

# Study on the Mechanism of Zhuang Medicine Longzuan Tongbi Decoction in Regulating Toll-like Receptor Signaling Pathway to Intervene in the Proliferation of Fibroblast-like Synoviocytes

Estudio sobre el Mecanismo de la Decocción de Longzuan Tongbi de la Medicina Zhuang en la Regulación de la Vía de Señalización del Receptor Tipo Toll para Intervenir en la Proliferación de Sinoviocitos Similares a Fibroblastos

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**SUMMARY:** This study explores the regulatory effects of Zhuang medicine Longzuan Tongbi Decoction on the Toll-like receptor (TLR) signaling pathway, aiming to identify new methods for preventing and treating rheumatoid arthritis (RA). Sixty Wistar rats were divided into six groups: normal, model, methotrexate (MTX), and high, medium, and low-dose decoction groups. RA models were induced with a mixture of bovine type II collagen and incomplete Freund's adjuvant. The intervention groups were treated for 28 days, with body weight and toe volume measured throughout. Pathological changes in the ankle joints were evaluated through HE staining, while enzyme-linked immunosorbent assay (ELISA) and Western blot analysis were used to detect TLR4, FOS, JUN, and CCL5 expression levels in serum and synovial tissues. Additionally, real-time PCR assessed mRNA levels of these markers. Primary fibroblast-like synoviocytes (FLS) from RA models were cultured with medicated serum, and proliferation and apoptosis were analyzed. Results showed that compared to the model group, the decoction groups exhibited reduced synovial hyperplasia, joint damage, and expression of TLR4, FOS, JUN, and CCL5. *In vitro*, the decoction inhibited FLS proliferation and reduced key protein and mRNA levels. The findings suggest that Longzuan Tongbi Decoction may inhibit abnormal FLS proliferation and mitigate joint pathology by modulating the TLR signaling pathway, highlighting its potential therapeutic effects in RA.

**KEY WORDS:** Zhuang medicine Longzuan Tongbi decoction; Toll-like receptor signaling pathway; Fibroblast-like synoviocytes; Rheumatoid arthritis.

## INTRODUCTION

Rheumatoid Arthritis (RA) is a systemic autoimmune disease that primarily affects the synovial joints, leading to chronic synovitis, which eventually causes joint destruction and loss of function. The pathological mechanism of this disease involves interactions between various types of cells, among which fibroblast-like synoviocytes (FLS) play a crucial role. These cells exhibit an aggressive phenotype, producing inflammatory mediators that lead to synovitis and cause damage to

cartilage and bone (Mousavi *et al.*, 2021). Currently, Western medicine does not have a specific treatment or drug for RA. Most treatments involve a combination of anti-inflammatory, analgesic, and immunosuppressive therapies to control the condition. However, the cure rate is low, and the disease is prone to relapse. Long-term medication use also results in more adverse effects (Tang *et al.*, 2021). With ongoing research and exploration, increasing data suggest that herbal formulations may be

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more effective in treating RA. Zhuang medicine Longzuan Tongbi Decoction, formulated by renowned Guangxi TCM physician Professor Pang Yuzhou, is based on Zhuang medical theory and is targeted at the etiology and pathogenesis of "exuberant pathogenic factors." This decoction has the effects of dispelling wind and cold, eliminating dampness and toxins, promoting blood circulation, and clearing the meridians. It expels pathogens, unblocks the stagnation in the dragon, fire, and grain paths, and restores the synchronous operation of the three energies—heaven, human, and earth—thereby achieving therapeutic effects. Previous clinical studies have shown that the effectiveness of Zhuang medicine Longzuan Tongbi Decoction in treating RA patients reaches 85.0 % (Pang *et al.*, 2013). Comprehensive analysis of domestic and international literature and network pharmacology methods has found that the active ingredients of Zhuang medicine Longzuan Tongbi Decoction are closely related to target genes such as TLR4, FOS, JUN, and CCL5 in the pathogenesis of RA. These genes are key factors in the Toll-like receptor signaling pathway that regulate FLS proliferation and intervene in the disease signaling pathway of RA (Huang *et al.*, 2019). Based on this, our research team has employed methods such as Western Blot, qPCR, and Elisa to detect the expression of TLR4, FOS, JUN, and CCL5, which are closely related to the Toll-like receptor signaling pathway, in the serum and synovial tissue of RA rats after intervention with Zhuang medicine Longzuan Tongbi Decoction. This provides more real and reliable data for further exploration of the bone-protective mechanism of Zhuang medicine Longzuan Tongbi Decoction.

## MATERIAL AND METHOD

**Instruments.** PV-200 Toe Volume Measuring Instrument (Chengdu Taimeng Software Co., Ltd.); ST16R High-Speed Refrigerated Centrifuge (ThermoFisher, USA); MIR-154L-PC Low-Temperature Incubator (PHcbi, Japan); JX-CL Automatic Sample Freezing Grinder (Shanghai Jingxin Industrial Development Co., Ltd.); DM2500 Research-grade Microscope (Leica, Germany); Synergy H1 Multi-Mode Microplate Reader (BioTek, USA); ES-60C Constant Temperature Incubator Shaker (Hangzhou MIO Instrument Co., Ltd.); Chemidoc CD Touch Chemiluminescent Imaging System (BioRad, USA); 1658001 Mini Vertical Electrophoresis Cell (BioRad, USA); EasyCycler Gradient 96 PCR System (Analytikjena, Germany); LightCycler® 96 PCR Instrument (Roche, Switzerland).

**Drugs and Reagents.** Zhuang medicine Longzuan Tongbi Decoction consists of the main herbs *Toddalia asiatica* (TA), *Kadsura coccinea* (KC), *Alangium chinense* (AC), *Sinomenium acutum* (SA), *Bauhinia championii* (BC),

*Spatholobus suberectus* (SS), *Zanthoxylum nitidum* (ZN), and *Ficus hirta* Vahl (FHV). Longzuan Tongbi Granules were produced by Beinianle Pharmaceutical Factory affiliated with Guangxi University of Chinese Medicine (Batch No. 20180508); Methotrexate tablets were produced by Tonghua Maoxiang Pharmaceutical Co., Ltd. (MTX, 2.5 mg/tablet, Batch No. H2202267); Bovine type II collagen and incomplete Freund's adjuvant (IFA) were purchased from Sigma.

**Animals.** Sixty SPF-grade male Wistar rats, weighing approximately 170-230 g, were provided by the Animal Experiment Center of Guangxi Medical University. The rats were housed in the SPF-grade animal experiment facility of our institution at a temperature of 20-25 °C and humidity of 40 %-60 %. After one week of adaptive feeding, they were randomly divided into a blank group, model group, MTX group, low-dose Zhuang medicine Longzuan Tongbi Decoction (referred to as Zhuang Decoction) group, medium-dose Zhuang Decoction group, and high-dose Zhuang Decoction group, with 10 rats in each group (El-Tedawy *et al.*, 2020).

## Methods

**Animal Model Preparation.** Except for the blank group, the remaining 50 rats were used to establish CIA models following the method described in reference (Gertel *et al.*, 2015). A 4 mg/L solution of bovine type II collagen was mixed on ice with an equal volume of 1g/L incomplete Freund's adjuvant to prepare an emulsion. On the day of immunization, 0.2 ml of the emulsion was subcutaneously injected into the tail base of the rats in the model group, while 0.2 ml of physiological saline was injected into the tail base of the rats in the blank group. A booster immunization was administered one week later.

**Drug Administration.** Starting from the 10th day after successful modeling, the high, medium, and low-dose Zhuang Decoction groups were given the Zhuang medicine Longzuan Tongbi Decoction (granules) in solutions of 1.08 g/100g-d-1, 0.54 mg/100g-d-1, and 0.27 mg/100g-d-1, respectively, by oral gavage. The MTX group was given 0.9 mg·kg<sup>-1</sup> based on the adult dosage standard in the 2020 edition of the "Chinese Pharmacopoeia," adjusted for rats using a surface area ratio (1:6.3). The model and blank groups were given an equal volume of distilled water by oral gavage. The gavage volume was calculated as 1 mL/100 g, and administration continued for 28 days (Qin *et al.*, 2023).

**General Observation of Rats.** The general conditions of the normal and model rats, including diet, urination and defecation, emotional changes, activity levels, coat glossiness, and limb alterations, were observed.

**Sample Collection Time and Methods.** Blood: After drug administration, rats were anesthetized, and blood was collected from the abdominal aorta to separate the serum, which was stored at -80 °C for future use. Synovium: After blood collection, the rats were euthanized, the ankle joint cavity was opened, and the synovial tissue was extracted and frozen at -80°C (El-Tedawy *et al.*, 2020).

**Arthritis Evaluation.** Arthritis evaluation was mainly performed using the Wood's arthritis index scoring method. Specific details are as follows: damage to each paw was graded on a scale of 0-4, with a total score calculated for all four paws. Normal = 0 points; redness and swelling in one digit = 1 point; mild redness and swelling in two or more digits or the entire paw = 2 points; severe redness and swelling of the paw = 3 points; severe redness, swelling, and lack of elasticity in the paw = 4 points (Kaur *et al.*, 2023).

**HE Staining to Observe Synovial Pathological Thickening in the Ankle Joint.** The ankle joint tissue was fixed in 4 % paraformaldehyde solution for 48 h, and then transferred to a decalcification solution. After routine embedding, sectioning, drying, deparaffinization, and HE staining, the pathological sections of the ankle joint synovium in each group of rats were observed under a light microscope. The pathological scores of the tissue were based on overall observations of cartilage damage, bone damage, inflammatory cell infiltration, synovial hyperplasia, and pannus, with scores ranging from 0 to 3 (0=normal, 1=mild, 2=moderate, 3=severe) (Kaur *et al.*, 2023).

**ELISA to Detect TLR4, FOS, JUN, and CCL5 Levels in Rat Serum.** ELISA was performed according to the steps provided in the ELISA kit, with absorbance measured at 450 nm using a microplate reader. A standard curve was constructed, and the sample concentrations were calculated based on the standard curve (Kaur *et al.*, 2023).

**Western Blot to Detect the Expression of TLR4, FOS, JUN, and CCL5 Proteins in Synovial Tissue.** Tissue proteins were extracted using strong RIPA lysis buffer, and protein concentration was adjusted with a BCA kit. After denaturation, the proteins were transferred onto a PVDF membrane. The membrane was blocked with 5 % skim milk for 1 hour, incubated overnight at 4 °C with primary antibodies (all 1:1000), washed with 1×TBST solution (5 min × 3 times), incubated with secondary antibodies (1:20000) at room temperature for 1 hour, washed with 1×TBST (5 min × 3 times), and then visualized. Image J software was used to read the relative levels of target proteins (Curson *et al.*, 2023).

**Real-Time Quantitative PCR (qPCR) to Detect TLR4 mRNA, FOS mRNA, JUN mRNA, and CCL5 mRNA Levels in Synovial Tissue.** RNA was extracted from tissue using Trizol, and RNA concentration was measured with a UV spectrophotometer before being reverse transcribed into cDNA. Primers for the reference gene GAPDH and the genes TLR4, FOS, JUN, and CCL5 were designed based on NCBI data, as shown in **Table I**. The RT-PCR reaction program was as follows: 95 °C for 30 s of pre-denaturation; 95 °C for 10 s, 60 °C for 30 s, for a total of 40 cycles; melting curve analysis followed the default instrument settings. The relative expression levels of the target genes' mRNA were calculated using the 2<sup>-ΔΔCt</sup> method, with GAPDH as the reference gene (Curson *et al.*, 2023).

Table I. List of RT-PCR Primers.

Name	Primer Sequence	Product Length
rat-TLR4-F	ACAGGGCACAAAGGAAGTAGC	149
rat-TLR4-R	GTTCTCACTGGGCCTTAGCC	
rat-FOS-F	GGTTTCAACGCGGACTACGA	140
rat-FOS-R	GCGCAA AAGTCCTGTGTGTT	
rat-JUN-F	GCCACCGAGACCGTAAAGAA	84
rat-JUN-R	TAGCACTCGCCCAACTTCAG	
rat-CCL5-F	ACTGAGCAACCCCCTACTCC	111
rat-CCL5-R	CCTGTGAAGAGCACACCTCC	
rat-gapdh-F	CTCTCTGCTCCTCCCTGTTC	90
rat-gapdh-R	TCACACCGACCTTCACCATC	

**Primary Cell Isolation and Culture.** Under sterile conditions, synovial tissue was rapidly extracted, washed with PBS to remove debris and blood, and immersed in 20 % FBS culture medium. The tissue fragments were evenly spread on the bottom of the culture flask with a pipette, and 2 ml of culture medium was added. After 6-7 h in the incubator, once the tissue fragments adhered to the surface, the culture medium was replaced every three days. When RA-FLS grew abundantly around the tissue fragments, the tissue was removed with a pipette, and the culture continued. When the cells reached 90 % confluency, 1 ml of trypsin was added to digest the RA-FLS cells, and routine passaging continued. Cells from passages 3-5 were used for subsequent experiments (Curson *et al.*, 2023).

**CCK-8 Assay to Detect RA-FLS Proliferative Activity.** RA-FLS cells in the logarithmic growth phase were observed under an inverted microscope. The culture medium was removed and the cells were washed twice with sterile 0.9 % saline. Then, 100 µl of sterile trypsin was added for digestion. When the cells became round and bright, 2 ml of 20 % fetal

bovine serum culture medium was added to stop the digestion. The cells were counted under a microscope, and a suspension with a cell concentration of  $1 \times 10^5/\text{ml}$  was prepared in the culture medium. The cell suspension was added to a 96-well plate, with 100  $\mu\text{l}$  per well. Each group had four replicates, and the surrounding wells were filled with sterile PBS as isolation wells. The plate was incubated in a 5 %  $\text{CO}_2$ , 37 °C incubator for 12 h. The culture medium was then replaced with 5 % fetal bovine serum pre-treatment for 24 h, followed by the addition of medicated serum of different concentrations. A blank control group was also set up, and incubation continued for 48 h. Before the end of the culture, 10  $\mu\text{l}$  of CCK-8 reagent was added to each well, mixed, and incubated in a 5 %  $\text{CO}_2$ , 37 °C incubator for an additional 2 h. The OD value of each well was measured at 490 nm using a microplate reader. The experiment was repeated three times, and the average value was taken (Yang *et al.*, 2022).

**TLR4, FOS, JUN, CCL5 Protein Expression Detection.** The expression of these proteins was detected using Western Blot. Cells growing to approximately 75 % confluency were divided according to the experimental design into a blank control group and Longzuan Tongbi Decoction groups (high, medium, and low doses). Different concentrations of Longzuan Tongbi Decoction were added to the Longzuan Tongbi Decoction groups, while the blank control group received normal rat serum. The cells were then incubated in a 37 °C, 5 %  $\text{CO}_2$  incubator for 24 h, after which the cells were harvested for further analysis. Protein extraction and detection followed the kit's procedures strictly (Yang *et al.*, 2022).

**Statistical Analysis.** Experimental data were processed using SPSS21.0 software. One-way ANOVA was used to analyze differences between groups. Data conforming to a normal distribution were expressed as mean  $\pm$  standard deviation ( $\bar{x} \pm s$ ). Differences were considered statistically significant at  $P < 0.05$ .

## RESULTS

### General Condition of Rats

**Pathological Changes in the Ankle Joints of Rats in Each Group.** As shown in **Figures 1 and 2A**, the ankle joints of the rats in the normal group showed no significant changes. In the model group, the ankle joints were noticeably swollen, with significant cartilage destruction, synovial thickening, and infiltration of inflammatory cells (Figs. 1 and 2B). In the MTX group, the ankle joints were swollen, the cartilage cells were irregularly arranged, synovial thickening was observed, and some inflammatory cell infiltration was present (Figs. 1 and 2C). In the LZTBL group, the ankle joints were significantly swollen, with cartilage destruction, synovial thickening, and partial inflammatory cell infiltration (Figs. 1 and 2D). In the LZTBM group, the ankle joints were swollen, the cartilage cells were irregularly arranged, synovial cell proliferation was observed, and partial inflammatory cell infiltration was present (Figs. 1 and 2E). In the LZTBH group, the ankle joints showed mild swelling, the cartilage cells were irregularly arranged, synovial cell proliferation was not evident, and there was no significant inflammatory cell infiltration (Figs. 1 and 2F).

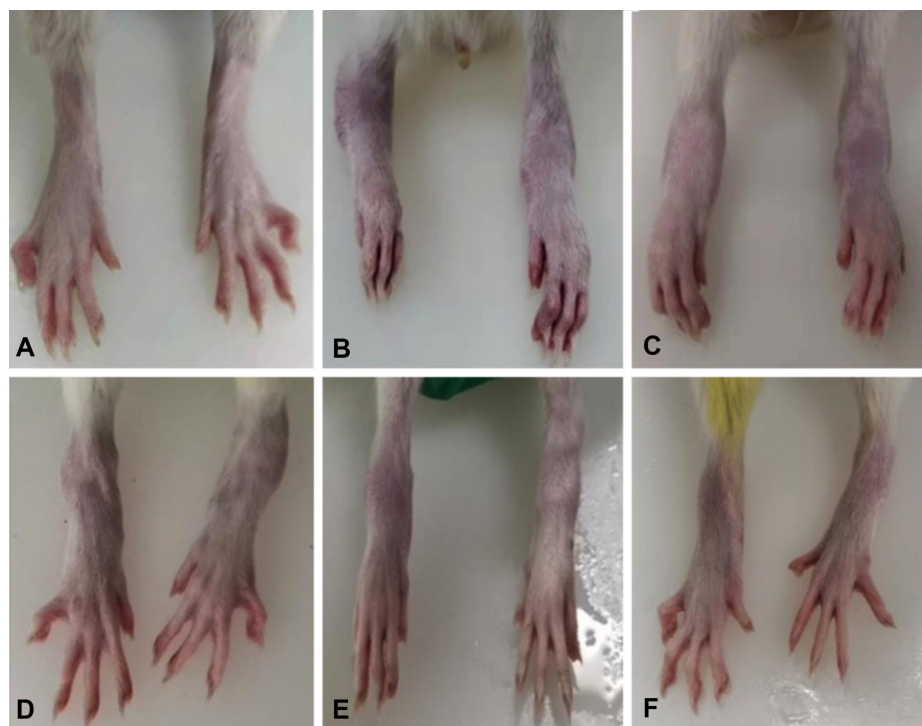


Fig. 1. Morphological changes in the ankle joints of rats in each group. Blank (A), Model (B), Low-dose (C), Medium-dose (D), High-dose (E), and MTX (F) groups.

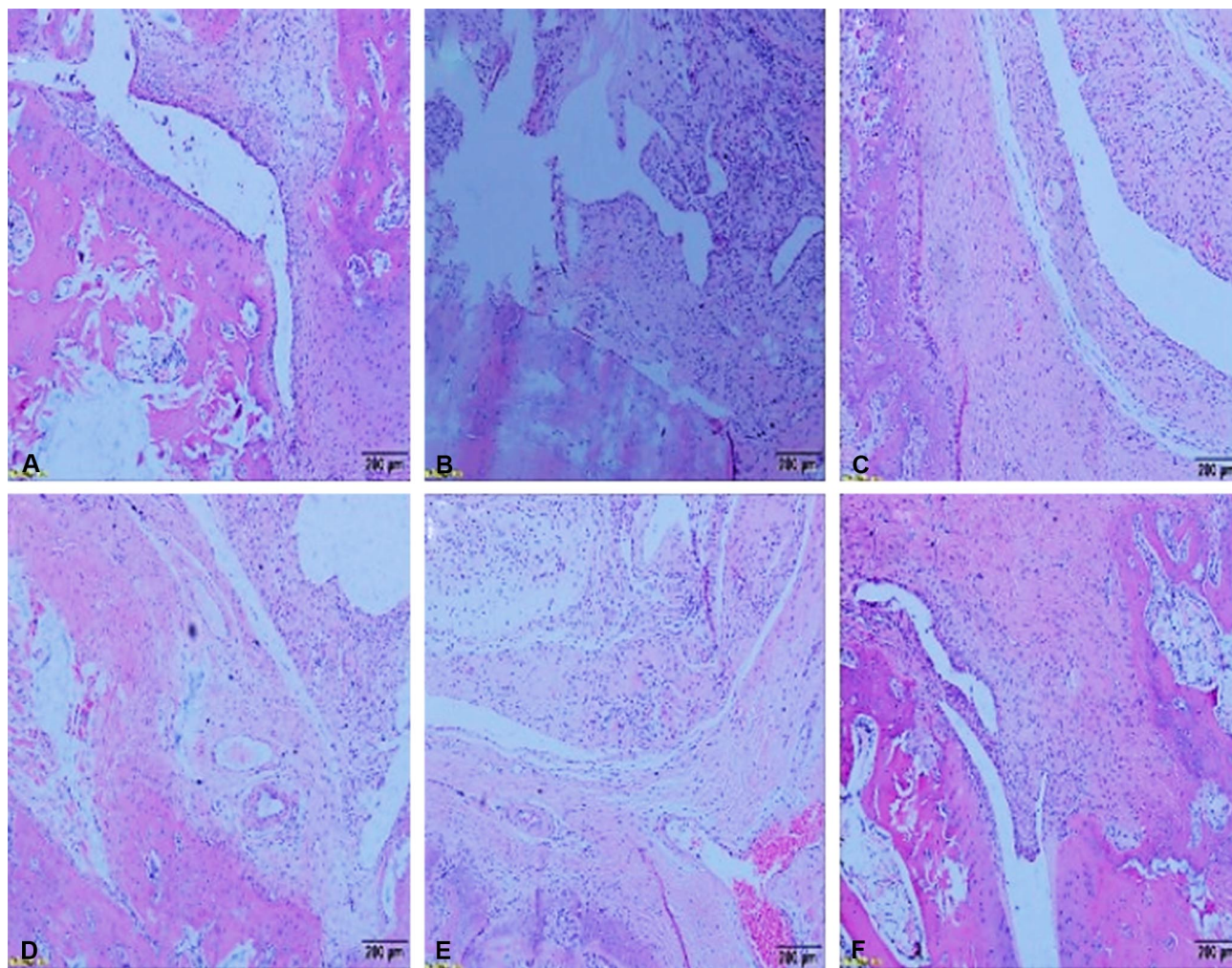


Fig. 2. Pathological changes in the ankle joints of rats in each group. Blank (A), Model (B), Low-dose (C), Medium-dose (D), High-dose (E), and MTX (F) groups.

### Effect of Longzuan Tongbi Decoction on the Levels of TLR4, FOS, JUN, and CCL5 in the Serum of CIA Rats.

As shown in Table II and Figure 3, compared with the blank group, the levels of TLR4, FOS, JUN, and CCL5 in the serum of CIA rats in the model group were significantly elevated ( $P < 0.05$ ). After 28 days of treatment, the LZTBL, LZTBM,

LZTBH groups, as well as the MTX group, exhibited a significant reduction in serum levels of TLR4, FOS, JUN, and CCL5 compared to the model group ( $P < 0.05$ ). Additionally, a dose-dependent trend was observed, with higher concentrations of Longzuan Tongbi Decoction leading to greater reductions in these serum biomarkers ( $P < 0.05$ ).

Table II. Effect of Longzuan Tongbi Decoction on the Expression of TLR4, FOS, JUN, and CCL5 Proteins in the Serum of CIA Rats ( $\bar{x} \pm s$ ,  $n=10$ ,  $\text{ng} \cdot \text{mL}^{-1}$ )

Group	CCL5 (ng/ml)	FOS (ng/ml)	JUN (ng/ml)	TLR4 (ng/ml)
Blank group	1.06±0.14	3.10±0.52	146.43±20.80	1.49±0.18
Model group	4.08±0.62	11.12±1.48	590.09±92.39	7.66±0.93
Low-dose group	3.17±0.40	8.15±1.17	457.05±55.82	6.28±0.83
Medium-dose group	2.22±0.27	6.45±0.87	320.98±41.10	3.85±0.48
High-dose group	1.41±0.18	4.01±0.57	212.23±26.28	2.09±0.25
MTX group	1.46±0.18	4.40±0.67	223.30±27.93	2.17±0.29

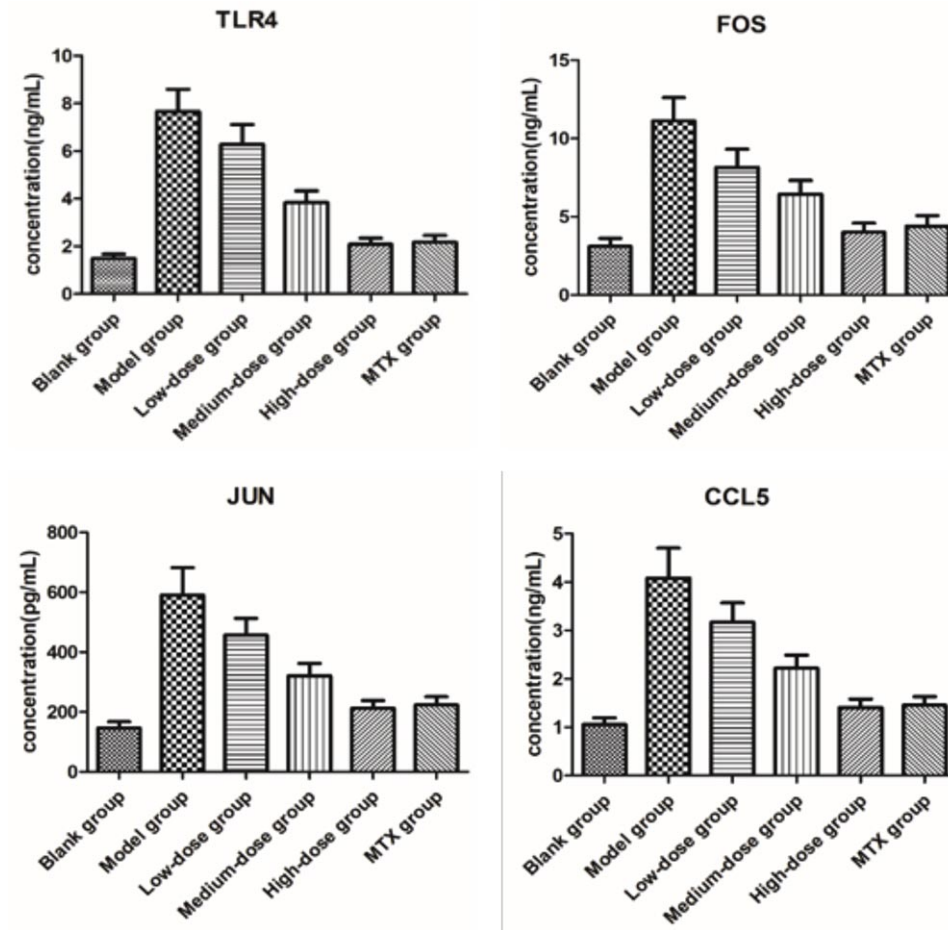


Fig. 3. Effect of Longzuan Tongbi Decoction on the Expression of TLR4, FOS, JUN, and CCL5 Proteins in the Serum of CIA Rats ( $\bar{x} \pm s$ , n=10)

**Effect of Longzuan Tongbi Decoction on the Expression of TLR4, FOS, JUN, and CCL5 Proteins in the Synovial Tissue of CIA Rats.** As shown in Table III and in Figure 4, compared with the blank group, the expression levels of TLR4, FOS, JUN, and CCL5 proteins in the synovial tissue of CIA rats in the model group were significantly elevated ( $P < 0.05$ ). After 28 days of treatment, the LZTBL, LZTBM, LZTBH

groups, as well as the MTX group, exhibited significantly reduced expression levels of TLR4, FOS, JUN, and CCL5 proteins in synovial tissue compared to the model group ( $P < 0.05$ ). Additionally, a dose-dependent reduction in protein expression was observed, with higher concentrations of Longzuan Tongbi Decoction leading to greater decreases in TLR4, FOS, JUN, and CCL5 levels ( $P < 0.05$ ).

Table III. Effect of Longzuan Tongbi Decoction on the Expression of TLR4, FOS, JUN, and CCL5 Proteins in the Synovial Tissue of CIA Rats ( $\bar{x} \pm s$ , n=10)

Group	CCL5	FOS	JUN	TLR4
Blank group	0.48±0.05	0.26±0.03	0.34±0.05	0.30±0.05
Model group	1.45±0.18	0.92±0.11	0.94±0.11	0.98±0.11
Low-dose group	1.26±0.13	0.77±0.09	0.75±0.09	0.78±0.09
Medium-dose group	0.91±0.11	0.63±0.07	0.63±0.08	0.59±0.07
High-dose group	0.77±0.09	0.48±0.07	0.46±0.06	0.47±0.05
MTX group	0.79±0.09	0.50±0.06	0.47±0.06	0.50±0.07

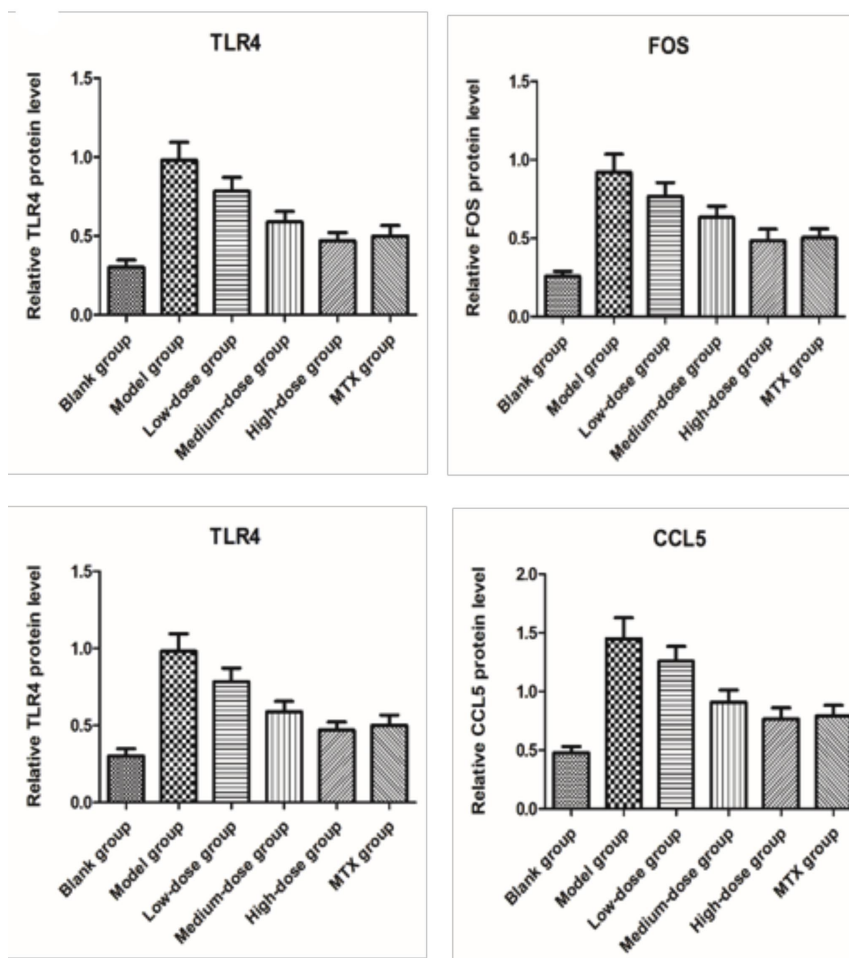
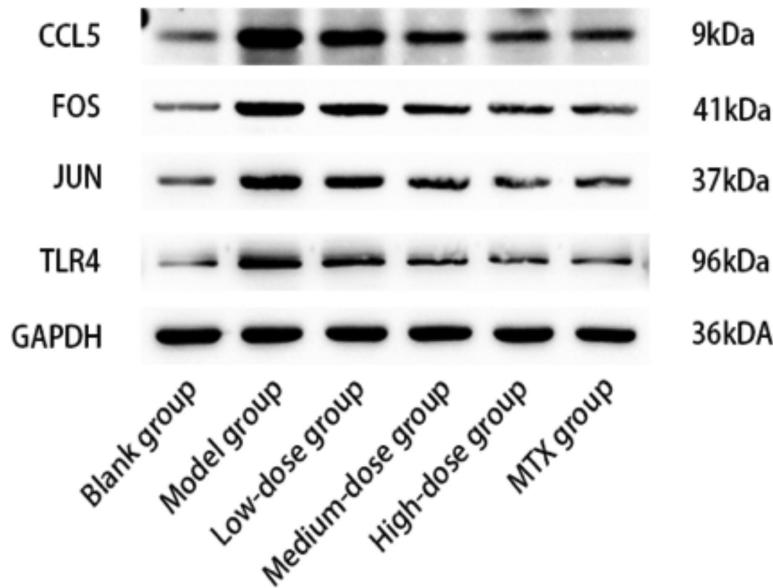


Fig. 4. Effect of Longzuan Tongbi Decoction on the Expression of TLR4, FOS, JUN, and CCL5 Proteins in the Synovial Tissue of CIA Rats ( $x \pm s$ ,  $n=10$ )

**Effect of Longzuan Tongbi Decoction on the Expression of TLR4, FOS, JUN, and CCL5 mRNA in the Synovial Tissue of CIA Rats.** As shown in Table IV and Figure 5, compared with the blank group, the expression levels of TLR4, FOS, JUN, and CCL5 mRNA in the synovial tissue of CIA rats in the model group were significantly elevated ( $P < 0.05$ ). After 28 days of treatment, the expression levels of TLR4, FOS, JUN, and CCL5 mRNA in the synovial tissue of rats in the LZTBL, LZTBM, LZTBH groups, as well as the MTX group, were significantly reduced compared to the model group. Furthermore, the mRNA expression levels of TLR4, FOS, JUN, and CCL5 showed a decreasing trend with increasing concentrations of Longzuan Tongbi Decoction ( $P < 0.05$ ).

**Effect of Medicated Serum on the Expression of TLR4, FOS, JUN, and CCL5 in Fibroblast-Like Synoviocytes of CIA Rats.** As shown in Table V and Figure 6, compared with the blank group, the expression levels of TLR4, FOS, JUN, and CCL5 mRNA in the synovial tissue of CIA rats in the model group were significantly elevated ( $P < 0.05$ ). After 28 days of treatment, the expression levels of TLR4, FOS, JUN, and CCL5 mRNA in the synovial tissue of rats in the LZTBL, LZTBM, LZTBH groups, as well as the MTX group, were significantly reduced compared to the model group. Furthermore, the mRNA expression levels of TLR4, FOS, JUN, and CCL5 showed a decreasing trend with increasing concentrations of Longzuan Tongbi Decoction ( $P < 0.05$ ).

Table IV. Effect of Longzuan Tongbi Decoction on the Expression of TLR4, FOS, JUN, and CCL5 mRNA in the Synovial Tissue of CIA Rats ( $\bar{x} \pm s$ , n=10)

Group	CCL5	FOS	JUN	TLR4
Blank group	1.01±0.12	1.01±0.11	1.01±0.12	1.01±0.16
Model group	2.53±0.36	3.39±0.40	2.51±0.38	3.57±0.49
Low-dose group	2.06±0.24	2.66±0.35	2.00±0.28	2.91±0.33
Medium-dose group	1.70±0.22	1.96±0.25	1.58±0.21	2.17±0.28
High-dose group	1.32±0.17	1.45±0.17	1.24±0.17	1.62±0.20
MTX group	1.36±0.15	1.49±0.20	1.28±0.12	1.76±0.25

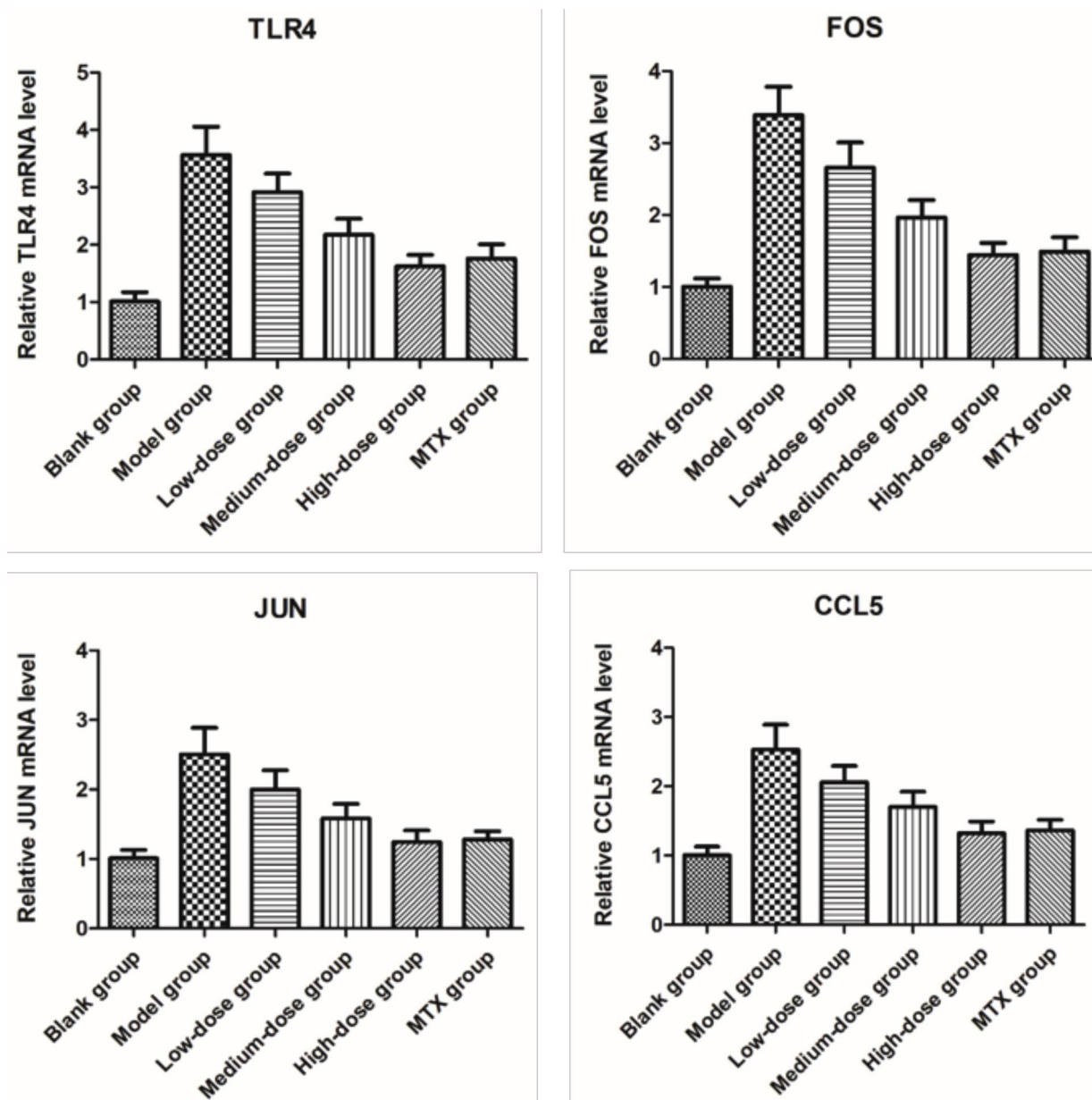


Fig. 5. Effect of Longzuan Tongbi Decoction on the Expression of TLR4, FOS, JUN, and CCL5 Proteins in the Synovial Tissue of CIA Rats ( $\bar{x} \pm s$ , n=10)



Tabla V. Effect of Medicated Serum on the Expression of TLR4, FOS, JUN, and CCL5 in Fibroblast-Like Synoviocytes of CIA Rats ( $\bar{x} \pm s$ , n=10).

Group	CCL5	FOS	JUN	TLR4
Control group	1.49±0.19	0.93±0.11	1.07±0.13	1.34±0.15
Low-dose group	1.16±0.14	0.63±0.08	0.81±0.10	0.83±0.11
Medium-dose group	0.91±0.12	0.48±0.07	0.61±0.08	0.57±0.07
High-dose group	0.65±0.08	0.29±0.03	0.40±0.05	0.38±0.04
MTX group	0.67±0.08	0.31±0.04	0.42±0.05	0.40±0.05

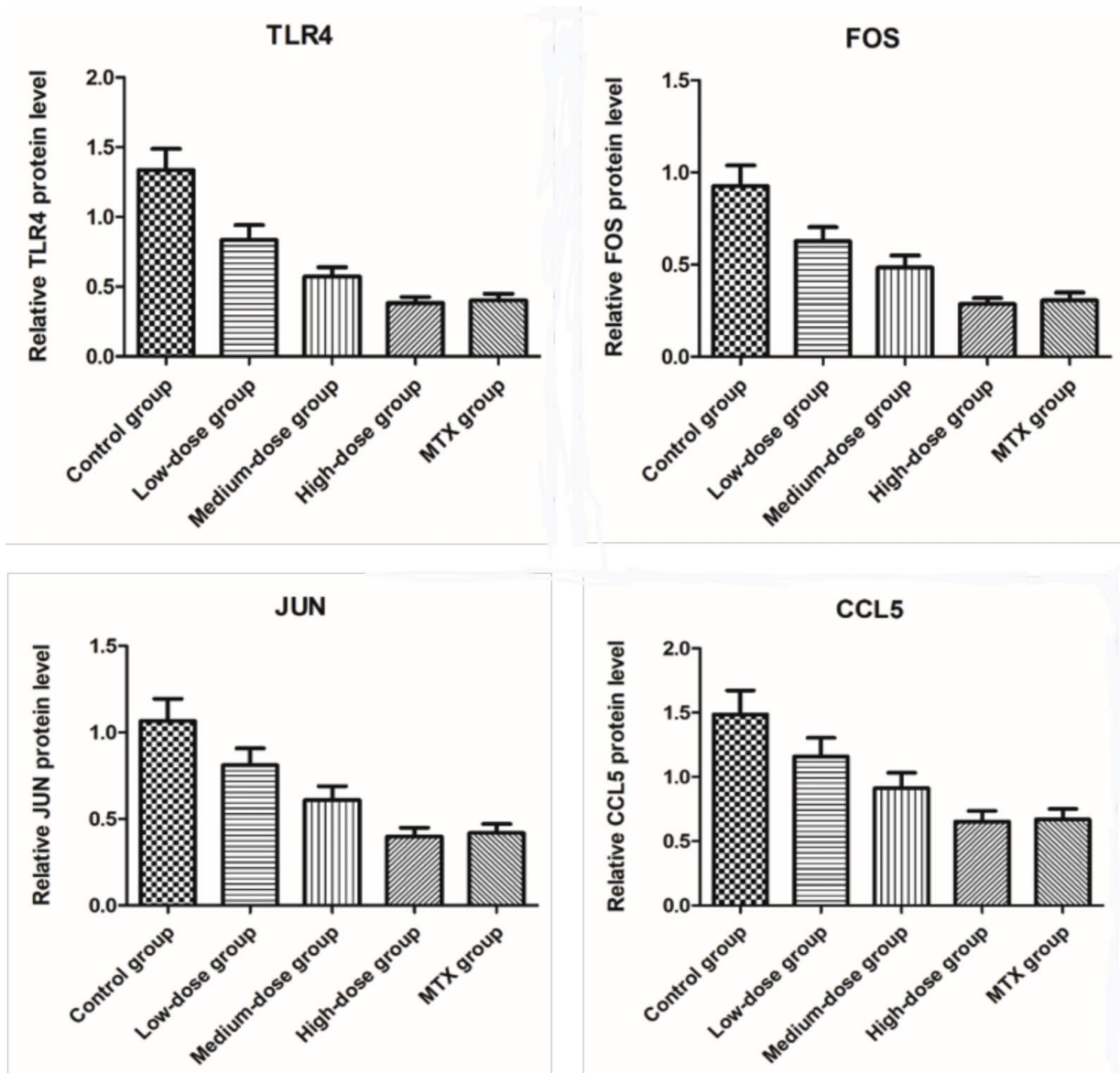


Fig. 6. Effect of Medicated Serum on the Expression of TLR4, FOS, JUN, and CCL5 in Fibroblast-Like Synoviocytes of CIA Rats ( $\bar{x} \pm s$ , n=10).

## DISCUSSION

Rheumatoid arthritis (RA) is a common systemic immune disease in rheumatology, with a complex etiology linked to factors such as environmental changes, infections, and immune system dysfunction. The disease has a high incidence rate, with women being more affected than men, and it manifests with symptoms such as joint swelling, significant pain, and restricted movement. As the disease progresses, it can lead to severe complications such as pleuritis and pericarditis, reducing quality of life and threatening life (Topless *et al.*, 2021). Currently, the primary clinical treatment for RA is Western medicine, but the effectiveness of Western medicine alone is not significant and has many side effects. With the deepening research on traditional Chinese medicine (TCM) and ethnic medicine in the field of RA, an increasing number of TCM treatments have demonstrated unique advantages (Liu *et al.*, 2014). In Zhuang medicine, the pathogenesis of RA is essentially viewed as "network obstruction causing paralysis," often due to the invasion of pathogenic factors such as wind, cold, dampness, and heat, which block the "Dragon Road" and "Fire Road" networks, leading to poor circulation of qi and blood and causing pain in the muscles, bones, and joints. In Zhuang medicine, this condition is referred to as "Gunk," which falls under the category of "Flourishing Disease." Treatment should focus on "expelling pathogens and detoxifying, and unblocking the two roads" to synchronize the three energies of the human body and achieve a state of "yin-yang harmony" (Wang *et al.*, 2022). Longzuan Tongbi Decoction, a Zhuang medicine formula, is an experienced prescription for treating RA. Has found that the total alkaloids in *Toddalia asiatica* (TA), *Alangium chinense* (AC), *Zanthoxylum nitidum* (ZN), and *Spatholobus suberectus* (SS); the total flavonoids in *Sinomenium acutum* (SA) and *Bauhinia championii* (BC); and the volatile oils and lignans in *Zanthoxylum nitidum* (ZN) all have anti-inflammatory and analgesic effects (Zhang *et al.*, 2012). Therefore, Longzuan Tongbi Decoction mainly treats RA through anti-inflammatory, analgesic, and immune-regulating effects (Yao *et al.*, 2022).

There are 10 types of TLRs in humans, with 8 of them present in joint synovial cells. The expression levels of TLR2, TLR3, TLR4, and TLR5 are elevated in RA synovium and are involved in regulating the inflammatory cytokines of RA synovial cells, exacerbating joint inflammation (Luo *et al.*, 2020). The worsening of joint inflammation further stimulates the abnormal proliferation and activation of FLS, leading to erosion of the joint cartilage. Studies have shown that in the disease signaling pathways of RA, TLR2/4 in synovial cells, under the regulation of the Toll-like receptor signaling pathway (hsa04620), synthesize

factors such as FOS, JUN, and TNFSF11, which further secrete factors like IL6, IL-1b, CCL5, and CSF2, exacerbating joint inflammation and inducing the abnormal proliferation and activation of FLS. This can form a "Toll-like receptor signaling pathway—synovium—bone" axis.

## CONCLUSION

In conclusion, Zhuang medicine Longzuan Tongbi Decoction exerts its bone-protective effect by targeting TLR4, FOS, JUN, and CCL5, regulating the "Toll-like receptor signaling pathway—synovium—bone" axis, and intervening in the proliferation of fibroblast-like synoviocytes.

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**LU, X.; WEI, S.; WU, L.; LIAO, L.; NONG, Y. & HUANG, A.** Estudio sobre el mecanismo de la Decocción de Longzuan Tongbi de la medicina Zhuang en la regulación de la vía de señalización del receptor tipo Toll para intervenir en la proliferación de sinoviocitos similares a fibroblastos. *Int. J. Morphol.*, 43(1):99-109, 2025.

**RESUMEN:** Este estudio explora los efectos reguladores de la Decocción de Longzuan Tongbi de la medicina Zhuang en la vía de señalización del receptor tipo Toll (TLR), con el objetivo de identificar nuevos métodos para prevenir y tratar la artritis reumatoide (AR). Sesenta ratas Wistar se dividieron en seis grupos: normal, modelo, metotrexato (MTX) y grupos de decocción de dosis alta, media y baja. Los modelos de AR se indujeron con una mezcla de colágeno bovino tipo II y adyuvante incompleto de Freund. Los grupos de intervención fueron tratados durante 28 días, y se midió el peso corporal y el volumen de los dedos durante todo el tratamiento. Los cambios patológicos en las articulaciones del tobillo se evaluaron mediante tinción HE, mientras que el ensayo inmunoabsorbente ligado a enzimas (ELISA) y el análisis Western blot se utilizaron para detectar los niveles de expresión de TLR4, FOS, JUN y CCL5 en suero y tejidos sinoviales. Además, la PCR en tiempo real evaluó los niveles de ARNm de estos marcadores. Los sinoviocitos primarios similares a fibroblastos (FLS) de modelos de AR se cultivaron con suero medicado y se analizaron la proliferación y la apoptosis. Los resultados mostraron que, en comparación con el grupo modelo, los grupos de decocción exhibieron una hiperplasia sinovial reducida, daño articular y expresión de TLR4, FOS, JUN y CCL5. *In vitro*, la decocción inhibió la proliferación de FLS y redujo los niveles de proteínas clave y ARNm. Los hallazgos sugieren que la Decocción Longzuan Tongbi puede inhibir la proliferación anormal de FLS y mitigar la patología articular modulando la vía de señalización de TLR, lo que destaca sus posibles efectos terapéuticos en la AR.

**PALABRAS CLAVE:** Decocción de Longzuan Tongbi de la medicina Zhuang; Vía de señalización del receptor tipo Toll; Sinoviocitos similares a fibroblastos; Artritis reumatoide.

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