Iranian Journal of Basic Medical Sciences
ijbms.mums.ac.ir

The effect(s) of berberine from *Berberis vulgaris* L. (Berberidaceae) on treating type 1 diabetes mellitus in streptozotocin-induced diabetic rats

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ARTICLE INFO

Article type:
Original

Article history:
Received: Aug 27, 2025
Accepted: Feb 2, 2026

Keywords:
Berberine
Diabetes mellitus type 1
Streptozocin
Gamma-aminobutyric acid
Gastrins
Islets of langerhans

ABSTRACT

Objective(s): *Berberis vulgaris* (*B. vulgaris*) L. (Berberidaceae), deeply rooted in Iranian traditional medicine, exhibits significant antidiabetic potential attributed to its berberine content. This plant has been historically used for its glucose-lowering, anti-oxidant, and anti-inflammatory properties. This research investigated berberine's capacity to regenerate pancreatic β-cells in type 1 diabetic rats, specifically examining its dose-dependent effects on metabolic recovery, histopathological restoration, and molecular mechanisms underlying β-cell regeneration.

Materials and Methods: Two groups of streptozotocin-induced diabetic rats received daily oral berberine (50 and 100 mg/kg) for 60 days. We measured berberine half-life and gamma aminobutyric acid (GABA) levels using high-performance liquid chromatography (HPLC) with ultraviolet-visible (Uv/Vis) and fluorescence detection (FD). Fasting blood sugar (FBS), lipid profiles, liver enzymes, insulin, and gastrin were assessed. Pancreatic histopathology (islet damage) and pancreatic and duodenal homeobox 1 (Pdx1) expression were analyzed.

Results: Berberine exhibited a 60-min half-life, with blood concentration declining to 0.63 and 0.95 µmol/l within 90 min. Treatment significantly elevated GABA levels (3.0 and 3.8 mg/dl vs diabetic group: 0.44 mg/dl) and reduced FBS by 50% (327 and 296 mg/dl vs diabetic group: 616 mg/dl). Gastrin levels increased to 8.70 and 8.93 pg/ml (vs diabetic group: 5.96 pg/ml). Histopathology revealed reduced islet shrinkage and vacuolization. Pdx1 expression was increased in the treated groups compared with diabetic controls.

Conclusion: Berberine from *B. vulgaris* effectively stimulates pancreatic β-cell regeneration, as evidenced by restored histoarchitecture, up-regulated Pdx1, and sustained glycemic control despite rapid clearance. This validates its traditional antidiabetic use and positions berberine as a promising disease-modifying agent.

▶Please cite this article as:
Salmanizadeh H, Yazdanparast R, Ghanadian M. The effect(s) of berberine from *Berberis vulgaris* L. (Berberidaceae) on treating type 1 diabetes mellitus in streptozotocin-induced diabetic rats. Iran J Basic Med Sci 2026; 29: 613-622. doi: https://dx.doi.org/10.22038/ijbms.2026.90772.19575

Introduction

Type 1 diabetes mellitus (T1DM), commonly referred to as juvenile diabetes, early-onset diabetes, insulin-dependent diabetes, and complex disease, is a long-lasting autoimmune condition and one of the most widespread hormone and metabolism-related illnesses. In T1DM, the body's immune system attacks the pancreas's insulin-making cells, causing them to stop producing insulin. T1DM accounts for only 10% of all diabetes cases, with 10-15% having a family link (1). The International Diabetes Federation (IDF) reported that in 2022, approximately 8.75 million people worldwide had T1DM, including 1.52 million aged under 20 years. T1DM has two forms: type 1A (autoimmune type, 70-90%) shows immune-related, self-attacking antibodies, while type 1B (unknown cause type, idiopathic) has an unclear specific origin (2, 3).

T1DM results from the interplay of genes, environmental factors, age, the immune system, and metabolism (1, 4). Genetically, more than 50 T1DM risk loci have been identified (1, 5). Environmental triggers include virus infections (*enteroviruses*, in particular coxsackieviruses) (1), pregnancy infections (congenital rubella syndrome), bacteria (1), cow's milk, grains, and wheat proteins, lack of vitamin D and omega-3 fatty acids (1, 5, 6), nitrate exposure, and location. T1DM mainly affects children aged 4 to 6 and 10 to 14. It involves immune tolerance, cellular immunity, and humoral immunity. High blood sugar and ketoacidosis (7) are two possibly life-threatening complications in T1DM patients. High blood sugar leads to small blood vessel problems (eye, nerve, and kidney damage) and large blood vessel issues (hardening and blocking arteries) (2, 7).

A key emerging strategy in diabetes therapy focuses on targeting the core transcription factor essential for the

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identity, function, and survival of pancreatic β-cells. Pdx1 is crucial for pancreatic development, β-cell differentiation, and the proper functioning of mature β-cells. Pdx1 dysfunction is a well-known contributor to the development of T1DM and type 2 diabetes mellitus (T2DM), significantly contributing to β-cell failure and cell death (8, 9, 10). As a result, therapeutic strategies aimed at restoring or enhancing Pdx1 expression and activity have gained attention, as they offer a promising approach to reverse β-cell dysfunction and promote regeneration. Pdx1 plays a central role in β-cell regeneration through two key mechanisms: it promotes the proliferation of existing β-cells and facilitates the transdifferentiation of other pancreatic cell types, such as α-cells, into functional, insulin-producing β-like cells (11). This ability to reprogram underscores its therapeutic potential, as non-β-cells can be converted into functional, insulin-secreting cells to help restore normal blood sugar levels (11). Therefore, increasing Pdx1 expression is an effective strategy to improve β-cell function and slow the progression of diabetes, positioning it as a prime target for the development of novel antidiabetic drugs (10).

In addition to key transcription factors, intra-islet signaling molecules play a crucial role in determining β-cell fate. While GABA is primarily known as a major inhibitory neurotransmitter in the central nervous system, it is also produced and stored abundantly within pancreatic β-cells (12). Within the islets of Langerhans, GABA functions as a paracrine and autocrine messenger, modulating hormone secretion. GABA's significance in T1DM stems from its dual capabilities. First, it has immunomodulatory effects that can protect β-cells from autoimmune destruction by reducing T-cell responses. Second, compelling preclinical evidence shows that GABA can induce the transdifferentiation of glucagon-producing α-cells into functional insulin-producing β-cells, representing a fundamental strategy for restoring lost β-cell mass (8, 12, 13). Consequently, enhancing GABAergic signaling in the pancreas has emerged as a promising therapeutic approach to both curb autoimmune progression and stimulate β-cell regeneration in T1DM.

T1DM treatment includes immunosuppressive drugs, antigen-based tests, pancreas transplantation, and cell therapy (2, 7). A new approach to treat T1DM is to regrow insulin-producing cells or convert other pancreatic cells (e.g., glucagon-producing cells) into insulin-producing cells, which has been studied using various approaches, including plant-based medicines. Natural plant components are valuable for finding new treatments. *Berberis vulgaris*, known as barberry in Iran, exhibits antidiabetic, anticancer, anti-inflammatory, anti-oxidant, antibacterial, analgesic, and hepatoprotective effects. This plant is part of the *Berberidaceae* family and the *Berberis* genus. It has particular potential for treating T1DM due to its berberine content (14). Berberine is found in different parts of *B. vulgaris*, especially its roots. Berberine can lower blood sugar, fight oxidative stress and inflammation, reduce blood pressure, and improve lipid profile (14).

In this study, we investigated the effects of berberine on streptozotocin (STZ)- induced diabetes using multiple experimental methods. Our findings showed that berberine significantly reduced blood glucose levels, enhanced pancreatic function by increasing GABA and gastrin levels, and increased Pdx1 expression. Additionally, histological analysis revealed notable regeneration of pancreatic islets in diabetic rats treated with berberine. These results indicate that berberine improves glycemic control and promotes pancreatic repair, underscoring its potential as a therapeutic agent for managing T1DM.

Materials and Methods

Isolation of herbal compound

We obtained berberine from the roots of *B. vulgaris*. The plant was harvested from Shams Abad Valley, located 60 km south of Chaharmahal and Bakhtiari Province, at an altitude of 2300 meters. The scientific name was verified with World Flora Online (www.worldfloraonline.org) on August 15, 2025. Two thousand mg of air-dried roots were ground into powder using an electric mill (100 mesh) and then subjected to classical percolation with methanol (approximately 10 liters). The plant powder was initially soaked in the solvent for three days in a percolation vessel and subsequently percolated at room temperature for four days at a flow rate of around 3 ml/min. The methanol extract was then evaporated using a rotary evaporator operated under reduced pressure at 40 °C. Alkaloids, in their free base state, are less soluble in pure water but have good solubility when ionized in acidic solutions. The extract syrup was first treated with 5% hydrochloric acid and then filtered to efficiently extract the alkaloids. The clear filtered solution was then precipitated at 4 °C overnight. The resulting yellowish precipitate was identified as berberine (15). The resulting yellowish precipitate was collected, dried, and confirmed as berberine by HPLC. The HPLC system was calibrated with a commercially available berberine hydrochloride standard (Sigma), and the purified berberine exhibited approximately 90% purity based on HPLC peak-area comparison with the standard. The yield of purified berberine was approximately 20 mg from 2000 mg of dried root powder (about 1%). For pharmacokinetic studies, including the determination of berberine half-life, the HPLC system was calibrated with the same berberine standard to ensure accurate quantification.

Experimental rats

The study involved 40 Wistar rats (250 ± 5 g) divided into five treatment groups (n = 8 per group). The first group served as a non-diabetic, normal control, the second was non-diabetic but treated with berberine, the third was diabetic, received the vehicle only treatment, the fourth was diabetic and received a daily 50 mg/kg berberine through gavage, and the fifth was diabetic and received a daily 100 mg/kg berberine. Diabetes was induced in the rats using a dose of 65 mg/kg STZ. FBS levels between 600 and 700 mg/dl were recorded in the rats one to four days after induction.

Berberine preparation for gavage

The solution for daily gavage of berberine was prepared by dissolving 6 g of berberine in an aqueous vehicle containing 4 g of sodium carboxymethyl cellulose (CMC-Na) and 0.06% (v/v) tween 80, with the final volume adjusted to 200 ml using distilled water. The mixture was stirred for approximately an hour to produce a homogeneous oral solution. This solution was used for the daily 500 (50 mg/kg dose) and 1000-microliter (100 mg/kg dose) gavages administered to two groups of 8 rats each for 60 days. The berberine used for gavage was purified as described in the isolation of herbal compound section, and its identity and purity were confirmed by HPLC analysis using a commercially available berberine hydrochloride standard.

Based on HPLC results, the purified compound showed approximately 90% purity.

HPLC-UV standardization for berberine half-life

HPLC was employed to standardize berberine content using a Shimadzu Nexera X2 system (Japan). This system included an LC-30AD pump, a 20AD Prominence dual-absorbance UV/Vis detector (operating at 239 nm), a CTO-20A Prominence column oven (at 30 °C), and LabSolution software. A 40 µl supernatant sample was injected into a Macherey-Nagel 4.6/150 mm nucleodur C18, gravity 5 µm column (Germany) using a Nexera X2 SIL-30A autosampler. Gradient elution was performed at a 0.9 ml/min flow rate over an 18-minute run time. The mobile phases were 10 mM KH2PO4 (pH 5) (buffer A) and buffer A plus 70% (v/v) acetonitrile (pH 5) (buffer B). Berberine (Sigma Aldrich, USA) served as the standard for calibration curves. The correlation between interaction and the maximum standard area was assessed using least-squares regression (R2). For *in vivo* comparison, blood sugar levels were recorded every 15 min from 0 to 90 'min' post-gavage in the 50 and 100 mg/kg berberine treatment groups to determine optimal effect time.

HPLC-FD standardization for GABA concentration

GABA content was standardized using HPLC with Shimadzu and Knauer Azura systems (Japan, Germany). This setup included a P 6.1L Azura pump (Germany), RF-20A prominence Shimadzu (Japan) fluorescence detector (330 nm excitation, 450 nm emission), CTO-20A prominence column oven Shimadzu (Japan) at 30 °C, and Clarity software version 5 (Germany). A 25 µl derivatized sample (mouse plasma derivatized with 100 ul borate buffer 0.1 mol/l, 50 ul o-phthalaldehyde (OPA), and 25 ul 3-mercaptopropionic acid (MPA) was injected into a Macherey-Nagel 4.6/150 mm nucleodur C18, gravity 5 µm column (Germany) via Nexera X2 SIL-30A autosampler. Gradient elution occurred at 1.3 ml/min over 35 min. Mobile phases were 15 mmol/l potassium dihydrogen phosphate and 5 mmol/l dipotassium hydrogen phosphate (buffer A) and a mixture of 50 ml ultrapure water, 250 ml acetonitrile, and 200 ml methanol (v/v) (solvent B). GABA (Sigma-Aldrich, USA) was used as the calibration standard. Correlation between interaction and the maximum standard area used least squares (R2 value) (16).

Blood biochemical factors assay

Serum levels of various blood markers were analyzed using colorimetric test kits (Biorexfars, Isfahan, Iran) and an Olympus AU640 autoanalyzer (Japan). These markers included FBS, random blood sugar (BS), total cholesterol (Chol), triglyceride (TG), high-density lipoprotein (HDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), low-density lipoprotein (LDL), gamma glutamyltransferase (GGT), and insulin (Ins)

Gastrin level measurement

Gastrin, a non-classical islet peptide, was measured in serum using electrochemiluminescence immunoassay. The gastrin kit, used with the Immulite 2000 XPi immunoassay system (Siemens Healthineers, Germany), was employed for this purpose.

Pancreas tissue histopathological evaluation

For histopathological analysis, pancreas samples from all five rat groups were collected and fixed in 10% neutral-buffered formalin. These samples were then embedded in paraffin, thinly sectioned, and stained with hematoxylin and eosin (H&E). The endocrine and exocrine pancreatic cells were examined for pathological changes.

Real-time polymerase chain reaction (qPCR) analysis

The total DNA was extracted from formalin-fixed rat pancreas tissue using the GeneAll® Exgene™ formalin-fixed paraffin-embedded (FFPE) tissue DNA kit. Samples were collected from 8 rats per experimental group; for the analysis, 3 representative samples per group were selected. This method is advantageous because it does not require organic solvents, making it suitable for molecular diagnosis of T1DM. The procedure begins by mixing 180 µL of formalin protein lysis (FPL) buffer thoroughly, then adding 20 µL of proteinase K solution (20 mg/ml) and thoroughly mixing. The sample is subsequently incubated at 56 °C for one hour, then at 90 °C for another hour. An optional step involves treating with RNase A to ensure RNA-free DNA. Next, 200 µL of buffer-fragment precipitation (FPB) is added and mixed thoroughly, followed by 200 µL of absolute ethanol, which is then mixed thoroughly again. The resulting mixture is transferred to a silica-based spin (SV) column and centrifuged. After this, the column is washed with buffer binding wash (BW) and tris-based wash (TW), and finally, the DNA is eluted with buffer elution (E) or distilled water (GeneAll®, www.geneall.com). qPCR was performed in triplicate for each of the 3 representative samples per group. Gene expression was normalized to β-actin using the ΔΔCt method. Data presented as mean fold change ± standard error of the mean (SEM).

Docking-protein structure preparation

The three-dimensional (3D) structure of PDX1 (PDB ID: 2H1K) was sourced from the Protein Data Bank (PDB) (http://www.rcsb.org/pdb). This structure was then prepared using Chimera 1.7, which involved adding partial charges to hydrogen atoms and correcting any missing residues. These modifications were crucial for optimizing the protein structure for subsequent molecular docking analyses.

Docking-ligand preparation

Initially, the 3D structure of berberine was retrieved from the PubChem database (pubchem.ncbi.nlm.nih.gov) (Table 1). The structure was then converted to PDB format using Biovia Discovery Studio (DS). This PDB format structure was subsequently employed in the present study. Berberine underwent docking assessments using Autodock software. The results, including experimentally determined metrics such as inhibition percentage and docking score (expressed in kcal/mol). Following this, a ligand was subjected to docking within the binding sites of the PDX1 (PDB ID: 2H1K) using Autodock software integrated into DS 2.5. This software employs a shape-based methodology to precisely

Table 1. Chemical data of berberine (Molecular Weight: MW)

No.	Compound	Sources	MW (g/mol)	Molecular formula	PubChem ID
1	Berberine	*B. vulgaris*	336.4	$C_{20}H_{18}NO_4^+$	2353

dock substrates into the PDX1 protein. The resulting docked poses were evaluated for docking efficiency, based on the potential docking energy.

Docking- docking scoring

To analyze the binding interactions between berberine and PDX1 (PDB ID: 2H1K), we performed molecular docking using AutoDock (version 1.5.6). We prepared the enzyme and substrate by removing water, adding partial charges, and including polar hydrogen atoms via Gasteiger and Kollman methods. A 125 × 115 × 120 grid map with 0.346 Å spacing was created to examine binding and active site residues. We employed the Lamarckian genetic algorithm for docking. The best conformation, identified by the lowest docking score, was chosen for further study. We generated a two-dimensional (2D) diagram of ligand interactions with binding-site residues using DS. We calculated the binding energy for each ligand based on ten conformations from each docking run.

Statistical analysis

We repeated the experiments three times and analyzed data with SPSS software, version 22.0. Significant differences were evaluated for each parameter using Duncan's test (P<0.05).

Results

HPLC-UV/Vis calibration and time-dependent blood glucose assessment for estimating berberine half-life

The half-life and pharmacokinetics of berberine from *B. vulgaris* were investigated using HPLC-UV/Vis. Blood samples from rats were collected at 0, 30, 60, and 90 min post-gavage to determine the concentration of berberine and its effect on blood glucose levels. Additionally, blood glucose levels were measured after berberine gavage from time 0 to 75 min. HPLC-UV/Vis method identified the berberine peak at a 10-minute retention time, with a 5% identification window on either side. We created a calibration curve from 0.20, 0.50, and 0.012 mM berberine standards in LabSolution, adjusted the curve, and performed linear regression. The resulting equation was y=3.25760e+007*x-14789.8, where "x" is berberine concentration (µg/ml), with a correlation coefficient (R^2) of 0.9991 (Figure 1). The effect of berberine on FBS levels in diabetic rats was evaluated over 60 days following oral gavage with two doses: 50 and 100 mg/kg. In rats given 50 mg/kg of berberine daily, we observed a 0.63 mol/l decrease from 30 to 90 min. For those given 100 mg/kg, the decrease was 0.95 mol/l (Figure 1). In the 50 mg/kg dose group, blood glucose levels decreased from 585 mg/dl at baseline to 379 mg/dl at 75 min, a reduction of approximately 35.1%. The most significant drop occurred between 45 and 60 min, from 424 mg/dl to 340 mg/dl (19.8% decrease). In the 100 mg/kg dose group, blood glucose levels decreased from 590 mg/dl to 328 mg/dl, representing a 44.3% reduction. The largest decrease was observed between 45 and 60 min, with levels dropping from 405 mg/dl to 278 mg/dl (31.4% decrease). Both doses showed a progressive reduction in blood sugar levels, with the 100 mg/kg dose demonstrating

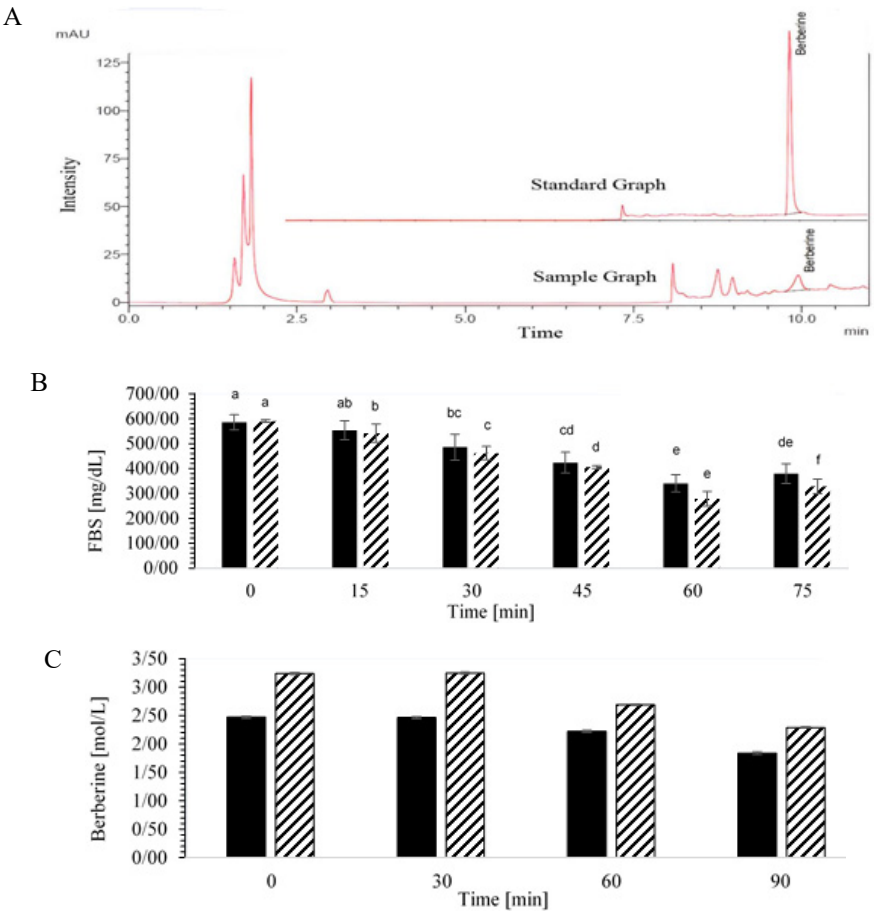

Figure 1. HPLC-UV/Vis calibration and time-dependent effects of berberine on rat blood sugar levels following oral gavage
(A) Representative HPLC-UV/Vis chromatograms of berberine standard and purified sample. The y-axis shows milli-absorbance units (mAU), while the x-axis shows retention time. (B) Time-course profile used to estimate the half-life of the berberine effect following oral administration. (C) Blood glucose levels were measured at 0, 15, 30, 45, 60, and 75 min after oral gavage with berberine at doses of 50 and 100 mg/kg. Data are presented as mean ± SEM. Statistical significance (P<0.05) was determined using Duncan's multiple range test.

a greater effect. The peak reductions for both doses were observed between 45 and 60 min post-gavage, indicating a dose-dependent relationship where higher doses resulted in more significant glucose-lowering effects.

Quantitative analysis of blood GABA by HPLC-FD

We measured GABA using HPLC- FD. The GABA calibration graph was obtained using five concentrations: 200, 300, 400, 500, and 1000 mg/l. Comparing results across five rat groups, the average GABA levels in these groups were as follows: 5.19 mg/dl for normal rats, 5.03 mg/dl for normal rats treated with berberine, 0.46 mg/dl for diabetic rats, 3.33 mg/dl for diabetic rats treated with 50 mg//kg of berberine, and 3.89 mg/dl for diabetic rats treated with 100 mg/kg of berberine (Figure 2). In terms of percentage change, diabetic rats exhibited a drastic reduction in GABA levels, showing approximately a 91.1% decrease compared to normal controls. Treatment with 50 and 100 mg/kg of berberine resulted in significant increases in GABA levels: the 50 mg/kg dose showed a 625.0% increase, and the 100 mg/kg dose demonstrated a 746.7% increase compared with diabetic rats. These findings suggest that berberine has a restorative effect on GABA levels, which are severely depleted in diabetic rats, potentially contributing to the therapeutic effects observed in the study.

In vivo antidiabetic effects of berberine on biochemical parameters

To assess the impact of berberine on pancreatic recovery and beta-cell restoration in diabetic rats, we measured and analyzed blood biochemical parameters, with FBS being the most critical. Figure 3 shows that diabetic rats lost approximately 40 g of weight, whereas rats treated with berberine (50 and 100 mg/kg) maintained weights close to normal. Insulin production increased substantially in treated rats, likely due to islet restoration. Cholesterol, TG, and LDL levels decreased, whereas HDL increased in the treated groups compared with the diabetic group, indicating improved clinical outcomes. ALP, AST, and ALT, which typically increase in diabetes, showed some decrease in treated rats. GGT levels increased in diabetic rats and decreased in treated groups. (Figure 3). The data demonstrate that berberine treatment, especially at higher doses (100 mg/kg), significantly improved various health parameters in diabetic rats, including blood glucose control, lipid metabolism, liver function, pancreatic enzyme activity, and insulin secretion. Based on these improvements, berberine appears to mitigate the adverse effects of T1DM. Berberine reduced FBS by approximately 50.9% (50 mg/kg) and 52.0% (100 mg/kg) compared with the diabetic group. It decreased Chol by 13.2% and 32.4%, TG by 59.0% and 58.8%, and LDL by 15.0% and 34.2%, respectively. Berberine treatment resulted in a reduction in AST by 31.9% and 44.1%; ALT by 28.4% and 57.5%; ALP by 10.5% and 16.4%; and GGT by 37.6% and 55.3%. However, berberine increased HDL by 34.5% and 77.3%. Additionally, berberine increased insulin levels by 8.3-fold (50 mg/kg) and 13.3-fold (100 mg/kg) compared with diabetic rats. The observed percentage improvements suggest that berberine may be a promising adjunct therapy for managing T1DM, particularly for improving metabolic and organ health. These results are valuable and support the therapeutic potential of berberine in the treatment of diabetes.

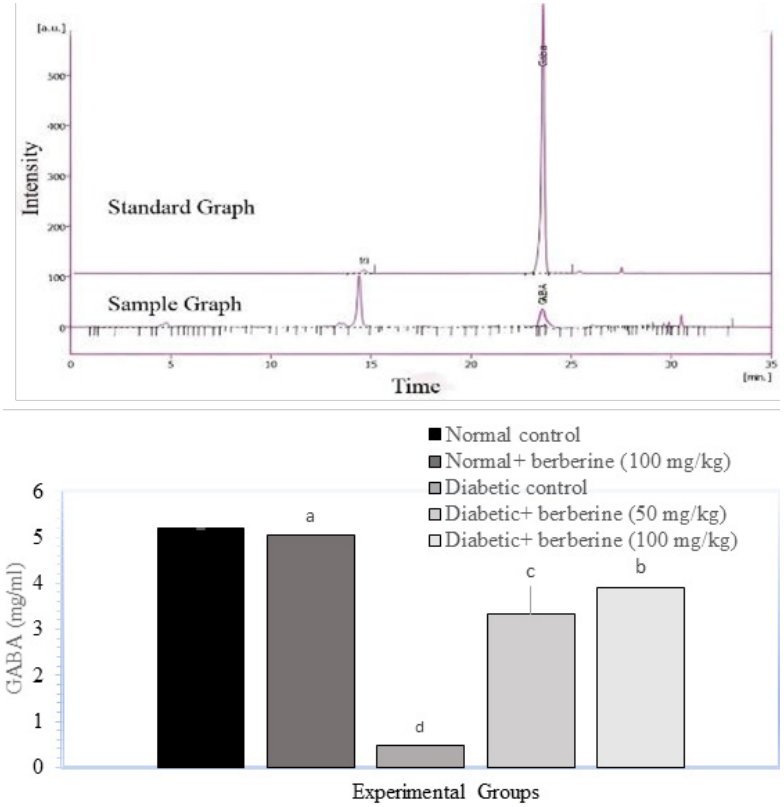

Figure 2. Quantitative analysis of rat blood gamma aminobutyric acid (GABA) levels by HPLC-FD
(A) Representative HPLC-FD chromatograms of GABA standard and purified sample. The y-axis shows absorbance units (a.u.), while the x-axis shows retention time. (B) Blood GABA concentrations in normal rats (5.19 mg/ml), normal rats with berberine gavage (5.03 mg/ml), diabetic rats (0.46 mg/ml), diabetic rats treated with 50 mg/kg berberine for 60 days (3.33 mg/ml), and diabetic rats treated with 100 mg/kg berberine for 60 days (3.89 mg/ml). Data are presented as mean ± SEM. Statistical significance was determined using Duncan's multiple range test (P<0.05)

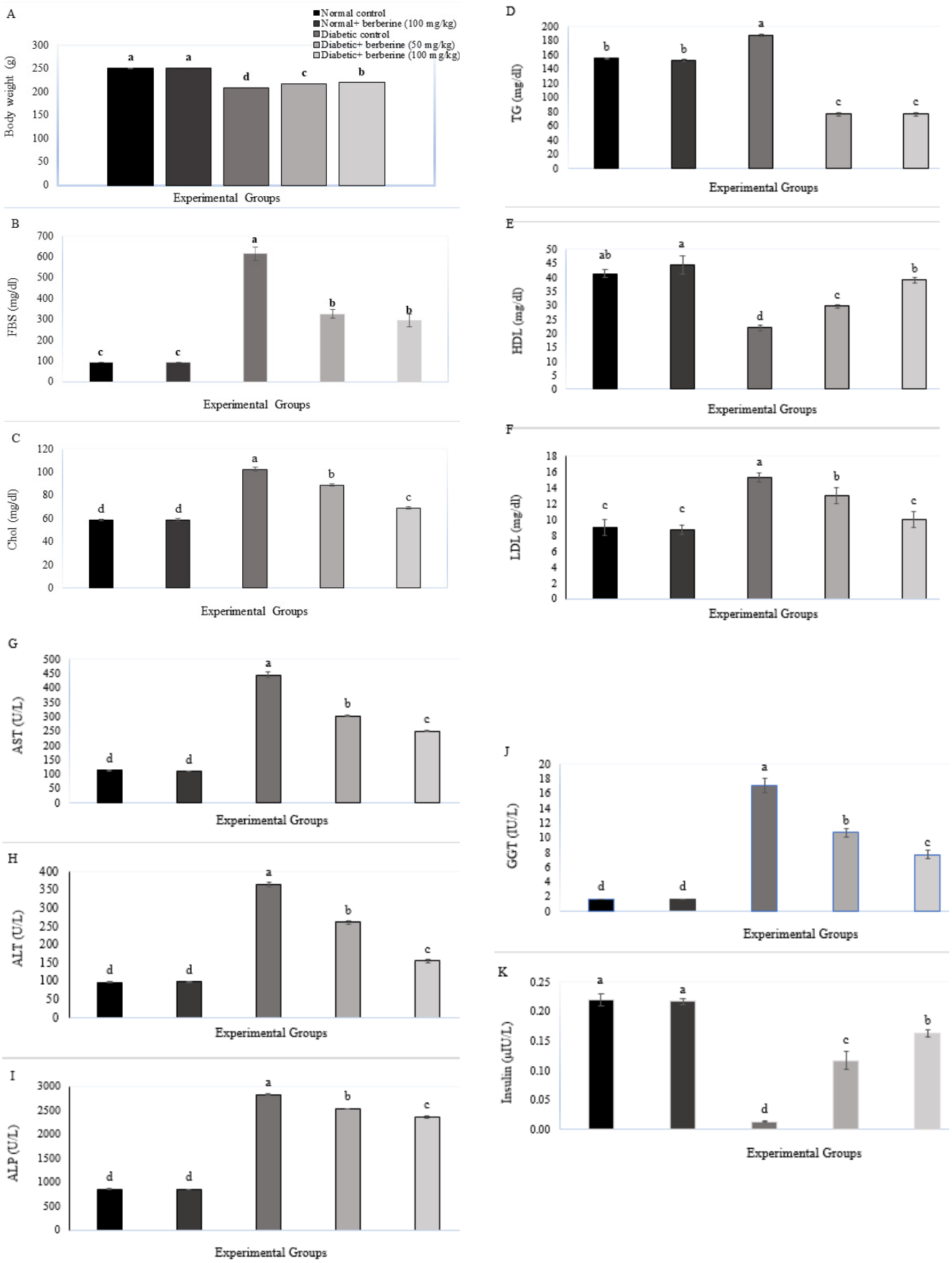

Figure 3. Effects of berberine on biochemical parameters in rats
(A) Body weight (g). (B) FBS (mg/dl). (C) Chol (mg/dl). (D) TG (mg/dl). (E) HDL (mg/dL). (F) LDL (mg/dl). (G) AST (U/l). (H) ALT (U/l). (I) ALP (U/l). (J) GGT (IU/l). (K) Ins (µU/ml, micro-international units per milliliter) groups; normal control, normal+ berberine, diabetic control, diabetic+ 50 mg/kg berberine, diabetic+ 100 mg/kg berberine, respectively. FBS: Fasting blood sugar; Col: Cholesterol; TG: Triglyceride; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma glutamyltransferase; Ins: Insulin

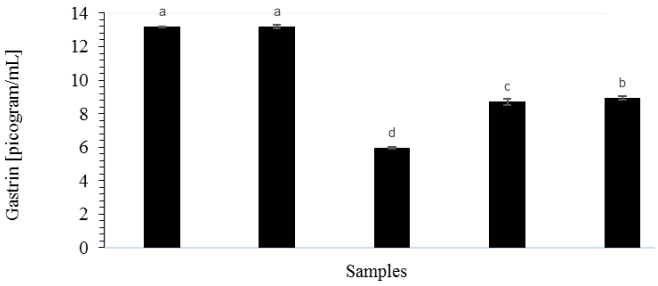

Figure 4. Electrochemiluminescence measurement of blood gastrin levels
Bar graph shows blood gastrin concentrations in normal rats (13.21 pg/ml), normal rats treated with berberine (13.21 pg/ml), diabetic rats (5.96 pg/ml), and diabetic rats treated with 50 mg/kg berberine (8.70 pg/ml), diabetic rats treated with 100 mg/kg of berberine (8.93 pg/ml). Data are presented as mean ± SEM. Statistical significance was determined using Duncan's multiple range test (P<0.05).

Gastrin level assessment

Gastrin promotes β-cell growth, neogenesis, and insulin content in rat models. We measured plasma gastrin levels in five rat groups using electrochemiluminescence. The average gastrin levels were: 13.18 pg/ml in normal rats, 13.21 pg/ml in normal rats treated with berberine, 5.96 pg/ml in diabetic rats, 8.70 pg/ml in diabetic rats treated with 50 mg/kg of berberine, and 8.93 pg/ml in diabetic rats treated with 100 mg/kg of berberine. Diabetic rats showed a significant 54.7% decrease in gastrin levels compared to normal controls. Berberine treatment increased gastrin levels in diabetic groups: a 46.3% increase at 50 mg/kg and a 50.3% increase at 100 mg/kg compared with diabetic controls (Figure 4). These results indicate that berberine helps restore gastrin levels in diabetes, potentially aiding gastrointestinal and metabolic health.

Berberine's impact on pancreas regeneration: Histological evaluation

We performed pancreatic pathology using H&E staining to assess the pancreatic condition. Normal tissue showed healthy endocrine and exocrine components, consistent with references. Diabetic tissue exhibited Langerhans islet shrinkage, destruction, severe degeneration, atrophy, and vacuolization. Pancreatic tissue treated with 50 mg/kg berberine showed near-normal histology with slightly smaller islets and minor exocrine degeneration. The 100 mg/kg berberine treatment yielded similar results (Figure 5 A-H). Berberine treatment, administered at 50 and 100 mg/kg, appears to promote pancreatic tissue regeneration. Histological findings suggest that berberine may reduce damage to the pancreatic islets and improve overall pancreatic architecture, approaching a normal state. The slight degeneration observed in the exocrine tissue may indicate that a protective response is still developing alongside berberine treatment.

qPCR analysis of blood samples

We examined Pdx1 gene expression via qPCR and attempted to detect PDX1 protein in the bloodstream, which proved challenging due to its low levels. Results showed reduced Pdx1 expression in diabetic rats. Berberine treatment increased Pdx1 expression, likely due to pancreatic recovery and regeneration (Figure 6). Rats receiving 50 mg/kg of berberine showed increased Pdx1 levels compared to untreated controls. Those treated with 100 mg/kg had even higher Pdx1 expression, indicating a dose-dependent effect. qPCR analysis of blood samples from diabetic rats showed a significant decrease in Pdx1 expression to 0.031-fold compared to normal rats, indicating a 96.9% reduction

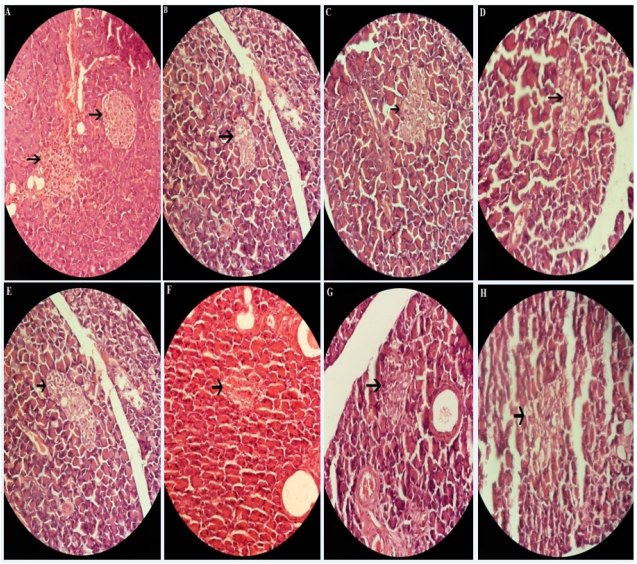

Figure 5. Rat pancreas histological evaluation (H&E staining) after exposure to berberine treatments.
(A) Normal tissue: marked endocrine tissue, surrounding exocrine tissue, matching references. (B) Diabetic tissue: Langerhans islet shrinkage, destruction (marked), severe degeneration, atrophy, pancreatic vacuolization. (C, D, E) 50 mg/kg berberine-treated tissue: near-normal islet histology, slightly smaller islets, and brief exocrine degeneration. (F, G, H) 100 mg/kg berberine-treated tissue: similar to C.

(P<0.001). Treatment with berberine at 50 mg/kg and 100 mg/kg doses restored Pdx1 expression by 806% (0.281-fold vs normal, P<0.01), 28.1% of normal levels. There was no significant difference in effectiveness between the two doses (P>0.05), indicating consistent results.

qPCR analysis of total DNA from FFPE tissue

We directly analyzed pancreatic tissue samples to assess Pdx1 gene expression. Results indicated increased gene expression following berberine treatment, contributing to pancreatic recovery and regeneration (Figure 7). To evaluate the effect of berberine on Pdx1 gene expression in pancreatic tissue, we analyzed samples from rats in different experimental groups. Using qPCR on total DNA extracted from FFPE pancreatic tissue, we assessed Pdx1 expression levels. We observed a significant reduction in Pdx1 expression in STZ-diabetic rats, consistent with STZ-induced damage and pancreatic dysfunction. This decline was expected in the

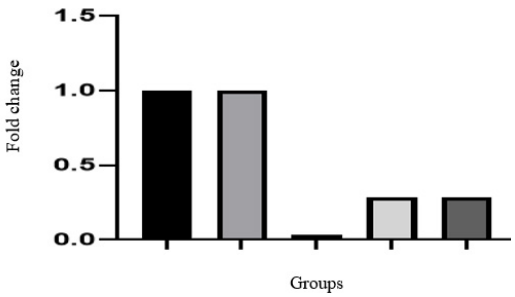

Figure 6. qPCR for Pdx1 in rat blood. Five groups (from left to right); normal rats, normal rats treated with berberine, diabetic rats, diabetic rats treated with 50 mg/kg of berberine, diabetic rats treated with 100 mg/kg of berberine
Fold change in target gene expression normalized to β-Actin (ΔΔCt method). Diabetic rats (0.031-fold change) exhibited markedly reduced expression (96.9%) compared to normal controls (1.00-fold change). Berberine gavage at 50 and 100 mg/kg partially reversed expression (0.281-fold change) to 28.1% of normal levels, with no dose-dependent difference. Data represent mean fold change ± SEM (n = 8 biologically independent samples per group, each measured in triplicate).
qPCR: Real-time polymerase chain reaction; Pdx1: Duodenal homeobox 1

Table 2. Berberine"s docking score and total hydrogen bonds formed during docking with pancreatic duodenal homeobox 1 (Pdx1, PDB ID: 2H1K)

Berberine	-7.40	-2.19	-0.93	-4.32

compound	2H1K			
	Docking score (Kcal/mol)	H-bond	Electrostatics	van der Waals
Berberine	-7.40	-2.19	-0.93	-4.32

diabetic group due to STZ-induced destruction of pancreatic β-cells. In pancreatic tissue, Pdx1 expression in diabetic rats was reduced to 0.06-fold, representing a 94% decrease (P<0.001). Berberine treatment showed a dose-dependent response: 50 mg/kg: 350% improvement (0.27-fold vs normal, P<0.05); 100 mg/kg: 533.3% improvement (0.38-fold vs normal, P<0.01); 27% and 38% of normal levels, respectively. The difference between the two doses was statistically significant (P<0.05), indicating a specific pharmacodynamic effect on pancreatic tissue.

Docking score analysis

Computational methods have revolutionized drug discovery, making molecular docking an essential technique. This approach offers valuable insights into ligand-receptor interactions, shedding light on potential drug effects. In our study, the berberine-2H1K complex showed a docking score of -7.4 kcal/mol (Table 2). The complex's interactions fall into four categories: van der Waals forces, conventional hydrogen bonds, π–cation interactions, and π-π stacking interactions. We observed two conventional hydrogen bonds with Arg52 and Leu21 residues. van der Waals forces were noted with Ile26, Ser27, Arg30, Pro29, Glu60, Phe22, Asn23, Phe20, Trp48, and Lys24 residues. A π–cation interaction was seen with the Lys24 residue, while a π-π stacking interaction was detected with the Trp56 residue (Figure 8 A-H).

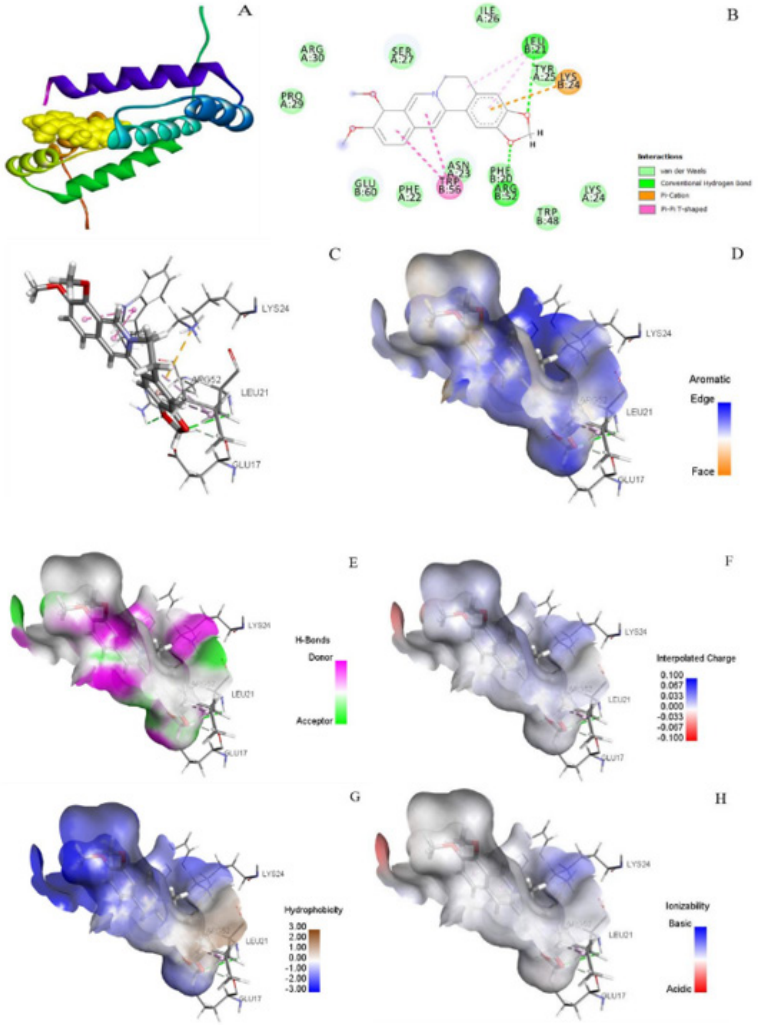

Figure 8. Berberine"s docking score and total hydrogen bonds formed during docking with pancreatic duodenal homeobox 1 (Pdx1, PDB ID: 2H1K)
(A) 3D structure of the target protein, highlighting the berberine binding site. (B) 2D interaction diagram illustrating key molecular interactions between berberine and nearby amino acid residues, including hydrogen bonds, π–cation interactions, and π–π stacking interactions. (C) 3D representation of berberine within the binding pocket, showing the interacting residues. (D) Aromatic interaction surface of the binding site, indicating edge and face orientations. (E) Regions of hydrogen bond donors and acceptors within the binding pocket. (F) Electrostatic potential surface of the binding site, illustrating charge distribution. (G) Hydrophobicity surface map of the binding pocket. (H) Ionizability surface indicating basic and acidic regions surrounding the ligand.
2D: Two-dimensional; 3D: Three-dimensional

Discussion

In this study, we examined the effects of berberine from *B. vulgaris* on T1DM using a STZ-induced rat model. Our results showed that berberine effectively improves antidiabetic outcomes, significantly lowering FBS levels in a dose-dependent manner, particularly at the higher dose of 100 mg/kg. Berberine appears to enhance insulin sensitivity or decrease hepatic glucose production; however, its glucose-lowering effect is transient, suggesting that repeated doses may be required to sustain benefits, as blood glucose levels rebounded after 60 min. Additionally, berberine treatment led to increased levels of GABA and gastrin, suggesting potential β-cell recovery and pancreatic regeneration. Histological analysis also indicated improved pancreatic tissue structure, which may help reverse some damage caused by STZ-induced diabetes. Berberine has been shown to significantly improve metabolic parameters such as glucose level, lipid profiles, liver function, and insulin secretion. These results align with previous studies that highlight its effectiveness in addressing diabetic dyslipidemia and protecting against organ damage. The improvements in TG, Chol, liver enzymes, and insulin secretion are dose-dependent, indicating berberine's therapeutic potential. Our qPCR analysis revealed that berberine increased Pdx1 expression, a gene important for pancreatic β-cell function. This suggests that berberine may aid in pancreatic regeneration and support β-cell recovery in diabetes. Additionally, molecular docking studies indicate a strong binding affinity between berberine and its target protein, supporting its potential as a diabetes treatment. These findings are consistent with existing research highlighting berberine's antidiabetic effects, including improved glucose metabolism and insulin sensitivity. While promising results have been observed in preclinical models, clinical studies are necessary to confirm berberine's efficacy and safety in humans. The transient effects noted in our study suggest that combining berberine with other treatments or using sustained-release formulations may be needed for long-term glucose control.

Other studies have examined berberine's effects on these biochemical factors, often in combination with other compounds. However, their results showed less pronounced effects than those observed in our research. While much research has focused on berberine's impact on T2DM, our study addresses specifically its effects on T1DM. For example, oral berberine administration in diabetic and normal rats significantly reduced blood glucose levels within 3-7 days, affecting HbA1c, serum lipid profile, and body weight (14, 17). In another study, intraperitoneal injection of 50 and 100 mg/kg berberine for four weeks in streptozotocin-induced diabetic rats improved FBS, hepatic gluconeogenesis, glucose 6-phosphatase activity, weight gain, and TG (15, 18). A four-month study using 150 and 300 mg/kg berberine in T2DM rats showed increased insulin expression, beta cell regeneration, and anti-oxidant enzyme activity. Other researchers reported reductions in FBS and malondialdehyde levels, along with increases in superoxide dismutase and catalase activities, in T2DM mice following two weeks of treatment with 100 mg/kg of berberine (15, 18). Berberine has also been shown to up-regulate glucose transporter 4 and to increase plasma insulin levels and β-cell mass in STZ-diabetic rats (12, 15, 18). Clinical trials have also demonstrated berberine's effectiveness. In one study, 500 mg/kg of berberine combined with two other compounds was administered to 50 hypercholesterolemia patients for six weeks, reducing the atherogenic profile, Chol, LDL, and TG (18, 19). Another study of 40 hyperlipidemic patients using 500 mg/kg of berberine, combined with two other compounds, reported a 20-25% reduction in TG and LDL levels (18, 19). A two-month study on 97 diabetic patients revealed decreases of 25.9%, 18.1%, and 17.6% in FBS, HbA1c, and TG levels, respectively, in the berberine-treated group (18, 19). Berberine also significantly reduced FBS and TG levels in the patient groups suffering from chronic hepatitis (B and C). In hypercholesterolemia patients, a three-month oral berberine treatment reduced Chol, TG, and LDL by 29%, 35%, and 25%, respectively (18, 19). Hyperlipidemic hamsters showed 40% and 42% reductions in cholesterol and LDL levels, respectively (17). In newly diagnosed and poorly controlled T2DM patients, 500 mg/kg of berberine twice daily for three months significantly decreased FBS, HbA1c, TG, and LDL (18, 19). A meta-analysis of 2569 patients with T2DM demonstrated the effectiveness of berberine in treating diabetes, hyperlipidemia, and hypertension, with no serious adverse effects (18, 19). Our study showed improvements in both endocrine and exocrine functions. We found that the optimal effect of berberine on reducing blood glucose levels in rats was observed 60 min after gavage. Research has shown that berberine is more stable and has a longer half-life in the pancreas than in plasma, maintaining constant levels for at least 2 hours. This leads to improved pancreatic recovery (18, 20).

The wide distribution of berberine in the pancreas suggests its potential clinical utility in treating T1DM. These findings collectively highlight berberine's potential as a therapeutic agent for diabetes, particularly T1DM (15). Its ability to improve biochemical markers, including blood sugar, lipid profiles, and insulin levels, along with its pancreatic-protective properties, makes it a promising candidate for further research and potential clinical applications. However, further studies are required to better comprehend its mechanisms of action and optimize its application in diabetes treatment. Research indicates that GABA protects against T1DM in rats. It enhances insulin secretion in beta cells by influencing SIRT1 activity and inhibits glucagon release from alpha cells in the islets of Langerhans (12, 21). In various mouse models, GABA treatment preserves beta-cell mass, prevents diabetes onset, and regenerates beta cells in severely diabetic mice. In transplanted pancreas models, GABA promotes cell proliferation, reduces apoptosis, increases beta-cell mass, and improves hyperglycemia (15).

Our study shows that berberine increases GABA in the pancreas. This GABA, acting in an autocrine manner, positively affects beta cells, improving pancreatic islets, increasing beta-cell mass, and improving T1DM outcomes. Pdx1 is crucial for pancreatic development; its loss causes pancreatic agenesis in mice and humans (8, 9, 22). The Pdx1 gene encodes a vital factor that regulates insulin gene expression, beta-cell proliferation, and maturation. Efforts have been made to produce recombinant PDX1 protein for diabetes treatment (8, 9, 22). In our qPCR analysis, Pdx1 gene expression decreased after pancreatic damage. Berberine treatment increased PDX1 protein expression by modulating PDX1 gene expression. Given PDX1's known effects, pancreatic beta-cell regeneration and improved glycemic control in diabetic rats are expected. In qPCR

analysis, blood reflects overall transcriptional recovery, while pancreatic tissue shows specific, dose-responsive mechanisms influenced by its unique bioavailability and regulatory pathways. Our molecular docking studies revealed that the berberine-2H1K complex had a docking score of -7.4 kcal/mol, indicating a strong interaction. These findings further support berberine's potential as a therapeutic agent for T1DM, demonstrating its ability to influence key factors like GABA and Pdx1, which are crucial for pancreatic health and function. The molecular docking results also suggest a strong interaction between berberine and its target, potentially explaining its effectiveness in improving diabetic conditions.

Conclusion

Our findings provide robust evidence that berberine effectively ameliorates hyperglycemia in T1DM rats by improving pancreatic β-cell function and stimulating islet regeneration. These results highlight berberine's considerable promise as a natural candidate for diabetes therapy, potentially targeting the underlying pathophysiology. Further studies are essential to decode the molecular mechanisms involved and to validate these therapeutic benefits in clinical trials.

Acknowledgment

The authors would like to thank the financial support of this investigation.

Ethicts Statment

All experimental procedures involving animals were approved by the Ethics Committee of the School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran. Approval Number: IR.MUI.AEC.1404.052. The study was conducted in accordance with internationally accepted principles for the care and use of laboratory animals, including the European Community guidelines (EEC Directive 86/609/EEC) and the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1985). Every effort was made to minimize animal suffering and reduce the number of animals used.

Authors' Contributions

H S conducted experiments, designed the methodology, carried out the investigation, collected, processed, and curated the data, and wrote the manuscript. R Y supervised the study, administered the project, and edited the manuscript. M Gh validated the methods and reviewed and edited the manuscript.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Declaration

The authors have not used AI tools or technologies to prepare this manuscript.

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