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Evaluation of the effect of phellodendrin application on rats creating an experimental model of non-compression lumbar disc herniation on the NF-κB-related inflammatory signaling pathway

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Abstract

Objective To explore the therapeutic effects of phellodendrine on non-compression lumbar disc herniation (NCLDH).

Methods The Sprague Dawley rat model of NCLDH was established via autologous caudal nucleus pulposus transplantation. Behavioral observations and neurological function scoring were conducted in Sprague Dawley rats, and the serum levels of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) were measured via enzyme-linked immunosorbent assay (ELISA). Real-time quantitative polymerase chain reaction (RT–qPCR) was used to detect the expression of nuclear factor kappa-B (NF-κB) p65 mRNA in L5 nerve roots and surrounding tissues. Western blotting was used to assess the protein expression of NF-κB p65 and TNF-α. Immunofluorescence and immunohistochemical analyses were performed to investigate the distribution and expression of the NF-κB p65 protein in the L5 nerve and its surrounding tissues.

Results In this animal study, phellodendrine was found to downregulate the expression of p65 mRNA, decrease the release of inflammatory factors, and alleviate motor dysfunction caused by lumbar disc herniation(LDH). Therefore, the phellodendrine technique has potential value for the treatment of NCLDH.

Conclusion In this animal experiment, phellodendrine was found to significantly reduce the expression level of p65 mRNA, decrease the release of inflammatory cytokines, and alleviate lumbar disc pain.

Clinical trial number Not applicable.

Keywords Non-compression lumbar disc herniation, Phellodendrine, Nuclear factor-kappa B, Tumor necrosis factor-alpha, Interleukin-1 beta

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Introduction

LDH is a relatively common degenerative disease in clinical practice and is a key factor that induces pain symptoms in the lumbar region and legs. It predominantly affects individuals aged 20–50 years, with an increasing trend towards younger age groups [1]. Various mechanisms underlying LDH-induced symptoms have been extensively studied, and evidence suggests that even in the absence of mechanical compression, nucleus pulposus tissue can cause excitability changes in the dorsal root ganglion (DRG) [2]. Researchers have utilized neurophysiological techniques in rodent models to investigate the role of the DRG in radicular pain associated with LDH [3]. They reported that applying nucleus pulposus tissue to nerve roots under noncompressive conditions led to increased excitability and mechanical hypersensitivity in the DRG compared with those of controls treated with fat on the nerve roots. Moreover, it was observed to increase intraneural fluid pressure, decrease DRG blood flow, and trigger excitability, which are closely associated with subsequent immune and inflammatory responses. In a porcine model in which the autologous nucleus pulposus was exposed to spinal nerve roots, T lymphocytes were found to be stimulated to produce CD4+Th2 cells that produce IL-4 three weeks after exposure to the systemic immune system, promoting autoimmune reactions within the intervertebral disc via IL and TNF-α release, resulting in pain occurrence [4]. In rats, autologous nucleus pulposus implantation near nerve roots without compression results in increased levels of the inflammatory factors IL-17 and IL-23 and decreased pain thresholds, suggesting that the nucleus pulposus outside the intervertebral disc elevates inflammatory factors and damages the surrounding nerve roots, leading to pain [5].

Currently, clinical interventions for NCLDH primarily focus on alleviating pain, reducing inflammation, and promoting neural function recovery. However, existing treatment methods, including medication, physical therapy, and rehabilitation exercises, while able to alleviate patients' symptoms to some extent, often fail to achieve a radical cure and are limited by long treatment cycles and unstable effects. Therefore, exploring and developing new, more effective treatments is crucial for improving the prognosis of NCLDH patients.

Phellodendrine possesses pharmacological activities, such as antioxidant, anti-inflammatory, and immunomodulatory properties [6–8]. It is one of the main active ingredients in phellodendron plants. Phellodendrine reduces the release of key mediators involved in inflammatory responses, such as tumor necrosis factor TNF-α, IL-6, and IL-1β, thereby mitigating inflammation. Additionally, it exerts neuroprotective effects, mitigates neuronal injury and degenerative changes, and promotes neural repair. However, the use of phellodendrine for treating NCLDH has not yet been reported.

NF-κB is considered a critical nuclear transcription factor present in all animal cells. The NF-κB transcription factor family members include p65 (RelA), RelB, c-Rel, NF-κB1 (p105/p50), and p100/p52 (NF-κB2) proteins, which bind to DNA to regulate downstream gene transcription [9, 10]. In a rat model of NCLDH, the intrathecal injection of lipoxin A4 resulted in decreased expression of the NF-κB p65 protein and reduced expression of the inflammatory factor TNF-α while increasing TGF-β activity, thereby relieving radicular neuropathic pain. This mechanism may involve the modulation of inflammatory factors and the inhibition of the NF-κB pathway to alleviate pain [11].

This study focused on the preliminary mechanism of action of phellodendrine in treating NCLDH models. In recent years, numerous studies have demonstrated the unique advantages of natural plant extracts and their active components in anti-inflammatory, analgesic, and neuroprotective aspects. As a compound with extensive pharmacological activities, phellodendrine has been proven to possess significant anti-inflammatory and immunomodulatory effects, making it a promising candidate for treating inflammation-related diseases.

Materials and methods

Reagent

BCA Protein Concentration Assay Kit was provided from Shanghai Biyun Tian Biotechnology (Shanghai, China; Cat. No.: P0009). Western and IP Cell Lysis Buffer were obtained from Shanghai Biyun Tian Biotechnology (Shanghai, China; Cat. No.: P0013). HRP goat anti-mouse IgG (H+L) was acquired from abclonal (Wuhan, China; Cat. No.: AS003), and goat anti-rabbit IgG (H+L) HRP was provided from Affbiotech (Liyang, China; Cat. No.: S0001). P65 antibody was from HUABIO (Hangzhou, China; Cat. No.: HA721307). Molpure® Cell/Tissue Total RNA Kit was purchased from YEASEN (Shanghai, China; Cat. No.: 19221ES50). PrimeScript RT Reagent Kit were acquired from BaoRui Medical Biotechnology (Peking, China; Cat. No.: RR047A). TB Green TM Premix Ex TaqTM II (Tli RNaseH Plus) was from BaoRui Medical Biotechnology (Peking, China; Cat. No.: RR820A). DAPI staining reagent, Rat IL-1β ELISA Kit, and Rat TNF-α ELISA Kit were from Union-Bio(Shanghai, China).

Animals

Twenty-four male Sprague Dawley rats weighing between 230 and 250 g were purchased from Chengdu Dashuo Experimental Animal (Chengdu, China) with an animal experiment production licence number: SCXK (Sichuan) 2020-0030. The animals were housed in the animal facilities of Chengdu University Affiliated Hospital. These

Sprague Dawley rats were fed standard laboratory chow and provided with water ad libitum, with the room temperature maintained between 25 °C and 28 °C. All housing and experimental procedures strictly adhered to the relevant regulations and guidelines for animal experiments and were conducted in accordance with ethical principles to ensure the welfare of the animals. The animal experiments received ethical approval from the Ethics Committee of Chengdu University Affiliated Hospital.

Twenty-four male SD rats were randomly divided into a normal group, a model group, and a treatment group. On the second day after modelling, The rats in the treatment group were gavaged daily with phellodendrine at a dosage of 48.2 mg/kg [12]. The normal control group and model control group received double-steamed water treatment, with both groups being administered 0.1 mL of it every day. During the adaptation period, the mice had free access to food and water. This treatment regimen was continued for 14 consecutive days.

Establishment of the lumbar intervertebral disc degeneration model

The control group did not undergo modelling treatment, whereas the model group and treatment group Sprague Dawley rats underwent autologous transplantation of the nucleus pulposus from their tail to establish a NCLDH model. In accordance with the methods of He Yuancheng [13], this study adopted a technique for establishing a NCLDH model. First, the body weights of the rats were measured, and anaesthesia was induced via the intraperitoneal injection of 1% pentobarbital (40 mg/kg). After confirming the effect of anaesthesia, the intervertebral space between the spinous processes of lumbar vertebrae 4/5 was selected as the puncture center. The area of the back hair was shaved with a razor, with dimensions of 4 cm in length and 3 cm in width. The Sprague Dawley rat was subsequently immobilized on the operating table, and disinfection was performed at the root of the tail, approximately 1 cm from the base. The tail was then ligated and severed to expose the transparent gel-like nucleus pulposus tissue inside. Five pieces of nucleus pulposus tissue were collected and placed in a clean container. Next, 50 μL of physiological saline was added to the nucleus pulposus tissue, which was prepared as a suspension by grinding and stirring. Finally, 20 μL of autologous nucleus pulposus suspension and 30 μL of a 2% lidocaine solution were injected separately into the puncture center on the back. This method is used to establish a NCLDH model in rats for research purposes.

Table 1 PCR primers used for cDNA

Genes	Primers
β-actin	F: 5′-ctacctcatgaagatcctgacc-3′ R:5′-cacagcttctctttgatgtcac-3′
P65	F:5′-ggctacacaggaccaggaacag-3′ R:5′-aggaactatggatactgcggtctg-3′

Neurological function measurement

Neurological scoring is based on the method provided by Siegal, which assesses the motor coordination, limb sensation, reflex strength, balance ability and other aspects of the animals through behavioral observation of Sprague Dawley rats. Sprague Dawley rats were observed on the 2nd day after modelling and on the 14th day after intervention. The scoring principles are as follows: normal general condition, normal gait is recognized as level 0 and scored as 2 points; Sprague Dawley rats with basically normal gait are scored as level 1 and scored as 4 points; weakness of one or both hind limbs and mild difficulty in crawling are scored as level 2 and scored as 6 points; weakness of one or both hind limbs, weakness of hind limbs and obvious instability in crawling are scored as level 3 and scored as 8 points; instability while standing but able to move are scored as level 4 and scored as 10 points; and paralysis is scored as level 5 and scored as 12 points. Paralysis was classified as grade 5 with 12 points.

RT-qPCR

After Sprague Dawley rats were euthanized via decapitation on the 14th day of intervention, the L5 nerve and surrounding tissues were dissected. Total RNA was extracted via the Molpure® Cell/Tissue Total RNA Kit (YEASEN, Shanghai, China; Cat. No.: 19221ES50). The purity of the RNA was assessed, with A260/A280 ratios ranging between 1.8 and 2.0. The total RNA was then mixed in a 20 μL reaction mixture and reverse transcribed into cDNA. Real-time quantitative PCR amplification was conducted via the QuantStudioTM 3 Real-Time PCR System (Thermo Fisher Scientific, US). The PCR amplification conditions were as follows: initial denaturation at 95 °C for 30 s, denaturation at 95 °C for 5 s, annealing at 55 °C for 30 s, and extension at 72 °C for sufficient elongation, with a total of 45 cycles. The QuantStudioTM Design & Analysis SE Software from Thermo Fisher Scientific (US) is used to analyze the CT (Threshold cycle) values of each sample during the PCR process, and the relative mRNA expression level of P65 is calculated using the 2^-ΔΔCT method. Table 1 lists the rat-specific sequences of primers used for detecting p65 and mRNA via PCR analysis.

ELISA

After centrifugation of rat left atrial arterial blood, serum samples were obtained, and the levels of IL-1β and TNFα were detected via an ELISA s following the protocols of the manufacturer.

Western blot analysis

After Sprague Dawley rats were euthanized via decapitation on the 14th day of intervention, the L5 nerve and surrounding tissues were dissected. The samples were then transferred to 2 mL grinding tubes, The sample was then removed and put into a 2 mL grinding tube, followed by the addition of 2 pieces of 3 mm grinding beads and RIPA lysate (Shanghai Biyun Tian, Shanghai, China; Cat. No.: P0013) with a mass ratio of sample to lysate at 1:10. The tubes were placed in a high-speed low-temperature tissue grinder (temperature: -20°C) and subjected to grinding four times for 60 s each time. After grinding, the tubes were removed and allowed to sit at 4°C for 30 minutes for lysis. After 30 minutes, the tubes were centrifuged (temperature: 4°C, speed: 12000 rpm) for 10 minutes. Following centrifugation, the supernatant was collected, and the protein concentration was determined via a Bicinchoninic AcidAssay (BCA) protein quantification kit from Shanghai Biyun Tian Biotechnology Co., Ltd (Shanghai, China; Cat. No.: P0009). The samples were then subjected to high-temperature denaturation at 95°C for 15 minutes and stored at -80°C after denaturation. The samples were loaded onto a 10% sodium dodecyl sulfate (SDS)–polyacrylamide gel (PAGE) (saiguobio, Guangzhou, China; Cat. No.: 3250GR500) and electrophoresed to transfer the proteins onto a polyvinylidene fluoride(PVDF)(Sigmaaldrich, Wuxi, China; Cat. No.: ISEQ00010) membrane. The PVDF membrane was blocked with 5% skim milk for 2 hours. After blocking, the PVDF membrane was incubated with primary antibodies (dilution ratio: p65 1:1000; Tubulinβ 1:2000) overnight at 4°C with gentle shaking. The PVDF membrane was then washed three times with Tris-buffered saline containing Tween-20 (TBST) for 5 minutes each. Next, the PVDF membrane was incubated with secondary antibodies (dilution ratio: 1:5000) for 2 hours at room temperature with gentle shaking. After incubation, the PVDF membrane was washed three times with TBST for 10 minutes each time. The PVDF membrane was then fully immersed in a mixture containing 'Torchlight' Hypersensitive ECL Western HRP Substrate (zen-bio, Chengdu, China; Cat. No.: 17046) in a culture dish under dark conditions. The bands were visualized via Tanon Fluorescence Imaging System software V2.0 (Shanghai, China), and the results were scanned and analysed via Gel-Pro Analyser4 software (Media Cybernetics, US), with the integrated optical density (IOD) representing the target protein.

Immunofluorescence

After Sprague Dawley rats were euthanized by decapitation on the 14th day of intervention, the L5 nerve and surrounding tissues were dissected and fixed in 4% formaldehyde. The samples were then embedded in paraffin and sectioned, and the sections were immersed in citrate buffer (pH 6.0) for antigen retrieval, followed by microwave treatment for 20 min. After cooling, the sections were washed three times with phosphate-buffered saline (PBS) (zsbio, Peking, China; Cat. No.: ZLI-9062) for 5 min each. Endogenous peroxidase activity was blocked by incubating the sections in 3% hydrogen peroxide at room temperature for 25 min, followed by washing the slides in PBS (pH 7.4) on a shaker 3 times, each time for 5 min. Serum blocking was performed using bovine serum albumin at room temperature for at least 30 min. Primary antibody was applied by gently removing the blocking solution and adding prediluted primary antibody to the sections, which were then placed flat in a humidified chamber and incubated overnight at 4 °C. After incubation, the sections were washed three times with PBS for 5 min each, followed by the addition of secondary antibody for 30 min at 37 °C. The sections were subsequently washed again with PBS three times for 5 min each. The 4',6-diamidino-2-phenylindole (DAPI) (Servicebio, Wuhan, China; Cat. No.: G1012) staining was performed by incubating the sections with DAPI at room temperature for 10 min, followed by washing with PBS three times for 5 min each. Finally, the sections were mounted with anti-fade mounting medium. OlyVIA scanning software (Olympus, Japan) was used to capture images of the sections. Each section was observed at low magnification to visualize the entire tissue, followed by capturing images at 200x magnification from three different fields. DAPI staining was used to label the cell nuclei in blue, while positive expression of p65 was visualized in green. The Halo Data Analysis System (lndica Labs, US) was used to calculate the number of positive cells in each image.

Immunohistochemistry

The samples were embedded in paraffin and made into sections for storage. First, the paraffin sections were dewaxed, a process that included the sequential use of xylene I, xylene II and xylene III (zhiyuanhx, Tianjin, China; Cat. No.: 202150101), each solvent for 15 min, followed by treatment of the sections with anhydrous ethanol I and anhydrous ethanol II (haixinghuagong, Chengdu, China; Cat. No.: GB678-90), each continuously for 5 min, 85% alcohol and 75% alcohol for 5 min each, and finally, washing of the treated sections with distilled water. The next stage was the antigen repair stage, where the sections were placed in citrate buffer (zsbio, Peking, China; Cat. No.: ZLI-9065) with a pH of 6.0 and repaired via a repaireer at 80 °C for 20 min. After repair, the sections were washed three times with PBS solution for 5 min each. Finally, to block endogenous peroxidase activity, the sections were soaked in 3% hydrogen peroxide solution for 10 min at room temperature, followed

by three washes with PBS solution for 5 min each. The tissue samples were washed for 5 min; next, to seal possible nonspecific binding sites, goat serum was added to the samples for sealing, and this step lasted for 20 min at room temperature. After that, the first antibody was added, and the samples were incubated overnight at 4 °C. The next day, the samples were washed three times with PBS for 5 min each time. To continue, the second antibody was added, and the samples were incubated at 37 °C for 30 min. The samples were again washed three times with PBS for 5 min each. Next, the DAB (zsbio, Peking, China; Cat. No.: ZLI-9018) color development process was carried out by adding drops of freshly prepared DAB color development solution to the tissue samples and controlling the time of color development at room temperature until the appearance of brownish–yellow color positivity, after which the sections were washed with distilled water to stop color development. Finally, to highlight the nuclei of the samples, the sections were immersed in hematoxylin for 3 min, followed by rinsing with water and waiting for the sections to return blue in water before rinsing thoroughly with running water. Dehydration was then performed by placing the sections in anhydrous ethanol at concentrations of 75%, 85%, and 95% and immersing them at each concentration for 10 min. The sections were subsequently transferred to xylene and immersed for 10 min to complete the dehydration step, after which they were finally sealed with neutral gum. Images of the sections were captured via CaseViewer(3DHISTECH, Hungary), a digital section viewing software, and the average protein concentration was determined via ImageJ software (National Institutes of Health, US).

Reaults

Neurological function scores of the rats in each group

On the 2nd day after the model was established (Tables 2 and 3), when the model group and treatment group were compared with the normal group, the neurological function scores of the NCLDH model group were significantly greater than those of the normal group ($P=0.001$; $P<0.001$). On the 14th day of the intervention (Table 4), when the neurological function scores of the model group were compared with those of the treatment group, the neurological function scores of the treated group were significantly lower than those of the model group ($P<0.001$). Phellodendrine reduced the neurological function score and relieved pain in NCLDH model rats.

Serum TNF-α and IL-1β concentrations in the rats in each group

The serum TNF-α and IL-1β concentrations were significantly greater in the model group than in the normal group ($P=0.02$; $P=0.012$), suggesting that rat transplantation of autologous nucleus pulposus to the vicinity of nerve roots can increase the release of inflammatory factors. After dissipation, the serum concentrations of TNF-α and IL-1β in the treatment group were significantly greater than those in the model group ($P=0.03$; $P=0.035$), suggesting that phellodendrine can control inflammation in NCLDH (Fig. 1A and B).

Table 2 Neurological function scores of the rats on day 2 after modelling ($\overline{x} \pm s$)

groups	average value ± standard deviation
normal($n=8$)	2.000 ± 0.000
mould($n=8$)	9.750 ± 1.669##

Note: Compared with the normal group,#$P<0.05$, ##$P<0.01$

Table 3 Neurological function scores of the rats on day 2 after modelling ($\overline{x} \pm s$)

Groups	Average value ± standard deviation
Normal($n=8$)	2.000 ± 0.000
Treatment ($n=8$)	9.500 ± 2.329###

Note: Compared with the normal group, #$P<0.05$, ##$P<0.01$, and ###$P<0.001$

Table 4 The effects of 14 days of phellodendrine elimination intervention on neurological function scores in rats in NCLDH model ($\overline{x} \pm s$)

Groups	*n*	Average value ± standard deviation
Normal	8	2.000 ± 0.000
Mould	8	8.250 ± 1.982###
Treatment	8	3.000 ± 1.069***

Note: Compared with the normal group, #$P<0.05$, ##$P<0.01$, and ###$P<0.001$; compared with the model group, *$P<0.05$, **$P<0.01$, and **$P<0.001$

RT–PCR analysis of NF-κB P65 mRNA expression in the nerve roots and tissues of the rats in each group

Compared with that in the normal group, the expression of NF-κB P65 mRNA in the L5 nerve roots and surrounding tissues of the rats in the model group was significantly greater ($P<0.001$). These findings suggest that the stimulation caused by the implantation of nucleus pulposus near nerve roots in rat models may increase the expression of NF-κB p65mRNA. After treatment with phellodendrine, the relative expression level of NF-κB P65 mRNA in the rats in the treatment group was significantly lower than that in the model group ($P=0.001$). These findings indicate that phellodendrine can affect the expression of NF-κB P65mRNA. (Fig. 2)

Western blot analysis of the relative protein expression of NF-κB P65 and TNF-α in the nerve roots and tissues of the rats in each group

The effect of phellodendrine on the NF-κB P65 and TNF-α in LDH was clarified. The relative protein expression levels of P65 and TNF-α in the nerve roots and surrounding tissues of the rats in the model group were significantly greater than those in the normal group

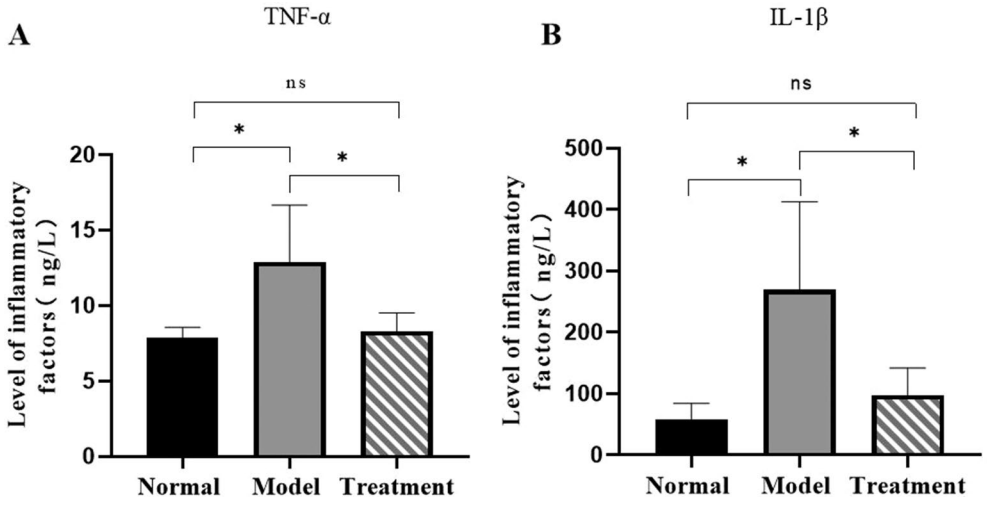

Fig. 1 Effects of phellodendrine on serum inflammation due to the factors TNF-α (**A**) and IL-1β (**B**) in NCLDH patients. Note: * $P<0.05$, * * $P<0.01$

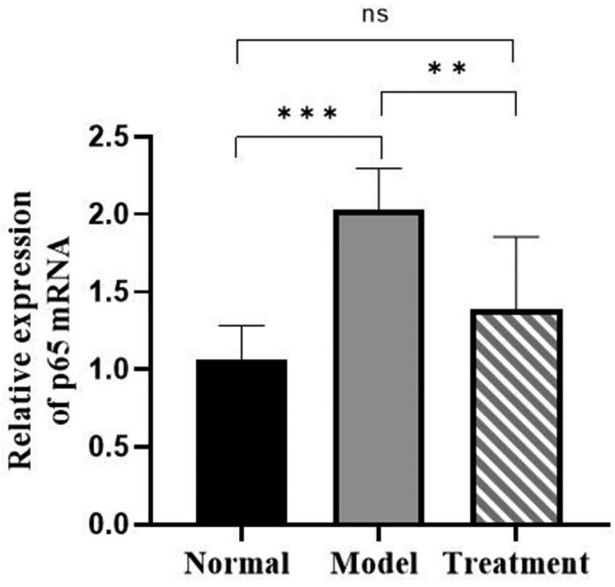

Fig. 2 Relative expression of p65 mRNA in nerves and surrounding tissues. Note: * $P<0.05$, * * $P<0.01$, * * $P<0.001$

($P<0.001$; $P<0.001$). Compared with those in the model group, the relative protein expression levels in the nerve roots and surrounding tissues of the rats in the treatment group were significantly lower ($P=0.002$; $P<0.001$). These findings indicate that phellodendrine can downregulate the expression of proteins in L5 nerve roots and surrounding tissues, affecting the NF-κB P65 and TNF-α (Fig. 3A and B).

Immunofluorescence and immunohistochemistry analyses of relative NF-κB P65 protein expression in the nerve roots and tissues of the rats in each group

The **immunofluorescence** (Fig. 4A and B) and **immunohistochemistry** (Fig. 4C and D) results revealed that, compared with that in the normal group, the protein expression of NF-κB p65 in the L5 nerve and surrounding tissues of the rats in the model group was significantly greater ($P<0.001$; $P<0.001$). Compared with that in the model group, the protein expression of NF-κB p65 in the L4–L5 dorsal root ganglia and surrounding tissues of the rats in the treatment group was significantly lower ($P<0.001$; $P<0.001$).

Discussion

This study aims to explore the role and preliminary mechanism of phellodendrine in NCLDH, providing theoretical support for the clinical application of this drug. Our research found that phellodendrine can significantly reduce the levels of TNF-α and IL-1β inflammatory cytokines in serum, downregulate the transcription of p65 mRNA, and alleviate pain in NCLDH rats.Inflammation plays a pivotal role in lumbar radicular pain. Previous studies have shown that inflammation in DRG and the spinal cord induced by zymosan or nucleus pulposus can lead to mechanical allodynia. Autologous NCLDH implantation into rat DRGs can induce the NCLDH model, which is a relatively mature model [14–16]. Autologous nucleus pulposus implantation serves as the herniated tissue and acts as an exogenous nociceptive antigen, triggering a robust immunoneuroinflammatory response around the LDH site [16]. Stimulation of the herniated tissue results in central sensitization, which is considered the primary cause of neuropathic pain. Our findings suggest that autologous nucleus pulposus implantation near the DRG can alter rat behavior to some extent, inducing pain.

Previous studies have shown that TNF-α can promote the production of downstream inflammatory factors, leading to accelerated disc degeneration [17]. Some studies have indicated that the level of IL-1β increases in

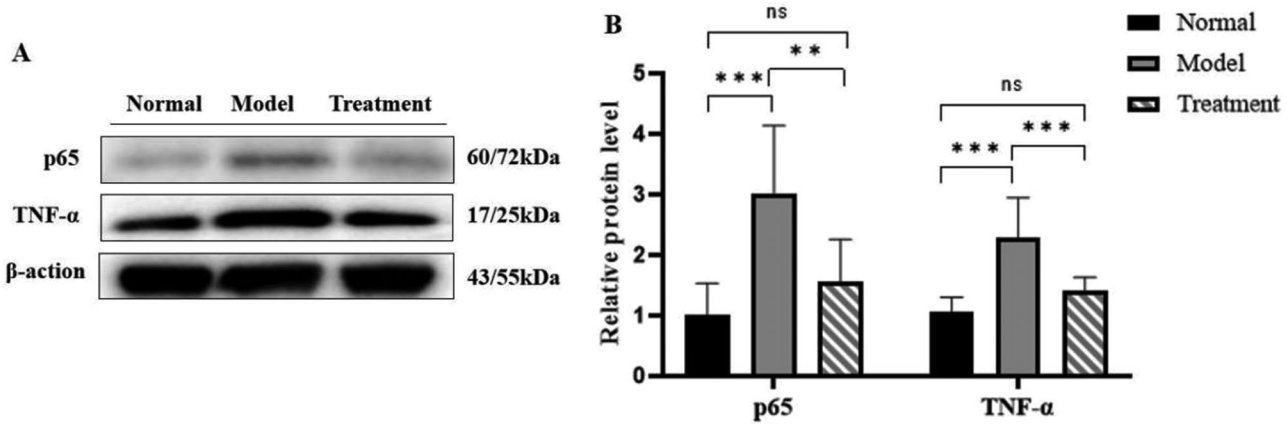

Fig. 3 Effect of phellodendrine on NF- κ B p65 and TNF-α in NCLDH. A, P65, TNF-α, β - action Gels and Blots images. B, Relative protein expression of NF- κ B p65 and TNF-α. Note: * $P < 0.05$, * * $P < 0.01$, * * $P < 0.001$

nucleus pulposus cells stimulated with TNF-α. Furthermore, TNF-α can stimulate nucleus pulposus cells to produce other cytokines and chemokines, thereby recruiting and activating immune cells to further enhance the inflammatory state [18, 19]. IL-1β, a member of the IL-1 family, is highly active in promoting inflammatory factors and is associated with various proinflammatory mediators. Previous studies have confirmed its involvement in nucleus pulposus cell inflammation, cell apoptosis, extracellular matrix (ECM) degradation, and oxidative stress. TNF-α mainly regulates immune and inflammatory responses. It is an important inflammatory mediator that plays a key role in the body's resistance to infection, regulating immune cell activity, and mediating the inflammatory process [20]. TNF-α can trigger inflammatory responses by activating the NF-κB signalling pathway, inducing the expression of other cytokines, and promoting the migration and activation of inflammatory cells. Its excessive or abnormal production is closely related to the occurrence and development of various inflammatory diseases, including rheumatoid arthritis, inflammatory bowel disease, and cardiovascular diseases [21–23]. TNF-α is also considered an important mediator of neuropathic pain, directly or indirectly affecting the excitability and sensitivity of neurons, thereby participating in the regulation of pain transmission and the generation of pathological pain [24–26].

With the deepening understanding of the pathological mechanisms of disc degeneration, increasing evidence suggests that TNF-α is a crucial activating factor for disc degeneration and is associated with pathological processes such as metabolic disorders, inflammation, cell apoptosis, and extracellular matrix degradation within intervertebral discs. Therefore, anti-TNF-α therapy is an effective therapeutic target for alleviating disc degeneration, especially for inhibiting extracellular matrix degradation and reducing inflammatory responses [27, 28]. Our study revealed that phellodendrine can reduce the levels of IL-1β and TNF-α in the serum of NCLDH model rats.

NF-κB plays a pivotal role in multiple cellular immune responses and inflammatory pathways. In the cytoplasm, IκB proteins keep NF-κB in inactive state under basal conditions [29]. Phosphorylation of IκB is induced by NF-B activation both internally and externally [30]. NF-κB activation can enhance the transcription, expression, and release of IL-2, IL-1β, and TNF-α, subsequently leading to the reactivation of NF-κB [31]. Our research indicates that phellodendrine can significantly reduce the levels of inflammatory cytokines TNF-α and IL-1β, and downregulate the protein expression of NF-κB p65.

During the implementation of this study, we closely monitored the average weight changes in rats and found that they exhibited a stable and healthy growth trend. This fully demonstrates that the experimental animals were in good growth condition and received proper feeding management. All rats were fed according to standardized feeding procedures, with the pellets or feed undergoing rigorous screening and formulation to provide comprehensive and balanced nutritional support tailored to their physiological needs. It is particularly noteworthy that during the drug administration process, we conducted in-depth investigations and analyses of potential food-drug interactions. By consulting expert opinions and conducting preliminary experimental validations, we confirmed that there were no significant food-drug interactions between the pellets or feed used in this study and the administered drugs. However, this does not exclude the possibility of interactions occurring under certain specific conditions. Therefore, in future studies, we will continue to follow research progress in this field and take necessary measures to reduce or eliminate potential food-drug interactions, ensuring the accuracy and reliability of experimental results.

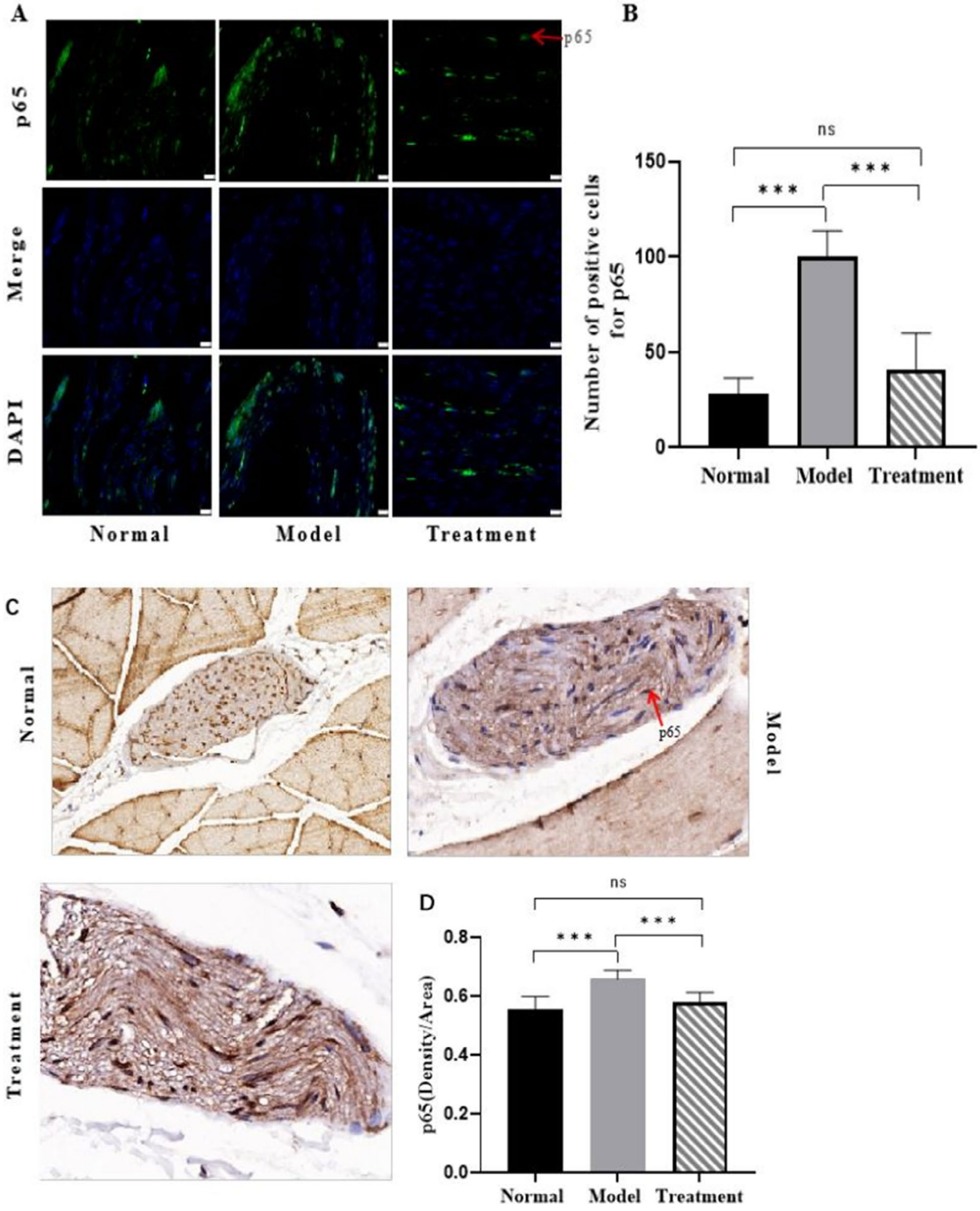

Fig. 4 (**A**), Immunofluorescence image of the rats; the nuclei are blue, and the positive expression of p65 is green (scale bar = 50 μm), magnification 20×; (**B**), the number of P65-positive cells in the rats in each group, compared with the normal group, ***$P < 0.001$; compared with the model group, ***$P < 0.001$; C, the immunohistochemistry image of the rats, where p65 is present, the area where P65 is present will show a tan or brown color (produced by DAB color development); the area that does not contain the target antigen will not show a tan color but will show a background color (light blue, light blue–purple, or slightly whitish), and the color of the nucleus: blue or blue–purple (hematoxylin staining) (scale bar = 50 μm), magnification of 20×; D, the expression of P65 protein in the rats in each group, P65: compared with the normal group, ***$P < 0.001$; compared with the model group, ***$P < 0.001$

Nerve root tissue and serum were successfully obtained, and the desired goal was achieved. Nerve function was evaluated, and the concentrations of inflammatory factors in the serum were measured via an enzyme-linked assay. The protein expression of p65 in nerves and tissues was analysed by immunofluorescence, immunohistochemistry and protein blotting. This experiment validated the method from the molecular level to the cellular level to the tissue level, and the evidence was sufficiently conclusive and highly credible. However, this study has several limitations: only the normal group, model group and treatment group were used in this study, and the sham operation group was not used. Although the sham-operated group is informative for assessing the impact of the surgical operation itself on the experimental results, its absence may have a limited impact on the overall study results, considering that the surgical operation has a relatively small impact on the main study indices, such as inflammation and pain. However, to more accurately distinguish the effects of surgical operation and disease modelling, the inclusion of a sham operation group should still be considered in future studies; in this experiment, only a single dose of phellodendrine was used for treatment, and different concentration gradients were not set up. Drug dose gradient experiments can more comprehensively assess the dose-dependent effects of drugs and help to determine the optimal therapeutic dose. Therefore, the absence of a gradient experiment on drug dose may limit the full assessment of the treatment effect, and future studies may consider further experimental validation at different doses; this study did not include a control group of other drugs, such as the use of standard anti-inflammatory drugs or other conventional therapeutic drugs. Such a control group would have provided a more comprehensive assessment of efficacy by providing a comparison of the effects of phellodendrine n with those of existing treatments. The lack of these control groups may limit the understanding of the therapeutic effects of phellodendrine; therefore, future studies may consider including these control groups to further validate the efficacy of phellodendrine and its comparison with other treatments. In this study, we conducted Western blot and PCR analyses using an integral anatomical region encompassing the L5 nerve and its adjacent tissues. This design choice was based on our hypothesis of potential close interactions between nerves and surrounding tissues. Although we did not physically separate neural tissue from surrounding tissue, we believe that this holistic analysis approach can provide more comprehensive information, contributing to uncovering complex biological processes within this region. At the same time, we recognize the potential issues arising from this design, namely the inability to directly distinguish specific differences between neural tissue and surrounding tissue. To address this issue, we maintained the integrity of the L5 nerve and its adjacent tissues as much as possible during sampling and employed rigorous quality control measures in experimental analyses. Nevertheless, we must cautiously interpret the experimental results and avoid over-inferring specific differences between different tissue components. In short, these deficiencies may have some impact on the interpretation and extrapolation of the study results.

Conclusion

In this animal experiment, the administration of phellodendrine was found to effectively decrease the expression level of p65 mRNA and reduce the levels of inflammatory factors. Furthermore, it was confirmed through behavioral tests and other methods that berberine could alleviate lumbar disc pain.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13018-024-05313-7.

Supplementary Material 1
Supplementary Material 2
Supplementary Material 3
Supplementary Material 4
Supplementary Material 5
Supplementary Material 6
Supplementary Material 7
Supplementary Material 8
Supplementary Material 9
Supplementary Material 10
Supplementary Material 11
Supplementary Material 12
Supplementary Material 13
Supplementary Material 14
Supplementary Material 15
Supplementary Material 16

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Not applicable.

Author contributions
P.T. and J.M. wrote the main manuscript text, P.T. and D.W prepared Tables 1, 2, 3 and 4; Figs. 1, 2, 3 and 4. All authors reviewed the manuscript.

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

Declarations

Ethics approval and consent to participate
The animal study was reviewed and approved by the Ethics Committee of Chengdu University Affiliated Hospital (No.2022-12-18).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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