**Pentamethylquercetin Protects Against Diabetes-Related Cognitive Deficits in Diabetic Goto-Kakizaki Rats**

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**Abstract.** Diabetic patients have a significantly higher risk of developing all forms of dementia. Pentamethylquercetin (PMQ) has been proven to have potential as an anti-diabetic agent. Nevertheless, whether PMQ can improve diabetes-induced cognitive dysfunction has not been investigated. To address this, we evaluated the effectiveness and underlying mechanisms of PMQ for ameliorating diabetes-related cognitive dysfunction in vivo and in vitro. Our results showed that Goto-Kakizaki (GK) rats displayed impairment in their learning abilities and memory capabilities. Furthermore, GK rats reflected cognitive dysfunction in proportion to the intensity of insulin resistance index. In addition, dendritic spine density and the % cell viability significantly decreased in hippocampus neurons. High glucose conditions induced hippocampal neurons damage, inflicted dendritic spine dysontogenesis, and reduced Akt/cAMP response element-binding protein activation. Treatment with PMQ in GK rats significantly ameliorated cognitive deficits and neuronal damage and increased dendritic spine density, at least in part, by improving insulin resistance and metabolic disorders. Furthermore, PMQ significantly activated the Akt/cAMP response element-binding protein pathway and increased the expression of memory-related proteins in the downstream part of the Akt/cAMP response element-binding protein pathway, such as synaptophysin and glutamate receptor 1. In addition, PMQ inhibited high glucose-induced cellular toxicity. LY294002 appeared to partly inhibit PMQ-mediated protective effects in hippocampal neurons. The results suggest that insulin resistance could predominantly reduce Akt/cAMP response element-binding protein activation in the brain, which is associated with a higher risk of cognitive dysfunction. PMQ could provide a new potential option for the prevention of cognitive dysfunction in diabetes.

**Keywords:** Akt/cAMP response element-binding protein pathway, cognitive deficits, dendrite, diabetes, pentamethylquercetin

**INTRODUCTION**

With the increase of global population life expectancy and change of lifestyle, the morbidities of diabetes mellitus are increasing at an alarming rate and have become major public health problems [1]. Many epidemiological studies have shown that the development of diabetes mellitus leads to a higher risk...
of developing all forms of dementia, including vascular dementia and Alzheimer’s disease (AD) [2–5]. Although cognitive deficits associated with diabetes have been reproduced in studies of rodent models of diabetes [6–9], understanding the mechanism(s) responsible for cognitive dysfunction in diabetes is of paramount scientific and medical importance. Dendritic spine morphology plays a central role in brain development, plasticity, and cognitive function. Several studies have demonstrated that diabetes can cause a reduced number of dendritic spines and a decreased total length of dendrites of pyramidal neurons of the hippocampus in streptozotocin (STZ)-treated rats [10], a diet-induced insulin resistance [11] model and db/db mouse [12]. However, there is no report whether the morphology and density of dendritic spines are abnormal in type 2 diabetic Goto-Kakizaki (GK) rats, which are spontaneously diabetic, non-obese rodents from selectively breeding Wistar rats showing high levels of blood glucose in oral glucose tolerance tests and display several characteristics of type 2 diabetes mellitus. Akt/cAMP-responsive element-binding protein (CREB) pathway is involved in the learning and memory processes, and when the Akt/CREB pathway has been inhibited in the hippocampus, the expression of memory-related proteins are significantly down-regulated, ultimately lead to behavioral deficits [13–17]. However, whether Akt/CREB pathway activity is inhibited in GK rats remains confusing and unresolved.

Polymethoxylated flavones (PMF) are a group of highly methoxylated phenolic compounds that are abundant in nature. Numerous reports have demonstrated that a few PMF family members possess health beneficial effects such as improvement in insulin resistance [18–20], and anti-inflammatory [21], anti-Akt/CREB pathway, and display several characteristics of type 2 diabetes mellitus. Akt/cAMP-responsive element-binding protein (CREB) pathway is involved in the learning and memory processes, and when the Akt/CREB pathway has been inhibited in the hippocampus, the expression of memory-related proteins are significantly down-regulated, ultimately lead to behavioral deficits [13–17]. However, whether Akt/CREB pathway activity is inhibited in GK rats remains confusing and unresolved.

Twelve-week-old (male) GK rats were purchased from Shanghai Slac Laboratory Animal Co. LTD (China), while age-matched healthy Wistar rats were purchased from the Center of Experimental Animals (Tongji Medical College, Huazhong University of Science and Technology, China). The animals were maintained in a temperature- and humidity-regulated room (22 ± 2°C; 50 ± 5%, respectively) with controlled lighting (12-h light/dark cycle). The experimental protocol was approved by the Animal Care and Welfare Committee of Huazhong University of Science and Technology. All rats were acclimatized to their environment for at least 1 week prior to the beginning of the study.

PMQ was synthesized by the Food and Drug Evaluation Center of Tongji Medical College at Huazhong University of Science and Technology at a purity of 99.5% as examined by HPLC [31]. The rats were randomized into six groups as follows (n = 9–12): GK group; PMQ groups (GK rats given PMQ at 2.5, 5, 10 mg kg\(^{-1}\) of PMQ for 16 consecutive weeks. Tap water was provided ad libitum. Food and water intakes were measured every 2 days, and rats were weighed weekly.

### Biochemical parameters assay

During the experiment, blood glucose levels of the rats were monitored at the 4th, 8th, and 16th week after the beginning of intervention. Blood glucose levels were measured with a portable glucometer (Roche Group, Switzerland). Briefly, blood was withdrawn from the rats using tail vein rupture method, and drop of blood was placed on the glucometer strip loaded
in the glucometer for blood glucose determination. Serum insulin concentration was measured by radioimmunoassay (the 4th and 16th week after treatment). Oral glucose tolerance test (OGTT) was performed at the end of the study (the 16th week after PMQ and MET treatment). Briefly, rats were fasted overnight (12 h) and glucose was administered orally to the rats at a dose of 2.5 g kg\(^{-1}\) body weight. Serum glucose levels of 0, 0.5, 1, and 1.5 h were measured. The results of OGTT were also expressed as integrated area under the curve (AUC) for glucose concentrations. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by the following equations: 

\[
\text{HOMA-IR} = \frac{\text{fasting glucose (mmol/l)} \times \text{fasting insulin (mU/ml)}}{22.5} \tag{32}
\]

Most of the PMQ treatment groups (2.5, 5, and 10 mg/kg) were selected for further Golgi, Nissl, and immunohistochemical staining and western blotting analysis by biochemical parameters.

**Assessment of learning and memory by Morris water maze**

The learning and memory abilities of rats in the different groups were assessed by Morris water maze [33–35] on the 16th week. The water-maze apparatus (Chinese Academy of Medical Sciences, Institute of Materia Medica, Beijing, China) consisted of a large circular pool (214 cm in diameter, 50 cm in height, filled to a depth of 30 cm with water at 22 ± 1°C), which was divided into four equally spaced quadrants: North (N), South (S), East (E), and West (W). The water was made opaque with black colored dye. A circular platform (10 cm in diameter) was placed at the SW quadrant 2 cm below the surface of the water. Each rat was subjected to four consecutive trials on each day with a gap of 5 min and allowed 120 s to locate the submerged platform. Then, it was allowed to stay on the platform for 15 s. If it failed to find the platform within 120 s, it was guided gently onto the platform and allowed to remain there for 15 s. Escape latency time was noted as the index of acquisition or learning. The rat was subjected to acquisition trials for five consecutive days. The platform was removed and each rat was allowed to explore the pool for 15 s on the sixth day, then the visible platform was raised above the water and each rat was allowed to explore the pool for 15 s. The time spent in all four quadrants was noted as the index of retrieval. The swimming velocity was measured. All the trials were completed between 9:00 a.m. and 17:00 p.m.

**Golgi staining and morphological analysis of dendritic spines**

To analyze dendritic spine morphology in brain, FD Rapid GolgiStain Kit (FD Neuro Technologies, Ellicott City, MD, USA) was used. After completion of the behavioral tests (the 16th week after treatment), rats were deeply anesthetized with 10% chloral hydrate (n = 5 per group), and the brains of the rats were immersed in Solution A and B for 2 weeks at room temperature and transferred into Solution C for 24 h at 4°C. Brains were sectioned at 200 µm thickness in the coronal plane at the level of hippocampus using a VT1000S Vibratome (Leica, Bannockburn, IL, US). Dendritic images were acquired by Axioplan 2 (Zeiss, Oberkochen, Germany) under bright-field microscopy. Spine width, length, and linear density were measured using Neurol software [36]. All morphological analysis was done blind to experimental conditions.

**Measurement of neuronal density by Nissl staining**

The brains were fixed with 4% paraformaldehyde overnight and embedded in paraffin before coronal sections (5 µm thick) of the dorsal hippocampus were cut using a rotary microtome (n = 5 per group). The brain sections were stained with toluidine blue. CA1 subregions of bilateral hippocampi from each animal were captured and quantitative analysis of cells was performed using Imaging-Pro-Plus (LEIKA DMLB).

**Immunohistochemical staining**

After dehydrating and embedding in paraffin, 5-µm thick sections were prepared (n = 3 per group). Briefly, endogenous peroxidase was quenched with 0.3% H\(_2\)O\(_2\) in distilled water for 10 min. Nonspecific immunoglobulin binding sites were blocked with normal goat serum for 1 h., and then the sections were incubated with rat synaptophysin (SYP) or GluR1 monoclonal antibody (Wuhan Boster, China) at 4°C overnight. The sections were then incubated for 1 h with secondary antibody at room temperature. Immunoglobulin complexes were visualized on incubation with DAB. Results were recorded with a magnifying digital camera and quantified by mean optical intensity in five random microscopic fields per section at a magnification of ×10.
Primary hippocampal neuron cultures

Primary cultures of rat hippocampal neurons were prepared from the hippocampus of E18-E19 Wistar rat embryos. In brief, the rats were decapitated and the hippocampus was carefully isolated. After being washed twice using D-Hank’s buffer, the hippocampus was digested with equal volumes of 0.125% trypsin/ethylene diamine tetraacetic acid (EDTA) for approximately 15 min at 37°C. The hippocampus was then washed with Hank’s solution containing 10% fetal bovine serum (Biochrom, Cambridge, UK) to stop digestion. Then, neurons were plated in six-well plates (8.75 x 10^4 cells/cm^2) or in 96 well plate plates (0.85 x 10^4 cells/cm^2). The cultured neurons were incubated with DMEM in a humidified 5% CO_2 incubator at 37°C for 6 h, and the medium was then replaced with Neurobasal/B27 medium. Neurobasal/B27 medium containing 25 mM glucose (control condition) is intended to give optimal growth and long-term survival to rat embryonic hippocampal neurons. After 7 days in culture, cells were incubated with 50, 75, 100, or 125 mM of glucose or with 75 mM mannitol (plus 75 mM mannitol in normal medium), which was used as an osmotic control, and maintained for a further 24, 48, 72, and 96 h. The optimal time point and glucose concentration were selected for further study as a representation of hyperglycemic condition. At day 8, hippocampal neurons were incubated with various concentrations of PMQ (0.1, 0.3, 1, 3, and 10 μM; PMQ was dissolved in dimethylsulphoxide) or vehicle under this optimal time point and glucose concentration. To study the mechanism involved in PMQ effects on high glucose-induced neurotoxicity, hippocampal neurons were incubated under this optimal time point and glucose concentration with PMQ (1 μM), either with or without LY294002 (a specific inhibitor of PI3 kinase; 50 μM; Beyotime Institute of Biotechnology, China).

Cell viability assay and cell imaging

Cell viability was determined using a modified 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT) (Sigma, St Louis, MO) assay. Briefly, cells were seeded onto flat-bottomed 96-well culture plates. After removing the medium, MTT solution (5 mg/ml in phosphate buffer saline) was added for 4 h resulting in the production of formazan dye, which was then solubilized in 200 μl dimethyl sulfoxide. The absorbance was then quantified by measuring optical density at 570 nm (630 nm as a reference). Cell images were taken using a Zeiss Observer.Z1 microscope.

Western blotting analysis

The hippocampus samples and hippocampal neurons were lysed in RIPA buffer containing 150 mM NaF, 2 mM sodium orthovanadate, and protease inhibitors (protease inhibitor mixture; Roche). A 25% homogenate of total lysate (30 μg) was loaded and blotted. Primary antibodies against phospho-Akt (Ser473), phospho-CREB (Ser133) (Cell Signalling Technology, Inc., Beverly, MA, USA), GluR1, and SYP (Wuhan Boster, China) were used. The proteins were detected using horseradish peroxidase-conjugated anti-rabbit, or anti-mouse secondary antibodies. The optical density values of bands were measured with NeuroJ software and were normalized using anti-total-Tubulin, anti-total-CREB, anti-total-Akt (Wuhan Boster, China), and anti-Tubulin (Cell Signalling Technology, Inc.) as an internal control (optical density detected protein / optical density internal control).

Statistical analysis

The data of behavior, body weight, and glucose levels were made with two-way ANOVA with repeated measures, the factors being treatment and training day. The other data were analyzed with one-way ANOVA followed by Tukey’s Honestly Significant Difference post hoc test. Results were expressed as mean ± S.E.M. Statistical significance was considered at p < 0.05 in all the cases.

RESULTS

Pentamethylquercetin ameliorates polyphagia, polydipsia, weight loss, and hyperglycemia in metabolic features of the GK rats

The effectiveness of dietary PMQ in alleviating metabolic disorder was assessed in GK rats, and common metabolic disorder indices associated with diabetes were measured. Our data showed that GK rats exhibited significantly reduced weight gain and increased food and water intake (p < 0.01 versus Wistar group; Fig. 1A, D). Moreover, GK rats also exhibited increased levels of blood glucose, fasting insulin, and glucose intolerance and showed a significant increase in insulin resistance index (p < 0.01 versus Wistar...
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Fig. 1. Effect of pentamethylquercetin (PMQ) treatment on body weight, food and water intake, and metabolic features of GK rats. A) Daily food (left) and water (right) intakes of the rats at 16 weeks after intervention. Significant differences were present in the Wistar rats, PMQ-treated groups, and Metformin (MET) group compared to the Goto-Kakizaki (GK) group after 16 weeks of treatment. B) Glucose levels of the rats at 16 weeks post intervention were significantly lower in the PMQ-treated GK rats compared to the GK rats. C) Fasting insulin levels of the rats at the 8th week after intervention were significantly increased in the GK group compared to all PMQ-treated groups. D) Body weights of the rats in the PMQ (10 mg kg\(^{-1}\))-treated group, MET group, and Wistar groups were significantly increased compared to the GK group after 4 weeks of treatment (\(p<0.01\)). E) At 16 weeks after treatment, the PMQ-treated groups, MET group, and Wistar groups had relatively lower glucose levels at 120 min compared to GK rats (PMQ 2.5, 5 mg kg\(^{-1}\), \(p<0.05\); PMQ 10 mg kg\(^{-1}\), \(p<0.01\); MET, \(p<0.05\); Wistar, \(p<0.01\)). F) PMQ treatment significantly decreased the AUC of GK rats by the 16th week after treatment. All data are represented as mean ± S.E.M for 9–12 rats in each group, *\(p<0.05\) and **\(p<0.01\) versus GK group, *\(p<0.01\) versus Wistar group.

PMQ improves diabetes-induced cognitive dysfunction

In the Morris water maze, GK rats displayed significantly increase escape latencies and swimming distance in the hidden platform paradigm (Fig. 2A-B), while spending less time in the target quadrant in a probe trial (Fig. 2C) compared with the Wistar rats (\(p<0.01\)). PMQ treatment (2.5, 5, and 10 mg/kg) significantly decreased escape latency and swimming distance and increased the time spent in target quadrant compared to GK rats group (\(p<0.01\)). Treatment with MET (300 mg/kg) in GK rats showed similar
Fig. 2. Effects of pentamethylquercetin (PMQ) on diabetes-induced cognitive dysfunction. Escape latency (A) and swimming distance (B) during training trials compared to Wistar rats ($p < 0.01$). PMQ treatment (2.5, 5, and 10 mg kg$^{-1}$) significantly decreased escape latency and swimming distance compared to GK rats ($p < 0.01$). Treatment with MET (300 mg kg$^{-1}$) showed similar results. C) Swimming time spent in each quadrant in the probe trial on day 6 (T, target; L, left; O, opposite; R, right). PMQ treatment at all doses significantly prevented memory impairment, as indicated by the increase in the time spent in target quadrant ($p < 0.01$). D) Representative path tracings of the probe test. E) Insulin resistance index in all groups. F) Correlation analysis revealed a positive correlation between insulin resistance index and escape latency. Each value represents mean ± S.E.M. for 6–8 animals, *$p < 0.05$ and **$p < 0.01$ versus GK group; ***$p < 0.01$ versus Wistar group.

results, as shown in Fig. 2A–C. The performance of all groups in the trial with the visible platform was not significantly different (data not shown), indicating that visual function did not differ among the different groups. Furthermore, velocities of all the groups were similar (data not shown) indicating that motor performance was unaffected by the hyperglycemia and/or treatments. Together, these results show that GK rats have memory impairment and PMQ at all doses significantly prevented the memory impairment. To evaluate a possible association between metabolic changes and cognitive behavior, the correlation of insulin resistance levels with memory was assessed. Our results demonstrated that the escape latency time varied in proportion to the insulin resistance ($r = 0.946, p < 0.01$), which suggests that memory performance may rely on levels of insulin resistance index (Fig. 2F).
PMQ attenuates hippocampus neuronal damages and enhances hippocampus dendritic spine density of the GK rats

Given that treatment with PMQ diminished cognitive dysfunction resulting from diabetes, the neuroprotective effects of PMQ on neurons and dendritic spine morphology was evaluated by measuring the neuronal cell viability, the loss of neuronal cells, dendritic spine density, and length in CA1 hippocampal region after 16 weeks of treatment. CA1 pyramidal neurons of all groups from four hemispherical sections were counted and averaged. As shown in Fig. 3, in the CA1 sectors of GK rats group, cells were sparsely arranged and the cell shapes were fuzzy; cells with eumorphism were significantly reduced; the % cell viability was significantly lower ($p < 0.01$ versus Wistar group; Fig. 3A1-E). Nevertheless, in the PMQ (10 mg/kg) group, and MET groups, in contrast, cell shapes were clear and the cell structure was compact; cells were relative large and had abundant cytoplasm and Nissl bodies. The % cell viability was significantly higher ($p < 0.01$ versus GK group; Fig. 3A1-E).

As shown in Fig. 3F-G, the Golgi-Cox impregnation procedure clearly filled the dendritic shafts and spines of pyramidal neurons of hippocampus ($n = 4$ rats/group). The dendritic spines density was significantly decreased in the CA1 hippocampus of the GK rats compared with the Wistar rats ($p < 0.01$). The administration of PMQ (10 mg/kg) and MET significantly increased the dendritic spine density compared with the GK rats group ($p < 0.01$ versus GK group; $n = 56$ neurons/group; Fig. 3H). However, the dendritic length of the pyramidal cells of the CA1 hippocampus was not significantly different in all groups (Fig. 3I).
PMQ enhances Akt/CREB signaling pathways activity and increases synaptophysin and GluR1 expression in the hippocampus of GK rats

The CREB protein is a member of the large family structurally related transcription factors, which regulated the expression of memory-related proteins in the healthy brain. On the basis of this, we further examined Akt/CREB pathway activity and the expression of SYN and GluR1. The basal expression of SYN and GluR1 and Akt/CREB pathway activity were lower in the GK rat group (p < 0.01 versus Wistar group), but were significantly improved by 16 weeks treatment with PMQ or MET (p < 0.01 versus GK group) (Fig. 4).

PMQ reversed high glucose-induced cellular toxicity and enhanced hippocampal neurons viability

Hippocampal neurons play a crucial role in dictating cognitive function. To understand high glucose-induced cellular toxicity and the neuroprotective effect of PMQ on neurons during the progression of diabetes-induced cognitive dysfunction, cultured primary hippocampal neurons were used. As shown in Fig. 5, when incubating cells for 24 h, we did not find any detrimental effect of high glucose on cell viability at concentrations ranging from 50 to 125 mM, when incubated for 48, 72, and 96 h, cell activities were inhibited. At 72 h with 100 mM of glucose, the hippocampal neuron activity dropped to 58.8% (p < 0.01 versus control group). So this time point and glucose concentration were selected for further study as a representation of high glucose condition. To support the in vivo findings reported above, hippocampal neurons grown in high glucose media were treated with PMQ. As expected, the cells treated with 0.1, 0.3, 1, 3, and 10 μM of PMQ in the presence of 100 mM glucose for 72 h exhibited higher viability than the high glucose group (1 and 3 μM of PMQ: p < 0.01; 10 μM of PMQ: p < 0.05; Fig. 5E). Similarly, we have found that the high glucose condition can lead to shortening dendrite of hippocampal neurons, decreasing the numbers of the branches, and influencing the normal growth of neuronal cells, while PMQ (1 μM) can ameliorate the pathological changes. Pretreatment with LY294002
resulted in a partial loss of PMQ-mediated protective effects in hippocampal neurons (Fig. 5F).

**High glucose-induced hippocampal neuron growth inhibition and damage may be reversed by activation of Akt/CREB signaling pathways**

To further explore molecular mechanism(s) of how PMQ relieves high glucose-induced hippocampal neuron growth inhibition and damage, we studied the expression of p-Akt, p-CREB, SYP, and GluR1 protein. Consistent with our *in vivo* data, high glucose down-regulated p-Akt, p-CREB, SYP, and GluR1 protein (*p* < 0.01 versus control group; Fig. 6). Concurrent treatment with 1 μM PMQ resulted in elevations in p-Akt, p-CREB, SYP, and GluR1 protein (*p* < 0.01 versus vehicle group; Fig. 6). When hippocampal neurons were pretreated with LY294002 for 30 min, PMQ did not increase the levels of p-Akt, p-CREB, SYP, or GluR1 protein. Taken together, these results suggest that PMQ is able to confer neuroprotection in diabetic conditions by enhancing Akt/CREB pathway activity.

**DISCUSSION**

Multiple studies have reported that patients with diabetes mellitus have an increased risk of developing AD compared to age- and gender-matched controls. Meanwhile, accumulating evidence suggests that AD is closely related to dysfunction of both insulin signaling and glucose metabolism in the brain, prompting some investigators to refer to AD as type 3 diabetes or an insulin-resistant brain state [37–40]. Some scholars have reported that antihyperglycemics and insulin sensitizers reduce cognitive dysfunction in the diabetic condition [41, 42]. The precise mechanisms underlying type 2 diabetes mellitus-related cognitive dysfunction or the development of dementia, especially AD-type dementia, remain to be elucidated; however, several hypothetical mechanisms have been proposed.

Type 2 diabetes mellitus is characterized by insulin resistance and/or hyperinsulinemia. It is generally agreed that insulin located within the brain is mostly of pancreatic origin, having passed through the blood-brain barrier. Brain insulin signaling plays crucial roles in the regulation of food intake, body weight,
Fig. 6. Pentamethylquercetin (PMQ) increases the levels of p-Akt, p-CREB, SYP, and GluR1 in the hippocampal neuronal cells (n = 3, PMQ 1 μM). A) Representative immunoblot for p-Akt (Ser473), total-Akt (t-Akt), p-CREB (Ser133), total-CREB (t-CREB), SYP, GluR1, and α-tubulin in all treated groups. B) Relative density analysis of the phosphorylated Akt and CREB and SYP and GluR1 protein bands. The relative density is expressed as the ratio. Each value represents mean ± S.E.M, #p < 0.05 and ##p < 0.01 versus high glucose (HG) + vehicle (Vh) group, ∗∗p < 0.01 versus Control group.

and reproduction, as well as in learning and memory [43]. Defective insulin signaling is associated with decreased cognitive ability and development of dementia, including AD. It was demonstrated that insulin signaling in the CNS prevents the pathologic binding of amyloid-β oligomers. Disruption of insulin signaling has made cortical and hippocampal neurons more vulnerable to amyloid-β and tau toxicity, thus accelerating neuronal dysfunction. In the nervous system, decreased Akt signaling is a feature of neuronal dysfunction. Akt signaling is crucial for cell survival and normal cell function [5]. Some scholars speculate that insulin resistance accelerates neuronal dysfunction by preventing neurons from responding to the neurotrophic properties of insulin and rendering them more susceptible to a variety of injurious stimuli [5, 44–46]. In the present study, we demonstrate that GK rats display lowered learning performance on the Morris water maze, indicating impairment in their learning abilities and memory capabilities. In particular, we show that GK rats display cognitive dysfunction in proportion to the intensity of insulin resistance index. Given that insulin-stimulated Akt phosphorylation is decreased in insulin resistance and the Akt/CREB signaling pathways play a major role in synaptic plasticity and cognitive functions, we examined Akt/CREB pathway activity. Our results show that Akt/CREB activities are inhibited in the pyramidal neurons of the GK hippocampus and that the expression of memory-related proteins in the downstream part of the Akt/CREB pathway, such as SYP and GluR1, is significantly down-regulated.

High glucose concentration, a major pathological characteristic of diabetes, may have toxic effects on neurons in the brain through oxidative stress, which can inflict cellular damage and dendritic spine dysontogenesis and activate a number of cellular stress-sensitive pathways to induce hippocampal neuron dysfunction. The maintenance of chronic high glucose also leads to the enhanced formation of advanced glycation endproducts, which have potentially toxic effects on neurons. It was demonstrated that insulin-stimulated Akt phosphorylation is reduced in high glucose conditions in cortical neuron cultures [44, 47]. This process is implicated as an underlying mechanism in diverse neurodegenerative diseases, including AD. In vitro, we researched high glucose-induced cellular toxicity on neuronal cells. Our results demonstrate that incubating with elevated glucose levels is able to induce hippocampal neuron damage, inflict dendritic spine dysontogenesis, and reduce Akt/CREB activation. Our present experimental data demonstrate that insulin resistance predominantly reduces Akt/CREB activity in the brain, and it is associated with a higher risk of cognitive dysfunction. Overall, we provide evidence supporting the harmful impact of diabetes on the brain, impacting synaptic plasticity and cognitive function (Fig. 7).

We have evaluated whether PMQ has a protective effect against diabetes-induced cognitive dysfunction
and high glucose-induced cellular toxicity and investigated the potential mechanism underlying its action. Importantly, our results demonstrate that treatment with PMQ might improve glucose metabolism and insulin resistance, increase dendritic spine density and the % cell viability in hippocampus neurons, enhance Akt/CREB activation, ameliorate diabetes-induced cognitive dysfunction, and reverse high glucose-induced cellular toxicity. LY294002 (inhibitor of PI3 kinase) appears to partly inhibit PMQ-mediated protective effects in hippocampal neurons. It is intriguing to compare the cognitive function effects of PMQ with Metformin. Based on the data presented here, PMQ and Metformin share a number of beneficial features, e.g., the improvement of serum glucose, insulin resistance, and the learning and memory behaviors. These results suggest that PMQ and Metformin may ameliorate diabetes-induced cognitive dysfunction by improving insulin resistance. On the basis of these results, we speculate that the mechanism for PMQ to ameliorate cognitive deficits and neuronal damage may be because of its capacity in ameliorating insulin resistance of the hippocampal neuron, reversing deregulation of glucose metabolism and activating the Akt/CREB pathway in the hippocampus of GK rats. Meanwhile, the antioxidant properties of PMQ may also be involved. A diagram of the neuroprotective effects of PMQ against diabetes-induced cognitive dysfunction in the GK rat is shown in Fig. 7.
In conclusion, our study suggests that PMQ exerts its beneficial effects on cognitive dysfunction of GK rats, which may be attributed to its anti-diabetic properties. Therefore, PMQ could be recommended as a possible candidate for the prevention and therapy of cognitive deficits in type 2 diabetes mellitus and AD. However, further studies are needed to elucidate the exact mechanism of PMQ in treating cognitive dysfunction.

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