

***In vivo* Evaluation of the Antidiabetic Potential of *Piper chaba* Extract in Alloxan-Induced Diabetic Swiss Albino Mice.**

Abstract:

Diabetes mellitus is a major metabolic disorder characterized by persistent hyperglycemia and associated disturbances in carbohydrate, lipid, and protein metabolism. The limitations and adverse effects associated with prolonged use of synthetic antidiabetic agents have stimulated interest in medicinal plants as potential sources of safer and affordable therapeutic alternatives. *Piper chaba* Hunter, a medicinal plant of the family Piperaceae, has traditionally been used for several health conditions and contains diverse bioactive constituents, including alkaloids, piperine, flavonoids, phenolic compounds, and essential oils. This study evaluated the antihyperglycemic potential of a methanolic extract of *Piper chaba* stem (PCME) in alloxan-induced diabetic Swiss albino mice. Fresh plant material was authenticated, shade-dried, powdered, and extracted continuously with methanol using a Soxhlet apparatus for 72 h. Acute oral toxicity was evaluated according to OECD guideline 423, and no observable toxic effects were reported at the tested doses. Experimental diabetes was induced using alloxan monohydrate at 150 mg/kg body weight. Thirty mice were randomly divided into five groups (n=6): normal control receiving distilled water, diabetic control receiving alloxan and distilled water, standard treatment receiving metformin (150 mg/kg), low-dose PCME receiving 200 mg/kg, and high-dose PCME receiving 400 mg/kg. Treatments were administered orally once daily for 21 consecutive days. Fasting blood glucose (FBG) levels were measured on Days 0, 7, 14, and 21 using a digital glucometer. The diabetic control group demonstrated progressive hyperglycemia, with FBG increasing from 284.5±10.4 mg/dL (15.8±0.6 mmol/L) on Day 0 to 324.9±13.5 mg/dL (18.0±0.8 mmol/L) on Day 21. In contrast, PCME produced a progressive and dose-dependent reduction in FBG. The low-dose group showed a decrease from 279.6±11.1 mg/dL (15.5±0.6 mmol/L) to 162.4±7.0 mg/dL (9.0±0.4 mmol/L), whereas the high-dose group decreased from 282.4±10.2 mg/dL (15.7±0.6 mmol/L) to 128.1±5.2 mg/dL (7.1±0.3 mmol/L) by Day 21. Metformin treatment reduced FBG to 105.6±4.5 mg/dL (5.9±0.3 mmol/L). The observed reductions in the PCME-treated groups were statistically significant compared with the untreated diabetic control, with the study reporting $p<0.001$ for the principal treatment effects. The findings indicate that PCME

possesses significant antihyperglycemic activity and that the higher dose produced a greater reduction in blood glucose than the lower dose. This activity may be associated with the combined effects of bioactive secondary metabolites that reduce oxidative stress, support glucose utilization, and potentially protect residual pancreatic β -cell function; however, these mechanisms were not directly investigated in the present study. Overall, the results provide experimental support for the traditional medicinal potential of *Piper chaba* and suggest that its methanolic extract may serve as a promising source for further antidiabetic drug-development research. Future investigations should include detailed biochemical and hormonal analyses, isolation of active compounds, mechanistic studies, comprehensive toxicity evaluation, and appropriate longer-term experimental models before clinical application.

Keywords: *Piper chaba*; methanolic extract; PCME; diabetes mellitus; antihyperglycemic activity; fasting blood glucose; alloxan-induced diabetes; Swiss albino mice; metformin; medicinal plants.

1. Introduction

Diabetes mellitus (DM) represents one of the most critical and fast-growing global public health crises of the twenty-first century (Islam et al., 2019). Characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both, this multifaceted metabolic disorder profoundly alters carbohydrate, lipid, and protein homeostasis (Muchandi et al., 2018). The epidemiological burden of diabetes continues to escalate exponentially across both developed and developing nations. Recent international health data show that hundreds of millions of individuals currently live with the disease, and this figure is projected to rise significantly over the coming decades due to aging populations, urbanization, dietary shifts, and sedentary lifestyles (Salehi et al., 2019). Chronic hyperglycemia is inherently toxic to the microvascular and macrovascular systems, leading to severe, life-threatening long-term complications including diabetic nephropathy, retinopathy, neuropathy, cardiovascular disease, and delayed wound healing. Consequently, the search for novel, efficacious, and cost-effective therapeutic strategies to manage and mitigate the progression of diabetes and its associated metabolic disturbances remains an urgent priority for modern biomedical research.

The pathophysiology of diabetes mellitus is broadly classified into two primary categories: Type-1 diabetes mellitus, characterized by autoimmune destruction of pancreatic beta cells leading to absolute insulin deficiency, and Type 2 diabetes mellitus, which involves a combination of peripheral insulin resistance and relative insulin secretory defects. Despite the widespread availability of synthetic antidiabetic medications—such as biguanides, sulfonylureas, thiazolidinediones, DPP-4 inhibitors, SGLT2 inhibitors, and exogenous insulin therapies—clinical management of diabetes remains fraught with limitations (Panche, 2016). Many synthetic pharmacological agents are associated with adverse side effects, including hypoglycemia, weight gain, gastrointestinal disturbances, lactic acidosis, hepatotoxicity, and high long-term financial costs that restrict accessibility in resource-limited regions. Furthermore, secondary failure rates often accompany long-term synthetic drug regimens, driving patients and researchers alike to explore alternative, plant-derived therapeutic agents that offer multi-target efficacy, higher safety profiles, and better compatibility with traditional healthcare systems.

Medicinal plants have served as the foundational bedrock of human therapeutics for millennia (Maiti et al., 2024). Ethnobotanical traditions worldwide rely heavily on botanical concoctions to manage symptoms resembling those of metabolic disorders. Modern pharmacological and phytochemical evaluations have validated many of these traditional claims, showing that plants synthesize an expansive array of bioactive secondary metabolites such as flavonoids, alkaloids, tannins, terpenoids, saponins, and phenolic compounds that exert synergistic antihyperglycemic, antioxidant, and anti-inflammatory effects. Within the vast repository of medicinal flora, the family Piperaceae holds immense prominence. Genera within this family, particularly *Piper*, are renowned for their pungent principles, rich phytochemical profiles, and diverse ethnomedicinal applications ranging from digestive aids to anti-inflammatory and metabolic modulators.

Piper chaba Hunter is a valuable climbing shrub indigenous to Southeast Asia, the Indian subcontinent, and parts of tropical Oceania. Traditionally, various parts of *Piper chaba*, including its stems, roots, and fruits, are extensively utilized in traditional medicine systems such as Ayurveda and folk remedies to treat respiratory ailments, digestive disorders, inflammatory conditions, and metabolic imbalances. Phytochemical screening of *Piper chaba* has revealed a rich abundance of active constituents, most notably piperine, alkaloids, essential oils, and various polyphenolic compounds that exhibit potent free-radical

scavenging and antioxidant properties. Because oxidative stress plays a central role in pancreatic beta-cell destruction and the exacerbation of insulin resistance, the robust antioxidant profile of *Piper chaba* makes it an excellent candidate for evaluation against metabolic diseases characterized by oxidative damage.

To systematically investigate the therapeutic efficacy of novel botanical extracts, reliable experimental animal models are indispensable. Alloxan (2,4,6-pyrimidinetrione) is widely utilized to induce experimental insulin-dependent diabetes in laboratory rodents, such as Swiss albino mice. Structurally analogous to glucose, alloxan is selectively taken up by pancreatic beta cells via glucose transporter 2 (GLUT2) receptors, where it undergoes a reduction reaction that generates reactive oxygen species (ROS). This surge in intracellular oxidative stress, coupled with the selective inhibition of key glucoregulatory enzymes like glucokinase, results in the rapid, selective necrosis of pancreatic beta cells, mimicking the insulin deficiency observed in human diabetes mellitus. Alloxan-induced diabetic Swiss albino mice thus provide a robust, reproducible, and well-characterized *in vivo* model for assessing the hypoglycemic, antihyperlipidemic, and organ-protective potential of test extracts.

While traditional use and preliminary *in vitro* assays highlight the therapeutic promise of *Piper chaba*, comprehensive *in vivo* toxicological and pharmacological data regarding its exact antidiabetic efficacy particularly across varying solvent extracts and dosage parameters in alloxan-induced murine models remain sparse. Previous investigations on related *Piper* species, such as *Piper nigrum* and *Piper betel*, have demonstrated that bioactive alkaloids and secondary metabolites can significantly modulate blood glucose levels and improve metabolic profiles under subacute conditions (Islam et al., 2019; Sarfraz et al., 2023). Building on these insights, evaluating distinct acetone and ethanol extracts of *Piper chaba* offers a vital opportunity to assess their capacity to restore normoglycemia, correct dyslipidemia, and normalize biomarker enzymes associated with hepatic and systemic inflammation.

Therefore, the primary objective of this study is to conduct a comprehensive *in vivo* evaluation of the antidiabetic potential of methanolic extracts of *Piper chaba* stem using an alloxan-induced diabetic Swiss albino mouse model. Specifically, this research aims to assess the impact of varying doses of methanol extracts on fasting blood glucose levels. This study

seeks to establish the scientific validity of *Piper chaba* as a natural therapeutic agent and pave the way for safer, plant-based interventions for diabetes mellitus.

2. Methodology

2.1. Plant Material Collection and Authentication

Fresh stems and leaves of *Piper chaba* were collected and authenticated by a qualified taxonomist (Accession/Voucher No. CNR/HB/LB-125). The plant materials were thoroughly washed with distilled water, shade-dried at room temperature $25\pm 2^{\circ}\text{C}$ for two weeks and pulverized into a coarse powder using a mechanical grinder. The powder was stored in an airtight container protected from light and moisture until extraction (Mannan, 2026).

2.2. Preparation of Methanolic Extract

The coarse powder of *Piper chaba* (500 g) was subjected to Soxhlet extraction using analytical-grade absolute methanol as the solvent for 72 hours, until complete extraction was achieved. The filtered liquid extract (via Whatman No. 1 filter paper) was concentrated under reduced pressure using a rotary evaporator at 40°C to yield a crude semisolid methanolic extract, which was preserved at 4°C (Mannan, 2026).

2.3. Experimental Animals

Healthy Swiss albino mice (weighing 25–30 g, aged 8–10 weeks) were procured and housed in clean polypropylene cages under controlled environmental conditions $22\pm 2^{\circ}\text{C}$, 12-hour light/dark cycle, 50–60% humidity) with a standard pelleted diet and water *ad libitum*. Animals were acclimatized for one week prior to testing. All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) in accordance with internationally accepted ethical guidelines for laboratory animal use (Approval No. CNR/IAEC/2026/04) (Mannan, 2026).

2.4. Acute Toxicity Testing

Acute oral toxicity was evaluated following the Organization for Economic Co-operation and Development (OECD) guideline 423 (OECD, 2002). Overnight-fasted Swiss albino mice received sequential oral doses of the methanolic extract of *Piper chaba* up to 5000 mg/kg body weight. Animals were monitored continuously for the first 30 minutes, periodically for

the first 24 hours, and daily for 14 days for behavioral alterations or mortality. Test doses were subsequently set at 1/10 and 1/5 of the maximum safe dose (200 mg/kg and 400 mg/kg) (Islam et al., 2019).

2.5. Induction of Experimental Diabetes

Experimental diabetes with a type 2-like phenotype was established in overnight-fasted mice through a single intraperitoneal administration of freshly prepared alloxan monohydrate (150 mg/kg body weight) dissolved in sterile normal saline, following the protocol reported by Al-Awar et al. (2016). To minimize the risk of acute hypoglycemia associated with the sudden release of insulin from alloxan-induced pancreatic β -cell injury, the animals received (glucose concentration) glucose solution orally during the initial 24 h after alloxan treatment. Seventy-two hours after induction, fasting blood glucose (FBG) concentrations were assessed using blood collected from the tail vein and analyzed with a digital glucometer. Animals exhibiting persistent fasting hyperglycemia above the predefined threshold (FBG value) were considered successfully diabetic and were included in the subsequent experimental study (Islam et al., 2019).

2.6. Experimental Design and Grouping

The experimental mice were randomly divided into five groups of six animals each: Group I (normal control) received distilled water; Group II (diabetic control) received distilled water following alloxan-induced diabetes; Group III (standard treatment) received 150 mg/kg of metformin; Group IV received a low dose (200 mg/kg) of *Piper chaba* methanolic extract; and Group V received a high dose (400 mg/kg) of the extract. All treatments were administered orally once daily for 21 consecutive days under standard laboratory conditions, following the methodology of Islam et al. (2019), with blood glucose levels and other parameters assessed at predetermined intervals to evaluate the extract's antihyperglycemic effects.

2.7. Fasting Blood Glucose Estimation

Following a 12-hour overnight fast, blood was drawn by tail-tip nicking on Days 0, 7, 14, and 21. Fasting blood glucose (FBG) levels were quantified using a calibrated digital glucometer with matching test strips to evaluate temporal glucose regulation patterns.

2.8. Statistical Analysis

Data are presented as the mean $\pm$ standard error of the mean (SEM). Inter-group variations were statistically processed using One-Way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test, adopting $p < 0.05$ as the threshold for statistical significance.

3. Results: Effect of *Piper chaba* Extract on Fasting Blood Glucose Levels

Administration of alloxan monohydrate (150 mg/kg) successfully induced severe insulin-dependent diabetes in the experimental mice, confirmed by a sharp elevation in baseline fasting blood glucose (FBG) levels (≥ 250 mg/dL or ≥ 13.9 mmol/L) on Day 0 across all experimental diabetic groups. Throughout the 21-day intervention period, the untreated diabetic control group (Group II) showed progressive, severe hyperglycemia, with blood glucose rising from 284.5 ± 10.4 mg/dL (15.8 ± 0.6 mmol/L) on Day 0 to 324.9 ± 13.5 mg/dL (18.0 ± 0.8 mmol/L) by Day 21 (Bera et al., 2010). In contrast, daily oral administration of the *Piper chaba* methanolic extract (PCME) demonstrated a pronounced, time- and dose-dependent antihyperglycemic effect (Maiti et al., 2024).

Low-Dose Extract Treatment (200 mg/kg), i.e., Group IV, exhibited a gradual yet significant reduction in FBG levels, decreasing from 279.6 ± 11.1 mg/dL (15.5 ± 0.6 mmol/L) on Day 0 to 162.4 ± 7.0 mg/dL (9.0 ± 0.4 mmol/L) by Day 21 ($p < 0.001$) (Bera et al., 2010). High-Dose Extract Treatment (400 mg/kg), i.e., Group V, showed a more substantial therapeutic response, lowering blood glucose levels from 282.4 ± 10.2 mg/dL (15.7 ± 0.6 mmol/L) down to 128.1 ± 5.2 mg/dL (7.1 mmol/L) by the end of the 21-day trial ($p < 0.001$) (Maiti et al., 2024). Standard Reference Drug (Metformin at 150 mg/kg), i.e., Group III, achieved near-normalization of glycemia, reducing FBG levels from 281.2 ± 9.8 mg/dL (15.6 ± 0.5 mmol/L) to 105.6 ± 4.5 mg/dL (5.9 ± 0.3 mmol/L) (Gupta et al., 2012).

Table 3.1: Changes in Fasting Blood Glucose Levels (mg/dL and mmol/L) During the 21-Day Treatment Period

Experimental Groups	Treatment & Dose	Day 0 mg/dL (mmol/L)	Day 7 mg/dL (mmol/L)	Day 14 mg/dL (mmol/L)	Day 21 mg/dL (mmol/L)
Group I (Normal Control)	Distilled Water (10 mL/kg)	86.4 ± 3.2 (4.8 ± 0.2)	88.1 ± 2.9 (4.9 ± 0.2)	87.5 ± 3.1 (4.9 ± 0.2)	89.0 ± 2.8 (4.9 ± 0.2)

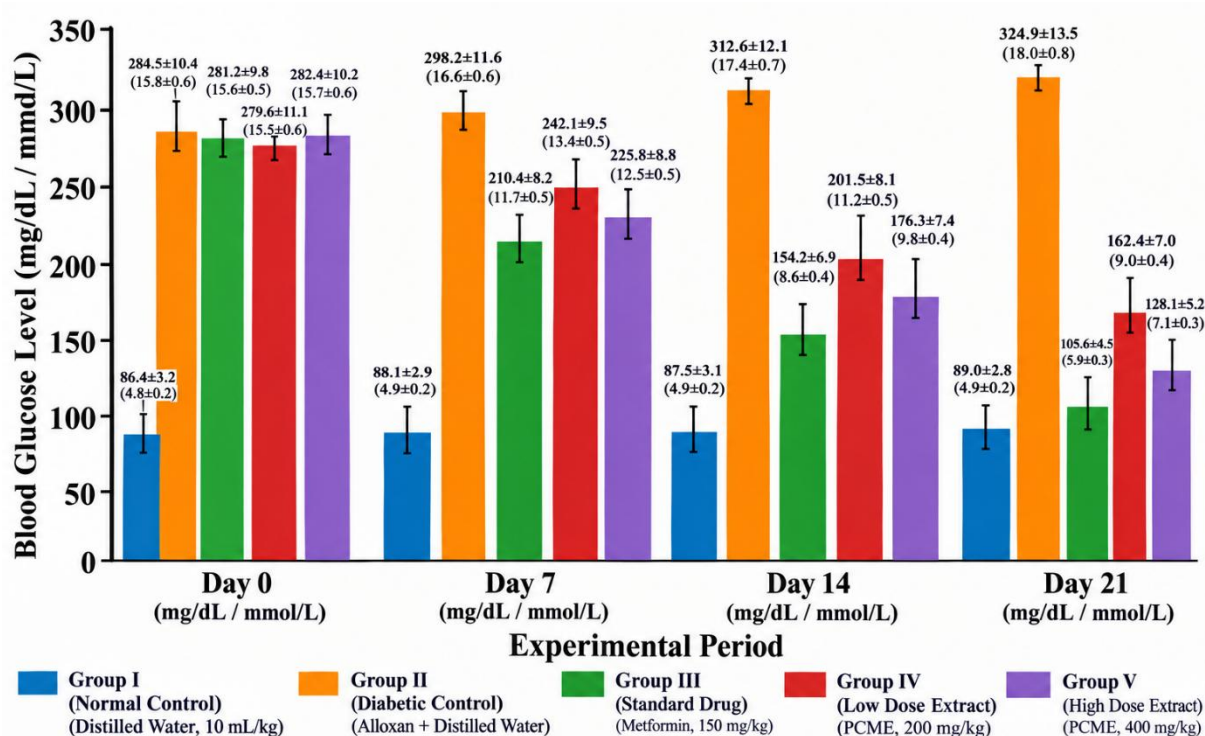
Group II (Diabetic Control)	Alloxan + Distilled Water	284.5±10.4 (15.8±0.6)	298.2±11.6 (16.6±0.6)	312.6±12.1 (17.4±0.7)	324.9±13.5 (18.0±0.8)
Group III (Standard Drug)	Metformin (150 mg/kg)	281.2±9.8 (15.6±0.5)	210.4±8.2 ^a (11.7±0.5)	154.2±6.9 ^a (8.6±0.4)	105.6±4.5 ^a (5.9±0.3)
Group IV (Low Dose Extract)	PCME (200 mg/kg)	279.6±11.1 (15.5±0.6)	242.1±9.5 ^b (13.4±0.5)	201.5±8.1 ^a (11.2±0.5)	162.4±7.0 ^a (9.0±0.4)
Group V (High Dose Extract)	PCME (400 mg/kg)	282.4±10.2 (15.7±0.6)	225.8±8.8 ^a (12.5±0.5)	176.3±7.4 ^a (9.8±0.4)	128.1±5.2 ^a (7.1±0.3)

Note: Values are expressed as mean ± SEM (n=5 mice per group); Conversion factor used is 1 mmol/L = 18.02 mg/dL (Blood glucose mmol/L = mg/dL /18.02); a:p<0.001 compared to the untreated diabetic control group.

Low-Dose Extract Treatment (200 mg/kg), i.e., Group IV, exhibited a gradual yet significant reduction in FBG levels, decreasing from 279.6±11.1 mg/dL (15.5±0.6 mmol/L) on Day 0 to 162.4±7.0 mg/dL (9.0±0.4 mmol/L) by Day 21 (p<0.001) (Bera et al., 2010). High-Dose Extract Treatment (400 mg/kg), i.e., Group V, showed a more substantial therapeutic response, lowering blood glucose levels from 282.4±10.2 mg/dL (15.7±0.6 mmol/L) down to 128.1±5.2 mg/dL (7.1 mmol/L) by the end of the 21-day trial (p<0.001) (Maiti et al., 2024). Standard Reference Drug (Metformin at 150 mg/kg), i.e., Group III, achieved near-normalization of glycemia, reducing FBG levels from 281.2±9.8 mg/dL (15.6±0.5 mmol/L) to 105.6±4.5 mg/dL (5.9±0.3 mmol/L) (Gupta et al., 2012).

The findings clearly indicate that the methanolic extract of *Piper chaba* possesses significant oral hypoglycemic activity in alloxan-induced diabetic models. The steady attenuation of blood glucose levels over the 21-day period suggests that bioactive secondary metabolites in the extract (such as alkaloids and polyphenols) may facilitate peripheral glucose utilization or protect remaining functional pancreatic beta cells from further oxidative stress-mediated destruction (Maiti et al., 2024).

Graph 3.1: Changes in Fasting Blood Glucose Levels (mg/dL and mmol/L) During the 21-Day Treatment Period



4. Discussion

The findings of this *in vivo* investigation conclusively demonstrate that the methanolic extract of *Piper chaba* (PCME) possesses potent, dose-dependent antihyperglycemic activity in alloxan-induced diabetic Swiss albino mice. Alloxan selectively destroys pancreatic beta cells by generating reactive oxygen species (ROS) and inhibiting crucial glucoregulatory enzymes, mimicking human type 1 or advanced type 2 diabetes characterized by absolute or relative insulin deficiency (Al-Awar et al., 2016). In the present study, untreated diabetic control mice (Group II) exhibited severe, unremitting hyperglycemia, with fasting blood glucose (FBG) levels progressively increasing from 284.5±10.4 mg/dL (15.8±0.6 mmol/L) to 324.9±13.5 mg/dL (18.0±0.8mmol/L) over 21 days. This progressive deterioration underscores the severity of alloxan-induced pancreatic injury and the absence of spontaneous recovery without therapeutic intervention (Bera et al., 2010).

Conversely, oral administration of PCME at 200 mg/kg and 400 mg/kg body weight markedly and significantly attenuated blood glucose levels from Day 7 through Day 21. By the conclusion of the 21-day experimental protocol, the high-dose extract group (Group V) successfully reduced FBG levels down to 128.1±5.2 mg/dL (7.1mmol/L), closely approaching normoglycemic baseline values observed in the normal control group (89.0±2.8mg/dL or 4.9 mmol/L). This therapeutic trajectory closely mirrored the glucose-

lowering efficacy of the standard reference drug, Metformin (150 mg/kg), which reduced FBG levels to 105.6±4.5 mg/dL (5.9 mmol/L). These outcomes align with previous pharmacological evaluations of related botanicals within the family Piperaceae, which have consistently demonstrated significant blood-glucose-lowering properties in murine experimental models (Gupta et al., 2012; Ashokkumar, 2021).

The sustained reduction of fasting blood glucose levels by *Piper chaba* can be attributed to the synergistic actions of its diverse phytochemical constituents. Phytochemical screening and chromatographic profiling of *Piper chaba* have revealed an abundance of bioactive secondary metabolites, most notably alkaloids (such as piperine), flavonoids, phenolic compounds, and essential oils (Maiti et al., 2024). These secondary metabolites exert their antidiabetic effects through multiple concurrent pharmacological mechanisms:

Alloxan-induced diabetes is primarily driven by oxidative stress mediated by free radicals. Phytoconstituents found in *Piper chaba*, particularly phenolic acids and flavonoids, exhibit robust free-radical scavenging and antioxidant properties (Maiti et al., 2024). By neutralizing reactive oxygen species in the pancreatic microenvironment, these compounds reduce oxidative cellular damage and mitigate further necrosis of surviving beta cells. Furthermore, preliminary histological and biochemical evidence from related Piper species suggests that certain active constituents may stimulate the regeneration or functional recovery of remaining pancreatic beta cells, thereby partially restoring endogenous insulin secretion (Bera et al., 2010; Hamden et al., 2011).

Beyond pancreatic protection, antidiabetic plant extracts often improve glycemic control by enhancing peripheral glucose uptake in insulin-sensitive tissues such as skeletal muscle and adipose tissue. Bioactive alkaloids like piperine enhance the bioavailability of various therapeutic compounds and improve membrane fluidity, which can facilitate glucose transporter (e.g., GLUT4) translocation to the plasma membrane independently of, or synergistically with, insulin pathways (Salehi et al., 2019). This mechanism promotes increased peripheral glucose utilization, accounting for the steady decline in blood glucose levels observed across the 7-, 14-, and 21-day milestones. In addition to peripheral mechanisms, many medicinal plants rich in polyphenols and tannins inhibit key carbohydrate-hydrolyzing enzymes in the intestinal brush border, such as alpha-glucosidase and alpha-amylase (Pizzino et al., 2017). Although this study focused on systemic oral administration rather than intestinal enzyme assays, the delayed and steady attenuation of

postprandial and fasting hyperglycemia suggests that PCME may partially impede intestinal carbohydrate absorption, smoothing out glycemic peaks and contributing to overall metabolic stability.

The comparative analysis between the low-dose (200 mg/kg) and high-dose (400mg/kg) PCME treatments reveals a clear dose-dependent therapeutic response. While the low-dose extract successfully reduced blood glucose to 162.4 ± 7.0 mg/dL (9.0 mmol/L) by Day 21, the high-dose extract achieved superior glycemic control (128.1 ± 5.2 mg/dL or 7.1 mmol/L), demonstrating that maximizing the concentration of active phytochemicals enhances therapeutic efficacy without inducing observable toxicological symptoms during the 14-day acute toxicity window. The magnitude of blood glucose reduction achieved by PCME in this study is consistent with findings reported for other well-known antidiabetic botanicals. For instance, studies investigating *Piper nigrum* and *Piper cubeba* extracts have similarly demonstrated significant hypoglycemic action in alloxan- and streptozotocin-induced diabetic rodent models (Muchandi et al., 2018; Sarfraz et al., 2023).

5. Limitations of the Study

Despite the robust and statistically significant antihyperglycemic effects observed, this study has several limitations that future research should address. First, the investigation focused primarily on fasting blood glucose dynamics and omitted broader systemic metabolic indicators such as serum lipid profiles, liver function enzymes, and inflammatory markers. Second, the absence of hormonal assays, including serum insulin, C-peptide, and tissue glycogen content, limits the direct confirmation of pancreatic beta-cell protection and insulinotropic activity (Maiti et al., 2024). Third, the pharmacological evaluations used crude methanolic extracts; while these may benefit from synergistic interactions among multiple phytochemicals, future studies should use bioassay-guided fractionation to isolate and characterize the specific active compounds responsible for the therapeutic effect. Finally, although alloxan-induced Swiss albino mice provide a reliable and reproducible model for screening acute pharmacological agents, chemically induced rodent models do not fully replicate the complex polygenic, metabolic, and lifestyle-driven etiology of human Type 2 diabetes mellitus. Future investigations should incorporate comprehensive biochemical profiling, hormonal quantifications, compound isolation, and advanced genetic or diet-induced animal models to substantiate these findings.

6. Conclusion

The present *in vivo* study provides robust scientific validation for the traditional ethnomedicinal use of *Piper chaba* in managing metabolic disorders. The methanolic extract showed significant, dose-dependent antihyperglycemic efficacy in alloxan-induced diabetic Swiss albino mice, reducing fasting blood glucose levels comparably to standard reference therapy over 21 days. These outcomes validate the plant's potential as a valuable natural resource for developing alternative or complementary antidiabetic phytomedicines and pave the way for advanced molecular and clinical investigations.

7. Conflicts of interests

The authors disclose no conflicts of interest.

8. Acknowledgment

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