

基于代谢组学探讨桔梗汤对脂多糖诱导的小鼠急性肺损伤的保护作用

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摘要: 桔梗汤作为治疗肺部疾病的经典方剂之一, 其化学组成与作用机制尚不明确。本研究采用高分辨质谱技术检测桔梗汤中的化学成分, 通过代谢组学技术探究桔梗汤对急性肺损伤 (acute lung injury, ALI) 模型小鼠肺组织代谢物的影响。以差异倍数 (fold change, FC) > 1.5 或 FC < 0.67, 并且 $P < 0.05$ 为条件筛选潜在生物标志物并进行代谢通路富集分析。桔梗汤经质谱分析共鉴定出 40 个化合物, 包括三萜皂苷类成分、黄酮类成分、糖苷类成分等。动物实验经天津中医药大学实验动物伦理委员会批准 (批准号: TCM-LAEC2021106)。研究发现, 桔梗汤可改善 ALI 模型小鼠的肺系数及肺组织形态, 降低肺泡灌洗液中丙二醛、肿瘤坏死因子- α 、白介素-6 的含量, 减少肺组织中髓过氧化物酶含量。代谢组学结果表明, 桔梗汤可调节与 ALI 相关的 22 个代谢物, 其中白三烯 D4、二十二碳五烯酸、次黄嘌呤、L-5-羟脯氨酸等代谢物主要与机体炎症反应和氧化应激相关, 且被富集于谷胱甘肽代谢、嘌呤代谢和初级胆汁酸生物合成等通路。本研究通过代谢组学分析桔梗汤治疗 ALI 的潜在作用机制, 为桔梗汤的临床应用提供了重要的理论依据。

关键词: 桔梗汤; 急性肺损伤; 代谢组学; 作用机制

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The effect of Jiegeng decoction on lipopolysaccharide-induced acute lung injury in mice based on metabolomics

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Abstract: One of the traditional prescriptions for treating lung diseases, Jiegeng decoction (JGT), is still unknown in terms of its chemical makeup and mechanism. In this study, Q-Exactive-Orbitrap MS technology was used to identify the chemical constituents of JGT, and metabolomics was used to examine the effect of JGT on metabolites in the lung tissue of mice with acute lung injury (ALI) model. The potential biomarkers were screened by fold change (FC) > 1.5 or FC < 0.67 and $P < 0.05$, and enriched for metabolic pathways. A total of 40 compounds, including triterpenoid saponins, flavonoids and glycosides, were identified by mass spectrometry analysis of JGT. All animal experiments were approved by the Experimental Animal Ethics Committee of Tianjin University of Traditional Chinese Medicine (No. TCM-LAEC2021106). The results showed that JGT improved the lung coefficient, and lung tissue morphology of mice with ALI, lowered the levels of malondialdehyde (MDA), tumor necrosis factor α (TNF- α), and interleukin 6 (IL-6) in bronchoalveolar lavage fluid (BALF), and reduced myeloperoxidase (MPO) content in lung tissue. The metabolomic results showed that JGT could regulate 22 metabolites associated with ALI, among which leukotriene D4, docosapentaenoic acid, hypoxanthine, L-5-oxoproline, and other metabolites were mainly associated with the body's inflammatory response and oxidative stress, and were enriched in the

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pathways of glutathione metabolism, purine metabolism, and primary bile acid biosynthesis. This study analyzed the potential mechanism of JGT in the treatment of ALI through metabolomics, providing an important theoretical basis for the clinical application of JGT.

Key words: Jiegeng decoction; acute lung injury; metabolomics; mechanism

急性肺损伤 (acute lung injury, ALI) 可由脓毒症、肺炎、烧伤等多种因素诱发, 是一种以肺水肿、组织微循环发生阻碍、血管通透性增加、急性缺氧为特征的呼吸道疾病^[1]。临床上主要使用糖皮质激素和阿司匹林进行治疗, 但治疗效果均不理想, 且还会产生一些不良反应^[2]。ALI 在全球的发病率及病死率都很高, 尤其是近年来严重急性呼吸综合征冠状病毒 2 以肺为攻击的主要目标器官, 导致严重的 ALI 和急性呼吸窘迫综合征, 给全球的医疗、经济等造成重大影响^[3,4]。中药复方作为中医临床用药的主要形式, 在我国此次抗击 2019 年冠状病毒病中, 被广泛应用于临床, 并取得了积极的治疗效果^[5,6]。

桔梗汤 (Jiegeng decoction, JGT) 作为经典复方之一, 最初记载于张仲景的《伤寒论·辨少阴病脉证并治》和《金匱要略》中, 全方由桔梗、甘草两味药组成^[7]。桔梗行瘀而排脓, 甘草泄热而保中, 桔梗甘草二者相配主治肺痈^[8]。临床上桔梗汤随证灵活加味对肺部等其他方面疾病均有较好的治疗效果^[9-11]。近年来, 有研究采用蛋白质组、脂质组探究桔梗汤对脂多糖 (lipopolysaccharide, LPS) 诱导的 ALI 模型小鼠的保护作用^[12,13], 但其作用机制还有待进一步研究。

由于目前临床诊断及方法存在局限性, 肺癌通常在晚期才被诊断出来^[14]。代谢组学作为组学领域的重要分支, 主要研究小分子代谢物的变化^[15]。基于代谢组学筛选出差异的代谢物可作为潜在生物标志物, 用于评价肺部疾病的早期、发展及预后, 为新靶点干预提供依据^[16]。与血清、肺泡灌洗液等生物样本相比, 对肺组织进行相关代谢组的研究更有利于揭示呼吸系统疾病的发病机制^[17]。本文首先对桔梗汤的化学成分进行定性分析。其次, 通过生化指标以及病理症状评价桔梗汤对于 LPS 诱导的 ALI 模型小鼠的治疗效果, 并对肺组织进行代谢组研究。旨在探究桔梗汤抗 ALI 可能的作用机制, 为桔梗汤在临床上治疗肺部疾病提供实验基础和理论依据。

材料与方

药物与试剂 桔梗 (安徽, 200501) 和甘草 (甘肃, 20200801) 均购买于天津市南开区北京同仁堂药店, 经天津中医药大学中医药研究院吴红华副研究员鉴定

为桔梗科植物桔梗 (*Platycodon grandiflorus* (Jacq.) A. DC.) 的根、豆科植物甘草 (*Glycyrrhiza uralensis* Fisch.) 的根茎; 桔梗皂苷 D (20022601)、桔梗皂苷 D2 (20110904)、甘草素 (19021410)、异甘草素 (19031103)、甘草苷 (20022701)、异甘草苷 (20022803)、甘草酸 (20022509)、甘草次酸 (20042710) 对照品购自四川维克奇生物科技有限公司 (桔梗皂苷 D2 纯度 $\geq 96\%$, 其他对照品纯度 $\geq 98\%$); LPS (L4268), 美国 Sigma-Aldrich 公司; PBS (pH 7.4) (02-024-1ACS), 以色列 Biological Industries 生物科技公司; 地塞米松 (DXMS, D829854), 上海麦克林生化科技有限公司; 髓过氧化物酶 (myeloperoxidase, MPO) 试剂盒 (A044-1-1) 与超氧化物歧化酶 (superoxide dismutase, SOD) 试剂盒 (A001-3-2), 南京建成生物工程有限公司; 丙二醛 (malondialdehyde, MDA) 试剂盒 (ZC-S0343)、肿瘤坏死因子- α (tumor necrosis factor α , TNF- α) 试剂盒 (ZC-39024)、白介素-6 (interleukin 6, IL-6) 试剂盒 (ZC-37988), 上海茁彩生物科技有限公司; 甲醇、乙腈 (色谱纯), 美国 Fisher 公司; 甲酸, 美国 ACS 恩科化学。

仪器 Centrifuge5424R 高速低温离心机 (德国 Eppendorf 公司); AX-205 十万分之一电子天平 (瑞士 Mettler Toledo 公司); Infinite 200 PRO 多功能酶标仪 (瑞士 TECAN 公司); Tissuelyser-32L 多样品组织研磨仪 (上海净信实业发展有限公司); Q-Exactive-Orbitrap MS 四级杆-静电场轨道阱高分辨质谱仪 (美国 Thermo Fisher Scientific 公司)。

药物的配置 取桔梗药材 250 g, 甘草药材 500 g 加 10 倍量水, 浸泡 0.5 h, 加热回流 2 h, 将药渣再加八倍量水加热回流提取 1.5 h, 合并两次药液, 减压浓缩, 冻干, 得桔梗汤冻干粉。

称取桔梗汤粉末 5 mg, 加入 1 mL 水涡旋混匀, 14 000 r·min⁻¹ 离心 10 min, 取上清, 得到质量浓度为 5 mg·mL⁻¹ 的全方溶液; 精密称取各对照品 1 mg, 加 1 mL 水涡旋混匀, 配制成质量浓度 1 mg·mL⁻¹ 的单标溶液, 将 8 个单标溶液分别取 100 μ L 再加水至 1 mL, 涡旋、离心, 取上清, 得到质量浓度为 100 μ g·mL⁻¹ 的混标溶液。

称取一定量桔梗汤冻干粉, 加水配制成含生药 1.365、2.73、4.095 g·mL⁻¹ 的桔梗汤低、中、高剂量溶液; 称

取一定量的地塞米松, 配制质量浓度为 $0.2 \text{ mg} \cdot \text{mL}^{-1}$ 的溶液。

桔梗汤的化学成分分析 使用Phenomenex Kinetex[®] C18 100A (100 mm \times 2.1 mm, 1.7 μm) 色谱柱进行分离。柱温 $35 \text{ }^\circ\text{C}$, 流速 $0.3 \text{ mL} \cdot \text{min}^{-1}$, 进样量 $3 \mu\text{L}$, 流动相由乙腈(A)和0.1% 甲酸水(B)组成。洗脱梯度: 0~5 min, 5% A; 5~8 min, 23% A; 8~10 min, 23%~30% A; 10~20 min, 30%~45% A; 20~25 min, 45%~95% A; 电喷雾离子源, 正负离子检测模式; 质子扫描范围 m/z 为100~1 500; 喷雾电压为 $-3.0 \text{ kV}/+3.5 \text{ kV}$; 鞘气流量为 $35 \text{ L} \cdot \text{h}^{-1}$; 辅助气 $10 \text{ L} \cdot \text{h}^{-1}$; 毛细管温度 $350 \text{ }^\circ\text{C}$; 二级扫描分辨率为17 500。

实验动物 SPF级雌性BALB/C小鼠(18~20 g), 60只, 购自斯贝福(北京)生物技术有限公司, 许可证号: SCXK(京)2019-0010, 饲养于天津中医药大学动物中心。适应性喂养1周, 自由饮水摄食。实验方案均已通过天津中医药大学实验动物伦理委员会批准(批准号: TCM-LAEC2021106)。

分组、模型建立与给药 将60只BALB/C雌性小鼠, 随机分为6组: 空白组(control group, CON)、模型组(model group, LPS)、阳性药组(positive drug group, DXMS)、桔梗汤低剂量组(low-dose group of JGT, JGT-L)、中剂量组(middle-dose group of JGT, JGT-M)、高剂量组(high-dose group of JGT, JGT-H)。以灌胃的方式给药, 给药量为 $10 \text{ mL} \cdot \text{kg}^{-1}$, CON组和LPS组给予等量生理盐水。在灌胃给药或生理盐水15 min后, 采用三溴乙醇($350 \text{ mg} \cdot \text{kg}^{-1}$)将小鼠麻醉。小鼠经气管软骨环间隙向气管内缓慢注射LPS($2 \text{ mg} \cdot \text{kg}^{-1}$) $30 \mu\text{L}$ 建立ALI模型。CON组注射等体积生理盐水, 操作方法同上。

标本采集 造模6 h后, 称量体重, 摘眼球取血, 血液于室温下静置2 h, $4 \text{ }^\circ\text{C}$ 、 $3 500 \text{ r} \cdot \text{min}^{-1}$ 离心10 min, 分离血清, $-80 \text{ }^\circ\text{C}$ 储存。进行3次支气管肺泡灌洗, 每次 0.5 mL PBS, 将获得肺泡灌洗液(bronchoalveolar lavage fluid, BALF), $4 \text{ }^\circ\text{C}$ 、 $1 500 \text{ r} \cdot \text{min}^{-1}$ 离心10 min, 取上清。根据试剂盒说明, 测定BALF中SOD、MDA的含量; 采用酶联免疫法测定BALF中TNF- α 、IL-6的含量。摘取肺, 称肺湿重, 计算肺系数, 肺系数(%) = 肺湿重(g)/体重(kg) $\times 100\%$ 。切取部分肺用于苏木紫-伊红(hematoxylin and eosin, HE)染色, 在光学显微镜下观察病理变化。准确称取肺组织制成5%的组织匀浆, 按试剂盒要求测定其中MPO的含量。

统计学处理 采用GraphPad Prism 8软件分析数据, 使用单因素方差分析(one-way ANOVA)检验组间差异是否具有显著性, 数据用平均值 \pm 标准差(Mean \pm SD)表达, 以 $P < 0.05$ 表示具有统计学差异。

代谢组学样品制备 称取CON、LPS、JGT-M组的肺组织50 mg, 加入1.5 mL预冷的 $\text{CH}_3\text{OH}/\text{H}_2\text{O}$ (2:1), 使用组织研磨仪研磨, $4 \text{ }^\circ\text{C}$ 、 $12 700 \text{ r} \cdot \text{min}^{-1}$ 离心10 min, 将上清液取出并氮吹干, 加入 $100 \mu\text{L}$ $\text{CH}_3\text{OH}/\text{H}_2\text{O}$ (2:1)复溶, 涡旋3 min, 离心, 取上清待测。取等量样本混合即得质量控制样本(quality control, QC), 每10个样品后插入一个QC, 用于评价系统稳定性。

肺组织代谢组学分析 使用ACQUITY UPLC HSS T3 (100 mm \times 2.1 mm, 1.8 μm) 色谱柱进行分离。柱温 $45 \text{ }^\circ\text{C}$, 流速 $0.3 \text{ mL} \cdot \text{min}^{-1}$, 进样量 $5 \mu\text{L}$, 流动相由0.1% 甲酸水(A)和0.1% 甲酸甲醇(B)组成。洗脱梯度: 0~2 min, 0.1% B; 2~6 min, 0.1%~25% B; 6~10 min, 25%~80% B; 10~12 min, 80%~90% B; 12~21 min, 90%~99.9% B; 质谱条件同上。

代谢组学数据处理 原始谱图首先使用AbfConverter软件转换成abf格式, 之后导入MS-DIAL 4.6软件中, 设置具体参数, 进行归一化等处理, 最终得到不同离子模式下代谢组学数据。将所得数据整理后导入SIMCA 14.1软件进行多维统计分析, 包括主成分分析和偏最小二乘判别分析。利用MetaboAnalyst 5.0 (<https://www.metaboanalyst.ca/>) 网站进行单因素统计分析, 计算代谢物的FC值和P值, 通过筛选得到具有显著性差异的离子($\text{FC} > 1.5$ 或 $\text{FC} < 0.67$, 并且 $P < 0.05$)。之后使用MS-DIAL软件内置数据库及HMDB数据库进行差异代谢物的鉴定。将CON组vs LPS组、JGT-M组vs LPS组所获得的上下调差异代谢物分别取交集, 得到潜在差异代谢物, 并进行代谢通路富集分析。

结果

1 桔梗汤中化学成分的定性分析

使用Thermo Scientific[™] Xcalibu[™]系统编辑质谱方法、采集并处理数据, 预测化学式, 将对应的二级碎片与文献中已知化学成分的碎片信息进行比对, 推测其中可能存在的化学成分。桔梗汤共鉴定了40个化合物(图1), 经标准品比对确证了8个。化合物负、正离子模式下的质谱信息如下(表1)。

2 药效学研究

2.1 一般状态观察及肺系数 LPS组与CON组相比, 小鼠活动减弱, 肺部出现湿罗音, 呼吸急促。JGT-H组可能因为给药浓度大导致小鼠精神状态相对较差, 其余给药组与LPS组相比小鼠状态均明显好转。如图2A所示, 与CON组相比, LPS组肺系数显著升高($P < 0.05$); 与LPS组相比, DXMS组与JGT-H组可显著降低肺系数($P < 0.05$), JGT-L组和JGT-M组肺系数也均降低, 但差异无统计学意义。

Table 1 Identification of chemical compounds of JGT. *Verification with reference standards; MS: Mass spectrometry

No.	t_R /min	Detected (m/z)	Mode	MS/MS (m/z)	Error	Compound	Formula
1	0.99	191.018 9	[M-H] ⁻	173.008 3	-4.323×10 ⁻⁶	Citric acid	C ₆ H ₈ O ₇
2	1.12	117.018 2	[M-H] ⁻	99.007 2; 73.028 0	-9.673×10 ⁻⁶	Butanedioic acid	C ₄ H ₆ O ₄
3	3.09	211.044 8	[M-H] ⁻	165.054 3; 121.064 3; 119.048 8; 93.033 0; 59.012 4	-3.572×10 ⁻⁶	2-[(4-Hydroxyphenyl)methyl] propanedioic acid	C ₁₀ H ₁₀ O ₅
4	3.16	165.054 6	[M-H] ⁻	—	-6.77×10 ⁻⁶	Ethylparaben	C ₉ H ₁₀ O ₃
5	3.9	625.177 1	[M+HCOO] ⁻	417.119 0; 255.065 5; 135.007 2; 119.048 6	-0.493×10 ⁻⁶	Liquiritigenin-7,4'-diglucoside	C ₂₇ H ₃₂ O ₁₄
6	4.86	563.140 7	[M-H] ⁻	353.065 8; 365.065 7; 383.076 5; 425.087 6; 443.097 3; 455.097 5; 473.107 6; 503.119 7; 545.128 4	0.127×10 ⁻⁶	Schaftoside	C ₂₆ H ₂₈ O ₁₄
7	5.42	577.155 8	[M-H] ⁻	559.140 9; 503.117 9; 457.114 1; 413.087 3; 383.076 3; 353.065 8	-0.829×10 ⁻⁶	6-C-Rhamnosyl-8-C- glucosylapigenin	C ₂₇ H ₃₀ O ₁₄
8	5.50	433.113 6	[M-H] ⁻	271.060 5; 119.048 6	-0.97×10 ⁻⁶	5-Hydroxyliquiritin	C ₂₁ H ₂₂ O ₁₀
9	5.53	549.161 1	[M-H] ⁻	429.105 2; 255.065 6	-0.481×10 ⁻⁶	Liquiritin apioside	C ₂₆ H ₃₀ O ₁₃
10	5.54	491.119 3	[M+HCOO] ⁻	283.060 5; 268.037 0	-0.406×10 ⁻⁶	Calycosin-7-O-β-D-glucoside	C ₂₂ H ₂₂ O ₁₀
11	5.61	433.113 6	[M-H] ⁻	271.060 6; 119.048 7	-0.97×10 ⁻⁶	Prunin	C ₂₁ H ₂₂ O ₁₀
12*	5.68	255.065 4	[M-H] ⁻	135.007 2; 119.048 6	-2.282×10 ⁻⁶	Liquiritigenin	C ₁₅ H ₁₂ O ₄
13	5.72	579.171 0	[M-H] ⁻	255.065 6	-1.604×10 ⁻⁶	Liquiritigenin-7,4''-diglucoside	C ₂₇ H ₃₂ O ₁₄
14*	5.73	417.119 4	[M-H] ⁻	255.065 5; 135.007 2	0.706×10 ⁻⁶	Liquiritin	C ₂₁ H ₂₂ O ₉
15	5.79	707.182 7	[M-H] ⁻	605.149 5; 563.136 6; 545.127 4; 473.107 0; 443.097 3; 383.076 2; 353.065 6; 97.075 8	-0.265×10 ⁻⁶	Apigenin-6-C-β-xylopyranosyl-8-C- (6''-O-(3-hydroxy-3- methylglutaroyl)-β-glucopyranoside)	C ₃₂ H ₃₆ O ₁₈
16	7.06	441.176 7	[M-H] ⁻	—	0.181×10 ⁻⁶	Lusitanicoside	C ₂₁ H ₃₀ O ₁₀
17	7.15	723.502 7	[M-H] ⁻	—	-3.554×10 ⁻⁶	[1-Tetradecanoyloxy-3-[(2R,4R,5R)- 3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl]oxypropan-2-yl](9Z,12Z, 15Z)-octadeca-9,12,15-trienoate	C ₄₁ H ₇₂ O ₁₀
18	7.35	549.161 8	[M-H] ⁻	297.076 4; 269.081 1; 255.065 5	0.794×10 ⁻⁶	Licraside	C ₂₆ H ₃₀ O ₁₃
19	7.65	267.066 2	[M-H] ⁻	252.041 9	3.799×10 ⁻⁶	Formononetin	C ₁₆ H ₁₂ O ₄
20*	7.84	417.119 4	[M-H] ⁻	255.065 3; 153.017 8; 135.007 1; 119.048 6	0.706×10 ⁻⁶	Isoliquiritoside	C ₂₁ H ₂₂ O ₉
21*	8.84	255.065 5	[M-H] ⁻	153.017 8; 135.007 2; 119.048 6	-2.282×10 ⁻⁶	Isoliquiritigenin	C ₁₅ H ₁₂ O ₄
22*	11.19	1 385.621 8	[M-H] ⁻	—	-0.908×10 ⁻⁶	Platycodin D2	C ₆₃ H ₁₀₂ O ₃₃
23*	11.23	1 223.569 8	[M-H] ⁻	409.134 2; 469.155 2; 541.176 4; 635.378 5; 681.383 3; 723.396 2; 1 133.538 5	-0.355×10 ⁻⁶	Platycodin D	C ₅₇ H ₉₂ O ₂₈
24	11.30	1 369.626 6	[M-H] ⁻	—	-1.127×10 ⁻⁶	Polygalacin D2	C ₆₃ H ₁₀₂ O ₃₂
25	11.31	823.412	[M-H] ⁻	351.055 9; 289.056 0	-0.193×10 ⁻⁶	Uralsaponin C/Licorice-saponin J2	C ₄₂ H ₆₄ O ₁₆
26	11.44	1 207.575 6	[M-H] ⁻	—	0.232×10 ⁻⁶	Polygalacin D	C ₅₇ H ₉₂ O ₂₇
27	11.62	1 279.559 9	[M-H] ⁻	—	-0.128×10 ⁻⁶	PlatycodinK/L	C ₅₉ H ₉₂ O ₃₀
28	11.66	999.444 1	[M-H] ⁻	837.380 7; 497.114 3; 321.081 4	-0.147×10 ⁻⁶	24-Hydroxy-licoricesaponin A3	C ₄₈ H ₇₂ O ₂₂
29	11.70	853.386 5	[M-H] ⁻	501.317 2; 351.056 0; 193.034 2	0.19×10 ⁻⁶	22-Hydroxy-licoricesaponin G2	C ₄₂ H ₆₂ O ₁₈
30	12.20	849.355 2	[M-H] ⁻	289.055 9; 351.056 0; 453.297 9; 629.332 6	0.191×10 ⁻⁶	Uralsaponin D	C ₄₂ H ₅₈ O ₁₈
31	12.24	1 279.559 9	[M-H] ⁻	—	-0.128×10 ⁻⁶	Platyconic acid B	C ₅₉ H ₉₂ O ₃₀
32	14.44	819.380 6	[M-H] ⁻	289.055 8; 351.056 0; 193.034 0	-0.316×10 ⁻⁶	Licorice-saponin E2	C ₄₂ H ₆₀ O ₁₆
33	15.01	863.407 1	[M-H] ⁻	289.054 7; 351.056 1	0.031×10 ⁻⁶	22β-Acetoxyl-glycyrrhaldehyde	C ₄₄ H ₆₄ O ₁₇
34	15.30	985.465 4	[M-H] ⁻	663.371 7; 497.113 9; 321.081 9	0.424×10 ⁻⁶	Yunganoside D1	C ₄₈ H ₇₄ O ₂₁
35	15.35	953.474 5	[M-H] ⁻	193.034 3	-0.58×10 ⁻⁶	Uralsaponin T	C ₄₈ H ₇₄ O ₁₉
36*	15.79	471.345 3	[M+H] ⁺	453.334 5	-6.336×10 ⁻⁶	Glycyrrhetic acid	C ₃₀ H ₄₆ O ₄
37*	15.81	821.396 5	[M-H] ⁻	469.333 1; 351.056 0	-0.011×10 ⁻⁶	Glycyrrhizic acid	C ₄₂ H ₆₂ O ₁₆
38	16.63	807.417 7	[M-H] ⁻	351.055 9; 333.044 4; 289.056 4; 193.034 1; 113.022 9	0.564×10 ⁻⁶	Licoricesaponine B2	C ₄₂ H ₆₄ O ₁₅
39	16.81	969.470 3	[M-H] ⁻	497.113 9; 321.081 8	0.24×10 ⁻⁶	Albizzasaponin B	C ₄₈ H ₇₄ O ₂₀
40	22.45	351.087 4	[M-H] ⁻	283.096 9; 265.086 2; 241.086 1; 199.075 2	-0.033×10 ⁻⁶	Licoisoflavone B	C ₂₀ H ₁₆ O ₆

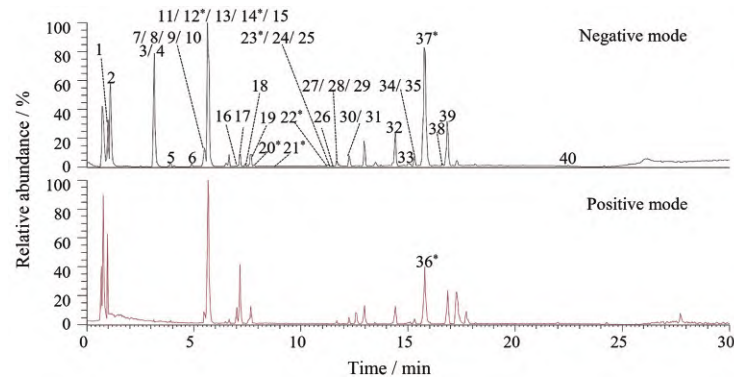


Figure 1 Total ion chromatograms of Jiegeng decoction (JGT). The peak numbers were in accordance with Table 1. Asterisk (*) indicates the compound which is verified to reference standards

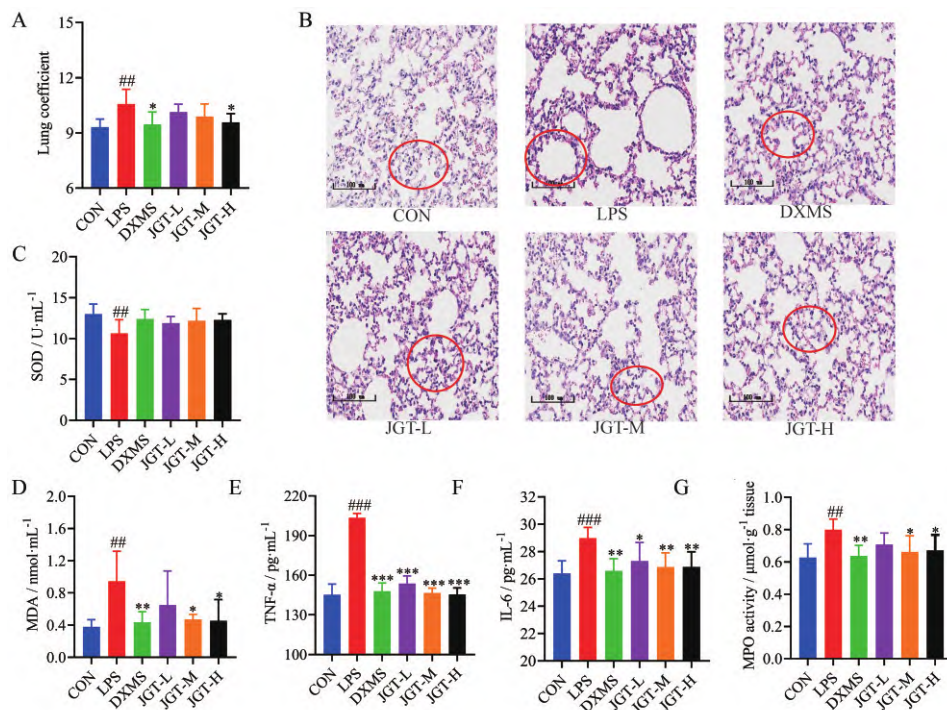


Figure 2 Comparison of the lung coefficients of mice in each group (A), pathological results of lipopolysaccharide (LPS)-induced acute lung injury (ALI) (B), comparison of biochemical indexes of mice in each group (C-G). C, D, E, F: The amount of SOD, MDA, TNF- α , IL-6 in each group BALF. G: MPO content in lung tissue of each group. SOD: Superoxide dismutase; MDA: Malondialdehyde; TNF- α : Tumor necrosis factor α ; IL-6: Interleukin 6; MPO: Myeloperoxidase; HE $\times 100$. CON: Control group; LPS: Lipopolysaccharide (model group); DXMS: Dexamethasone (positive drug group); JGT-L, JGT-M, JGT-H: Total extract of JGT low-dose group, middle-dose group, high-dose group. $n = 6$, $\bar{x} \pm s$. ## $P < 0.01$, ### $P < 0.001$ vs CON; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs LPS

2.2 病理学影响 HE染色结果显示(图2B), CON组小鼠肺组织结构清楚、无渗出液,肺泡腔没有出现水肿及炎细胞浸润等病理现象,细胞核居中,细胞间连接紧密,结构完整;LPS组小鼠肺组织结构紊乱,细胞间隙增宽,肺泡壁明显增厚且肺泡腔融合(红色圈),出现明显的炎性浸润和间质水肿。此外,还有大量炎性细胞出现,纤维组织增生,肺组织细胞质皱缩,细胞核不规则并有黏连;与LPS组相比较,JGT-L组仍有部分细胞黏连,肺泡

间隔增宽,肺泡腔部分融合;JGT-M组与JGT-H组细胞核居中,细胞连接紧密;总体上来看,各给药组对肺组织水肿、肺泡壁增厚、炎性细胞浸润均有不同程度的减轻。

2.3 生化指标检测 与CON组相比,LPS组SOD含量显著降低($P < 0.05$);相较于LPS组,各给药组SOD含量均有升高趋势,但差异无统计学意义。与CON组相比LPS组MDA水平显著升高($P < 0.05$);与LPS组相比较,各给药组MDA水平均有降低趋势,且DXMS组、

JGT-M组及JGT-H组MDA水平显著降低 ($P < 0.05$)。与CON组相比, LPS组中TNF- α 和IL-6水平显著升高 ($P < 0.05$), 给药后均显著降低 ($P < 0.05$)。与CON组相比, LPS组MPO值显著升高 ($P < 0.05$); 与LPS组相比, DXMS组、JGT-M组及JGT-H组MPO值均显著降低 ($P < 0.05$), JGT-L组MPO水平虽有降低趋势, 但差异无统计学意义 ($P > 0.05$)。具体结果见图2C~G。

3 JGT干预下的ALI模型小鼠肺组织代谢组学分析

3.1 肺组织样本的多元统计分析 药效学结果显示JGT-M组治疗效果最佳, 因此本部分仅对JGT-M组进行代谢组学研究。由于各组在正、负离子模式下谱图的出峰时间和峰高具有差异, 推测各组的代谢产物也具有差异(图3)。

主成分分析得分图显示, 正负离子模式下, QC样本均在 ± 2 倍标准差的范围内, 说明分析平台重复性良好, 试验数据稳定可靠(图4A、B)。偏最小二乘判别分析发现CON组、LPS组、JGT-M组之间能明显区分开,

且各组内有较好的聚类效果(图4C、D)。为避免模型过度拟合, 进行200次模型验证分析(图4E、F)。 R^2 和 Q^2 左边的模拟值均低于右边的真实值, 回归线斜率为正, 说明本实验建立的模型可靠, 可用于差异代谢物的筛选。

3.2 差异代谢物的筛选 以 $FC > 1.5$ 或 $FC < 0.67$, 并且 $P < 0.05$ 为条件进行差异代谢物的筛选。CON组 vs LPS组共筛选鉴定出155个差异代谢物, 其中上调83个, 下调72个; JGT-M组 vs LPS组共筛选鉴定出82个差异代谢物, 其中上调49个, 下调33个。给药组表现出向CON组明显回调的成分就是潜在差异代谢物。故将CON组 vs LPS组与JGT-M组 vs LPS组上下调的差异代谢物分别取交集, 最终上调得到8个潜在差异代谢物, 下调得到14个潜在差异代谢物(表2)。

3.3 差异代谢物的相关性及其代谢通路分析 将筛选得到的22个潜在差异代谢物进行通路富集分析, 结果如图5所示。主要被富集到初级胆汁酸生物合成、嘌呤代谢、柠檬酸循环等通路中。

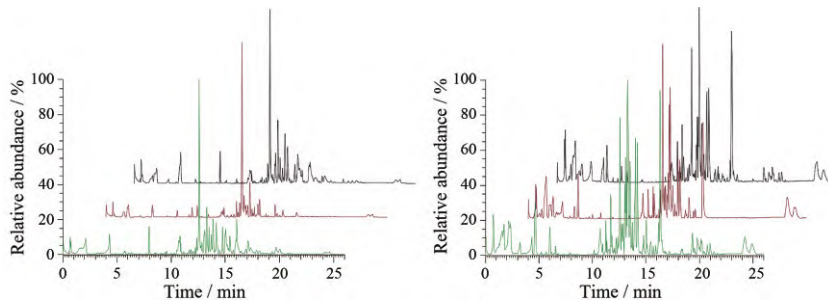


Figure 3 Total ion chromatograms of each group of samples induced by LPS

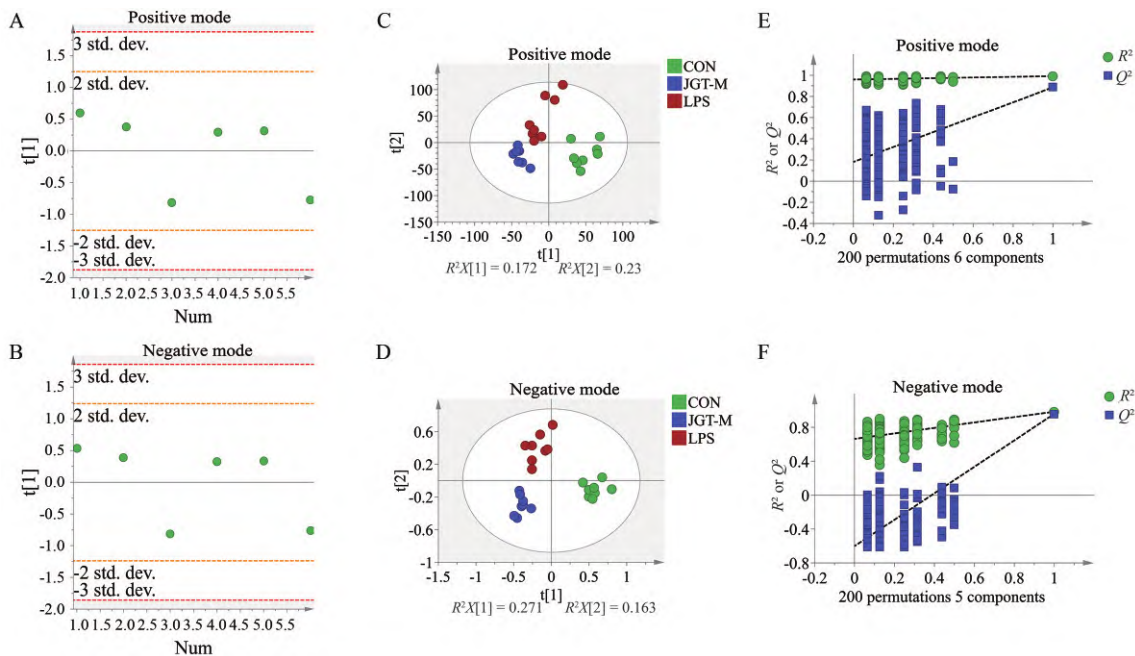
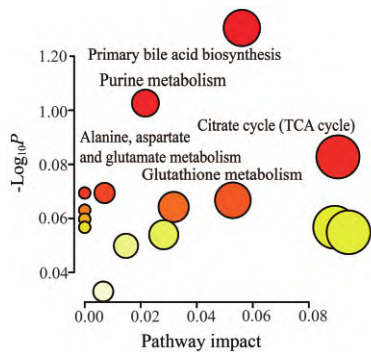


Figure 4 PCA scores plots of QC sample (A, B); PLS-DA scores plots (C, D) and displacement test diagrams (E, F) of CON, MOD, JGT-M

Table 2 Information of potential biomarkers. ↑ and ↓ represent higher and lower level

No.	t_R /min	Detected(m/z)	Mode	Metabolite	Formula	Trend
1	0.801	325.041 7	[M+H] ⁺	Uridine 5'-monophosphate	C ₉ H ₁₃ N ₂ O ₉ P	↓
2	1.000	259.960 5	[M+H] ⁺	<i>N</i> -Acetyl- <i>O</i> -methyltyrosine	C ₁₂ H ₁₅ NO ₄	↓
3	2.273	209.048 5	[M+H] ⁺	Kynurenine	C ₁₀ H ₁₂ N ₂ O ₃	↓
4	4.316	284.098 2	[M+H] ⁺	Guanosine	C ₁₀ H ₁₃ N ₅ O ₅	↓
5	16.836	583.288 2	[M-H] ⁻	Bilirubin	C ₃₃ H ₃₆ N ₄ O ₆	↓
6	17.149	495.2517	[M-H] ⁻	Leukotriene D4	C ₂₅ H ₄₀ N ₂ O ₆ S	↓
7	17.985	504.266 7	[M+Na] ⁺	Glycocholic acid	C ₂₆ H ₄₃ NO ₆	↓
8	18.471	255.232 3	[M-H] ⁻	Palmitic acid	C ₁₆ H ₃₂ O ₂	↓
9	18.566	447.244 8	[M+H] ⁺	Hyochoic acid	C ₂₄ H ₄₀ O ₅	↓
10	18.722	369.295 6	[M+Na] ⁺	Docosapentaenoic acid	C ₂₂ H ₃₄ O ₂	↓
11	20.271	431.251 3	[M+H] ⁺	Deoxycholic acid	C ₂₄ H ₄₀ O ₄	↓
12	20.337	681.357 8	[M+H] ⁺	26-Deoxycimicifugoside	C ₃₇ H ₅₄ O ₁₀	↓
13	22.203	392.281 5	[M+H] ⁺	<i>N</i> -(3-Hydroxytetradecanoyl)- <i>L</i> -phenylalanine	C ₂₃ H ₃₇ NO ₄	↓
14	24.173	369.350 9	[M+H] ⁺	Cholesterol	C ₂₇ H ₄₆ O	↑
15	0.795	290.133 3	[M+H] ⁺	Ophthalmic acid	C ₁₁ H ₁₉ N ₃ O ₆	↑
16	0.841	191.018 8	[M-H] ⁻	Citric acid	C ₆ H ₈ O ₇	↑
17	0.895	175.001 3	[M+K] ⁺	Hypoxanthine	C ₅ H ₄ N ₄ O	↑
18	1.078	128.033 9	[M-H] ⁻	<i>L</i> -5-Oxoproline	C ₅ H ₇ NO ₃	↑
19	4.161	212.102 6	[M+H] ⁺	2,3-Dideoxycytidine	C ₉ H ₁₃ N ₃ O ₃	↑
20	11.984	506.285 2	[M+K] ⁺	Taurolithocholic acid	C ₂₆ H ₄₅ NO ₅ S	↑
21	12.041	373.194 4	[M-H] ⁻	Nortrachelogenin	C ₂₀ H ₂₂ O ₇	↑
22	13.493	331.264 1	[M-H] ⁻	Docosatetraenoic acid	C ₂₂ H ₃₆ O ₂	↑

**Figure 5** Pathway bubble diagram of differential metabolites in LPS-induced ALI model

讨论

中药复方大多数由多味药组成,各味药之间相互作用,机制复杂,成为中药复方研究过程中的难点^[18]。本研究对桔梗汤的化学成分进行分析,共鉴定出了40个化学成分。其中桔梗皂苷D、桔梗皂苷D2通过激活核因子E2相关因子2,抑制核因子- κ B信号通路减轻肺部炎症^[19]。甘草素可以保护人体肺细胞A549免受 α -溶血素介导的损伤^[20]。异甘草素通过激活过氧化物酶体增殖物激活受体 γ ,抑制核因子- κ B活化减轻LPS诱导的肺部炎症^[21]。甘草苷可通过调控JNK/核转录因子Nur77/c-Jun信号通路对LPS诱导的ALI起到治疗作用^[22]。甘草酸能通过抑制炎症因子及调节相关信号通路对ALI起到保护作用^[23-28]。甘草次酸能通过抑制ROS-PI3K/AKT通路介导的NOD样受体蛋白3炎症小体的激活,从而对ALI起到改善作用^[29]。这些成分均

是治疗ALI的活性成分且不同类型的活性成分可能相互作用,共同发挥桔梗汤抗ALI的药理作用。

过度的炎症反应是ALI的主要特征之一,通过抑制炎症反应可对ALI模型小鼠起到保护作用^[30]。白三烯D4 (leukotriene D4, LTD4) 是一种常见的半胱氨酸-白三烯,可以与靶细胞上的特定膜受体结合从而引起炎症反应^[31]。炎症反应的消退与促炎症消退介质的产生有关,二十二碳五烯酸 (docosapentaenoic acid) 作为一种多不饱和脂肪酸,是合成促炎症消退介质的底物^[32]。ALI与嘌呤代谢之间存在一定的关联,在ALI模型中次黄嘌呤和黄嘌呤减少^[33,34]。黄嘌呤氧化酶是一种重要嘌呤分解代谢酶,可将次黄嘌呤和黄嘌呤分解成尿酸并释放超氧化物^[35]。尿酸已被证明是肺部的主要“危险信号”,可通过激活肺部NALP3炎症小体,从而引起肺部炎症及纤维化^[36]。本研究中,给药后LTD4、docosapentaenoic acid水平明显下调,次黄嘌呤水平明显增加,推断桔梗汤可能通过抑制炎症反应,促进炎症消退治疗ALI。

氧化应激是LPS诱导的ALI的致病机制之一。谷胱甘肽 (glutathione, GSH) 是细胞中一种重要的抗氧化剂,可通过清除羟基自由基、超氧化物,维护细胞内的氧化还原平衡^[37,38]。GSH在抗肺部炎症性疾病上也具有重要作用^[39]。在LPS诱导的大鼠ALI模型中,GSH水平会出现异常^[40]。棕榈酸 (palmitic acid, PA) 是一种重要的长链饱和脂肪酸,可作为肺癌诊断及预后的生物学标志物^[41]。还有研究指出PA可激活内质网应激加剧

肺纤维化^[42]。在本研究中,给药后PA及内源性GSH生物合成的中间体L-5-羟脯氨酸(L-5-oxoproline)^[43]均显著回调。说明桔梗汤可通过干预L-5-羟脯氨酸、PA的水平调节氧化应激,进而改善ALI模型小鼠的代谢紊乱。

胆汁酸(bile acids, BA)水平与炎症和肺部疾病之间的关系已有报道^[44]。给药干预后,初级胆汁酸合成通路上的相关代谢物均有回调,说明桔梗汤可能通过调节胆汁酸代谢改善ALI模型小鼠的代谢异常。柠檬酸循环(citrate cycle, TCA)是机体将糖或其他物质氧化而获得能量的主要代谢通路,是机体内三大营养素的最终代谢通路^[45]。作为TCA循环中重要的中间产物,ALI模型小鼠体内的柠檬酸含量发生显著变化,给药后其含量回调至正常水平。代谢组学研究结果表明,桔梗汤可能通过调节三羧酸循环改善ALI模型小鼠的代谢紊乱。此外,在本研究还富集至丙氨酸、天冬氨酸和谷氨酸代谢途径。有研究指出丙氨酸、天冬氨酸和谷氨酸代谢与坏死性凋亡有关,且丙氨酸、天冬氨酸和谷氨酸代谢的丙酮酸随后会参与TCA循环^[46]。因此,桔梗汤可能通过调节氨基酸代谢和能量代谢从而对ALI模型小鼠起到保护作用。

综上所述,桔梗汤可通过降低炎症因子水平,调节机体氧化应激从而减轻LPS诱导的肺组织损伤。此外,初级胆汁酸生物合成、柠檬酸循环、嘌呤代谢、谷胱甘肽代谢等通路可能是ALI的潜在治疗途径。该研究将为桔梗汤在临床上治疗ALI提供重要的理论依据。

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利益冲突: 所有作者均不存在任何利益冲突。

References

- [1] Wang XJ, Yang BC, Li YY, et al. AKR1C1 alleviates LPS-induced ALI in mice by activating the JAK2/STAT3 signaling pathway [J]. *Mol Med Rep*, 2021, 24: 833.
- [2] Mokra D. Acute lung injury - from pathophysiology to treatment [J]. *Physiol Res*, 2020, 69: S353-S366.
- [3] Camporota L, Cronin JN, Busana M, et al. Pathophysiology of coronavirus-19 disease acute lung injury [J]. *Curr Opin Crit Care*, 2022, 28: 9-16.
- [4] Wang S, Yao XH, Ma S, et al. A single-cell transcriptomic landscape of the lungs of patients with COVID-19 [J]. *Nat Cell Biol*, 2021, 23: 1314-1328.
- [5] Ji XW, Meng XR, Zhu X, et al. Research and development of Chinese anti-COVID-19 drugs [J]. *Acta Pharm Sin B*, 2022, 12: 4271-4286.
- [6] Wang YY, Wang X, Li YX, et al. Xuanfei Baidu Decoction reduces acute lung injury by regulating infiltration of neutrophils and macrophages via PD-1/IL17A pathway [J]. *Pharmacol Res*, 2022, 176: 106083.
- [7] Fan WJ, Ren JN, Zhan XJ, et al. Research progress on mechanism of Jiegeng Decoction and its active components in treatment of acute lung injury [J]. *Chin Tradit Herb Drugs (中草药)*, 2022, 53: 1230-1239.
- [8] Zhu HJ, Huang KF, Wang WL, et al. Application law of platycodon root in classical prescription [J]. *China J Tradit Chin Med Pharm (中华中医药杂志)*, 2019, 34: 4391-4393.
- [9] Qian YJ, Jiang SL, Li B, et al. Therapeutic effects of modified Jiawei Jiegeng Decoction on patients with stable bronchiectasis and its impacts on inflammatory cells and factors in bronchoalveolar lavage fluid [J]. *Guangxi Med J (广西医药)*, 2022, 44: 385-388.
- [10] Zhang Y, Gu YF. Clinical observation of effect of Platycodon decoction combined Zhizhu pill on chronic functional constipation [J]. *Mod J Integr Tradit Chin West Med (现代中西医结合杂志)*, 2016, 25: 2069-2071.
- [11] Fang F, Cai CS. A clinical study on the treatment of pediatric bronchopneumonia by adding Jiawei Jiegeng Decoction combined with Baohe pill [J]. *J Chin Med Mater (中药材)*, 2018, 41: 1754-1756.
- [12] Tao J, Nie Y, Hou YY, et al. Chemomics-integrated proteomics analysis of Jie-Geng-Tang to ameliorate lipopolysaccharide-induced acute lung injury in mice [J]. *Evid Based Complement Alternat Med*, 2016, 2016: 7379146.
- [13] Yang R, Qian WJ, Peng LX, et al. Phospholipidomics study of Jiegeng Decoction for LPS-induced acute lung injury in mice based on UHPLC-Q-Exactive Orbitrap/MS [J]. *Acta Pharm Sin (药学报)*, 2019, 54: 144-150.
- [14] Tang YQ, Li Z, Lazar L, et al. Metabolomics workflow for lung cancer: discovery of biomarkers [J]. *Clin Chim Acta*, 2019, 495: 436-445.
- [15] Zhou Y, Xie T, Shi C, et al. Potential mechanism of platycodon grandiflorum in the prevention of acute lung injury based on non-target serum metabolomics [J]. *J Nanjing Univ Tradit Chin Med (南京中医药大学学报)*, 2021, 37: 737-743.
- [16] Ge P, Zhang JQ, Zhang GX, et al. Research progress on application of metabolomics in acute lung injury [J]. *Chin Crit Care Med (中国危重病急救医学)*, 2021, 33: 1266-1271.
- [17] Bernatchez J A, Mccall L I. Insights gained into respiratory infection pathogenesis using lung tissue metabolomics [J]. *PLoS Pathog*, 2020, 16: e1008662.
- [18] Liu B. Study on Compatibility Regularity of Drug Pair of Platycodin and Glycyrrhiza in Pharmacodynamics (II) (甘草、桔梗"药对"药效学环节之配伍机理研究(II)) [D]. Beijing: Peking Union Medical College, 2006.
- [19] Gao W, Guo Y, Yang HX. Platycodin D protects against cigarette

- smoke-induced lung inflammation in mice [J]. *Int Immunopharmacol*, 2017, 47: 53-58.
- [20] Wang LQ, Yang R, Yuan BC, et al. The antiviral and antimicrobial activities of licorice, a widely-used Chinese herb [J]. *Acta Pharm Sin B*, 2015, 5: 310-315.
- [21] Zhang WB, Wang G, Zhou SJ. Protective effects of isoliquiritigenin on LPS-induced acute lung injury by activating PPAR-gamma [J]. *Inflammation*, 2018, 41: 1290-1296.
- [22] Zhou HL. Anti-endotoxin Activity Screening of Traditional Chinese Medicine by Using Zebrafish Models and Mechanism Study of Liquiritin on Attenuating Lipopolysaccharide-induced Acute Lung Injury (基于斑马鱼模型的中药抗内毒素活性筛选及甘草苷抑制 LPS 诱导急性肺损伤作用机制研究) [D]. Luzhou: Southern Medical University, 2020.
- [23] Li YJ, Du JY, Li JC, et al. Research progress on protective effects of glycyrrhizic acid and its derivatives against sepsis-induced organ dysfunction [J]. *Pharmacol Clin Chin Mater Med (中药药理与临床)*, 2022, 38: 221-224.
- [24] Lee SA, Lee SH, Kim JY, et al. Effects of glycyrrhizin on lipopolysaccharide-induced acute lung injury in a mouse model [J]. *J Thorac Dis*, 2019, 11: 1287-1302.
- [25] Qu LH, Chen C, He W, et al. Glycyrrhizic acid ameliorates LPS-induced acute lung injury by regulating autophagy through the PI3K/AKT/mTOR pathway [J]. *Am J Transl Res*, 2019, 11: 2042-2055.
- [26] Yao L, Sun TL. Glycyrrhizin administration ameliorates *Streptococcus aureus*-induced acute lung injury [J]. *Int Immunopharmacol*, 2019, 70: 504-511.
- [27] Kong DL, Wang ZF, Tian J, et al. Glycyrrhizin inactivates toll-like receptor (TLR) signaling pathway to reduce lipopolysaccharide-induced acute lung injury by inhibiting TLR2 [J]. *J Cell Physiol*, 2019, 234: 4597-4607.
- [28] Ni YF, Kuai JK, Lu ZF, et al. Glycyrrhizin treatment is associated with attenuation of lipopolysaccharide-induced acute lung injury by inhibiting cyclooxygenase-2 and inducible nitric oxide synthase expression [J]. *J Surg Res*, 2011, 165: e29-e35.
- [29] Wang K, Zhang Y, Cao Y, et al. Glycyrrhetic acid alleviates acute lung injury by PI3K/AKT suppressing macrophagic Nlrp3 inflammasome activation [J]. *Biochem Biophys Res Commun*, 2020, 532: 555-562.
- [30] Li Y, Huang J, Foley NM, et al. B7H3 ameliorates LPS-induced acute lung injury via attenuation of neutrophil migration and infiltration [J]. *Sci Rep*, 2016, 6: 31284.
- [31] Meshram D, Bhardwaj K, Rathod C, et al. The role of leukotrienes inhibitors in the management of chronic inflammatory diseases [J]. *Recent Pat Inflamm Allergy Drug Discov*, 2020, 14: 15-31.
- [32] Fattori V, Zaninelli TH, Rasquel-Oliveira FS, et al. Specialized pro-resolving lipid mediators: a new class of non-immunosuppressive and non-opioid analgesic drugs [J]. *Pharmacol Res*, 2020, 151: 104549.
- [33] Shi X, Chen GN, Wei J, et al. UHPLC-Q-TOF MS-based metabolic analysis for the therapeutic efficacy of "Xuebijing Injection" against sepsis-induced acute lung injury [J]. *Evid Based Complement Alternat Med*, 2018, 2018: 8514619.
- [34] Xiong ZL, Wang YM, Lang L, et al. Tissue metabolomic profiling to reveal the therapeutic mechanism of Reduning injection on LPS-induced acute lung injury rats [J]. *RSC Adv*, 2018, 8: 10023-10031.
- [35] Schmidt HM, Kelley EE, Straub AC. The impact of xanthine oxidase (XO) on hemolytic diseases [J]. *Redox Biol*, 2019, 21: 101072.
- [36] Gasse P, Riteau N, Charron S, et al. Uric acid is a danger signal activating NALP3 inflammasome in lung injury inflammation and fibrosis [J]. *Am J Respir Crit Care Med*, 2009, 179: 903-913.
- [37] Niu BY, Liao KX, Zhou YX, et al. Application of glutathione depletion in cancer therapy: enhanced ROS-based therapy, ferroptosis, and chemotherapy [J]. *Biomaterials*, 2021, 277: 121110.
- [38] Chen Y, Dong H, Thompson DC, et al. Glutathione defense mechanism in liver injury: insights from animal models [J]. *Food Chem Toxicol*, 2013, 60: 38-44.
- [39] Qian MZ, Lou YY, Wang YX, et al. PICK1 deficiency exacerbates sepsis-associated acute lung injury and impairs glutathione synthesis via reduction of xCT [J]. *Free Radic Biol Med*, 2018, 118: 23-34.
- [40] Tao Q, Zhang ZD, Qin Z, et al. Aspirin eugenol ester alleviates lipopolysaccharide-induced acute lung injury in rats while stabilizing serum metabolites levels [J]. *Front Immunol*, 2022, 13: 939106.
- [41] Qi SA, Wu Q, Chen ZP, et al. High-resolution metabolomic biomarkers for lung cancer diagnosis and prognosis [J]. *Sci Rep*, 2021, 11: 11805.
- [42] Chu SG, Villalba JA, Liang XL, et al. Palmitic acid-rich high-fat diet exacerbates experimental pulmonary fibrosis by modulating endoplasmic reticulum stress [J]. *Am J Respir Cell Mol Biol*, 2019, 61: 737-746.
- [43] Lan XY, Sun ZW, Xu GL, et al. Bone marrow mesenchymal stem cells exert protective effects after ischemic stroke through upregulation of glutathione [J]. *Stem Cell Rev Rep*, 2022, 18: 585-594.
- [44] Wang TY, Lin S, Liu R, et al. Acute lung injury therapeutic mechanism exploration for Chinese classic prescription Qingzao Jiufei Decoction by UFLC-MS/MS quantification of bile acids, fatty acids and eicosanoids in rats [J]. *J Pharm Biomed Anal*, 2020, 189: 113463.
- [45] Kang W, Suzuki M, Saito T, et al. Emerging role of TCA cycle-related enzymes in human diseases [J]. *Int J Mol Sci*, 2021, 22: 13057.
- [46] Mao KM, Luo P, Geng W, et al. An integrative transcriptomic and metabolomic study revealed that melatonin plays a protective role in chronic lung inflammation by reducing necroptosis [J]. *Front Immunol*, 2021, 12: 668002.