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TF-LNPS-Based Delivery of Emodin Alleviates Hippocampal Neuronal PANoptosis in Diabetic Encephalopathy Rats via the HDAC4/NF-κB Pathway

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Research Article

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ABSTRACT

Previous studies have demonstrated that Polygonum multiflorum Thunb. ameliorates diabetic encephalopathy through multiple pathways; however, the exploration of the efficacy of its individual components remains relatively limited. Emodin, as the main active ingredient of Polygonum multiflorum, has significant anti-inflammatory effects, reduces oxidative stress, and has certain neuroprotective functions. However, its application in the treatment of diabetic encephalopathy has not been reported. In this study, the rat model of type 1 diabetes was used, and emodin was given by gavage. The results showed that emodin could significantly improve the learning and memory of diabetic rats, and reduce the level of inflammation in their bodies. Further data proved that emodin effectively reduced the PANoptosis phenomenon of hippocampal neurons by inhibiting the activation of HDAC4/NF-κB

signaling pathway. When emodin is used as a medicine, it has limitations such as poor water solubility, low availability and rapid metabolism, which greatly limits its medicine transformation value. In this study, TF-LNPS nanocarriers were innovatively used to encapsulate emodin to optimize its medicine delivery performance. The experimental results showed that TF-LNPs-Emodin could effectively penetrate the blood-brain barrier and exert its effect on the central nervous system. This is the first report of TF-LNPs delivery system for the treatment of diabetic encephalopathy, showing excellent application and transformation potential, and providing important theoretical basis and practical reference for the subsequent development of relevant medicine carriers.

Keywords: Diabetic encephalopathy，Emodin，HDAC4/NF-κB pathway，PANoptosis

INTRODUCTION

The latest diabetes world atlas report released by the International Diabetes Federation on April 7, 2025 shows that the number of adult diabetic patients aged 20-79 years has exceeded 589million worldwide. In the past 2024, the number of deaths caused by diabetes and its complications was 3.4 million[1]. The prevention and control of diabetes and its complications has been extremely serious. Diabetic encephalopathy (DE) is a serious complication of cognitive dysfunction caused by long-term hyperglycemia in diabetic patients. Its clinical manifestations are the decline of learning and memory ability and mental and psychological problems[2]. Since the concept of "diabetic encephalopathy" was put forward in the 1960s, the research on its pathogenesis has made some progress, mainly focusing on the changes

of brain vascular function, oxidative stress, advanced glycation end products (AEGs), reduced neurotransmitter synthesis, blood brain barrier (BBB) dysfunction and other aspects[3]. The incidence and prevalence of dementia in people with diabetes mellitus have increased sharply, and the probability of dementia increases in proportion with the increase of the duration of diabetes mellitus, which brings a huge burden on the medical resources of the society[4]. In recent years, with the rising incidence of diabetes, diabetic encephalopathy has also attracted more and more attention. At present, epidemiological studies on diabetic encephalopathy show that about 20% - 40% of diabetic patients have cognitive dysfunction to varying degrees, and with the extension of the course of the disease and the growth of age, the risk of the disease increases significantly[5].

In 2019, Malireddi et al[6] first proposed the concept of "PANoptosis", which includes apoptosis, pyroptosis, necroptosis and other programmed death modes, and attracted extensive attention. Its characteristics are pyroptosis, apoptosis and necroptosis, but it cannot be characterized by any one of them alone[7]. For a long time, it has been believed that the death pathways of cells are parallel without overlap. In fact, in recent years, more and more studies have shown that pyroptosis, apoptosis and necroptosis are closely related and can regulate each other[8]. PANoptosis is an inflammatory programmed cell death pathway activated by specific triggers and regulated by the PANoptosome complex. The PANoptosome complex provides a molecular scaffold for the simultaneous participation of key molecules from pyroptosis, apoptosis and necroptosis. Therefore, this complex has the key

characteristics of pyroptosis, apoptosis and necroptosis[9]. Initially, the research of PANoptosis focused on infectious diseases in which pathogenic microorganisms invade host cells. In recent years, it has been found that it plays an important role in the occurrence and development of neurodegenerative diseases, immune related diseases, tumor diseases and bone metabolic diseases[10]. However, there is no relevant report of PANoptosis in diabetic encephalopathy at present, combined with the complex pathogenesis of diabetic encephalopathy itself; Therefore, it is of great significance to study the role of PANoptosis in diabetic encephalopathy.

At present, the treatment of diabetic encephalopathy mainly relies on medicines that improve blood glucose control, such as insulin, metformin, etc[11]. These medicines can effectively control blood glucose levels, but have no specific therapeutic effect on cognitive decline and other neurodegenerative changes caused by diabetic encephalopathy[12]. Therefore, it is urgent to find specific medicines for diabetic encephalopathy. As a huge data source for medicine development, traditional Chinese medicine has rich active ingredients and unique pharmacological mechanisms. For thousands of years, traditional Chinese medicine has accumulated a lot of clinical experience and theoretical basis in the treatment of various diseases, and has become an important resource for modern medicine research[13]. Traditional Chinese medicine has unique advantages in the treatment of complex diseases such as diabetic encephalopathy due to its multi-component and multi-target characteristics[14]. Our team has confirmed in previous studies that Polygonum multiflorum can improve the symptoms of diabetic encephalopathy through a variety of ways[3]. Polygonum

multiflorum Thunb is a traditional Chinese medicine, which has many effects, such as tonifying liver and kidney, strengthening muscles and bones, and benefiting essence and blood. In the study of diabetic encephalopathy, Polygonum multiflorum was found to play its therapeutic role by regulating blood glucose, reducing oxidative stress, inhibiting inflammatory response and improving neurological function. However, the discussion on the efficacy of its single component is relatively limited, and it is not clear which component plays a major role in the treatment of diabetic encephalopathy, which limits its further promotion in clinical application. Emodin is one of the main active ingredients in Polygonum multiflorum Thunb. It has anti-inflammatory effect, reducing oxidative stress reaction and certain neuroprotective function[15]. Although emodin has shown its potential therapeutic effect in the study of other diseases[16], its application in the treatment of diabetic encephalopathy has not been reported. In particular, studies on emodin in improving PANoptosis are also relatively rare. Therefore, it is planned to carry out research on emodin protection against diabetic encephalopathy, to prove its anti-inflammatory and neuroprotective functions, as well as the potential molecular mechanism of improving neuronal PANoptosis.

In recent years, the application of nano medicine delivery system in the treatment of brain diseases has shown great potential, especially in overcoming the blood-brain barrier and improving medicine targeting[17, 18]. For Alzheimer's disease and Parkinson's disease, nanoparticles can be targeted to deliver medicines through specific ligands or antibodies liposomes are nanoscale medicine carriers composed of

natural lipids[19, 20]. Because they are similar to cell membrane components and have excellent biocompatibility and biodegradability, they show significant advantages in the field of brain medicine delivery[21]. Although these medicine delivery tools have performed well in the treatment of neurological diseases, most of them are traumatic diseases and solid tumors[22-24], and drug delivery strategies specifically targeting diabetic encephalopathy have not yet been reported. Patients with diabetic encephalopathy need more efficient medicine delivery methods to ensure that active ingredients such as emodin can accurately reach the brain tissue and play its anti-inflammatory, antioxidant and neuroprotective roles. Our team plans to carry out relevant research and select specific modified nanocarriers with emodin as the medicine content. This modified nanocarrier will improve its biocompatibility and targeting, reduce the clearance rate in the blood circulation, and increase the ability to penetrate the blood-brain barrier. By optimizing the design and preparation methods of nanocarriers, we hope to achieve efficient targeted delivery and therapeutic effect of medicines, provide effective medicine carrier basis for the treatment of diabetic encephalopathy, and open up a new way for future clinical medication.

METHODS and MATERIALS

1. Animal Models

Fifty healthy SPF (Specific Pathogen Free) 10-week-old Sprague-Dawley (SD) rats were purchased and housed in an SPF animal facility under standard conditions of 24°C with a 12-hour light-dark cycle. Twenty-four healthy SPF (Specific Pathogen

Free) 15-week-old C57BL/6 mice were purchased and housed in an SPF animal facility under standard conditions of 24°C with a 12-hour light-dark cycle.All procedures conformed to the 3R principles (Reduction, Replacement, Refinement) to minimize animal suffering and ensure ethical use. The study adheres to established animal welfare guidelines, and all experiments were approved by the Institutional Animal Care and Use Committee.This study was approved by the Animal Ethics Committee of Guizhou Medical University (Approval No. 2402598).

2. Common Materials and Reagents

For the study on the treatment of diabetic encephalopathy in rats using emodin, the following common materials and reagents were used:Emodin-Pure emodin from Aladdin (Shanghai, China)；Streptozotocin (STZ)-STZ from Sigma (St. Louis, MO, USA)；Glucose-Glucose solution from Tianjin Pharma Reagent (Tianjin, China)；Protein Assay Kit-BCA Protein Assay Kit from Thermo Fisher (Waltham, MA, USA)；ELISA Kits for Cytokines-ELISA kits from Abcam (Cambridge, UK)；Dihydroethidium (DHE)-DHE from Beyotime (Shanghai, China)；Transmission Electron Microscopy (TEM) Staining Kit-TEM staining kit from Alfa Aesar (Ward Hill, MA, USA)；Hematoxylin and Eosin (H&E) Staining Kit-H&E staining kit from Alfa Aesar (Ward Hill, MA, USA).All reagents were of high purity and stored according to the manufacturer's instructions to ensure optimal performance in the experiments.

3. Cell Models and Culture

This study used the hCMEC/D3 human brain microvascular endothelial cell

model to simulate the blood-brain barrier[25, 26] and HT-22 hippocampal neurons for in vitro mechanism exploration[27, 28]. The cells were cultured according to previously reported methods. For the high-glucose damage model, cells in the logarithmic growth phase were selected for the experiments. During the cell treatment period, inhibitors, activators, and rhein were administered. All operations were performed in a sterile environment to prevent cell contamination. To ensure the accuracy of the experimental results, all experiments were repeated three times.

4. Behavioral Experiments

Morris Water Maze Test：The Morris Water Maze test was used to evaluate the spatial memory and learning abilities of the experimental rats[29, 30]. The maze was a circular pool with a diameter of 120 cm and a depth of 30 cm, maintained at a water temperature of 22±1°C, and filled with opaque black paint to obscure the bottom. A submerged platform, serving as the hidden target, was located in one quadrant of the maze, with a diameter of 10 cm. The experiment consisted of four stages: adaptation, learning, memory consolidation, and spatial memory testing. Rats were trained twice daily for five consecutive days. During the training phase, latency time (time taken from the start to reach the platform) and swimming paths were recorded to assess learning and memory stability. In the testing phase, the platform was removed, and the rats were observed for 60 seconds to record the number of times they crossed the original platform location and the time spent there, reflecting their spatial memory capability.

Place Navigation Test：The Place Navigation test is a behavioral experiment

designed to evaluate the spatial learning and memory capabilities of rats. The test was conducted in an open square testing chamber with a 2.5 mm thick layer of white wood chips on the floor, and multiple corners were adorned with brightly colored visual landmarks. A hidden platform, 10 cm × 10 cm in size and 2 cm high, was placed in the center of the chamber, covered with 1 cm of wood chips to match the color of the floor, making it invisible to the rats. The experiment consisted of two phases: a training phase, with twice-daily trials for five consecutive days, where rats were required to find and stay on the hidden platform without any cues. The second phase was the testing phase, conducted 24 hours after training, with the platform removed. The number of times rats entered the original platform location and their total exploration time were recorded to assess the retention of spatial memory.

5. Cell Viability Assay

Cell viability was assessed using the CCK-8 reagent kit to evaluate the effect of different stimuli on the cell models. HT-22 hippocampal neurons and vascular endothelial cells were seeded in 96-well plates at a density of 1 × 10^4 cells/well. After allowing the cells to adhere and reach a suitable confluence, they were treated with the respective stimuli for 24 hours. Subsequently, 10 μL of CCK-8 solution was added to each well, and the plates were incubated for an additional 1 hour at 37°C in a humidified 5% CO2 atmosphere. Absorbance was measured at 450 nm using a microplate reader. Each experiment was performed in triplicate to ensure data reliability. The viability was calculated as the percentage of absorbance relative to the control group

6. HE Staining of Hippocampi

At the end of the experimental duration, rats were euthanized humanely using a ketamine-xylazine cocktail administered intraperitoneally. The hippocampi were carefully removed, fixed in 4% formalin for 24 hours, and then transferred to 70% ethanol for storage. Subsequently, the tissues were dehydrated through a series of ethanol concentrations, embedded in paraffin, and sectioned into 4 μm thick slices using a microtome. The sections were then placed on glass slides and stained with hematoxylin and eosin (HE) following standard procedures. Briefly, the sections were first deparaffinized with xylene, hydrated through ethanol gradients, stained with hematoxylin for 5 minutes, rinsed with water, bleached with 0.5% hydrogen peroxide, stained with eosin for 3 minutes, and dehydrated again with ethanol. Finally, the sections were cleared with xylene and mounted with coverslips using mounting medium. The HE-stained sections were observed under a light microscope to assess pathological damage in the hippocampi. Quantitative analysis of the damage was performed using ImageJ software, and the results were compared across different experimental groups.

7.Immunohistochemistry

Using the paraffin-embedded tissue sections prepared in Section 6, immunohistochemical staining was performed on the hippocampi to determine the expression of target proteins, following the methods described by previous studies. First, 4 μm-thick paraffin sections were deparaffinized and rehydrated using xylene and ethanol gradients. Antigen retrieval was carried out by incubating the sections in

10 mM EDTA solution at 95-98°C for 15-20 minutes using a microwave oven. The sections were then rinsed three times with PBS, each wash lasting 3 minutes. To block endogenous peroxidase activity, the sections were incubated in 3% H2O2 solution for 15 minutes, followed by three PBS rinses. Next, sections were incubated with normal goat serum for 1 hour to reduce non-specific background staining. The primary antibody specific to the target protein (diluted according to the manufacturer's instructions) was then added, and sections were incubated overnight at 4°C. The following day, sections were rinsed three times with PBS and incubated with the appropriate secondary antibody (horse anti-rabbit or horse anti-mouse IgG conjugated with horseradish peroxidase, depending on the primary antibody species) at room temperature for 1 hour. The sections were rinsed three more times with PBS, and DAB staining was performed for 5-10 minutes to visualize the target protein. Staining was terminated with tap water, and sections were counterstained with hematoxylin for 5 minutes. After dehydrating with ethanol and clearing with xylene, sections were mounted with neutral resin. Stained sections were observed under a light microscope and imaged. Quantitative analysis of protein expression intensity and positive cell percentage was performed using image analysis software, and the results were compared with the control group.

8.Immunofluorescence

Immunofluorescence was used to detect the expression and localization of key proteins in the hippocampus or cell models. Following previously reported methods, the samples underwent detailed staining procedures. First, 4 μm-thick paraffin

sections or cell culture samples were deparaffinized and rehydrated. Cell culture samples were directly fixed with 4% paraformaldehyde for 15 minutes and then permeabilized with 0.1% Triton X-100 solution for 5 minutes. Both sections and cell samples were rinsed three times with PBS, each wash lasting 3 minutes, to remove residual fixative and permeabilization agent. Subsequently, samples were incubated with normal goat serum for 1 hour to reduce non-specific background staining. Next, samples were treated with the appropriate diluted primary antibody (according to the manufacturer's instructions) and incubated overnight at 4°C. The following day, samples were rinsed three more times with PBS and then incubated with a fluorescein-labeled secondary antibody (such as Alexa Fluor 488-conjugated goat anti-rabbit IgG or Alexa Fluor 594-conjugated goat anti-mouse IgG) at room temperature for 1 hour. Samples were rinsed three more times with PBS and subsequently counterstained with DAPI for 10 minutes to stain the nuclei. Finally, samples were mounted with glycerol-based mounting medium and observed under a fluorescence microscope to record the expression and localization of the target protein. Immunofluorescence images were processed and analyzed using image analysis software to quantitatively assess the intensity of protein expression and the proportion of positive cells, and were compared with the control group.

9.ELISA

Enzyme-Linked Immunosorbent Assay (ELISA) is a semiquantitative method used for detecting specific proteins in serum and tissue samples. In this study, we employed this technique to measure inflammation factors and HDAC levels. First,

serum or tissue samples were diluted appropriately and added to 96-well microplates pre-coated with specific antibodies, and incubated at room temperature for 1 hour to allow target proteins to bind to the antibodies. Next, the samples were removed, and the microplates were washed three times with wash buffer for 5 minutes each to eliminate unbound proteins. After that, enzyme-labeled secondary antibodies were added, and the plates were incubated for another hour. The microplates were washed three more times before the addition of substrate solution, and the colorimetric reaction was allowed to proceed for approximately 15 minutes. The colorimetric reaction was stopped by adding stop solution, and the optical density (OD) values of each well were immediately read. The obtained OD values were quantified using a standard curve to semiquantitatively assess the expression levels of inflammation factors and HDAC. All experiments were repeated three times to ensure data reliability.

10.Preparation and Characterization of TF-LNPs Materials

To prepare transferrin (TF)–modified liposomal nanoparticles (TF-LNPs) for encapsulating emodin and delivering it to the brain to treat neuronal damage, we followed a series of steps. Initially, we prepared blank liposomal nanoparticles (LNPs) using the thin film hydration method. This involved dissolving cholesterol (Chol) and phospholipids (DSPC/DSPE-PEG2000) in chloroform, rotating to evaporate the organic solvent, and forming a lipid film. The lipid film was subsequently dispersed in phosphate-buffered saline (PBS) and subjected to multiple freeze-thaw cycles and ultrasonication to produce LNPs. Emodin was then dissolved in DMSO and added to

the liposomal solution, followed by incubation to ensure the medicine was fully encapsulated within the liposomes, resulting in TF-LNPs-Emodin.The prepared TF-LNPs-Emodin underwent comprehensive characterization. First, the particle size distribution of LNPs was analyzed using dynamic light scattering (DLS) to confirm its nanoscale size. Second, transmission electron microscopy (TEM) was employed to capture morphological images of the LNPs, evaluating its uniformity and structural integrity. Third, Fourier-transform infrared spectroscopy (FTIR) was used to analyze the molecular structure of TF-LNPs-Emodin, verifying the successful modification with TF and the encapsulation of emodin. Finally, ultraviolet-visible spectroscopy (UV-Vis) was utilized to determine the encapsulation efficiency of emodin, ensuring that a sufficient dose of the medicine was loaded within the liposomes. These characterization results indicated that the prepared TF-LNPs-Emodin exhibited appropriate particle size, uniform morphology, and high medicine loading capacity, making it suitable as a medicine delivery vehicle for treating neuronal damage.

11. Assessment of Acute Toxicity of Liposomal Nanoparticles

To evaluate the acute toxicity of liposomal nanoparticles, C57BL/6 mice were randomly divided into four experimental groups (n = 6 per group). The nanoparticles were administered via tail vein injection at predetermined doses. Following the designated treatment periods, mice were anesthetized and euthanized for sample collection.

12. Blood Collection and Analysis

Whole blood samples were obtained via cardiac puncture and processed for

hematological and inflammatory marker analysis. Complete blood count (CBC) was performed to assess leukocyte populations, including WBC (white blood cell count), neutrophils (NEUT), lymphocytes (LYMPH), and their absolute values (NEUT Abs, LYMPH Abs). Plasma levels of pro-inflammatory cytokines (e.g., TNF-α, IL-6, IL-1β, IL-10) were quantified using ELISA to evaluate systemic immune responses.

13.Assessment of Liver and Kidney Function

To evaluate the potential toxicity of liposomal formulations, hepatic and renal function biomarkers were quantitatively analyzed in serum samples. Blood was collected from the orbital venous plexus of anesthetized mice at predetermined time points post-administration and centrifuged at 3,000 × g for 10 min to isolate serum.Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured using commercial assay kits (e.g., Sigma-Aldrich, USA) following the manufacturer’s protocols. Elevated ALT and AST levels are indicative of hepatocellular injury.Blood urea nitrogen (BUN) and creatinine concentrations were determined using standardized enzymatic assays (e.g., BioAssay Systems, USA). These biomarkers reflect glomerular filtration rate and renal excretory function.

14.Network Pharmacology and Molecular Docking

This study employed network pharmacology databases to analyze the potential targets and biological functions of emodin and used molecular docking techniques to predict its binding interactions. Initially, relevant information about emodin, including its chemical structure, known targets, and mechanisms of action, was collected from databases such as PubChem, medicineBank, and The Human Protein Atlas.

Subsequently, network pharmacology analysis of emodin was performed using the PharmMapper platform to identify potential targets. These targets were further analyzed for biological functional enrichment using the DAVID bioinformatics tool to understand the potential biological effects of emodin.

Additionally, to predict the binding interactions between emodin and its potential targets, molecular docking technology was utilized. Specifically, a three-dimensional structural model of emodin was constructed using ChemAxon's MARVIN Sketch software, and three-dimensional structural files of the potential target proteins were obtained from the PDB database. Then, molecular docking simulations were carried out using AutoDock Vina software to optimize the binding poses of emodin with the target proteins and calculate the binding energies, thereby assessing the stability of the emodin–target protein binding interactions.By analyzing emodin's potential targets and binding interactions, this study provided important insights into its pharmacological effects and facilitated the development of potential therapeutic strategies.

12.Western Blot Analysis

Western Blot analysis was employed to quantitatively assess the expression of specific key proteins in animal models and cell models. Initially, total protein was extracted from animal tissues and cell cultures, and protein concentration was determined using the BCA protein quantitation kit. Equal amounts of protein samples were then loaded onto SDS-polyacrylamide gels for electrophoresis separation, followed by transfer of the proteins onto PVDF membranes. The membranes were

subsequently blocked with 5% non-fat milk to eliminate non-specific binding sites. Primary antibodies specific to the target proteins were added, and the membranes were incubated overnight at 4°C. The next day, the membranes were washed three times with TBST for 10 minutes each, then incubated with the corresponding secondary antibodies for 1 hour at room temperature. Another three washes were performed, and the membranes were developed using ECL chemiluminescence reagents. Western blot images were captured using a gel imaging system. Finally, ImageJ software was used to analyze the grayscale intensities of the images, quantifying the relative expression levels of the target proteins. All experiments were repeated three times to ensure the reliability of the results.

13.In Vivo Imaging

In vivo imaging was employed to evaluate the distribution of the prepared TF-LNPs-Emodin in animal models. For the experiment, TF-LNPs-Emodin was labeled with a near-infrared fluorescent dye to enable observation in live animals. The labeled nanoparticles were administered via tail vein injection to mice. Post-injection, the animals were placed in a near-infrared imaging system, with imaging performed every 2 hours for a total duration of 24 hours. Images were captured using the IVIS Lumina imaging and analyzed with LivingImage software to quantify the fluorescence intensity in various organs. Additionally, samples of blood and tissue were collected and analyzed to further verify the specific distribution of TF-LNPs-Emodin in the body. All experiments were repeated three times to ensure the accuracy and reliability of the results.

14.Surface Plasmon Resonance (SPR) Analysis

Surface Plasmon Resonance (SPR) technology was employed to detect the direct binding between emodin and histone deacetylase 4 (HDAC4) protein. The experiment began by immobilizing HDAC4 protein on a CM5 sensor chip using standard amine coupling methods. Specifically, a high-intensity self-assembled monolayer was deposited on the CM5 chip surface, then activated with N-hydroxysuccinimide (NHS) and N-(3-dimethylaminopropyl) N′-ethylcarbodiimide hydrochloride (EDC) to couple the protein. Subsequently, injection experiments were performed using increasing concentrations of emodin solution, monitoring the sensor response. The system used a flow injection with an injection time of 60 seconds and a flow rate of 30 μL/min. By analyzing the Equilibrium Binding (ECB) model, the binding constant (KD) of emodin with HDAC4 protein was determined, thereby assessing the binding affinity. All experiments were repeated three times to ensure the reliability of the results.

15.Statistical Analysis

Statistical analysis was conducted to evaluate the significance and reliability of the experimental data. All data were processed using SPSS 25.0 software. Quantitative data are presented as mean ± standard deviation (Mean ± SD), and group comparisons were performed using one-way analysis of variance (One-Way ANOVA). If the ANOVA results showed significant differences, further multiple comparisons were conducted using Tukey’s HSD (Honestly Significant Difference) to identify specific differences between groups. Qualitative data were analyzed using the Chi-Square Test. Survival curve comparisons were performed using the Log-Rank

Test. All statistical tests were set at a significance level (α) of 0.05, with P-values less than 0.05 considered statistically significant. Results are presented in figures and tables for clear interpretation.

RESULT

1. Emodin improves cognitive function of rats with diabetes encephalopathy

The rat model of type 1 diabetes mellitus was replicated by the previous research method of the research group. The results showed that the random blood glucose of rats in T1DM group was ≥ 16.7mmol/l, and it was stable for more than 3 weeks, suggesting that the rat model of type 1 diabetes mellitus was successfully replicated (Fig. 1A); Then, on the basis of the successful T1DM rat model, the intervention of emodin was started at the 4th week. After 20 weeks of intervention with emodin, it was found that the body weight of rats in the T1DM group was always maintained at a low level, while the body weight of rats in the low and high groups was increased compared with that in the T1DM group, and the body weight of rats in the high group was increased more significantly(Fig. 1B). Morris water maze was used to test the learning and memory ability to evaluate the cognitive function of rats in each group. The experimental data of Morris platform covert period showed that compared with the control group, the time for T1DM group rats to find the platform was prolonged (P<0.05); Compared with the T1DM group, the time to find the platform in the low

group was reduced, but there was no statistical significance (P>0.05); The time to find the platform of rats in the high group decreased (P < 0.05), compared with rats in the conrol group, the number of crossing the platform of rats in the T1DM group decreased significantly (P<0.001), and compared with rats in the T1DM group, the number of crossing the platform of rats in the low (P<0.05) and high groups (P<0.01) increased significantly(Fig. S1AB). There were no significant changes in the time to find the platform and the number of times to cross the platform in the low and high groups (P>0.05)(Fig. 1C). The results showed that emodin could improve the learning and memory ability of rats with diabetic encephalopathy. The hippocampal tissues of SD rats were stained with HE and Nissl(Fig. 1D E). Compared with the control group, the neurons in the hippocampal CA1 region of the T1DM group were loosely arranged and the number of neuronal Nissl was reduced, which may be one of the reasons for the poor performance of the water maze in the T1DM group. Compared with the T1DM group, the neurons in the hippocampal CA1 region of the high group were more closely arranged and more numerous, suggesting that emodin has a certain protective effect on the hippocampal tissue of diabetic rats.

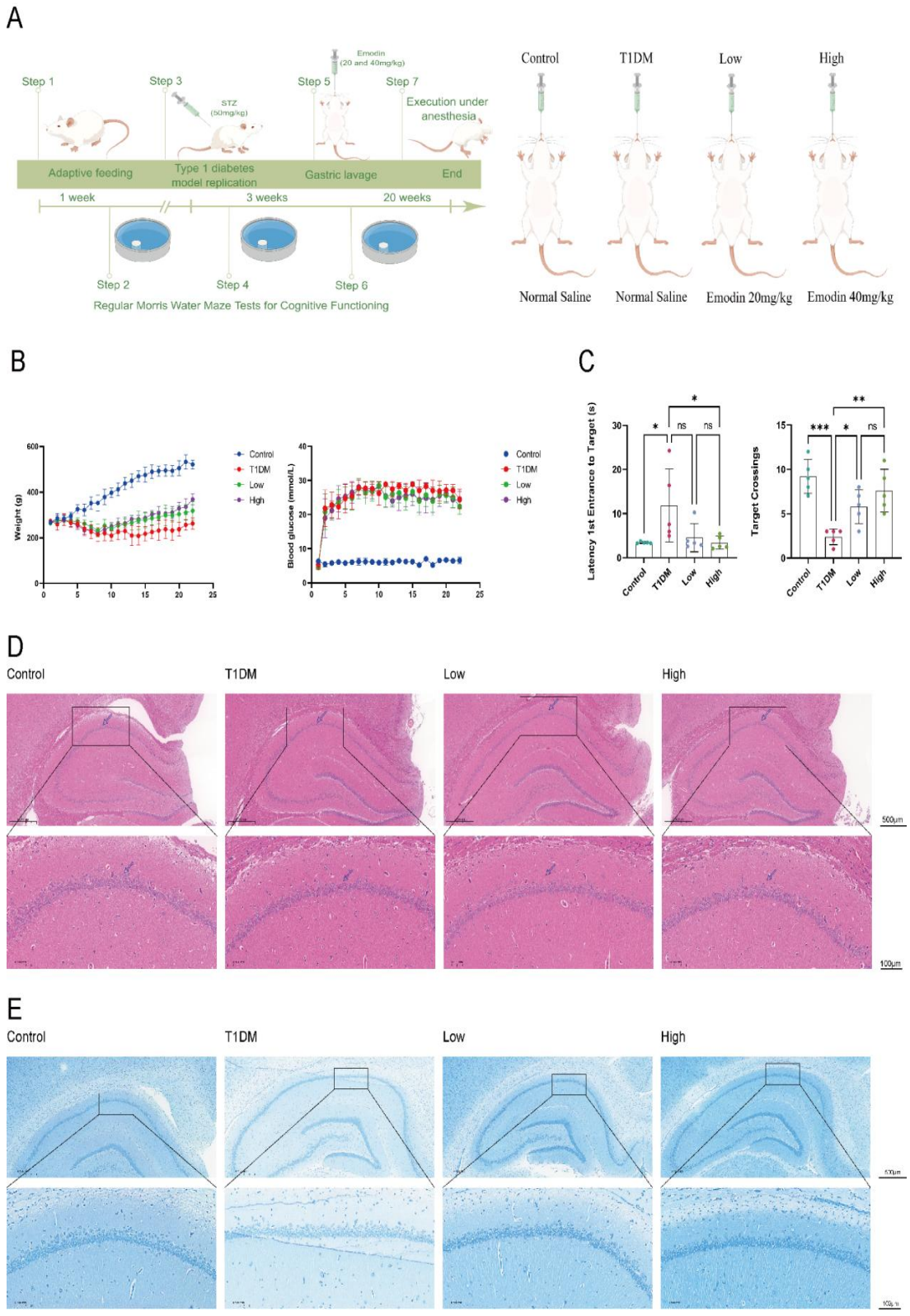


Fig. 1 Effect of emodin on general condition of diabetic encephalopathy rats.

A: Schematic diagram and grouping of the whole process of animal model. B: Blood glucose and body weight of SD rats. C: SD water maze performance (escape latency

and number of platform crossings). D-E: Hematoxylin eosin staining and Nissl staining of hippocampus in SD rats(scale: 200 μm, 100 μm)

2. Emodin alleviates the level of PANoptosis in the model of diabetes encephalopathy

ZBP1, an innate immune sensor, has been identified as an important initiating molecule of innate immune response and PANoptosis; In addition, Caspase-8, as the promoter of apoptosis, is the first discovered intermediate regulatory point of the pathway of apoptosis and necrosis. At the same time, Caspase-8 is also the upstream of NLRP3 inflammasome activation, which can promote the occurrence of pyroptosis. ZBP1 and Caspase-8 together constitute the core regulatory axis of PANoptosis-ZBP1 initiates PANoptosis signals, and caspase-8 precisely regulates the type of cell death and the intensity of inflammatory response by integrating multi-path cross-talk. We first detected the expression of ZBP1 protein, a PANoptotic Receptor, in the hippocampus of rats in each group, and found that ZBP1 expression increased in the T1DM group, while ZBP1 expression in the hippocampus of low and high groups decreased to varying degrees(Fig. 2A). Then we detected the expression of caspase-8 protein, and the result was consistent with that of ZBP1. Further detection of apoptosis protein, pyroptosis protein and necroptosis protein in the hippocampus showed the same results as before(Fig. 2BCD). These results indicate that PANoptosis occurs in the hippocampus of diabetic encephalopathy rats, and emodin can alleviate this mode of cell death. Subsequently, we further verified the results of animal models in cell experiments. First, we used CCK-8 experiment to verify the toxicity of emodin

on HT-22 cells. The results showed that the survival rate of cells was still maintained at a high level at the concentration of 100 μm used in the experiment, and emodin intervention could improve the survival rate of HT-22 cells under high glucose(Fig. S2AB). Subsequently, cell proteins were collected to detect the expression of ZBP1. It was found that ZBP1 expression increased in cells of Hg group, while ZBP1 expression decreased in he group. The results of Caspase-8 protein and phenotypic protein downstream of PANoptosis were also consistent with ZBP1(Fig. 2EFGH).

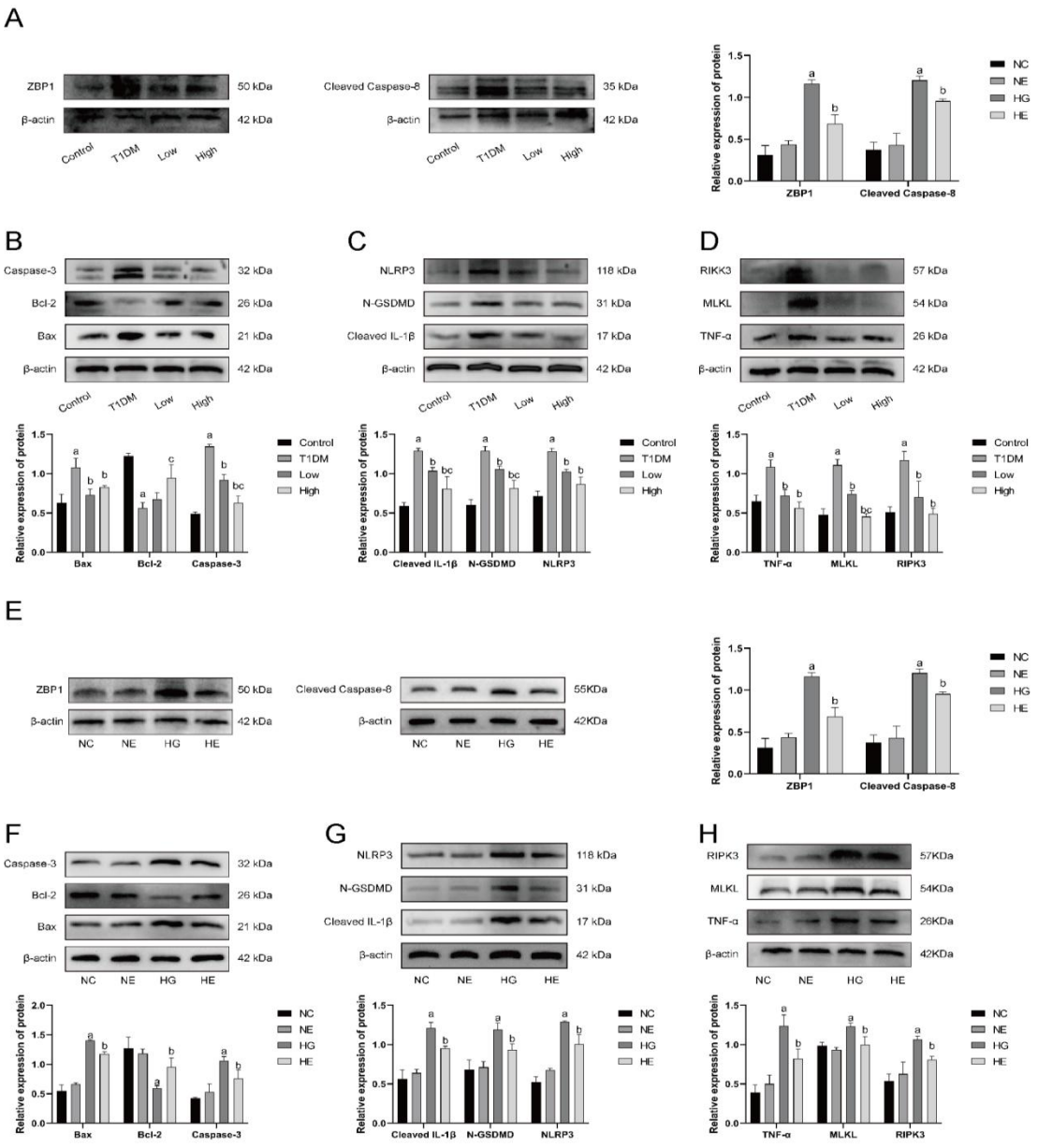


Fig. 2 Effect of Emodin on PANoptosis related protein in diabetic encephalopathy model.

A: Expression of ZBP1and Cleaved Caspase-8 protein in hippocampus of SD rats. B-D: Changes of apoptosis, pyroptosis and necroptosis related proteins in the hippocampus of SD rats. E: Expression of ZBP1and Cleaved Caspase-8 protein in hippocampus of HT-22 cells. F-H: Changes of apoptosis, pyroptosis and necroptosis related proteins in the hippocampus of HT-22 cells.

3. Emodin can bind to HDAC4 and reduce its activity

In order to clarify the specific molecular targets of emodin in regulating the neurons of diabetic encephalopathy, network pharmacology was adopted. First, the genecards database was searched for genes related to emodin, diabetes and Alzheimer's disease. Veen diagram showed that there were 93 genes among the three(Fig. 3A). Through go and KEGG enrichment analysis, the results showed that the neural signaling pathway may be an important target of emodin action(Fig. 3BCD); Combined with our previous research on the improvement of cognitive dysfunction in diabetic encephalopathy rats by Radix Polygoni Multiflori, it was found that Radix Polygoni Multiflori improved hippocampal apoptosis and cognitive function by down regulating the high expression of HDAC4 in the hippocampus of diabetic encephalopathy rats; Emodin is one of the effective components in the preparation of Polygonum multiflorum Thunb. Therefore, whether emodin can improve PANoptosis in diabetic encephalopathy rats by intervening HDAC4 is

worthy of further study. In the prediction of the binding between emodin and HDAC4 using molecular docking technology, the binding energy between emodin and HDAC4 was found to be -7.8 kcal/mol, showing a good binding relationship between emodin and HDAC4(Fig. 3E). Subsequently, SPR experiments were used to verify the corresponding intensity of emodin and HDAC4, and the results showed that the response intensity of emodin and HDAC4 was directly proportional to the concentration of emodin(Fig. 3F). Subsequently, we detected the activity of HDACs in the serum and hippocampus of the animal model by ELISA, and the results showed that the activity was increased in the T1DM group, but decreased in the low and high groups(Fig. 3G).

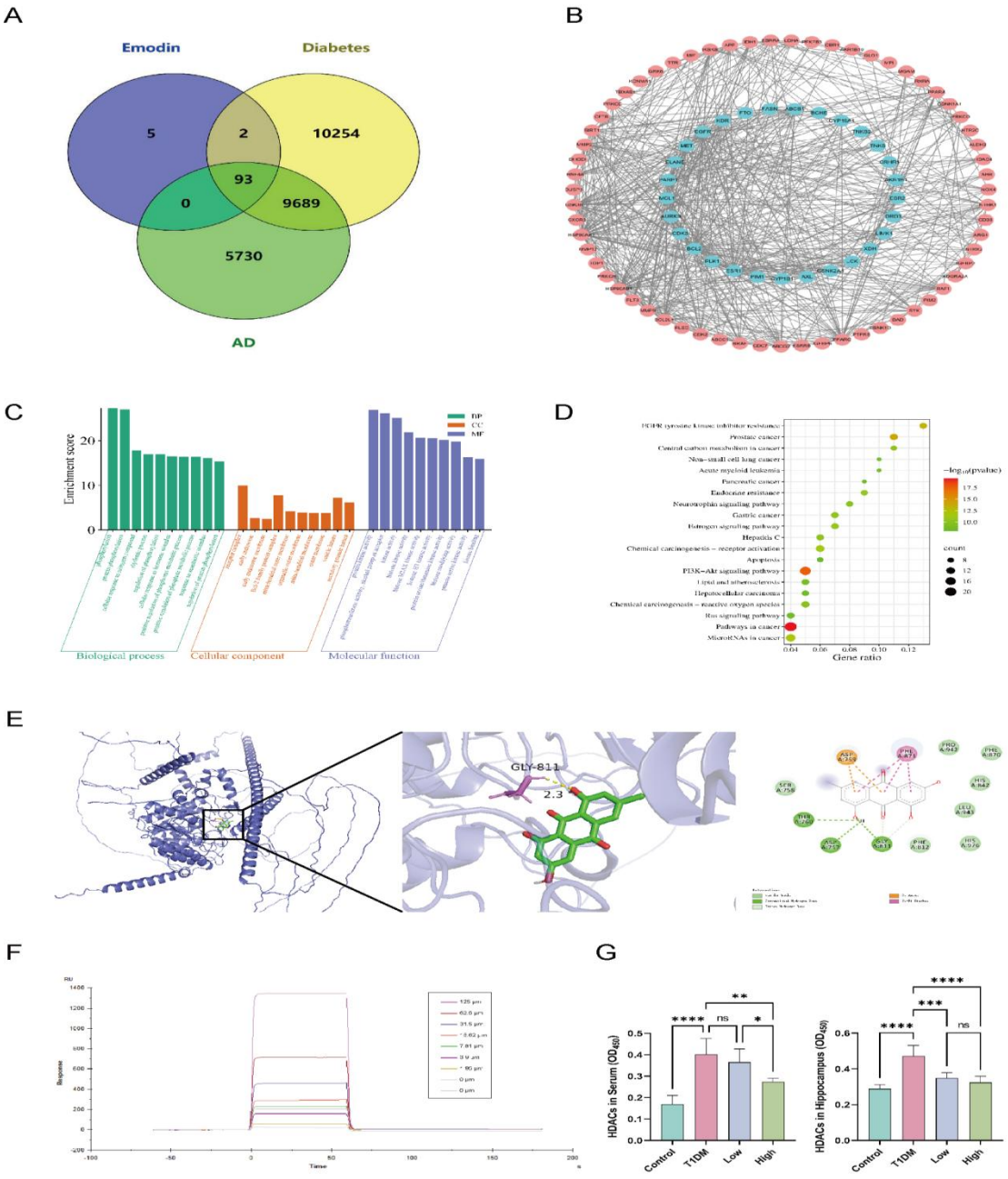


Fig. 3 Network pharmacology of emodin and its impact on HDAC4.

A: Venn diagram of emodin and DE targets predicted by network pharmacology. B: Network diagram of common targets of emodin and DE related genes. C-D: GO and KEGG enrichment analysis of cross targets between emodin and DE.

4. Emodin reduces HDAC4/NF-κB pathway in diabetes encephalopathy model

The expression of HDAC4 protein in hippocampal tissue of diabetic rats and after intervention with emodin was detected by Western blot. Compared with the control group, the expression of HDAC4 in T1DM group was significantly increased, and compared with the T1DM group, the expression of HDAC4 in low group(P>0.05) and high group (P<0.01) was significantly decreased(Fig. 4AB). In the previous results, it was found that some inflammatory factors were highly expressed in diabetic encephalopathy rats and neuronal cells under high glucose. Subsequently, we detected the canonical signal NF-κB pathway of inflammation and found that it was highly expressed in cells and animal models, and the expression of related proteins decreased after the intervention of emodin(Fig. 4AB). Studies have reported that HDAC4 and NF-κB (p65) exist in the nucleus and cytoplasm, and they mainly play a role by entering the nucleus. Subsequently, we detected the expression of the two proteins in the nucleoplasm of HT-22 cells by nucleocytoplasmic separation experiment, and compared with the cells treated with HDAC4 inhibitor LMK-235(Fig. 4CD). The results showed that under high glucose, HDAC4 and p65 were more expressed in the nucleus, and after adding lmk-235, an inhibitor of HDAC4, the entry of HDAC4 and p65 into the nucleus was reduced; On this basis, we added LPS to induce inflammation in cells and found that the nuclear entry of HDAC4 and p65 was restored. This suggests that emodin can inhibit the hdc4/nf- κ B signaling axis.

To explore whether downregulation of HDAC4 could improve the occurrence of PANoptosis in HT-22 cells under high glucose, we used double immunofluorescence to detect the expression of HDAC4 and ZBP1 in HT-22 cells, and compared the

lmk-345 inhibitor group with emodin treatment group. The results showed that the expression of HDAC4 and ZBP1 in HT-22 cells decreased after adding HDAC4 inhibitors lmk-235 and emodin, indicating that downregulation of HDAC4 also reduced the expression of PANoptosis receptor ZBP1(Fig. 4E). Subsequently, using immunohistochemistry, we also found that the expression of HDAC4 and ZBP1 in the hippocampus of Diabetic Rats Intervened by emodin in the animal model were decreased(Fig. 4FG).

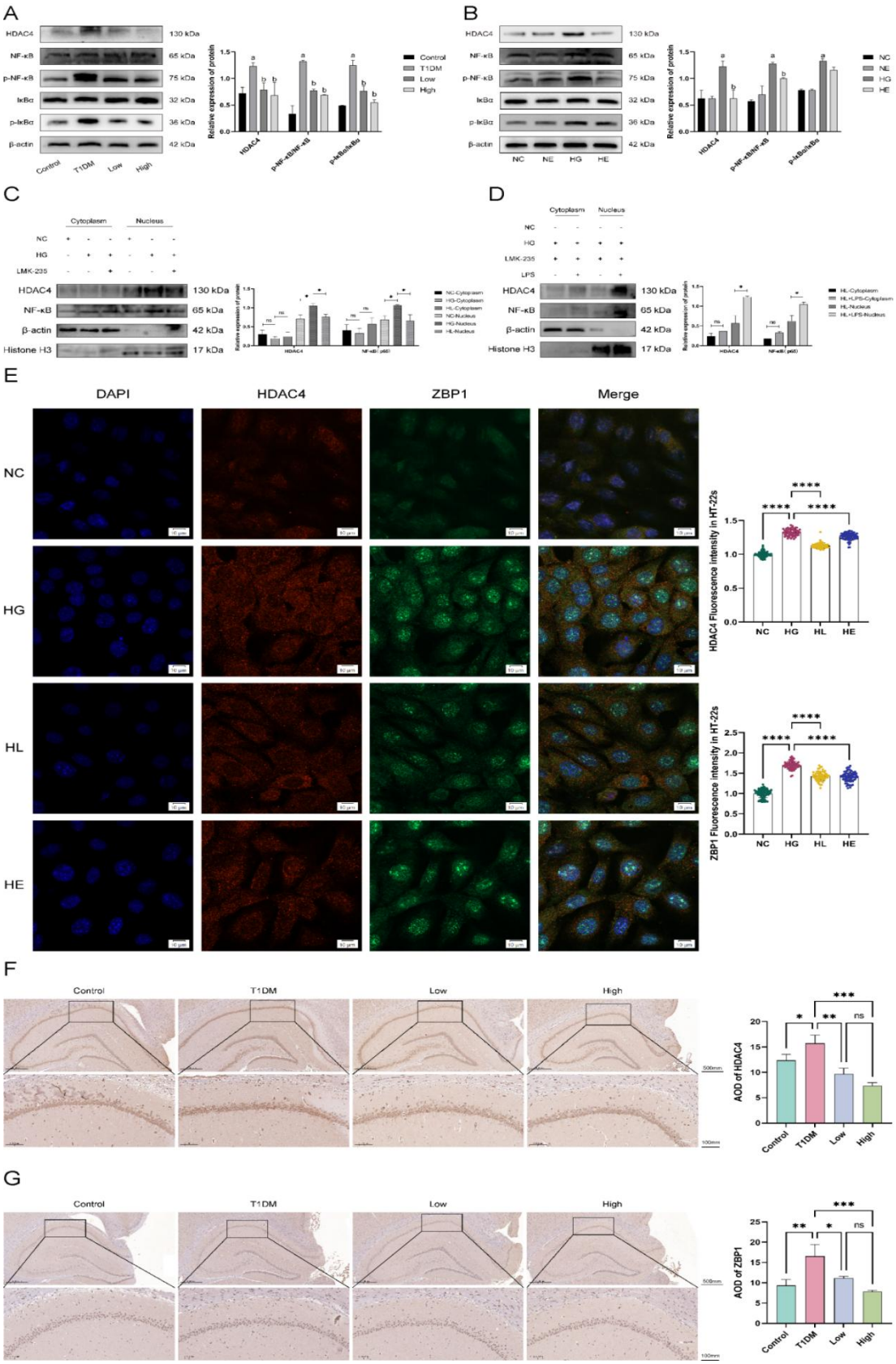


Fig. 4 The effect of emodin on the HDAC4/NF-κB pathway.

A: Expression of HDAC4/NF-κB pathway proteins in DE animal models. B:

Expression of HDAC4/NF-κB pathway proteins in DE cell models. C-D: Nuclear-cytoplasmic separation experiments verified the expression of HDAC4 and NF-κB (p65) proteins. E: IF verification of the expression of HDAC4 and ZBP1 in HT-22 cells. F-G: IHC detection of HDAC4 and ZBP1 expression in the hippocampus of DE animal model

5. The characterization and biosafety of TF-LNPs-Emodin are stable and reliable

Brain disease treatment faces major challenges due to the strict restriction of blood-brain barrier, and traditional medicines are difficult to effectively deliver to brain tissue[31]. Liposomes, as biocompatible nanocarriers, have become the key strategy to break through this bottleneck by virtue of their modifiability, medicine loading ability and potential to penetrate the BBB[32, 33]. As a natural active ingredient, emodin has outstanding performance in anti-inflammatory, antioxidant and neuroprotection[34-36], but its poor water solubility and low BBB penetration rate restrict its clinical application[37]. Emodin encapsulated in liposomes can not only improve its physicochemical properties, but also enhance the brain delivery efficiency with the help of targeted ligand modification (such as transferrin, angiopep-2) or physical assisted technology (such as focused ultrasound).

We identified whether liposomes could be used for brain medicine delivery by detecting the morphology, particle size, zeta potential, polydispersity index and other characteristics, detected its biological safety by hemolysis rate, and detected its delivery efficiency of emodin by Transwell cell culture device and animal in vivo

imaging. We constructed transferrin functionalized liposomes (TF-LNPs) for brain delivery of emodin. Firstly, the encapsulation efficiency of emodin and the coupling efficiency of transferrin were detected(Fig. 5A). Subsequently, the liposomes were observed as uniform spherical nanoparticles by transmission electron microscopy. Further, dynamic light trichrome (DLS) was used to detect the characterization of liposomes(Fig. 5B). The study showed that nanoparticles with a particle size > 200nm were easily captured by Kupffer cells or other phagocytes, while nanoparticles with a particle size less than 100 nm had a high probability of aggregation. This may cause aggregates to block the blood flow and trigger embolism the particle size of LNPs in each group is in the range of 100-200nm, which is very important for prolonging its circulation time in vivo and enhancing the permeability through the blood-brain barrier. Polydispersity index is an index to measure the uniformity of particle size distribution. The low value obtained (about 0.15) indicates that nanoparticles are monodisperse, with low variability and no aggregation trend. Zeta potential reflects the repulsive force between particles, which directly affects the stability and aggregation trend of particles in suspension, and then affects the particle size distribution(Fig. 5B). The higher the absolute value of zeta potential is, the more stable the dispersion of nanoparticles is, and there is no trend of aggregation. The zeta value of free LNPS is -41.50 ± 1.41mv(Fig. 5B). After adding transferrin to the surface of nanoparticles, the negative charge of zeta potential decreases. Because transferrin is a protein with positive net charge, the negative charge of nanoparticles will decrease slightly, but there is still -25.93 ± 1.17 mv to maintain strong suspension

stability(Fig. 5B).Then CCK-8 assay was used to evaluate the effect of TF-LNPs-Emodin on the viability of HT-22 and hCMEC/D3 cells. The cell viability rates at the experimental dose were 94.80% ± 1.87% and 95.88% ± 6.24%, respectively. At twice the experimental concentration, the viability rates were 93.69% ± 3.02% and 74.88% ± 9.61%(Fig. 5D).Subsequently, hemolysis assays were performed to assess the in vivo safety of the liposomes. At the experimental dose, the hemolysis rates of Free-LNPs-3, LNPs-Emodin-3, and TF-LNPs-Emodin-3 were 0.36% ± 0.15%, 1.03% ± 0.12%, and 0.90% ± 0.09%(Fig. 5C) . These results indicate that the LNPs exhibit good biocompatibility.

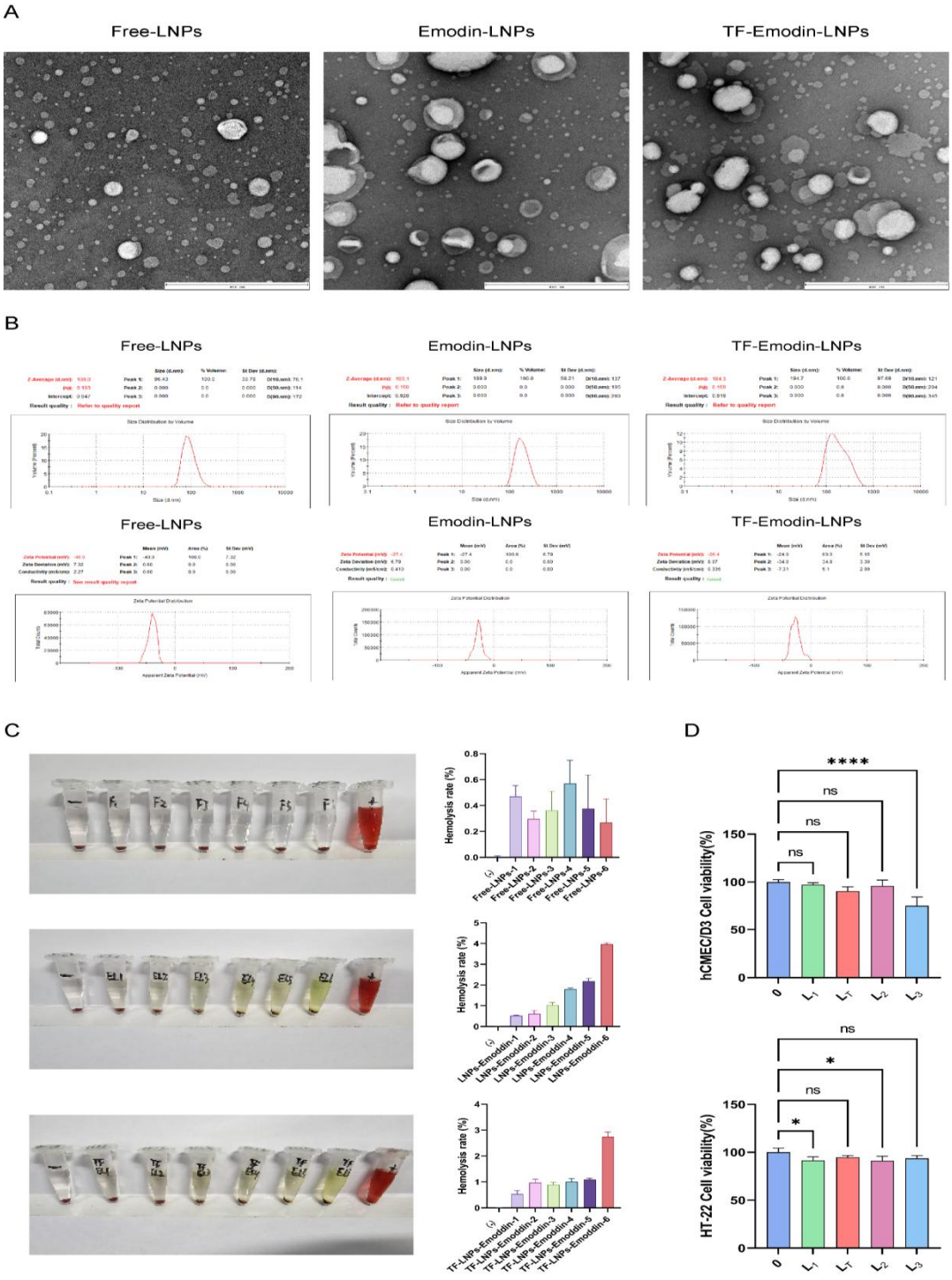


Fig. 5 Characterization and Biosafety of TF LNPs.

A: TEM detection of morphology of LNPs. B: Characterization of LNPs detected by DLS. C: Red blood cell hemolysis assay for detecting the hemolysis rate of LNPs. D: CCK-8 detection of the effect of TF-LNPs-Emodin on cells survival rate.

6. Systematic Safety Evaluation of TF-LNPs

Consistent with hemolysis assay findings, the prepared liposomal formulations exhibited excellent biocompatibility in vivo. Histopathological examination of major organs (heart, liver, kidneys, and hippocampus) showed no structural abnormalities in emodin-loaded liposome-treated mice compared to controls (Fig. 6A). Hematological analysis revealed no significant alterations in blood cell counts (WBC, RBC, platelets) across treatment groups (Fig. 6B), confirming the absence of hematotoxicity. Furthermore, liposome administration did not induce systemic inflammation, as evidenced by stable plasma levels of both pro-inflammatory (TNF-α, IL-6, IL-1β) and anti-inflammatory (IL-10) cytokines (Fig. 6C). Serum biomarkers of hepatic (ALT, AST) and renal (BUN, creatinine) function remained within normal ranges (Fig. 6DE), demonstrating no adverse effects on organ function. Taken together, these results establish that the engineered liposomes possess an optimal safety profile, with no detectable acute toxicity, immunostimulation, or organ impairment in murine models.

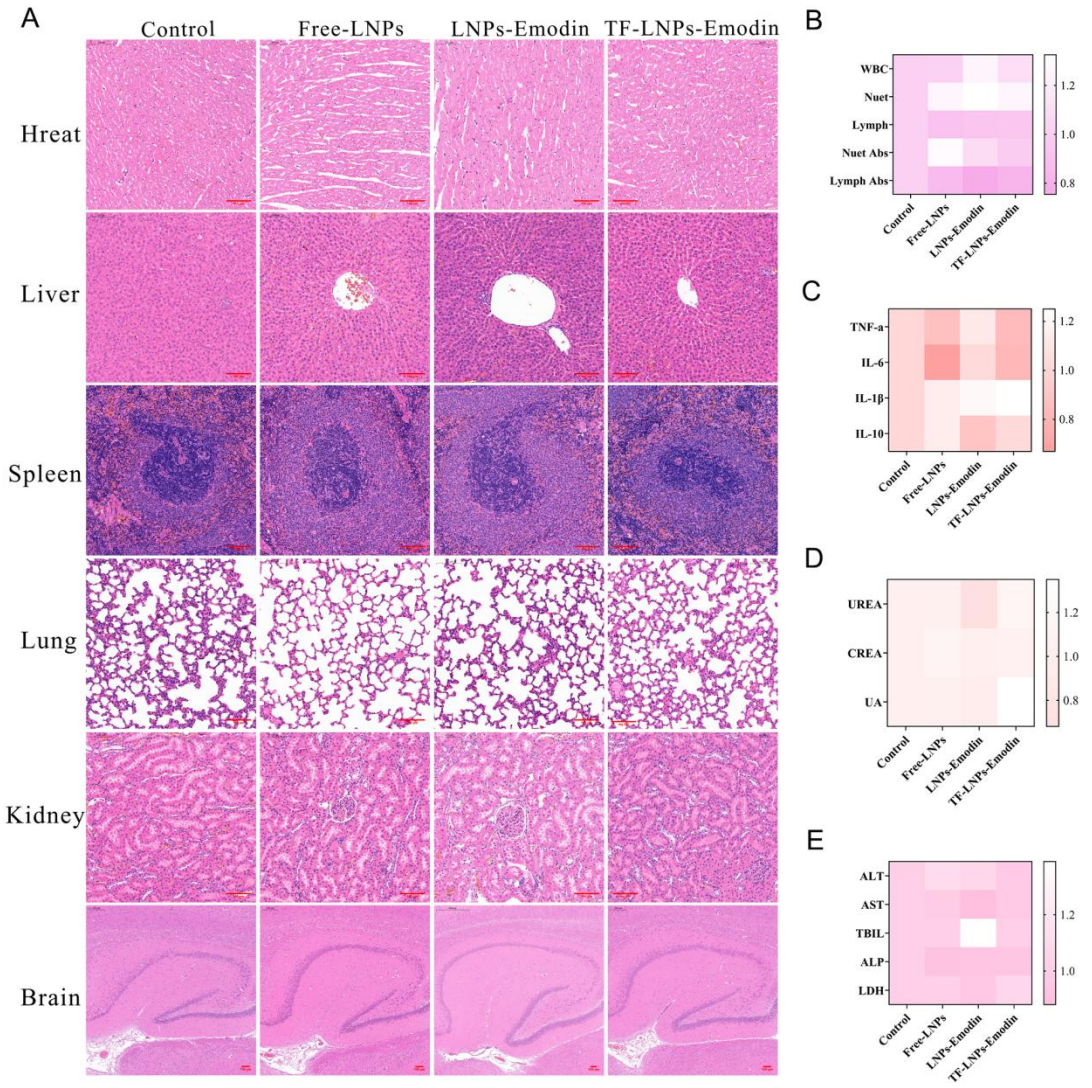


Fig. 6 Safety Evaluation of Liposomal Formulations in Mice.

A: Histopathological Analysis (H&E Staining).B: Hematological Analysis. C: Inflammatory Cytokine Levels. D: Renal Function Biomarkers..E: Hepatic Function Biomarkers..

7. TF-LNPs can effectively increase the BBB permeability of emodin and enhance its therapeutic effect on neuronal cells

As mentioned earlier, TfR expression on the luminal side of brain capillary endothelial cells (BECs) at the blood-brain barrier is abundant. During transport,

molecules combine with specific domains of TfR on the side of BECs to trigger endocytosis events, forming transport vesicles inside the cells, and cargo molecules in the vesicles are transported to the brain. We expect that cargo molecules can be accurately localized to hippocampal neurons after being transported to the brain. We detected the TfR expression of neuronal cells under high glucose and found that the TfR expression of hippocampal neurons in the in vitro and in vivo models was more abundant in the high glucose group(Fig. 7 AB). This suggests that we can target cargo molecules to the hippocampus in the diabetic encephalopathy model through the transport of TFR. hCMEC/D3 monolayer cell is a very reliable BBB model, which is commonly used for the cell model of medicine molecule transport across the BBB due to its good correlation with the permeability in vivo; We used hCMEC/D3 endothelial cells to replicate the BBB model in vitro. When the cell transmembrane resistance (TEER) of the model was ≥ 130 $\Omega \cdot cm^2$, the BBB model was co cultured with HT-22 cells in the Transwell device(Fig. 7CD). Then free emodin, LNPs emodin and TF-LNPs emodin were added to the device respectively, and the culture medium at the lower side of the device was collected after 1, 2, 3, 4 and 5h, respectively, and the concentration of emodin was detected by Liquid chromatography. The results showed that the concentration of emodin in TF-LPNs-Emodin group was the highest in both conventional and high glucose medium, which indicated that the modification of TF made liposomes carry more emodin across the blood-brain barrier to reach hippocampal neuronal cells(Fig. 7EF). We collected the proteins of HT-22 cells at the lower side of the device to detect the changes of PANoptosis related proteins, and

compared with the cells in which emodin was directly added to the culture medium in the BBB withdrawal model. We found that the expression of PANoptosis related proteins in the TF-LNPs emodin group was reduced to a level closer to that in the emodin group in the BBB withdrawal model(Fig. 7G). In addition, we also detected the distribution of LNPs emodin and TF-LNPs emodin in animals by in vivo imaging. The results showed that TF-LNPs emodin was more enriched in mouse brain(Fig. 7HI). Therefore, we can conclude that the constructed TF-LNPs emodin can be used to protect emodin during the transport of emodin into the brain through the BBB, enhance the penetration of emodin through the BBB, and target the hippocampus.

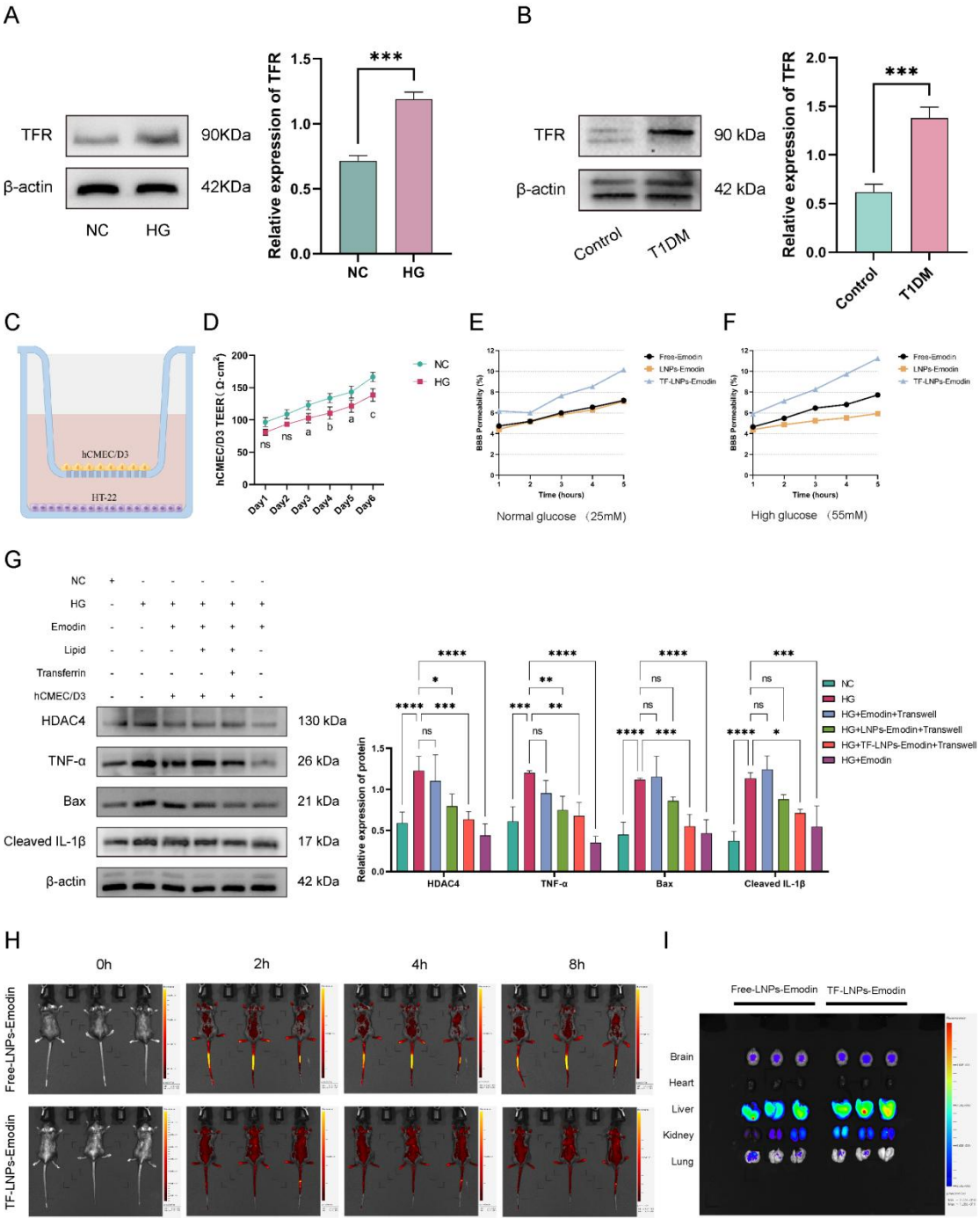


Fig. 7 The BBB delivery efficiency of TF-LNPs-Emodin and its improvement effect on PANoptosis.

A-B: TFR protein expression in DE model. C: Establishment of BBB in vitro model. D: TEER values of hCMCE/D3 cells. E: BBB permeability of TF-LNPs-Emodin under conventional sugar conditions. F: BBB permeability of TF-LNPs-Emodin under

high glucose conditions. G: The effect of TF-LNPs-Emodin on PANoptosis proteins in HT-22 cells under high glucose conditions. H-I: Animal live imaging detection of TF distribution in vivo.

DISCUSSSION：

As a traditional Chinese medicine ingredient, emodin has been widely used in anti-inflammatory, antioxidant and other therapeutic fields for a long time[36, 38, 39]. However, its potential role in improving cognitive function has rarely been reported. This study revealed for the first time the significant effect of emodin on improving cognitive function, which broke through the therapeutic scope of traditional Chinese medicine ingredients and further expanded its application prospect in the field of neuroprotection. Emodin not only significantly improved cognitive dysfunction, but also exhibited its unique protective mechanism by inhibiting neuronal PANoptosis. These results are different from the existing literature. Existing studies have shown that curcumin, as a natural antioxidant, increases the content of brain-derived neurotrophic factor (BDNF) by enhancing the wnt/β-catenin signaling pathway, thereby improving the neurogenesis and spatial localization ability of Alzheimer's disease mice[40]. Baicalein inhibited the formation of PANoptosome by reducing ROS generation from mitochondria and improved PANoptosis in multiple organ injury in mice with inflammatory disease[41]. Although these studies revealed the neuroprotective effects of a variety of traditional Chinese medicine monomers in specific apoptotic pathways or disease models, there is no literature detailing the

extensive inhibitory effect of emodin on neuronal PANoptosis.

PANoptosis refers to a new way of cell death in which a variety of programmed cell death occur simultaneously under specific conditions[10, 42]. PANoptosis may lead to the non-specific death of a large number of neurons, seriously affecting brain function[43, 44]. The mechanism of PANoptosis in neurodegenerative diseases is complex, usually related to chronic inflammation, oxidative stress, metabolic disorders and other factors, which can trigger a wide range of neuronal apoptosis signals, thereby accelerating the disease process[44]. In the study of diabetic encephalopathy, PANoptosis of hippocampal neurons was first reported by our team. Through animal models and pathological analysis, we found that PANoptosis appeared in diabetic encephalopathy, which may be one of the important reasons for cognitive dysfunction. On this basis, we candidate emodin as a potential therapeutic medicine for research. Through in vitro cell experiments and animal model experiments, we systematically evaluated the anti PANoptosis effect of emodin under diabetic conditions and found that it could significantly reduce the rate of neuronal apoptosis. Molecular biology experiments further confirmed that emodin down regulated the expression of PANoptosis related genes Bax and Caspase-3, and up-regulated the expression of anti apoptosis gene Bcl-2, thus exerting its neuroprotective effect. These results not only revealed the potential mechanism of emodin in improving cognitive function, but also provided a new theoretical basis for its application in neurodegenerative diseases.

In terms of molecular mechanism, our previous study found that HDAC4 was

highly expressed in neurons of diabetic encephalopathy rats, and demonstrated its relationship with neuronal apoptosis. This finding laid a foundation for further exploring the function of HDAC4 in neurons. In this study, we further expanded the functional study of HDAC4 and revealed the key mechanism of its regulation of neuronal pan apoptosis through the NF-κB pathway. Previous literature has reported the important role of histone acetylation in apoptosis[45].For example, Xu C et al found that histone deacetylase mediated silencing of PSTPIP2 expression could promote the occurrence of PANoptosis in renal tubular cells of aristolochic acid nephropathy (AAN) mice[46].In another study, the use of histone acetylase inhibitors could prevent DNA damage induced neuronal apoptosis by changing the acetylation level of tumor suppressor gene p53[47].

This study is the first to demonstrate that high expression of HDAC4 leads to pan apoptosis of hippocampal neurons by regulating NF-κB pathway activation through systematic experimental methods. Using inhibitors and rescue experiments, we observed that the high expression of HDAC4 significantly enhanced the activity of NF KB, which in turn promoted the expression of a variety of apoptosis related genes. In combination with other people's studies, HDAC4 plays a deacetylation role and affects the acetylation level of NF-κB, thereby inhibiting the expression of NF-κB inhibitory protein IκB and activating the NF-κB pathway. A large number of studies have found that histone deacetylases (HDACs) have significant potential as disease therapeutic targets[48-51]. Harnessing the HDAC–histone deacetylase enzymes, inhibitors and how these can be utilised in tissue engineering[52]. This article

summarizes the history, current situation and future prospects of HDAC inhibitors (HDACi) in the treatment of a variety of diseases. HDACi has broad application potential for cancer, cardiovascular disease and neurodegenerative disease by regulating histone acetylation levels and affecting gene expression. At present, HDAC inhibitors have been applied in the treatment of neurodegenerative diseases. For example, Sodium butyrate can cross the blood-brain barrier to regulate brain function, increase histone and Specificity protein-1 acetylation and protected against 3-nitropropionic acid neurotoxicity, and then improve the weight and exercise performance of Huntington disease model mice[53]. Our team's research shows that HDAC4 plays a key role in the process of apoptosis and pan apoptosis in diabetic encephalopathy. Combined with the results of other research teams on the impact of HDAC4 on neuronal synaptic plasticity and chromatin state, we speculate that HDAC4 may be an important therapeutic target for neuronal damage in diabetic encephalopathy. The development of targeted inhibitors against HDAC4 is expected to become a new and effective therapeutic strategy.

Therefore, our team took HDAC4 as the target, explored the relationship between emodin and HDAC4 using network pharmacology and molecular docking technology, and proved its binding ability with surface plasmon resonance (SPR) technology. The data shows that emodin can become an inhibitor of HDAC4 and has a certain degree of binding ability, which is another in-depth discovery of traditional Chinese medicine monomer components by our team. Why does the research of Shouwu and other systematic traditional Chinese medicines provide new ideas. This

method of combining network pharmacology and molecular docking technology provides a scientific basis for the research of single components of traditional Chinese medicine. Our research has also systematically promoted this field, from the multi-component and multi-target effects of traditional Chinese medicine to the in-depth mechanism research of single components, providing an important theoretical basis and practical strategies for the modernization of traditional Chinese medicine. Emodin, as an inhibitor of HDAC4, indeed shows a certain application potential, which can regulate gene expression in neurodegenerative diseases and diabetic encephalopathy, reduce nerve injury and improve cognitive function, providing a new therapeutic approach for these diseases. However, emodin as a drug still has some significant limitations, such as poor water solubility and low bioavailability, which limits its clinical application. In addition, its metabolic pathways and potential toxic and side effects in vivo have not been fully defined, and it does not have a strong value of chemical transformation[54]. Further research is needed to solve these problems in order to better play its therapeutic potential.

In order to solve the shortcomings of emodin, we have extensively investigated various drug carrier delivery systems. Compared the application of other small molecule carriers in the treatment of neurological diseases[55, 56], such as curcumin and some monomer components of traditional Chinese medicine. The common carriers in these studies include peptide nanoparticles, liposomes and polymer nanoparticles[57]. Curcumin can significantly improve the permeability of blood-brain barrier by modifying transferrin on the surface of liposomes, but its bioavailability has not been

studied[58]; Other monomeric components of traditional Chinese medicine, such as resveratrol, also showed better efficacy when delivered through modified nanoparticles[59]. After comprehensive evaluation, liposomes nanoparticles (LNPs) were finally selected as delivery vehicles. Different modified LNPs have different targeting to disease organs, which has been shown by several studies. For example, LinTT1 peptide modified liposomes enhance the targeting to tumor tissues[60], while dual-targeting galactose-functionalized hyaluronic acid modified LNPs can effectively target the liver to improve the delivery efficiency of silybin and alleviate alcoholic liver injury[61]. In addition, studies have shown that menthol-modified quercetin liposomes can achieve efficient delivery to neurons and improve the learning and memory ability of Alzheimer's disease model mice[62], which further enhances our confidence in selecting LNPs for the treatment of neurological diseases. The reason why we chose to use transferrin (TF)-modified LNPs is that transferrin, as a naturally occurring protein in the blood, has efficient receptor-mediated endocytosis on the surface of hepatocytes, and can cross the blood-brain barrier to achieve highly targeted delivery to neural tissues. Previous studies have shown that transferrin (TF) - modified liposomes can significantly improve the distribution and stability of gene therapeutics or natural compounds in the central nervous system[63]. For example, a study using TF modified liposomes to deliver rapamycin to treat traumatic brain injury showed that TF modified liposomes exhibited superior biodistribution and lower systemic toxicity[64]. In addition, TF modified liposomes have also been applied to deliver anti apoptotic natural compounds into Alzheimer's disease models, showing

good targeting efficiency and therapeutic effect[65]. Our results also confirmed the effectiveness of transferrin modified liposomes in delivering emodin for the treatment of neurological diseases. When the drug is encapsulated into the carrier, the bioavailability of emodin is significantly improved, and it can show lasting efficacy in the target region. Medicines such as TF-LNPs delivery not only enhance the targeting, but also improve its half-life and biological activity in vivo[66]. To some extent, our team solved the application shortcomings of emodin, combined with the current effective drug delivery vehicles, to ensure that it can better pass through the blood-brain barrier to reach the treatment area, and can maximize its ability to inhibit HDAC4 without being limited by low bioavailability. Unfortunately, our team tried to prove that TF LNPS have targeted binding effect with hippocampal neurons, but imaging showed that TF LNPS were evenly distributed in the brain, and there was no obvious targeted delivery effect on the hippocampus. In the later stage, we will further optimize the structure and function of LNPs to achieve more precise medicine delivery and higher therapeutic effect.

EXPECTATION：

Our team first reported a drug delivery material suitable for the treatment of diabetic encephalopathy, which marks an important breakthrough in the targeted delivery of traditional Chinese medicine monomer components (such as emodin). Transferrin modified lipid nanoparticles (TF-LNPs) not only overcome the limitations of low bioavailability and poor water solubility of emodin, but also achieve efficient

targeted delivery to its neural tissue. This delivery system significantly improved the efficacy of emodin, and also provided a new strategy for the treatment of diabetic encephalopathy. In order to further expand the application scope of this delivery system, we are exploring more targeted modifications to achieve precise control of its targeted delivery. At present, we are conducting screening work to find suitable neuronal membrane proteins for docking to further limit the delivery of emodin to the hippocampus. Through precise targeted delivery, it is hoped to improve the specificity of emodin to the hippocampus, so as to more effectively treat neurodegenerative diseases related to hippocampal injury.

In the future, it is hoped that this delivery technology can be applied to other central nervous system diseases and enhance the application value and potential of traditional Chinese medicine in modern medicine. It is worth noting that in addition to showing significant targeting to the nervous system, we also found that this TF-LNPs had a significant aggregation effect in the liver. This unexpected finding opens up another important research prospect for us. Combined with our previous research findings that Polygonum multiflorum can effectively improve nonalcoholic fatty liver, the targeting ability of this delivery system in the liver provides a basis for its clinical transformation.

CONCLUSION：

This study confirmed that emodin can effectively inhibit HDAC4/NF-κB signaling pathway, significantly reduce the level of hippocampal neuron pan apoptosis and

improve cognitive dysfunction in diabetic encephalopathy rats. Further explore the application of TF-LNPs as a drug delivery system in diabetic encephalopathy. TF-LNPs can improve the targeting and bioavailability of emodin, further enhance its therapeutic effect, and provide a new strategy for subsequent clinical intervention of diabetic encephalopathy.

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Conflict of Interest.

The authors declare that they have no competing interests.

Author Contributions

Yongjie Xu, Wei Pan, and Liying Zhu conceived the study and drafted the manuscript. Huiru Yang, Yiqiong Zhang, and Mi Liu performed data analysis and

visualization. Changyudong Huang, Yinxue Zhong, and Haizhi Li conducted the investigation and provided resources. Hongtao Yang and Chengcheng Li contributed to validation and manuscript review. Di Chen carried out the data collation. Xing Li supervised the project and acquired funding. All authors reviewed and approved the final manuscript.

Consent for Publication

All authors have provided their explicit consent for the publication of this manuscript.

Availability of Data and Materials

The datasets supporting the conclusions of this study can be obtained from the corresponding author upon justified request.

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