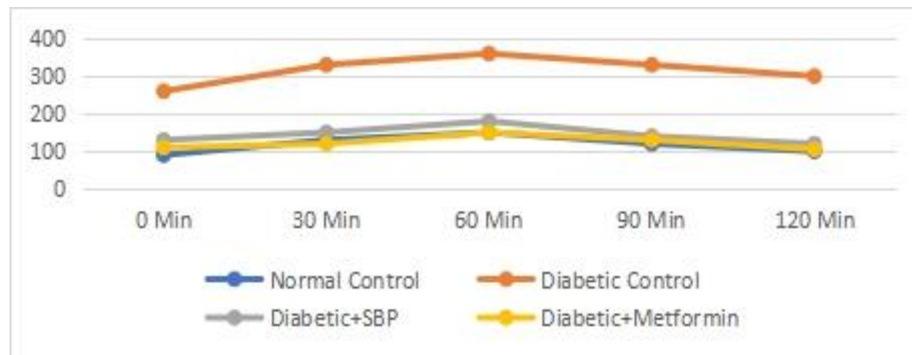

Figure 3: The oral administration influence of SBP on OGTT in Control and Experimental Groups

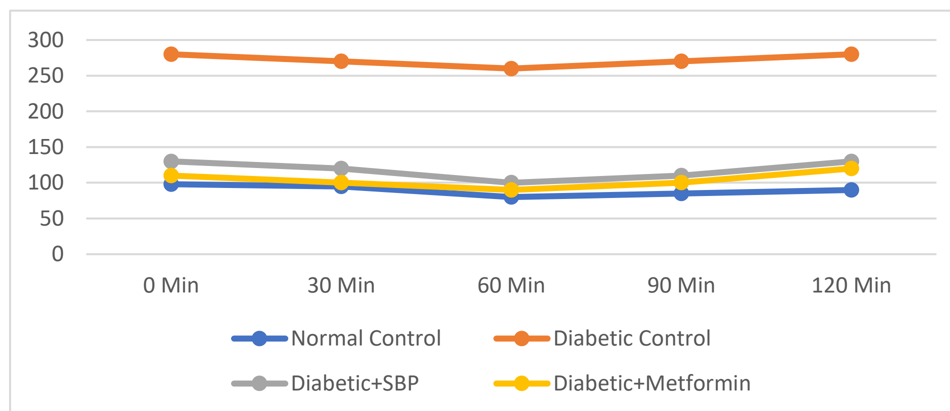

Figure 4: The Impact of Oral Administration of Stingless Bee Pollen (SBP) on ITT in Control and Experimental Groups of Rats

In control groups, the highest point of the blood glucose level was 60 minutes after an oral glucose challenge as well as slowly declined, returning to nearly normal after 120 minutes. This pattern indicates normal glucose homeostasis maintenance. However, in HFD-STZ-induced T2DM rats, blood glucose peaked at 60 minutes and remained elevated for 60 minutes. Oral management of stingless bee pollen and metformin significantly reduced the fasting times at 30 and 60 minutes than untreated T2DM rats. Moreover, in a group of T2DM rats treated with stingless bee pollen and metformin, blood glucose levels back to baseline after 120 minutes following an oral glucose challenge. This suggests that the stingless bee pollen-treated rats could maintain normal blood glucose levels (euglycemia) [14].

The (OGTT) oral glucose tolerance test is highly valuable as it utilizes fasting blood glucose and serves as a practical tool to simplify and streamline the T2DM diagnosis. Chronic hyperglycaemia is crucial in the T2DM complications onset and advancement [15]. Like the OGTT, the ITT monitors glucose concentrations over time but responds to insulin boluses rather than glucose boluses. After a bolus of insulin by intraperitoneal injection, blood glucose concentrations are monitored every 15 to 30 minutes for 60 to 90 minutes. The extent to which blood glucose levels drop after an insulin bolus indicates the effects of insulin throughout the body.

The HOMA-IR of normal subjects, T2DM patients, and T2DM patients treated with stingless bee pollen are shown in Fig.5 HOMA-IR was significantly increased in T2DM rats and significantly decreased after taking stingless bee pollen. Insulin resistance is a typical metabolic defect that precedes overt β-cell dysfunction as well as mainly related to insulin-mediated resistance to peripheral glucose handling and compensatory hyperinsulinemia. HOMA-IR has proven to be a powerful tool for the insulin-resistance surrogate

assessment [16]. As a more convenient way to measure insulin resistance, a homeostatic model to assess insulin resistance was developed and widely used in clinical and epidemiological studies [17].

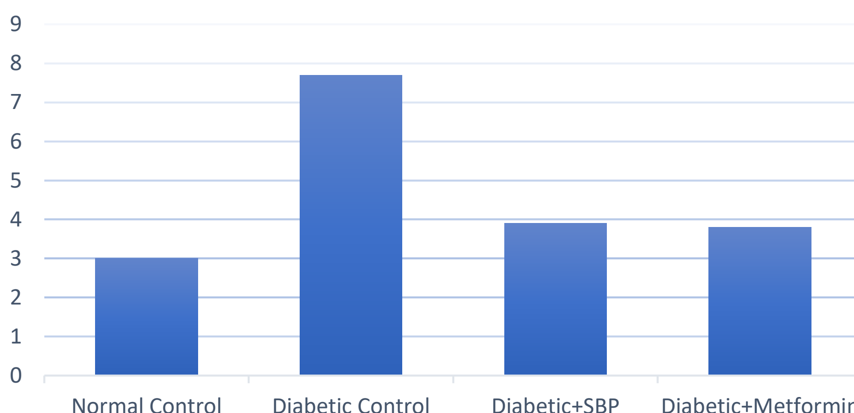

Figure 5: HOMA-IR on Stingless Bee Pollen (SBP) Treated and Experimental Groups

Table 1 shows the stingless bee pollen impacts on glycosylated haemoglobin, fasting blood glucose, plasma insulin, as well as urine glucose in HFD-STZ T2DM rats. The study found that fasting blood glucose and HbA1c levels were higher in T2DM than in normal groups. Oral administration of stingless bee pollen to T2DM rats ameliorated altered FBG and HbA1c levels. Plasma insulin levels were modestly reduced in HFD-STZ-induced T2DM rats. Insulin improved in T2DM rats with stingless bee pollen and metformin. Moreover, the study found that their urine sugar was missing in the stingless bee pollen and metformin-treated rats.

Table 1: Stingless Bee Pollen (SBP) Effect on The Fasting Blood Glucose Levels, HbA1C, Plasma Insulin, as well as Urine Sugar in The Experimental Group after a Month Experiment

Groups	Fasting Blood Glucose (mg/dl)	HbA1C (%Hb)	Plasma insulin (μU/ml)	Urine Sugar
Normal Control	83.30±4.76	5.08±0.25	18.68±0.26	Nil
Diabetic Control	290.18±9.05[a*]	12.40±0.41 [a*]	10.19±0.36 [a*]	+++
Diabetic + SBP	132.11±5.90[b*]	7.05±0.21[b*]	12.14±0.43[b*]	Nil
Diabetic + Metformin	119.27±6.81[b*]	6.71±0.18[b*]	14.39±0.43[b*]	Nil

Values are given as average ± SEM for 6 rats in each group. Post hoc test LSD observed one-way ANOVA. Values with diverse superscript letters in the similar parameter are very different, $P<0.05$.
[a]Diabetic rats were compared with control rats.
[b]Diabetic + Stingless Bee Pollen and T2DM + Metformin treated were compared to T2DM rats.

The trustworthy indicator for the T2DM diagnosis as well as prediction is blood glucose. In DM, blood glucose levels are markedly elevated due to decreased glucose used by diverse tissues, typical of reduced insulin [18]. Increased insulin secretion increases glucose utilization by extrahepatic tissues, thereby reducing blood glucose levels [19]. Elevated plasma insulin levels are strongly related to increased insulin-mediated resistance to glucose uptake [20].

Non-enzymatic and irreversible covalent binding of excess glucose to circulating haemoglobin leads to the HbA1C formation, a key parameter for evaluating long-term glycaemic control and estimating diabetic intricacies development [21]. HbA1c was introduced into the clinic in the 1980s and has become a cornerstone of clinical practice [22]. HbA1c indicates mean plasma glucose after 8 to 12 weeks. Glycosylated haemoglobin remains in the blood for the remaining life of the red blood cells (120 days). For these reasons, the determination of glycated haemoglobin has become an important and reliable tool in diagnosing and prognosis diabetes [23].

The formation of HbA1c is a two-step process in which glucose first binds to the β chain's N-terminal valine to create an unstable aldimine, which then carried out an Amadori rearrangement to shape stable ketamine. The first reaction is fast and reversible, while the second is irreversible. Depending on the glucose concentration in the environment, aldimines are formed and dissociated rapidly, and ketamine is formed more slowly. The reduce HbA1c levels in the treated rats indicated the maintenance of normoglycemia in T2DM rats [24].

Renal glucose thresholds are of great interest to physiologists concerned with renal function and are equally important to clinicians in the study and management of T2DM urine glucose. Below the glucose threshold of the kidneys, there is no sugar in the urine, and above this level, sugar appears in the urine in large quantities, both in total and percentage concentrations, as blood glucose gradually rises. Glucose in the urine is reflected in the renal threshold for glucose excretion. The renal threshold for glucose excretion was generalized, setting the threshold at ~10 mmol/L [25].

The stingless bee pollen effects on serum uric acid, blood urea, plasma protein, as well as serum creatinine levels in both groups are displayed in Table 2. It was found that total protein levels were reduced in STZ-induced T2DM rats. It was found that serum uric acid, blood urea, as well as serum creatinine levels increased in STZ-induced T2DM rats. After oral administration of stingless bee pollen, these biochemical markers returned to near-normal levels.

Table 2: Stingless Bee Pollen (SBP) Effect on Total Protein Level, Serum Uric Acid, Blood Urea, Serum Creatinine of Control and Experimental Groups

Groups	Total Protein (gr/dl)	Blood Urea (mg/dl)	Serum Uric Acid (mg/dl)	Serum Creatinine (mg/dl)
Normal Control	9.05±0.21	20.645±0.38	2.36±0.19	0.42±0.07
Diabetic Control	5.76±0.37[a*]	45.30±2.11[a*]	5.75±0.40[a*]	1.02±0.02[a*]
Diabetic + SBP	7.34±0.21[b*]	27.23±1.25[b*]	2.21±0.06[b*]	0.56±003[b*]
Diabetic + Metformin	7.50±0.33[b*]	31.80±1.62b*	2.05±0.08[b*]	0.51±0.02[b*]

Values are shown as average ±S.D. for 6 rats in each group. One-way ANOVA with post hoc test L.S.D. statistical importance was compared within the following groups.
[a]Diabetic rats were compared with control groups.
[b]Diabetic + Stingless Bee Pollen and T2DM +Metformin treated T2DM rats were compared to T2DM rats

Reduced serum protein levels in diabetic patients is due to the oxidative phosphorylation inhibition, resulting in reduced protein synthesis, increased catabolic processes, and reduced protein absorption [6]. Urea and creatinine are metabolism products contain a lot of nitrogen. Urea is the main metabolite produced by dietary protein and tissue protein turnover. Creatinine is a muscle creatine catabolism product. It is made in the muscles through non-enzymatic changes in creatine and phosphocreatine [26]s. The liver is important in creatinine production through the guanidine glycine methylation. Urea is an organic compound that is influential in metabolizing nitrogenous compounds [26].

The accumulation of purines is the uric acid main source through the activity of xanthine oxidase. These accumulated purines indicate raised oxidative stress, strongly associated with diabetes and its vascular complications. Therefore, high circulating uric acid may indicate that the body is attempting to guard against the free radicals harmful impacts by growing endogenous antioxidants production including uric acid [27].

Figure 6 shows the influence of Stingless Bee pollen on ALT, AST, as well as ALP in the serum of management and rat experimental groups. Activities of AST, ALP, and ALT marker enzymes in the plasma can indirectly estimate the integrity of liver tissue and the extent of damage after exposure to certain pharmacological agents such as STZ and Alloxan. These enzymes are usually liver characteristics whose

plasma engagement above the homeostatic limit could be associated with various disorders that influence the liver tissue's operational integrity [28].

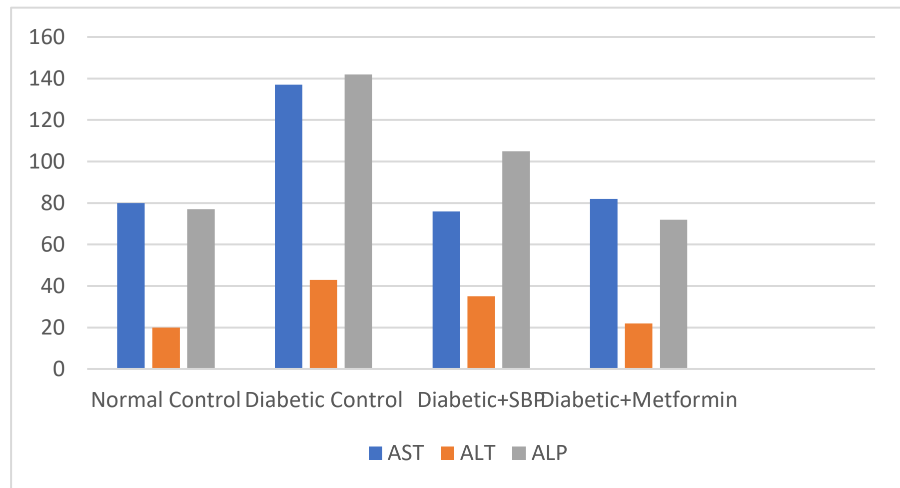

Figure 6: The Effect of Stingless Bee Pollen (SBP) on AST, ALT, and ALP in Control Serum and Rats Experimental Groups

Liver ALP is mustered fastly in the blood, and its plasma levels can raise during early times of liver hurt. A raise in ALP in diabetes can develop leaking out from the tissue into the bloodstream as an impact on the liver [29]. The high movements of physiological enzymes were restored to near-normal levels in Stingless Bee pollen and metformin-treated T2DM rats. It revealed that Stingless Bee pollen-treated T2DM rats showed the compound tissue-protective and non-toxic nature [30].

Table 3 shows the glycogen scope classes and glycogen synthase actions and phosphorylase in both groups' liver tissues. The glycogen levels and synthase were reduced in T2DM rats. In contrast, therapy with Stingless Bee pollen, and metformin in T2DM groups, restored the glycogen level in liver tissues. Elevated glycogen phosphorylase activity is reverted to the normal level in Stingless Bee pollen and metformin-treated T2DM groups [31].

Table 3: Impact of Stingless Bee Pollen (SBP) on The Glycogen Synthase and Phosphorylase Levels in Liver Tissues of Control and Experimental Groups

Groups	Glycogen	Glycogen Synthase	Glycogen Phosphorylase
Normal Control	65.73±2.08	812.43±7.39	618.22±2.68
Diabetic Control	27.57±2.35[a*]	473.13±51.23[a*]	872.26±3.03[a*]
Diabetic + SBP	45.63±2.13[b*]	733.09±8.73[b*]	673.11±4.11[b*]
Diabetic + Metformin	47.81±3.01[b*]	736.26±8.89[b*]	668.23±3.36[b*]

Units are described as mg/g wet tissue for glycogen, μ mol of UDP formed/h/mg protein for Glycogen synthase, and μ mol of Pi liberated/h/mg of protein for Glycogen phosphorylase. Values are given as average ±S.D. for 6 rats in each group. One-way ANOVA with post hoc test L.S.D. Statistical significance was reached within the groups as follows.
[a]Diabetic rats were compared with control groups.
[b]Diabetic + Stingless Bee Pollen and T2DM +Metformin treated T2DM rats were compared to T2DM rats

Table 4 shows the impact of the extract toward the activities of pyruvate kinase, hexokinase, and lactate dehydrogenase in the liver tissue of experimental rats. The levels of hexokinase as well as pyruvate kinase were greatly reduced in the liver tissue of streptozotocin-induced T2DM rats. Conversely, the lactate dehydrogenase activity in the experimental groups was increased. However, after oral treatment with Stingless Bee pollen, the levels of lactate dehydrogenase returned to normal [32]. The oral Stingless Bee pollen administration to T2DM rats resulted in the normalization of enzyme activities in the liver tissue, comparable to the effects identified in rats treated with metformin [33].

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Table 4: Effect of Stingless Bee Pollen (SBP) on TheHexokinase Levels, Pyruvate Kinase and Lactate Dehydrogenase in Liver Tissues of Control and Rats Experimental Groups

Groups	Hexokinase	Pyruvate Kinase	Lactate Dehydrogenase
Normal Control	267.28±4.78	218.30±4.57	213.10±2.76
Diabetic Control	134.45±4.27^{a*}	115.53±4.62^{a*}	458.23±39.34^{a*}
Diabetic + SBP	212.63±10.16^{b*}	180.13±3.67^{b*}	301.56±5.01^{b*}
Diabetic + Metformin	217.30±9.64^{b*}	176.48±1.78^{b*}	282.13±3.12^{b*}

Units are expressed as μ mol of Glucose-6-Phosphate formed/h/mg of protein for Hexokinase, mU/mg of protein for Pyruvate Kinase, μmol of pyruvate formed /h/mg/ of protein for lactate dehydrogenase. Values are given as average ±S.D. for 6 rats in each group. One-way ANOVA with post hoc test L.S.D. statistical significance was compared within the following groups.
a. Diabetic rats were compared with control groups.
b Diabetic + Stingless Bee Pollen and T2DM +Metformin treated T2DM rats were compared to T2DM rats

Carbohydrate metabolism dysfunctions as well as the persistent actions of physiological systems to restore balance exert excessive strain on the endocrine system, causing the endocrine regulation deterioration. This progressive decline in endocrine control further aggravates metabolic disruptions by altering enzymes in carbohydrate metabolism, ultimately contributing to the development of diabetes [34].

The main intracellular storage form of glucose, known as glycogen, serves as an insulin activity reflection in various tissues. Insulin stimulates glycogen deposition within cells by glycogen synthase stimulation as well as glycogen phosphorylase inhibition. In the case of selective destruction of β-cells caused by STZ in the pancreas, which results in a significant insulin decline, it is expected that glycogen levels in tissues would decrease since they rely on insulin to facilitate glucose uptake [30]. Generally, the key symptoms of type 2 diabetes, such as improved hepatic glucose, decreased hepatic glycogen synthesis, and impaired glycolysis, contribute to hyperglycaemia [35].

Insulin plays a critical role in regulating glucose utilization within cells through various mechanisms. One important effect of insulin is its ability to enhance hepatic glycolysis by increasing the activity and quantity of key enzymes. Hexokinase, a vital enzyme responsible for converting glucose to glucose-6-phosphate, is crucial for maintaining glucose homeostasis [36]. In the liver, hexokinase serves as a significant regulatory enzyme in glucose oxidation. In individuals with diabetes, the absence of insulin greatly impairs or deactivates hepatic hexokinase since its proper function relies on insulin. This impairment leads to a notable decrease in the glucose oxidation rate through glycolysis, eventually resulting in hyperglycaemia. However, when Stingless Bee pollen was orally administered to T2DM rats induced by an HFD-STZ, a remarkable restoration of hexokinase activity was observed, subsequently promoting glucose oxidation [37].

Glucose-6-phosphatase as a crucial enzyme is involved in maintaining glucose balance as it catalyses the final biochemical reactions in glycogenolysis as well as gluconeogenesis. In the gluconeogenic pathway, fructose-1,6-bisphosphatase serves as a critical enzyme [38]. In the condition of T2DM, hepatic glucose production is elevated and is related to impaired suppression of fructose-1,6-bisphosphatase, the gluconeogenic enzyme. This occurs due to insulin impairment, as insulin normally acts as a suppressor of this enzyme [38].

As a critical enzyme involved in the final steps of glycolysis, LDH (lactate dehydrogenase) is accountable for transforming pyruvate to lactate, which generates energy under anaerobic conditions [39]. The reduced LDH activity in tissues is significant as it ensures that a substantial portion of pyruvate as well as NADH produced by glycolysis is oxidized by the mitochondria. Interestingly, increased levels of LDH in experimental T2DM rats are related to inadequate insulin secretion in reaction to glucose stimulation. This indicates that increased LDH activity disrupts normal glucose metabolism. However, when T2DM rats were treated with Stingless Bee pollen, the activity of LDH reverted to near-normal levels, likely restoring the

balance of pyruvate as well as NADH. This restoration promotes glucose's mitochondrial oxidation, potentially facilitating improved glucose metabolism [39].

Conclusion

This study concluded a role for Stingless Bee pollen in maintaining normoglycemia through the modulation of insulin resistance and regulation of carbohydrate metabolism enzymes. Furthermore, these data also proved the tissue protective properties of Stingless Bee pollen. Further analyses were conducted to comprehend the molecular mechanisms implicated in the regulation of normoglycemia by administering Stingless Bee pollen.

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