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Insulinomimetic Effects of Tyrosine Kinase Inhibitors via PPAR-γ/Akt-Mediated β-Cell Preservation in Type 2 Diabetes Mellitus

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Abstract

Introduction:

Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance and progressive β-cell dysfunction. Despite advances in treatment, there remains a need for novel therapeutic strategies that enhance glycemic control while simultaneously targeting associated metabolic abnormalities.

Aim

To investigate the anti-diabetic potential of selected tyrosine kinase inhibitors (TKIs) via modulation of peroxisome proliferator-activated receptor gamma (PPAR-γ) and protein kinase B (Akt) signaling cascade.

Methods

Initially *in vitro* activity of selected TKIs was evaluated in mature 3T3-L1 adipocytes using cell viability and glucose uptake assays. For *in vivo* studies, db/db mice were randomly allocated in to five groups; disease control (DC), db/db mice treated with pioglitazone, upadacitinib, ruxolitinib, erlotinib (25 mg/kg p.o. once daily) and treated for four weeks. C57/BL6 mice were taken as normal control (NC). Blood glucose levels, body weight, food intake and % HbA1c levels were monitored. Biochemical analyses including estimation of triglyceride (TG), total cholesterol (TC), alanine aminotransferase (ALT), aspartate aminotransferase (AST), adiponectin, leptin, uncoupling protein-1 (UCP-1) and c-peptide was done using enzyme-linked immunosorbent assay (ELISA) and colorimetric assays. Expression of phosphorylated Akt was assessed using western blot technique. Histopathological analysis of pancreas, liver and adipose tissues was performed to determine structural alterations.

Results

Erlotinib significantly enhanced glucose uptake in differentiated 3T3-L1 adipocytes ($p < 0.01$). In db/db mice, treatment with all TKIs significantly reduced fasting blood glucose and HbA1c levels when compared to disease control (all $p < 0.05$). Unlike pioglitazone, which increased body weight, ruxolitinib ($p < 0.01$) and erlotinib ($p < 0.001$) significantly reduced body weight. In addition, significant decrease in food intake was also seen after 4 weeks in erlotinib ($p < 0.01$) and upadacitinib ($p < 0.05$). Chronic administration of upadacitinib, ruxolitinib and erlotinib ($p < 0.001$) significantly improved cholesterol and triglyceride, antioxidant parameters, adiponectin, leptin, UCP-1 and C-peptide levels. Notably, erlotinib demonstrated Akt activation ($p < 0.001$), preservation of pancreatic β-cell islet area, and reduced adipocyte size.

Conclusions

In this study, erlotinib demonstrated the most robust antidiabetic activity, with upadacitinib also showing significant metabolic benefits. These findings suggest these drugs might be considered as potential therapeutic agents for type 2 diabetes mellitus.

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia resulting from progressive pancreatic β-cell dysfunction leading to relative insulin deficiency and insulin resistance. Impaired β-cell function and reduced insulin secretion can lead to persistent rise in blood glucose levels, which might result in severe complications. Such complications include obesity, cardiovascular disease and chronic kidney disease (Zhang et al. 2021; Suzuki et al. 2024).

Amongst the molecular signaling cascades involved in glucose homeostasis, peroxisome proliferator activated receptor gamma (PPAR-γ) and protein kinase B (Akt) play a significant role in enhancement of insulin sensitivity, promoting β-cell survival and regulating glucose as well as lipid metabolism. Upregulation of PPARγ enhances insulin responsiveness and further activates downstream Akt pathway, which supports β-cell viability, glucose uptake and homeostasis. As a result, therapeutic strategies targeting the PPARγ–Akt pathway (Teixeira et al. 2022; Dutta et al. 2023) have gained substantial interest as a promising strategy for preserving β-cell function and improving outcomes in T2DM (Savova et al. 2023; Suzuki et al. 2024; Yang et al. 2024).

Despite the availability of multiple medications for type 2 diabetes mellitus (T2DM), including oral hypoglycemic agents and insulin; maintaining continuous glycemic control remains a challenge. Consequently, it results into acute risk conditions and long-term complications. And hence, preservation of β-cell function is an emerging strategy for delaying disease progression and improving glycemic control (Yang et al. 2024). Despite continuous research and therapeutic advancements, including the development of newer formulations and technologies, current therapies remain suboptimal.

Tyrosine kinases play a prominent role in upregulation of c-Jun N-terminal kinase (JNK), thereby promoting the phosphorylation of the insulin receptor and its substrates on serine residues (Shimobayashi et al. 2018). These phosphorylated residues further phosphorylate and activate their respective target proteins which leads to the activation of various downstream signaling cascades. Thus, inhibiting tyrosine kinases can be a promising strategy to improve insulin sensitivity in T2DM (Roskoski 2024a). Among the downstream signaling cascades, the phosphatidylinositol-3 kinase (PI3K)/Akt pathway is a key regulator of β-cell survival, proliferation, as well as insulin secretion. Upregulation of Akt promotes β-cell survival and glucose homeostasis, whereas its downregulation contributes to β-cell dysfunction and the pathological progression resulting in T2DM. Evidence suggests that stimulation of Akt via tyrosine kinase inhibitors (TKI) might preserve β cell function, which can further lead to better clinical management of T2DM. TKIs are multi-targeted class of drugs primarily approved for the

treatment of different types of malignancies. However, small molecule TKI have demonstrated modulation of glycemic control and glucose metabolism, which justifies their potential for management of T2DM. In previous studies, it was observed that several TKIs, such as imatinib, sunitinib, dasatinib, nilotinib and neratinib demonstrated antidiabetic properties (Althubiti 2022; Janssen et al. 2023). TKIs bind to a chain of receptor and proteins which modulates glucose metabolism. For instance, TKIs can target both non-receptor tyrosine kinase like proto-oncogene tyrosine-protein kinase Src and Abelson proto-oncogene (Abl) and receptor tyrosine kinases such as fibroblast growth factor receptor (FGFR), epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR); all of which participate in glucose metabolism. Several clinical studies have demonstrated significant improvements in both type 1 diabetes mellitus as well as T2DM, following TKI administration. The mechanism underlying this effect is supported by various *in-vitro* and *in-vivo* studies (Fountas et al. 2015). According to recent clinical study reports and experimental data, imatinib is in phase 2 clinical trial for the use in Type 2 Diabetes (Gitelman et al. 2021). Recent study reports show that upadacitinib can improve glucose metabolism by reducing low-grade inflammation, potentially restoring insulin function (Grygiel-Górniak and Skoczek 2023). Studies have shown that erlotinib can improve insulin sensitivity and reduce inflammation in β-cells and produce prolonged remission of type 2 diabetes associated with metabolic syndrome. This suggests that erlotinib might have therapeutic potential in improving glycemic control (Brooks 2012). Furthermore, ruxolitinib treatment has been associated with rapid reductions in unusual insulin requirements and significant improvements in HbA1c levels (Wegehaupt et al. 2020).

Therefore, the present study aimed to compare the antidiabetic potential of upadacitinib, ruxolitinib, and erlotinib with PPAR-γ agonist, pioglitazone. *In-vitro* glucose uptake assay was conducted using 3T3-L1 cell line to evaluate the glucose uptake, while *in vivo* experiment using db/db mice was used to assess TKIs effects on glucose metabolism and insulin sensitivity. The study also investigated the role of TKIs in modulating the PI3K/Akt signalling pathway, highlighting their potential as novel therapeutic candidates for T2DM.

2. Material and methods

2.1 Cell culture and differentiation

3T3-L1 pre-adipocytes were obtained from the National Centre for Cell Science (NCCS), Pune, India and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS and 1% antibiotic cocktail (normal media) at 37°C in a humidified incubator at 5% CO2. Preadipocyte differentiation was initiated 48 h after the cells attained confluency, by exposure to a differentiation medium (500 µM 3-isobutyl-1-methylxanthine (IBMX), 1 µM dexamethasone (DEX), and 10 µg/ml Insulin) for 72 h. Subsequently, the differentiation medium was replaced with the post-differentiation medium supplemented with only 10 µg/ml Insulin for 96 h, with media renewal every 48 h. Thereafter, cells were maintained in normal medium containing the treatment for an additional 2 days.

2.2. Cell viability assay

3T3-L1 preadipocytes were seeded in 96-well plates at a density of 1×10^5/well. The preadipocytes were incubated overnight and subsequently exposed to varying concentrations of pioglitazone, upadacitinib, ruxolitinib, and erlotinib for 24 h. Following treatment, a 5 mg/ml MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazonium bromide) stock solution was added to each well at a final concentration of 50 µg/100 µl/well, and the plates were incubated for an additional 4 h. The resulting formazan crystals were dissolved in dimethyl sulfoxide (DMSO), and absorbance was measured at 550 nm (Dutta et al. 2023).

% cell viability= $(Absorbance_{treated}/Absorbance_{control}) X 100$

2.3 Glucose Uptake Assay

In a 96-well plate, 3T3-L1 pre-adipocyte cells were initiated to mature adipocytes as above described procedure. After maturation, the mature adipocytes were cultured with test drugs (pioglitazone, upadacitinib, ruxolitinib and erlotinib) at 3 µM concentration for 2 days. Followed by treatment, cells were fasted for 2 h. Cellular glucose uptake was assessed using fluorescent labeled glucose derivative, 2-NBDG (2-[7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino]-2-deoxy-D-glucose). Glucose uptake was initiated by adding 20 µM of 2-NBDG to each well in glucose-free media for 30 minutes. After incubation, the cells were washed twice with PBS, and then 2-NBDG uptake was measured using a fluorescence spectrophotometer (Cytation-5) at excitation and emission wavelengths 485 nm and 535 nm, respectively (Spandana et al. 2023).

2.4. *In-vivo* studies

2.4.1 Animals and experimental design

All *in vivo* study experiments were performed at Sun Pharma Industries Pvt. Ltd., Vadodara, Gujarat, following approval from the Institutional Animal Ethics Committee having project proposal no. 1094/85/2024. C57BL/6J wild-type and db/db mice (8–10 weeks of age; 30-50g body weight) were procured from the Laboratory Animal Resources Department of Sun Pharma Advanced Research Company Ltd., Vadodara, Gujarat. db/db mice which showed elevation of fasting blood glucose levels (200mg/dl or above), were used for the study. All the mice were housed in standard laboratory conditions at a temperature 18℃-26℃, relative humidity 30–70%, and a 12-hour light/12-hour dark cycle in the experimental room at Sun Pharma Industry Limited, Vadodara, Gujarat. Each ventilated cage housed two to three mice. All animals were allowed free access to diet and water. The mice were acclimatized for one week before initiation of the actual study.

In this study, 36 animals were randomly divided into six treatment groups: Normal Control (NC); Disease Control (DC), db/db mice treated with pioglitazone (25 mg/kg, orally), upadacitinib (25 mg/kg, orally), ruxolitinib (25 mg/kg, orally), erlotinib (25 mg/kg, oral), for 4 weeks (n = 6 per group) with matched basal blood glucose levels. All the treatments were administered once daily orally for four weeks. Body weight and food intake were measured once in a week till end of the study.

2.4.2 Measurement of biochemical markers in db/db mice

2.4.2.1. Blood glucose and glycated hemoglobin levels

On Day 0, 10 µL of blood was collected from retro-orbital plexus under mild isoflurane anesthesia. Blood glucose level was measured using glucometer (One Touch™ Ultra™; LIFESCAN, Johnson & Johnson) and % HbA1C was measured on Day 0 and Day 28 using A1CNow + ® kits (PTS Diagnostics). Blood was also sampled on Day 0,14 and 28 to compare the hypoglycemic effect and % HbA1C between treatment groups (Spandana et al. 2023).

2.4.2.2. Lipid parameters

Twenty-four hours after the last dose of treatment (Day 29) blood was collected, plasma and serum were separated for the measurement of insulin (Rat/mouse Insulin ELISA Kit, Millipore, EZRMI-13K), triglycerides (TG, Triglyceride Colorimetric Assay Kit, Item No. 10010303, Cayman Chemical) and Total Cholesterol (TC, Sigma, Cat No. MAK045-1KT). At the end of the study, pancreas, liver, white adipose tissue and, brown adipose tissue, were isolated for further molecular biology studies.

Adiponectin (Mouse Adiponectin EISA Kit, Cat. No. EZMADP-60K, Millipore, USA), Leptin (Leptin Mouse/Rat ELISA Kit, REF: 22-LEPMS-E01, ALPCO, Salem, NH) and C-peptide (Mouse C-peptide ELISA, REF: 80-CPTMS-E01, ALPCO, Salem, NH) were measured in serum using ELISA kits in accordance to the Manufacturer's recommended protocols. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured in serum samples using Sigma-Aldrich ELISA kit (Thakur and Mehta 2022).

2.4.2.3. Assessment of thermogenic markers

Tissue homogenate was prepared for the estimation of UCP-1 (uncoupling protein-1) levels in brown adipose tissue samples using ELISA kits (Mouse UCP-1 (Uncoupling Protein-1, Mitochondrial) ELISA Kit, Cat. No.: E-EL-M0717, Elabscience). All the steps were followed as per the manufacturer's guidelines.

2.4.2.4. Anti-oxidant parameters

A number of experimental studies suggest that reactive oxygen species (ROS) is increased in diabetic state which leads to various diabetic microvascular and macrovascular complications. This elevated oxidative stress is due to increase in mitochondrial ROS and is thought be associated with hyperglycemia and insulin resistance. Assessment of antioxidant parameters were carried out using liver tissue homogenate. Superoxide dismutase (SOD) and catalase were determined by method described as per manufacturer's instructions (SOD ELISA Kit: #MBS2707323, MYBioSource; Catalase Assay Kit: #707002, Cayman Chemical) (Weydert and Cullen 2010; Chavan et al. 2016).

2.4.3 Western Blot Analysis

Total protein was extracted from harvested liver tissues, lysed using RIPA buffer. Protein quantification was performed using BCA method (Pierce™ BCA Protein Assay kit, Thermo Fisher Scientific, Catalog No. 23227). After quantification, protein samples were mixed with loading buffer and denatured at 95℃ for 5 minutes. For each SDS-PAGE run, 40 µg of protein was loaded to separate p-Akt, t-Akt, and β-actin, followed by the transfer of separated proteins onto PVDF membrane (Millipore). Membrane was blocked with 5% Bovine Serum Albumin (BSA) in TBST for 1.5-h at room temperature (RT), followed by subsequent wash with TBST and TBS. The membrane was incubated with primary antibody (1:1000) overnight at 4℃, and then the PVDF membrane was washed three times with TBST. Following this, the membrane was incubated with anti-rabbit secondary antibody (1:12000) for 2h at room temperature. After three additional washes with TBST, the membrane was incubated with ECL (Western Blotting Substrate) to develop protein bands. Images were captured using iBright 1500 Imaging System and band densitometry was analyzed using ImageJ software. The antibodies used are as follows: Phospho-Akt (Ser473) Antibody (9271, CST, USA), Akt Antibody (9272, CST, USA), and Anti-Rabbit IgG (7074, CST, USA), HRP-linked Antibody (7074, CST, USA) (Chavan et al. 2016).

2.4.4. Histopathological Analyses

Histopathological analysis was performed on pancreas, liver, white adipose tissue, heart and kidneys from all animals in the normal control, disease control and treatment groups. Animal tissues were fixed in 10% phosphate-buffered formalin. The organs/tissues were processed for routine paraffin embedding, sectioned at4-6 µm thickness, and mounted on glass microscope slides and then stained with hematoxylin and eosin (H&E) stain. Additionally, histomorphometry analysis was performed for the measurement of pancreas area of Islets of Langerhans and adipocyte size for white adipose tissue. Histo-microphotographs were captured using Zeiss Axiocam 208 color camera and Zen blue software was used to measure the islet area and size of white adipose tissue cells (Chavan et al. 2016).

2.5. Statistical Analysis

Statistical Analysis was performed using software GraphPad Prism 9.0. Datasets were tested using one-way ANOVA and two-way ANOVA, as appropriate. The comparison between groups were performed using One-way ANOVA and multiple comparisons were done using Dunnett's and Tukey's multiple comparison post-hoc tests. Two-way ANOVA was performed for food intake data analysis. All data are presented as mean ± standard error of mean (SEM) for *in-vivo* studies and mean± standard deviation (SD) for *in-vitro* results. p value < 0.05 was deemed as statistically significant.

3. Results

3.1 *In vitro* cell viability and glucose uptake assays

In 3T3-L1 cell line, MTT assay demonstrated that pioglitazone, upadacitinib, ruxolitinib, and erlotinib maintained cell viability above 80% at all tested concentrations (0.5, 1, 2, 4 and 8 µM), indicating minimal cytotoxicity **(**Fig. 1a**)**.

At 3 μM concentration, erlotinib significantly increased ($p < 0.01$) glucose uptake in differentiated adipocytes as compared to the control group. Moreover, pioglitazone significantly increased ($p < 0.05$) glucose uptake at 3 μM concentration compared with the control group (Fig. 1b).

3.2 Effects of TKIs on Food intake and Body Weight in db/db mice

The disease control group showed a significantly increased body weight than the normal control group ($p < 0.001$), after 4 weeks. Treatment with upadacitinib, ruxolitinib, and erlotinib significantly reduced body weight compared with the disease control group. In contrast, pioglitazone showed a significant increase in body weight as compared to the disease control group (Fig. 2a).

The disease control group showed significant increase in food intake compared with the normal control group ($p < 0.01$). Among the treatment groups, Upadacitinib ($p < 0.05$) and erlotinib ($p < 0.01$) significantly reduced food intake compared with the disease control group. Pioglitazone and ruxolitinib did not alter food intake as compared with the disease control group (Fig. 2b).

3.3. Effect of TKI on Glycemic control

The disease control group showed a significant rise in blood glucose levels compared with the normal control group ($p < 0.0001$). Pioglitazone ($p < 0.0001$), ruxolitinib ($p < 0.01$) and erlotinib ($p < 0.001$) showed significant reduction in blood glucose levels compared with the disease control group. Upadacitinib did not show a significant reduction (Fig. 3a).

HbA1c levels were significantly increased in the disease control group compared with the normal control group ($p < 0.001$). Erlotinib and pioglitazone significantly reduced HbA1c levels compared with the disease control group ($p < 0.001$). No significant changes were observed with upadacitinib, ruxolitinib groups (Fig. 3b).

The disease control group showed a significant decrease in insulin levels compared with the normal control group ($p < 0.01$). Treatment with pioglitazone ($p < 0.05$), erlotinib ($p < 0.01$) and upadacitinib ($p < 0.05$) significantly increased insulin levels compared with the disease control group. Ruxolitinib did not show any significant change in insulin levels (Fig. 3c).

3.4. Effect of TKI on metabolic biochemical markers

3.4.1. Lipid profile and hepatic function markers

The disease control exhibited significantly elevated triglyceride (TG; $p < 0.01$) and total cholesterol (TC; $p < 0.001$) levels compared with the normal control. Pioglitazone significantly reduced TG ($p < 0.01$), whereas upadacitinib ($p < 0.01$), ruxolitinib ($p < 0.05$), and erlotinib ($p < 0.001$) significantly reduced TC. Erlotinib also significantly lowered TG ($p < 0.05$) compared with the disease control (Fig. 4a,b).

3.4.2. Hepatic function markers

The disease control demonstrated significantly increase in alanine aminotransferase (ALT; $p < 0.05$) and aspartate aminotransferase (AST; $p < 0.01$) levels compared with the normal control. Pioglitazone, ruxolitinib, and erlotinib significantly reduced ALT ($p < 0.05$), while erlotinib also significantly decreased AST ($p < 0.05$) compared with the disease control (Fig. 4c,d).

3.4.3. Serum adipokines

The disease control group showed significant decrease in adiponectin ($p < 0.001$) and increase in leptin ($p < 0.01$) levels compared with the normal control group. Pioglitazone significantly increased adiponectin ($p < 0.05$) and leptin levels ($p < 0.05$) compared with the disease control. Upadacitinib, ruxolitinib, and erlotinib demonstrated no significant changes in adiponectin or leptin levels. Erlotinib produced slight increase in adiponectin levels **(**Fig. 4e-f**)**.

3.4.4. Brown Adipose tissue UCP-1 and C-peptide levels

The disease control group exhibited significantly decreased BAT UCP-1 ($p < 0.05$) and C-peptide levels ($p < 0.01$) compared with the normal control group. Pioglitazone significantly and restored C-peptide levels ($p < 0.05$), but showed no significant alterations in UCP-1 levels compared with the disease control group. Upadacitinib significantly increased UCP-1 ($p < 0.01$), but did not significantly modulate C-peptide levels. Erlotinib demonstrate significant rise in UCP-1 ($p < 0.001$) and C-peptide ($p < 0.05$) levels. but showed no significant change in C-peptide levels. Ruxolitinib significantly increased ($p < 0.05$) UCP-1 levels but did not significantly alter C-peptide levels **(**Fig. 4g-h**)**.

3.5. Effect of TKIs on oxidative stress

The disease control group exhibited a significant reduction in SOD activity ($p < 0.01$) and catalase activity ($p < 0.05$) compared with the normal control. Pioglitazone significantly increased SOD ($p < 0.01$) but showed no significant increase in catalase activity relative to the disease control group. Upadacitinib did not show a significant improvement in SOD or catalase activity. Ruxolitinib ($p < 0.05$) and erlotinib ($p < 0.01$) significantly restored SOD activity in comparison to the disease control. Similarly, catalase activity was also significantly increased by erlotinib ($p < 0.001$) and ruxolitinib ($p < 0.05$) compared with the disease control **(**Fig. 5a-b**)**.

3.6. Effect of TKIs on Akt phosphorylation

The disease control group exhibited a significant decrease in the hepatic p-Akt/t-Akt ratio compared with the normal control group ($p < 0.01$). Treatment with pioglitazone significantly increased the p-Akt/t-Akt ratio compared with the disease control group ($p < 0.05$). Erlotinib also demonstrated as significant increase in p-Akt/t-Akt ratio ($p < 0.001$) compared to disease control. Although upadacitinib and ruxolitinib showed a trend towards increase in p-Akt/t-Akt ratio, these changes were not statistically significant. Representative Western blot images and densitometric analysis are shown in Fig. 6 **(a-b)**.

3.7. Effect of TKIs on pancreatic islet area

Histopathological analyses of the pancreatic tissue demonstrated intact islet architecture in the normal control group; whereas the disease control group showed marked pancreatic damage characterized by cellular necrosis, apoptosis, proliferation of dilated ductal structures, reduced islet area, and irregular morphology of the Islets of Langerhans. Quantitative analysis confirmed a significant reduction in pancreatic islet area in the disease control group compared with the normal control group ($p < 0.001$). Treatment with upadacitinib ($p < 0.05$), ruxolitinib ($p < 0.05$), and erlotinib ($p < 0.01$) showed significant increase in the pancreatic islet area compared with the disease control group. Histological examination further demonstrated improved islet architecture with well-defined nuclei and restoration of normal pancreatic morphology following treatment with TKIs **(**Fig. 7a-b**)**.

3.8. Effect of TKIs on adipocyte size

Histological analysis of adipose tissue revealed marked adipocyte hypertrophy in the disease control group compared with the normal control ($p < 0.001$). Pioglitazone showed no significant improvement in adipocyte size compared with the disease control. In contrast, upadacitinib ($p < 0.01$), ruxolitinib ($p < 0.01$) and erlotinib ($p < 0.001$) significantly reduced adipocyte size compared with the disease control group **(**Fig. 8a-b**)**.

3.9. Effect of TKIs on hepatic steatosis

Histopathological analyses of liver sections showed normal hepatic architecture in the normal control group, whereas the disease control group demonstrated marked hepatic steatosis with lipid vacuolation. Pioglitazone-treated animals showed steatosis with increased liver whereas treatment with upadacitinib, ruxolitinib, and erlotinib reduced hepatic steatosis and improved liver histology. Quantitative analysis demonstrated a significant increase in liver weight in the disease control group compared with the normal control ($p < 0.001$). Pioglitazone treatment significantly increased liver weight compared with the disease control ($p < 0.01$). Upadacitinib, ruxolitinib, and erlotinib showed lower liver weights than disease control, but it was non-significant **(**Fig. 9a-b**)**.

4. Discussion

T2DM is a progressive metabolic disorder characterized by chronic hyperglycaemia due to impaired insulin secretion or peripheral insulin resistance. Persistent hyperglycaemia leads to dysregulated lipid metabolism, oxidative stress, inflammation as well as pancreatic β-cell dysfunction. This collectively leads to cardiometabolic complications and end-organ damage. Evidence suggests that multiple signalling cascades, including receptor tyrosine kinases, EGFR, JAK/STAT, PI3K/Akt, and PPAR-γ, play a rather important role in regulation of insulin sensitivity along with glucose homeostasis(Dutta et al. 2023). Consequently, modulation of these cascades is found to be a promising strategy for improvement of metabolic dysfunction in T2DM (Batista et al. 2021; Ou et al. 2024).

From the existing experimental models, the db/db mouse is extensively used genetic model for studying obesity associated with T2DM. These mice possess a spontaneous mutation in the leptin receptor gene, which leads to defective signalling, obesity, insulin resistance, progressive β-cell dysfunction, as well as hyperglycaemia that resemble T2DM characteristics. Unlike other chemically induced diabetic models (such as streptozotocin (STZ) or alloxan), that induce β-cell destruction and mimic insulin deficiency, db/db mice model demonstrates diabetes pathology via progressive obesity leading to insulin resistance. Therefore, db/db model is found to be a robust model for evaluating therapeutic interventions (Kimura et al. 2015; Malek and Davis 2016).

Repurposing clinically approved TKIs has recently gained considerable attention for accelerating drug development for T2DM. Most TKIs competitively inhibit ATP binding within the catalytic domain of receptor and non-receptor tyrosine kinases, thereby modulating multiple downstream signalling cascades involved in inflammation, oxidative stress, insulin signalling, and cellular metabolism. Previous studies have demonstrated their ability to suppress NF-κB activation, JNK signalling, nitric oxide production, and c-Abl-mediated cellular injury (Ou et al. 2024). Emerging preclinical and clinical evidence suggests that several TKIs can improve insulin sensitivity and glucose metabolism in both T2DM (S et al. 2022; Roskoski 2024b; Thomson et al. 2026).

The selected TKIs were screened in differentiated 3T3-L1 adipocytes to assess their cytotoxicity and glucose uptake potential. All compounds demonstrated minimal cytotoxicity which supports their suitability for subsequent pharmacological investigations. Enhanced glucose uptake in adipocytes is an indicator of improved insulin sensitivity (Guan et al. 2021). Among TKIs, erlotinib significantly enhanced glucose uptake at 3 μM. These findings showed preliminary evidence for further *in vivo* validation.

Furthermore, *in vivo* studies were performed using db/db mice. Pioglitazone was considered as the positive control as it acts through PPAR-γ activation and is widely used for T2DM management (Yang et al. 2010; Kimura et al. 2015; Spandana et al. 2023). All TKIs were administered at 25 mg/kg for four weeks for estimating antidiabetic effects and assessing any dose related toxicity. Although previous studies have reported much higher doses of erlotinib, ruxolitinib, and upadacitinib in other pathologies (Li et al. 2018; Zhou et al. 2025), the current study used lower and uniform doses to assess whether metabolic benefits could be achieved with reduced drug exposure. In a study, db/db mice were treated with 80mg/kg erlotinib, which led to reduced insulin resistance (Li et al. 2018).

Insulin resistance is the primary pathological feature of T2DM that contributes to impaired glucose metabolism. Previous studies have demonstrated that inhibition of EGFR and JAK pathways inhibit inflammatory responses (Sheng et al. 2021), improves insulin signaling and enhances glucose utilization (Li et al. 2018; Batista et al. 2021; Zhou et al. 2025). The improvements in blood glucose, HbA1c, and insulin observed in the present study following treatment with erlotinib, upadacitinib and ruxolitinib suggest restoration of insulin signaling rather than only providing symptomatic relief.

Obesity-linked insulin resistance is usually accompanied by excessive food intake, increased body weight, and altered metabolism. Hyperphagia due to impaired leptin signaling is a typical feature of

db/db mice which leads to rapid weight gain (Kimura et al. 2015; Malek and Davis 2016). Consequently, decrease in body weight gain and food intake following TKI treatment suggests improvement in metabolic function. Reduction in body weight was observed with upadacitinib, ruxolitinib, and erlotinib.

Metabolic dysfunction in T2DM also include hypertriglyceridemia, hypercholesterolaemia, and hepatic impairment. Elevated TG and TC promote lipid deposition and aggravate insulin resistance, while increased serum ALT and AST demonstrate hepatic metabolic stress (Ye et al. 2025). The improvement in lipid profile and liver enzymes following TKI treatment suggests that modulation of tyrosine kinase pathway may improve both lipid metabolism as well as hepatic function. In previous study, it was observed that improvement in ALT and AST parameters, led to upregulation of PI3K/Akt/GSK-3β signaling which further reduced blood sugar levels in db/db mice model (Liang et al. 2018). Our findings are in-line with previous studies demonstrating that EGFR inhibition ameliorates insulin resistance and metabolic dysfunction in diabetes (Li et al. 2018).

Adipose tissue functions regulates systemic insulin sensitivity and energy homeostasis. Adiponectin enhances insulin signalling along with fatty acid oxidation. On the other hand, leptin controls appetite and glucose metabolism via hypothalamic and other peripheral signalling pathways (Buffier et al. 2018). Relatively stable adipokine profile was observed following TKI treatment which suggests that their metabolic effects may not depend on modulation of adipokine secretion. Instead, their effects might be associated with intracellular signalling pathways. Furthermore, increased UCP-1 expression, following TKI treatment, suggests enhanced thermogenic action in brown adipose tissue, which may reduce adiposity. In addition, restoration of C-peptide levels support preservation of endogenous β-cell secretory function. In a study, similar effects were observed. A new PPAR-α/γ dual agonist MHY908 reduced serum TG, TC and insulin levels and increased adiponectin levels. In addition, it also improved hepatic steatosis and reduced insulin resistance in db/db mice model (Park et al. 2013).

Oxidative stress also leads to β-cell dysfunction along with progression of insulin resistance. Excessive formation of reactive oxygen species lead to impaired insulin signalling. Increase in SOD and catalase activity, following TKI therapy, with particularly erlotinib treatment, suggests attenuation of oxidative stress. These findings are consistent with previously reported evidence (Brooks 2012).

PI3K/Akt signalling cascade plays a central role in maintenance of glucose homeostasis (Savova et al. 2023). Downregulation of Akt signaling is a pathological hallmark of insulin resistance and can lead to hyperglycaemia (Guan et al. 2021). The present study investigated Akt phosphorylation in hepatic tissue. Erlotinib markedly increased hepatic Akt phosphorylation suggesting that upregulation of the Akt signaling may have improved glucose uptake, insulin sensitivity, as well as glycaemic control.

The findings were further supported by histopathological analyses. Untreated db/db mice demonstrated degeneration of islets, increased white adipocyte size and hepatic steatosis. Improvement of pancreatic structure along with restoration of islet area after subsequent TKI treatment suggests conservation of β-cell integrity. Similarly, the decrease in adipocyte size following treatment with upadacitinib, ruxolitinib, and erlotinib indicates improved adipose tissue structure and reduced lipid accumulation. In addition,

attenuation of hepatic steatosis is also observed. Collectively, these findings suggest that selected TKIs improve glucose homeostasis via modulation of insulin signalling.

5. Study limitations and future perspectives

This study has few limitations which needs to be acknowledged. This study was conducted using a single T2DM model. In addition, only one treatment dose was evaluated for a relatively short duration of 4 weeks. And hence, assessment of long-term efficacy along with safety with TKIs require further investigations. Additional downstream mediators, including insulin receptor substrate-1 (IRS-1), glucose transporter 2/4 (GLUT2/GLUT4), glycogen synthase kinase-3 beta (GSK-3β), AMP-activated protein kinase (AMPK), and inflammatory signalling pathways should be evaluated in further studies. Moreover, other assessments such as oral glucose tolerance tests and insulin tolerance tests would have further strengthened the mechanistic conclusions. Future studies should validate these findings in other experimental models, investigate effect of dose and evaluate the long-term metabolic profiles of TKIs in order to promote their clinical translation.

6. Conclusion

In conclusion, selected TKIs, particularly erlotinib, improve glucose homeostasis in db/db mice through modulation of multiple signaling cascades. TKIs improved insulin sensitivity, restored metabolic function, exerted antioxidant effects, preserved pancreatic islet structure, reduced adipocyte size and hepatic steatosis, and stimulated hepatic Akt phosphorylation. These findings support the therapeutic potential of TKIs as repurposed agents for the management of T2DM.

Declarations

7. **Acknowledgments**

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8. Author contributions

ER and SP conceptualized and designed the study. ER conducted the research, performed data analysis, and drafted the manuscript. SV contributed to the *in vitro* experiments. VB and SP supervised the research and provided critical review of the manuscript. All authors reviewed and approved the final manuscript.

9. Data availability

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

10. Declarations

Ethics approval

All animal related experimental procedures were conducted in accordance with the guidelines of the Institutional Animal Ethics Committee (IAEC) and were approved under Project Proposal No. 1094/85/2024.

Clinical trial number

Not applicable

Competing interests

The authors declare no financial or non-financial competing interests.

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Figures

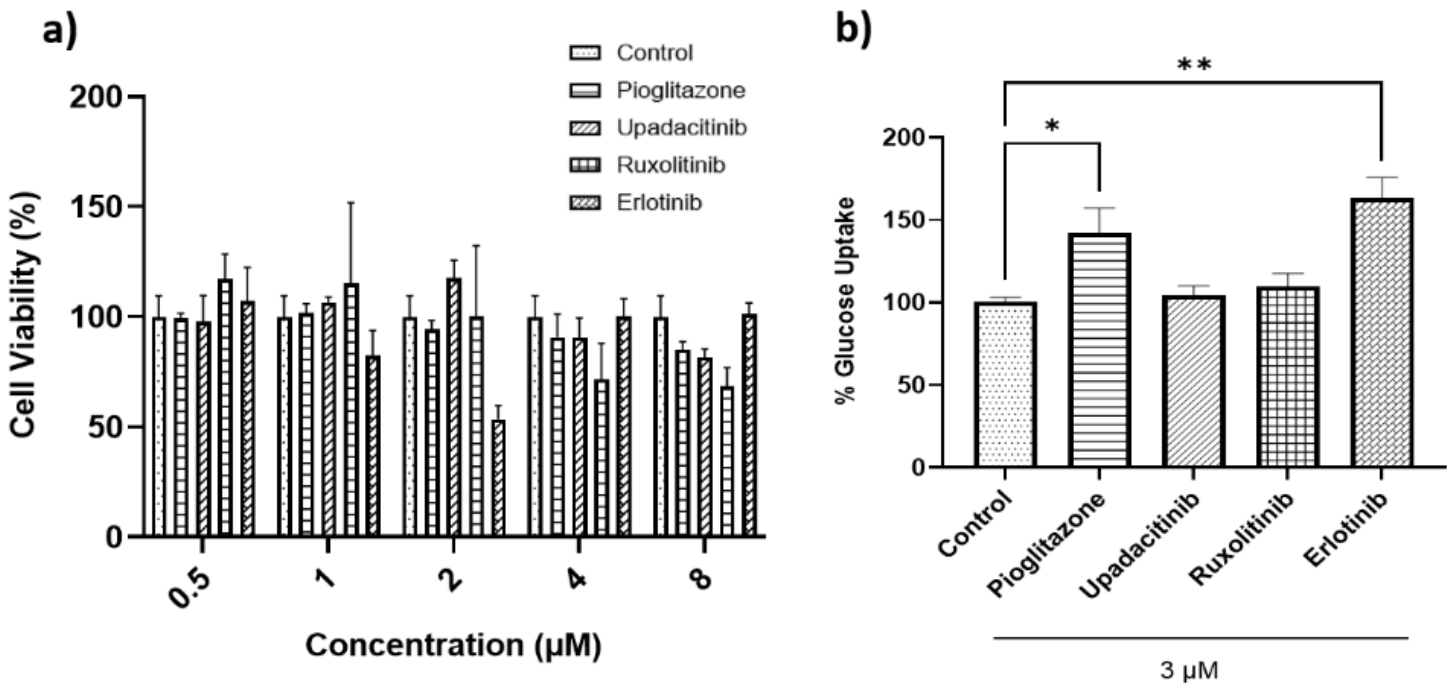


Figure 1

Effect of TKIs and PPAR-γ agonist on 3T3-L1 adipocytes a) Cell viability assessed by the MTT assay; b) Glucose uptake in differentiated 3T3-L1 adipocytes following treatment with TKIs and the PPAR-γ agonist at 3 μM. Compared to control *p< 0.05. Data are presented as mean ± SD.

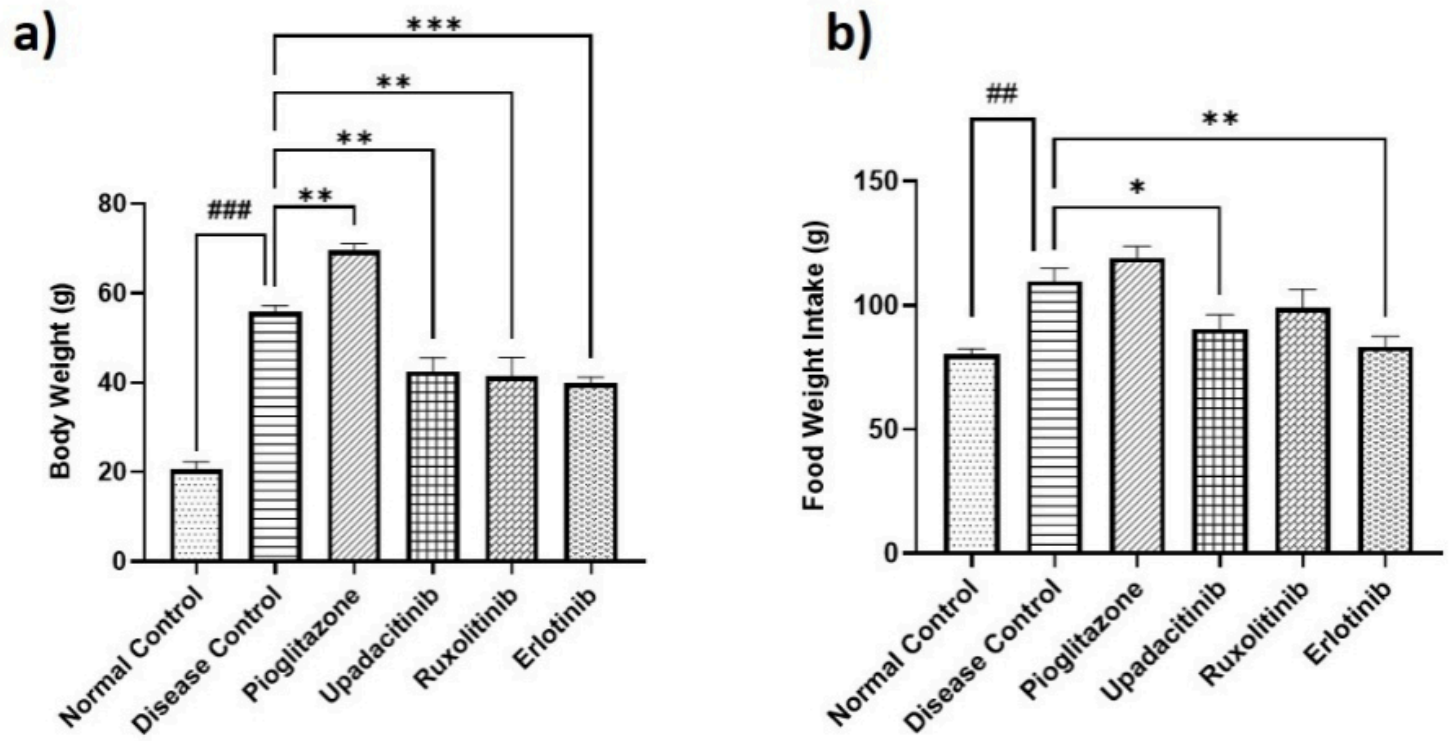


Figure 2

Effect of TKIs on body weight and food intake after 4 weeks of treatment. a) Body weight; b) Food intake. Compared to normal control, ##p<0.01; ###p<0.001; Compared to disease control *p< 0.05, **p<0.01, ***p<0.001. n=6; Data are presented as mean ± SD.

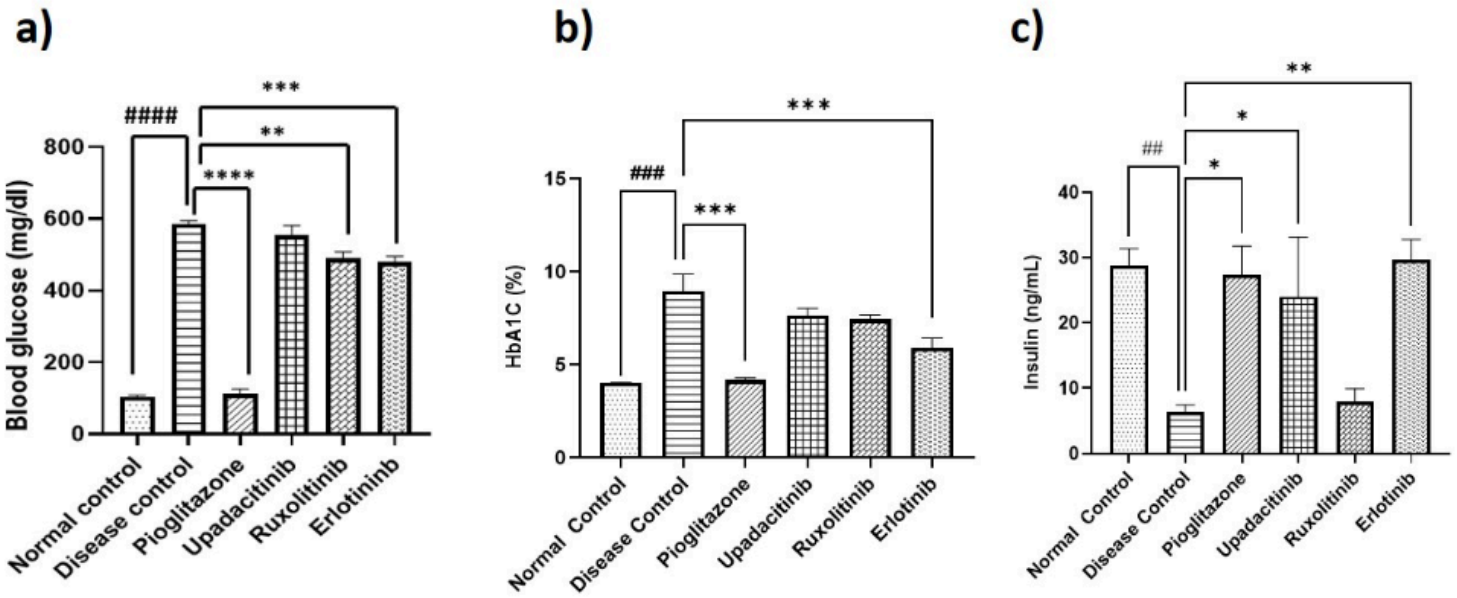


Figure 3

Effect of TKIs on blood glucose, Hb1Ac after 4 weeks of treatment. a) Fasting blood glucose; b) Hb1Ac levels c) Insulin. Compared to normal control, ##p<0.01; ###p<0.001; Compared to disease control *p<0.05, **p<0.01, ***p<0.001. n=6; Data are presented as mean ± SD.

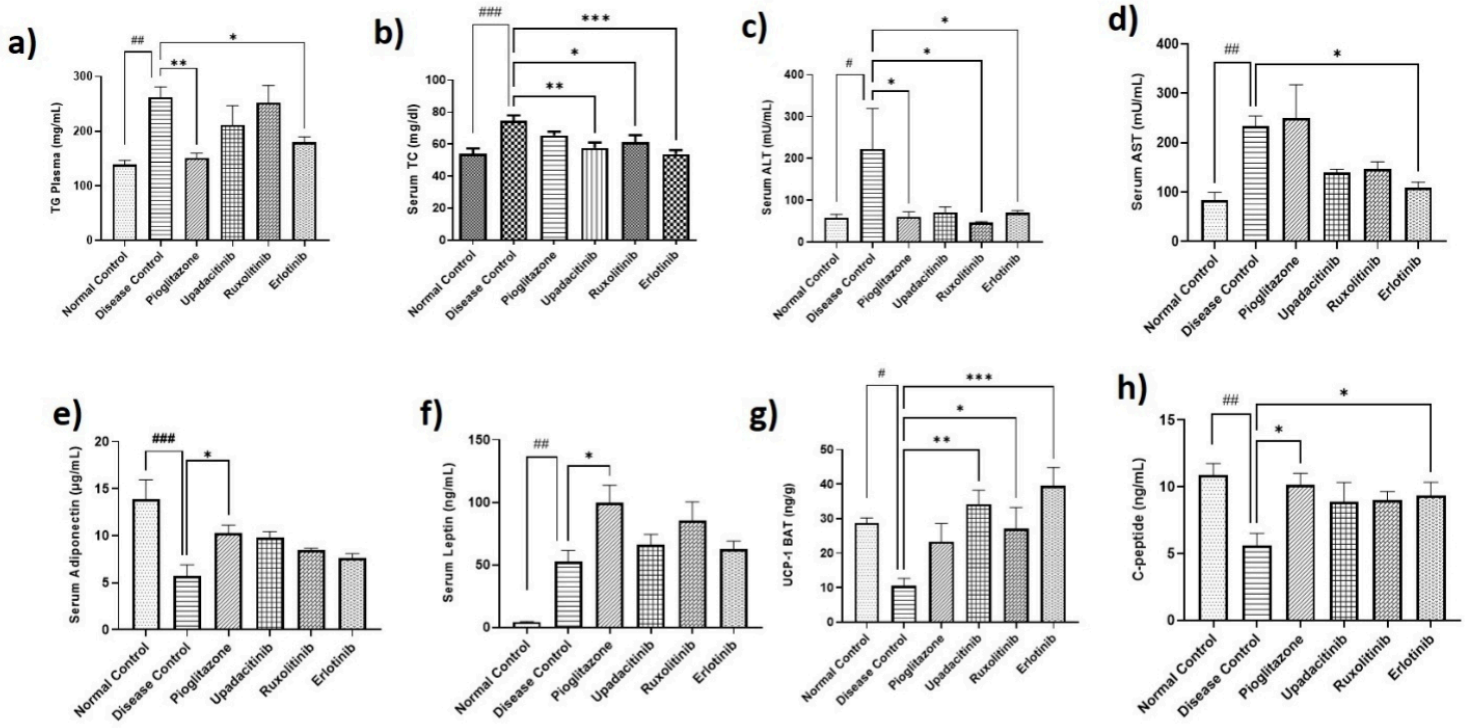


Figure 4

Effect of TKIs on serum and tissue biochemical markers. a) Triglyceride levels b) Total Cholesterol c) Serum ALT d) Serum AST e) adiponectin f) leptin g) UCP-1 h) C-peptide. Compared to normal control, #p<0.05, ##p<0.01; ###p<0.001; Compared to disease control *p< 0.05, **p<0.01, ***p<0.001. n=6; Data are presented as mean ± SEM.

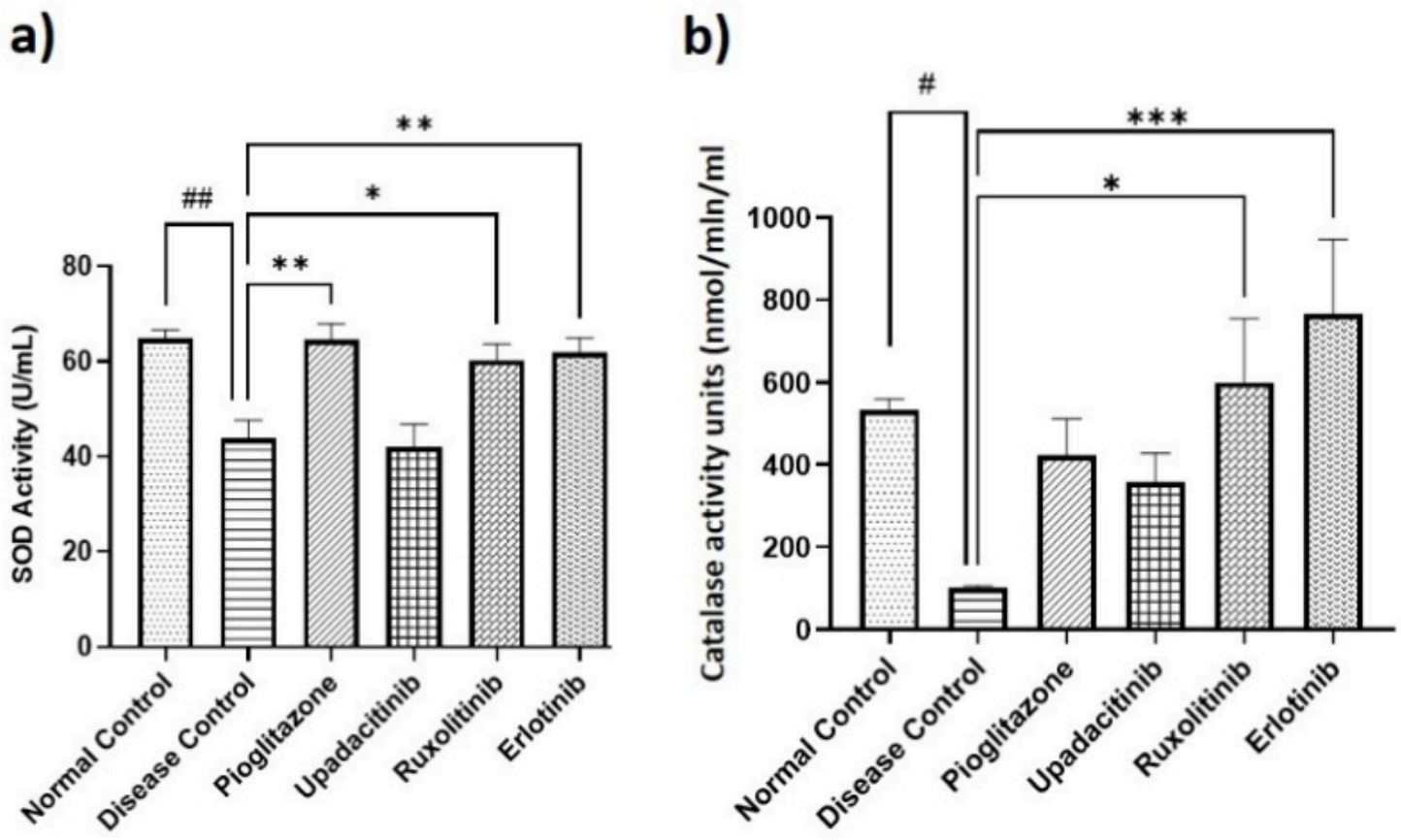


Figure 5

Effect of TKIs on oxidative stress markers. a) SOD activity b) Catalase activity Compared to normal control, #p<0.05, ##p<0.01; Compared to disease control *p< 0.05, **p<0.01, ***p<0.001. n=6; Data are presented as mean ± SEM.

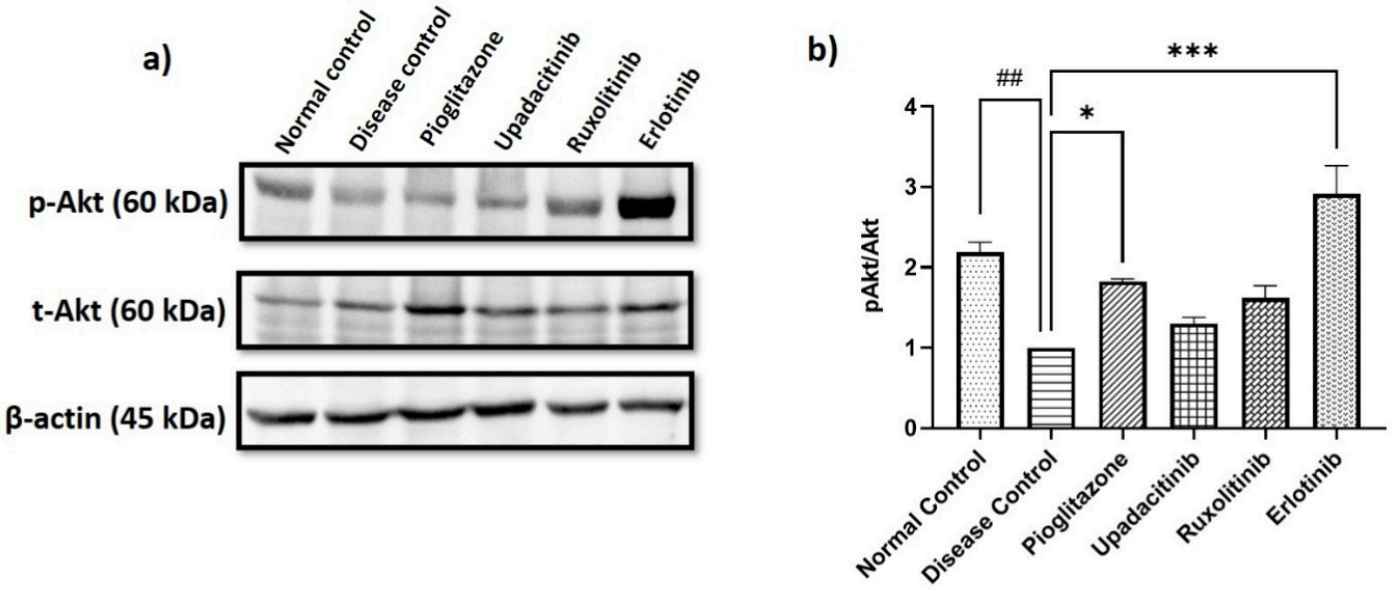


Figure 6

Effect of TKIs on p-Akt expression in hepatic tissue. a) Representative images of p-Akt and t-Akt b) Quantification of p-Akt/t-Akt ratio. Compared to normal control ##p<0.01; Compared to disease control *p< 0.05, ***p<0.001. n=3; Data are presented as mean ± SEM.

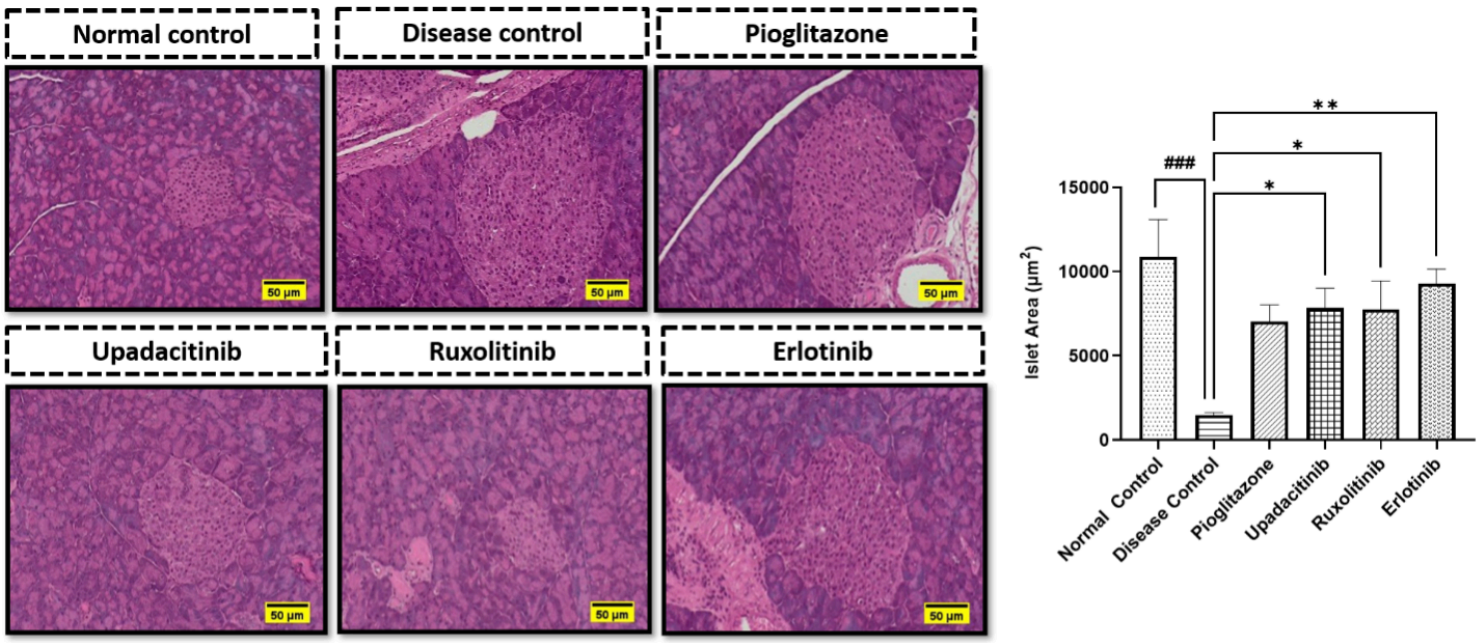


Figure 7

Effect of TKIs on pancreatic histopathology and islet area. a) Representative images H&E-stained pancreatic tissue (Normal control group exhibited intact pancreatic islet architecture; disease control group showed lipid droplet accumulation and disrupted islet morphology; pioglitazone-treated group showed mild vacuolar degeneration of pancreatic islet cells; treatment with TKIs demonstrated improved

pancreatic islet morphology after 4 weeks of treatment); b) Quantitative analysis of pancreatic islet area. Compared to normal control, ### p<0.001; Compared to disease control, * p<0.05 and ** p<0.01. n=3; Data are presented as mean ± SEM.

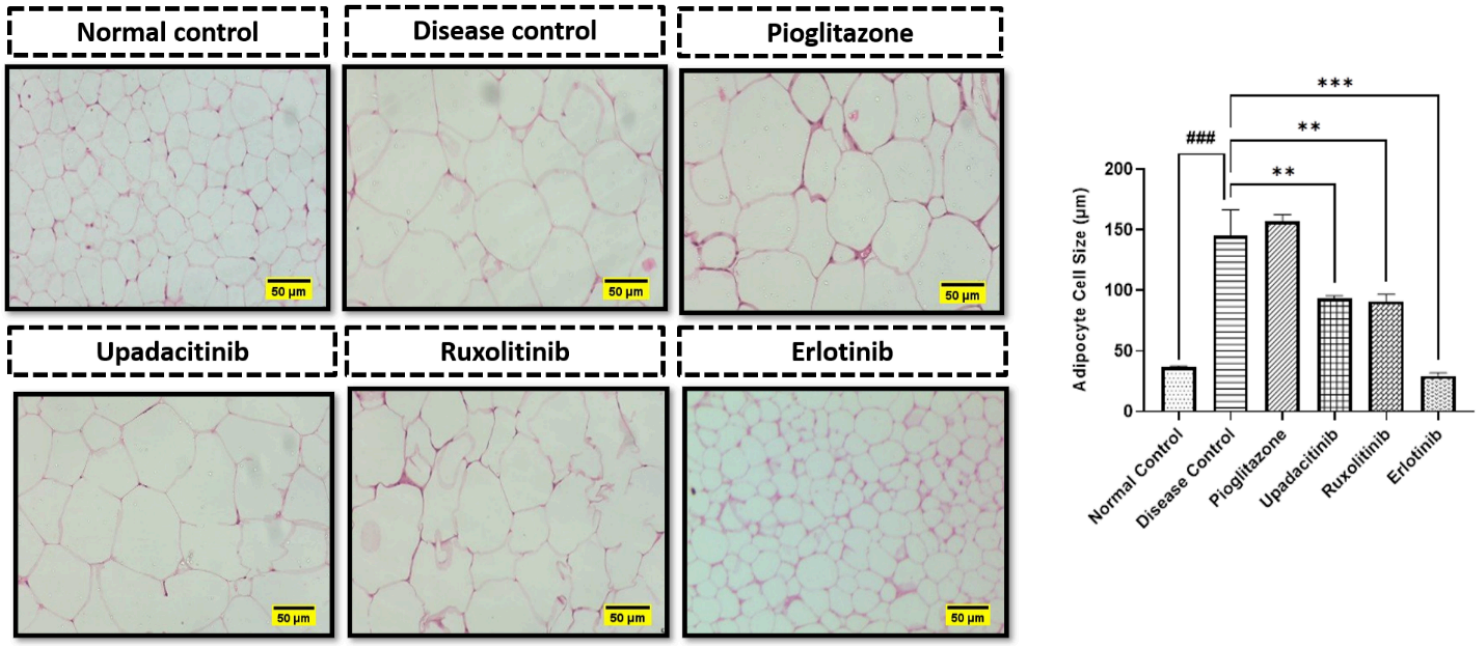


Figure 8

Effect of TKIs on white adipose tissue histology and adipocyte area after 4 weeks of treatment. a) Representative images of H&E-stained sections of white adipose tissue (WAT). (Normal control group demonstrated normal adipocyte architecture; disease control and pioglitazone-treated groups showed enlarged adipocytes; treatment with upadacitinib and ruxolitinib reduced adipocyte size compared with the disease control and pioglitazone-treated groups. Erlotinib presented smaller adipocyte size compared with all treatment groups as well as the disease control group); b) Quantitative analysis of white adipocyte area. Compared to normal control, ### p<0.001; Compared to disease control, * p<0.05 and ** p<0.01. n=3; Data are presented as mean ± SEM.

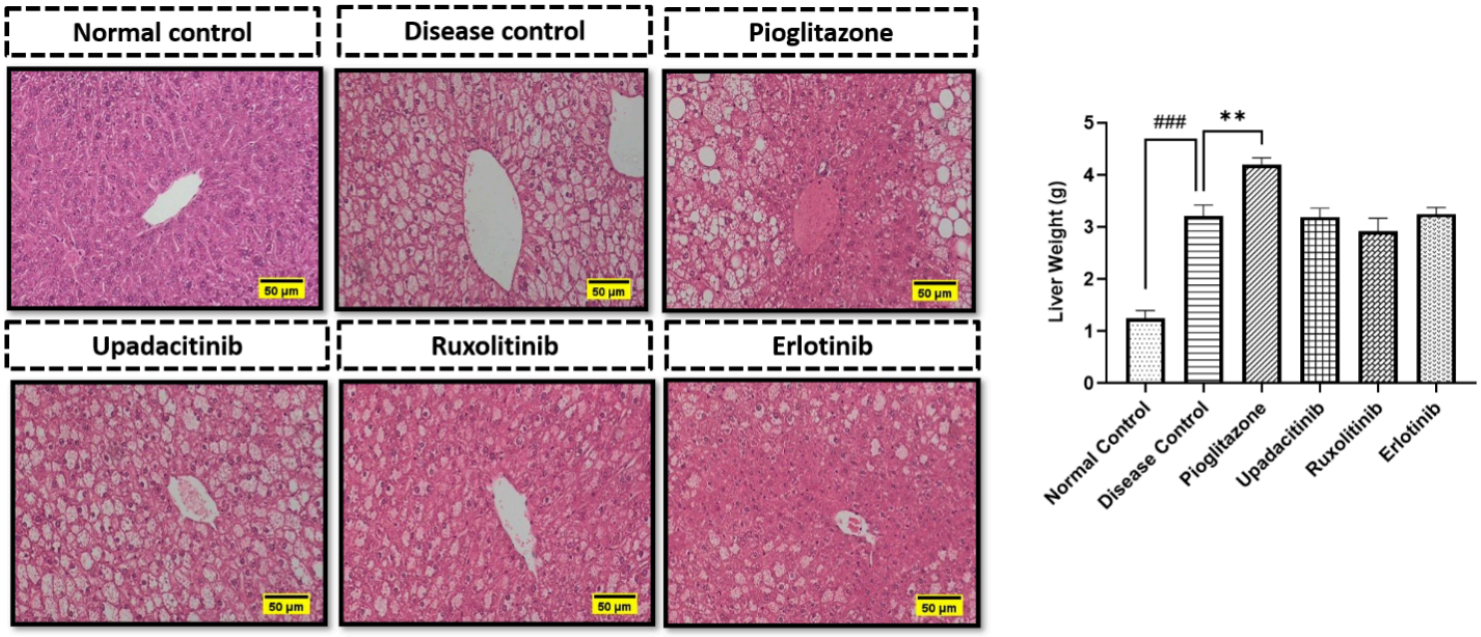


Figure 9

Effect of TKIs on liver histopathology after 4 weeks of treatment. a) Representative images of H&E-stained liver sections. b) Liver weight of the experimental groups. Compared to normal control, ### $p<0.001$; Compared to disease control, * $p<0.05$ and ** $p<0.01$. n=3; Data are presented as mean ± SEM.

Supplementary Files

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- SupplementaryFile.pdf