Praeruptorin C alleviates cognitive impairment in Type 2 diabetic mice through restoring PI3K/AKT/ GSK3β pathway

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Abstract

Diabetic encephalopathy is a common complication of diabetes, leading to cognitive impairment. In this study, we investigated the therapeutic effects of Praeruptorin C (Pra-C), an active component of traditional Chinese herb medicine *Peucedanum praeruptorum*, on cognitive impairment in a mouse model of type 2 diabetes. Our results showed that Pra-C administration significantly reduced body weight and fasting blood glucose levels, relieved learning and memory behavioral deficits in diabetic model mice. The effects of Pra-C on learning and memory ability were further confirmed by late-phase LTP (L-LTP) facilitation, which was recorded by Med64 electrophysiology recording system. Pra-C also restored the activation of PI3K/AKT/GSK3β signaling pathway in hippocampus of mice suffering from diabetes. Taken together, this study demonstrates that Pra-C alleviates cognitive impairment in diabetic mice by restoring PI3K/AKT/GSK3β activation.

Introduction

Diabetes mellitus (DM), characterized by high blood glucose levels, is one of the most important public health challenges worldwide for a long time. The prevalence of diabetes has been increasing over recent decades. The International Diabetes Federation reported 1 in 11 adults suffering diabetes and estimated 700 million patients in 2045 [1]. Diabetic patients have a high risk of developing serious complications affecting the heart, eye, kidney, and nerve [2].

Diabetic encephalopathy (DE), one of the main complications of DM, is characterized by brain infarcts [3], brain atrophy in cortex and hippocampal volume [4], cerebrovascular disfunction and morphological change [5], and impairment of synaptic plasticity [6]. Notably, an increasing amount of studies have revealed the link between diabetes and neurodegenerative disorders, such as Alzheimer's disease (AD), with changes in cognitive impairment [7−9]. Furthermore, animal studies also showed learning and memory deficits in different DM models mice, such as obesity by high-fat diet (HFD) feeding, pancreatic β cells damage by streptozotocin injection, or genetic construction (d*b*/d*b* mice) [10−12].

Type 2 Diabetes mellitus (T2DM) is characterized as insufficient function of pancreatic β cells or insulin resistance. Besides exogenous insulin, a number of drugs exert therapeutic effects on hyperglycemia in T2DM [13−15]. It has been drawing a lot of attention on developing new drugs on DE of T2DM [16−18], the best is hitting two birds with one stone, treating hyperglycemia and DE at the same time[19].

Praeruptorin C (Pra-C) is derived from the dried roots of *Peucedanum praeruptorum* (Peucedani Radix), a traditional Chinese herbal medicine for treating cough, bronchitis, and furuncle [20]. Monomer composition studies show the anti-inflammatory activity and anti-osteoporosis effects of Pra-C [21, 22]. Our previous studies revealed its neuroprotective effect in excitatory toxicity *in vitro* [23] and behavioral improvement in a Huntington's disease mouse model *in vivo* [24]. Given its beneficial effects on neuron apoptosis and neurodegenerative model animals, we hypothesize that Pra-C may provide therapeutic benefits for treating cognitive impairment related diseases, such as DE. The aim of present study is to

investigate its therapeutic effect of Pra-C on cognitive impairment in diabetic model mice and reveal underlying mechanisms.

Materials And Methods

Materials

Streptozotocin (STZ) was obtained from Sigma (St. Louis, MO, USA). Blood glucose test strip and glucometer were delivered from Roche (Changsha, Hunan, China). Primary antibodies anti-phosphor-Ser 9 GSK3β (ab131097), GSK3β (ab32391) and β-actin (ab8226) were purchased from Abcam (Cambridge, UK). Anti-phosphor-Tyr607 PI3K (#17366), PI3K (#4228), phosphor-Ser308 AKT (#4060), and AKT (#9272) were from Cell Signaling Technology (Danvers, MA, USA). Secondary antibodies conjugated with horseradish peroxidase (HRP) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Pra-C was purchased from Shanghai PureOne Biotechnology (Shanghai, China) with 98% of purity. All chemicals and reagents used were of standard biochemical quality.

Animals And Establishment Of Type 2 Diabetic Model

Male C57BL/6 mice aged 5–7 weeks were got from the Experimental Animal Center of the Air Force Military Medical University (Xi'an, China). Mice were fed under standard laboratory conditions (12 h light/12 h dark, temperature 22–26 °C, air humidity 55–60%) with water and mice chow *ad libitum*. The experimental procedures were approved by the Institutional Animal Care and Use Committee of The Fourth Military Medical University, and are complied with the Protection of Animals and Principles of laboratory animal care" (NIH publication No. 86 – 23, revised 1985). The number of animals used and their suffering were greatly minimized. To induce T2DM model, mice got HFD (60% kcal fat, Ke'ao Biotech, Xi'an, China) feeding for 4 weeks, a single dose of STZ (60 mg/kg, dissolved in 0.1 M citrate buffer) was intraperitoneally injected after 12 h fasting, while other control mice were given regular food and same volume of 0.1 M citrate buffer injection. Model mice then continuously fed with HFD for 1 week and got blood glucose test by single tail tip pricks. HFD fed mice with fasting blood glucose levels over 13 mM were considered the successful establishment of T2DM. Blood glucose levels were measured every 2 weeks. All mice were allowed to acclimate for 24 h of laboratory environment before any room change.

Pra-C Treatment

After establishment of T2DM model induction, mice were randomly divided into 2 groups, T2DM and Pra-C treatment, and were continuously fed with HFD. Pra-C was dissolved in 0.9% saline and administrated intraperitoneally (i.p., 3 mg/kg/d) at 9 a.m. once a day for 4 weeks from 5th week, while T2DM group mice got equal volume of saline. Pra-C dose was selected base on previous study [24]. Control mice were fed with conventional chow. Behavior tests were performed 3 weeks after treatment. Fasting blood glucose and body weight were detected before final sacrifice.

New Objection Recognition (NOR) Test

NOR test was performed in a sound-proof polyvinyl chloride box (25 × 25 cm bottom, 25 cm height) with a digital camera on the roof. On Day 1, single mouse was placed in the box to acclimate the new environment for 10 min. On Day 2, mouse was put into box with two cylinders (5 cm in diameter, 5 cm height) fixed in symmetrical corner of the box (training trial). On Day 3, every group was equally divided into two groups randomly. For the new object recognition, one cylinder was replaced with a cone (5 cm in diameter, 5 cm height) (testing trial). For the object-place recognition test, the former cylinders were fixed in parallel corners with one in the former location as the training trial and the other in a different location. In both training and testing trials, mice freely explored for 10 min, and time spent exploring each object or location was recorded and scored by software (Jiliang, Shanghai, China). After each single experiment, the box and objects were wiped with 75% ethanol. The proportion of exploration time on new object or new object-location was defined as the "Descrimination index" expressed by the ration of (TN-TF)/(TN + TF) (TN: time spent on exploring the new object or location; TF: time spent on exploring the familiar object or location).

Morris Water Maze (MWM) Test

MWM test was conducted in a circular tank (120 cm in diameter and 50 cm deep) with white wall decorated by different bold marks in four directions. An escape platform (10 cm in the diameter) was erected in the midway position between the center and wall of the tank. Before experiment, opaque water (24 ± 1 °C, with unharmed white coating) was filled in the tank to a height 1.0 cm above the platform. Mice were put into water and trained for four trials (different starting positions) per day with 10 min inter-trial intervals for four consecutive days. During each trial, mice were allowed to explore until they found the hidden platform and held on the platform for 20 s before returning to a holding cage. Mice failed to find the platform in 60 s were guided to the platform by a wooden stick. Probe test was performed 24 h after the final training trial, mice were put into the same height water filled tank with undersurface hidden platform for 90 s and their swim path was recorded.

Western Blot Analysis

Western blot analysis was conducted as described previously [25]. After behavior tests and blood glucose test, mice were anesthetized with urethane (1.5 g/kg) and then rapidly decapitated. Brain was immediately placed on ice; hippocampus and hypothalamus were removed, respectively collected in 1.5 mL tubes and stored at -80 °C until further analysis. Tissues were homogenized by RIPA lysis buffer and separated by centrifugation at 13,000 rpm for 20 min at 4 °C, and supernatant was collected. All proteins were quantified by BCA protein assay Kit (Pierce Biotech, USA) and equal amounts (30 μg) of protein were subjected to western blot analysis. Samples were separated via 10% SDS-PAGE gel and electrotransferred onto PVDF membranes (Millipore, USA), which were blocked with primary antibodies at

4 °C overnight, washed 3 times with Tris-phosphate buffer containing 0.05% Tween 20 (TBST) for 10 min of each time and were further incubated with HRP-conjugated secondary antibodies. Bands were developed using enhanced chemiluminescence detection (Genshare Biological, China) and imaged by Tanon imaging system (Tanon 4200, China).

Field Electrophysiological Recording

Hippocampal slices from mice were prepared as previously described [26]. In short, slices (300 μm) were cut in oxygenated solution (in mM): 250 Sucrose, 2.5 KCl, 0.5 $CaCl_2$, 6 $MgSO_4$, 1.2 NaH_2PO_4, 25 $NaHCO_3$, and 10 D-glucose. After cutting, the slices recovered at 34 °C in ACSF (in mM) as follows: 124 NaCl, 4.4 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 1 NaH_2PO_4, 25 $NaHCO_3$, and 10 D-glucose. After 10 min, the slices were placed in ACSF at room temperature for an additional 1–2 h, gassed with 95% O_2-5% CO_2. A commercial MED64 recording system (Panasonic Alpha-Med Sciences, Osaka, Japan) was used to record field excitatory postsynaptic potentials (fEPSPs), and the procedure was similar to previous study [27]. Briefly, single hippocampal slice was placed in a probe (MED-P515A, 8 × 8 array; interpolar distance, 150 μm), positioned on the Schaffer collateral-CA1 pathway of the dorsal hippocampus, with oxygenated ACSF (30.0 °C, 1–2 ml/min) perfusion. After a recovery period of at least 1 h for the slices in the recording chamber, biphasic constant-current pulse stimulation (0.2 ms) was applied to the stimulation channel to evoke the fEPSPs in the channels closest to the stimulation site. Stable baseline responses were recorded for at least 40 min, L-LTP was induced by a strong theta burst stimulation (TBS, 5 bursts at 5 Hz, repeated 5 times at 10 s intervals, four pulses at 100 Hz for each burst) [28], then test stimulus was repeatedly given once per minute for at least 3 h. The fEPSPs were measured and analysed using Mobius software (Panasonic Alpha-Med Sciences, Tokyo, Japan).

Data Analyses

All data were presented as mean ± SEM. The statistical significance of differences between groups were analyzed with One-way analysis of variance (ANOVA) followed by LSD and S-N-K(s) t-tests. In all cases, the criterion for statistical significance was $p < 0.05$.

Results

Effects of Pra-C on body weight and fasting blood glucose

Obesity and high saturated fats are risks and symptoms of T2DM, described to have harmful effects in the brain, and performing negatively correlated with cognitive behaviors [29]. Persistent high blood glucose level is associated with greater chance of atrophy in hippocampus [30], and accordingly, successive control of blood glucose level improves learning and memory in type 2 diabetic patients [31]. T2DM model was induced by chronic HFD feeding and injection of STZ to damage pancreatic β cells. HFD and STZ injection induced significant gain of body weight (Fig. 1A) and increment of fasting blood

glucose levels (Fig. 1B) compared with control ones. Treatment of Pra-C (i.p., 3 mg/kg/d) four attenuated these changes (Figure. 1a and 1b), although not to the control group levels. These results indicate the positive effects of Pra-C on body weight and fasting blood glucose levels in T2DM.

Pra-C Relieves Cognitive Impairment In T2DM Mice

Learning and memory deficits are the main cognitive abnormalities affecting T2DM patients [32], and the cognitive dysfunction takes place even at the early phase of diabetes [33]. To test whether Pra-C could relieve cognitive impairment, we performed novel object recognition (NOR) and Morris water maze (MWM) tests. The interests in exploring new object is the nature instinct of rodents, compared with familiar ones, rodents would spend more time sniff or touch the unharmful novel objects, unless the rodents forget what they have already met. In NOR test (Fig. 2A), T2DM model mice showed shorter exploring time on novel object, while mice with Pra-C treatment spent significantly longer time than the models (Fig. 2B). The ability of spatial learning and memory is tested by both novel location recognition (NLR) and MWM tests. In NLR test (Fig. 2A), model mice showed lower discrimination index (DI) compared with control ones. Mice with Pra-C treatment showed significantly a higher level of DI than non-treated model mice (Figure. 2C). In MWM test, the time for control mice to find the platform was gradually decreased in four training days, but the escape latency significantly prolonged in T2DM mice. After treatment of Pra-C, model mice showed a marked shortness in time of the escape performance (Fig. 3A and 3B). On the MWM testing day, the T2DM mice had shorter dwelling time in target quadrant than control group, while Pra-C treated group spent more time swimming in the target quadrant compared with model mice (Fig. 3C). These behavioral results indicate that Pra-C improves the learning and memory abilities of T2DM mice, manifesting its alleviating effects on cognitive impairment in diabetes.

Pra-C Rescues PI3K/AKT/GSK3β Pathway Activation Of T2DM Mice

The PI3K/AKT/GSK3β signaling pathway plays an important role in glucose homeostasis, cell proliferation, and protein synthesis mediated by glucose-related hormones like insulin and leptin in the digestive system and central nervous system [34]. All of above actions are important for learning and memory abilities, and studies indicate the down-regulation of this pathway in different issues of diabetic animals, and restoration is beneficial to relieve of diabetic symptoms [35–37]. As illustrated in Fig. 4, T2DM mice showed significantly lower levels of phosphor-Tyr607 PI3K, phosphor-Ser407 AKT, and phosphor-S9 GSK3β in hippocampus, while the total levels of these proteins remained. Pra-C treatment markedly restored the phosphorylation levels of all these proteins. Present results suggest that Pra-C alleviates cognition impairment in T2DM mice may through restoring the activation of PI3K/AKT/GSK3β signaling pathway.

Pra-C Reverses Impairment Of L-LTP Facilitation In T2DM Mice

The hippocampus, a vital brain region involved in learning ability and memory formation, is particularly affected by hyperglycemia mediated dysregulation of metabolism and osmolarity [38]. Long-term potentiation (LTP), a cellular model of information storage in the brain, has long been linked with learning and memory ability [39]. Diabetic animals exhibit impaired facilitation of LTP at Schaffer collateral−CA1 synapses in hippocampus [40, 41]. Here we tested late-phase LTP (L-LTP), a temporal phase of LTP dependent on protein synthesis, in hippocampal CA1 region by MED64 recording system. Hippocampus slices from all three groups showed elevated fEPSP slope to TBS in Schaffer collateral axons compared with baseline recording slopes (Fig. 5B). We found that the L-LTP could maintain at a high level for at least 3 h in slices from control mice. However, fEPSP slopes of slices from T2DM mice decreased significantly after 2 h from LTP induction. Slices from Pra-C treatment group showed lower slope compared with control group at the end of recording, but still at a significantly higher level than those from T2DM mice (Fig. 5C). These results indicate a rescue effect of Pra-C on L-LTP induction in T2DM mice.

Discussion

To our knowledge, this is the first study to clarify alleviated effects on cognitive impairment in diabetic mice. The results showed that Pra-C treatment reduced body weight and blood glucose levels, relieved cognitive impairment in T2DM model mice. This beneficial effect of Pra-C may be through restoration of PI3K/AKT/GSK3β signaling activation in hippocampus and alleviation of impairment of L-LTP in T2DM model mice.

Epidemiological evidences indicate the link between diabetes and neurodegenerative diseases with cognitive impairment [7]. Different strategies targeting DE have been considered, however treatment still remains a challenge. Traditional Chinese herb medicine has been used for a long time in China and other Asia countries, exerting great therapeutic effects on chronic diseases like diabetes and getting accepted by more and more western countries [42]. The richness and diversity of nature herbs provide huge opportunity to develop leading drugs for different diseases. Several natural herbs, including quercetin, berberine, and curcumin, have been reported to attenuate cognitive deficits in diabetic animals. With the technical development of isolation and purification, more compounds with medicinal properties would be found.

In present study, we demonstrate that Pra-C, one of the effective constituents derived from the dried roots of *P. praeruptorum*, is beneficial for alleviating cognitive impairment in T2DM model mice. Our previous study showed that Pra-C exhibits neuroprotective effects by down-regulation of GluN2B-containing *N*-methyl-D-aspartate receptors [23]. GluN2B receptors in hypothalamic agouti-related peptide (AgRP) neurons play a critical role in central control of energy balance and genetic deletion of GluN2B in AgRP neurons reduces body, fat mass, blood glucose balance, and food intake [43]. Present results show that

Pra-C treatment reduces body weight and fasting blood glucose levels in diabetic mice, which may due to the inhibition of GluN2B by Pra-C in AgRP neurons. DE induced by hyperglycemia impairs learning and memory abilities in diabetic patients and animals [6, 10], increased the risks of occurrence and progression of neurodegenerative diseases [7]. Our previous study showed that Pra-C improved the behavioral performance in Huntington's disease model mice by regulating neurotransmitter balance [24]. In present study, T2DM model mice show poor ability in discriminating novel object and impaired spatial memory in novel location recognition test and spent more time to find the platform in MWM trainings and less time in target quadrant during hidden platform testing, indicating cognitive impairment. Treatment with Pra-C improved the ability in discriminating novel object and novel location and performance in MWM testing. These behavioral results show that Pra-C treatment significantly improves learning and memory ability in T2DM mice.

PI3K/AKT signaling plays central role in regulating protein synthesis, cell proliferation, differentiation, and metabolism in physiological and morbid conditions, such as obesity and T2DM. High glucose levels leads to decrement of activated PI3K and AKT in skeletal muscle, adipose tissue, liver, pancreas, and brain [35]. Dysfunction of PI3K/AKT signaling pathway leads to abnormal GSK-3β activity, promoting tau hyperphosphorylation and axonopathy in T2DM-induced DE [44]. Down-regulation of PI3K/AKT/GSK3β pathway activity in hippocampus was found in HFD/STZ induced T2DM mice. Restoration of the phosphorylation levels of this pathway by Pra-C may be partially responsible for the alleviating cognitive impairment.

LTP, a putative mechanism for learning and memory, is the most widely used paradigm to study molecular and cellular events underlying synaptic transmission [45]. Multiple studies suggest that LTP has two main temporal phases, i.e., early-phase LTP (E-LTP) and late-phase LTP (L-LTP). The former is considered protein synthesis-independent, and the latter depends on new protein synthesis [46]. In present study, L-LTP is impaired in CA1 region from T2DM mice. Treatment of Pra-C restores L-LTP in hippocampus CA1 region, which may be due to the restoration of protein synthesis through PI3K/AKT/GSK3β pathway.

In summary, present findings demonstrate that Pra-C exhibits the therapeutic effects in cognitive impairment by restoring PI3K/AKT/GSK3β signaling in T2DM mice. Further study is needed to elucidate the underlying mechanisms for controlling glucose metabolism and neuronal functions.

Abbreviations

ACSF: artificial cerebrospinal fluid;EPSP: excitatory postsynaptic potential;DE: diabetic encephalopathy; DM: diabetic mellitus; HFD: high fat diets; LTP: long-term potentiation; Pra-C: Praeruptorin C; STZ: streptozotocin.

Declarations

Ethics approval and consent to participate

All of the experiments involving mice were conducted using protocols approved by the Animal Care and Use Committee of the Fourth Military Medical University.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Author's contributions

Guarantors of integrity of entire study, L.Y.; experimental studies, D. S., Y. Z., and T. Y.; literature research and data acquisition, D. S., Y. Z., T. Y., S. C., J. L. and W. L.; study design and data analysis, Y. S.; manuscript drafting and editing, L. Y.. All authors read and approved the final manuscript.

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References

1. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, et al. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract. 2018;138:271–81.
2. Baena-Diez JM, Penafiel J, Subirana I, Ramos R, Elosua R, Marin-Ibanez A, et al. Risk of Cause-Specific Death in Individuals With Diabetes: A Competing Risks Analysis. Diabetes Care. 2016;39:1987–95.
3. Ward R, Valenzuela JP, Li W, Dong G, Fagan SC, Ergul A. Poststroke cognitive impairment and hippocampal neurovascular remodeling: the impact of diabetes and sex. American journal of physiology Heart circulatory physiology. 2018;315:H1402-H13.
4. Schmidt R, Launer LJ, Nilsson LG, Pajak A, Sans S, Berger K, et al. Magnetic resonance imaging of the brain in diabetes: the Cardiovascular Determinants of Dementia (CASCADE) Study. Diabetes.

2004;53:687–92.

5. Chen R, Shi J, Yin Q, Li X, Sheng Y, Han J, et al. Morphological and Pathological Characteristics of Brain in Diabetic Encephalopathy. Journal of Alzheimer's disease. 2018;65:15–28.

6. Fried PJ, Schilberg L, Brem AK, Saxena S, Wong B, Cypess AM, et al. Humans with Type-2 Diabetes Show Abnormal Long-Term Potentiation-Like Cortical Plasticity Associated with Verbal Learning Deficits. Journal of Alzheimer's disease. 2017;55:89–100.

7. Zhang J, Chen C, Hua S, Liao H, Wang M, Xiong Y, et al. An updated meta-analysis of cohort studies: Diabetes and risk of Alzheimer's disease. Diabetes Res Clin Pract. 2017;124:41–7.

8. Secnik J, Cermakova P, Fereshtehnejad SM, Dannberg P, Johnell K, Fastbom J, et al. Diabetes in a Large Dementia Cohort: Clinical Characteristics and Treatment From the Swedish Dementia Registry. Diabetes Care. 2017;40:1159–66.

9. Simo R, Ciudin A, Simo-Servat O, Hernandez C. Cognitive impairment and dementia: a new emerging complication of type 2 diabetes-The diabetologist's perspective. Acta diabetologica. 2017;54:417–24.

10. Rom S, Zuluaga-Ramirez V, Gajghate S, Seliga A, Winfield M, Heldt NA, et al. Hyperglycemia-Driven Neuroinflammation Compromises BBB Leading to Memory Loss in Both Diabetes Mellitus (DM) Type 1 and Type 2 Mouse Models. Mol Neurobiol. 2019;56:1883–96.

11. Yan S, Du F, Wu L, Zhang Z, Zhong C, Yu Q, et al. F1F0 ATP Synthase-Cyclophilin D Interaction Contributes to Diabetes-Induced Synaptic Dysfunction and Cognitive Decline. Diabetes. 2016;65:3482–94.

12. Taylor SL, Trudeau D, Arnold B, Wang J, Gerrow K, Summerfeldt K, et al. VEGF can protect against blood brain barrier dysfunction, dendritic spine loss and spatial memory impairment in an experimental model of diabetes. Neurobiol Dis. 2015;78:1–11.

13. Ahren B. Glucagon-like peptide-1 receptor agonists for type 2 diabetes: A rational drug development. Journal of diabetes investigation. 2019;10:196–201.

14. Pfeiffer AF, Klein HH. The treatment of type 2 diabetes. Deutsches Arzteblatt international. 2014;111:69–81. quiz 2.

15. Horii T, Iwasawa M, Kabeya Y, Atuda K. Polypharmacy and oral antidiabetic treatment for type 2 diabetes characterised by drug class and patient characteristics: A Japanese database analysis. Scientific reports. 2019;9:12992.

16. Zhou J, Zhang Z, Zhou H, Qian G. Diabetic Cognitive Dysfunction: From Bench to Clinic. Current medicinal chemistry 2019.

17. Kosaraju J, Murthy V, Khatwal RB, Dubala A, Chinni S, Muthureddy Nataraj SK, et al. Vildagliptin: an anti-diabetes agent ameliorates cognitive deficits and pathology observed in streptozotocin-induced Alzheimer's disease. The Journal of pharmacy pharmacology. 2013;65:1773–84.

18. McClean PL, Parthsarathy V, Faivre E, Holscher C. The diabetes drug liraglutide prevents degenerative processes in a mouse model of Alzheimer's disease. The Journal of neuroscience. 2011;31:6587–94.

19. Pipatpiboon N, Pintana H, Pratchayasakul W, Chattipakorn N, Chattipakorn SC. DPP4-inhibitor improves neuronal insulin receptor function, brain mitochondrial function and cognitive function in

rats with insulin resistance induced by high-fat diet consumption. Eur J Neurosci. 2013;37:839–49.

20. Sarkhail P, Shafiee A, Sarkheil P. Biological activities and pharmacokinetics of praeruptorins from Peucedanum species: a systematic review. BioMed research international. 2013;2013:343808.

21. Yu PJ, Jin H, Zhang JY, Wang GF, Li JR, Zhu ZG, et al. Pyranocoumarins isolated from Peucedanum praeruptorum Dunn suppress lipopolysaccharide-induced inflammatory response in murine macrophages through inhibition of NF-kappaB and STAT3 activation. Inflammation. 2012;35:967–77.

22. Liu X, Chin JF, Qu X, Bi H, Liu Y, Yu Z, et al. The Beneficial Effect of Praeruptorin C on Osteoporotic Bone in Ovariectomized Mice via Suppression of Osteoclast Formation and Bone Resorption. Front Pharmacol. 2017;8:627.

23. Yang L, Li XB, Yang Q, Zhang K, Zhang N, Guo YY, et al. The neuroprotective effect of praeruptorin C against NMDA-induced apoptosis through down-regulating of GluN2B-containing NMDA receptors. Toxicology in vitro. 2013;27:908–14.

24. Wang L, Wang J, Yang L, Zhou SM, Guan SY, Yang LK, et al. Effect of Praeruptorin C on 3-nitropropionic acid induced Huntington's disease-like symptoms in mice. Biomedicine pharmacotherapy. 2017;86:81–7.

25. Yang L, Yang Q, Zhang K, Li YJ, Wu YM, Liu SB, et al. Neuroprotective effects of daphnetin against NMDA receptor-mediated excitotoxicity. Molecules. 2014;19:14542–55.

26. Yang L, Wang M, Guo YY, Sun T, Li YJ, Yang Q, et al. Systemic inflammation induces anxiety disorder through CXCL12/CXCR4 pathway. Brain Behav Immun. 2016;56:352–62.

27. Yang Q, Yang L, Zhang K, Guo YY, Liu SB, Wu YM, et al. Increased coupling of caveolin-1 and estrogen receptor alpha contributes to the fragile X syndrome. Ann Neurol. 2015;77:618–36.

28. Liu MG, Song Q, Zhuo M. Loss of Synaptic Tagging in the Anterior Cingulate Cortex after Tail Amputation in Adult Mice. The Journal of neuroscience. 2018;38:8060–70.

29. Mueller K, Sacher J, Arelin K, Holiga S, Kratzsch J, Villringer A, et al. Overweight and obesity are associated with neuronal injury in the human cerebellum and hippocampus in young adults: a combined MRI, serum marker and gene expression study. Translational psychiatry. 2012;2:e200.

30. Cherbuin N, Sachdev P, Anstey KJ. Higher normal fasting plasma glucose is associated with hippocampal atrophy: The PATH Study. Neurology. 2012;79:1019–26.

31. Ryan CM, Freed MI, Rood JA, Cobitz AR, Waterhouse BR, Strachan MW. Improving metabolic control leads to better working memory in adults with type 2 diabetes. Diabetes Care. 2006;29:345–51.

32. Mollon J, Curran JE, Mathias SR, Knowles EEM, Carlisle P, Fox PT, et al. Neurocognitive impairment in type 2 diabetes: evidence for shared genetic aetiology. Diabetologia 2020.

33. Whitmer RA. Type 2 diabetes and risk of cognitive impairment and dementia. Curr Neurol Neurosci Rep. 2007;7:373–80.

34. Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, et al. Role of leptin in the neuroendocrine response to fasting. Nature. 1996;382:250–2.

35. Huang X, Liu G, Guo J, Su Z. The PI3K/AKT pathway in obesity and type 2 diabetes. Int J Biol Sci. 2018;14:1483–96.

36. Shin JH, Kim KM, Jeong JU, Shin JM, Kang JH, Bang K, et al. Nrf2-Heme Oxygenase-1 Attenuates High-Glucose-Induced Epithelial-to-Mesenchymal Transition of Renal Tubule Cells by Inhibiting ROS-Mediated PI3K/Akt/GSK-3beta Signaling. Journal of diabetes research 2019;2019:2510105.

37. Jere SW, Houreld NN, Abrahamse H. Role of the PI3K/AKT (mTOR and GSK3beta) signalling pathway and photobiomodulation in diabetic wound healing. Cytokine Growth Factor Rev. 2019;50:52–9.

38. Heng LJ, Yang RH, Jia D. Diabetes impairs learning performance through affecting membrane excitability of hippocampal pyramidal neurons. Behav Brain Res. 2011;224:250–8.

39. Bliss TV, Collingridge GL. Expression of NMDA receptor-dependent LTP in the hippocampus: bridging the divide. Mol Brain. 2013;6:5.

40. Winocur G, Greenwood CE, Piroli GG, Grillo CA, Reznikov LR, Reagan LP, et al. Memory impairment in obese Zucker rats: an investigation of cognitive function in an animal model of insulin resistance and obesity. Behavioral neuroscience. 2005;119:1389–95.

41. Stranahan AM, Norman ED, Lee K, Cutler RG, Telljohann RS, Egan JM, et al. Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats. Hippocampus. 2008;18:1085–8.

42. Cyranoski D. Why Chinese medicine is heading for clinics around the world. Nature. 2018;561:448–50.

43. Uner A, Goncalves GH, Li W, Porceban M, Caron N, Schonke M, et al. The role of GluN2A and GluN2B NMDA receptor subunits in AgRP and POMC neurons on body weight and glucose homeostasis. Molecular metabolism. 2015;4:678–91.

44. Wang S, He B, Hang W, Wu N, Xia L, Wang X, et al. Berberine Alleviates Tau Hyperphosphorylation and Axonopathy-Associated with Diabetic Encephalopathy via Restoring PI3K/Akt/GSK3beta Pathway. Journal of Alzheimer's disease: JAD. 2018;65:1385–400.

45. Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature. 1993;361:31–9.

46. Huang EP. Synaptic plasticity: going through phases with LTP. Current biology. 1998;8:R350-2.
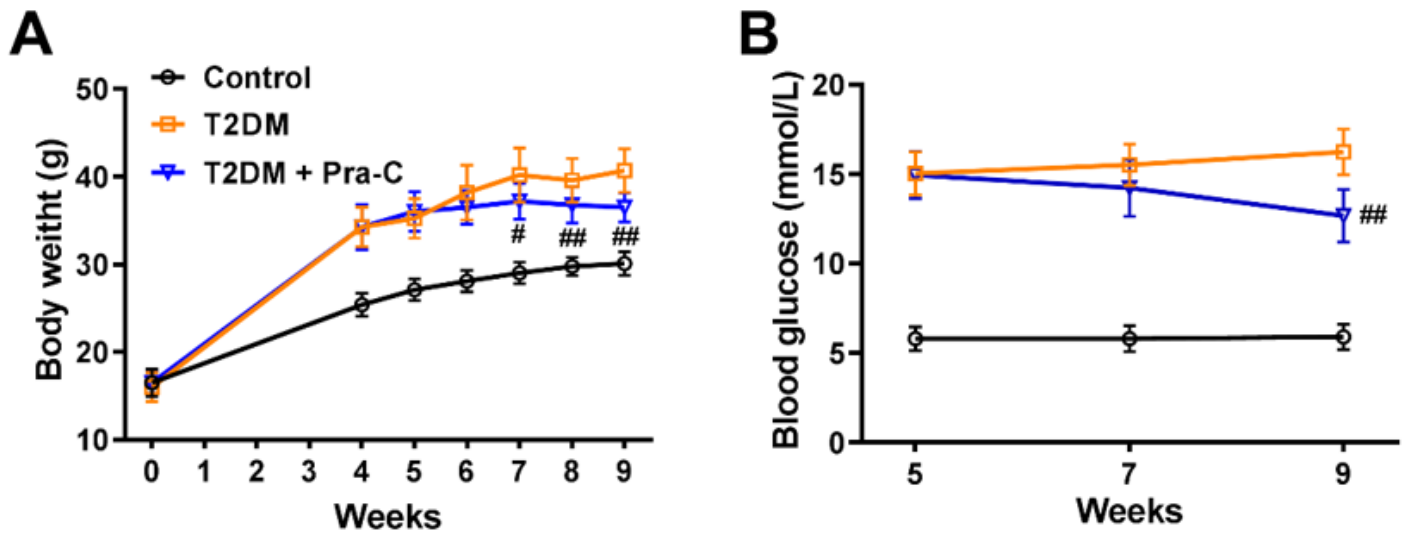
Figures

Figure 1

Pra-C reduced body weight and fasting blood glucose in T2DM mice (a) Changing of body weight detected weekly after 4 weeks of HFD feeding. (b) Levels of four-hour fasting blood glucose every two weeks after establishment of T2DM model. Values are expressed as mean ± SEM (n = 12 in each group). #p◻0.05, ##p◻0.01 vs. T2DM group.
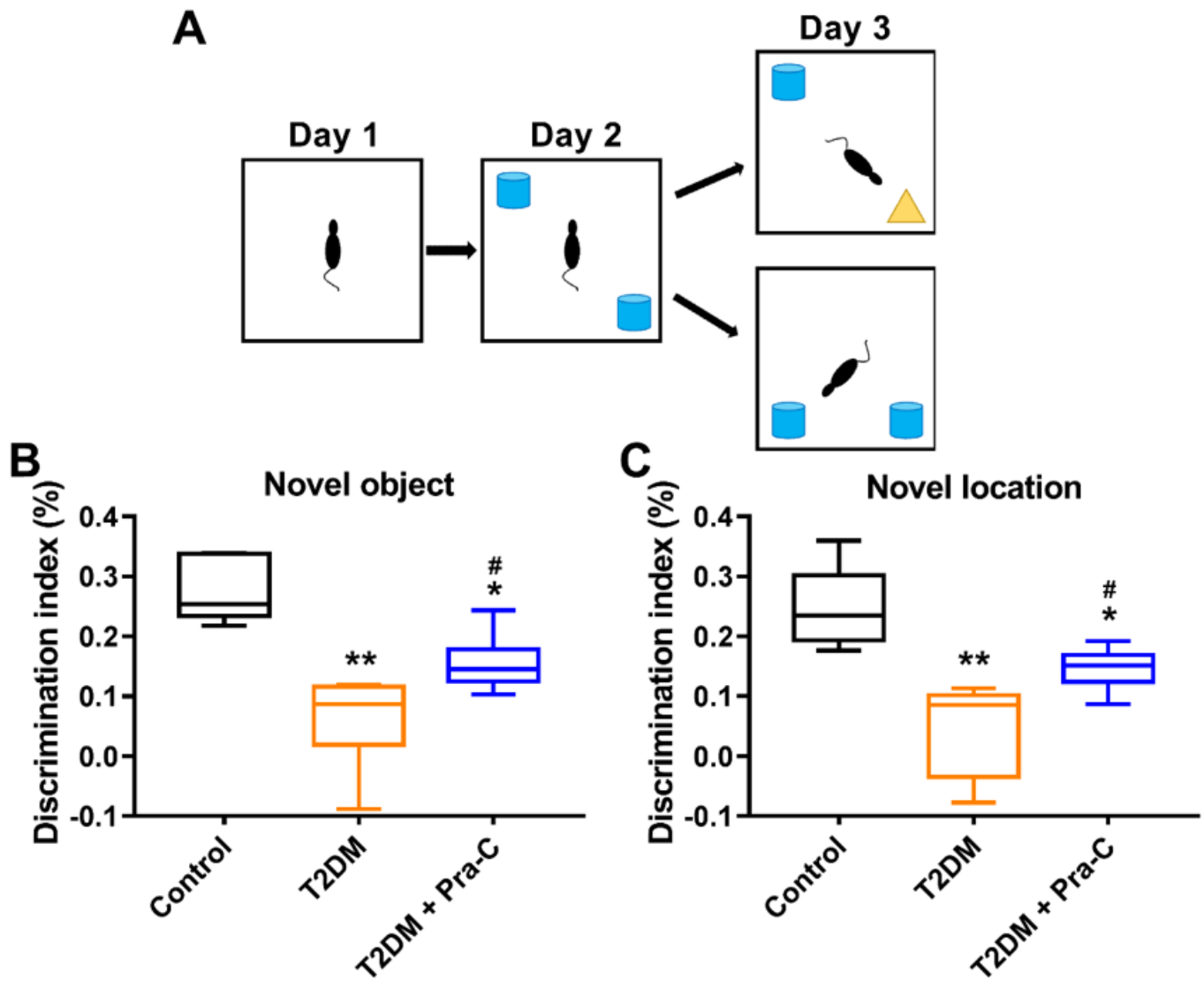
Figure 2

Improvement of novel object recognition by Pra-C in T2DM mice (a) Schematic of the novel object recognition test, mice of each group were randomly average assigned to different tests on day 3. Summary of discrimination index for a novel object (b) and novel location (c). Values are expressed as mean ± SEM (n = 6 in each group).*p⬚0.05,**p⬚0.01 vs. control;#p⬚0.05 vs. T2DM.
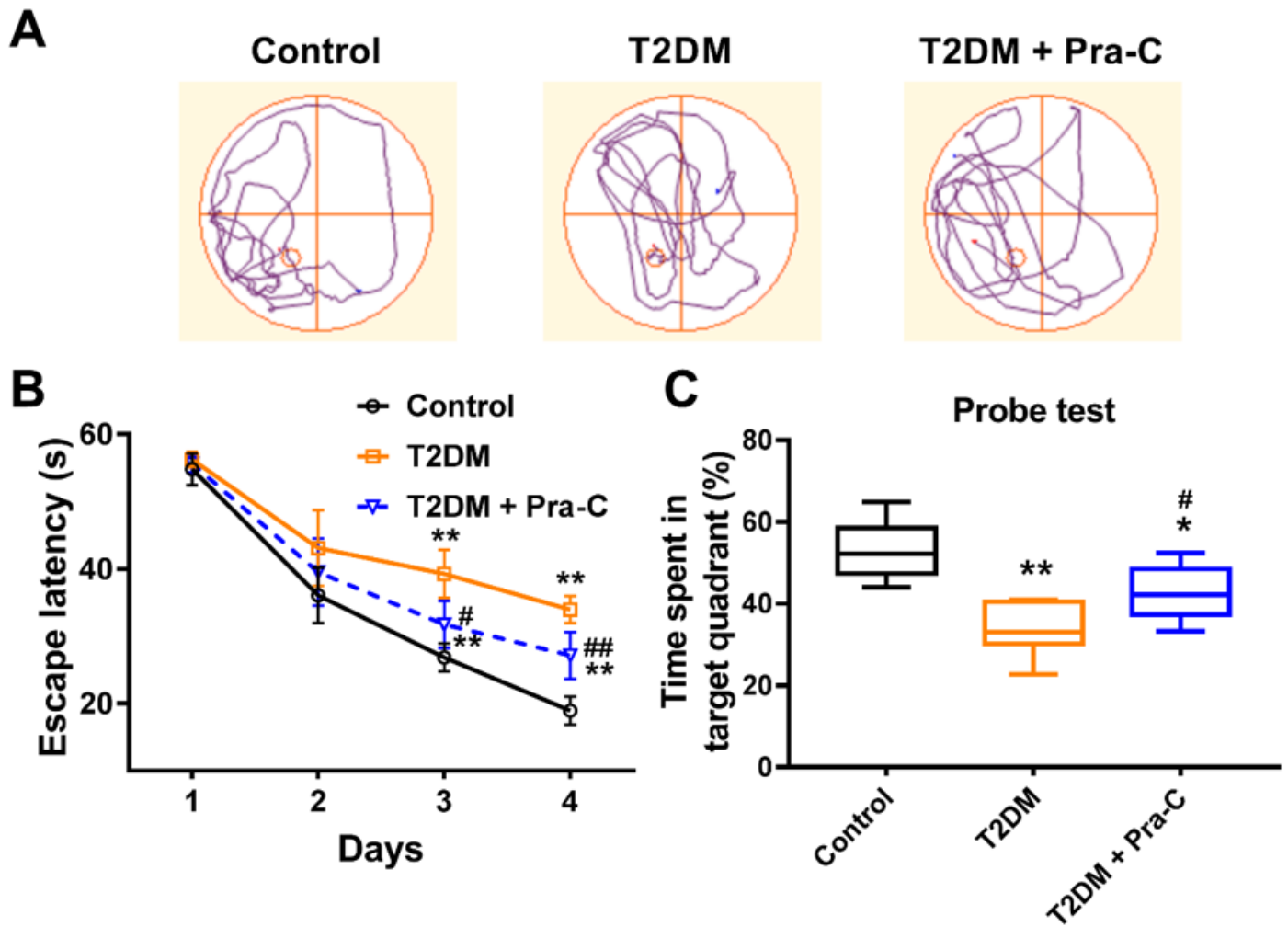
Figure 3

Improvement of spatial learning and memory by Pra-C in T2DM mice (a) Representative swimming paths of mice from different groups in water maze during probe test. (b) Latency of mice to located platform position during learning phase of water maze test. (c) Percentage of time spent in the target quadrant of water maze during probe test. Values are expressed as mean ± SEM (n = 12 in each group).*p⬚0.05, **p⬚ 0.01 vs. control; #p⬚0.05, ##p⬚0.01 vs. T2DM.
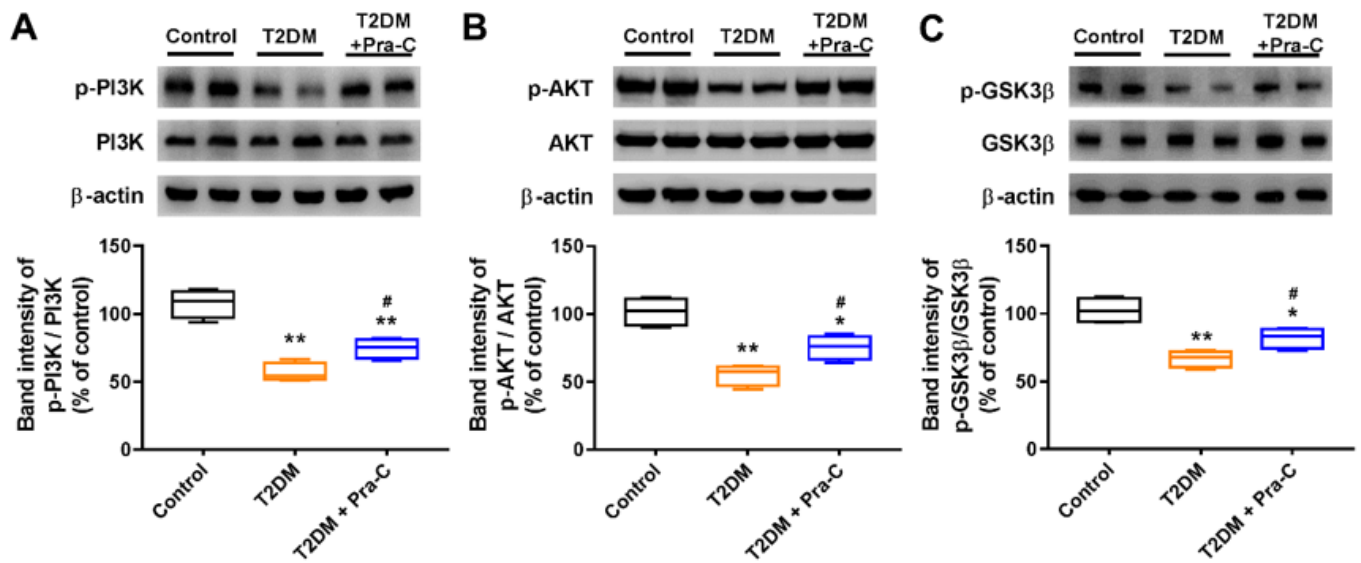
Figure 4

Pra-C restored PI3K/AKT/GSK3β signaling pathway in the hippocampus Phosphorylation levels of Tyr607 PI3K (a), Ser308 AKT (b), and Ser 9 GSK3β (c) and the correspondent total form proteins. Upper: representative bands of Western blot. Lower: Band intensities quantified as percentage of values from control mice. Values are expressed as mean ±SEM (n = 6 for each group).*p◻0.05, **p◻0.01 vs. control;#p◻ 0.05 vs. T2DM.
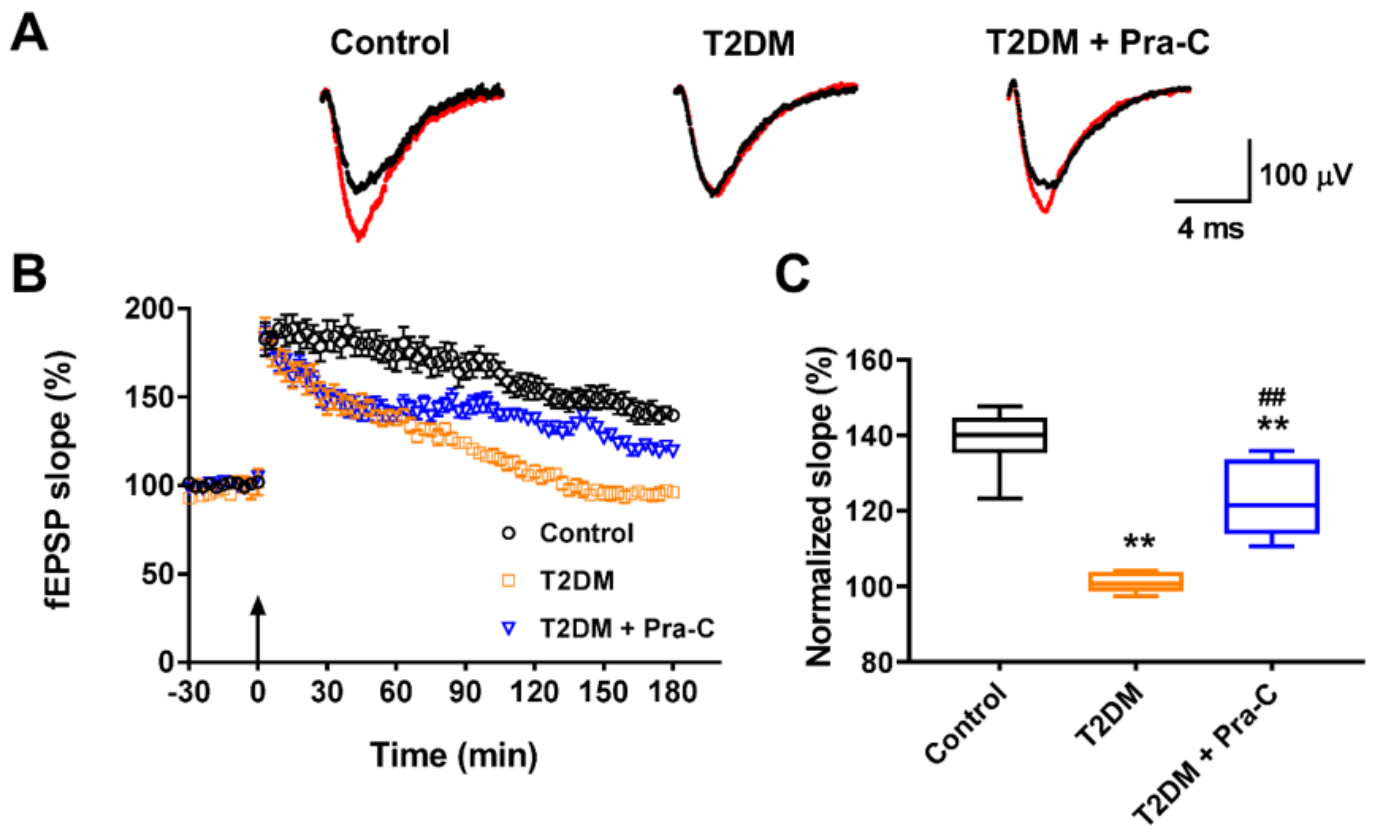
Figure 5

Pra-C rescued L-LTP in hippocampal CA1 of T2DM mice. (a) Representative average traces of last 5 min of baseline (black) and last 5 min of whole recording (red). (b) fEPSP slope recorded from hippocampal CA1 region. Arrow indicates the time point of TBS training. (c) Average fEPSP slope of last 30 min during whole recording. Values are expressed as mean ± SEM (n = 8 slices/4 mice for each group). **p◻0.01 vs. control; ##p◻0.01 vs. T2DM.