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Original Article

Establishment and evaluation of a rat model of cervical spondylosis with Yin deficiency syndrome

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Abstract: Objective: To establish and evaluate a rat model of cervical spondylosis (CS) with yin deficiency syndrome. Methods: Thirty-six male Sprague-Dawley rats were randomly assigned to the control group, CS group, and CS with Yin deficiency syndrome (YCS) group (n = 12 per group). The control group underwent daily routine care for 37 days. The CS group underwent induction of cervical static-dynamic imbalance, followed by 30 days of standard care. The YCS group underwent cervical static-dynamic imbalance induction, followed by 7 days of sleep deprivation to model Yin deficiency cervical spondylosis. Behavioral performance, heart rate, blood pressure, mechanical pain thresholds, serum cAMP, cGMP, cAMP/cGMP levels, and the expression of collagen-II, Bcl-2, Bax, and Bcl-2/Bax in cervical intervertebral discs were analyzed at various time points. Results: Following CS induction, modeled rats exhibited significant changes in intervertebral disc structure, including misalignment of the annulus fibrosus, atrophy of the nucleus pulposus, rough cartilaginous endplate boundaries, and disc degeneration. Mechanical pain thresholds decreased. In the YCS group, compared with the CS and control groups, rats showed heightened excitability, dull fur, reddish mouth, lips, nose, paws, and tail, resistance to handling, slower weight gain, initial heart rate elevation followed by decline, and progressive blood pressure reduction. Serum cAMP and cAMP/cGMP ratios were significantly elevated, while cGMP levels were reduced. Collagen-II, Bcl-2, and Bcl-2/Bax levels decreased, and Bax levels increased. Conclusion: The established rat model of Yin deficiency syndrome aligns with clinical and traditional Chinese medicine characteristics, making it a promising model for studying Yin deficiency syndrome.

Keywords: Yin deficiency syndrome, cervical spondylosis, male rats, model evaluation

Introduction

Cervical spondylosis (CS), characterized by cervical pain and activity limitation, is a degenerative disease of the cervical intervertebral discs. Its secondary pathological changes progressively affect the function of peripheral nerves, blood vessels, and surrounding tissues [1]. Based on clinical manifestations and pathological features, CS can be classified into cervical, nerve root, spinal cord, and other subtypes [2, 3]. The condition is common, primarily resulting from occupational factors and lifestyle-related issues [4]. With increasing work-related stress, the prevalence of CS has risen annually, affecting younger populations and imposing significant physical, psychological, and economic burdens on individuals, families, and society [5].

In traditional Chinese medicine (TCM), CS falls under the categories of “arthralgia”, “Xiang paralysis”, and “Xiang Qiang”. TCM recognizes the complexity and variability of CS symptoms. Clinical observations have noted that some patients with CS experience neck, shoulder, and arm pain that worsens at night, accompanied by yin deficiency symptoms such as hot flashes, night sweats, irritability, insomnia, and a fine pulse [6]. This indicates a strong correlation between CS and yin deficiency according to TCM theory.

TCM treatments, including acupuncture, tuina, and the internal and external application of herbal medicines, have demonstrated unique advantages in managing CS by alleviating symptoms and restoring yin-yang balance. However,

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the absence of animal models tailored to the characteristics of TCM evidence has hindered in-depth research into the mechanisms underlying TCM therapies. This limitation has also restricted the broader clinical application of TCM in CS management [7-9]. In this context, the present study aims to establish an animal model of CS with TCM-specific characteristics of yin deficiency. This model will serve as an essential experimental platform to explore the TCM pathogenesis of CS, validate the efficacy and safety of TCM treatments, and provide a scientific basis for further studies.

Materials and methods

Animals and materials

Animals: Thirty-six male Sprague-Dawley rats (200 ± 20 g) were obtained from Jinan Pengyue Laboratory Animal Breeding Co., Ltd. (SCXK-Lu2019-0003, Shandong, China). The rats were housed in a clean room with controlled conditions: temperature of 23°C ± 2°C, relative humidity of 60% ± 10%, and a 12-hour light-dark cycle. All animals were ad libitum fed a standard solid diet, with three rats per cage. A 7-day acclimatization period preceded the experiment.

Ethical statement: The experimental protocol was approved by the Ethics Committee of the First Affiliated Hospital of Shandong First Medical University & Shandong Provincial Qianfoshan Hospital ([2019] no. S177). All animal procedures complied with the ARRIVE guidelines and relevant ethical regulations. Rats were euthanized by intraperitoneal injection of sodium pentobarbital (150 mg/kg) prior to autopsy.

Reagents and equipment: Key materials included cAMP and cGMP kits (Wuhan Elabscience, E-EL-0056c, E-EL-0083c), 3% sodium pentobarbital, and hematoxylin-eosin (HE) stain (Beijing Dingguo Changsheng Biotechnology Co., Ltd.). Equipment used included a paraffin sectioning machine (LEICA RM2235, Germany), biological tissue spreading and baking machine (Beijing Dingguo Changsheng Biotechnology Co., Ltd.), biological tissue spreader (PHY-III, Wuhan Zhongtian Hongbo Medical Equipment Co., Ltd.), tabletop cryogenic centrifuge (Thermo Scientific Heraeus Multifuge X1R, USA), biochemical analyzer (HERA. Multiftige X1R), an ultra-low temperature freezer (-80°C, Thermo Scientific FORMA700 SERIES), an optical microscope (OLYMPUS FSX 100), a sleep deprivation instrument (Beijing Xinxin XR-XS107), hair clippers (HC1066, Philips, Netherlands), a small animal noninvasive blood pressure detector (Beijing Softlung BP-2010A), an enzyme marker (BioTek Epoch, USA), BCA protein test kit (P0012, Beo Tianmei Biotechnology Co., Ltd., Shanghai, China). Antibodies used included anti-collagen-II (1:1000 dilution, ab188570, Abcam), anti-Bcl-2 (1:500 dilution, ab196495, Abcam), anti-Bax (1:1000 dilution, ab32503, Abcam), GAPDH (1:2000 dilution, E-AB-20059, Elabscience, Wuhan, China), and HRP-conjugated goat anti-rabbit IgG (1:5000 dilution, EF0002, Sparkjade, Shandong, China).

Methods and models

Grouping: Rats were randomly divided into three groups (n = 12 per group): the control group, the CS group, and the CS with yin deficiency syndrome (YCS) group.

Model establishment: In the control group, rats underwent standard feeding for 37 days without additional interventions. In the CS group, after a 7-day acclimatization period, rats were anesthetized with 3% sodium pentobarbital (30 mg/kg, intraperitoneally) and fixed in a prone position. Following sterilization, a midline incision (2-2.5 cm) was made on the back of the neck, from the atlanto-occipital joint to the interspinous process of the second thoracic vertebra. The skin, subcutaneous tissues, and deep cervical muscles (e.g., the cervical pincer, cephalic, and cervical longus muscles) were carefully dissected to expose the cervical vertebrae. The supraspinous and interspinous ligaments of the C2-C7 vertebrae were severed. After hemostasis, the incision was sutured. Post-surgery, rats were fed normally for 30 days. In the YCS group, in addition to the procedures outlined for the CS group, rats were subjected to continuous sleep deprivation for 7 days following the surgery. The model combined cervical spine static-dynamic imbalance and sleep deprivation to simulate the characteristics of yin deficiency in TCM.

Specimen collection and index testing

Sleep deprivation: Total sleep deprivation (TSD) in the YCS group was induced using a sleep

Table 1. Morphologic grading criteria for Miyamoto intervertebral discs

Classification	Histomorphology
Level 1	Normal intervertebral disc, the annulus fibrosus and nucleus pulposus are arranged regularly, the cartilage endplate is divided into growth cartilage and articular cartilage layers, and the tide mark is clear (1 point)
Level 2	Fibrous annulus laminae structure disappears, cartilage endplates proliferate (2 points)
Level 3	The nucleus pulposus is wrinkled or disappeared (3 points)
Level 4	Fissures or splitting of the annulus fibrosus (4 points)
Level 5	Herniated intervertebral discs or bony encumbrance formation (5 points)

deprivation instrument [10]. This apparatus consisted of a transparent plexiglass chamber (464 × 300 × 180 mm) with a linear circulating stirrer at the base, which physically disturbed the rats to prevent sleep. The stirrer moved at approximately 25.4 mm/s, ensuring that food and water remained accessible. The control and CS groups were placed in similarly confined spaces without inducing sleep deprivation.

Behavioral measurements: Behavioral changes in the three groups were observed and recorded at four time points: 0 h, 72 h, 120 h, and 168 h of sleep deprivation. Behavioral parameters included mental activity, fur appearance, coloration of the nose, lips, paws, and tail, irritability, resistance to handling, and urination/defecation patterns.

Detection of heart rate and blood pressure: Heart rate and blood pressure were measured at 0 h, 72 h, 120 h, and 168 h of sleep deprivation using a small animal non-invasive blood pressure detector. Measurements were conducted in a quiet room with soft lighting and a maintained temperature of 25°C from 9:00 am to 6:00 pm. The tail-cuff method was used, and values were averaged from three consecutive readings after the rats were calm.

Pain threshold measurement: Pain thresholds were evaluated using the Von Frey test [11] at the same four time points. Hair was removed from the neck and back areas of the rats, which were then acclimatized in a wire mesh box for 30 minutes. Vertical mechanical stimulation was applied with a Von Frey filaments for 8 seconds. Positive responses, such as head withdrawal or scratching, were recorded. The minimum force (threshold) required to elicit three positive responses out of five trials was noted, with a maximum threshold set at 15 g.

Body weight measurement: The body weights of the rats was recorded at 0 h, 72 h, 120 h, and 168 h of sleep deprivation using an electronic balance.

Serum cAMP, cGMP, and cAMP/cGMP determination: Blood samples were collected from the femoral vein at the four time points. Blood was allowed to stand for 24 h and centrifuged at 4°C for 25 minutes at 5000 rpm. The supernatant was analyzed for cAMP and cGMP using ELISA. For example, 50 μL of biotinylated detection antibody was added to each well, followed by washing and incubation with HRP-conjugated antibodies. The reaction was terminated, and optical density was measured at 450 nm.

Hematoxylin-eosin (HE) staining: Intervertebral discs (C4/5, C5/6) were extracted from rats at 0 h of sleep deprivation. Discs were rinsed with PBS, fixed in 10% formaldehyde, decalcified with EDTA for 14 days, and processed for HE staining. Observations were made under a 40× optical microscope and scored according to the Miyamoto grading standard [12] (**Table 1**).

Ultrastructure examination: Intervertebral discs (without vertebral bodies) were prepared for ultrastructural examination. Samples were rinsed, fixed, embedded, and sectioned. After staining with uranyl acetate and lead citrate, the ultrastructure of the nucleus pulposus was observed under an electron microscope.

Immunoblotting of collagen-II, Bcl-2, Bax, and Bcl-2/Bax: Total protein from intervertebral disc tissues was extracted using RIPA buffer containing phenylmethylsulfonyl fluoride. Protein concentrations were determined using the BCA Protein Assay Kit. Proteins (30 μg) were separated by SDS-PAGE and transferred to PVDF membranes. Membranes were blocked and incubated with primary antibodies (anti-

collagen-II, Bcl-2, Bax, GAPDH) overnight at 4°C. After washing, membranes were incubated with HRP-conjugated secondary antibodies for 2 hours. Protein bands were visualized using enhanced chemiluminescence and quantified with ImageJ software.

Statistical analysis

Statistical analyses were performed using SPSS 27.0 (IBM Corp., Armonk, NY, USA), and GraphPad Prism 8.0.1 (San Diego, CA, USA) was used for graphing. Data are expressed as mean ± standard error ($\bar{x} \pm s$). Normally distributed data were analyzed using independent samples t-tests (for two groups) or one-way ANOVA/two-way repeated-measures ANOVA with post hoc Tukey tests (for three or more groups). Non-normally distributed data were assessed with the Kruskal-Wallis test. A $P < 0.05$ was considered statistically significant.

Results

Observation of behavioral performance in rats

In the control group, rats remained active and exhibited normal behavior throughout the experiment, with strong resistance to grasping, with pale red and glossy lips, nose, and tail, and well-formed, dry stools.

At 0 h of sleep deprivation, rats in all three groups displayed no significant behavioral changes compared to the control group. They were mentally alert, with moist white fur, red and shiny lips, noses, and tails, moderate resistance to handling, dry and shaped stools, and normal urination.

After 72 h of sleep deprivation, rats in the YCS group exhibited increased excitability and exploratory behavior, heightened sensitivity to external stimuli (sound and light), slightly dull fur, reddish and glossy lips, nose, paws, and tail, strong resistance to handling, and biting. Urine was yellow and odorous, and stools were dry.

At 120 h of sleep deprivation, rats in the YCS group showed signs of mental exhaustion and reduced activity. The were slightly emaciated, with fur that was fluffy, whitish, and slightly yellowish. Lips, nose, and tail appeared dull in color. Resistance to handling was minimal, urine output decreased, and stools were soft and sticky.

After 168 h of sleep deprivation, rats in the YCS group appeared depressed, frequently yawning, and dozing. Rats appeared emaciated, and their fur was erect, and light ivory in color. Lips, noses, and tails were pale and greenish-purple. Grip resistance was weak, and stools were loose (**Figure 1**).

Detection of heart rate and blood pressure indexes

Heart rate: As shown in the heart rate trend (**Figure 2A**), the heart rate of rats in the YCS group initially increased and then decreased over the observation period. At 0 h of sleep deprivation, there were no significant differences in heart rate among the three groups. From 72 h to 120 h of sleep deprivation, the heart rate of the YCS group was significantly higher than that of the CS group and the control group ($P<0.01$). No significant differences were observed between the CS and control groups ($P>0.05$). At 168 h of sleep deprivation, the heart rate of the YCS group decreased to pre-sleep deprivation levels, showing a significant reduction compared to 72 h ($P<0.01$) (**Figure 2B**).

Blood pressure: Blood pressure trends (**Figure 3A**) indicated a progressive decrease in the YCS group over the experimental period. At 0 h of sleep deprivation, there were no significant differences in blood pressure among the three groups ($P>0.05$). From 72 h to 168 h, blood pressure in the YCS group was significantly lower than in the CS and control groups (all $P<0.01$), while no significant differences were observed between the CS and control groups (all $P>0.05$) (**Figure 3B**).

Changes in mechanical pain: The mechanical pain thresholds of the YCS and CS groups decreased significantly on the 30th day post-surgery compared to the control group ($P<0.01$) (**Figure 4A**). Throughout the sleep deprivation period, the mechanical pain thresholds of the YCS and CS groups progressively decreased, with significant differences from the control group ($P<0.01$). At 0 h of sleep deprivation, no significant differences were observed between the YCS and CS groups ($P>0.05$). However, at 120-168 h of sleep deprivation, the YCS group

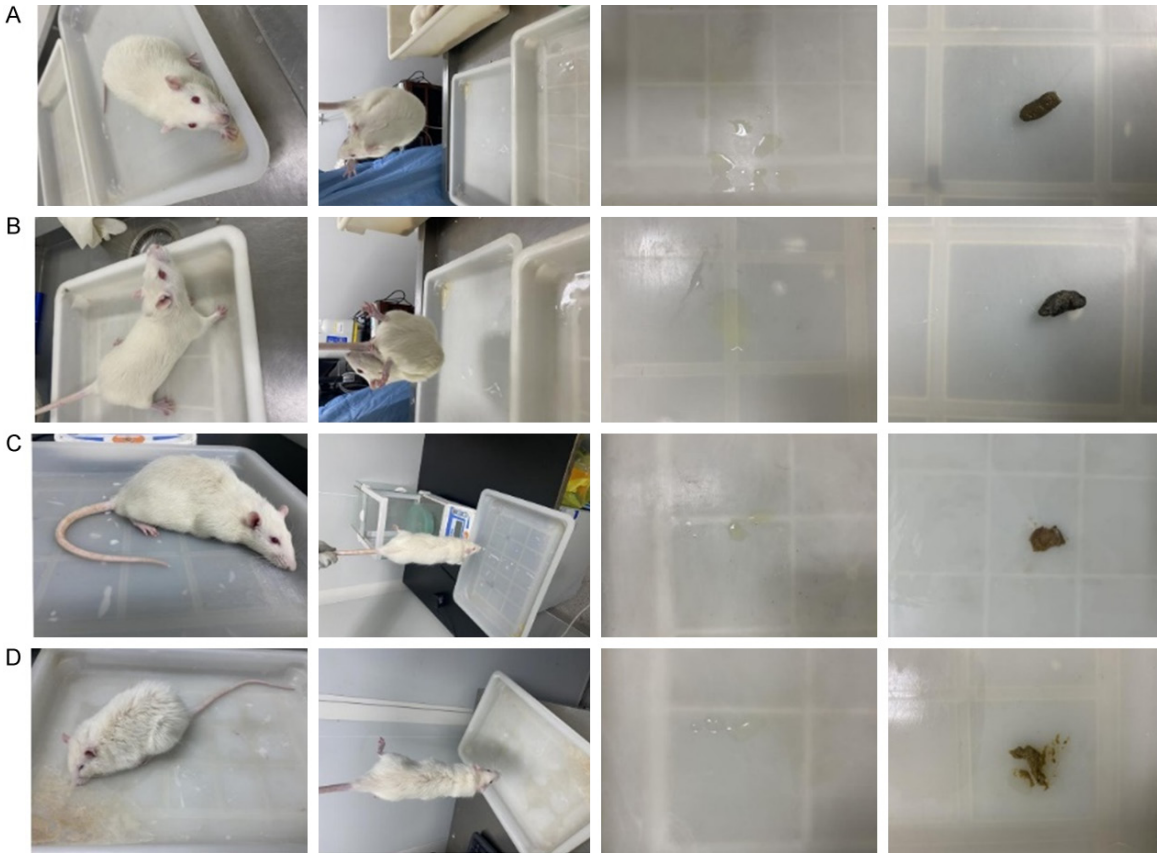

Figure 1. Representative figures of the behavioral performance of rats. A. Behavioral performance figures of rats in the control group. B. Behavioral performance figures of rats in the YCS group after 72 h of sleep deprivation. C. Behavioral performance figures of rats in the YCS group after 120 h of sleep deprivation. D. Behavioral performance figures of rats in the YCS group after 168 h of sleep deprivation. CS, Cervical spondylosis; YCS, CS with yin deficiency syndrome.

exhibited significantly lower mechanical pain thresholds compared to the CS group at the same time points (all P<0.01) (**Figure 4B**).

Body weight measurement

At 0 h of sleep deprivation, no significant differences in body weight were observed among the three groups (P>0.05). Between 72 h and 168 h of sleep deprivation, the CS and control groups exhibited significant weight gains compared to the YCS group at the same time points (all P<0.01). In the YCS group, weight increased only slightly during this period, likely due to the induction treatment. By the end of the induction period, rats in the CS and control groups showed significant weight increases, whereas the YCS group did not (P<0.01). There was no significant difference in weight gain between the CS and control groups (P>0.05) (**Figure 5**).

Serum cAMP, cGMP, and cAMP/cGMP levels

At 0 h of sleep deprivation, there were no significant differences in serum cAMP, cGMP, or cAMP/cGMP levels among the three groups (all P>0.05). At 72 h, the YCS group showed significantly higher cAMP and cAMP/cGMP levels and significantly lower cGMP levels compared to the CS and control groups (all P<0.01). At 120 h, cAMP and cAMP/cGMP levels in the YCS group remained significantly higher compared to the other groups (all P<0.05). No significant differences were observed between the CS and control groups (all P>0.05). At 168 h, cAMP levels in the YCS group were not significantly different (P>0.05), but cGMP levels were elevated, and cAMP/cGMP levels decreased compared to the CS and control groups (all P<0.01). No significant differences were noted between the CS and control groups at this time (all P>0.05) (**Figure 6**).

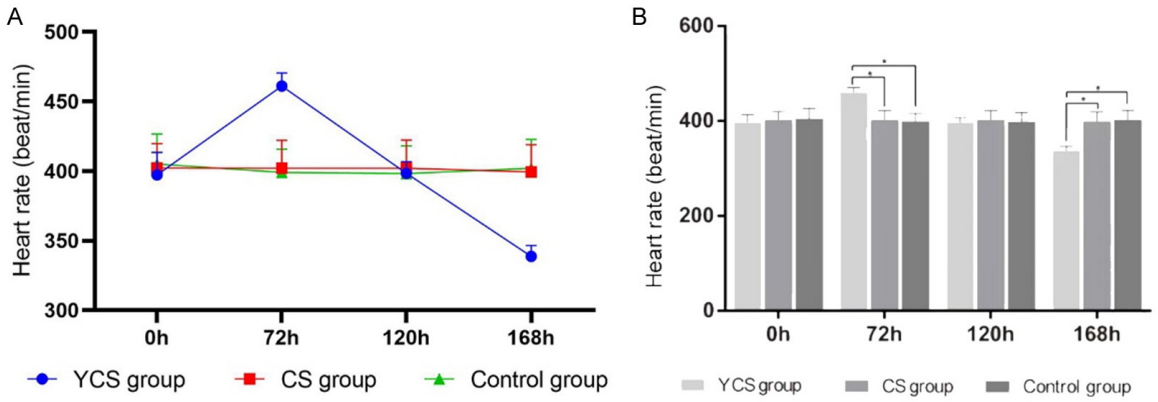

Figure 2. Changes in the heart rate of rats in each group. A. Trend of heart rate changes in each group of rats. B. Comparison of heart rate of rats in each group with different sleep deprivation times. *P<0.05. CS, Cervical spondylosis; YCS, CS with yin deficiency syndrome.

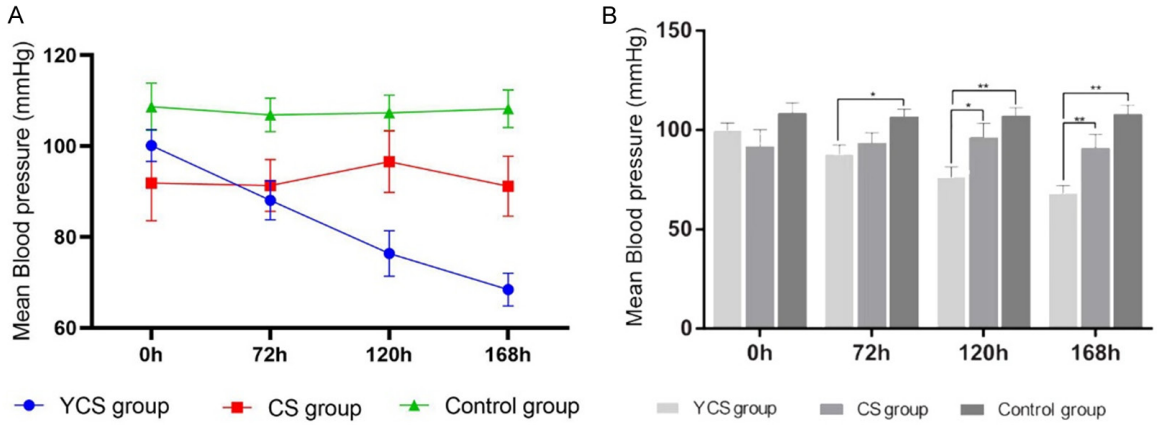

Figure 3. Changes in the blood pressure of rats in each group. A. Trend of blood pressure changes in each group of rats. B. Comparison of blood pressure of rats in each group with different sleep deprivation times. *P<0.05, **P<0.01. CS, Cervical spondylosis; YCS, CS with yin deficiency syndrome.

Histomorphological observation of intervertebral discs under HE staining

HE-stained cervical intervertebral discs were observed at 100× magnification (**Figure 7**). In the control group, the annulus fibrosus and nucleus pulposus were regularly arranged, and the cartilaginous endplates were clear. In contrast, the CS and YCS groups exhibited significant morphological changes, including misaligned annulus fibrosus, atrophy of the nucleus pulposus, and rough cartilaginous endplate borders (**Figure 7A-C**).

According to Miyamoto grading, at 168 h of sleep deprivation, the control group had significantly lower scores compared to the CS and YCS groups (P<0.01), and no significant differences were observed between the YCS and CS groups (P>0.05) (**Figure 7D**).

Ultrastructure of nucleus pulposus observed by transmission electron microscopy

The ultrastructure of the nucleus pulposus was examined in the three groups (**Figure 8**). In the control group, cells were round or oval, with distinct cell membranes. The nuclei were centrally located, with smooth and intact nuclear membranes, uniformly distributed chromatin, and minimal heterochromatin. The cytoplasm contained abundant organelles, including rough endoplasmic reticulum, mitochondria, matrix vesicles, and halos (**Figure 8C**).

In the CS group, cells exhibited irregular shapes, uneven nuclear membranes, increased het-

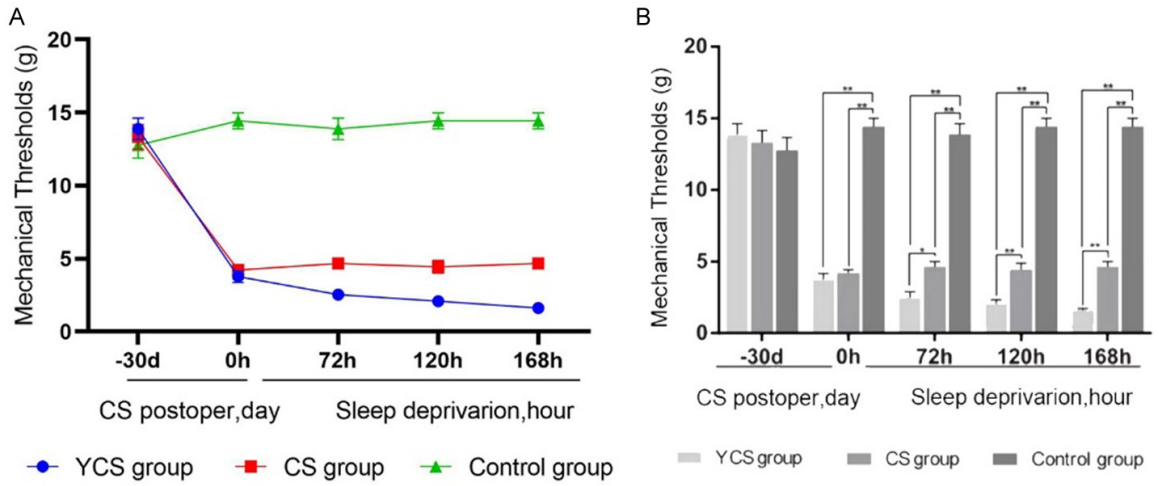

Figure 4. Changes in the mechanical pain of rats in each group. A. Trend of mechanical pain changes in each group of rats. B. Comparison of mechanical pain of rats in each group with different sleep deprivation times. *P<0.05, **P<0.01. CS, Cervical spondylosis; YCS, CS with yin deficiency syndrome.

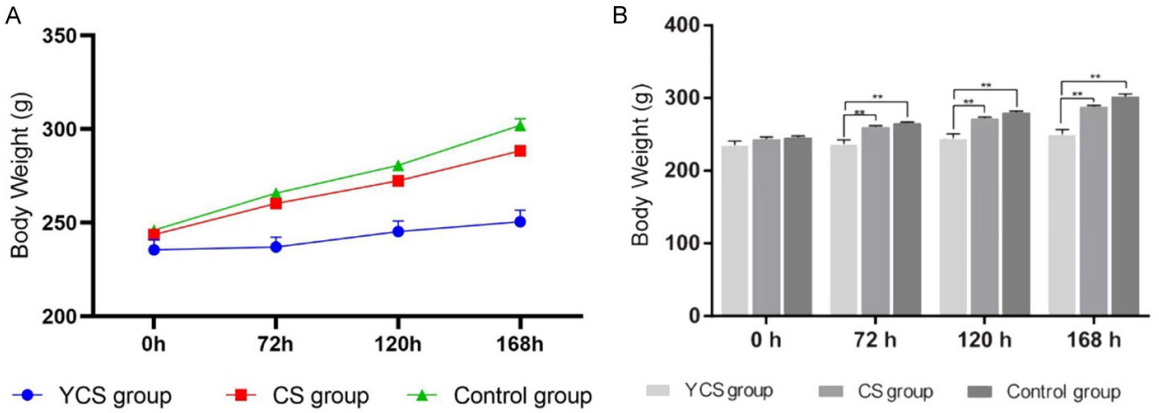

Figure 5. Changes in body weight of rats in groups with different sleep deprivation times. A. Trends of body weight changes in rats of each group with different sleep deprivation times. B. Comparison of mechanical pain of rats in each group with different sleep deprivation times. **P<0.01. CS, Cervical spondylosis; YCS, CS with yin deficiency syndrome.

erochromatin, reduced cytoplasmic content, and decreased organelle abundance. Observations included swollen mitochondria, rough endoplasmic reticulum, and thickened envelopes (**Figure 8B**).

In the YCS group (168 h of sleep deprivation), the ultrastructure was similar to that of the CS group, showing comparable degenerative features (**Figure 8A**).

Expression levels of collagen-II, Bcl-2, Bax, and Bcl-2/Bax in cervical intervertebral discs

Immunoblotting revealed that collagen-II levels, which are negatively correlated with intervertebral disc degeneration, were significantly higher in the control group than in the CS and YCS groups (P<0.01) (**Figure 9A**). No significant difference was observed between the YCS and CS groups (P>0.05) (**Figure 9B**).

Bcl-2, an anti-apoptotic protein, was significantly higher in the control group than in the CS and YCS groups (P<0.01). In the YCS group, Bcl-2 levels decreased continuously at 72 h, 120 h, and 168 h of sleep deprivation, with levels significantly lower than in the CS group (P<0.01) (**Figure 9C**).

Bax, a pro-apoptotic protein, was lower in the Control group than in the CS and YCS groups

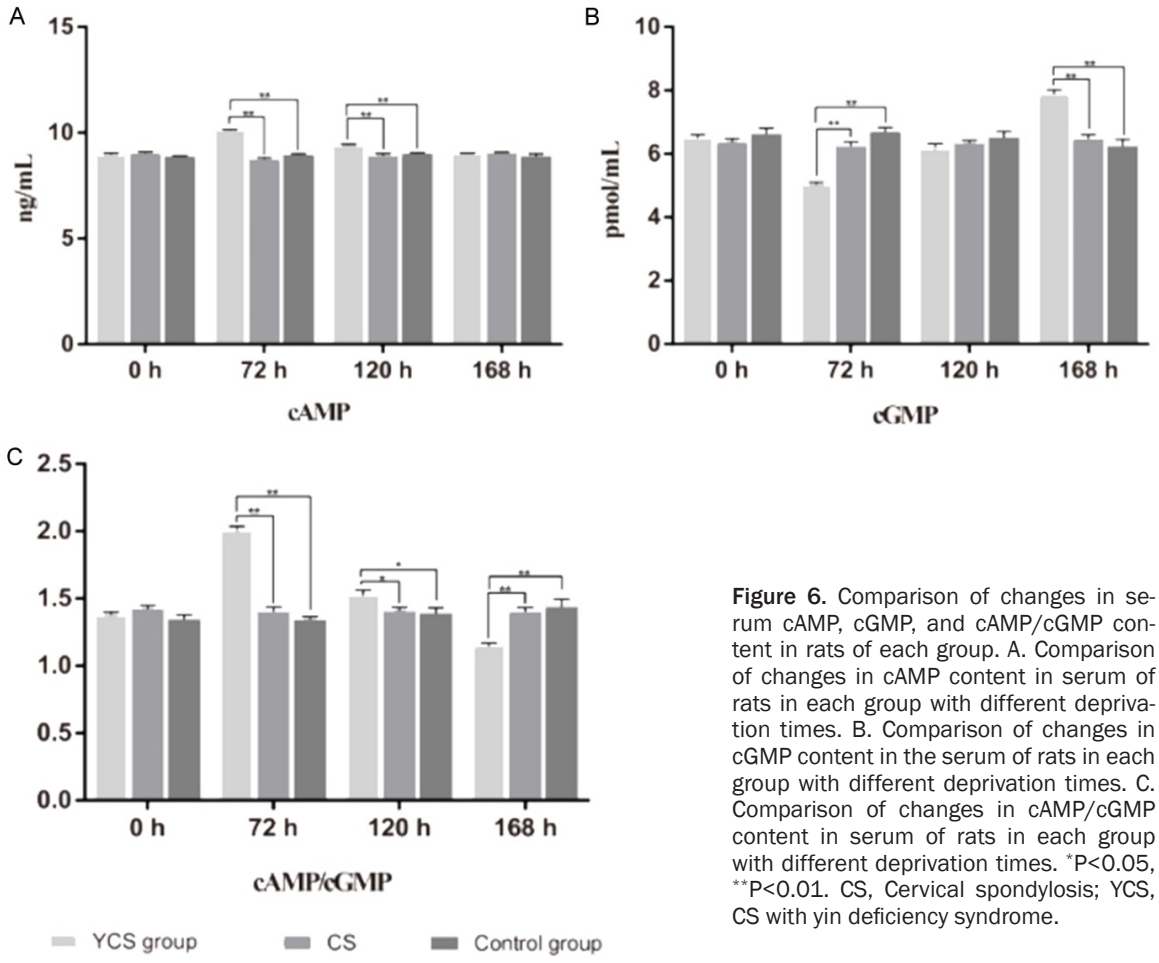

Figure 6. Comparison of changes in serum cAMP, cGMP, and cAMP/cGMP content in rats of each group. A. Comparison of changes in cAMP content in serum of rats in each group with different deprivation times. B. Comparison of changes in cGMP content in the serum of rats in each group with different deprivation times. C. Comparison of changes in cAMP/cGMP content in serum of rats in each group with different deprivation times. *P<0.05, **P<0.01. CS, Cervical spondylosis; YCS, CS with yin deficiency syndrome.

(P<0.01). Bax levels were consistently higher in the YCS group than in the CS group at all observed time points (**Figure 9D**).

The Bcl-2/Bax ratio, which reflects the balance between anti- and pro-apoptotic signals, was highest in the control group (P<0.01). In the YCS group, the ratio decreased progressively at 72 h, 120 h, and 168 h of sleep deprivation, with values significantly lower than those in the CS group (all P<0.05) (**Figure 9E**).

Discussion

Significant progress has been made in developing animal models of CS. Among these, the model based on cervical dynamic imbalance induced by cutting and separating the posterior cervical muscles and ligaments is well-established, reproducible, and widely used [7, 13, 14]. Previous studies have confirmed the reliability and feasibility of this model. Compared to the control group, the CS group demonstrated significantly lower Miyamoto grading values, increased apoptosis, and reduced mechanical pain thresholds. In recent years, TCM has demonstrated unique advantages in the treatment of CS [15, 16]. However, the integration of specific TCM syndrome type evidence into CS models remains underexplored.

Yin deficiency is a distinctive concept in TCM [17]. It arises from insufficient rest, overwork, or prolonged illness, manifesting as symptoms such as insomnia, hot flashes, and fatigue. Yin deficiency syndrome is associated with more severe and persistent CS symptoms [18]. Clinical studies indicate that TCM therapies focusing on nourishing yin and calming the mind are particularly effective in managing these symptoms [19]. Based on this, this study attempted to establish a rat model of CS with yin deficiency through sleep deprivation.

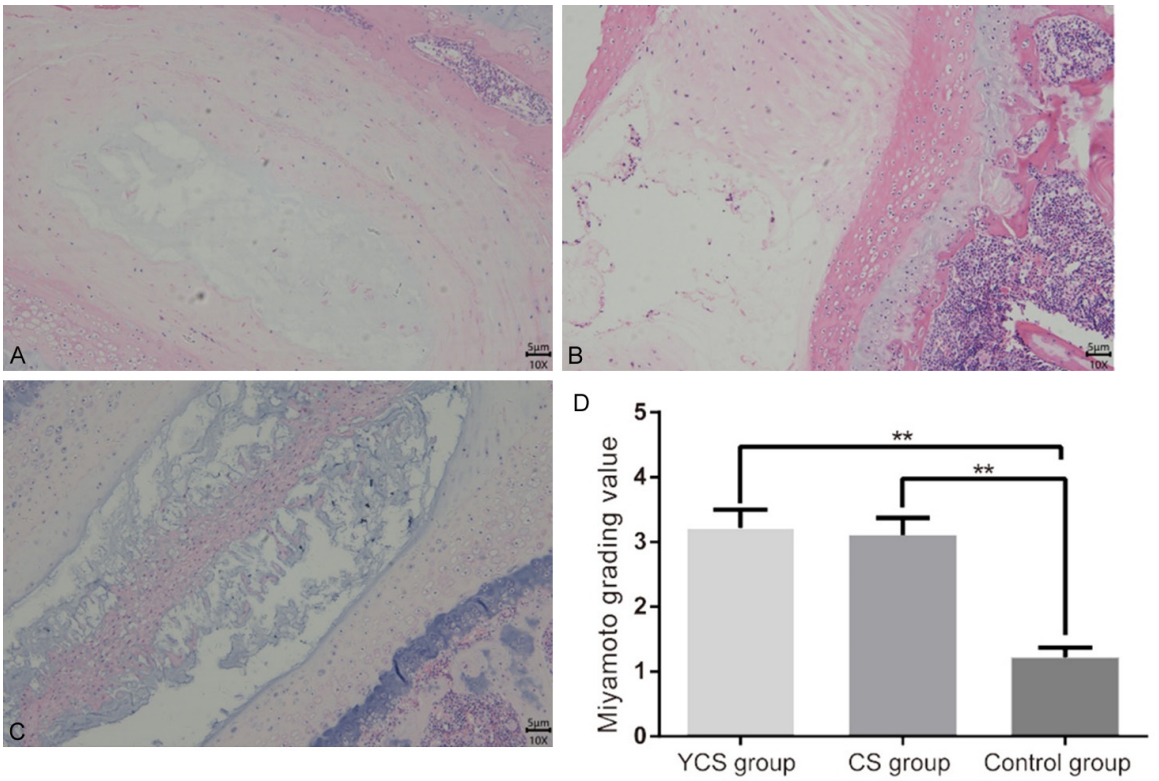

Figure 7. Morphological observation of rat cervical intervertebral disc tissue. A-C. The figures of HE staining results of intervertebral disc tissues of rats in the YCS group, CS group, and control group, respectively (HE staining, 40×, scale bar shows 5 μm). D. Comparison of Miyamoto grading values in each group of rats. **P<0.01. CS, Cervical spondylosis; YCS, CS with yin deficiency syndrome.

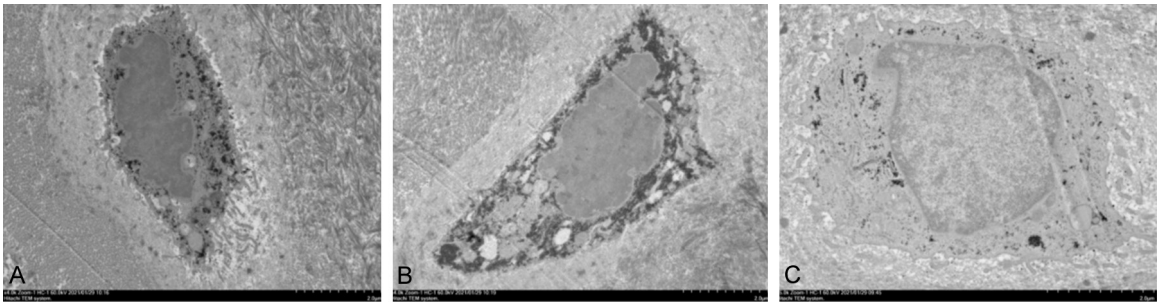

Figure 8. Ultrastructure of the nucleus pulposus in each group of rats under transmission electron microscopy. A-C. Transmission electron microscopy figures of the ultrastructure of the nucleus pulposus in YCS, CS, and control rats, respectively. CS, Cervical spondylosis; YCS, CS with yin deficiency syndrome.

Numerous evaluation criteria have been established for Yin deficiency syndrome models, including behavioral observations, biomarkers, and factors derived from proposed hypotheses [20, 21]. In this study, the YCS group exhibited significantly reduced body weight compared to the CS and control groups at 72-168 h of sleep deprivation. Before the sleep intervention, behavioral characteristics of the YCS group were normal. However, after 72 h of sleep deprivation, the YCS group showed irritability, dehydration, and signs of internal heat, aligning with the clinical symptoms of Yin deficiency. Prolonged sleep deprivation led to a transition from pure Yin deficiency symptoms to a combination of Yin and Yang deficiency symptoms.

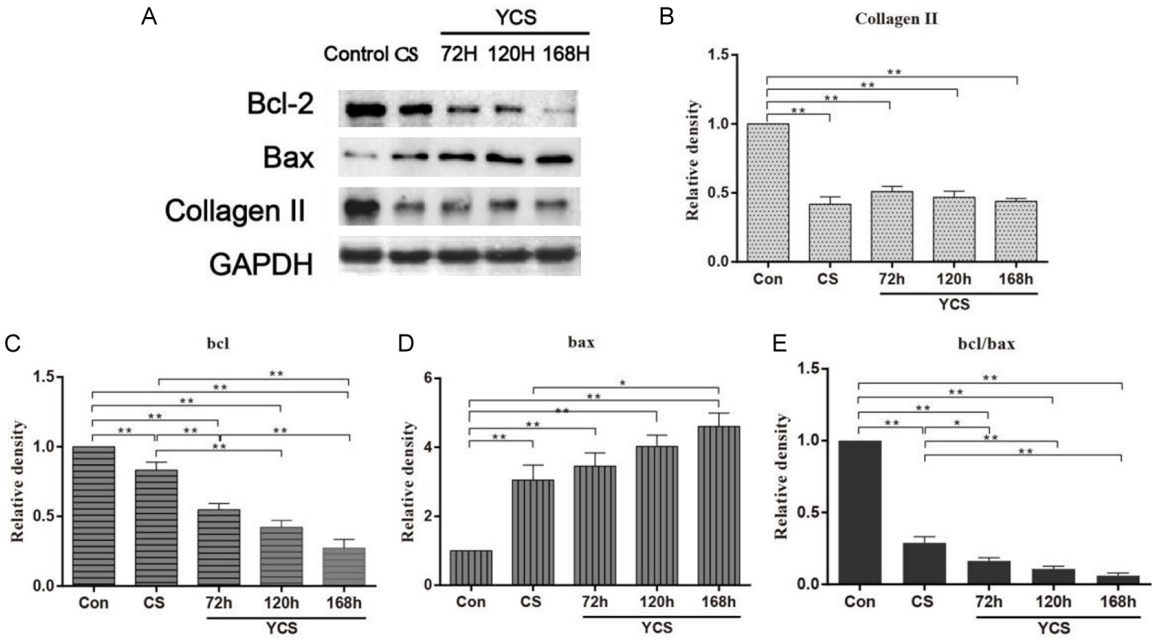

Figure 9. Comparison of the expression levels of collagen-II, Bcl-2, and Bax and Bcl-2/Bax in the cervical intervertebral disc tissues of rats in each group. A. Protein electrophoretic banding of collagen-II, Bcl-2, Bax, and Bcl-2/Bax in cervical disc tissues of rats in various groups. B-E. Comparative results of protein expression levels of collagen-II, Bcl-2, and Bax and Bcl-2/Bax in cervical intervertebral disc tissues of rats in each group, in turn. $^{*}P<0.05$, $^{**}P<0.01$. CS, Cervical spondylosis; YCS, CS with yin deficiency syndrome.

In TCM, pulse characteristics - including speed, strength, and depth - are key to diagnosing and categorizing conditions [22]. Yin deficiency syndrome is typically associated with a fine and rapid pulse. Although rat models cannot directly replicate these measurements, heart rate and blood pressure can serve as analogs for pulse characteristics [23]. An increased heart rate indicates a faster pulse, while lower blood pressure corresponds to a finer pulse. Our findings were consistent with this hypothesis. At 72 h of sleep deprivation, rats exhibited increased heart rates and decreased blood pressure, followed by continuous reductions in both parameters as sleep deprivation progressed.

cAMP and cGMP are critical second messengers that regulate cellular functions through antagonistic interactions, akin to the Yin-Yang theory in TCM [24]. This concept was supported by Goldberg's "Yin-Yang hypothesis of biological regulation" based on cAMP and cGMP [25]. Studies have shown that patients with Yin deficiency exhibit elevated cAMP levels and an increased cAMP/cGMP ratio, whereas those with Yang deficiency show reduced ratios [26]. In this study, ELISA results indicated significantly higher cAMP levels in the YCS group at 72 h of sleep deprivation compared to the CS and Control groups. By 168 h, cAMP/cGMP levels in the YCS group had decreased, suggesting a shift from Yin deficiency symptoms to combined Yin-Yang deficiency. These findings were consistent with the observed clinical signs in the rats.

Neck pain is a hallmark symptom of cervical spondylosis and a critical indicator of its severity [27]. Yin deficiency syndrome significantly decreased the nociceptive threshold in the YCS group compared to the CS group. Prolonged sleep deprivation further decreased the nociceptive threshold in the YCS group, mirroring the severe nocturnal pain experienced by cervical spondylosis patients with Yin deficiency. Furthermore, apoptosis of intervertebral disc cells was more pronounced in the YCS group, although changes in collagen-II levels were minimal. This suggests that Yin deficiency exacerbates cellular apoptosis rather than structural degradation of the intervertebral discs.

This study compared the Yin deficiency syndrome cervical spondylosis model with the conventional cervical spondylosis model in terms of behavior, heart rate, blood pressure, mechanical pain threshold, disc degeneration, and

protein expression. The findings align closely with clinical observations, making this model a valuable tool for exploring cervical spondylosis pathogenesis and evaluating potential treatments. There are some limitations to this study. Despite efforts to replicate human cervical spondylosis and Yin deficiency syndrome, physiological and pathological differences between rats and humans remain. Additionally, the sample size was relatively small, and future studies with larger cohorts are necessary to enhance the reliability and generalizability of the results. In conclusion, we successfully established rat models of CS with Yin deficiency syndrome that closely mimic the clinical characteristics described in TCM. These models offer a reliable platform for investigating the pathogenesis of CS with Yin deficiency syndrome and evaluating therapeutic interventions.

Acknowledgements

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Disclosure of conflict of interest

None.

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