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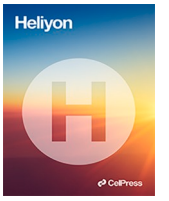

Research article

The effect and mechanism of palmar ginseng in type 2 diabetic cognitive impairment

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ABSTRACT

Objective: To investigate the therapeutic effect of palmar ginseng on cognitive impairment in rats with type 2 diabetes, evaluate its neuroprotective effects, and explore its underlying mechanism.
Methods: A rat model of diabetic cognitive impairment (DCI) was established by feeding with homemade high-fat, high-sugar chow combined with intraperitoneal injection of streptozotocin (STZ). Rats were continually fed high-fat, high-sugar chow for 60 days after successful induction of the model. Palmar ginseng was administered via gavage. The Morris test was performed after 30 days of treatment. At the end of the test, blood samples were collected, and the activities of IL-6, IL-10, TNF-α, and IL-1β in rat serum. Pathological changes in hippocampal tissues were observed by Haematoxylin–eosin (HE) staining of the brain, activation of microglia in hippocampal tissues was detected by immunofluorescence, and the expression of PI3K/Akt/mTOR and JAK2/STAT3 proteins in the hippocampal tissues by Western blot.
Results: During the administration of palmar Ginseng, the body weight and blood glucose levels of DCI rats were measured weekly, with results showing that Palmar Ginseng effectively reduced blood glucose levels and body weight of DCI rats. Behavioural tests in the water maze indicated that palmar ginseng effectively improved the learning and memory ability of DCI rats. HE and immunofluorescence staining showed that palmar ginseng improved DCI in rats, ameliorated hippocampal neuronal damage, and improved microglial activation. ELISA showed that palmar ginseng significantly reduced the expression of pro-inflammatory factors in the serum of DCI rats. Increased expression of anti-inflammatory factors was observed, and Western blot analysis showed that Palmar Ginseng regulated PI3K/Akt/mTOR and JAK2/STAT3 protein expression, promoted the phosphorylation of PI3K/Akt/mTOR, and inhibited JAK2/STAT3 protein phosphorylation in rat hippocampal tissues as well as in BV2 cells.
Conclusions: Palmar ginseng may improve the onset and development of DCI by upregulating the phosphorylation of proteins in the PI3K/Akt/mTOR pathway.

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1. Introduction

Diabetes Mellitus (DM) is a metabolic syndrome characterised by chronically high blood glucose levels [1]. Persistently high levels of blood glucose can cause systemic vascular damage and a variety of cardiac, renal, and neurological complications [2]. DM and its complications affect the metabolism, structure, and function of the brain. Clinical studies have found that approximately 60–90 % of individuals with DM develop neuropathy to varying degrees, which in turn triggers cognitive dysfunction [3]. Diabetic Encephalopathy (DE) is a major chronic complication of DM, and is mainly characterised by acquired cognitive impairment and behavioural deficits, which some researchers refer to as diabetic cognitive impairment (DCI). Clinical manifestations include memory loss and decreased language expression and comprehension, which may be accompanied by apathy and slow movement [4]. Research has further shown that the risk of dementia in diabetic patients is about two times higher than that of normal individuals [5]. China has the fastest growing prevalence of diabetes, and the largest number of people with this disease [6]. As the number of people with diabetes increases, the prevalence of cognitive impairment has also tended to increase significantly. Currently, most treatments for DCI, such as donepezil and memantine, focus on improving cognitive impairment by inducing glycaemic control [7,8], However, improper use may lead to adverse reactions, including hypoglycaemic reactions, cardiovascular disease, and lactic acidosis. There is currently a lack of targeted drugs for the treatment of DCI. Traditional Chinese medicines (TCM), including "Qi Fuyin" [9] and "Yu Yetang" [10], have been documented as treatments for DCI since ancient times, with both showing efficacy. Modern pharmacological studies have shown that TCM, such as ginseng, have a better therapeutic effect on DCI [11,12]. Therefore, the development of new potential TCM drugs to combat cognitive impairment in diabetes is of great relevance. *Gymnadenia conopsea* (L.) R. Br., also known as palmer ginseng, is a TCM herb that was first mentioned in the 'Four Medical Classics'. Modern pharmacological studies have shown that palmer ginseng exerts neuroprotective effects and improves memory. For example, the Tibetan medicine "Yangzong Sanbao", the main ingredient of which is palmar ginseng, was found to treat cardiovascular diseases, inducing a significant improvement in myocardial blood supply and heart rate. This medicine has a bidirectional regulatory effect on blood pressure and heart rate and is especially effective for hypertension [13]. The "Jinsha Pill" of Mongolian medicine is used to treat chronic nephritis, uraemia, and other urological diseases. It has been shown restore kidney function in a shorter period, achieving a lower likelihood of recurrence [14]. "Compound palm ginseng drops" can be used as an adjunct treatment for diabetic nephropathy [15]. "Yishouwan" (composed of 20 herbs, including Palmer ginseng and Crocus sativus) can nourish the liver and kidneys, strengthen the brain, and brighten the eyes. It is used to treat forgetfulness, dizziness, palpitations, insomnia, tiredness, and weakness caused by insufficiency of the liver and kidneys and deficiency of the qi and blood [16]. This study aimed to investigate the effect of palmar ginseng on cognitive impairment in patients with type 2 DM, and to provide a theoretical basis for subsequent research to develop the clinical use of the drug.

2. Materials and method

2.1. Experimental reagents

Streptozotocin (STZ) was purchased from Sigma-Aldrich (St. Louis, MO, USA; no. S0130). Antibodies against PI3k (AF8241) and p-PI3k (AF3242) were purchased from Affinity; antibodies against Akt (9272S) and p-Akt (4060S), JAK2 (3230S), p-JAK2 (3776S), STAT3 (9139S), and p-STAT3 (9145S) were purchased from CST; antibodies against TNF-α (MM-0180R1), IL-6 (MM-0190R1), IL-1β (MM-0047R1), and IL-10 (MM-0195R1) were purchased from Jiangsu Enzyme Immunity Industry Co.; antibodies against mTOR (10024436), p-mTOR (10020406), and GAPDH (10017731) were purchased from Proteintech. Sheep anti-mouse (BA1050), sheep anti-rabbit (BA1054), and ultrasensitive ECL chemiluminescent solutions (AR1197) were purchased from Wuhan Boster Bioengineering Co. Tris (#T8060), SDS (#S8010), cyclic acid (#G8200) were purchased from Solebrite Technology & Biology Co., Ltd., while anhydrous ethanol, and methanol were purchased from Tianjin Fuyu Fine Chemical Co. The BCA protein concentration determination kit, SDS-PAGE gel electrophoresis kit, and RIPA lysis buffer reagents were purchased from Biyuntian Biotechnology Co. Oolyvinylidene fluoride (PVDF) membranes (Millipore, USA), DMEM high-glucose medium, and penicillin-streptomycin double antibiotic were

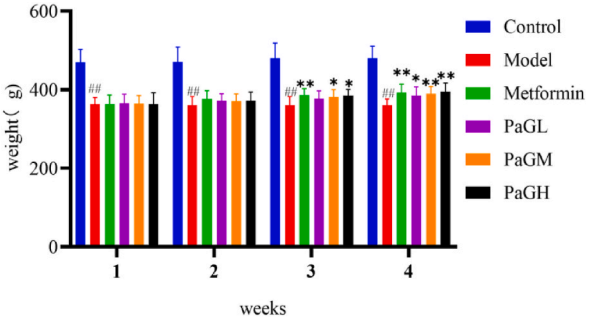


Fig. 1. Effects of Palmar Ginseng on weight changes in rats with Diabetic Cognitive Impairment.
Body weight in the Control group stabilized, while body weight in the Model group decreased. The administered group showed an increase in body weight.

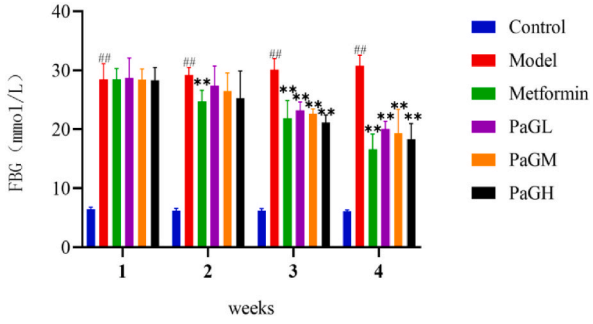


Fig. 2. Effects of Palmar Ginseng on blood glucose changes in rats with Diabetic Cognitive Impairment.
The normal level of fasting blood glucose in the Control group, was significantly elevated in the model group. There was a significant decrease in the administered group.

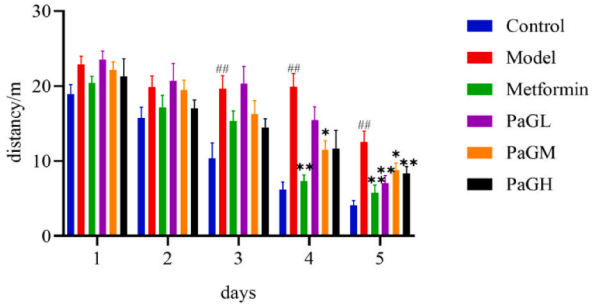


Fig. 3. Effect of palmer ginseng on water maze test performance in rats with diabetic cognitive impairment.

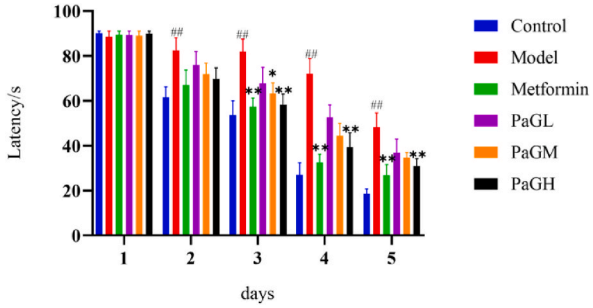


Fig. 4. Effect of palmer ginseng on water maze test performance in rats with diabetic cognitive impairment.

purchased from Bioland Biotechnology Co., Ltd. CCK-8 (20230128) was purchased from 7 Sea-Cell Counting Kit, while dimethyl sulfoxide (DMSO), and glucose were purchased from Tianjin Kermel Chemical Reagent Co.

All experiments in this study were reviewed by the Laboratory Animal Ethics Committee of Shaanxi University of Chinese Medicine. The agreement number was SUCMDL20230310005.

2.2. Establishment of a rat model of diabetic cognitive impairment

Animals were acclimatised for one week, and eight were randomly selected as the control group. The control group of rats were fed regular rat maintenance chow, while the rest were fed a homemade high-fat and high-sugar diet, comprising basic feed (59 %), white granulated sugar (20 %), lard (18 %), and barnyard egg (3 %) for one month, after which they were injected intraperitoneally with 35 mg kg^{-1} STZ. Fasting blood glucose levels were measured by collecting blood from the tail vein using a Roche glucometer 72 h after fasting for 8 h before the test. Successful modelling of DM was defined as a blood glucose concentration $\geq$16.7 mmol/L.

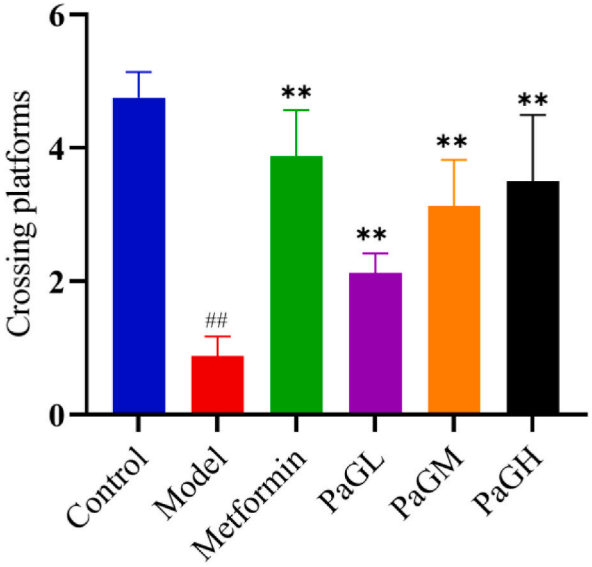


Fig. 5. Effect of palmer ginseng on water maze test performance in rats with diabetic cognitive impairment.
Excellent performance in the water maze test for rats in the Control group，Poorly performance in the water maze test for rats in the Model group, Better water maze test scores in each dosing group compared to the model group. Excellent performance was observed in the water maze test for rats in the Control group, while the rats in the model group showed poor performance. Additionally, the rats in each dosing group showed better water maze test scores compared to those in the model group. (Compared with the blank group，$^{\#}P < 0.05$，$^{\#\#}P < 0.01$.compared with the model group，$^{*}P < 0.05$，$^{**}P < 0.01$).

2.3. Animal grouping

After successful modelling, the rats were maintained on the high-fat and high-sugar diet for 60 d. Two rats were randomly selected 60 d later to examine the pathology of the CA1 region of the hippocampus, finding that the cellular morphology of the CA1 region of the hippocampus was altered compared with that of the control group. The remaining rats were then subjected to the water maze test over 5 days, and rats with a latency of more than 50 s on day 5 were considered to have DCI. Those classified as having DCI in the water maze test were randomly divided into the Model group (Model), Metformin group (Metformin: 0.02 g/(kg·d), rat administration equivalent dose of 1.2 g/(kg·d), representing a human administration dose of 12 g), and the palmar ginseng low-dose group (PaG_M: 0.6 g/(kg·d)) and Palmar Ginseng high-dose group (PaG_H: 2.4 g/(kg·d)). Eight animals from each group were treated via oral gavage for 30 d. During the administration period, animals were fed normal feed (corn 25.7 %, maize 30.6 %, soya bean 13.0 %, soybean meal 5.0 %, rice husk 6.0 %, bran 10.0 %, fishmeal 6.0 %, and other premixes 3.7 %). Their body weights and blood glucose levels were measured weekly, and they were provided ad libitum access to food and water.

2.4. Morris water maze

Animals were subjected to the Morris water maze experiment for 6d after 30 days of drug administration from 31d to 36d. Rats were placed in the water facing the wall of the pool from two different marked points for 90 s each time. Swimming distance and latency were recorded, and the number of times the rats crossed the platform in 90 s was recorded on day 6 when the station was withdrawn.

2.5. Haematoxylin-eosin (HE) staining

The brain tissue was fixed in 4 % paraformaldehyde, followed by dehydration in an ethanol gradient, transparency was achieved using a xylene gradient, and routine paraffin embedding. Paraffin-embedded brain tissue sections (3 μm) were de-paraffinised using xylene and stained with haematoxylin for 3–5 min. Sections were then exposed to a graded acidic ethanol solution and clarified by dehydration with anhydrous ethanol and xylene. Eosin staining was performed for 5 min, followed by dehydration and transparency assays. The samples were then sealed with neutral gum and examined under a light microscope to observe changes in the morphology and number of histiocytes in the CA1 area of the hippocampus. Visualisation was performed using a Leica optical microscope. Visualisation magnification: 200×.

2.6. Immunofluorescence

Paraffin sections were de-paraffinised and antigenically repaired. They were then incubated overnight at 4 °C with a primary

antibody against Iba-1 (1:3000). The sections were then closed, and a secondary antibody was added. DAPI was used to re-stain the nuclei and quench tissue autofluorescence. After sealing the slices, they were scanned microscopically to observe and count the expression of the microglial marker Iba-1 in the CA1 region of the hippocampus. DAPI fluoresces blue, whereas Iba-1 fluoresces red. Immunoblotting was performed using a 200× microscope.

2.7. Effect of different concentrations of glucose on BV2 cell damage

The BV2 mouse microglial cell line was purchased from the Wuhan Punosai Life Science and Technology Co. After five passages, the CCK-8 assay was used to detect the effect of different concentrations of GLC on the viability of BV2 cells, which were collected from the logarithmic growth phase, digested, blocked, and centrifuged. The cells were inoculated uniformly at a density of 2×10^5 cells/mL in a 96-well plate, and four replicate wells were set up in parallel for each cell group. The cells were then incubated for 24 h. When cell growth was stabilized, BV2 cells were treated with different concentrations of glucose (800, 400, 200, 100, 50, 25, 12.5, 6.25, and 3.125 mM) for 24, 48, or 72 h. After the treatment, 10 μL of CCK-8 reagent was added to each well. The optical density (OD) of each group of cells was measured at 450 nm using an enzyme labelling instrument. Cell viability was calculated as follows: (the mean value of OD in the control group – the mean value of OD in experimental group)/mean value of OD in the control group × 100.

2.8. Effect of lyophilised powder of palmar ginseng on BV2 cells

When BV2 cells reached the logarithmic phase of growth, the cell density was adjusted to 2×10^5 cells/mL in a 96-well plate with approximately 20,000 cells/well. Four replicate wells were established in parallel for each cell group. The cells were incubated at 37 °C in a 5 % CO_2 incubator for 24 h. The medium was replaced with different concentrations of lyophilised palmer ginseng powder. The final concentrations of the lyophilised powders were 40, 20, 10, 5, 2.5, 1.25, 0.625, and 0.312 mg/mL. Four replicates were performed for each concentration. After 24 h of incubation, 10 μL of CCK-8 solution was added to each well. The optical density (OD) of the cells in each group was measured at 450 nm using an enzyme marker, and cell viability was calculated as above.

2.9. Western blot

Frozen hippocampal tissue was used for protein extraction. The extracted proteins were electrophoresed using SDS-PAGE, and transferred onto a PVDF membrane, which was then incubated overnight at 4 °C with the corresponding primary antibody. The blots were then washed with TBST and incubated with the secondary antibody, and incubated for 1 h at room temperature. Specific bands were visualised using an Ultrasensitive ECL Chemiluminescence Kit and Chemiluminescence Imaging System. The relative expression

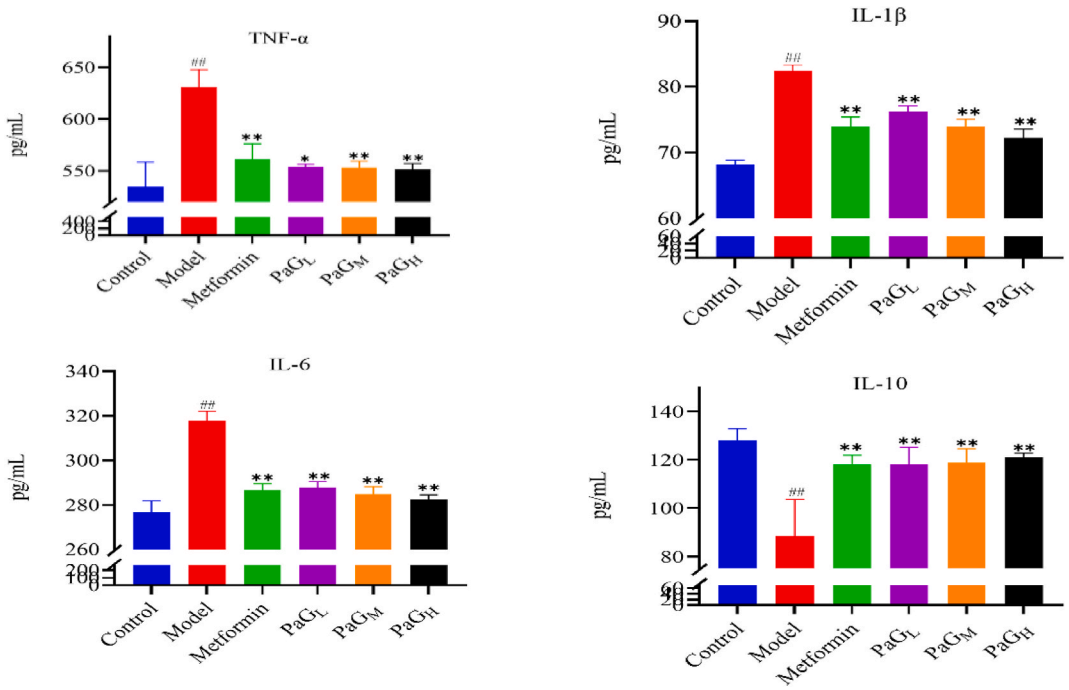


Fig. 6. Effect of palmar ginseng on serum inflammatory factors in rats with diabetic cognitive impairment.
There was a decrease in pro-inflammatory factors and increase in anti-inflammatory factors in the serum of rats in the administered group compared to the model group. (Compared with the blank group , $^{\#}P < 0.05$, $^{\#\#}P < 0.01$.compared with the model group , $^{*}P < 0.05$, $^{**}P < 0.01$).

of target proteins was calculated using ImageJ with GAPDH (or total protein if the target protein was phosphorylated) as a reference. This process was repeated thrice. The Chemiluminescent imaging system was sourced from Shaanxi Leyue Biotechnology Co. The GBOX-Chemi-XRQ instrument was used. Each protein sample was loaded at 35 μg. The antibody:diluent ratios were as follows: primary antibody, 1:1000; secondary antibodies, 1:10,000. The SDS-PAGE gels were prepared at the following percentages: 5 % for the gel concentrate and 8 % for the separating gel. The chemical reflection kit was obtained from Wuhan Dr. De Biological Co. (lot:19A17b72).

2.10. Statistical analysis

All data were analyzed using SPSS 26.0, and the results are expressed as the mean ± standard error of the mean (mean ± SD). Multiple comparisons were performed using one-way analysis of variance (ANOVA), and $P < 0.05$ or $P < 0.01$ was considered statistically significant. Statistical analyses were performed using the GraphPad Prism 8.0 software (GraphPad Software, Inc., CA, USA).

3. Results

3.1. Palmar ginseng reduces body weight and elevates blood glucose levels in DCI rats

Fig. 1 shows the body weights of rats with T2DM treated with DCI before and after four weeks of gavage with palmer ginseng. The body weight of the rats in the model group (which comprised DCI rats that were not administered any drugs) was significantly lower before drug administration than that of the control group ($P < 0.01$). However, the body weights of the treatment groups showed no significant difference from that of the model group. There was a significant increase in the body weight in each dosing group before and after gavage. A decreasing trend was observed in the model group ($P < 0.05$), and the increase in body weight of the PaG_H group was more pronounced. This indicated that P. ginseng could inhibit continuous weight loss in DCI rats to a certain extent.

Fig. 2 shows the fasting blood glucose levels in rats with T2DM treated with DCI before and after four weeks of gavage with palmer ginseng. Fasting blood glucose levels in the model group before drug administration were compared with those in the control group, showing significantly higher levels in the model group ($P < 0.01$). However, there was no significant difference between fasting blood glucose levels in each administration group with those in the model group. When comparing the fasting blood glucose levels before and after gavage, a decreasing trend was observed in each administration group, whereas the model group showed an increasing trend ($P < 0.05$). The fasting blood glucose of the PaG_H group, except for the metformin group, declined most significantly, indicating that PaG_H could inhibit the continuous increase in blood glucose in DCI rats to a certain degree, indicating a good glucose-lowering effect.

3.2. Palmar ginseng improves morris water maze test performance in DCI rats

Since its development, the Morris water maze test has arisen as a popular tool for assessing spatial learning and memory. This test primarily assesses the effectiveness of the hippocampus-dependent aspects of learning and memory. Therefore, we chose the Morris water maze test to examine cognition in rats [17,18]. The results of these tests are shown in Figs. 3–5 With an increase in training time, the avoidance latency and exploration distance of the rats in each group showed decreasing trends. From the second day, the escape latency of the model group was significantly higher than that of the control group and all of the administered groups ($P < 0.05$). This suggests that spatial learning and memory abilities were significantly impaired in the model group, whereas learning and memory

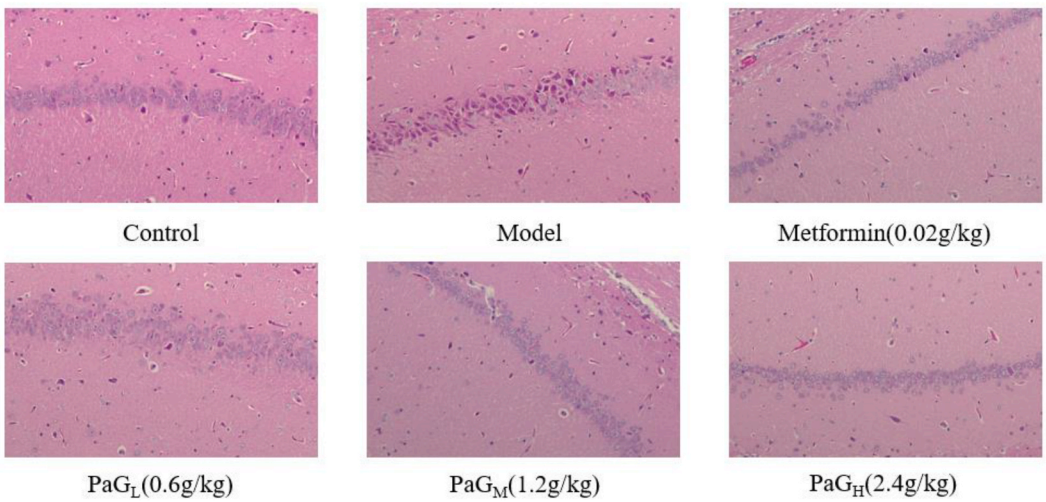


Fig. 7. Effect of Palmar Ginseng on pathological changes in CA1 region of hippocampus in rats with Diabetic Cognitive Impairment. The hippocampal region of each group of rats was observed under a 200× microscope.

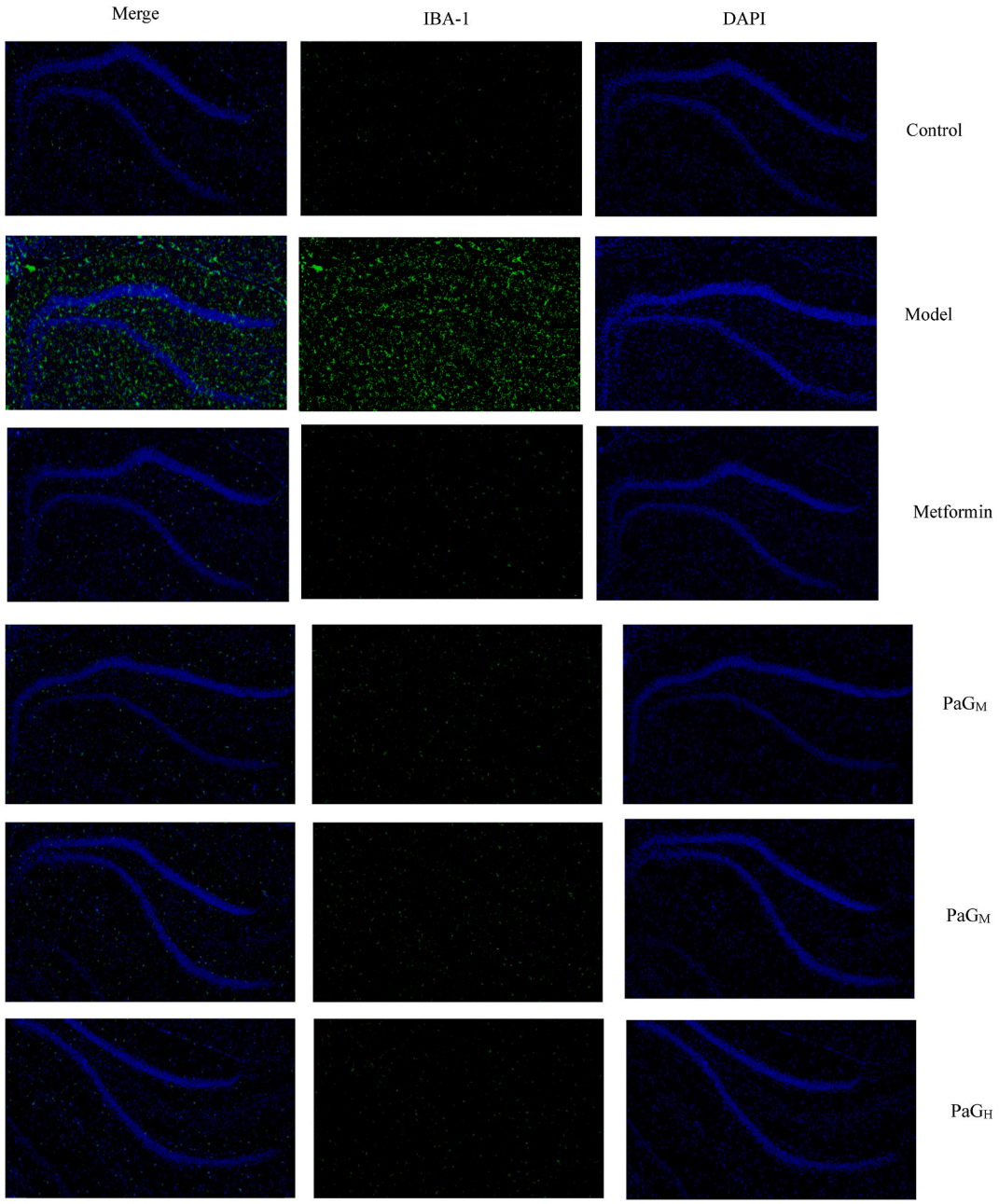
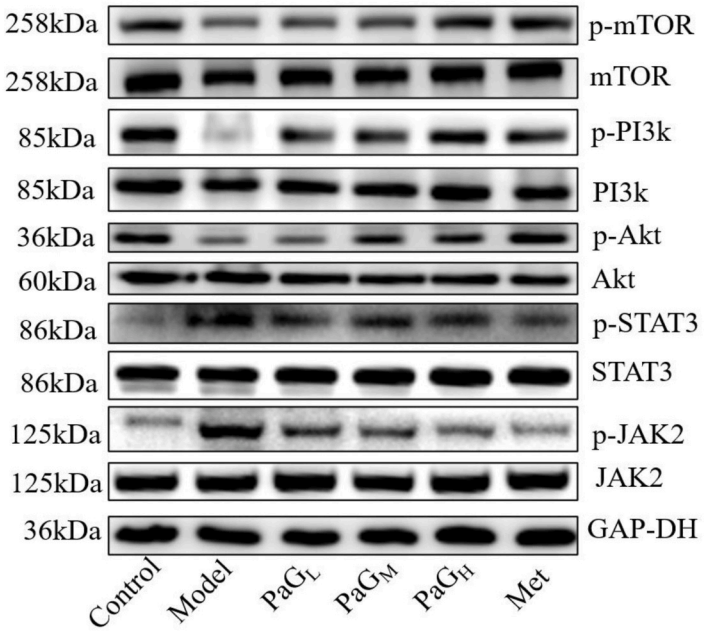


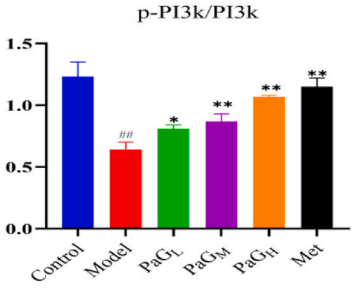
Fig. 8. Effects of palmar ginseng on microglia activation in the hippocampus of rats with diabetic cognitive impairment
Activation of microglia in the hippocampus of rats in each group observed by immunofluorescence staining.

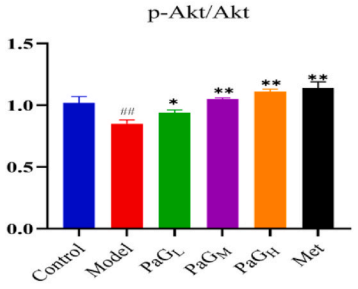
abilities improved after intervention with palmer ginseng and metformin. The number of times the model group crossed the target platform was significantly lower than that of the control group on day 6 after the platform was removed ($P < 0.05$). The performance of each administration group in traversing the platform was significantly higher than that of the model group ($P < 0.05$). In conclusion, the spatial learning and memory abilities of T2DM DCI rats were significantly improved after intervention with palmer ginseng.

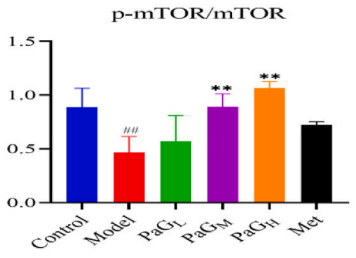
3.3. Palmar ginseng reduces the expression of inflammatory factors in the serum of DCI rats

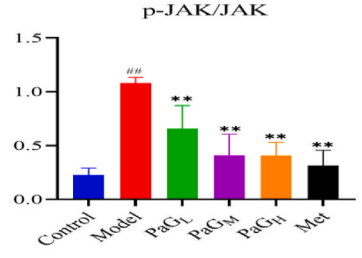
The expression of inflammatory factors in the serum of rats in each group was measured using ELISA. Pro-inflammatory factors such as TNF-α, IL-1β, and IL-6 were found to be significantly higher in the Model group compared to the control group ($P < 0.05$). In contrast, their levels were significantly reduced in each of the palmer ginseng administration groups compared to the model group ($P < 0.05$). The expression of the anti-inflammatory factor IL-10 was significantly reduced in the model group ($P < 0.05$), whereas it was











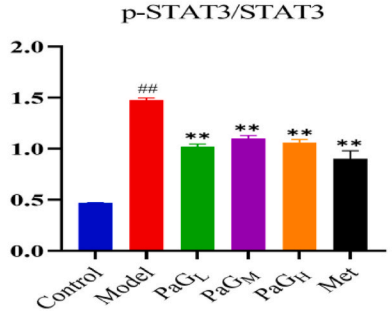


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Fig. 9. Effect of palmar ginseng on protein expression of PI3k/Akt/mTOR pathway in rats with diabetic cognitive impairment.
Palmer ginseng activates the PI3K/Akt/mTOR pathway to improve cognitive impairment in diabetes. (Compared with the blank group , $^{\#}P < 0.05$, $^{\#\#}P < 0.01$.compared with the model group , $^{*}P < 0.05$, $^{**}P < 0.01$).

elevated in each of the palmer ginseng-treated groups ($P < 0.05$). Thus, the improvement of T2DM in DCI rats by palmer ginseng was associated with the suppression of inflammation and an increase in anti-inflammatory factors (see Fig. 6).

3.4. Palmar ginseng improves hippocampal CA1 area pathology in DCI rats

The hippocampus is an important part of the human brain that plays important roles in learning, memory, and spatial navigation. One important function of the brain is the encoding and integration of sensory information, which leads to memory formation. The hippocampal CA1 is primarily responsible for processing time-related information. We therefore selected the hippocampal CA1 area as an important indicator of whether cognitive impairment was improved [19]. HE staining revealed that the number of neurones in the CA1 area of the hippocampus of rats in the control group and the number of neurones in the CA1 area of the model group was reduced. The staining was blurred, and some neurones were sparsely arranged with a wrinkled cytosolic structure. In contrast, the administration of P. ginseng and metformin resulted in an increased number of neurones with a tight arrangement and better morphology, suggesting that palmar ginseng intervention can effectively attenuate neuronal damage in T2DM DCI rats, and play a role in protecting neurones (see Fig. 7).

3.5. Palmar ginseng improves hippocampal microglia activation in DCI rats

IBA-1, a marker of microglial activation, is associated with neuroinflammation caused by T2DM and DCI. Microglial activation in the hippocampus was confirmed by immunofluorescence staining. In the figure, IBA-1 is shown in green and DAPI in blue figure. The hippocampal DG region of the model group showed a significant enrichment of activated microglia ($P < 0.01$). However, both doses of palmer ginseng significantly reduced their numbers ($P < 0.05$). Thus, palmer ginseng may protect the hippocampus from inflammatory damage by inhibiting microglia activation, and thereby protecting the hippocampus from inflammatory damage (see Fig. 8).

3.6. Palmar ginseng improves PI3k/Akt/mTOR signalling pathway-related protein expression in hippocampal tissues of DCI rats

Expression levels of the PI3k/Akt/mTOR signalling pathway in the hippocampal tissues of each group were detected by immunoblotting. Compared with the control group, the expression of p-PI3k, p-Akt, and p-mTOR proteins was significantly downregulated in the model group ($P < 0.01$). Additionally, the expression of p-STAT3 and p-JAK2 was significantly increased ($P < 0.01$). In comparison, each dosing group of palmar ginseng was able to significantly upregulate the expression of p-PI3k, p-Akt, and p-mTOR proteins ($P < 0.05$, $P < 0.01$) and significantly downregulate the expression of p-STAT3 and p-JAK2 proteins ($P < 0.01$), as compared to the model group (see Fig. 9).

3.7. Effect of different concentrations of glucose on BV2 cell damage

As shown inFig. 10, BV2 cell activity showed concentration- and time-dependent changes following the administration of various concentrations of glucose. Higher concentrations of glucose were toxic to BV2 cells, resulting in a decrease in viability compared to the Control group, where the activity of 100 mM glucose on the cells was (0.9566 ± 0.0241), In order to reduce cell viability without causing massive cell death, 24 h treatment with 100 mM glucose was selected as the modelling concentration and time for the intervention of BV2 cells$

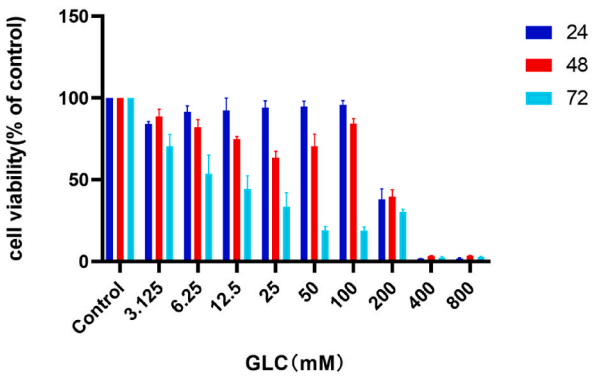

Fig. 10. Effect of different concentrations of glucose on the viability of BV2 cells.
100 mM high glucose was used to induce BV2 cells for 24 h in order to establish a high glucose model.

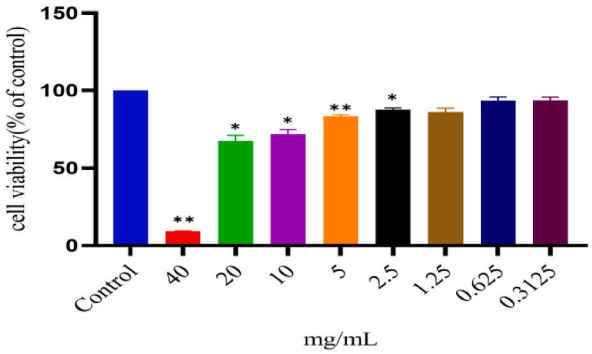

Fig. 11. Effect of different concentrations of Palmer Ginseng on BV2 cell.
The cellular activities in the low dose group of Palmer ginseng (PaG_L: 5 mg/mL) were all higher than those in the high glucose modelling group. The cellular activities in the high dose group of Palmer ginseng (PaG_H: 10 mg/mL) were all higher than those in the high glucose modelling group.

3.8. Effect of different administration concentrations of palmer ginseng on BV2 cell viability

As shown inFig. 11, the activity of BV2 cells exhibited concentration- and time-dependent effects following the administration of different concentrations of palmer ginseng extracts. Various concentrations of glucose were found to be toxic to BV2 cells compared to the control group. Among the different concentrations of palmar ginseng, 5 mg/mL showed the highest activity (0.8356 ± 0.081) on the cells. To avoid excessive cell death, a low dose of 5 mg/mL of palmar ginseng was selected for intervention in BV2 cells, whereas a high dose of 10 mg/mL was chosen for this experiment.

3.9. Effects of different concentrations of palmar ginseng on high glucose-induced BV2 cell activity

As shown inFig. 12, the activity of BV2 cells induced by GLC was significantly lower than that of the control group ($P < 0.01$). PaG_L (5 mg/mL) and PaG_H (10 mg/mL) levels were both higher than those in the model group ($P < 0.05$). Cell growth was significantly inhibited in the GLC induced group, whereas it was alleviated in all groups after administration of PaG_H.

3.10. Effect of lyophilised palmar ginseng powder on the expression of PI3k/Akt pathway-related proteins in high glucose-induced BV2 cells

The expression levels of the PI3k/Akt/mTOR signalling pathway in the BV2 cells of each group were detected by immunoblotting, as shown in F ig. 16. The expressions of p-PI3k, p-Akt, and p-mTOR proteins were significantly downregulated in the model group compared to that in the control group ($P < 0.01$), while the expressions of p-STAT3 and p-JAK2 were significantly increased ($P < 0.01$). Compared with the model group, each dosing group of palmer ginseng showed up-regulation in the expression of p-PI3k, p-Akt, and p-

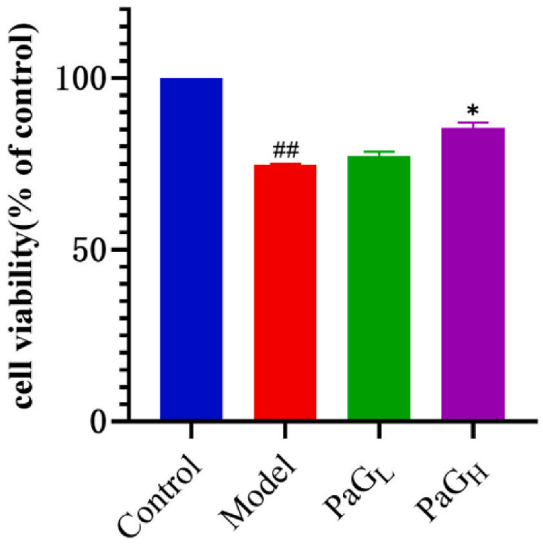

Fig. 12. Effect of different concentrations of palmer ginseng on high glucose-induced BV2 cell activity.
Administration of palmar ginseng after establishing a cell model does not cause massive cell death.

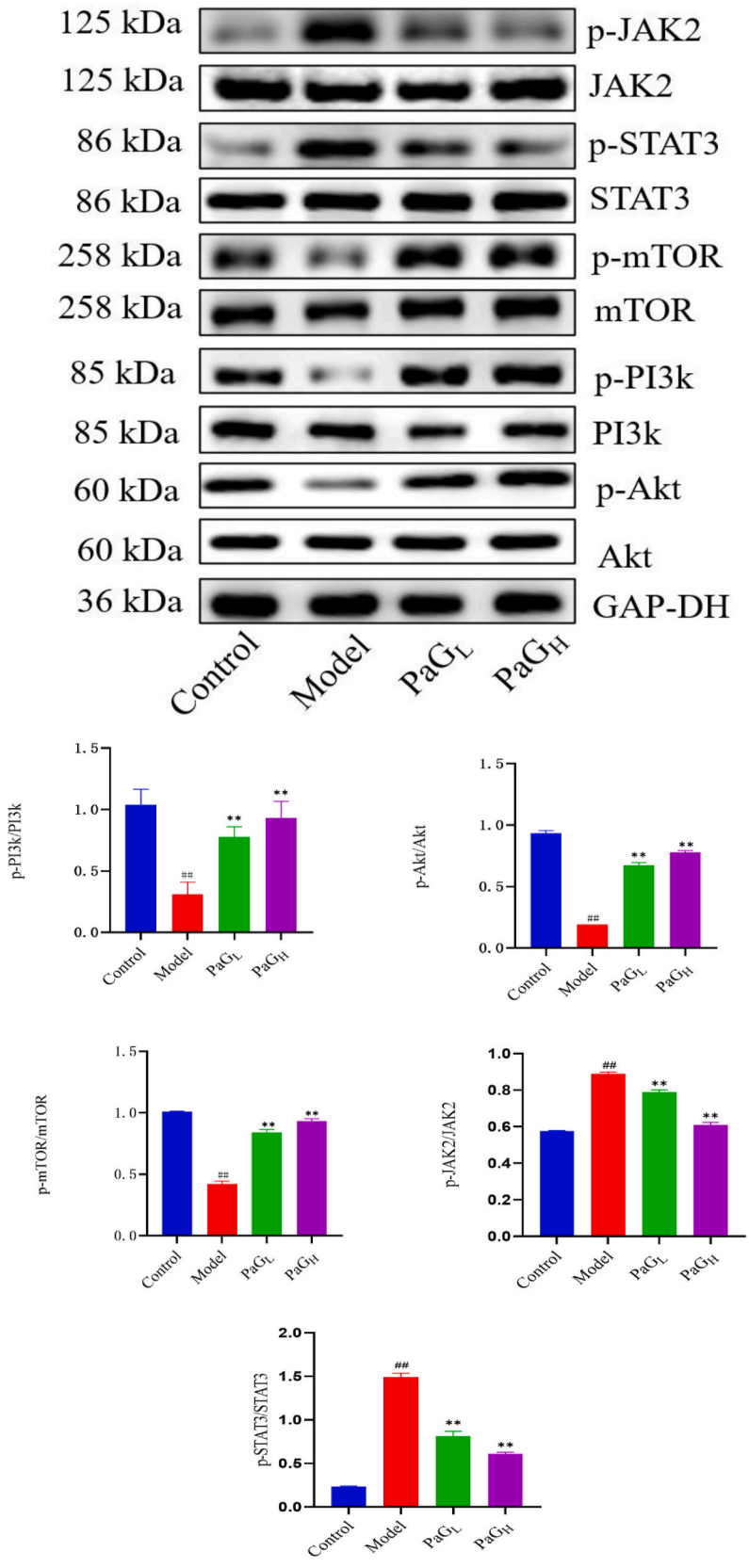

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Fig. 13. Effect of palmar ginseng on protein expression of PI3k/Akt/mTOR pathway in GLC-induced BV2 cell injury.
Palmer ginseng activates the PI3K/Akt/mTOR pathway to improve microglia activation.

mTOR proteins ($P < 0.05$ or $P < 0.01$), and significant down-regulation of the expression of p-STAT3 and p-JAK2 proteins ($P < 0.01$) (see Fig. 13).

4. Disscussion

Chinese medicine posits that diabetes is a type of "thirst-quenching syndrome". While there is no mention of DE in the Chinese medical literature, based on its clinical manifestations and characteristics, it can be categorised as a combination of "thirst-quenching", "dullness", and "forgetfulness". Modern medical research has demonstrated that the development of DE involves multiple mechanisms and targets. These mechanisms are related to a variety of factors, including the deficiency of neurotrophic factors, insulin resistance, inflammatory response, and oxidative stress [20,21]. This corresponds to the multi-component and multi-target characteristics of TCM. Modern pharmacological studies have shown that most polysaccharide components in TCM exert hypolipidemic and hypoglycaemic effects [22,23]. Polysaccharides are the main active components of palmer ginseng, which show antioxidant, anti-inflammatory, and immunofunction-enhancing effects. In addition [24], palmar ginseng improves cognitive impairment caused by various factors. Li et al. showed that palmer ginseng extract improves scopolamine-induced cognitive impairment in rats. Zhang et al. [25] further demonstrated that palmar ginseng extracts also improved cognitive deficits induced by d-galactose (d-Gal) and sodium nitrite ($NaNO_2$) in senescent mice. Pan et al. [26] found that palmar ginseng exerted neuroprotective effects by regulating the PI3k/Akt signalling pathway. However, this is the first study to explore the effects and mechanisms of palmar ginseng on the cognitive impairment induced T2DM, based on previous studies. However, one previous study found that administration of palmar ginseng to DCI rats through gavage improved cognitive impairment by modulating the PI3K/AKT pathway [27].

The rats in this study generally gained weight faster during the early stages of the study. They had vigorous, white, and shiny fur, and normal fasting blood glucose levels. All rats in the model group, excluding those in the control group, exhibited diabetic characteristics. This was evident from the significant decrease in body weight and the presence of scanty and dry fur. The rats' yellowish colour, significant elevation in fasting blood glucose, sluggish movement, and rising threshold of the stress response with prolonged feeding time suggest that this batch of animal models better replicates the occurrence and development of human type 2 diabetic encephalopathy. After treatment with palmer ginseng by gavage, the blood glucose levels of rats in each administration group, except for the model group, decreased. This indicates that palmer ginseng has a strong hypoglycaemic effect. The water maze test is a classic behavioural method used to study learning and memory in rodents. The results of the present study revealed a significant decrease in evasion latency and exploration distance, and an increase in the number of platform crossings in the rats of each dosing group of palms ginseng compared to the model group. This indicates that inflammatory factors are strongly associated with the development of cognitive impairment in diabetes. Detection of pro-inflammatory factor activity in the serum of DCI rats using ELISA revealed that the pro-inflammatory factor activities of rats in the administered group were significantly lower than those in the model group. The anti-inflammatory factor content was also higher than that in the model group, suggesting that palmer ginseng reduced the expression of pro-inflammatory factors and increased the levels of anti-inflammatory factors in rats. This indicated that palmer ginseng possesses anti-inflammatory properties.

Numerous studies have shown that activation of the PI3k/Akt pathway reduces obesity and insulin resistance. As such, the PI3k/Akt pathway is closely associated with the development of diabetic encephalopathy. Both insulin and insulin-like growth factors bind to receptors associated with the PI3k/Akt signalling pathway, generating a number of secondary responses linked to neural protrusion growth, synaptic plasticity regulation, and memory-related gene transcription. This is known as the neuronal survival information transduction pathways [28].

The JAK/STAT signalling pathway is a common element of numerous cytokine signalling pathways, and is widely involved in cell proliferation/differentiation, inflammation, and apoptosis. It is also a key pathway regulating microglial polarisation [29], with its activation promoting microglia polarisation to the M1 type [30,31]. In contrast, IL-10 can mediate anti-inflammatory responses by inhibiting the expression of IL-6, and TNF-α. This may be related to the inhibition of the JAK/STAT signalling cascade, which restricts the secretion of pro-inflammatory factors, and increases the levels of anti-inflammatory factors such as IL-4 and IL-10 [32]. This process leads to a shift in microglial polarisation towards a protective M2 phenotype. In contrast to the M1-type pro-inflammatory state, the M2 phenotype has tissue-repairing and immuno-inflammatory suppressive effects. This shift is further characterised by a transition from the M1 to the M2 state [33], which attenuates the inflammatory process of neurodegeneration caused by abnormal microglia [34].

The results of the present study revealed that palmar ginseng regulated the expression of proteins in the PI3K/Akt and JAK2/STAT3 pathways, specifically promoting the phosphorylation of PI3K/Akt and inhibiting the phosphorylation of JAK2/STAT3. Palmar ginseng may ameliorate the onset and progression of diabetic cognitive dysfunction by upregulating the phosphorylation of PI3K/Akt, and downregulating the phosphorylation of JAK2/STAT3.

Microglia are progenitor cells that perform immune functions in the central nervous system (CNS). Growing evidence has suggested that microglia play a key role in triggering neurodegenerative diseases. In the present study, we demonstrated that palmer ginseng suppresses microglial activation in the brains of DCI rats, and attenuates GLC-induced microglial damage by activating the PI3k/Akt pathway [35]. In conclusion, palmer ginseng can effectively reduce blood glucose levels and improve diabetes-induced cognitive impairment in rats. Its mechanism of action may be related to its antioxidant activity and regulation of the PI3K/Akt pathway of

Palmer ginseng. However, the present study did not discuss the potential mechanisms of action of palmer ginseng in the treatment of DCI. We plan to conduct further experiments to systematically investigate the specific components of palmar ginseng for the treatment of DCI using experimental methods, such as network pharmacology and molecular docking. Overall, this study provides a scientific basis for modern pharmacological research.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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CRediT authorship contribution statement

Shi Yong: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology. **Zhang Yuhan:** Validation, Conceptualization. **Cao Shanshan:** Conceptualization. **Wang Xin:** Data curation. **Shi Leilei:** Formal analysis. **Jiping Liu:** Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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