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Research article

Therapeutic potential of *Canna edulis* RS3-resistant starch in alleviating neuroinflammation and apoptosis in a Parkinson's disease rat model

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ABSTRACT

This study aimed to investigate the effects of Miao medicinal *Canna edulis* RS3-resistant starch on behavioral performance and substantia nigra neuron apoptosis-related indicators in a rat model of Parkinson's disease (PD). Among the experimental groups, except for the control group, we induced PD rat models by subcutaneous injection of rotenone in the neck and back. After model induction, a 28-day drug intervention was conducted. Various techniques have been employed, including behavioral analysis, Real-time Polymerase Chain Reaction (RT-PCR), western blotting, enzyme-linked immunosorbent assay (ELISA), immunofluorescence, and terminal deoxynucleotidyltransferase-mediated UTP nick-ends. labeling (TUNEL) and Nissl staining to investigate the effect of *Canna edulis* RS3-resistant starch on the substantia nigra and neuronal apoptosis-related markers in the brains of PD model rats. Our study revealed that *Canna edulis* RS3, a resistant starch, significantly reduced the climbing time of PD model rats, prolonged their hanging time, lowered the expression levels of the inflammatory factors IL-1β, IL-6, and TNF-α, increased the number of TH-positive neurons in the substantia nigra, and decreased the levels of IL-1β, IL-6, and TNF-α. Furthermore, *Canna edulis* RS3 elevated the protein expression levels of

Abbreviations: PD, Parkinson's disease; IL, interleukin; TNF, tumor necrosis factor; TUNEL, terminal deoxynucleotidyl transferase-mediated nick-end labeling; NF-κB, Nuclear factor kappa B; TNF-α, Tumor necrosis factor-α; RT-PCR, Reverse transcription polymerase chain reaction; WB, Western blot; TLR-4, Toll-like receptor4; NLRP3, NOD-like receptor thermal protein domain associated protein 3; TH, tyrosine hydroxylase; DAB, 3' diaminobenzidine tetrahydrochloride; α-syn: synuclein. Bcl-2, B-cell lymphoma-2; BAX, BCL2-Associated X; ELISA, enzyme-linked immunosorbent assay.

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tyrosine hydroxylase (TH) and Bcl-2 while reducing those of Bax, TLR4, NLRP3,and p-P65, and mitigated apoptosis and morphological changes in dopaminergic neurons in the substantia nigra region. Our results suggest that *Canna edulis* RS3-resistant starch may offer therapeutic benefits for PD patients with PD by potentially influencing inflammation and apoptosis in the dopaminergic system.

1. Introduction

Parkinson's disease (PD) is a chronic and progressive neurodegenerative disorder primarily characterized by motor impairment, elevated muscle tension, and unstable posture [1]. Globally, it is the second most prevalent neurodegenerative disease, affecting more than six million individuals [2]. Although PD is commonly found among those aged 50 and above, it is worth noting that occasional occurrences in younger individuals are also observed [3]. Genetic factors play a significant role in PD, as demonstrated by the discovery of over 90 genetic risk sites [4]. Moreover, environmental and behavioral factors are thought to affect the development and progression of PD in variouspopulations [5]. The distinctive pathological feature of PD is the accumulation of synuclein proteins, primarily in the substantia nigra [6], forming intracellular Lewy bodies and neuritic aggregates [7]. Notable aspects of the brains of patients with PD involve either persistent loss of dopaminergic neurons or abnormal accumulation of such neurons within the substantia nigra.

Currently, the fundamental therapeutic approach for PD is dopamine replacement therapy with levodopa as the established gold standard treatment [8]. However, while adverse drug reactions intensify over time, the treatment efficacy decreases. Additionally, patients might experience motor complications, including dyskinesia, wearing-off phenomenon, and on-off fluctuations, while treatment aims to impede disease progression. Owing to the intricate pathogenesis of PD, a complete cure remains a clinical challenge. Research has shown that traditional Chinese medicine has a significant improvement effect on tremors, limb stiffness, constipation, and sleep disorders in Parkinson's patients [9,10]. Traditional Chinese medicine has increasingly gained trust among patients due to its distinct therapeutic effects, in contrast to the strict restrictions and recommendations linked to Western medicine in this context.

Canna edulis, scientifically named as Canna edulis Ker, is a perennial herbaceous plant with tuberous roots and purple stems. It can grow up to 3 m in height and is also called a dry yam, banana yam, lotus tuber, ginger yam, and champion tuber [11]. In regions such as Xingyi and Shuicheng in Guizhou, Canna edulis is traditional Miao medicine. The tuberous roots invigorate the spleen and stomach, reduce inflammation and swelling, and nourish the body. Canna edulis contains a unique linear form of resistant starch, comprising 17.5 %–27 %, making it an innovative source [12]. Unlike regular starch, resistant starch undergoes slow digestion and absorption in the human body. It resists enzymatic digestion and is fermented by beneficial bacteria in the colon, resulting in short-chain fatty acid production. Similar to dietary fiber, these short-chain fatty acids promote the growth of beneficial colon microorganisms and mediate various physiological functions, including preventing colon cancer, improving insulin resistance, and reducing blood lipid levels [13]. Early studies indicated that resistant starch can alleviate inflammatory responses [14]. Nonetheless, the uroprotective effect of *Canna edulis* RS3's resistant starch on dopaminergic neurons in the substantia nigra of PD rats remains unreported.

The TLR4/NF-κB/NLRP3 pathway is important for regulating apoptosis and inflammation [15,16]. Neuroinflammation and apoptosis play important roles in the occurrence and development of Parkinson's disease [17,18]. Therefore, the primary objective of this study was to investigate the neuroprotective potential of resistant starch from the Miao medicine *Canna edulis* RS3 on dopaminergic neurons in the substantia nigra of PD rats using a rotenone-induced rat model by regulating the TLR4/NF-κB/NLRP3 pathway. Through this research, we uncovered the mechanisms of resistant starch in *Canna edulis* RS3 and established a solid theoretical foundation and experimental basis for its potential application in PD treatment.

2. Material and methods

2.1. Materials

Herein, we used *Canna edulis* RS3 RS3-resistant starch (purity 98 %) obtained from Yilitai Technology Co., Ltd., located in Xingyi City, Guizhou Province, China, for our experimental purposes. Anti-TH (ab137869, 1:1000), anti- Bcl-2 (A0208, 1:1000), anti-Bax (A0207, 1:1000), anti-TLR4 (A0528, 1:1000), anti-p-P65 (AP0124, 1:1000), anti- NLRP3 (A5652, 1:1000), anti-α-syn (A20407, 1:1000), anti-Caspase-3 (A17900, 1:1000), anti-GAPDH (A0347, 1:1000), horseradish peroxidase(HRP) conjugated secondary goat anti-rabbit IgG, and anti-mouse IgG secondary antibodies (AS014, 1:2000) were purchased from ABclonal (Wuhan, China). Enhanced chemiluminescence (ECL) for easy detection of horseradish peroxidase (HRP) on immunoblots was obtained from EngreenBiosystems (Beijing, China).

2.2. Animals

Sixty healthy male SD rats served as experimental subjects and were housed in standard animal facilities with access to food and water. They were housed in standard cages with regulated cycles of temperature and humidity light/dark periods of 12 h, undergoing an adaptation feeding period. The experiment lasted one week, during which strict adherence to regulations governing the management and protection of experimental animals was maintained to ensure their well-being and health. All procedures involving

animal experiments followed the guidelines of the Animal Ethics Committee of Guizhou University of Traditional Chinese Medicine and received approval from the same committee (Approval No. 20220079).

2.3. Construction of PD rat model

PD rat models were established by subcutaneously injecting rotenone (2 mg/kg) at the nape of the neck, which was dissolved in sunflower oil at a concentration of 2 mg/mL [19]. The injection was administered once daily at the nape of the neck for 28 days.

2.4. Experimental groups and treatment

The intervention with *Canna edulis* RS3-resistant Starch began on day 1 of modeling, following one week of adaptive feeding. The *Canna edulis* RS3 Resistant Starch intervention was conducted once daily from 8:00 a.m. to 11:30 a.m. for one month. The rats were randomly divided into six groups, each consisting of ten rats. The groups were as follows.

Control Group: Normal rats received daily gavage of distilled water (equal volume to the herbal medicine group) for 28 days without modeling.

Model Group: Rats in the maintained PD model received daily gavage of distilled water (equal volume to the herbal medicine group) for 28 days.

Canna edulis RS3 Resistant Starch-H Group: Rats received *Canna edulis* RS3-resistant starch solution (4.5 g/30 mL) by gavage at a doses of 4.5 g/kg once daily for 28 days.

Canna edulis RS3 Resistant Starch-M Group: Rats received *Canna edulis* RS3-resistant starch solution (2.25 g/30 mL) by gavage at a doses of 2.25 g/kg once daily for 28 days.

Canna edulis RS3 Resistant Starch-L Group: rats received *Canna edulis* RS3-resistant starch solution (1.125 g/30 mL) by gavage at a dose of 1.125 g/kg once daily for 28 days.

Pramipexole Group: Rats received pramipexole at doses of 0.05 mg/kg [14] by gavage once daily for 28 days.

2.5. Sample preparations

After 28 days of treatment, the rats in each group were anesthetized with 1 % sodium pentobarbital (50 mg/kg; Wuhan Dinghui Chemical Co., Ltd., Wuhan, China). Subsequently, mice were euthanized by cervical dislocation. The right internal carotid artery was isolated, and 5 mLof blood was collected, centrifuged, and the resultant supernatant was placed into two sterile test tubes, sealed, and stored at −20 °C. Nissl staining, immunohistochemistry, and western blotting were performed on the substantia nigra tissue to detect the effect of *Canna edulis* RS3 Resistant Starch on the expression of PD-related genes and proteins in this tissue.

2.6. Behavioral analysis

Pole climbing test: After establishing the PD model, we conducted a climbing pole experiment for behavioral assessment. According to a previously described method [20], the rats were held by the tail upside down at the top of a pole, with their heads downwards. Consequently, a stopwatch was used to record their time and progress as they climbed from the top to the bottom of the pole, ensuring that their hind limbs reached the ground. This process was repeated three times with a 5-min interval between each repetition. The experiment was repeated if there was a pause (continuous stoppage) or backward climbing during crawling. The scoring criteria were as follows:0 points, the rat smoothly descended in one attempt using all limbs; 0.5 points: the rat descended step-by-step in a spiral with hind limb sliding; 1 point, the rat descended with intermittent pauses but held onto the metal pole; 1.5 points: the rat fell after sliding; 2.0 points: the rat could not grip the pole and fell directly.

Tail suspension test: According to the previously described method [21], in the hanging experiment, a metal wire was suspended

Table 1
Primer sequences for qRT−PCR analysis.

Primers	Sequences
TLR4-F	TCCAGAGCCGTTGGTGTATC
TLR4-R	GAAGATGTGCCTCCCCAGAG
NF-κB-F	ACGACGATCCTTTCGGAACT
NF-κB-R	TCCTCTCTGTTTCGGTTGCTC
IL-1β-F	TGACTCGTGGGATGATGACG
IL-1β-R	CAGACAGCACGAGGCATTTT
IL-6-F	TCCTACCCCAACTTCCAATGC
IL-6-R	GGTCTTGGTCCTTAGCCACT
IL-10-F	ATAAAAGGGGGACACCGGGC
IL-10-R	CTCATAACCCATGGCTTGGC
TNF-α-F	CGTCGTAGCAAACCACCAAG
TNF-α-R	GTGAGGAGCACGTAGTCGG
GAPDH-F	TGGTGCTGAGTATGTCGTGG
GAPDH-R	GGCGGAGATGATGACCCTTT

at either 1 m or 50 cm above the ground. The time before the rat touched the ground after suspension was recorded. If a rat fell within 3 s or only managed to grip the metal wire with one paw without falling, it was considered a failure and the trial was repeated. The experiment was repeated three times with a 5-min interval between each trial. The scoring criteria were as follows: landing time within 0–4 s, 0 points; landing time within 5–9 s, 1 point; landing time within 10–14 s, 2 points; landing time within 15–19 s, 3 points; landing time within 20–24 s, 4 points; landing time within 25–29 s, 5 points; landing time exceeding 30 s, 6 points.

2.7. qRT-PCR detection of the mRNA expression of TLR4, NF-κB, IL-1β, IL-10, IL-6, and TNF-α

Using qRT-PCR, total RNA was extracted from the substantia nigra tissue of each group using an RNA extraction kit, according to the manufacturer's instructions. Reverse transcription (RT) was performed using a reverse transcription kit following the manufacturer's instructions. PCR was performed using fluorescent amplification reagents, DEPC-treated water, and other reagents. The reaction involved pre-denaturation at 95 °C for 30 s, followed by 40 PCR cycles (95 °C for 5 s and 60b°C for 30 s), and fluorescence values collected during the extension stage of each cycle. After completion of the PCR amplification reaction, melt curve analysis was conducted to ensure the quality of the PCR products, ultimately obtaining the expression levels of IL-1β, IL-10, IL-6, TNF-α, TLR4, and p-p65 mRNA in the substantia nigra region.The primer series are depicted in Table 1.

2.8. Immunofluorescence detection of tyrosine hydroxylase (TH) and αsynuclein(α-Syn) expression in dopaminergic neurons

After dewaxing and rehydration of paraffin sections, antigen retrieval was performed by boiling the sections for 30 min, followed by cooling at room temperature. Subsequently, the sections were washed thrice with PBS for 5 min each. Tissues were then incubated with Spontaneous Fluorescence Quencher A solution at room temperature for 30 min, followed by three 5-min PBS washes. After blocking with goat serum at room temperature for 60 min, the primary antibodies (TH, 25859-1-AP, diluted 1:200, Proteintech, Wuhan, China) and (α-Syn, RP00700, diluted 1:200, ABclonal, Wuhan, China)were then applied and left to incubate overnight at 4 °C. The specimens were washed three times with PBS for 5 min each. The secondary antibody (FITC Goat Anti-Rabbit IgG (H + L), A22414, ABclonal, diluted 1:200) was incubated at room temperature in the dark for 1.5 h. Subsequently, the specimens were washed thrice with PBS for 5 min each. Following the washes, DAPI was added and the cells were incubated in the dark for 5 min to stain the nuclei. The specimens were washed three times with PBS for 5 min each to remove the excess DAPI. Tissue Self-Quenching Solution B was incubated at room temperature for 5 min, followed by a 5-min wash with distilled water before sealing with an anti-fluorescence quencher. Finally, image acquisition of the sections was performed using a Mshot MF53 inverted microscope (Guangzhou Mshot Photoelectric Technology Co., Ltd).

2.9. Western blot analysis of the protein expression of TH, NLRP3, caspase-3,.α-Syn, Bcl-2, Bax, TLR4, and p-P65 in the substantianigra

RIPA lysis buffer containing phenylmethylsulfonyl fluoride (PMSF) and a protease inhibitor cocktail was prepared. Small tissue fragments or cell samples were placed in 2 mL grinding tubes, along with RIPA lysis buffer and steel beads, and thoroughly disrupted with a tissue homogenizer. The ground tubes were vortexed for 1 min and left to stand on ice for 10 min. This step was repeated thrice to ensure complete tissue lysis. The homogenized tissue suspension was transferred to new 1.5 mL centrifuge tubes and centrifuged at 4 °C and 13000 rpm for 20 min. The supernatants were transferred to prechilled 1.5 mL EP tubes and stored at −80 °C for later use. The gel plates were removed from the refrigerator and placed in an electrophoresis chamber. Samples were prepared by loading denatured total protein onto a gel. Electrophoresis was conducted with voltage and runtime adjustments, as required. Membranes were prepared by placing them in transfer buffer with the gel, and protein transfer was performed under constant current for a specified duration. Subsequently, the membranes were removed for blocking and washing. The primary antibody was diluted with dilution buffer and incubated overnight, followed by washing to remove the excess primary antibodies. The secondary antibody was diluted in blocking solution and incubated, followed by additional washing steps to remove excess secondary antibodies from the membrane. Detection was performed using an ECL exposure solution. The results were quantified by densitometry using Image J software.

2.10. TUNEL detection of apoptosis of dopaminergic neurons in the substantianigra

The paraffin sections were initially deparaffinized and rehydrated, followed by 2 min of dual-steam watering. Subsequently, the excess liquid was removed by rinsing with PBS. Subsequently, the sequence of steps were performed: incubation in proteinase K working solution (37 °C for 20 min), treatment with H2O2 (room temperature for 15 min), equilibration buffer exposure (room temperature for 10 min), TdT buffer incubation (37 °C for 1 h), and streptavidin-HRP reaction (37 °C for 30 min). After each incubation, sections were washed with PBS for 5 min to remove any surplus liquid. Upon completion of the incubation steps, DAB staining was performed until a positive reaction appeared, and then the reaction was terminated using distilled water. Hematoxylin staining was performed, followed by rinsing and washing steps. After DAB staining, dehydration was initiated, involving sequential exposure to 95 % ethanol for 2 min, 100 % anhydrous ethanol for 2 min, and treatment with xylene for 5 min, followed by repeated exposure to fresh xylene for an additional 5 min. Finally, a neutral resin was used for mounting. Following these procedures, microscopic inspection and image capture were performed using an aMshot MF53 inverted microscope. Microscopic examination revealed that the apoptotic positive cells had brownish-yellow stained nuclei, whereas the normal negative cells had blue-stained nuclei.

2.11. Enzyme linked immunosorbent assay (ELISA) detection of IL-10, IL-1β, IL-6, and TNF-α expression in the substantia nigra

IL-10, IL-1β, IL-6, and TNF-α levels in the substantia nigra were measured using the following rat-specific ELISA kits: **IL-10** ELISA kit (RX302092R), IL-1β ELISA kit (RX302869R), TNF-α ELISA kit (RX302058R),and IL-6 ELISA kit (RX302856R), all from Ruixing Biotechnology Co., Ltd., Henan, China. Subsequently, the kits were equilibrated at room temperature. Rat substantia nigra samples were prepared as 10 % tissue homogenates using a physiological saline solution on ice. The supernatant was collected after centrifugation at 2000 rpm for 15 min at a radius of 10 cm. IL-1β, IL-6, and TNF-α expression levels in the substantia nigra of each group of mice were determined according to the respective ELISA kit instructions.

2.12. Nissl staining

After dewaxing and rehydration of the paraffin sections, the sections were washed three times with dual distilled water for 2 min each. Subsequently, staining was performed by immersing the sections in cresyl violet Stain and incubating them at 56 °C for 1 h, followed by rinsing with distilled water. After incubation, the sections were immediately placed in a Nissl Differentiation medium for differentiation until the background approached colorlessness under microscopic observation, at which point differentiation was terminated. The sections were dehydrated with absolute ethanol, cleared with xylene, and mounted using neutral mounting medium. Finally, microscopic inspection and image acquisition were performed using a Mshot MF53 microscope. Microscopic examination revealed violet-stained Nissl bodies on a colorless or light blue background.

2.13. Statistical methods

In this study, we established a comprehensive database of all research results and conducted statistical analyses using the SPSS software package (version 26.0). For comparisons involving multiple groups of continuous data and repeated measurements, we employed one-way analysis of variance, followed by post hoc pairwise comparisons using the SNK-Q test, with a significance level set at $p < 0.05$. These statistical techniques allowed us to precisely evaluate data variance and ascertain statistical significance.

3. Results

3.1. Behavioral test results

Rats received *Canna edulis* RS3-resistant starch solution (1.125–4.50 g/30 mL) by gavage once daily for 28 days at doses ranging from 1.125 to 4.50 g/kg. The pole climbing experiments were conducted on five groups of rats before and after model construction and

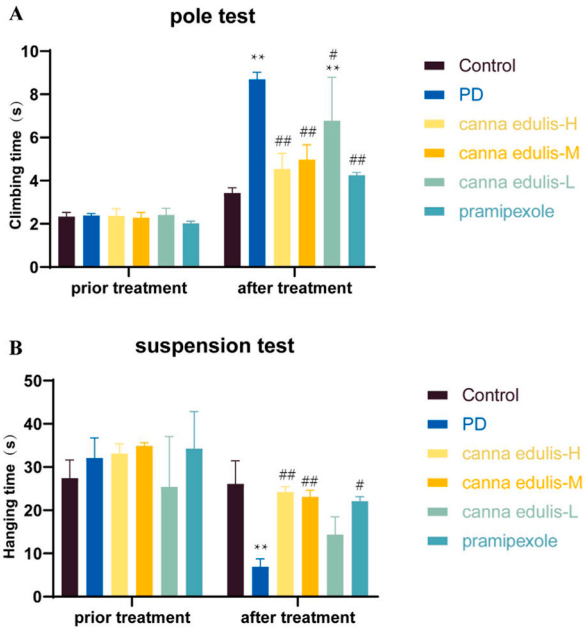

Fig. 1. Behavioral assay findings of PD model rats. Rats received *Canna edulis* RS3-resistant starch solution (1.125–4.50 g/30 mL) by gavage for 28 days at doses of 1.125–4.50 g/kg once daily. The pole climbing time (A) and hanging time (B) of the rats were evaluated, and data from three independent experiments are presented as the mean ± SD. ** implies $p < 0.01$ compared with the control group, and ## illustrates $p < 0.05$ compared with the PD group.

following medication to assess their motor abilities. Our results revealed no significant differences in pole climbing times among the six groups of rats before model construction ($p > 0.05$) (Fig. 1A). However, after constructing the PD model, the pole climbing time of model group rats was significantly increased compared than in the control group ($p < 0.01$). Over the next 28 days, *Canna edulis* and Pramipexole were administered. The results indicated that unlike the model group, the pole climbing time was significantly decreased in the *Canna edulis*-H, *Canna edulis*-M, pramipexole ($p < 0.01$), and *Canna edulis*-L groups ($p < 0.05$) after treatment, indicating a significant improvement in motor abilities as a result of the medication.

We conducted a hanging test on six groups of rats to evaluate their motor performance. Before model construction, there were no significant differences in the hanging times among the six groups of rats ($p > 0.05$) (Fig. 1B). However, after constructing the PD model, the hanging time of the model group rats significantly decreased ($p < 0.01$). In the 28 days after the medication intervention, we observed a significant increase in the hanging time in the *Canna edulis*-H, *Canna edulis*-M ($p < 0.01$), and pramipexole groups ($p < 0.05$). There was no significant difference in the hanging time between the *Canna edulis*-L group ($p > 0.05$) and the model group. These results suggest that Canna edulis can improve motor abilities and motor performance in PD.

3.2. TLR4, NF-κB, IL-1β, IL-10, IL-6, and TNF-α mRNA expression in the substantianigra of PD rats

TLR4 can regulate the inflammatory responses induced by various factors by activating NF-κB activity [22]. We analyzed the effect of Canna edulis on substantia nigra inflammation by regulating the TLR4/NF-κ B pathway. Rats were orally administered *Canna edulis* RS3-resistant starch solution (1.125–4.50 g/30 mL) once daily for 28 days at doses ranging from 1.125 to 4.50 g/kg qRT-PCR was used to assess the mRNA expression of the inflammatory factors TLR4, NF-κB, IL-10, IL-1β, IL-6, and TNF-α in the substantia nigra of PD model rats. The model group showed a significant increase in the mRNA expression of TLR4, IL-1β, IL-6, IL-10, NF-κB, and TNF-α ($p < 0.01$) (Fig. 2A–F). Compared to the model group, the expression levels mRNA expression of TLR4, IL-1β, IL-6, NF-κB, and TNF-α were significantly decreased and the expression levels mRNA expression of IL-10 were significantly increased in the *Canna edulis* RS3-resistant starch-H, *Canna edulis*-M, *Canna edulis*-L, and pramipexole groups ($p < 0.01$) (Fig. 2A–F).

3.3. TH distribution in the substantia nigra

Rats received *Canna edulis*RS3-resistant starch solution (1.125–4.50 g/30 mL) by gavage once daily for 28 days at doses of

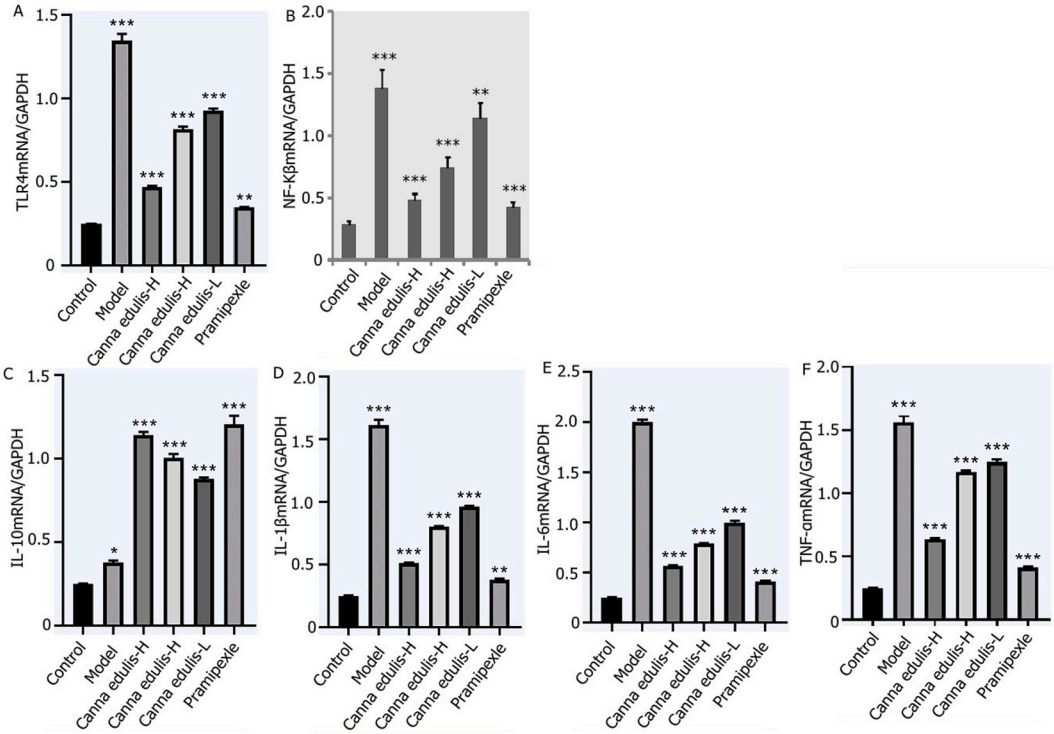

Fig. 2. TLR4, NF-κB, IL-10, IL-1β, IL-6, and TNF-α mRNA expressionin the substantia nigra of experimental rats in each group. Rats received *Canna edulis* RS3-resistant starch solution (1.125–4.50 g/30 mL) once daily for 28 days at doses of 1.125–4.50 g/kg. The mRNA expression levels of TLR4(A), NF-κB(B), IL-10(C), IL-1β(D), IL-6(E), and TNF-α(F) in the substantia nigra of experimental rats were evaluated using RT-PCR. Data from three independent experiments are presented as the mean ± SD. *** demonstrates $p < 0.001$ compared with the control group, ## reveals $p < 0.01$, ### reveals $p < 0.001$ compared with the PD group.

1.125–4.50 g/kg. Using immunofluorescence detection, we observed a significant reduction in TH-positive neurons in the substantia nigra of the control group. However, the nerve fiber density was elevated, and the synaptic terminals appeared normal. Compared to the control group, the model group exhibited a significant decrease in TH-positive neurons in the substantia nigra (Fig. 3A–B), along with sparse nerve fibers and blurry synaptic terminals. After drug intervention, TH-positive neuron counts were significantly increased

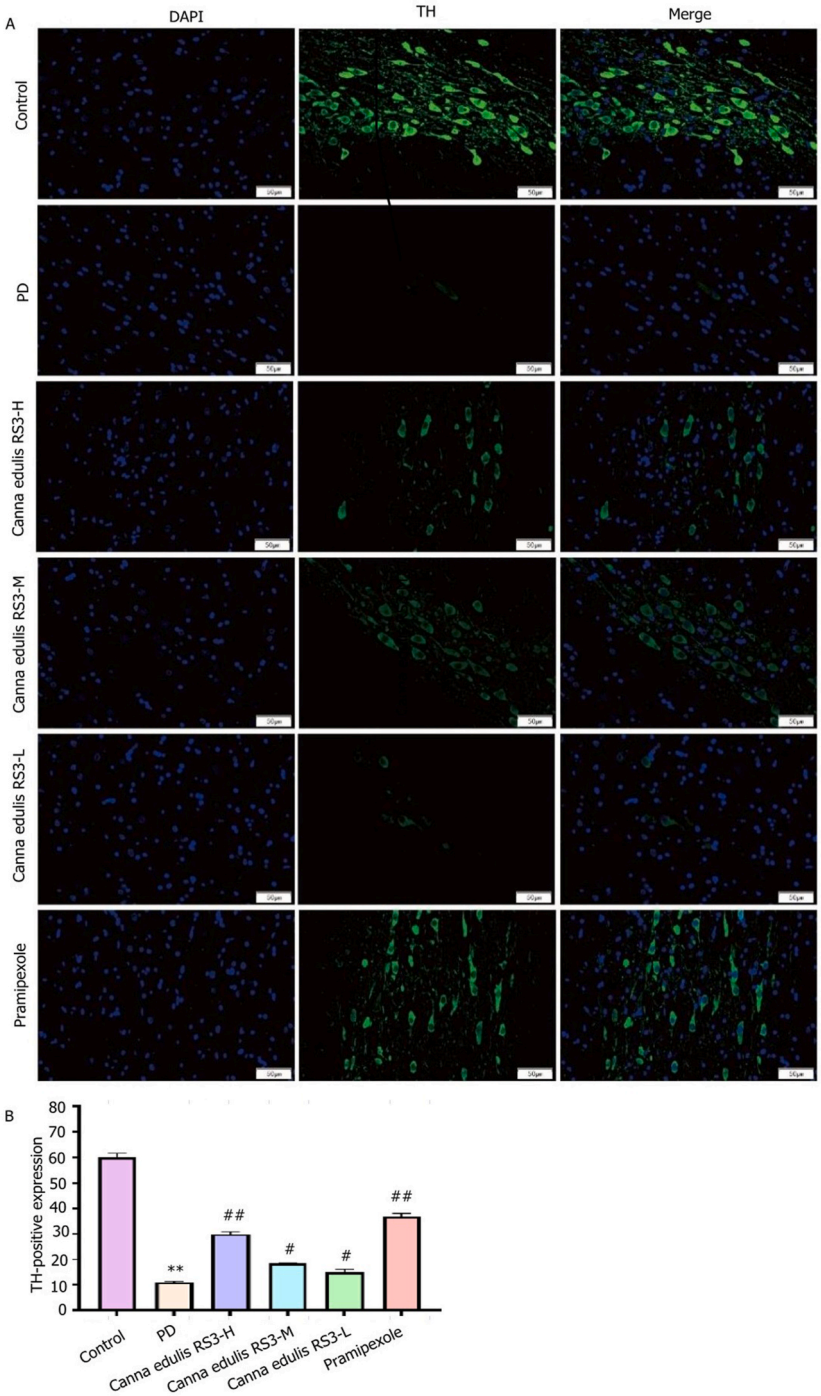

Fig. 3. Immunohistochemical analysis of the TH distribution in the substantianigra. Rats received *Canna edulis* RS3-resistant starch solution (1.125–4.50 g/30 mL) by gavage once daily for 28 days atlevels of 1.125–4.50 g/kg. A: The TH distribution in the substantia nigra of experimental rats in each group was evaluated by immunofluorescence (scale bar = 50 μm); B: The TH positive expression was measured. Data from three independent experiments are presented as the mean ± SD. ** represents $p < 0.01$ compared with the control group, # displays $p < 0.05$, ## displays $p < 0.01$ compared with the model group.

in the *Canna edulis*-H, *Canna edulis*-M, and pramipexole groups compared to the model group (Fig. 3A–B). However, the nerve fibers remained relatively sparse and the synaptic terminals appeared normal. The *Canna edulis*-L group showed no significant change in the number of TH-positive neurons (Fig. 3A–B). These findings suggest that medium and high doses of *Canna edulis* herbal treatment can improve TH distribution in the substantia nigra of PD model rats.

3.4. Changes in IL-1β, IL-10, IL-6, and TNF-α expression in the substantianigra

Neuroinflammation promotes the development of Parkinson's disease [23]. To analyze the effects of Canna edulis on neuroinflammation. Rats were administered *Canna edulis* RS3-resistant starch solution (1.125–4.50 g/30 mL) by oral gavage once daily for 28 days at concentrations of 1.125–4.50 g/kg. IL-1β, IL-10, IL-6, and TNF-α expression in the substantia nigra of experimental rats in each group was evaluated using ELISA. Compared to the control group, the model group exhibited a significant increase in IL-1β, IL-10, TNF-α ($p < 0.01$), and IL-6 ($p < 0.001$)expression (Fig. 4A–D). After 28 days of pharmaceutical intervention, the *Canna edulis*-H group, *Canna edulis*-M, *Canna edulis*-L, and pramipexole groups displayed a significant decrease in IL-1β, TNF-α, and IL-6 expression and a significant increase in IL-10 expression, with significant differences compared to the model group ($p < 0.01$(Fig. 4A–D).

3.5. Protein expression of TH, Bcl-2, Bax,caspase-3, α-Syn, NLRP3, TLR4, and p-P65 in the substantianigra

Neuroinflammation promotes apoptosis of nerve cells. Neuroinflammation promotes the apoptosis of nerve cells [24]. TLR4/NF-κB/NLRP3 is an important inflammatory and apoptotic regulatory pathway [25]. We analyzed the effect of Canna edulis on apoptotic protein in nerve cells by regulating TLR4/NF-κB/NLRP3**.** Rats received *Canna edulis* RS3-resistant starch solution (1.125–4.50 g/30 mL) by oral gavage for 28 days at doses of 1.125–4.50 g/kg once daily. Protein expression of TH, Bcl-2, Bax, caspase-3, α-syn, NLRP3, TLR4, and p-P65 in the substantia nigra was measured by western blotting. Our findings revealed that in the model group, TH and Bcl-2 protein expression levels significantly decreased ($p < 0.01$) (Fig. 5A–C). However, following drug treatment, there was a significant increase in the expression of these proteins ($p < 0.01$). Conversely, Bax, α-syn, NLRP3, caspase-3, TLR4, and p-P65 expressions was examined (Fig. 5A–D-H). Compared to the model group, drug treatment significantly reduced the expression of these proteins, whereas the model group showed a significant increase ($p < 0.01$) (Fig. 5A–D-H).

3.6. Apoptosis of substantianigra dopaminergic neurons

We analyzed the effect of Canna edulis on nerve cell apoptosis. Rats were administered *Canna edulis* RS3-resistant starch solution (1.125–4.50 g/30 mL) by gavage once daily for 28 days at doses of 1.125–4.50 g/kg. The apoptotic rate of dopaminergic neurons in the substantia nigra was measured by TUNEL staining. In the substantia nigra control group and the *Canna edulis*-H, *Canna edulis*-M, *Canna edulis*-L, and Pramipexole groups, the cell nuclei exhibited uniform and diffuse blue fluorescence, apoptosis of substantia nigra neurons is significantly reduced (Fig. 6A–B). In the model group, we observed numerous apoptotic cells characterized by nuclear condensation, chromatin aggregation within the nucleus (resulting in dense staining), and subsequent nuclear fragmentation, leading to apoptotic body formation, apoptosis of substantia nigra neurons is significantly increased (Fig. 6A–B).

3.7. Changes in the morphology of substantianigra dopamine neurons

To analyze the effect of Canna edulis on the morphology of substantianigra dopamine neurons in the substantia nigra. Rats received *Canna edulis* RS3-resistant starch solution (1.125–4.50 g/30 mL) by oral gavage for 28 days at doses of 1.125–4.50 g/kg once daily. Our

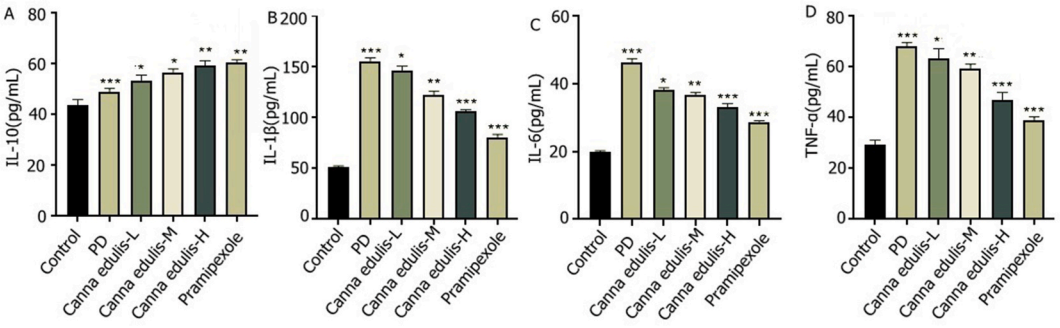

Fig. 4. Expression of IL-10, IL-1β, IL-6, and TNF-α in the substantianigra. Rats received *Canna edulis* RS3-resistant starch solution (1.125–4.50 g/30 mL) by gavage once daily for 28 days with amounts of 1.125–4.50 g/kg. IL-10(A), IL-1β (B), IL-6 (C), and TNF-α (D) expression levels in the substantia nigra of experimental rats in each group were evaluated by ELISA. Data from three independent experiments are presented as the mean ± SD. *** represents $p < 0.001$ compared with the control group, #displays $p < 0.05$, ## displays $p < 0.01$ compared with the model group.

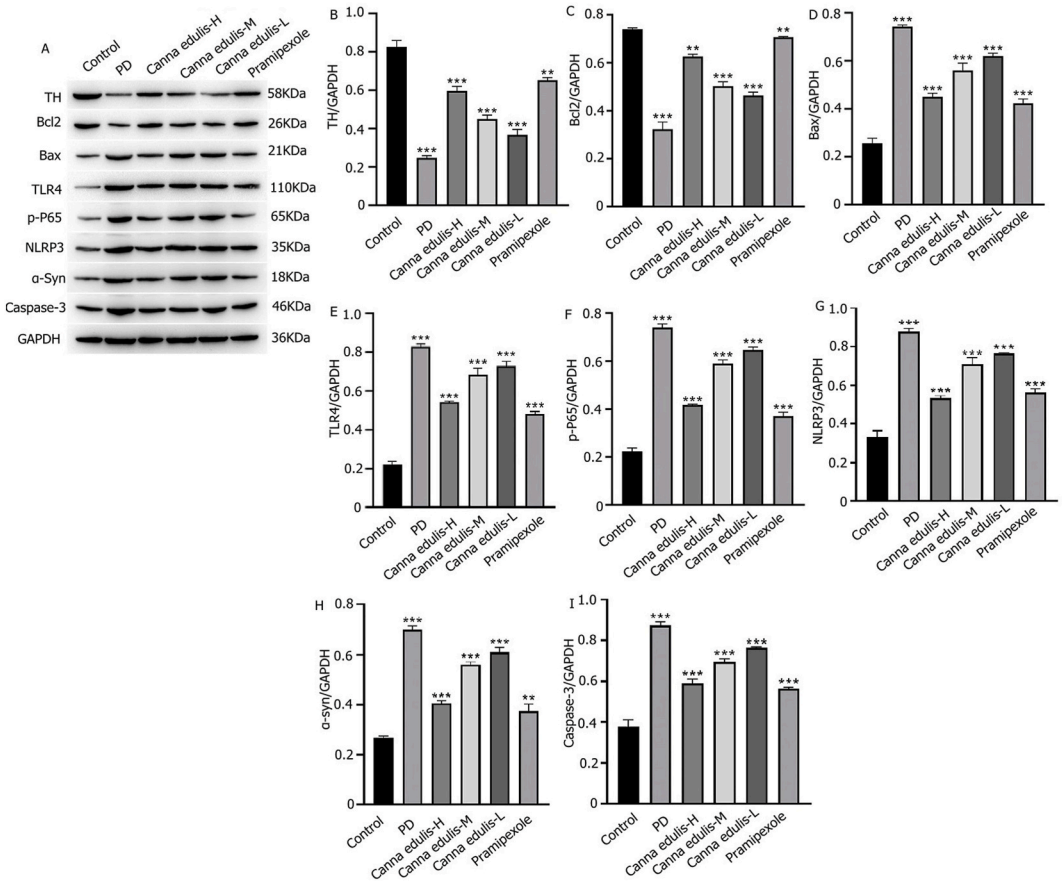

Fig. 5. Protein expression of TH, Bcl-2, Bax, caspase-3, α-Syn, NLRP3, TLR4, and p-P65 in the substantianigra. Rats received *Canna edulis* RS3-resistant starch solution (1.125–4.50 g/30 mL) by gavage once daily for 28 days at doses of 1.125–4.50 g/kg. A: TH, Bcl-2, Bax, caspase-3, α-Syn, NLRP3, TLR4, and p-P65 protein levels in the substantianigra of experimental rats were evaluated by Western blot. B–H: Statistical analysis of TH, Bcl-2, Bax, caspase-3, α - Syn, NLRP3, TLR4, and p-P65 proteins expression in the substantianigra of experimental rats. Data from three independent experiments are presented as the mean ± SD. ** represents $p < 0.05$, *** represents $p < 0.01$ compared with the control group, # reveals $p < 0.05$, ## reveals $p < 0.01$ compared with the model group.

Nissl staining results indicated that unlike the control group, the model group had significantly fewer neurons in the substantia nigra pars compacta, with the remaining neurons exhibiting cytoplasmic swelling and vacuolar degeneration(Fig. 7A–B). Following pharmaceutical intervention, the *Canna edulis*-H, *Canna edulis*-M, and pramipexole groups displayed increased numbers of dopaminergic neurons in the substantia nigra striatum region, improved cytoplasmic swelling, and reduced vacuolar degeneration(Fig. 7A–B).

3.8. The α-syn distribution in the substantianigra

To analyze the effect of Canna edulis on α-syn distribution in the substantia nigra**.** Rats were administered *Canna edulis* RS3-resistant starch solution (1.125–4.50 g/30 mL) by gavage once daily for 28 days at doses of 1.125–4.50 g/kg. Immunofluorescence assays were used to assess the α-syn distribution in the substantia nigra of each group. Compared to the control group, the model group exhibited a significant increase in α-syn-positive neurons (Fig. 8A–B). Following drug intervention, the *Canna edulis*-H, *Canna edulis*-M, and pramipexole groups exhibited a significant reduction in α-syn-positive neurons compared to the model group, while the *Canna edulis*-L group showed no significant change (Fig. 8A–B). These findings suggest that medium and high doses of *Canna edulis* herbal treatment can decrease α-syn distribution in the substantia nigra of PD model rats.

4. Discussion

PD greatly affects patients' quality of life. Current PD treatments primarily rely on medications and deep brain stimulation [26]; however, these approaches cannot completely halt disease progression. Therefore, there is a growing need to develop novel therapeutic strategies. This study established PD rat models and utilized *Canna edulis* RS3-resistant starch for intervention, with the aim of unveiling its therapeutic effects and mechanisms in PD. Our findings demonstrated that *Canna edulis* RS3-resistant starch significantly

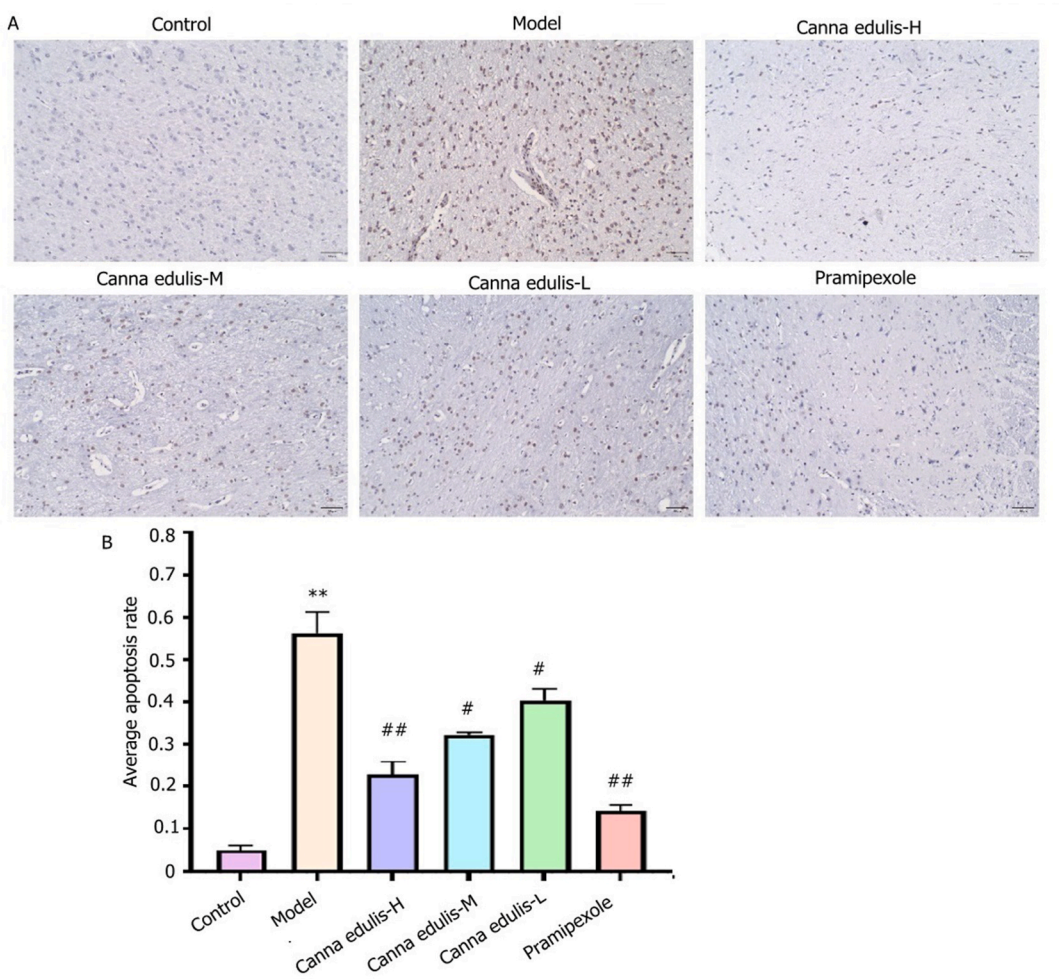

Fig. 6. Apoptotic rate of substantianigra dopaminergic neurons. Rats received *Canna edulis* RS3-resistant starch solution (1.125–4.50 g/30 mL) by gavage once daily for 28 days at doses of 1.125–4.50 g/kg. A: Apoptotic activities of substantianigra dopaminergic neurons of experimental rats in all groups were assayed by TUNEL staining (scale bar = 50 μm). B: Statistical analysis of mortality rate of substantia nigra dopaminergic neurons. of experimental rats in all groups. Data from three independent experiments are presented as the mean ± SD. ** represents $p < 0.01$ compared with the control group, # reveals $p < 0.05$, ## reveals $p < 0.01$ compared with the model group.

alleviated motor impairments in PD rats, prolonging the time the rats spent climbing and hanging. This suggests the potential of *Canna edulis* RS3-resistant starch to enhance motor function and coordination in PD rats, thus offering a promising therapeutic strategy. Moreover, this study indicated that PD symptoms impair sensorimotor behavior, reducing the ability of mice to descend from a pole or hang on a wire for extended periods [27]. Zhang et al. [28] conducted a study on arctigenin, the primary active compound from the traditional Chinese medicine ArctiiFructus, revealing its neuroprotective effect on fisetin-induced PD. The results indicated that rats treated with arctigenin exhibited significantly reduced deadlock time and enhanced motor activity scores in behavioral tests, indicating better behavior in rats with PD. This aligns with the results of the behavioral tests in PD rats and *Canna edulis* RS3-resistant starch treatment in this study. Further experimental results suggested that the therapeutic effects of *Canna edulis* RS3-resistant starch may be closely associated with its anti-inflammatory properties.

Neuroinflammation is a prominent factor in the development and progression of neurodegenerative diseases such as PD [29]. In the PD model, the expression levels of IL-1β, IL-6, and TNF-α were significantly elevated. However, treatment with *Canna edulis* RS3-resistant starch reduced the expression of these inflammatory factors and alleviated the inflammatory response in PD rats. This may be attributed to the anti-inflammatory activity of *Canna edulis* RS3-resistant starch, which inhibits the production and release of inflammatory factors, regulates immune system function, and ameliorates the pathological inflammatory state in PD rats. Furthermore, the results of WB blotting revealed that *Canna edulis* RS3-resistant starch could regulate the activity of the TLR4/NF-κBsignaling pathway. This pathway is crucial in the inflammatory response, particularly in PD pathogenesis, and may reduce the generation and release of inflammatory factors, potentially mitigating the inflammatory response within the substantia nigra of PD rats. Moreover, this investigation ascertained that the Zishen Pingchuan decoction can ameliorate inflammation in PD mice by inhibiting the JNK pathway, thereby providing protection to dopamine neurons [30].

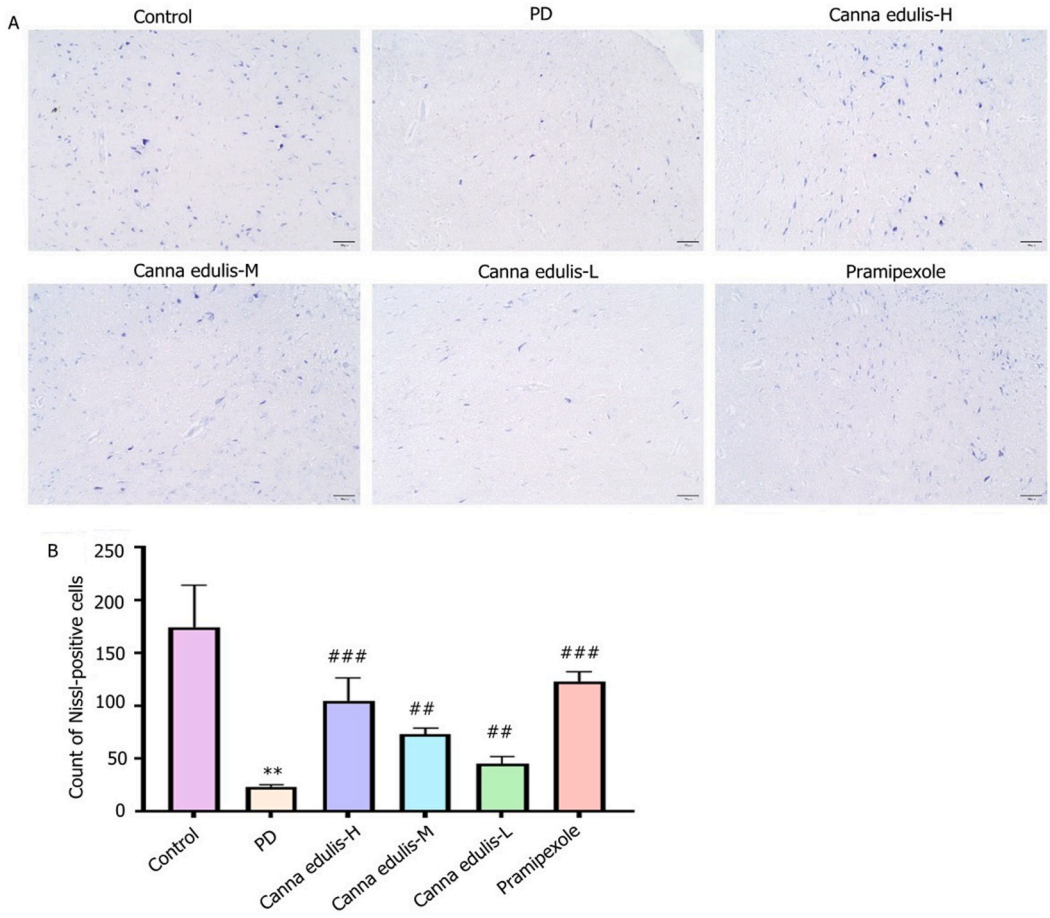

Fig. 7. Changes in the morphology of substantianigra dopamine neurons. Rats received *Canna edulis* RS3-resistant starch solution (1.125–4.50 g/30 mL) by gavage once daily for 28 days at doses of 1.125–4.50 g/kg. A: The number of neurons in the substantia nigra pars compacta in each group was assayed by Nissl staining (scale bar = 50 μm). B: Statistical analysis of neurons in the substantia nigra pars compacta in each group. Data from three independent experiments are presented as the mean ± SD. ** represents $p < 0.01$ compared with the control group, ## reveals $p < 0.01$, ### reveals $p < 0.001$ compared with the model group.

Since tyrosine hydroxylase (TH) catalyzes L-DOPA formation, the rate-limiting step in DA biosynthesis, PD can be considered a TH deficiency syndrome in the striatum [31]. Additionally, *Canna edulis* RS3-resistant starch positively influenced TH distribution in the substantia nigra and dopaminergic neuron functionality. PD primarily results from dopaminergic neuron degeneration in the substantia (SN). Previous research suggests that *Canna edulis* RS3-resistant starch potentially increases the number of TH-positive neurons in the substantia nigra and enhances dopamine levels. This effect could be attributed to the ability of *Canna edulis* RS3-resistant starch to protect dopaminergic neurons from oxidative stress and apoptosis. Furthermore, *Canna edulis* RS3-resistant starch modulates Bcl-2 and Bax protein expression, affecting the apoptotic pathway and preserving dopaminergic neuron integrity. Additionally, the modified Dihuangyinzi decoction demonstrated a protective effect on dopaminergic neurons in PD mice through its anti-apoptotic mechanism [32]. Conversely, *Cistanchetubulosa*[exerts neuroprotective effects on dopaminergic neurons by inhibiting apoptosis and promoting neurotrophic factor production from glial cells [33].

Inflammatory vesicles are vital protein complexes of the immune system. Among receptor proteins, NLRP3 is activated by agonists with different sources, chemical compositions, and structural properties [34]. Activation of the NLRP3 inflammasome leads to the release of pro-inflammatory factors, including IL-1β, IL-6, and TNF-α [35]. These proinflammatory factors stimulate peripheral inflammatory responses dominated by macrophages and trigger glial cell-mediated central nervous system inflammatory responses, which is a key pathological mechanism in PD [36]. TLR4/NF-κB is a classic inflammation-related signaling pathway. Microglia and astrocytes are two important cell types involved in the immune response in the central nervous system [37]. Upon activation, the TLR4/NF-κB pathway stimulates NLRP3 inflammasome to release many proinflammatory cytokines. This exacerbates the damage to dopaminergic neurons in the inflammatory microenvironment [38]. In this study, *Canna edulis* RS3 blocked TLR4/NF-κB65 activity, decreased NLRP3 activation, attenuated proinflammatory factor release, and ameliorated dopaminergic neuron injury.

α-Synuclein, a high-risk gene for PD, greatly influences the development and progression of PD [39]. TLR4 activation can induce mitochondrial dysfunction and promote pathological changes in α-Syn aggregation [40]. Moreover, α-syn aggregation can induce

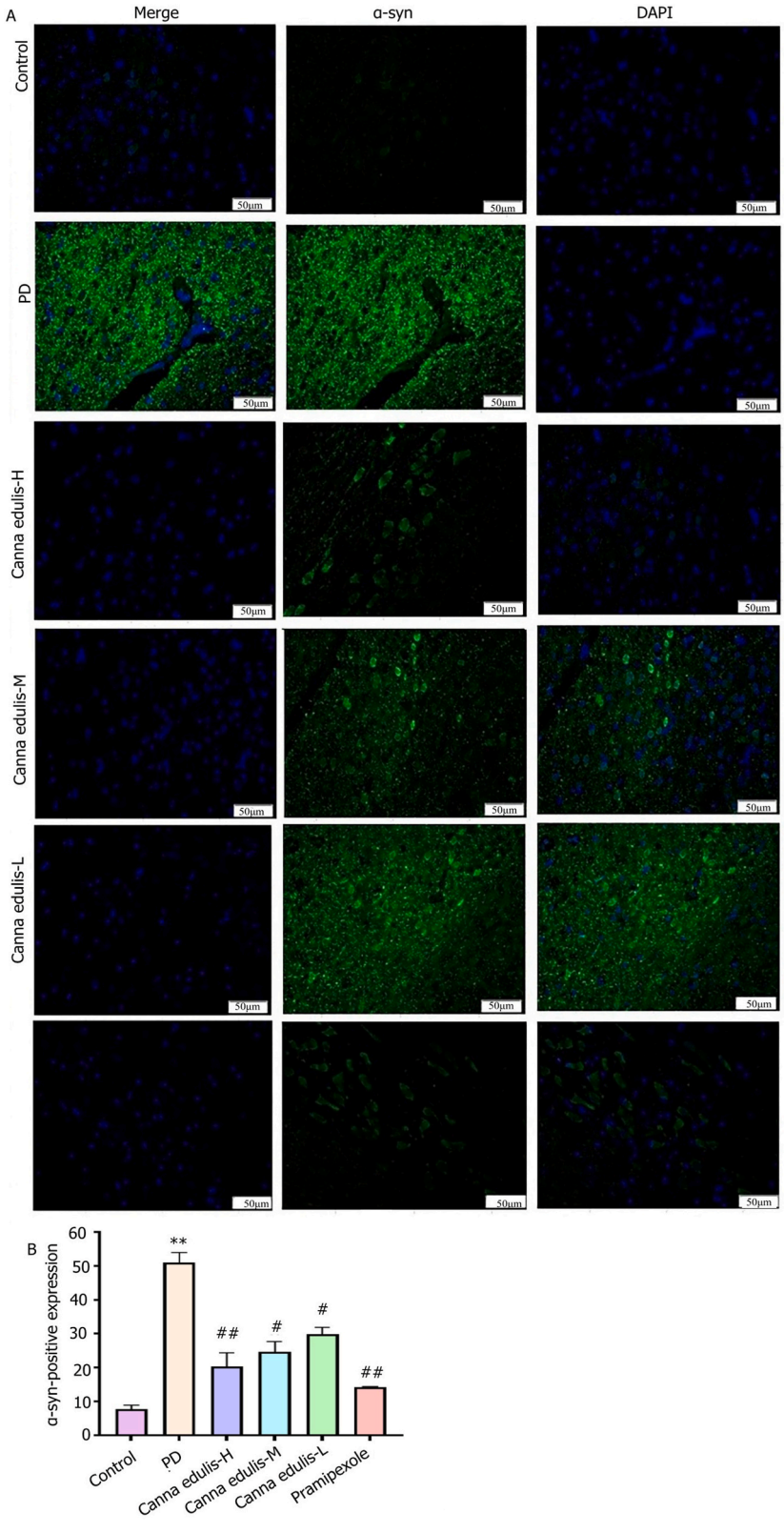

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Fig. 8. Immunohistochemical analysis of the α-syn distribution in the substantianigra. Rats received *Canna edulis* RS3-resistant starch solution (1.125–4.50 g/30 mL) by gavage for 28 days at doses of 1.125–4.50 g/kg once daily. A: The α-syn distribution in the substantia nigra in each group was assayed by immunofluorescence. Scale bar = 50 μm. B: Statistical analysis of the **α-syn** positive expression in the substantianigra. Data from three independent experiments are presented as the mean ± SD. ** represents $p < 0.01$ compared with the control group, #reveals $p < 0.05$, ## reveals $p < 0.01$ compared with the model group.

NLRP3 activation and recruit more pro-inflammatory factors for release [41,42]. This persistent pro-inflammatory and oxidative stress response in the central nervous system fosters further pathological α-syn aggregation. Abnormal α-syn aggregation in the substantia nigra is the primary cause of degeneration and death of dopaminergic neurons [39], ultimately leading to PD development. This study demonstrated that*Canna edulis* RS3 inhibited TLR4 activity, suppressed NLRP3 activation, and decreased pathological α-syn aggregation, and thus showed promise in PD treatment.

Substantial evidence has associated PD with the overexpression and abnormal aggregation of α-syn. As a characteristic biomarker of PD, α-Syn is invaluable for the diagnosis and tailoring of effective therapeutic strategies [39]. Our findings suggest that*Canna edulis* RS3 decreases pathological α-syn aggregation, indicating its therapeutic potential in PD.

4.1. Limitations of the study

It is imperative to note that the insights gained from our study require further validation. Detailed exploration of Canna edulis RS3-resistant the therapeutic mechanisms, active ingredients, dosage effects, potential side effects, and safety is essential. We believe that these investigations will enhance innovative drug development and therapeutic approaches for treating PD. The regulation of banana RS3 in the substantia nigra of PD models may extend beyond inflammation and apoptosis, encompassing autophagy, pyroptosis, and ferroptosis. Therefore, the exact mechanism remains unclear and warrants further investigation. The study primarily focused on the effects of Canna edulis RS3-resistant starch, and its outcomes may not account for potential interactions with other medications or therapies that patients with PD might concurrently receive. There are species differences between the use of rat Parkinson's disease models and human Parkinson's disease models; PD manifests in different stages, and the therapeutic effects of Canna edulis RS3-resistant starch may differ. We studied a relatively short observation period of 28 d. However, long-term studies can provide valuable insights into the sustainability and durability of the observed effects.

5. Conclusions

Our results revealed that *Canna edulis* RS3-resistant starch can modulate the TLR4/NF-κB/NLRP3 signaling pathway. *Canna edulis* RS3-resistant starch holds promise for treating PD by potentially navigating various mechanisms. In addition to mitigating inflammatory responses and protecting dopaminergic neurons, it appears to influence the TLR4/NF-κB/NLRP3 signaling pathways. Consequently, the *in vivo* benefits of Canna edulis RS3 in a PD context of PD, as described here, support its potential as a novel therapeutic modality for PD and related neurodegenerative conditions.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

CRediT authorship contribution statement

Qian-Kun He: Writing – original draft, Supervision, Project administration, Investigation, Formal analysis. **Xue-Yong Wang:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Funding acquisition, Data curation. **Wei Hu:** Validation, Supervision, Project administration, Methodology, Funding acquisition, Data curation. **Jing Cai:** Software, Project administration, Investigation, Formal analysis, Data curation. **Peng Chen:** Visualization, Project administration, Methodology, Data curation, Conceptualization. **Ming-Wei Liu:** Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Yuan-Hua Wu:** Writing – review & editing, Software, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to

influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e38072.

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