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Polyherbal blend of *Nigella sativa*, *Cinnamomum zeylanicum*, and *Cassia angustifolia* restores pancreatic β-Cell transcription factors (*Pdx-1* and *MAFA*) and glycemic control in alloxan-induced diabetic rats

Aqsa Rehman[1], Ayesha Yasin[1], Arslan Shaukat[1], Shahzad Irfan[1*], Laaraib Nawaz[2,*]

[1]*Department of Physiology, Faculty of Life Sciences, Government College University Faisalabad, Punjab, Pakistan*
[2]*University Institute of Physical Therapy, Faculty of Allied Health Sciences, University of Lahore, Punjab, Pakistan*

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**** Corresponding Authors:***
shahzadirfan@gcuf.edu.pk
Fax: no fax
shahzadirfan@gcuf.edu.pk

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Abstract

Objective: Diabetes mellitus (DM) is a metabolic disorder characterized by persistent hyperglycemia, either due to impairment in insulin production or insulin action. Emerging evidence highlights the therapeutic potential of medicinal herbs over conventional medicine. Our study investigated the anti-diabetic and antioxidant potential of *Nigella sativa*, *Cinnamomum zeylanicum*, and *Cassia angustifolia* in a polyherbal formulation in a diabetic rat model.

Materials and methods: Wistar albino rats (N=36, male, 6-8 weeks, 150-180 g) were kept in standard conditions throughout the experiment. Hyperglycemia was induced by a single intraperitoneal. injection of alloxan (120 mg/kg bw) in 30 rats. After 72 hr, hyperglycemic rats fasting blood glucose(FBG) ≥150 mg/dl) were randomly divided into 5 groups (n=6): hyperglycemic positive control (PC), standard control (metformin 200 mg/kg bw), low dose extract treatment (LDE, 75 mg/kgbw), medium dose extract treatment group (MDE, 150 mg/kg bw), and high dose extract treatment group (HDE, 300 mg/kg bw) for 28 days. Negative control (n=6) received standard diet. After regular monitoring of body weight and FBG, serum markers (glucose, insulin, lipid profile, oxidative stress markers, and liver enzymes) were evaluated. The pancreas was processed for histopathology and expression levels of *Pdx-1* and *MAFA*.

Results: Results showed significant improvements in body weight, FBG, lipid profile, liver enzymes, and antioxidant status in treatment groups versus the PC group, supported by preserved pancreatic histology and upregulation of *Pdx-1* and *MAFA*, which are essential for β-cell function.

Conclusion: The polyherbal formulation effectively ameliorated hyperglycemia and enhanced β-cell regeneration by modulating oxidative stress and key transcriptional factors.

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Introduction

Diabetes mellitus (DM) is a chronic, multifactorial metabolic disorder mainly attributed to persistent hyperglycemic levels due to either insufficient insulin production, impaired insulin action, or both (Nawaz et al. 2024). Despite the availability of various therapeutic options, the global prevalence of diabetes continues to escalate, with an estimated 578 million adults projected to be affected by 2030 (Arokiasamy et al. 2020), underscoring the urgent need for more effective and sustainable interventions. Central to the pathogenesis of diabetes is the dysfunction and eventual loss of pancreatic β-cells which are essential for the production and secretion of insulin in response to elevated blood glucose (Kaneto et al. 2022). Moreover, oxidative stress and an insufficient antioxidant defense system, is a major contributors to β-cell dysfunction (Eguchi et al. 2021). The impact of reactive oxygen species is particularly profound on the transcriptional machinery that governs β-cell identity and insulin gene expression (Nawaz et al. 2024).

Current pharmacological treatments mainly provide glycemic control without reversing disease progression or preventing complications (Association 2022). Thus, herbal medicines have gained widespread interest as complementary or alternative therapies due to their multitargeted mechanisms, bioactive phytochemicals, and relatively low toxicity (Mubeen et al. 2025). Plants such as *Nigella sativa*, *Cinnamomum zeylanicum*, and *Cassia angustifolia* are rich in antioxidants, anti-inflammatory agents, and insulin-sensitizing compounds. They offer the added benefit of modulating oxidative stress, improving β-cell function, and enhancing insulin signaling pathways (Ahmad et al. 2013; Kumar et al. 2014; Ranasinghe et al. 2013). The rationale for selecting *N. sativa*, *C. zeylanicum*, and *C. angustifolia* lies in their well-documented antidiabetic, antioxidant, and regenerative properties, especially targeting oxidative stress, pancreatic β-cell health, and glycemic control (Ibrahim et al. 2024; Li et al. 2015; Li et al. 2025) Together, these three herbs offer a synergistic phytotherapeutic potential by targeting multiple facets of diabetes pathology such as oxidative stress, inflammation, β-cell regeneration, and glucose metabolism, which justifies their integration into a polyherbal formulation for managing diabetes and its complications. Unlike single-compound pharmacology, polyherbal formulations offer a multitargeted approach, addressing complex disorders such as diabetes by modulating multiple biochemical and molecular pathways simultaneously (Parasuraman et al. 2014; Patwardhan et al. 2005). The key knowledge gap lies in understanding how a synergistic blend of these herbs can promote pancreatic β-cell regeneration and modulate transcription factors like *PdxX-1* and *MAFA* which are essential for β-cell survival and function. Hence, this study aims to evaluate the modulation of key β-cell transcriptional factors (*Pdx-1* and *MAFA*) involved in insulin gene expression (*INS-I* and *INS-II*) and β-cell survival. Nonetheless, the key research gap and novelty of the current study lies in understanding the regenerative potential of the synergic blend of these specific herbs in pancreatic β-cells and how it modulates the important transcriptional factors like *Pdx-1* and *MAFA* which are crucial for survival and function of β-cells in insulin gene expression, as this aspect is still unexplored.

Materials and Methods

Chemicals

Alloxan monohydrate (Sigma-Aldrich, A7413, Germany), TRIzol reagent (Invitrogen™, 15596018, USA), cDNA synthesis kit (Thermo Fisher Scientific, K1621, USA), and SYBR green (Thermo Fisher Scientific, 2307541, USA).

Collection and extraction of plant material

The seeds of *N. sativa*, barks of *C. zeylanicum*, and leaves of *C. Angustifolia* were procured and identified from the Department of Botany, Govt College University Faisalabad (GCUF; 280-bot-21). Herbs were shade-dried and manually ground to make powder by using a pestle and mortar. To prepare the individual extracts, 80 g of each powdered material was used. Aqueous extracts of *N. sativa* and *C. zeylanicum* were made by soaking in 320 ml of distilled water (1:4 w/v ratio) at 25℃ for 24 hr. Afterwards, the mixtures were manually stirred every two hours to enhance extraction. The ethanolic extract of *C. angustifolia* was prepared by soaking 80 g of powder in 320 ml of 70% ethanol (1:4 w/v ratio). Lastly, the mixture was filtered, and the filtrate was subjected to a rotary apparatus (Scilogex RE100-Pro; USA) at 30 RPM at 47℃ temperature for one hour. Afterwards, the filtrate was stored for experiments. Lastly, the extracts of the above-mentioned herbs were combined in a 1/1/1 ratio to make a polyherbal formulation. This ratio was selected based on previous studies exploring therapeutic roles in diabetes management, comparable effective dose ranges, and precedent in polyherbal formulation research, where equal proportions have been employed to ensure synergistic or additive actions while maintaining formulation simplicity (Alhamhoom et al. 2023; Anwar et al. 2022). This ratio was selected based on previous studies exploring therapeutic roles in diabetes management. The chosen doses of 75, 150, and 300 mg/kg bw represent a low, medium, and high dose based on the reported effective doses for individual constituent extracts in antidiabetic experiments in rodents and to establish a clear dose-response understanding (Osman et al. 2017; Singh et al. 2020).

Animal experimentation and groups

Male Wistar rats (N=36, 150-180 g, age 6-8 weeks) were procured and kept at the animal research station of GCUF, at standard conditions: temperature 26±2℃, 40–60% ambient humidity, and 12 hr light/dark cycle. Animals were conditioned for one week. To induce hyperglycemia, after 12 hr of fasting, rats received a single intraperitoneal injection of alloxan monohydrate (120 mg/kg bw in normal saline) (Muzaffar et al. 2023). After 72 hr, rats having fasting blood glucose (FBG) ≥150 mg/dl were considered hyperglycemic, and were randomly and equally divided (n=6) into 5 groups: positive control hyperglycemic group (PC), metformin-treated standard control group (STD, 200 mg/kg bw), low-dose extract-treated group (LDE 75 mg/kg bw), medium-dose extract-treated group (MDE, 150 mg/kg bw), and high-dose extract-treated group (HDE, 300 mg/kg bw) 1 ml volume orally once daily. Another group of healthy normal controls was added (NC, n=6) to compare the effects. After 28 days of extract administration, rats were decapitated by neck dislocation. Afterwards, blood samples were processed for biochemical analyses and pancreatic tissue was harvested for histopathology and gene expression analyses.

Physical, biochemical and histological analyses

Body weight and fasting blood glucose measurement

The body weight of each rat was observed at day 0, 14, and 28 during the trial.

Blood glucose and serum insulin determination

Whereas FBG levels were monitored weekly at regular intervals by using a glucometer (OnCall® Ez-II; SN303S0014E09, UK). Serum insulin concentration was determined by using an ELISA kit (Calbiotech; catalogue IN374S, El Cajon, CA, USA).

Oxidative stress markers

Total oxidative status (TOS) and the total antioxidant capacity (TAC) and malondialdehyde (MDA) levels in the serum were determined by the calorimetric (BIOLAB-310; Biosciences, Singapore) method as previously described (Maqbool et al. 2021; Nawaz et al. 2024), with minor modifications. Briefly, serum TAC levels were determined by quantifying the bleaching of ortho-dianisidine dye and results are expressed as mmol Trolox equivalent/L. Serum TOS levels were quantified against H_2O_2 standard and results are expressed as µmol H_2O_2 equivalent/L/L. Lastly, MDA levels were assessed by using the thiobarbituric acid reactive substances (TBARS) assay, and measurement was taken at 532 nm.

Liver function markers

Serum AST (Aspartate transaminase) and ALT (Alanine transaminase) levels were measured using the commercially available kit (AST: Crescent® Diagnostic kit; Cat.#15204C; and ALT: Cat.#17504; Jeddah). Final measurements for both assays were determined through a spectrophotometer (BIOLAB-310; Biosciences, Yishun, Singapore).

Serum lipid profile

Total cholesterol (TC) and triglycerides were measured by the commercially available kit (Crescent® Diagnostics; catalog#35401; Jeddah). Final readings were taken by using a spectrophotometer (BIOLAB-310; Biosciences, Yishun, Singapore). The low-density lipoproteins (LDL-c) was measured by using Friedewald's equation (Cicero et al. 2021):

$$LDL - cholesterol = Total\ cholesterol - \left(\frac{Triglycerides}{5}\right) - HDL - cholesterol \quad Eq..1$$

Tissue analyses

Histopathology and gene expression analyses of *INS-I* and *INS-II* genes, *Pdx1*, and *MAFA* transcriptional factors were carried out by following the described protocols (Mukhtar et al. 2025). For pancreatic histopathology, tissues were fixed in 10% formalin, embedded in paraffin, and subsequently sectioned at 4-5 µm. Later on, sections were stained with hematoxylin and eosin and examined under a light microscope at 40X magnification. For gene expression analyses of *INS-I, INS-II*, *Pdx-1*, and *MAFA*. These levels were normalized with *β-actin.* Primers for these genes are listed in Table 1.

Table 1. Primers listed below were designed by Macrogen, South Korea

Genes	Sequence	
PDX-1	Forward	*CGTAGTAGCGGGACAACGAG*
	Reverse	*CCCGAGGTTACGGCACAAT*
MAFA	Forward	*GACCTGATGAAGTTCGAGGTG*
	Reverse	*GGGCGTCGAGGATAGCGA*
INS-1	Forward	*CTGGGAAATGAGGTGGAAAA*
	Reverse	*TCCACAAGCCACGCTTCTG*
INS-II	Forward	*AGAAAGGTTTGGTACCTGGAATAGAGC*
	Reverse	*GTAGAAGAAGCTTCGCTCCCCACA*

Statistical analysis

GraphPad Prism software (version 8.0.263; USA) was used to perform statistical analyses. Two-way ANOVA (time x treatment) was applied for body weight and fasting blood glucose (FBG) analysis, while one-way ANOVA followed by Tukey's post hoc test was employed to compare differences between multiple groups.

Results

Polyherbal formulation reverses alloxan-induced weight loss in diabetic rats

Body weight results revealed (Figure 1) a significant ($p \leq 0.05$) reduction in body weight in the PC group as compared to the NC group. Whereas treatment with polyherbal formulation resulted in significant ($p \leq 0.05$) restoration of body weight in the medium and high dose treatment groups compared with PC group on the 28th day. These results indicate that the polyherbal formulation significantly reversed alloxan-induced weight loss in diabetic rats, with medium and high doses showing greater efficacy than the low dose and standard treatment.

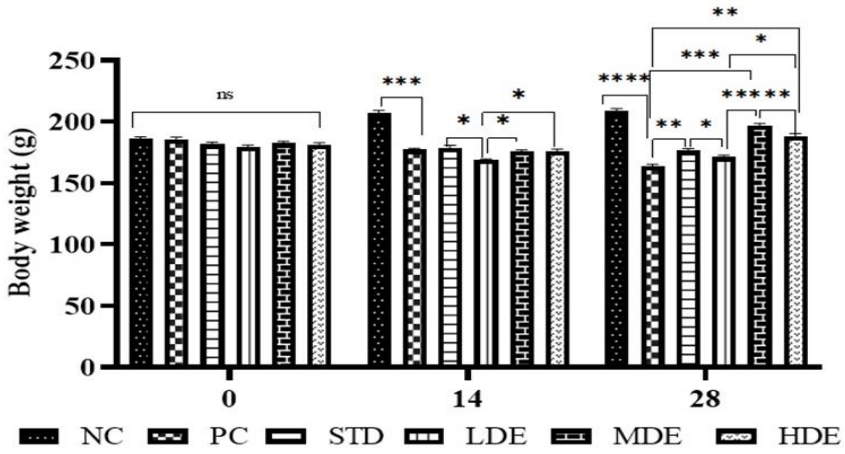

Figure 1. Polyherbal formulation treatment of *N. sativa*, *C. zeylanicum* and *C. angustifolia* restored body weight (g) in hyperglycemic rats. Graph represents progression of body weight measured on day 0, 14, and 28 after induction in negative control (NC) and hyperglycemic positive control group (PC), metformin treated standard control group (STD), low-dose extract treated group (LDE), medium dose extract treated group (MDE), and high dose extract treated group (HDE). ****$p<0.0001$, ***$p<0.001$, **$p<0.01$, *$p<0.05$, ns=non-significant.

Polyherbal formulation lowers fasting blood glucose in diabetic rats

Fasting blood glucose (FBG) results revealed (Table 2) a significant ($p \leq 0.05$) elevation in FBG levels in the PC group as compared to the NC group, indicating alloxan-induced hyperglycemia. Whereas, treatment with the polyherbal formulation resulted in a significant ($p \leq 0.05$) better restoration of FBG levels in the MDE group compared with the LDE, HDE, and STD groups, confirming its strong antihyperglycemic potential.

Polyherbal formulation restores insulin levels and exhibits dose-dependent hepatoprotective effects by normalizing liver enzymes in diabetic rats

Serum insulin results revealed (Table 3) a significant ($p \leq 0.05$) reduction in serum insulin levels in the PC group as compared to the NC group. Whereas treatment with the polyherbal formulation effectively reversed these effects in a dose-dependent manner, with medium and high doses significantly normalizing insulin levels, demonstrating potent antihyperglycemic and insulin-restorative properties. Results also revealed that serum liver enzymes AST and ALT were significantly ($p \leq 0.05$) elevated in the PC group as compared to the negative control group, indicating that alloxan effectively induced hepatic damage due to diabetic stress. Furthermore, treatment with the polyherbal formulation effectively restored liver enzyme levels toward normal in a dose-dependent manner; the MDE and HDE groups showed superior effects over the LDE group, indicating their hepatoprotective potential.

Table 2. Effect of the polyherbal formulation on fasting blood glucose (mg/dl) levels

	0day	7th day	14th day	21st day	28th day	Overall Mean±SEM
NC	90±6.08[a]	79.83±3.68[d]	81.66±3.81[e]	81.16±3.67[e]	77.16±2.78[d]	81.96±4.53**E**
PC	86.16±6.08[a]	269.83±12.44[a]	285.16±14.23[a]	331.66±14.2[a]	367.83±13.2[a]	268.12±12.11**A**
STD	87.33±5.2[a]	259.33±18.2[b]	177.5±12.2[c]	135.5±7.8[c]	121.5±5.51[b]	156.23±6.46**C**
LDE	82.16±5.78[a]	276±12.91[a]	206.16±11.09[b]	147.5±10.22[b]	115.66±5.96[b]	165.49±5.78**B**
MDE	81.33±4.35[a]	234.66±12.24[c]	159.33±12.23[d]	122.5±11.21[d]	83.59±5.99[c]	136.28±6.98**D**
HDE	84.33±5.46[a]	253.67±5.25[b]	167.05±6.86[c]	117.66±5.36[d]	95.83±6.32[c]	143.71±7.32**D**

Data is presented as mean±SEM (n=6 per group). Superscripts [a-e] indicate significant differences between groups within the same time point; superscripts A-D indicate significant differences between overall means across groups ($p \leq 0.05$). Abbreviations: NC, negative control; PC, positive control (hyperglycemic); STD, standard control (metformin 200 mg/kg); LDE, low-dose extract (75 mg/kg); MDE, medium-dose extract (150 mg/kg); HDE, high-dose extract (300 mg/kg); FBG, fasting blood glucose; SEM, standard error of the mean.

Table 3. Effect of the polyherbal formulation on serum insulin and hepatic enzymes.

	NC	PC	STD	LDE	MDE	HDE
Insulin (ng/µl)	16.87±0.77[a]	7.67±0.45[d]	13.01±0.58[b]	9.64±0.83[c]	13.07±0.60[b]	15.01±0.44[a]
AST (IU/L)	26.33±4.91[d]	133.33±12.67[a]	37.66±1.27[d]	87.33±6.53[b]	53.66±5.32[c]	51.33±6.43[c]
ALT (IU/L)	39.66±3.45[d]	179.66±8.92[a]	56.66±6.32[c]	93.66±5.66[b]	51.33±5.78[c]	48.01±5.43[c]

Data is presented as mean±SEM (n=6 per group). Superscripts [a-d] indicate significant differences between groups within the same time point ($p \leq 0.05$). Abbreviations: AST, aspartate transaminase; ALT, alanine transaminase; IU/L, international units per liter; NC, negative control; PC, positive control (hyperglycemic); STD, standard control (metformin 200 mg/kg); LDE, low-dose extract (75 mg/kg); MDE, medium-dose extract (150 mg/kg); HDE, high-dose extract (300 mg/kg).

Polyherbal formulation alleviates alloxan-induced oxidative stress by enhancing antioxidant status in diabetic rats

Results revealed (Table 4) a significant ($p \leq 0.05$) reduction in TAC levels with concomitant increase in TOS and MDA levels in the hyperglycemic PC group as compared to the NC group. Whereas treatment with polyherbal formulation resulted in significant ($p \leq 0.05$) restoration of TAC levels in the LDE group, MDE group, HDE group, and STD group. While the levels of TOS and MDA were significantly suppressed in all treatment groups, MDE and HDE showed better effects than LDE, which implies that the polyherbal formulation possesses strong antioxidant potential in a dose-dependent manner.

Polyherbal formulation significantly ameliorates alloxan-induced dyslipidemia by improving lipid profile parameters in a dose-dependent manner in diabetic rats

Lipid profile results revealed significant alloxan-induced dyslipidemia reflected by significantly ($p \leq 0.05$) raised serum levels of total cholesterol (TC), triglycerides, and LDL-c (Table 5) in the PC group, with concomitant decrease in high-density

lipoproteins (HDL-c) levels as compared to the NC group. Whereas, treatment with polyherbal formulation significantly ($p \leq 0.05$) improved the lipid profile in a dose-dependent manner, demonstrating its potent antihyperlipidemic effects.

Polyherbal formulation enhances expression of key β-Cell transcription factors (*Pdx-1* and *MAFA*), supporting β-Cell regeneration and functional recovery in diabetic rats

Gene expression analysis (Figure 2) revealed significant ($p \leq 0.05$) downregulation in the expression levels of key β-cell transcriptional factors critical for insulin gene regulation and signaling: *Pdx-1/β-actin* and *MAFA/β-actin* in the PC group as compared to the NC group. Whereas treatment with polyherbal formulation resulted in significant ($p \leq 0.05$) upregulation in a dose-dependent manner, suggesting enhanced β-cell regeneration and functional recovery at the molecular level.

Table 4. Effect of the polyherbal formulation on oxidative stress markers

	NC	PC	STD	LDE	MDE	HDE
TAC mmol Trolox equiv./L	1.63±0.16^{a}	0.46±0.09^{d}	1.25±0.11^{b}	0.97±0.04^{c}	1.21±0.04^{b}	1.34±0.13^{b}
TOS µmol H_2O_2 equiv./L	22.57±6.45^{d}	105.01±9.76^{a}	78.24±7.53^{b}	61.55±9.83^{c}	34.84±6.54^{d}	34.76±3.65^{d}
MDA mmol/L	1.07±0.02^{d}	2.05±0.06^{a}	1.42±0.04^{c}	1.58±0.04^{b}	1.34±0.05^{c}	1.31±0.04^{c}

Data are mean ± SEM (n=6). Superscripts (a-d) indicate significant differences between groups ($p \leq 0.05$). Abbreviations: TAC, total antioxidant capacity (mmol Trolox equivalent/L); TOS, total oxidant status (µmol H_2O_2 equivalent/L); MDA, malondialdehyde (mmol/L); NC, negative control; PC, positive control (hyperglycemic); STD, standard control (metformin 200 mg/kg); LDE, low-dose extract (75 mg/kg); MDE, medium-dose extract (150 mg/kg); HDE, high-dose extract (300 mg/kg).

Table 5. Effect of the polyherbal formulation on lipid profile

	NC	PC	STD	LDE	MDE	HDE
TC (mg/dl)	116.43±5.63^{d}	205.76±8.92^{a}	138.02±8.47^{c}	173.90±9.23^{b}	137.09±7.89^{c}	133.26±7.29^{c}
Tg(mg/dl)	99.33±4.56^{d}	211.01±4.50^{a}	142.13±4.87^{b}	144.66±7.89^{b}	110.30±6.98^{c}	118.33±5.43^{c}
HDL-c(mg/dl)	69.66±4.87^{a}	41.33±5.85^{b}	66.33±4.98^{a}	59.01±6.77^{a}	63.01±5.72^{a}	63.33±6.75^{a}
LDL-c(mg/dl)	32.60±3.64^{d}	162.01±8.62^{a}	46.33±6.32^{c}	65.13±6.23^{b}	58.73±4.56^{b}	52.20±5.60^{c}

Data are mean ± SEM (n=6). Superscripts (a-d) indicate significant differences between groups (p$P \leq 0.05$). Abbreviations: TC, total cholesterol; Tg, triglycerides; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; NC, negative control; PC, positive control (hyperglycemic); STD, standard control (metformin 200 mg/kg); LDE, low-dose extract (75 mg/kg); MDE, medium-dose extract (150 mg/kg); HDE, high-dose extract (300 mg/kg).

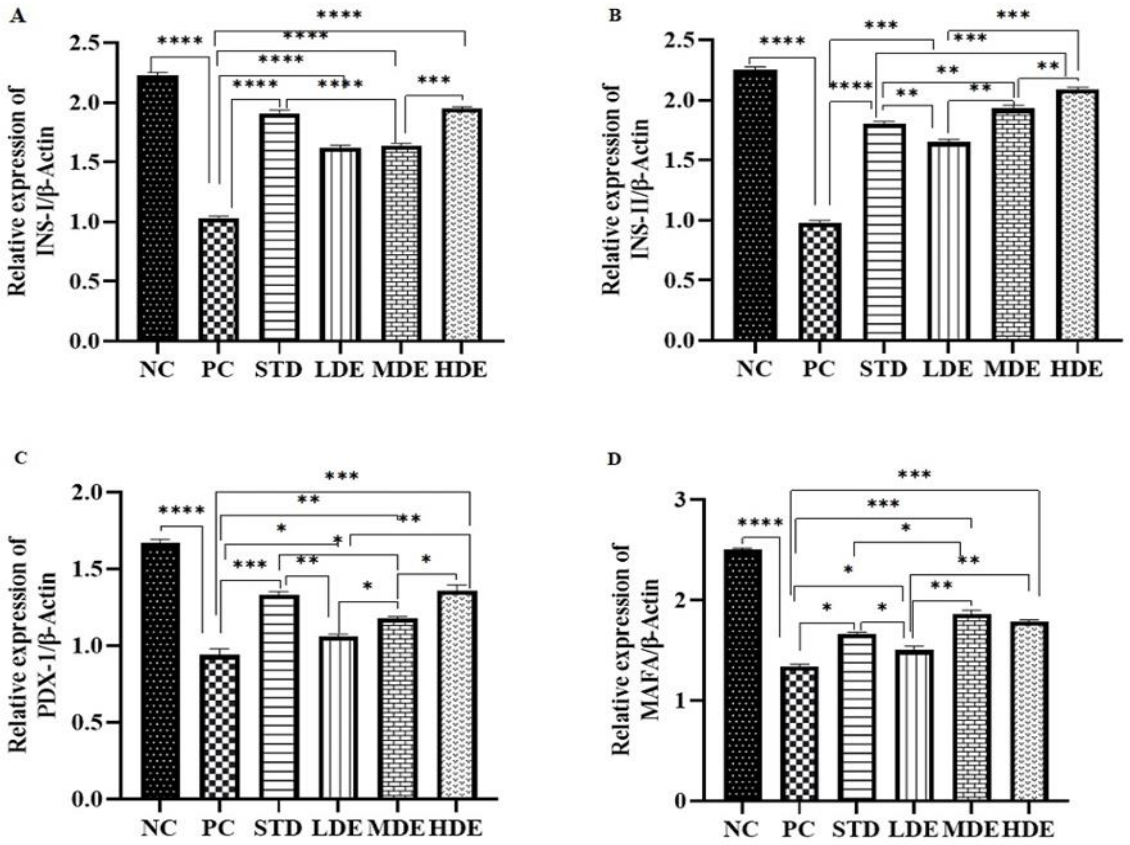

Figure 2. Polyherbal formulation treatment of *N. sativa*, *C. zeylanicum* and *C. angustifolia* upregulated expression levels of insulin signaling genes and transcriptional factors in hyperglycemic rats. After 28 days, rats were sacrificed for gene expression analysis of (A) relative expression of *INS-I/β-actin* gene, (B) relative expression of *INS-II/β-actin* gene, (C) relative expression of *Pdx-1/β-actin* transcriptional factor, and (D) relative expression of *MAFA/β-actin* transcriptional factor in negative control (NC) and hyperglycemic positive control group (PC), metformin treated standard control group (STD), low-dose extract treated group (LDE), medium dose extract treated group (MDE), and high dose extract treated group (HDE). Data is mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001.

Polyherbal formulation exhibits dose-dependent histological restoration of pancreatic islets and exocrine tissue, with marked regeneration in medium and high-dose groups

The histopathological images of pancreatic tissue sections (Figure 3) demonstrate the morphological changes in islets of Langerhans (yellow arrow) and surrounding exocrine tissue (green arrow) across different treatment groups. The negative control showed intact islets of Langerhans with well-defined boundaries and dense cellularity. The positive control group exhibited marked degeneration of islets with disrupted architecture and reduced cellular density. The metformin-treated standard control group showed partial restoration of islet architecture with improved cellular integrity. The acinar cells also appear moderately preserved, suggesting therapeutic recovery by standard drug treatment. The low-dose extract-treated group also showed mild regeneration in the islets but remained disorganized compared to normal. Acinar tissue also exhibits limited recovery, indicating only slight protective effects at this dose. Whereas the medium-dose extract-treated group showed significant improvement in islet morphology, with denser and more organized islet cells. Lastly, the high-dose extract-treated group exhibited near-complete restoration of islet structure, closely resembling the normal architecture seen in the negative control.

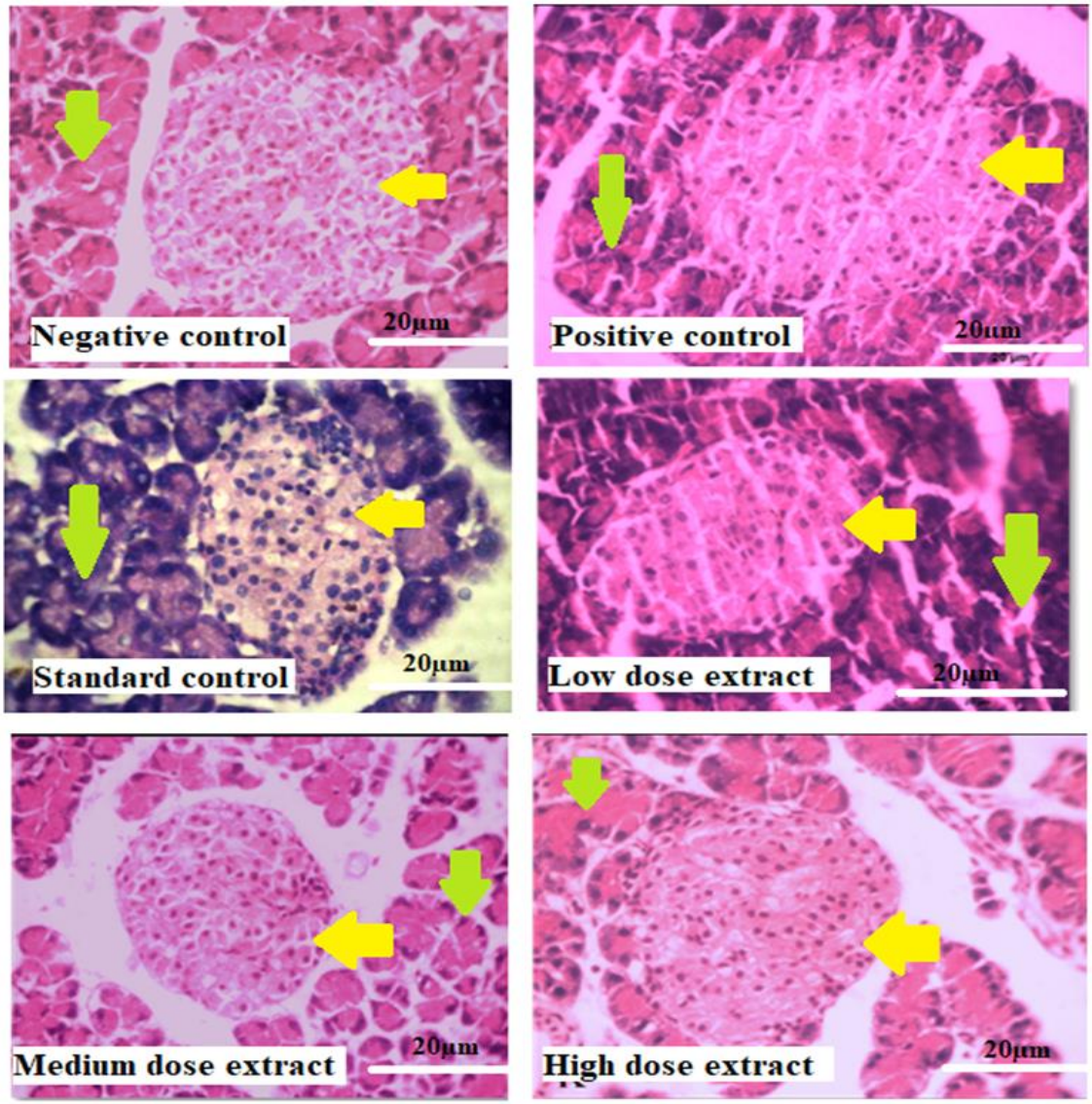

Figure 3. In healthy rat pancreas, representative H&E-stained histopathology sections (40× magnification) are exhibiting normal histological structures such as islets of Langerhans, with scattered β cells is shown in yellow arrows and exocrine pancreas is showing in green arrow. These features were disrupted in the hyperglycemic rat pancreases (deformed margins and targeted β-cell degeneration) and restored by polyherbal formulation of *N. sativa*, *C. zeylanicum* and *C. angustifolia* or metformin treatment in negative control, hyperglycemic positive control group, standard control, low dose extract treatment group, medium dose extract treatment group, and high dose extract treatment group. Scale bar = 20 µm.

Discussion

The current study demonstrated that the polyherbal formulation of *N. sativa*, *C. zeylanicum*, and *C. angustifolia* not only possesses potent antidiabetic and antioxidant effects but also uniquely promotes regeneration of β-cell by upregulating the key transcriptional factors involved in β-cell normal functioning and insulin secretion.

The therapeutic efficacy of a polyherbal formulation of *N. sativa*, *C. zeylanicum*, and *C. angustifolia* in an alloxan-induced diabetic rat model was explored. Diabetes mellitus is a complex metabolic disorder characterized by persistent hyperglycemia,

oxidative stress, and progressive pancreatic β-cell dysfunction, often causing multi-organ pathologies (Grover et al. 2021). Considering the limitations and adverse effects of conventional antidiabetic drugs (Stein et al. 2013), this study aimed to assess the potential of a plant-based, multitargeted therapy. The polyherbal formulation was selected for its rich composition of bioactive compounds with proven anti-inflammatory, antioxidant, and insulin-sensitizing properties (Patel 2025).

Alloxan administration caused a significant ($p \leq 0.05$) drop in body weight (Figure 1) in the hyperglycemic positive control group (PC), which is primarily attributed to insulin deficiency, which impairs glucose uptake. The lack of intracellular glucose availability leads to catabolism of fats and proteins for energy, resulting in muscle wasting and adipose tissue degradation (Dimitriadis et al. 2011). Treatment with the polyherbal formulation significantly restored body weight in a dose-dependent manner. This improvement is likely due to enhanced insulin sensitivity and secretion facilitated by the bioactive compounds such as thymoquinone, cinnamaldehyde, and sennosides (Choi et al. 2006; Karandrea et al. 2017; Li et al. 2015). These compounds reduce hyperglycemia and restore normal metabolic homeostasis by improving glucose uptake, reducing oxidative stress, and preserving pancreatic β-cell function. Improved glycemic control also reduces proteolysis and lipolysis, thus contributing to weight stabilization (Tamura et al. 2020; Whyte et al. 2004). Moreover, a significant ($p \leq 0.05$) elevation in FBG levels (Table 2), and a decrease in serum insulin levels were observed in the positive control group (Table 3), reflecting the hallmark of diabetes mellitus induced by alloxan. Upon cellular uptake, alloxan generates free oxygen radicals, leading to oxidative DNA damage and irreversible β-cell necrosis (Li et al. 2002; Queiroz et al. 2021). This antihyperglycemic effect can be attributed to the synergistic action of the formulation's phytoconstituents, cinnamaldehyde from *C. zeylanicum* mimics insulin and facilitates glucose uptake (Gaique et al. 2023), while sennosides from *C. angustifolia* modulate glucose metabolism and reduce postprandial spikes (Olofinsan et al. 2023). From a clinical standpoint, improving endogenous insulin secretion while lowering glucose levels is crucial for long-term diabetes control (Laxmi et al. 2025). Importantly, it was observed that polyherbal formulation was well-tolerated, as evidenced by the absence of mortality or toxicity signs, observed normalization of liver enzymes and body weight, suggesting a favorable safety profile within the studied dose range.

The significant reduction in TAC and the concurrent rise in TOS and MDA levels in the positive control group (Table 4) demonstrate the oxidative burden imposed by alloxan-induced diabetes (Ceretta et al. 2012). This excessive production of free radicals overwhelms the endogenous antioxidant systems and impairs insulin secretion (Wang et al. 2023). Treatment with the polyherbal formulation significantly reversed these oxidative changes in a dose-dependent fashion. These improvements can be attributed to the phytoconstituents with strong antioxidant activity: Thymoquinone in *N. sativa* scavenges free radicals and enhances endogenous antioxidant enzymes like catalase and superoxide dismutase (Demirkıran and Özdem 2025; Isaev et al. 2023). Anthraquinones in *C. angustifolia* possess free radical neutralizing properties and contribute to redox homeostasis (Zhao and Zheng 2023). The demonstrated antioxidant potential of the polyherbal blend highlights its value not only in protecting pancreatic β-cells but also in alleviating systemic oxidative injury.

The significant elevation of serum AST and ALT levels in the positive control group (Table 3) reflects hepatic injury caused by hyperglycemia, as it triggers oxidative stress (Anwar et al. 2023), and mitochondrial dysfunction in hepatocytes,

thereby increasing leakage of intracellular hepatic enzymes into the bloodstream (Mihajlovic and Vinken 2022). Polyherbal formulation significantly restored AST and ALT levels in a dose-dependent fashion. These hepatoprotective effects can be attributed to thymoquinone in *N. sativa*, known for its membrane-stabilizing and radical scavenging properties, which protect hepatocytes (Alasmari 2021), and Cinnamaldehyde and eugenol from *C. zeylanicum*, which inhibit hepatic inflammation and oxidative damage (Gulec Peker and Kaltalioglu 2021). The observed normalization of liver enzymes suggests that the polyherbal formulation can provide supportive hepatoprotection in diabetic conditions, making it a promising adjunct to conventional therapy.

Alloxan-induced hyperglycemia led to an abrupt hyperlipidemia accompanied by a marked reduction in HDL-c in the positive control group (Table 5), reflecting the characteristic metabolic disturbance of diabetes mellitus (Bahiru et al. 2021). Hyperglycemia impairs insulin signaling and results in increased lipolysis, which contributes to lipid accumulation (Huang and Lee 2022). The polyherbal treatment significantly reversed these abnormalities in a dose-dependent manner. This lipid-regulatory effect is likely due to the synergistic action of bioactive compounds in the these herbs: thymoquinone in *N. sativa* enhances lipid metabolism and exhibits hypolipidemic effects by modulating hepatic enzymes like HMG-CoA reductase (Ibrahim et al. 2024). Anthraquinones in *C. angustifolia* alleviate lipid accumulation by regulating brown adipose tissue and liver function (Li et al. 2025). The ability of the polyherbal formulation to correct lipid imbalance highlights its role in comprehensive diabetes care, especially for patients intolerant to statins or those seeking natural alternatives.

The integrity of pancreatic β-cells is central to maintaining insulin secretion and glucose homeostasis (Benáková et al. 2021). In the current study, alloxan administration significantly downregulated the expression of two key β-cell transcription factors, *PDX-1* and *MAFA*, in the positive control group (Figure 2), accompanied by evident histological damage (Figure 3), including degenerated islets of Langerhans, reduced cellularity, and disrupted acinar architecture. This outcome reflects the known diabetogenic mechanism of alloxan, which selectively targets β-cells via the GLUT2 transporter, inducing DNA fragmentation and oxidative stress-mediated apoptosis (Wickramasinghe et al. 2021). *PDX-1* plays a crucial part in the development of the pancreas and insulin gene regulation (Ebrahim et al. 2022), while *MAFA* is required for β-cell maturation and glucose-stimulated insulin secretion (GSIS). Downregulation of these transcription factors under oxidative conditions translates into β-cell dysfunction and diminished insulin biosynthesis, exacerbating diabetic pathology. Furthermore, treatment with the polyherbal formulation resulted in dose-dependent upregulation of *PDX-1* and *MAFA* expression, with the medium- and high-dose groups exhibiting restoration to normal levels. The transcriptional recovery may be attributed to thymoquinone and cinnamaldehyde which counteract oxidative stress, stabilize *PDX-1* expression, and enhance β-cell survival (Kang et al. 2019; Soleimani-Dodran et al. 2022). This finding positions the formulation as a viable candidate for functional pancreatic restoration in diabetic patients (Table 6).

Table 6. Summary of the therapeutic effects and proposed mechanisms, and comparison with previous literature.

Therapeutic Effect	**Demonstrated Outcome**	**Proposed Molecular Mechanism**	**Comparison with Previous Studies**
Antihyperglycemic	↓ Fasting Blood Glucose, ↑ Serum Insulin	• Insulin Mimetic & Sensitizing Action: Cinnamaldehyde from *C. zeylanicum* mimics insulin. β-Cell regeneration and upregulation of *Pdx-1* and MAFA	Individual herbs are known for their hypoglycemic effects (Ahmad et al., 2013; Kumar et al., 2014). Our study newly demonstrates the synergistic effect of the blend and its specific action on β-cell transcription factors.
Antioxidant	↑ TAC, ↓ TOS, ↓ MDA	• Free Radical Scavenging: Thymoquinone and anthraquinones neutralize ROS. • Enhancement of Endogenous Defenses.	The antioxidant capacity of thymoquinone is well-established (Isaev et al. 2023). Our study confirms and extends this by showing the combined formulation's systemic effect in a diabetic model.
Lipid-Lowering	↓ TC, ↓ Tg, ↓ LDL-c, ↑ HDL-c	• Inhibition of HMG-CoA reductase by Thymoquinone. • Regulation of adipose tissue by Anthraquinones.	Thymoquinone's hypolipidemic effect has been reported (Majdalawieh et al. 2021). Our work provides novel evidence for the lipid-correcting synergy of the three-herb combination.
Gene expression Modulator (β-Cell)	↑ *Pdx-1*, and *MAFA* Gene Expression	• Transcriptional Activation via oxidative stress counteraction.	Previous studies reported *N. sativa*'s effect on *Pdx-1* (Alaryani 2024) however *C. zeylanicum* and *C. angustifolia* have not been explored. Our novel finding is the significant, dose-dependent upregulation of *both Pdx-1* and *MAFA* by the polyherbal blend.
Hepato-protective	↓ AST, ↓ ALT	• Membrane Stabilization & Anti-inflammatory effects of thymoquinone and cinnamaldehyde.	Hepatoprotection from *N. sativa* and cinnamon is known (Alasmari 2021; Gulec Peker and Kaltalioglu 2021). Our study demonstrates this protective effect specifically in the context of alloxan-induced diabetic hepatotoxicity.
Structural protection (Pancreas)	Restoration of Islet of Langerhans architecture	• Reduced apoptosis and driven regeneration via *Pdx-1/MAFA*.	While β-cell protection by herbs is known (Wickramasinghe et al. 2021), the near-complete histological restoration with our high-dose formulation is a significant finding.

Arrows indicate direction of change (↑ increase, ↓ decrease). Abbreviations: ROS, reactive oxygen species; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; *Pdx-1*: pancreatic and duodenal homeobox-1, MAFA: MAF bZIP transcription factor A, FBG: fasting blood glucose, TAC: total antioxidant capacity, TOS: total oxidant status, MDA: malondialdehyde, AST: aspartate aminotransferase, ALT: alanine transaminase.

Despite the promising results, the current study has certain limitations. Firstly, the sample size (n=6 per group), while consistent with preliminary efficacy studies in rodent models, may limit the ability to detect subtler effects. Future studies with larger cohorts are warranted to confirm these results. Secondly, the study was exclusively conducted on male rats to avoid the potential confounding effects of the estrous cycle on metabolic parameters. Thirdly, the 28-day duration was enough to demonstrate the significant therapeutic effects but does not provide information on long-term sustainability of treatment. Lastly, clinical trials to validate the translatory aspect of this polyherbal formulation for human diabetic management are needed.

This study demonstrates that the polyherbal formulation of *N. sativa*, *C. zeylanicum*, and *C. angustifolia* exerts potent antidiabetic, antioxidant, and β-cell regenerative effects in alloxan-induced diabetic rats. These findings highlight its multitargeted therapeutic potential and dose-dependent efficacy. Future studies should explore its long-term safety, optimize dosing, and validate these effects in clinical trials to support its development as a natural, adjunct therapy for diabetes management.

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Conflicts of interest

The authors had no competing interests.

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Authors' Contributions

A.R. designed the experiments and collected data, A.Y. experimentation and data curation, L.N. data analyses and manuscript preparation, A.S. Final draft preparation, S.I. final review and editing.

Abbreviations

Pdx-1: pancreatic and duodenal homeobox-1, MAFA: MAF bZIP transcription factor A, FBG: fasting blood glucose, TAC: total antioxidant capacity, TOS: total oxidant status, MDA: malondialdehyde, AST: aspartate aminotransferase, ALT: alanine transaminase.

References

Ahmad A, Husain A, Mujeeb M, et al. (2013) A review on therapeutic potential of Nigella sativa: A miracle herb. Asian Pac. J. Trop. Biomed. 3(5):337-352

Alaryani FS (2024) Examining the pancreatic regenerative potential of Nigella sativa in diabetic rats: Evaluation of histological changes and transcription factors pdx-1 and neurog-3 levels. Mater. Express. 14(3):376-384

Alasmari A (2021) Cardioprotective and nephroprotective effects of Quercetin against different toxic agents. Eur. Rev. Med. Pharmacol. Sci. 25(23)

Alhamhoom Y, Ahmed SS, Salahuddin M, et al. (2023) Synergistic Antihyperglycemic and Antihyperlipidemic effect of Polyherbal and Allopolyherbal formulation. Pharmaceuticals 16(10):1368

Anwar H, Nawaz L, Saddiqa A, et al. (2023) Oxidative Stress as a Triggering Mechanism of Various Diseases The Role of Natural Antioxidants in Brain Disorders. Springer, p 71-87

Anwar S, Kausar MA, Parveen K, et al. (2022) Polyherbal formulation: The studies towards identification of composition and their biological activities. J. King Saud Univ.-Sci. 34(7):102256

Arokiasamy P, Salvi S, Selvamani Y (2020) Global burden of diabetes mellitus. Handbook of global health:1-44

Association AD (2022) Introduction: standards of medical care in diabetes—2022. Diabetes care 45(Supplement_1):S1-S2

Bahiru E, Hsiao R, Phillipson D, Watson KE (2021) Mechanisms and treatment of

dyslipidemia in diabetes. Curr. Cardiol. Rep. 23:1-6

Benáková Š, Holendová B, Plecitá-Hlavatá L (2021) Redox homeostasis in pancreatic β-cells: from development to failure. Antioxidants 10(4):526

Ceretta LB, Réus GZ, Abelaira HM, et al. (2012) Increased oxidative stress and imbalance in antioxidant enzymes in the brains of alloxan-induced diabetic rats. J. Diabetes Res. 2012(1):302682

Choi SB, Ko BS, Park SK, Jang JS, Park S (2006) Insulin sensitizing and α-glucoamylase inhibitory action of sennosides, rheins and rhaponticin in Rhei Rhizoma. Life Sci. 78(9):934-942

Cicero AF, Fogacci F, Patrono D, et al. (2021) Application of the Sampson equation to estimate LDL-C in children: Comparison with LDL direct measurement and Friedewald equation in the BLIP study. Nutr. Metab. Cardiovasc. Dis. 31(6):1911-1915

Demirkıran T, Özdem T (2025) Antioxidant Effects of Thymoquinone in Lower Extremity Reperfusion Injury in Rats: An Examination of SOD and MDA Levels. Eurasian J. Med. Investig. 9(1):24

Dimitriadis G, Mitrou P, Lambadiari V, Maratou E, Raptis SA (2011) Insulin effects in muscle and adipose tissue. Diabetes Res. Clin. Pract. 93:S52-S59

Ebrahim N, Shakirova K, Dashinimaev E (2022) PDX1 is the cornerstone of pancreatic β-cell functions and identity. Front. Mol. Biosci. 9:1091757

Eguchi N, Vaziri ND, Dafoe DC, Ichii H (2021) The role of oxidative stress in pancreatic β cell dysfunction in diabetes.Int. J. Mol. Sci. 22(4):1509

Gaique TG, Boechat SK, Neto JGO, et al. (2023) Cinnamaldehyde supplementation acts as an insulin mimetic compound improving glucose metabolism during adolescence, but not during adulthood, in healthy male rats. Hormones 22(2):295-304

Grover A, Sharma K, Gautam S, Gautam S, Gulati M, Singh SK (2021) Diabetes and its complications: therapies available, anticipated and aspired. Curr. Diabetes Rev. 17(4):397-420

Gulec Peker EG, Kaltalioglu K (2021) Cinnamaldehyde and eugenol protect against LPS-stimulated oxidative stress and inflammation in Raw 264.7 cells. J. Food Biochem. 45(12):e13980

Huang J-K, Lee H-C (2022) Emerging evidence of pathological roles of very-low-density lipoprotein (VLDL). Int. J. Mol. Sci. (8):4300

Ibrahim KG, Hudu SA, Jega AY, et al. (2024) Thymoquinone: A comprehensive review of its potential role as a monotherapy for metabolic syndrome. Iran. J. Basic Med. Sci. 27(10):1214

Isaev NK, Genrikhs EE, Stelmashook EV (2023) Antioxidant thymoquinone and its potential in the treatment of neurological diseases. Antioxidants 12(2):433

Kaneto H, Kimura T, Shimoda M, et al. (2022) Molecular mechanism of pancreatic β-cell failure in type 2 diabetes mellitus. Biomedicines 10(4):818

Kang GG, Francis N, Hill R, Waters D, Blanchard C, Santhakumar AB (2019) Dietary polyphenols and gene expression in molecular pathways associated with type 2 diabetes mellitus: A review. Int. J. Mol.Sci. 21(1):140

Karandrea S, Yin H, Liang X, Slitt AL, Heart EA (2017) Thymoquinone ameliorates diabetic phenotype in Diet-Induced Obesity mice via activation of SIRT-1-dependent pathways. PloS one 12(9):e0185374

Kumar V, Anwar F, Ahmed D, et al. (2014) Paederia foetida Linn. leaf extract: an antihyperlipidemic, antihyperglycaemic and antioxidant activity. BMC Complement. Med. Ther. 14:1-16

Laxmi P, Gomez S, Markose AM, Chandrashekhar V, Suman K, Pathrose B (2025) Therapeutic potential of amla and cinnamon bark in diabetes mellitus: a phytochemical and mechanistic perspective. Plant Arch. 25(1):869-881

Li J-E, Futawaka K, Yamamoto H, et al. (2015) Cinnamaldehyde contributes to insulin sensitivity by activating PPARδ, PPARγ, and RXR. Am. J. Chin. Med. 43(05):879-892

Li Y, Nishimura T, Teruya K, et al. (2002) Protective mechanism of reduced water against alloxan-induced pancreatic β-cell damage: Scavenging effect against reactive oxygen species. Cytotechnology 40:139-149

Li Y, Wu R, Li X, et al. (2025) Anthraquinones of Cassiae Semen alleviate lipid

accumulation in obesity by regulating brown adipose tissue and liver function. Chin. Herb. Med.

Majdalawieh AF, Yousef SM, Abu-Yousef IA (2021) Thymoquinone, a major constituent in Nigella sativa seeds, is a potential preventative and treatment option for atherosclerosis. Eur. J. Pharmacol. 909:174420

Maqbool J, Anwar H, Iqbal J, et al. (2021) Methanolic extract of Fennel (Foeniculum vulgare) escalates functional restoration following a compression injury to the sciatic nerve in a mouse model. Food Sci. Nutr. 9(2):701-710

Mihajlovic M, Vinken M (2022) Mitochondria as the target of hepatotoxicity and drug-induced liver injury: molecular mechanisms and detection methods. Int. J Mol.Sci. 23(6):3315

Mubeen B, Hasnain A, Atif S, et al. (2025) Phytochemicals as multi-target therapeutic agents for oxidative stress-driven pathologies: mechanisms, synergies, and clinical prospects. Phyton 94(7):1941

Mukhtar A, Mukhtar I, Muzaffar H, et al. (2025) Nutraceutical Impact of Pumpkin Seed Oil on Expression Levels of EZH-2 and KRT-14 Genes against DSS-induced Inflammatory Bowel Disease in the Rat Model. Cell Biochem. Biophys. 83(2):2185-2199

Muzaffar H, Qamar I, Bashir M, Jabeen F, Irfan S, Anwar H (2023) Gymnema sylvestre supplementation restores normoglycemia, corrects dyslipidemia, and transcriptionally modulates pancreatic and hepatic gene expression in alloxan-induced hyperglycemic rats. Metabolites 13(4):516

Nawaz L, Grieve DJ, Muzaffar H, Iftikhar A, Anwar H (2024) Methanolic Extract of Phoenix Dactylifera Confers Protection against Experimental Diabetic Cardiomyopathy through Modulation of Glucolipid Metabolism and Cardiac Remodeling. Cells 13(14):1196

Olofinsan KA, Salau VF, Erukainure OL, Islam MS (2023) Senna petersiana (Bolle) leaf extract modulates glycemic homeostasis and improves dysregulated enzyme activities in fructose-fed streptozotocin-induced diabetic rats. J. Ethnopharmacol. 303:115998

Osman N, Jambi E, Aseri N (2017) Assessment of antidiabetic and antioxidant activities of Cassia angustifolia and Feoniculum vulgare in diabetic rats. Int. J. Pharm. Res. Allied Sci. 6(2)

Parasuraman S, Thing GS, Dhanaraj SA (2014) Polyherbal formulation: Concept of ayurveda. Pharmacogn. Rev. 8(16):73

Patel J (2025) Preparation and Standardization of some herbal Antidiabetic drugs in Polyherbal Formulation. Asian. J. Pharm. (AJP) 19(01)

Patwardhan B, Warude D, Pushpangadan P, Bhatt N (2005) Ayurveda and traditional Chinese medicine: a comparative overview. Evid.-Based Complement. Altern. Med. 2(4):465-473

Queiroz LA, Assis JB, Guimarães JP, et al. (2021) Endangered lymphocytes: The effects of alloxan and streptozotocin on immune cells in type 1 induced diabetes. Mediat. Inflamm. 2021(1):9940009

Ranasinghe P, Pigera S, Premakumara GS, Galappaththy P, Constantine GR, Katulanda P (2013) Medicinal properties of 'true'cinnamon (Cinnamomum zeylanicum): a systematic review. BMC Complement. Altern. Med. 13:1-10

Singh R, Parasuraman S, Kathiresan S (2020) Antioxidant and antidiabetic activities of methanolic extract of bark of Cinnamomum zeylanicum in diabetic rats. Free Rad. Antiox. 10(1):16-23

Soleimani-Dodran M, Alipanah-Moghadam R, Jeddi F, Babaei M, Salimnejad R, Bahreini E (2022) Effect of hydroalcoholic seed extract of Nigella sativa on hepatic and pancreatic factors of Nrf2 and FGF21 in the regulation of insulin transcription factors of MafA and PDX-1 in streptozotocin-treated diabetic rats. Nutr. Metab. 19(1):64

Stein SA, Lamos EM, Davis SN (2013) A review of the efficacy and safety of oral antidiabetic drugs. Expert Opin. Drug Saf. 12(2):153-175

Tamura Y, Omura T, Toyoshima K, Araki A (2020) Nutrition management in older adults with diabetes: a review on the importance of shifting prevention strategies from metabolic syndrome to frailty. Nutrients 12(11):3367

Wang B, Wang Y, Zhang J, et al. (2023) ROS-induced lipid peroxidation modulates cell death outcome: mechanisms behind apoptosis, autophagy, and ferroptosis. Arch. Toxicol. 97(6):1439-1451

Whyte M, Jackson N, Shojaee-moradie F, et al. The effect of insulin therapy on glucose production rate, glucose uptake, lipolysis and proteolysis in non-surgical ICU patients. In: Diabetologia-Volume 47, Issue 1 Supplement, August 2004-Proceedings of the 40th EASD Annual Meeting of the European Association for the Study of Diabetes, 2004. vol 47. p A216-A216

Wickramasinghe ASD, Kalansuriya P, Attanayake AP (2021) Herbal Medicines Targeting the Improved β-Cell Functions and β-Cell Regeneration for the Management of Diabetes Mellitus. Evid.-Based Complement. Altern. Med. 2021(1):2920530

Zhao L, Zheng L (2023) A Review on Bioactive Anthraquinone and Derivatives as the Regulators for ROS. Molecules 28(24):8139