

BASIC INVESTIGATION

Anti-inflammatory and analgesic effects of Yaotuitong Capsules in experimental rats with chemically induced radicular neuritis

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Abstract

OBJECTIVE: The aims of this study were to determine the anti-inflammatory and analgesic effects of Yaotuitong (translation: low back and leg pain) capsules, a Chinese herbal preparation, and the histological changes it induces in experimental rats with chemically induced radicular neuritis.

METHODS: Wistar rats were randomly divided into normal, model, Western medicine, and traditional Chinese medicine groups ($n=24$ per group). We surgically duplicated a chemical radicular neuritis model to simulate lumbar intervertebral disc protrusion. Granuloma formation was measured on postoperative days (PODs) 3, 7, 14, and 21. Prostaglandin E_2 and 5-hydroxytryptamine (inflammation mediators) levels in the surrounding tissue and the histology of the nerve root were determined on PODs 7 and 14.

RESULTS: Yaotuitong capsules significantly reduced prostaglandin E_2 ($P<0.01$) and 5-hydroxytryptamine ($P<0.01$) levels in tissue surrounding the nerve root. It also inhibited granuloma formation ($P<0.05$).

CONCLUSION: Yaotuitong capsules have anti-in-

flammatory and analgesic effects that can alleviate the discomfort of lumbar intervertebral disc protrusion.

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Key words: Yaotuitong capsules; Radiculitis; Inflammation mediators

INTRODUCTION

Yaotuitong (translation: low back and leg pain) capsules are a Chinese herbal preparation based on a Yaotuitong decoction developed by Professor Jianfu Zhang (Department of Orthopedics, Traditional Chinese Medical University of Henan Province, Zhengzhou, China).^{1,2} According to traditional Chinese medical theory, Yaotuitong capsules can alleviate impediments to the flow of *Qi*, promote blood circulation, remove meridian obstructions, and eliminate pain. It was therefore thought that it might be used to treat the herniated lumbar disc. We planned to study the effects and the mechanism of action of the Yaotuitong capsules on lumbar disc herniation. Therefore, based on previous anti-inflammatory and analgesic studies,³ we designed a chemically induced radicular neuritis rat model⁴ that simulated lumbar disc herniation. The resulting granuloma formation, levels of inflammatory mediators in tissue surrounding the nerve root, and histopathological changes were evaluated in this model and compared to the same parameters in three other groups of rats.

METHODS

A total of 96 Wistar rats weighing 200-240 g (irrespective of sex) were obtained from the Experimental Animal Center of Henan University of Traditional Chinese Medicine in Zhengzhou, China. The rats were random-

ly divided into four groups: normal rats, untreated model rats, rats treated with Western medicine, and rats treated with traditional Chinese medicine (24 rats per group). All of the rats were housed in separate cages with free access to water and food.

The equipment used included a Sartorius-2452 analytical balance (Sartorius AG, Gottingen, Germany) and a Shimadzu-510 fluorescence photometer (Shimadzu, Tokyo, Japan)

Model preparation

The model rats⁴ were depilated dorsally 2 days prior to the operation. On the day of surgery, they were anesthetized with sodium pentobarbital (50 mg/kg) via intraperitoneal injection, and a 3 cm midline incision was made on the dorsal skin under strict aseptic conditions using the fourth and fifth lumbar spinous processes as the center. The muscle was bluntly dissected to the paraspinal muscles, and the right lamina and articular process of the fourth and fifth lumbar spinous processes were removed. Using a surgical microscope, the L5 nerve root was carefully exposed. We then placed filter paper (0.6 cm diameter) that had been treated with 0.5% formalin for 24 h and rinsed with sterile saline before use on the L5 nerve root axilla. The incision was closed with sutures in a routine manner. Postoperatively, each rat was given 40,000 units of gentamicin intramuscularly daily for 3 days to prevent infection.

Drugs and administration

The Hospital of Henan University of Traditional Chinese Medicine in Zhengzhou, China provided the Yaotuitong capsules (batch no. 080422), from which we prepared a decoction: 1 g crude drug/mL. Aspirin (batch no. 080701; Xinxiang Zhongjie Pharmaceutical, Xinxiang, China) was formulated into a 0.5% suspension. The four groups of rats were given the following drugs intragastrically beginning 2 days preoperatively until death: traditional Chinese medicine group — Yaotuitong capsules, 24 g raw drug/kg (according to human-to-rat body surface area conversion, this dose was equivalent to four times the usual dose); Western medicine group — aspirin 0.1 g/kg; normal and model groups — 0.9% sodium chloride injection (1 mL/kg).

Outcome measures

To determine stimulus-induced granuloma development, six rats from each group, randomly selected, were killed on days 3, 7, 14, and 21 after preparing the model (postoperative days, or PODs). The surgical wound was reopened to remove the filter paper from the model rats. The granuloma was weighed to 1 µg using the Sartorius analytical balance model 2452.

The levels of the inflammatory mediators in tissue surrounding the nerve root were determined as follows. Two samples of tissue surrounding the nerve root were selected, accurately weighed, and ground into powder after surface moisture was absorbed with filter paper.

Prostaglandin E₂ (PGE₂) was soaked in saline and then saponified. The absorbance at 278 nm was determined and expressed as the absorbance value per gram.⁵ A second sample of tissue surrounding the nerve root was selected to extract 5-hydroxytryptamine (5-HT) using alkaline n-butanol. The relative fluorescence intensity of the extraction solution was determined using a fluorometric assay with o-phthalaldehyde, with the results expressed as the relative fluorescence intensity per gram.⁶

For the histological examination, samples of the L5 nerve root at the surgical side were removed on PODs 7 and 14. They were fixed in 10% formalin and then stored at 4°C for histological analysis.

Data were expressed as the mean ± SD and were analyzed using analysis of variance. Values between groups were compared using the *t*-test. Statistical analysis was performed using SPSS version 13.0 software (SPSS, Chicago, IL, USA).

RESULTS

Effects of Yaotuitong capsules on stimulus-induced granuloma

Granuloma development was significant around the stimulus area 14 days after the model was prepared ($P < 0.01$ versus the development at both 3 days and 7 days). There was significantly less granuloma formation in the traditional Chinese medicine group than in the model group on PODs 14 and 21 ($P < 0.05$). Although there was also less granuloma development in the Western medicine group than in the model group, there was even less in the traditional Chinese medicine group than in the Western medicine group. The difference, however, was not statistically significant. It was clear that the Yaotuitong capsule preparation effectively inhibited granuloma formation around the nerve root (Table 1).

Effect of Yaotuitong capsules on inflammatory mediator levels in tissue surrounding the nerve root

Prostaglandin E₂ and 5-HT levels were significantly increased in local tissue in the model group ($P < 0.01$), suggesting chemical inflammation around the nerve root. Western medicine had strong, rapid anti-inflammatory and analgesic effects ($P < 0.05$ or $P < 0.01$), especially regarding 5-HT. Evidence that traditional Chinese medicine was inhibiting PGE₂ appeared on POD 7 and decreased 5-HT levels earlier ($P < 0.01$) (Tables 2, 3).

Histological examination of the nerve root

Histologically, there was less inflammation of the nerve root tissue in the Western medicine group than in the model group, but it did not prevent degeneration and necrosis of the nerve roots. In contrast, traditional Chinese medicine significantly delayed the process of degeneration and necrosis of the nerve roots and played a protective role in the rats with chemically induced inflammation and nerve root injury (Figure 1, 2).

Table 1 Effect of Yaotuitong capsules on inflammation-induced granuloma formation ($\bar{x} \pm s, n=6$)

Group	Inflammatory granuloma formation at PODs 3 - 21 (mg)			
	3 days	7 days	14 days	21 days
Model	10.18±1.18	10.08±2.03	21.73±4.78 ^{aa}	40.32±6.78
Western medicine	11.70±2.54	8.90±0.76	18.08±4.54	34.97±3.84
Traditional Chinese medicine	9.22±1.51	9.18±2.46	16.00±2.26 ^b	30.98±5.60 ^b

Notes: ^a $P<0.05$, ^{aa} $P<0.01$, compared with the model group before and after surgical preparation of the model; ^b $P<0.05$, ^{bb} $P<0.01$, compared with the model group at the same time point.

Table 2 Effect of Yaotuitong capsules on PGE₂ levels in tissue surrounding the nerve root ($\bar{x} \pm s, n=6$)

Group	PGE ₂ levels on PODs 3 - 21 after preparation of the model (absorbance value)			
	3 days	7 days	14 days	21 days
Normal	1.84±0.35	1.86±0.27	1.91±0.42	1.88±0.26
Model	3.31±0.12 ^a	3.51±0.43 ^a	3.14±0.34 ^a	2.87±0.37 ^a
Western medicine	2.84±0.31 ^{ab}	2.61±0.56 ^{ab}	2.76±0.40 ^{ab}	2.55±0.28 ^d
Traditional Chinese medicine	3.04±0.42 ^a	2.83±0.41 ^{ac}	2.85±0.25 ^{db}	2.45±0.24 ^{dc}

Notes: PGE₂: prostaglandin E₂, ^d $P<0.01$, ^a $P<0.001$, compared with the normal group at the same time point; ^c $P<0.05$, ^b $P<0.01$, compared with the model group at the same time point

Table 3 Effect of Yaotuitong capsules on 5-HT Levels in tissue surrounding the nerve root ($\bar{x} \pm s, n=6$)

Group	5-HT levels on PODs 3 - 21 after preparation of the model (relative fluorescence intensity/g)			
	3 days	7 days	14 days	21 days
Normal	26±5	26±4	26±5	26±5
Model	50±9 ^a	46±4 ^a	41±4 ^a	39±6 ^c
Western medicine	34±8 ^b	33±8 ^b	35±4 ^{cd}	30±7 ^d
Traditional Chinese medicine	38±8 ^{cb}	32±7 ^b	39±7 ^c	33±6

Notes: 5-HT: 5-hydroxytryptamine, ^c $P<0.01$, ^a $P<0.001$ compared with the normal group at the same time point; ^d $P<0.05$, ^b $P<0.01$, compared with the model group at the same time point

DISCUSSION

Chemical radiculitis⁷ and intervertebral foramen venous obstruction⁸ are significant factors in the pathological process of producing radicular pain in patients with lumbar disc herniation. Strong evidence indicates that rupture of the annulus fibrosus and the exposed nucleus pulposus stimulates the dural sac and nerve root sleeve, leading to chemically induced radicular neuritis and pain.⁹ Venous occlusion and microcirculation disorders impede the supply of oxygen and nutrients to tissues, resulting in accumulation of metabolites. They also increase capillary permeability, exacerbate the inflammatory reaction, and lead to local inflammation-induced granuloma formation. The keys to treating lumbar disc herniation are to improve the microcirculation, inhibit inflammation around the nerve root, reduce the production/accumulation of inflammatory- and pain-inducing chemicals in local tissue, and prevent damage to nerve roots.

Yaotuitong capsules are a pure traditional Chinese

Medicine preparation modified from Zhang's decoction. It can alleviate impediments to the flow of Qi, promote blood circulation, remove meridian obstruction, and relieve pain. With these actions, it is considered an optimal therapy for lumbar disc herniation.^{1,2} The anti-inflammatory and analgesic effects of Yaotuitong capsules were studied preliminarily in 1999 to determine its mechanism(s) of action. Hotplate pain tests and acetic acid-induced writhing tests in mice confirmed its good analgesic effects at middle and high doses. Also, xylene-induced ear swelling tests in mice demonstrated good anti-inflammatory effects with middle- and high-dose Yaotuitong capsules, especially for chemically induced inflammation.¹ A study conducted from September 2001 to March 2004^{11,12} showed that Yaotuitong capsules can effectively protect motor and sensory fibers from nucleus pulposus-induced nerve root injury. The underlying mechanisms may be related to reduced levels of nitric oxide and interleukin and inhibition of inflammation.

Prostaglandin E₂ and 5-HT are important mediators of inflammation. Because their concentrations in tissue directly reflect the degree of inflammation and pain, they

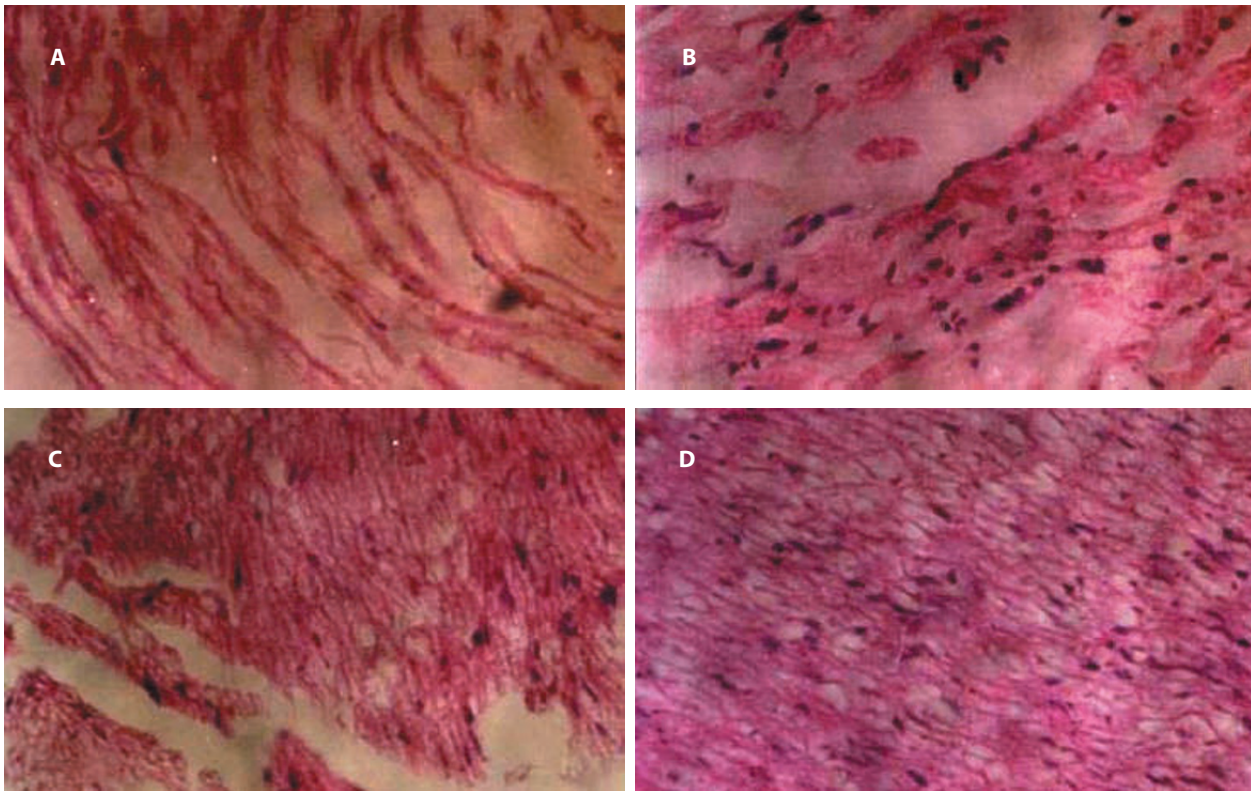


Figure 1 Histological image of nerve root in rats on postoperative day (POD) 7 ($\times 400$)

A Normal group: Nerve roots show intact structure and tight arrangement of fibers. Long Fei's nodules are visible. B Model group: Nerve root fibers appear edematous and thicker, axons have partially disappeared, and most of the myelin sheath has disintegrated. C Western medicine group: Axons have partially disappeared, and demyelination of nerve fibers is observed. D Traditional Chinese medicine group: Partial nerve fiber demyelination is visible.

A Model group: Nerve root degeneration and necrosis are found with inflammatory infiltration. B Western medicine group: Discontinuous axonal remnants are visible. C Traditional Chinese medicine group: Nerve cells have survived in the ganglia.

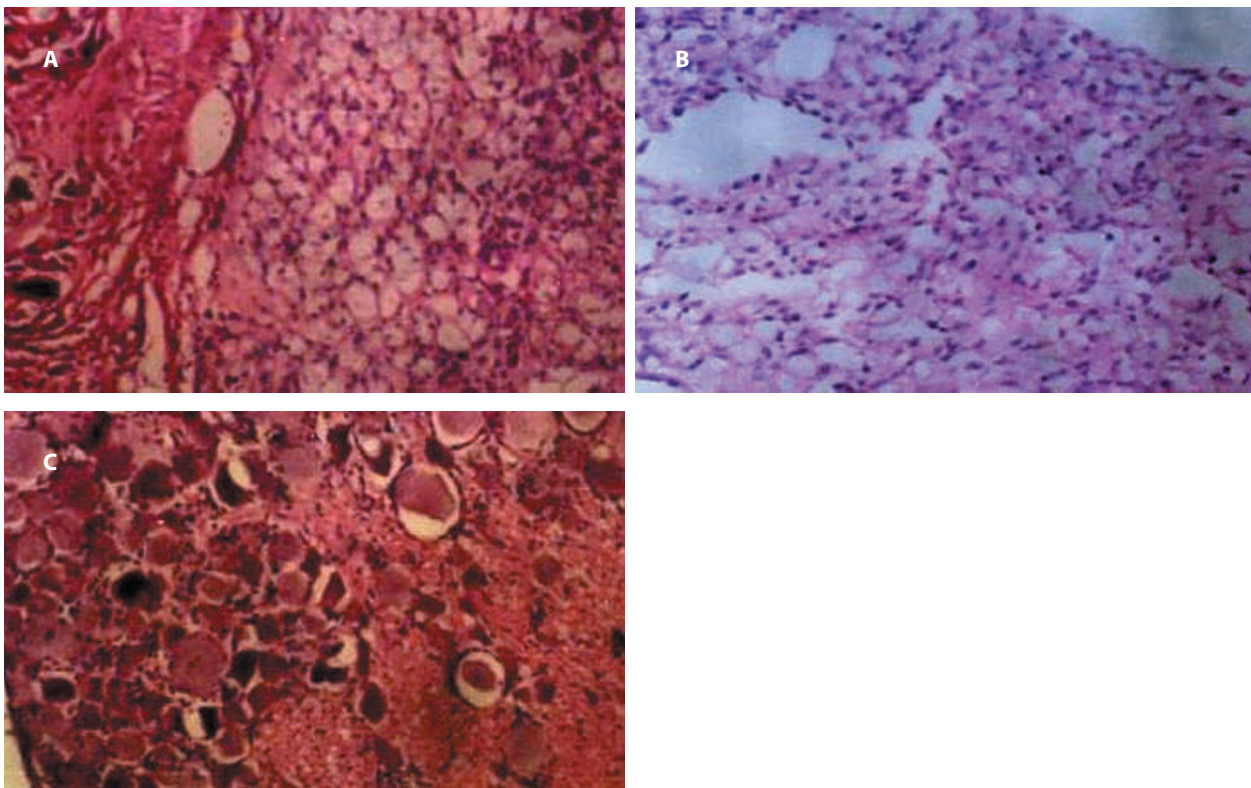


Figure 2 Pathological image of nerve root in rats on POD 14 ($\times 400$)

serve as important hallmarks of anti-inflammatory and analgesic efficacy. In this study, we successfully replicated a chemical radicular neuritis model in rats to simulate lumbar disc herniation. Stimulus-induced granuloma development and the concentrations of these inflammatory mediators in the nerve root and surrounding tissue were determined at several time points after the model was prepared. Results of this study showed that PGE₂ and 5-HT levels were significantly elevated in local nerve root tissue after the surgery to prepare the model rats. Inflammatory granuloma formation was visible, suggesting that the pathological process of lumbar disc herniation involves chemically induced radicular neuritis. Yaotuitong capsules effectively reduced the levels of inflammatory mediators in nerve roots and surrounding tissue, prevented inflammatory granuloma formation, and inhibited inflammation. Histopathological analysis of the nerve root confirmed that Yaotuitong capsules significantly delayed the process of nerve root degeneration and necrosis and protected nerve roots against injury caused by chemically induced inflammation.

The pharmacological effects of Yaotuitong capsules are determined by its active ingredients, which include the following: Ma Qian Zi (*Semen Strychni*), Yan Hu Suo (*Rhizoma Atracylodes*), Ru Xiang (*Frankincense*), Mo Yao (*Myrrh*), Dan Shen (*Radix Salviae Miltiorrhiae*), Quan Xie (*Scorpio*), Wu Gong (*Scolopendra*), Cang Zhu (*Rhizoma Atracylodes*), Bai Shao (*Radix Paeoniae Alba*), Dang Gui (*Radix Angelicae Sinensis*), Hong Hua (*Flos Carthami*), Chi Shao (*Radix Paeoniae Rubra*), and Gan Cao (*Radix Glycythizae*). Studies have shown that strychnine and other alkaloids in *Semen Strychni* selectively excite spinal cord tissue, improve skeletal myasthenia, and adjust the imbalance between the vertebrae and the medullary spinal cord.¹³ *Rhizoma Atracylodes* has analgesic effects in modern pharmacological studies with a potency of total alkaloids of approximately 40% of morphine's efficacy and an analgesic effect greater than that of aspirin.¹⁴ *Myrrh* and *Radix Salviae Miltiorrhiae* dilate capillaries, reduce blood viscosity, and improve the microcirculation.¹⁴ *Radix Salviae Miltiorrhiae* delays degeneration of lumbar intervertebral discs; its mechanism may be related to improving the local microcirculation of the intervertebral disc tissue, reducing venous stasis, and regulating the immune response.¹⁴ β -Amine alcohol in *Rhizoma Atracylodes* can alleviate cell hypoxia.¹⁵ Total glucosides of peony have immunomodulatory and significant analgesic effects.¹⁴ Methanol extract of *Glycyrrhizae* FM100 in *Radix Glycythizae* reacts synergistically with total glucosides of peony to create antispasmodic and analgesic effects.¹⁵ Pharmacological studies also found steroid hormone-like anti-inflammatory effects of *Radix Glycythizae*, where the anti-inflammatory potency of glycyrrhetic acid was one-tenth that of hydrocortisone.¹⁴ Safflower can expand blood vessels; it antagonizes epinephrine- and norepinephrine-induced vascular con-

traction while improving the local microcirculation around the disc.¹⁴ All of the ingredients in the Yaotuitong prescription can alleviate chemical inflammation of the nerve root.

In summary, Yaotuitong capsules can reduce the effects of chemically induced radicular neuritis. It exhibits anti-inflammatory and analgesic effects and effectively alleviates inflammation-induced nerve root injury. These effects may explain the pharmacological mechanisms underlying the response to treatment of lumbar disc herniation with Yaotuitong capsules. Our findings provide experimental evidence to support clinical application and promotion of the use of Yaotuitong capsules.

REFERENCES

- 1 Xing QC, Huang ZJ, Zhang JF. Observation on the preparation and clinical effects of Yaotuitong capsule. *China Journal of Chinese Medicine* 2011; 26 (10): 1211-1212.
- 2 Wu QJ, Zhang JF. The therapeutic effect of Yaotuitong capsule on the low back pain. *Zhong Yi Zheng Gu* 2006; 18(11): 31-32.
- 3 Xing QC, Zhang JF, Xu BJ. Experimental study of anti-inflammatory and analgesic effect of Yaotuitong capsule. *Zhong Yi Zheng Gu* 2000;12(6): 8-10.
- 4 Jiang WZ, Fan M, Chen YP. Clinical and experimental study of discogenic back and leg pain treated with the principle of promoting blood circulation and removing blood stasis. *Zhong Guo Gu Shang* 1991; 4 (1): 8-10.
- 5 Chen Q. *Methodology of Traditional Chinese Medicine Pharmacology*. Beijing: People's Medical Publishing House; 1993: 371-372.
- 6 Kuang PG. mensuration of 5-HT in tissue. *Journal of Chinese PLA Postgraduate Medical School* 1982; 2: 81-86.
- 7 Murphy RW. Nerve roots and spinal nerves in degenerative disc disease. *Clin Orthop Relat Res* 1997; (129): 46-60.
- 8 Hoyland JA, Freemont AJ, Jayson MI. Intervertebral foramen venous obstruction: A cause of periradicular fibrosis? *Spine* 1989; 14(6): 558-568.
- 9 Yoshizawa H, Kobayashi S, Morita T. Chronic nerve root compression: Pathophysiologic mechanism of nerve root dysfunction. *Spine* 1995; 20 (4): 397-407.
- 10 Liu WG, Xu XM, Zhang JF, Wen ZJ, Cao YJ, Ai JW. The influence of capsules for lumbago and scelalgia on rat nerve-root function after epidural nucleus pulposus autografting. *Zhong Yi Zheng Gu* 2004; 16(8): 4-6.
- 11 Ai JW, Huang CL, Zhang JF, Cao YJ, Liu WG. The effect of "Yaotui Tong Jiaonang" on the inflammatory cells in rats with lumbar intervertebral disc hernia. *Zhong Yi Zheng Gu* 2003; 15(8): 7-9.
- 12 Guo XZ. Therapeutic effect of compound powder of *Semen Strychni* for the treatment of lumbar intervertebral disc protrusion in 40 cases. *Zhong Yi Za Zhi* 1980; 7: 44.
- 13 Shen YJ. *Pharmacology of Traditional Chinese Medicine*. Shanghai: Shanghai Scientific and Technical Publishers; 1997: 131-158.
- 14 Jiangsu New Medical College. *Dictionary of Traditional Chinese Medicine*. Shanghai: Shanghai Scientific and Technical Publishers; 1985: 1066-1069.