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Antidiabetic efficacy and antioxidant potential of *Tinospora cordifolia* and *Moringa oleifera* against streptozotocin-induced type 2 diabetes in Wistar rats

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ABSTRACT

Introduction: *Tinospora cordifolia* (TC) and *Moringa oleifera* (MO) are widely used in traditional medicine for managing hyperglycaemia and oxidative stress due to their rich phytochemical composition. This study evaluated their antidiabetic and antioxidant potential, individually and in combination, in streptozotocin (STZ)-induced type 2 diabetic Wistar rats.
Methods: Authenticated plant materials were extracted and subjected to phytochemical screening and gas chromatography–mass spectrometry (GC-MS) profiling for bioactive constituents. Six groups of experimental animals were created: diabetic control, normal control, diabetic + TC, diabetic + metformin (200 mg/kg b.w.), diabetic + MO, and diabetic + TC + MO (each at 150 mg/kg b.w.). Serum biochemical parameters, oxidative stress enzyme markers, and histopathological alterations in the pancreas and kidney were assessed for each group following standard methods.
Results: Diabetic rats showed marked hyperglycaemia, dyslipidaemia, impaired insulin levels, organ dysfunction, and elevated oxidative stress compared to the normal rats. The combined formulation demonstrated superior efficacy, reducing fasting blood glucose by 64.3% and HbA1c by 39.1%, while increasing serum insulin by 253% comparing diabetic controls ($p < 0.05$). Oxidative stress was significantly reduced, evidenced by decreased malondialdehyde (MDA) (38.3%) and enhanced catalase, reduced glutathione (GSH), and superoxide dismutase (SOD) levels. Histological findings revealed improved pancreatic islet integrity, β-cell preservation, and reduced renal damage.
Conclusion: The combined formulation produced greater improvements in biochemical, oxidative, and histological parameters despite each herb being administered at half the dose used in individual treatments, indicating a possible synergistic interaction and supporting its potential as an herb-based therapeutic approach for managing diabetes and associated oxidative damage.

Implication for health policy/practice/research/medical education:
The study highlights the therapeutic relevance of *Tinospora cordifolia* (TC) and *Moringa oleifera* (MO) in managing type 2 diabetes mellitus (T2DM) through their antihyperglycaemic and antioxidant effects. These findings provide a scientific basis for future phytochemical standardization, mechanistic studies, and controlled clinical trials to validate efficacy and safety in humans.

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Introduction

Persistent hyperglycemia is an indicator of type 2 diabetes mellitus (T2DM), a chronic metabolic disease characterized by decreased insulin action, increased insulin resistance, and progressive pancreatic β-cell dysfunction. It currently affects over 537 million adults worldwide, with India bearing a disproportionate share of the burden and often described as a major global hotspot for diabetes prevalence (1). The combination of rapid urbanisation, nutrition transitions, sedentary lifestyle, and

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genetic predisposition has escalated T2DM prevalence in both urban and rural settings, even among younger populations (2,3).

At the cellular level, chronic hyperglycaemia promotes overproduction of ROS (reactive oxygen species) through glucose autoxidation and mitochondrial electron transport chain disruption, while endogenous antioxidant defence systems, viz., catalase (CAT), superoxide dismutase (SOD), and reduced glutathione (GSH), become impaired (4). This oxidative imbalance contributes to insulin resistance, β-cell apoptosis, and the onset of diabetic complications, including diabetic neuropathy, retinopathy, nephropathy, cardiovascular disease, and pancreatic histopathological damage (5). Despite the availability of oral hypoglycaemic agents such as metformin, sulfonylureas, and thiazolidinediones, their prolonged efficacy is often constrained by adverse reactions, diminished responsiveness, and economic burden (6). As a result, there is renewed interest in medicinal plants that may offer multifaceted biological activity, fewer side effects, and lower cost.

Tinospora cordifolia (TC, Guduchi/Amṛtā) is described in Ayurveda as a Rasāyana drug used to promote vitality, resilience, and longevity, and classically indicated for febrile, metabolic, and inflammatory conditions; contemporary reviews of Ayurvedic sources and pharmacology consistently note its adaptogenic, immunomodulatory, and antipyretic use across traditional practice (7,8). *Moringa oleifera* (MO, Śigru/drumstick) is likewise recorded in Indian traditional medicine for a broad range of infirmities; Ayurvedic and ethnomedicinal accounts emphasise its leaves, pods, and seeds for anti-inflammatory, wound-healing, and nutritive applications, with historical documentation of use in classical Indian literature and continued dietary or therapeutic use in community practice (9,10). In traditional systems, combining plant extracts (polyherbal formulations) is a long-established strategy aimed at achieving synergistic or complementary therapeutic effects.

Epidemiological and clinical investigations have demonstrated that diets abundant in flavonoids are linked to a reduced risk of developing T2DM, consistent with the biochemical pathways by which phytochemicals enhance insulin responsiveness and improve antioxidant defences (11,12). The growing focus on plant-based therapeutics stems from their multifaceted actions, including stimulation of insulin secretion, enhancement of peripheral insulin responsiveness, inhibition of carbohydrate-digesting enzymes, suppression of hepatic gluconeogenesis, and modulation of inflammatory and oxidative processes (13). Furthermore, polyherbal formulations may potentiate therapeutic efficacy through synergistic interactions among diverse phytoconstituents, as evidenced by emerging preclinical and clinical findings demonstrating improved glycaemic control and lipid metabolism (14,15).

Given the distinctly different phytochemical profiles of TC (alkaloids, diterpenoid lactones) and MO (flavonoids, phenolics), a combination may offer broader antidiabetic and antioxidant potential than either plant alone. Although both plants have been individually studied and occasionally evaluated together *in vitro*, existing studies remain limited in several ways: (i) many have not examined the combination in a nicotinamide–streptozotocin (NA–STZ) model that closely mimics human T2DM *in vivo* model; (ii) few studies have simultaneously integrated biochemical, oxidative stress, lipid, and histopathological endpoints; and (iii) little attention has been given to whether combined extracts can achieve enhanced efficacy despite each being administered at a lower dose. Furthermore, prior work rarely correlates Gas chromatography–mass spectrometry (GC-MS)-identified phytoconstituents with antidiabetic mechanisms in combined formulations. These gaps underscore the need for a more comprehensive evaluation of this polyherbal approach. The model of STZ-induced T2DM in Wistar rats provides a reliable experimental platform, closely mimicking human T2DM pathophysiology, including partial β-cell destruction, insulin resistance, and oxidative damage (16).

Under this contemplated background, current research has been undertaken to study the antidiabetic as well as oxidative stress-modulating effects of the stem of TC and leaves of MO, administered individually and in combination, in STZ-induced T2DM Wistar rats through assessment of glycaemic and lipid parameters, serum biochemical content, antioxidant enzyme profiles, and histopathological changes in pancreas and kidney. By integrating biochemical and morphological outcomes, the purpose of this study is to provide scientific support for the conventional application of certain plants and support their development into safe and effective phyto-herbal formulations for diabetes management.

Materials and Methods

Collection of plant materials and preparation of extracts

Fresh stems of TC and leaves of MO were obtained from the campuses of IIT Guwahati and Cotton University, Kamrup district, Assam, between April and October 2024. The plant materials were taxonomically authenticated at the Department of Botany, Gauhati University, where voucher specimens were deposited under accession numbers GUBH020711 (*T. cordifolia*) and GUBH020712 (*M. oleifera*).

The stem of TC and leaves of MO were washed and dried for 7-10 days in a shaded environment (23-27 °C ± 2 °C). The shade-dried samples were powdered in an electric stainless-steel grinder. The shade-dried and powdered plant material (50 g) was extracted using a Soxhlet extraction apparatus with 250 mL of solvent (methanol, double-distilled water, ethanol) at its boiling temperature (~65 °C) for 6 hours (17). Rotary evaporator was used to concentrate the extracts at lower pressure, and they were then kept at 4 °C until they were needed again. Following formula was used to determine extraction yields (%):

Extraction yield (%) = [weight of dried extract/weight of plant material] × 100

Aqueous, methanolic, and ethanolic extraction yield values of TC stem extracts were 8.4%, 14.2%, and 19.1%, respectively, and for the leaves of MO were 12.6%, 20.3%, and 24.5%, respectively. Among the three solvents, the methanolic extract was selected for *in vivo* bioassays in Wistar rats based on preliminary phytochemical profiling, which indicated a higher yield of bioactive constituents, consistent with methanol's polarity range and extraction efficiency for phenolic and antioxidant compounds.

Chemicals

All of the analytical-grade chemicals and reagents used in the investigation were purchased from Merck Ltd. (Mumbai, India) and Sigma-Aldrich (St. Louis, MO, USA).

Qualitative phytochemical screening

Following conventional protocols, a preliminary phytochemical study of the chosen herbs' aqueous, methanolic, and ethanolic extracts was carried out to identify the main groups of bioactive chemicals (18,19).

GC-MS analysis of methanolic extracts

About 10 mg of each dried methanolic extract was dissolved in 1 mL of methanol, vortexed for 10 minutes, sonicated for 60 minutes, then centrifuged at 10,000 rpm for 10 minutes at 4 °C to perform GC–MS analysis. To enhance the identification of polar chemicals, the supernatant underwent derivatization after being vacuum-dried. In order to perform oxidation, 90 µL of o-methylhydroxylamine hydrochloride was added to pyridine, and it was then incubated for 90 minutes at 60 °C. Two hundred microliters of N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) containing one percent trimethylchlorosilane (TMCS) were then used for sialylation, which was carried out for 120 minutes at 60 degrees Celsius. Using a vacuum, the derivatized samples were reconstituted in 300 µL of n-hexane and thoroughly mixed. For compound identification, a 5977B mass selective detector (Agilent Technologies, Palo Alto, CA, USA) was connected to an Intuvo 9000 gas chromatograph by injecting a 1 µL aliquot.

Experimental animals

A total of 36 healthy adult male Wistar rats weighing between 85 and 105 g and aged between 10 and 12 weeks were procured from the Assam Agricultural University's College of Veterinary Science in Khanapara, India. The animals were housed in vented polypropylene cages with a 12-hour light/12-hour dark cycle, 22–25 °C, and 60–70% relative humidity for seven days to acclimate them. There were an unlimited supply of water and a standard rat pellet diet.

Induction of type 2 diabetes mellitus

Diabetes type 2 was brought on as previously mentioned (20). After an overnight fast of 8 hours, rats received nicotinamide (230 mg/kg, intraperitoneally), followed 15 minutes later by STZ (65 mg/kg, intraperitoneally) prepared in ice-cold 0.1 M citrate buffer (pH 4.5). Animals with fasting blood glucose levels more than 200 mg/dL were classified as diabetics. Measurements were made ten days after induction, after a 6-hour fast.

Experimental design

Rats (n = 6) were divided into six groups at random. As the standard control, group I was given a regular diet, unlimited water, and no test drugs. Diabetes was induced in groups II–IV using single intraperitoneal doses of nicotinamide (230 mg/kg) and STZ (65 mg/kg). Group II served as the diabetic control (negative control). Group III (diabetic+met) received metformin at 200 mg/kg body weight/day orally (positive control) using an oral gavage needle. Groups IV and V were treated orally with methanolic extracts of TC and MO, respectively, at a dose of 150 mg/kg body weight/day. Group VI (diabetic+TC+MO) received a combined formulation of TC and MO in equal ratio (1:1) at a total dose of 150 mg/kg/day (75 mg/kg each) per animal orally. Treatments were administered daily for four weeks using an oral gavage needle. For oral administration, dried methanolic extracts of the selected herbs were reconstituted using distilled water as a medium to ensure uniform solubility and dosing. Fresh suspensions were prepared daily for animal treatment.

Collection of serum and tissue samples

Sodium pentobarbital (50 mg/kg b.w.) was used to anesthetize the animals at the conclusion of the treatment period, and cervical dislocation was used to end their lives. Serum was obtained by centrifuging blood from the retro-orbital plexus for 10 minutes at 4 °C at 5000 rpm. The kidney and pancreas tissues were removed, cleaned in cold saline, and stored at -80 °C for histological and biochemical examinations.

Biochemical assays

Fasting blood glucose (mg/dL) was monitored weekly using a glucometer (One Touch Horizon, Singapore). After four weeks, glycated hemoglobin (HbA1c) (%) and serum creatinine (µmol/L) were estimated using commercial kits (Crystal Chem, USA). Serum insulin (mmol/L) was quantified using an ELISA kit (Mercodia, USA). Hepatic enzymes, viz., alanine aminotransferase (ALT) (U/L), and aspartate aminotransferase (AST) (U/L) were assayed with kits from HiMedia (India). Serum alkaline phosphatase

(ALP) (U/L) has been analysed using LabAssay™ (Wako) ALP Assay Kit. Bilirubin (mg/dL), Total protein (g/dL), and BUN (mg/dL) were determined using rat ELISA kits from MyBioSource, India. Serum uric acid (mg/dL) was determined using a Siemens kit (India), while triglycerides (mg/dL) and total cholesterol (mg/dL) were analysed using Merck kits (Germany). Total lipid content (mg/dL) was quantified by the sulfo-phospho-vanillin (SPV) method (Atlas Medical, India). All assays were performed according to the manufacturer's protocols. Blood urea was calculated utilizing the formula:

Urea (mg/dL) = BUN (mg/dL) × 2.14

Estimation of pancreatic oxidative stress

Indices of oxidative stress were measured in pancreatic tissue. By assessing thiobarbituric acid–reactive compounds (TBARS), with malondialdehyde (MDA) represented as nmol/g tissue in whole-tissue homogenates, lipid peroxidation was evaluated (21). After centrifuging pancreatic homogenates at 15,000 × g for 30 minutes at 4 °C, the supernatant was utilized for tests involving antioxidant enzymes. Measured colorimetrically by tracking the breakdown of H_2O_2 at 620 nm, CAT activity was expressed as µmol H_2O_2 consumed/min/mg protein (22). Using a commercial kit from Fluka Analytical in Switzerland, the activity of SOD was measured and expressed as a percentage of inhibition (23). Reduced GSH levels were determined according to a standard protocol and expressed as mmol/g wet tissue (24). Protein content was quantified using the Lowry method employing Folin–Ciocalteu reagent (25).

Histopathological examination

The tissues of the kidney and pancreas were preserved for 24 hours in Carnoy's fixative, then cleaned, graded ethanol-dehydrated, xylene-cleared, and paraffin-embedded. A Leica DM750 microscope equipped with a Leica ICC50 W digital imaging system (Leica Microsystems, Germany) was used to analyze sections that were 5 µm thick. They were then sliced using a rotary microtome, stained with hematoxylin and eosin (H&E), and mounted in DPX.

Statistical analysis

Mean ± SEM was used to express all data (n = 6). The Shapiro-Wilk test was used to determine whether the data distribution was normal, and Levene's test was used to determine whether the variances were homogeneous. One-way analysis of variance (ANOVA) and Bonferroni's post-hoc test were used to compare the groups statistically, utilizing software, namely, IBM SPSS Statistics version 25 and GraphPad Prism version 10.2.3 (GraphPad Software, San Diego, CA, USA). A value of $P < 0.05$ was considered statistically significant.

Results

Qualitative phytochemical screening

Preliminary phytochemical screening of TC and MO demonstrated the presence of multiple classes of secondary metabolites, including alkaloids, tannins, phenolics, flavonoids, glycosides, terpenoids, and saponins at varying levels, with methanolic extracts showing stronger reactions (greater color intensity), indicative of higher relative concentration (Table 1).

Table 1. Qualitative phytochemical analysis of *Tinospora cordifolia* (TC) and *Moringa oleifera* (MO) across various solvent extracts

Name of the compound	Phytochemical test	TC (Aqueous)	TC (Methanol)	TC (Ethanol)	MO (Aqueous)	MO (Methanol)	MO (Ethanol)
Flavonoid	Lead acetate test	-	+++	++	+	+++	++
	Alkaline reagent test	-	+++	++	+	+++	++
Alkaloid	Dragendroff's test	-	++	+	+	++	+
	Mayer's test	-	+	-	+	+	-
	Wagner's test	-	++	+	+	++	+
Tannin	Ferric chloride test	-	++	+	+	+++	++
Terpenoid	Salkowski's test	+	++	+	+	++	+
Saponin	Foam test	+	++	++	++	++	++
Phenol	Ferric chloride test	-	++	++	+	+++	++
Anthraquinone	Borntrager's test	-	-	-	-	-	-
	Modified Borntrager's test	-	-	-	-	-	-
Glycoside	Keller-Killiani's test	+	+	+	+	++	+
	Legal's test	+	+	+	+	++	+
Carbohydrate	Molisch's test	-	+	+	+	++	-
	Fehling's test	-	-	+	+	++	+
	Benedict's test	-	-	-	+	++	+
Protein and amino acid	Xanthoproteic test	+	+	-	+	++	-
	Ninhydrin test	+	+	+	+	++	+

–: absent, +: low intensity, ++: moderate intensity, and +++: high intensity
Intensity levels represent visual qualitative differences based on standard phytochemical screening tests.

Bioactive phytochemical constituents in methanolic extracts of selected herbs

Phytochemical profiling of the methanolic extracts of TC and MO by GC-MS identified several chemical constituents with different retention times, as shown in the chromatograms (Figure 1). Bioactive marker molecules included phenolic derivatives, fatty acids, sterols, organic acids, and secondary metabolites. Table 2 summarizes the main chemicals, retention periods, and biological activities.

For some compounds, the relative peak area was extremely low and close to the detection limit; in such cases, values below the limit of detection are indicated as <LOD. The reported Area sum % values represent the percentage of the total ion chromatogram contributed by each identified compound. In addition, several compounds appear as trimethylsilyl (TMS) derivatives, as derivatization with MSTFA was used to improve the detection of polar metabolites. Therefore, some empirical formulas in Table 2 (e.g., those containing silicon, Si) correspond to silylated derivatives generated during GC–MS analysis, rather than the native forms present in the plant matrix.

Modulation of fasting blood glucose (FBG), glycated haemoglobin, and serum insulin after TC and MO administration

Animals in the diabetes control group had significantly higher FBG than the normal control group on days 1, 7, 14, 21, and 28 ($P < 0.05$). At day 7, neither the metformin-treated nor the herb-treated groups showed a significant attenuation of FBG versus diabetic control (Figure 2A). A significant drop in FBG ($P < 0.05$, $P < 0.01$) was observed in all four treatment groups after the treatment period. Consistent with glycaemic trends, HbA1c levels were markedly elevated in diabetic control rats ($P < 0.01$) and were significantly reduced following treatment with metformin, TC, MO, or their combined formulation (Figure 2B). Serum insulin levels, which were significantly diminished in diabetic control animals ($P < 0.05$), showed a notable increase in all treated groups, reaching values comparable to those observed in the metformin-treated rats (Figure 2C). Among all interventions, the combined TC + MO formulation demonstrated the greatest efficacy across glycaemic and insulin parameters.

Improvement of serum biochemical parameters and lipid profile after TC and MO administration

The liver and kidney function and lipid profile of all experimental groups after 4 weeks of treatment are presented in Tables 3 to 5, respectively. Serum AST, ALP, ALT, and bilirubin measurements were considerably higher in diabetic rats (group II) compared to normal rats (group I) ($P < 0.01$), demonstrating hepatic impairment linked to diabetes. In contrast, diabetic rats treated with metformin (group III) and with administration of both herbs, either individually (groups IV and V) or in combination (group VI), showed a marked reduction

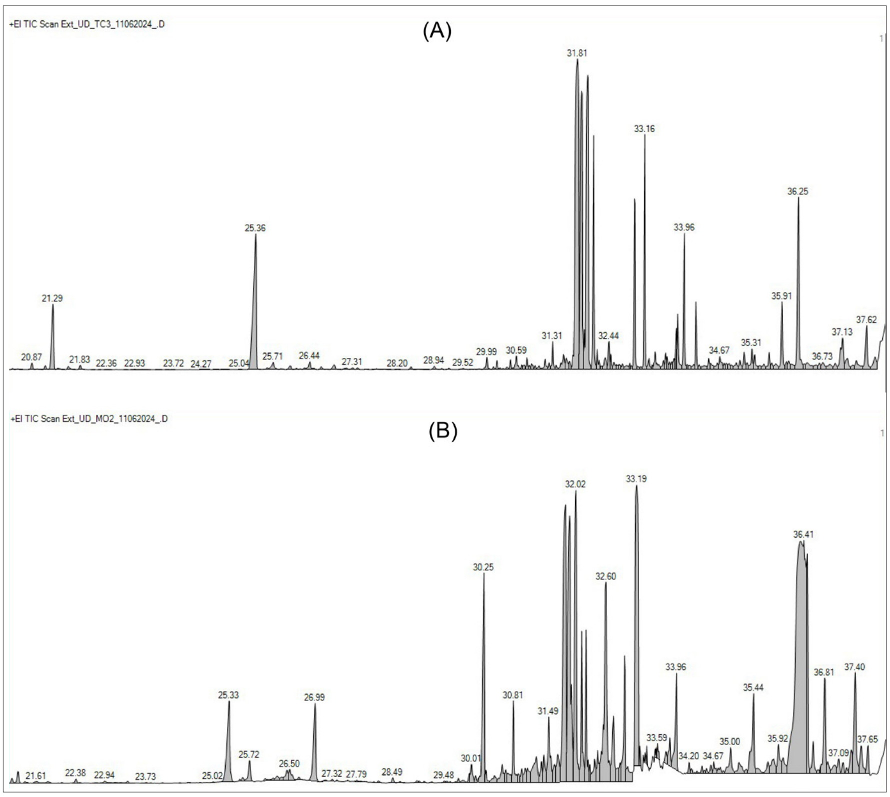


Figure 1. Gas chromatography - mass spectrometry (GC-MS) chromatogram of methanolic extracts of *Tinospora cordifolia* (A) and *Moringa oleifera* (B).

Table 2. Principal bioactive marker components detected in the methanolic extract of *Tinospora cordifolia* and *Moringa oleifera* through GC-MS

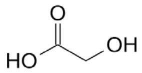
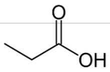
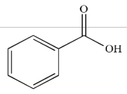
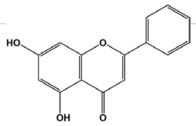
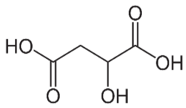
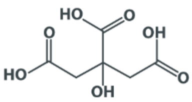
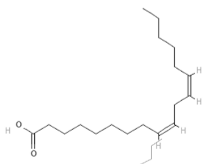
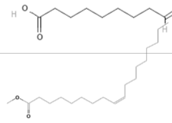
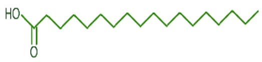
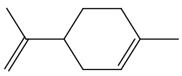
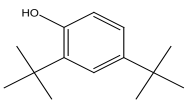
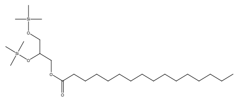
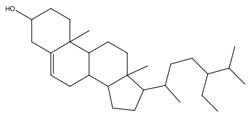
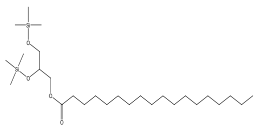
Compound name	Chemical structure	Retention time	Area sum %	Biological activity
		Tinospora cordifolia		
Glycolic acid ($C_2H_4O_3$)		14.1	0.01	Decreases intercellular cohesiveness, keratolytic, and exfoliating. Increases collagen, hyaluronic acid, and fibroblast proliferation. Antibacterial action is pH-dependent (26-29).
Propanoic acid ($C_3H_6O_2$)		14.41	0.01	Decreases food consumption, hepatic and plasma fatty acid levels, and moderate immunosuppression. Insulin sensitivity and MRSA efficacy improved (30,31).
Benzoic acid ($C_7H_6O_2$)		19.05	<LOD	Functions as an antimicrobial preservative with antifungal, bacteriostatic, and anti-inflammatory activities. Its derivatives exhibit antioxidant potential, with activity influenced by pH (32,33).
Itaconic acid ($C_5H_6O_4$)		21.59	0.1	Acts as an immunometabolite with multifaceted anti-inflammatory, antioxidant, antimicrobial, and antiviral effects. Mechanisms include activation of the Nrf2/ATF3 pathway, inhibition of TET2 and succinate dehydrogenase, induction of reactive oxygen species via the pentose phosphate pathway, and suppression of NLRP3 inflammasome activation (34-36).
Malic acid ($C_4H_6O_5$)		25.36	5.3	Serves as a key tricarboxylic acid (TCA) cycle intermediate, supporting cellular energy metabolism and maintaining acid–base homeostasis. Regulates digestive enzyme activity, including α-glucosidase, α-amylase, and lipase (37,38).
Citric acid ($C_6H_8O_7$)		30.89	0.14	Primary TCA-cycle intermediate involved in energy production and metabolic control. Stabilises hydroxyapatite crystals and acts as a metal-ion chelator and physiological pH buffer to mineralize bone (39,40).
Linoleic acid ($C_{18}H_{32}O_2$)		33.79	0.55	An essential polyunsaturated fatty acid serving as a precursor of eicosapentaenoic (EPA) and docosahexaenoic acids (DHA). Exhibits anti-inflammatory, cardioprotective, and neuroprotective properties, and supports immune modulation while reducing cardiovascular risk (41-43).
9-Octadecenoic acid (Oleic acid) ($C_{18}H_{34}O_2$)		33.82	0.88	Possesses hypolipidemic and anti-inflammatory effects by reducing LDL cholesterol and improving endothelial function. Protects against insulin resistance and oxidative stress, and exhibits antimicrobial activity (44-46).
Stearic acid ($C_{18}H_{36}O_2$)		33.96	1.92	A saturated fatty acid that maintains membrane integrity and serves as an energy source. Exhibits neutral effects on serum cholesterol levels and mild antibacterial and antifungal properties (47-49).
		Moringa oleifera		
Limonene ($C_{10}H_{16}$)		12.86	0.01	Demonstrates broad pharmacological properties including antimicrobial, antioxidant, anti-inflammatory, and anticancer activities (50-52).
2,4-Di-tert-butylphenol ($C_{14}H_{22}O$)		25.75	0.07	Has high antioxidant and broad-spectrum antibacterial properties against germs and fungi. It also reduces inflammation *in vitro* and *in vivo* (53-55).
Octadecanoic acid ($C_{18}H_{36}O_2$)		35.36	0.54	Acts as a structural lipid within biological membranes, serving as an energy source with mild antibacterial and antifungal activities. Exerts neutral effects on cholesterol metabolism (46-48).
α-Linolenic acid ($C_{21}H_{38}O_2Si$)		33.8	0.94	An essential omega-3 fatty acid that acts as a precursor of EPA and DHA, mediating anti-inflammatory, cardioprotective, and neuroprotective responses. Enhances immune modulation and reduces the risk of cardiovascular disorders (40-42).

Table 2. Continued

Compound name	Chemical structure	Retention time	Area sum %	Biological activity
1-Monopalmitin ($C_{25}H_{54}O_4Si_2$)		35.75	0.47	Functions as a mild surfactant and food emulsifier, inhibits intestinal P-glycoprotein activity, and serves as a biomarker for metabolic stress (56,57).
β-Sitosterol ($C_{29}H_{50}O$)		35.96	0.73	Exerts anti-inflammatory and antioxidant actions through inhibition of NF-κB and MAPK signaling pathways and modulation of cytokine expression (58,59).
Glycerol monostearate ($C_{27}H_{58}O_4Si_2$)		37.53	0.32	Improves formulation oil-binding and structural stability as an oleogelator. Increases viscosity and hardness in bigels, facilitates controlled bioactive release, and may potentiate reproductive toxicity when co-exposed with phthalates (60-62).

in these enzyme levels. The combined treatment (TC + MO) exhibited the most pronounced improvement, with AST, ALT, and ALP activities nearly returning to normal values (93.5 ± 1.13, 45.41 ± 0.36, and 95.45 ± 1.23 U/L, respectively; $P < 0.01$ vs. diabetic control). Similarly, serum bilirubin concentration, which increased significantly in diabetic rats (1.62 ± 0.12 mg/dL), was markedly reduced following treatment, with the lowest level observed in the combined treatment group (0.79 ± 0.06 mg/dL). Serum total protein levels, which were significantly decreased in diabetic control animals (4.3 ± 0.3 g/dL, $P < 0.05$), improved notably in all treated groups. The combined administration of TC and MO restored total protein levels close to the control group (7.2 ± 0.2 g/dL, $P < 0.01$ vs. diabetic control).

The diabetes control group had much higher BUN (28.72

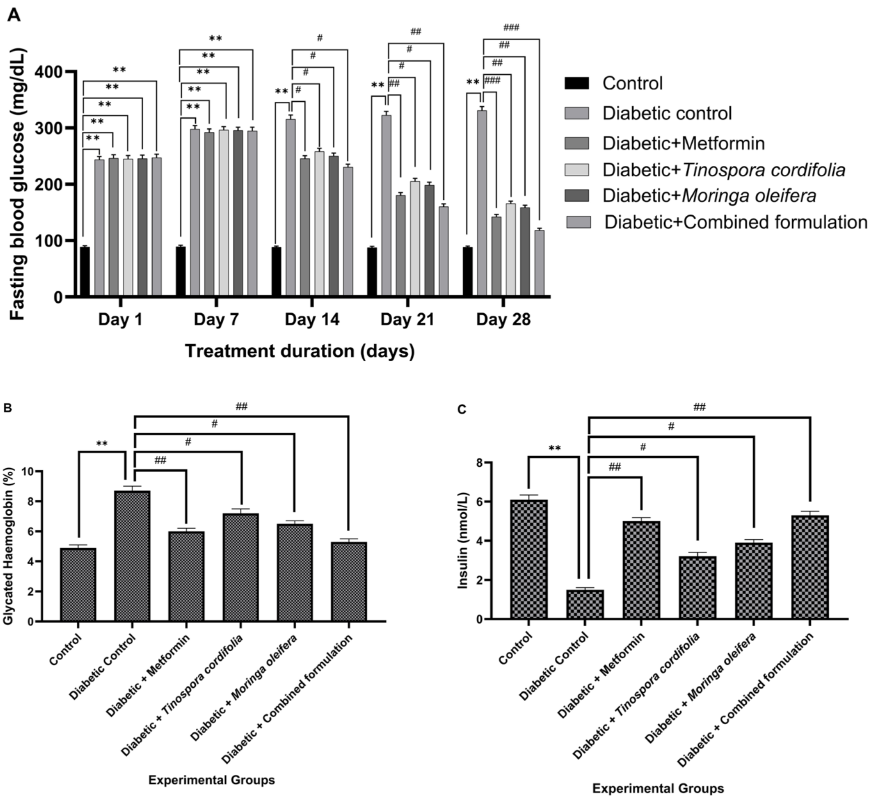


Figure 2. Effect of *Tinospora cordifolia* (TC) and *Moringa oleifera* (MO) on (A) fasting blood glucose, (B) glycated haemoglobin, and (C) serum insulin levels after 4 weeks of treatment. Data are shown as Mean ± SEM (n = 6). Statistical comparisons were made between control and diabetic control (single asterisk mark), treatment groups and diabetic control (hash mark), and individual herb groups and combination group (double asterisk mark), using one-way ANOVA followed by Bonferroni's post hoc test. *$P < 0.05$ vs. control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. diabetic control group; and **$P < 0.05$ vs. combined formulation group, indicating statistical significance.

Table 3. Effect of *Tinospora cordifolia* (TC) and *Moringa oleifera* (MO) on blood serum liver function profile in diabetic rats after 4 weeks of treatment

Parameters	Group I (Control)	Group II (Diabetic control)	Group III (Diabetic + met) (200 mg/kg)	Group IV (Diabetic+TC) (150 mg/kg)	Group V (Diabetic+MO) (150 mg/kg)	Group VI (Diabetic+TC+MO) (150 mg/kg)
Aspartate transaminase (U/L)	82.4 ± 1.12	152.7 ± 1.15**	96.8 ± 1.10$^{##}$	125 ± 1.23$^{#}$	112 ± 1.55$^{#}$	93.5 ± 1.13$^{##}$
Alanine transaminase (U/L)	35.8 ± 0.66	85.6 ± 1.20**	60.7 ± 0.54$^{#}$	55.34 ± 0.53$^{#}$	58.78 ± 0.47$^{#}$	45.41 ± 0.36$^{##}$
Alkaline phosphatase (U/L)	81.15 ± 1.47	153.89 ± 1.47**	112.32 ± 1.56$^{##}$	115.47 ± 1.10$^{#}$	110.08 ± 0.28$^{#}$	95.45 ± 1.23$^{##}$
Bilirubin (mg/dL)	0.67 ± 0.05	1.62 ± 0.12*	1.03 ± 0.08$^{#}$	1.11 ± 0.10$^{#}$	1.05 ± 0.09$^{#}$	0.79 ± 0.06$^{##}$
Total protein (g/dL)	7.8 ± 0.2	4.3 ± 0.3*	6.2 ± 0.2$^{#}$	6.9 ± 0.3$^{#}$	6.7 ± 0.2$^{#}$	7.2 ± 0.2$^{##}$

Values are presented as mean ± SEM (n = 6). Statistical comparisons were made between control and diabetic control (asterisk mark) as well as treatment groups and diabetic control (hash mark) using one-way ANOVA followed by Bonferroni's post hoc test. Superscripts $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control group and $^{#}P < 0.05$, $^{##}P < 0.01$ vs. diabetic control group, indicate statistical significance.

Table 4. Effect of *Tinospora cordifolia* (TC) and *Moringa oleifera* (MO) on blood serum kidney function profile in diabetic rats after 4 weeks of treatment

Parameters	Group I (Control)	Group II (Diabetic control)	Group III (Diabetic + met) (200 mg/kg)	Group IV (Diabetic+TC) (150 mg/kg)	Group V (Diabetic+MO) (150 mg/kg)	Group VI (Diabetic+TC+MO) (150 mg/kg)
Blood urea nitrogen (mg/dL)	18.68 ± 1.2	28.72 ± 1.03*	19.24 ± 1.1$^{#}$	20.14 ± 1.6$^{#}$	21.23 ± 1.4$^{#}$	19.86 ± 1.2$^{#}$
Serum creatinine (μmol/L)	55.6 ± 1.2	82.9 ± 1.1**	65.2 ± 1.8$^{#}$	66.7 ± 1.1$^{#}$	63.4 ± 1.02$^{#}$	61.7 ± 1.5$^{#}$
BUN/ Serum creatinine ratio	0.33	0.34	0.29	0.30	0.33	0.32
Urea (calculated) (mg/dL)	39.97 ± 1.2	61.46 ± 1.03**	41.17 ± 1.1$^{#}$	43.09 ± 1.6$^{#}$	45.4 ± 1.4$^{#}$	42.5 ± 1.2$^{#}$
Urea/serum creatinine ratio	0.72	0.74	0.63	0.64	0.71	0.68
Serum uric acid (mg/dL)	1.62 ± 0.08	2.43 ± 0.12*	1.81 ± 0.09$^{#}$	1.77 ± 0.05$^{#}$	1.75 ± 0.03$^{#}$	1.71 ± 0.10$^{#}$

Values are presented as mean ± SEM (n = 6). Statistical comparisons were made between control and diabetic control (asterisk mark) as well as treatment groups and diabetic control (hash mark) using one-way ANOVA followed by Bonferroni's post hoc test. Superscripts $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control group and $^{#}P < 0.05$, $^{##}P < 0.01$ vs. diabetic control group, indicate statistical significance.

Table 5. Effect of *Tinospora cordifolia* (TC) and *Moringa oleifera* (MO) on blood serum lipid profile in diabetic rats after 4 weeks of treatment

Parameters	Group I (Control)	Group II (Diabetic control)	Group III (Diabetic + met) (200 mg/kg)	Group IV (Diabetic+TC) (150 mg/kg)	Group V (Diabetic+MO) (150 mg/kg)	Group VI (Diabetic+TC+MO) (150 mg/kg)
Total cholesterol (mg/dL)	78.4 ± 1.6	132.7 ± 1.9**	93.6 ± 1.0$^{#}$	94.51 ± 1.3$^{#}$	91.14 ± 1.7$^{#}$	89.6 ± 1.1$^{##}$
Total triglycerides (mg/dL)	92.5 ± 1.4	138.9 ± 1.7*	114.8 ± 1.2$^{#}$	111.4 ± 1.5$^{#}$	107.2 ± 1.2$^{#}$	102.3 ± 1.6$^{#}$
Total lipids (mg/dL)	440.8 ± 10.5	628.7 ± 14.9**	504.3 ± 10.2$^{#}$	518.5 ± 13.4$^{#}$	509.7 ± 11.2$^{#}$	512.6 ± 10.8$^{##}$

Values are presented as mean ± SEM (n = 6). Statistical comparisons were made between control and diabetic control (asterisk mark) as well as treatment groups and diabetic control (hash mark) using one-way ANOVA followed by Bonferroni's post hoc test. Superscripts $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control group and $^{#}P < 0.05$, $^{##}P < 0.01$ vs. diabetic control group, indicate statistical significance.

± 1.03 mg/dL; $P < 0.05$) and serum creatinine (82.9 ± 1.1 μmol/L; $P < 0.01$) levels than the normal control (18.68 ± 1.2 mg/dL and 55.6 ± 1.2 μmol/L, respectively), indicating possible renal dysfunction associated with hyperglycemia. A corresponding increase was also noted in the calculated urea level (61.46 ± 1.03 mg/dL) and serum uric acid (2.43 ± 0.12 mg/dL; $P < 0.05$). Administration of metformin (200 mg/kg), TC (150 mg/kg), MO (150 mg/kg), and particularly their combined formulation (TC + MO; 150 mg/kg) significantly ameliorated these alterations, showing near-normal levels of BUN, urea, creatinine, and uric acid compared to diabetic control ($P < 0.05$). Among the "treated groups," the TC + MO combination (group VI) exhibited the most pronounced renoprotective effects with BUN and serum creatinine levels of 19.86 ± 1.2 mg/dL and 61.7 ± 1.5 μmol/L, respectively, values that closely resembled those of the normal control. The BUN/creatinine and urea/creatinine ratios remained within the physiological range across treated groups, suggesting restoration of renal filtration efficiency.

Diabetic control rats exhibited significant increases in total cholesterol, triglycerides, and total lipid content ($P < 0.01$), compared to the control group, confirming hyperlipidaemia associated with diabetes. Administration of metformin (200 mg/kg) markedly reduced these elevated lipid parameters ($P < 0.05$ vs. diabetic control).

Similarly, treatment with TC (150 mg/kg), MO (150 mg/kg), and the combined formulation of TC + MO (150 mg/kg each) produced a significant ($P < 0.05$) decrease in total cholesterol, triglycerides, and total lipids compared to the diabetic control group. Among the "treated groups," the combined TC + MO formulation exhibited the most pronounced improvement, bringing lipid parameters closer to normal control levels.

Attenuation of pancreatic oxidative stress after TC and MO administration

Diabetic control rats had significantly higher pancreatic MDA levels ($P < 0.05$) compared to the control group, indicating increased lipid peroxidation (Figure 3A). Treatment with TC, MO, and their combined formulation (TC+MO) markedly reduced MDA levels relative to the diabetic control ($P < 0.05$), suggesting improved oxidative status. When compared to the control group, diabetic rats showed significantly ($P < 0.01$) lower levels of pancreatic GSH activity, SOD activity, and pancreatic CAT activity. Oral administration of TC, MO, metformin, and especially the TC + MO combination significantly restored these antioxidant enzyme levels toward normal values (Figure 3B-3D).

Histopathological restoration of pancreatic architecture

Histopathological evaluation of pancreatic tissues after H&E staining revealed marked differences among experimental groups (Figure 4). The normal control group exhibited intact pancreatic architecture with well-defined islets of Langerhans (IsL) containing uniformly granular endocrine cells and compact exocrine acini (Ac) without vacuolation or nuclear pyknosis (Figure 4A). In contrast, diabetic control rats showed severe islet degeneration, including shrunken and irregular islets, cytoplasmic vacuolation, degranulation, and pyknotic nuclei, along with acinar vacuolation, interstitial edema, and mild peri-islet inflammation (Figure 4B–4C). Treatment with metformin led to partial recovery, characterized by larger, more cellular islets and reduced vacuolar and degenerative changes, though mild edema persisted (Figure 4D). Remarkably, TC and MO treatments, particularly their combined formulation, produced near-normal histoarchitecture with well-circumscribed islets, restored cellular density and granularity, and minimal vacuolar alteration (Figure 4E–4G).

Histopathological improvement of renal tissue

Significant morphological differences between

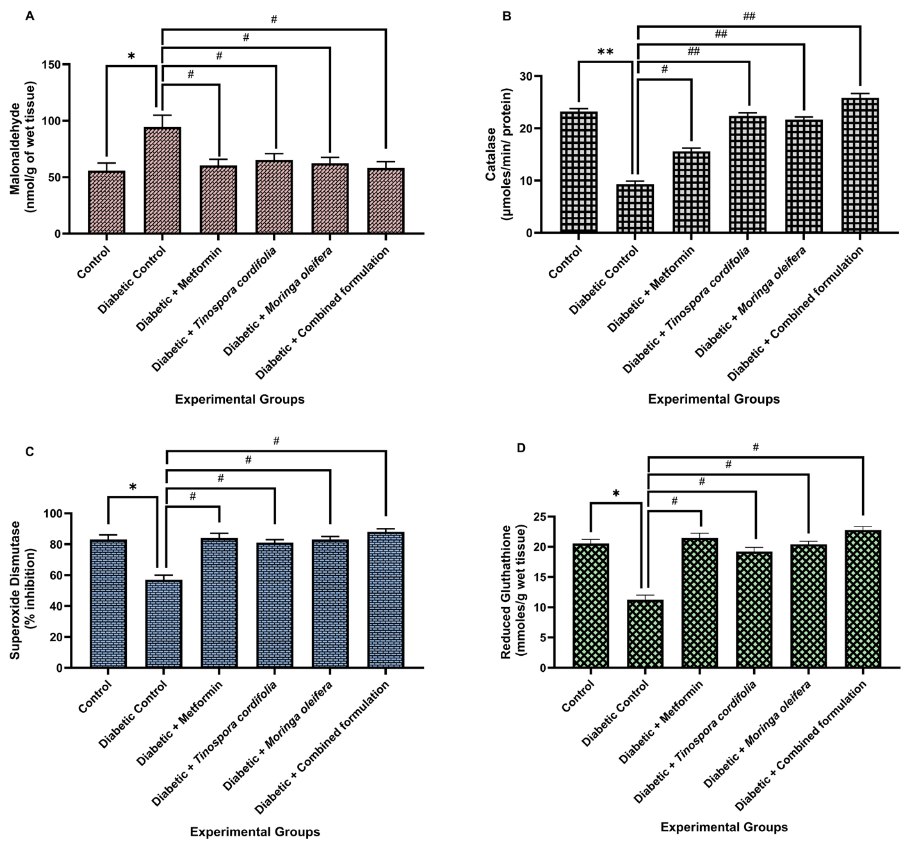


Figure 3. Effect of *Tinospora cordifolia* (TC) and *Moringa oleifera* (MO) on oxidative stress parameters in the pancreas after 4 weeks. (A) malondialdehyde (MDA), (B) Catalase (CAT) activity, (C) Superoxide Dismutase (SOD) activity, (D) Reduced Glutathione (GSH) activity. [Data are shown as Mean ± SEM (n = 6). Statistical comparisons were made between control and diabetic control (asterisk mark) as well as treatment groups and diabetic control (hash mark) using one-way ANOVA followed by Bonferroni's post hoc test. *$P < 0.05$, **$P < 0.01$ vs. control group and #$P < 0.05$, ##$P < 0.01$ vs. diabetic control group, indicating statistical significance].

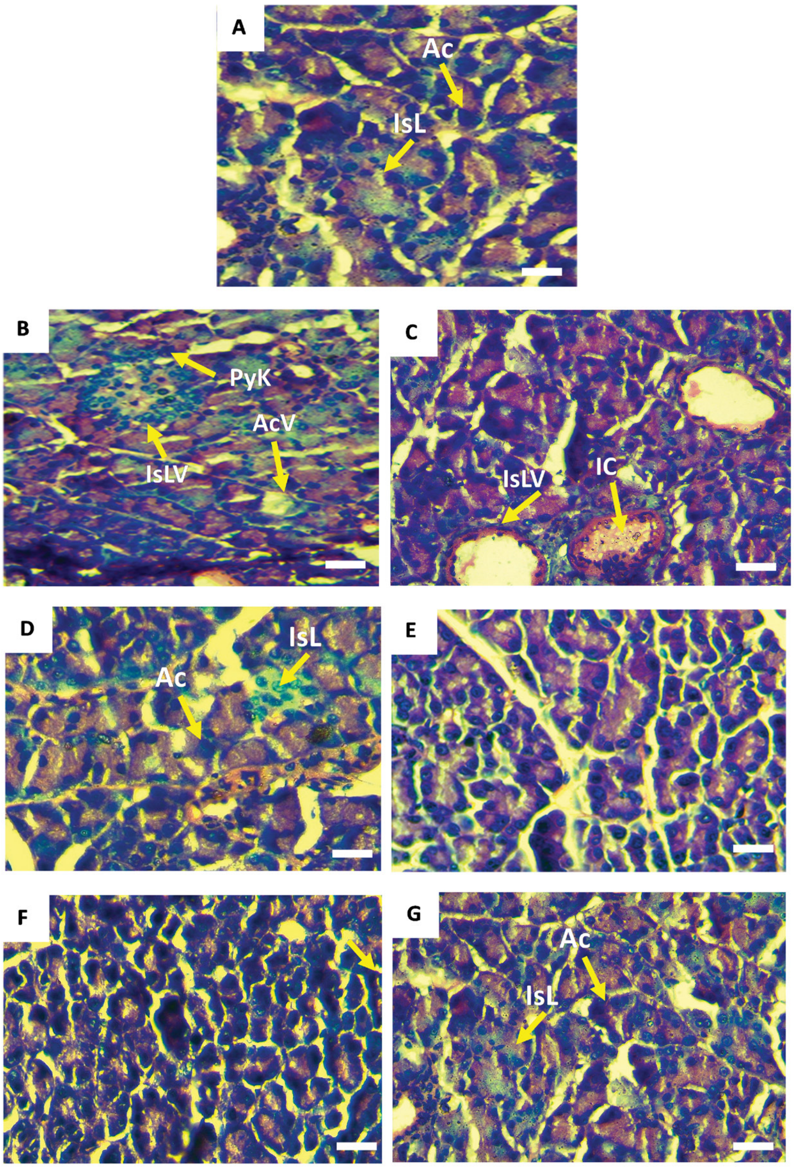


Figure 4. Histopathology photomicrographs of pancreatic tissue showing the effect of *Tinospora cordifolia* (TC), *Moringa oleifera* (MO), and their combination on islet architecture in STZ-induced diabetic rats (H&E, 40X, scale bar = 50 µm). (A) Normal control; (B, C) Diabetic control; (D) Diabetic+Met; (E) Diabetic+TC; (F) Diabetic+MO; (G) Diabetic+TC+MO. [IsL: Islets of Langerhans, Ac: Acinar cells, IsLV: Islets of Langerhans vacuolation or degeneration, PyK: pyknotic nuclei, AcV: acinar cell vacuolation].

experimental groups were found by histopathological analysis of kidney tissues stained with H&E (Figure 5). The control rats showed well-organized tubular epithelium with intact glomeruli, patent capillary loops, and clear Bowman's spaces. Tubules were structurally preserved with uniform epithelial lining and minimal interstitial space, lacking edema or inflammation (Figure 5A). In contrast, diabetic control rats exhibited pronounced tubulointerstitial damage characterized by enlarged glomeruli, mesangial proliferation, irregular Bowman's spaces, tubular degeneration, epithelial desquamation, proteinaceous casts, and inflammatory infiltration within the interstitium (Figure 5B–5C). Metformin-treated rats showed partial renal recovery with more regular glomeruli, restored Bowman's spaces, and reduced tubular degeneration and inflammation, although mild mesangial expansion persisted (Figure 5D). Treatment with TC and MO, either individually or in combination, demonstrated near-normal renal histology with well-defined glomeruli, minimal mesangial expansion, patent Bowman's spaces, and preserved tubular epithelium, indicating substantial protection against diabetic renal injury (Figure 5E–5G).

Discussion

Complex metabolic and cellular problems characterize type 2 diabetes, involving persistent hyperglycaemia, oxidative stress, impaired insulin signalling, β-cell degeneration, and progressive organ damage. Because a single pharmacologic agent often cannot address this multifactorial pathology, nutritional therapies with multi-target activities merit investigation. Accordingly, this study evaluated a di-herb formulation composed of TC stem and MO leaves (1:1) for its ability to attenuate hyperglycemia, NA–STZ type 2 diabetes model oxidative stress, and tissue damage.

Over 28 days, the formulation produced a significant decline in fasting blood glucose and HbA1c as well as partially restored circulating insulin toward control values, findings that align with prior reports of TC and MO improving glycaemic control and β-cell function in rodent models. Mechanistic studies of TC indicate that it mitigates oxidative stress, promotes insulin secretion, inhibits gluconeogenic enzymes (e.g., fructose-1,6-bisphosphatase and glucose-6-phosphatase), restores hepatic glycogen, and stimulates glucose uptake in peripheral cells, actions that collectively reduce hyperglycaemia. These antidiabetic mechanisms have been demonstrated in "*in vivo* and *in vitro* studies" of TC as well as its constituents (e.g., palmatine, tinosporaside) (63-65). MO extracts are rich in polyphenols and other bioactives, which have been repeatedly shown to lower blood glucose, enhance insulin sensitivity, protect islet morphology, and reduce markers of oxidative stress across numerous preclinical studies and systematic reviews (66). Meta-analyses and recent reviews report consistent antihyperglycaemic and antioxidative effects of MO leaf and seed extracts in rodent models and, in several small clinical studies, beneficial effects on glycaemia in humans (67,68).

Several bioactive compounds identified through GC–MS analysis support the biological outcomes observed

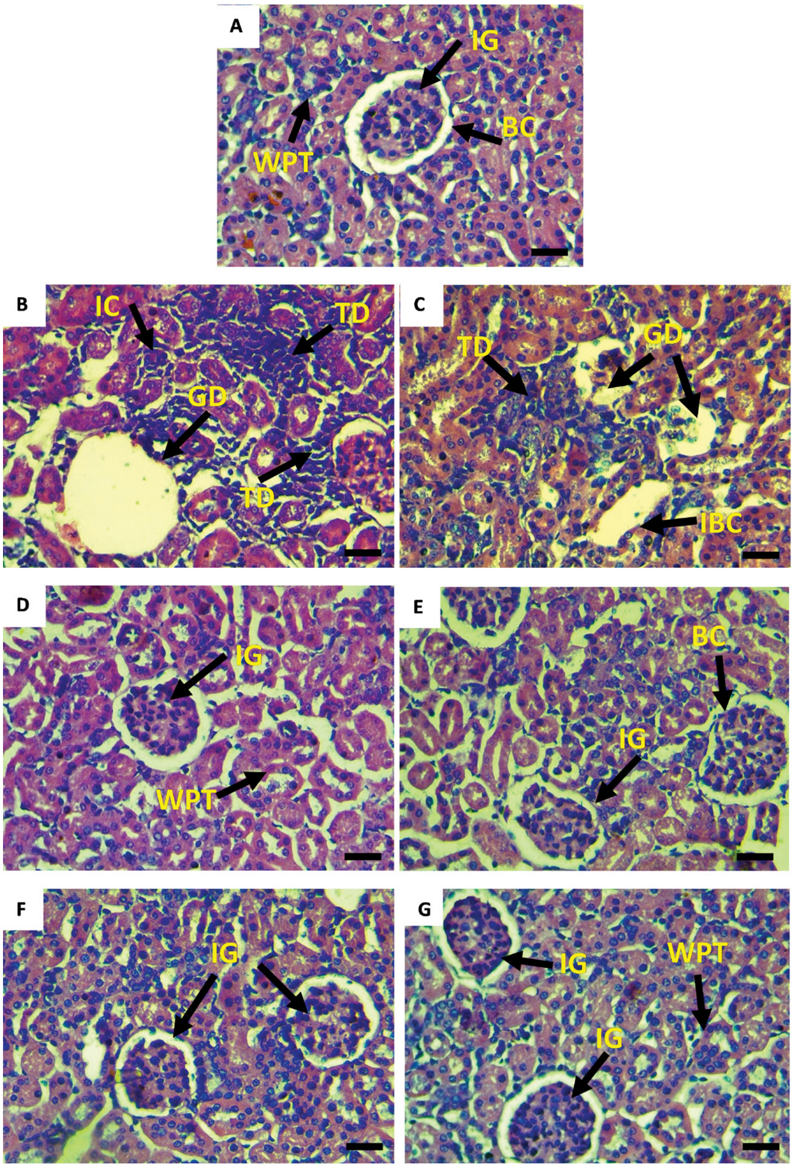


Figure 5. Histopathological photomicrographs of renal tissue showing the effect of *Tinospora cordifolia* (TC), *Moringa oleifera* (MO), and their combination on kidney morphology in streptozotocin-induced diabetic rats (H&E, 40X, scale bar = 50 μm). (A) Normal control; (B, C) Diabetic control; (D) Diabetic+Met; (E) Diabetic+TC; (F) Diabetic+MO; (G) Diabetic+TC+MO. [IG – intact glomerulus, BC – bowman's capsule, WPT – well preserved tubules, GD – glomerulus degeneration, TD – tubule disintegration, IBC – irregular bowman's capsule].

in the study viz., (i) β-Sitosterol (*Moringa oleifera*) improves insulin sensitivity and suppresses oxidative and inflammatory signaling via NF-κB and MAPK pathways, consistent with reductions in MDA and improvements in SOD, CAT, and GSH (69), (ii) Linoleic and α-linolenic acids, detected in both extracts, modulate hepatic lipid metabolism, activate PPAR pathways, and reduce oxidative lipid damage, explaining improvements in lipid profile and hepatic enzymes (40-42), (iii) Itaconic acid (*Tinospora cordifolia*) is a well-known immunometabolite that activates Nrf2, suppresses NLRP3 inflammasome activity, and reduces ROS generation, supporting the observed antioxidant restoration (33-35), (iv) 2,4-Di-tert-butylphenol (*Moringa oleifera*) is a potent antioxidant and ROS scavenger, offering plausible biochemical support for preserved pancreatic and renal tissue morphology (52-54). These compound-specific mechanisms collectively strengthen the biological rationale for the multi-target improvements achieved by the combined herbal formulation. Certain individual phytochemicals can exert pronounced biological effects even at relatively low concentrations when they are embedded within a complex phytochemical matrix, where additive and synergistic interactions among constituents may enhance overall bioactivity beyond that of isolated compounds. This concept is increasingly supported by work on natural product mixtures and functional foods, which demonstrates that whole-extract or multi-compound preparations often show greater efficacy than equivalent doses of single purified agents due to matrix-driven synergy (70,71).

An important finding of the current study is that the combined formulation produced superior therapeutic effects despite each component being administered at half the dose used in individual treatments. This suggests a true synergistic or additive interaction, consistent with classical polyherbal principles and supported by emerging pharmacological studies showing enhanced AMPK activation, PI3K-linked glucose uptake, and complementary antioxidant responses when multiple phytochemicals act on convergent metabolic pathways (14,69).

Diabetic controls developed marked dyslipidaemia (elevated triglycerides and total cholesterol), consistent with perturbations in intestinal absorption and hepatic lipid handling previously reported in STZ models. Treatment with TC, MO, and metformin significantly corrected these lipid abnormalities. The lipid-lowering effects of these botanicals have been attributed to combined antioxidant activity, interference with intestinal cholesterol uptake, modulation of hepatic cholesterol metabolism, and the presence of phytosterols and polyphenols that influence lipoprotein metabolism. These mechanisms are supported by both mechanistic and preclinical studies of TC and MO phytoconstituents (69). Hepatic transaminases were reduced following TC and MO treatment, consistent with hepatoprotective effects reported for both herbs in diabetic and chemically induced liver-injury models. Similarly, serum creatinine declined with treatment, aligning with previous demonstrations that MO can ameliorate diabetic nephropathy and improve renal histology and function indices in rodents. The observed reduction in serum uric acid is biologically plausible given documented antioxidant and xanthine-oxidase-modulating activities of MO phenolics that can influence urate metabolism and oxidative stress pathways (72,73). Metformin's multifaceted glycaemic actions involving chief inhibition of hepatic gluconeogenesis through AMP-activated protein kinase (AMPK) activation and improved peripheral glucose uptake are well established and provide a benchmark for comparative efficacy (74,75). In our study, the terminal glycaemic outcomes with TC+MO were broadly comparable to those seen with metformin, suggesting overlapping or complementary mechanisms (e.g., AMPK/Phosphatidylinositol 3-kinase (PI3K) pathway engagement and enhanced peripheral glucose utilization). Preclinical work on TC derivatives (e.g., tinosporaside) and extracts supports PI3K- and AMPK-linked enhancement of glucose utilization in muscle and other tissues, which helps explain this similarity (76,77).

At the pancreatic level, the combined formulation significantly decreased MDA and restored endogenous antioxidant defenses (SOD, CAT, GSH), indicating correction of the redox imbalance characteristic of diabetes. Preservation of islet architecture histologically coincided with these biochemical advancements, while fewer pyknotic nuclei, consistent with prior reports that TC and MO protect β-cells and attenuate diabetes-associated oxidative injury. Although we did not isolate individual phytocompounds in this study, GC–MS profiling of the formulation revealed a polyphenol- and sterol-rich phytochemical matrix. Polyphenols are well documented to mitigate hyperglycaemia-induced oxidative stress through ROS scavenging and modulation of redox-sensitive signaling, while phytosterols reduce Low-density lipoprotein-cholesterol (LDL-C) by limiting intestinal absorption and altering hepatic cholesterol homeostasis, which are the mechanistic pathways that plausibly underlie our observed lipid-lowering and antioxidant effects (78,79). These phytochemical-driven mechanisms have broad support in the literature on plant-derived antidiabetic agents.

Based on the findings of this study, TC and MO and their combined formulation significantly improved liver and kidney function markers and ameliorated oxidative stress in "diabetic rats." According to these findings, the therapeutic effect is not limited to glycemic regulation but extends to the protection of vital organs. However, the current study is restricted to short-term evaluation in an *in vivo* model, and the underlying molecular mechanisms were not explored. The study did not isolate individual bioactive compounds; thus, the exact molecular contributors remain undefined. There is a need for further studies involving mechanism-based assays (AMPK, PI3K, Nrf2), longer treatment durations, transcriptomic, proteomic, and metabolomic profiling to determine whether these results translate into therapeutic potential in humans.

Conclusion

The findings demonstrate that the combined herbal formulation of TC and MO confers significant multi-organ protection in STZ-induced type II diabetic Wistar rats. The formulation effectively improved glycemic control, lipid metabolism, and antioxidant balance while restoring normal architecture in pancreatic and renal tissues. Histopathological observations confirmed preservation of islet cytoarchitecture and improvement in glomerulotubular integrity, supporting its cytoprotective and regenerative potential. GC–MS profiling revealed a phytochemical matrix rich in polyphenols, flavonoids, and phytosterols, suggesting that these bioactive compounds collectively mediate the observed antihyperglycemic, antioxidant, and hypolipidemic effects. The synergistic combination of TC and MO appears to enhance glucose homeostasis, reduce oxidative and inflammatory stress, and protect vital organs against diabetic damage. Overall, the results demonstrate this di-herbal formulation's potential as a viable phytotherapeutic option for type II diabetes mellitus management and prevention. More clinical, pharmacokinetic, and mechanistic research is necessary to confirm its safety and effectiveness and to help it become a standardized plant-based medication.

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Authors' contribution

Conceptualization: Uma Dutta.
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Funding acquisition: Sonali Dey.
Investigation: Sonali Dey.
Methodology: Uma Dutta and Sonali Dey.
Resources: Uma Dutta.
Software: Uma Dutta and Sonali Dey.
Supervision: Uma Dutta.
Validation: Uma Dutta and Sonali Dey.
Visualization: Uma Dutta and Sonali Dey.
Writing–original draft: Sonali Dey.
Writing–review & editing: Uma Dutta and Sonali Dey.

Data availability statement

All data are securely retained by the authors and can be shared upon request.

Conflict of interests

The authors declare that they have no conflict of interest.

Declaration of AI-assisted Tools in the Writing Procedure

No AI software has been used to prepare the manuscript.

Ethical considerations

Animal experimentation ethical approval was given by the Institutional Animal Ethics Committee, Cotton University (Approval No. 16/IAEC/CU/27/11/2024) following the evaluation of the research protocol. All experiments and handling of animals were done as per the standard protocol (ARRIVE guidelines). All procedures were strictly followed as per the ethical guidelines.

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References

1. International Diabetes Federation (IDF). IDF Diabetes Atlas. 10th ed. Brussels: IDF; 2023. p. 1-150.
2. Hu FB. Globalization of diabetes: the role of diet, lifestyle, and genes. Diabetes Care. 2011;34(6):1249-57. doi: 10.2337/dc11-0442.
3. Nanditha A, Susairaj P, Satheesh K, Raghavan A, Snehalatha C, Ramachandran A. The rising prevalence of type 2 diabetes among the youth in southern India-An ancillary analysis of the Secular TRends in DiabEtes in India (STRiDE-I) study. J Diabetes. 2024;16(7):e13576. doi: 10.1111/1753-0407.13576.
4. Evans JL, Goldfine ID. Alpha-lipoic acid: a multifunctional antioxidant that improves insulin sensitivity in patients with type 2 diabetes. Diabetes Technol Ther. 2000;2(3):401-13. doi: 10.1089/15209150050194279.
5. Robertson RP, Harmon JS. Diabetes, glucose toxicity, and oxidative stress: a case of double jeopardy for the pancreatic islet β cell. Free Radic Biol Med. 2006;41(2):177-84. doi: 10.1016/j.freeradbiomed.2005.04.030.
6. Sharma R, Sharma A, Singh V, Patel DK. In-vitro α-amylase, α-glucosidase inhibitory activities and in-vivo antihyperglycemic potential of different dosage forms of *Tinospora cordifolia*. Front Pharmacol. 2021;12:642300. doi: 10.3389/fphar.2021.642300.
7. Upadhyay AK, Kumar K, Kumar A, Mishra HS. *Tinospora cordifolia* (Willd.) Miers: a review on its phytochemical and therapeutic potential. Indian J Pharm Sci. 2010;72(5):546-53. doi: 10.4103/0250-474X.78519.
8. Choudhry N, Singh S, Siddiqui MB, Khatoon S. Impact of seasons and dioecy on therapeutic phytoconstituents of *Tinospora cordifolia*, a Rasayana drug. Biomed Res Int. 2014;2014:902138. doi: 10.1155/2014/902138.
9. Pareek A, Pant M, Gupta MM, Kashania P, Ratan Y, Jain V, et al. *Moringa oleifera*: an updated comprehensive

review of its pharmacological activities, ethnomedicinal, phytopharmaceutical formulation, clinical, phytochemical, and toxicological aspects. Int J Mol Sci. 2023;24(3):2098. doi: 10.3390/ijms24032098.

10. Senthilkumar A, Karuvantevida N, Rastrelli L, Kurup SS, Cheruth AJ. Traditional Uses, Pharmacological Efficacy, and Phytochemistry of *Moringa peregrina* (Forssk.) Fiori. -A Review. Front Pharmacol. 2018;9:465. doi: 10.3389/fphar.2018.00465.
11. Liu YJ, Zhan J, Liu XL, Wang Y, Ji J, He QQ. Dietary flavonoids intake and risk of type 2 diabetes: a meta-analysis of prospective cohort studies. Clin Nutr. 2014;33(1):59-63. doi: 10.1016/j.clnu.2013.03.011.
12. Thompson AS, Hollis JL, Cassidy A, et al. Higher habitual intakes of flavonoids and flavonoid-rich foods are associated with a lower incidence of type 2 diabetes in the UK Biobank cohort. Nutr Diabetes. 2024;14:32. doi: 10.1038/s41387-024-00288-0.
13. Alam S, Sarker MMR, Islam MT, Mohamed IN, Mohamed M, Ahmad S, et al. Antidiabetic phytochemicals for drug discovery and development. Front Endocrinol. 2022;13:800714. doi: 10.3389/fendo.2022.800714.
14. Suvarna R, Shenoy RP, Hadapad BS, Nayak AV. Effectiveness of polyherbal formulations for the treatment of type 2 Diabetes mellitus - A systematic review and meta-analysis. J Ayurveda Integr Med. 2021;12(1):213-222. doi: 10.1016/j.jaim.2020.11.002.
15. Pandya S, Savaliya C, Thummar K, Gothwad A, Panchabhai T, Nagore D. Validation of standardized polyherbal formulation in the management of type 2 diabetes mellitus: A randomized, double-blind, placebo-controlled trial. J Diabetes Metab Disord. 2022;22(1):495-506. doi: 10.1007/s40200-022-01171-4.
16. Masiello P, Broca C, Gross R, Roye M, Manteghetti M, Hillaire-Buys D, et al. Experimental NIDDM: development of a new model in adult rats administered streptozotocin and nicotinamide. Diabetes. 1998;47(2):224-29. doi: 10.2337/diab.47.2.224.
17. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: a review. Int Pharm Sci. 2011;1(1):98-106.
18. Trease GE, Evans WC. Pharmacognosy. 13th ed. London: ELBS/Bailliere Tindall; 1989. p. 345-6.
19. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Germany: Springer Netherlands; 1998. p. 302.
20. Furman BL. Streptozotocin-induced diabetic models in mice and rats. Curr Protoc. 2021;1(4):e78. doi: 10.1002/cpz1.78.
21. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 1979;95(2):351–358. doi: 10.1016/0003-2697(79)90738-3.
22. Takahara S, Hamilton HB, Neel JV, Kobara TY, Ogura Y, Nishimura ET. Hypocatalasemia: a new genetic carrier state. J Clin Invest. 1960;39(4):610-9. doi: 10.1172/JCI104075.
23. Khatua TN, Padiya R, Karnewar S, Kuncha M, Agawane SB, Kotamraju S, Banerjee SK. Garlic provides protection to mice heart against isoproterenol-induced oxidative damage: role of nitric oxide. Nitric Oxide. 2012;27(1):9-17. doi: 10.1016/j.niox.2012.03.004.
24. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82(1):70-7. doi: 10.1016/0003-9861(59)90090-6.
25. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193(1):265-75.
26. Fartasch M, Teal J, Menon GK. Mode of action of glycolic acid on human stratum corneum: ultrastructural and functional evaluation of the epidermal barrier. Arch Dermatol Res. 1997;289(7):404-9. doi: 10.1007/s004030050212.
27. Kim SJ, Won YH. The effect of glycolic acid on cultured human skin fibroblasts: cell proliferative effect and increased collagen synthesis. J Dermatol. 1998;25(2):85-9.
28. Bernstein EF, Lee J, Brown DB, Yu R, Van Scott E. Glycolic acid treatment increases type I collagen mRNA and hyaluronic acid content of human skin. Dermatol Surg. 2001;27(5):429-33. doi: 10.1046/j.1524-4725.2001.00234.x.
29. Valle-González ER, Jackman JA, Yoon BK, Mokrzecka N, Cho NJ. pH-dependent antibacterial activity of glycolic acid: implications for anti-acne formulations. Sci Rep. 2020;10(1):7491. doi: 10.1038/s41598-020-64545-9.
30. Al-Lahham SH, Peppelenbosch MP, Roelofsen H, Vonk RJ, Venema K. Biological effects of propionic acid in humans: metabolism, potential applications, and underlying mechanisms. Biochim Biophys Acta. 2010;1801(11):1175-83. doi: 10.1016/j.bbalip.2010.07.007.
31. Ahmed MA, Azam F, Rghigh AM, Gbaj A, Zetrini AE. Structure-based design, synthesis, molecular docking, and biological activities of 2-(3-benzoylphenyl)propanoic acid derivatives. J Pharm Bioallied Sci. 2012;4(1):43-50. doi: 10.4103/0975-7406.92728.
32. Park ES, Moon WS, Song MJ, Kim MN, Chung KH, Yoon JS. Antimicrobial activity of phenol and benzoic acid derivatives. Int Biodeterior Biodegrad. 2001;47(4):209-14. doi: 10.1016/S0964-8305(01)00058-0.
33. Issa HM, Mohammed DH. A critical review on the journey of benzoic acid in the pharmaceutical industry. Environ Anal Health Toxicol. 2025;40(1):e2025007. doi: 10.5620/eaht.2025007.
34. Zhu X, Guo Y, Liu Z, Yang J, Tang H, Wang Y. Itaconic acid exerts anti-inflammatory and antibacterial effects via promoting pentose phosphate pathway to produce ROS. Sci Rep. 2021;11(1):18173. doi: 10.1038/s41598-021-97352-x.
35. Yang W, Wang Y, Tao K, Li R. Metabolite itaconate in host immunoregulation and defense. Cell Mol Biol Lett. 2023;28(1):100. doi: 10.1186/s11658-023-00503-3.
36. Al Mazraani RAAD, Malys N, Maliene V. Itaconate and its derivatives as anti-pathogenic agents. RSC Adv. 2025;15(6):4408-20. doi: 10.1039/D4RA08298B.
37. Alakolanga AGAW, Kumar NS, Jayasinghe L, Fujimoto Y. Antioxidant property and α-glucosidase, α-amylase and lipase inhibiting activities of Flacourtia inermis fruits: characterization of malic acid as an inhibitor. J Food Sci Technol. 2015;52(12):8383-8. doi: 10.1007/s13197-015-1937-6.
38. Arslan ME. Anticarcinogenic properties of malic acid on glioblastoma cell line through necrotic cell death mechanism. Manas J Eng. 2021;9(1):22-9. doi: 10.51354/mjen.848282.
39. Nangare S, Vispute Y, Tade R, Dugam S, Patil P. Pharmaceutical applications of citric acid. Future J Pharm

Sci. 2021;7(1):54. doi: 10.1186/s43094-021-00203-9.
40. Książek E. Citric acid: properties, microbial production, and applications in industries. Molecules. 2023;29(1):22. doi: 10.3390/molecules29010022.
41. Kim KB, Nam YA, Kim HS, Hayes AW, Lee BM. α-Linolenic acid: nutraceutical, pharmacological and toxicological evaluation. Food Chem Toxicol. 2014;70:163-78. doi: 10.1016/j.fct.2014.05.009.
42. Dittrich M, Jahreis G, Bothor K, Drechsel C, Kiehntopf M, Blüher M, et al. Benefits of foods supplemented with vegetable oils rich in α-linolenic, stearidonic or docosahexaenoic acid. Eur J Nutr. 2015;54(6):881-93. doi: 10.1007/s00394-014-0764-2.
43. Yuan Q, Xie F, Huang W, Hu M, Yan Q, Chen Z, et al. The review of alpha-linolenic acid: sources, metabolism, and pharmacology. Phytother Res. 2022;36(1):164-88. doi: 10.1002/ptr.7295.
44. Perdomo L, Beneit N, Otero YF, Escribano Ó, Díaz-Castroverde S, Gómez-Hernández A, et al. Protective role of oleic acid against cardiovascular insulin resistance and atherosclerotic processes. Cardiovasc Diabetol. 2015;14:75. doi: 10.1186/s12933-015-0237-9.
45. Santa-María C, López-Enríquez S, Montserrat-de la Paz S, Geniz I, Reyes-Quiroz ME, Moreno M, et al. Update on anti-inflammatory molecular mechanisms induced by oleic acid. Nutrients. 2023;15(1):224. doi: 10.3390/nu15010224.
46. Ramadan AMAA, Zidan SAH, Shehata RM, El-Sheikh HH, Ameen F, Stephenson SL, et al. Antioxidant, antibacterial, and molecular docking of methyl ferulate and oleic acid. Sci Rep. 2024;14(1):3183. doi: 10.1038/s41598-024-52045-z.
47. Bonanome A, Grundy SM. Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. N Engl J Med. 1988;318(19):1244-8. doi: 10.1056/NEJM198805123181905.
48. Schneider CL, Cowles RL, Stuefer-Powell CL, Carr TP. Dietary stearic acid reduces cholesterol absorption and increases endogenous cholesterol excretion. J Nutr. 2000;130(5):1232-8. doi: 10.1093/jn/130.5.1232.
49. Tahlan S, Kumar P, Narasimhan B. Synthesis, antimicrobial evaluation, and QSAR studies of stearic acid derivatives. Drug Res. 2014;64(2):98-103. doi: 10.1055/s-0033-1353173.
50. Mukhtar YM, Adu-Frimpong M, Xu X, Yu J. Biochemical significance of limonene and its metabolites: future prospects for designing and developing highly potent anticancer drugs. Biosci Rep. 2018;38(6):BSR20181253. doi: 10.1042/BSR20181253.
51. Anandakumar P, Kamaraj S, Vanitha MK. D-limonene: a multifunctional compound with potent therapeutic effects. J Food Biochem. 2021;45(1):e13566. doi: 10.1111/jfbc.13566.
52. Devi NS, Bhattacharya B, Sharma A, Singh I, Kumar P, Huanbutta K, et al. From citrus to clinic: limonene's journey through preclinical research, clinical trials, and formulation innovations. Int J Nanomedicine. 2025;20:4433-60. doi: 10.2147/IJN.S514247.
53. Zhao F, Wang P, Lucardi RD, Su Z, Li S. Natural sources and bioactivities of 2,4-di-tert-butylphenol and its analogs. Toxins (Basel). 2020;12(1):35. doi: 10.3390/toxins12010035.
54. Ayswarya S, Radhakrishnan M, Manigundan K, Gopikrishnan V, Soytong K. Antioxidant activity of 2,4-di-tert-butylphenol isolated from plant growth promoting endophytic Streptomyces KCA-1. Int J Agric Technol. 2022;18(6):2343-52.
55. Rouvier F, Abou L, Wafo E, Andre P, Cheyrol J, Khacef MM, et al. Identification of 2,4-di-tert-butylphenol as an antimicrobial agent against *Cutibacterium acnes* bacteria from Rwandan propolis. Antibiotics (Basel). 2024;13(11):1080. doi: 10.3390/antibiotics13111080.
56. Konishi T, Satsu H, Hatsugai Y, Aizawa K, Inakuma T, Nagata S, et al. A bitter melon extract inhibits the P-glycoprotein activity in intestinal Caco-2 cells: monoglyceride as an active compound. Biofactors. 2004;22(1-4):71-4. doi: 10.1002/biof.5520220113.
57. Bunka F, Pavlinek V, Hrabe J, Rop O, Janis R, Krejci J. Effect of 1-monoglycerides on viscoelastic properties of processed cheese. Int J Food Prop. 2007;10(4):819-28. doi: 10.1080/10942910601113756.
58. Rashed K. Beta-sitosterol medicinal properties: a review article. J Sci Innov Technol. 2020;9:208-12.
59. Zhang P, Liu N, Xue M, Zhang M, Liu W, Xu C, et al. Anti-inflammatory and antioxidant properties of β-sitosterol in copper sulfate-induced inflammation in zebrafish (Danio rerio). Antioxidants (Basel). 2023;12(2):391. doi: 10.3390/antiox12020391.
60. Gao HT, Xu R, Cao WX, Zhou X, Yan YH, Lu L, et al. Food emulsifier glycerin monostearate increases internal exposure levels of six priority controlled phthalate esters and exacerbates their male reproductive toxicities in rats. PLoS One. 2016;11(8):e0161253. doi: 10.1371/journal.pone.0161253.
61. Lu Y, Zhong Y, Guo X, Zhang J, Gao Y, Mao L. Structural modification of O/W bigels by glycerol monostearate for improved co-delivery of curcumin and epigallocatechin gallate. ACS Food Sci Technol. 2022;2(6):975-83. doi: 10.1021/acsfoodscitech.2c00044.
62. Malvano F, Albanese D, Cinquanta L, Liparoti S, Marra F. A comparative study between beeswax and glycerol monostearate for food-grade oleogels. Gels. 2024;10(4):214. doi: 10.3390/gels10040214.
63. Sangeetha MK, Balaji Raghavendran HR, Gayathri V, Vasanthi HR. *Tinospora cordifolia* attenuates oxidative stress and distorted carbohydrate metabolism in experimentally induced type 2 diabetes in rats. J Nat Med. 2011;65(3-4):544-50. doi: 10.1007/s11418-011-0538-6.
64. Joladarashi D, Chilkunda ND, Salimath PV. Glucose uptake-stimulatory activity of *Tinospora cordifolia* stem extracts in Ehrlich ascites tumor cell model system. J Food Sci Technol. 2014;51(1):178-82. doi: 10.1007/s13197-011-0480-3.
65. Mishra A, Sharma K, Pandey J, Dev K, Kadan S, Sahai M, et al. Tinosporaside from *Tinospora cordifolia* Encourages Skeletal Muscle Glucose Transport through Both PI-3-Kinase- and AMPK-Dependent Mechanisms. Molecules. 2023;28(2):483. doi: 10.3390/molecules28020483.
66. Mthiyane FT, Dludla PV, Ziqubu K, Mthembu SXH, Muvhulawa N, Hlengwa N, et al. A review on the antidiabetic properties of *Moringa oleifera* extracts: focusing on oxidative stress and inflammation as main therapeutic targets. Front Pharmacol. 2022;13:940572. doi: 10.3389/fphar.2022.940572.
67. Watanabe S, Okoshi H, Yamabe S, Shimada M. *Moringa oleifera* Lam. in diabetes mellitus: a systematic review and

meta-analysis. Molecules. 2021;26(12):3513. doi: 10.3390/molecules26123513.

68. Gupta R, Mathur M, Bajaj VK, Katariya P, Yadav S, Kamal R, et al. Evaluation of antidiabetic and antioxidant activity of *Moringa oleifera* in experimental diabetes. J Diabetes. 2012;4(2):164-71. doi: 10.1111/j.1753-0407.2011.00173.x.
69. Tran N, Pham B, Le L. Bioactive compounds in anti-diabetic plants: from herbal medicine to modern drug discovery. Biology (Basel). 2020;9(9):252. doi: 10.3390/biology9090252.
70. Caesar LK, Cech NB. Synergy and antagonism in natural product extracts: when 1 + 1 does not equal 2. Nat Prod Rep. 2019;36(6):869-888. doi: 10.1039/c9np00011a.
71. Sonam KS, Guleria S. Synergistic antioxidant activity of natural products. Ann. Pharmacol. Pharm. 2017;2(8):1086.
72. Tang Y, Choi EJ, Han WC, Oh M, Kim J, Hwang JY, et al. *Moringa oleifera* from Cambodia ameliorates oxidative stress, hyperglycemia, and Kidney Dysfunction in Type 2 Diabetic Mice. J Med Food. 2017;20(5):502-510. doi: 10.1089/jmf.2016.3792.
73. Balkrishna A, Haldar S, Varshney A. OECD-407 Driven 28-day-repeated-dose non-clinical safety evaluation of *Tinospora cordifolia* (Giloy) stem aqueous extract in Sprague-Dawley rats under GLP compliance. Front Pharmacol. 2023;14:1095083. doi: 10.3389/fphar.2023.1095083.
74. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, et al. Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest. 2001;108(8):1167-74. doi: 10.1172/JCI13505.
75. Foretz M, Guigas B, Bertrand L, Pollak M, Viollet B. Metformin: from mechanisms of action to therapies. Cell Metab. 2014;20(6):953-66. doi: 10.1016/j.cmet.2014.09.018.
76. Sharma BR, Park CM, Kim HA, Kim HJ, Rhyu DY. *Tinospora cordifolia* preserves pancreatic beta cells and enhances glucose uptake in adipocytes to regulate glucose metabolism in diabetic rats. Phytother Res. 2019;33(10):2765-2774. doi: 10.1002/ptr.6462.
77. Arunachalam K, Yang X, San TT. *Tinospora cordifolia* (Willd.) Miers: Protection mechanisms and strategies against oxidative stress-related diseases. J Ethnopharmacol. 2022;283:114540. doi: 10.1016/j.jep.2021.114540.
78. Cedó L, Farràs M, Lee-Rueckert M, Escolà-Gil JC. Molecular Insights into the Mechanisms Underlying the Cholesterol- Lowering Effects of Phytosterols. Curr Med Chem. 2019;26(37):6704-6723. doi: 10.2174/0929867326666190822154701.
79. Castellanos-Jankiewicz A, Del Bosque-Plata L, Tejero ME. Combined effect of plant sterols and dietary fiber for the treatment of hypercholesterolemia. Plant Foods Hum Nutr. 2014;69(2):93-100. doi: 10.1007/s11130-014-0419-8.