Tauroursodeoxycholic Acid Protect Against Type 2 Diabetes Through Suppression of Pancreatic TBP-2 and Preservation of Thioredoxin Activity

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Abstract

Endoplasmic reticulum (ER) stress contributes to the development of type 2 diabetes mellitus (T2DM) by inducing thioredoxin-interacting protein (TXNIP/TBP-2), a key mediator of oxidative stress, mitochondrial dysfunction, and pancreatic β-cell injury. This study investigated whether tauroursodeoxycholic acid (TUDCA) and ursodeoxycholic acid (UDCA), two bile acids with ER stress–reducing properties, protect against T2DM through modulation of the pancreatic TBP-2/thioredoxin pathway. Combined TUDCA and UDCA supplementation markedly reduced diabetes incidence in high-fat diet (HFD)– and streptozotocin-induced diabetic rats (34% vs. 75% in untreated animals) and attenuated body weight gain. Mechanistically, rats protected from diabetes exhibited significantly lower pancreatic TBP-2 expression and reduced mitochondrial accumulation of TBP-2 compared with untreated diabetic rats. Preservation of both total and reduced thioredoxin activity was observed in supplemented animals, and thioredoxin activity was inversely associated with pancreatic and mitochondrial TBP-2 levels. These findings indicate that suppression of TBP-2 expression and inhibition of its mitochondrial translocation are closely linked to improved pancreatic redox homeostasis and resistance to diabetes development. In conclusion, TUDCA and UDCA protect against diabetogenesis by limiting TBP-2–mediated oxidative stress and preserving thioredoxin function, identifying the TBP-2/thioredoxin axis as a potential therapeutic target for T2DM.

Keywords: Tauroursodeoxycholic acid (TUDCA); Ursodeoxycholic acid (UDCA); Thioredoxin-interacting protein (TXNIP/TBP-2); mitochondrial translocation; type 2 diabetes

1. Introduction

Type 2 diabetes mellitus (T2DM) is a progressive metabolic disorder characterized by insulin resistance, pancreatic β-cell dysfunction, and chronic hyperglycemia, affecting millions worldwide (International Diabetes Federation, 2021). Its global prevalence continues to rise, largely driven by obesity and consumption of HFD, which promote metabolic stress and accelerate β-cell failure. HFD-induced obesity is associated with elevated circulating free fatty acids, inflammatory mediators, and adipokines that impair insulin signaling and glucose homeostasis, thereby increasing the risk of diabetes development (Kolb & Martin, 2017; Kahn et al., 2000).

Growing evidence indicates that endoplasmic reticulum (ER) stress plays a central role in the pathogenesis of T2DM, particularly in obesity-associated insulin resistance and β-cell dysfunction. Accumulation of misfolded proteins within the ER activates the unfolded protein response (UPR), which initially functions as an adaptive mechanism but becomes deleterious when chronically activated. Sustained ER stress induces C/EBP homologous protein (CHOP), a pro-apoptotic transcription factor that contributes to β-cell death and diabetes progression (Cnop et al., 2012; Ozcan et al., 2004). Genetic or pharmacological suppression of CHOP has been shown to preserve ER homeostasis, reduce oxidative stress, and protect against obesity- and insulin resistance–induced diabetes (Song et al., 2008).

ER stress is closely linked to mitochondrial dysfunction through physical and functional coupling at mitochondria-associated membranes (MAMs). Disruption of ER–mitochondrial communication perturbs calcium signaling, redox balance, and lipid metabolism, amplifying cellular stress responses (Rowland & Voeltz, 2012; Hayashi et al., 2009; Arruda et al., 2014). One critical downstream mediator of ER stress is thioredoxin-interacting protein (TXNIP), also

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known as TBP-2. In obese T2D pancreas, CHOP highly accumulates inside the cell nucleus which is not seen in T1D pancreas (Huang et al., 2007). CHOP promotes the overexpression of TBP-2 inside the nucleus and induce mitochondrial translocation of TBP-2, where it inhibits thioredoxin activity, enhances reactive oxygen species (ROS) production, activates inflammasome signaling, and triggers mitochondrial-dependent β-cell death (Dutta et al., 2005; Saxena G et al., 2010; Choi EH et al., 2023; Park SJ et al., 2022). In a condition of excessive oxidative stress, TBP-2 inhibits glucose transporters and cellular glucose uptake. TBP-2 causes glucose transporters to be localized in the lysosomes for degradation (Qualls-Histed SJ et al., 2023). The cells suffer from glucose transporters on their membrane and a condition "insulin resistance" develops. TBP-2 inhibits pancreatic insulin transcription and production via microRNA-204 inhibiting STAT 3 and transcription factor MAFA (Xu G et al., 2013). TBP-2 also increases glucagon production by the alpha cells in the pancreas and increases gluconeogenesis and glucose production in the liver (Lu B et al., 2022). TBP-2 causes insulin producing beta cells to dedifferentiate and to lose their identity (Amo-Shiinoki K et al., 2025). Dedifferentiated beta cells do not produce any pancreatic insulin hormones (Talchai C et al., 2012)

Bile acids have emerged as important metabolic regulators beyond their classical role in lipid digestion. Tauroursodeoxycholic acid (TUDCA) and ursodeoxycholic acid (UDCA) are well-established chemical chaperones that alleviate ER stress and suppress CHOP induction (Park et al., 2022; Yoon et al., 2016; Malo et al., 2010; Paridaens et al., 2017; Chung et al., 2015; Cao et al., 2016; Mueller et al., 2018; Morales et al., 2023; Habib et al., 2025). Experimental studies have demonstrated that TUDCA improves insulin sensitivity, reduces β-cell apoptosis, and delays diabetes onset in diet-induced and streptozotocin (STZ)-based models of T2DM (Ozcan et al., 2006; Yoon et al., 2016; Malo et al., 2010). UDCA, which is rapidly conjugated to TUDCA in vivo, has similarly been shown to exert cytoprotective and anti-inflammatory effects that support metabolic homeostasis. Combined supplementation with TUDCA and UDCA has been reported to exert potent anti-obesogenic and anti-diabetic effects, including reduced weight gain, improved glycemic control, and delayed diabetes development in HFD-fed animals (Bhowmick et al., 2025).

Despite these promising findings, the molecular mechanisms underlying diabetes resistance or delay following TUDCA and UDCA supplementation remain incompletely understood, particularly with respect to pancreatic TBP-2 expression and its mitochondrial localization. Whether modulation of the ER stress–CHOP–TBP-2 axis contributes to the protective effects of these bile acids in diet-induced T2DM has not been fully elucidated.

The current work represents a secondary mechanistic analysis performed on pancreatic tissues collected from the previously published animal experiment in a HFD–fed, streptozotocin-induced rat model of T2DM supplemented with TUDCA and UDCA. We specifically examined whether combined bile acid treatment reduces total and mitochondrial TBP-2 levels and restores thioredoxin activity in TUDCA and UDCA supplemented rats that were reported to resist or delay diabetes development. By elucidating the role of ER stress modulation in preventing TBP-2–mediated mitochondrial dysfunction, this study aims to provide mechanistic insight into the therapeutic potential of TUDCA and UDCA for the prevention and management of T2DM.

2. Materials and methods

2.1 Chemicals and reagents

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Tauroursodeoxycholic acid (TUDCA; CAS No. 14605-22-2; purity >98%) was obtained from TCI (Tokyo, Japan). Ursodeoxycholic acid (UDCA) tablets were purchased from a local pharmacy and manufactured by Biopharma Ltd. (Bangladesh). Streptozotocin (STZ; CAS No. 18883-66-4; purity 98%) was purchased from Sisco Research Laboratories Pvt. Ltd. (SRL, Maharashtra, India). Citric acid monohydrate and trisodium citrate, used for the preparation of 0.1 M citrate buffer (pH 4.5), were obtained from Smart Lab (Indonesia) and Merck Life Science Pvt. Ltd. (India), respectively. Sodium chloride (NaCl; CAS No. 7647-14-5; purity ≥99.5) was purchased from Merck Life Science Pvt. Ltd. (India). Tris(hydroxymethyl)aminomethane (Tris; CAS No. 77-86-1; purity 99.9%), Triton X-100 (CAS No. 9002-93-1), ethylenediaminetetraacetic acid (EDTA; CAS No. 60-00-4; purity 98%), potassium phosphate monobasic (KH_2PO_4; CAS No. 7778-77-0; purity 99%), magnesium chloride ($MgCl_2$; CAS No. 7791-18-6; purity 99%), HEPES (CAS No. 7365-45-9; purity 99%), and D-mannitol (CAS No. 69-65-8; purity 98.5%) were purchased from Sisco Research Laboratories Pvt. Ltd. (SRL, Maharashtra, India). Sucrose (CAS No. 57-50-1; purity 99.9%) was obtained from MolyChem (Maharashtra, India). Phosphate-buffered saline (PBS; Cat. No. SO10081000) was purchased from Scharlab (India).

A 100× protease inhibitor cocktail (Cat. No. P2949) was obtained from TCI (Tokyo, Japan). Bovine serum albumin (BSA; CAS No. 9048-46-8; purity ≥98%) and dithiothreitol (DTT; CAS No. 3483-12-3; purity 99%) were purchased from Research-Lab Fine Chem Industries (Mumbai, India). Biuret reagent was purchased from Loba Chemie Pvt. Ltd. (India). Human insulin suspension (30/70) was obtained from Square Pharmaceuticals Ltd. (Bangladesh). A Rat TXNIP (TBP-2) enzyme-linked immunosorbent assay (ELISA) kit (Cat. No. CSB-EL025383RA) was purchased from Cusabio (Wuhan, China).

Standard rodent chow and beef tallow were used for the preparation of the experimental diets as previously described (Bhowmick et al., 2025). A glucose solution was administered following streptozotocin injection to prevent severe hypoglycemia. Ultrapure deionized water was used for the preparation of all buffers and reagent solutions.

The following buffers and solutions were freshly prepared as required: 0.1 M citrate buffer (pH 4.5) for streptozotocin administration, phosphate-buffered saline (PBS), non-denaturing lysis buffer containing Tris-HCl, NaCl, Triton X-100, and EDTA, mitochondrial isolation buffer containing mannitol, sucrose, KH_2PO_4, $MgCl_2$, HEPES, and EDTA, and potassium phosphate assay buffer for determination of thioredoxin activity.

Blood glucose concentrations were measured using a digital glucometer (Quick Check, Taiwan) and compatible Quick Check blood glucose test strips. Unless otherwise stated, all chemicals and reagents used in this study were of analytical or molecular biology grade and were obtained from standard commercial suppliers.

2.2 Animal Experiments

A total of 19 male Swiss albino rats were used for this study. The animal experiment, dietary intervention, diabetes induction protocol, and physiological outcomes from this cohort were reported previously (Bhowmick et al., 2025). The present study represents a secondary mechanistic analysis performed using pancreatic tissue samples collected and preserved from the same experimental animals.

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2.3 Tissue collection

Pancreatic tissues were collected from experimental rats immediately after sacrifice in accordance with institutional ethical guidelines (**Fig. 3.**). The excised pancreas was rapidly rinsed in ice-cold phosphate-buffered saline (PBS) to remove excess blood, blotted dry, snap-frozen in liquid nitrogen, and stored at −80 °C until further processing.

2.4 Non-denaturing extraction of total protein from rat pancreas

2.4.1 Preparation of non-denaturing lysis buffer

A non-denaturing lysis buffer was freshly prepared with the following composition: 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, and 1% Triton X-100. A protease inhibitor cocktail was added immediately before use to prevent protein degradation. Additionally, 2 mM EDTA was optionally included as a chelating agent to inhibit metal-dependent proteases. The buffer was kept ice-cold throughout the procedure.

2.4.2 Protein extraction procedure

Frozen pancreatic tissue (~200 mg) was homogenized in 10 volumes of ice-cold lysis buffer using a glass-Teflon homogenizer. Homogenization was performed on ice to preserve protein integrity. The homogenate was incubated on ice for 20–30 minutes with intermittent gentle vortexing to facilitate efficient lysis and protein solubilization under non-denaturing conditions.

Following incubation, the lysate was centrifuged at 12,000 × g for 20 minutes at 4 °C to pellet cellular debris and insoluble material. The clear supernatant containing total soluble proteins was carefully collected without disturbing the pellet.

2.4.3 Protein quantification and storage

Protein concentration was determined using biuret reagent, following the manufacturer's instructions. Aliquots of the extracted protein were stored at −80 °C to avoid repeated freeze–thaw cycles.

2.5.1 Isolation of Rat Pancreatic Mitochondria

Rat pancreatic mitochondria were isolated via differential centrifugation following established protocols with minor modifications. All procedures were performed at 4°C to maintain organelle integrity. Briefly, frozen pancreatic tissue was transferred to a pre-chilled glass homogenizer containing mitochondria isolation buffer (220 mM mannitol, 70 mM sucrose, 5 mM KH_2PO_4, 5 mM $MgCl_2$, 2 mM HEPES, and 1 mM EDTA; pH 7.4). The sample was subjected to 20 strokes with a chilled pestle to ensure uniform homogenization.

The resulting homogenate was centrifuged at 1,000 ×g for 10 min at 4°C to sediment nuclei and intact cellular debris. The supernatant was collected and subjected to an additional clarification step at 1,000 ×g for 10 min. To pellet the mitochondrial fraction, the clarified supernatant was

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centrifuged at 10,000 ×g for 10 min at 4°C. The pellets were resuspended in 500 µL of isolation buffer, pooled, and washed via a final centrifugation step at 10,000 ×g for 10 min.

2.5.2 Mitochondrial Protein Extraction

To extract soluble mitochondrial proteins, the final pellet was resuspended in 1 mL of non-denaturing lysis buffer (50 mM Tris-HCl, pH 8.0; 150 mM NaCl; 1% Triton X-100; 2 mM EDTA) supplemented with a protease inhibitor cocktail. The suspension was vortexed briefly and incubated on ice for 10 min to ensure complete membrane disruption.

The lysate was then clarified by high-speed centrifugation at 14,000 rpm for 20 min at 4°C. The protein-rich supernatant was harvested and stored at –80°C for downstream applications. Total mitochondrial protein concentration was quantified using biuret reagent.

2.5.3 Quantitation of Total and Mitochondrial Protein Using Biuret Method

The concentrations of total pancreatic protein and isolated mitochondrial protein were determined by the Biuret method using bovine serum albumin (BSA) as the standard. A series of BSA standard solutions with known concentrations were prepared in distilled water to generate a standard calibration curve.

Briefly, an appropriate volume of protein sample or BSA standard was taken into separate test tubes and mixed with Biuret reagent. The reaction mixtures were incubated at room temperature for 20–30 min to allow the formation of a violet-colored copper–protein complex under alkaline conditions. Following incubation, the absorbance of each sample and standard was measured spectrophotometrically at 540 nm against a reagent blank.

A standard curve was constructed from the absorbance values of the BSA standards, and the concentrations of total pancreatic protein and mitochondrial protein samples were calculated from the linear regression equation of the standard curve. Protein concentrations were expressed as mg/mL.

2.6 Quantitation of Total and Mitochondrial TBP-2 Protein Using ELISA Method

The concentrations of total and mitochondrial thioredoxin-binding protein-2 (TXNIP/TBP-2) were determined using a commercially available rat TBP-2 ELISA kit (Cat.no. CSB-EL025383RA, Cusabio, Wuhan, China) according to the manufacturer's instructions. Total pancreatic protein extracts and mitochondrial protein extracts obtained from rat pancreas and isolated pancreatic mitochondria were used for the assay.

Briefly, all reagents, standards, and protein samples were brought to room temperature before analysis. A series of TBP-2 standards with known concentrations were prepared to generate a standard calibration curve. Appropriate volumes of standards (500pg, 1,000pg, 2,000pg and 3,000pg) and 1.0 mg diluted protein samples were added into the antibody-coated ELISA plate wells. The plate was then incubated at 37ºC for 2 hours to allow binding of TBP-2 protein to the immobilized capture antibodies.

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After incubation, the liquid of each well were removed. Subsequently, 100µL biotin-antibody (1X) was added to each well and incubated for 1 hour at 37ºC. After washing 3 times, 100 µL HRP-avidin (1X) was added to each well and incubated for 1 hour at 37ºC. After washing 5 times 90 µL TMB substrate was added to each well and incubated at 37ºC for 15 minutes. To stop the reaction, 50 µL stop solution was added to each well and finally the colorimetric measurement was done using a microplate reader at 450nm within 5 minutes.

A standard curve was constructed from the absorbance values of the TBP-2 standards, and the concentrations of total and mitochondrial TBP-2 proteins in the samples were calculated accordingly.

2.7 Determination of Total and Reduced Thioredoxin Level in Diabetic and Non-Diabetic Pancreas

Total and reduced thioredoxin (Trx) levels in pancreatic tissues of diabetic and non-diabetic rats were determined using the insulin disulfide reduction assay based on the ability of thioredoxin to catalyze the reduction of insulin disulfide bonds, resulting in precipitation of the insulin B chain. Pancreatic tissues were homogenized in ice-cold extraction buffer and centrifuged to obtain clear supernatants for analysis. Protein concentrations of the samples were determined prior to the assay.

For determination of total thioredoxin level, the assay mixture contained dithiothreitol (DTT), which reduced oxidized thioredoxin into its active reduced form, thereby enabling measurement of total thioredoxin present in the sample. For measurement of reduced thioredoxin level, the assay was performed in the absence of DTT so that only endogenous reduced thioredoxin activity was measured.

Briefly, the reaction mixture contained 100mM potassium phosphate buffer (pH 7), 2 mM EDTA, 0.13 mM insulin solution, and pancreatic protein sample (2.0 mg). For total thioredoxin assay, 1mM DTT was added to the reaction mixture, whereas DTT was omitted for determination of reduced thioredoxin. The absorbance measurements were recorded by the Spectrophotometer at 650 nm for 15 minutes following a 10 minute incubation period at 30°C and the resultant increase in 15 minutes were compared among different experimental rat groups.

Reduced thioredoxin levels were obtained directly from assays performed without DTT and incubating only for 5 minutes, while total thioredoxin levels were obtained from assays containing DTT.

2.8 Statistical analysis

All data are presented as the mean ± standard error of mean (SEM). Statistical significance was assessed using GraphPad Prism software (version 10.5.0; GraphPad Software, San Diego, CA, USA). Comparisons between two groups were performed using an unpaired two-sample Student's t-test. For comparisons involving more than two groups, a one-way analysis of variance (ANOVA) was used, followed by Tukey's post-hoc test to identify specific group differences. Pearson analyses were performed to evaluate the strength and direction of relationship across the data sets. A p-value of less than 0.05 was considered statistically significant.

3. Results

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3.1 Previously Published Effects of TUDCA and UDCA on Body Weight Gain and Glycemic Control

The physiological and metabolic outcomes of the experimental cohort used in the present study were reported previously (Bhowmick et al., 2025). Briefly, supplementation with tauroursodeoxycholic acid (TUDCA) and ursodeoxycholic acid (UDCA) significantly attenuated body weight gain in HFD-fed rats and produced a markedly leaner phenotype compared with untreated HFD controls. In addition, TUDCA and UDCA supplementation improved glycemic control, as evidenced by lower fasting blood glucose (FBG) and postprandial blood glucose (PBG) concentrations throughout the experimental period.

The incidence of streptozotocin-induced T2DM was substantially reduced in the supplemented group. By Day 54, none of the supplemented animals developed diabetes, whereas 50% of untreated animals met the criteria for diabetes. Following the second streptozotocin administration, diabetes incidence increased to 75% in the untreated diabetic group by Day 90, compared with only 33.3% in the TUDCA- and UDCA-supplemented group. Furthermore, diabetic animals receiving supplementation exhibited significantly lower postprandial blood glucose levels than untreated diabetic animals.

Collectively, these findings demonstrated that combined TUDCA and UDCA supplementation exerts significant anti-obesogenic and anti-diabetic effects in HFD-fed, streptozotocin-treated rats. These previously published observations provided the physiological basis for the present investigation, which was undertaken to explore the underlying molecular mechanisms involving pancreatic TXNIP (TBP-2), mitochondrial TBP-2 accumulation, and thioredoxin activity.

3.2 Validation of Protein Quantification Assays

A standard calibration curve was generated using bovine serum albumin (BSA) to determine total pancreatic and mitochondrial protein concentrations by the Biuret method (**Fig. 1.**). The assay exhibited a strong linear relationship between absorbance and protein concentration, confirming its suitability for protein quantification.

Similarly, a TBP-2 standard calibration curve was established using purified TBP-2 protein for ELISA-based quantification (**Fig. 2.**). The resulting standard curve showed excellent linearity across the tested concentration range, confirming the reliability and reproducibility of the ELISA method used in this study.

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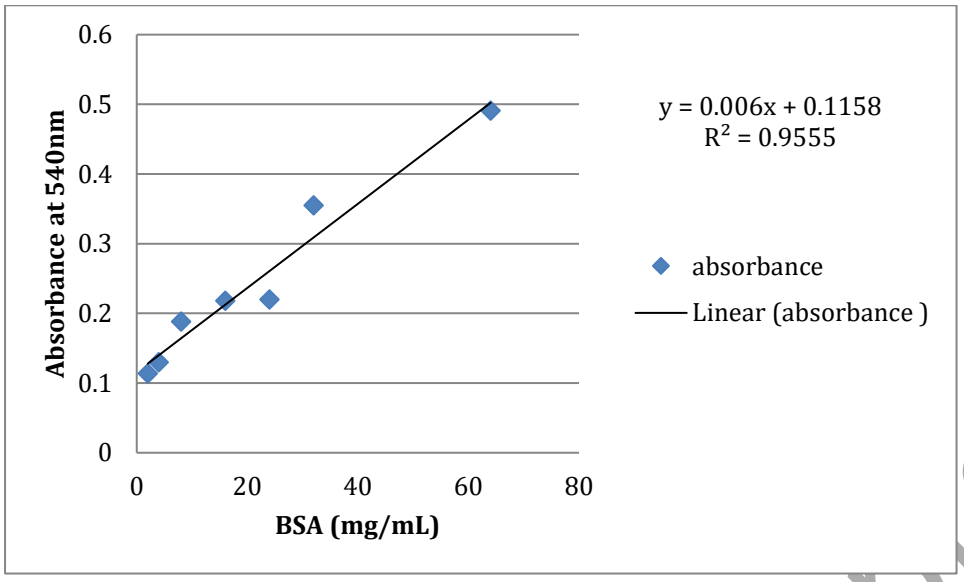

Fig. 1. Bovine serum albumin (BSA) standard calibration curve used for quantification of total pancreatic and mitochondrial protein concentrations by the Biuret method.

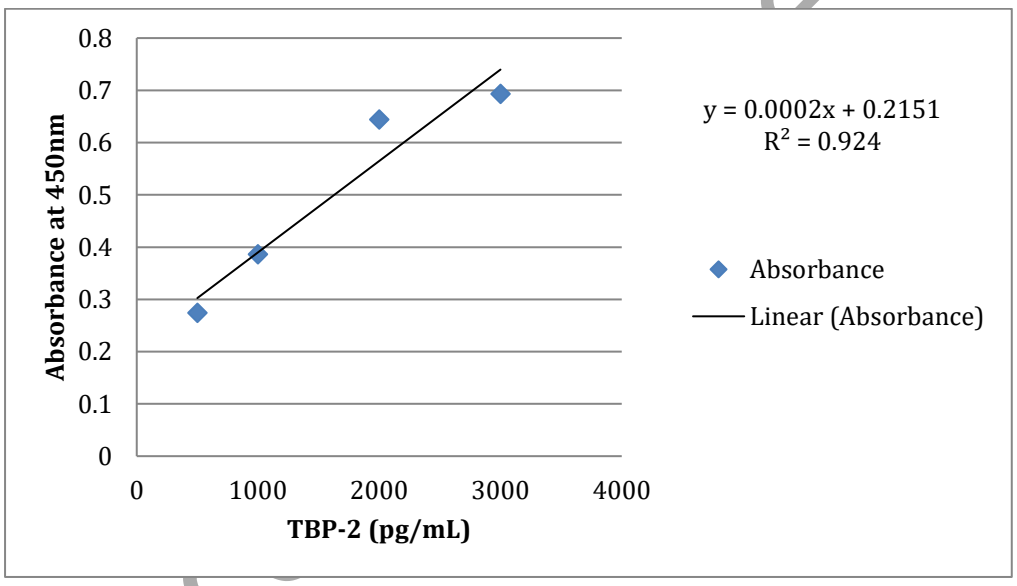

Fig. 2. Standard calibration curve generated using purified TBP-2 protein for ELISA-based quantification of pancreatic TBP-2 levels.

3.3 Total Pancreatic TBP-2 Protein Expression

Quantitative ELISA analysis revealed substantial alterations in pancreatic thioredoxin-binding protein-2 (TBP-2/TXNIP) expression among the experimental groups. As shown in **Fig. 4.**, TUDCA and UDCA supplementation highly modulated pancreatic TBP-2 expression. The treatment group exhibited TBP-2 protein levels of 2148.10 ± 473.13 pg/mL, whereas untreated diabetic rats exhibited levels of 3427.25 ± 14.03 pg/mL. The treatment group comprised both rats that developed diabetes and those that resisted disease development following sullimentation.

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Comparative analysis of diabetic and non-diabetic pancreatic tissues further demonstrated marked differences in TBP-2 expression (**Fig. 5.**). Non-diabetic pancreatic tissues exhibited TBP-2 levels of 1540.45 ± 436.17 pg/mL, whereas diabetic tissues exhibited levels of 3405.97 ± 17.83 pg/mL. Overall, these findings indicate significant alterations in pancreatic TBP-2 expression associated with diabetes status and TUDCA/UDCA supplementation.

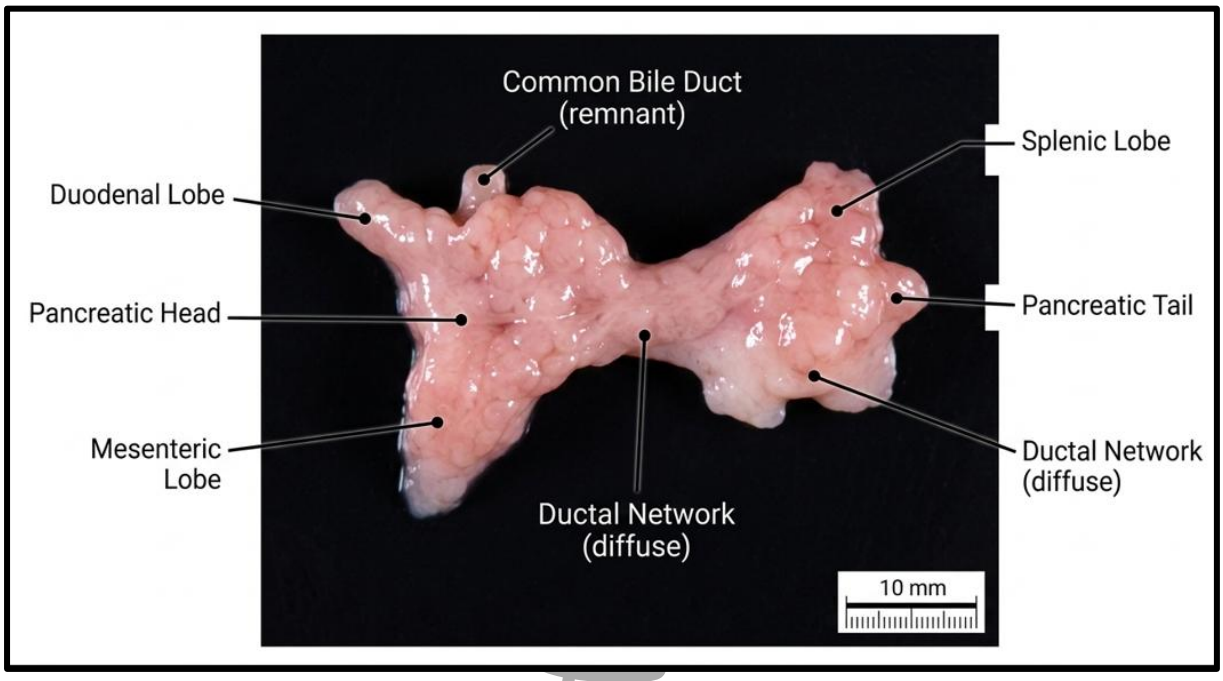

Fig. 3. Isolated pancreas from experimental rats

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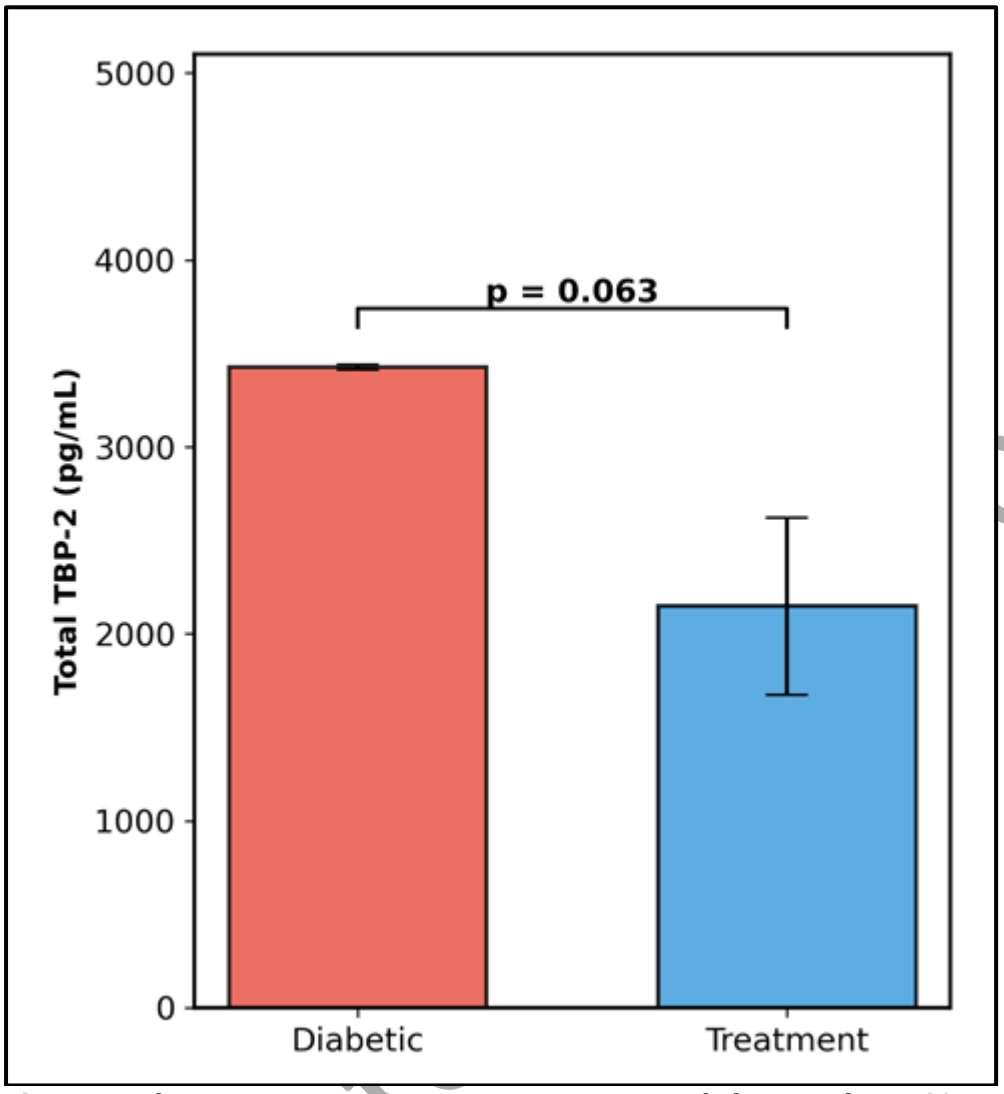

Fig. 4. Total pancreatic TBP-2 protein expression in diabetic and TUDCA- and UDCA-treated rats. Diabetic rats (n=4) exhibited elevated TBP-2 levels, whereas TUDCA and UDCA supplementation (n=6) showed strong trend toward reduction of pancreatic TBP-2 expression.

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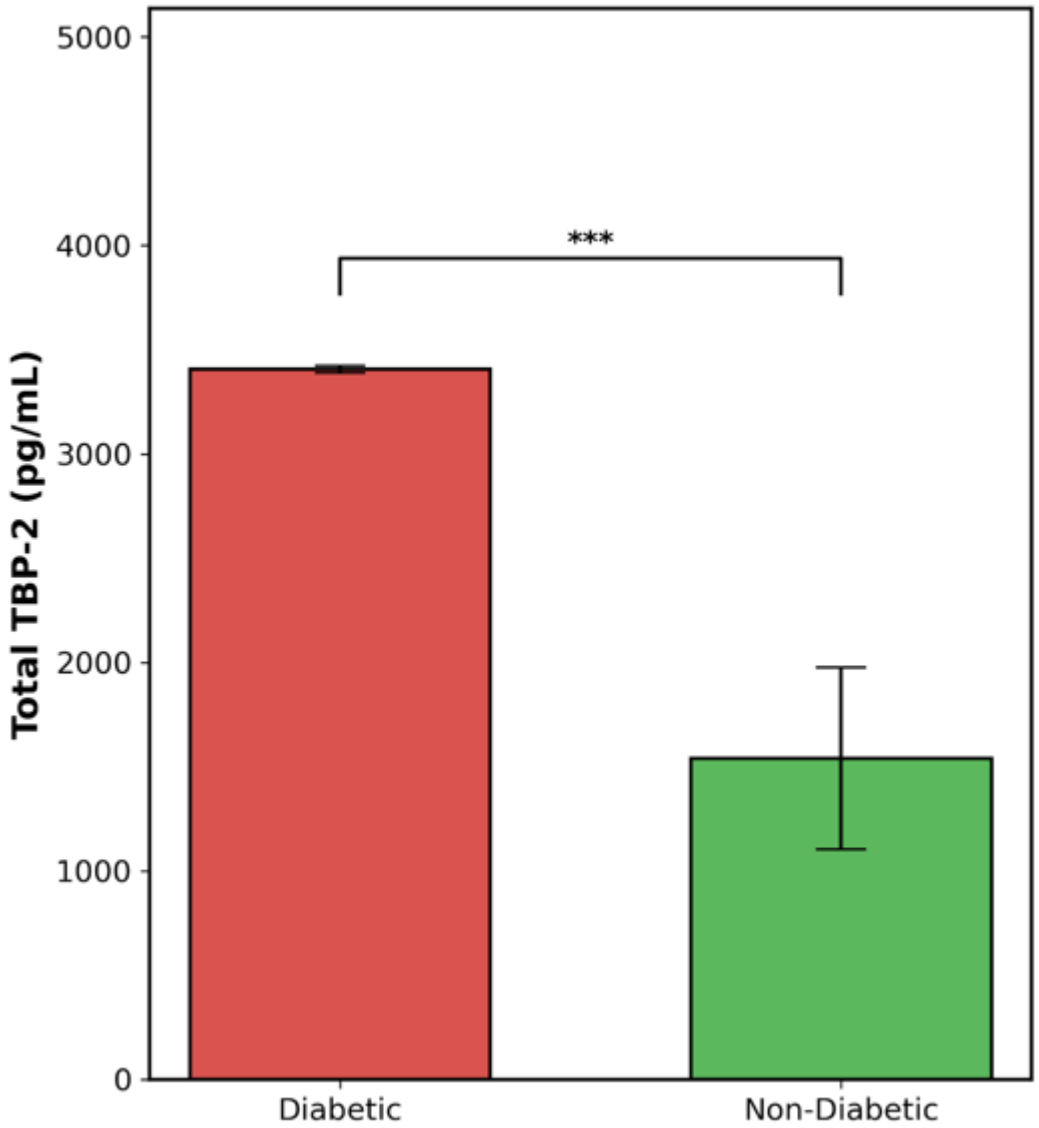

Fig. 5. Comparative analysis of total pancreatic TBP-2 protein levels in diabetic (n=6) and non-diabetic (n=4) pancreatic tissues. Diabetic pancreas demonstrated marked TBP-2 overexpression relative to non-diabetic pancreas.

3.4 Mitochondrial Localization of TBP-2 in Pancreatic Tissue

To determine whether diabetes influences intracellular trafficking of TBP-2, mitochondrial protein fractions were isolated from pancreatic tissues and analyzed for TBP-2 content.

As shown in **Fig. 6.**, rats in the diabetic group exhibited significantly greater mitochondrial accumulation of TBP-2 (175.00 ± 14.43 relative densitometric units) than rats receiving TUDCA and UDCA supplementation (81.67 ± 15.90 relative densitometric units). These results indicate that TUDCA and UDCA supplementation significantly reduced the mitochondrial localization of TBP-2.

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The reduction in mitochondrial TBP-2 accumulation suggests that bile acid supplementation may inhibit diabetes-associated translocation of TBP-2 into mitochondria and thereby contribute to the preservation of pancreatic cellular integrity.

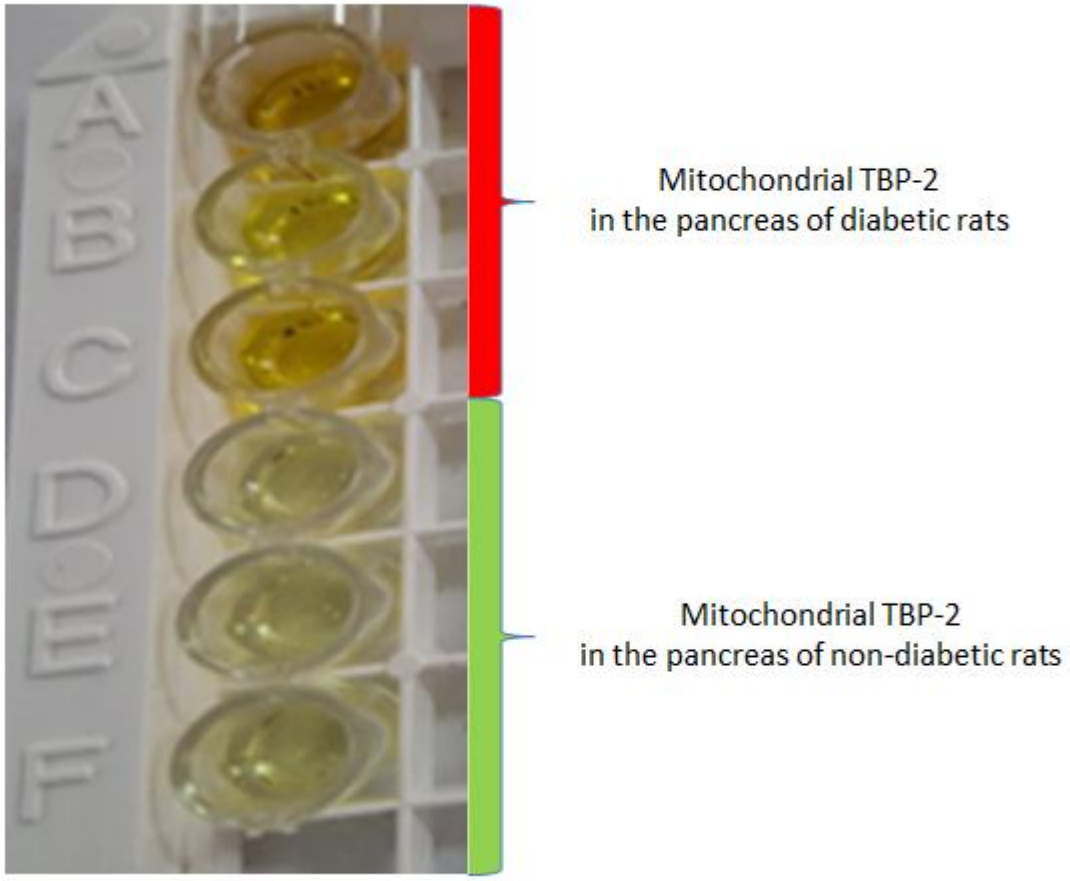

Fig. 6. Relative mitochondrial TBP-2 protein accumulation in pancreatic tissue of diabetic (n=3) and non-diabetic rats (n=3). Diabetic rats showed enhanced mitochondrial localization of TBP-2 compared with control animals.

3.5 Total and Reduced Thioredoxin Activity in Pancreatic Tissue

Total and reduced thioredoxin activities were assessed to evaluate pancreatic redox status (**Fig. 7. and 8.**). Significant differences were observed among the experimental groups.

Untreated diabetic rats exhibited markedly reduced total thioredoxin activity (0.0640 ± 0.0125 absorbance units) compared with rats receiving TUDCA and UDCA supplementation (0.1850 ± 0.0276 absorbance units), indicating impaired antioxidant defense mechanisms in diabetic pancreatic tissue.

Similarly, differences in reduced thioredoxin (free –SH) activity were observed between the groups. The untreated diabetic group exhibited low level of reduced thioredoxin (free –SH)

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activity of 0.0655 ± 0.0334 absorbance units, whereas rats in the treatment group exhibited activity of 0.2970 ± 0.0840 absorbance units.

Overall, these findings demonstrate that TUDCA and UDCA supplementation significantly altered thioredoxin-dependent antioxidant activity and pancreatic redox homeostasis.

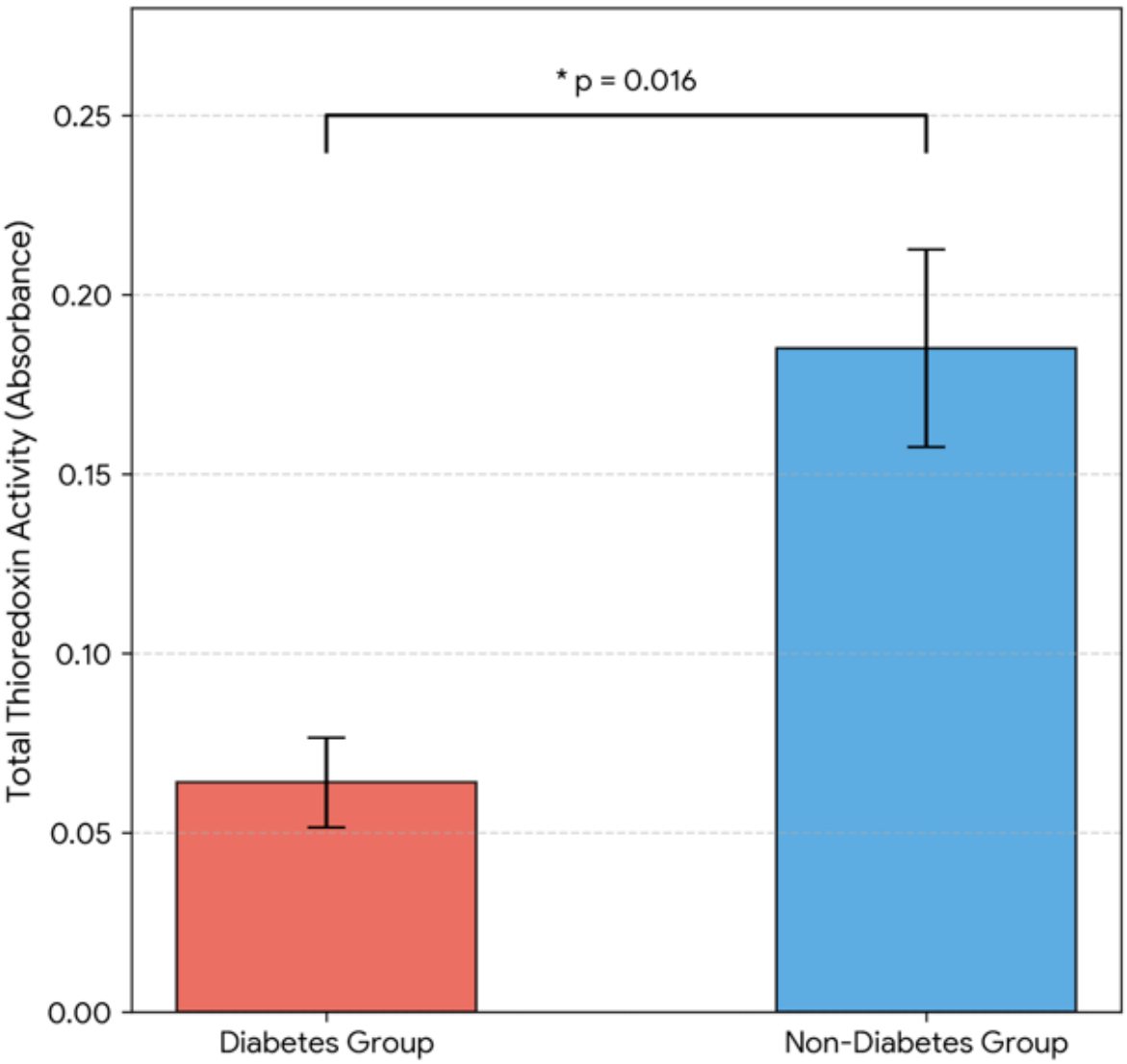

Fig. 7. Total thioredoxin activity (oxidized + reduced thioredoxin) in pancreatic tissue of control diabetic (n=3) and TUDCA- and UDCA-treated rats (n=3). Diabetic rats exhibited significantly reduced total thioredoxin activity, whereas supplementation preserved thioredoxin antioxidant function.

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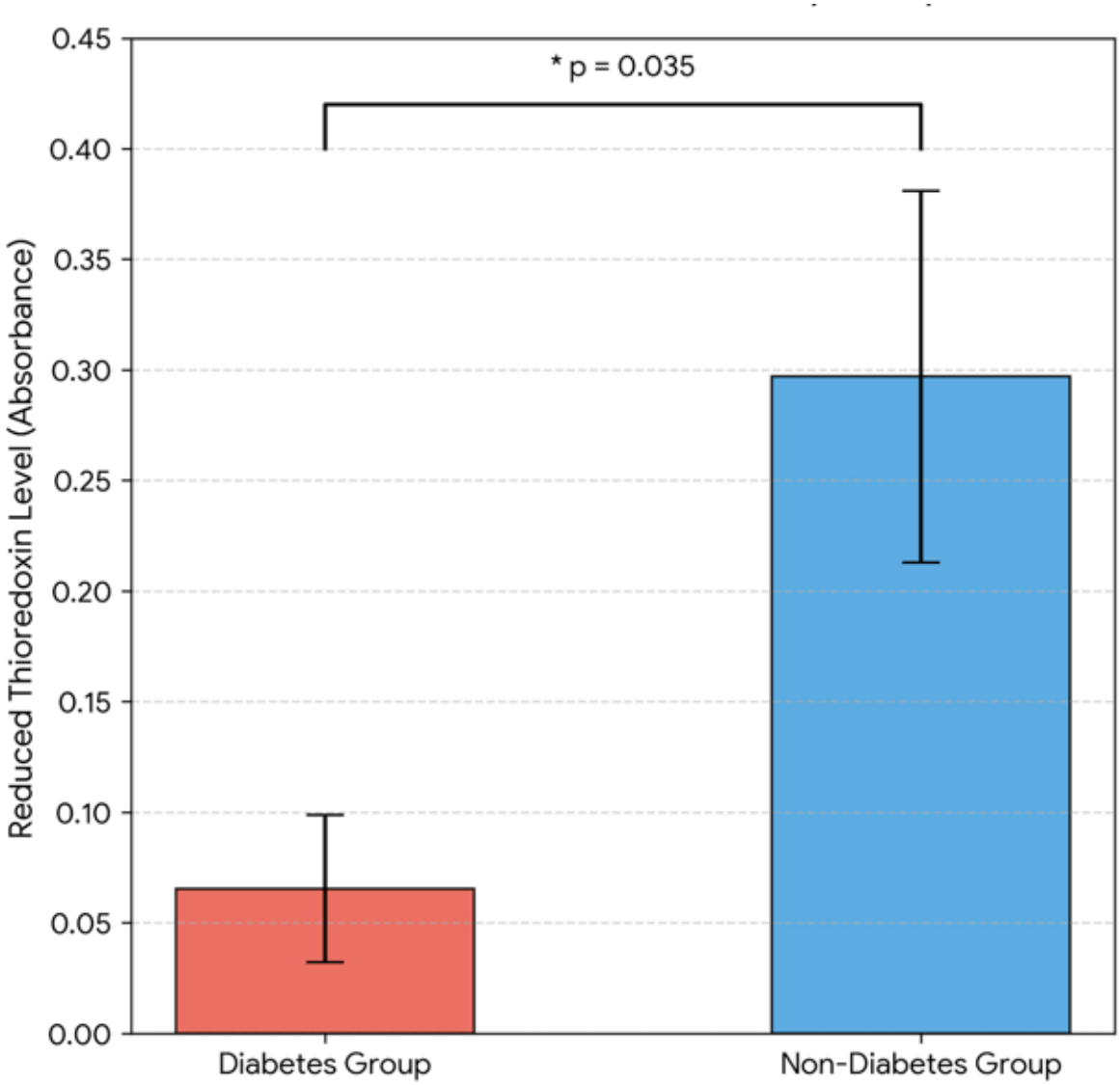

Fig. 8. Reduced thioredoxin (free –SH) activity in pancreatic tissue of control diabetic (n=4) and TUDCA- and UDCA-treated rats (n=3). TUDCA and UDCA supplementation significantly restored reduced thioredoxin activity compared with untreated diabetic rats.

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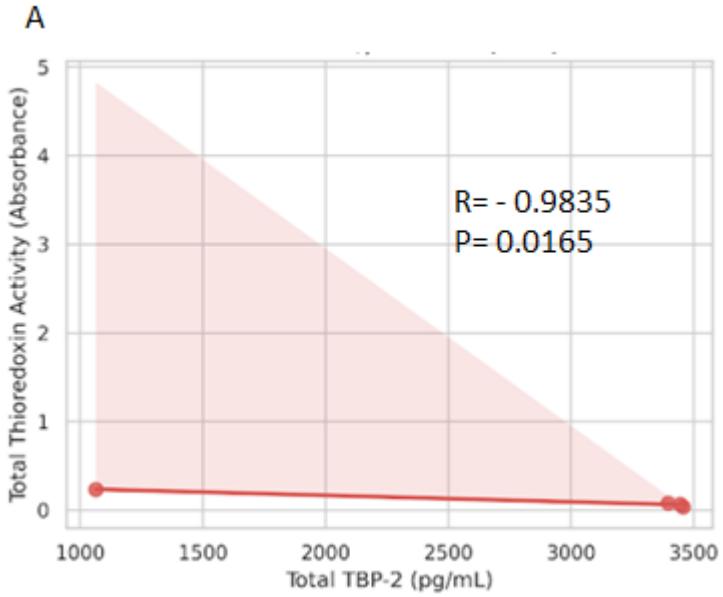
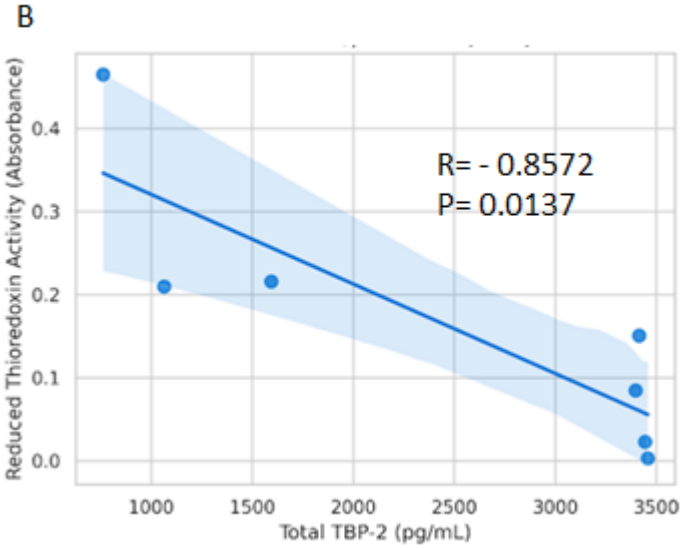
3.6 Relationship between TBP-2 suppression and thioredoxin activation





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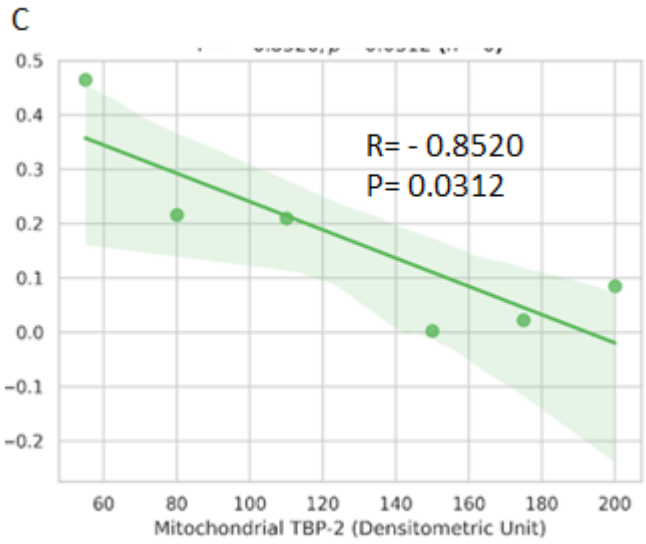

Fig. 9. Pearson correlation analyses between TBP-2 expression levels and thioredoxin activity. Elevated pancreatic and mitochondrial TBP-2 expression is significantly associated with decreased thioredoxin activity.

An inverse relationship was observed between pancreatic TBP-2 expression and thioredoxin activity across experimental groups. Elevated TBP-2 levels in diabetic pancreatic tissue were associated with significantly reduced thioredoxin activity, whereas suppression of TBP-2 expression in TUDCA- and UDCA-treated rats coincided with restoration of thioredoxin antioxidant function.

Similarly, increased mitochondrial accumulation of TBP-2 in diabetic rats corresponded with severe impairment of reduced thioredoxin activity, while diminished mitochondrial localization of TBP-2 in supplemented animals was associated with preservation of thioredoxin-dependent antioxidant defense.

Collectively, these findings suggest that suppression of pancreatic TBP-2 expression and inhibition of mitochondrial TBP-2 translocation contribute to maintenance of pancreatic redox homeostasis and protection against oxidative stress during diabetes development.

4. Discussion

This study demonstrates that combined supplementation with tauroursodeoxycholic acid (TUDCA) and ursodeoxycholic acid (UDCA) exerts potent anti-obesogenic and anti-diabetic effects in HFD-fed and streptozotocin (STZ)-induced diabetic rats, while simultaneously suppressing pancreatic thioredoxin-interacting protein (TXNIP/TBP-2) expression and inhibiting its mitochondrial localization. Most importantly, the present findings provide mechanistic evidence that resistance or delayed progression to diabetes in TUDCA- and UDCA-treated rats is associated with reduced pancreatic TBP-2 accumulation and preservation of thioredoxin (Trx) activity. These findings strongly support the hypothesis that pharmacological

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inhibition of mitochondrial translocation of TBP-2 represents a promising therapeutic strategy for preventing β-cell dysfunction and progression of T2DM.

A major observation of the study was that TUDCA- and UDCA-treated rats exhibited significantly lower diabetes incidence compared with untreated diabetic animals. By day 54, 50% of rats in the untreated diabetic group had already developed diabetes, whereas none of the treated rats met diabetic criteria. By day 90, diabetes incidence increased to 75% in untreated diabetic rats but remained limited to only 34% in the supplemented group. In addition, treated animals maintained substantially lower fasting blood glucose (FBG) and postprandial blood glucose (PBG) levels throughout the experimental period. These results are consistent with earlier reports demonstrating that TUDCA improves insulin sensitivity and restores glucose homeostasis under conditions of obesity-associated insulin resistance and ER stress (Ozcan et al., 2006; Kars et al., 2010). UDCA has similarly been shown to improve hepatic insulin sensitivity and suppress gluconeogenesis (Staels et al., 2010). Furthermore, activation of TGR5 signaling by bile acids stimulates glucagon-like peptide-1 (GLP-1) secretion, which enhances insulin release and improves postprandial glucose regulation (Thomas et al., 2009). The markedly reduced incidence and severity of diabetes observed in the present study therefore suggest that combined bile acid supplementation effectively preserves metabolic homeostasis under diabetogenic conditions.

The anti-obesogenic effects observed in the supplemented group may also have contributed substantially to improved metabolic outcomes. Rats receiving TUDCA and UDCA exhibited significantly lower body weight gain and a visibly leaner phenotype compared with HFD-fed control animals. Obesity-induced metabolic stress is closely linked to chronic ER stress, inflammation, insulin resistance, and β-cell dysfunction (Kahn & Flier, 2000; Cnop et al., 2012). Previous studies have demonstrated that bile acids regulate lipid metabolism and energy expenditure through FXR and TGR5-mediated signaling pathways (Chiang, 2017; Lefebvre et al., 2009; Pols et al., 2011). Recent evidence further showed that ketogenic diet-associated increases in circulating TUDCA protect against obesity and metabolic dysfunction (Li et al., 2024). Therefore, the reduced body weight gain observed in the present study likely reflects attenuation of obesity-induced ER stress and metabolic overload, thereby contributing indirectly to reduced diabetes progression.

The most significant mechanistic finding of this study was the marked reduction of pancreatic TBP-2 expression and mitochondrial TBP-2 accumulation in TUDCA- and UDCA-treated rats that resisted or delayed diabetes development. TXNIP/TBP-2 is increasingly recognized as a critical mediator linking ER stress, oxidative stress, mitochondrial dysfunction, inflammation, and β-cell death in T2DM (Saxena et al., 2010; Choi & Park, 2023). Under chronic ER stress, activation of the unfolded protein response (UPR) induces the pro-apoptotic transcription factor C/EBP homologous protein (CHOP), which promotes both TBP-2 expression and its translocation to mitochondria (Park et al., 2022). Mitochondrial accumulation of TBP-2 subsequently increases reactive oxygen species (ROS) generation, activates inflammasome signaling, impairs mitochondrial function, and promotes apoptosis (Saxena et al., 2010). In the present study, untreated diabetic rats exhibited marked mitochondrial accumulation of TBP-2, whereas TUDCA- and UDCA-supplemented rats showed substantially reduced mitochondrial localization of TBP-2. These findings are consistent with previous reports implicating CHOP-dependent TBP-2 mitochondrial translocation and suggest that this pathway may contribute to the observed effects.

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Another key finding was the preservation of pancreatic thioredoxin activity in TUDCA- and UDCA-treated animals. Diabetic rats exhibited significantly reduced total and reduced thioredoxin activity, indicating impaired antioxidant defense and severe oxidative stress within pancreatic tissues. In contrast, treated rats maintained significantly higher total thioredoxin and reduced thioredoxin (free –SH) activity. Importantly, pancreatic TBP-2 levels were inversely correlated with thioredoxin activity. These findings are biologically significant because TBP-2 directly binds to and inhibits thioredoxin, thereby suppressing its antioxidant function and promoting oxidative injury (Chen et al., 2010). Restoration of thioredoxin activity following TUDCA and UDCA supplementation therefore suggests improved redox homeostasis and enhanced protection against oxidative β-cell damage. Since pancreatic β-cells possess relatively weak intrinsic antioxidant defenses, preservation of thioredoxin activity may be critically important for maintaining β-cell survival and insulin secretion under diabetic conditions.

TBP-2 also contributes to diabetes progression through several additional mechanisms. Previous studies have shown that TXNIP impairs glucose transporter trafficking and promotes lysosomal degradation of GLUT1, thereby contributing to insulin resistance (Qualls-Histed et al., 2023). TBP-2 additionally suppresses insulin gene transcription through miR-204-mediated inhibition of STAT3 and MAFA (Xu et al., 2013), increases glucagon secretion and hepatic gluconeogenesis (Lu et al., 2022), and promotes β-cell dedifferentiation and loss of β-cell identity (Talchai et al., 2012; Amo-Shiinoki et al., 2025). Therefore, suppression of pancreatic TBP-2 expression by TUDCA and UDCA may interrupt multiple interconnected diabetogenic pathways simultaneously. The reduced diabetes incidence observed in the supplemented group strongly supports the concept that inhibition of TBP-2 activation preserves β-cell function and delays metabolic deterioration.

The findings of this study also reinforce the growing importance of ER–mitochondrial crosstalk in metabolic disease. ER and mitochondria communicate through mitochondria-associated membranes (MAMs), which regulate calcium signaling, lipid trafficking, apoptosis, and mitochondrial function (Hayashi et al., 2009; Arruda et al., 2014). Chronic ER stress disrupts MAM integrity and promotes mitochondrial dysfunction, thereby amplifying oxidative stress and insulin resistance. Since CHOP-dependent TBP-2 mitochondrial translocation occurs within this ER–mitochondrial stress axis, the present findings suggest that bile acid-mediated suppression of ER stress may stabilize mitochondrial function and preserve cellular metabolic homeostasis.

Several limitations of the present study should be acknowledged. First, the relatively small sample size may limit the statistical power and generalizability of the findings. Additionally, insufficient tissue remained after mitochondrial isolation to perform **all planned** experiments and assays on the same animals. Nevertheless, the study yielded statistically significant results, which partially mitigate concerns associated with the limited sample size in this exploratory investigation. Second, CHOP expression was not measured directly in this study, although it was considered an upstream regulator of TBP-2 based on previous reports. While pancreatic TBP-2 expression, its mitochondrial localization, and thioredoxin activity were assessed, several additional mechanistic parameters—including insulin levels, HOMA-IR, inflammatory cytokines, reactive oxygen species (ROS), lipid profiles, and histopathological alterations—were not evaluated. Third, separate TUDCA-only and UDCA-only treatment groups were not included, limiting the ability to distinguish their individual contributions to the observed effects. Future studies involving larger cohorts and more comprehensive molecular and histopathological analyses will be important for confirming and extending the present findings.

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In conclusion, combined TUDCA and UDCA supplementation markedly reduced pancreatic TBP-2 expression, inhibited mitochondrial accumulation of TBP-2, preserved pancreatic thioredoxin activity, and significantly delayed or prevented diabetes development in HFD- and STZ-induced diabetic rats. The inverse relationship between TBP-2 expression and thioredoxin activity highlights the critical role of the ER stress–CHOP–TBP-2 axis in oxidative β-cell injury and diabetes progression. These findings identify TBP-2 expression and mitochondrial localization as potential mechanistic targets associated with diabetes resistance in this experimental model. Further studies are required to determine whether these mechanisms are relevant to human T2DM. Inhibitor molecules like, "MitoBlock-6" which inhibits **Erv1/ALR** (essential for respiration and viability/augmenter of liver regeneration) and blocks the mitochondrial translocation TBP-2 may be investigated in future to clearify and explore the mitochondrial contributions in the pathology of diabetogenesis.

5. Conclusion

In conclusion, supplementation with tauroursodeoxycholic acid (TUDCA) and ursodeoxycholic acid (UDCA) in a HFD and streptozotocin-induced rat model of T2DM resulted in reduced body weight gain, improved fasting and postprandial glucose regulation, and a lower incidence of diabetes. Combined TUDCA and UDCA supplementation markedly reduced pancreatic TBP-2 expression and its mitochondrial translocation in rats that resisted or delayed diabetes development. These results suggest that targeting mitochondrial translocation of TXNIP may offer a promising strategy for the prevention and management of T2DM.

Declaration by authors

The authors declare no conflict of interest.

Ratan Hossain conducted this research work (thesis) as a partial requirement for the completion of his M.Sc. degree in Biochemistry and Molecular Biology from Gopalganj Science and Technology University, under the guidance and supervision of Dr. Khokon Kumar Dutta from the same department. The project also received significant contributions from all other co-authors, including project design, facilities, data analysis, and guidance. The authors used ChatGPT (OpenAI, San Francisco, CA, USA) solely to improve grammar, language clarity, and formatting of the manuscript. All study design, data collection, analysis, interpretation, and intellectual content were conducted by the authors.

Disclosure of ethical statements:

Approval of the research protocol

N/A

Informed Consent

N/A

Approval date of Registry and the Registration No. of the study/trial

N/A

Animal Studies

This study complies with the ARRIVE guidelines

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