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Ginkgolide B restores pancreatic β -cell identity and attenuates oxidative stress in streptozotocin-induced diabetic rats *via* Nrf2/Keap1 pathway activation

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ABSTRACT

Objective: To investigate the efficacy of ginkgolide B, a major active constituent isolated from *Ginkgo biloba*, on pancreatic β -cell integrity and Nrf2/Keap1 signaling in a streptozotocin (STZ)-induced diabetes model of rats.

Methods: Sprague-Dawley rats were injected with STZ along with nicotinamide. After confirmation of hyperglycemia, diabetic rats ($n=8$ /group) received no treatment, or ginkgolide B (10 or 30 mg/kg). Healthy rats served as normal control. After 21 days of treatment, rats were sacrificed, and plasma samples were obtained to assess the biochemical, hepatic, renal, oxidative stress, lipid profile, apoptotic, and inflammatory parameters. Pancreatic tissue was used for histopathological observation and detection of gene expression of *NFE2L2*, *KEAP1*, *NQO1*, *HO-1*, *Ins1*, *MAFA*, *Ng3*, *Pdx1*, and *Pax4*.

Results: Ginkgolide B-treated rats showed significant improvement in diabetes markers, *i.e.*, fasting plasma glucose, insulin, and glucagon. It also significantly improved oxidative stress, inflammation, as well as lipid profile, antiapoptotic, liver, and kidney function markers as compared with the model group. Ginkgolide B improved β -cell function, activated Nrf2 signaling, and upregulated β -cell-related genes *Ins1*, *Pdx1*, *MAFA*, *Ng3*, and *Pax4*. Histopathological results showed nearly normal islet architecture in ginkgolide B-treated groups.

Conclusions: Ginkgolide B shows antidiabetic effect. It can alleviate oxidative damage and inflammation, and upregulate genes related to regeneration and insulin signaling.

KEYWORDS: *Ginkgo biloba*; Ginkgolide B; Diabetes mellitus; Nrf2/Keap1 pathway; Oxidative stress; β -cell regeneration

1. Introduction

Diabetes mellitus (DM: 5A11.0, 5A11.1) is among the most challenging global health issues of this century[1]. It is a chronic and complex metabolic disorder and affects individuals across all age groups. DM is characterized by significantly increased blood glucose triggered by relative insulin deficiency[2]. It leads to a number of complications, such as coronary artery disease, cerebrovascular accidents, nephropathy, neuropathy, ocular dysfunction, and impaired wound healing. These issues raise patient morbidity and mortality and pose a threat to global public health[3]. Abnormal

Summary

Question: Could ginkgolide B treat hyperglycemia in rats?

Findings: Ginkgolide B ameliorated oxidative stress, inflammation, and metabolic disorders by improving liver and kidney function, restoring lipid and protein profiles, and modulating Nrf2 and key genes related to insulin signaling and β -cell regeneration.

Meaning: Ginkgolide B could significantly improve diabetes and protect organs at molecular level.

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levels of liver enzymes and dyslipidemia are also linked with DM[4]. Hyperglycemia in DM conditions induces chronic inflammation, pancreatic β -cell dysfunction, and excessive generation of reactive oxygen species[5]. Such oxidative stress also impairs the synthesis and release of insulin by disrupting the expression of key β -cells-specific genes and transcription factors, including pancreatic and duodenal homeobox1 (*Pdx1*), neurogenin-3 (*Ngn3*), v-maf musculoaponeurotic fibrosarcoma oncogene homolog A (*MAFA*), paired box gene 4 (*Pax4*), and insulin-1 (*Ins1*)[6]. The nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway plays a central role in cellular defense against oxidative stress through the transcriptional activation of antioxidant enzymes such as NAD(P)H quinone oxidoreductase-1 (NQO1) and heme oxygenase-1 (HO-1)[7]. Under physiological conditions, Nrf2 activity is negatively regulated by Kelch-like ECH-associated protein 1 (Keap1). Dysregulation of this pathway significantly contributes to β -cell injury and the progression of diabetes[8].

Medicinal plants have been used for the treatment of different diseases for centuries. Even today, a large number of people rely on herbal medicines, especially in developing countries, owing to their easy availability, lower cost, and a long history of traditional use[9]. Many plant-derived compounds have also shown beneficial effects against chronic diseases, including DM[10].

Ginkgolide B is a major active constituent isolated from *Ginkgo biloba*[11]. Previous studies have reported that ginkgolide B possesses antioxidant[12,13], anti-inflammatory[14], cardioprotective[15,16], and cell-protective activities[17]. Evidence also suggests that ginkgolide B can enhance the body's antioxidant defense system by regulating the Nrf2 signaling pathway and increasing the expression of protective antioxidant genes such as *HO-1* and *NQO1*[18]. These biological properties render ginkgolide B a promising candidate for protecting pancreatic β -cells against diabetes-induced damage. Furthermore, ginkgolide B has been shown to ameliorate metabolic and vascular complications associated with oxidative stress and inflammation[19].

Therefore, the present study was designed to investigate the therapeutic potential of ginkgolide B in restoring pancreatic β -cell identity and function in streptozotocin-induced diabetic rats, with a focus on its regulatory effects on β -cell-associated genes (*Ins1*, *Pdx1*, *Ngn3*, *MAFA*, and *Pax4*) and modulation of the Nrf2/Keap1 antioxidant signaling pathway.

2. Methods

2.1. Procurement of ginkgolide B

Ginkgolide B was purchased from Merck KGaA, Darmstadt, Germany (CAS number: 15291-77-7; Catalog number: G6910); purity $\geq 90\%$ (HPLC), batch No. MKCZ2454. It was stored at -20°C in amber vials until use.

2.2. Experimental animals and diabetes induction

A total of 32 Sprague-Dawley male rats, aged (8 ± 1) weeks and weighing (200 ± 10) g were taken from the Laboratory Animal Facility of the University of Lahore. The animals were kept under standard laboratory conditions, at (26 ± 2) $^{\circ}\text{C}$, relative humidity of 40%-60%, and a 12-hour light/dark cycle.

To induce diabetes, rats were fasted overnight before treatment. Nicotinamide (110 mg/kg body weight) dissolved in normal saline was injected intraperitoneally. After 15 min, a freshly prepared solution of streptozotocin (65 mg/kg body weight) in cold 0.1 M citrate buffer (pH 4.5) was injected intraperitoneally. This combination was used to establish the diabetic model. After 72 h, blood glucose levels were measured using a glucometer (Glucocard 01-mini, Arkray Factory Inc., Japan) to verify hyperglycemia (250 mg/dL)[20]. These rats were divided into three groups: treatment groups receiving 10 and 30 mg/kg body weight of ginkgolide B (GB-1 and GB-2) and the model group without treatment. The dosages were chosen based on previous studies on the antioxidant qualities of ginkgolide B[19,21,22]. Eight healthy rats were housed under the same conditions as the negative control group (NC) and did not receive any treatment.

After the 21-day treatment, the rats were fasted for 12 h, then anesthetized with inhaled isoflurane in an induction chamber until a deep surgical plane of anesthesia was attained, as confirmed by loss of pedal and corneal reflexes. Following adequate anesthesia, euthanasia was performed by rapid decapitation using a sharp guillotine, in accordance with internationally accepted guidelines for the humane use of laboratory animals[23]. Heparin, an anticoagulant, was added to vacutainers containing jugular vein blood. It was centrifuged at 2000 *g* for 10 min to extract the serum and subsequently stored at $-(20 \pm 4)^{\circ}\text{C}$ for further analysis. For gene expression studies, a fresh slice of pancreatic tissue was preserved in TRIzol and stored at $-(80 \pm 5)^{\circ}\text{C}$; another slice was kept at room temperature in formalin for histopathological evaluation[24].

2.3. Ethical statement

All animal experimental procedures involving animals were approved by the Institutional Animal Ethics Committee of the University of Lahore (Approval No: IMBB/BBBC/24/006-C; dated January 10, 2024) and were conducted in accordance with the guidelines of the National Institutes of Health. ARRIVE 2.0 guidelines were followed in this study to guarantee repeatability and transparency in research involving animals[25].

2.4. Assessment of biochemical parameters

2.4.1. Serum antidiabetic parameters

Commercial ELISA kits from Elabscience (USA) were utilized to test serum levels of insulin (Cat. No. E-EL-R2466) and

glucagon (Catalog No. E-EL-R0425) in accordance with the kits' recommendations. The coefficient of variance for both kits was under 10%. The Bioclin[®] Glucose Mono-reagent testing kit was used to measure serum glucose levels.

2.4.2. Serum liver and kidney function markers

Liquiform method kits from Randox Laboratories Ltd. (BT29 4QY; Crumlin, County Antrim, UK) were used to measure aspartate aminotransferase (AST; Cat. No. AS3804), alanine aminotransferase (ALT; Cat. No. AL3801), total protein (Cat. No. TP4001), albumin (Cat. No. AB3800), globulin (Cat. No. IG8044), urea (Cat. No. UR3825), and creatinine (Cat. No. CR8316).

2.4.3. Oxidative stress markers

Serum total antioxidant capacity (TAC) and total oxidant status (TOS) were measured according to the methods described by Mustafa *et al.*[24]. Serum glutathione levels were determined using a commercially available Glutathione ELISA Kit (Elabscience, Cat. No. E-EL-0026) according to the manufacturer's instructions. The results were expressed as µg/mL.

2.4.4. Pro-inflammatory markers

Serum concentrations of tumor necrosis factor-alpha (TNF-α; E-EL-R2856), nuclear factor-kappa B p105 subunit (NF-κB p105; E-EL-R0673), cyclooxygenase-2 (COX-2/PTGS2; E-EL-R0792), interleukin-6 (IL-6; E-EL-R0015), and interleukin-1 beta (IL-1β; E-EL-R0012) were determined using commercially available ELISA kits (Elabscience[®], USA) according to the manufacturer's instructions.

2.4.5. Evaluation of apoptotic markers

Serum levels of B-cell lymphoma 2 (Bcl-2; CSB-E08854r), Bcl-2-associated X protein (Bax; CSB-E11987r), Caspase-3 (CSB-E08857r), and Caspase-9 (CSB-E08863r) were determined using rat-specific ELISA kits (CUSABIO Technology LLC, Houston, TX, USA) according to the manufacturer's instructions.

2.4.6. Serum lipid profile

Serum concentrations of triglycerides (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) were determined using commercial assay kits from Crescent Diagnostic Systems (Jeddah, Saudi Arabia) (Cat. No. CS611-4, CS603-10, and CS607-2, respectively). Low-density lipoprotein cholesterol (LDL-C) levels were estimated using the Friedewald formula[26]:

$$\text{LDL-C} = \text{TC} - \text{HDL-C} - (\text{TG}/5)$$

2.5. Histopathology of the pancreas

For histopathological evaluation, pancreatic tissues were collected and fixed in 10% neutral buffered formalin, followed by routine

paraffin embedding. Tissue blocks were sectioned at a thickness of 4–5 µm using a rotary microtome (BK-MT268M, Biobase Biodustry Co., Ltd., Shandong, China). The sections were mounted on albumin-coated glass slides and processed for hematoxylin and eosin (H&E) staining after deparaffinization and rehydration. Following staining, the slides were mounted with DPX mounting medium and examined under a light microscope (XSZ-107BN, Hinotek Group Limited) for assessment of pancreatic histoarchitecture[24].

2.6. Gene expression analysis

Total RNA was isolated from pancreatic tissue using the TRIzol reagent method, and its purity and concentration were determined using a NanoDrop spectrophotometer[24]. Complementary DNA (cDNA) was synthesized from the recommended amount of RNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA) according to the manufacturer's protocol. Quantitative real-time PCR (qRT-PCR) was carried out on an iQ5 Real-Time PCR Detection System (Bio-Rad Laboratories, USA) using Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific, USA).

The relative mRNA expression of *Ins1*, *MAFA*, *Pax4*, *Pdx1*, *Ngn3*, *NQO1*, *HO-1*, *NFE2L2*, and *Keap1* was determined using *β-actin* as the internal reference gene. Primer sequences are provided in Supplementary Table. PCR amplification was performed under the following conditions: initial denaturation followed by 39 cycles of denaturation at 95 °C for 15 s, annealing at 58 °C for 30 s, and extension at 72 °C for 20 s. All primers were synthesized by Invitrogen (Karlsruhe, Germany). Relative gene expression was calculated using the 2^{−ΔΔCt} method.

2.7. Statistical analysis

Statistical analysis was performed using SPSS software version 28.0 (IBM Corp., Armonk, NY, USA). Data are presented as mean ± standard deviation (SD). The normality of data distribution was assessed using the Shapiro-Wilk test. Differences among experimental groups were evaluated by one-way analysis of variance (ANOVA), followed by Tukey's *post hoc* test. A *P*-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Effect of ginkgolide B on serum biochemical factors

3.1.1. Effect of ginkgolide B on serum glucose, insulin and glucagon levels

The model group showed significantly elevated insulin, glucose, and glucagon levels compared with the normal control group

($P < 0.05$). Treatment with ginkgolide B significantly improved these parameters in both treatment groups ($P < 0.05$) (Figure 1).

3.1.2. Effect of ginkgolide B on lipid profile, and liver and kidney function markers

Serum biochemical parameters are presented in Table 1. The model group showed significantly elevated creatinine, urea, ALT, and AST compared with the normal control group ($P < 0.05$). Treatment with ginkgolide B significantly reduced these parameters ($P < 0.001$) (Table 1).

Lipid profile analysis revealed significantly increased TC, TG, and LDL-C along with reduced HDL-C in the model group ($P < 0.05$). Ginkgolide B treatment significantly improved these parameters ($P < 0.05$) (Table 1).

Similarly, total protein, albumin, and globulin levels were significantly reduced in diabetic rats compared with normal controls ($P < 0.05$), which were significantly increased by ginkgolide B

treatment ($P < 0.05$) (Table 1).

3.1.3. Effect of ginkgolide B on apoptotic, pro-inflammatory and oxidative stress markers

The diabetic rats in the model group showed lower Bcl-2 levels and higher levels of Bax, Caspase-3, and Caspase-9 compared with the normal control group ($P < 0.05$). Treatment with ginkgolide B significantly increased Bcl-2 levels and decreased levels of Bax, Caspase-3, and Caspase-9 ($P < 0.001$) (Table 2).

Inflammatory markers including NF- κ B, TNF- α , IL-1 β , IL-6, and COX-2 were significantly elevated in diabetic rats ($P < 0.05$). These values were significantly reduced following ginkgolide B treatment, particularly at 30 mg/kg ($P < 0.05$) (Table 2).

For oxidative stress markers, glutathione and TAC were significantly reduced in the model group, while TOS was significantly increased ($P < 0.05$). Ginkgolide B significantly increased glutathione and TAC, and reduced TOS compared with diabetic controls ($P < 0.001$) (Table 2).

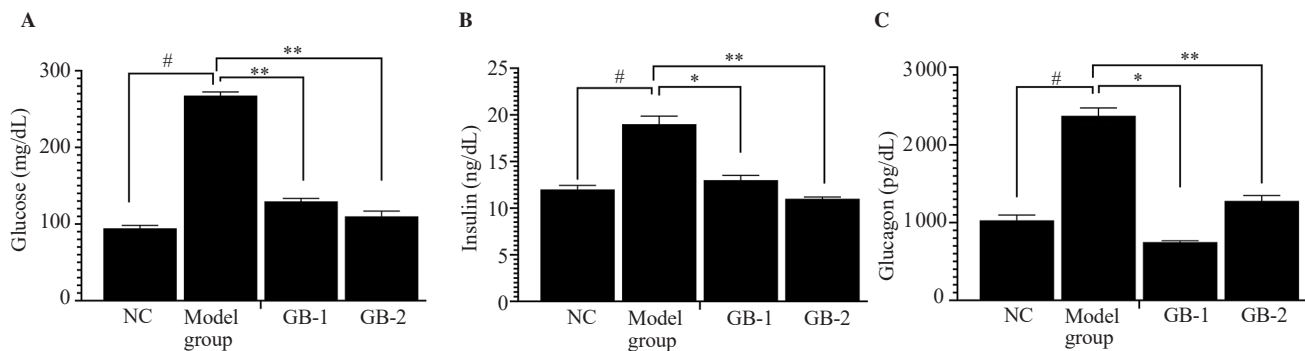


Figure 1. Effect of ginkgolide B on fasting plasma glucose (A), insulin (B), and glucagon (C) in streptozotocin-induced diabetic rats. Values are expressed as mean $\pm$ SD ($n = 8$). # $P < 0.05$ compared with the normal control group; * $P < 0.05$, ** $P < 0.001$ compared with the model group. NC: normal control; GB-1: 10 mg/kg body weight of ginkgolide B; GB-2: 30 mg/kg body weight of ginkgolide B.

Table 1. Effect of ginkgolide B on renal function, liver enzymes, lipid profile, and serum protein levels in streptozotocin-induced diabetic rats.

Parameters	Normal control	Model	Ginkgolide B 10 mg/kg	Ginkgolide B 30 mg/kg
Creatinine (mg/dL)	0.60 $\pm$ 0.08	1.80 $\pm$ 0.02 [#]	1.40 $\pm$ 0.03 ^{**}	1.20 $\pm$ 0.01 ^{**}
Urea (mg/dL)	28.40 $\pm$ 1.17	80.30 $\pm$ 3.50 [#]	51.30 $\pm$ 1.50 ^{**}	42.90 $\pm$ 2.59 ^{**}
ALT (U/L)	29.37 $\pm$ 1.17	78.31 $\pm$ 1.50 [#]	46.11 $\pm$ 2.50 ^{**}	37.41 $\pm$ 2.59 ^{**}
AST (U/L)	52.09 $\pm$ 3.78	148.58 $\pm$ 4.78 [#]	68.35 $\pm$ 4.01 ^{**}	52.09 $\pm$ 6.90 ^{**}
Total cholesterol (mg/dL)	288.00 $\pm$ 13.45	329.00 $\pm$ 9.78 [#]	314.00 $\pm$ 2.41 [*]	207.00 $\pm$ 2.79 ^{**}
Triglycerides (mg/dL)	177.00 $\pm$ 29.78	328.00 $\pm$ 27.39 [#]	154.00 $\pm$ 2.16 ^{**}	153.00 $\pm$ 1.39 ^{**}
HDL-C (mg/dL)	136.00 $\pm$ 12.44	80.00 $\pm$ 40.06 [#]	112.00 $\pm$ 6.43 [*]	134.00 $\pm$ 12.41 ^{**}
LDL-C (mg/dL)	189.00 $\pm$ 20.24	314.00 $\pm$ 49.86 [#]	190.00 $\pm$ 6.00 ^{**}	135.00 $\pm$ 12.50 ^{**}
Total protein (g/dL)	6.50 $\pm$ 0.17	4.50 $\pm$ 0.39 [#]	5.90 $\pm$ 0.05 ^{**}	6.30 $\pm$ 0.11 ^{**}
Albumin (g/dL)	3.80 $\pm$ 0.17	2.10 $\pm$ 0.19 [#]	3.20 $\pm$ 0.05 ^{**}	3.60 $\pm$ 0.11 ^{**}
Globulin (g/dL)	2.80 $\pm$ 0.17	2.10 $\pm$ 0.04 [#]	2.20 $\pm$ 0.05 [*]	2.60 $\pm$ 0.11 ^{**}

Values are presented as mean $\pm$ SD ($n = 8$). Statistical analysis was performed using one-way ANOVA followed by Tukey's *post hoc* test. # $P < 0.05$ compared with the normal control group; * $P < 0.05$, ** $P < 0.001$ compared with the model group. ALT: alanine aminotransferase; AST: aspartate aminotransferase; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.

Table 2. Effect of ginkgolide B on apoptotic, inflammatory, and oxidative stress markers.

Parameters	Normal control	Model	Ginkgolide B 10 mg/kg	Ginkgolide B 30 mg/kg
Bax (ng/mg protein)	1.84 ± 0.09	10.17 ± 0.48 [#]	4.96 ± 0.22 ^{**}	4.11 ± 0.18 ^{**}
Bcl-2 (ng/mg protein)	15.94 ± 0.71	5.03 ± 0.44 [#]	10.58 ± 0.52 ^{**}	13.36 ± 0.57 ^{**}
Caspase-3 (pg/mL)	1.29 ± 0.08	12.68 ± 0.69 [#]	7.03 ± 0.26 ^{**}	5.34 ± 0.17 ^{**}
Caspase-9 (pg/mL)	2.05 ± 0.12	12.87 ± 0.61 [#]	6.48 ± 0.21 ^{**}	4.76 ± 0.22 ^{**}
NF-κB (ng/g tissue)	15.22 ± 0.96	57.63 ± 2.34 [#]	34.17 ± 1.37 ^{**}	28.41 ± 1.08 ^{**}
TNF-α (ng/g tissue)	7.46 ± 0.69	45.28 ± 1.43 [#]	20.05 ± 0.81 ^{**}	14.56 ± 0.63 ^{**}
IL-1β (ng/g tissue)	24.71 ± 1.11	66.92 ± 1.73 [#]	39.14 ± 1.28 ^{**}	34.61 ± 1.36 ^{**}
IL-6 (ng/g tissue)	4.38 ± 0.91	38.42 ± 1.45 [#]	26.31 ± 1.07 ^{**}	15.88 ± 1.02 ^{**}
COX-2 (ng/g tissue)	12.14 ± 1.03	56.48 ± 2.11 [#]	30.47 ± 1.13 ^{**}	23.76 ± 1.19 ^{**}
Glutathione (μg/mL)	552.25 ± 28.44	259.75 ± 24.86 [#]	729.48 ± 16.51 ^{**}	879.50 ± 18.19 ^{**}
TAC (mM Vit. C equiv./L)	1.63 ± 0.20	0.76 ± 0.08 [#]	1.45 ± 0.20 ^{**}	2.88 ± 0.07 ^{**}
TOS (μM H ₂ O ₂ equiv./L)	19.43 ± 2.72	30.95 ± 1.34 [#]	24.28 ± 0.82 ^{**}	21.77 ± 0.61 ^{**}

Values are presented as mean ± SD (*n* = 8). Statistical analysis was performed using one-way ANOVA followed by Tukey's *post hoc* test. [#]*P* < 0.05 compared with the normal control group; ^{*}*P* < 0.05, ^{**}*P* < 0.001 compared with the model group. NF-κB: nuclear factor kappa B; TNF-α: tumor necrosis factor-alpha; IL-1β: interleukin-1 beta; IL-6: interleukin-6; COX-2: cyclooxygenase-2; TAC: total antioxidant capacity; TOS: total oxidant status.

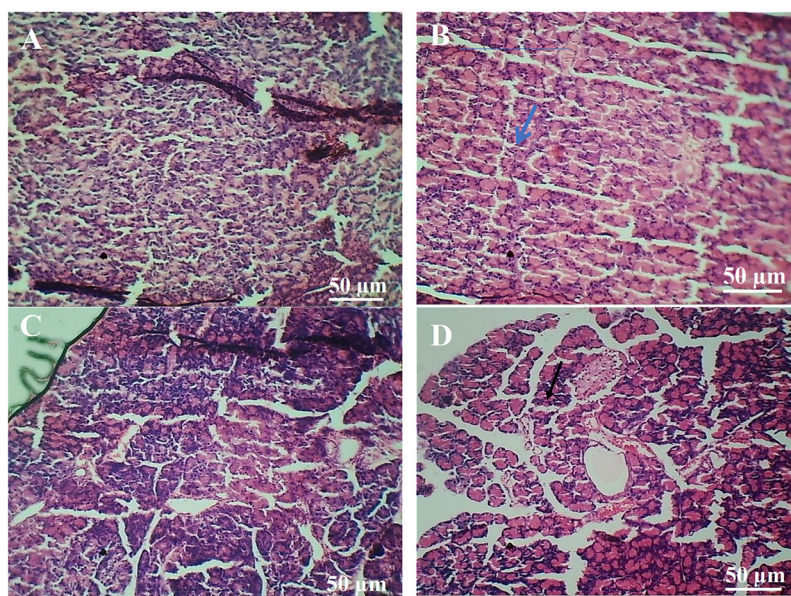


Figure 2. Histological evaluation of pancreatic tissue following ginkgolide B treatment in streptozotocin-induced diabetic rats. Hematoxylin and eosin (H&E)-stained pancreatic sections (40× magnification; scale bar = 50 μm). (A) The normal control group showing normal pancreatic architecture with intact islets of Langerhans and well-organized acinar cells; (B) The model group showing marked shrinkage of islets, cellular degeneration, vacuolization, and mild inflammatory cell infiltration (blue arrow); (C) The GB-1 (ginkgolide B 10 mg/kg) group exhibiting moderate improvement in islet structure and acinar organization; (D) GB-2 (ginkgolide B 30 mg/kg) group displaying nearly normal pancreatic architecture with well-preserved islets of Langerhans and intact exocrine tissue (black arrow).

3.2. Effect of ginkgolide B on pancreatic histopathology

Microscopic examination of pancreatic tissue showed clear differences among the experimental groups. The normal control group (Figure 2A) displayed normal pancreatic structure with well-defined islets of Langerhans and regularly arranged acinar cells. No tissue injury, inflammation, or cellular damage was observed.

The model group (Figure 2B) presented pathological lesions. The islets of Langerhans shrank with irregular morphological distortion (blue arrow). Peripheral acinar cells underwent degeneration and vacuolization, along with mild inflammatory cell infiltration.

Treatment with ginkgolide B at 10 mg/kg alleviated pancreatic structural abnormalities (Figure 2C), with improved retention of

islet integrity and reduced cellular injury and vacuolization. The GB-2 treatment group achieved the most evident amelioration (Figure 2D), manifesting nearly normal pancreatic architecture with well-maintained islets of Langerhans and minimal tissue damage. The black arrow highlights healthy islet cells and well-organized exocrine pancreatic parenchyma.

3.3. Regulatory effect of ginkgolide B on the key genes in the pancreatic tissue of rats

The diabetic model group exhibited significant downregulation of the β-cell-related genes *Ins1*, *Pdx1*, *Ngn3*, *MAFA*, and *Pax4* compared with the normal control group (*P* < 0.05). Treatment with

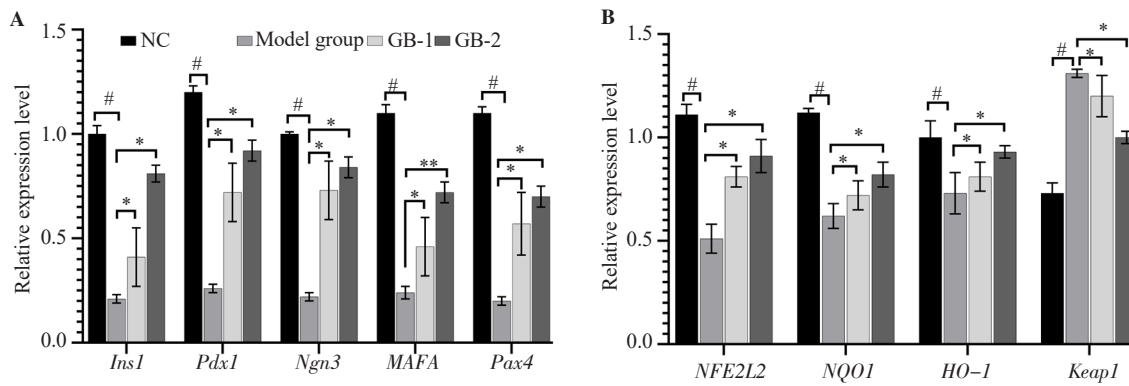


Figure 3. Effect of ginkgolide B on pancreatic gene expression in streptozotocin-induced diabetic rats. (A) Relative mRNA expression of β -cell function and regeneration markers (*Ins1*, *Pdx1*, *Ngn3*, *MAFA*, and *Pax4*). (B) Relative mRNA expression of antioxidant signaling pathway genes (*NFE2L2*, *NQO1*, *HO-1*, and *Keap1*). Values are expressed as mean $\pm$ SD ($n = 8$). [#] $P < 0.05$ compared with the normal control group; ^{*} $P < 0.05$, ^{**} $P < 0.001$ compared with the model group.

ginkgolide B restored the expression of these genes in a dose-dependent manner, with the higher-dose group showing greater improvement ($P < 0.05$) (Figure 3A).

Similarly, the expression of *NFE2L2*, *NQO1*, and *HO-1* was significantly reduced in the diabetic model group ($P < 0.05$). Administration of ginkgolide B significantly increased the expression of these genes, with a more significant effect observed in the GB-2 group ($P < 0.05$). In contrast, *Keap1* expression was elevated in diabetic rats and was reduced following ginkgolide B treatment, particularly in the higher dose group ($P < 0.05$) (Figure 3B).

4. Discussion

DM is characterized by progressive β -cell dysfunction[27]. Additionally, some other factors such as persistent oxidative stress and chronic inflammation exacerbate disease progression[28]. These factors gradually impair glucose homeostasis and trigger pancreatic damage. In the present study, STZ-induced diabetic rats showed several pathological changes, *e.g.*, pancreatic morphological lesions, abnormal biochemical indices, oxidative status, elevated inflammatory factors, altered apoptotic biomarkers, and dysregulated β -cell-related genes. Treatment with ginkgolide B ameliorated most of these abnormalities, with greater beneficial effects observed at the higher dose.

Previous histological investigations have confirmed severe pancreatic lesions in the model group rats, including shrunken and distorted islets of Langerhans and disrupted normal cell structure. Muzaffar *et al.*[4] and Mustafa *et al.*[24] likewise observed islet shrinkage, cell degeneration, and β -cell loss in diabetic animals. In comparison, the GB-1 and GB-2 groups showed retained intact pancreatic and islet structure alongside improved blood glucose

levels, suggesting that ginkgolide B protects functional β -cells and promotes recovery.

Oxidative stress is a major contributor to β -cell dysfunction. In this study, diabetic rats showed impaired antioxidant capacity and increased oxidative stress levels. Ginkgolide B restored glutathione and TAC and reduced TOS. She *et al.* reported that ginkgolide B attenuated oxidative damage and ferroptosis through activation of Nrf2 signaling[18]. Recent studies and reviews confirmed that ginkgolide B enhances antioxidant enzyme activity, reduces lipid peroxidation, and increases HO-1 expression in different disease models[12,29].

We also evaluated the Nrf2/Keap1 pathway to clarify the antioxidant mechanism. Nrf2 mediates major cellular defense, with *NQO1* and *HO-1* as important downstream genes. Keap1 inhibits this pathway under normal conditions. Diabetic rats showed inhibited Nrf2 activity and increased Keap1 expression, suggesting impaired antioxidant defense. Ginkgolide B reversed these changes and enhanced the expression of Nrf2-associated genes in the GB-1 and GB-2 groups. Consistently, Liu *et al.* reported that ginkgolide B triggers Nrf2 signaling and increases the expression of antioxidant genes to resist oxidative cellular injury[30]. Similarly, a recent study by Ma *et al.* demonstrated that ginkgolide B attenuated oxidative damage through activation of the Nrf2/HO-1 pathway, thereby reinforcing endogenous antioxidant defenses[31]. Collectively, these studies prove that ginkgolide B consistently exerts antioxidant activity by activating Nrf2-dependent antioxidant signaling. Therefore, the upregulation of *Nrf2*, *NQO1*, and *HO-1* together with suppression of *Keap1* observed in the GB-1 and GB-2 groups in the present study suggests that activation of the Nrf2/Keap1 pathway may be a major mechanism underlying the antioxidant and cytoprotective effects of ginkgolide B in diabetic rats.

Excessive oxidative stress also promotes inflammatory responses

and accelerates β -cell loss[32]. Accordingly, diabetic rats showed marked elevations in NF- κ B, TNF- α , IL-1 β , IL-6, and COX-2. These findings are consistent with previous reports demonstrating activation of inflammatory pathways in diabetic pancreatic tissues. Ginkgolide B significantly reduced all detected inflammatory markers. Liu *et al.* also confirmed that ginkgolide B treatment inhibits NF- κ B-mediated inflammatory signaling, supporting its anti-inflammatory property[30]. The suppressed inflammation in our study is likely related to recovered redox balance and reduced oxidative stress.

Persistent oxidative stress and inflammation eventually trigger apoptosis. This was evident by decreased Bcl-2 expression and increased Bax, Caspase-3, and Caspase-9 levels in diabetic animals. Ginkgolide B restored this balance to support cell survival by increasing Bcl-2 and reducing pro-apoptotic markers. These findings suggest that preservation of β -cell viability may be one of the important mechanisms contributing to the antidiabetic effects of ginkgolide B.

We also examined the expression of *Ins1*, *Pdx1*, *Ngn3*, *MAFA*, and *Pax4* to further assess β -cell status. These genes participate centrally in insulin synthesis, β -cell maturation, and maintenance of β -cell identity. Diabetes markedly suppressed their expression, while ginkgolide B restored their gene expression. Similar observations have been reported for several plant-derived compounds[7]. For example, supplementation with *Gymnema sylvestre* reduced oxidative stress and improved pancreatic function in alloxan-induced hyperglycemic rats through modulation of key β -cell-related genes. The authors reported restoration of genes involved in insulin synthesis, β -cell differentiation, and maturation, including *Ins1*, *Pdx1*, *Ngn3*, *MAFA*, and *Pax4*. Likewise, Al-Adsani *et al.* also proved that garlic extract (*Allium sativum*) significantly increased the expression of *Pdx1*, *Ngn3*, and *MAFA* in pancreatic tissue. The authors suggested that upregulation of these genes contributed to β -cell maturation and recovery of insulin-producing capacity in diabetic rats. Given the well-documented antioxidant activity of garlic extract, the study validates that plant-derived compounds can improve β -cell function by reducing oxidative stress and restoring key transcription factors involved in β -cell regeneration and insulin synthesis[33]. More recently, Raza *et al.* reported that manool restored the expression of these β -cell-related genes together with improved glucose homeostasis[1]. These findings indicate that reduction of oxidative stress may facilitate recovery of β -cell-specific transcription factors and preservation of insulin synthesis and secretion.

The protective effects of ginkgolide B were not limited to pancreatic tissue. Diabetic rats also developed dyslipidemia and impaired liver and kidney function. Ginkgolide B improved TC, TG, LDL-C, HDL-C, ALT, AST, creatinine, and urea levels. Consistent with our

results, accumulating evidence has demonstrated that ginkgolide B alleviates renal and hepatic injury and improves metabolic disorders through antioxidant and anti-inflammatory activities[34]. Therefore, the systemic improvements observed in the present study are plausibly attributed to enhanced glycemic homeostasis and reduced oxidative damage.

The present findings suggest that ginkgolide B effectively preserves β -cell function and alleviates diabetes-associated tissue injury. However, this study was limited to an experimental animal model and a short treatment duration. Future studies should include protein-level validation of the Nrf2 pathway, long-term safety assessment, and clinical evaluation in diabetic patients.

In conclusion, ginkgolide B could improve pancreatic morphology, restore β -cell-related gene expression, reduce oxidative stress, inflammation, and apoptosis, and improve metabolic abnormalities in diabetic rats. These protective effects appear to be mediated, at least in part, through activation of the Nrf2/Keap1 pathway and preservation of β -cell function.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding author upon request.

Authors' contributions

JN and IM conceived and designed the study. NSK, SARS, and SZ were responsible for data collection and experimental work. IM, NSK, and RA performed data analysis and interpretation. MS and IM drafted the manuscript. RA and IM critically revised the manuscript for important intellectual content. All authors reviewed and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

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Supplementary Table. Sequences of the primers.

<i>Ins1-F</i>	5'-GCAAGCAGGTCATTGTTTCAAC-3'
<i>Ins1-R</i>	5'-AAGCCTGGGTGGGTTTGG -3'
<i>Pdx1-F</i>	5'-TCCCGAATGGAACCGAGACT-3'
<i>Pdx1-R</i>	5'-TTCATCCACGGGAAAGGGAG-3'
<i>MAFA-F</i>	5'-CGCACCCGACTTCTTTCTGT-3'
<i>MAFA-R</i>	5'-CTCAGAGTCCGAACCGAGG-3'
<i>Pax4-F</i>	5'-AGATGTTCCAGTGACACCACA-3'
<i>Pax4-R</i>	5'-CACAGGAAGGAGGGAGTGG-3'
<i>Ngn3-F</i>	5'-CACCGCTGCTTGACTCTGACC-3'
<i>Ngn3-R</i>	5'-CACTCCCCTCTTCTACCCTTTG-3'
<i>NFE2L2-F</i>	5'-CACATCCAGACAGACACCAGT-3'
<i>NFE2L2-R</i>	5'-CTACAAATGGGAATGTCTCTGC-3'
<i>NQO1-F</i>	5'-CAGTTCCCATTTGTATTGGTTTG-3'
<i>NQO1-R</i>	5'-TGGAAAGCAAGGTCTTCTTATT-3'
<i>HO-1-F</i>	5'-GAGGAACACAAAGACCAGAG-3'
<i>HO-1-R</i>	5'-TGGGATGAACTAGTGCTGA-3'
<i>Keap1-F</i>	5'-CAACTTCGCTGAGCAGATTGGC-3'
<i>Keap1-R</i>	5'-TGATGAGGGTCACCAGTTGGCA-3'
<i>Beta-actin-F</i>	5'-TCCTGAGCGCAAGTACTCTGT-3'
<i>Beta-actin-R</i>	5'-CTGATCCACATCTGCTGGAAG-3'