Effect of *Elephantopus scaber* L. Extract on Acute Pleurisy in Rats

Yan ZHANG[1], Jianting GAO[2], Xinchao SHI[2], Hailong LIU[1*], Xin HE[2]

1. Institute of Animal Husbandry and Veterinary Medicine, Hainan Academy of Agricultural Sciences, Haikou 571100, China 2. College of Veterinary Medicine, Hebei Agricultural University, Baoding 071000, China

Abstract [**Objectives**] To study the anti-inflammatory effect of *Elephantopus scaber* L. extract on acute pleurisy induced by carrageenan in rats, and to explore its anti-inflammatory mechanism. [**Methods**] The active sites of *E. scaber* L. were extracted by ethanol reflux method. The extracts of different concentrations of *E. scaber* L. were used as the study object, and dexamethasone was used as the positive control drug. The anti-inflammatory effects of *E. scaber* L. extracts were studied by measuring the levels of malondialdehyde (MDA), prostaglandin E_2 (PGE_2), tumor necrosis factor (TNF-α), interleukin-1β (IL-1β) in pleural fluid and serum nitric oxide (NO), MDA, PEG_2, TNF-α, IL-1β in rats with acute pleurisy induced by carrageenan. [**Results**] *E. scaber* L. extracts in three doses could reduce the levels of inflammatory factors in pleural fluid and serum, and inhibit acute pleurisy in rats. It was speculated that the anti-inflammatory mechanism was related to the inhibition of the release of inflammatory factors and the antioxidant effect of extracts of three doses of *E. scaber* L. [**Conclusions**] This experiment provides a basis for the development and application of *E. scaber* L.

Key words *Elephantopus scaber* L., Extract, Acute pleurisy, Anti-inflammatory

1 Introduction

Elephantopus scaber L. is a medicinal plant in the family of Compositae, mainly distributed in Guangdong, Zhejiang and Fujian, China. Its whole plant can be used as medicine[1]. It has the effects of clearing away heat and toxic materials, reducing swelling and diuresis, and is often used for the treatment of cold, acute tonsillitis, conjunctivitis, edema and other diseases[2]. Inflammation is a common comprehensive pathological process in humans and animals. It is a defense-oriented pathological reaction of living tissues with vascular system to inflammatory factors, and it can be divided into acute inflammation and chronic inflammation. Acute inflammation refers to the response of the body in a short time after being stimulated by inflammatory factors. Pleurisy is one of the most common inflammatory diseases, and many life-threatening diseases, such as tuberculosis and pneumonia, are related to pleurisy[3]. Rat pleurisy model induced by carrageenan is an ideal acute inflammatory model, which is often used to test the anti-inflammatory effect of drugs[4]. For rodents, carrageenan injected into thoracic cavity can activate kinase, promote the release of inflammatory factors, activate inflammatory cells (mainly neutrophils) to gather in inflammatory sites. Therefore, with the help of this model, the anti-inflammatory effect of drugs can be studied by measuring the level of inflammatory factors and MDA content.

In this study, the active substances of *E. scaber* L. were extracted by ethanol reflux method, and the effects of different concentrations of extracts on acute pleurisy in rats were investigated, and its anti-inflammatory mechanism was preliminarily discussed, which provided a basis for its research and development and application.

Received: December 13, 2023 Accepted: January 5, 2024
Supported by the Special Project of Technological Innovation of Hainan Provincial Research Institute (SQKY2022-0006) &Natural Science Foundation of Hainan Province (321MS0809).
* Corresponding author. E-mail: liuhailong423@126.com

2 Materials and methods

2.1 Materials

2.1.1 Test animals and plants. Healthy Kunming male rats, purchased from Sibeifu (Beijing) Biotechnology Co., Ltd., weighed (250 ± 20.0) g, and the production license number was SCXK (Beijing) 2019-0010.

The purchased rats were fed adaptively for one week, during which it was kept ventilated, and they were forced to fast for 12 h but could drink water before the start of the experiment. *E. scaber* L. was collected in Dongshan Town, Haikou City, Hainan Province in March 2022.

2.1.2 Pharmaceuticals and instruments. (i) Pharmaceuticals. Dexamethasone Sodium Phosphate Injection (5 mg/mL) (Shanxi Zhaoyi Co., Ltd.); Carrageenan (Shanghai Macklin Biochemical Technology Co., Ltd.); Heparin Sodium Injection (Tianjin Biochemical Pharmaceutical Co., Ltd.); Anhydrous Ethanol (Tianjin Huihang Chemical Technology Co., Ltd.); 0.9% Normal Saline Injection (Shiyao Yinhu Pharmaceutical Co., Ltd.); Tween-80 and other solvents (Fuchen Chemical Reagent Co., Ltd.); Rat Nitric Oxide (NO) ELISA Detection Kit (Shanghai Yiyan Biotechnology Co., Ltd.); Elisa Kit for Detecting Malondialdehyde (MDA), Prostaglandin E_2 (PGE_2), Tumor Necrosis Factor (TNF-α) and Interleukin-1β (IL-1β) in Rats (Shanghai Zhenke Biotechnology Co., Ltd.).

(ii) Instruments. Extracting and Concentracting Tank (Beijing Jinfu Renhao Technology Development Co., Ltd.); RE-5205 Rotary Evaporator (Shanghai Yarong Biochemical Instrument Factory); SHD-III Circulating Water Multi-purpose Vacuum Pump (Sunshine Science and Education Instrument Factory, Baoding High-tech Zone); TGL-16B Centrifuge (Shanghai Anting Scientific Instrument Factory).

2.2 Methods

2.2.1 Preparation of ethanol extract from *E. scaber* L. The effective components of *E. scaber* L. were extracted by ethanol re-

flux extraction. 2 600 g of *E. scaber* L. was weighed, refluxed and extracted with 95% ethanol for 2 times. 1∶15 ethanol was added for extraction for 2 h at the first time and 1∶10 ethanol for extraction for 1 h at the second time; the primary extracts were combined, concentrated under reduced pressure, and ethanol was recovered. After absorption occurred in the concentration bottle, a proper amount of total ethanol extract was taken. The rest of the liquid medicine was concentrated to extract, 500 mL of hot water was added, and stirred well for later use.

2.2.2 Animal model establishment and administration. The experimental animals were randomly divided into 5 groups. The control group was divided into negative control group (5% Tween-80) and positive control group (0.5 mg/kg dexamethasone); the experimental group was divided into low dose group (7.5 g/kg), medium dose group (15 g/kg) and high dose group (30 g/kg). The rats were given intragastric administration (1 mL/hg) once a day for 7 d; 30 min after the last administration, the rats were anesthetized by intraperitoneal injection of 10% chloral hydrate at a dose of 0.4 mL/hg[5], fixed in supine position, shaved at the right thoracic cavity, and disinfected by alcohol wiping; according to the dose of 0.2 mL/hg[6], 1% sterile carrageenan solution was injected into the right pleural cavity[7] between the 7th and 8th ribs under the right upper limb to cause inflammation[8-9]. In order to avoid lung injury, this operation should be completed in a short time[10].

2.2.3 Sampling and determination. After 4 h of inflammation, the rats were anesthetized, and blood was collected from orbital sinuses before killing. The whole blood was centrifuged at 5 000 r/min for 5 min, and the content of inflammatory factors NO, MDA, PGE_2, TNF-α and IL-1β in the supernatant was determined according to the instructions of the kit.

The rats were fixed in supine position, wiped and disinfected with alcohol, and then the skin of chest and abdomen was cut with surgical scissors to expose the muscle layer. 2 mL of saline pleural lotion was injected (including 5 U/mL heparin) into the thoracic cavity[11-12], and it was shaken gently to mix the fluid in the thoracic cavity evenly; the pleural exudate was centrifuged at 5 000 r/min for 5 min, and the supernatant was taken. The content of MDA, PGE_2, TNF-α and IL-1β was determined according to the instructions of the kit.

2.2.4 Statistics and analysis. SPSS 19.0 was used for statistical analysis. The experimental data were expressed by (mean ± standard deviation). *T*-test and one-way ANOVA were used. $P<0.05$ indicated that the difference was statistically significant.

3 Results and analysis

3.1 Extract content of *E. scaber* L. The results showed that the extraction rate of ethanol extract of *E. scaber* L. was 30.58%, and 1 g of the extract was equivalent to 3.27 g of the original drug. In this study, the solvent of the extract was selected as the highest dose, and the medium and low dose solvents were diluted accordingly.

3.2 Content of inflammatory factors in serum The content of inflammatory factors in serum is shown in Table 1. Compared with the negative control group, the content of serum NO, MDA, PGE_2, TNF-α and IL-1β in the positive control group was significantly decreased ($P<0.05$ or $P<0.01$); *E. scaber* L. ethanol extracts in different doses of groups could reduce the content of serum inflammatory factors in different levels. Compared with the negative control group, the high dose, medium dose and low dose groups could significantly reduce the serum TNF-α content ($P<0.05$ or $P<0.01$), but there was no significant difference between the high dose group and the positive control group; the content of serum NO, MDA and IL-1β in high and medium dose groups was significantly decreased ($P<0.05$ or $P<0.01$), and the effects of high dose group on MDA, TNF-α and IL-1β were significantly better than those in medium dose group; only the content of PGE_2 in serum was significantly reduced in the high dose group.

Table 1 Content of inflammatory factors in serum of rats ($\bar{x}\pm s$, $n=6$)

Group	NO//μmol/mL	MDA//nmol/mL	PGE_2//pg/mL	TNF-α//pg/mL	IL-1β//pg/mL
Negative control	(93.317±2.897)	(4.286±0.584)	(358.975±33.805)	(277.065±24.906)	(31.671±3.817)
Positive control	(71.262±3.534)**	(2.202±0.764)**	(238.075±27.315)**	(178.968±29.582)**	(19.712±3.533)*
Low dose	(88.867±3.695)	(3.868±0.663)	(357.942±28.801)	(276.276±24.755)*	(31.186±3.095)
Medium dose	(86.282±3.552)*	(3.388±0.393)*	(314.762±32.506)	(222.211±38.628)*	(25.944±3.649)*
High dose	(84.426±5.429)*	(2.853±0.456)**	(238.620±29.557)**	(182.460±24.413)**	(21.280±2.084)**

Note: Compared with the negative control group, "*" means $P<0.05$, and "**" means $P<0.01$. The same below.

3.3 Content of inflammatory factors in pleural fluid The results of inflammatory factors in pleural fluid are shown in Table 2. Compared with the negative control group, the content of MDA, PGE_2, TNF-α and IL-1β in pleural fluid in the positive control group was significantly decreased ($P<0.05$ or $P<0.01$); in the three dose groups of ethanol extract of *E. scaber* L., the content of TNF-α in pleural fluid was significantly decreased; the content of MDA, PGE_2 and IL-1β in pleural fluid was significantly decreased ($P<0.05$ or $P<0.01$) in medium dose group and high dose group, and the effect of high dose group on PGE_2 was significantly higher than that of medium dose group, but not significantly different from that of positive control group.

Table 2 Content of inflammatory factors in pleural fluid of rats ($\bar{x} \pm s$, $n = 6$)

Group	MDA // nmol/mL	PGE_2 // pg/mL	TNF-α // pg/mL	IL-1β // pg/mL
Negative control	(3.655 ± 0.342)	(343.611 ± 20.600)	(246.675 ± 30.137)	(33.439 ± 3.337)
Positive control	(1.680 ± 0.470) **	(174.097 ± 32.499) **	(157.778 ± 32.838) *	(16.004 ± 3.620) **
Low dose	(3.494 ± 0.701)	(326.046 ± 30.582)	(231.975 ± 32.826) *	(30.410 ± 3.135)
Medium dose	(2.578 ± 0.331) **	(297.509 ± 34.586) *	(192.264 ± 28.318) *	(25.037 ± 2.477) **
High dose	(1.819 ± 0.489) **	(185.318 ± 19.852) **	(164.605 ± 33.451) *	(16.963 ± 3.832) **

4 Discussion and conclusions

Acute pleurisy induced by carrageenan in rats is widely used in screening and evaluating anti-inflammatory drugs, and it is an ideal model of acute exudative inflammation[13]. Inflammatory stimulation can cause the secretion of a large number of inflammatory mediators, and it is easy to detect the level of factors related to inflammatory reaction[14-16]. Therefore, the content of inflammatory mediators in rat pleurisy can be used as an important index to measure inflammation.

NO can regulate inflammatory reaction. When inflammation occurs, stimulants promote the synthesis and release of NO, and then promote the inflammatory reaction; MDA can reflect the reaction between free radicals and lipids; PGE_2 plays an important role in inflammatory reaction, and can promote the role of other factors and make white blood cells gather in inflammatory areas, thus causing inflammatory reactions such as tissue edema and congestion[17]; TNF-α, as a pro-inflammatory factor, can induce or activate a variety of inflammatory mediators in vivo through a variety of biological pathways, and inflammatory mediators work together to cause tissue damage and apoptosis[18-19]; IL-1β, as one of the most important cytokines involved in inflammatory reaction, can increase the secretion of PGE_2, participate in mediating acute inflammatory reaction, and promote leukocyte aggregation in local inflammatory sites.

In this experiment, the ethanol extract of *E. scaber* L. could decrease the content of NO, PGE_2, TNF-α, IL-1β and MDA in serum and pleural fluid, indicating that the mechanism of the ethanol extract of *E. scaber* L. inhibiting acute pleurisy in rats may be related to its anti-oxidation and inhibition of the release of inflammatory factors.

Wan *et al.*[20] found that high concentration of *E. scaber* L. ethanol extract can be used as a hepatoprotective drug for liver injury induced by ethanol, and it is safe and has no toxic and side effects in rats. In order to explore the anti-inflammatory effect of *E. scaber* L. water extract, Wen Xianmin *et al.*[21] established a rat toe swelling model induced by carrageenan, and compared the swelling degree of the right hind toe and inhibition rate at different time after administration of carrageenan in each group. The results showed that the toe swelling was inhibited when the dose was 0.45 g/kg (approximately equivalent to 38.67 g/kg crude drug). In order to test the median lethal dose (LD_{50}) of different solvent extracts of *E. scaber* L., the acute toxicity test of different solvent extracts of *E. scaber* L. was carried out in rats. The results showed that at the dose of 5 000 mg/kg (approximately equivalent to 16.35 g/kg crude drug), the rats did not die[22]. The inflammatory model rats were given *E. scaber* L. extract at a dose of 0.4 g/kg (approximately equivalent to 8.25 g/kg crude drug). It was found that compared with the control group, the administration group had obvious inhibitory effect on carrageenan-induced inflammation[23]. The extract used in this experiment was sticky, and the high dose group used the extract solution. In order to ensure the flowing state of the liquid medicine, the final dose groups divided into low dose group (7.5 g/kg according to the crude drug quantity), medium dose group (15 g/kg according to the crude drug quantity) and high dose group (30 g/kg according to the crude drug quantity).

In this experiment, the results showed that the ethanol extract of *E. scaber* L. could inhibit the content of NO, MDA, PGE_2, TNF-α and IL-1β in pleural fluid and serum of rats with acute pleurisy induced by carrageenan. The anti-inflammatory effect was in the order of high dose > medium dose > low dose, suggesting that its anti-inflammatory mechanism might be related to its inhibition of the release of inflammatory factors and its antioxidant effect.

References

[1] WANG JN, LIAO CX, HUANG GK, *et al.* Effect of *Elephantopus scaber* L. extract on vasculogenic mimicry of hepatocellular carcinoma[J]. The Chinese Journal of Clinical Pharmacology, 2022,38(7): 652-656. (in Chinese).

[2] WANG JN. Research progress of sesquiterpene lactones and their antitumor activities in *Elephantopus scaber* Linn.[J]. Guangdong Chemical Industry, 2021, 48(14): 115-117. (in Chinese).

[3] BAYIR Y, UN H, CADIRCI E, *et al.* Effects of Aliskiren, an RAAS inhibitor, on a carrageenan-induced pleurisy model of rats[J]. Anais da Academia Brasileira de Ciências,2019, 91(1): e20180106.

[4] YANG ZP, GUAN YF, LI J, *et al.* Chrysin attenuates carrageenan-induced pleurisy and lung injury via activation of SIRT1/NRF2 pathway in rats[J]. European Journal of Pharmacology, 2018, 836: 83-88.

[5] HUANG C, ZHANG D, XIN WY, *et al.* Study of methyl salicylate glycosides in acute pleurisy model in rats[J]. Chinese Pharmacological Bulletin, 2013, 29(3): 328-332. (in Chinese).

[6] ZHAO B, YANG XY, QU XL, *et al.* *Scutellaria baicalensis* Georgi stems and leaves improve acute pleurisy in rats by inhibiting TRPV1 pathway [J]. Pharmacology and Clinics of Chinese Materia Medica, 2022, 38(4): 112-116. (in Chinese).

[7] ALLEGRA M, TESORIERE L, LIVREA MA, *et al.* Cactus pear fruit extract exerts anti-inflammatory effects in carrageenin-induced rat pleurisy [J]. Acta Horticulturae, 2015(1067): 19-25.

[8] HU R, LI ZL, YANG L, *et al.* Anti-inflammatory effect of imperatorin from radix *Angelica dahurica* on rats with acute pleurisy[J]. Journal of Emergency in Traditional Chinese Medicine, 2012, 21(4): 576-577. (in Chinese).

(To page 56)

[12] ROSENFELDT MT, RYAN KM. The multiple roles of autophagy in cancer[J]. Carcinogenesis, 2011, 32(7): 955 – 963.
[13] RABANAL-RUIZ Y, OTTEN EG, KOROLCHUK VI. mTORC1 as the main gateway to autophagy[J]. Essays in Biochemistry, 2017, 61(6): 565 – 584.
[14] WANG B, HUANG X, LIANG H, *et al.* PLK1 inhibition sensitizes breast cancer cells to radiation via suppressing autophagy[J]. International Journal of Radiation Oncology Biology Physics, 2021, 110(4): 1234 – 1247.
[15] CAI XP, CHEN LD, SONG HB, *et al.* PLK1 promotes epithelial-mesenchymal transition and metastasis of gastric carcinoma cells[J]. American Journal of Translational Research, 2016, 8(10): 4172 – 4183.
[16] FERRAROTTO R, GOONATILAKE R, YOO SY, *et al.* Epithelial-mesenchymal transition predicts Polo-like kinase 1 inhibitor-mediated apoptosis in non-small cell lung cancer[J]. Clinical Cancer Research, 2016, 22(7): 1674 – 1686.
[17] OTSU H, IIMORI M, ANDO K, *et al.* Gastric cancer patients with high PLK1 expression and DNA aneuploidy correlate with poor prognosis[J]. Oncology, 2016, 91(1): 31 – 40.
[18] FANG L, LIU Q, CUI H, *et al.* Bioinformatics analysis highlight differentially expressed CCNB1 and PLK1 genes as potential anti-breast cancer drug targets and prognostic markers[J]. Genes (Basel), 2022, 13(4): 654.
[19] WANG B, HUANG X, LIANG H, *et al.* PLK1 Inhibition sensitizes breast cancer cells to radiation via suppressing autophagy[J]. International Journal of Radiation Oncology, 2021, 110(4): 1234 – 1247.
[20] RAN Z, CHEN W, SHANG J, *et al.* Clinicopathological and prognostic implications of polo-like kinase 1 expression in colorectal cancer: A systematic review and meta-analysis[J]. Gene, 2019(721): 144097.
[21] YU Z, DENG P, CHEN Y, *et al.* Inhibition of the PLK1-Coupled cell cycle machinery overcomes resistance to oxaliplatin in colorectal cancer[J]. Advanced Science, 2021, 8(23): e2100759.
[22] GAO P, HAO JL, XIE QW, *et al.* PELO facilitates PLK1-induced the ubiquitination and degradation of Smad4 and promotes the progression of prostate cancer[J]. Oncogene, 2022, 41(21): 2945 – 2957.
[23] ZHANG Z, CHENG L, LI J, *et al.* Targeting Plk1 sensitizes pancreatic cancer to immune checkpoint therapy[J]. Cancer Research, 2022, 82(19): 3532 – 3548.
[24] WANG J, HU K, GUO J, *et al.* Suppression of KRas-mutant cancer through the combined inhibition of KRAS with PLK1 and ROCK[J]. Nature Communications, 2016(7): 11363.
[25] DUFIES M, VERBIEST A, COOLEY LS, *et al.* Plk1, upregulated by HIF-2, mediates metastasis and drug resistance of clear cell renal cell carcinoma[J]. Communications Biology, 2021, 4(1): 166.
[26] RUDOLPH D, STEEGMAIER M, HOFFMANN M, *et al.* BI 6727, a Polo-like kinase inhibitor with improved pharmacokinetic profile and broad antitumor activity[J]. Clinical Cancer Research, 2009, 15(9): 3094 – 3102.

(From page 56)

[9] TANG XN, HUANG JC, HE M, *et al.* Study on the effect of ethanol extract of calonyction acculeatum beans on acute pleurisy in rats and its mechanism[J]. Beijing Journal of Traditional Chinese Medicine, 2009, 28(8): 641 – 643. (in Chinese).
[10] SUN AJ, PANG SQ, XU XX, *et al.* Effects of ethanol extract from *Schefflera arboricola* Hayata flower on rats in pleurisy model[J]. Journal of Chinese Medicinal Materials, 2015, 38(3): 595 – 597. (in Chinese).
[11] QU S, KAN H, LI JH, *et al.* Study on the anti-inflammatory effect of Gancao Fuzi Decoction based on rat pleurisy model[J]. Journal of Changchun University of Chinese Medicine, 2021, 37(1): 67 – 70. (in Chinese).
[12] ELISABETTA C, SILVANA M, ROSA C, *et al.* The Ecto-5'-Nucleotidase/CD73 inhibitor, α, β-Methylene adenosine 5'-Diphosphate, exacerbates Carrageenan-Induced pleurisy in rat[J]. Frontiers in Pharmacology, 2019(10): 775.
[13] LU S, WANG L, ZHANG W, *et al.* Ulcerative colitis with acute pleurisy: A case report and review of the literature[J]. Medicine, 2017, 96(30): e7630.
[14] PETBOROM P, DECHATES B, MUANGNOI P. Differentiating tuberculous pleuritis from other exudative lymphocytic pleural effusions[J]. Annals of Palliative Medicine, 2020, 9(5): 2508 – 2515.
[15] ARPINAR YB, SATICI C, KOSAR AF. Adenosine deaminase cutoff value when diagnosing tuberculous pleurisy in patients aged 40 years and older[J]. The Clinical Respiratory Journal, 2020, 15(1): 109 – 115.
[16] WU J, HAN Y, LI BH. Anti-inflammatory effects of Qingwei Huanglian pill on rats with acute pleurisy[J]. Asia-Pacific Traditional Medicine, 2014, 10(17): 8 – 10. (in Chinese).
[17] CHEN JA, LI DF, YUAN L, *et al.* Research progress on role of prostaglandin E2/prostacyclin receptor signaling pathway in lung diseases[J]. International Journal of Respiration, 2020, 40(12): 944 – 949. (in Chinese).
[18] ZHU P, YAN DM, ZHENG W. Chrysophanol improves LPS-induced inflammation in mice by inhibiting macrophage expression of inflammatory factors[J]. Chinese Journal of Laboratory Diagnosis, 2021, 25(1): 98 – 101. (in Chinese).
[19] WU HF. Inflammatory cytokine network and disease[J]. Journal of Qinghai Medical College, 2003(4): 267 – 270. (in Chinese).
[20] WAN YH, YEAP SK, CHAI LH, *et al.* Hepatoprotective activity of *elephantopus scaber* on Alcohol-Induced liver damage in mice[J]. Evidence-based Complementary and Alternative Medicine: eCAM, 2012, 2012(13): 417953.
[21] WEN XM, YANG MN, HU TK. Animal experimental study on the anti-inflammatory and analgesic effects of the aqueous extract of *Elephantopus scaber* L.[J]. Yunnan Journal of Traditional Chinese Medicine and Materia Medica, 2015, 36(12): 71 – 72. (in Chinese).
[22] HIRADEVE SM, RANGARI VD. Immunomodulatory study of different solvent extract of *Elephantopus scaber* Linn.[J]. Drug Research, 2017, 67(4): 239 – 243.
[23] HE CG, DONG LW, RUAN XP, *et al.* Experimental study on the antibacterial and anti-inflammatory effects of the whole herb extract of *Elephantopus scaber* L.[J]. Chinese Journal of Traditional Medical Science and Technology, 2008(3): 191 – 192. (in Chinese).