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Phytochemical profiling, antidiabetic and cardio-protective efficacy of a polyherbal blend in Type 2 diabetic Wistar Rats

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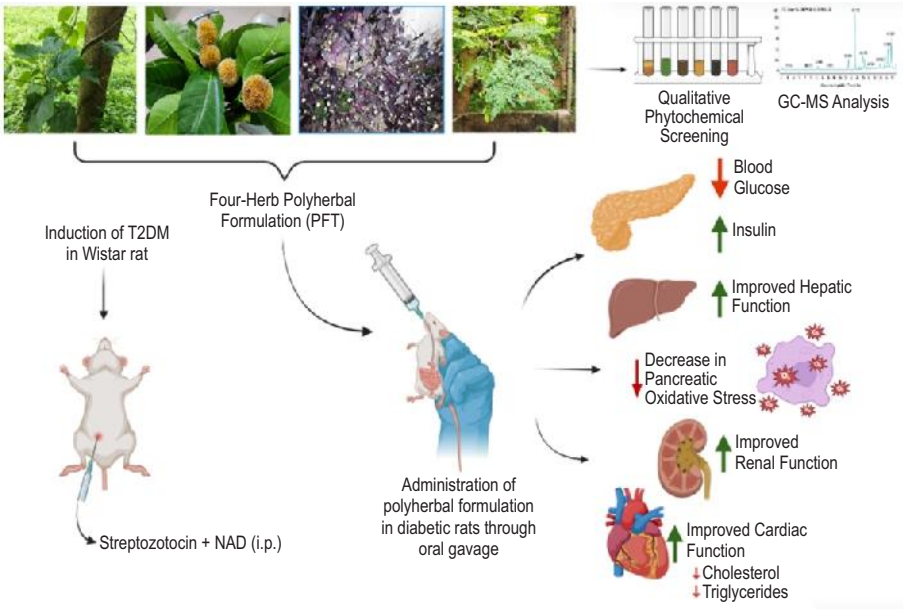
Abstract

Aim: This study investigated the efficacy of a novel phytotherapeutic formulation comprising the stem of *Tinospora cordifolia* and leaves of *Neolamarckia cadamba*, *Alternanthera brasiliana* and *Moringa oleifera* against streptozotocin (STZ)-induced T2DM in Wistar rats.



Methodology: Plant materials were collected, authenticated, extracted, and analysed through qualitative phytochemical screening, and GC–MS for bioactive compounds. Wistar rats were divided into four groups: healthy control, diabetic control, metformin-treated (200 mg kg^{-1} b.wt.), and formulation-treated (150 mg kg^{-1} b.wt.). Body weight, food intake, serum biochemical parameters, marker oxidative stress enzyme profile, echocardiography profile, and histopathology of the pancreas and heart were assessed for each group following the standard methods.

Results: The phytotherapeutic formulation significantly reduced fasting blood glucose levels in diabetic rats compared with diabetic controls ($p < 0.01$) and showed comparable efficacy to metformin. Body weight loss in diabetic rats was markedly attenuated in the formulation group. STZ-induced T2DM caused elevated glucose, triglycerides, total cholesterol, creatinine, SGOT, and SGPT, alongside decreased insulin levels ($p<0.05$). Treatment with the formulation effectively restored these biochemical parameters and increased serum insulin in a time-dependent manner.

Interpretation: The polyherbal formulation exhibited potent antihyperglycemic, lipid-lowering, and organ-protective effects in T2DM rats, supporting its potential for further clinical evaluation.

Key words: Diabetes mellitus, Hyperglycemia, Phytoactive compounds, Polyherbal formulation, Streptozotocin

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Introduction

Type 2 diabetes mellitus (T2DM) is a chronic, non-communicable metabolic disorder caused by impaired insulin action and a progressive decline in β-cell function, leading to persistent hyperglycaemia (American Diabetes Association, 2025). Sustained hyperglycaemia drives oxidative stress and low-grade inflammation that underlie both microvascular (retinopathy, nephropathy, neuropathy) and macrovascular complications (coronary, cerebrovascular, peripheral arterial disease) (Caturano *et al.,* 2023; Li *et al.,* 2023). In parallel, hepatic steatosis is highly prevalent in people with T2DM and further amplifies cardiometabolic risk (Younossi *et al.,* 2024). T2DM continues to escalate globally and now affects roughly one in nine adults, with projections indicating a substantial rise by 2050 (International Diabetes Federation, 2025). Beyond dysglycemia, T2DM markedly elevates cardiovascular risk; individuals with T2DM have ~1.5–2-fold higher risk of cardiovascular disease (CVD) and experience excess CVD morbidity and mortality (Ahmad *et al.,* 2024). Given that cardiac complications are a principal driver of diabetes-related deaths, cardiometabolic readouts should be integral to antidiabetic drug or formulation evaluation (Wong and Sattar, 2023).

Lifestyle modification (medical nutrition therapy, physical activity, weight management) remains foundational, but many individuals ultimately require pharmacotherapy to achieve glycaemic targets (ADA, 2025). While contemporary agents (e.g., metformin, SGLT2 inhibitors, GLP-1 receptor agonists) improve outcomes, access, tolerability, adherence, and residual risk continue to motivate the search for complementary, affordable interventions—including evidence-based botanicals. Observational and trial data also suggest that diets richer in flavonoid-containing foods are associated with lower incident T2DM, aligning with phytochemical mechanisms that improve insulin sensitivity and antioxidant capacity (Liu *et al.,* 2014; Thompson *et al.,* 2024). Herbal medicines provide multi-target phytochemicals (flavonoids, phenolic acids, alkaloids, polysaccharides) that may modulate insulin secretion or action, intestinal carbohydrate handling, gluconeogenesis, oxidative stress, and inflammation. Polyherbal formulations can leverage synergy among such constituents and are increasingly investigated in diabetes models and clinical studies (Jain *et al.,* 2025).

Four ethnomedicinal species *viz., Moringa oleifera* (Moringaceae), *Tinospora cordifolia* (Menispermaceae), *Alternanthera brasiliana* (Amaranthaceae), and *Neolamarckia cadamba* (Rubiaceae) provide complementary phytochemicals (flavonoids, phenolic acids, alkaloids, polysaccharides) with documented antidiabetic and antioxidant actions. In diabetic rodent models, *M. oleifera* has been found to improve glycaemia, lipids, and redox–inflammatory signaling (Watanabe *et al.,* 2021). *T. cordifolia* has been reported to exert antihyperglycaemic effects and support β-cell regeneration (Rajalakshmi and Anita, 2016). *A. brasiliana*, widely used in Brazilian folk medicine, has shown robust antioxidant and anti-inflammatory activity and, importantly, recent combined *in vitro* and *in vivo* evidence of antihyperglycaemic effects (Faloye *et al.,* 2024). *N. cadamba* extracts have been found to lower glucose and improve metabolic status in diabetic rats (Munira *et al.,* 2020). These convergent actions provide a biologically plausible rationale for a polyherbal strategy targeting multiple nodes of cardiometabolic dysfunction. Despite advances in diabetes pharmacotherapy, evidence for botanical, especially polyherbal, interventions remain fragmented. Under this contemplated background, the current research work is aimed at evaluating the antidiabetic efficacy of a four-herb polyherbal formulation comprising *Moringa oleifera*, *Neolamarckia cadamba*, *Alternanthera brasiliana* and *Tinospora cordifolia* in STZ-induced type 2 diabetic Wistar rats, integrating phytochemical profiling, biochemical assays, multiorgan histology, and cardiac (ECHO) assessments.

Materials and Methods

Collection of plant materials and preparation of extracts: *Tinospora cordifolia, Neolamarckia cadamba, Alternanthera brasiliana,* and *Moringa oleifera* were collected from the IIT Guwahati and Cotton University campuses, Kamrup district, Assam, between April and October 2024. The plant materials were authenticated and voucher specimens were deposited in the Herbarium of the Department of Botany, Gauhati University (accession nos. GUBH020711, GUBH20713, GUBH020715, and GUBH020712, respectively). Stems of *T. cordifolia* and leaves of the remaining species were washed, shade-dried for 7–10 days (23–27 ± 2 °C), and powdered using a stainless-steel grinder. The powdered samples were extracted with double-distilled water, ethanol, and methanol using the Soxhlet method at room temperature (Tiwari *et al.,* 2011). Extracts were concentrated to dryness using a rotary evaporator and used for phytochemical screening.

Qualitative phytochemical screening: In order to determine the presence of bioactive components in the aqueous, methanolic and ethanolic extract of the selected herbs, phytochemical screening tests were conducted according to the standard procedures of Trease and Evans (1989), Sofowora (1993), and Harborne (1998).

GC-MS analysis of methanolic extracts: A 10 mg sample was extracted with 1 ml methanol by vortexing (10 min), sonication (60 min), and centrifugation (10,000 rpm, 10 min, 4°C). The supernatant was vacuum-dried and derivatized for GC–MS analysis. Methoximation was performed with 90 µl o-methylhydroxylamine hydrochloride in pyridine at 60°C for 90 min, followed by silylation with 200 µl MSTFA containing 1% TMCS at 60°C for 120 min. The derivatized extract was vacuum-concentrated, reconstituted in 300 µl n-hexane, and vortexed. A 1 µl aliquot was injected into a GC–MS system (Intuvo 9000 GC coupled with 5977B MSD, Agilent Technologies, USA).

Experimental animals: Adult male Wistar rats (n=24; 10–12 weeks old, weighing. 85–105g) were purchased from the College

of Veterinary Science, Assam Agricultural University, Khanapara. Prior to the experiment, the rats were acclimatized for seven days in ventilated polypropylene cages under standardized conditions (22–25°C, humidity 60–70%, 12 hr light: 12 hr dark cycle). They were provided with standard rodent pellet diet and water ad libitum and monitored regularly.

Induction of Type 2 Diabetes: Rats were initially injected with a single intraperitoneal dose of 230 mg kg^{-1} b.wt. nicotinamide in physiological saline and 15 min later with 65 mg kg^{-1} b.wt. STZ in 0.1M citrate buffer (pH 4.5), following the method of Furman (2021). After 10 days of nicotinamide + STZ injection, fasting blood glucose level (BGL) was examined. Rats with BGL >200 mg dl^{-1} were selected as Type 2 diabetic.

Experimental design: Adult Wistar rats were randomly allocated to four groups (n = 6 per group). Group I (Control) received standard diet and water ad libitum without any test substances. Diabetes was induced in Groups II–IV using single intraperitoneal doses of nicotinamide (230 mg kg^{-1}) and streptozotocin (65 mg kg^{-1}). Group II served as the diabetic control (negative control). Group III (Metformin-treated) received metformin at 200 mg kg^{-1} b.wt. per day orally (positive control) using oral gavage needle. Group IV (Polyherbal-treated) received a 1:1:1:1 polyherbal formulation of *Tinospora cordifolia, Neolamarckia cadamba, Alternanthera brasiliana* and *Moringa oleifera* at 150 mg kg^{-1} b.wt. per day orally using oral gavage needle. The experiment was carried out for a period of 4 weeks.

Estimation of food intake and Body-weight gain: Body weight and food intake were monitored for all the groups throughout the study. Individual body weights were recorded on day 0 and then weekly. The weekly quantity of diet consumed by each group was measured, and per-rat food intake was calculated for all the groups.

Collection of serum and tissue samples: After 4 weeks of feeding and drug administration, the rats were anesthetized with sodium pentobarbital (50 mg kg^{-1}) and sacrificed by cervical dislocation. Blood was drawn from the retro-orbital plexus using capillary tubes immediately prior to sacrifice. Serum was separated by centrifugation at 5000 rpm for 10 min at 4 °C. Pancreas and heart were excised and stored at −80 °C for further biochemical assessments.

Biochemical assays for serum parameters: Blood samples from all groups were analyzed at different time intervals to assess different metabolic parameters. Fasting blood glucose (FBG) was measured weekly, directly from a small drop of blood from tail vein using glucometer (One Touch Horizon, Singapore). After 4 weeks, the glycated hemoglobin (HbA1c) and serum creatinine was determined using kits from Crystal Chem, USA. Estimation of serum insulin levels was done by ELISA kit (Mercodia, USA). SGOT and SGPT activities were assayed using the kits from HiMedia, India. Serum uric acid was measured using a kit from Siemens, India. Total cholesterol and total triglyceride levels were measured using the kits from Merck, Germany. Total lipids were quantified using the Sulfo-phospho-vanillin (SPV) method using a kit from Atlas Medical, India. All the assays were run following the manufacturer's instructions on the kits.

Estimation of pancreatic oxidative stress: Oxidative stress in pancreatic tissue was evaluated by measuring thiobarbituric acid–reactive substances (TBARS) as an index of lipid peroxidation and antioxidant markers including reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and hydrogen sulphide (H_2S). TBARS levels were determined in whole-tissue homogenates following Ohkawa *et al.* (1979) and expressed as nmol MDA g^{-1} tissue. For GSH, SOD, CAT, and H_2S analyses, the tissue homogenates were centrifuged at 15,000 × g for 30 min at 4 °C, and the supernatant was used. CAT activity was estimated colorimetrically by monitoring H_2O_2 decomposition at 620 nm (Takahara *et al.,* 1960) and expressed as µmoles of H_2O_2 consumed min^{-1} $protein^{-1}$. SOD activity was measured using a commercial assay kit (Fluka Analytical, Switzerland) based on inhibition of xanthine oxidase–generated superoxide (Khatua *et al.,* 2012) and expressed as % inhibition. GSH levels were estimated by the Ellman method (1959) and expressed as mmol g^{-1} wet tissue. H_2S concentration was measured according to Cai *et al.,* (2007) and expressed as µmol g^{-1} tissue. All the chemicals were procured from Sigma (USA). Protein content was determined by the Lowry method using Folin–Ciocalteu reagent (Lowry *et al.,* 1951).

Estimation of echocardiographic LV systolic function and structural parameters: Transthoracic echocardiography was performed 4 weeks post-treatment, prior to sacrifice, in lightly anesthetized Wistar rats using a FUJIFILM VisualSonics Vevo LAZR-X 3100 small-animal imaging system. Anesthesia was induced with isoflurane (3–4%) and maintained at 1–2% in oxygen (0.6–1.0 L min^{-1}). Animals were positioned supine on a heated platform with ECG monitoring and maintained at ~37 °C. Thoracic hair was removed, and two-dimensional and M-mode images were acquired using a 13-MHz linear transducer (MX400) with ultrasound gel. Heart rate was maintained within the physiological range (≈300–400 bpm). Standard parasternal long-axis and short-axis (papillary-muscle level) views were obtained, with apical four-chamber views acquired when required. Cine loops and M-mode recordings of ≥3 consecutive cardiac cycles were stored. LV ejection fraction, fractional shortening, stroke volume, LV mass, and cardiac output were quantified offline using Vevo LAB software (v5.10.0) following vendor-validated measurement protocols; cardiac output was calculated as heart rate × stroke volume.

Histopathology: Pancreas and heart tissues from each group were fixed in Carnoy's solution for 24 hr, washed thoroughly, dehydrated through graded alcohols, cleared in xylene, and embedded in paraffin. Sections (5 µm) were cut using a rotary microtome, stained with hematoxylin and eosin (H&E), dehydrated, and mounted with DPX. Histological observations were made using a Leica DM750 microscope equipped with a Leica ICC50 W digital camera (Leica Microsystems, Germany).

Statistical analysis: Data are expressed as mean±SEM. Statistical analysis was performed using One-way ANOVA followed by Tukey's post hoc test for multiple comparisons in GraphPad Prism version 10.2.3 (GraphPad Software, San Diego, CA, USA)(Liang *et al.,* 2023). Differences were considered statistically significant at $P < 0.05$.

Results and Discussion

Phytochemical screening of the selected herbs *viz., Tinospora cordifolia, Neolamarckia cadamba, Alternanthera brasiliana,* and *Moringa oleifera* demonstrated the presence of alkaloids, phenolics, tannins, flavonoids, glycosides, terpenoids, and saponins at varying levels, with methanolic extracts showing stronger reactions (greater color intensity), indicative of higher relative concentration (Fig. 1). Phytochemical profiling of methanolic extracts of the selected herbs by GC-MS identified several chemical constituents with different retention times. It revealed a diverse profile of bioactive marker compounds, representing different classes such as organic acids, fatty acids, sterols, phenolic derivatives, and secondary metabolites. The principal compounds identified, along with their retention times and known biological activities, are summarized in Table 1. The GC–MS profiling of methanolic extracts from *Tinospora cordifolia, Neolamarckia cadamba, Alternanthera brasiliana,* and *Moringa oleifera* revealed multiple bioactive marker compounds associated with glucose homeostasis, immunomodulation, and oxidative-stress mitigation. In *Tinospora cordifolia*, the dominant metabolites included malic acid (5.3%), a TCA-cycle intermediate and inhibitor of carbohydrate-hydrolyzing enzymes, along with oleic acid (0.88%) and linoleic acid (0.55%), both known for recent evidence of improved insulin sensitivity and vascular protection (Arslan, 2021; Yuan *et al.,* 2022; Ramadan *et al.,* 2024). Quinic acid (2.27%), detected in *Neolamarckia cadamba*, has been recently validated for enhancing mitochondrial function and insulin secretion, while protocatechuic acid (0.05%) and caffeic acid (0.28%) exhibit modern support for antihyperglycemic and chemopreventive potential (Aijaz *et al.,* 2022; Benali *et al.,* 2024; Cadena-Iñiguez *et al.,* 2024). *Alternanthera brasiliana* showed sterol biomarkers stigmasterol (0.65%) and β-sitosterol (0.3%), recently reported to suppress inflammatory signaling via NF-κB/MAPK pathways (Goswami *et al.,* 2023; Zhang *et al.,* 2023). In *Moringa oleifera*, α-linolenic acid (0.94%) and β-sitosterol (0.73%) were prominent, alongside limonene (0.01%), which has been newly evidenced for redox-balance restoration and broad antimicrobial activity (Yuan *et al.,* 2022; Zhang *et al.,* 2023; S Devi *et al.,* 2025). The GC–MS profile indicating a polyphenol- and phytosterol-rich matrix provides a mechanistic basis: dietary polyphenols mitigate diabetes-linked oxidative stress and improve carbohydrate metabolism, while phytosterols lower LDL-cholesterol by limiting intestinal absorption and modulating hepatic feedback, which are consistent with the observed antioxidant and lipid-lowering effects (De Smet *et al.,* 2012; Bahadoran *et al.,* 2013; Nakano *et al.,* 2019).

Across groups, the daily food intake remained comparable with no statistically significant differences between the normal control (NC) and diabetic control (DC) groups, or among the metformin-treated (MT) and polyherbal formulation–treated (PFT) diabetic groups (Fig. 2A). Control rats showed progressive weight gain, whereas diabetic rats showed a decline in body weight. Treatment with metformin and polyherbal formulation partially ameliorated this loss, but not significant when compared to diabetic control groups (Fig. 2B,C). Across days 1, 7, 14, 21 and 28, the rats from the diabetic control group exhibited significantly elevated fasting blood glucose relative to the normal control ($p < 0.05$). At day 7, neither the metformin-treated nor the polyherbal formulation–treated groups showed a

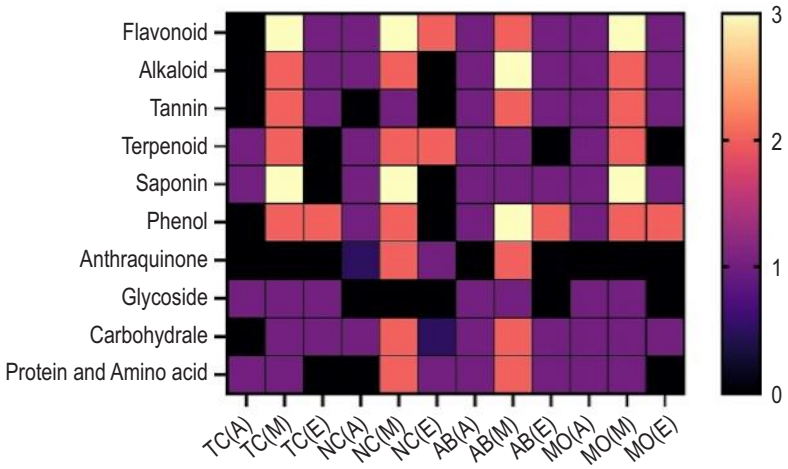

Fig. 1: Heatmap analysis of phytochemicals detected in aqueous, (A) methanolic (M) and ethanolic (E) extracts of *Tinospora cordifolia* (TC), *Neolamarckia cadamba* (NC), *Alternanthera brasiliana* (AB) and *Moringa oleifera* (MO) in varying colour intensities. [0 – no intensity; 1 – low intensity; 2 – moderate intensity and 3 – high intensity].

Table 1: Principal bioactive marker components detected in the methanolic extract of selected herbs through GC-MS

Compound Name	Chemical formula	Retention Time	Area Sum %
	Tinospora cordifolia		
Itaconic acid	$C_5H_6O_4$	21.59	0.1
Malic acid	$C_4H_6O_5$	25.36	5.3
Citric acid	$C_6H_8O_7$	30.89	0.14
Linoleic acid	$C_{18}H_{32}O_2$	33.79	0.55
9-Octadecenoic acid (Oleic acid)	$C_{18}H_{34}O_2$	33.82	0.88
	Neolamarckia cadamba		
Malic acid	$C_4H_6O_5$	25.26	0.05
2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	25.82	0.01
Protocatechuic acid	$C_7H_6O_4$	31.34	0.05
Quinic acid	$C_7H_{12}O_6$	31.68	2.27
Caffeic acid	$C_9H_8O_4$	33.41	0.28
α-Linolenic acid	$C_{18}H_{30}O_2$	33.83	0.19
	Alternanthera brasiliana		
Malic acid	$C_4H_6O_5$	25.26	0.05
2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	25.82	0.01
Protocatechuic acid	$C_7H_6O_4$	31.34	0.05
Quinic acid	$C_7H_{12}O_6$	31.68	2.27
Caffeic acid	$C_9H_8O_4$	33.41	0.28
α-Linolenic acid	$C_{18}H_{30}O_2$	33.83	0.19
	Moringa oleifera		
Limonene	$C_{10}H_{16}$	12.86	0.01
Octadecanoic acid	$C_{18}H_{36}O_2$	35.36	0.54
α-Linolenic acid	$C_{21}H_{38}O_2Si$	33.8	0.94
β-Sitosterol	$C_{29}H_{50}O$	35.96	0.73
Glycerol monostearate	$C_{27}H_{58}O_4Si_2$	37.53	0.32

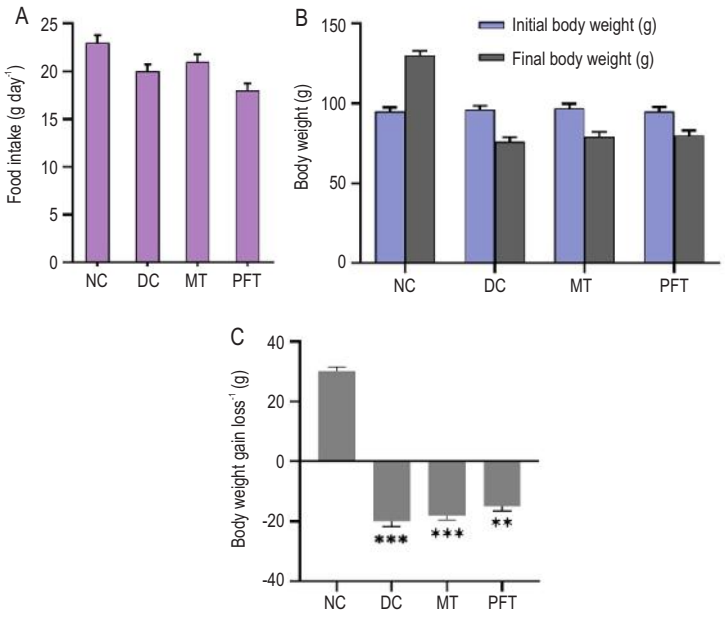

Fig. 2: Effect of polyherbal formulation on food intake and body weight gain. Bar graph showing (A) average daily food intake from first day to the end of 4 weeks; (B) showing initial and final body weight of rats from all groups and (C) showing body weight gain or loss from all groups during the experimental period. Data are of six replicates ± SEM; ∗∗∗p < 0.001 vs. control.[NC – Normal control; DC – Diabetic control; MT – Metformin treated; PFT – Polyherbal formulation treated].

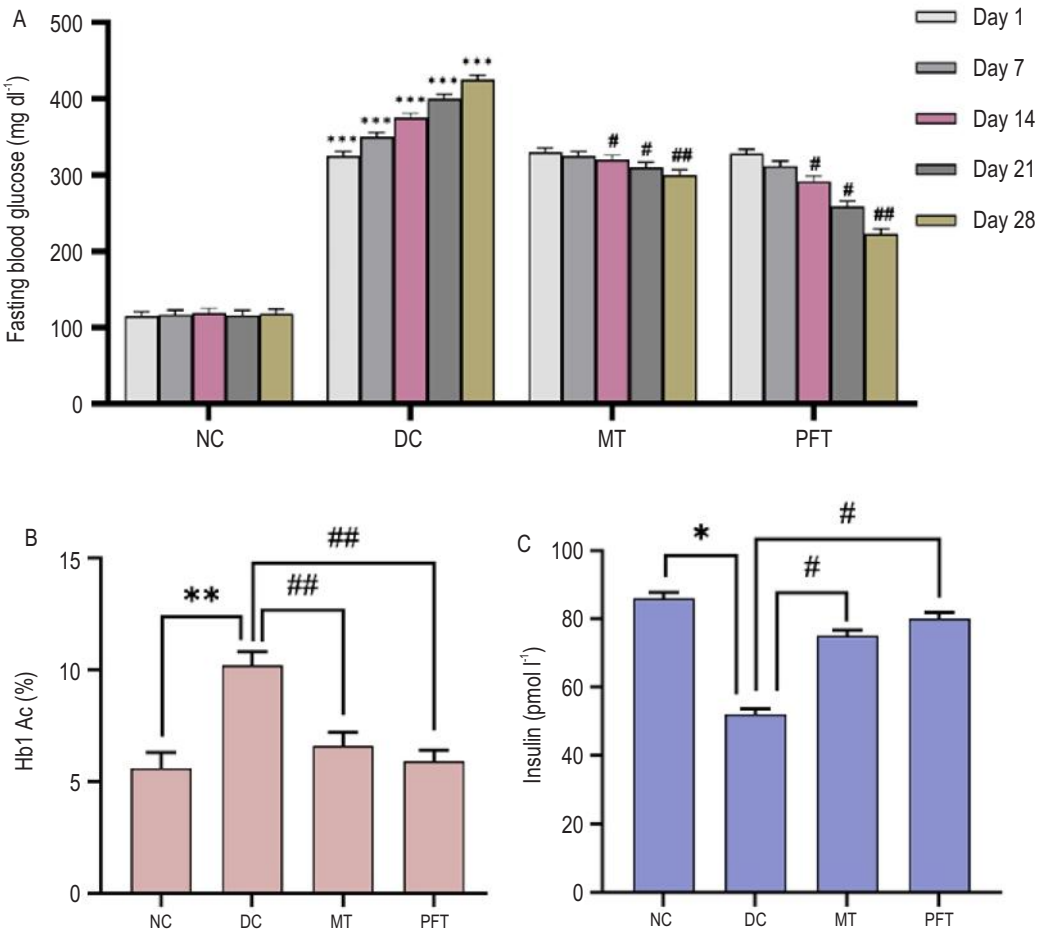

Fig. 3: Effect of polyherbal formulation on blood glucose, glycated hemoglobin, and serum insulin levels (A) Blood glucose levels on days 1, 7, 14, 21 and 28. (B) Glycated hemoglobin levels in blood after 4 weeks (C) Serum insulin levels of six replicates ± SEM; ∗∗∗p < 0.001, ∗∗p < 0.01, ∗p < 0.05 vs. control group; #p < 0.05, ##p < 0.01 vs. diabetic control group. [NC – Normal Control; DC – Diabetic Control; MT – Metformin treated; PFT – Polyherbal formulation treated].

significant attenuation of fasting blood glucose versus diabetic control (Fig. 3A). However, after 4 weeks, significant fasting blood glucose ($p < 0.05$, $p < 0.01$) decrease was observed in both the treatment groups. After 4 weeks, the increase in glycated hemoglobin observed in diabetic control group ($p < 0.01$) was significantly decreased in both metformin-treated and polyherbal formulation–treated groups ($p < 0.01$) (Fig. 3B). Similarly, decrease in serum insulin observed in diabetic control rats ($p < 0.05$) was significantly increased following polyherbal formulation–treated administration ($p < 0.05$ vs DC), with values comparable to the metformin group (Fig. 3C).

The dissociation between unchanged food intake and reduced body-weight gain in diabetic controls is characteristic of the nicotinamide–streptozotocin (NA–STZ) model, in which partial β-cell loss and insulinopenia promote proteolysis and lipolysis (Masiello *et al.,* 1998; Yan, 2022). Accordingly, interventions that improve glycemic control typically attenuate, but do not normalize, weight gain over 4–5 weeks, consistent with the partial protection observed with metformin and the polyherbal formulation. Over 28 days, significant reduction in fasting blood glucose and HbA1c, along with restoration of circulating insulin, align with reports showing that polyherbal formulations enhance glucose handling and β-cell function without excessive weight gain (Petchi *et al.,* 2014; Khan *et al.,* 2023).

Metformin similarly improves glycemia via suppression of hepatic gluconeogenesis and AMPK-mediated pathways, often with modest effects on body weight, supporting the comparable terminal glycemic outcomes between polyherbal formulation–treated and metformin (DeFronzo *et al.,* 2015; Di Magno *et al.,* 2022). These effects are consistent with the known antihyperglycemic actions of the formulation's constituents, including *Tinospora cordifolia, Moringa oleifera, Neolamarckia cadamba,* and *Alternanthera brasiliana*. Collectively, these findings suggest that polyherbal formulation–treated acts through

Table 2: Serum biochemical parameters and lipid profile in all the experimental groups

Parameters	Group I Normal Control	Group II Diabetic Control	Group III Metformin-treated (200 mg kg^{-1})	Group IV Polyherbal formulation-treated (150 mg kg^{-1})
SGOT (U/L)	82.4 ± 1.12	152.7 ± 1.15***	96.8 ± 1.10$^{\#\#}$	93.5 ± 1.13$^{\#\#}$
SGPT (U/L)	46.8 ± 0.66	98.6 ± 1.20***	60.7 ± 0.54$^{\#}$	52.3 ± 0.53$^{\#}$
Serum uric acid (mg dl^{-1})	1.62 ± 0.08	2.43 ± 0.12***	1.81 ± 0.09$^{\#}$	1.71 ± 0.10$^{\#}$
Serum creatinine (µmol l^{-1})	55.6 ± 1.2	82.9 ± 1.1***	65.2 ± 1.8$^{\#}$	61.7 ± 1.5$^{\#}$
Total triglycerides (mg dl^{-1})	92.5 ± 1.4	168.9 ± 1.7***	114.8 ± 1.2$^{\#}$	102.3 ± 1.6$^{\#\#}$
Total cholesterol (mg dl^{-1})	78.4 ± 1.6	132.7 ± 1.9***	93.6 ± 1.0$^{\#}$	89.6 ± 1.1$^{\#\#}$
Total lipids (mg dl^{-1})	440.8 ± 10.5	628.7 ± 14.9***	504.3 ± 10.2$^{\#\#}$	512.6 ± 10.8$^{\#\#}$

Values are presented as Mean of six replicates ± SEM. Superscripts indicate significant differences across groups. ***p < 0.001 vs. control group; #p < 0.05, ##p < 0.01 vs. diabetic control group.

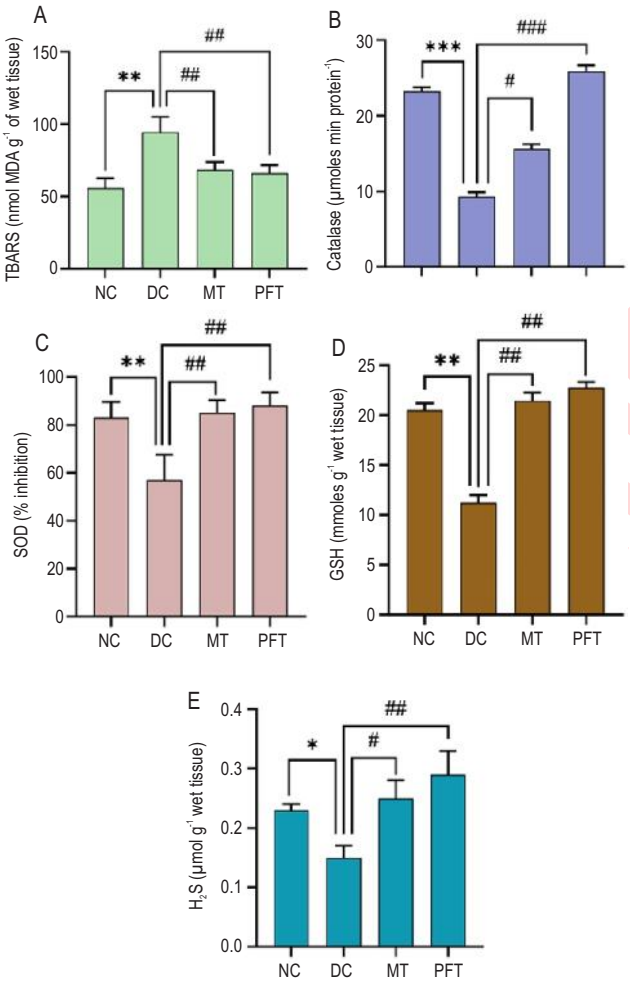

Fig. 4: Effect of polyherbal formulation administration on oxidative stress parameters in pancreas after 4 weeks. (A) TBARS (B) Catalase activity (C) SOD activity (D) GSH activity (E) H2S activity. Data are shown as Mean of six replicates ± SEM; *p < 0.05, **p<0.01,***p < 0.001 vs. control group; #p < 0.05, ##p < 0.01, ###p < 0.001 vs. diabetic control group. [NC – Normal Control; DC – Diabetic Control; MT – Metformin treated; PFT – Polyherbal formulation treated].

complementary mechanisms involving β-cell preservation and improved peripheral insulin sensitivity. Diabetic controls displayed dyslipidemia with elevated triglycerides and total cholesterol, which was significantly corrected by both polyherbal formulation–treated and metformin (e.g., a 39.4% reduction in triglycerides and a 32.5% reduction in total cholesterol) (Table 2). Dyslipidemia observed in STZ-diabetic controls is consistent with prior reports of hypertriglyceridemia and hypercholesterolemia driven by altered intestinal and hepatic lipid metabolism (Kusunoki *et al.,* 2000). The marked correction of triglycerides and total cholesterol by both the polyherbal formulation and metformin aligns with hypolipidemic effects documented for the individual botanicals in diabetic rodent models (Tan and Kim, 2013). Mechanistically, a phytochemical milieu enriched in polyphenols and phytosterols could contribute via antioxidant actions and interference with intestinal cholesterol absorption and hepatic cholesterol handling (Li *et al.,* 2022; Miszczuk *et al.,* 2024).

The marked fall in transaminases from diabetic-control levels (e. .g., SGOT 152.7 ± 1.15 U/L; 93.5 ± 1.13 and SGPT 98.6 ± 1.20 U/L to 52.3 ± 0.53) with polyherbal formulation administration is consistent with hepatoprotection in STZ models; both *Moringa oleifera* extracts and metformin have independently lowered ALT/AST and improved hepatic histology in diabetic rats (Omodanisi et al. 2017). The concurrent drop in creatinine (e.g from 82.9 ± 1.1 to 61.7 ± 1.5 µmol l^{-1}) supports nephroprotection; low-dose Moringa seed powder ameliorated diabetic nephropathy, improving renal function indices and renal histology (Al-Malki and El Rabey, 2015). The decrease in serum uric acid (~2.43→1.71 mg dl^{-1}) is biologically plausible given hyperuricemia's oxidative-stress/XO axis and the xanthine-oxidase-inhibiting and urate-transporter-modulating actions of *Moringa* phenolics (Luo *et al.,* 2022). Pancreatic TBARS levels were significantly ($p < 0.001$) increased in diabetic group when compared with the control group. However, a significant ($p < 0.05$) decrease in pancreatic TBARS levels was observed after administration of polyherbal formulation and metformin (MT and PFT groups) compared to diabetic control group (Fig. 4A). In diabetic rats, significantly ($p<0.001$) diminished levels of

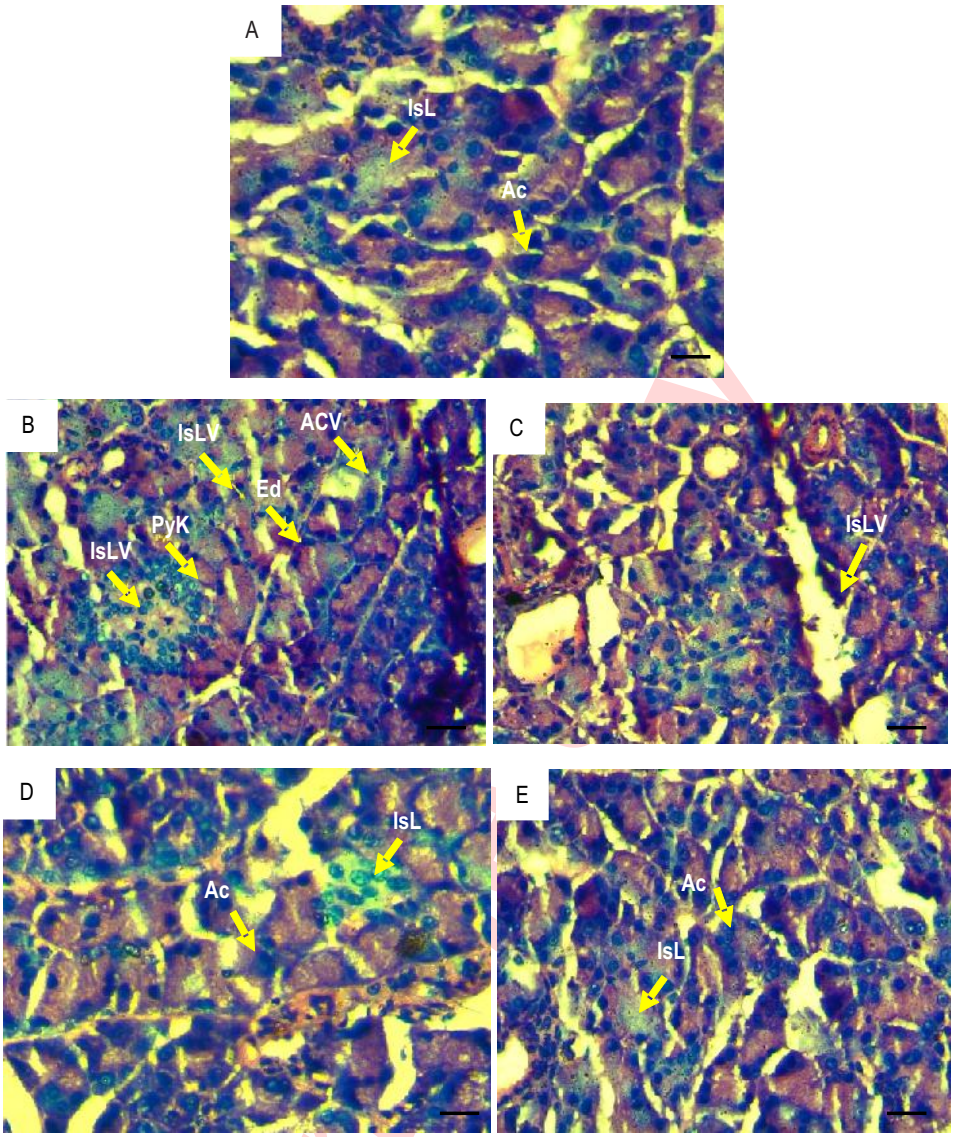

Fig. 5: Photomicrographs of rat pancreas, H&E, 400X. Scale bar - 50µm. (A) Normal control pancreas; (B,C) Diabetic control pancreas; (D) Metformin-treated pancreas; (E) Polyherbal-formulation-treated pancreas [IsL – Islets of Langerhans, Ac – Acinar cells, IsLV - Islets of Langerhans vacuolation or degenration, PyK – pyknotic nuclei, Ed – Interstitial edema, AcV – acinar cell vacuolation]

pancreatic CAT, SOD, GSH and H_2S was observed when compared with the normal rats. The oral administration of polyherbal formulation–treated and metformin significantly ($p<0.01$) increased the enzymic antioxidant activities and their levels in the pancreas of diabetic rats compared with the diabetic control rats (Fig. 4B-E). Polyherbal formulation–treated significantly reduced pancreatic TBARS and restored the antioxidant defense mechanism (SOD, CAT, GSH), indicating correction of the redox imbalance characteristic of diabetes (Giacco and Brownlee, 2010; Tiwari *et al.,* 2013). Restoration of pancreatic H_2S further supports redox rebalancing; tissue and circulating H_2S bioavailability are frequently reduced in diabetes, and normalization is associated with improved vascular and metabolic outcomes (Feng *et al.,* 2022).

Echocardiography showed that diabetic rats developed marked systolic dysfunction and ventricular remodeling, evidenced by increased LVIDs, LVESV and LVEDV, along with reduced SV, EF, FS, CO and LV mass compared with normal controls ($p < 0.001$). Treatment with metformin (200 mg kg^{-1}) and the polyherbal formulation (PFT, 150 mg kg^{-1}) significantly improved the cardiac function versus diabetic controls, increasing HR, SV, EF, CO, LVESV and LV mass ($p < 0.05$) and reducing LVIDs ($p < 0.01$). Polyherbal formulation–treated produced

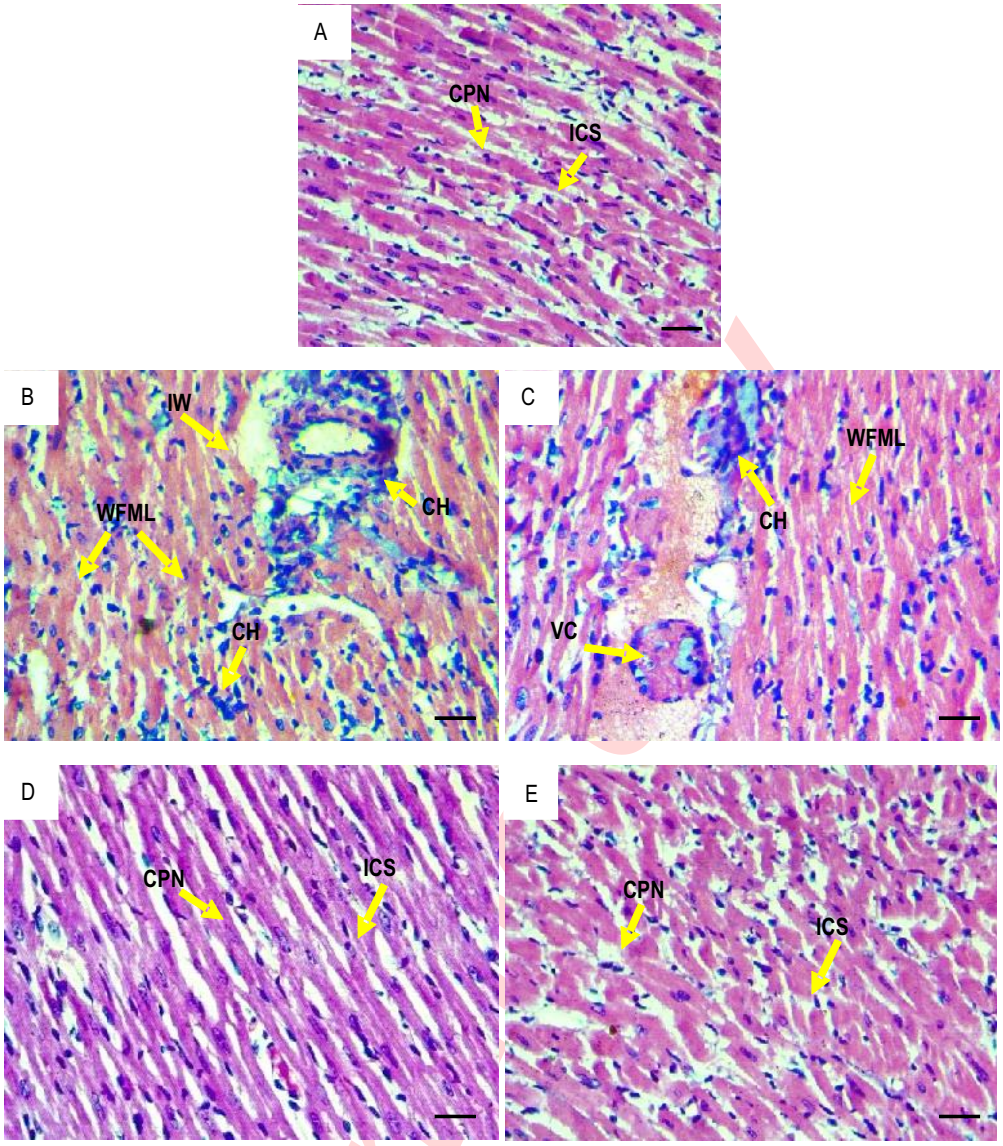

Fig. 6: Photomicrographs of rat heart, H&E, 400X. Scale bar - 50µm (A) Normal control heart; (B,C) Diabetic control heart; (D) Metformin-treated heart; (E) Polyherbal-formulation-treated heart[ICS – intact cross striations, CPN – centally placed nuclei, WFML – wavy fibers with myofibrillar loss, IW - interstitial widening, CH - cytoplasmic hypereosinophilia, VC - vascular congestion].

marginally improvement in the SV, EF and CO than metformin. Although LVIDd and FS showed an upward trend, these changes were not statistically significant. Overall, polyherbal formulation–treated shifted echocardiographic indices toward control values, indicating partial reversal of diabetic cardiomyopathy. These findings are consistent with the previous reports linking improved glycemic control and reduced oxidative stress to restored systolic function and ventricular geometry in diabetic hearts (Cosyns *et al.,* 2008; Bugger and Abel, 2014), suggesting that the systemic metabolic benefits observed contributed to myocardial protection over the 4-week treatment period. Histopathological changes were observed in the pancreas and heart after H & E staining in all groups of animals (Fig. 5,6). The normal control pancreas showed preserved architecture with well-defined islets of Langerhans composed of evenly granular endocrine cells and closely packed exocrine acini, with scant interstitium and no evidence of vacuolation or nuclear pyknosis (Fig. 5A). In the STZ-induced diabetic control group, the islets were shrunken and irregular, exhibiting loss of granularity, cytoplasmic vacuolation, and numerous pyknotic, hyperchromatic nuclei. Surrounding acini showed patchy vacuolation, interstitial edema, and mild peri-islet inflammatory infiltration (Fig. 5B–C). Treatment groups demonstrated histological recovery relative to diabetic controls. Metformin

Table 3: Echocardiographic indices measured in all experiment groups after 4 weeks

Parameters	Units	Group I Normal Control	Group II Diabetic Control	Group III Metformin-treated (200 mg kg^{-1})	Group IV Polyherbal formulation-treated (150 mg kg^{-1})
Heart Rate (HR)	BPM	345.3 ± 2.31	273.5 ± 2.15***	319.72 ± 2.18###	331.48 ± 3.27###
Left Ventricular Internal Diameter in systole (heart contraction) (LVIDs)	mm	3.6 ± 0.15	5.4 ±0.17***	4.53 ± 0.11##	4.31 ± 0.16##
Left Ventricular Internal Diameter in diastole (heart relaxation) (LVIDd)	mm	6.6 ± 0.33	7.9 ± 0.25*	7.18 ± 0.18	7.02 ± 0.21
Left Ventricular End-Systolic Volume (LVESV)	µl	65.19 ± 3.85	129.6 ± 2.95***	89.27 ± 3.27###	81.37 ± 3.57###
Left Ventricular End-Diastolic Volume (LVEDV)	µl	192.01 ± 4.19	228.2 ± 3.87***	209.34 ± 3.59#	203.56 ± 4.42##
Stroke Volume (SV)	µl	126.81 ± 2.73	98.6 ± 3.28***	120.07 ± 2.46##	122.19 ± 2.81###
Ejection Fraction (EF)	%	66.04 ± 3.35	43.2 ± 2.33**	57.36 ± 3.18#	60.03 ± 3.46##
Fractional Shortening (FS)	%	45.63 ± 2.17	31.64 ± 2.34**	36.91 ± 1.93	38.60 ± 2.54
Cardiac Output (CO)	ml min^{-1}	43.79 ± 1.32	26.96 ± 0.98***	38.39 ± 1.53###	40.50 ± 1.74###
Mass of the left ventricle (LV mass)	mg	734.69 ± 6.34	624.5 ± 5.42***	684.36 ± 5.25###	699.82 ± 6.12###

Values are presented as Mean of six replicates ± SEM. Superscripts indicate significant differences across groups.∗p<0.05, ∗∗p<0.01,∗∗∗p < 0.001 vs. control group; #p < 0.05, ##p < 0.01, ###p<0.001 vs. diabetic control group.

partially restored islet size and cellularity with reduced nuclear pyknosis and acinar vacuolation, although mild edema and focal degeneration persisted (Fig. 5D). The polyherbal formulation produced the most marked improvement, with well-circumscribed, densely cellular islets, restored granularity, and near-normal acinar architecture with minimal residual vacuolation (Fig. 5E). The normal control myocardium displayed well-aligned cardiomyocyte bundles with intact cross-striations, centrally placed nuclei, and minimal endomysial space, without edema, inflammation, or necrosis (Fig. 6A).

In STZ-induced diabetic controls, myocardial degeneration was evident, characterized by wavy fibers, myofibrillar loss, hypereosinophilia, interstitial edema, vascular congestion, mononuclear inflammatory infiltrates, and focal myocyte necrosis with disrupted fiber alignment (Fig. 6B–C). Metformin treatment resulted in partial architectural recovery, with improved fiber alignment and reduced edema and inflammation, though occasional vacuolated myocytes persisted (Fig. 6D). The polyherbal formulation restored myocardial architecture close to normal, showing compact, parallel fibers, minimal endomysial spaces, and only negligible residual vacuolation (Fig. 6E).

Histopathological studies sections showed better islet cytoarchitecture with fewer pyknotic nuclei, ameliorated, and attenuated myocardial vacuolation in treated groups, mirroring reports that *Moringa oleifera* and *Tinospora cordifolia* preserve β-cell structure and improve pancreatic and cardiac histology in diabetic models (Abd El Latif *et al.,* 2014; Sharma *et al.,* 2019).

The present study demonstrates that a four-plant polyherbal formulation provides concurrent pancreatic and cardiac histoprotection in stz-induced type II diabetic Wistar rats while improving glycemic, lipid, and systolic indices. The work introduces a GC–MS-validated polyphenol- and phytosterol-rich matrix as a plausible multi-target cardiometabolic protective intervention, offering a novel, low-cost, enzyme-linked antioxidant therapeutic lead for early diabetic injury. These findings highlight mechanistic novelty and translational relevance for herb-based drug-lead development in type II diabetes. However, further *in-vitro* studies and clinical validation are required to validate the potential of this formulation.

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