

Comparative Analysis of Pioglitazone and Tirzepatide on Body Weight, Glucose Levels, Neuroinflammation, and Oxidative Stress in Diabetic Rats

Ahmad Alhowail¹, Mohammed F Aldawsari², Maha Aldubayan¹

¹Department of Pharmacology and Toxicology, College of Pharmacy, Qassim University, Buraydah, 51452, Saudi Arabia; ²Department of Pharmaceutics, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-kharj, 11942, Saudi Arabia

Correspondence: Ahmad Alhowail, Email aalhowail@qu.edu.sa

Introduction: Type 2 diabetes mellitus (T2DM) is a widespread metabolic illness that compromises cognitive function by inducing inflammation and oxidative damage. Diabetes mellitus is treated with many types of medications, including tirzepatide (TZP) and pioglitazone (PIO), which have also been shown to enhance cognitive deficits associated with the condition. This study intends to investigate the neuroprotective effects of TZP and PIO on type 2 diabetic mellitus (T2DM) via mitigating neuroinflammation and oxidative stress, along with enhancing cognitive impairment in rats as models with T2DM.

Methods: A total of six distinct groups of sixty albino rat males ($n = 10$) were allocated at random: Saline, TZP, PIO, T2DM, T2DM+TZP, and T2DM+PIO. Intramuscular doses of streptozotocin (50 mg/kg) and nicotinamide (120 mg/kg) precipitated T2DM. The TZP and PIO therapies persisted for a duration of 15 days. The survival percentage, body weight, behavioral assessments (Y-maze, novel object recognition (NOR)), glucose concentrations, inflammatory mediators tumor necrosis factor alpha (TNF- α), interleukin 6 (IL-6), and interleukin-1 beta (IL-1 β), as well as oxidative stress biomarkers superoxide dismutase (SOD), glutathione peroxidase (GPx), malondialdehyde (MDA), and lipid peroxidation were evaluated following the conclusion of the treatments.

Results: The results demonstrate that diabetes decreased survival rates, body weight, cognitive function, increased glucose levels, neuroinflammation, and oxidative stress. The TZP and PIO increased survival rates and cognitive function as well as decreased glucose levels, neuroinflammation, and oxidative stress in diabetic rats, with PIO demonstrating a more pronounced effect on neuroinflammation and oxidative stress, contrasted with TZP.

Discussion: This study concluded that TZP and PIO enhanced cognitive impairment in diabetic rats, with PIO demonstrating superior efficacy in contrast to TZP.

Keywords: diabetes, inflammation, cognitive impairment, oxidative stress, insulin, pioglitazone, tirzepatide

Introduction

Type 2 Diabetes mellitus (T2DM) is a metabolic illness specified by impaired insulin receptor activation and insulin resistance, resulting in chronic hyperglycemia.¹ Chronic hyperglycemia results from the tissues' inability to respond to insulin and effectively metabolize glucose.² Insulin is essential for controlling physiological functions, including cellular energy production necessary for metabolism.³ T2DM hinders the metabolizing process of carbohydrates, fats, and proteins, which ended in problems that impact the function of various essential organs.⁴ By 2035, the global population of individuals with diabetes is projected to reach 357 million, according to the Diabetes Atlas released in 2021 by the International Diabetes Federation. The progressive impacts of T2DM encompass issues that disturb the morphology and function of various physiological structures and systems, including the brain, which later leads to dementia, cerebrovascular injury, nephropathy, eyesight damage, and erectile dysfunction.^{5,6} The insulin signaling pathways that occur after receptor activation in both the brain and peripheral tissues play a crucial role in memory encoding.⁷ T2DM is claimed to

generate neuroinflammation and oxidative stress, which are known to impact brain function and cause cognitive impairment.⁸

Inflammation and oxidative stress play serious roles in cognitive decline and are linked to various conditions leading to the gradual degeneration of the nervous system, like Alzheimer's illness and multiple forms of dementia.^{9,10} Pro-inflammatory cytokines that are systemic in nature, for example, TNF, IL-6, and IL-1 β , carry a capacity to navigate the blood-brain barrier, prompting central inflammation and resulting in neuroinflammation that provokes oxidative stress.¹¹ Oxidative stress appears when an accumulation of free radicals exceeds the regulatory capacity of antioxidants, resulting in cellular membranes injury via degradation of lipids, oxidation of proteins, and damage to DNA.¹² Reduced levels of endogenous enzymatic antioxidants SOD, GPx, and catalase, together with elevated ROS, lipid peroxidation, and MDA, are linked to neurotoxicity and cognitive deterioration.¹³ Consequently, the interaction between inflammation and oxidative stress is intricate and reciprocal. Inflammatory processes can induce oxidative stress, and oxidative stress can activate inflammatory pathways, eventually resulting in cognitive impairments.^{11,14}

Pioglitazone (PIO) is a substance categorized as a thiazolidinedione originated. This compound acts as an activator for the Peroxisome Proliferator-Activated Receptor (PPAR).¹⁵ The PIO has obtained authorization to manage T2DM linked to insulin resistance.¹⁶ Activating PPAR agonists like PIO enhances reactivity to insulin, the ingestion of glucose, and the breakdown of lipids.¹⁷ Moreover, PIO has demonstrated efficacy in the treatment of neurodegenerative illnesses, positioning it as a potential therapeutic agent for neurodegenerative illnesses.^{18,19} An earlier published study showed that PIO positively impacts cognitive impairment by activating the cholinergic system and reducing neuroinflammation in rat models of neurodegeneration and chemobrain.^{20,21}

Tirzepatide (TZP) is classified as a receptor agonist that targets glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP). TZP has recently received approval for use in the treatment of obesity and diabetes mellitus type 2.²² It has been noted for its potential in facilitating weight loss and lowering glucose levels.^{22,23} Furthermore, TZP reduces the inflammation in the brain caused by elevated glucose levels.²⁴ However, there was no adequate evidence linking the TZP treatment to diabetes, neuroinflammation, and oxidative stress.

Therefore, this study aimed to explore the chance of PIO or TZP therapies in enhancing body weight and mitigating neuroinflammation and oxidative stress induced by diabetes mellitus type 2, offering a promising prospect for progress in controlling diabetes.

Materials and Methods

The layout of the complete study is displayed in [Figure 1](#).

Chemicals

Tirzepatide (TZP) (Mounjaro 7.5 Mg/0.5mL Prefilled Pen 4s) was acquired from Eli Lilly Saudi Arabia Ltd in Riyadh, Saudi Arabia. Pioglitazone hydrochloride (PIO) (Glados[®]) was sourced from Tabuk Pharmaceuticals Co., located in Tabuk, Saudi Arabia. The streptozotocin (STZ) and nicotinamide were acquired from Cayman Chemical located in Ann Arbor, MI, USA.

Animals and Treatment Protocol

The experimental procedures applied in this study were approved by of the Ethics Panel for Educational institutions and the Deanship of Graduate Studies and Scientific Research at Qassim University, Saudi Arabia [Approval ID 2020-CP-12/23-67-05]. Ten male Sprague-Dawley rats per group were randomly allocated to one of six categories: saline, TZP, PIO, type 2 diabetes mellitus (T2DM), T2DM+TZP, or T2DM+PIO. The subjects were all 12 weeks of their age. The rats in the saline group experienced a muscle injection of a vehicle solution, specifically normal saline. The TZP group was given an amount of 1.35 mg/kg every three days.²⁵ The PIO group gained a daily administration of 2 mg/mL via their drinking water.¹¹ Hyperglycaemia was generated in the other groups via an intramuscular injection of STZ at an amount of 50 mg/kg and nicotinamide at an amount of 120 mg/kg.²⁶ The appearance of Hyperglycaemia was verified through monitoring the blood glucose levels of the rats via an Accu-Chek Glucometer from Roche, Germany) shortly after a 72-hour time period and once more on day 4 soon afterwards the ultimate injection.²⁶ Rats exhibiting fasting blood glucose

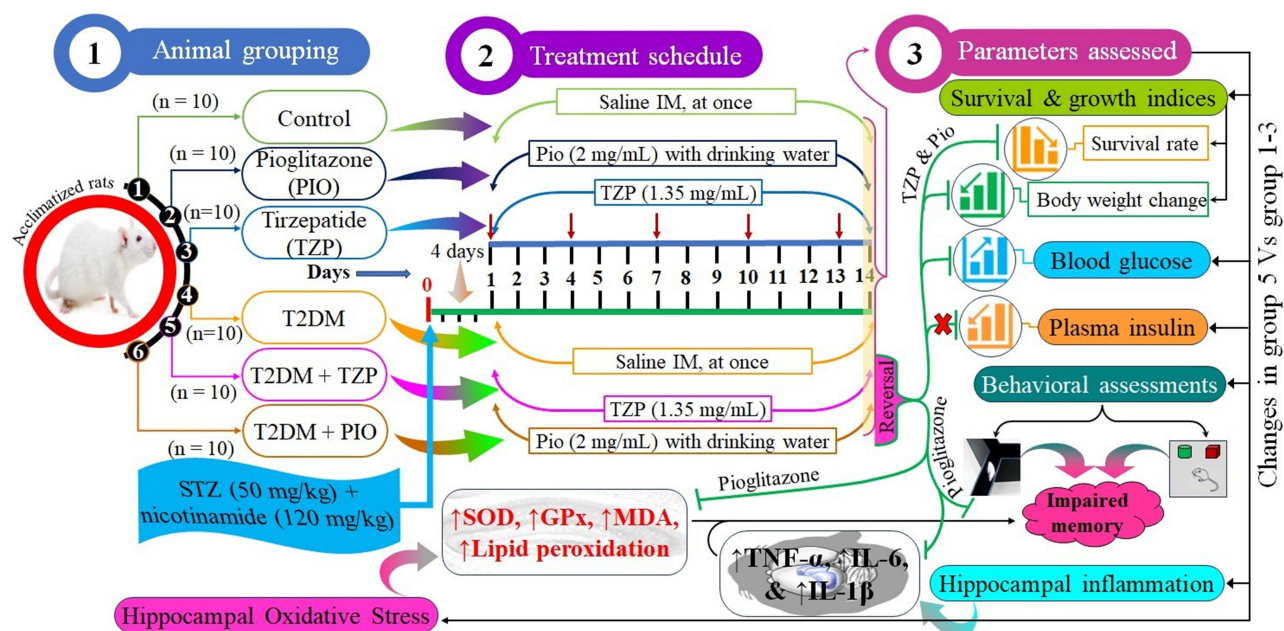


Figure 1 The design of the study and its results (schematic).

concentrations exceeding 200 mg/dl were classified as diabetic and utilized as subjects for subsequent investigations. The two groups that received treatments, either pioglitazone or tirzepatide, continued for two weeks following the induction of hyperglycemia.

Survival Rate and Body Weight

The daily observation of the survival rate yielded essential insights into the ongoing study. The cages were sanitized every two days, and any deceased animals were promptly disposed of. Monitoring body weight provided valuable information regarding general health conditions. Consequently, body weight was measured consistently every three days, facilitating the identification of subtle changes and aiding in the detection of potential health issues.

Rats' Behavioral Evaluation

The health and condition of the animals were assessed daily for mortality rates, while body weight and glucose concentrations were measured every three days. Subsequently, all the rats underwent behavioral assessments and biochemical assays at the study's endpoint.

Y-Maze

The Y-maze assessment task is utilized as a method for examining spatial working memory, which relies on the functionality of the hippocampus. The evaluation of unplanned fluctuation behavior was conducted using a Y maze, which consists of three wooden arms, each separated by 120° angles and measuring 50×10 × 18 cm. The arms were painted brown to improve visibility, and the apparatus was positioned on the floor. Overhead lighting was used to ensure even light distribution. This configuration supports spontaneous alternation, as rats have a natural tendency to explore new environments. When presented with the option to enter two arms, they generally opt for the one that has not been previously explored. This behavior enables the rat to recall which section of the arm has been visited less frequently, thus assessing working spatial memory, a type of short-term memory. During the exercise session, the rats were permitted to explore two arms at their own pace for a total of 15 minutes. In the subsequent session, which lasted 3 minutes, the rats were permitted to explore the maze, including the unfamiliar arm. The sessions were separated by a 3-hour interval. Test

sessions were documented to calculate the animals' arm utilization time and rate of entry, with the requirement that at least half of the animal's body had to be inside an arm.²⁷

Novel Object Recognition

Ennaceur and Delacour's NOR test relies on rats' extra robust exploratory behavior with novel stimuli over familiar ones. The NOR test addresses recognition memory through rats' typical exploratory behavior in a non-rewarded, relevant paradigm. Each examination session covers two attempts of the most common tests. In the initial trial (gaining), animals were provided with two matching objects and free to inspect the instrument. In the second trial (preservation), participants experienced two objects: one familiar and one unusual. Object recognition is determined by rodents' length of time looking at known vs new objects. Novel objects are explored more by rats than familiar ones. A 40-cm-diameter wooden box with an open top was used for testing. The unusual object was a black and white bottle that was the exact size as the adaptation objects, two black teacups. Rats were tolerable to discover the two teacups for 10 minutes before returning to their cages during training. A new object displaced one teacup in the subsequent session, 3 hours later and 3 minutes long. Video camera and stopwatch recorded moments spent exploring the unique thing.¹⁰

Glucose Assessment

The insulin test was conducted following a 3-hour fasting period, as previously outlined. Blood samples were gathered from rats experiencing chemotherapy and saline rats via the tail's vein in rats at 10-minute intervals over the course of 1 hour after an injection into the peritoneum of insulin (2 U kg⁻¹). Plasma glucose levels were measured using blood samples analyzed with glucose monitoring devices from the ACCU-CHEK company.²⁸

ELISA

Brain samples from six groups (control, TZP, PIO, T2DM, T2DM + TZP, and T2DM + PIO) were homogenized using a Q-sonica homogenizer at 30 Hz pulses for 20 seconds, mixed with N-PER from Thermo Scientific, and centrifugated at 10,000× g for 15 minutes. The top part has been conserved at -80 °C. Sample protein has been determined using the bicinchoninic acid test (Pierce, Waltham, MA, USA). ELISA was applied to assess insulin (cat. no. RK09278, ABclonal Technology, Woburn, MA, USA), TNF-α (cat. no. RK00029, ABclonal Technology, Woburn, MA, USA), IL-1β (cat. no. RK00009, ABclonal Technology, Woburn, MA, USA), IL-6 (cat. no. RK00020, ABclonal Technology, Woburn, MA, USA), SOD (cat. no. RK07054, ABclonal Technology, Woburn, MA, USA), GPx (cat. no. RK15281, ABclonal Technology, Woburn, MA, USA), MDA (cat. no. RK03696, ABclonal Technology, Woburn, MA, USA), and lipid peroxidation (MBS763367, MyBioSource, San-Diego, CA, USA) in samples, following manufacturers techniques. The plates had been examined at 450 nm using the ELx800 Optical density Microplate Reader (BioTek Company of Instruments, Winooski, VT, USA). The color intensity was contrasted with the standard and control to measure TNF-α, IL-6, IL-1β, SOD, GPx, MDA, lipid peroxidation, and amounts of insulin in the samples. Statistics have been employed to analyze the data.

Statistical Analysis

The GraphPad Prism 10 program was utilized to evaluate the data. The outcomes were given as the median with the median and standard error of the mean (SEM) suggested as a spectrum. Tukey analysis was performed after one-way ANOVA was used to examine each group's survival percentage, body weight changes, Y-maze, NOR, and ELISA data. A *p*-value underneath 0.05 was interpreted as proof of significance in statistics.

Results

TZP and PIO Effect on Survival Rate of Diabetic Rats

Type 2 diabetes mellitus (T2DM) impacted the survival rate of rats; nonetheless, it was observed that the rats administered both TZP and PIO exhibited a reduced incidence of mortality (Figure 2). After a period of two weeks,

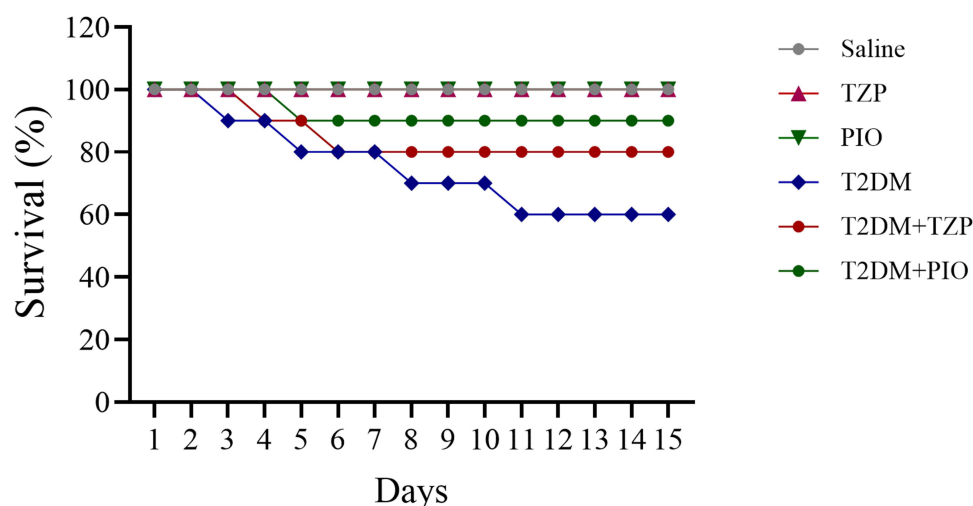


Figure 2 Effect of TZP and PIO on diabetic rats on the survival rate.

40% of the rats with diabetes succumbed. In a study, it was detected that 20% of diabetic rats treated with TZP and 10% of those treated with PIO succumbed after a two-week period.

TZP and PIO Effect on Body Weight of Diabetic Rats

The body mass of diabetic rodents, along with those administered TZP or PIO, exhibited a significant drop in contrast with the control group (Figure 3). However, neither TZP nor PIO administered separately indicated any effect on body weight in contrast with the saline group.

TZP and PIO Effect on Impaired Memory of Diabetic Rats of Performance Regarding Y Maze Task

Figure 4 conveys an in-depth analysis of the outcomes displayed by rats in the Y maze task, with the goal of assessing spatial recall abilities. When contrasted with the saline group, there is an obvious drop in the total sum of entrances and the total length of time consumed in the new arm by the diabetic rats. Nonetheless, TZP or PIO indicates a recovery in the quantity of

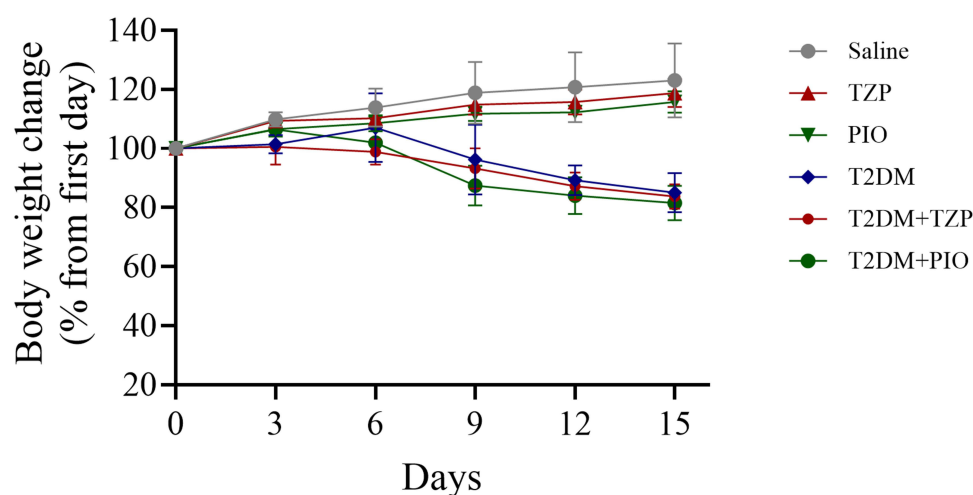


Figure 3 Impact of TZP and PIO on the body-weight in diabetic rats. The graphs illustrate the mean body weight for each group, tracked since the initial day through to the conclusion of the study. The findings indicated that there were no notable alterations in body weight throughout the study duration for the saline, TZP, or PIO groups, nor were there significant changes observed in the diabetic, T2DM+TZP, or T2DM+PIO cohorts. The data can be seen as the average divided by the standard error of the mean. (n=6).

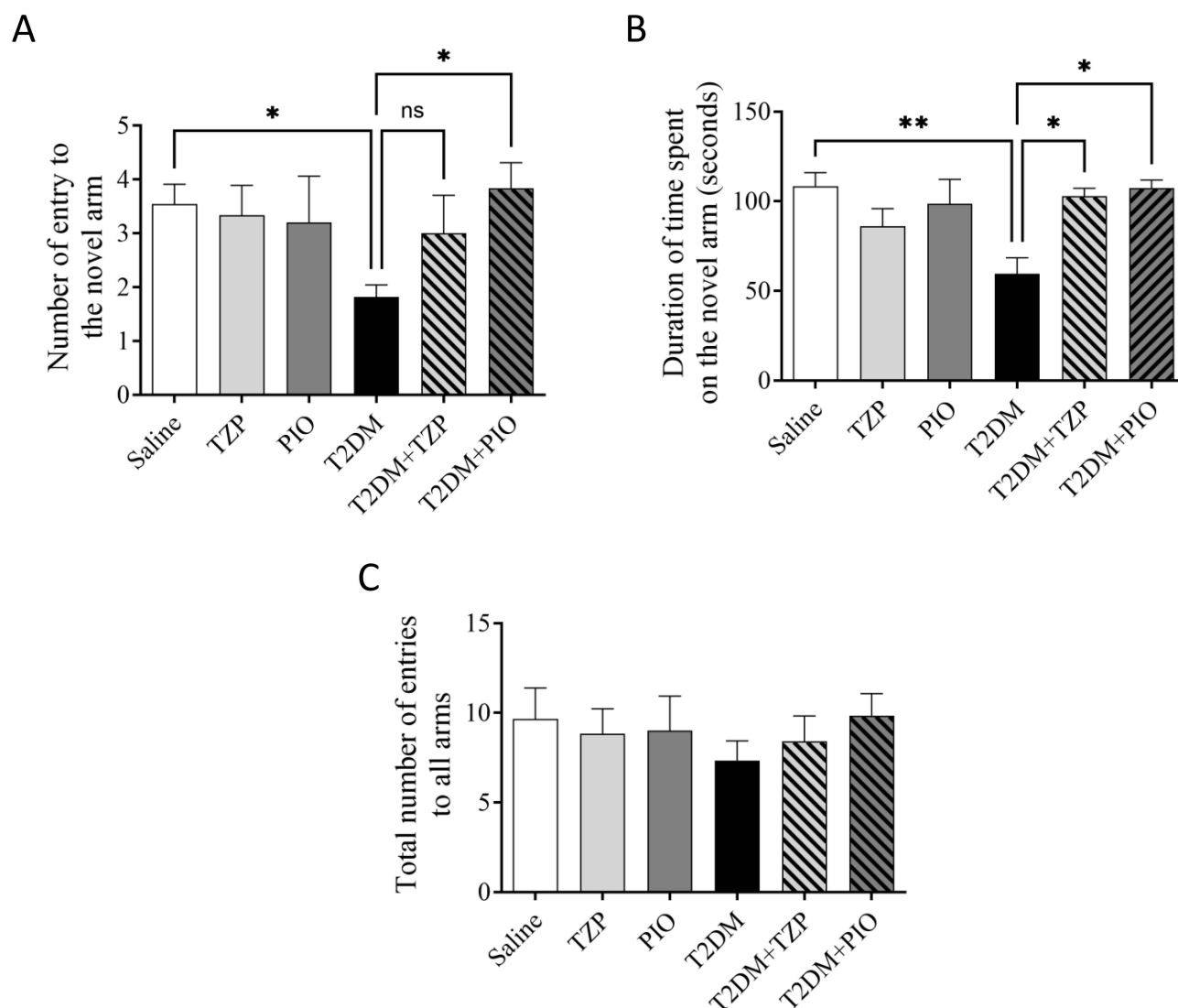


Figure 4 Impact of TZP and PIO on diabetes-induced Y-maze regarding the frequency of entrances into the new arm (A), the total amount of time allocated in the new arm (B), and cumulative entries across all arms (C). Contrasted with the saline group, those in the T2DM group entered the new arm far less frequently ($*p < 0.05$) and for shorter periods of time ($**p < 0.01$), suggesting cognitive deficits. The administration of TZP and PIO led to improvements in cognitive deficits, with PIO demonstrating superior efficacy over TZP by significantly enhancing cognitive function. The data exist as the average $\pm$ standard error of the average. (n=6).

entrances and length in the unusual arm, alongside the group obtaining PIO therapy displaying a significant improvement when contrasted with the diabetic group versus TZP. The total number of entries was not noteworthy.

TZP and PIO Effect on Impaired Memory of Diabetic Rats of Novel Object Recognition

Figure 5 summarizes rats' performance in the NOR task, which was carried out to assess spatial memory. In contrast with the saline group, a reduction in exploration time towards the novel object has been noted. However, TZP or PIO enhances the exploration time in the new object, with the PIO-treated group showing a substantial improvement in against with the diabetic group. The combination of PIO demonstrated a more significant effect than TZP.

TZP and PIO Effect on the Amount of Glucose of Diabetic Rats

The concentrations of glucose that exist in the bloodstream were tracked on days 1, 4, 7, 10, and 13. Rats suffering from diabetes exhibited elevated levels of blood glucose contrasting with the standard saline group. The administration of TZP

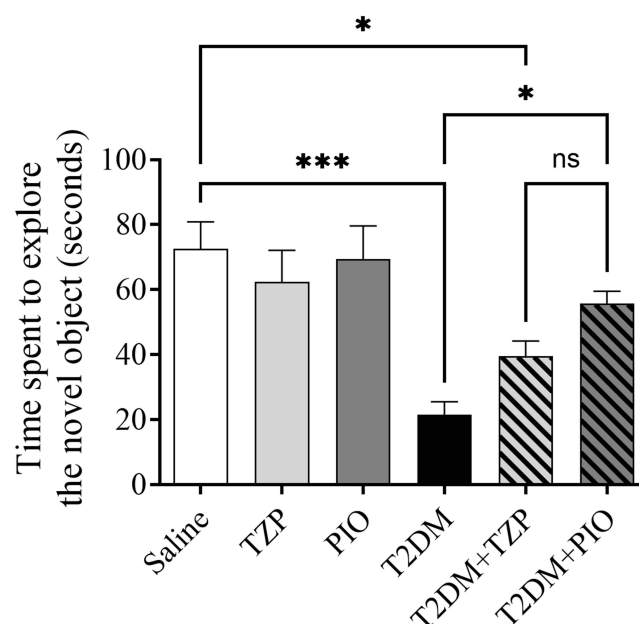


Figure 5 Impact of TZP and PIO on dementia caused by diabetes in rat models. The data is presented as SEM. Asterisks denote significant differences: * an apparent variance among the saline group and the one with diabetes (* $p < 0.05$, *** $p < 0.001$ vs saline group), and between the diabetic and PIO treated groups related to the group those with diabetes (* $p < 0.05$ in contrast to the diabetic group). (n=6).

or PIO individually did not result in any variation in glucose levels. Nonetheless, the groups treated with TZP or PIO exhibited a progressive enhancement in glucose levels (Figure 6A). In addition, plasma insulin levels were reduced pointedly in the diabetic group and did not improve after TZP and PIO medical treatments (Figure 6B).

TZP and PIO Effect on Neuroinflammation of Diabetic Rats

The quantities of inflammatory mediators like TNF- α , IL-6, and IL-1 β were markedly elevated in diabetic rats. TZP or PIO treatments decline these inflammatory cytokines, with a notable reduction observed specifically in TNF- α and IL-1 β through PIO medical treatment (Figure 7).

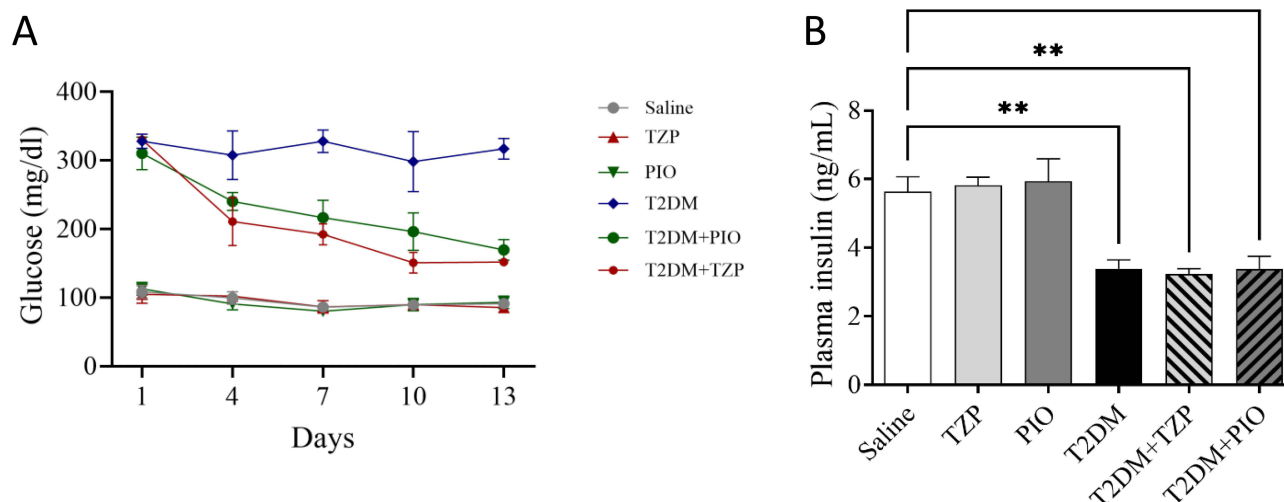


Figure 6 Impact of TZP and PIO on glucose concentrations (A) and plasma insulin levels (B) in diabetic rats. The data point out the mean $\pm$ SEM of the amount of glucose recorded on days 1, 4, 7, 10, and 13, alongside the plasma insulin concentrations noticed in the saline group (** $p < 0.01$ against the diabetic groups). (n=6).

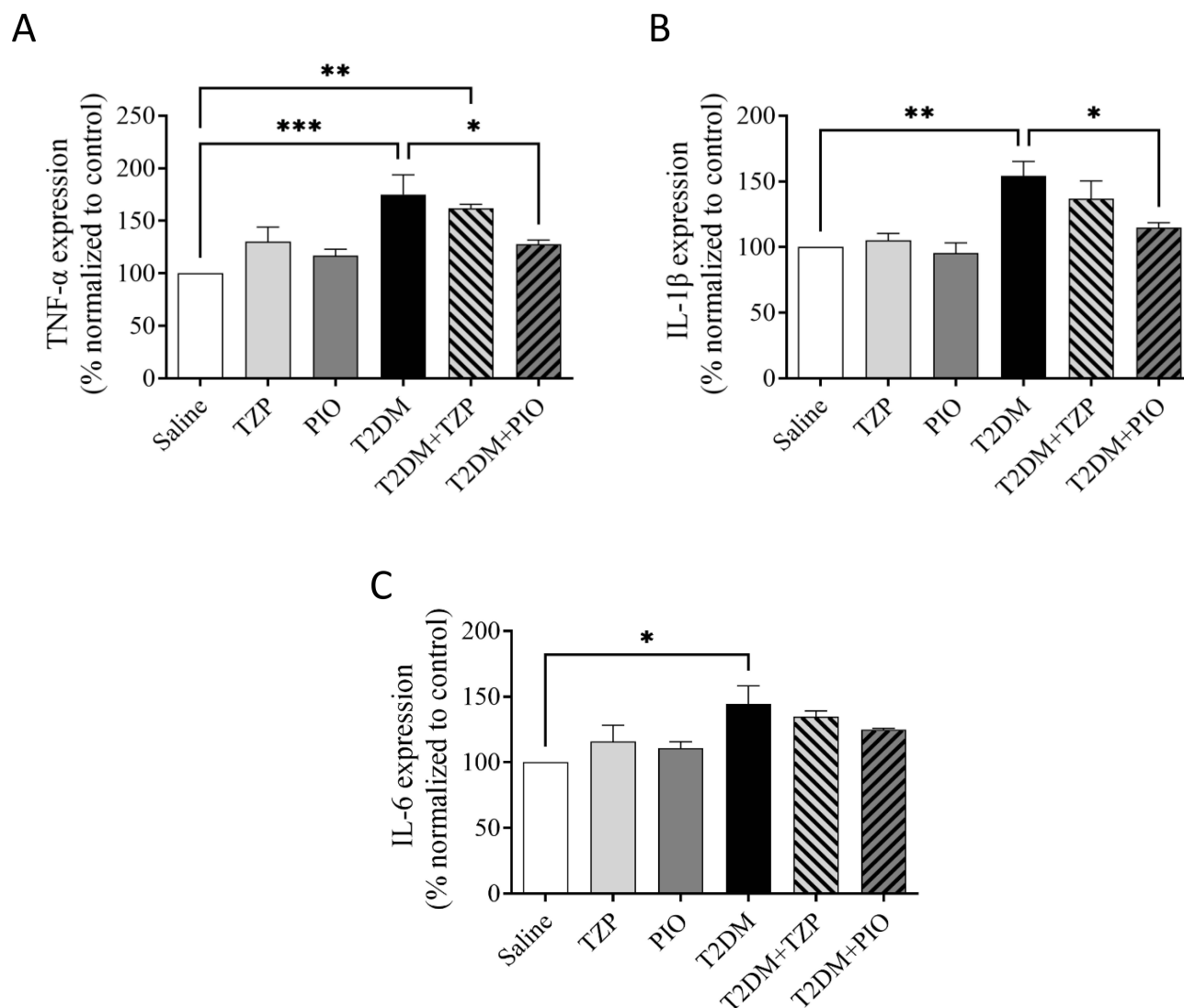


Figure 7 Impacts of TZP or PIO regarding expressed levels of TNF- α , IL-6, and IL-1 β proteins in the brains of diabetic rats. (**A–C**) Rats with diabetic mellitus indicated a substantial increase in TNF- α , IL-6, and IL-1 β levels, which were greatly dropped after delivered of PIO. The observed expressed volumes of (**A**) TNF- α , (**B**) IL-1 β , and (**C**) IL-6 were identified to be higher than those in the saline group. Bars suggest the average $\pm$ the SEM (* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$). ($n=6$).

TZP and PIO Effect on Oxidative Stress of Diabetic Rats

The concentrations of oxidative stress indicators SOD, GPx, MDA, and lipid peroxidation showed significant increases in diabetic rats. TZP or PIO treatments in diabetic rats demonstrated enhancement exclusively with PIO treatment (Figure 8).

Discussion

Recent research indicates that PIO, in contrast to TZP, enhances cognitive function. The effectiveness of PIO in mitigating cognitive dysfunction is attributed to its superior insulin-sensitizing and anti-inflammatory properties, particularly in neurodegenerative conditions such as Alzheimer's and Parkinson's diseases.¹⁵ Furthermore, clinical investigations have demonstrated that cognitive impairments in individuals with mild cognitive impairment associated with Alzheimer's disease and diabetes improve with PIO treatment.²⁹ Similarly, animal studies have corroborated that PIO enhances cognitive performance in models of Alzheimer's disease and diabetes.³⁰ In contrast, the precise mechanism by which TZP ameliorates cognitive dysfunction remains incompletely understood; however, GLP-1 activation may mitigate inflammation and oxidative stress while promoting synaptic plasticity.^{31,32} To date, no clinical studies have elucidated the

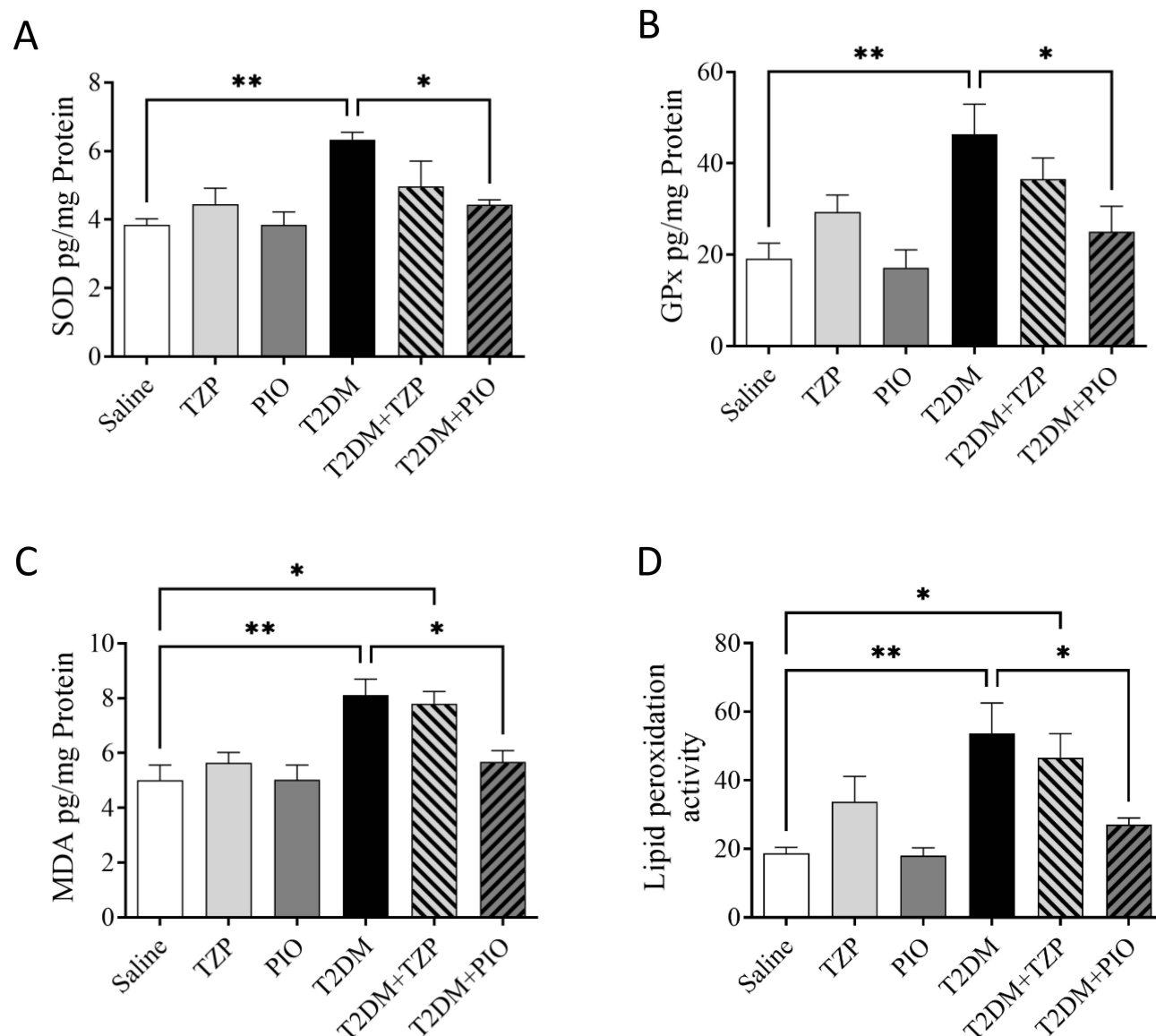


Figure 8 Impact of TZP or PIO on the concentrations of SOD, GPx, MDA, and lipid peroxidation in the cerebral tissue of diabetic rats. **(A–D)** T2DM rats exhibited markedly elevated levels of SOD, GPx, MDA, and lipid peroxidation, and this increase was significantly attenuated with PIO treatment but not with TZP. **(A)** SOD, **(B)** GPx, **(C)** MDA, and **(D)** lipid peroxidation were analyzed in comparison to the saline control. The bars point to the mean $\pm$ SEM (* p < 0.05 and ** p < 0.01). (n=6).

impact of TZP on cognitive impairment. Nonetheless, TZP has been documented to improve cognitive impairment in diabetic animal models by augmenting insulin sensitivity and diminishing neuroinflammation.^{25,33} Existing research evaluated the efficacy of the anti-diabetic therapies TZP and PIO in preventing cognitive deficits associated with diabetes in rat models. Through the reduction of blood glucose concentrations and enhancement of cognitive function, TZP and PIO have demonstrated their potential to elevate the psychological health of individuals suffering from diabetes.^{34,35} PIO has demonstrated a reduction in blood glucose concentrations and body weight, while also lowering the risk of developing Alzheimer's disease.¹⁵ Furthermore, recent findings indicate that TZP has the potential to alleviate the cardiotoxic effects associated with chemotherapy.²⁴ In contrast, earlier studies on PIO have demonstrated its efficacy in enhancing memory function impaired by diabetes and neurodegenerative conditions.¹⁵ Consequently, the current research seeks to evaluate the efficacy of TZP and PIO in enhancing cognitive deficits, neuroinflammatory responses, and oxidative stress associated with diabetes.

The onset of diabetes in rats was achieved through intramuscular injection of streptozotocin and nicotinamide.²⁸ Saline, TZP, or PIO groups alone exhibited a survival rate of 100% throughout the study. Diabetic rats exhibited a mortality rate of 40% after two weeks of hyperglycemia induction. Conversely, diabetic rats receiving TZP exhibited a 30% mortality rate, while those treated with PIO showed a 20% mortality rate. The findings indicate that both TZP and PIO may offer a safeguard against diabetes and may improve management of its complications. In the body weight analysis, neither TZP nor PIO interventions altered body weight compared to the saline group. However, the body weight of diabetic rats was markedly drop than that of the saline group, and neither TZP nor PIO treatment succeeded in reversing this decrease in body weight. Consequently, therapies like TZP and PIO may reduce hyperglycemia and improve survival rates, but do not improve weight loss caused by diabetes.

The Y-maze and the NOR were employed to evaluate cognitive functions indicative of the preservation of neuronal integrity and synaptic activity.³⁶ These assessments are reliant on the hippocampus and were performed to investigate the mechanisms of short-term memory.³⁷ Diabetic rats exhibited a decline in memory encoding, as proved by a decrease in the frequency of entrances and the time taken to discover the new arm in the Y-maze test when contrasted with control rats. This observation highlighted the challenge faced by diabetic rats in distinguishing the new arm from the start and known arms. While diabetic rats receiving TZP or PIO exhibited enhancements in both the frequency of entrances and the duration spent in the novel arm relative to controls, the saline-treated with PIO demonstrated superior effectiveness in Y-maze tasks associated with the diabetic cohort contrasted with those treated with TZP. Likewise, the findings from the NOR test indicated that diabetic rats spent a shorter time analyzing the new object in comparison to the control rats. Nonetheless, the time for discovery saw considerable enhancement in PIO, whereas TZP did not exhibit similar improvements. In comparison, the PIO treatment demonstrated superior outcomes in behavioral evaluations relative to the TZP treatment.

The function of insulin and its receptors plays a crucial role for controlling the digestion of glucose, which matters for most effective brain energy expenditure and strengthens synaptic remodeling, therefore impacting learning and memory capabilities.^{38,39} Receptors for insulin are predominantly embedded in the brain's nervous system, foremost throughout the brain's hippocampus, which is vital for the creation of short-term recall.⁴⁰ Insulin signaling modulates neuronal energy production, playing a crucial role in memory encoding.⁴¹ The administration of insulin led to a notable enhancement in cognitive function among individuals with type 2 diabetes mellitus.⁴² Insulin attaches to its receptors, initiating the activation of insulin receptor substrate 1 (IRS-1), which in turn stimulates the phosphoinositide 3-kinase (PI3K)/protein kinase B (PKB, known as Akt) signaling pathway.⁴³ This procedure facilitates the movement of glucose transporter 4 (GLUT4) to the cell membrane, hence decreasing hyperglycemia.⁴⁴ Both TZP and PIO treatments have been recognized for their ability to enhance GLUT4 trafficking as antihyperglycemic agents.^{45,46} It is crucial to acknowledge that prolonged elevated glucose levels in the blood can harm the circulatory vessels throughout the brain, resulting in ischemia and consequently leading to cognitive decline.⁴⁷ Our findings demonstrated that hyperglycemia, induced by STZ and nicotinamide to create a diabetic model in rats, resulted in cognitive decline in these subjects. The treatments with TZP and PIO demonstrated a notable reduction in blood glucose levels when contrasted with rats that did not receive any diabetic treatment. However, upon assessing plasma insulin levels, it is noted that insulin levels were significantly reduced in diabetic rats, which also did not exhibit improvement following TZP or PIO treatments. Consequently, this finding suggests that TZP and PIO lower hyperglycemia through enhanced cellular glucose uptake instead of elevating insulin levels.

Higher levels of glucose in the body may promote the forming of pro-inflammatory cytokines that consist of TNF- α , IL-6, and IL-1 β .⁴⁸ These cytokines, when increased in peripheral tissues, have the potential to cross the blood-brain barrier, resulting in the activation of microglia and astrocytes and sustaining inflammatory processes within the central nervous system (CNS).^{49,50} The rise of TNF- α , IL-6, and IL-1 β within the CNS can contribute to the formation of reactive oxygen species (ROS) due to impaired mitochondrial function, elevate glutamate concentrations by disrupting its uptake and clearance, and compromise the integrity of the blood-brain barrier (BBB).⁵¹ This compromise permits peripheral immune cells to enter the CNS, thereby intensifying inflammation and oxidative stress in the brain.^{52,53} Furthermore, the activation of TNF receptors has been associated with the initiation of apoptotic pathways via caspase-8, leading to apoptosis, which further worsens cognitive impairment.⁵⁴ The current investigation highlights a significant

divergence in the anti-inflammatory properties of PIO and TZP. PIO treatment markedly decreased key inflammatory markers, namely TNF- α and IL-1 β , suggesting a beneficial effect on neuroinflammatory pathways linked to cognitive impairment. Conversely, TZP did not exhibit a notable reduction in these inflammatory markers, despite its recognized effectiveness as an antidiabetic treatment. This contrast emphasizes the intricate and often unpredictable nature of the inflammatory mechanisms.

Our findings were further extended by assessing oxidative stress in diabetic rats subsequent to an evaluation of neuroinflammation. This assessment involved quantifying the levels of enzymatic antioxidants, specifically superoxide dismutase (SOD) and glutathione peroxidase (GPx), alongside oxidative damage biomarkers such as malondialdehyde (MDA) and lipid peroxidation.¹³ SOD functions as an antioxidant enzyme, converting superoxide radicals into hydrogen peroxide, while GPx facilitates the reduction of hydrogen peroxide to water.⁵⁵ Consequently, both SOD and GPx are pivotal in modulating oxidative stress. A reduction in these enzymes results in the accumulation of superoxide radicals, potentially leading to oxidative neuronal damage.^{56,57} Elevated malondialdehyde (MDA) levels signify increased lipid peroxidation, a consequence of fatty acid oxidation in cellular membranes and oxidative stress.⁵⁸ This process undermines membrane integrity and neuronal function, ultimately affecting neurotransmission and resulting in cognitive impairments.⁵⁹ Our results corroborated the presence of oxidative stress, evidenced by increased levels of the antioxidants SOD and GPx, as well as oxidative stress biomarkers, including MDA and lipid peroxidation, in diabetic rats. This indicates the occurrence of oxidative stress, which was significantly mitigated by PIO, in contrast to the TZP-treated group. These findings robustly confirmed the link between heightened neuroinflammation and oxidative stress, as demonstrated by increased levels of TNF- α , IL-6, and IL-1 β , in conjunction with elevated SOD, GPx, MDA, and lipid peroxidation in diabetic rats. This suggests an attempt by enzymatic antioxidants to counteract oxidative stress, a process effectively achieved by PIO but not in the TZP-treated group.

This investigation has particular advantages and limitations. This study clarified the distinctions in efficacy between TZP and PIO in the management of diabetes. The impact of diabetes on cognitive decline is discussed, highlighting its connection to neuroinflammation and oxidative stress, along with the potential protective effects of TZP or PIO. The animals used were of the same strain, age, and body weight, and the experiment was conducted simultaneously among the research cohorts to minimize confounding variables. Additionally, it is important to highlight that several doses were given to replicate the real-world treatment scenario in human subjects.

In conclusion, our results confirmed the hypothesis that elevated blood sugar levels lead to cognitive deficits by affecting cognitive performance in Y-maze and NOR tasks, along with heightened neuroinflammation and oxidative stress within the brain. Moreover, the molecular evaluations after cognitive decline indicated neuronal toxicity through increased inflammatory markers, for example, TNF- α , IL-6, and IL-1 β , together with oxidative stress as demonstrated by elevated levels of SOD, GPx, MDA, and lipid peroxidation in the brain. PIO treatment successfully mitigated the cognitive impairments and reduced the elevated levels of neuroinflammation and oxidative stress biomarkers. Conversely, the ineffectiveness of TZP highlights the superior efficacy of PIO compared to TZP. Extra inspection is necessary to inspect the impact of diabetes on neurotransmission, particularly regarding the expression of neurotransmitters and receptors in the glutamatergic and dopaminergic systems.

Data Sharing Statement

Obtainable at the reasonable request.

Institutional Review Board Approval

The work was legalized by the Institutional Committee on Animal Protection and Use of Qassim University's Deanship for Scientific Research (approval number 2020-CP-12/23-67-05), and followed to the NIH Procedures for the Care and Use of Laboratory Animals for all animals utilized in the experiments.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report that no conflict of interest exists.

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