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Dose-dependent antidiabetic, antihyperlipidemic, and hepatoprotective effects of *Piper betle* leaf powder suspension in streptozotocin-induced diabetic mice

Md. Nazmul Hosen[1], Md. Siddiqul Islam[1], Md. Ashraf Zaman Faruk[2*], Md. Saifur Rahman[3,4], Md. Anwar Hossain[1], Md. Khairul Islam[3,4], Md. Mahbubul Alam Sarker[3,4], Md. Rizianul Islam[1], Md. Mowdudul Hasan Talha[1]

[1]Department of Pharmacology and Toxicology, Sylhet Agricultural University, Sylhet-3100, Bangladesh
[2]Division of Pharmacology, Graduate School of Medical and Dental Sciences, Niigata University, Niigata 951-8510, Japan
[3]Department of Veterinary and Animal Sciences, University of Rajshahi, Rajshahi-6205, Bangladesh
[4]Department of Livestock Services, Ministry of Fisheries and Livestock, Government of the People's Republic of Bangladesh, Dhaka-1215, Bangladesh

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ABSTRACT

Introduction: *Piper betle* (PB) is rich in bioactive compounds and traditionally used for its antihyperglycemic and hepatoprotective properties. This study examined the dose-dependent hypoglycemic, hypolipidemic, and hepatoprotective responses to a suspension of PB leaf powder in streptozotocin (STZ)-induced diabetic mice.

Methods: Thirty Swiss albino mice were allocated into six groups: a healthy control (T0), a diabetic control (T1), three treatment groups receiving 50, 100, and 200 mg/kg body weight (BW) of PB leaf powder suspension (T2-T4), and a gliclazide-treated positive control (T5). A single intraperitoneal injection of STZ (50 mg/kg) induced diabetes. Fasting blood glucose (FBG), serum lipid profiles, including high-density lipoprotein (HDL) and low-density lipoprotein (LDL), and hepatic biomarkers, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were measured periodically.

Results: Compared with the untreated diabetic control group (T1), PB-treated mice exhibited a dose-dependent reduction in FBG levels ($P < 0.05$). Treatment also significantly improved lipid profiles ($P < 0.05$), lowering LDL and increasing HDL concentrations. In addition, hepatic dysfunction was attenuated ($P < 0.05$), indicated by the restoration of ALT and AST activities. The 200 mg/kg dose showed the highest efficacy, comparable to gliclazide ($P > 0.05$).

Conclusion: Oral administration of PB leaf suspension exhibits significant antidiabetic, antihyperlipidemic, and hepatoprotective effects in STZ-induced diabetic mice, supporting its potential as an alternative therapeutic agent for diabetes management.

Implication for health policy/practice/research/medical education:
The study indicates that PB leaf powder suspension significantly improves glycemic control, lipid profile, and hepatic function in an STZ-induced diabetic mouse model, suggesting its promise as a plant-derived medicinal candidate for diabetes prevention and management. To support its translational applicability, further research, including mechanistic studies and well-designed clinical trials, would be necessary.

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Introduction

Diabetes mellitus (DM) has emerged as a major critical metabolic disorder affecting the world in the 21st century. It is characterized by persistent hyperglycemia caused by impaired insulin secretion, defective insulin action, or a combination of both (1). This condition leads to disturbances in carbohydrate, protein, and lipid metabolism, and is strongly related to classical clinical manifestations, including weight loss, polyuria, polyphagia, and polydipsia (2). Chronic hyperglycemia in

***Corresponding author**: Md. Ashraf Zaman Faruk, Email: faruk.unibd@yahoo.com

DM may eventually cause long-term harm, malfunction, and failure of multiple organs, particularly in nerves, kidneys, eyes, heart, and vascular system (3). The International Diabetes Federation (IDF) estimates that 589 million people between the ages of 20 and 79 years are currently living with diabetes worldwide, accounting for about 11.1% of the adult population. By 2050, this figure is projected to increase to approximately 853 million (4). In South Asia, particularly Bangladesh, the burden of diabetes is escalating rapidly owing to genetic predisposition, rapid urbanization, and sedentary lifestyles. Notably, present IDF reports indicate that Bangladesh ranks second among South Asian countries, with an estimated diabetes prevalence of 13.2%. Furthermore, recent estimates indicate that diabetes prevalence in Bangladesh has increased from 1.8 million cases in 2000 to approximately 13.9 million in 2024 (5).

Current management strategies for diabetes primarily involve exogenous insulin and oral hypoglycemic agents such as biguanides (e.g., metformin) and sulfonylureas (e.g., gliclazide). Although effective, prolonged use of these synthetic drugs is often associated with secondary complications, including weight gain, gastrointestinal disturbances, and hepatic toxicity (6-8). Consequently, there is a growing global interest in ethnopharmacology and plant-based medicinal strategies that may provide effective glycemic regulation with fewer adverse effects.

Piper betle (PB), commonly known as "Betel leaf" or "Paan", is a perennial climbing vine of the Piperaceae family and is widely cultivated across South and Southeast Asia. In traditional Ayurvedic and folk medicine, PB has long been utilized for its carminative, stimulant, antiseptic, and wound-healing properties (9). Recent phytochemical analyses have revealed that PB leaves contain several biologically active constituents, including polyphenols, flavonoids, alkaloids, and volatile organic compounds such as chavibetol and eugenol (10,11). Beyond its traditional use, accumulating evidence suggests that these constituents confer antioxidant, anti-inflammatory, and hepatoprotective properties (12). Although earlier studies have reported that PB has been traditionally used for metabolic disorders, comprehensive experimental evaluations addressing its simultaneous effects on glycemic control, lipid metabolism, and liver function in diabetic models remain relatively limited.

Streptozotocin (STZ)-induced diabetes is a well-established experimental model that mimics insulin-deficient diabetes by selectively destroying pancreatic β-cells. Therefore, the current research was designed to evaluate whether oral administration of PB leaf powder suspension could dose-dependently improve glycemic control, lipid metabolism, and hepatic function in an STZ-induced diabetic mouse model, thereby providing experimental validation for its traditional therapeutic use in diabetes management.

Materials and Methods

Study setting and ethical approval

The experimental study was carried out at the Department of Pharmacology and Toxicology, Sylhet Agricultural University, Bangladesh, from March to June 2020 under controlled environmental conditions for animal trials. All experimental protocols involving animal subjects were examined and authorized by the Animal Experimentation and Ethics Committee (AEEC) of Sylhet Agricultural University (Approval #AUP2020012) and were conducted following institutional regulations for the care and handling of laboratory animals.

Preparation of PB leaf powder

Mature and healthy PB leaves were procured from local markets in Sylhet, Bangladesh, and visually inspected to ensure the quality. The leaves were washed thoroughly with deionized water to remove surface contaminants and dried in a controlled cabinet dryer at 60 °C for 72 hours (not freeze-dried). The plant sample was taxonomically verified and authenticated by the Bangladesh National Herbarium at Dhaka (Voucher specimen no. DACB 120586). The dried material was subsequently pulverized using a mechanical grinder and sieved through an 80 μm mesh to obtain a uniform coarse powder. The powder was preserved in airtight containers at 25 °C until further use.

Preparation of PB leaf powder suspension

For oral administration, the required amount of PB leaf powder was freshly suspended in distilled water prior to dosing. The suspension was prepared by thoroughly mixing the calculated quantity of leaf powder with distilled water to achieve a final concentration of 50, 100, and 200 mg/kg BW for each animal. The formulation was administered orally at a fixed volume of 10-15 mL per 100 gm BW (equivalent to 100-150 mL/kg). The mixture was vortexed thoroughly to ensure homogeneous dispersion. Fresh suspensions were prepared daily to maintain consistency and stability of the administered material.

Animal husbandry and environmental control

Thirty adult male Swiss albino mice (8th week old, weighing 25-30 gm) were procured from the animal center of the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B), Dhaka, Bangladesh. Male animals were selected to reduce physiological variability related to hormonal changes occurring during the female estrous cycle, which can affect both glucose and lipid metabolism. Animals were housed under controlled laboratory settings with a 12-hour light/12-hour dark photocycle at 20 ± 2°C and were allowed unlimited access to standard formulated food and deionized water.

The mice were fed a commercially available laboratory rodent pellet diet supplied by ICDDR,B, containing approximately 55-60% carbohydrates, 12-18% protein,

4-6% fat, 3-5% crude fiber, and 5-6% ash, with an estimated energy content of 300-500 kcal per 100 gm, formulated to meet the nutritional requirements of laboratory rodents. Animal rooms were maintained under hygienic conditions, and routine laboratory sanitation procedures were followed to minimize environmental contamination.

Induction of experimental diabetes

Diabetes was experimentally induced through a single intraperitoneal administration of STZ at 50 mg/kg BW, prepared freshly in ice-cold 0.1 M citrate buffer (pH 4.5) (13,14). This dose is widely used in rodent models to induce pancreatic β-cell damage and hyperglycemia (15). To prevent acute hypoglycemic shock, mice were supplied with 5% glucose solution for 24 hours after STZ administration. Fasting blood glucose (FBG) levels were measured from the tail vein using a glucometer on days 2, 3, and 7 post-injections. Animals with FBG levels exceeding 250 mg/dL (13.88 mmol/L) on day 7 were deemed diabetic and selected for subsequent experiments. This threshold has been widely used in experimental studies to define hyperglycemia in STZ-induced diabetic rodents (16,17).

Experimental design and treatment regimen

Diabetic and non-diabetic mice were randomly divided into six cohorts (n = 5):

- Group T0: Normoglycemic control receiving 0.9% saline.
- Group T1: Diabetic control (STZ-induced) without treatment.
- Groups T2, T3, T4: Diabetic mice treated orally with PB leaf powder suspension at doses of 50, 100, and 200 mg/kg BW, respectively.
- Group T5: Diabetic mice treated with the reference drug Gliclazide (2 mg/kg BW).

PB suspension and gliclazide were administered orally by gavage once daily for 45 consecutive days at a standardized dosing volume of 10 mL/100 gm BW. All animals survived throughout the experimental period, and no mortality was observed during the 45-day treatment period.

Biochemical analysis

Sterile microcapillary tubes were used to collect blood from the tail vein at baseline (day 0) and at predetermined intervals (days 15, 30, and 45) throughout the experiment. For each collection, approximately 0.2-0.3 mL of blood was withdrawn for biochemical analysis. The collected blood samples were centrifuged at 3000 rpm for 10 minutes to separate the serum. The resulting serum samples were then used for the determination of lipid profile parameters (HDL and LDL) and hepatic enzyme activities (ALT and AST) using standard enzymatic methods commonly applied in clinical chemistry (18).

Statistical analysis

The mean ± standard deviation (SD) is used to express all experimental data. One-way ANOVA was carried out for statistical analysis, followed by Tukey's multiple group comparisons post-hoc test. To minimize potential bias, biochemical analysis was performed in a blinded manner. Serum samples were coded prior to analysis, and the investigator conducting the biochemical assays was unaware of the treatment group allocation during the measurements. Group comparisons were performed separately at each time point. Statistical significance was defined as a probability value of less than 0.05. SPSS software version 21 was the tool used for all statistical analysis.

Results

Effects of PB leaf suspension on glycemic control

Administration of PB leaf suspension resulted in a significant and dose-dependent improvement in FBG levels during the 45-day experimental period (Table 1). Diabetic control mice (T1) exhibited a progressive elevation in FBG levels, reaching 16.31 ± 0.52 mmol/L on day 45. In contrast, PB-treated groups displayed a gradual reduction in glucose concentrations over time.

Mice receiving the highest PB dose (T4; 200 mg/kg) demonstrated a significant decline in FBG levels from 14.58 ± 0.49 mmol/L at baseline to 11.03 ± 0.12 mmol/L on day 45 ($P < 0.05$ vs. diabetic control). The standard antidiabetic drug gliclazide (T5) produced the most

Table 1. Effect of *Piper betle* (PB) leaf powder on fasting blood glucose (FBG) levels (mmol/L) in streptozotocin-induced diabetic mice during the 45-day treatment period

Treatment	Day 0	Day 15	Day 30	Day 45
T0 (Normal)	5.31 ± 0.28^{a}	5.28 ± 0.25^{a}	5.41 ± 0.34^{a}	5.26 ± 0.19^{a}
T1 (Diabetic)	14.40 ± 0.19^{b}	14.90 ± 0.21^{d}	15.71 ± 0.30^{d}	16.31 ± 0.52^{d}
T2 (PB 50 mg/kg BW)	14.53 ± 0.36^{b}	13.95 ± 0.36^{c}	13.56 ± 0.48^{c}	12.96 ± 0.59^{c}
T3 (PB 100 mg/kg BW)	14.62 ± 0.50^{b}	13.64 ± 0.43^{c}	12.88 ± 0.41^{b}	12.47 ± 0.36^{b}
T4 (PB 200 mg/kg BW)	14.58 ± 0.49^{b}	13.22 ± 0.31^{b}	12.08 ± 0.29^{b}	11.03 ± 0.12^{b}
T5 (Gliclazide 2 mg/kg BW)	14.39 ± 0.49^{b}	10.74 ± 0.35^{a}	7.94 ± 0.41^{a}	7.63 ± 0.53^{a}

Values are presented as mean ± SD (n = 5). Different superscript letters within the same column differ significantly among treatment groups at $P < 0.05$, as determined by one-way ANOVA followed by Tukey's post hoc multiple comparison test.

pronounced hypoglycemic response, lowering glucose levels to 7.63 ± 0.53 mmol/L ($P < 0.05$ vs. diabetic control).

Effects of PB leaf suspension on serum lipid profile

PB suspension exerted significant modulatory effects on STZ-induced dyslipidemia, as reflected in changes in serum LDL (Table 2) and HDL (Table 3) concentrations.

Reduction of LDL cholesterol

Untreated diabetic mice (T1) showed a continuous rise in LDL cholesterol levels, increasing from 94.58 ± 0.55 mg/dL at baseline to 106.27 ± 0.30 mg/dL by day 45 ($P < 0.05$ vs. normal control). In contrast, PB treatment attenuated this elevation in a dose-dependent manner. The highest dose (T4; 200 mg/kg) lowered LDL levels to 76.73 ± 0.42 mg/dL at the end of the experiment ($P < 0.05$ vs. diabetic control), indicating substantial improvement in lipid metabolism.

Elevation of HDL cholesterol

PB-treated groups showed higher serum HDL concentrations than the diabetic control group. While HDL levels declined progressively in untreated diabetic mice, PB supplementation produced a gradual increase throughout the experimental period. The T4 group displayed an increase in HDL concentration from 30.02 ± 0.45 mg/dL at baseline to 45.06 ± 0.61 mg/dL on day 45 ($P < 0.05$ vs. diabetic control), approaching the effect observed in the gliclazide-treated group (49.26 ± 0.41 mg/dL) ($P < 0.05$ vs. diabetic control).

Hepatoprotective effects of PB leaf suspension

Administration of STZ to induce diabetes significantly elevated hepatic enzyme activities, indicating hepatocellular injury (Tables 4 and 5). In untreated diabetic mice (T1), ALT and AST levels improved progressively, reaching 85.82 ± 0.78 U/L and 153.51 ± 0.93 U/L, respectively, by

Table 2. Effect of *Piper betle* (PB) leaf powder on serum low-density lipoprotein cholesterol levels (mg/dL) in streptozotocin-induced diabetic mice during the 45-day treatment period

Treatment	Day 0	Day 15	Day 30	Day 45
T0 (Normal)	45.03 ± 0.62^{a}	45.67 ± 0.60^{a}	45.89 ± 0.54^{a}	45.97 ± 0.45^{a}
T1 (Diabetic)	94.58 ± 0.55^{b}	97.79 ± 0.25^{d}	103.78 ± 0.86^{d}	106.27 ± 0.30^{d}
T2 (PB 50 mg/kg BW)	94.56 ± 0.59^{b}	92.79 ± 0.36^{c}	90.77 ± 0.56^{c}	88.51 ± 0.68^{c}
T3 (PB 100 mg/kg BW)	93.90 ± 0.44^{b}	89.97 ± 0.35^{b}	87.56 ± 0.60^{b}	84.07 ± 0.80^{b}
T4 (PB 200 mg/kg BW)	94.86 ± 0.60^{b}	88.85 ± 0.72^{b}	83.91 ± 0.46^{b}	76.73 ± 0.42^{b}
T5 (Gliclazide 2 mg/kg BW)	95.11 ± 0.66^{b}	80.90 ± 0.50^{a}	72.00 ± 0.29^{a}	62.72 ± 0.61^{a}

Values are presented as mean ± SD (n = 5). Different superscript letters within the same column differ significantly among treatment groups at $P < 0.05$, as determined by one-way ANOVA followed by Tukey's post hoc multiple comparison test.

Table 3. Effect of *Piper betle* (PB) leaf powder on serum high-density lipoprotein cholesterol levels (mg/dL) in streptozotocin-induced diabetic mice during the 45-day treatment period

Treatment	Day 0	Day 15	Day 30	Day 45
T0 (Normal)	56.31 ± 0.40^{a}	55.58 ± 0.59^{a}	56.93 ± 0.37^{a}	57.11 ± 0.55^{a}
T1 (Diabetic)	27.08 ± 0.28^{d}	25.88 ± 0.50^{c}	23.03 ± 0.69^{c}	21.80 ± 0.55^{c}
T2 (PB 50 mg/kg BW)	29.10 ± 0.30^{c}	30.08 ± 0.41^{c}	31.50 ± 0.40^{c}	32.76 ± 0.55^{c}
T3 (PB 100 mg/kg BW)	31.62 ± 0.44^{b}	33.25 ± 0.54^{b}	35.17 ± 0.59^{b}	37.02 ± 0.35^{b}
T4 (PB 200 mg/kg BW)	30.02 ± 0.45^{c}	35.62 ± 0.60^{b}	40.15 ± 0.57^{b}	45.06 ± 0.61^{b}
T5 (Gliclazide 2 mg/kg BW)	29.40 ± 0.79^{c}	42.13 ± 0.70^{a}	45.85 ± 0.55^{a}	49.26 ± 0.41^{a}

Values are presented as mean ± SD (n = 5). Different superscript letters within the same column differ significantly among treatment groups at $P < 0.05$, as determined by one-way ANOVA followed by Tukey's post hoc multiple comparison test.

Table 4. Effect of *Piper betle* (PB) leaf powder on serum alanine aminotransferase activity (U/L) in streptozotocin-induced diabetic mice during the 45-day treatment period

Treatment	Day 0	Day 15	Day 30	Day 45
T0 (Normal)	45.02 ± 0.64^{a}	45.35 ± 1.01^{a}	44.11 ± 0.67^{a}	45.03 ± 0.45^{a}
T1 (Diabetic)	78.09 ± 0.94^{b}	80.43 ± 0.77^{d}	83.87 ± 0.56^{d}	85.82 ± 0.78^{d}
T2 (PB 50 mg/kg BW)	78.05 ± 0.32^{b}	77.57 ± 0.45^{c}	76.91 ± 0.30^{c}	75.88 ± 0.38^{c}
T3 (PB 100 mg/kg BW)	78.07 ± 0.62^{b}	74.75 ± 0.57^{b}	72.72 ± 0.65^{b}	71.42 ± 0.68^{b}
T4 (PB 200 mg/kg BW)	78.44 ± 0.83^{b}	76.00 ± 0.13^{b}	71.86 ± 0.56^{b}	68.85 ± 0.63^{b}
T5 (Gliclazide 2 mg/kg BW)	78.38 ± 0.89^{b}	63.10 ± 0.91^{a}	57.19 ± 0.31^{a}	54.70 ± 0.43^{a}

Values are presented as mean ± SD (n = 5). Different superscript letters within the same column differ significantly among treatment groups at $P < 0.05$, as determined by one-way ANOVA followed by Tukey's post hoc multiple comparison test.

Table 5. Effect of *Piper betle* (PB) leaf powder on serum aspartate aminotransferase activity (U/L) in streptozotocin-induced diabetic mice during the 45-day treatment period

Treatment	Day 0	Day 15	Day 30	Day 45
T0 (Normal)	87.80 ± 0.69[a]	88.10 ± 0.31[a]	88.21 ± 0.50[a]	88.48 ± 0.43[a]
T1 (Diabetic)	143.93 ± 0.54[d]	145.95 ± 0.60[d]	149.23 ± 0.65[d]	153.51 ± 0.93[d]
T2 (PB 50 mg/kg BW)	146.26 ± 0.62[c]	144.18 ± 0.67[c]	141.04 ± 0.62[c]	139.47 ± 0.70[c]
T3 (PB 100 mg/kg BW)	143.73 ± 0.78[b]	140.57 ± 0.90[b]	137.79 ± 0.89[b]	135.96 ± 0.67[b]
T4 (PB 200 mg/kg BW)	144.66 ± 0.61[b]	139.10 ± 0.58[b]	135.23 ± 0.45[b]	131.78 ± 0.42[b]
T5 (Gliclazide 2 mg/kg BW)	144.68 ± 0.74[a]	121.94 ± 0.72[a]	113.74 ± 0.38[a]	107.78 ± 0.33[a]

Values are presented as mean ± SD (n = 5). Different superscript letters within the same column differ significantly among treatment groups at $P < 0.05$, as determined by one-way ANOVA followed by Tukey's post hoc multiple comparison test.

day 45 ($P < 0.05$ vs. normal control). Treatment with PB powder suspension reduced these elevated enzyme levels in a dose-dependent manner. Mice receiving 200 mg/kg PB (T4) demonstrated a substantial reduction in ALT and AST levels to 68.85 ± 0.63 U/L and 131.78 ± 0.42 U/L, respectively, at the end of the study period ($P < 0.05$ vs. diabetic control). These results suggest that PB powder effectively attenuated STZ-induced hepatic dysfunction and promoted restoration of hepatic enzymatic balance, with outcomes trending toward those observed in the positive control group.

Discussion

In this experiment, PB treatment produced a dose-dependent improvement in FBG, serum lipid profile, and hepatic enzyme markers, indicating restoration of systemic metabolic homeostasis. These physiological improvements suggest that PB may exert its therapeutic actions through several complementary biological mechanisms, including modulation of glucose metabolism, antioxidant defence, and lipid regulatory pathways.

The dose-dependent reduction in FBG level observed in groups T2, T3, and particularly T4 (200 mg/kg) aligns with the primary objective of diabetes management. STZ-induced diabetes is characterized by selective damage of pancreatic β-cells, resulting in insulin deficiency and potent hyperglycemia (19). The observed glycemic improvement suggests that PB may exert antihyperglycemic effects potentially through insulin-mimetic activity, enhanced peripheral glucose utilization, and modulation of hepatic glucose-metabolizing enzymes. These outcomes are consistent with previous experimental studies reporting that PB leaf extract significantly reduces blood glucose and glycosylated hemoglobin levels, suppresses hepatic gluconeogenic enzymes, and modulates aldose reductase activity in diabetic models (20-23). Collectively, these effects may contribute to improved glucose homeostasis and attenuation of hyperglycemia. Notably, the comparable efficacy of the 200 mg/kg dose to gliclazide (T5) suggests the involvement of bioactive phytochemicals in regulating glucose metabolism. Phytochemical profiling has revealed that PB leaves are enriched with flavonoid and phenolic constituents, which are well known for their antioxidant and therapeutic activities (11,24). Emerging evidence indicates that plant-derived polyphenolic compounds can modulate key metabolic pathways by enhancing insulin signalling through the PI3K/Akt cascade and improving peripheral glucose uptake, while their antioxidant capacity may protect pancreatic β-cells from oxidative stress-mediated injury associated with diabetic conditions (25,26).

Chronic hyperglycemia is frequently related to dyslipidemia, characterized by elevated LDL and reduced HDL concentrations, which potentially increase cardiovascular risk in diabetic patients. In this study, the diabetic control (T1) exhibited severe lipid abnormalities, which were markedly reversed by PB treatment. The improvement in lipid profile observed in PB-treated groups suggests a potential regulatory effect on lipid metabolism pathways. One possible mechanism involves the inhibition of the key cholesterol-biosynthetic enzyme HMG-CoA reductase, the rate-limiting enzyme responsible for endogenous cholesterol synthesis (27). In addition, the antihyperlipidemic effect of PB may partly be attributed to its phenolic constituent eugenol, which has been experimentally shown to reduce triglycerides, total cholesterol, and LDL levels while increasing HDL concentrations in hypercholesterolemic animal models (28). These observations are consistent with earlier reports demonstrating that aqueous PB extract reduces lipid peroxidation and tissue lipid accumulation while enhancing endogenous antioxidant defences (29). Additionally, Thirumalai et al showed that methanolic PB extracts significantly reduced serum cholesterol, triglycerides, LDL, and VLDL concentrations in rats with diet-induced hyperlipidemia (30). The elevation of HDL observed in the T4 group is particularly noteworthy, given HDL's cardioprotective role in reverse cholesterol transport, which may reduce cardiovascular risk.

The liver is a primary target of STZ-induced oxidative stress, often leading to hepatocellular injury and leakage of ALT and AST into the circulation. These results depict a significant elevation of these markers in the diabetic control group, indicating hepatic dysfunction. However, treatment with the PB leaf powder suspension effectively attenuated these elevations in a dose-responsive manner. The observed hepatoprotective effect is likely attributable to the potent antioxidative and radical-neutralizing

capacities of PB-derived phytochemicals, which reduce lipid peroxidation and stabilize hepatocyte membranes. By neutralizing reactive oxygen species (ROS), PB may prevent mitochondrial dysfunction and hepatocellular injury, thereby preserving normal liver function (29,31). The effectiveness of PB in maintaining cellular architecture and reestablishing enzymatic balance in the liver was further highlighted by studies conducted by De et al (31). Beyond diabetic models, PB has also shown strong hepatoprotective efficacy in CCl4-induced liver injury by restoring key antioxidant enzymes like SOD, CAT, and GST to reduce oxidative damage, while effectively suppressing profibrotic markers like α-SMA and TIMP2 (32,33). Collectively, these data suggest that the bioactive constituents in the PB powder suspension, most likely polyphenols and flavonoids, offer a comprehensive protective barrier against metabolic and chemical-induced hepatic stress, validating its traditional use as a potent regenerative agent. This may be the reason why the T4 group showed a restoration of liver function markers toward baseline levels.

In summary, the diverse hypoglycemic, hypolipidemic, and hepatoprotective properties of PB leaf highlights its potential as an adjunct therapy for diabetes management. Although this study confirms its effectiveness in an animal model, it is still limited by the lack of molecular mechanistic information, histological proof of liver and pancreas, and an oxidative stress profile. Future investigations should prioritize the isolation of particular bioactive phytochemicals and understanding of the signalling pathways involved in PB-mediated metabolic regulation, including insulin signalling, oxidative stress pathways, lipid profile (cholesterol and triglycerides), and lipid metabolism genes. Additionally, validation in alternative diabetic models and well-controlled clinical studies will be essential to establish its translational relevance.

Conclusions

These findings suggest that the PB leaf powder suspension possesses significant antidiabetic potential in STZ-induced diabetic mice. Dose-dependent oral administration of the suspension effectively improved glycemic control, serum lipid profiles, and hepatic enzyme activities, with the highest dose showing effects close to those observed with the standard antidiabetic drug gliclazide. These findings suggest that PB leaf may serve as an alternative therapy for diabetes and its associated metabolic complications. Further studies are warranted to confirm its safety, efficacy, and clinical applicability.

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Authors' contribution

Conceptualization: Md. Nazmul Hosen.
Data curation: Md. Nazmul Hosen, Md. Rizianul Islam.
Formal analysis: Md. Nazmul Hosen, Md. Saifur Rahman, Md. Ashraf Zaman Faruk.
Funding acquisition: Md. Siddiqul Islam.
Investigation: Md. Nazmul Hosen, Md. Mowdudul Hasan Talha, Md. Saifur Rahman.
Methodology: Md. Nazmul Hosen, Md. Siddiqul Islam.
Project administration: Md. Anwar Hossain.
Resources: Md. Siddiqul Islam.
Software: Md. Nazmul Hosen, Md. Khairul Islam, Md. Mahbubul Alam Sarker.
Supervision: Md. Siddiqul Islam.
Validation: Md. Siddiqul Islam.
Visualization: Md. Anwar Hossain.
Writing-original draft: Md. Ashraf Zaman Faruk.
Writing-review & editing: Md. Ashraf Zaman Faruk.

Conflicts of interest

The authors declare no competing financial or non-financial interests that could have influenced the research presented in this manuscript.

Ethical issues

All animal-based experimental procedures were evaluated and formally authorized by the Animal Experimentation and Ethics Committee (AEEC) of Sylhet Agricultural University (Approval No. AUP2020012). The experiments were carried out in strict compliance with the university's established guidelines and ethical standards for the humane care and use of laboratory animals.

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