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Original Article

Dendrobium mixture on depressive symptoms via inhibition of the NLRP3/caspase-1 pathway in models of diabetes

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Abstract: Objective: To investigate the expression of NLRP3/caspase-1 signaling pathway both in patients with diabetes mellitus complicated with depression (DD) and in a DD rat model under the influence of Dendrobium Mixture. Methods: RT-PCR was used to detect the expression of genes related to the NLRP3/caspase-1 pathway in: 20 DD patients, 20 patients with diabetes mellitus (DM), 20 patients with depression (DE), and 20 healthy subjects as the normal control group (NG). Subsequently, a DD rat model, and rat NIT-1 islet β cells and hippocampal neural stem cells (NSCs) were constructed for *in vivo* and *in vitro* experiments. Open-field experiments were performed to detect the behavior of the rats. According to individual quality, the DD rats were randomly divided into 4 groups: diabetes complicated with depression model (DD), positive medicine model (PM), positive medicine combined with Dendrobium model (PD) and NLRP3 inhibitor group (MCC950). Another 10 healthy rats were used as the normal group (NG). NIT-1 islet β cells and NSCs were cultured (NG), in normal culture medium with 150 nm glucose and 200 μM corticosterone to simulate a high glucose and high ketone microenvironment (GC), high glucose and high ketone microenvironment plus positive medicine group (GC + PM), and high-glucose-high-ketone microenvironment plus positive medicine and dendrobium mixture (GC + DM). RT-PCR assay was used to detect the expression of NLRP3/caspase-1 related-pathway genes in cells of rats in each group. Cell proliferation and apoptosis rate were detected by MTT assay and flow cytometry, respectively. Results: In DD patients, the expression of NLRP3/caspase-1 pathway related genes was significantly increased, which was more significant than in DM and DE patients (*P<0.05, **P<0.01, ***P<0.001). Dendrobium Mixture significantly reduced the NLRP3/caspase-1 pathway related gene expression in the PM group and improved the symptoms of DD rats (*P<0.05, **P<0.01, ***P<0.001). The results of MTT assay and flow cytometry showed that Dendrobium mixture can promote the proliferation and anti-apoptotic effects on pancreatic islets β cells and NSCs (***P<0.001). Conclusion: Dendrobium mixture can significantly improve the symptoms of diabetes complicated with depression and promote cell proliferation, which may be closely related to the inhibition of the NLRP3/caspase-1 signaling pathway.

Keywords: Dendrobium mixture, diabetes complicated with depression, NLRP3/caspase-1 signaling pathway, islet β cells, hippocampal neurons

Introduction

According to statistics, 422 million people have been diagnosed with diabetes worldwide [1-3]. Diabetes is a chronic metabolic disease and is often accompanied by complications [4]; among which, the incidence of concurrent depression is 15.3 to 36%, which is much higher than that of non-diabetic patients [2, 3, 5, 6]. Concurrent depression can have unpredictable consequences on the treatment of diabetes. Patients with poor glycemic control may have exacerbated depressive symptoms and may have increased risk of micro and macro vascular complications. In addition, the quality of life of patients with diabetes complicated with depression (DD) can also be seriously affected, which can burden the family and economic stability.

The NLRP3 inflammasome is an intracellular polyprotein complex, which is composed of NLRP3 and caspase-1 precursors, and plays the role of an extracellular signaling molecule

in the innate immune system [7]. Metabolic responses to stress can activate the NLRP3 inflammasome, which regulates the release of the pro-inflammatory cytokines IL-1β and IL-18 and induces inflammatory reactions [8-10]. Studies have shown that the NLRP3 inflammasome, as a signal transduction pathway for inflammatory responses and metabolic diseases, may play the same proximal role in diabetes and depression [11, 12].

Dendrobium mixture is a commonly prescribed formula for the treatment of diabetes. It has been reported that Dendrobium mixture has anti-inflammatory and immunomodulatory effects as well as protective effects on the nervous system and hippocampal neurons in diabetic rats [13-15]. Therefore, we speculate that Dendrobium mixture can protect neurons by inhibiting the inflammatory response, thus having a good effect in the treatment of DD. In this study, we used a rat model of DD to explore the effects of Dendrobium mixture on DD through regulation of the NLRP3/caspase-1 signaling pathway using dendrobium mixture to improve DD in rats, and attempted to clarify its underlying molecular mechanism in the treatment of DD.

Materials and methods

Sample sourcing

Inclusion criteria: Patients diagnosed with typical diabetes symptoms (polyuria, polydipsia, weight loss and fasting blood glucose ≥ 8.0 mmol/L) in our hospital; Patients with depression and other comorbidities who meet the diagnostic criteria of the 10th edition of the International Classification of Diseases (Patients who meet both diagnostic criteria above). Serum samples from 20 patients with DD, 20 patients with diabetes mellitus (DM), 20 patients with depression (DE), and 20 healthy subjects as normal control group (NG) in our hospital. All patients had no complications. All subjects participating in the study signed an informed consent. This study was approved by the hospital ethics committee.

Cell culture

Rat NIT-1 islet β cells were purchased from Peking Union Medical College, Chinese Academy of Medical Sciences, cultured in RPMI 1640 medium containing 10% FBS and 100 U double antibody at 37°C, and 5% CO_2. The NIT-1 islet β microenvironment was induced by high glucose and ketones. Then, 150 nm glucose and 200 μM corticosterone were added to the medium to simulate the growth conditions of islet cells in DD. NIT-1 islet β cells were divided into four groups on basis of culture method: normal culture (NG), normal culture medium + 150 nm glucose and 200 μM corticosterone (GC), high glucose and high ketone microenvironment + positive medicine (GC + PM) and high glucose and high ketone microenvironment + Dendrobium mixture + positive medicine (GC + DM).

Hippocampal neural stem cells (NSCs) isolation: Rats were anesthetized with 30 mg/kg sodium pentobarbital. The hippocampus was carefully removed from the brain, and sliced after measuring weight and volume. The tissue was then digested with 0.25% trypsin and 0.2% collagenase for 15 minutes at 25°C, which was terminated by adding Dulbecco's Modified Eagle's Medium (DMEM)/F12 (Hyclone, Logan, UT, USA) containing 10% fetal bovine serum (Gibco, Grand Island, NY, USA) and 1% penicillin/streptomycin. After centrifugation at 250 g for 5 minutes, the cells were collected and filtered through a 200 mesh sieve. Cells were then resuspended in DMEM/F12 containing 10% fetal bovine serum, 1% L-glutamine, 1% B27 (Gibco), and 1% penicillin/streptomycin. The cell density was adjusted to 3.0×10^5/mL and seeded on a plate pre-coated with poly-L-lysine. After 4 hours of incubation at 37°C, 5% CO_2, they were transferred to a basal medium (Gibco) containing 2% B27, 1% glutamine, and 1% penicillin/streptomycin. NSCs were cultured as follows: normal culture medium (NSCs), normal culture medium + 150 nm glucose and 200 μM corticosterone (NSCs + GC), high glucose and high ketone microenvironment + positive medicine (NSCs + GC + PM), and high sugar and high ketone microenvironment + Dendrobium mixture + positive medicine (NSCs + GC + DM).

Experimental animals and model building

Wistar rats (180-220 grams) were housed at 22°C, 55 ± 5% humidity, and food and water were freely available. DD model construction: Citric acid buffer (pH=4.6) containing 35 mg/

kg streptozotocin (Streptozotocin, STZ, Sigma, St. Louis, MO) was injected intraperitoneally for 5 days, and chronic unpredictable stress was applied for 28 consecutive days (cage tilt 45°C for 24 h; inversion of day/night light cycle for 24 h; ice water bath at 4°C for 5 min, noise stimulus for 8 h, wet litter for 24 h). After 1 week, Roche blood glucose meter was used to measure the blood glucose in the tail vein blood of rats. Blood glucose level > 16.7 mmol/L, and obvious abnormalities observed on Open-field behavioral test were evidence of a successful DD modeling. Subsequently, the rats were divided into four groups: a diabetic-complicated depression model group (DD, N=10), positive medicine group (PM, N=10), positive medicine + Dendrobium mixture group (PD, N=10), and DD rats were injected intraperitoneally with daily 100 mg/kg of NLRP3 inhibitor (MCC950, N=10). Another 10 healthy Wistar rats were taken as the normal group (NG group, N=10).

Animal administration

Two g/ml Dendrobium mixture was prepared with Dendrobium, Pheretima, Radix Puerariae, Rehmannia glutinosa, Rhizoma Anemarrhenae, Astragalus, Salvia miltiorrhiza, Schisandra chinensis, Achyranthes bidentata, and Hedyotis diffusa Willd by hot-water extraction followed by ethanol precipitation. The positive medicine was metformin (0.18 g/kg) combined with fluoxetine (1.8 mg/kg). The PM group was administered via oral gavage with metformin (0.18 g/kg) and fluoxetine (1.8 mg/kg); The PD group was administered 10 g/kg of Dendrobium mixture + PM; MCC950 group was intraperitoneally injected with 100 mg/kg NLRP3 inhibitor MCC950 daily, and DD group and NG group were administered with equal volume of 0.9% NaCl solution by oral gavage. All treatments lasted 30 days.

RT-PCR assay

Total RNA was extracted using a serum extraction kit (Life Technologies, US) according to the suggested protocol [16]. RT-PCR was used to detect the expression of NLRP3/caspase-1 pathway related genes in the serum of DD patients. The reverse transcription reaction was performed using a reverse transcription kit (Thermo Fisher Scientific, US). The reverse transcription system consists of 10 μl total RNA extract, 4 μl 5X buffer, 2 μl 10 mM dNTP, 1 μl primer, 1 μl Revert Aid RT, and 1 μl RNase inhibitor and 1 μl ddH_2O, which was incubated at 42°C for 60 min and 70°C for 5 min. The synthesized cDNA was stored at 80°C until use. All reactions were detected using Roche LightCycler 480 (Roche, Switzerland) under the following conditions: 95°C for 30 seconds, 95°C for 5 seconds, and 60°C for 30 seconds over 45 cycles. With melt-curve analysis, the appropriate magnification of the product is determined. Primers were as follows: NLRP3 Forward: 5'-GTGGAGATCCTAGGTTTCTCTG-3', NLRP3 Reverse: 5'-CAGGATCTCATTCTCTTGGATC-3'; caspase-1 Forward: 5'-AAGGTCCTGAGGGCAAAGAG-3', caspase-1 Reverse: 5'-GTGTTGCAGATAATGAGGGC-3'; IL-1β Forward: 5'-CCCTGCAGCTGGAGAGTGTGG-3', IL-1β Reverse: 5'-TGTGCTCTGCTTGAGAGGTGCT-3'; IL-18 Forward: 5'-ACAACCGCAGTAATACGGAGCA-3', IL-18 Reverse: 5'-TGTGCTCTGCTTGAGAGGTGCT-3'; GAPDH Forward: 5'-CAGTGCCAGCCTCGTCTCAT-3', GAPDH Reverse: 5'-AGGGGCCATCCACAGTCTTC-3'.

MTT assay detects cell proliferation

Cells were seeded into a 96-well plate at a density of 5000 cells per well. Cells were transfected with liposome 2000 as transfection reagent. Cell counting kit (CCK8; 7Sea Biotech, Shanghai, China) was used to detect cell proliferation. The OD value at 490 nm was measured at 24 h, 48 h, and 72 h after transfection using a FLUOstar OPTIMA mini plate (BMG Labtech GmbH, Ortenberg, Germany).

Flow cytometry detects apoptosis

Cells from each group were resuspended in 100 μL of 1 Annexin binding buffer and incubated with 5 μL of propidium iodide and 5 μL of FITC-Annexin V (BD, Franklin, NJ, USA). After 20 minutes of incubation in the dark at room temperature, 400 μL of Annexin binding buffer was added, and the fluorescence intensity of propidium iodide and Annexin V was detected by flow cytometry (BeamCyte).

Data analysis

SPSS17.0 software was used for analysis. Unpaired student-t test and one-way analysis of variance were used to compare the differences between two groups or different groups. Two-tailed $P<0.05$ was considered statistically sig-

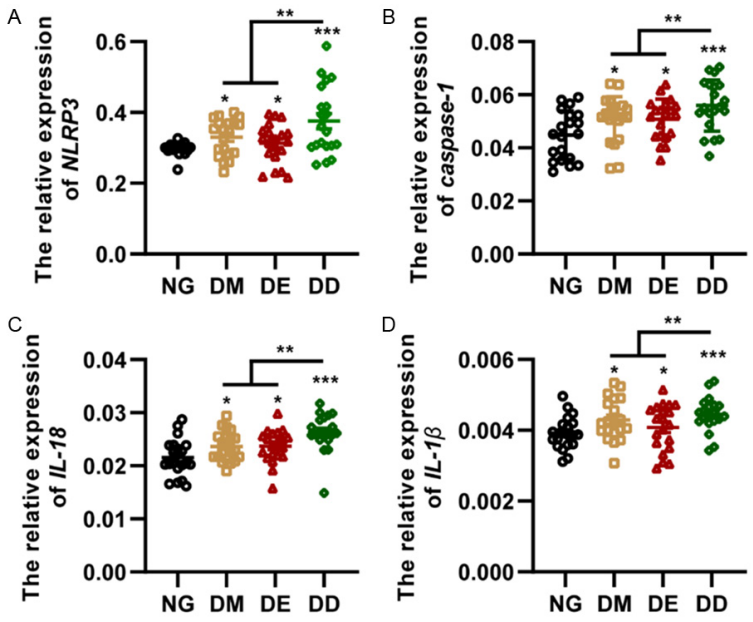

Figure 1. NLRP3/caspase-1 pathway-associated gene expression is elevated in DD patients. (A-D) Increased expression of NLRP3 (A), caspase-1 (B), IL-18 (C) and IL-1β (D) in the DD group. n=20 for each. *P<0.05, **P<0.01, ***P<0.001. n.s., not significant. One-way ANOVA followed by Tukey's multiple comparisons test. Error bars indicate SEM.

nificant. GraphPad Prism 8 was used for figure illustration.

Results

NLRP3/caspase-1 pathway-related genes are highly expressed in patients with DD

In order to investigate whether the NLRP3/caspase-1 pathway is related to DD, we first collected serum from patients with DD, DM, and DE and NG, and detected the expression of NLRP3/caspase-1 pathway-related genes (*NLRP3, caspase-1, IL-18 and IL-1β*). We found that compared with NG, the expression of NLRP3/caspase-1 pathway-related genes was increased in patients with DM and DE (**Figure 1A-D**, *P<0.05), and the increase was particularly pronounced in the DD group (**Figure 1C**, ***P< 0.001). These results indicate that the NLRP3/caspase-1 pathway is related to both DM and DE, and particularly DD.

Overexpression of NLRP3/caspase-1 pathway-related genes in DD rats

To further explore the relationship between the NLRP3/caspase-1 pathway and DD, we constructed DD rats. First, biochemical tests and Open-field behavioral experiments found that the blood glucose level of rats in the DD group was much higher than that in the NG group, while the amount of activity was much lower than that in the NG group (**Figure 2A** and **2B**, ***P< 0.001). RT-PCR showed that the expression of NLRP3/caspase-1 pathway related genes was significantly higher in DD rats than in NG group (**Figure 2C**, ***P<0.001). These results indicate that the DD model was successfully constructed, and the activation of the NLRP3/caspase-1 pathway correlated with DD.

Dendrobium mixture down-regulated expression of NLRP3/caspase-1 pathway-related genes to ameliorate the condition of DD rats

We found that the expression of NLRP3/caspase-1 pathway-related genes in the PD group was significantly reduced, while the PM group exhibited no significant change (**Figure 3A-D**, ***P<0.001). Moreover, compared with the PM group, the PD group was significantly improved with respect to the blood glucose level and mobility of DD rats (**Figure 3E** and **3F**, **P<0.01). These results indicate that Dendrobium mixture may improve DD symptoms by down-regulating the expression of genes related to the NLRP3/caspase-1 pathway, while positive drugs do not show similar effects.

Dendrobium promotes NIT-1 islet β proliferation

We used glucose and corticosterone to establish a high-glucose and high-ketone microenvironment for islet cell proliferation in DD model cells. It was found that the proportion of NIT-1 islet β-cell apoptosis was high in the GC group compared with the NG group (**Figure 4A** and **4B**, ***P<0.001). In the GC + DM group and the GC + PM group, the apoptosis rate was inversed, which was significantly pronounced in GC + DM group (**Figure 4A** and **4B**, **P<0.01).

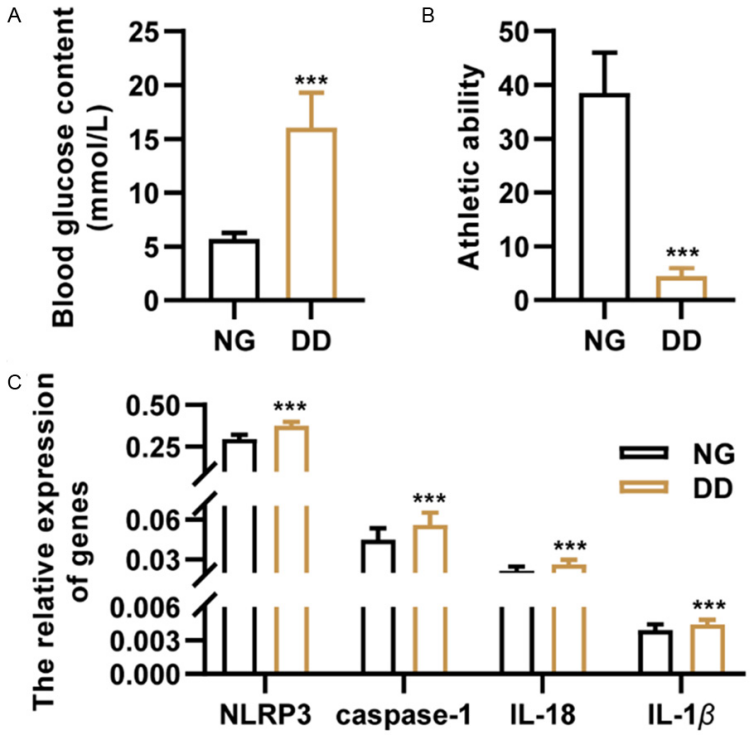

Figure 2. The expression of NLRP3/caspase-1 pathway-related genes is elevated in DD model rats. A. Increased blood glucose levels in DD rats; B. Significant decline in activity level in DD rats; C. Expressions of NLRP3, caspase-1, IL-18 and IL-1β in DD rats. n=10 for each. ***P<0.001. Mann-Whitney U test.

MTT assay found that the cell proliferation in the GC + DM group was not significantly different from that in the NG group, while the cell proliferation in the GC group and the GC + PM group was delayed (**Figure 4C**, ***P<0.001). RT-PCR assay had shown that the expression of NLRP3/caspase-1 pathway genes of NIT-1 islet β cells in the GC group and GC + PM group was activated (**Figure 4D**, ***P<0.001), and NLRP3 protein concentration was increased. The expression of NLRP3/caspase-1 pathway was inhibited in the G.C + DM group (**Figure 4D**). The above results show that Dendrobium mixture can promote the growth of NIT-1 islet β cells in the high glucose and ketone microenvironment, and offset the NIT-1 islet β cells apoptosis induced by high glucose and ketone microenvironment.

Protective effect of dendrobium mixture on hippocampal neural stem cells

In patients with depression, apoptosis in hippocampal NSCs was increased and hippocampal atrophy was observed. We dissected the brains from the rat models of each group and found that compared with the NG group, the hippocampus in the DD group and the PM group atrophied with decreased weight and volume, but the hippocampus in the PD group showed no obvious changes (**Figure 5A** and **5B**, **P<0.01, ***P<0.001). MTT assay revealed that there was no significant difference in the cell proliferation between the NSCs + GC + DM group and the NSCs group, while the cell proliferation NSCs + GC group and the NSCs + GC + PM group was delayed (**Figure 5C**, ***P<0.001). A glucose and ketone microenvironment for the growth of NSCs cells was established using glucose and corticosterone to simulate the growth conditions of NSCs in DD. The results showed that compared with the NG group, the apoptosis rate of NSCs was higher in the NSCs + G.C group (**Figure 5D** and **5E**, ***P<0.001). The apoptosis rate was offset in the NSCs + GC + DM group and the NSCs + GC + PM group, particularly in the NSCs + GC + DM group (**Figure 5D** and **5E**, **P<0.01, ***P<0.001).

RT-PCR demonstrated that the expression of NLRP3/caspase-1 pathway-related genes of NSCs in the NSCs + GC group and NSCs + GC + PM group was activated, and the NLRP3 protein concentration was also increased (**Figure 5F**, ***P<0.001). However, the expression of the NLRP3/caspase-1 pathway was suppressed in the NSCs + GC + DM group (**Figure 5F**). The above results showed that Dendrobium mixture can promote the growth of NSCs in the high glucose and ketone microenvironment, improving the apoptosis rate of NSCs.

NLRP3 inhibitor (MCC950) improves DD symptoms

We injected NLRP3 inhibitor MCC950 into DD rats. We found that MCC950 can significantly

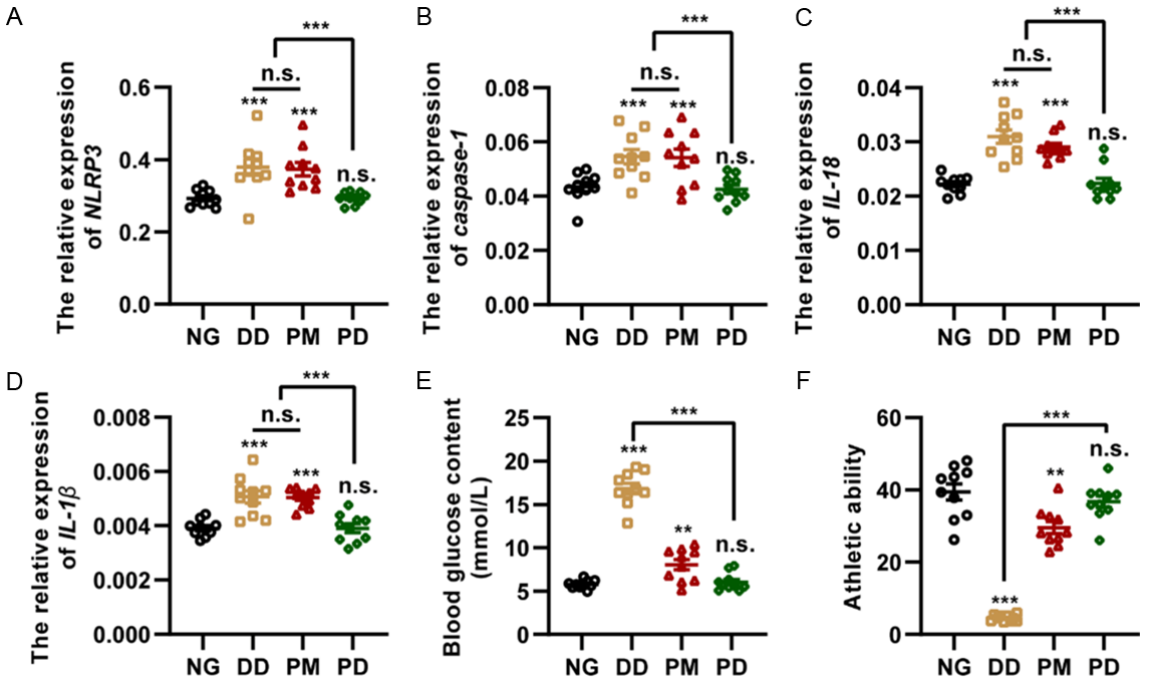

Figure 3. Dendrobium Mixture reduces gene expression of NLRP3/caspase-1 pathway and improves symptoms of DD rats. (A-D) Dendrobium mixture can effectively reduce the expression of genes related to the NLRP3/caspase-1 pathway; (E, F) Dendrobium mixture can reduce blood glucose (E) and improve exercise capacity (F) in DD rats. n=10 for each. **P<0.01, ***P<0.001. n.s., not significant. One-way ANOVA followed by Tukey's multiple comparisons test. Error bars indicate SEM.

improve the symptoms of DD rats, increase mobility, and lower blood glucose (**Figure 6A** and **6B**, *P<0.05). Injecting MCC950 into NIT-1 islet β cells and NSCs in a high ketone microenvironment can promote cell proliferation (**Figure 6C** and **6D**, ***P<0.001). These results indicate that the NLRP3/caspase-1 pathway plays a proximal role in the occurrence and development of DD.

Discussion

Depression is twice as common in patients with diabetes as in healthy people and the increase in the prevalence of depression is closely related to diabetes complications [4, 17]. Depression in patients with diabetes may be caused by decreased quality of life due to treatment, or it may be caused by biochemical changes accompanying the diabetes [18, 19]. The NLRP3/caspase-1 pathway is activated when exposed to external stimuli such as a high-glucose environment, leading to an inflammatory response [9, 20-22]. Previous studies have found that Dendrobium mixture has a good effect on the regulation of the inflammatory response and protection of nerve cells [14, 23-25]. Therefore, this study used Dendrobium Mixture to treat DD rats, and for the first time proved that Dendrobium Mixture can significantly improve the condition of DD rats by inhibiting the expression of NLRP3/caspase-1 pathway.

In this study, PCR assay found that the related gene expression of the NLRP3/caspase-1 pathway was abnormally elevated in DD patients, which has not been observed in previous studies of DD. We then constructed the rat DD model and obtained similar results. Therefore, it is speculated that the NLRP3/caspase-1 pathway plays a very important role in DD. The combination of Dendrobium mixture with metformin and fluoxetine significantly reduced the expression of NLRP3/caspase-1 pathway-related genes. The above results proved that Dendrobium mixture can regulate the expression of NLRP3/caspase-1 pathway-related genes.

The main manifestations of patients with DD are increased blood glucose levels, decreased mobility, communication problems, etc.; and with the worsening of diabetes, the symptoms of depression become more prominent [26, 27]. In this study, the blood glucose of DD rats was significantly increased in the model ani-

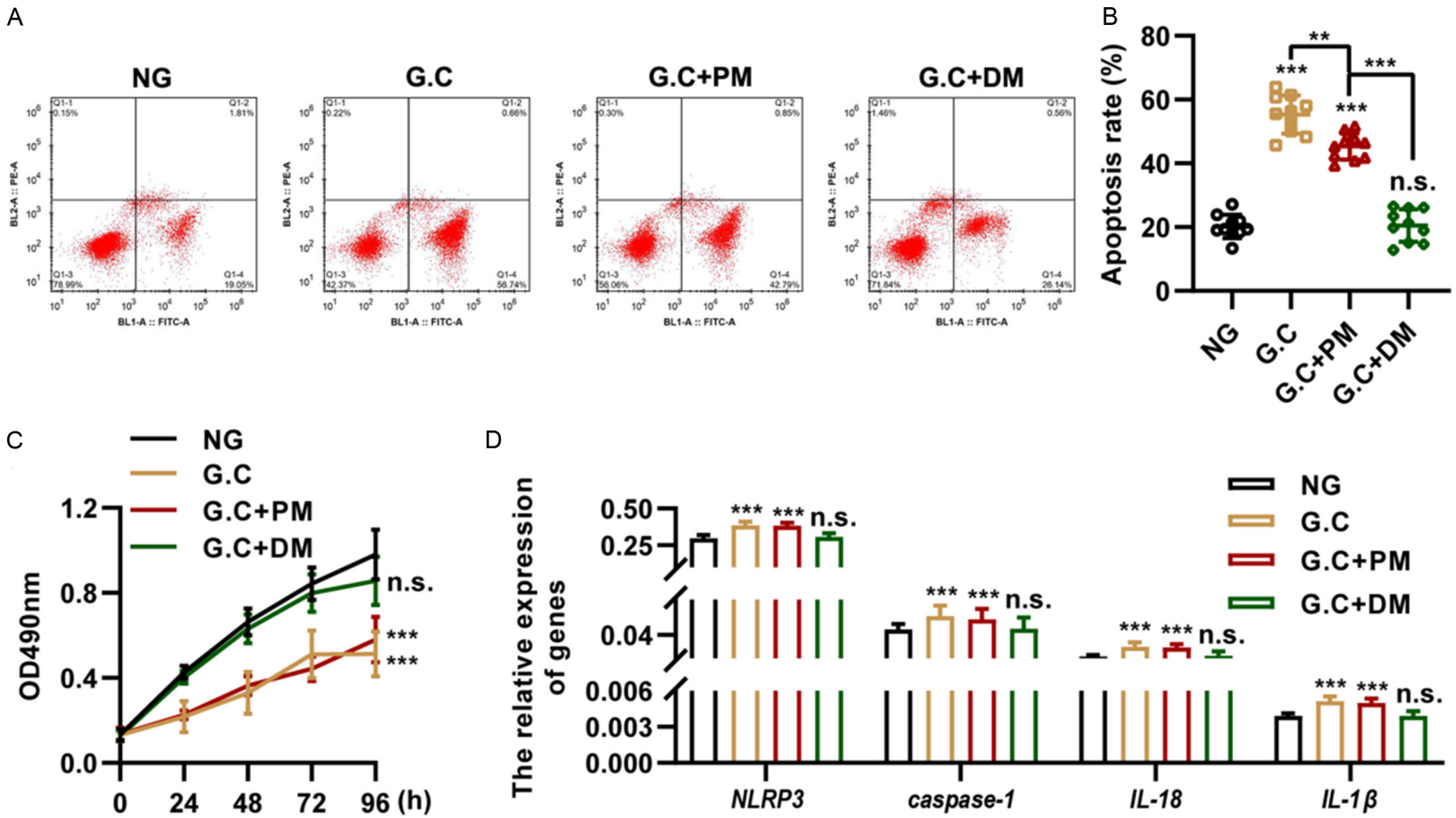

Figure 4. Dendrobium mixture promotes NIT-1 islet β-cell proliferation by down-regulating gene expression of NLRP3/caspase-1 pathway. A and B. Flow cytometry detects the apoptosis of NIT-1 islet β cells in each group; C. Dendrobium mixture promotes the proliferation of NIT-1 islet β cells under high glucose and ketone conditions; D. RT-PCR detects gene expression of NLRP3/caspase-1 pathway in NIT-1 islet β cells. n=10 for each. **P<0.01, ***P<0.001. n.s., not significant. One-way ANOVA followed by Tukey's multiple comparisons test and chi-square test. Error bars indicate SEM.

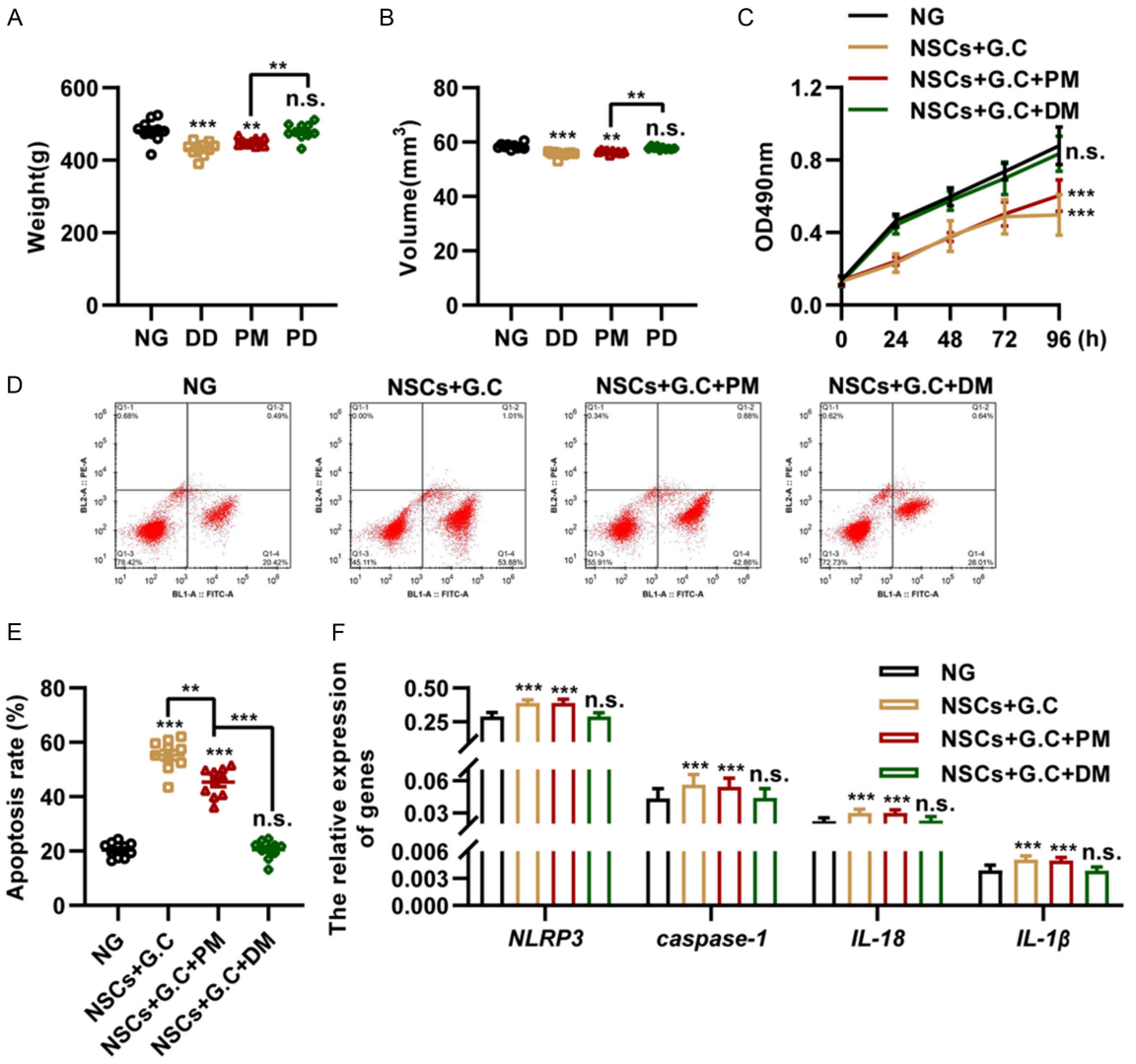

Figure 5. Dendrobium mixture promotes NSCs proliferation by down-regulating gene expression of NLRP3/caspase-1 pathway. A and B. Measure the weight and volume of hippocampus in each group of rats; C. Dendrobium mixture promotes the proliferation of NSCs in vitro; D and E. Dendrobium mixture reduces the apoptosis of NSCs; F. RT-PCR detects NLRP3/caspase-1 pathway-related gene expression. n=10 for each. **P<0.01, ***P<0.001. n.s., not significant. One-way ANOVA followed by Tukey's multiple comparisons test and chi-square test. Error bars indicate SEM.

mals, while their activity level was significantly decreased compared with healthy rats (**Figure 3F**). Islet β cells are insulin-secreting cells, responsible for blood glucose regulation. In this study, glucose and corticosterone were used to simulate the microenvironment of pancreatic β-cell NIT-1 in DD patients with high levels of glucose and ketones *in vitro*, and explore the effects of Dendrobium mixture on pancreatic NIT-1 β cells. The NLRP3/caspase-1 pathway related gene expression was up-regulated, and cell proliferation was promoted, and also the apoptotic rate was reduced. Administration of positive medicine alone did not gain the same results. We hypothesized that Dendrobium mixture protects islet β cells by reducing the inflammatory response of the NLRP3/caspase-1 pathway, and that the expression of the NLRP3/caspase-1 pathway has a major effect on the islet β cell apoptosis. Therefore, Dendrobium mixture can restore blood glucose levels in DD rats, which may be related to the protection of islet β cells.

The subgranular region of the dentate gyrus in the hippocampus is one of the major growth and aggregation regions of NSCs in the adult rat brain [10, 28]. NSCs mainly exist here, and

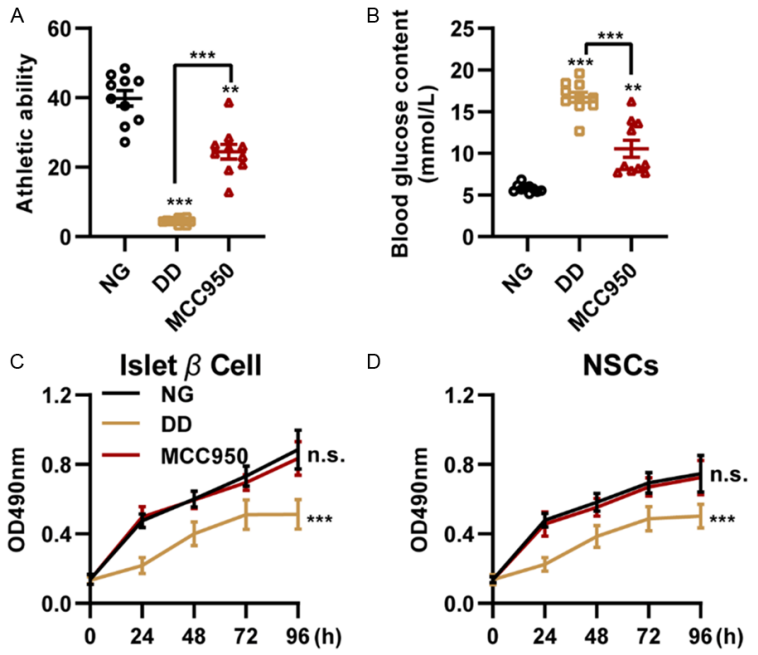

Figure 6. NLRP3 inhibitor MCC950 improves DD disorders and promotes cell proliferation. (A and B) MCC950 enhances the exercise capacity of DD rats (A), reduces blood glucose levels in DD rats (B); (C and D) MCC950 promotes the proliferation of islet β cells and NSCs. n=10 for each. **P<0.01, ***P<0.001. n.s., not significant. One-way ANOVA followed by Tukey's multiple comparisons test and chi-square test. Error bars indicate SEM.

thus proliferate and migrate to the granular cell layer, differentiate into granular cells, slowing down the senescence, apoptosis and death of neural cells, and play an important role in maintaining learning, memory and physical functions [29]. Many studies have proved that hippocampal damage has a close relationship with depression [30-32]. Therefore, we hypothesize that the reduction of NSCs in the hippocampus is the main causative factor that leads to a decrease in the vitality of depressive rats.

Our experiments further explored whether the Dendrobium mixture has a protective effect on the hippocampus in the DD environment. We found that the use of Dendrobium mixture can significantly improve the activity level of DD rats (**Figure 3F**). Subsequently, we isolated rat hippocampal NSCs and made a few passages of primary culture then cloned and cultured single cells. These cultured cells were neural stem cells with a purification rate of 98%. With BrdU immunofluorescence staining and MTT experiments on NSCs, it was confirmed that high glucose culture reduced the proliferation ability of NSCs, and Dendrobium mixture can protect NSCs cultured in high glucose and high ketone environment.

These results indicated that Dendrobium mixture can promote the proliferation of NSCs under high levels of glucose and ketone and has anti-apoptotic effects. Compared with the NG group, the use of positive medicine alone did not protect NSCs, and the expression of the NLRP3/caspase-1 pathway was not inhibited in the positive medicine group. However, Dendrobium mixture can effectively inhibit the expression of NLRP3/caspase-1 inflammatory pathway in NSCs and protect the integrity of hippocampus. Finally, *in vivo* experiments using the NLRP3 inhibitor MCC950 have been found to improve symptoms in DD rats, indicating that the NLRP3/caspase-1 inflammatory pathway is involved in the occurrence of DD.

This study has verified synthetically that the improvement of DD disease by Dendrobium mixture is achieved via the NLRP3/caspase-1 pathway. However, there are still some shortcomings, such as whether the effect of Dendrobium mixture on the NLRP3/caspase-1 pathway is unique in DD, and whether it also affects the expression of other genes. At the same time, there are problems such as insufficient verification of results. It is hoped that these problems can be solved in further research.

Dendrobium mixture improves the proliferation of islet β cells and hippocampal neural stem cells, and prevents the high glucose and high ketone environment from inducing apoptosis. By inhibiting the NLRP3/caspase-1 pathway, it lowers blood glucose, improves exercise capacity, and improves the symptoms of diabetic depression in DD rats.

Disclosure of conflict of interest

None.

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