Evaluation of The Antioxidant, Hypolipidemic, and Pancreatic Histological Alterations Effects of Tirzepatide in High-Fat Diet and Streptozotocin-Induced Type 2 Diabetic Rats

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Article Info:

Received 29 Aug 2025
Revised 30 Nov 2025
Accepted 18 Jan 2025
Published 31 Mar 2026
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DOI: https://doi.org/10.32947/ajps.v26i1.1347

Abstract:

Background: Type 2 diabetes mellitus (T2DM) is a complicated and multifaceted metabolic disease defined by insulin resistance, dyslipidemia, oxidative stress, and β-cell dysfunction. Tirzepatide (TZP), a dual GIP and GLP-1 receptor agonist, offers potent glucose-lowering and weight-loss.

Objective: To assess the effect of different doses of TZP on lipid profile, SOD activity, and histopathological changes of the pancreas in a high-fat diet and streptozotocin-induced T2DM.

Methods: Thirty male Wistar rats were divided into five groups (n=6): Group I, control group, Groups II-V were induced with diabetes by feeding with a high-fat diet for six weeks with a single injection of streptozocin (35 mg/kg). Groups III- V were treated with TZP at three doses: low, medium, and high (0.44, 0.88, and 1.32 mg/kg) subcutaneously once weekly for 4 weeks. Blood and pancreas samples were then collected for biochemical and histopathological analyses.

Result: The results showed that all doses of TZP-treated groups significantly lowered ($p<0.0001$) cholesterol, TG, LDL, and VLDL, and increased SOD and HDL levels in a dose-response manner compared to the T2DM rats. while all doses of the TZP-treated groups revealed pronounced improvements in pancreatic architecture, especially at the higher doses.

Conclusion: Treatment of T2DM rats with TZP ameliorated their lipid profile, improving pancreatic architecture and augmenting antioxidant activity.

Keywords: Type 2 diabetes, High-Fat diet, Streptozocin, Tirzepatide, lipid profile, SOD.

تقييم التأثيرات المضادة للأكسدة، والخافضة للدهون، والتغيرات النسجية البنكرياسية لدواء تيرزيباتايد في نموذج الجرذان المصابة بداء السكري من النوع الثاني المستحدث بالنظام الغذائي عالي الدهون والستربتوزوتوسين

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الخلاصة:

الخلفية: داء السكري من النوع الثاني (T2DM) هو متلازمة أيضية معقدة ومتعددة الاوجه تتميز بمقاومة الانسولين، واضطراب دهون الدم، والاجهاد التأكسدي،واختلال وظيفية البيتا. تيرزيباتيد،المنشط المزدوج لمستقبلي GIP و GLP-1 يمتاز بفعالية عالية في خفض مستوى الجلوكوز وفي تعزيز فقدان الوزن.

الهدف:تقيم تأثير التيرزيباتيد على صورة الدهون، ونشاط انزيم SOD، والتغيرات النسيخية في البنكرياس في نموذج داء السكري من النوع الثاني المستحث بنظام غذائي عالي الدهون وحقن Streptozocin.

المنهجية: تم استخدام ثلاثين جرذ من الذكور وقسمت الى خمسة مجموعات كل منها يحتوي ستة ،جرذان المجموعة الاولى مجموعة الضابطة،و خضعت المجموعات من II الىV لتحفيز السكري من النوع الثاني من خلال تغذيتها بنظام عالي الدهون لمد ستة اسابيع، تلاها حقن منفردة منStreptozocin بجرعه35ملغم/كغم.ثم تمت معالجة المجموعات من III إلىV باستخدام تيرزيباتايد بثلاث جرعات: منخفضة، ومتوسطة، وعالية(0.44، 0.88، و1.32 ملغم/كغم) مرة واحدة اسبوعيا لمدة أربعة أسابيع وتم جمع عينات الدم والبنكرياس بعد انتهاء فترة العلاج لاجراء التحاليل البيوكيمائية والفحوصات النسجية.

النتائج: أظهرت النتائج أن جميع الجرعات من التيرزيباتيد أدت الى انخفاض ملحوظ في مستويات الكوليستيرول، والدهون الثلاثية والبروتين الدهني منخفض الكثافة، والبروتين الدهني المخفض الكثافة جدا وزيادة في مستويات انزيم السوبر اوكسيد ديسميوتيز والكوليسترول الجيد مقارنة بجرذان داء السكري من النوع الثاني.ومن جهة اخرى أظهرت المجموعات المعالجة بالتيرزيباتيد تحسن واضحا في البنية النسيجية للبنكرياس، لاسيما في جرعات العالية.

الاستنتاج: أدى علاج الجرذان المصابة بداء السكري من النوع الثاني بواسطة تيرزيباتايد إلى تحسين صورة الدهون، وتحسين بنية البنكرياس، وتعزيز النشاط المضاد للأكسدة.

الكلمات المفتاحية: داء السكري من النوع الثاني، النظام الغذائي عالي الدهون، الستربتوزوتوسين، التيرزيباتيد، صورة الدهون، إنزيم السوبر أوكسيد ديسميوتاز

Introduction

Diabetes mellitus (DM) has been identified as a significant worldwide healthcare problem, with its prevalence consistently increasing, particularly in countries with poor or moderate incomes. Type 2 diabetes mellitus (T2DM) constitutes the primary type of diabetes, comprising 90-95% of all diabetes instances (1). T2DM is a metabolic disease that arises from a confluence of lifestyle and genetic aspects. In addition to hyperglycemia, it is characterized by insulin resistance. Insulin resistance significantly contributes to the pathophysiology of T2DM and is critical with associated metabolic disorders, including dyslipidemia. diabetic dyslipidemia is characterized by elevated triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and lowered high-density lipoprotein cholesterol (HDL-C) levels(2). The most important key characteristic of T2DM pathophysiology is a disparity between Reactive oxygen species (ROS) production and the antioxidant defense system produced by the body(3). Excessive ROS produced by hyperglycemia results in oxidative damage, a diminished antioxidant response, and compromised DNA repair mechanisms, which are critical factors in the pathophysiology of T2DM and the onset of later complications (4). Superoxide dismutase (SOD) is a first-line defense antioxidants, which transform superoxide radicals to molecular oxygen and hydrogen peroxide. In diabetes, SOD activity is often reduced, leading to extreme oxidative stress, and cellular damage can occur alongside diminished pancreatic beta-cell activity (5).

Hyperglycemia in T2DM is largely caused by the progressive loss of pancreatic beta-cell mass. Lipotoxicity, glucotoxicity, and ROS exacerbate pancreatic β-cell dysfunction in both humans and experimental models, ultimately resulting in structural and functional damage to the pancreatic tissue. Rats subjected to a high-fat diet (HFD) and treated with streptozotocin (STZ) have gained popularity in recent years as models for T2DM, mimicking pathophysiological conditions close to T2DM [6] .Among the currently available treatment options, the incidence of T2DM is increasing rapidly. Thus, there is a growing trend in research focused on the discovery and progress of new medications for both the prevention and the treatment of T2DM. Tirzepatide (TZP), defined as a 'twincretin', is a first dual agonist of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 receptor agonist (GLP-1) receptors, and is classified as an imbalanced dual agonist. TZP exhibits an affinity for the GIP receptor comparable to that of endogenous GIP, and has roughly five times less affinity for the GLP-1 receptor compared to endogenous GLP-1 [7]. TZP has received approval for treating T2DM and obesity, able to substantially lower glycemic levels and enhance insulin sensitivity [8]. This study set out to determine the effect of three different doses of TZP, focusing on lipid profile, antioxidant activity (SOD level), and histopathological changes of the pancreas in a HFD and STZ-induced T2DM.

Materials and Methods

Animals and Experimental Design

The study started following the approval of the scientific committee of the Department of Pharmacology and Toxicology at Mustansiriyah University/College of Pharmacy (Approval number: 87, Reference number: 190, Date: 8 October 2024). This study involved thirty untreated adult male Wistar rats, weighing between 100 to 120 grams. Rats were randomly assigned to five groups, each with six rats. They were kept in plastic containers within a well-ventilated setting. The containers had woodchip bedding, and the rats had no restrictions on access to chow and water. The animals were acclimated to the aforementioned environmental circumstances for a week before the start of the studies.

I. **The control group (n=6):** The rats were kept as healthy controls, fed with a regular pellet, and intraperitoneally (IP) injected with citrate buffer at the same period of induction with STZ, and then subcutaneously (SC) injected with Deionized water through the experimental treatment
II. **Induction Group (n=6):** The rats were subjected to HFD for six weeks, and afterward administered a single IP injection of STZ (35 mg/kg) [9].
III. **Low dose TZP group (n=6):** The rats were subjected to an HFD for six weeks, and afterward administered a single IP injection of STZ (35 mg/kg). After confirming diabetes, the rats were treated with TZP at a dose of 0.44 mg/kg SC once weekly for the next 4 weeks.
IV. **Medium dose TZP group (n=6):** The rats were subjected to an HFD for six weeks, and afterward administered a single IP injection of STZ (35 mg/kg). After confirming diabetes, the rats were treated with TZP at a dose of 0.88 mg/kg SC once weekly for the next 4 weeks.
V. **High dose TZP group (n=6):** The rats were subjected to an HFD for six weeks, and afterward administered a single IP injection of STZ (35 mg/kg). After confirming diabetes, the rats were treated with TZP at a dose of 1.32 mg/kg[10,11] SC once weekly for the next 4 weeks. All the rat groups remained on their respective

diets at the termination of the study period to ensure the stability of the model.

Preparation of the drug and doses

STZ pure drug was purchased from Meryer (Shanghai, China), and was prepared freshly for every rat according to the rat body weight by the dissolution of STZ in cold citrate buffer (pH 4.5) and covered with aluminum foil, and injected into the rats within five minutes after the dissolution of STZ.TZP was purchased from Macklin (Shanghai, China). The animal doses of TZP used in this study were determined by converting human equivalent doses (5, 10, and 15 mg for adults, assuming a 70 kg body weight) to rat doses using standard dose conversion formulas based on body surface area. A stock solution was prepared based on the manufacturer's guidelines by dissolving 15 mg of the pure drug in 15 mL of deionized water; 0.5 mL contained 0.5 mg of the drug. It was given by SC injection at doses of 0.44mg, 0.88mg, and 1.32mg /kg/week for 28 days.

Induction of type 2 diabetes mellitus

The diabetic model was developed according to Binh et al. (2013) (9) by the conjugation of an HFD and a single low-dose STZ . The rats undergo dietary manipulation by consuming the HFD for six weeks(9). The HFD consisted of 60% of calories from fat (animal fat), 26.2% from carbohydrates, and 13.8% from protein, with a total caloric content of around 5,200 Kcal/kg. Followed by a single IP. Injection of a low dose of STZ, 35mg/kg(9). To confirm diabetes, 3 days post-administration of STZ, fasting blood glucose was assessed from a tail-vein blood sample using a One Touch glucometer and test strip. A tiny drop of blood obtained from the rats' tail vein was placed on the test strip, which had been inserted into the glucometer following activation. we selected rats exhibiting fasting blood glucose levels above 250mg/dl as the diabetic model.

Sample collection

Upon the end of the investigation, the rats were anesthetized via IP administration of pentobarbital 200 mg/kg (Dolethal Solution for Injection, Vetoquinol UK Ltd, United Kingdom) (12,13). Blood specimens were obtained from the animals via cardiac puncture. Isolating the serum required centrifugation at 5,000 rpm for 12 minutes after blood collection. Before evaluation, the serum was frozen in 2 ml Eppendorf tubes and kept at -18 °C. Following euthanasia of the rat, the pancreas was promptly excised, rinsed with water, and then preserved in neutral buffered formalin (10%) and prepared for histological examination.

A. Assessment of lipid profile

The colorimetric test kits used for detecting total cholesterol (TC), triglycerides (TG), and high-density lipoprotein (HDL-C) levels in serum samples were obtained from commercially accessible diagnostic kits (Linear, Chemical, Spain). Determinations of serum (LDL-C) applying this formula LDL-c (mg/dL) = [TC (mg/dL) − HDL-c (mg/dL) − TG (mg/dL)]/5 (14), and VLDL was estimated using the formula VLDL (mg/dl) = TG (mg/dl) ÷ 5 (15).

B. Assessment of serum superoxide dismutase level

SOD serum levels have been assessed using the enzyme-linked immunosorbent assay (ELISA) method utilizing the sandwich enzyme-linked immunosorbent assay approach. Rat ELISA SOD kit was purchased from My BioSource, USA (Cat: MBS036924).

C. Assessment of Histopathological Changes

This study used the conventional paraffin-embedded method to prepare the pancreatic tissues for microscopic examination. The steps comprised (16): I. Fixation of pancreatic

tissues: Tissues were covered with neutral buffered formalin (NBF). II. Pancreas tissue preparation: To ensure that all the fixative was removed from the pancreas tissue blocks, they were rinsed with Phosphate-Buffered Saline (PBS). To prepare the pancreas tissues for paraffin wax-embedding media, the tissues were first dried using an automated tissue processor (LEICA TP1020-Germany) with escalating concentrations of ethanol to getting rid of water. Subsequently, xylene was used to replace ethanol during tissue clearance to eliminate any remaining dehydrating alcohol. Then, using a vacuum oven to infiltrate the tissue, xylene allowed the paraffin wax to penetrate the cells. III. Embedding of pancreatic tissues: Blocks of pancreatic tissues were embedded in cassettes using liquid paraffin. IV. Sectioning of pancreas tissues: The tissue blocks were sliced into pieces and subsequently mounted on slides. V. Preparation and staining of slides: Heat from a dry oven was applied to the tissue slides to soften the paraffin and enhance tissue adherence. A stain called Hematoxylin and Eosin (H&E) was used to color the slides. Negatively charged cellular components were stained blue with hematoxylin, whereas positively charged components were stained red with eosin. VI. Preparation of Permanently Mounted Sections. The pancreas sections were scrutinized for morphological alterations, which covered inflammatory infiltration, degeneration with necrosis of acini, necrosis of islet cells, congestion & hemorrhage, Atrophy, Islet depletion, and hypertrophy of islets base on ordinal scoring system scale of 0-4 (0= None, 1= minimal (Rarely sporadic alterations), 2=Mild (dispersed), 3=Moderate(confluent), 4= Severe (generalize)[17].

Statistical analysis

A statistical analysis was conducted using the GraphPad Prism software 10.5. Results from experiments are shown as mean ± standard deviation. One-way analysis of variance was used to compare parametric data among groups, followed by a Tukey multiple comparisons post hoc test for pairwise group comparisons. * Indicates p-value <0.05, ** indicates p-value <0.01, *** indicates p-value <0.001, **** indicates p-value <0.0001, and ns indicates no significant difference.

Results

A. Effect of Tirzepatide on lipid profile

Cholesterol levels were significantly ($p<0.0001$) raised in the induction group than in the control group, and significantly ($p<0.0001$) declined in all TZP treatment groups, compared to the induction group. This finding is supported by the data in Table 1and Figure A. TG levels were substantially($p<0.0001$) enhanced in the induction group than in the control group, and significantly($p<0.0001$) lower in all groups receiving TZP than in the induction group. The data in Table 1and Figure B support this finding. VLDL cholesterol levels were significantly ($p<0.0001$) raised in the induction group in contrast with the control group and notably declined ($p<0.0001$) in all groups receiving TZP than in the induction group, as seen in Table 1and Figure C. HDL levels were greatly lower in the induction group than in the control group ($p<0.0001$) and significantly higher in the low, medium, and high TZP doses groups($p <0.05$, $p<0.001$, $p<0.0001$, respectively), compared to the induction group, as seen in Table 1 and Figure D. LDL levels were markedly ($p<0.0001$) elevated in the induction group compared to the control group and considerably($p<0.0001$) reduce in all groups receiving TZP, compared to the induction group, as seen in Table 1and Figure E.

Table 1: Assessment of lipid profile changes in all groups.

Groups	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Control	80.8±4.49	75.8±8.98	39.7±4.63	26±6.19	15.2±1.8
Induction	180±9.99	186±8.78	20.3±1.63	123±9.17	37.1±1.76
Low-dose TZP	139±9.15	136±8.78	26.7±1.75	85.1±8.01	27.1±1.76
Medium-dose TZP	138±5.54	127±6.31	29±3.58	84±7.59	25.5±1.26
High-dose TZP	121±5.27	112±7.34	31.8±2.86	66.5±7.22	22.5±1.47
p-value	<0.001	<0.001	<0.001	<0.001	<0.001

*An ordinary one-way ANOVA test was utilized (Tukey's multiple comparisons test was employed as a post hoc test). n = 6 rats per group. Data were expressed as Mean ±SD. TC = total Cholesterol, TG =Triglyceride, HDL =High-Density Lipoprotein, Low-Density Lipoprotein, and VLDL =Very Low-Density Lipoprotein.

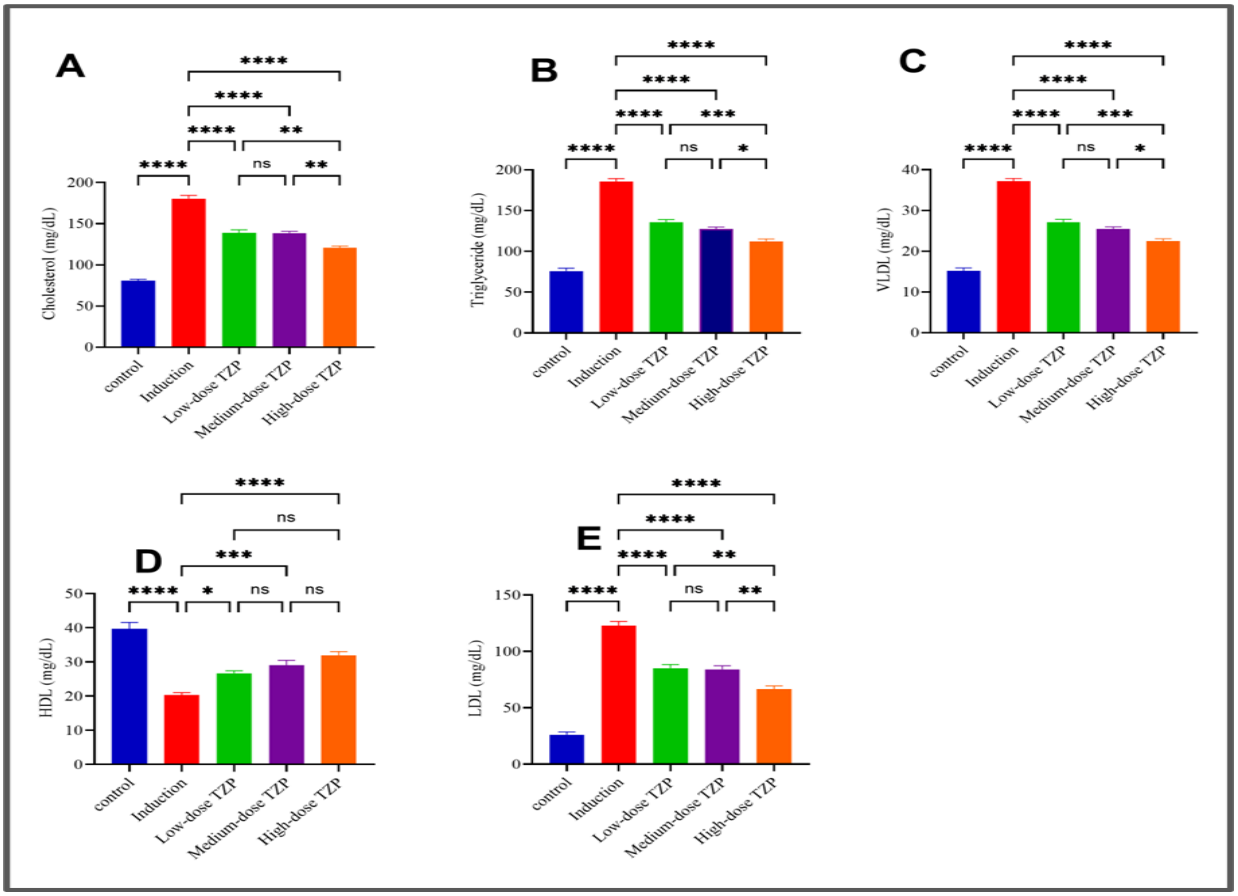

Figure 1: Assessment of lipid profile in different experimental groups.

A) Effect on cholesterol levels,
B) effect on triglyceride levels,
C) effect on VLDL levels,
D) effect on HDL levels,
E) effect on LDL levels. n = 6 rats per group. Data presented as mean ± SEM (one-way ANOVA with post-hoc Tukey test used for pairwise comparisons). * Denotes p-value <0.05, ** denotes p-value <0.01, * indicate p-value <0.001, **** denotes p-value <0.0001, ns no significant difference.**

B. Effect of Tirzepatide on superoxide dismutase (SOD) level

Serum SOD level was markedly ($p<0.0001$) lower in the induction group than in the control group. Treatment with TZP increased SOD levels in a dose-dependent pattern: TZP low dose ($p <0.05$), TZP medium dose ($p<0.01$), and TZP high dose ($p<0.0001$), compared to the induction group. This finding is supported by the data in Table 2 and Figure 2.

Table 2: Assessment of Superoxide dismutase (SOD) level changes in all groups.

Groups	SOD (U/mL) Mean ±SD
Control	133±14.8
Induction	67.3±7.87
Low-dose TZP	85.2±7.77
Medium-dose TZP	90.3±3.34
High-dose TZP	127±10.3
p-value	<0.001

An ordinary one-way ANOVA test was utilized (Tukey's multiple comparisons test was employed as a post hoc test). n = 6 rats per group. Data were expressed as Mean ±SD

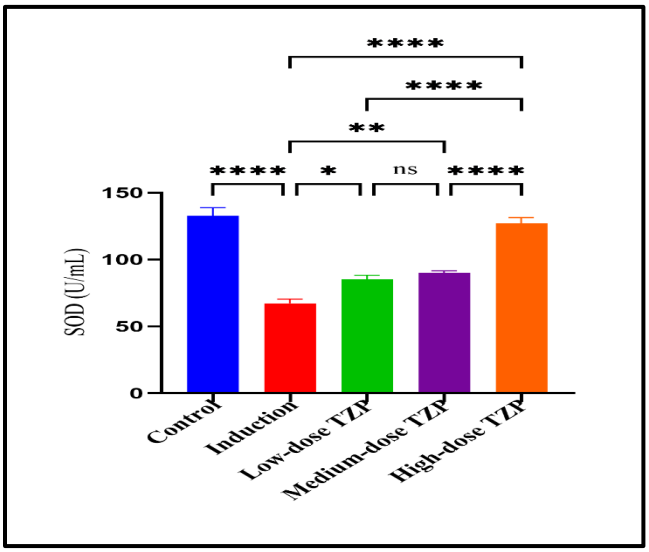

Figure 2: Assessment of SOD in different experimental groups. Data presented as mean ± SEM (one-way ANOVA with post-hoc Tukey test used for pairwise comparisons), n = 6 rats per group. * Indicates p-value <0.05, ** indicate p-value <0.01, ** indicates p-value <0.0001, ns indicates no significant difference**.

C. Effect of Tirzepatide on pancreatic tissues

Light microscopic examinations of pancreatic tissues revealed that the control group had an apparently normal appearance of the pancreatic exocrine acini lining cells and endocrine islet cells, as shown in Figure 3A. Pancreas sections of the induction group showed severe deterioration of pancreatic lobules with marked degeneration and necrosis of the exocrine acinar cells and cellular depletion of pancreatic islet cells, associated with necrosis and severe atrophy of acini, associated with an increased area of interlobular tissue, also degeneration with necrosis of islet cells, as illustrated in Figure

3B. Most histopathological sections of TZP low dose revealed little atrophy of acinar cells, and there was only degeneration without necrosis of islet cells, as illustrated in Figure 3C. All histopathological sections of TZP medium dose showed normal appearance of acinar cells and normal islet cells; a small number of sections displayed certain mild fat degeneration (steatosis) within acinar cells, as illustrated in Figure 3D. All histopathological figures of TZP at high dose revealed a normal appearance of acinar cells and normal islet cells, as compared to those of the control group, as shown in Figure 3E.

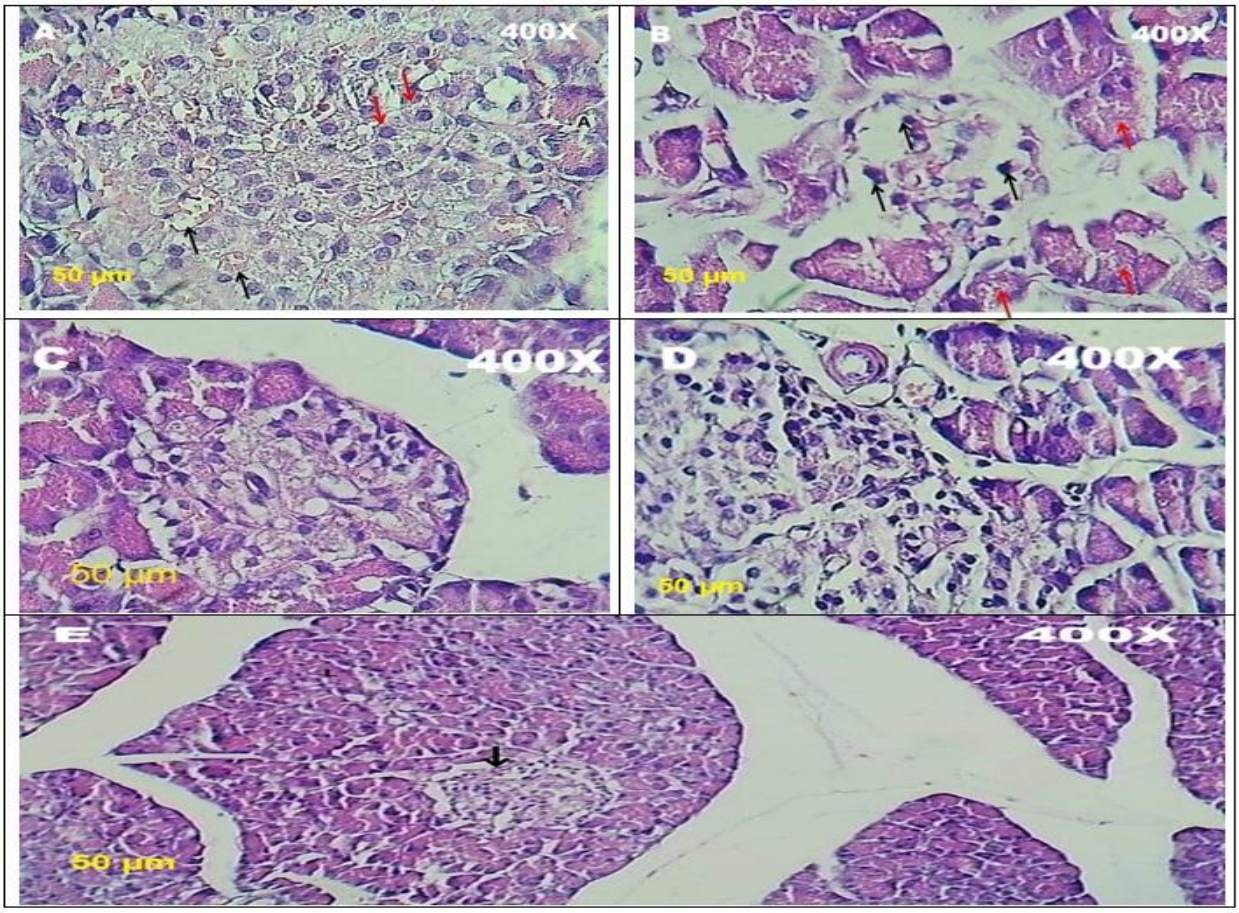

Figure 3: Effect of TZP on pancreas tissue of diabetic rats fed with HFD. A) control group: it shows the normal appearance of islet cells (Red arrows), (S), sinusoidal capillaries (black arrows) & acinus (A). B) Induction group: shows severe degeneration of acini linin cells (Red arrows) and necrosis with severe nuclear pyknosis of islet cells with atrophy of islets (Black arrows). C) Low-dose TZP group: exhibits mild atrophy of acini and slight degeneration of islet cells; and shows minimal degeneration of islet cells without necrosis. E) Medium-dose TZP group: it appears to have a normal appearance of the acinus lining cells & islet cells. F) High-dose TZP group: it displays a normal appearance of acinar cells (a) & normal appearance of islet cells (Black arrow). H&E stain at 400x magnification.

Table (3): The semiquantitative score of pancreas sections

Groups	Control	Induction	TZP-Low dose	TZP-Medium dose	TZP-High dose
Pancreas					
Inflammatory infiltration	0	1	0	0	0
Degeneration with necrosis of acini	0	3	1	0	0
Necrosis of islet cells	0	4	1	0	0
Congestion & hemorrhage	0	1	0	0	0
Atrophy	0	3	1	0	0
Fatty changes	0	3	2	1	0
Islet depletion	0	1	0	0	0
Hypertrophy of the islet	0	0	0	0	0

The ordinal scoring system scale of 0-4 (0= None, 1= minimal (Rarely sporadic alterations), 2=Mild (dispersed), 3=Moderate(confluent), 4= Severe (generalize)

The semiquantitative score of pancreas sections, as displayed in Table 3, The control group exhibited a semiquantitative score of 0 across all evaluated parameters. The diabetes-induction group scored 4 for necrosis of islet cells,3 for degeneration with necrosis of acini, Fatty change, and atrophy. Additionally, assigning a score of 1 for inflammatory infiltration, islet depletion, and Congestion & hemorrhage. The group treated with TZP0.44 mg/kg revealed atrophy, degeneration with necrosis of acini, a score of 1, and scored 2 for Fatty change. The group treated with TZP at a dosage of 0.88 mg/kg received a score of 0 in all evaluated parameters, and scored 1 for the Fatty change parameter. The group treated with TZP at a dosage of 1.32 mg/kg received a score of 0 in all evaluated parameters.

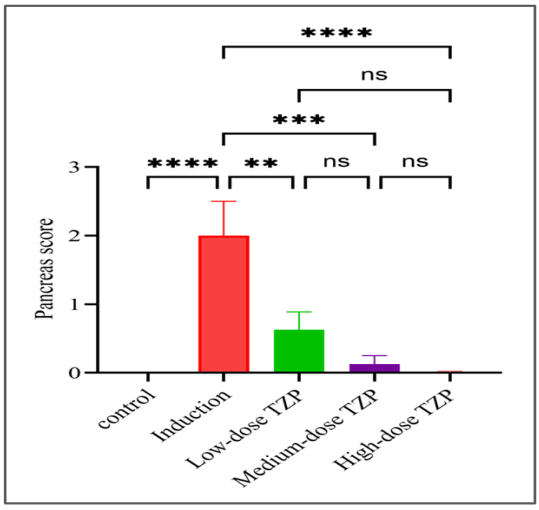

Figure 4: Histogram of pancreas histopathological score. Data presented as mean ± SEM (one-way ANOVA with post-hoc Tukey test used for pairwise comparisons). n = 6 rats per group **indicate p-value <0.01, ****denotes p-value <0.0001, ns denotes no significant difference.

Discussion

Type 2 diabetes mellitus constitutes a major problem in the field of public health that significantly affects morbidity, mortality, and healthcare spending internationally (18) .This study utilized a male rat as an animal model of T2DM by incorporating HFD and STZ. This model suggests that the consumption of an HFD can lead to hyperinsulinemia, Insulin resistance, and lipotoxicity, arising from increased free fatty acids in plasma. However, it does not cause sustained hyperglycemia or diabetes. Therefore, inducing diabetes would require administering a low dose of STZ, which results in initial β-cell malfunction. The synergistic effect of these two primary stresses aims to mimic the natural evolution of T2DM, a rise from insulin resistance to hyperglycemia and hypoinsulinemia at a faster rate compared to human illness (19). Diet is the main element in feeding any experimental animal. The current research revealed that the induction group, whose rats were fed an HFD and received STZ, resulted in a considerable rise in cholesterol, TG, LDL, and VLDL levels, and a significant decline in HDL levels when compared to the control group. This finding, along with previous studies (20–22). Chronic dyslipidemia can lead to significant cardiovascular problems; therefore, managing the lipid profile is crucial in the management of diabetes. Under appropriate conditions, insulin stimulates the enzyme lipoprotein lipase, which catalyzes the hydrolysis of TG. In a diabetic condition, insufficient activation of lipoprotein lipase occurs due to insulin insufficiency, leading to hypertriglyceridemia (23). This study indicated that treatment with all TZP doses showed a significant decrease in TC, TG, LDL, and VLDL levels and an increase in HDL levels when compared to the control group, which is consistent with which is consistent with recent study by Alathary AJ, Kadhim ZJ .2024 that have shown that TZP-treated obese rats exhibited a significant lowering in VLDL and TG levels (24). Another recent meta-analysis studies revealed that treatment with TZP showed a remarkable decrease in TG, TC, VLDL, and LDL levels and an increase in HDL levels(25,26).TZP exhibits significant lipid-modulating effects; this property can manifest in one of two ways: either by activating GIPR on adipocytes or by triggering insulin's lipogenic effect (27). Because TZP improves lipid metabolism, there is less free fatty acid in the circulation. Overall, enhanced glycemic control typically yields beneficial effects on lipoprotein levels among people with diabetes, resulting in decreased cholesterol and TG levels due to diminished circulating VLDL and increased breakdown of LDL via decreased glycation and upregulation of the receptor for LDL (28). Oxidative stress serves as an important contributor to the progression of numerous diseases, including DM. Oxidative stress refers to a condition resulting from an unbalance between the antioxidant levels and free radical concentrations (29). Endogenous antioxidant enzymes, such as SOD, are essential for eliminating ROS and safeguarding cells from the damaging consequences of oxidative stress(30). The current study proved that the induction of diabetes by HFD+STZ results in reduced serum SOD levels, compared to the control group, in line with previous studies(31,32). Reduced levels of SOD show an increased requirement for antioxidant defense. T2DM, Excess ROS produced by hyperglycemia and HFD, results in oxidative damage, lipid peroxidation, and reduced antioxidant enzyme activity(33). The excessive generation of ROS and diminished synthesis of SOD proteins may result in feedback inhibition or oxidative inactivation of enzymatic proteins, consequently reducing

SOD activity. Treatment with TZP at all dosages substantially increased SOD levels compared to the induction group in a dose-dependent manner; This agrees with a recent study done by Yang et al.(2025), showing that TZP increased the levels of SOD in diabetic nephropathy (DN)[34]. Another recent study indicated that the hepatic SOD levels in obese rats administered TZP tended to rise [24]. Tian et al.(2025) showed that TZP increases SOD levels and exerts a beneficial impact on DN[35].TZP has the power to stabilize glucose levels and mitigate hyperglycemia-induced oxidative stress[36]. TZP is a dual agonist for the GIP-1 and GIP receptors, which interact with G proteins. The activation of this receptor elevates adenylate cyclase (AC) levels, enhancing cAMP production. It stimulates many pathways, including those involving cAMP response element-binding protein (CREB) and protein kinase A (PKA). CREB binding initiates the transcription of the SOD gene, resulting in increased quantities of mRNA and, subsequently, proteins. As a result, the TZP therapy elevated the levels of the antioxidant enzyme SOD, aiding in the TZP antioxidant effect. A single IP dose of STZ (35 mg/kg) in HFD-fed rats effectively replicates the pancreatic histopathology of T2DM, including lobular deterioration, acinar cell necrosis, and islet cell depletion with fatty changes. Treatment with TZP, especially at the high doses, showed the normal appearance of acinar cells and normal islet cells of pancreatic tissue, which suggests that TZP has a protective effect against the STZ+HFD effect. This finding is consistent with a recent study by Iwamoto et al. (2024), which indicated that TZP has a favorable impact on the pancreatic β-cells of obese T2DM mice[37].The treatment of TZP markedly elevated SOD levels, improved lipid metabolism, and enhanced TZP's ability to lower blood glucose and improve insulin resistance in T2DM-induced rats, resulting in significant improvements in pancreatic integrity and maintaining the overall normalcy of pancreatic histology in the DM rat model. This study found that treatment with TZP in diabetic rats could reduce oxidative stress levels by increasing SOD levels. TZP can improve the lipid profile, and more importantly, found that TZP treatment might alleviate pancreatic injury induced by HFD/ STZ.

Conclusion

Treatment of T2DM rats with TZP ameliorated their lipid profile, improving pancreatic architecture and augmenting antioxidant activity.

Study Limitations

This study has many limitations, including the use of a small sample size and short duration. This study focuses on the histopathological and biochemical changes without assigning the molecular pathway that explains TZP's ameliorative effects.

Reference

1- Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Res Clin Pract. 2022;183:109119.
2- Goel S, Garg PK, Malhotra V, Madan J, Mitra S, Grover S. Dyslipidemia in Type II Diabetes Mellitus-An assessment of the main lipoprotein abnormalities. Bangladesh J Med Sci. 2016;15(1):99–102.
3- Bhatti JS, Sehrawat A, Mishra J, Sidhu IS, Navik U, Khullar N, et al. Oxidative stress in the pathophysiology of type 2 diabetes and related complications: Current therapeutics strategies and future perspectives. Free Radic Biol Med. 2022;184:114–34.

4- Sakamoto-Hojo ET, Lima J, Xavier D. Oxidative Stress, DNA Damage and Repair Pathways in Patients with Type 2 Diabetes Mellitus. In: Siderova M, editor. Rijeka: IntechOpen; 2019.
5- Omoruyi F, Sparks J, Stennett D, Dilworth L. Chapter 40 - Superoxide dismutase as a measure of antioxidant status and its application to diabetes. In: Preedy VRBTD (Second E, editor. Academic Press; 2020. p. 409–17.
6- Skovsø S. Modeling type 2 diabetes in rats using high fat diet and streptozotocin. J Diabetes Investig. 2014;5(4):349–58.
7- Willard FS, Douros JD, Gabe MBN, Showalter AD, Wainscott DB, Suter TM, et al. Tirzepatide is an imbalanced and biased dual GIP and GLP-1 receptor agonist. JCI insight. 2020;5(17).
8- Chavda VP, Ajabiya J, Teli D, Bojarska J, Apostolopoulos V. Tirzepatide, a new era of dual-targeted treatment for diabetes and obesity: a mini-review. Molecules. 2022;27(13):4315.
9- Binh DV, Dung NTK, Thao LTB, Nhi NB, Chi P V. Macro-and microvascular complications of diabetes induced by high-fat diet and low-dose streptozotocin injection in rats model. Int J Diabetes Res. 2013;2(3):50–5.
10- Guo X, Lei M, Zhao J, Wu M, Ren Z, Yang X, et al. Tirzepatide ameliorates spatial learning and memory impairment through modulation of aberrant insulin resistance and in fl ammation response in diabetic rats. 2023;(August):1–14.
11- Hegab II, El-Horany HE, Abd-Ellatif RN, Nasef NA, Okasha AH, Emam MN, et al. Adropin/Tirzepatide Combination Mitigates Cardiac Metabolic Aberrations in a Rat Model of Polycystic Ovarian Syndrome, Implicating the Role of the AKT/GSK3β/NF-κB/NLRP3 Pathway. Vol. 26, International Journal of Molecular Sciences. 2025.
12- Zatroch KK, Knight CG, Reimer JN, Pang DSJ. Refinement of intraperitoneal injection of sodium pentobarbital for euthanasia in laboratory rats (Rattus norvegicus). BMC Vet Res. 2017;13(1):60.
13- Pang D, Laferriere C. Review of Intraperitoneal Injection of Sodium Pentobarbital as a Method of Euthanasia in Laboratory Rodents. J Am Assoc Lab Anim Sci. 2020 May;59(3):346.
14- Knopfholz J, Disserol CCD, Pierin AJ, Schirr FL, Streisky L, Takito LL, et al. Validation of the friedewald formula in patients with metabolic syndrome. Cholesterol. 2014;2014:261878.
15- Obineche EN, Abdulle AM, Lakhani MS, Gillett MPT. Estimation of plasma very low density lipoprotein-cholesterol levels in uremia. Clin Exp Nephrol. 2002;6:38–42.
16- A protective effect of Ivermectin on vasculitis rat model: Histopathological study. Al Mustansiriyah J Pharm Sci. 2025 Oct;25(4 SE-Reviews):515–24.
17- Meyerholz DK, Beck AP. Fundamental Concepts for Semiquantitative Tissue Scoring in Translational Research. ILAR J. 2018 Dec;59(1):13–7.
18- Impact of Pyridoxine Supplement on Oxidative Stress in Type 2 Diabetic Patients. Al Mustansiriyah J Pharm Sci. 2024 Jan;24(1 SE-Articles):89–104.
19- Halande MP, Patil PA, Garge V, Dhar H. Anti-diabetic effects of a phytomedicinal formulation in streptozotocin and high-fat diet-induced diabetic nephropathy. Indian J Physiol Pharmacol. 68.
20- Naidu PB, Ponmurugan P, Begum MS, Mohan K, Meriga B, RavindarNaik R, et al. Diosgenin reorganises hyperglycaemia and distorted tissue lipid profile in high-fat diet–streptozotocin-induced diabetic rats. J Sci Food Agric. 2015 Dec;95(15):3177–82.
21- Pratiwi RY, Elya B, Setiawan H,

Solawati A, Rosmalena. Alterations in Body Weight, Blood Glucose Levels, and Lipid Profiles in High-Fat Diet-Low Dose Streptozotocin-Induced Diabetic Rats. Pharmacogn J. 2021;13(6s).
22- Kurek JM, Król E, Krejpcio Z. Steviol Glycosides Supplementation Affects Lipid Metabolism in High-Fat Fed STZ-Induced Diabetic Rats. Nutrients. 2020 Dec;13(1):112.
23- Pandey KB, Mishra N, Rizvi SI. Protein oxidation biomarkers in plasma of type 2 diabetic patients. Clin Biochem. 2010;43(4–5):508–11.
24- Alathary AJ, Kadhim ZJ. Tirzepatide protects against hepatic oxidative stress in high-fat induced obesity in male rats. Maaen J Med Sci. 2024;3(3):6.
25- Lv X, Wang H, Chen C, Zhao Y, Li K, Wang Y, et al. The effect of tirzepatide on weight, lipid metabolism and blood pressure in overweight/obese patients with type 2 diabetes mellitus: a systematic review and meta-analysis. Diabetes, Metab Syndr Obes. 2024;701–14.
26- Kanbay M, Copur S, Siriopol D, Yildiz AB, Gaipov A, van Raalte DH, et al. Effect of tirzepatide on blood pressure and lipids: A meta-analysis of randomized controlled trials. Diabetes, Obes Metab. 2023 Dec;25(12):3766–78.
27- Kim SJ, Nian C, Karunakaran S, Clee SM, Isales CM, McIntosh CHS. GIP-Overexpressing Mice Demonstrate Reduced Diet-Induced Obesity and Steatosis, and Improved Glucose Homeostasis. PLoS One. 2012 Jul;7(7):e40156.
28- Schofield JD, Liu Y, Rao-Balakrishna P, Malik RA, Soran H. Diabetes Dyslipidemia. Diabetes Ther Res Treat Educ diabetes Relat Disord. 2016 Jun;7(2):203–19.
29- Hammadi NA, Waheed HJ. Nebivolol effect on oxidative biomarkers in Tamoxifen-Induced Hepatotoxicity in Female White Albino Rats: In Vivo Study. Al Mustansiriyah J Pharm Sci. 2025;25(2):203–11.
30- Mendoza-Pérez JA, Fregoso-Aguilar TA, Hernandez Navarro BCC. Endogenous Antioxidants: A Review of their Role in Oxidative Stress. In: Morales-Gonzalez JA, Morales-González A, Madrigal-Santillan EO, editors. Rijeka: IntechOpen; 2016.
31- Gilani SJ, Bin-Jumah MN, Al-Abbasi FA, Nadeem MS, Afzal M, Sayyed N, et al. Fustin Ameliorates Elevated Levels of Leptin, Adiponectin, Serum TNF-α, and Intracellular Oxidative Free Radicals in High-Fat Diet and Streptozotocin-Induced Diabetic Rats. ACS Omega. 2021 Oct;6(40):26098–107.
32- Das S, Behera JP, Rojaramani Y, Mohanty RR. Effects of resveratrol on oxidative stress in high fat diet /streptozocin induced diabetic wistar albino rats. Int J Basic Clin Pharmacol. 2019 Feb;8(3 SE-Original Research Articles):482–7.
33- Kumawat M, Sharma TK, Singh I, Singh N, Ghalaut VS, Vardey SK, et al. Antioxidant Enzymes and Lipid Peroxidation in Type 2 Diabetes Mellitus Patients with and without Nephropathy. N Am J Med Sci. 2013 Mar;5(3):213–9.
34- Yang Y, Wang Y, Zhou Y, Deng J, Wu L. Tirzepatide alleviates oxidative stress and inflammation in diabetic nephropathy via IL-17 signaling pathway. Mol Cell Biochem. 2025;480(2):1241–54.
35- Tian Y, Tian R, Juan H, Guo Y, Yan P, Cheng Y, et al. GLP-1/GIP dual agonist tirzepatide normalizes diabetic nephropathy via PI3K/AKT mediated suppression of oxidative stress. Int Immunopharmacol. 2025 Jan;146:113877.

36- Batiha GES, Al-Kuraishy HM, Al-Gareeb AI, Ashour NA, Negm WA. Potential role of tirzepatide towards Covid-19 infection in diabetic patients: a perspective approach. Inflammopharmacology. 2023 Aug;31(4):1683–93.

37- Iwamoto Y, Kimura T, Dan K, Iwamoto H, Sanada J, Fushimi Y, et al. Tirzepatide, a dual glucose-dependent insulinotropic polypeptide/glucagon-like peptide 1 receptor agonist, exhibits favourable effects on pancreatic β-cells and hepatic steatosis in obese type 2 diabetic db/db mice. Diabetes, Obes Metab. 2024 Dec;26(12):5982–94.