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Article

Trelagliptin Ameliorates Memory Decline in Diabetic Rats through the AMPK/AKT/GSK-3β Pathway in the Cerebral Cortex

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Cite This: *ACS Omega* 2025, 10, 15673−15680

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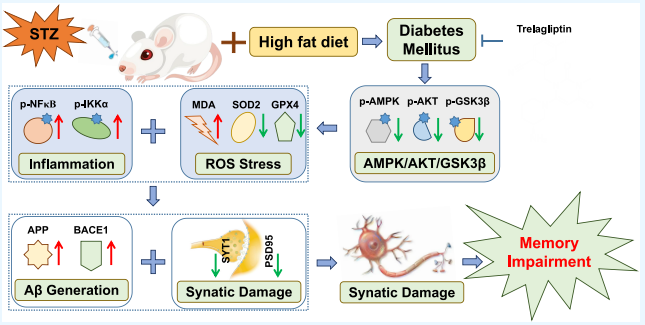
ABSTRACT: Examining how hypoglycemic medications affect brain function is one of the best approaches to addressing cognitive impairment. In this study, trelagliptin, a dipeptidyl peptidase-4 (DPP4) inhibitor, was utilized to assess memory loss in diabetic rats through fear conditioning tests. Trelagliptin restored fear memory in diabetic rats that had been disrupted over a relatively long period (24 h) or extended period (5 days). Moreover, trelagliptin treatment reduced the higher incidence of neuronal cell death in the cerebral cortex, as observed via Nissl or hematoxylin and eosin staining. Subsequent analyses revealed that diabetic rats exhibited elevated levels of inflammatory cytokines (p-IKKα and p-NFκB) and a trend toward oxidative damage, indicated by malondialdehyde (MDA), superoxide dismutase 2 (SOD2), and glutathione peroxidase 4 (GPX4) detection. However, administration of trelagliptin reversed these markers to baseline levels. Additionally, trelagliptin activated p-AMPK, p-AKT, and p-GSK-3β. Notably, trelagliptin upregulated the expression of postsynaptic density protein 95 (PSD95) and synaptotagmin 1 (SYT1) while downregulating amyloid precursor protein (APP) and beta-site amyloid precursor protein cleaving enzyme 1 (BACE1). These findings suggest that trelagliptin alleviates cognitive impairment in diabetic rats, likely through AMPK-AKT-GSK-3β-mediated mitigation of oxidative stress, enhancement of synaptic plasticity, and reduction of Aβ accumulation.



INTRODUCTION

Diabetes mellitus (DM), a multifaceted chronic metabolic disease and a significant global public health concern, is characterized by hyperglycemia.[1] As the most prevalent metabolic disorder, diabetes affected 38.1 million adults in the United States as of 2024, representing 14.7% of the adult population according to the US Centers for Disease Control.[2] Clinical observations spanning over a century have established that diabetes can induce central nervous system complications, evidenced by frequent patient reports of impaired memory and attention.[3] A recent longitudinal study demonstrated that DM shows significant association with cognitive deterioration during the first year of mild cognitive impairment monitoring, along with increased risk of developing Alzheimer's disease (AD).[4] African epidemiological investigations reveal a 43.99% prevalence rate of cognitive impairment among diabetic populations.[5]

The pathophysiology of AD and diabetes-related cognitive impairments demonstrates numerous commonalities. Studies indicate that oxidative stress and inflammation represent overlapping neurodegenerative pathways observed in both AD and diabetes, with diabetes being a modifiable risk factor for AD.[6] Evidence suggests that insulin resistance plays a significant role in this pathophysiology.[7] Notably, studies reveal that certain antidiabetic medications ameliorate cognitive impairment. For example, metformin therapy in 18-month-old mice demonstrated improved cognitive performance by modulating microglia activity, proinflammatory cytokines, and autophagy pathways.[8] Similarly, sodium-glucose cotransporter 2 (SGLT2) inhibitors and dipeptidyl peptidase-4 (DPP4) inhibitors have been shown to protect type 2 diabetes mellitus (T2DM) mice from AD-like pathology and cognitive decline through distinct mechanisms involving brain insulin signaling.[9]

It is critical to investigate specific brain regions whose damage leads to cognitive dysfunction. Studies have demonstrated that diabetic cognitive impairment is strongly associated with the hippocampus. Vascular-mediated hippocampal injury has been linked to memory loss and cognitive

Received: February 5, 2025
Revised: March 27, 2025
Accepted: April 4, 2025
Published: April 11, 2025

decline in both cerebrovascular illnesses and healthy aging.[10] T2DM-accelerated cognitive impairment may result from altered brain oscillation patterns and reduced hippocampal neurogenesis.[11] However, the role of the cerebral cortex in diabetic cognitive dysfunction should not be overlooked. Research indicates that progressive cognitive decline in type 2 diabetes patients is at least partially attributable to diabetes-induced disruption of the cellular and molecular architecture of the cerebral cortex.[12] Furthermore, structural changes in the posterior cingulate cortex, a region critical for cognition, were observed in T2DM patients prior to the onset of clinically detectable cognitive impairment.[13]

Numerous phenotypic traits associated with cognitive impairment have been documented, including altered expression of Aβ-related proteins, neuronal loss, and synaptic plasticity abnormalities. Research demonstrates that multiple pathways contribute to cognitive decline. For instance, ranolazine may enhance learning and long-term memory in diabetic mice by reducing proinflammatory mediators.[14] Dietary alkaloids exert therapeutic effects on cognitive impairment through mechanisms involving glucose metabolism modulation, gut microbiota regulation, vascular pathology alleviation, and suppression of neuroinflammation and oxidative stress.[15] Additionally, advanced glycation end products (AGEs) may exacerbate cognitive dysfunction by amplifying inflammatory cascades, particularly in individuals with comorbid arterial hypertension, diabetes mellitus, or hypercholesterolemia.[16] Current evidence highlights the urgent need for further investigations to develop targeted pharmacotherapies and to elucidate the precise mechanisms underlying diabetic cognitive impairment.

Trelagliptin is an orally active DPP4 inhibitor approved in Japan for treating T2DM. Unlike other agents in its class, trelagliptin can be administered once weekly, which improves patient compliance.[17] In previous studies, trelagliptin exerted effects on PI3K/AKT/GSK-3β and inflammation pathways in the hippocampus of rats, though its role in the cerebral cortex remains unexplored. This study evaluated diabetic rats' memory function via fear conditioning experiments and assessed cortical histological injury using Nissl or hematoxylin−eosin (HE) staining. Reactive oxygen species, inflammation markers, synaptic plasticity, and Aβ-related proteins were analyzed to investigate trelagliptin's neuroprotective effects in diabetic rats, which may help elucidate its cognitive-enhancing mechanisms.

MATERIALS AND METHODS

Animal Treatment. A total of 36 male Sprague−Dawley (SD) rats (7−8 weeks old, 160−180 g) were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. [certificate: SCXK (Beijing) 2016-0011]. The rats were housed in a specific pathogen-free (SPF) environment (22 ± 1 °C, 50 ± 10% humidity) with free access to water and a standard diet (40% corn, 20% soybean meal, 7% fish meal, 23% flour, 3.5% yeast, 4% composite mineral salts, 0.2% multivitamins, 0.8% composite amino acids, and 1.5% edible oil; Wuhan Wanqianjiaxing Biotechnology Co., Ltd.). A 12 h light/dark cycle and separate cage feeding were maintained. After a two-week acclimatization period, DM was induced by feeding a high-fat (HF) diet (66.5% standard diet, 10% lard, 20% sucrose, 2.5% cholesterol, and 1% sodium cholate; Beijing Ke'ao Xieli Feed Co., Ltd.) followed by intraperitoneal streptozotocin (STZ) injections (35 mg/kg for two consecutive days). Control rats received equivalent volumes of the solvent (0.1 mM sodium citrate buffer, pH 4.4).

Postmodeling, DM rats were randomized into DM (n = 10) and DM+Trelagliptin (n = 10) groups, while control rats were allocated to CON (n = 8) and CON+Trelagliptin (n = 8) groups. Trelagliptin (>99% purity, Perfemiker, Shanghai) was administered weekly via oral gavage (40 mg/kg) for three months.[18] Fear conditioning tests were conducted between 8:00 and 18:00 prior to euthanasia, with fasting initiated 12 h before sacrifice. All procedures adhered to national guidelines for the welfare of laboratory animals and experimental protocols.

Histopathological Examination. Following immediate detachment, brain tissues were fixed in 10% neutral buffered formalin and subsequently embedded in paraffin. The paraffin-embedded tissues were sectioned into 3−5 μm slices using a microtome. Histological staining procedures (HE and Nissl staining) were performed by Wuhan Servicebio Technology Co., Ltd. (Wuhan, China). Tissue sections were examined under an optical microscope (Olympus BX53, Tokyo, Japan) with digital image acquisition. Quantitative analysis of neuronal death was conducted through blind cell counting across multiple tissue sections.

Western Blotting. The cortex of rats was ground and suspended in a RIPA lysate containing cocktail and phosphatase inhibitors, and then, the supernatants were collected and proteins were subsequently separated by SDS-PAGE as previously described.[18] The samples were incubated with antibodies as shown in Table 1.

Table 1. Antibody List

name of antibody	manufacturer	catalog
GAPDH	Servicebio	GB15002-100
β-tubulin	ABclonal	A12289
β-actin	ABclonal	AC006
SOD2	ABclonal	A21805
GPX4	ABclonal	A11243
p-IKKα	ABclonal	AP0505
IKKα	ABclonal	A2062
p-NFκB	Affinity	AF3390
NFκB	Cell Signaling Technology	Q04206
AKT	Proteintech	60203-2-lg
p-AKT	ABclonal	AP1266
AMPK	ABclonal	A1229
p-AMPK (pT172)	Abclonal	AP1171
GSK-3β	ABclonal	A2081
p-GSK-3β	ABclonal	AP0039
PSD95	Affinity	#AF5283
SYT1	Affinity	#AF6223
APP	ABclonal	A162655
BACE1	ABclonal	A5266

Fear Conditions. Fear memory assessment was conducted as previously described with minor modifications.[19] After 3 days of acclimatization, rats were allowed to freely explore the fear conditioning chamber before receiving the tone cue. The fear conditioning experiment was performed as illustrated in Figure 1A. On the first day, three tones (85 dB, 2.7 kHz, 18 s on/off cycle) paired with foot shocks (0.8 mA for 2 s) were delivered to the animals; a 30 s recovery period was provided after each shock. Three hours later, the same procedures without shocks were repeated to test short-term memory. For

https://doi.org/10.1021/acsomega.5c00535
ACS Omega 2025, 10, 15673−15680

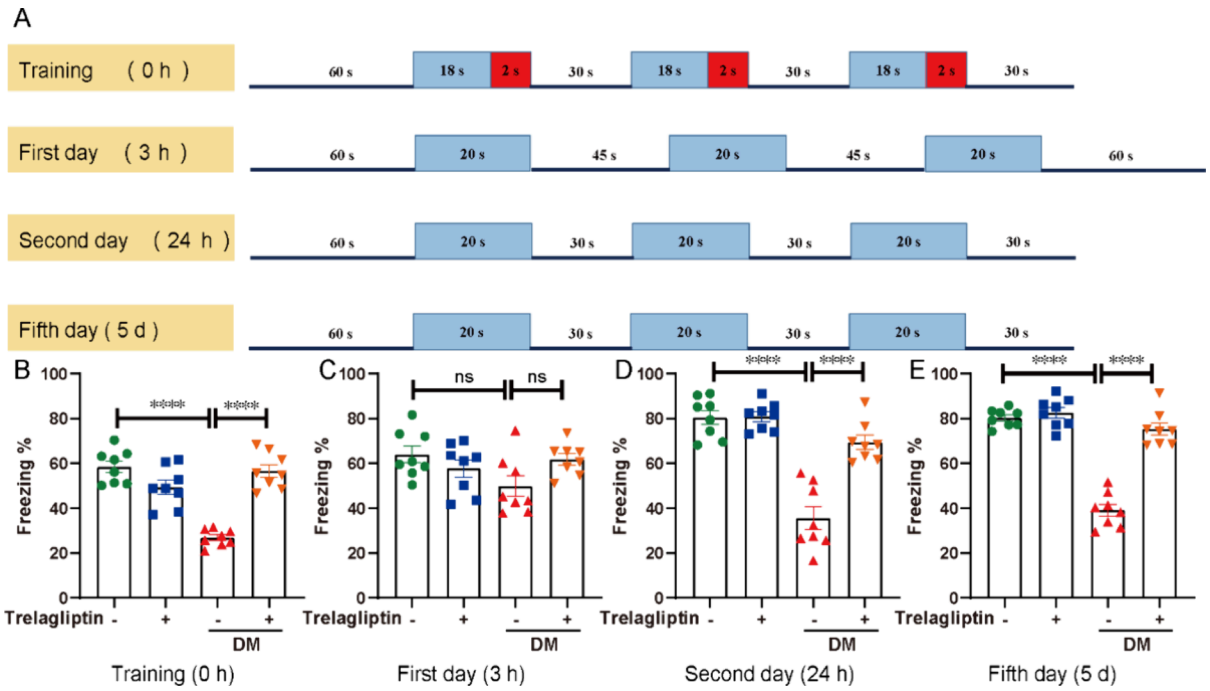


Figure 1. Trelagliptin relieved DM-induced fear memory deficit of rats. (A) Experimental procedure for the training, first day, second day, and fifth day. The blue box labeled 18 or 20 s represents the time for tone stimuli (85 dB, 2.7 kHz, 18 s), and the red box labeled 2 s represents the time for tone−foot stimulus pairings (0.8 mA, 2 s). (B) Freezing percent in the training experiment. The freezing time corresponds to learning sensitivity, and less learning sensitivity was seen in DM rats. (C) Freezing percent in the first day. The freezing time corresponds to short-term memory, and no difference was seen among groups. (D) Freezing percent in the second day. (E) Freezing percent in the fifth day. In (D) and (E), the freezing time corresponds to long-term memory, and the DM group showed a decreased long-term memory (****P < 0.0001).

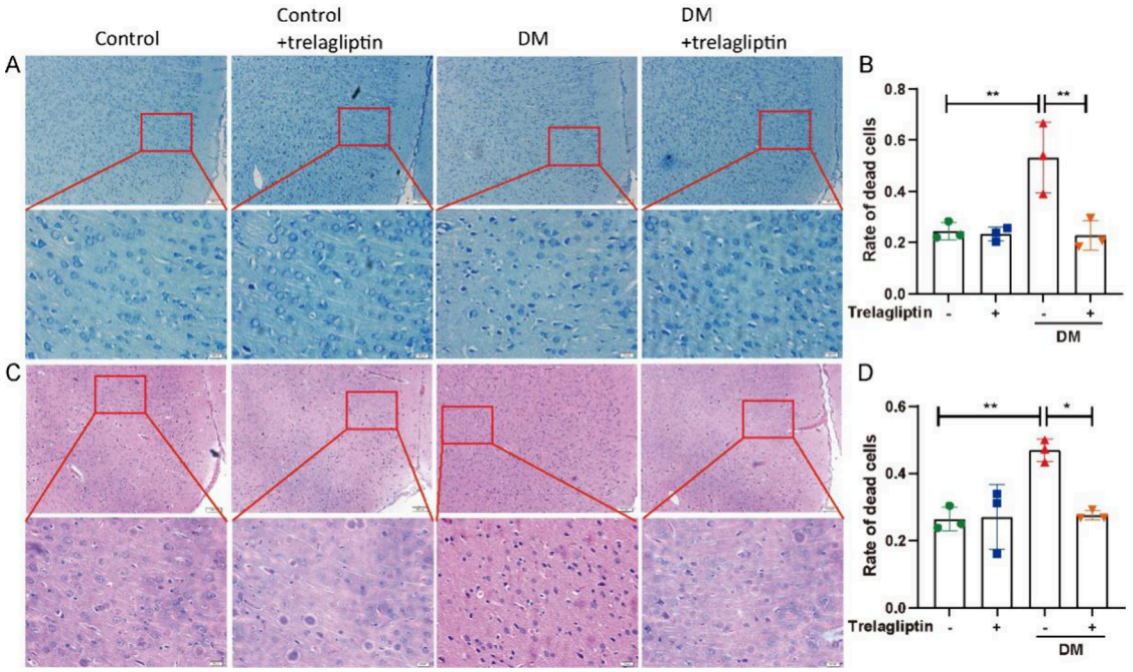


Figure 2. Trelagliptin rescued neuronal cell necrosis in the cerebral cortex of DM rats. (A) Nissl staining in the rat cerebral cortex and (B) statistical analysis for dead cells in Nissl staining. The aberrant morphology and wrinkled dead cells were more noticeable in the DM group. (C) HE staining in the rat cerebral cortex and (D) statistical analysis for dead cells in HE staining. The nuclei retract in dead cells, while the cytoplasm increased eosinophilic acid and turned dark red, which was more noticeable in the DM group (*P < 0.05, **P < 0.01).

contextual fear extinction testing, rats were placed in the chamber on the following day with three modified tones (85 dB, 2.7 kHz, 20 s on/off cycle) at 45 s intervals, without shocks. On day 5, animals were reintroduced to the chamber containing a new floor configuration and multicolored wall panels. Identical 20 s tones and 30 s rest periods were administered after a 2 min acclimatization period, maintaining shock-free conditions. Freezing behavior was scored using

https://doi.org/10.1021/acsomega.5c00535
ACS Omega 2025, 10, 15673−15680

AniLab software and instrument. All equipment was systematically cleaned with 75% ethanol and periodically dried with lint-free towels to eliminate odor cues.

MDA and SOD2 Detection. In accordance with the manufacturer's instructions, kits (Abokine, Wuhan, China) were used to detect the malondialdehyde (MDA) content and the superoxide dismutase 2 (SOD2) activity.

Statistical Analysis. After analyzing and expressing the experimental data as means ± SD, GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA) was used for testing and graphing. The statistical significance values were performed by one-way ANOVA followed by a post hoc Tukey's test. P values <0.05 were considered significant.

RESULTS

Trelagliptin Relieved DM-Induced Fear Memory Deficit of Rats. As shown in Figure 1A, cue and contextual fear conditioning experiments were used to assess memory deficits in rats. Among the four groups, DM rats exhibited the shortest percentage of freezing time during training, indicating reduced learning sensitivity compared to the other groups (Figure 1B). However, 3 h post-training on day 1, no significant differences in short-term memory were observed (Figure 1C). Fear memory was subsequently evaluated on days 2 and 5 to test long-term memory retention. Results demonstrated that rats in the DM group showed significant memory decline on both days, whereas trelagliptin treatment reversed these deficits (Figure 1D,E). These findings suggest that trelagliptin may partially ameliorate DM-induced memory impairment, although the precise underlying mechanism remains unclear and requires further investigation.

Trelagliptin Rescued Neuronal Cell Necrosis. Both Nissl and HE stainings were employed to evaluate neuronal status in the cerebral cortex, given the potential association between memory impairment and neuronal death. In DM rats, both staining methods revealed darker chromatic profiles compared to the Control and Control+Trelaglitpin groups, indicating increased neuronal cell death (Figure 2A,C). However, trelagliptin treatment induced marked histological improvements, as demonstrated by quantitative analysis and reduced necrotic cell counts in the photomicrographs (Figure 2B,D).

Trelagliptin Protected DM Rats from Oxidative Damage. To investigate the protective mechanism of trelagliptin, oxidative damage markers and oxidoreductase activity were assessed. Brain MDA levels in DM rats were significantly elevated compared with both Control and Control +Trelagliptin groups, serving as an indirect biomarker of oxidative damage (Figure 3A). Concomitant with MDA accumulation, the SOD activity showed a marked reduction, potentially exacerbating oxidative injury in DM rats (Figure 3B). Western blot analysis further revealed decreased expression of both SOD2 and glutathione peroxidase 4 (GPX4) in DM rats. Notably, trelagliptin intervention normalized MDA concentrations while restoring SOD2 activity, SOD2 expression, and GPX4 levels (Figure 3), indicating cortical antioxidant effects in DM rats.

Trelagliptin Relieved Inflammatory Effects in DM Rats. Inflammation significantly influences disease onset and progression, potentially contributing to cognitive decline. Given the established roles of IKKα and NFκB in inflammatory signaling across multiple pathologies, we analyzed the phosphorylated forms (p-IKKα and p-NFκB).

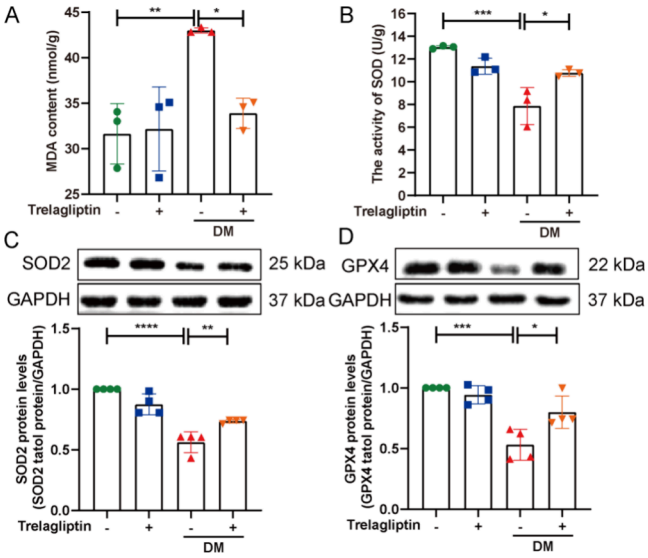


Figure 3. Trelagliptin protected rats from oxidative injury in the cerebral cortex of DM rats. (A) MDA (malondialdehyde) content in the rat cerebral cortex, which indicates that the level of lipid peroxidation in the membrane was elevated in the DM group. (B) Activity of SOD2 (superoxide dismutase 2) in the rat cerebral cortex. (C) Western blot analysis of SOD2 in the rat cerebral cortex. The results of (B) and (C) indicate a decreased antioxidant activity in the DM group. (D) Western blot analysis of GPX4 (glutathione peroxidase 4) in the rat cerebral cortex. The decreased expression of GPX4 partly explains the elevated lipid peroxidation in the DM group (*P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001).

While total IKKα and NFκB expression remained comparable across all four experimental groups, phosphorylated isoforms were markedly elevated in DM rats relative to both the Control and Control+Trelagliptin groups. Conversely, trelagliptin-treated DM rats exhibited significant suppression of p-IKKα and p-NFκB (Figure 4). These findings demonstrate that trelagliptin attenuates cortical inflammation in DM rats, which may mechanistically underlie its memory-enhancing effects, although detailed pathways require further elucidation.

Trelagliptin Relieved Cognition Impairment by Activating the AMPK/AKT/GSK-3β Pathway. Western blot analysis was conducted to assess AMPK, AKT, and

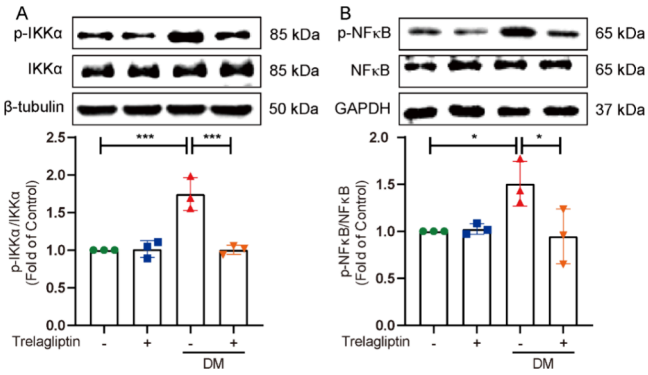


Figure 4. Trelagliptin relieved inflammatory effects in the cerebral cortex of DM rats. (A) Western blot analysis of p-IKKα and IKKα. IKKα was activated in the DM group, and the NFκB signaling pathway is promoted by an increased ratio of p-IKKα to IKKα. (B) Western blot analysis of p-NFκB and NFκB. The increasing ratio of p-NFκB to NFκB increases inflammation (*P < 0.05, ***P < 0.001).

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ACS Omega 2025, 10, 15673−15680

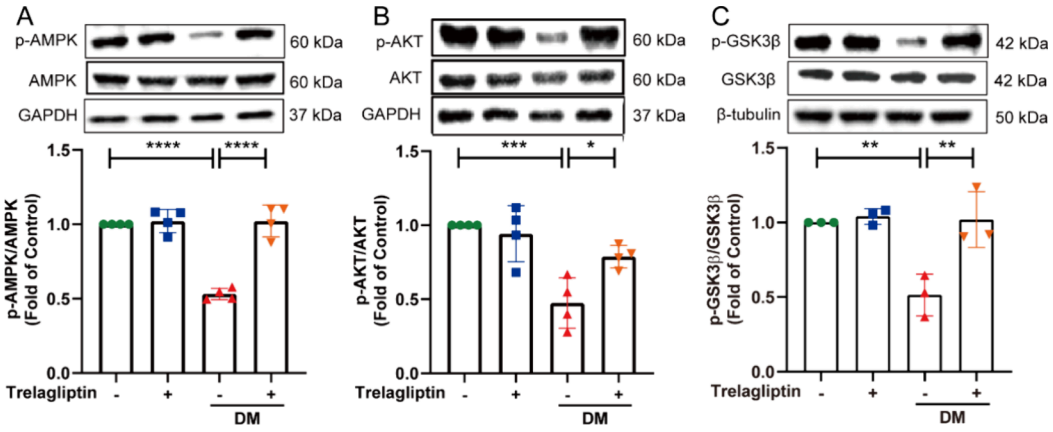

Figure 5. Trelagliptin relieved cognitive impairment by activating the AKT/AMPK/GSK-3β pathway in DM rats. (A) Western blot analysis of p-AKT and AKT; the activity of AKT was inhibited in the DM group. (B) Western blot analysis of p-AMPK and AMPK; the activity of AMPK was inhitited in the DM group. (C) Western blot analysis of p-GSK-3β and GSK-3β; the activity of GSK-3β was inhibited in the DM group (*P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001).

GSK-3β expression, aiming to identify signaling pathways modulating cognitive function in rats. Phosphorylated isoforms (p-AMPK, p-AKT, and p-GSK-3β) exhibited significant reductions in the cerebral cortex of DM rats, whereas total protein levels remained stable (Figure 5). Trelagliptin treatment effectively reversed these phosphorylation deficits. These results suggest that trelagliptin ameliorates memory impairment in DM rats through activation of the AMPK/AKT/GSK-3β signaling axis.

Trelagliptin Improved Synaptic Plasticity in Diabetic Rats. Synaptic plasticity, a partial biomarker of cognitive capacity, is mediated by postsynaptic density protein 95 (PSD95), a critical regulator of experience-dependent plasticity and learning processes.[20] Synaptotagmin 1 (SYT1), a synaptic vesicle membrane protein, facilitates vesicle trafficking and exocytosis.[21] Our study quantified cortical PSD95 and SYT1 expression in experimental groups. DM rats exhibited significantly reduced PSD95 levels compared to those of both Control and Control+Trelagliptin groups, with trelagliptin administration normalizing this deficit (Figure 6A). A comparable pattern emerged in SYT1 expression, where trelagliptin reversed diabetes-induced SYT1 downregulation (Figure 6B). These findings collectively indicate that trelagliptin enhances synaptic plasticity in diabetic rats, potentially counteracting memory dysfunction through this mechanism.

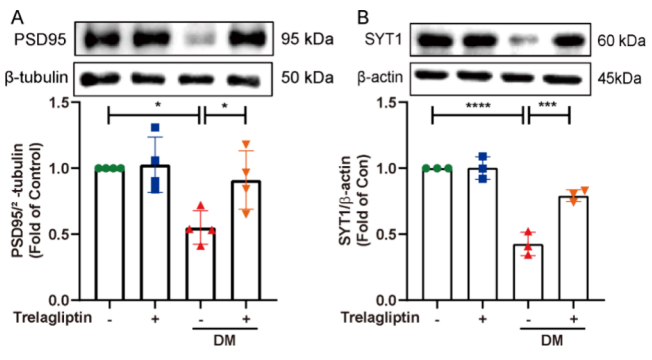

Figure 6. Trelagliptin improved synaptic plasticity in the cerebral cortex of DM rats. (A) Western blot analysis of PSD95 (postsynaptic density protein 95), an essential postsynaptic scaffold protein that decreased in the DM group. (B) Western blot analysis of SYT1 (synaptotagmin 1), the primary regulator of synaptic transmission that functions as an integrin in the synaptic vesicular membrane; its expression decreased in DM rats (*P < 0.05, ***P < 0.001, and ****P < 0.0001).

Trelagliptin Relieved the Accumulation of APP in Diabetic Rats. The amyloid precursor protein (APP), primarily recognized as the molecular precursor of amyloid-β (Aβ) peptides, undergoes proteolytic processing mediated by beta-site APP cleaving enzyme 1 (BACE1), the rate-limiting enzyme for Aβ production. Our data revealed significant upregulation of BACE1 in DM rats, which was effectively attenuated by trelagliptin treatment (Figure 7B). APP accumulation, serving as a biomarker for neurodegeneration severity, showed a significant elevation in the DM group. Notably, trelagliptin administration reduced APP content to levels comparable to both Control and Control+Trelagliptin groups (Figure 7A). These findings collectively suggest that fear memory deficits in diabetic rats may be mediated through APP/BACE1 dysregulation, a pathological cascade that can be modifiable by trelagliptin intervention.

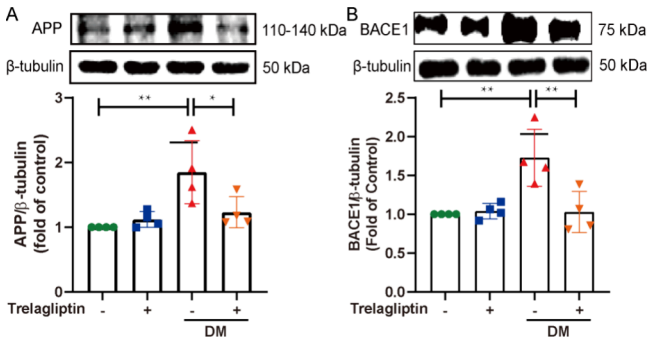

Figure 7. Trelagliptin relieved the accumulation of APP (amyloid precursor protein) in the cerebral cortex of DM rats. (A) Western blot analysis of APP. (B) Western blot analysis of BACE1 (beta-site amyloid precursor protein cleaving enzyme 1). The elevated expression of APP and BACE1 in DM rats may partly explain the decreased learning sensitivity and long-term memory deficit of DM rats (*P < 0.05, **P < 0.01).

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ACS Omega 2025, 10, 15673−15680

CONCLUSIONS

Trelagliptin, a long-acting DPP4 inhibitor with a weekly dosing regimen, demonstrates therapeutic potential in diabetic cognitive impairment. Our findings revealed two neuroprotective mechanisms: (1) cortical neuronal apoptosis was significantly attenuated by trelagliptin treatment, accompanied by restored fear memory retention in diabetic rats; (2) this protection appears mediated through AMPK/AKT/GSK-3β pathway activation, which mitigated oxidative stress damage. Furthermore, trelagliptin administration reduced amyloid-beta accumulation and enhanced synaptic plasticity markers, both contributing to improved cognitive recovery in the diabetic cohort.

DISCUSSION

Growing evidence indicates that T2DM induces cognitive decline beginning as early as the prediabetic stage, with poor glycemic control exacerbating these impairments, particularly in learning domains.[22] Protein−protein interaction network analysis has further identified tightly connected gene clusters influencing memory and cognition among comorbid genes shared by AD and T2DM. These findings not only provide substantial genetic data for future investigations but also reinforce the biological overlap between these two conditions.[23] The conceptualization of AD as "type 3 diabetes" stems from evidence implicating cerebral insulin resistance as a key pathophysiological mechanism.[24] However, this association remains controversial, as post-mortem neuropathological analyses suggest distinct pathological correlations between AD and diabetes-related cognitive decline.[25] Our longitudinal rodent studies demonstrate that diabetic rats exhibit consistent cognitive deficits in both fear conditioning tests (Figure 1) and Morris water maze performance.[18] These experimental results partially support the proposed AD-DM connection while highlighting the complexity of their relationship.

Investigating the mechanisms underlying the relationship between AD and diabetes is a critical yet challenging task. Given the lack of effective medications for treating AD, numerous approaches have been explored. One promising strategy involves examining how conventional hypoglycemic medications influence cognitive function. The effects of various drugs, including traditional Chinese medicine, on the nervous system have been studied, and plausible mechanisms have been proposed. (1) SGLT2 inhibitors ameliorate cognitive impairment in T2DM rats by reducing neuroinflammation, oxidative stress, and mitochondrial dysfunction while enhancing neural plasticity. Additionally, SGLT2 inhibitors may delay the onset or progression of neurodegenerative diseases by restoring mTOR to a physiologically active state.[26] (2) DPP4 inhibitors significantly reduce cognitive impairment in patients with type 2 diabetes mellitus.[27] DPP4 may bind to PAR2 in the hippocampus, leading to mitochondrial dysfunction that ultimately contributes to cognitive deficits in diabetics. DPP4 inhibitors block this process.[28,29] (3) Glucagon-like peptide-1 (GLP-1) receptor agonists are under investigation for their neuroprotective effects on synaptic plasticity, cognition, and cell survival.[30] GLP-1 analogs are considered viable therapeutic candidates for AD.[31] Eight mechanisms are reducing oxidative stress, neuronal apoptosis, neurotoxicity, β-amyloid and tau protein, and neuroinflammation and also promoting insulin signal transduction, neurogenic and synaptic plasticity, and direct central effects.[32] (4) Naofucong, a traditional Chinese herbal formulation, improves cognitive impairment in diabetic rats by inhibiting the ERK/JNK/p38 MAPK pathway, upregulating insulin-degrading enzymes, reducing Aβ deposition, suppressing APP and p-tau expression, and alleviating neuronal damage and oxidative stress.[33] In our study, the DPP4 inhibitor trelagliptin demonstrated neuroprotective effects by rescuing neurons from apoptosis, alleviating oxidative stress and neuroinflammation, enhancing synaptic plasticity, and reducing Aβ deposition in the cerebral cortex. The AKT/AMPK/GSK-3β pathway was also implicated in this process. Collectively, these findings underscore the therapeutic potential of the DPP4 inhibitors.

The relationship among oxidative stress, neuroinflammation, and cognitive impairment holds particular significance in studying cognitive dysfunction in diabetes. Our findings align with a recent investigation demonstrating elevated oxidative stress and MDA accumulation alongside reduced glutathione peroxidase (GPX) activity in T2DM mice with cognitive deficits.[34] Furthermore, heightened oxidative stress may trigger neuroinflammation, and compound danshen dripping pills have been shown to alleviate both inflammation and cognitive dysfunction in KK-Ay mice, a spontaneous model of T2DM.[35,36] In our study, trelagliptin treatment significantly reduced phosphorylation levels of inflammatory mediators (p-IKKα and p-NFκB; Figure 4), indicating attenuated neuroinflammation, a result consistent with prior findings. Histopathological evidence from HE and Nissl staining (Figure 2), combined with upregulated expression of synaptic markers PSD95 and SYT1 (Figure 6) in diabetic rats, strongly suggests that oxidative stress and neuroinflammation mediate synaptic loss and neurodegeneration.[37]

However, this study has limitations, and many unresolved questions remain, requiring further research to fully elucidate the underlying regulatory mechanisms. First, although oxidative stress and elevated membrane lipid peroxidation were observed in the cerebral cortex of diabetic rats, it remains unclear whether the observed cell death involves ferroptosis, let alone the precise mechanism by which neurons are spared. Second, is the neuroprotective effect specific to trelagliptin or common to other DPP4 inhibitors? As a DPP4 inhibitor, omarigliptin has been reported to improve neurological function after intracerebral hemorrhage (ICH) in mice, with its neuroprotective effects potentially linked to antiferroptosis activity via regulation of the GLP-1 receptor post-ICH injury.[38] Additionally, DPP4 has been identified as a key protein that promotes intracellular lipid peroxidation and iron accumulation, triggering ferroptosis in tumor cells, a finding suggesting broader potential mechanisms for DPP4 inhibitors.[39,40] In conclusion, our study did not determine whether trelagliptin, as a DPP4 inhibitor, shares a similar or distinct mechanism of action compared with other inhibitors in its class, highlighting the need for further investigation.

ASSOCIATED CONTENT

Data Availability Statement

The data used to support the findings of this study are available from the corresponding author upon request or from https://data.mendeley.com/datasets/9bfyv9h7ws/1.

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https://doi.org/10.1021/acsomega.5c00535
ACS Omega 2025, 10, 15673−15680

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Author Contributions

†Y.Y., T.S., and X.G. contributed equally to this work. Each author made an important scientific contribution to the study. Y.Y., J.H., and X.L. performed the experiments and data analysis; X.G. and T.S. contributed to manuscript writing. Q.C. conducted software implementation and analysis; X.Y. and C.O. participated in manuscript review and editing; M.L. oversaw project design, funding acquisition, and supervision.

Funding

This work was supported by the National Natural Science Foundation of China (82073852 and 82270892), the Natural Science Foundation of Hubei Province (2024AFB378), the Joint supported by Hubei Provincial Natural Science Foundation and Xianning Innovation and Development Project (2025AFD406), the Hubei Provincial Department of Education Youth Talent Scientific Research Program (Q20232803), and the Hubei University of Science and Technology School-Level Fund (2022HX141 and 2023HX177).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We gratefully acknowledge the support and contributions that facilitated this research. First and foremost, we extend our sincere gratitude to Prof. Chao Liu for her invaluable guidance, critical insights, and continuous encouragement throughout this project. Her expertise and patience were instrumental in shaping the direction of this work. We would also like to thank Prof. Qing Yao for her technical assistance in animal handling and the histochemical analysis.

■ ABBREVIATIONS

AD:Alzheimer's disease; APP:amyloid precursor protein; BACE1:beta-site amyloid precursor protein cleaving enzyme 1; DM:diabetes mellitus; DPP4:dipeptidyl peptidase-4; GLP-1:glucagon-like peptide-1; GPX4:glutathione peroxidase 4; HE:hematoxylin-eosin; MDA:malondialdehyde; PSD95:post-synaptic density protein 95; SGLT2:sodium-dependent glucose transporters 2; SOD2:superoxide dismutase 2; SYT1:synaptotagmin 1; T2DM:type 2 diabetes mellitus

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https://doi.org/10.1021/acsomega.5c00535
ACS Omega 2025, 10, 15673−15680