Anti-inflammatory Effect of Polysaccharide from *Embelia parviflora* Wall. on Rheumatoid Arthritis in Rats

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Abstract [**Objectives**] To investigate the anti-inflammatory effect of *Embelia parviflora* Wall. polysaccharide on rheumatoid arthritis (RA) in rats. [**Methods**] RA rat model was induced by type II collagen. After successful modeling, the rats were divided into model group, positive group, low, medium and high dose of *E. parviflora* Wall. polysaccharide groups, and normal control group. Body mass, toe volume and arthritis index were measured, and thymus index and spleen index were calculated. The levels of interleukin-1β, interleukin-6 and tumor necrosis factor-α in serum and synovial tissue of ankle joint were detected by ELISA. [**Results**] Compared with the normal control group, the pathological changes such as synovial hyperplasia and unclear layer were observed in the model group, the body mass was decreased ($P<0.05$), the toe volume, arthritis index, thymus and spleen index were increased ($P<0.05$), and the levels of IL-1β, IL-6 and TNF-α in serum and ankle synovial tissue were increased ($P<0.05$). Compared with the model group, the histopathological changes in synovium of ankle joint in the positive group and the medium and high dose groups of *E. parviflora* Wall. polysaccharide were significantly improved, and the body mass was increased ($P<0.05$). The toe volume, arthritis index, thymus index and spleen index were decreased ($P<0.05$). The levels of IL-Iβ, IL-6 and TNF-α in serum and synovial tissue of ankle joint were increased ($P<0.05$), while there was no significant difference between the low dose group of *E. parviflora* Wall. polysaccharide and the model group ($P>0.05$). [**Conclusions**] *E. parviflora* Wall. polysaccharide can reduce the body's inflammatory response and improve RA, which may be related to the inhibition of the activation of inflammatory cytokines IL-1β, IL-6 and TNF-α.

Key words Polysaccharide from *Embelia parviflora* Wall., Rheumatoid arthritis (RA), Anti-inflammatory effect

1 Introduction

Rheumatoid arthritis (RA) is a chronic, progressive autoimmune disease that primarily affects the joints, but may also affect multiple systems throughout the body[1]. RA is mainly characterized by symmetrical arthritis, often accompanied by joint swelling and morning stiffness[2], and it can induce the activation of inflammatory factors and the polarization of tissue macrophages[3]. RA is prevalent worldwide and affects people of all ages[4]. *Embelia parviflora* Wall. is the dry ground part of the *Embelia parviflora* Wall. of Dangguiteng of Myrsinaceae, also known as Xiaohua Suantengzi, Daliwang, *etc.* *E. parviflora* Wall. has the functions of relaxing muscles and tendons, activating collaterals, eliminating dampness and relieving pain. *E. parviflora* Wall. contains sugars, glycosides, flavonoids, saponins, organic acids, *etc.*[5-8]. Polysaccharides have anti-inflammatory, antioxidant, anticoagulant, and tumor cell growth inhibitory effects[9-10]. *E. parviflora* Wall. polysaccharide may improve RA by inhibiting the expression of inflammatory cytokines, but the specific role of *E. parviflora* Wall. polysaccharide in the process of RA has not been reported. Therefore, we intended to establish a rat model of RA and explore the effect of *E. parviflora* Wall. polysaccharide on RA and its possible mechanism.

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2 Materials and methods

2.1 Materials

2.1.1 Experimental animals. Sixty male SD rats of SPF grade, aged 8 weeks and weighing 300 - 350 g, were provided by the Laboratory Animal Center of Guangxi Medical University, with the animal production license number of SCXK Gui 2014-0002. Before the experiment, the rats were adaptively fed in a clean animal room for one week at an ambient temperature of (25 ± 2) ℃ and a humidity of about 65%. During the experiment, the rats were fed freely with standard rodent feed and purified water in strict accordance with the requirements of laboratory animal ethics.

2.1.2 Main reagents and instruments. Bovine type II collagen was purchased from American Chondrex Company. Complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA) were purchased from American Sigma Company. Tripterygium glycosides tablets were purchased from Zhejiang DND Pharmaceutical Co., Ltd. HE staining kit was purchased from Beijing Solarbio Science & Technology Co., Ltd. Rat interleukin-6 (IL-6), interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) ELISA kits were purchased from Shanghai Jonln Biotechnology

Co., Ltd. Automatic microplate reader was provided by American Thermo Fisher Scientific.

2.2 Methods

2.2.1 Animal model preparation and grouping. The rat model of RA was induced by type II collagen. The specific operation is as follows: Acetic acid was used as a solvent, bovine type II collagen was prepared into a solution with a concentration of 2 mg/mL, and the solution was mixed with CFA at a ratio of 1∶1 to prepare emulsion I, and is mixed with IFA at a ratio of 1∶1 to prepare emulsion II. On the first day, 0.1 mL of emulsion I was injected subcutaneously into the root of the tail of rats, and on the seventh day, 0.1 mL of emulsion II was injected subcutaneously into the root of the tail of rats. Two weeks after modeling, the hind toes of the rats were red and swollen, and the volume of the ankle joint increased significantly, suggesting that the modeling was successful. Successfully modeled rats were randomly divided into model group, positive group, *E. parviflora* Wall. polysaccharide low, medium and high dose groups, and normal control group, with 12 rats in each group[11]. After grouping, the positive group was given 6.25 mg/kg[12] tripterygium glycosides tablets by gavage. The rats in the low, medium and high dose groups of *E. parviflora* Wall. polysaccharide were given *E. parviflora* Wall. polysaccharide at the doses of 75, 150 and 300 mg/kg, respectively. The normal control group and the model group were given the same amount of saline by gavage, once a day for 4 weeks.

2.2.2 Body mass measurement. The body mass of rats in each group was measured every 7 d from the second immunization (day 0 of drug intervention).

2.2.3 Toe swelling test. The water volume method was used to measure the right hindfoot volume of rats in each group every 7 d from the second immunization (day 0 of drug intervention).

2.2.4 Arthritis index detection. The arthritis index of rats in each group was observed and calculated every 7 d from the second immunization (day 0 of drug intervention) by 5-grade scoring method[12]. The sum of all arthritis indexes of rats was the arthritis index value of each rat, and the highest score was 16 points.

2.2.5 Thymus and spleen index detection. Took the spleen and thymus tissue, removed the fat, absorbed the liquid on the surface with filter paper and weighed it accurately, and calculated the proportion of it to the body mass (mg/g).

2.2.6 HE staining. The synovial tissue of ankle joint was taken and fixed in 4% paraformaldehyde for 24 h, and then dehydrated by gradient ethanol, cleared by xylene, immersed in liquid paraffin, and embedded in paraffin. After that, continuous 5 μm sections were made and stained by HE, and observed under the optical microscope.

2.2.7 ELISA detection. Ankle joint synovial tissue homogenate and abdominal aorta whole blood were centrifuged at 3 000 r/min for 15 min at 4 ℃, and the supernatant and serum were extracted separately. According to the instructions of the kit, the levels of inflammatory factors IL-1β, IL-6 and TNF-α in serum and ankle joint synovial tissue of rats in each group were detected.

2.2.8 Statistical processing. SPSS 22.0 statistical software was used for data analysis, and the results were expressed as $\bar{x} \pm s$. One-way ANOVA was used to compare the data between groups, and *LSD-t* method was used to compare the data between groups. When $P < 0.05$, it means the difference was statistically significant.

3 Results and analysis

3.1 Comparison of body mass of rats in each group Compared with the normal control group, the body mass of rats in the model group decreased ($P < 0.05$). Compared with the model group, the body mass of rats in the positive drug group, medium and high dose groups of *E. parviflora* Wall. polysaccharide increased after one week of treatment ($P < 0.05$), and there was no significant difference in the low dose group of *E. parviflora* Wall. polysaccharide ($P > 0.05$), as shown in Table 1.

Table 1 Comparison of body mass of rats in each group ($\bar{x} \pm s$, g, $n = 12$)

Group	0 d	7 d	14 d	21 d	28 d
Normal control	335.2 ± 7.58	360.1 ± 11.06	397.1 ± 10.32	430.8 ± 12.46	458.3 ± 13.08
Model	254.7 ± 8.13 *	275.4 ± 8.02 *	298.3 ± 7.25 *	331.9 ± 10.50 *	353.9 ± 8.57 *
Positive	284.1 ± 8.26	305.9 ± 7.25 #	351.9 ± 8.63 #	380.3 ± 9.72 #	420.3 ± 12.21 #
A	259.3 ± 7.02	281.9 ± 6.85	304.8 ± 9.52	339.5 ± 10.18	350.6 ± 11.93
B	274.2 ± 6.92	305.7 ± 8.41	325.5 ± 7.34	370.2 ± 10.51	389.5 ± 11.84
C	298.1 ± 10.15 #	330.6 ± 9.62 #	360.7 ± 9.50 #	395.8 ± 11.92 #	435.1 ± 12.86 #

NOTE A, B, and C are low, medium and high dose groups of *E. parviflora* Wall. polysaccharide; compared with the normal control group, $^{*}P < 0.05$, $^{**}P < 0.01$; compared with the model group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, the same below.

3.2 Comparison of toe swelling degree of rats in each group Compared with the normal control group, the degree of toe swelling in the model group was increased ($P < 0.05$); compared with the model group, the toe swelling degree of rats in the positive drug group and the medium and high dose groups of *E. parviflora* Wall. polysaccharide decreased after one week of treatment ($P < 0.05$), while there was no significant difference in the low dose group of *E. parviflora* Wall. polysaccharide ($P > 0.05$), as shown in Table 2.

Table 2 Comparison of toe swelling degree of rats in each group ($\bar{x} \pm s$, mm, $n = 12$)

Group	0 d	7 d	14 d	21 d	28 d
Normal control	1.28 ± 0.32$^{\#\#}$	1.33 ± 0.28$^{\#\#}$	1.39 ± 0.31$^{\#\#}$	1.45 ± 0.40$^{\#\#}$	1.53 ± 0.61$^{\#\#}$
Model	5.17 ± 2.05**	4.98 ± 1.62**	4.85 ± 1.54**	4.72 ± 1.51**	4.59 ± 1.18**
Positive	4.21 ± 1.16*	3.57 ± 1.34$^{*\#}$	2.91 ± 1.60$^{\#}$	2.35 ± 1.12$^{\#}$	1.78 ± 0.82$^{\#}$
A	4.81 ± 1.02*	4.73 ± 1.82*	4.61 ± 1.50*	4.45 ± 1.21*	4.19 ± 1.65*
B	4.73 ± 1.52*	3.82 ± 1.40$^{*\#}$	3.21 ± 1.10$^{*\#}$	2.43 ± 0.81$^{\#}$	1.84 ± 0.76$^{\#}$
C	4.52 ± 1.36*	3.63 ± 1.60$^{*\#}$	3.05 ± 0.62$^{*\#\#}$	2.23 ± 0.95$^{\#}$	1.61 ± 0.82$^{\#}$

3.3 Comparison of arthritis index of rats in each group

Compared with the normal control group, the arthritis index of the model group increased ($P < 0.05$); compared with the model group, the arthritis index of rats in the positive drug group and the medium and high dose groups of *E. parviflora* Wall. polysaccharide decreased after one week of treatment ($P < 0.05$), while there was no significant difference in the low dose group of *E. parviflora* Wall. polysaccharide ($P > 0.05$), as shown in Table 3.

Table 3 Comparison of arthritis index of rats in each group ($\bar{x} \pm s$, points, $n = 12$)

Group	0 d	7 d	14 d	21 d	28 d
Normal control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Model	15.82 ± 3.16**	13.65 ± 2.64**	12.71 ± 2.50**	10.15 ± 3.06**	8.77 ± 1.83**
Positive	13.16 ± 4.10*	9.02 ± 2.31$^{*\#}$	6.43 ± 1.60$^{*\#}$	3.55 ± 1.32$^{\#\#}$	2.42 ± 1.54$^{\#\#}$
A	14.09 ± 3.72*	12.86 ± 3.60*	11.92 ± 3.51 *	9.08 ± 2.27*	8.12 ± 2.53*
B	13.32 ± 3.30$^{*\#}$	12.21 ± 2.42$^{*\#}$	11.35 ± 3.06$^{*\#}$	8.47 ± 2.61$^{*\#}$	7.01 ± 2.42*
C	12.03 ± 3.31$^{*\#}$	9.37 ± 2.69$^{*\#}$	6.83 ± 2.25$^{*\#}$	3.72 ± 0.92$^{\#\#}$	1.46 ± 0.65$^{\#\#}$

3.4 Comparison of thymus and spleen indexes of rats in each group

Compared with the normal control group, the thymus and spleen indexes in the model group were increased ($P < 0.05$); compared with the model group, the thymus and spleen indexes in the positive drug group and the medium and high dose groups of *E. parviflora* Wall. polysaccharide decreased after one week of treatment ($P < 0.05$), while there was no significant difference in the low dose group of *E. parviflora* Wall. polysaccharide ($P > 0.05$), as shown in Table 4.

Table 4 Comparison of thymus and spleen indexes of rats in each group ($\bar{x} \pm s$, mg/g)

Group	Thymus index	Spleen index
Normal control	0.92 ± 0.06$^{\#}$	2.18 ± 0.71$^{\#}$
Model	1.80 ± 0.16*	3.94 ± 0.52*
Positive	1.03 ± 0.10$^{\#}$	2.26 ± 0.32$^{\#}$
A	1.65 ± 0.43	3.75 ± 1.45*
B	1.43 ± 0.65$^{\#}$	3.58 ± 1.02$^{*\#}$
C	1.05 ± 0.26$^{\#}$	3.21 ± 0.76$^{*\#}$

3.5 Histopathological changes of synovium of ankle joint in rats of each group

In the normal control group, the structure of synovial tissue was normal, and there was no hyperplasia on the surface of synovial tissue. The synovial tissue of rats in the model group and the low dose group proliferated, with unclear layers and a large number of inflammatory cell infiltration. The proliferation of synovial tissue and the infiltration of inflammatory cells were significantly improved in the positive drug group and the medium and high dose groups of *E. parviflora* Wall. polysaccharide.

3.6 Levels of IL-1β, IL-6 and TNF-α in serum and synovial tissue of ankle joint of rats in each group

Compared with the normal control group, the levels of IL-1β, IL-6 and TNF-α in serum and synovial tissue of ankle joint in the model group were increased ($P < 0.05$). Compared with the model group, the levels of IL-1β, IL-6 and TNF-α in serum and ankle joint synovial tissue of rats in the positive drug group, medium and high dose groups of *E. parviflora* Wall. polysaccharide were decreased ($P < 0.05$), while there was no significant difference in the low dose group of *E. parviflora* Wall. polysaccharide ($P > 0.05$), as shown in Table 5.

Table 5 Levels of IL-1β, IL-6 and TNF-α in serum and synovial tissue of ankle joint of rats in each group ($\bar{x} \pm s$, ng/L, $n = 12$)

Group	Serum			Synovial tissue of ankle joint		
	IL-1β	IL-6	TNF-α	IL-1β	IL-6	TNF-α
Normal control	42.08 ± 4.55	28.16 ± 3.50	83.20 ± 5.39	64.31 ± 6.42	41.37 ± 5.12	59.83 ± 6.11
Model	118.25 ± 8.41**	79.12 ± 7.20**	221.73 ± 15.20**	301.05 ± 15.10**	135.72 ± 8.91**	186.95 ± 12.73**
Positive	54.83 ± 6.20$^{\#\#}$	41.25 ± 4.13$^{\#}$	111.30 ± 10.27$^{\#\#}$	94.05 ± 5.62$^{\#\#}$	69.02 ± 7.26$^{\#}$	78.25 ± 5.30$^{\#\#}$
A	105.12 ± 9.75	70.02 ± 6.40	203.16 ± 10.31	295.18 ± 11.37	134.02 ± 8.92	175.11 ± 9.83
B	86.32 ± 7.05$^{\#}$	56.07 ± 5.80$^{\#}$	167.13 ± 8.36$^{\#}$	221.07 ± 10.25$^{\#}$	110.05 ± 9.16$^{\#}$	139.80 ± 8.21$^{\#}$
C	48.98 ± 5.12$^{\#}$	28.19 ± 4.21$^{\#}$	96.85 ± 7.82$^{\#}$	78.37 ± 6.50$^{\#}$	53.42 ± 4.21$^{\#}$	84.29 ± 5.06$^{\#}$

4 Discussion

In this study, RA rat model was established by type II collagen induction. We found that after administration of *E. parviflora* Wall. polysaccharide, the body mass of RA rats was significantly higher than that of the model group, the toe swelling and arthritis index were significantly reduced, and the synovial tissue structure of ankle joint tended to be normal, suggesting that *E. parviflora* Wall. polysaccharide can protect the synovial tissue of rat joint and improve RA.

Spleen and thymus are important immune organs in the body. Thymus index and spleen index can reflect the immune level and state of the body to a certain extent. The experimental results showed that the thymus index and spleen index in the model group were significantly higher than those in the normal control group, indicating the activation of the immune system in RA rats, the changes in local antigens, and the release of inflammatory factors in synovial tissue, leading to the occurrence of synovial inflammation. IL-1β is an osteoclast activating factor, and it is often synthesized and secreted simultaneously with TNF-α to destroy articular cartilage and cause joint injury[13]. IL-6 is an important pro-inflammatory factor, and it is involved in a variety of human inflammatory reactions and diseases, and also promotes the proliferation and differentiation of immune cells[14]. IL-1β, IL-6 and TNF-α stimulate chondrocytes and synoviocytes to secrete collagenase and other substances, destroy the surrounding environment of chondrocytes, and are the main inflammatory mediators in RA[15]. The results showed that the levels of IL-1β, IL-6 and TNF-α in serum and ankle synovial tissue of RA rats in the model group were significantly higher than those in the normal control group. After administration of *E. parviflora* Wall. polysaccharide, the levels of IL-1β, IL-6 and TNF-α were significantly decreased, and the thymus index and spleen index were significantly decreased, which indicated that *E. parviflora* Wall. polysaccharide canreduce the inflammatory response in RA rats by regulating the immune level. In summary, *E. parviflora* Wall. polysaccharide can play a therapeutic and protective role in RA rats by reducing the body's inflammatory response.

References

[1] DI MATTEO A, BATHON JM, EMERY P. Rheumatoid arthritis[J]. Lancet, 2023, 402(10416): 2019 – 2033.

[2] DÍAZ-GONZÁLEZ F, HERNÁNDEZ-HERNÁNDEZ MV. Rheumatoid arthritis[J]. Journal of Clinical Medicine (Barc), 2023, 161(12): 533 – 542.

[3] CUTOLO M, CAMPITIELLO R, GOTELLI E, *et al*. The role of M1/M2 macrophage polarization in rheumatoid arthritis synovitis[J]. Frontiers in Immunology, 2022(13): 867260.

[4] GRAVALLESE EM, FIRESTEIN GS. Rheumatoid arthritis-common origins, divergent mechanisms[J]. New England Journal of Medicine, 2023, 388(6): 529 – 542.

[5] QUE ZL, PANG DQ, CHEN Y, *et al*. HPLC fingerprints of *Embelia parviflora* from different growing areas[J]. Chinese Traditional Patent Medicine, 2020, 42(2): 415 – 422. (in Chinese).

[6] PANG DQ, QUE ZL, CHEN Y, *et al*. Infrared spectral characteristics of *Embelia parviflora* Wall. from different habitats and different parts[J]. Journal of Liaoning University of Traditional Chinese Medicine, 2019, 21(3): 67 – 70. (in Chinese).

[7] QUE ZL, LIU D, CHEN Y, *et al*. Study on Chemical constituents of *Embelia parviflora* Wall.[J]. Chinese Journal of Ethnomedicine and Ethnopharmacy, 2017, 26(5): 5 – 9. (in Chinese).

[8] PANG DQ, QUE ZL, CHEN Y, *et al*. Optimisation of the extraction process and antioxidant activity of polysaccharides from *Embelia parviflora* Wall. by response surface methodology[J]. Lishizhen Medicine and Materia Medica Research, 2019, 30(1): 22 – 25. (in Chinese).

[9] HOU Y, JIN C, WEN C, *et al*. Research progress on the mechanism of action and targeting of polysaccharides in traditional Chinese medicine [J/OL]. World Science and Technology-Modernisation of Traditional Chinese Medicine: 1 – 33[2024-05-05]. http://kns.cnki.net/kcms/detail/11.5699.R.20240429.1629.002.html. (in Chinese).

[10] YUE TJ, LU YN, DING WL, *et al*. Research progress on the protective mechanism of Chinese herbal polysaccharides on animal bone health [J/OL]. Journal of Henan Agricultural University: 1 – 14[2024-05-05]. https://doi.org/10.16445/j.cnki.1000-2340.20240425.002. (in Chinese).

[11] WEI JC, TANG YL, QIN SH, *et al*. *Laggera alata* attenuates inflammatory response by regulating macrophage polarization in rheumatoid arthritis mice[J]. Molecular Biotechnology, 2023. https://doi.org/10.1007/s12033-023-00808-w.

[12] ZHU J, WU LL, ZHANG QS, *et al*. Therapeutic effect of ethanol extract of *Pterocarya hupehensis* Skan on collagen-induced arthritis rats and its mechanism[J]. Chinese Pharmacological Bulletin, 2019, 35(1): 117 – 123. (in Chinese).

[13] FAN WC, LU LT. Effect of Danggui Sini Decoction on inflammatory factor expression and TLR/Beclin-1 signaling pathway in rats with rheumatoid arthritis[J]. Pharmacy and Clinics of Chinese Materia Medica, 2020, 11(2): 27 – 30. (in Chinese).

[14] LI DW, MU BC, DO DQ, *et al*. Effects of Gancao Fuzi Decoction on IL-1β, IL-10 and TNF-α in serum and synovial fluid of collagen-induced arthritis rats[J]. China Journal of Traditional Chinese Medicine and Pharmacy, 2020, 35(12): 6355 – 6358. (in Chinese).

[15] CHEN Y, LIU W, WU JJ. Effect of Shexiang Wulong pill combined with Methotrexate Capsules on IL-1, IL-6 and TNF-α in rheumatoid arthritis patients with cold-stagnancy[J]. Drug Evaluation Research, 2019, 42(8): 1609 – 1612. (in Chinese).