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Costus pictus methanolic extract alleviates diabetes in Streptozotocin-induced diabetic Sprague Dawley rats

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ABSTRACT

Costus pictus, commonly referred to as the "insulin plant," has gained interest for its antidiabetic and organ-protective properties. The objective of the study was to evaluate the claim of *C. pictus* leaf extract on glycaemic control, organ weights, feed intake, and biochemical parameters in a streptozotocin-induced diabetic rat model. Male Wistar rats were divided into four groups: Group 1 – Normal Control; Group 2 – Diabetic Untreated; Group 3 – Diabetic + Low-Dose *C. pictus* (50 mg/kg); and Group 4 – Diabetic + High-Dose *C. pictus* (250 mg/kg). The study was conducted as a therapeutic trial. Body weight, feed intake, organ weights, and serum biomarkers including glucose, liver enzymes, renal parameters, lipids, and proteins were monitored over six weeks. Statistical comparisons were performed using ANOVA followed by Tukey's test. Diabetic control rats showed significant hyperglycaemia, organomegaly (notably in liver, kidney, and testis), and elevations in SGPT, ALP, urea, creatinine, and cholesterol. Treatment with *C. pictus* significantly reduced glucose levels by 33–37%, improved feed intake patterns, and restored liver, kidney, and testis weights toward normal. High-dose treatment exhibited superior efficacy in lowering SGPT (by 76.5 U/L), ALP (by 267 U/L), urea (by 35.2 mg/dL), and creatinine (by 0.29 mg/dL). Both low and high doses of *C. pictus* significantly improved serum albumin and total protein levels, with high dose showing greater therapeutic benefits. *C. pictus* effectively ameliorates hyperglycaemia and associated hepatic and renal dysfunctions in diabetic rats, with high-dose treatment providing enhanced benefits. These findings support its potential as a plant-based adjunct in diabetes management.

Keywords: methanolic extract of *C. pictus*, Streptozotocin induced diabetes, SD/NIN rat, Glucose

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INTRODUCTION

In 2024, an estimated 11.10% of the global population, about 589 million adults were living with diabetes, a figure projected to increase to 12.95% or roughly 853 million adults by 2050 (Genitsaridi et al., 2025). In 2019, an estimated 77 million people in India were living with diabetes, a number projected to exceed 134 million by 2045. Nearly 57% of these cases remain undiagnosed. Type 2 diabetes, which constitutes the vast majority, is associated with multi-organ damage, manifesting as both microvascular and macrovascular complications (Pradeepa & Mohan, 2021). The disease arises from impaired insulin secretion, insulin resistance, or both, resulting in chronic hyperglycaemia that disrupts carbohydrate, lipid, and protein metabolism, and drives microvascular complications such as retinopathy, nephropathy, and neuropathy, as well as macrovascular outcomes including coronary artery disease and stroke (Zakir et al., 2023). Despite the availability of conventional pharmacotherapies such as metformin, sulfonylureas, and insulin, long-term efficacy is often undermined by side effects, hypoglycaemia risk, high cost, and progressive β-cell decline (Gieroba, Kryska, & Sroka-Bartnicka, 2025). These limitations, coupled with the cultural acceptability, accessibility, and multitargeted mechanisms of plant-derived compounds, have driven growing interest in phytopharmaceutical interventions, with over hundreds of species reported to exhibit antidiabetic activity through antioxidant defence, insulin sensitization, and β-cell protection (Tran, Pham, & Le, 2020).

Medicinal plants have long been integral to traditional healthcare systems worldwide, and many are now being systematically evaluated for their therapeutic potential in diabetes management. These botanicals often exert multiple beneficial effects, including improvement of glycemic control, lipid profile modulation, attenuation of oxidative stress, and reduction of chronic inflammation which are key factors in preventing diabetes-related complications (Ansari et al., 2023; Parikh, Parikh, & Kothari, 2014). Among them, *C. pictus* D. Don, popularly known as the "insulin plant," has gained attention for its promising pharmacological profile. Native to Central and South America but widely cultivated in southern India, particularly Kerala, *C. pictus* belongs to the family Costaceae and is rich in bioactive constituents such as diosgenin, β-sitosterol glucoside, flavonoids, and steroidal saponins (Rani, Priya, Singh, & Bansal, 2022; Saranya, Balakrishnaraja, & Jadhav, 2024). These phytochemicals have been reported to stimulate pancreatic β-cell regeneration, enhance GLUT4 translocation in skeletal muscle, suppress hepatic gluconeogenesis, and scavenge free radicals, collectively improving insulin sensitivity and mitigating oxidative stress.

Preclinical studies have demonstrated that supplementation with *C. pictus* can produce marked reductions in fasting blood glucose up to 66% in streptozotocin-induced diabetic rats along with significant hepatoprotective effects, as evidenced by reductions in SGPT and ALP levels, and partial normalization of lipid parameters (Rani et al., 2022). Its multifaceted mode of action, coupled with safety and cultural acceptance, makes *C. pictus* a strong candidate for integration into dietary or therapeutic regimens aimed at diabetes management, particularly in resource-limited settings where affordable long-term solutions are needed.

The present study was undertaken to evaluate the anti-hyperglycemic, hepatoprotective, and reno-protective potential of methanolic extracts of *C. pictus* rhizomes in a streptozotocin-induced diabetic rat model. Specifically, we aimed to assess the dose-dependent effects of the extract on glycaemic regulation, biochemical markers of liver and kidney function, organ weights, and feed intake patterns, thereby providing experimental evidence for its possible inclusion as a Phyto-pharma intervention in diabetes care.

METHODS

Study Design

The study was conducted to evaluate the anti-diabetic therapeutic activity of the methanol-based extract of *C. pictus* rhizomes in a streptozotocin-induced diabetic (SID) rat model. Under ethical approval (ICMR-NIN Animal Ethics number: P10F/IAEC/NIN/2017/MSV/SD), a total of 44 Sprague Dawley adult male (11-12 weeks old) rats were used for the study and were randomly allotted into four groups **(Figure 1).**

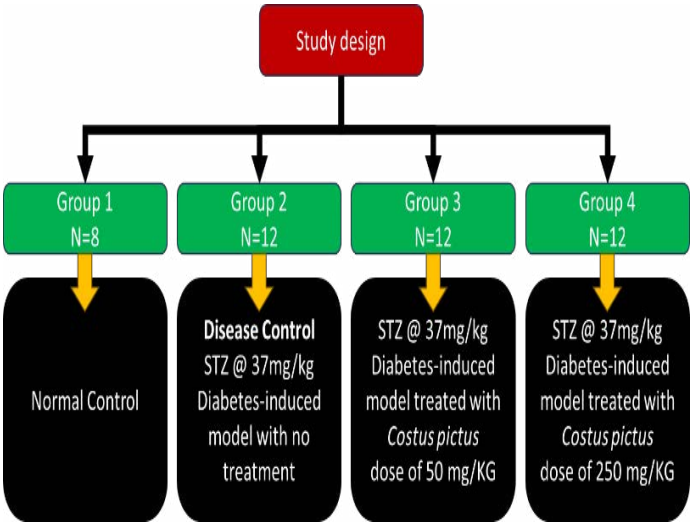


Figure 1: Study Design for Evaluating the Effects of Costus pictus in a Streptozotocin (STZ)-Induced Diabetes Model

Group 1 served as the normal control, consisting of non-diabetic rats (n = 8), maintained without any induction of diabetes or treatment. Group 2 comprised diabetic control rats (n = 12), which were induced with STZ to develop diabetes but received no further treatment. Groups 3 and 4 included diabetic rats treated with *C. pictus* extract of authenticated plant at two different doses: Group 3 received a low dose of 50mg/kg body weight (n = 12), while Group 4 was administered a high dose of 250mg/kg body weight (n = 12).

Due to preliminary study, reference control was not included. Animals received standard pellet diet from NIN consisting 14% crude protein and 4% crude fibre. The methanolic extract was administered daily to test groups (3 and 4) through oral gavaging (0.5ml/animal – concentration for groups varies but volume for dosing was maintained same) for a period of six weeks daily. This design allowed for the assessment of the dose-dependent effects of the extract on diabetic parameters.

Plant Botanical and Morphological Information.

C. pictus D. Don, widely recognized as spiral ginger or the insulin plant (Figure 2a-f), belongs to the family Costaceae. It is a perennial rhizomatous herb valued for its extensive medicinal applications, particularly in traditional systems of medicine for managing diabetes and related metabolic conditions. The plant is characterized by sub-sessile leaves that are elliptic to obovate in shape, providing a lush, green appearance (Figure 2f). The inflorescence appears as prominent spikes with large bracts positioned sub-terminally, adding to its distinct morphology. The flowers are vibrant, with their size ranging between 5–6 cm in length, and exhibit a cup-shaped labellum with a yellow crest bearing intricate lines, enhancing their ornamental and botanical significance (Figure 2a, e).

The fruits of *C. pictus* are capsular in structure, containing five black seeds encased in a fleshy white aril, which supports the plant's reproductive efficiency (Figure 2b). The rhizomes, a critical component of the plant, serve as a rich source of bioactive compounds (Figure 2c), including diosgenin (a steroidal sapogenin), tigogenin, dioscin, gracillin, and β-sitosterol glucoside. These phytochemicals contribute to its diverse pharmacological properties, such as antidiabetic, antihyperglycemic, astringent, and expectorant activities. The rhizomes also play a significant role in traditional treatments for jaundice, pneumonia, rheumatism, constipation, and diarrhoea, reflecting the plant's versatility in therapeutic applications (Figure 2d). This study utilizes the rhizomes of *C. pictus* to explore its efficacy in managing SID in a rat model, highlighting the relevance of this plant in modern pharmacological investigations.

Figure 2: Botanical information of *Costus pictus* used in this study

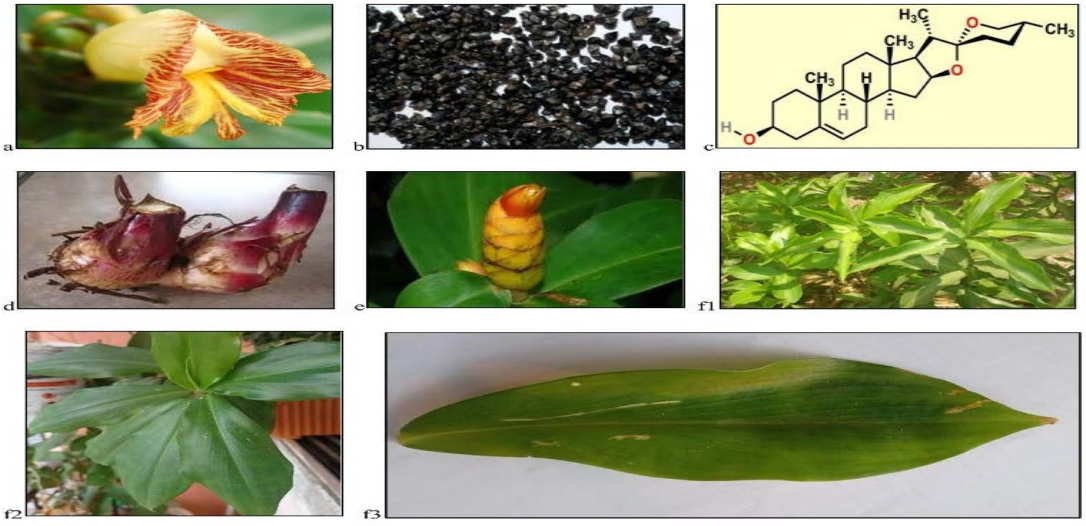


Figure 2 provides detailed botanical information on *Costus pictus* used in this study. Panel (a) shows the vibrant flower of *Costus pictus*, characterized by its yellow hue and striking red streaks. Panel (b) highlights the small, dark, and textured seeds of the plant, indicative of its reproductive mechanism. Panel (c) presents the chemical structure of a bioactive compound, likely representing a steroidal or medicinal molecule derived from *Costus pictus*. Panel (d) displays the thick, reddish-purple rhizomes, showcasing the plant's underground parts. Panel (e) depicts a cone-like bud in its early blooming stage with an orange-yellow color. Panels (f1) and (f2) illustrate the dense green foliage and mature broad leaves with a smooth texture, respectively. Lastly, panel (f3) focuses on a single lanceolate-shaped leaf, highlighting its visible veins and natural wear patterns.

Figure 2: Botanical information of Costus pictus used in this study

Legend: It provides detailed botanical information on *Costus pictus* used in this study. Panel (a) shows the vibrant flower of *Costus pictus*, characterized by its yellow hue and striking red streaks. Panel (b) highlights the small, dark, and textured seeds of the plant, indicative of its reproductive mechanism. Panel (c) presents the chemical structure of a bioactive compound, likely representing a steroidal or medicinal molecule derived from *Costus pictus*. Panel (d) displays the thick, reddish-purple rhizomes, showcasing the plant's underground parts. Panel (e) depicts a cone-like bud in its early blooming stage with an orange-yellow color. Panels (f1) and (f2) illustrate the dense green foliage and mature broad leaves with a smooth texture, respectively. Lastly, panel (f3) focuses on a single lanceolate-shaped leaf, highlighting its visible veins and natural wear patterns.

Plant Procurement, Authentication and Methanolic Extraction preparation

The rhizomes and other parts of *C. pictus* were collected (Figure 2) from various regions of Hyderabad, Kerala and authenticated by experts at Osmania University. The plant material was carefully rinsed with flowing tap water, allowed to air dry completely, and then ground into a coarse powder. Approximately 200 g of the powdered rhizomes were subjected to Soxhlet extraction using methanol as the solvent. The methanolic extract was concentrated under reduced pressure using a rotary evaporator, yielding a semisolid residue. The residue was weighed and stored at 4°C for further analysis and use. For administration, the extract was dissolved in a 1% Tween 80 solution to prepare the desired doses.

Animal Ethics and Housing Conditions

The rats were kept in individual cages under a controlled environment (temperature @22 ± 2°C and relative humidity 45-55%, and a 12-hour light/dark cycle). A standard stock diet and water were provided ad libitum throughout the study. The rats were acclimatised for one week before initiating the experiments.

Experimental Design

Diabetes in SD rats was induced in three groups (2, 3, & 4) by a single intraperitoneal (i/p) injection of streptozotocin (STZ @ 37 mg/kg body weight, Product code: S0130-1G, ≥75% α-anomer basis, ≥98% purity by HPLC, Sigma-Aldrich) dissolved in citrate buffer (pH 4.5). After 72 hours, blood glucose levels were measured at five time-points using a glucometer (Accu-Chek, Roche Diabetes Care India Private Limited), and rats with fasting blood glucose levels of 200 mg/dL or higher were considered diabetic and included in the study.

Blood Glucose Analysis

The treatment period spanned six weeks, during which several parameters were systematically monitored to evaluate the effects of diabetes and *C. pictus* extract treatment. Blood glucose levels were measured weekly by collecting blood samples via retro-orbital sinus puncture under mild anaesthesia(isoflurane), using a portable glucometer for analysis.

Body Weight and Feed Intake

Body weight was recorded weekly for each rat to assess the physical impact of diabetes and treatment. Additionally, daily food intake was carefully measured to identify any changes associated with metabolic alterations.

Necropsy and Organ Collection

At the conclusion of the six-week period, the rats were fasted overnight, and final blood samples were collected for a detailed biochemical analysis. Subsequently, the animals were euthanized using humane method using CO2 asphyxiation, and tissues were harvested for histopathological evaluation.

Serum Biochemistry

Plasma samples were analysed using an autoanalyzer (Cobos c 311 analyser, Roche) to assess key biochemical parameters. These included the lipid profile (total cholesterol and triglycerides) to evaluate metabolic disturbances, and liver and renal function tests, such as alanine transaminase (SGPT), aspartate transaminase (SGOT), alkaline phosphatase (ALP), creatinine, and urea, to determine the systemic effects of the treatment. Additionally, other parameters, such as total protein and albumin levels, were measured to provide insights into overall metabolic health and protein metabolism.

Histopathological Evaluation

The pancreas, liver, kidneys, and spleen were dissected, fixed in 10% formalin, and processed for histopathological analysis. Sections were stained with haematoxylin and eosin stains to examine structural changes and assess any regenerative effects of *C. pictus* extract on pancreatic β-cells.

Statistical Analysis

Statistical analysis was conducted to comprehensively evaluate the anti-diabetic potential of *C. pictus* methanolic extract and its impact on biochemical, physical, and gross morphology of visceral organs in an STZ-induced diabetic rat model. Data were expressed as mean ± standard deviation (SD) whereas statistical significance was assessed using GraphPad Prism Software (version 10.1.0). The two-way ANOVA was used to evaluate whether the effect of *C. pictus* treatment varied over time. For parameters measured only once at study termination (e.g., organ weights, end-point biochemical parameters), one-way ANOVA (Brown-Forsythe and Welch) was applied, followed by Dunnett T3 post-hoc test. The respective p-value < 0.05 was considered statistically significant, ensuring robust and reliable interpretation of the results.

RESULTS

The results of this study demonstrate the therapeutic efficacy of *C. pictus* extract in managing diabetic complications in rats. Significant improvements were observed in glucose levels, liver enzymes, organ weights, and feed intake, particularly at the high dose. These findings suggest dose-dependent modulation of metabolic and physiological parameters across treatment groups.

Body Weight

The progression of body weight revealed marked differences among experimental groups. At baseline, no significant variation was observed between groups ($p > 0.05$). By week 6, normal control rats (Group I) exhibited a modest gain of 4.2% (253.3 g to 263.9 g), whereas diabetic untreated rats (Group II) showed a significant reduction of 18.4% (247.2 g to 201.6 g; mean difference vs. Group I −62.3 g, 95% CI −71.3 to −53.3, $p < 0.0001$). *C. pictus*–treated animals displayed continued decline in body weight, with Group III (low dose) showing a 22.1% reduction (249.5 g to 194.4 g) and Group IV (high dose) showing a 20.8% reduction (250.6 g to 198.4 g). Although these reductions were substantial, the differences between treated groups and the diabetic untreated group were not statistically significant ($p > 0.05$).(Table 1 a and 1b)

Table 1a: Body Weight (in grams) Progression Over Time in STZ-Induced Diabetes Model with *Costus pictus* Treatment

Group	Baseline	3rd Week	4th Week	5th Week	6th Week
I	253.3 ± 5.6	265.1 ± 6.2	267.7 ± 4.2	260.8 ± 7.9	263.9 ± 5.9
II	247.2 ± 3.6	188.2 ± 21.2	207.6± 4.8	198.5 ± 5.6	201.6 ± 6.7
III	249.5 ± 4.7	215.8 ± 6.5	207.6 ± 8.3	199.2 ± 7.3	194.4 ± 5.9
IV	250.6 ± 5.3	245.4 ± 4.6	217.7 ± 6.0	203.5 ± 5.2	198.4 ± 6.9

Table 1b: Percentage Change (Baseline vs 6th wk) in Body Weight from Baseline to Week 6 in STZ-Induced Diabetes Model with *Costus pictus* Treatment

Group	Average Baseline Weight (g)	Average Weight at Week 6th (g)	Percent Change
I	253.3	263.9	+4.2
II	247.2	201.6	-18.4
III	249.5	194.4	-22.1
IV	250.6	198.4	-20.8

Feed Intake

Feed intake remained comparable across groups during the initial weeks but diverged toward the terminal phase. At week 6, the diabetic untreated group exhibited a slight decrease (−1.7%) compared to week 3, while the normal control group showed an increase of 10.2%. *C. pictus*–treated animals demonstrated an elevation in feed consumption, particularly at high dose, where Group IV recorded a 12.3% increase from week 3 to week 6. Statistical analysis revealed significant differences at week 6 between the normal control and both treatment groups (Group I vs. Group III, mean difference −2.95 g, $p < 0.0001$; Group I vs. Group IV, −1.89 g, $p < 0.0001$).(Table-2)

Table 2: Food Intake and Percentage Change (3rd wk vs 6th wk) Over Time in STZ-Induced Diabetes Model with *Costus pictus* Treatment

Group	3rd Week	4th Week	5th Week	6th Week	Percent change
I	15.39± 0.23	15.46± 0.41	15.99 ± 0.40	16.96± 0.36	10.20
II	15.84± 0.48	15.85± 0.33	15.71± 0.35	15.57± 0.46	-1.70
III	15.96± 0.52	15.67± 0.86	15.61± 0.40	16.07± 0.37	0.68
IV	15.37± 0.29	15.58± 0.31	18.32± 0.95	17.26 ± 0.77	12.29

Organ weights

Analysis of terminal organ weights showed selective alterations. The heart weight was reduced in diabetic untreated rats (0.9 g) compared to controls (1.1 g, $p = 0.003$), while liver weights also declined significantly in Group II (7.97 g vs. 8.32 g in controls, $p = 0.0047$). *C. pictus* treatment partially restored liver mass but did not significantly affect cardiac mass. Kidney weights were marginally increased in Group III (2.4 g) compared to controls (2.1 g, $p = 0.0267$). Pancreatic weight was significantly lower in treated groups (0.6 g vs. 0.8 g in controls, $p < 0.01$). Testicular atrophy was most severe in Group IV (1.9 g) compared to both diabetic untreated (3.4 g) and control (4.5 g) groups ($p < 0.0001$). No significant changes were observed in lung, brain, or spleen weights **(Table -3).**

Table 3: Terminal Organ Wet-Weight (grams) in STZ-Induced Diabetes Model with *Costus pictus* Treatment

Organ	Group - I	Group - II	Group - III	Group - IV
Heart	1.1±0.09	0.9±0.11	1±0.12	0.9±0.08
Liver	8.32±0.16	7.97±0.21	8.41±0.27	8.33±0.34
Lung	1.6±0.15	1.5±0.16	1.7±0.18	1.5±0.18
Kidney	2.1±0.16	2.3±0.12	2.4±0.19	2.3±0.25
Pancreas	0.8±0.13	0.7±0.14	0.6±0.08	0.6±0.13
Brain	1.7±0.15	1.6±0.17	1.7±0.09	1.6±0.17
Testis	4.5±0.28	3.4±0.36	3.0±0.35	1.9±0.25
Spleen	0.5±0.08	0.4±0.09	0.4±0.08	0.5±0.07

Blood Glucose

Weekly glucose monitoring showed stable values in the control group (70–90 mg/dL). In contrast, diabetic untreated rats maintained persistent hyperglycemia (baseline 307.9 mg/dL, rising to 315.9 mg/dL by week 6). Treatment with *C. pictus* resulted in a progressive and highly significant decline in glucose levels. Group III demonstrated a fall from 312.9 mg/dL at baseline to 158.1 mg/dL at week 6, while Group IV decreased from 349.7 mg/dL to 166.4 mg/dL over the same period. Both reductions were significant compared to diabetic untreated animals ($p < 0.0001$). Terminal serum glucose levels confirmed these results, with Group II remaining at 315.9 mg/dL compared to 158.1 mg/dL in Group III and 166.4 mg/dL in Group IV. No significant difference was observed between low and high doses ($p = 0.93$)(**Table-4)**

Table 4: Blood Glucose Levels (mg/dL) Across Groups over Time in an STZ-Induced Diabetes Model Treated with *Costus pictus*

Groups	**Baseline after Diabetes Induction** (mg/dL)	**3rd Week** (mg/dL)	**4th Week** (mg/dL)	**5th Week** (mg/dL)	**6th Week** (mg/dL)
I	70.63± 10.12	91.63± 3.55	87.25± 2.12	84.38± 3.68	79.88± 2.76
II	307.89±29.08	309.89±37.74	280.89±35.21	304.44±35.48	315.89±25.81
III	312.89±26.43	190.33±20.35	195±22.94	167.22±26.77	158.11±16.93
IV	349.67±20.48	241.11±54.56	223.44±53.33	223.44±60.22	166.44±37.09

Serum Biochemical Parameters

Serum biochemical analysis revealed pronounced alterations across groups (Figure 4, Table 5). Glucose levels at termination confirmed the weekly monitoring trends. Group I (control) maintained normoglycemia (79.88 ± 2.95 mg/dL), while diabetic untreated animals (Group II) exhibited severe hyper-glycemia (315.89 ± 26.96 mg/

dL), representing a 296% increase relative to controls ($p < 0.0001$). Both *C. pictus*–treated groups showed marked reductions: Group III (low dose) recorded 158.11 ± 17.68 mg/dL (50% reduction vs. Group II, $p < 0.0001$), and Group IV (high dose) 166.44 ± 38.74 mg/dL (47% reduction vs. Group II, $p < 0.0001$). No statistical difference was detected between low- and high-dose treatments ($p = 0.93$).

Serum triglycerides were significantly elevated in diabetic rats (42.50 ± 5.40 mg/dL) compared with controls (20.60 ± 5.00 mg/dL; mean difference −21.9 mg/dL, $p < 0.0001$). Treatment with *C. pictus* partially lowered triglycerides, with Group III at 34.90 ± 9.80 mg/dL ($p = 0.0029$ vs. controls, ns vs. diabetic) and Group IV at 39.30 ± 8.50 mg/dL ($p < 0.0001$ vs. controls, ns vs. diabetic). Cholesterol levels did not differ significantly between Groups I and II (61.9 ± 2.2 vs. 60.4 ± 2.8 mg/dL, $p = 0.70$), but Group IV displayed a modest elevation (67.3 ± 3.7 mg/dL; mean difference vs. controls −5.4 mg/dL, $p = 0.004$).

Hepatic enzymes showed notable elevations. SGOT remained statistically unchanged between control and diabetic untreated rats (100.9 ± 6.6 vs. 104.8 ± 9.0 U/L; $p = 0.83$). However, both treatment groups demonstrated significant increases, with Group III rising to 129.7 ± 25.9 U/L (mean diff. vs. control −28.8, $p = 0.016$) and Group IV to 135.9 ± 12.2 U/L (−35.0, $p < 0.0001$). SGPT showed the most striking alteration: levels nearly doubled in Group II compared with controls (113.6 ± 11.3 vs. 66.9 ± 3.3 U/L; mean diff. −46.7, $p < 0.0001$). *C. pictus* did not normalize these levels, as Group III reached 126.8 ± 19.2 U/L ($p < 0.0001$ vs. control) and Group IV 143.4 ± 11.1 U/L ($p < 0.0001$). ALP followed a similar pattern, rising from 143.0 ± 14.0 U/L in controls to 307.0 ± 39.0 U/L in Group II ($p < 0.0001$). Group III showed 293.0 ± 40.0 U/L ($p < 0.0001$), while Group IV peaked at 410.0 ± 31.0 U/L, a 186% increase vs. controls ($p < 0.0001$).

Protein metabolism was also altered. Total protein declined in Group II (5.73 ± 0.20 g/dL) compared to controls (6.36 ± 0.24 g/dL; mean diff. 0.63, $p = 0.0002$). Treatment partially restored values: Group III at 6.19 ± 0.28 g/dL (ns vs. control, $p = 0.0010$ vs. diabetic) and Group IV at 6.14 ± 0.12 g/dL (ns vs. control, $p < 0.0001$ vs. diabetic). Albumin levels were not significantly reduced in diabetic untreated rats (3.59 ± 0.19 g/dL vs. 3.68 ± 0.05 g/dL, $p = 0.56$), but both treatment groups showed a significant decrease (Group III 3.36 ± 0.14, $p < 0.0001$ vs. control; Group IV 3.42 ± 0.10, $p < 0.0001$ vs. control).

Renal markers revealed divergence between urea and creatinine. Serum creatinine was significantly lower in diabetic untreated rats (0.16 ± 0.02 mg/dL) compared to controls (0.27 ± 0.04 mg/dL; mean diff. 0.11, $p = 0.0005$). Groups III and IV recorded partial recovery (0.22 ± 0.02 and 0.22 ± 0.03 mg/dL, respectively; ns vs. control, but $p < 0.0001$ vs. diabetic). Urea, in contrast, was significantly elevated in Group II (29.2 ± 2.6 mg/dL vs. 21.0 ± 1.3 mg/dL in controls; $p < 0.0001$). This rise was further accentuated in Group III (33.7 ± 4.1 mg/dL; mean diff. vs. control −12.7, $p < 0.0001$; vs. diabetic −4.5, $p = 0.026$) and remained elevated in Group IV (27.9 ± 3.3 mg/dL; $p < 0.0001$ vs. control).(Table-5)

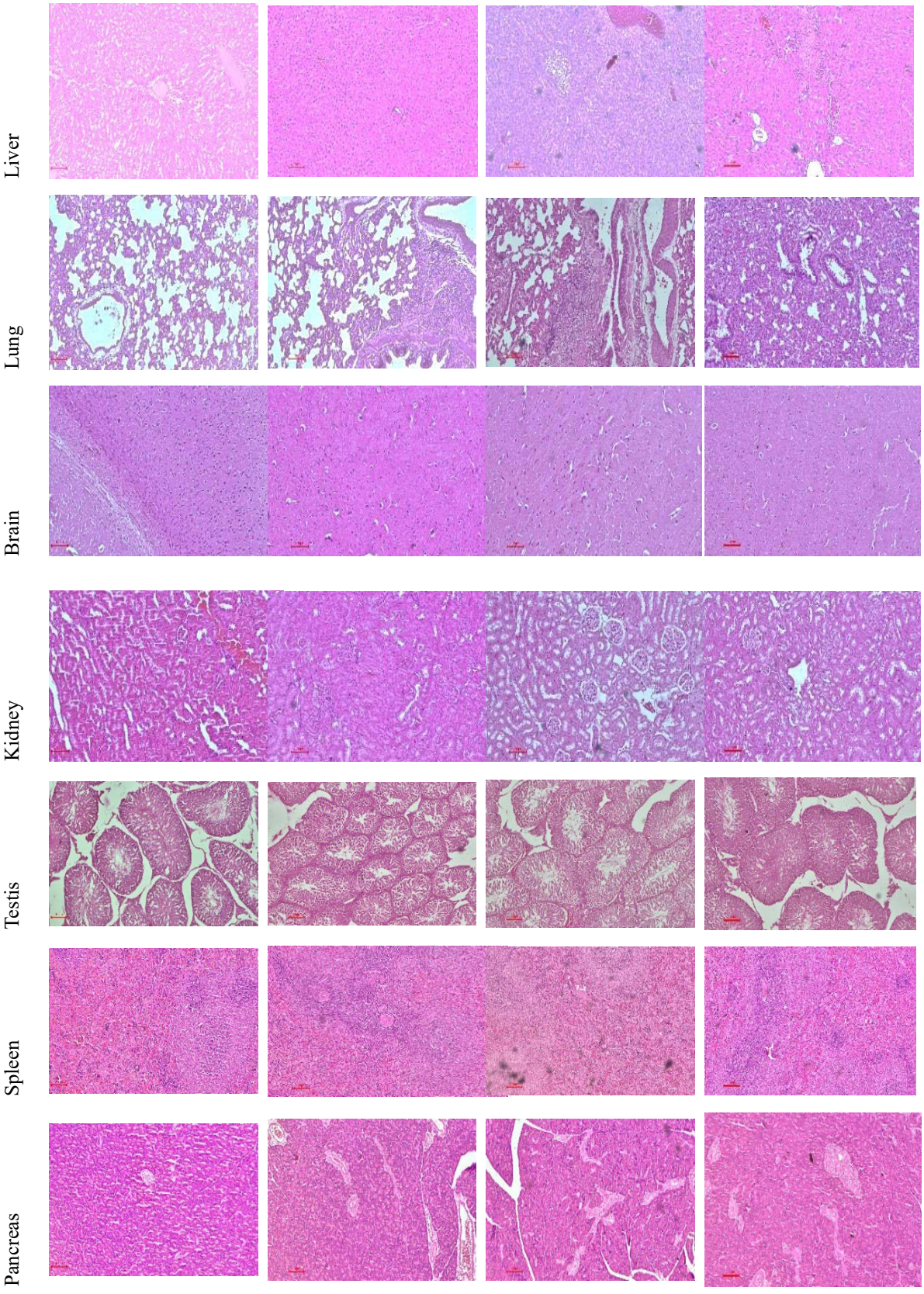
Table 5: Terminal Biochemical Parameters in STZ-Induced Diabetes Model with *Costus pictus* Treatment

Parameter	Group I	Group II	Group III	Group IV
Glucose (mg/dL)	79.88 ± 2.95	315.89 ± 26.96	158.11 ± 17.68	166.44 ± 38.74
Triglycerides (mg/dL)	20.60 ± 5.00	42.50 ± 5.40	34.90 ± 9.80	39.30 ± 8.50
SGOT (U/L)	100.90 ± 6.60	104.80 ± 9.00	129.70 ± 25.90	135.90 ± 12.20
SGPT (U/L)	66.90 ± 3.30	113.60 ± 11.30	126.80 ± 19.20	143.40 ± 11.10
ALP (U/L)	143.00 ± 14.00	307.00 ± 39.00	293.00 ± 40.00	410.00 ± 31.00
Albumin (g/dL)	3.68 ± 0.05	3.59 ± 0.19	3.36 ± 0.14	3.42 ± 0.10
Creatinine (mg/dL)	0.27 ± 0.04	0.16 ± 0.02	0.22 ± 0.02	0.22 ± 0.03
Total Protein (g/dL)	6.36 ± 0.24	5.73 ± 0.20	6.19 ± 0.28	6.14 ± 0.12
Cholesterol (mg/dL)	61.90 ± 2.20	60.40 ± 2.80	64.80 ± 4.20	67.30 ± 3.70
Urea (mg/dL)	21.00 ± 1.30	29.20 ± 2.60	33.70 ± 4.10	27.90 ± 3.30

Histological Observations

Histopathological examination revealed organ-specific changes across the treatment groups (**Figure 5**). The majority of animals in the normal control group displayed normal hepatic architecture. The diabetic control group showed mild pathological changes, primarily focal areas of necrosis (11.1%) and reduced normal histology incidence (66.6%). In the 50 mg/kg *C. pictus* group, 88.8% of livers appeared normal, with a single instance of focal necrosis (11.1%). The 250 mg/kg group showed one animal with focal necrosis (11.1%). These findings indicate that *C. pictus* did not exacerbate hepatic pathology compared with diabetic controls and may have preserved liver architecture at the lower dose.

Figure 5: Histology of Organs across four study groups (10x)



All kidneys from the normal control group were histologically normal. Chronic interstitial nephritis was present in 11.1% of diabetic controls and in 22.2% of animals receiving 50 mg/kg *C. pictus*. The 250 mg/kg group displayed both chronic interstitial nephritis (11.1%) and unilateral chronic interstitial nephritis (11.1%). Overall, renal lesions were mild and occurred at low frequencies, with no clear dose-dependent increase in severity.

Peribronchial lymphoid aggregates (PBLA) were the most frequent finding across all groups, including controls, affecting over half the animals. One case of chronic interstitial pneumonitis occurred in the high-dose group (11.1%), which was absent in the other groups. Given the distribution of PBLA across controls and treated groups, these changes are likely incidental rather than treatment-related.

All control animals had normal seminiferous tubules with active spermatogenesis. The diabetic control and low-dose groups maintained normal testicular histology, whereas the high-dose group showed one case (11.1%) of atrophied seminiferous tubules with absent spermatogenesis.

The overall histopathological profile suggests that the low dose (50 mg/kg) of *C. pictus* had the least adverse changes closely resembling the diabetic control group. The high dose (250 mg/kg) was associated with slightly more lesions, particularly in the kidneys and testes, but without marked or widespread pathological effects.

DISCUSSION

The present study demonstrates that *C. pictus* extract erts a significant anti-hyperglycaemic effect in streptozotocin (STZ)-induced diabetic rats, lowering fasting blood glucose by nearly 50% compared with untreated diabetic controls. This effect is consistent with prior reports indicating that *C. pictus* modulates glucose homeostasis through multiple mechanisms: stimulation of insulin secretion, enhancement of peripheral glucose uptake, inhibition of intestinal carbohydrate-digesting enzymes, and protection of pancreatic β-cells against oxidative stress (Ashwini, Bobby, *et al.*, 2015; Gireesh, *et al.*, 2009). These actions are particularly relevant in the STZ model, where diabetes arises from β-cell destruction mediated by GLUT2-dependent toxin uptake (Hajiaghaalipour, *et al.*, 2015), DNA alkylation, oxidative stress, and mitochondrial dysfunction, ultimately leading to insulin deficiency, hyperglycaemia, and systemic metabolic derangements (Zhang et al., 2023).

Both low- and high-dose groups treated with *C. pictus* showed a steady decline in glucose levels, with terminal values of 158.11 ± 17.68 mg/dL (*C. pictus* @ 50 mg/kg) and 166.44 ± 38.74 mg/dL (*C. pictus* @ 250 mg/kg), compared with >300 mg/dL in diabetic controls. Such reductions are patho-physiologically meaningful because restoration of euglycemia disrupts the cycle of glucotoxicity, oxidative stress, advanced glycation end-product (AGE) formation, and endothelial injury that drive diabetic complications (Mengstie et al., 2022).

The phytoconstituents of *C. pictus* shows notably flavonoids, phenolic acids, and steroidal saponins such as diosgenin which may be potentiating residual β-cell activity, enhancing GLUT4 translocation in muscle and adipose tissue, suppressing hepatic gluconeogenesis (via down-regulation of PEPCK and glucose-6-phosphatase), and inhibiting digestive enzymes such as α-amylase and α-glucosidase (Rani et al., 2022; Selvakumarasamy, *et al.*, 2021). Antioxidant properties of the extract may further protect metabolic tissues from reactive oxygen species generated during chronic hyperglycaemia.

Interestingly, triglyceride levels showed only modest, non-significant improvement. This suggests that lipid metabolism may require longer treatment, higher doses, or different extract preparations to achieve measurable benefits. Persistent hepatic overproduction of triglyceride-rich lipoproteins, driven by insulin resistance, may also explain the limited lipid-lowering effect observed here(Sparks, Sparks, & Adeli, 2012).

The Body Weight Dynamics in Untreated diabetic rats exhibited progressive weight loss, consistent with insulin-deficient catabolism involving proteolysis and lipolysis (Mitch et al., 1999; Moon et al., 2013). *C. pictus* altered this trajectory but did not completely restore body weight. By week 6, both treatment groups had lost 20–22% of baseline body weight, similar to diabetic controls. This indicates that while glucose control was achieved, the plant did not fully reverse catabolic muscle and fat breakdown.

Phytochemicals such as saponins and flavonoids may transiently enhance metabolic turnover or appetite regulation, contributing to this outcome. At higher doses, metabolic activation could exacerbate weight loss before stabilizing with improved glycaemic control (Marrelli, *et al.*, 2016; Stuby et al., 2019). The biphasic trajectory of weight change suggests a pharmacodynamic pattern where glucose homeostasis improves first, while anabolic recovery lags behind. By week six, however, body weight in this group recovered to near-normal levels, suggesting a biphasic pharmacodynamic effect: an initial catabolic phase followed by anabolic stabilization as glycaemic control improved and hepatic stress decreased (Saranya et al., 2024). These findings highlight the importance of dose optimization, as higher doses may transiently exacerbate weight loss before exerting restorative effects.

Diabetic hyperphagia, a hallmark of uncontrolled glycaemia (Banday, Sameer, & Nissar, 2020), was clearly evident in the untreated diabetic group. In contrast, feed intake in

the high-dose group followed a biphasic trend where it was initially elevated in week three, significantly suppressed in week five, and normalized by week six.

The transient anorectic effect observed in week five may reflect enhanced satiety signalling through leptin or ghrelin pathways (Klok, Jakobsdottir, & Drent, 2007). The normalization of intake without hyperphagia by week six indicates adaptive metabolic correction, likely linked to improved glycaemic control and hormonal signalling (Raj, et al., 2023). This suggests that *C. pictus* may help break the vicious cycle of polyphagia in diabetes, contributing to long-term metabolic stability.

The biochemical profile demonstrates that *C. pictus* exerts a dualistic effect: potent antihyperglycemic activity but mixed hepatic and renal responses. The normalization of blood glucose in both treatment groups, with nearly 50% reductions from diabetic untreated animals, confirms that the plant strongly augments glycemic control. This could be attributed to enhanced insulin sensitivity, facilitated glucose uptake, and reduced gluconeogenesis, consistent with known bioactive like diosgenin and flavonoidsDespite this, dyslipidemia was not fully corrected. Triglycerides declined modestly with treatment but remained significantly elevated compared to controls, while cholesterol showed a paradoxical increase at high dose. This suggests that while glucose regulation improved, lipid metabolism remained incompletely addressed, possibly due to persistent hepatic stress or incomplete insulin action on lipid turnover, which is seen in other studies as well (Vessal, Hemmati, & Vasei, 2003).The liver function markers raise caution. Elevations of SGOT, SGPT, and ALP, especially the striking ALP increase in high-dose treatment, indicate potential hepatocellular stress or enzyme induction. Such elevations could be secondary to phytochemical metabolism within the liver, suggesting dose-dependent hepatic burden. This pattern mirrors findings in some herbal hypoglycemic agents where efficacy comes at the cost of hepatic enzyme elevation.Protein metabolism was partially normalized. Total protein decline in diabetic untreated rats was significantly restored by treatment, indicating improved hepatic protein synthesis. However, albumin reduction in treated groups raises the possibility of altered hepatic synthetic pathways or early-onset hepatic stress.Renal indices revealed partial recovery of creatinine with *C. pictus*, suggesting some nephroprotective effects. However, persistent urea elevation, particularly in the low-dose group, suggests increased protein catabolism or incomplete renal clearance, hinting at ongoing nephron stress. This divergence between creatinine and urea highlights that while glomerular filtration may have improved, nitrogen metabolism remained dysregulated.Overall, the biochemical evidence underscores that *C. pictus* is highly effective in glucose regulation but may exert a metabolic cost, particularly at higher doses. Its therapeutic application will require careful dose optimization and possibly combination with hepatoprotective or nephroprotective adjuncts to maximize efficacy while minimizing organ stress.On Histopathology largely corroborated biochemical findings. At 50 mg/kg, liver histoarchitecture was preserved in nearly 90% of animals, indicating hepatoprotective potential. Focal necrosis in diabetic and treated groups likely reflected diabetes-related oxidative stress rather than extract toxicity. Renal lesions, including interstitial nephritis, occurred sporadically across groups but without dose-dependent worsening, aligning with typical STZ pathology. Pulmonary lesions were observed in all groups, including controls, and appear incidental. Testicular atrophy was limited to a single high-dose animal, suggesting an isolated event rather than a treatment-related trend. Overall, *C. pictus* was well tolerated, with minimal histological toxicity. Several unanswered questions emerge from this work. First, whether *C. pictus* primarily enhances insulin sensitivity or stimulates insulin secretion remains to be elucidated. Future studies should explore GLUT4 translocation, PPAR-γ activation (Dey & Mitra, 2013), and insulin signalling pathways (Rani et al., 2022). Second, the observed modulation of liver enzymes suggests possible antioxidant or anti-inflammatory actions at the cellular level, meriting further mechanistic evaluation. Third, the biphasic effects on appetite and body weight raise questions about central (hypothalamic) versus peripheral (gut hormone) regulation (Ashwini et al., 2015). Fourth, given emerging evidence linking polyphenols to gut microbiome modulation, the potential prebiotic role of *C. pictus* warrants exploration. Finally, reproductive outcomes remain inconclusive, requiring detailed hormonal profiling and fertility assessments.

Long-term safety and pharmacokinetic studies in higher-order models are also essential before translation to clinical use. This study establishes *C. pictus* as a promising botanical candidate for diabetes management, demonstrating significant anti-hyperglycaemic effects alongside improvements in metabolic balance, organ preservation, and feeding behaviour, with minimal toxicity. While the therapeutic potential is evident, further mechanistic and translational studies are necessary to refine dosing, identify active constituents, and validate long-term efficacy and safety.

CONCLUSION

This study demonstrates that methanolic leaf extract of *C. pictus* delivers robust, multi-targeted benefits against streptozotocin-induced diabetes in rats, achieving marked gly-

caemic reduction alongside normalization of hepatic and renal biomarkers, organ weights, and liver enzyme profiles, hallmarks of systemic metabolic stabilization. Rich in flavonoids, terpenoids, and steroidal saponins, the extract appears to enhance insulin secretion, improve glucose utilization, and confer hepatoprotective and reno-protective effects, while also reversing diabetes-related reproductive and nutritional deficits, as evidenced by restored testicular weight and serum proteins. The observed modulation of feed intake and body weight suggests a concurrent regulatory influence on energy metabolism and satiety. Collectively, these findings position *C. pictus* as a potent botanical candidate for integrated diabetes management, offering hypoglycaemic efficacy with organ-protective and homeostasis-restoring properties, and warranting further mechanistic and clinical exploration.

ABBREVIATIONS:

Conflict of interest: All authors in this manuscript do not have any conflict of interest in publishing the data.

AUTHOR'S CONTRIBUTION:

MMV– Conduct of the study, Study designing, Data collection, Funding Acquisition, Ethics Approval
SR – Plant authentication, Methanolic extract, Study design, Supervision and guidance in the project
PBP –Data Curation, Formal Analysis, Writing – Original Draft, Writing – Review & Editing
MVS – Histopathological photography, scoring and report
MS- Processing of histology samples
AP- Draft editing

Ethics approval: P10F/IAEC/NIN/2017/MSV/SD

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