Protective Effects of Xanthoxylin on Acute Lung Injury Induced by D-galactosamine/Lipopolysaccharide in Rats

Yuchen SHEN, Yuanxiang YANG, Liba XU, Zhonghua DAI*, Fengfeng XIE*, Hai LONG, Yujie LAN, Xiuqi YU

Guangxi University of Chinese Medicine, Guangxi Key Laboratory of Zhuang and Yao Ethnic Medicine, University Engineering Research Center of Development and Industrialization of Zhuang and Yao Ethnic Medicinal Materials, Guangxi, The Collaborative Innovation Center of Zhuang and Yao Ethnic Medicine, Guangxi Engineering Research Center of Ethnic Medicine Resources and Application, Nanning 530200, China

Abstract [**Objectives**] To investigate the protective effects of xanthoxylin on acute lung injury induced by D-Galactosamine (D-GalN) and Lipopolysaccharide (LPS) in rats. [**Methods**] Sixty male SD rats were randomly divided into a normal group, a model group, a silybin group (50 mg/kg), and three xanthoxylin groups (low-dose, medium-dose, high-dose as 60, 120, and 240 mg/kg), 10 rats per group. The rats were administered for 17 consecutive days, on day 14, all the rats except for the normal group were intraperitoneally injected with a D-GalN (400 mg/kg) /LPS (30 μg/kg) mixture once to establish acute lung injury models. At 72 h after modeling, their serum MCP-1 levels, IL-1β, IL-6, PCT, CRP, TNF-α levels in bronchoalveolar lavage fluid, and IL-1β, IL-6, TNF-α levels in lung tissue were measured, and lung tissue histological examination were checked by HE staining. [**Results**] Compared with the model group, the serum MCP-1 levels, IL-1β, IL-6, PCT, CRP, TNF-α levels in bronchoalveolar lavage fluid, and IL-1β, IL-6, TNF-α levels in lung tissue in xanthoxylin groups were significantly decreased ($P<0.05$ or $P<0.01$), and lung tissue injury were alleviated. [**Conclusions**] Xanthoxylin has protective effects on acute lung injury in rats, and it may be related to the increase of anti-inflammatory capacity and the promotion of lung tissue self-healing.

Key words Xanthoxylin, D-GalN/LPS, Acute lung injury (ALI), Protective effect

1 Introduction

Acute lung injury (ALI) is a form of acute and severe respiratory distress syndrome characterized by diffuse alveolar damage, pulmonary edema, hemorrhage, and abnormal fluid secretion[1-2]. In the livestock breeding industry, ALI is associated with significant morbidity and high mortality, substantially impacting productivity. The breeding environment often exposes animals to harsh conditions, making them susceptible to bacterial and viral infections. For instance, endotoxins released by Gram-negative bacteria such as *Escherichia coli* can enter the bloodstream, leading to enterogenous endotoxemia. This condition mediates severe pulmonary inflammatory responses, which play a key role in the development and progression of ALI[3-4]. At present, there are no specific pharmacological treatments for ALI, underscoring the importance of early diagnosis and timely intervention[5].

Xanthoxylin is an active component in various traditional Chinese medicines, such as *Phyllanthus niruri*[6-7], *Duchesnea indica*[8], *Persicaria capitata*[9], and pomegranate leaf[10], and exhibits significant anti-inflammatory effects. However, its potential role in combating acute lung injury (ALI) has not been reported to date. Therefore, this study investigates the protective effects of xanthoxylin on a D-GalN/LPS-induced ALI rat model, aiming to promote and apply xanthoxylin in the livestock and poultry industry for the prevention and treatment of ALI, and providing experimental and theoretical evidence.

2 Materials and methods

2.1 Animals Sprague Dawley (SD) rats, male, specific pathogen-free (SPF) grade, with body weights ranging from 185.6 g to 202.3 g at the start of the experiment, were supplied by Hunan SJA Laboratory Animal Co., Ltd. (Production License No. SCXK (Xiang) 2019-0004). The rats were housed in individually ventilated cages (IVC) within an SPF barrier facility at Guangxi University of Chinese Medicine [Laboratory Animal Use License No. SYXK (Gui) 2019-0001]. All experimental procedures were reviewed and approved by the Laboratory Animal Ethics Committee of Guangxi University of Chinese Medicine (Approval No. DW20231016-200). The experiment was carried out after 7 d of adaptive feeding.

2.2 Drugs and reagents Xanthoxylin (Lot No. Lf1129186229), supplied by Shanghai Haohong Biomedical Technology Co., Ltd.; Silybin (Batch No. MUST-23030607), supplied by Chengdu Must Bio-Technology Co., Ltd.; ELISA kits for PCT, IL-1β, IL-6, TNF-α, MCP-1, and CRP (with respective lot numbers AK000Z246505, AK102D229594, AK12BB2L5689, FU11X0L41225, AK078B8P4585, and AK00V0889630), supplied by Wuhan Elabscience Biotechnology Inc.; 0.9% NaCl Injection (Batch No. E23013128), produced by Shandong Chenxin Pharmaceutical Co., Ltd.; LPS (Batch No. 1031Q0312), supplied by Beijing Solarbio Science & Technology Co., Ltd.; D-GalN (Batch No.

Received: July 2, 2025 Accepted: September 12, 2025

Supported by Scientific Research Start-up Fund for Introduced PhD. Talents of Guangxi University of Chinese Medicine, 2023 (2023BS024); Basic Research Ability Enhancement Project for Young and Middle-aged Teachers in Guangxi Universities (2022KY0286); Guangxi Key Research and Development Program (Guike AB21196016); Guangxi Science and Technology Base and Talent Special Project (Guike AD21238031); Guangxi Key Discipline of Zhuang Medicine in Chinese Medicine (GZXK-Z-20-64).

Yuchen SHEN, master's degree candidate; * Corresponding author. Zhonghua DAI, doctoral degree, associate professor, master supervisor; Fengfeng XIE, doctoral degree, assistant researcher.

20230420), supplied by Shandong Xiya Chemical Co., Ltd.

2.3 Instruments ES 2220M Electronic Balance from Precisa (Switzerland); 5810R Refrigerated Centrifuge, from Eppendorf (Germany); Epoch2 Microplate Reader, from BioTek (USA); ECLIPSE Ni-U Upright Fluorescence Biological Microscope from Nikon (Japan).

2.4 Grouping and drug administration A total of 60 male SD rats were randomly divided into six groups ($n = 10$ per group): the normal control group, the model group, the silybin group (50 mg/kg)[11], and three xanthoxylin dose groups (60, 120, and 240 mg/kg). Rats in the silybin and xanthoxylin groups were administered corresponding concentrations of silybin or xanthoxylin by oral gavage, while those in the normal and model groups received an equal volume of 0.5% CMC-Na solution. The treatments were continued for 17 consecutive days. On day 14 of administration, after fasting for 14 – 16 h with free access to water, all rats except those in the normal group were intraperitoneally injected with a mixture of D-GalN (400 mg/kg) and LPS (30 μg/kg) one hour after the last drug administration to establish the acute lung injury (ALI) model[12].

2.5 Detection of serum MCP-1 level Seventy-two hours after modeling, the rats were fasted for 14 – 16 h with free access to water. Under sustained isoflurane anesthesia, blood was collected from the abdominal aorta without anticoagulant. The blood samples were centrifuged at 3 000 r/min for 10 min to obtain serum. The serum level of MCP-1 was measured strictly according to the instructions provided in the ELISA kit.

2.6 Detection of pathological changes of lung tissue by HE staining The left upper lung lobe was fixed in 10% neutral formalin, processed for histology, embedded, sectioned, and stained with hematoxylin and eosin (HE). The pathological changes in the lung tissue were then examined under a light microscope.

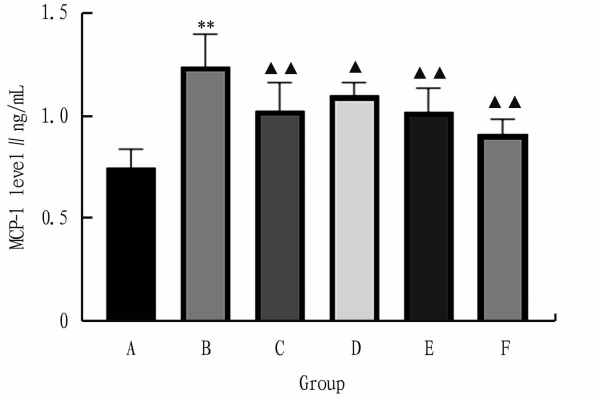
2.7 Detection of IL-1β, IL-6 and TNF-α in lung tissues The lung tissue from the left lower lobe was collected and weighed. A homogenate was prepared at a ratio of 1 ∶ 9 (lung weight expressed in g: ice-cold saline expressed in mL), followed by centrifugation at 3 000 r/min for 10 min to obtain the supernatant. The protein concentration in the supernatant was quantified using the BCA method. Levels of IL-1β, IL-6, and TNF-α in the lung tissue were measured according to the instructions provided in the assay kit.

2.8 Detection of IL-1β, IL-6, PCT, CRP and TNF-α in bronchoalveolar lavage fluid (BALF) The left main bronchus was ligated, and the right lung lobe was lavaged with ice-cold saline. The lung was gently shaken to fully lavage the alveolar spaces, with 0.6 mL administered per instillation. This lavage was repeated three times, and the collected fluid was pooled. Levels of IL-1β, IL-6, PCT, CRP, and TNF-α in the bronchoalveolar lavage fluid were measured according to the instructions provided in the assay kit.

2.9 Statistical analysis SPSS 24.0 was used for statistical analysis, expressed as ($\bar{x} \pm s$), and $P < 0.05$ was used as the criterion for statistical significance of data differences. Measurement data were analyzed by one-way ANOVA between multiple groups and *LSD* test between two groups.

3 Results and analysis

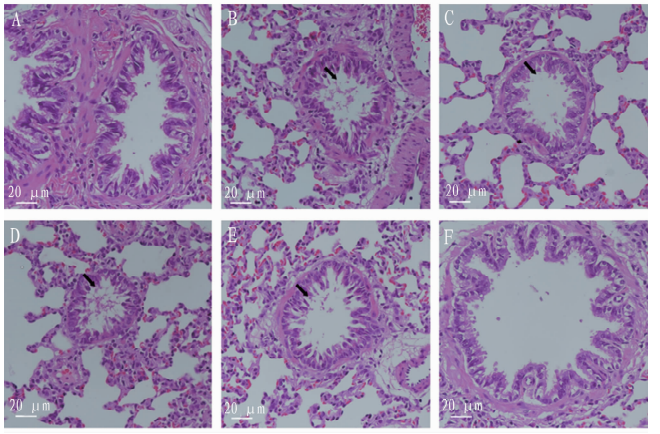
3.1 Effects on serum MCP-1 level As shown in Fig. 1, compared with the normal group, the serum MCP-1 level of rats in the model group was significantly increased ($P < 0.01$); compared with the model group, the serum MCP-1 level of rats in the silybin group and xanthoxylin groups was significantly decreased ($P < 0.05$ or $P < 0.01$).



NOTE A: normal group, B: model group, C: silybin group, D: xanthoxylin low dose group, E: xanthoxylin medium dose group, F: xanthoxylin high dose group; compared with normal group, $^{*}P < 0.05$, $^{**}P < 0.01$; Compared with the model group, $^{▲}P < 0.05$, $^{▲▲}P < 0.01$; the same below.

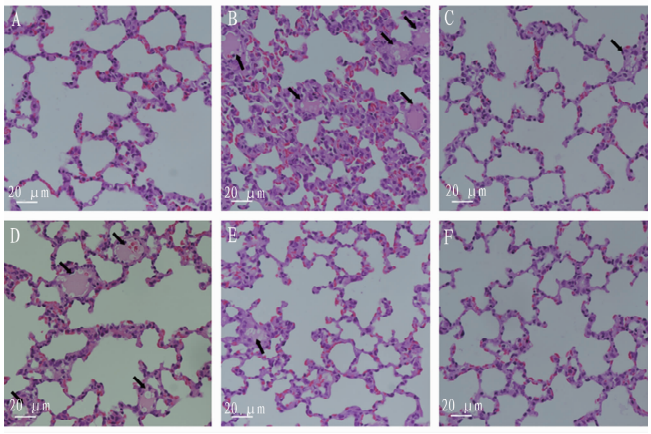
Fig. 1 Levels of serum MCP-1 in rats ($\bar{x} \pm s$, $n = 10$)

3.2 Effects on lung histopathological changes It can be observed from Fig. 2 and Fig. 3 that in the normal group, the lung tissue structure was well-defined, with a smooth bronchial mucosa, no abnormal cell shedding or secretions in the lumen, thin alveolar walls, and no evidence of hemorrhage or edema. In contrast, the model group exhibited blurred lung tissue architecture, disorganized cell arrangement, rough bronchial mucosal surfaces, extensive cell shedding and foreign secretions in the lumen, significantly thickened alveolar walls and septa, obscured alveolar structure, compressed and collapsed alveolar spaces, as well as interstitial edema, hyperemia, secretory exudation, and focal areas of consolidation. Compared with the model group, both the silybin and xanthoxylin groups showed significant improvement in lung pathology. The tissue structure was more distinct, the bronchial mucosa appeared smoother, luminal cell shedding and secretions were considerably reduced, and the thickness of alveolar walls and septa was significantly decreased. Alveolar compression and collapse were alleviated, and pulmonary interstitial edema, congestion, and secretory exudation were significantly attenuated.



NOTE The black arrow indicates that the surface of bronchial mucosa is rough, the cells in the lumen fall off, and there is abnormal secretion.

Fig. 2 Light microscopy of rat bronchial pathological changes (HE staining, 400 ×)



NOTE The black arrow indicates marked thickening of alveolar walls and septa, spatial compression, pulmonary interstitial edema, hyperemia, or abnormal secretion.

Fig. 3 Light microscopy of rat alveolar pathological changes (HE staining, 400 ×)

3.3 Effects on the levels of IL-1β, IL-6 and TNF-α in lung tissues From Fig. 4, it can be seen that compared with the normal group, the levels of IL-1β, IL-6 and TNF-α in the lung tissue of rats in the model group were significantly increased ($P < 0.01$); compared with the model group, the levels of IL-1β, IL-6 and TNF-α in the lung tissue of rats in silybin group and xanthoxylin groups were significantly decreased ($P < 0.05$ or $P < 0.01$).

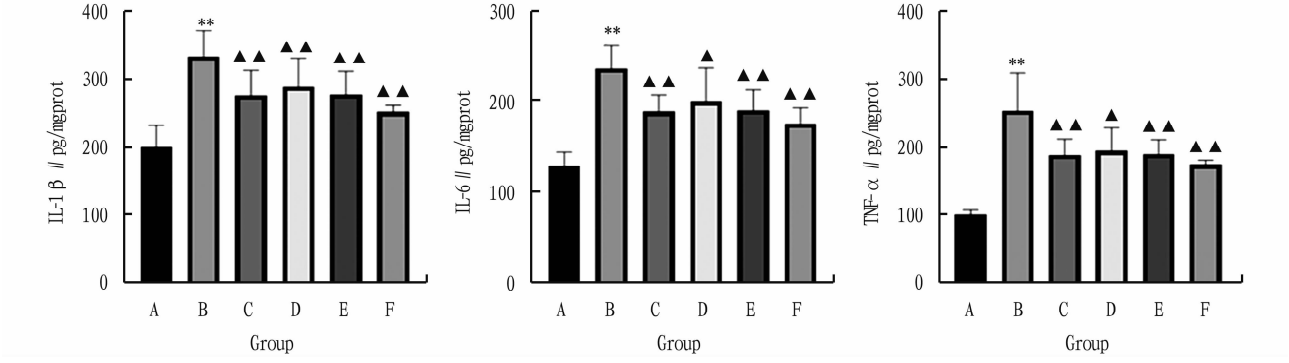


Fig. 4 Level of IL-1β, IL-6, and TNF-α in the lung tissues ($\bar{x} \pm s$, $n = 10$)

3.4 Effects on the levels of IL-1β, IL-6, PCT, CRP and TNF-α in BALF As shown in Fig. 5, compared with the normal group, the levels of IL-1β, IL-6, PCT, CRP and TNF-α in the BALF of the rats in the model group were significantly increased ($P < 0.01$); compared with the model group, the levels of IL-1β, IL-6, PCT, CRP and TNF-α in BALF of rats in silybin group and xanthoxylin groups were significantly decreased ($P < 0.05$ or $P < 0.01$).

4 Discussion

The occurrence and development of ALI is closely related to the abnormal inflammatory response in the lung, which is often accompanied by the infiltration of inflammatory cells and the abnormal release of pro-inflammatory mediators in the lung, including inflammatory mediators, pro-secondary inflammatory mediators and pro-infectious mediators[13]. LPS induces excessive inflammatory response, while D-GalN is a sensitizer. The combination of the two can promote the excessive release of inflammatory mediators, including TNF-α, IL-1β, IL-6 and other major pro-inflammatory cytokines, which mediate the occurrence and development of inflammatory response in multiple tissues and organs[14]. The results of this study showed that the levels of IL-1β, IL-6 and TNF-α in BALF and lung tissue of rats in the model group were significantly increased, indicating that a strong inflammatory reaction occurred in the lung tissue. The levels of IL-1β, IL-6 and TNF-α in BALF and lung tissue of rats in each dose group of xanthoxylin were significantly decreased, indicating that xanthoxylin can inhibit the excessive release of proinflammatory cytokines and has anti-inflammatory protective effect on acute lung injury induced by D-GalN/LPS in rats.

The serum MCP-1 is an important indicator for predicting the prognosis of severe pneumonia. It is a secondary inflammatory mediator that can chemotactic the distribution of specific leukocytes (such as monocytes and basophils)[15]. The results of this study showed that the serum MCP-1 level of rats in the model group was significantly increased, indicating that the body had a strong in-

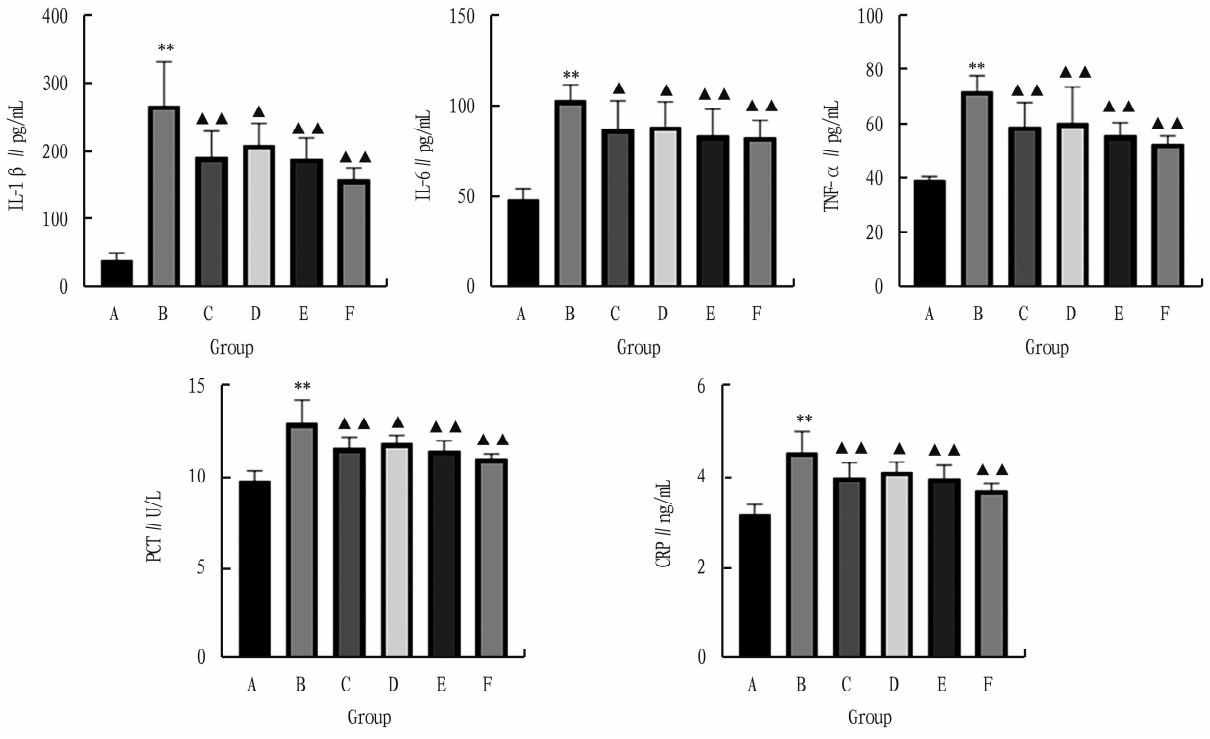


Fig. 5 Level of IL-1β, IL-6, PCT, CRP and TNF-α in the BALF ($\bar{x} \pm s$, $n = 10$)

flammatory response, secondary inflammation occurred and developed progressively, and the prognosis was poor, while the serum MCP-1 level of xanthoxylin groups was significantly decreased, indicating that xanthoxylin inhibited the excessive release of blood MCP-1. Inhibit the occurrence and development of secondary inflammation, and improve the systemic inflammatory microenvironment.

PCT and CRP can be used as sensitive markers to identify severe infection in specific parts of the body. In normal situation, it is undetectable or at low levels, but its release increases dramatically at sites of inflammation[16-17]. The results of this study showed that the levels of PCT and CRP in the BALF of rats in the model group were significantly increased, suggesting that the lungs were in severe infection and the inflammatory reaction was serious, while the levels of PCT and CRP in the BALF of rats in the xanthoxylin groups were significantly decreased, which suggests that xanthoxylin can inhibit the excessive release of PCT and CRP in the lung, and has a significant improvement on pulmonary infection.

Tissue repair is a collaborative process involving the exchange and alteration of tissue cells, extracellular matrix, and intracellular environmental components. It culminates in the restoration or improvement of tissue architecture, with the amelioration of pathological tissue structure serving as a key indicator for evaluating drug efficacy[18]. The typical clinicopathological features of acute lung injury (ALI) include pulmonary edema, hemorrhage, inflammatory cell infiltration, increased abnormal secretions, and diffuse necrosis of lung tissue[4]. Histological results revealed that in the model group, the alveolar structure was poorly defined, the bronchial mucosa appeared thickened and rough, and the alveolar spaces contained extensive necrotic cell debris and abnormal secretions. Alveoli were compressed, atrophic, and collapsed, accompanied by evident interstitial edema and congestion. Treatment with xanthoxylin significantly attenuated lung tissue injury in rats. The bronchial mucosa showed no abnormal thickening or roughness, and both necrotic cells and abnormal secretions within the alveoli were markedly reduced. Alveolar compression was substantially alleviated, and pulmonary interstitial edema and congestion were significantly ameliorated. These findings indicate that xanthoxylin promotes self-healing and repair processes in lung tissue.

In conclusion, xanthoxylin exerts a significant protective effect against D-GalN/LPS-induced acute lung injury (ALI) in rats. It reduces the levels of proinflammatory cytokines (IL-1β, IL-6, and TNF-α) in both BALF and lung tissue, and decreases the release of the secondary inflammatory mediator MCP-1 in serum. In addition, xanthoxylin lowers the levels of PCT and CRP in BALF and ameliorates pathological damage in lung tissue. These beneficial effects may be attributed to its anti-inflammatory, anti-secondary inflammatory, and anti-severe pulmonary infection properties, as well as its ability to promote lung tissue self-repair. With its broad developmental prospects as a therapeutic agent for ALI, xanthoxylin shows promise for clinical application in the prevention and treatment of this condition. This study provides a preliminary experimental foundation for its future use, although more in-depth mechanisms remain to be further investigated.

(To page 54)

363 – 371.
[11] LEE J, YANG SN. Effectiveness of extracorporeal shock wave therapy after botulinum toxin injection for post-stroke upper extremity spasticity: A randomized controlled study[J]. Toxins (Basel), 2024, 16(4): 197.
[12] LI TY, CHANG CY, CHOU YC, *et al.* Effect of radial shock wave therapy on spasticity of the upper limb in patients with chronic stroke: A prospective, randomized, single blind, controlled trial[J]. Medicine (Baltimore), 2016, 95(18): e3544.
[13] SANTAMATO A, NOTARNICOLA A, PANZA F, *et al.* SBOTE study: extracorporeal shock wave therapy versus electrical stimulation after botulinum toxin type a injection for post-stroke spasticity-a prospective randomized trial[J]. Ultrasound in Medicine and Biology, 2013, 39(2): 283 – 291.
[14] SENARATH ID, THALWAHTTE RD, PATHIRAGE M, *et al.* The effectiveness of radial extracorporeal shock wave therapy vs transcutaneous electrical nerve stimulation in the management of upper limb spasticity in chronic-post stroke hemiplegia-a randomized controlled trial[J]. PLoS One, 2023, 18(5): e0283321.
[15] WU YT, YU HK, CHEN LR, *et al.* Extracorporeal shock waves versus botulinum toxin type A in the treatment of poststroke upper limb spasticity: A randomized noninferiority trial[J]. Archives of Physical Medicine and Rehabilitation, 2018, 99(11): 2143 – 2150.
[16] YOON SH, SHIN MK, CHOI EJ, *et al.* Effective site for the application of extracorporeal shock-wave therapy on spasticity in chronic stroke: muscle belly or myotendinous junction[J]. Annals of Rehabilitation Medicine, 2017, 41(4): 547 – 555.
[17] MIHAI EE, DUMITRU L, MIHAI IV, *et al.* Long-term efficacy of extracorporeal shock wave therapy on lower limb post-stroke spasticity: A systematic review and meta-analysis of randomized controlled trials[J]. Journal of Clinical Medicine, 2020, 10(1): 86.
[18] YUAN H, WANG K, ZHANG QB, *et al.* The effect of extracorporeal shock wave on joint capsule fibrosis based on A2AR-Nrf2/HO-1 pathway in a rat extending knee immobilization model[J]. Journal of Orthopaedic Surgery and Research, 2023, 18(1): 930.

(From page 40)

References

[1] BAI T, JI P, ZHANG WF, *et al.* Protective effect of platycodon grandiflorum-glycyrrhiza on lipopolysaccharide-induced acute lung injury in mice[J]. Progress in Veterinary Medicine, 2025, 46(3): 82 – 86. (in Chinese).
[2] FANG Q, GAO R, GAO YJ, *et al.* Progress on animal models of acute lung injury[J]. China Animal Husbandry & Veterinary Medicine, 2010, 37(5): 50 – 54. (in Chinese).
[3] HUANG GX, LIU MJ, MA M, *et al.* Effects of *Callicarpa nudiflora* extract on growth performance, immune function, anti-inflammatory ability and intestinal barrier function of chickens infected with *Escherichia coli* [J]. Chinese Journal of Animal Nutrition, 2024, 36(12): 7754 – 7769. (in Chinese).
[4] HU MX, DENG YP, HE YY, *et al.* Protective effect of alcohol extract from *Curcuma kwangsiensis* on lung injury induced by LPS in mice[J]. China Animal Husbandry & Veterinary Medicine, 2024, 51(8): 3652 – 3661. (in Chinese).
[5] LIU C, XIAO K, XIE LX. Advances in the regulation of macrophage polarization by mesenchymal stem cells and implications for ALI/ARDS treatment[J]. Frontiers in Immunology, 2022, 13: 928134.
[6] WANG X, LI CD, PAN XB, *et al.* Simultaneous determination of galic acid, brervifolincaboxylic acid, corilagin, ellagic acid and rutin in *Phyllanthus niruri*. Linn. by HPLC[J]. Chinese Journal of Pharmaceutical Analysis, 2018, 38(9): 1641 – 1645. (in Chinese).
[7] NIU XF, HE LC, FAN T, *et al.* Protecting effect of brevifolin and 8,9-single-epoxy brevifolin of *Phyllanthus simplex* on rat liver injury[J]. China Journal of Chinese Materia Medica, 2006, 31(18): 1529 – 1532. (in Chinese).
[8] WANG YQ, SI JY, LIU XM, *et al.* Isolation, identification and quantitative determination of ellagic acid and brevifolincarboxylic acid in *Duchesnea indica* by RP-HPLCa[J]. Natural Product Research and Development, 2008, 20(4): 667 – 671. (in Chinese).
[9] CHEN XX, ZHANG LY, WAN JZ, *et al.* Simultaneous isolation and purification of gallic acid and brevifolincarboxylic acid from *Polygonum capitatum* by high-speed counter-current chromatography [J]. China Journal of Chinese Materia Medica, 2000, 35(15): 1957 – 1960. (in Chinese).
[10] WANG XL, XING DM, DING Y, *et al.* Determination and pharmacokinetic study of brevifolin in rat after ig administration of pomegranate leaf extract[J]. Chinese Pharmacological Bulletin, 2005, 21(3): 369 – 372. (in Chinese).
[11] REN WX, WANG JH, LIU M, *et al.* Effect and mechanism of silybin on improving pulmonary fibrosis and oxidative stress in mice by mediating BMP9/SMAD signaling pathways[J]. Medical Journal of Chinese People's Liberation Army, 2022, 47(12): 1201 – 1208. (in Chinese).
[12] RAISH M, AHMAD A, ALKHARFY KM, *et al.* Hepatoprotective activity of lepidium sativum seeds against D-galactosamine/lipopolysaccharide induced hepatotoxicity in animal model[J]. BMC Complementary and Alternative Medicine, 2016, 16(1): 501.
[13] DU LJ, LIN JH, YE JH, *et al.* Effect and mechanism of Yiqi tongfu xiere prescription on lipopoly saccharide-induced acute lung injury in mice[J]. Chinese Journal of Clinical Pharmacology and Therapeutics, 2025, 30(5): 599 – 607. (in Chinese).
[14] CEN W, CHEN ZL, GU N, *et al.* Prevention of AMI induced ventricular remodeling: inhibitory effects of heart-protecting musk pill on IL-6 and TNF-alpha[J]. Evidence-Based Complementary and Alternative Medicine, 2017, 2017: 3217395.
[15] ZHAO YG, LONG H. Relationship between changes of HMGB1, TLR4 and MCP-1 levels in peripheral blood and prognosis in patients with severe pneumonia-related sepsis before and after CVVHDF[J]. Journal of Tropical Medicine, 2023, 23(4): 516 – 521. (in Chinese).
[16] PONTRELLI G, DE CF, BUZZETTI R, *et al.* Accuracy of serum procalcitonin for the diagnosis of sepsis in neonates and children with systemic inflammatory syndrome: A meta-analysis[J]. BMC Infectious Diseases, 2017, 17(1): 302.
[17] STOCKER M, VAN HW, EL HS, *et al.* C-reactive protein, procalcitonin, and white blood count to rule out neonatal early-onset sepsis within 36 hours: A secondary analysis of the neonatal procalcitonin intervention study[J]. Clinical Infectious Diseases, 2021, 73(2): 383 – 390.
[18] XU LB, ZHAO DY, WEI ML, *et al.* Effect of weile capsules on acetic acid-induced chronic gastric ulcer in rats[J]. Chinese Traditional Patent Medicine, 2018, 40(6): 1377 – 1380. (in Chinese).