



Polyherbal Formulation Exhibits Protective Effects Against Streptozotocin-Induced Hepatic and Pancreatic Injury Through Experimental and Network Pharmacology Approaches

İsa Kiran¹ · Mustafa Cengiz² · Adnan Ayhancı¹

Received: 19 November 2025 / Accepted: 6 January 2026 / Published online: 20 January 2026
© The Author(s) 2026

Abstract

Diabetes mellitus is a chronic metabolic disease associated with progressive organ damage driven by hyperglycaemia-induced oxidative stress, inflammation, and cellular dysfunction. Cardiovascular complications remain a major cause of morbidity and mortality in diabetic patients. This study evaluated the antidiabetic, hepatoprotective, and pancreatic protective effects of a polyherbal formulation (PHF) comprising *Curcuma longa*, *Nigella sativa*, *Prunus mahaleb*, and *Vitis vinifera* in streptozotocin (STZ)-induced diabetic rats, integrating network pharmacology analysis with experimental validation. Network pharmacology analysis was performed to identify potential PHF-related targets and pathways associated with diabetes-related tissue injury. Rats were assigned to control, diabetic, metformin-treated, and PHF-treated (500 and 1000 mg/kg) groups. The 500 mg/kg dose was selected as an intermediate dose based on prior experimental studies reporting its biological relevance and safety in plant-derived formulations. Blood glucose, serum insulin, lipid profile, and pancreatic enzymes were measured. Hepatic and pancreatic tissues were examined using hematoxylin and eosin (H&E), Masson's trichrome (MT), periodic acid–Schiff (PAS) staining, and immunohistochemistry for endothelial nitric oxide synthase (eNOS), B-cell lymphoma 2 (Bcl-2), Bcl-2-associated X protein (Bax), caspase-3, tumour necrosis factor alpha (TNF- α), interleukin-1 beta (IL-1 β), and vascular endothelial growth factor (VEGF). PHF treatment, particularly at 1000 mg/kg, normalised blood glucose ($p < 0.001$), improved serum insulin, corrected dyslipidaemia, and restored amylase and lipase levels. Histological analysis demonstrated attenuation of steatosis, necrosis, fibrosis, and pancreatic β -cell loss. Immunohistochemical analysis showed reduced Bax and caspase-3 protein immunoreactivity and increased Bcl-2 and VEGF protein expression in PHF-treated groups. Network pharmacology analysis revealed 333 shared targets between PHF and diabetes-related injury, enriched in phosphoinositide 3-kinase/protein kinase B (PI3K/Akt), mitogen-activated protein kinase (MAPK), advanced glycation end product–receptor for AGE (AGE–RAGE), TNF, and metabolic signalling pathways. Hub genes including AKT1, IL6, TNF, STAT3, PTGS2, CASP3, and BCL2 showed reciprocal consistency between network pharmacology predictions and experimental immunohistochemical observations. To sum up, these findings support the potential of PHF as a multi-target therapeutic strategy against diabetes-induced hepatic and pancreatic injury.

Keywords Diabetes mellitus · Polyherbal formulation · Liver injury · B-cell regeneration · Network pharmacology · Angiogenesis

✉ İsa Kiran
isakiran065@gmail.com

✉ Mustafa Cengiz
m.cengiz@siirt.edu.tr; mustafacengizogu@gmail.com

¹ Department of Biology, Faculty of Science, Eskişehir Osmangazi University, Eskişehir 26040, Türkiye

² Department of Mathematics and Science Education, Faculty of Education, Siirt University, Siirt 56100, Turkey

Introduction

Diabetes mellitus (DM) is a complex metabolic disorder characterised by persistent hyperglycaemia, progressive β -cell dysfunction, impaired insulin action, and systemic metabolic abnormalities. Chronic oxidative stress, inflammatory signalling, mitochondrial dysfunction, and apoptotic pathways cause gradual damage to the liver

and pancreas, making these organs primary targets of diabetic disease. Hepatocytes develop steatosis, fibrosis, oxidative imbalance, and inflammatory activation, while pancreatic β -cells, which are especially susceptible to oxidative damage, exhibit DNA fragmentation, reduced insulin production, and apoptosis. Therefore, there is an urgent need for medicines that can simultaneously modulate metabolic, oxidative, apoptotic, and inflammatory pathways [1–7].

The treatment landscape for diabetes mellitus increasingly incorporates polyherbal formulations that enable synergistic interactions among multiple phytochemicals. *Curcuma longa* (curcumin) supports glycaemic regulation through antioxidative mechanisms and PI3K-Akt activation; *Nigella sativa* (thymoquinone) improves insulin sensitivity and reduces NF- κ B-mediated inflammation; *Vitis vinifera* (resveratrol) promotes mitochondrial stability and anti-apoptotic signalling; and *Prunus mahaleb* offers hepatoprotective antioxidant effects. Although these plants are individually well documented, their combined therapeutic potential, particularly for diabetic hepatic and pancreatic injury, remains insufficiently characterised [8, 9–15]. Metformin is a widely used first-line antidiabetic drug in clinical practice. It is often used as a positive control in streptozotocin (STZ)-induced diabetes models because, although it provides glycaemic regulation, its protective effect on tissues is limited [16]. In this study, metformin was used to evaluate the effects of a herbal formulation on glycaemic regulation and tissue protection, compared to this standard treatment. Despite the well-documented antidiabetic and antioxidant properties of individual constituents in this polyherbal formulation, including *Curcuma longa*, *Nigella sativa*, *Prunus mahaleb*, and *Vitis vinifera*, their combined therapeutic potential remains insufficiently characterised [17–19]. In particular, no comprehensive study has integrated in vivo biochemical, histological, and immunohistochemical validation within a systems-level network pharmacology framework to elucidate their collective efficacy. The existing experimental literature based on streptozotocin-induced diabetic animal models lacks mechanistic evidence demonstrating whether a polyherbal formulation can simultaneously protect hepatic and pancreatic tissues, modulate oxidative stress and apoptosis, suppress inflammatory signalling, and promote angiogenic repair [20, 21].

Furthermore, the coordinated regulation of interrelated metabolic pathways such as phosphoinositide 3-kinase/protein kinase B (PI3K–Akt), mitogen-activated protein kinase (MAPK), advanced glycation end product–receptor for AGE (AGE–RAGE), and tumour necrosis factor (TNF) signalling by these botanicals has not been systematically

investigated [21, 22]. Therefore, the present study integrates experimental validation with network pharmacology analysis to clarify the multi-targeted antidiabetic mechanisms of this polyherbal formulation.

Streptozotocin (STZ) induces pancreatic β -cell DNA alkylation, excessive reactive oxygen species (ROS) production, and pro-inflammatory cytokine release, thereby replicating key metabolic and histological features of type 1 diabetes. While metformin, the first-line antidiabetic therapy, effectively improves glycaemic regulation, it provides limited direct protection against diabetes-induced structural damage in hepatic and pancreatic tissues, underscoring the need for alternative multi-target therapeutic strategies [23–25]. Therefore, the present study integrates experimental validation with network pharmacology analysis to clarify the multi-targeted antidiabetic mechanisms of this polyherbal formulation.

Materials and Methods

Preparation of Polyherbal Formulation

Equal quantities of *Curcuma longa* rhizome, *Nigella sativa* seeds, *Prunus mahaleb* seeds and fruits, and *Vitis vinifera* seeds were cleaned, shade-dried at room temperature, and pulverised into a fine powder using a laboratory grinder. The powdered plant materials were thoroughly homogenised to obtain the polyherbal formulation (PHF). For aqueous extraction, 10 g of the PHF powder was mixed with 250 mL of distilled water and subjected to the decoction method by gentle boiling at low heat for 20 min. The extract was allowed to cool to room temperature and then filtered through Whatman No. 1 filter paper to remove particulate matter. The filtrate was freshly prepared prior to administration and stored at 4 °C for short-term use. The final extract was standardised based on total polyphenol and curcuminoid content using spectrophotometric methods, as previously described [26].

Experimental Animals

This study used commercially obtained adult male Wistar albino rats, weighing 200 to 250 g, supplied by Kobay Deney Hayvanları Laboratuvarı San. Tic. A.Ş. The rats were transported to the laboratory in polypropylene cages on the day of collection. They were given a seven-day acclimatisation period in laboratory conditions before the experiment. Upon arrival, the rats were kept under standardised conditions (temperature: 23 $\pm$ 3 °C, humidity: 35–60%, 12-hour light/dark cycle). Throughout the experiment, the rats had ad libitum access to standard rat chow and water. Body

weight changes, as well as food and water consumption, were systematically recorded for all groups throughout the study.

Diabetes Induction

Animals were initially weighed and counted. Streptozotocin (STZ), freshly prepared in chilled 0.1 M citrate buffer at pH 4.5, was administered intraperitoneally to overnight-fasted rats at a dose of 55 mg/kg body weight. To prevent hypoglycaemic mortality after STZ injection, subjects received a 5% w/v glucose solution in their drinking water for 3–4 h. Seven days after STZ injection, those with blood glucose levels above 200 mg/dL were selected for the study [27, 28, 29].

Experimental Groups

Fifty male Wistar albino rats were randomly allocated into five groups, each comprising ten rats. The groups were as follows:

Group I (Control) Rats received 1 mL of saline orally on the sixth day of the experiment.

Group II (Diabetic) On the first day of the experiment, 55 mg/kg streptozotocin (STZ) was administered intraperitoneally. At the end of the sixth day, 1 mL of saline was administered orally [30].

Group III (STZ+100 mg/kg MET) On the sixth day after STZ-induced diabetes, 100 mg/kg MET was administered orally as an antidiabetic agent [31].

Group IV (STZ+500 mg/kg PHF1) On the sixth day after STZ-induced diabetes, 500 mg/kg PHF1 was administered orally.

Group V (STZ+1000 mg/kg PHF2) On the sixth day after STZ-induced diabetes, 1000 mg/kg PHF2 was administered orally.

On the seventh day of the experiment, blood glucose levels were measured from 0.1 mL tail vein blood samples at 30, 60, 120, and 240 min in all groups. The 7-day experimental duration was deliberately selected to investigate early-phase diabetes-induced hepatic and pancreatic injury rather than advanced chronic damage. Previous studies have demonstrated that oxidative stress, inflammatory activation, mitochondrial dysfunction, and apoptotic signalling in the liver and pancreas emerge within the first week following streptozotocin administration, preceding overt fibrosis and

long-term structural remodelling [32, 33]. Accordingly, this early time window allows assessment of initial tissue injury and therapeutic responsiveness before irreversible pathological changes occur. Streptozotocin was administered on day 1 to induce diabetes, while treatment interventions were initiated on day 6 to allow sufficient establishment of hyperglycaemia. Blood glucose measurements were performed on day 7 at multiple time points to evaluate acute metabolic responses following treatment. This staggered timeline was designed to distinguish diabetes induction, treatment onset, and outcome assessment phases within a controlled early-injury model. All procedures were carried out in accordance with international ethical standards, including the ARRIVE guidelines, the U.K. Animals (Scientific Procedures) Act 1986, and the European Directive 2010/63/EU concerning the care and use of animals for research purposes.

Histological Investigations

Method for Histopathology

At the end of the experiment, rats were euthanized using ketamine/xylazine. Following cervical dislocation, the tissues were carefully dissected from all rats. The samples were subsequently processed histologically and embedded in paraffin. Sections of 4 µm thickness were cut from these blocks. The samples were stained with hematoxylin and eosin (HE, Sigma-Aldrich, St. Louis, MO, USA), Masson's Trichrome (MT), and periodic acid–Schiff (PAS) (Sigma-Aldrich, St. Louis, MO, USA). Ten randomly selected fields from each HE-stained section were examined at a magnification of ×40. A comprehensive review of recent literature includes works by Ahmed et al. [34] and Abid et al. [35].

HE Staining

Liver and pancreas samples were preserved in 10% neutral buffered formalin for 24 h and subsequently embedded in paraffin. Sections were stained with hematoxylin and eosin and examined using a light microscope. Histological images of tissues were obtained at magnifications of ×100.

Masson's Trichrome (MT) Staining

Masson's Trichrome staining was performed to evaluate collagen deposition in liver and pancreatic tissues. Paraffin-embedded tissue sections were deparaffinised, rehydrated, and sequentially stained with Weigert's iron hematoxylin, Biebrich scarlet–acid fuchsin, phosphomolybdic/phosphotungstic acid, and aniline blue. Sections were

then differentiated in acetic acid, dehydrated, cleared, and mounted according to standard histological procedures [36].

Periodic Acid–Schiff (PAS) Staining

PAS staining was utilized to evaluate glycogen content. In comparison to the control group, hepatocytes in the STZ group showed reduced PAS staining, indicating glycogen depletion, as well as sinusoidal dilatation, apoptosis, congestion, leukocyte infiltration, and accumulation of Kupffer cells. Treatment with PHF2, particularly at a dose of 1000 mg/kg, resulted in the restoration of glycogen levels and improved histopathological changes.

Immunohistochemistry (IHC)

Immunohistochemistry (IHC) was performed on formalin-fixed, paraffin-embedded tissue sections (5 μ m). Sections were deparaffinised in xylene and rehydrated through graded ethanol to distilled water. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide for 10 min. Heat-induced epitope retrieval was carried out using citrate buffer (10 mM, pH 6.0) or Tris–EDTA buffer (pH 9.0), depending on the antibody, by heating for 20 min followed by cooling at room temperature. Non-specific binding was blocked with 5% normal serum for 30 min.

Sections were incubated overnight at 4 °C with primary antibodies against Bax (mouse monoclonal, clone 6A7; Thermo Fisher Scientific, Waltham, MA, USA; catalog no. 33–6600), Caspase-3 (mouse monoclonal, clone 74T2; Thermo Fisher Scientific/Invitrogen, Waltham, MA, USA; catalog no. 43–7800), and Bcl-2 (mouse monoclonal, clone Bcl-2–100; Thermo Fisher Scientific, Waltham, MA, USA; catalog no. 13–8800). Primary antibodies against TNF- α , IL-1 β , VEGF, and eNOS were obtained from commercially available sources and used according to the manufacturers' recommendations. All primary antibodies were used at a dilution of 1:100. After washing, sections were incubated with appropriate biotinylated secondary antibodies for 30 min at room temperature, followed by streptavidin–horseradish peroxidase for 30 min. Immunoreactivity was visualised using 3,3'-diaminobenzidine (DAB) as chromogen and counterstained with haematoxylin. Negative controls were prepared by omitting the primary antibody, and positive controls were included according to the manufacturers' guidelines. Images were captured under identical acquisition settings for all groups. Semi-quantitative evaluation was performed using the H-score method by two independent observers blinded to group allocation. The immunohistochemical procedure was performed according to previously established and widely accepted protocols with minor modifications [37–39].

Biochemical Analyses

Serum concentrations of insulin, amylase, lipase, total cholesterol, triglycerides, low-density lipoprotein (LDL), high-density lipoprotein (HDL), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured in serum samples using commercially available rat-specific enzyme-linked immunosorbent assay (ELISA) kits (SunRed Biological Technology, Shanghai, China; Insulin: Cat. No. 201-11-0132; Amylase: Cat. No. 201-11-0187; Lipase: Cat. No. 201-11-0191; Total cholesterol: Cat. No. 201-11-0128; Triglycerides: Cat. No. 201-11-0155; LDL: Cat. No. 201-11-0141; HDL: Cat. No. 201-11-0142; ALT: Cat. No. 201-11-0171; AST: Cat. No. 201-11-0172). Analyses were performed using a microplate ELISA reader according to the manufacturer's instructions [40].

Network Pharmacology Analysis

A detailed network pharmacology study was conducted to elucidate the multi-target molecular processes potentially effective against STZ-induced hepatic and pancreatic injury in PHF (*Curcuma longa*, *Nigella sativa*, *Prunus mahaleb*, and *Vitis vinifera*). Potential therapeutic targets of the compounds were identified using SwissTargetPrediction (Homo sapiens, likelihood > 0) and standardised with the UniProt database. Genes associated with liver and pancreatic injury were obtained from the GeneCards database (with a corresponding score greater than 1) to establish a consolidated disease-associated gene cluster. By intersecting PHF targets and disease genes, 333 shared treatment-related targets were identified and subsequently converted into a protein–protein interaction (PPI) network using the STRING database (confidence level ≥ 0.40). This network analysis provided detailed insights into interaction density, node centrality, and functional protein modules that are pivotal in STZ-induced tissue damage.

Functional enrichment analysis was performed using ShinyGO v0.77. Gene Ontology Biological Process analysis revealed significant enrichment in fundamental biological systems, including oxidative stress response, inflammatory signalling pathways, regulation of apoptosis, glucose and lipid metabolism, and cellular response to insulin stimulation. Gene Ontology (GO) Molecular Function (MF) terms were defined by kinase activity, cytokine receptor binding, oxidoreductase activity, and enzyme regulatory functions, while GO Cellular Component (CC) analysis highlighted the prominence of membrane microdomains, cytosolic proteins, extracellular domains, and plasma membrane protein complexes. KEGG pathway analysis indicated that PHF targeted various signalling cascades, including PI3K–Akt, MAPK, AGE–RAGE signalling, apoptosis, metabolic

pathways, and neuroactive ligand-receptor interactions. The target–route interaction network, developed with Cytoscape, demonstrates that PHF exerts a multifaceted regulatory influence on STZ-induced hepatic and pancreatic damage via hexagonal route nodes and circular target gene nodes (Fig. 1).

Statistical Analysis

Data were analyzed using SPSS version 20.0 and Sigma Stat version 3.5. Continuous quantitative data are presented as mean±standard deviation, while qualitative data are presented as median and interquartile range (25th and 75th percentiles). For normally distributed continuous data, one-way ANOVA was performed, followed by Tukey's post-hoc

test for multiple comparisons. For non-normally distributed data, the Kruskal-Wallis test was used, with pairwise comparisons performed using the Dunn's post-hoc test. Statistical significance was defined as $p < 0.05$ for all comparisons.

Results

PHF Alleviates STZ-Induced Weight Reduction and Metabolic Dysregulation

STZ administration resulted in a significant reduction in body weight compared with the control group ($p < 0.05$), whereas both metformin and PHF treatments significantly attenuated this weight loss ($p < 0.05$ vs. STZ; Table 1).

Fig. 1 Workflow diagram of network pharmacology analysis

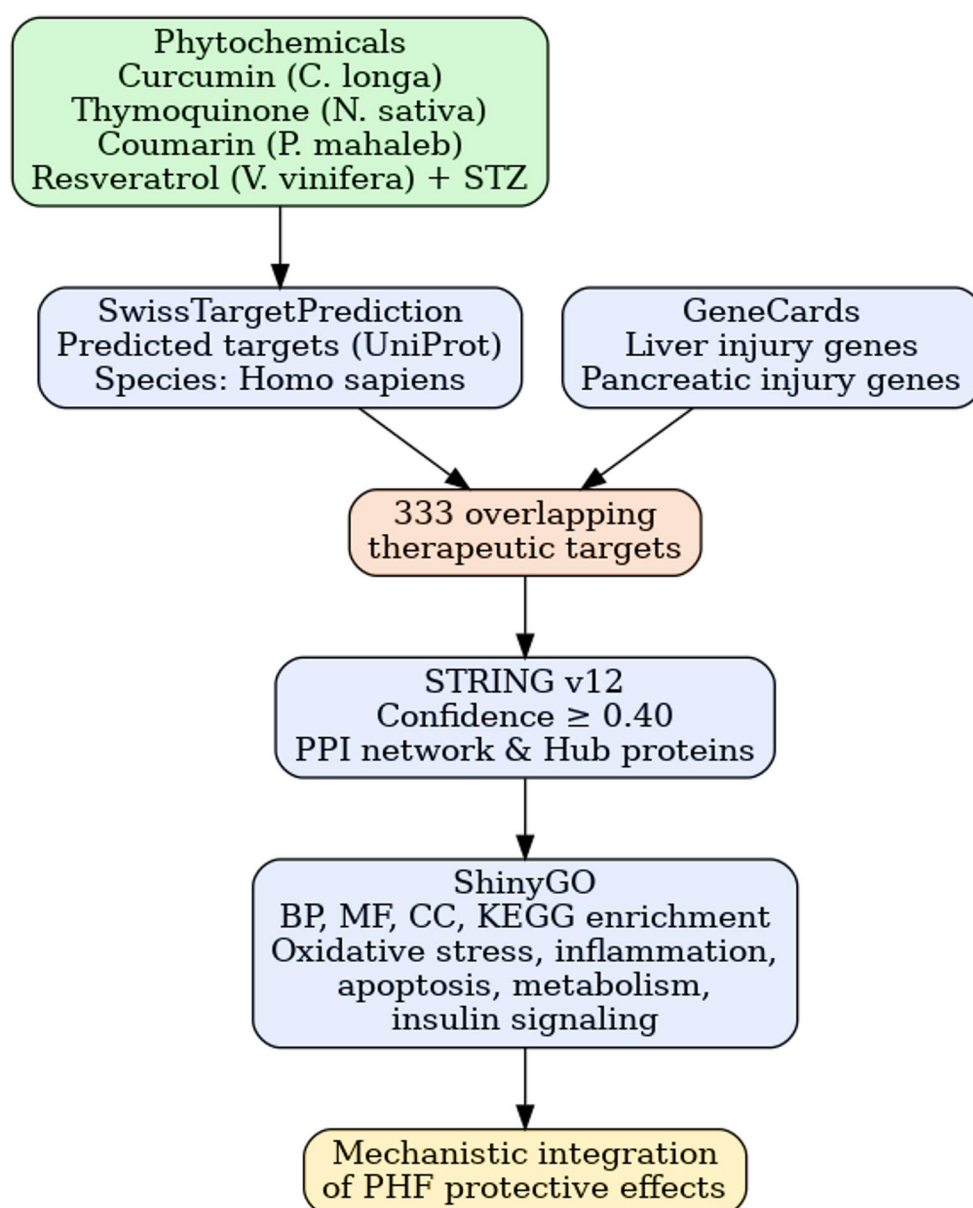


Table 1 Effects of STZ, metformin, and PHF treatments on body weight, food intake, and water consumption in the experimental groups

Groups	Body weight		Food (g/day)	Water (ml/day)
	1st	7th		
Control	203.5 ± 13.75	212.30 ± 12.14 ^b	33.70 ± 1.42	18.20 ± 2.15
STZ	205.00 ± 16.16	183.50 ± 13.95 ^a	39.80 ± 2.39 ^a	87.60 ± 4.58 ^a
STZ + MET	209.50 ± 13.22	195.50 ± 14.23 ^{ab}	36.50 ± 2.99 ^{ab}	77.40 ± 2.17 ^{ab}
STZ + PHF1	210.50 ± 12.57	200.00 ± 13.74 ^{ab}	33.20 ± 2.78	68.70 ± 2.83 ^{ab}
STZ + PHF2	210.00 ± 11.55	200.50 ± 10.29 ^{ab}	27.90 ± 2.81	60.20 ± 3.12 ^{ab}
<i>p</i>		0.001	0.001	0.001

a: significantly different from control; b: significantly different from STZ

Table 2 Effects of STZ, metformin, and PHF treatments on blood glucose levels (0–240 min) and serum insulin concentrations in the experimental groups

	0. min	30. min	60. min	120. min	240. min	Insulin (μIU/mL)
Control	52.50 ± 4.24	64.00 ± 5.58	59.88 ± 3.36	55.00 ± 6.72	52.75 ± 5.44	9.34 ± 1.06
STZ	405.40 ± 59.43 ^a	418.50 ± 61.80 ^a	402.80 ± 49.84 ^a	364.80 ± 49.74 ^a	321.80 ± 30.26 ^a	5.26 ± 0.45 ^a
STZ + MET	373.90 ± 24.97 ^a	384.30 ± 29.64 ^a	359.90 ± 26.32 ^{a, b}	292.80 ± 25.90 ^{a, b}	271.80 ± 14.04 ^{a, b}	6.24 ± 2.76 ^a
STZ + PHF1	349.30 ± 54.44 ^{a, b}	355.00 ± 53.49 ^{a, b}	308.70 ± 29.64 ^{a, b, c}	218.20 ± 44.91 ^{a, b, c}	164.60 ± 28.10 ^{a, b, c}	6.59 ± 0.51 ^a
STZ + PHF2	363.10 ± 37.21 ^a	349.50 ± 57.71 ^{a, b}	275.60 ± 56.38 ^{a, b, c}	170.80 ± 43.18 ^{a, b, c, d}	96.60 ± 15.37 ^{a, b, c, d}	6.30 ± 0.62 ^a

a: difference relative to control; b: significant difference relative to STZ; c: significant difference relative to STZ + MET; d: significant difference relative to STZ + PHF1

Among the treatment groups, PHF2 demonstrated the greatest preservation of initial body weight and was significantly more effective than PHF1 ($p < 0.05$). In parallel, STZ-induced increases in food and water consumption were significantly higher than controls ($p < 0.05$), while metformin partially reduced these alterations. Both PHF1 and PHF2 markedly decreased food and water intake compared with the STZ group ($p < 0.05$), with PHF2 showing a significantly stronger normalising effect, indicating restoration of metabolic homeostasis. Compared with the control group, STZ administration resulted in a significant reduction in body weight and a marked increase in food and water intake ($p < 0.05$). Metformin partially attenuated these alterations, whereas both PHF1 and PHF2 produced significantly greater improvements than metformin. Moreover, PHF2 showed a significantly stronger effect than PHF1 in normalising metabolic parameters.

PHF Improves Glycaemic Regulation and Increases Insulin Secretion

Blood glucose levels were significantly elevated in the STZ-induced diabetic group compared with the control group at all measured time points ($p < 0.05$; Table 2). Metformin treatment resulted in a partial but significant reduction in hyperglycaemia relative to the STZ group ($p < 0.05$). In contrast, both PHF1 and PHF2 produced significantly greater reductions in blood glucose levels than metformin ($p < 0.05$ vs. STZ and STZ + MET), with PHF2 showing significantly lower glucose levels than PHF1 at later time points

($p < 0.05$). Notably, glucose concentrations in the PHF2 group approached control values at the final measurement points. Serum insulin levels were significantly reduced in the STZ group compared with controls ($p < 0.05$). Treatment with PHF1 and PHF2 significantly increased serum insulin concentrations relative to the STZ group ($p < 0.05$), with a more pronounced improvement observed in the PHF2 group. These findings indicate improved glycaemic regulation and enhanced β -cell secretory function following PHF treatment.

PHF Ameliorates Dyslipidaemia and Improves Hepatic Enzyme Profiles

STZ-induced diabetes resulted in significant dyslipidaemia, characterised by increased triglycerides, total cholesterol, and LDL levels, along with a significant reduction in HDL compared with the control group ($p < 0.05$; Table 3). Hepatic enzyme activities (ALT and AST) were also significantly elevated in the STZ group relative to controls ($p < 0.05$). Metformin treatment partially improved lipid parameters and reduced ALT and AST levels compared with the STZ group ($p < 0.05$).

In contrast, both PHF1 and PHF2 produced significantly greater improvements in lipid profile and hepatic enzyme levels than metformin ($p < 0.05$ vs. STZ and STZ + MET). Notably, PHF2 was significantly more effective than PHF1 in normalising triglycerides, cholesterol fractions, and liver enzyme activities ($p < 0.05$), with ALT levels in the PHF2 group approaching control values at the 1000 mg/kg dose, indicating marked hepatoprotection.

Table 3 Statistical distribution of ALT and AST, lipid profile, amylase, and lipase enzyme levels in the experimental and control groups

	Control	STZ	STZ+MET	STZ+PHF1	STZ+PHF2
ALT (U/L)	64.86±6.62	101.29±13.43 ^a	90.00±14.33 ^a	68.71±7.91 ^{b, c}	63.57±8.48 ^{b, c}
AST (U/L)	165.00±7.98	190.71±18.59 ^a	170.14±6.34 ^{b, c}	171.86±7.84 ^{a, b, c}	165.29±11.31 ^{a, b, c, d}
Triglycerides (mg/dL)	40.43±1.62	51.14±5.61 ^a	52.86±7.22 ^a	44.29±5.94 ^{b, c}	37.14±2.27 ^{b, c, d}
Cholesterol (mg/dL)	39.71±2.43	50.57±4.31 ^a	34.71±2.75 ^{a, b}	32.29±1.38 ^{a, b}	30.43±3.21 ^{a, b}
LDL (mg/dL)	7.86±1.35	11.14±1.35 ^a	7.14±0.90 ^b	6.14±1.07 ^{a, b}	5.00±0.58 ^{a, b, c}
HDL (mg/dL)	36.71±3.35	18.86±2.67 ^a	34.43±1.90 ^b	38.14±3.80 ^b	44.14±6.09 ^{a, b, c, d}
Amylase (U/L)	1619.7±5.93	1039.5±86.57 ^a	1292.29±105.82 ^{a, b}	1391.71±100.34 ^{a, b}	1440.00±112.87 ^{a, b, c}
Lipase (U/L)	47.07±2.58	26.37±1.33 ^a	32.74±2.03 ^{a, b}	31.86±1.53 ^{a, b}	38.14±1.89 ^{a, b, c, d}

a: significant difference compared to control; b: significant difference compared to STZ; c: significant difference compared to STZ+MET; d: significant difference compared to STZ+PHF1

PHF Preserves Hepatic Structure and Reduces Fibrosis and Glycogen Depletion

Histopathological examination demonstrated marked steatosis, necrosis, hydropic degeneration, and sinusoidal dilatation in liver tissues from the STZ group compared with the control group ($p<0.05$; Fig. 2). Metformin treatment partially attenuated these histopathological alterations. In contrast, both PHF1 and PHF2 significantly improved liver histology compared with the STZ group ($p<0.05$), as evidenced by hematoxylin & eosin, Masson's trichrome, and PAS staining. Notably, PHF2 showed a significantly greater reduction in collagen deposition and a higher PAS staining intensity than PHF1 ($p<0.05$), indicating superior preservation of hepatic tissue structure.

PHF Reduces Hepatic Oxidative Stress and Apoptosis While Promoting Anti-Apoptotic Signalling Pathways

Immunohistochemical analysis revealed significantly increased eNOS, Bax, and Caspase-3 immunoreactivity and a significant decrease in Bcl-2 expression in liver tissues from the STZ group compared with the control group ($p<0.05$; Fig. 3). Metformin treatment partially reversed these alterations relative to the STZ group ($p<0.05$). In contrast, both PHF1 and PHF2 produced significantly greater normalisation of eNOS, Bax, Caspase-3, and Bcl-2 expression compared with metformin ($p<0.05$ vs. STZ and STZ+MET). Notably, PHF2 showed significantly stronger effects than PHF1 in restoring protein expression levels toward control values ($p<0.05$).

PHF Regulates Hepatic Inflammation and Promotes Angiogenic Recovery

Immunohistochemical analysis demonstrated significantly decreased TNF- α and increased IL-1 β immunoreactivity in liver tissues of the STZ group compared with the control group ($p<0.05$). Metformin treatment

partially normalised TNF- α and IL-1 β levels relative to the STZ group ($p<0.05$). In contrast, both PHF1 and PHF2 resulted in significantly greater modulation of TNF- α and IL-1 β expression compared with metformin ($p<0.05$ vs. STZ and STZ+MET). VEGF immunoreactivity was significantly reduced in the STZ group compared with controls ($p<0.05$) and was partially restored by metformin treatment. Both PHF1 and PHF2 significantly increased VEGF expression relative to the STZ and STZ+MET groups ($p<0.05$). Notably, PHF2 produced significantly higher VEGF immunoreactivity than PHF1 ($p<0.05$), indicating a dose-dependent effect (Fig. 4).

PHF Reinstates Pancreatic Architecture and Improves β -Cell Viability

Histopathological evaluation of pancreatic tissue revealed marked β -cell atrophy, irregular islet architecture, and nuclear degeneration in the STZ group compared with the control group ($p<0.05$; Fig. 5). Metformin treatment partially improved islet morphology relative to the STZ group ($p<0.05$). In contrast, both PHF1 and PHF2 treatments resulted in significantly greater preservation of islet architecture and β -cell integrity compared with metformin ($p<0.05$ vs. STZ and STZ+MET). PAS staining demonstrated a significant reduction in glycogen content within pancreatic β -cells in the STZ group compared with controls ($p<0.05$). Metformin moderately restored PAS positivity, whereas PHF1 and PHF2 significantly increased glycogen repletion compared with both the STZ and STZ+MET groups ($p<0.05$). Notably, PHF2 exhibited significantly higher PAS positivity than PHF1 ($p<0.05$), indicating a dose-dependent effect.

PHF Reduces Oxidative Damage and Apoptosis in the Pancreas While Promoting Cytoprotection

Immunohistochemical analysis of pancreatic tissue demonstrated a significant increase in eNOS, Bax, and Caspase-3 immunoreactivity, accompanied by a marked reduction

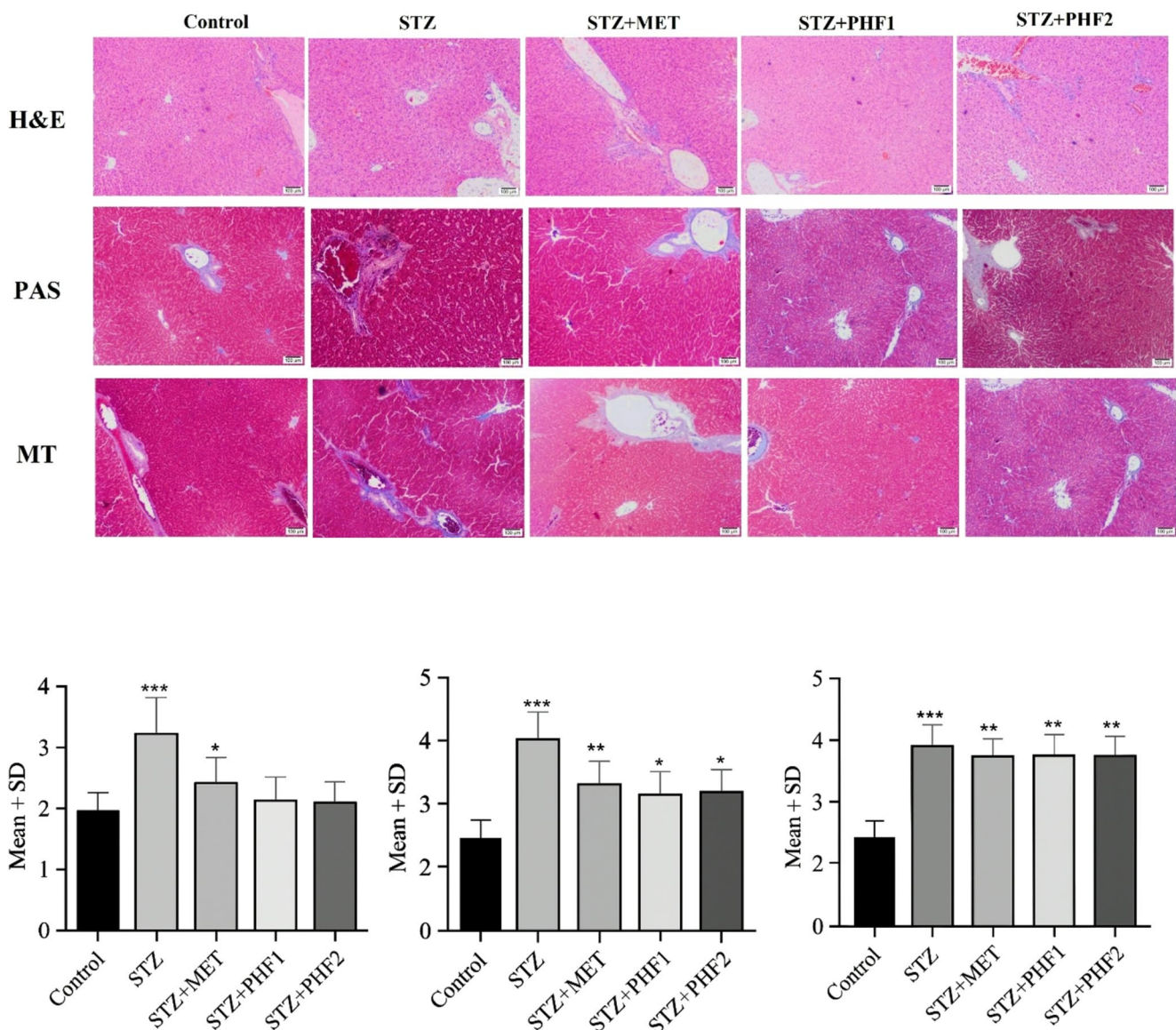


Fig. 2 Morphological changes in liver tissues were evaluated using haematoxylin & eosin (HE), Masson's trichrome (MT), and periodic acid–Schiff (PAS) staining techniques, and the mean±SD values of

histological scores were determined. The lower panel quantifies collagen deposition (MT staining) and glycogen content (PAS staining) in liver tissues

in Bcl-2 expression in the STZ group compared with the control group ($p < 0.05$, Fig. 6). Metformin treatment partially attenuated these alterations relative to the STZ group ($p < 0.05$). Both PHF1 and PHF2 treatments resulted in significantly greater reductions in eNOS, Bax, and Caspase-3 expression and significantly higher Bcl-2 levels compared with the STZ and STZ+MET groups ($p < 0.05$). Notably, PHF2 produced significantly stronger modulation of all evaluated markers than PHF1 ($p < 0.05$), indicating a dose-dependent reduction in oxidative stress and apoptotic signalling within pancreatic tissue.

PHF Inhibits Pancreatic Inflammatory Markers and Promotes VEGF-Mediated Microvascular Repair

Immunohistochemical analysis revealed that pancreatic TNF- α and IL-1 β immunoreactivity was significantly increased in the STZ group compared with the control group ($p < 0.05$; Fig. 7). Metformin treatment partially reduced TNF- α and IL-1 β levels relative to the STZ group ($p < 0.05$). Both PHF1 and PHF2 treatments resulted in significantly lower TNF- α and IL-1 β expression compared with the STZ and STZ+MET groups ($p < 0.05$). Notably,

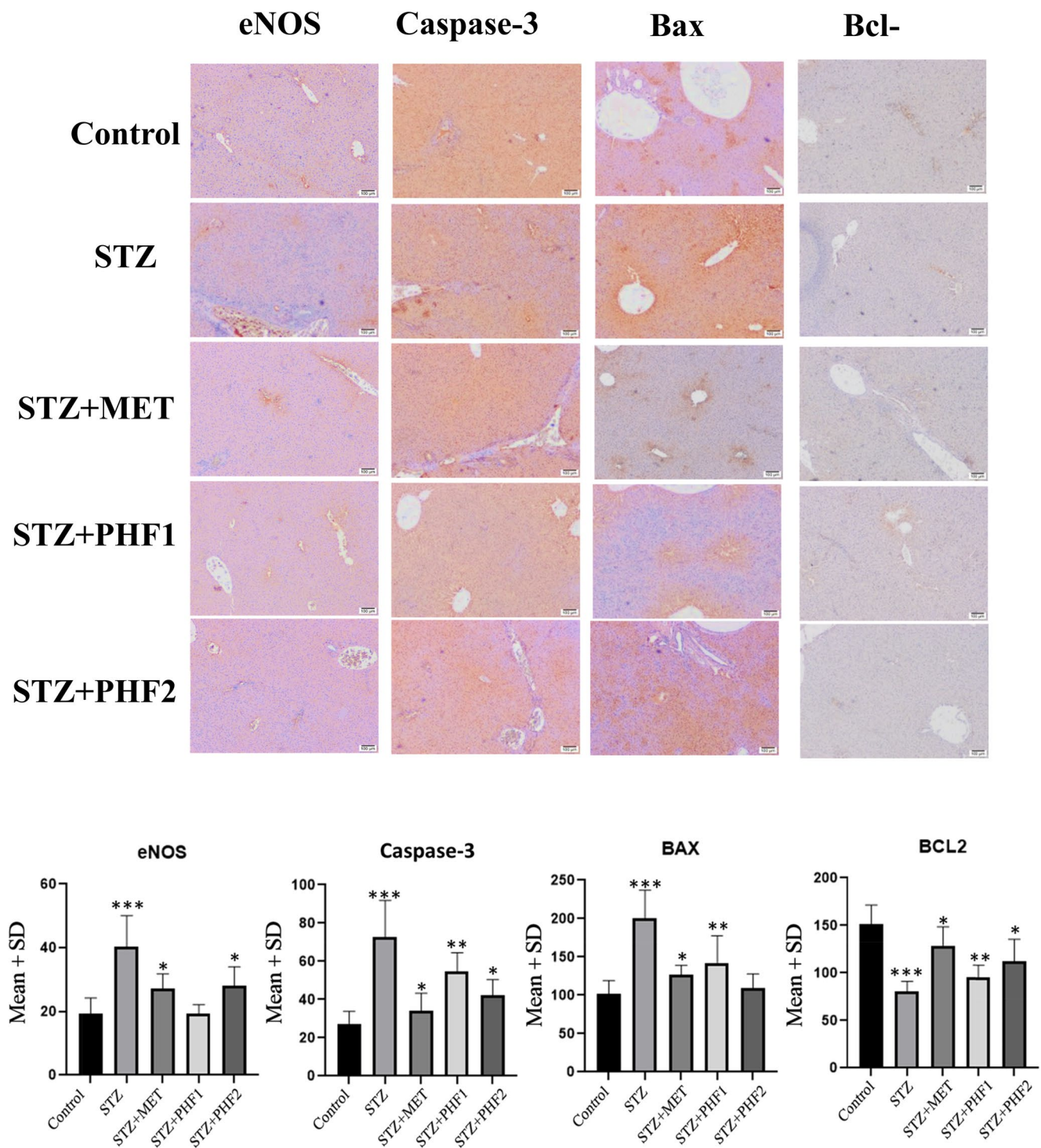


Fig. 3 Immunohistochemical analysis of eNOS, Caspase-3, Bax, and Bcl-2 in STZ-induced diabetic rat livers

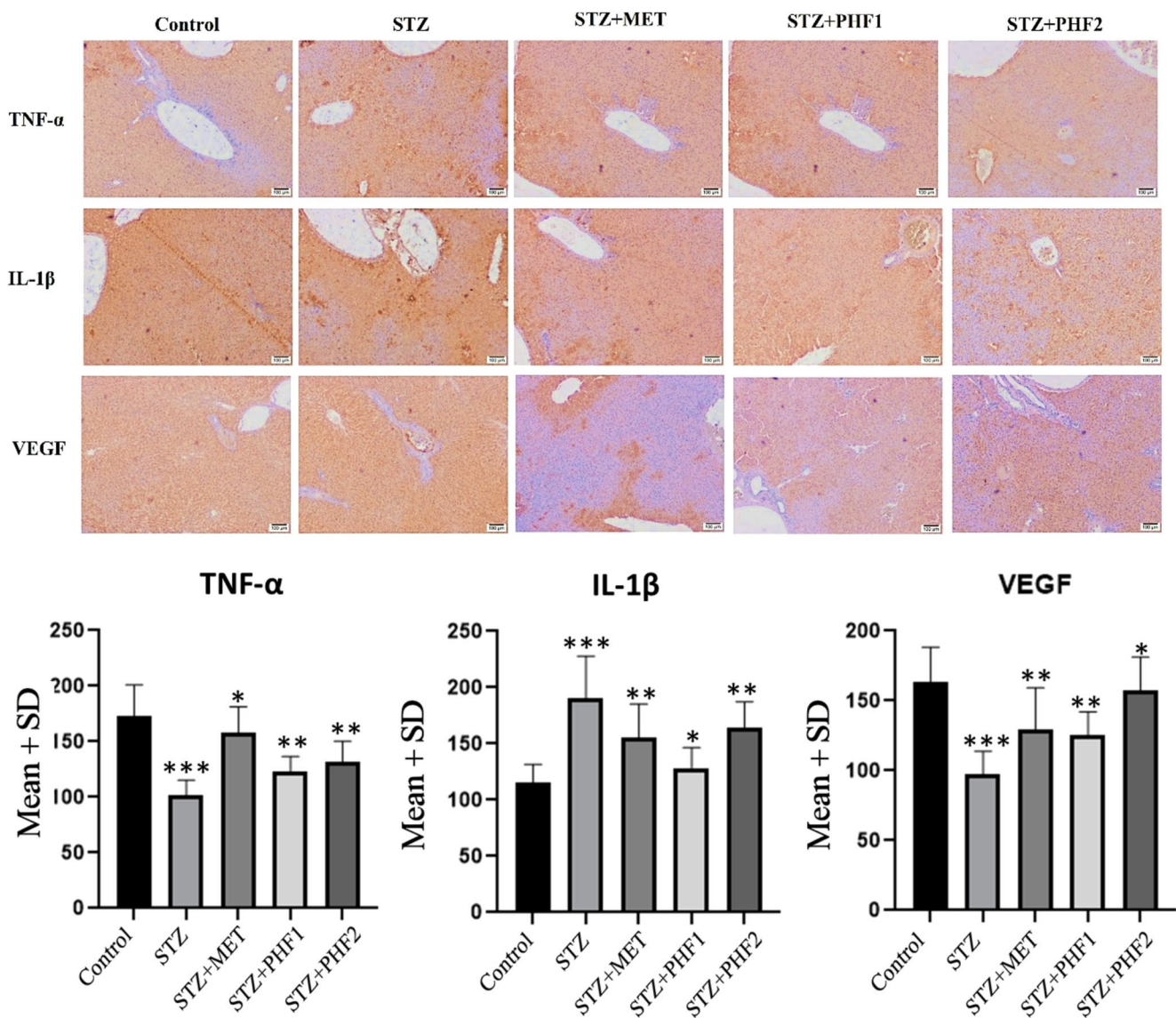


Fig. 4 Immunohistochemical analysis of inflammatory markers (TNF- α , IL-1 β) and the angiogenic marker (VEGF) in the livers of STZ-induced diabetic rats

PHF2 exerted a significantly stronger anti-inflammatory effect than PHF1 ($p < 0.05$), indicating a dose-dependent suppression of pancreatic inflammatory signalling. In contrast, VEGF immunoreactivity was markedly reduced in diabetic pancreatic tissue compared with controls ($p < 0.05$). PHF treatment significantly restored VEGF expression relative to the STZ group ($p < 0.05$), with PHF2 producing significantly higher VEGF levels than both PHF1 and metformin-treated groups ($p < 0.05$), suggesting enhanced microvascular repair.

Network Pharmacology Identifies Key PHF Targets Associated with Oxidative Stress, Apoptosis, Inflammation, and Metabolic Regulation

A total of 333 overlapping targets were identified between PHF constituents and diabetes-related hepatic or pancreatic injury (Fig. 8). STRING-based PPI analysis identified essential hub proteins – AKT1, TNF, IL6, STAT3, NFKB1, PTGS2, CASP3, BCL2, and GSK3B that serve as central regulators of inflammation, apoptosis, and metabolic signalling

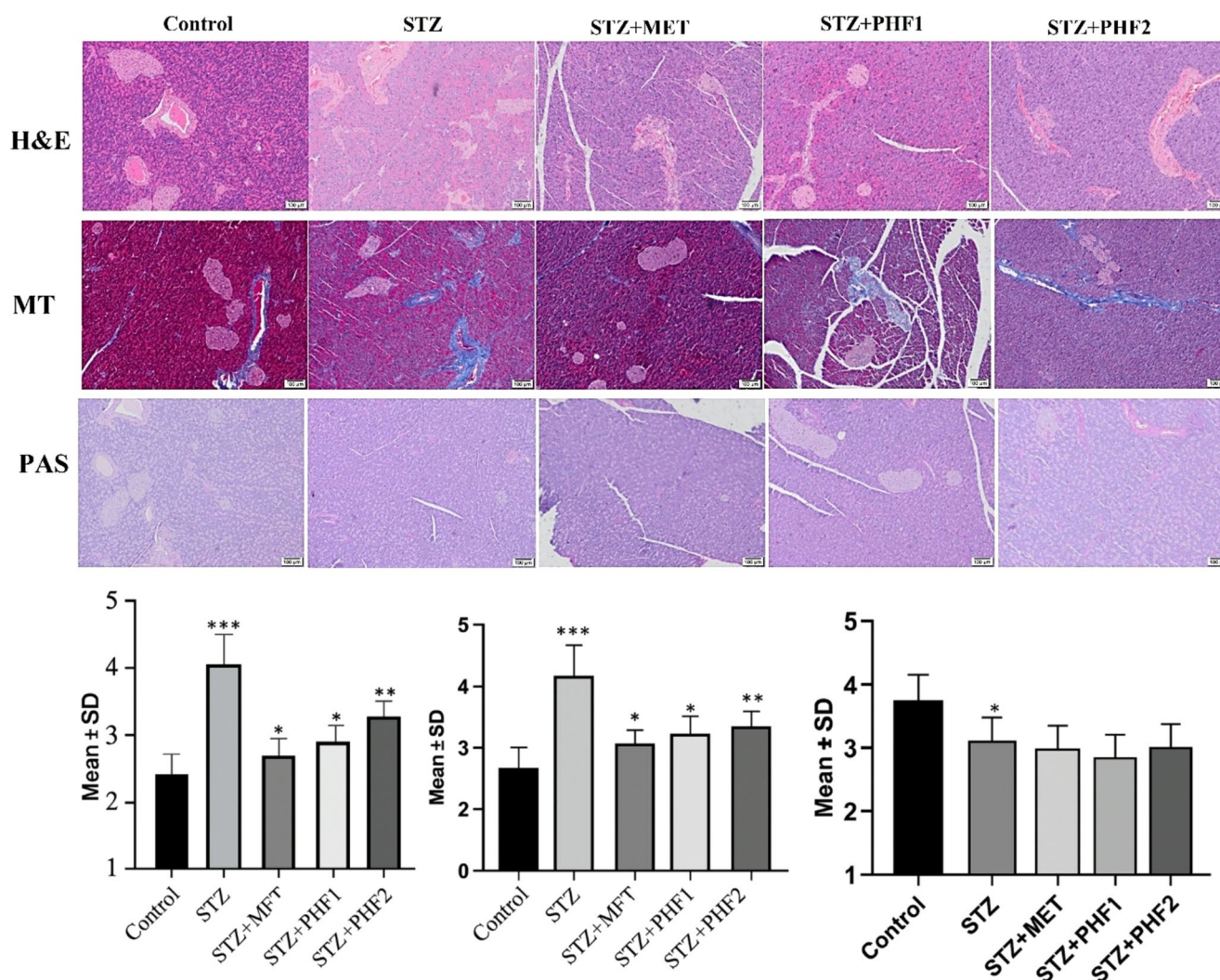


Fig. 5 Histopathological analysis of pancreatic tissue in STZ-induced diabetic rats. Diabetic rats showed β -cell atrophy, disorganized islets, nuclear necrosis, and swollen exocrine acini. PHF treatment (500 and

1000 mg/kg) dose-dependently restored islet architecture and β -cell integrity, indicating enhanced insulin secretion and antidiabetic activity, and the mean $\pm$ SD values of histological scores were determined

(Figs. 9). GO/KEGG enrichment analysis indicated significant involvement of the PI3K-Akt, MAPK, AGE-RAGE, TNF, calcium, and metabolic pathways (Fig. 10), consistent with the experimentally observed anti-inflammatory, anti-apoptotic, and antioxidant effects of PHF.

Discussion

This study presents a novel approach by integrating network pharmacology with experimental validation to evaluate the multi-target therapeutic effects of a polyherbal formulation (PHF) in the context of diabetes-induced hepatic and pancreatic injury. Unlike previous studies, this work systematically investigates the combined effects of *Curcuma longa*, *Nigella sativa*, *Prunus mahaleb*, and *Vitis vinifera* on glycaemic regulation, oxidative stress, inflammation, apoptosis, and

angiogenesis, providing new insights into their synergistic therapeutic potential. The streptozotocin (STZ)-induced diabetic model effectively replicates key characteristics of diabetes, including pancreatic β -cell destruction, persistent hyperglycaemia, insulin deficiency, dyslipidaemia, and hepatic injury, making it a valuable tool for assessing antidiabetic interventions [11, 41]. Consistent with earlier findings, STZ administration in this study led to significant increases in fasting blood glucose, notable decreases in serum insulin, and severe impairment of pancreatic histoarchitecture, demonstrating its recognised β -cell cytotoxic effects through mechanisms such as DNA alkylation, oxidative stress, and mitochondrial dysfunction [42, 43]. These changes indicate that STZ induces DNA alkylation and oxidative stress in β -cells, leading to apoptosis and reduced insulin secretion [44]. STZ also stimulates NF- κ B-mediated inflammatory pathways and impairs mitochondrial function

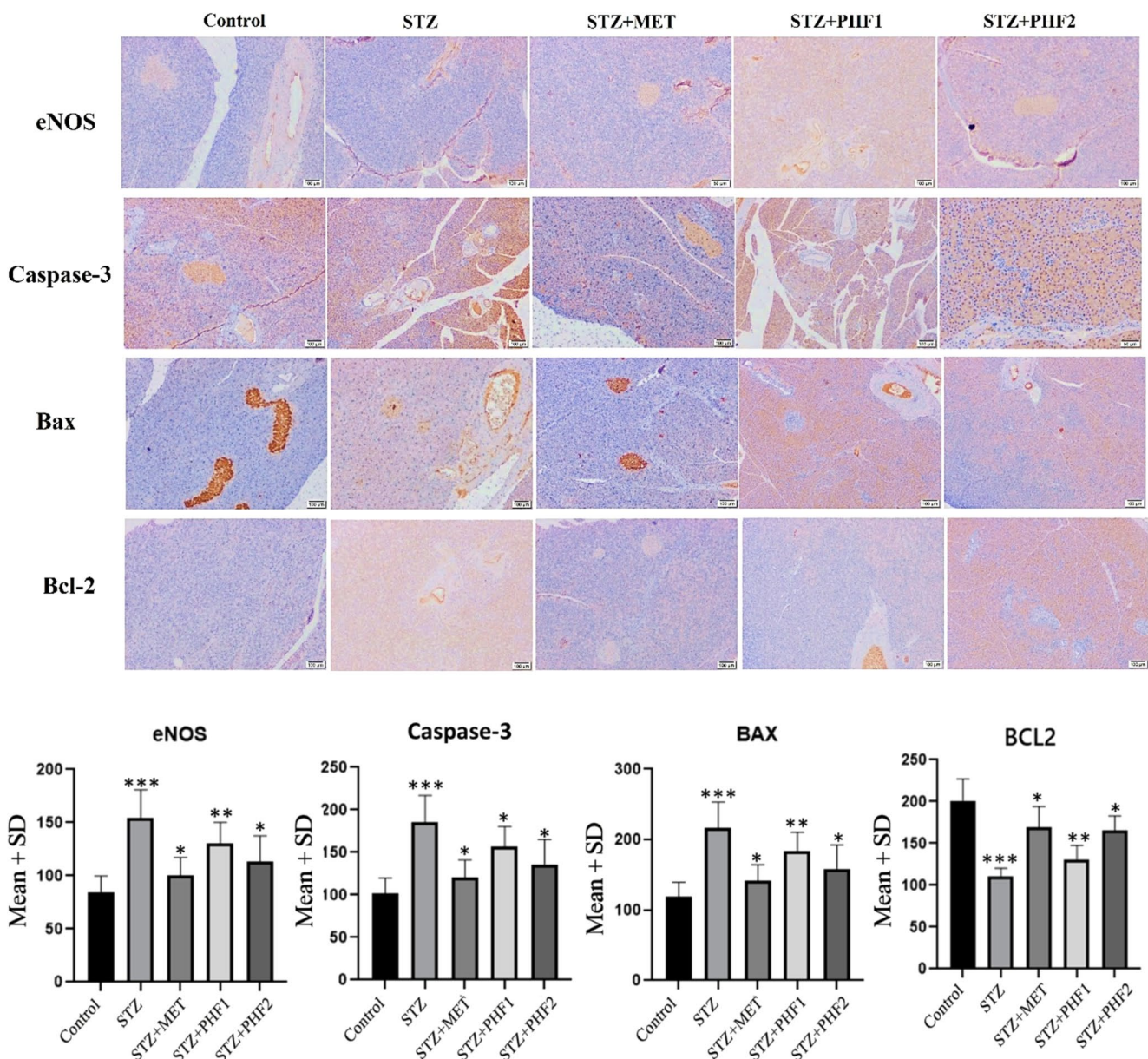


Fig. 6 Immunohistochemical analysis of pancreatic oxidative stress and apoptosis markers in STZ-induced diabetic rats. Diabetic pancreases showed increased eNOS, CASP3, and Bax, and decreased Bcl-2 expression. MET and PHF treatments (500 and 1000 mg/kg)

restored eNOS, CASP3, and Bax, and increased Bcl-2 levels, indicating reduced oxidative stress, anti-apoptotic effects, and improved β -cell survival

[44, 45], indicating the need for therapeutic strategies that can simultaneously attenuate oxidative, apoptotic, and inflammatory pathways.

Oral administration of the polyherbal formulation (PHF) significantly improved glycaemic control, particularly at the 1000 mg/kg dose, as shown by normalised fasting blood glucose levels and increased serum insulin concentrations. Histopathological and immunohistochemical analyses revealed partial β -cell regeneration, increased Bcl-2 expression, and reduced Bax and Caspase-3 levels, indicating PHF-mediated inhibition of mitochondrial apoptosis. These effects

are consistent with previous research showing that *Curcuma longa* supports β -cell viability through antioxidative and anti-inflammatory mechanisms, while *Nigella sativa* enhances insulin sensitivity and reduces oxidative damage [10, 46, 47]. Both plants activate the PI3K/Akt and ERK1/2 signalling pathways, which contribute to β -cell survival and proliferation [48, 49]. This finding is consistent with other studies demonstrating intracellular survival signalling induced by polyphenolic compounds [48, 49], consistent with other reports demonstrating intracellular survival signalling triggered by polyphenolic compounds [50, 51]. The

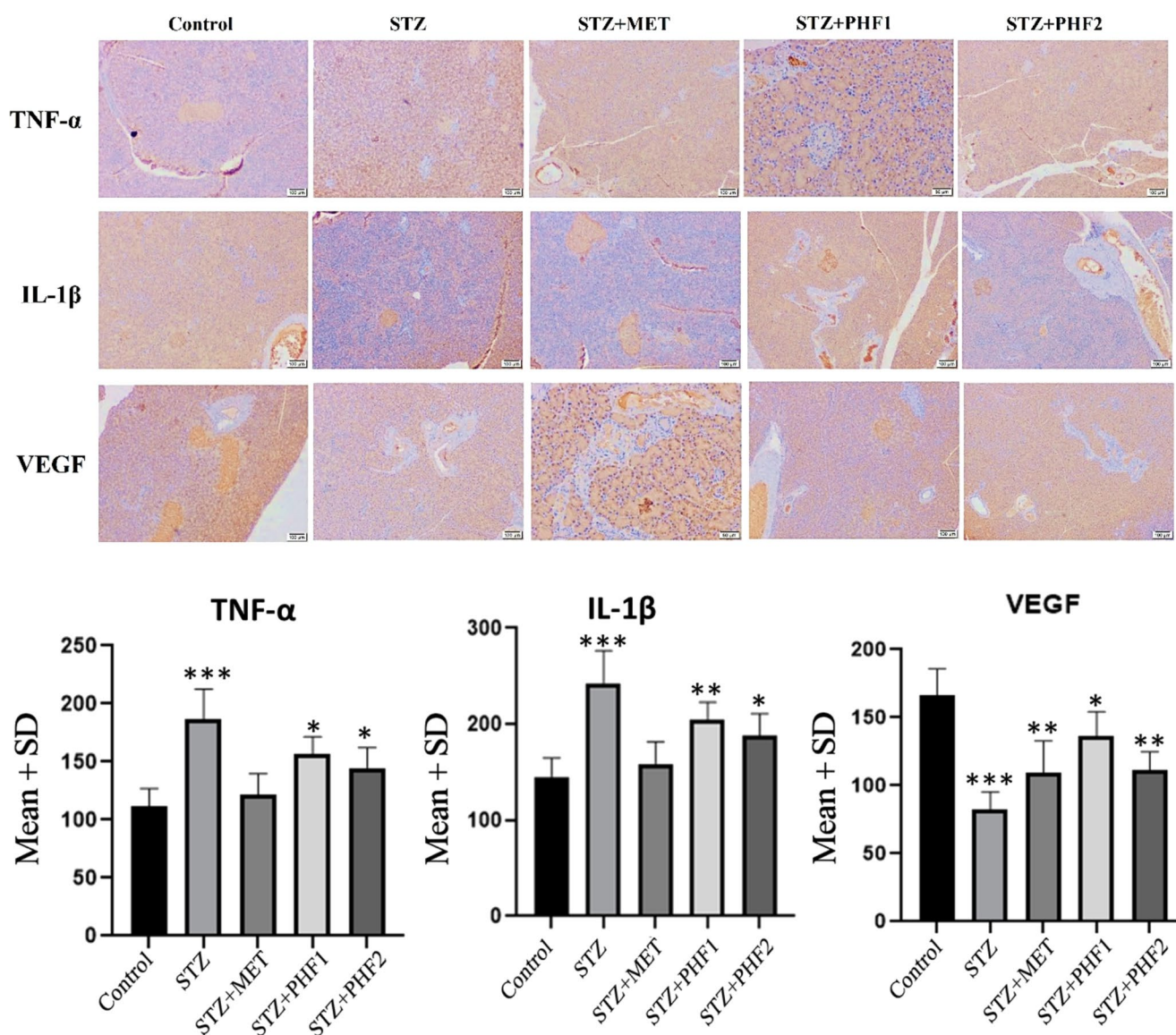


Fig. 7 Immunohistochemical analysis of pancreatic inflammation and angiogenesis markers. STZ-induced diabetes increased TNF- α and IL-1 β , and decreased VEGF expression in pancreatic tissue. Treat-

ment with MET and PHF (500 and 1000 mg/kg) significantly reduced TNF- α and IL-1 β levels and restored VEGF expression, indicating anti-inflammatory and pro-angiogenic effects

observed improvement in the glycaemic profile may reflect PHF-mediated ROS scavenging, suppression of TNF- α and IL-1 β , and enhancement of survival signalling pathways [52, 53].

In the liver, STZ caused significant hepatocellular damage, as indicated by increased ALT and AST levels, steatosis, necrosis, sinusoidal dilation, and fibrotic changes – results indicative of hepatic dysfunction associated with persistent hyperglycaemia and lipotoxicity [54, 55]. Treatment with PHF significantly improved these changes, restoring hepatic architecture and reducing apoptotic activity, presumably through the synergistic antioxidative and anti-inflammatory properties of its components. Previous research indicates that *Curcuma longa* and *Nigella sativa*

reduce hepatic oxidative stress, decrease inflammatory cytokines, and prevent hepatocyte apoptosis by activating the Nrf2/ARE signalling pathway and inducing antioxidant enzymes, including HO-1 and NQO1 [56, 57, 58]. The elevated levels of Bcl-2 and the decreased levels of Bax and Caspase-3 identified in our study provide further evidence for the inhibition of mitochondria-mediated apoptosis. These findings are consistent with reports indicating hepatoprotection conferred by plant-derived flavonoids and antioxidants [59–61].

PHF significantly improved diabetes-related dyslipidaemia by reducing triglycerides, total cholesterol, and LDL, while increasing HDL. Similar lipid-modulating effects have been attributed to the activation of the AMPK and

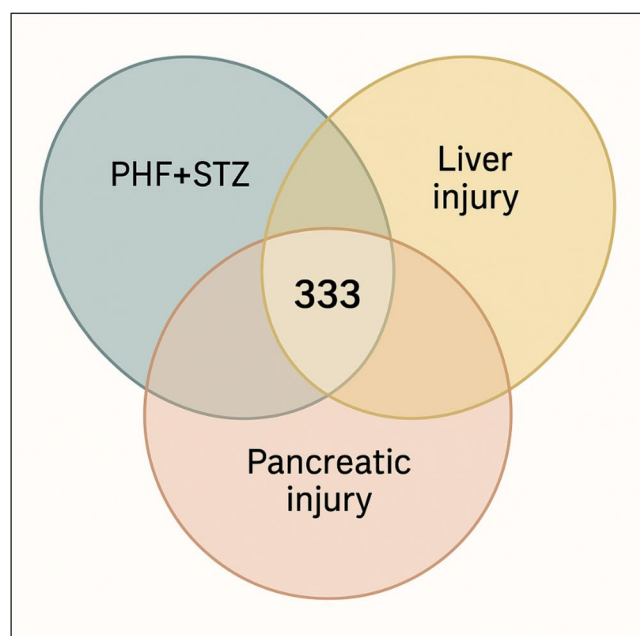


Fig. 8 Venn diagram illustrating the overlapping targets between PHF, liver injury, and pancreatic injury, highlighting 333 common targets

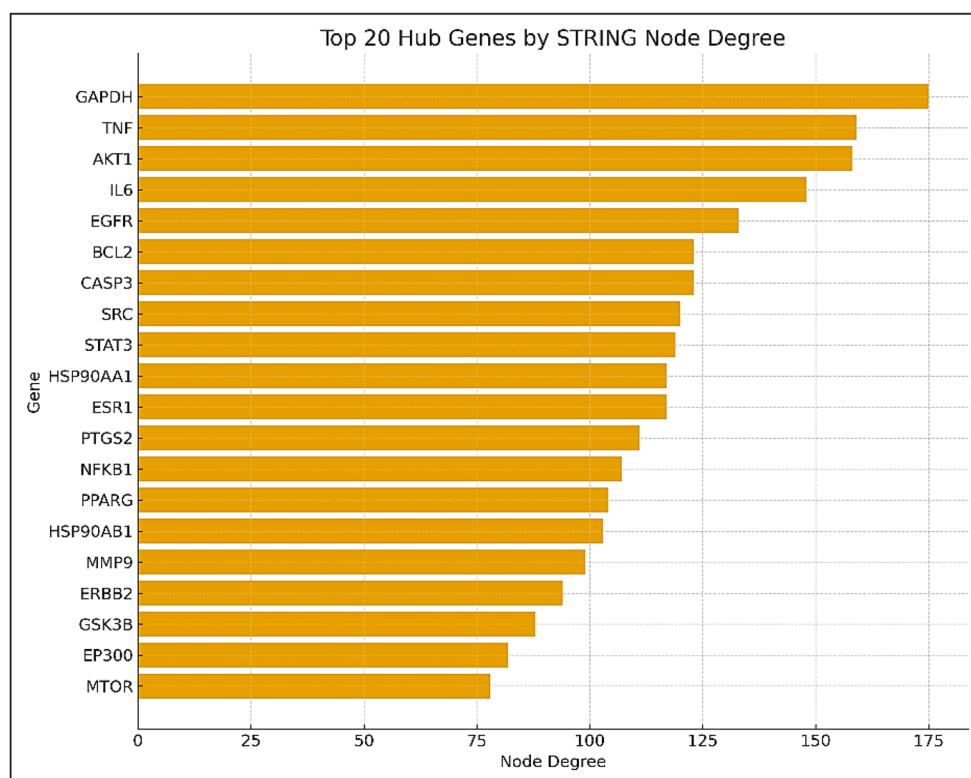
PPAR γ pathways, as demonstrated by studies on *Curcuma longa* and other polyphenols [62–64]. The reduced expression of TNF- α and IL-1 β in hepatic and pancreatic tissues indicates substantial anti-inflammatory efficacy, likely associated with the inhibition of NF- κ B signalling [53].

Collectively, these outcomes indicate that PHF offers extensive metabolic and anti-inflammatory benefits.

Network pharmacology provided further mechanistic insights by identifying 333 common targets associated with PHF, hepatic injury, and pancreatic injury. The PPI study indicated that hub proteins such as AKT1, TNF, IL6, EGFR, STAT3, NFKB1, PTGS2, CASP3, BCL2, and GSK3B play pivotal regulatory roles. These compounds are extensively characterised in relation to STZ-induced oxidative stress, inflammation, and apoptosis [65, 66]. GO-BP enrichment identified processes such as “response to chemical stimulus,” “response to oxygen-containing compound,” “regulation of apoptotic process,” and “metabolic process,” consistent with existing literature on curcumin-induced NFE2L2/HO-1 activation, thymoquinone-mediated cytokine suppression, and resveratrol-driven PI3K-Akt/SIRT1 signalling [67–69]. KEGG analysis indicated enrichment in the PI3K-Akt, MAPK, calcium, cAMP, TNF, and neuroactive ligand–receptor pathways, which are critical signalling pathways disrupted in diabetes [70, 71]. Moreover, the increased activity of STAT3, NFKB1, IL6, TNF, and PTGS2 in STZ animals is well documented [72–74]. and components of PHF, including curcumin, resveratrol, and thymoquinone, have been shown to inhibit these pathways [75, 76].

The network pharmacology results indicate that PHF exerts a multi-targeted regulatory effect by influencing oxidative stress, inflammation, apoptosis, angiogenesis, and

Fig. 9 Bar plot showing the top 20 hub genes identified by STRING analysis, ranked by node degree



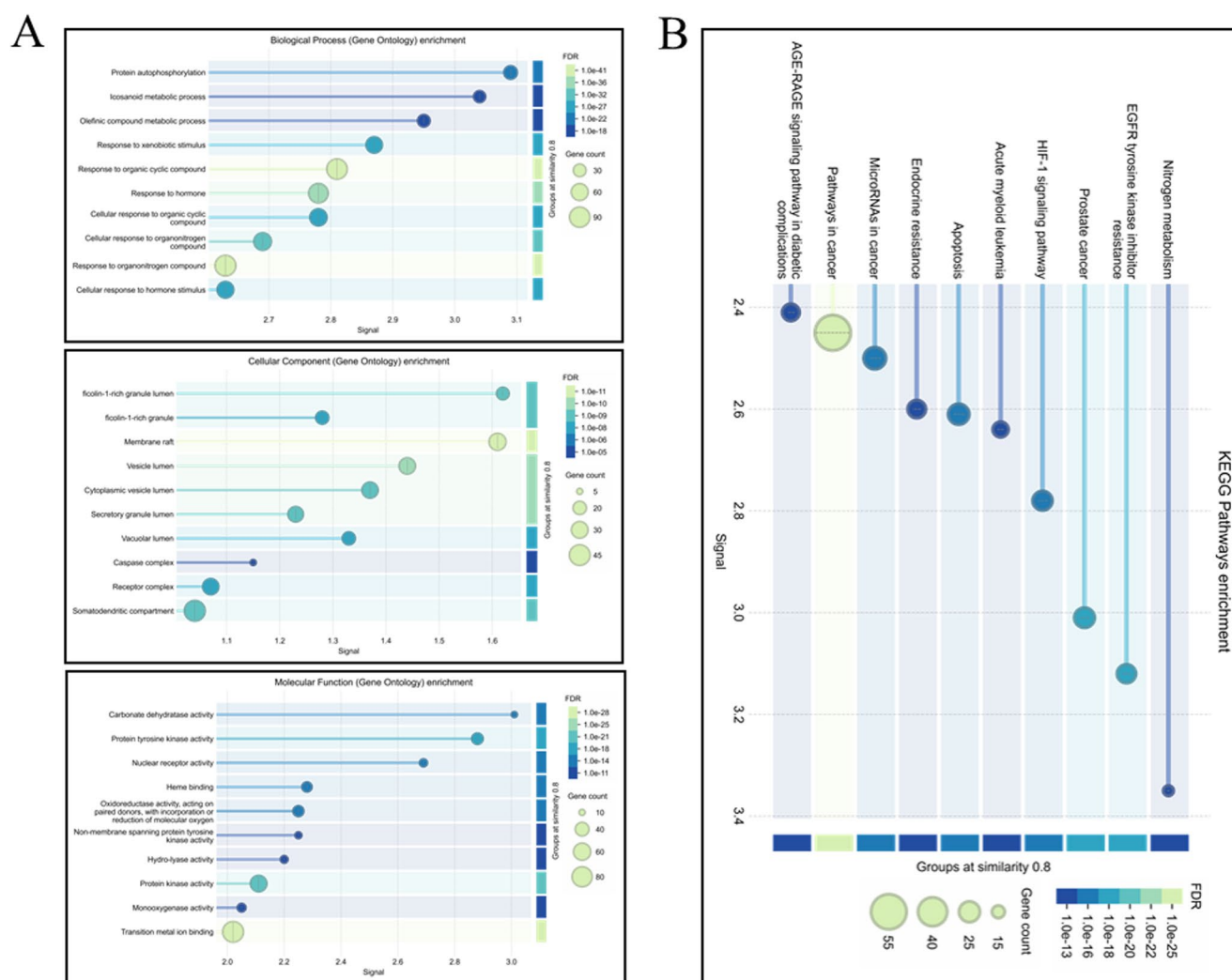


Fig. 10 BP, MF, CC, and KEGG pathway enrichment analysis of the 333 overlapping targets, showing significant biological processes and pathways involved

metabolic pathways. These integrative mechanisms reflect the traditional rationale underlying polyherbal therapies. The present study demonstrates that PHF promotes β -cell regeneration, restores liver and pancreatic function, normalises glycaemic and lipid profiles, reduces oxidative stress and inflammation, and improves microvascular repair. Although these findings strongly support PHF as a natural multi-target therapeutic candidate for diabetes treatment, further molecular and clinical studies are required to validate its translational potential.

Conclusion

The polyherbal formulation (PHF) containing *Curcuma longa*, *Nigella sativa*, *Prunus mahaleb*, and *Vitis vinifera* demonstrates significant antidiabetic and tissue-protective efficacy in STZ-induced metabolic dysfunction. PHF

restores glycaemic control, enhances insulin secretion, improves lipid metabolism, and preserves hepatic and pancreatic structural integrity. Alongside its experimentally confirmed antioxidant, anti-inflammatory, antiapoptotic, and pro-angiogenic properties, network pharmacology analysis indicates that PHF acts through a multi-target regulatory mechanism involving key molecular hubs such as AKT1, TNF, IL6, STAT3, PTGS2, and BCL2. Enrichment of PI3K-Akt, MAPK, calcium, cAMP, and metabolic pathways suggests that PHF modulates interconnected signalling networks disrupted by STZ toxicity. Overall, these findings show that PHF exerts a coordinated, systems-level therapeutic effect and represents a promising integrative strategy for the prevention and management of diabetes-associated hepatic and pancreatic injury.

Acknowledgements We would like to thank Eskişehir Osmangazi University for providing laboratory facilities for some of the experimental work of the study.

Author Contributions Isa Kıran : Investigation, Validation, Conceptualization, Writing - original draft, Writing - review & editing. Mustafa Cengiz : Writing - original draft, Conceptualization, Methodology, Software, Writing - review & editing, Adnan Ayhanci : Investigation, Methodology, Data curation, Formal analysis.

Funding Open access funding provided by the Scientific and Technological Research Council of Türkiye (TÜBİTAK).

Data Availability No datasets were generated or analysed during the current study.

Declarations

Ethics Approval and Consent to Participate The study was approved by the ESOGU Animal Experiments Ethics Committee (Protocol No: 954/2023).

Consent for Publication Not applicable.

Competing Interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Al-Shamsi M, Amin A, Adeghate E. Vitamin E decreases the hyperglucagonemia of diabetic rats. *Ann N Y Acad Sci*. 2006;1084:432–41.
- Al Hrouf A, Cervantes-Gracia K, Chahwan R, Amin A. Modeling liver cancer microenvironment using a novel 3D culture system. *Sci Rep*. 2022;12(1):8003.
- Jain A, Jangid T, Jangir RN, Bhardwaj GS. Antidiabetic activity of polyherbal formulations: a comprehensive review. *Protoplasma*. 2025;262(5):1031–52.
- Niu F, Liu W, Ren Y, Tian Y, Shi W, Li M, et al. β -cell neogenesis: a rising star to rescue diabetes mellitus. *J Adv Res*. 2024;62:71–89.
- Poznyak A, Grechko AV, Poggio P, Myasoedova VA, Alfieri V, Orekhov AN. The diabetes mellitus–atherosclerosis connection: the role of lipid and glucose metabolism and chronic inflammation. *Int J Mol Sci*. 2020;21(5):1835.
- TEMED. Diabetes mellitus ve Komplikasyonlarının Tani. Tedavi ve İzlem Kılavuzu. 2024.
- Zhao N, Yu X, Zhu X, Song Y, Gao F, Yu B, Qu A. Diabetes mellitus to accelerated atherosclerosis: shared cellular and molecular mechanisms in glucose and lipid metabolism. *J Cardiovasc Transl Res*. 2024;17(1):133–52.
- Ajele KW, Ugwu LE, Idemudia ES. Diabetes in Africa: Rising Prevalence, Premature Death, and Health System Challenges, 2024–2050. Premature Death, and Health System Challenges 2050. 2024.
- Habibah N, Rofi'ah LK, Bektı HS, Dewi NNA, Wilankrisna LA, Posmaningsih DAA. The efficacy of green grapefruit (*Vitis vinifera* L) extracts on reducing blood glucose in a diabetic rat model. *Med Lab Technol J*. 2024;10(2):80–94.
- Kanter M, Coskun O, Korkmaz A, Oter S. Effects of *Nigella sativa* on oxidative stress and β -cell damage in streptozotocin-induced diabetic rats. *Anat Rec Part A Discov Mol Cell Evol Biol*. 2004;279A(1):685–91.
- Konda PY, Nagalapuram R, Venkateswarlu JKM, Mohammad SA, Chippada AR. Pathophysiology of STZ-induced pancreatic β cell injury and dysfunction: traditional role of *Boswellia ovalifoliolata* Bal. & Henry on diabetes and dyslipidemia. *Comp Clin Pathol*. 2020;29(3):609–19.
- Mostafa TM, Hegazy SK, Elnaidany SS, Shehabeldin WA, Sawan ES. *Nigella sativa* as a promising intervention for metabolic and inflammatory disorders in obese prediabetic subjects: a comparative study of *Nigella sativa* versus both lifestyle modification and metformin. *J Diabetes Complications*. 2021;35(7):107947.
- Panahi Y, Khalili N, Sahebi E, Namazi S, Reiner Ž, Majeed M, et al. Curcuminoids modify lipid profile in type 2 diabetes mellitus: a randomized controlled trial. *Complement Ther Med*. 2017;33:1–5.
- Sharma RK, Jha N, Mishra A, Singh G, Sanwelka M, Gupta A. Therapeutic effectiveness of *Prunus dulcis* for nephroprotective and hepatoprotective in albino mice. *Curr Indian Sci*. 2024;2(1).
- Zarei M, Sahebi Vaighan N, Farjoo MH, Talebi S, Zarei M. Incretin-based therapy: a new horizon in diabetes management. *J Diabetes Metab Disord*. 2024;23(2):1665–86.
- Foretz M, Guigas B, Bertrand L, Pollak M, Viollet B. Metformin: from mechanisms of action to therapies. *Cell Metabol*. 2014;20(6):953–66.
- Bateni Z, Rahimi HR, Hedayati M, Afsharian S, Goudarzi R, Sohrab G. The effects of nano-curcumin supplementation on glycemic control, blood pressure, lipid profile, and insulin resistance in patients with the metabolic syndrome: a randomized, double-blind clinical trial. *Phytother Res*. 2021;35(7):3945–53.
- Kanter M, Akpolat M, Aktas C. Protective effects of the volatile oil of *Nigella sativa* seeds on β -cell damage in streptozotocin-induced diabetic rats: a light and electron microscopic study. *J Mol Histol*. 2009;40(5):379–85.
- Lam C-S, Xia Y-X, Chen B-S, Du Y-X, Liu K-L, Zhang H-J. Dihydro-resveratrol attenuates oxidative stress, adipogenesis and insulin resistance in in vitro models and high-fat diet-induced mouse model via AMPK activation. *Nutrients*. 2023;15(13):3006.
- Hopkins AL. Network pharmacology: the next paradigm in drug discovery. *Nat Chem Biol*. 2008;4(11):682–90.
- Li S, Zhang B. Traditional Chinese medicine network pharmacology: theory, methodology and application. *Chin J Nat Med*. 2013;11(2):110–20.
- Forbes JM, Cooper ME. Mechanisms of diabetic complications. *Physiol Rev*. 2013;93(1):137–88.
- Gezer A, Ustundag H, Mendil AS, Bedir G, Duysak L. Hepatoprotective effects of resveratrol on α -amanitin-induced liver toxicity in rats. *Toxicol*. 2024;247:107855.
- Lv J, Su M, Wang Y, Yang J, Liang Y, Chen L, et al. Yunvjian decoction mitigates hyperglycemia in rats induced by a high-fat diet and streptozotocin via reducing oxidative stress in pancreatic beta cells. *J Ethnopharmacol*. 2024;327:118045.
- Sheethal S, Jose SP, Ratheesh M, Rajan S, Sandya S, Samuel S, et al. Anti-diabetic effect of herbal formulation (Glymin Plus) on experimental model: possible role in GLP-1 associated pathway. *J Pharm Innov*. 2025;20(4):145.

26. Alberti A. Herstellung der Phyto-Arzneien therapeutische Anwendung der Pflanzlichen Drogen phytotherapie: Traditionelle und evidenzbasierte medizin. ESCOP, EMA Monografien. 2015.
27. Furman BL. Streptozotocin-induced diabetic models in mice and rats. *Curr Protoc Pharmacol*. 2015;70(1):5.47.41–45.47.20.
28. Phina UA, KI OE, Obiora UJ. Antidiabetic effect of methanol leaf extract of *Jatropha Gossypifolia* on normal and Streptozotocin-Induced Diabetic Male Albino Rats.
29. Wang W, Bai D, Wu C, Li H, Xie X, Ji W, et al. A protocol for constructing a rat wound model of type 1 diabetes. *JoVE*. 2023;192:e64914.
30. Karempudi VK, Gokul TA, Kumar KR, Veeramanikandan V, Ali D, Impellitteri F, et al. Protective role of *Pleurotus florida* against streptozotocin-induced hyperglycemia in rats: a preclinical study. *Biomed Pharmacother*. 2024;170:116005.
31. Jaikumkao K, Thongnak L, Htun KT, Pengrattanachot N, Phengpol N, Sutthasupha P, et al. Dapagliflozin and metformin in combination ameliorates diabetic nephropathy by suppressing oxidative stress, inflammation, and apoptosis and activating autophagy in diabetic rats. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*. 2024;1870(1):166912.
32. Ramesh B, Pugalendi K. Antioxidant role of umbelliferone in STZ-diabetic rats. *Life Sci*. 2006;79(3):306–10.
33. Szkudelski T. The mechanism of Alloxan and streptozotocin action in B cells of the rat pancreas. *Physiol Res*. 2001;50(6):537–46.
34. Adam GO, Rahman MM, Lee S-J, Kim G-B, Kang H-S, Kim J-S, et al. Hepatoprotective effects of *Nigella sativa* seed extract against acetaminophen-induced oxidative stress. *Asian Pac J Trop Med*. 2016;9(3):221–7.
35. Abid S, Mekhfi H, Ziyayt A, Legssyer A, Aziz M, Bnouham M. Beneficial effect of *Thymelaea hirsuta* on pancreatic islet degeneration, renal fibrosis, and liver damages as demonstrated in streptozotocin-induced diabetic rat. *Sci World J*. 2021;2021(1):6614903.
36. Bancroft JD, Gamble M. Theory and practice of histological techniques. Elsevier Health Sciences. 2008.
37. Ayhanci A, Cengiz M, Mehtap Kutlu H, Veyselova D. Protective effects of ellagic acid in D-galactosamine-induced kidney damage in rats. *Cytotechnology*. 2016;68(5):1763–70.
38. Botros SR, Matouk AI, Amin A, Heeba GH. Comparative effects of incretin-based therapy on doxorubicin-induced nephrotoxicity in rats: the role of SIRT1/Nrf2/NF- κ B/TNF- α signaling pathways. *Front Pharmacol*. 2024;15:1353029.
39. Ismail R, Habib HA, Anter AF, Amin A, Heeba GH. Modified citrus pectin ameliorates methotrexate-induced hepatic and pulmonary toxicity: role of Nrf2, galectin-3/TLR-4/NF- κ B/TNF- α and TGF- β signaling pathways. *Front Pharmacol*. 2025;16:1528978.
40. Alresheedi YS, Nour OA, Nader MA, Zaghloul MS. Targeting SIRT-1/AMPK/Nrf2 signaling pathway by tenofovir protected against. *Pharmaceutics: Cyclophosphamide-Induced Nephrotoxicity and Cardiotoxicity in Rats*; 2025.
41. Mohamed J, Nazratun Nafizah AH, Zariyantey AH, Budin SB. Mechanisms of diabetes-induced liver damage: the role of oxidative stress and inflammation. *Sultan Qaboos Univ Med J*. 2016;16(2):e132–141.
42. Nahdi AMTA, John A, Raza H. Elucidation of molecular mechanisms of streptozotocin-induced oxidative stress, apoptosis, and mitochondrial dysfunction in Rin-5F pancreatic β -cells. *Oxid Med Cell Longev*. 2017;2017(1):7054272.
43. Yavuz O, Dincel GC, Yildirim S, El-Ashram S, Al-Olayan E. Impact of apoptosis and oxidative stress on pancreatic beta cell pathophysiology in streptozotocin-induced type 1 diabetes mellitus. *Tissue Cell*. 2024;91:102552.
44. Manna P, Das J, Ghosh J, Sil PC. Contribution of type 1 diabetes to rat liver dysfunction and cellular damage via activation of NOS, PARP, I κ B α /NF- κ B, MAPKs, and mitochondria-dependent pathways: prophylactic role of Arjunolic acid. *Free Radic Biol Med*. 2010;48(11):1465–84.
45. Farid A, Moussa P, Youssef M, Haytham M, Shamy A, Safwat G. Melatonin relieves diabetic complications and regenerates pancreatic beta cells by the reduction in NF- κ B expression in streptozotocin induced diabetic rats. *Saudi J Biol Sci*. 2022;29(7):103313.
46. Balbaa M, El-Zeftawy M, Ghareeb D, Taha N, Mandour AW. *Nigella sativa* relieves the altered insulin receptor signaling in streptozotocin-induced diabetic rats fed with a high-fat diet. *Oxid Med Cell Longev*. 2016;2016(1):2492107.
47. Hamidpour R, Hamidpour S, Hamidpour M, Sohraby M, Hamidpour M. Turmeric (*Curcuma longa*): from a variety of traditional medicinal applications to its novel roles as active antioxidant, anti-inflammatory, anti-cancer, and anti-diabetes. *Int J Pharmacol Phytochem Ethnomed*. 2015;1(1):37–45.
48. Sadeghi E, Imenshahidi M, Hosseinzadeh H. Molecular mechanisms and signaling pathways of black cumin (*Nigella sativa*) and its active constituent, thymoquinone: a review. *Mol Biol Rep*. 2023;50(6):5439–54.
49. Wang H, Zhang K, Liu J, Yang J, Tian Y, Yang C, Li Y, Shao M, Su W, Song N. Curcumin Regulates Cancer Progression: Focus on ncRNAs and Molecular Signaling Pathways. *Frontiers in Oncology*. 2021;11.
50. Bera TK, De D, Chatterjee K, Ali KM, Ghosh D. Effect of diashis, a polyherbal formulation, in streptozotocin-induced diabetic male albino rats. *Int J Ayurveda Res*. 2010;1(1):18–24.
51. Chatterjee K, Ali KM, De D, Bera TK, Jana K, Maiti S, Ghosh A, Ghosh D. Hyperglycemia-induced alteration in reproductive profile and its amelioration by the polyherbal formulation MTEC (modified) in streptozotocin-induced diabetic albino rats. *Biomarkers Genomic Med*. 2013;5(1):54–66.
52. Jilakara VS, Jadhav AB, Chellammal HSJ, Jahnavi G, Khanam R, Babu MK, et al. Design and development of a polyherbal formulation containing *Gymnema sylvestre*, *Momordica charantia*, and *Trigonella foenum-graecum* for the treatment of diabetes mellitus. *Cuestiones Fisioter*. 2025;54(3):3269–83.
53. Karstoft K, Clark MA, Jakobsen I, Müller IA, Pedersen BK, Solomon TPJ, Ried-Larsen M. The effects of 2 weeks of interval vs continuous walking training on glycaemic control and whole-body oxidative stress in individuals with type 2 diabetes: a controlled, randomised, crossover trial. *Diabetologia*. 2017;60(3):508–17.
54. Mahfoz AM, Gawish AY. Insight into the hepatoprotective, hypolipidemic, and antidiabetic impacts of aliskiren in streptozotocin-induced diabetic liver disease in mice. *Diabetology & Metabolic Syndrome*. 2022;14(1):163.
55. Rehman Hu, Ullah K, Rasool A, Manzoor R, Yuan Y, Tareen AM, Kaleem I, Riaz N, Hameed S, Bashir S. Comparative impact of streptozotocin on altering normal glucose homeostasis in diabetic rats compared to normoglycemic rats. *Sci Rep*. 2023;13(1):7921.
56. Bhattamisra SK, Koh HM, Lim SY, Choudhury H, Pandey M. 2021. Molecular and Biochemical Pathways of Catalpol in Alleviating Diabetes Mellitus and Its Complications, *Biomolecules*.
57. Sun M, Zhao X, Li X, Wang C, Lin L, Wang K, Sun Y, Ye W, Li H, Zhang Y, Huang C. Aerobic exercise ameliorates liver injury in Db/Db mice by attenuating oxidative Stress, apoptosis and inflammation through the Nrf2 and JAK2/STAT3 signalling pathways. *J Inflamm Res*. 2023;16(null):4805–19.
58. Zhang Z, Huang Q, Zhao D, Lian F, Li X, Qi W. The impact of oxidative stress-induced mitochondrial dysfunction on diabetic microvascular complications. *Front Endocrinol*. 2023;14.
59. Bae C-S, Lee Y, Ahn T. Therapeutic treatments for diabetes mellitus-induced liver injury by regulating oxidative stress and inflammation. *Appl Microsc*. 2023;53(1):4.
60. Iksen, Witayateeraporn W, Hardianti B, Pongrakhananon V. Comprehensive review of Bcl-2 family proteins in cancer apoptosis: therapeutic strategies and promising updates of

- natural bioactive compounds and small molecules. *Phytother Res.* 2024;38(5):2249–75.
61. Shenoy TN, Abdul Salam AA. Therapeutic potential of dietary bioactive compounds against anti-apoptotic Bcl-2 proteins in breast cancer. *Crit Rev Food Sci Nutr.* 2025;65(25):4915–40.
 62. Cheng M, Ding F, Li L, Dai C, Sun X, Xu J, Chen F, Li M, Li X. Exploring the role of Curcumin in mitigating oxidative stress to alleviate lipid metabolism disorders. *Front Pharmacol.* 2025;16.
 63. Shehzad A, Ha T, Subhan F, Lee YS. New mechanisms and the anti-inflammatory role of Curcumin in obesity and obesity-related metabolic diseases. *Eur J Nutr.* 2011;50(3):151–61.
 64. Xu S, Ilyas I, Little PJ, Li H, Kamato D, Zheng X, Luo S, Li Z, Liu P, Han J, Harding IC, Ebong EE, Cameron SJ, Stewart AG, Weng J. Endothelial dysfunction in atherosclerotic cardiovascular diseases and beyond: from mechanism to pharmacotherapies. *Pharmacol Rev.* 2021;73(3):924–67.
 65. Bathina S, Das UN. Dysregulation of PI3K-Akt-mTOR pathway in brain of streptozotocin-induced type 2 diabetes mellitus in Wistar rats. *Lipids Health Dis.* 2018;17(1):168.
 66. Liu K, Yang Y, Zhou F, Xiao Y, Shi L. Inhibition of PI3K/AKT/mTOR signaling pathway promotes autophagy and relieves hyperalgesia in diabetic rats. *Neuroreport.* 2020. <https://doi.org/10.1097/WNR.0000000000001461>.
 67. Chai R, Fu H, Zheng Z, Liu T, Ji S, Li G. Resveratrol inhibits proliferation and migration through SIRT1 mediated post-translational modification of PI3K/AKT signaling in hepatocellular carcinoma cells. *Mol Med Rep.* 2017;16(6):8037–44.
 68. Lu H, Chang Z, Han W, Wang L, Hong G. [Curcumin reduces paraquat-induced oxidative injury in A549 cells by activation of the Nrf2-ARE pathway]. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi.* 2014;32(1):44–9.
 69. Umar S, Hedaya O, Singh AK, Ahmed S. Thymoquinone inhibits TNF- α -induced inflammation and cell adhesion in rheumatoid arthritis synovial fibroblasts by ASK1 regulation. *Toxicol Appl Pharmacol.* 2015;287(3):299–305.
 70. Ahmed M. Targeting aging pathways with natural compounds: a review of curcumin, epigallocatechin gallate, thymoquinone, and resveratrol. *Immun Ageing.* 2025;22(1):28.
 71. Bisht K, Wagner K-H, Bulmer AC. Curcumin, resveratrol and flavonoids as anti-inflammatory, cyto- and DNA-protective dietary compounds. *Toxicology.* 2010;278(1):88–100.
 72. Malik S, Suchal K, Khan SI, Bhatia J, Kishore K, Dinda AK, et al. Apigenin ameliorates streptozotocin-induced diabetic nephropathy in rats via MAPK-NF- κ B-TNF- α and TGF- β 1-MAPK-fibronectin pathways. *Am J Physiol-Renal Physiol.* 2017;313(2):F414–22.
 73. Mohamed NA, Ithmil MT, Elkady AI, Abdel Salam S. Tauroursodeoxycholic acid (TUDCA) relieves streptozotocin (STZ)-Induced diabetic rat model via modulation of Lipotoxicity, oxidative Stress, Inflammation, and apoptosis. *Int J Mole Sci.* 2024.
 74. Weng Q, Zhao M, Zheng J, Yang L, Xu Z, Zhang Z, et al. STAT3 dictates β -cell apoptosis by modulating PTEN in streptozotocin-induced hyperglycemia. *Cell Death & Differentiation.* 2020;27(1):130–45.
 75. Al-Hussaini H, Kittaneh RS, Kilarkaje N. Effects of trans-resveratrol on type 1 diabetes-induced up-regulation of apoptosis and mitogen-activated protein kinase signaling in retinal pigment epithelium of Dark Agouti rats. *European Journal of Pharmacology.* 2021;904: 174167.
 76. Hasan AA, Tatarskiy V, Kalinina E. Synthetic pathways and the therapeutic potential of Quercetin and Curcumin. *Int J Mole Sci.* 2022.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.