Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

# Repair effect of neurotrophic factor III (NT-3) on rats with spinal injury model and its mechanism

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**Article Info**

**Article history:**

**Received:** Novrmber15, 2023
**Accepted:** Janvier 20, 2024
**Published:** January 31, 2024

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**Abstract**

The present study aimed to study the repair effect of neurotrophic factor III (NT-3) on spinal injury model rats and its mechanism. Wistar rats with spinal injury were established by accelerated compression stroke after the operation and divided into control group, model group, and NT-3 intervention group. The motor function of rats in each group was evaluated at different postoperative time points (3, 7, 14 d). HE staining was used to detect the changes in tissue structure and morphology of the injured spinal column in each group. The changes of SOD, MDA and GSH in serum of rats were detected. The concentrations of inflammatory cytokines IL-1β, IL-6, IL-17 and TNF-α in serum were detected by enzyme-linked immunosorbent assay (ELISA). Western blot was used to detect the expression changes of anti-apoptotic protein (Bcl-2) and pro-apoptotic protein (Bax) in injured spinal tissue of rats in each group. Compared with model group, motor function score of NT-3 intervention group increased gradually, and had statistical significance at 7 and 14 days (5.29±1.62 vs 9.33±2.16, 5.92±1.44 vs 14.56±2.45, T =7.386, 9.294, P =0.004, 0.000). The levels of SOD and GSH in serum of NT-3 intervention group were significantly increased (t=9.117, 12.207, P=0.000, 0.000), while the level of MDA was significantly decreased (t=5.089, P=0.011). Serum levels of inflammatory cytokines IL-1β, IL-6, IL-17 and TNF-α in NT-3 intervention group were significantly decreased (T =6.157, 7.958, 6.339, 6.288, P=0.008, 0.005, 0.005, 0.007). In the NT-3 treatment group, Bax protein was significantly decreased (0.24±0.05 vs 0.89±0.12, T =8.579, P=0.001), and the relative expression of Bcl-2 protein was significantly increased (0.75±0.06 vs 0.13±0.05, T =9.367, P=0.001). Neurotrophic factor III can promote spinal injury repair in spinal injury model rats, and play a role by enhancing antioxidant stress ability, inhibiting inflammatory factors, promoting Bcl-2 and decreasing Bax expression.

**Keywords:** Spinal injury, Neurotrophic factor III, Damage repair, Oxidative stress, Inflammatory factors, Apoptosis

## 1. Introduction

Spinal cord injuries, often precipitated by traumatic events such as high-altitude falls, severe car accidents, or spinal fractures, constitute a prevalent and challenging concern in clinical medicine [1-3]. These injuries can result in a wide spectrum of physical impairments, ranging from spinal damage to movement restrictions and limb motor dysfunction. In severe cases, they may even pose a life-threatening risk to the affected individuals. As a growing global health issue, the number of patients grappling with spinal cord injuries has been steadily increasing year by year. Consequently, there is a pressing need to enhance the therapeutic outcomes for these patients while simultaneously mitigating the severe complications that often accompany spinal cord injuries [4].

Extensive research has illuminated several facets of spinal cord injuries, revealing their complex etiology. Microcirculation disturbances, oxidative stress, and spinal cord cell apoptosis have all been implicated in the progression of these injuries. Microcirculation disorders contribute to the compromise of blood flow and oxygen supply to the injured spinal cord, exacerbating tissue damage [5-9]. Oxygen-free radicals, as highly reactive molecules, play a central role in exacerbating the cellular damage associated with spinal injuries. They contribute to oxidative stress, which can further impede the healing process and lead to additional tissue damage. Apoptosis, the programmed cell death mechanism, has been observed in the context of spinal cord injuries, resulting in the loss of vital neural cells and further complicating the prospects of functional recovery [10].

In this landscape of spinal cord injury research, neurotrophin-III (NT-3) emerges as a promising candidate for therapeutic intervention [11]. NT-3 is a critical member of the neurotrophin family, a group of nerve growth factors that are widely distributed throughout the nervous system

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**Doi:** http://dx.doi.org/10.14715/cmb/2024.70.1.8

and its surrounding tissues. Existing studies have indicated that NT-3 holds the capacity to promote neural tissue repair in various injury contexts, yet there remains a relative scarcity of investigations into its potential efficacy in the specific domain of spinal cord injuries. Consequently, this study endeavors to bridge this knowledge gap by constructing a spinal injury model in rats to investigate the therapeutic effects of NT-3 and elucidate the underlying mechanisms [12,13].

Through a rigorous examination of NT-3's impact on spinal injuries, this study aims to contribute valuable insights into the potential utilization of NT-3 as a therapeutic agent. If successful, this research could lead to more effective treatment strategies for patients suffering from spinal cord injuries, thereby improving their prognosis and quality of life. Additionally, the findings of this study may shed light on novel avenues for the development of therapies that target microcirculation, oxidative stress, and apoptosis as they pertain to spinal cord injuries, further advancing the field of regenerative medicine and spinal injury management.

## 2. Materials and Methods

### 2.1. Experimental animals and Methods

Thirty-six healthy adult male Wistar rats with SPF (free of specific pathogens) grade were purchased from the Experimental Animal Center of Shanghai Academy of Life Sciences, Chinese Academy of Sciences (Animal Certificate No. 201800135), weighing (200±50) g. All rats were acculturated for 1 week in our laboratory for subsequent experiments. This study was approved by the Animal Ethics Committee of Yantaishan Hospital, Binzhou Medical University Animal Center.

Neurotrophic factor III was purchased from the American Sigma Company (St. Louis, MO, USA). The hematoxylin-eosin merlin staining kit was purchased from Beijing Dingguo Biotechnology Company (Beijing, China). Superoxide dismutase (SOD), malondialdehyde (MDA) and glutathione (GSH) detection kits were purchased from Nanjing Jiancheng Institute of Biology (Nanjing, China). Interleukin 1β (IL-1β), interleukin 6 (IL-6), interleukin 17 (IL-17), TNF-α ELISA kitPolyclonal rabbit anti-rat Bcl-2, Bax and GAPDH primary antibodies, monoclonal goat anti-rabbit IgG secondary antibodies were purchased from Abcam Company (Abcam, Cambridge, MA, USA).

### 2.2. Preparation and grouping of spinal injury model in rats

All rats were fed in the same environment and anesthetized with 3% pentobarbital sodium solution. In rats, a small amount of lamina was excised to create a lamina defect, and the model of spinal injury was established by the accelerated compression strike method. The rats were randomly divided into 3 groups: the control group only received wound incision surgery without spinal tissue injury; Rats in the model group underwent spinal injury modeling. The intervention group was given NT-3 drug injection according to $10000U \cdot Kg^{-1} \cdot d^{-1}$. The intraperitoneal injection was performed for continuous intervention for 14 days.

### 2.3. Motor function monitoring of rats in each group

At 3, 7, and 14 days after surgery, the motor function of rats in each group was scored according to the Basso-Beattie-Bresnahan (BBB) scoring standard.

### 2.4. HE staining of spinal tissue sections

Rats in each group were anesthetized and killed after 14 days of treatment. The spinal tissue of the injured section was exposed through the original surgical incision. A small amount of the injured spinal tissue was taken from the center of the injured point, fixed with 4% paraformaldehyde overnight, dehydrated, and embedded in paraffin for section. The spinal cord tissue sections of rats in each group were stained with hematoxylin and eosin. The morphological and structural changes of spinal cord cells were observed under a microscope to evaluate spinal cord injury recovery.

### 2.5. Oxidative stress index of rats in each group was detected

About 2 mL of arterial blood was collected for the rats in the three groups at the anesthesia time. The serum was collected by centrifugation at high speed after standing at room temperature for 30 min. An automatic biochemical analyzer detected the serum oxidative stress indexes SOD, MDA, and GSH.

### 2.6. ELISA detected changes in inflammatory factors

The ELISA kit detected serum levels of inflammatory cytokines IL-1β, IL-6, IL-17, and TNF-α.

### 2.7. The expression of apoptosis-related proteins in spinal tissue was detected by Western blot

About 100 mg of injured spinal tissue was taken from each group, 500μL of strong RIPA cell lysate was added, and total protein was extracted from tissue cells by homogenate. BCA kit was used to determine the total protein concentration in the injured spinal tissue of rats. Each group of samples was loaded with 30μg total protein and subjected to SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis), then transformed into PVDF membrane, closed, washed and incubated with polyclonal rabbit anti-rat BCL-2, Bax and GAPDH primary antibodies (1:1000). After incubation at 4°C overnight, monoclonal goat anti-rabbit IgG secondary antibody (1:5000) was washed for three times and incubated for 2 h. The relative expression levels of apoptosis-related proteins Bcl-2 and Bax in the spinal tissues of rats in each group were detected by chemiluminescence exposure development.

### 2.8. Statistical Analysis

Data were statistically analyzed by Statistic Package for Social Science (SPSS) 22.0 software (IBM, Armonk, NY, USA) and expressed as mean ± standard deviation ($\bar{x}$±s). One-way ANOVA was used for data of homogeneity of variance between multiple groups, and T-test was used for data between two groups. When $P<0.05$, the difference was considered statistically significant.

## 3. Results

### 3.1. Effect of NT-3 on motor function recovery after spinal injury in rats

BBB scores of motor function of rats in each group at 3, 7, and 14 days after spinal injury were shown in Table 1, Figure 1. The BBB scores of rats in the control group were

**Table 1.** Comparison of motor function scores in each group at different time after operation ($\bar{x}\pm s$).

| group | 3 d | 7 d | 14 d |
|---|---|---|---|
| Control group | 20.86±2.98 | 21.07±3.11 | 21.19±3.24 |
| Model group | 4.57±1.25 [a] | 5.29±1.62 [a] | 5.92±1.44 [a] |
| Intervention group | 5.24±1.46 [a] | 9.33±2.16 [ab] | 14.56±2.45 [ab] |

Note: Compared with the control group, [a] $P < 0.05$; Compared with the model group, [b] $P < 0.05$.

normal at different time points, and the BBB scores of rats in the model group were significantly decreased at 3, 7, and 14 days (t =15.235, 13.105, 12.954, P =0.000, 0.000, 0.000, respectively). Compared with the model group, THE BBB score of the NT-3 intervention group increased gradually, with statistical significance at 7 and 14 days (t =7.386 and 9.294, P =0.004 and 0.000, respectively).

### 3.2. Effects of NT-3 on tissue morphology and structure in rats with spinal injury

HE staining of spinal tissue sections showed that the morphology and structure of bone marrow cells in the spinal tissue of rats in control group were intact without bleeding, necrosis and edema. In the model group, there was obvious patchy bleeding in the injured spinal tissue, and a large number of spinal cord cells had degeneration and necrosis and edema. In the nT-3 intervention group, a small amount of bleeding, necrosis cells and tissue swelling were significantly reduced in the injured spinal tissue.

### 3.3. Effects of NT-3 on oxidative stress index in serum of spinal injury rats

After examination, it was found that NT-3 intervention could improve the antioxidant stress ability of spinal injury rats, and the results are shown in Table 2, Figure 2. According to statistics, compared with the control group, the levels of SOD and GSH in serum of model group and intervention group were significantly decreased (F=12.045, 16.248, P=0.000, 0.000), while the level of MDA was significantly increased (F=8.682, P=0.009). Compared with model group, serum SOD and GSH levels in NT-3 intervention group were significantly increased (t=9.117, 12.207, P=0.000, 0.000), while MDA levels were significantly decreased (t=5.089, P=0.011).

### 3.4. Effects of NT-3 on inflammatory cytokines in serum of spinal injury rats

ELISA showed that NT-3 intervention could reduce inflammatory factors in spinal injury rats, as shown in Table 3, and Figure 3. Statistically, compared with the control group, serum levels of inflammatory factors IL-1β, IL-6, IL-17 and TNF-α in model group and intervention group were significantly increased (F=8.246, 6.379, 8.476, 5.327, P=0.004, 0.006, 0.009, 0.001). However, compared

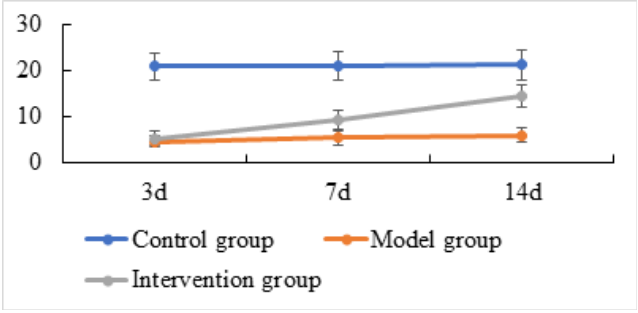


**Fig. 1.** Comparison of motor function scores in each group at different time after operation.

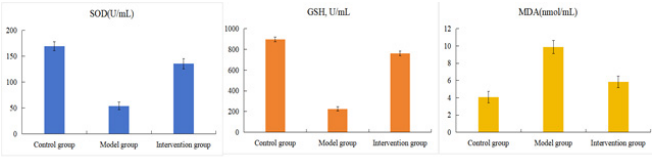


**Fig. 2.** Changes of oxidative stress indexes in serum of rats in each group.

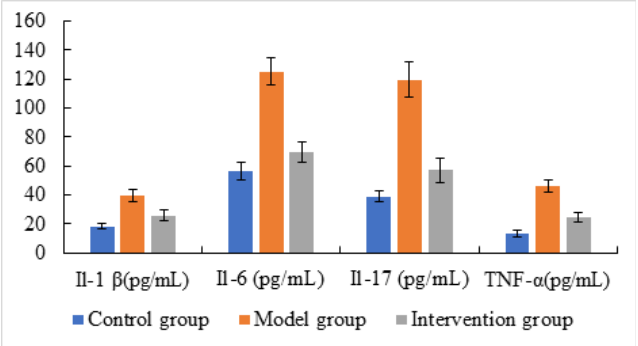


**Fig. 3.** Changes of serum inflammatory factors in each group.

**Table 2.** Changes of oxidative stress indexes in serum of rats in each group ($\bar{x}\pm s$).

| group | SOD(U/mL) | GSH, U/mL | MDA(nmol/mL) |
|---|---|---|---|
| Control group | 169.37±8.23 | 895.66±23.25 | 4.06±0.67 |
| Model group | 54.25±7.36 [a] | 225.15±20.97 [a] | 9.89±0.76 [a] |
| Intervention group | 135.57±9.77 [ab] | 759.36±22.34 [ab] | 5.83±0.65 [ab] |

Note: Compared with the control group, [a] $P < 0.05$; Compared with the model group, [b] $P < 0.05$.

**Table 3.** Changes of serum inflammatory factors in each group ($\bar{x}\pm s$).

| group | Il-1 β(pg/mL) | Il-6 (pg/mL) | Il-17 (pg/mL) | TNF-α(pg/mL) |
|---|---|---|---|---|
| Control group | 18.42±2.05 | 56.32±6.29 | 38.81±3.57 | 13.55±2.32 |
| Model group | 39.66±4.17 [a] | 125.13±9.34 [a] | 119.33±12.14 [a] | 45.81±4.27 [a] |
| Intervention group | 25.63±3.85 [ab] | 69.58±7.35 [ab] | 57.04±8.24 [ab] | 24.47±3.06 [ab] |

Note: Compared with the control group, [a] $P < 0.05$; Compared with the model group, [b] $P < 0.05$.

with model group, serum levels of inflammatory factors IL-1β, IL-6, IL-17 and TNF-α in NT-3 intervention group were significantly decreased (T =6.157, 7.958, 6.339, 6.288, P=0.008, 0.005, 0.005, 0.007).

### 3.5. Effects of NT-3 on the expression of apoptosis-related proteins in spinal injury rats

Western blot analysis showed that NT-3 could significantly reduce the expression of Bax protein and promote the expression of Bcl-2 protein in spinal tissue of rats with spinal injury. Statistically, compared with the control group, Bax protein was significantly increased (0.89±0.12 vs. 0.45±0.08, T =7.142, P=0.005), and the relative expression of Bcl-2 protein was significantly decreased (0.13±0.05 vs. 0.36±0.06, T =6.143, P=0.012) in the spinal tissue of rats with spinal injury model group. Compared with model group, Bax protein in nT-3 intervention group was significantly decreased (0.24±0.05 vs 0.89±0.12, T =8.579, P=0.001), and the relative expression of Bcl-2 protein was significantly increased (0.75±0.06 vs 0.13±0.05, T =9.367, P=0.001).

In order to investigate the molecular mechanism of NT-3 on cell apoptosis in spinal cord injury rats, TargetScan, starBase, and miRDB databases were used to predict the target intersection of miR-101a-3p, indicating that 121 genes may be the target genes of NT-3. The enrichment analysis results of the Kyoto Encyclopedia of Genes and Genomes (KEGG) showed that MAPK signaling pathway, PIK3/Akt/mTOR signaling pathway, and others may be involved in regulating the above processes (Figure 4). The enrichment analysis of Gene Ontology (GO) showed that 256 target genes were involved in biological processes such as macrophage activation, mitochondrial apoptosis, and protein phosphorylation (Figure 5).

## 4. Discussion

Due to falling injuries, traffic accidents, sports injuries and other factors, the incidence of clinical spinal injuries is increasing day by day and has become one of the refractory orthopedic diseases [14]. Neurotrophin III (NT-3) is considered to be a multifunctional cytotrophic factor, which plays a positive role in maintaining physiological functions and repairing tissue injury. Studies have shown that neurotrophin III can promote nerve repair in severe spinal cord injury, but whether it can promote the repair of spinal cord injury has not been determined [15]. In this study, the spinal injury model of rats was established by spinal injury shock. It was found that the motor function of rats after nT-3 induction intervention was significantly stronger than that of the model group at 7 and 14 days, and the spinal tissue structure and cell morphology of rats in the intervention group were significantly better than that of the model group in the pathological examination, indicating that NT-3 can play a role in promoting spinal repair in rats with spinal injury.

Studies have found that different degrees of direct or indirect pathogenic factors can cause spinal injury, and in addition to physical injury factors, oxidative stress is also one of the important mechanisms involved in spinal cord nervous system injury [16]. In this study, after nT-3 induction intervention, the activity levels of SOD and GSH in serum of rats with spinal injury significantly increased, while the level of MDA significantly decreased, indicating that NT-3 can improve the antioxidant stress ability

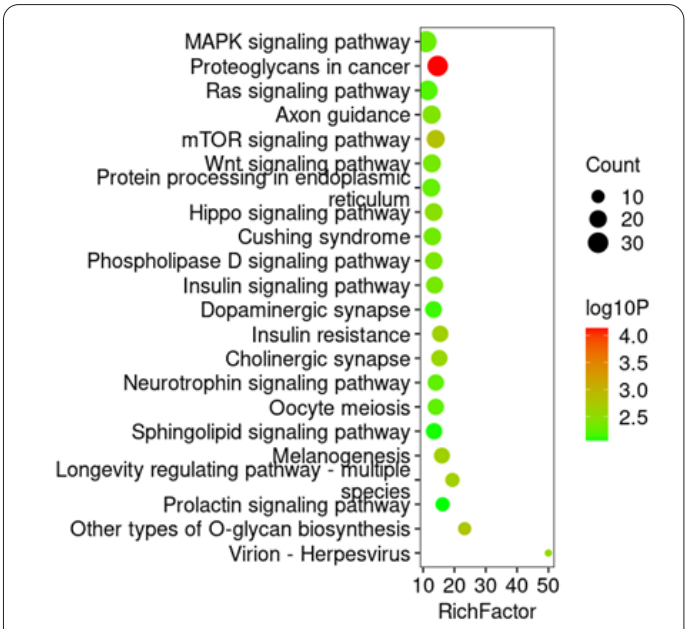


**Fig. 4.** Target gene KEGG enrichment results.

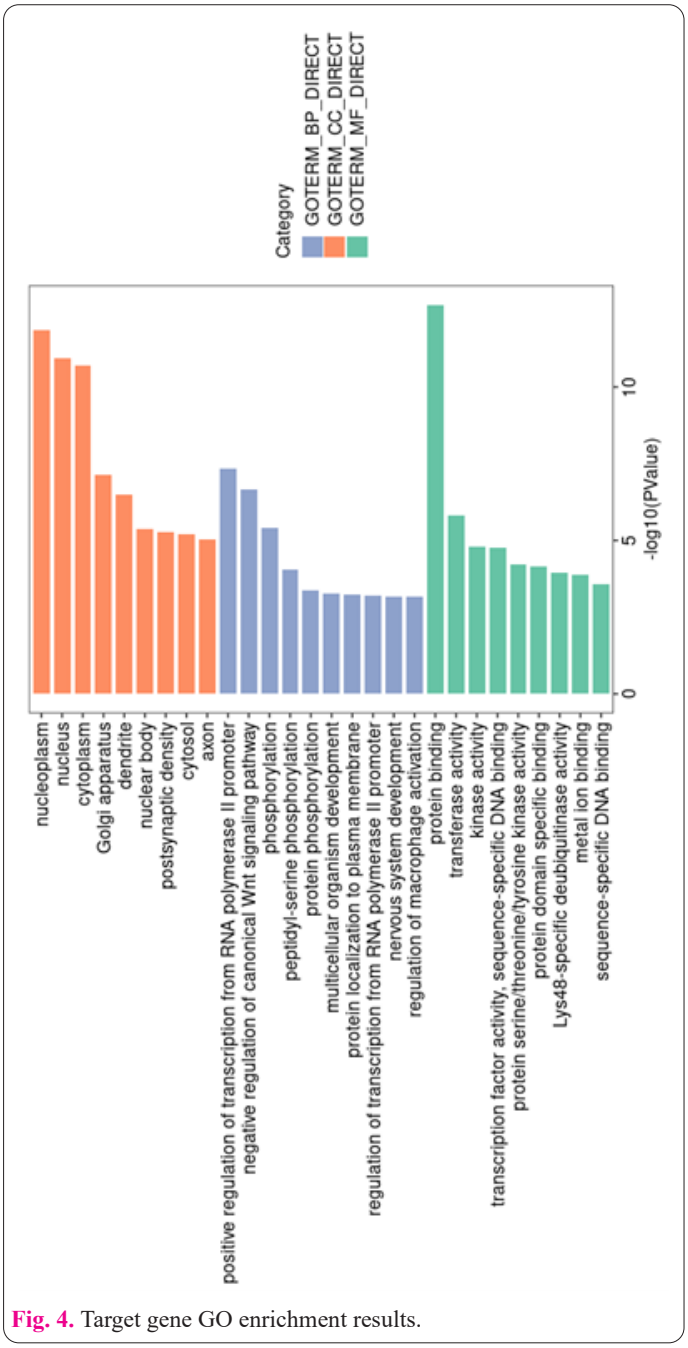


**Fig. 4.** Target gene GO enrichment results.

of rats with spinal injury.Il-1 β, IL-6, IL-17 and TNF-α are all important inflammatory factors related to immune regulation. Studies have shown that they can further induce secondary spinal injury on the basis of primary spinal injury, and even induce apoptosis of spinal cord cells, leading to functional spinal cord injury [17]. In this study, after nT-3 intervention on spinal injury rats, serum levels of inflammatory factors including IL-1β, IL-6, IL-17 and TNF-α were significantly decreased, suggesting that NT-3 can prevent the aggravation of spinal injury and promote the recovery of spinal function in spinal injury model rats by inhibiting inflammatory factors.

It has been found that a large number of nerve cells undergo apoptosis in animal models of spinal injury, thus affecting the tissue repair of the injured spine [18]. Bax and Bcl-2 are the genes most closely related to apoptosis, and Bcl-2 is an anti-apoptotic gene, which can inhibit various apoptotic pathways after spinal cord injury, while Bax can promote apoptosis [19]. In this study, Western blot analysis showed that BCL-2 protein significantly decreased and Bax protein significantly increased in the spinal tissue of spinal injury model rats, suggesting the existence of a large number of cell apoptosis after spinal injury. After NT-3-induced intervention, BCL-2 protein significantly increased and Bax protein significantly decreased, suggesting that NT-3 can inhibit apoptosis in spinal injury by increasing bcl-2 expression and decreasing Bax expression.

## 5. Conclusion

In conclusion, this study found that neurotrophin III can promote spinal injury repair in spinal injury model rats, and play a role by enhancing antioxidant stress ability, inhibiting inflammatory factors, promoting Bcl-2 and decreasing Bax expression.

## Conflict of interests

The author has no conflicts with any step of the article preparation.

## Consent for publications

The author read and approved the final manuscript for publication.

## Ethics approval and consent to participate

This study was approved by the Animal Ethics Committee of Yantaishan Hospital, Binzhou Medical University Animal Center.

## Informed consent

The authors declare not used any patients in this research.

## Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request

## Authors' contributions

Dexin Zou and Feng Chen designed the study and performed the experiments, Huimin Wang collected the data, Sibin Hao analyzed the data, Dexin Zou and Feng Chen prepared the manuscript. All authors read and approved the final manuscript.

## Funding

None.

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