

The protective effect and mechanism of Tibetan medicine *Aconitum tanguticum*(Maxim.) Stapf on acute lung injury

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Abstract

Ethnopharmacological relevance

Aconitum tanguticum(Maxim.) Stapf (ATS) is a rare Tibetan medicine belonging to the genus Ranunculaceae. This herb is mainly distributed in the high altitude's areas of Qinghai, Gansu and Tibet province in China. In Tibetan medicine, ATS is mainly used for lung inflammation disease, hepatitis, gastrointestinal diseases, influenza, fever caused by infectious diseases, food poisoning, snake and scorpion bite and yellow water disease. ATS contains anti-inflammatory, antiviral, and other pharmacological effects, according to recent investigations. Commonly utilized in the manufacture of Tibetan medicine is ATS. It is well-liked by individuals of many ethnic groups and has been utilized in Tibetan medicine for thousands of years.

Aim of the Study

This work seeks to examine the preventive effects of ATS, an alcoholic extract of the Tibetan medicinal *Aconitum tanguticum* (Maxim.) Stapf, on acute lung injury (ALI) in mice and to identify its possible mechanism.

Materials and methods

Alveolar epithelial cells A549 and mice were induced with lipopolysaccharide (LPS) to establish ALI models in vivo and in vitro, and to explore the pharmacological effect and therapeutic mechanism of ATS.

Results

ATS down-regulates the mRNA levels of inflammatory factors NFκB p65, TNF-α, IL-1β, IL-8, inhibits the release of ROS and inhibits the epithelial-mesenchymal transition process caused by sustained cell injury; promotes Keap1/Nrf2/HO-1 signaling pathway, reduced the degree of oxidative stress in vivo and the production of proteins linked to ferroptosis that are produced by LPS.

Conclusion

The Tibetan medicine ATS can reduce pulmonary hemorrhage and pulmonary edema in ALI mice, alleviate the degree of lung tissue lesions, inhibit the expression of inflammatory factors and inhibit cell apoptosis, play a protective role in ALI, and can be used as an effective drug for the design of ALI.

Introduction

When the new type of coronavirus pneumonia (COVID-19) develops into severe cases, it often manifests as ALI (Acute lung injury, ALI). Epithelial cells and capillary cells in the bronchi rupture, forming pulmonary edema and pulmonary fibrosis, leading to severe respiratory insufficiency, becoming typical clinical features of patients with severe COVID-19 infection (Li et al., 2020), which is a relatively difficult

type of disease. ALI clinical disease mortality is relatively high, involves extensive tissue inflammatory damage, and causes a series of pulmonary dysfunction, with a global mortality rate as high as 50% (Butt et al., 2016). The main feature of alveolar epithelial cells in ALI is the formation of a persistent inflammatory process, the release of numerous chemokines and pro-inflammatory cytokines, and the induction of further pathological processes, such as pulmonary fibrosis (Margaria et al., 2022). Compared with persistent pulmonary dysfunction, ALI Later pathological fibrosis significantly lowers the quality of life for critically ill individuals (Laskin et al., 2019). The prognosis of COVID-19 patients is directly impacted by the onset and development of ALI, but current clinical treatments limit the cure rate of ALI. Clinical attempts have been made to use negative fluid regulation (Liu et al., 2019), mechanical ventilation (Ferreira et al., 2018), use of glucocorticoids (Tu et al., 2017), NO ventilation (Baumann et al., 2008), and antibiotics (Walkey and Wiene, 2012) for treatment, but the quality of life of patients is extremely poor.

The pathogenesis of ALI is not fully understood. Cytokine storm or inflammatory storm is currently considered to be one of the most important pathophysiological processes of COVID-19. Cytokine storm can damage lung epithelial cells and lead to pathological manifestations outside the lung, and can induce secondary sepsis, usually leading to multiple Organ failure (Tang et al., 2021). In the early days of the COVID-19 epidemic, Tibet, Qinghai and other provinces that inherit Tibetan medicine have mentioned traditional Tibetan medicine such as Twelve Flavors Yishou Powder, Twenty-Five Flavors Lung Disease Capsules, Flu Pills, and Twenty-Five Flavors Main Medicine Powder in their prevention and control plans. The prescription of the drug ATS has a good preventive effect on COVID-19, especially the "inflammatory storm", which enriches the clinical application of Tibetan medicine in the treatment of coronavirus pneumonia (Tharpa and Norpu, 2020), and arouses people's concern about ATS research interests.

ATS is a kind of plant of the genus *Aconitum* in Ranunculaceae, and it is a rare medicinal material in Tibetan medicine. Modern studies have shown that ATS has pharmacological activities such as anti-inflammatory and anti-viral (Zhang et al., 2012). The anti-inflammatory and analgesic active ingredients in *Aconitum tangutata* are mainly concentrated in diterpenoid alkaloids (Yang et al., 2016). It is reported that the contents of Atisine and Heteratisine in *Aconitum tangutica* are 0.084% and 0.066% respectively (Gao, and Zeng, 2010); the content of hordenine is 0.0238% (Wang et al., 2013).

In the early stage, we applied network pharmacology to analyze and identify the potential core target of the anti-inflammatory effect of the chemical components of ATS. The ALI model of mice induced by lipopolysaccharide (LPS) in sepsis is consistent with the relevant symptoms of COVID-19 in pathological manifestations. Therefore, the anti-inflammatory target of Tanggut aconitum alcohol extract (ATS) was studied by LPS modeling method, and the anti-inflammatory mechanism and network were expounded. This study provides a theoretical basis for the anti-COVID-19 and other diseases induced by ALI therapy in ATS.

Materials and methods

ATS preparation

Dr. Qien Li of Qinghai University donated and identified the ATS (Specimen No. LQE-2019-066). A 150 g sample of dried ATS was obtained, crushed, put through a No. 30 sieve, and then immersed in 5 times as much ethanol with a volume fraction of 0.97 at ambient temperature for 24 hours. This process was done six times before being filtered to remove contaminants. To achieve 11.73 g of ATS extract, the solvent was evaporated using a rotary evaporator (Great Wall Science, Industry & Trade Co. Zhengzhou, China).

LC–MS/MS identification

80 % methanol was used as solvent to dissolve the appropriate amount of ATS, and ultrasonic (power 250 W, frequency 40 k Hz) for 30 min until the sample was completely dissolved. Then the sample was transferred to a 50 mL volumetric flask, and the volume was fixed to 50 mL. Cooling to room temperature, and then filtered by a 0.22 µm microporous membrane to obtain the test solution.

LC–MS/MS identification was conducted on ThermoFisher-UltiMate 3000 HPLC instrument. The chromatography-mass spectrometry parameters are as follows. Chromatographic parameter: The chromatographic column was AccucQreaQ100 * 2.1 mm, 2.6 µm. The mobile phase was A : acetonitrile, B : 0.1 % aqueous formic acid with a gradient elution program: 0~3 min 5%~10% A 3~7 min 10%~17% A 7~9 min 17%~20% A 9~11 min 20%~24% A 11~14 min 24%~28% A 14~16 min 28%~30% A 16~19 min 30%~35% A 19~21 min 35%~37% A 21~29 min 37%~55% A 29~33 min 55%~63% A 33~43 min 63%~95% A 43.1~50 min 95%~5% A. Flow rate : 0.2 mL / min ; column temperature : 25 °C. Mass spectrometric parameters: The ion source was a heated electrospray ion source (HESI) with positive and negative ion detection modes. Positive and negative spray voltage : 3200 V. Capillary temperature : 300.00 °C, gas volume flow : 40.00 Arb, auxiliary gas volume flow : 10.00 Arb ; the maximum injection current : 100.00 µA ; probe heater temperature : 320.00 °C ; scanning range m / z 80-1200. Finally, the Xclibwr 4.0 software is used to calculate the high-resolution accurate mass number, fit the molecular formula, and match it with the mzCloud database.

Evaluation of medication efficacy and cell culture

The Wuhan University Cell Bank (CCTCC) provided the alveolar epithelial cells A549. The cells were then cultivated in an incubator (Heal Force, Hong Kong) at 37° C using DMEM high-sugar culture medium in 10% FBS. A549 cells were seeded in 96-well dishes at a density of 5×10^3 cells per well and cultured until they reached the logarithmic growth phase. Subsequently, the cells were exposed to LPS at concentrations ranging from 1 to 100 µg/mL for a duration of 24 hours. Following this treatment, the culture media was replaced with DMEM supplemented with 2% FBS. To establish the ultimate mold-making concentration, cell viability was measured using a real-time label-free cell analyzer (Agilent Technologies, USA). To ascertain the range of supplied concentrations, cells were treated with final concentrations of 1-100 µg/mL ATS for 12 h, each, under standard medium conditions. Cell viability was then assessed using the MTT technique.

For the evaluation of cell proliferation by LPS, A549 cells were inoculated on E16 plates (ACEA, USA) at a density of 5 × 10³ cells/well. The xcelligence RTCA S16 system analyzes cell proliferation in real time, and the cell index (CI) is expressed by monitoring changes in the impedance of the electrode interface in real time.

In the control, model (LPS 100 g/mL), and medication administration groups, A549 cells were injected in 96-well plates at a rate of 100 L per well. The pretreatment of ATS (5~20 g/mL) was given to the medication administration group for 12 hours. Following this, LPS (100 g/mL) was introduced to the medium and cultured for 24 hours before being replaced with 2% FBS medium. The growth capacity of the cells was assessed using the MTT assay.

Monitoring cell morphology and detecting ROS

A549 cells were injected into 6-well plates and subsequently subjected to the aforementioned protocol. Following delivery, each batch of cells was rinsed with PBS to remove excess liquid, and subsequently, the cellular morphology was assessed using an inverted microscope (OPTeC, Chongqing, China).

Similar to before, A549 cells were injected into 6-well plates, groups were given medications, and then ROS were measured using a kit for detecting reactive oxygen species (Beyotime, Shanghai, China). After the aforementioned procedure, they were immediately treated with 10 M DCFH-DA and incubated for 30 min. The intracellular ROS levels were determined using Flow cytometry by analyzing the relative mean fluorescence intensity values of each set of cells.

Treatment of animals

Liaoning Changsheng Biotechnology Co. provided the SPF C57BL/6 mice (weight 20±2 g, 60 males; experimental animal license number SCXK(Liao)2020-0001). At South-Central Minzu University's Experimental Animal Center, the mice were kept in SPF-grade animal barriers. All procedures involving the use of experimental animals are carried out strictly in compliance with the rules established by the South-Central Minzu University's Animal Ethics Committee and the international NIH standards. According to the relevant documents, determine the ATS dosage that should be used. To make a suspension, 0.5% sodium carboxymethylcellulose (CMC) was added to make the medication solution. Before the experiment began, all of the animals were given a seven-day acclimatization period, and they were then randomly assigned into five groups:

The "Control" group, which consists of normal mice solely given CMC-Na treatment, is in (a); (b). Model group: "MOD" ALI mice that have been given CMC-Na; (c). Ferroptosis inhibitor group: For three days, 5 mg/kg of Fer-1 (also known as "Fer-1") was intraperitoneally given into ALI animals; (d). Low-dose ATS group: 350 mg/kg of ATS was given orally to ALI mice, known as "ATS-L"; (e). ATS group at high dose: "ATS-H" mice from the ALI group were gavaged with 700 mg/kg of ATS; Details on how to establish ALI model mice are described in Fig. 4-B.

Technique for administering drugs and obtaining lung tissue samples

The trial involved pre-dosing throughout the course of a 14-days dosing cycle. After 14 days of treatment, LPS (10 mg/kg) was intraperitoneally administered into the ATS group at low and high doses for modeling. On day 12 of the experiment, the ferroptosis inhibitor group received an intraperitoneal injection of Fer-1 (5 mg/kg), which acted for 24 hours over the course of three days. Six hours following the last injection, LPS (10 mg/kg) was administered for a 12-hour modeling period.

Upon completion of the modeling process, the mice were euthanized, and the left lung lobe was excised to assess the wet/dry weight ratio (W/D) of the mouse lung tissue. To facilitate histological examination, a portion of the right lung lobe was preserved in a 4% paraformaldehyde solution at ambient temperature. The remaining tissue was stored in an ultra-low temperature refrigerator (Haier, China) at -80 °C for future research purposes.

Wet to dry weight ratio of mouse lung tissue (W/D)

After the mice had been put to death, fresh lung tissue was removed, and any unwanted tissue remnants were removed and rinsed in saline. The Wet weight of the left lung lobe tissue was determined by removing excess water using filter paper. Subsequently, the tissue was subjected to a drying process at a constant temperature of 60 °C for a duration of 48 hours in a specialized oven (Ruihua Instrument Equipment Co., Wuhan, China) to obtain its Dry weight. Wet/Dry weight ratio (W/D) was determined for each mouse lung using the data that was collected.

Lung tissue histopathological staining

Fresh lung tissue was maintained for longer than 24 hours by soaking in 4% paraformaldehyde. To create 5 m paraffin sections, the tissue was excised and paraffin embedded. Hematoxylin and eosin (H&E) staining was employed to impart coloration to the sections. Subsequently, the staining of each segment was meticulously examined under a light microscope, enabling a comparative analysis of the pathological alterations in the lung tissue across different groups of mice.

Myeloperoxidase (MPO) activity was found in mouse lung tissue

Mice lung tissue was collected and ground quickly and at a low temperature. As directed by the kit's instructions (Nanjing Jiancheng Institute of Biological Engineering Co., Ltd.), we assessed the MPO activity in mouse lung tissue.

ROS fluorescence labeling and TUNEL

Xylene was used to soak tissue slices at ambient temperature. After that, they were dehydrated in an ethanol gradient and cleaned in PBS. Histochemical pens were used to draw circles on the tissue's edge to make further manipulation easier. After adding PBS to wash the sections, we added the membrane-breaking working solution and incubated for 20 min before adding the Proteinase K working solution at ambient temperature. For a 10-minute equilibration period, we introduced buffer dropwise. Recombinant TDTase, dUTP, and buffer were combined in a reaction solution at a volumetric ratio of 1:5:50 L. The

resulting mixture was subsequently incubated at a temperature of 37 °C for a duration of two hours. Following staining with DAPI for a period of 10 minutes at ambient temperature, the sections were sealed. Subsequently, the sections were examined under a fluorescent microscope, and images were captured using DAPI UV excitation wavelength of 330-380 nm, CY3 excitation wavelength of 510-561 nm, and 590 nm red light.

We prepared frozen mouse lung tissue samples, rehydrated them at ambient temperature, and dried them. A bursting agent was applied to the circle created by the histochemical pen, which was then burst for five minutes and cleaned. Dropwise addition of the ROS staining solution followed by 30 minutes of darkness and steady 37 °C temperature were required. The fluorescence microscope was utilized to capture images, employing specific excitation and emission wavelengths for each fluorophore (DAPI: excitation 330-380 nm, emission 420 nm; FITC: excitation 465-495 nm, emission 515-555 nm; CY3: excitation 510-560 nm, emission 590 nm). Prior to imaging, the nuclei were restained with DAPI, subjected to shaking to remove excess moisture, and sealed with an anti-fluorescent bursting agent.

Measurement of tissue iron content and oxidative stress indicators

The frozen reserve was used to extract the lung tissue. The supernatant of lung tissue homogenate was obtained after they were mixed in a high-speed, low-temperature tissue grinder with saline at a specific ratio. According to the kit's instructions (provided by Nanjing Jiancheng Institute of Biological Engineering Co., Ltd.), the SOD, GSH, CAT, and tissue iron levels were assessed.

Real-time RT-PCR

The mRNA expression levels of inflammation and EMT-associated genes in A549 cells were determined using qRT-PCR with GAPDH as the reference gene. Total RNA was extracted from cell samples using the TRNzol reagent, and cDNA was synthesized through reverse transcription using a kit. The qRT-PCR amplification process consisted of a preheated denaturation stage at 95 °C for 3 min, followed by cycling at 95 °C for 5 sec and 60 °C for 34 sec for 40 cycles.

The relative expression levels of TNF-, IL-1β, and IL-6 mRNA in the lung tissues of each group of mice were similarly determined by taking 20~30 mg of mouse lung tissues. Table 1 display the primer sequences that were utilized.

Tab. 1 qRT-PCR primer sequence

Gene (for A549)	primer 5'-3'	T
GAPDH	F: GCACCGTCAAGGCTGAGAAC R: GACTCCACGACATACTCAGCAC	61 °C
TNF- α	F: CCTCTCTCTAATCAGCCCTCTG R: GAGGACCTGGGAGTAGATGAG	58 °C
IL-1 β	F: ATGATGGCTTATTACAGTGGCAA R: GTCGGAGATTCGTAGCTGGA	57 °C
IL-8	F: ACTGAGAGTGATTGAGAGTGGAC R: AACCTCTGCACCCAGTTTTTC	60 °C
E-cadherin	F: CGAGAGCTACACGTTACGG R: GGGTGTCGAGGGAAAAATAGG	60 °C
MUC5AC	F: CAGCACAACCCCTGTTTCAA R: GCGCACAGAGGATGACAGT	59 °C
α -SMA	F: TGTGGCTATCCAGGCGGTGC R: TCTCGGCCAGCCAGATCCAGAC	65 °C
Gene (for mice)		
GAPDH	F: ACCCAGAAGACTGTGGATGG R: TCAGCTCAGGGATGACCTTG	57 °C
TNF- α	F: TTGACCTCAGCGCTGAGTTG R: CCTGTAGCCCACGTCGTAGC	59 °C
IL-1 β	F: CAGGATGAGGACATGAGCACC R: CTCTGCAGACTCAAACCTCCAC	58 °C
IL-6	F: ACTCACCTCTTCAGAACGAATTG R: CCATCTTTGGAAGGTTTCAGGTTG	58 °C

Western blot analysis

Using RIPA buffer, which contains a protease inhibitor cocktail, mouse lung tissue was homogenized and treated for 30 minutes. On ice, the entire surgery was carried out. Using a BCA assay kit from Cowin

Biotech Co.,Ltd. in Jiangsu, China, the total protein was extracted using differential centrifugation (13,000 g, 15 min at 4 °C). For electrophoresis, identical loads of denatured-treated proteins (40 g) were added to each group. After the electrophoresis was complete, 5% (w/v) skim milk was added to close the process. Anti-Keap1, Anti-Nrf2, Anti-HO-1, and Anti-GPX4 antibodies were used to react with the antigens at (1: 1000) 4 °C overnight, and secondary antibodies were used to react with the antigens at ambient temperature for 30 minutes. Using an ECL test kit (ABP Biosciences, USA), chemiluminescence was applied to immunoreactive strips.

After the strips had been processed, Image J software was able to identify their grayscale.

Statistical investigation

The statistical analysis was conducted using the SPSS software (Version 18.0). The data was presented as means ± standard deviation (SD). Multiple comparisons were performed using one-way analysis of variance (ANOVA) and Dunnett's test. Statistical significance was determined by a probability (*P*) value below 0.05.

Results

LC-MS / MS identification results

In order to clarify the chemical composition of ATS, LC-MS / MS analysis was utilized to identify the components of ATS. The primary mass spectrometry and secondary mass spectrometry information were compared with the mzcloud database and literature. A total of 20 compounds were identified, including 17 in positive ion mode and 3 in negative ion mode. The LC-MS / MS identification map was shown in Fig.1, and the compound information is shown in Table 2.

<Insert Fig. 1 here>

Tab. 2 Chemical composition information of ATS

No.	t _R / min	MW	MF	Ion mode	Fragment ion	Identification
1	1. 78	151.21	C ₉ H ₁₃ NO	Positive	121. 06, 103. 05	N-Methyltyramine
2	1. 81	313.4	C ₂₀ H ₂₇ NO ₂	Positive	296. 15, 121. 07	9-hydroxynominine / tongolinine
3	3. 56	345.4	C ₂₀ H ₂₇ NO ₄	Positive	310. 18, 247. 15	Tangutisine
4	3. 76	327.4	C ₂₀ H ₂₅ NO ₃	Positive	310. 18	Hetisinone
5	4. 01	137.18	C ₈ H ₁₁ NO	Positive	122. 07, 121. 08	Tyramine
6	5. 54	391.5	C ₂₂ H ₃₃ NO ₅	Positive	360. 22, 342. 21	Heteratisine
7	5. 84	165.23	C ₁₀ H ₁₅ NO	Positive	147. 047, 121. 06, 119. 05, 91. 05	Hordenine
8	7. 35	377.5	C ₂₁ H ₃₁ NO ₅	Positive	360. 25	heterophyllidine
9	7. 46	343.4	C ₂₀ H ₂₅ NO ₄	Positive	326. 21, 121. 07	tanguticuline E
10	7. 61	329.4	C ₂₀ H ₂₇ NO ₃	Positive	312. 20, 294. 19	Tanwusine
11	8. 14	433.5	C ₂₄ H ₃₅ NO ₆	Positive	342. 21, 307. 23	6-benzoylheteratisine
12	8. 42	343.5	C ₂₂ H ₃₃ NO ₂	Positive	326. 25	atisine
13	10. 65	371.5	C ₂₂ H ₂₉ NO ₄	Positive	312. 20	13-O-acetylhetisine / 11-acetylhetisine
14	11. 24	387.5	C ₂₂ H ₂₉ NO ₅	Positive	328. 19, 310. 18	Acorine
15	16. 17	476.6	C ₃₀ H ₄₀ N ₂ O ₃	Positive	459. 30, 121. 06	navirine B
16	17. 04	361.5	C ₂₁ H ₃₁ NO ₄	Positive	344. 19	heterophylline
17	18. 30	165.19	C ₉ H ₁₁ NO ₂	Positive	149. 13, 121. 07	phenylalanine

18	11. 33	1080.9	C ₄₅ H ₆₀ O ₃₀	Negative	917. 25, 771. 18, 609. 15, 301. 03	Quercetin-3-O-[β-D-glucose-(1→3)-(4-O-trans-p-coumaroyl)]-α-L-rhamnose-(1→6)-β-D-mannose-7-O-β-D-grape Glucosides
19	12. 36	1064.9	C ₄₈ H ₅₆ O ₂₇	Negative	609. 14, 301. 03	Quercetin-3-O-[β-D-glucose-(1→3)-(4-O-E-p-coumaroyl)]-α-L-rhamnose-(1→6)-β-D-galactoside-7-O-α-L-rhamnoside
20	13. 22	1048.9	C ₄₈ H ₅₆ O ₂₆	Negative	593. 15, 285. 04	Kaempferol-3-O- [β-D-glucose-(1→3)- (4-O-E-p-coumaroyl)]-α-L-rhamnose-(1→6)-β-D-glucose-7-O-α-L-rhamnose Glycosides

ATS inhibits LPS-induced A549 cell injury and the release of intracellular ROS

Detection of the effect of different concentrations of LPS on the viability of A549 cells by real-time unlabeled cell analyzers showed that LPS was the most significant suppressor of cell viability at 100 µg/mL, so it was the optimal modeling condition for this experiment; in order to evaluate the safety of the drug, the effect of ATS on cell viability was detected by MTT method, and the results showed that ATS did not show significant toxicity below 50 µg/mL, Fig. 2 (A, B); MTT results showed that ATS administration could reduce the damage of LPS to A549 cells, as shown in Fig. 2 (C). Through cell morphology, the LPS-treated group produced more stretched or elongated fusiform morphology and increased dead cells compared to the control group, and ATS was able to reverse this phenomenon, as shown in Fig. 2 (D).

After different concentrations of ATS acted on A549 cells for 12 h, A549 cells were stimulated with LPS for 24 h, and the intracellular ROS levels were detected after the cells were collected. The results are shown in Fig. 2(E). Compared with the normal control group, LPS can significantly up-regulate the intracellular ROS level ($P<0.01$); while pretreatment with various concentrations of ATS (5, 10, 20 µg/mL) can inhibit the ROS level in A549 cells induced by LPS ($P<0.01$, $P<0.001$), and the ROS level in the ATS (10 µg/mL) group decreased more significantly than that in the ATS (20 µg/mL) group.

<Insert Fig. 2 here>

ATS inhibits LPS-induced expression of NFκB p65, TNF-α, IL-1β, IL-8 and EMT-related factors mRNA expression induced by persistent inflammation

As shown in Fig. 3 (A~D), LPS can induce inflammatory injury in A549 cells, and intracellular NFκB p65, TNF-α, IL-1β, and IL-8 mRNAs were significantly expressed ($P<0.001$, $P<0.01$). The expression of inflammatory factors in ATS pretreated A549 cells was significantly reduced in a dose-dependent manner ($P<0.001$, $P<0.01$, $P<0.05$). In this experiment, the epithelial-mesenchymal transition indicators after the sustained inflammatory injury of the cells were also detected, as shown in Fig. 3 (E~G). After LPS continuously stimulated A549 cells for 24 h, the intracellular E-cadherin mRNA level was inhibited, and the α-smooth muscle actin and mucin mRNA levels increased ($P<0.01$, $P<0.5$), indicating that

persistent inflammation may induced the epithelial-mesenchymal transition of A549 cells; while the ATS administration group had an inhibitory effect on this process in a dose-dependent manner ($P<0.001$, $P<0.01$, $P<0.05$).

<Insert Fig. 3 here>

The impact of ATS on the pathomorphology in mice with ALI brought on by LPS

Fig. 5A displays the outcomes of the H&E staining of lung tissue. Fig. 4B depicts the animal modeling procedure; for further information, refer to the materials and techniques section. The lung tissue in the LPS model group had clear signs of inflammatory cell infiltration (red arrow), necrosis and exfoliation (black arrow), collapse of the alveolar cavity, and other pathological events when compared to the normal control group. The alveolar spacing was significantly widened, the alveolar wall thickness was increased, and the lung tissue structure was disturbed (green arrow). Compared with the LPS model group, the pathology of lung tissue in the low and high dose groups was relieved in different degrees, the inflammatory cell infiltration was relieved, and some alveolar structures were still intact. Compared with the model group, the pathological changes of lung tissue in the Fer-1 group were relieved, the infiltration of inflammatory cells was relieved, the alveolar cavity was reduced, and the alveolar structure was complete.

The results of Wet/Dry weight ratio (W/D ratio) of lung tissue of mice in each group are shown in Fig. 5B. Compared with the normal control group, the W/D ratio in the LPS modeling group was significantly increased ($P<0.01$); ATS could dose-dependently reduce the W/D ratio, and the high dose (700 mg/ kg) group had statistical significance ($P<0.05$); compared with LPS model group, Fer-1 group could also reduce the ratio, and the result was statistically significant ($P<0.05$). The results showed that LPS-induced ALI lung injury was achieved by reducing the degree of pulmonary edema in mice through ATS.

The results of MPO activity in mouse lung tissue are shown in Fig. 5C. Compared with the normal control group, the MPO activity in the lung tissue of the mice in the LPS model group was significantly increased ($P<0.001$); ATS could down-regulate the MPO activity in a dose-dependent manner, and the results were statistically significant ($P<0.05$, $P<0.01$); Notably, Fer-1 intervention also had a down-regulation effect on MPO activity ($P<0.001$). The results suggest that ATS can significantly inhibit the activation and migration of neutrophils in the lung tissue of mice, thereby reducing the lung inflammatory response.

Compared with the normal control group, the SOD and CAT content of the LPS modeling group decreased significantly ($P<0.01$). The experimental results are shown in Fig. 5E, F, which shows that after the establishment of the mouse ALI model using LPS, the antioxidant level in lung tissue was reduced and the oxidative damage to lung was aggravated. The antioxidant levels in lung tissue of mice modeled after ATS intervention were improved, and the intra-tissue SOD and CAT content showed a dose-dependent increase compared with the LPS modeling group ($P<0.05$, $P<0.01$). In addition, there were significant changes in GSH (Fig. 5G) and tissue iron content in the lung tissue of LPS-induced ALI mice Fig. 5D ($P<0.01$), but after the intervention of ATS and the specific iron death inhibitor Fer-1, the

reduction in GSH content was reversed, and the increase in iron content in lung tissue was also inhibited to varying degrees, and the experimental results were statistically significant ($P < 0.05$, $P < 0.01$).

<Insert Fig. 4 here>

<Insert Fig. 5 here>

The effect of ATS on apoptosis and ROS generation in lung tissue of ALI mice induced by LPS

Apoptosis is an important pathological feature of ALI. In order to investigate the effect of ATS on the apoptosis of ALI mouse lung tissue induced by LPS, we assessed the apoptosis of lung tissue by TUNEL staining, as shown in Fig. 6A, B, the number of TUNEL-positive cells (green fluorescence) in LPS model group increased significantly compared with that in normal control group, which indicated that the cells in lung tissues of mice suffered from lung injury were apoptotic, and the number of apoptotic cells in lung tissues was significantly lower than that in LPS model group regardless of low or high dose of Fer-1 or ATS.

DHE fluorescence probe was used in the experiment. When the cells took in DHE, they reacted with oxygen free radicals in the cells and showed red fluorescence at specific excitation wavelength. As shown in Fig. 6C and D, the oxygen free radicals in LPS group were significantly increased, while the fluorescence of oxygen free radicals in normal control group was hardly observed. The oxygen free radicals of ATS low and high dose groups were inhibited, and the red fluorescence was obviously weakened. Compared with LPS group, the red fluorescence of Fer-1 group was significantly inhibited, and the amount of oxygen free radicals was significantly lower than that of ATS group.

<Insert Fig. 6 here>

Regulatory effect of ATS on Keap1/Nrf2/GPX4/HO-1 protein expression and inflammatory factors in lung tissue of ALI mice induced by LPS

In lung tissue, Keap1/Nrf2/GPX4/HO-1 protein can enhance tissue antioxidant capacity and protect tissue cells from oxidative stress injury (Bellezza et al., 2018). GPX4 and Nrf2 can negatively regulate iron death by reducing ROS production and inhibiting intracellular iron uptake, respectively (Liu and Wang, 2019). Does ATS play an important role in ALI induced by LPS in mice? As shown in Fig. 7B~E, compared with the normal control group, the relative expression of Keap1 protein in lung tissue of LPS model mice was significantly increased ($P < 0.01$), and the relative expression of Nrf2, HO-1, GPX4 protein was significantly decreased ($P < 0.05$, $P < 0.01$). Compared with the LPS model, the relative expression of Keap1 protein was significantly decreased ($P < 0.05$, $P < 0.01$), and the relative expression of Nrf2, HO-1, GPX4 protein was significantly increased ($P < 0.05$, $P < 0.01$) in the ATS low and high dose and Fer-1 intervention groups. The results showed that ATS could protect mouse ALI by regulating Keap1/Nrf2/GPX4/HO-1 protein.

As shown in Fig. 7 (F~H), compared with the normal control group, the relative expression levels of TNF- α , IL-1 β , and IL-6 mRNA in the lung tissue of mice in the LPS model group were significantly increased ($P<0.001$, $P<0.001$, $P<0.01$); compared with the LPS modeling group, ATS could significantly inhibit the relative expression levels of TNF- α , IL-1 β , IL-6 mRNA in the lung tissue of mice induced by LPS ($P<0.05$, $P<0.01$), where The levels of IL-1 β and IL-6 mRNA in ATS low-dose and high-dose groups were dose-dependent. Compared with the LPS model group, the relative expression of inflammatory factor mRNA in the lung tissue of the mice in the Fer-1 group was significantly down-regulated ($P<0.001$, $P<0.01$). The results showed that the abnormal expression of inflammatory factors caused lung injury in mice, and ATS could reduce the lung injury by inhibiting the expression of inflammatory factors in the lung tissue of ALI mice induced by LPS.

<Insert Fig. 7 here>

Discussion

Patients with severe COVID-19 infection often develop secondary ALI, with high morbidity and mortality, and lack of effective treatment drugs. Late stage often leads to pulmonary fibrosis, poor prognosis, and seriously reduces the quality of life of patients. Finding an effective drug with a clear mechanism of action to treat ALI is an urgent problem to be solved in clinical treatment. The protective effect of ATS, an alcoholic extract of aconitum tangutica, on alveolar cell injury may be related to the inhibition of oxidative stress and the regulation of NF κ B pathway, and participates in the regulation of the release of epithelial-mesenchymal transition factor, by promoting the Keap1/Nrf2/HO-1 pathway. Signaling, inhibition of LPS-induced ferroptosis in lung cells.

When ALI occurs, macrophages and neutrophils are activated, a large number of cytokines are released, a variety of free radicals are generated, and severe inflammation is induced. Acute lung inflammation leads to the destruction of the alveolar epithelial barrier (Liou et al., 2017), leading to pulmonary fibrosis. It is very important to prevent the development of this pathological process and improve or even cure ALI. The severity of lung injury depends on the interaction between inflammatory cells and alveolar epithelial cells and the degree of epithelial cell damage. Therefore, the typical type II alveolar cells A549 were used in this experiment. These alveolar epithelial cells are involved in the pathogenesis of ALI (Shi and Ma, 2020). It is generally believed that alveolar epithelial cells produce cytokines and chemokines after inflammatory injury, and the continuous generation of ROS is considered to be a key factor in inducing inflammatory injury (Zhao et al., 2020). ROS activates NF κ B dimer to promote its transfer to the nucleus and promote its transcription. This leads to the expression of more pro-inflammatory cytokines (Jiao et al., 2021). The excessive and continuous expression of inflammatory factors can also induce pathological fibrosis, IL-1 β can activate EMT-related pathways (Zhang et al., 2021), TNF- α plays a role through various signaling pathways (Ding et al., 2022), and regulates alveolar cell apoptosis and fibrosis play a key role. According to reports (Zhang et al., 2020), NF κ B is a key central mediator in the process of EMT. The activation of NF κ B in cells can inhibit the expression of E-cadherin and downregulate its expression, which is considered to be a key initiation event in EMT (Aban et al., 2021), thereby promoting

lung Tissue fibrosis, but whether it affects the oxidative stress pathway needs to be further studied experimentally.

ATS can inhibit pulmonary hemorrhage and pulmonary edema in ALI mice, alleviate the degree of lung tissue lesions, inhibit the migration and activation of neutrophils, inhibit the expression of inflammatory factors and inhibit cell apoptosis. As shown in Fig. 8, by further investigating the potential mechanism of ATS on LPS-induced ALI in mice, we found that ATS promoted the signaling of the Keap1/Nrf2/HO-1 pathway and suppressed the expression of LPS-induced ferroptosis-related proteins and the level of oxidative stress *in vivo*.

Studies have shown that an important reason for the exacerbation of ALI is the unbalanced inflammatory response in the lung lesions, which can promote the excessive production of ROS (Li et al., 2021). Similarly, excessive ROS can also promote lung inflammation. Inflammation (Aali et al., 2017), thus leading to a vicious cycle of ALI. ATS inhibited the abnormal expression of inflammatory factors such as TNF- α , IL-1 β and IL-6 mRNA, and each dose group of ATS could reduce the number of apoptotic cells and alleviate the apoptotic injury of mouse lung tissue cells.

Many studies have shown that ferroptosis, an iron-dependent, lipid peroxidation-triggered PCD (von et al., 2023), occurs during ALI, and its key features are the generation of massive cellular ROS and dysregulated lipid peroxidation (Stockwell et al., 2020), ATS also has the potential to resist oxidative stress in animals, and the increase of the body's ability to resist oxidative stress will help inhibit the production of ROS and further inhibit the process of ferroptosis (Qiu, et al., 2020); in addition, we observed The iron content in the lung tissue of ALI mice increased while the GSH content was down-regulated. GSH is an important antioxidant in the human body (Park, 2018). As an important cofactor of GPX4, it depends on the regulation of GPX4 to catalyze the reduction of lipid peroxide (Lai et al., 2022) to further regulate the production of ROS. The essence of ferroptosis is the depletion of GSH (Liu et al., 2023), so GSH depletion is considered a sign of ferroptosis [90]. Interestingly, the phenomenon of GSH depletion in the lung tissue of ATS pretreated mice was inhibited, showing the same trend as Fer-1 treatment group.

Based on the above studies, we evaluated the protective effect of ATS on ALI *in vivo* and further investigated the potential mechanism of ATS on LPS-induced ALI in mice. ATS interferes with the activation of inflammatory cells in alveolar epithelial cells, prevents the interaction between inflammatory cells and alveolar epithelial cells, inhibits the generation of cytokine storm and apoptosis of alveolar epithelial cells; inhibits ferroptosis and ROS-related oxidative stress and lipid peroxidation, etc. Injury pathological process of alveolar epithelial cells, resulting in lung tissue protection. Our research deeply explores the protective mechanism of ATS on lung tissue from the aspects of cytokine storm, inflammatory pathological process, ferroptosis, oxidative stress, etc., enriches the research on the pathogenesis of ALI, and provides an experimental basis for the discovery of effective therapeutic drugs for ALI. The present investigation brings a deep knowledge of the pharmacological efficacy of RZP, which

may lead to further successful utilization of RZP for clinical therapy. The research results further provide a reference for the clinical research and product development of ATS in Tibetan medicine.

<Insert Fig.8 here>

Conclusion

Taken together, ATS inhibited the apoptosis of A549 cells, suppressed the release of ROS and inhibited the epithelial mesenchymal transition process caused by sustained cell injury. Meanwhile, ATS promoted the signaling of Keap1/Nrf2/HO-1 pathway and inhibited the expression of LPS-induced iron death-related proteins and oxidative stress levels. The present investigation brings a deep knowledge of the pharmacological efficacy of ATS, which may lead to further successful utilization of ATS for clinical therapy in acute lung injury.

Abbreviations

AKT1
Protein kinase B
ALI
Acute lung injury
Bcl-2
B cell lymphoma-2
CAT
Catalase
CMC-Na
Carboxymethylcellulose sodium
DHE
Dihydroethidium
DMSO
Dimethyl sulfoxide
EGFR
Epidermal Growth Factor Receptor
EMT
Epithelial-mesenchymal transition
Fer-1
Ferrostatin-1
GPX4
Glutathione peroxidase 4
GSH
Glutathione
H&E

Hematoxylin & eosin
HO-1
Heme oxygenase-1
HPLC
High pressure liquid chromatography
IL-1 β
Interleukin-1 β
IL-8
Interleukin-8
iNOS
Inducible nitric oxide synthase
JAK2
Janus kinase 2
Keap1
Kelch-like ECH-associated protein 1
LPS
Lipopolysaccharides
MMPs
Matrix metalloproteinases
MPO
Myeloperoxidase
MTT
3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-Tetrazolium bromide
NF- κ B
Nuclear factor-kappa B
Nrf2
Nuclear factor erythroid 2
PBS
Phosphate buffer saline
PCD
Programmed cell death
ROS
Reactive oxygen species
SOD
Superoxide dismutase
TNF- α
Tumor necrosis factor- α
TUNEL
Terminal deoxynucleotidyl transferase dUTP nick end-labeling
 α -SMA

α -smooth muscle actin

Declarations

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Author contributions

Xiang Meng: Methodology, writing—original draft, software, data curation. Yupeng Liu: Methodology, validation, software, data curation. Jiawei Dai: Writing—original draft. Xin Hu: Conceptualization, writing—review and editing. Yuan Bai: Methodology, validation. Muhammad Azhar: Writing—review and editing. Xianju Huang: Conceptualization, Methodology, writing—review and editing, funding acquisition.

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Availability of data and materials

The datasets used and/or analyzed among the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This experiment was approved by the Animal Ethics Committee of Central South University for Nationalities. Experimental animal license number SCXK(Liao)2020-0001. At South-Central Minzu University's Experimental Animal Center, the mice were kept in SPF-grade animal barriers. All procedures involving the use of experimental animals are carried out strictly in compliance with the rules established by the South-Central Minzu University's Animal Ethics Committee and the international NIH standards.

Consent for publication

All authors critically reviewed the content of the manuscript. The consent for publication was obtained from all authors.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

1. Aali, M., Caldwell, A, House K., et al., 2017. Iron chelation as novel treatment for lung inflammation in cystic fibrosis. *J. Med Hypotheses*. 104, 86–88.
2. Aban. C. E., Lombardi, A., Neiman, G., et al., 2021. Downregulation of E-cadherin in pluripotent stem cells triggers partial EMT. *J. Scientific Reports*. 11(1), 2048.
3. Baumann, H.J., Kluge, S., Balke, L., et al., 2008. Yield and safety of bedside open lung biopsy in mechanically ventilated patients with acute lung injury or acute respiratory distress syndrome. *J. Surgery*. 143(3), 426–433.
4. Bellezza, I., Giambanco, I., Minelli, A., et al., 2018. Nrf2-Keap1 signaling in oxidative and reductive stress. *J. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*. 1865(5), 721–733.
5. Butt, Y., Kurdowska, A., Allen, T.C., 2016. Acute Lung Injury: A Clinical and Molecular Review. *J. Arch Pathol Lab Med*. 140(4), 345–350.
6. Ding, Y., Hou, Y., Liu, Y., et al., 2022. MiR-130a-3p Alleviates Inflammatory and Fibrotic Phases of Pulmonary Fibrosis Through Proinflammatory Factor TNF- α and Profibrogenic Receptor TGF- β RII. *J. Frontiers in Pharmacology*. 13, 863646.
7. Ferreira, de, Camillis, M.L., Savi, A., Goulart, Rosa. R., et al., 2018. Effects of Mechanical Insufflation-Exsufflation on Airway Mucus Clearance Among Mechanically Ventilated ICU Subjects. *J. Respir Care*. 63(12), 1471–1477.
8. Gao, Y.M., Zeng, R., 2010. Determination of atisine and heterophyll aconitine in the Tibetan medicine Pangga by Hplc. *J. Chin J of Ethnomedicine and Ethnopharmacy*. 19(10), 188–190.
9. Jiao, B., Guo, S., Yang, X., et al., 2021. The role of HMGB1 on TDI-induced NLPR3 inflammasome activation via ROS/NF- κ B pathway in HBE cells. *J. International Immunopharmacology*. 98, 107859.
10. Lai, Y., Dong, J., Wu, Y., et al., 2022. Lipid Peroxides Mediated Ferroptosis in Electromagnetic Pulse-Induced Hippocampal Neuronal Damage via Inhibition of GSH/GPX4 Axis. *J. Int J Mol Sci*. 23(16), 9277.

11. Laskin, D.L., Malaviya, R., Laskin, J.D., 2019. Role of Macrophages in Acute Lung Injury and Chronic Fibrosis Induced by Pulmonary Toxicants. *J. Toxicol Sci.* 168(2), 287–301.
12. Li, J., Lu, K., Sun, F., et al., 2021. Panaxydol attenuates ferroptosis against LPS-induced acute lung injury in mice by Keap1-Nrf2/HO-1 pathway. *J. J Transl Med.* 19(1), 96.
13. Li, L., Huang, Q., Wang, D.C., et al., 2020. Acute lung injury in patients with COVID-19 infection. *J. Clin Transl Med.* 10(1), 20–27.
14. Liou, C.J., Huang, Y.L., Huang, W.C., et al., 2017. Water extract of *Helminthostachys zeylanica* attenuates LPS-induced acute lung injury in mice by modulating NF- κ B and MAPK pathways. *J. Journal of Ethnopharmacology.* 199, 30–38.
15. Liu, J., Yang, G., Zhang, H., 2023. Glyphosate-triggered hepatocyte ferroptosis via suppressing Nrf2/GSH/GPX4 axis exacerbates hepatotoxicity. *J. Sci Total Environ.* 862, 160839.
16. Liu, Q., Wang, K., 2019. The induction of ferroptosis by impairing STAT3/Nrf2/GPx4 signaling enhances the sensitivity of osteosarcoma cells to cisplatin. *J. Cell biology international.* 43(11), 1245–1256.
17. Liu, Y.P., Liu, L., Zeng, Y.X., 2019. Effect of negative fluid balance management model on cardiopulmonary function and prognostic outcome of patients with acute lung injury combined with infectious shock. *J. Chin General Practice Nursing.* 17(33), 4182–4184.
18. Margaria, J.P., Moretta, L., Alves-Filho, J.C., et al., 2022. PI3K Signaling in Mechanisms and Treatments of Pulmonary Fibrosis Following Sepsis and Acute Lung Injury. *J. Biomedicines.* 10(4), 756.
19. Park, W.H., 2018. Antiapoptotic effects of caspase inhibitors on H₂O₂-treated lung cancer cells concerning oxidative stress and GSH. *J. Mol Cell Biochem.* 441(1–2), 125–134.
20. Qiu, Y.B., Wan, B.B., Liu, G., et al., 2020. Nrf2 protects against seawater drowning-induced acute lung injury via inhibiting ferroptosis. *J. Respir Res.* 21(1), 232.
21. Shi, G.C., Ma, X.G., 2020. Effects of TNF- α on Proliferation and Apoptosis of Alveolar Type I Epithelial Cells in Rats. *J. Acta Med Universitatis Scientiae et Technologiae Huazhong.* 49(06), 673–676.
22. Stockwell, B.R., Jiang, X., Gu, W., 2020. Emerging Mechanisms and Disease Relevance of Ferroptosis. *J. Trends Cell Biol.* 30(6), 478–490.
23. Tang, G., Luo, Y., Lu, F., et al., 2021. Prediction of Sepsis in COVID-19 Using Laboratory Indicators. *J. Front Cell Infect Microbiol.* 10, 586054.
24. Tharpa, Gyaltsan., Norpu, Thundrup., 2020. Analysis of the COVID-19 Based on Tibetan Medicine“gnyn rims”Theory. *J. Asia-Pacific Tradit Med.* 16(07), 5–8.
25. Tu, G.W., Shi, Y., Zheng, Y.J., et al., 2017. Glucocorticoid attenuates acute lung injury through induction of type 2 macrophage. *J. J Transl Med.* 15(1), 181.
26. von, Krusenstiern, A.N., Robson, R.N., Qian, N., et al., 2023. Identification of essential sites of lipid peroxidation in ferroptosis. *J. Nat Chem Biol.*(in press)

27. Walkey, A.J., Wiener, R.S., 2012. Macrolide antibiotics and survival in patients with acute lung injury. *J. Chest.* 141(5), 1153–1159.
28. Wang, X.R., Li, J.P., Sun, X.D., 2013. Determination of hordenine in Tangut aconite by Hplc. *J. Practical J of Med & Pharm.* 30(07), 624–625.
29. Yang, L.H., Lin, M.L., Wang, Z.M., et al., 2016. Research Progress on *Aconitum tanguticum*. *J. Chin J of Exper Tradit Med Formu.* 22(10), 43–49.
30. Zhang, R., Xu, L., An, X., et al., 2020. Astragalus polysaccharides attenuate pulmonary fibrosis by inhibiting the epithelial-mesenchymal transition and NF- κ B pathway activation. *J. International Journal of Molecular Medicine.* 46(1), 331–339.
31. Zhang, S., Fan, Y., Qin, L., et al., 2021. IL-1 β augments TGF- β inducing epithelial-mesenchymal transition of epithelial cells and associates with poor pulmonary function improvement in neutrophilic asthmatics. *J. Respiratory Research.* 22(1), 216.
32. Zhang, Y.Z., Chang, J.H., Shao, C.L., et al., 2012. Progress of research on Tangut aconite. *J. J of Med & Pharm of Chin Minor.* 18(01), 70–73.
33. Zhao, S., Chen, F., Yin, Q., et al., 2020. Reactive Oxygen Species Interact With NLRP3 Inflammasomes and Are Involved in the Inflammation of Sepsis: From Mechanism to Treatment of Progression. *J. Frontiers in Physiology.* 11, 571810.

Figures

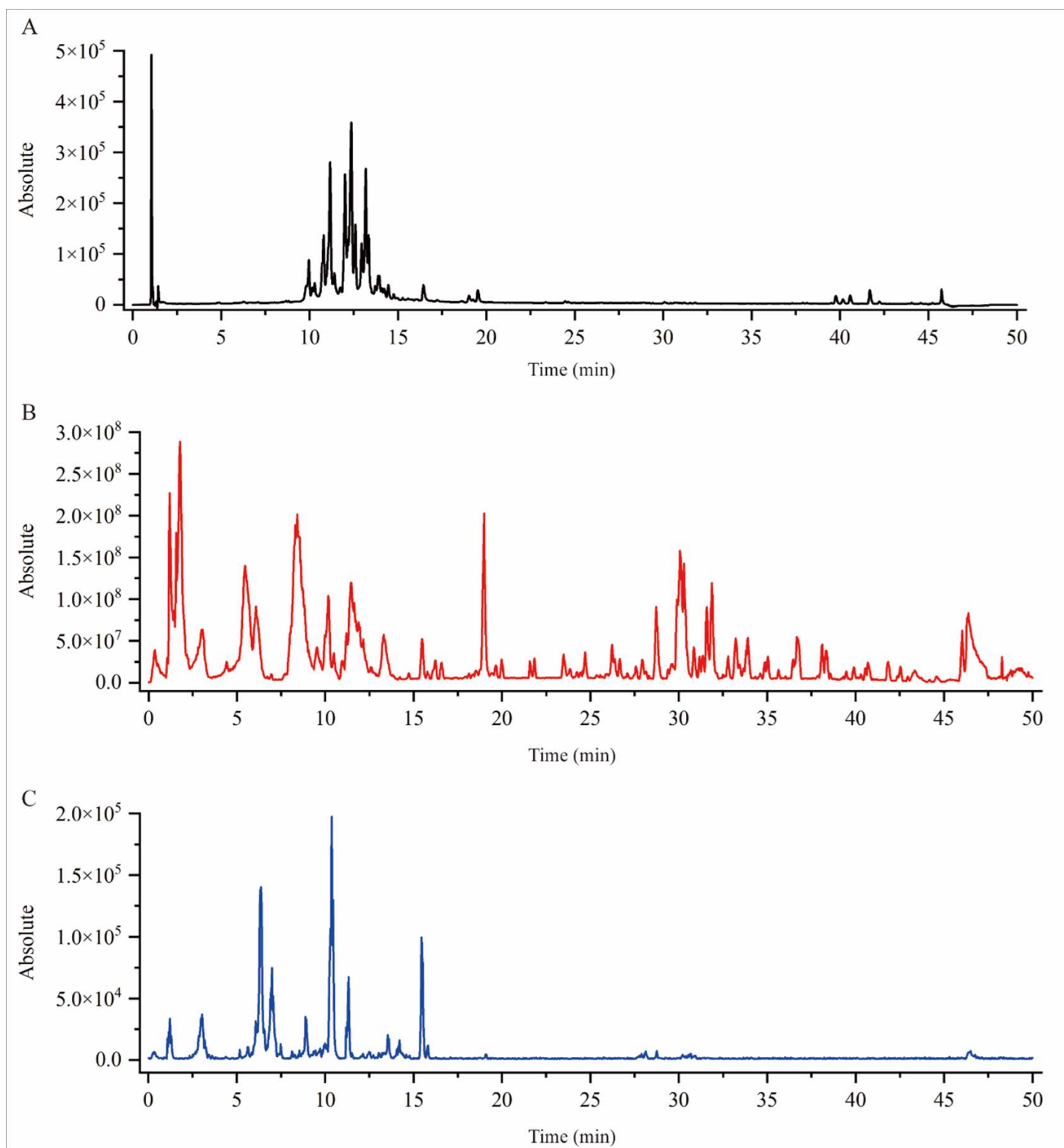


Figure 1

Total ion chromatogram, positive ion mode and negative ion mode of LC-MS / MS detection: A: Total ion chromatogram; B: Positive ion mode; C: Negative ion mode.

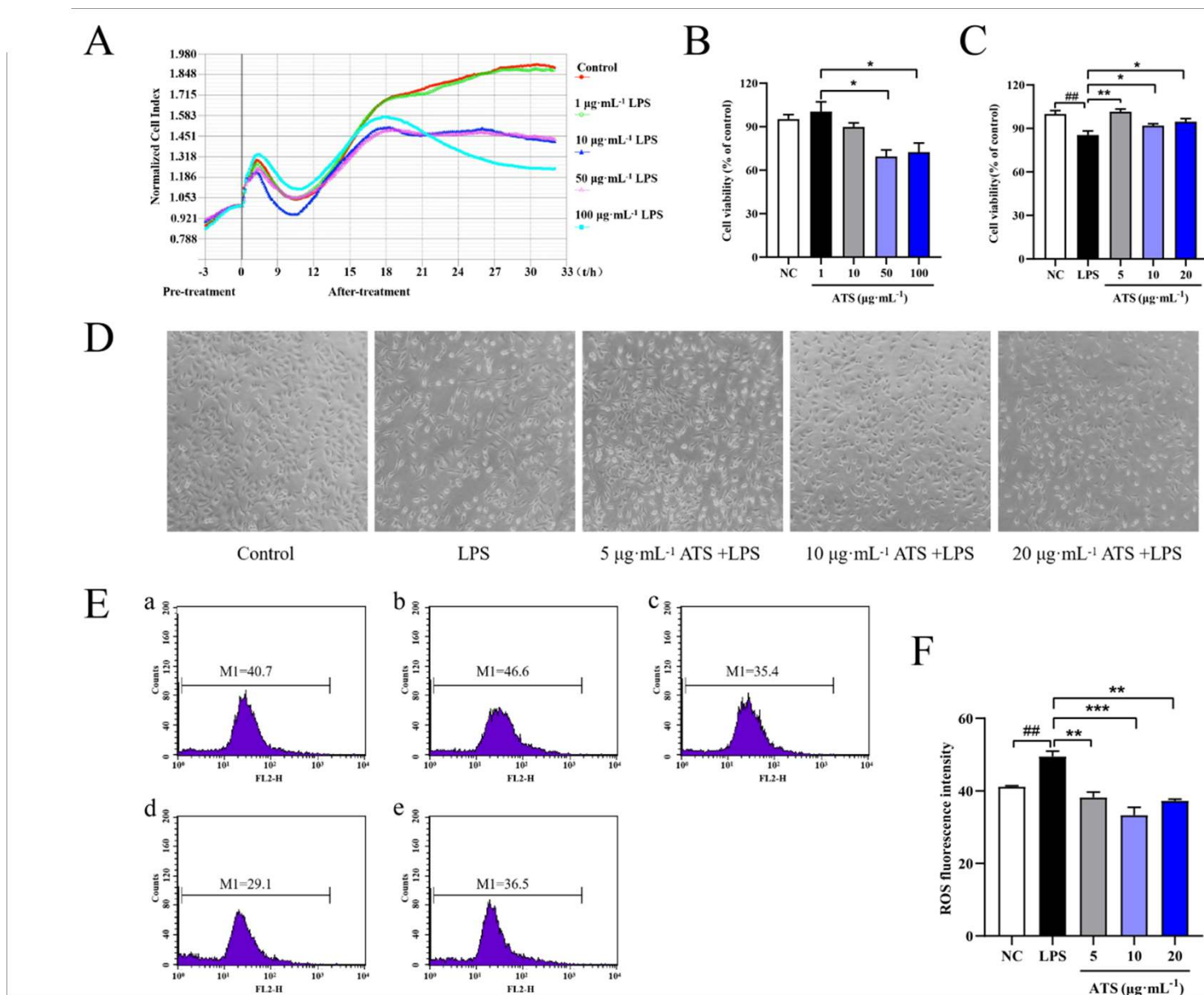


Figure 2

A: RTCA analysis of A549 cell proliferation on E-16 plate; B: Analysis of A549 cell proliferation; C: Effect of ATS on A549 cell viability induced by LPS; D: Change of cell morphology. $n=6$; * $P<0.05$, ** $P<0.01$ compared with control group; # $P<0.05$, ## $P<0.01$ compared with LPS group. E: Flow cytometry analysis of LPS-induced changes in intracellular ROS levels: a-NC group, b-LPS group, c-ATS (5 $\mu\text{g}/\text{mL}$) group, d-ATS (10 $\mu\text{g}/\text{mL}$) group, e-ATS (20 $\mu\text{g}/\text{mL}$) group; F: relative fluorescence intensity analysis; $n=3$; ## $P<0.01$, compared with normal control group; ** $P<0.01$, *** $P<0.001$ compared with LPS model group

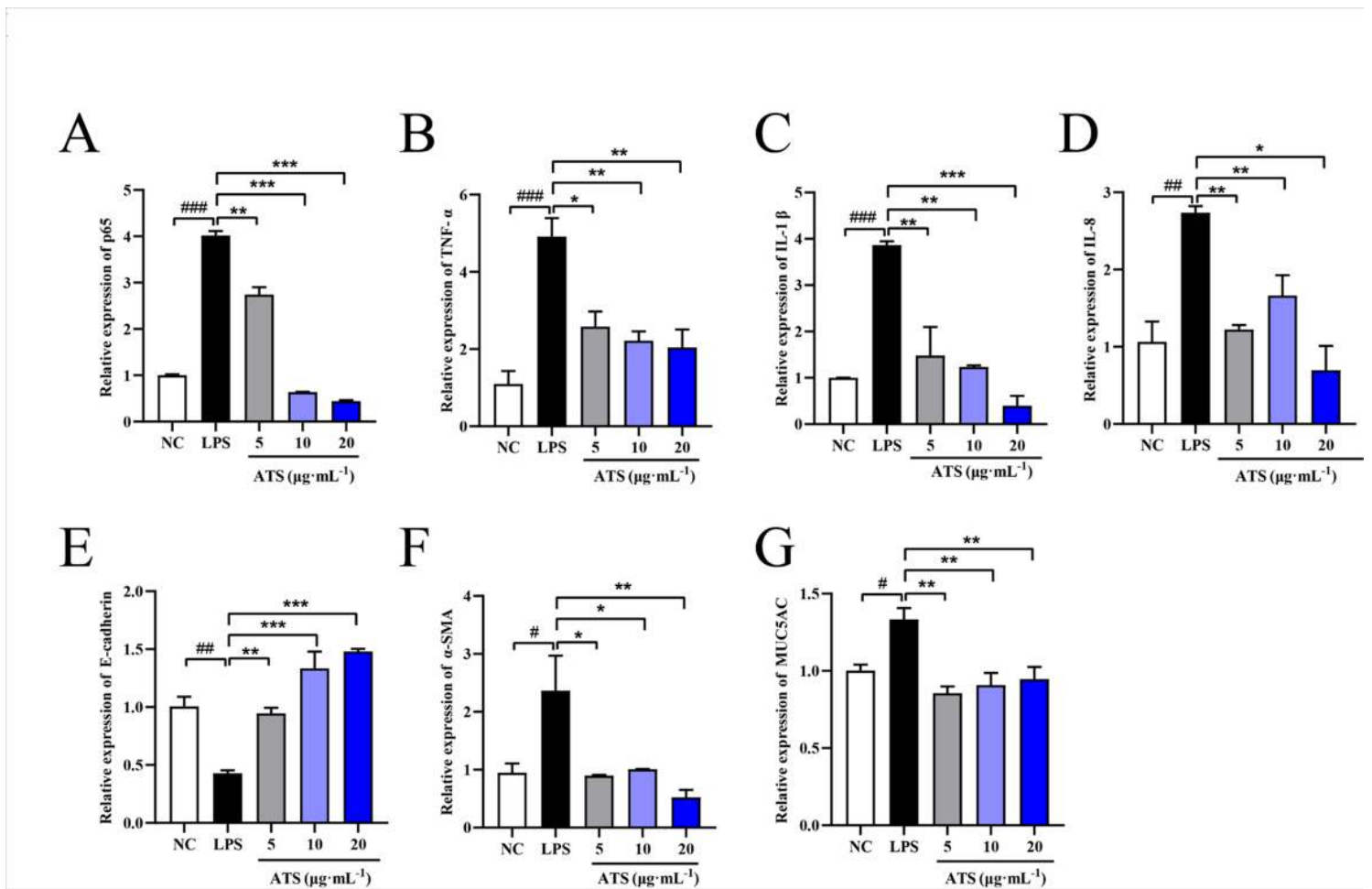


Figure 3

A~G shows the mRNA expressions of the following genes in cells: NF- κ B p65, TNF- α , IL-1, IL-8, E-cadherin, α -SMA, and MUC5AC, respectively. The normal control group (NC) and the model group (LPS) are represented by the letters, respectively. $n=5$; ## $P<0.01$, ### $P<0.001$ compared with normal control group; * $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared with LPS compared to the model group.

A



B

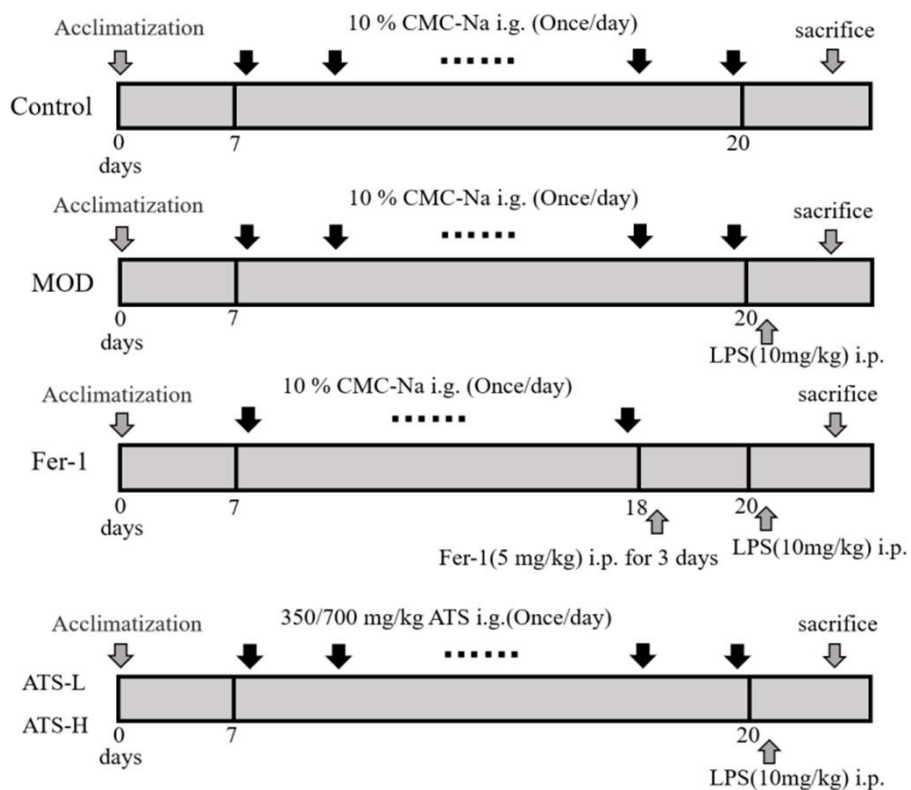


Figure 4

The plant of Tibetan medicine ATS (A) and the schematic diagram of animal treatment (B).

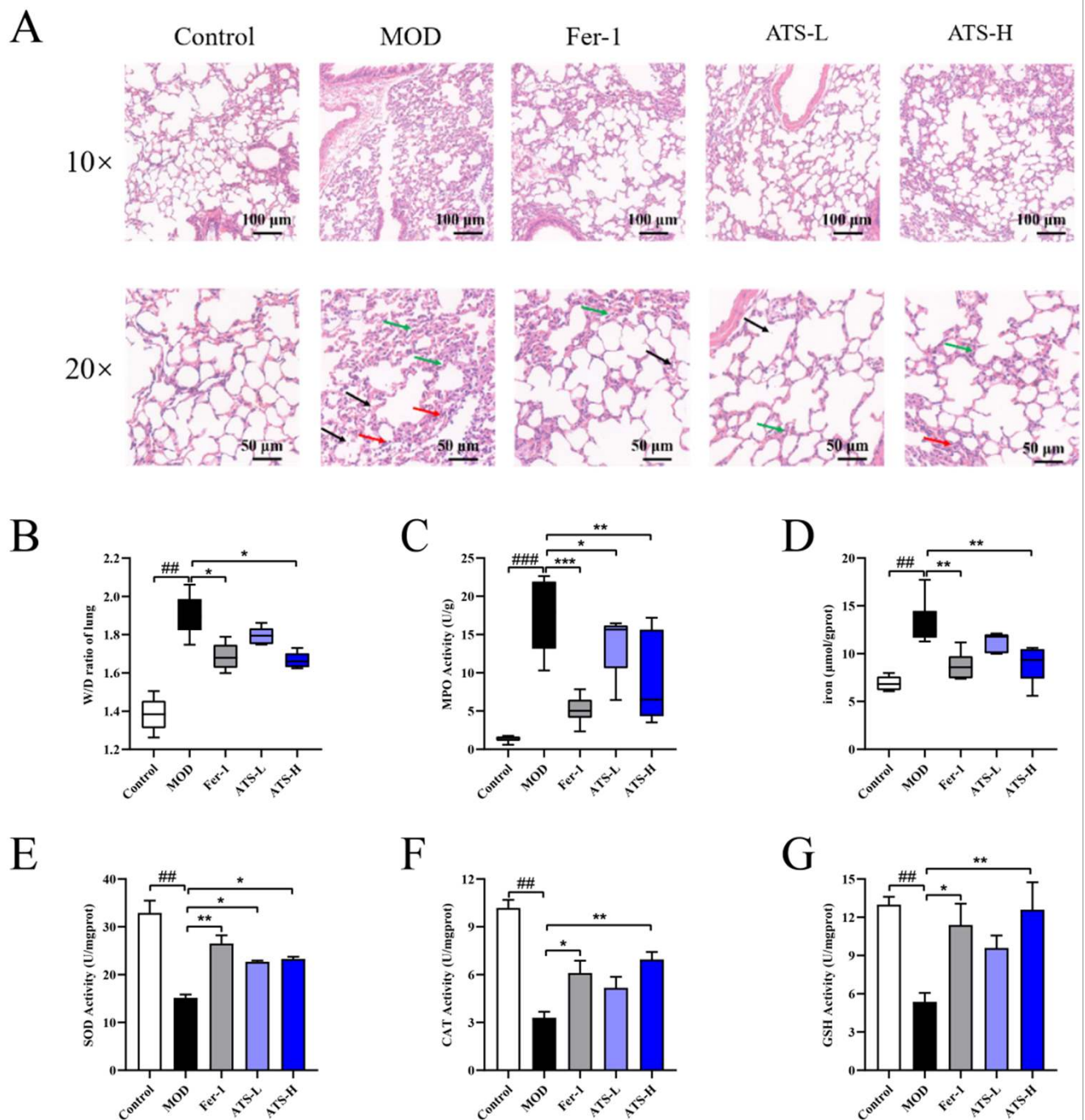


Figure 5

A~G: Effect of ATS on pathomorphology, W/D ratio, MPO, iron content, activity, SOD, CAT, GSH content in the lung tissue of LPS-induced ALI mice; n=6; compared with normal control group, ## $P<0.01$; compared with LPS model group, ** $P<0.01$, * $P<0.05$.

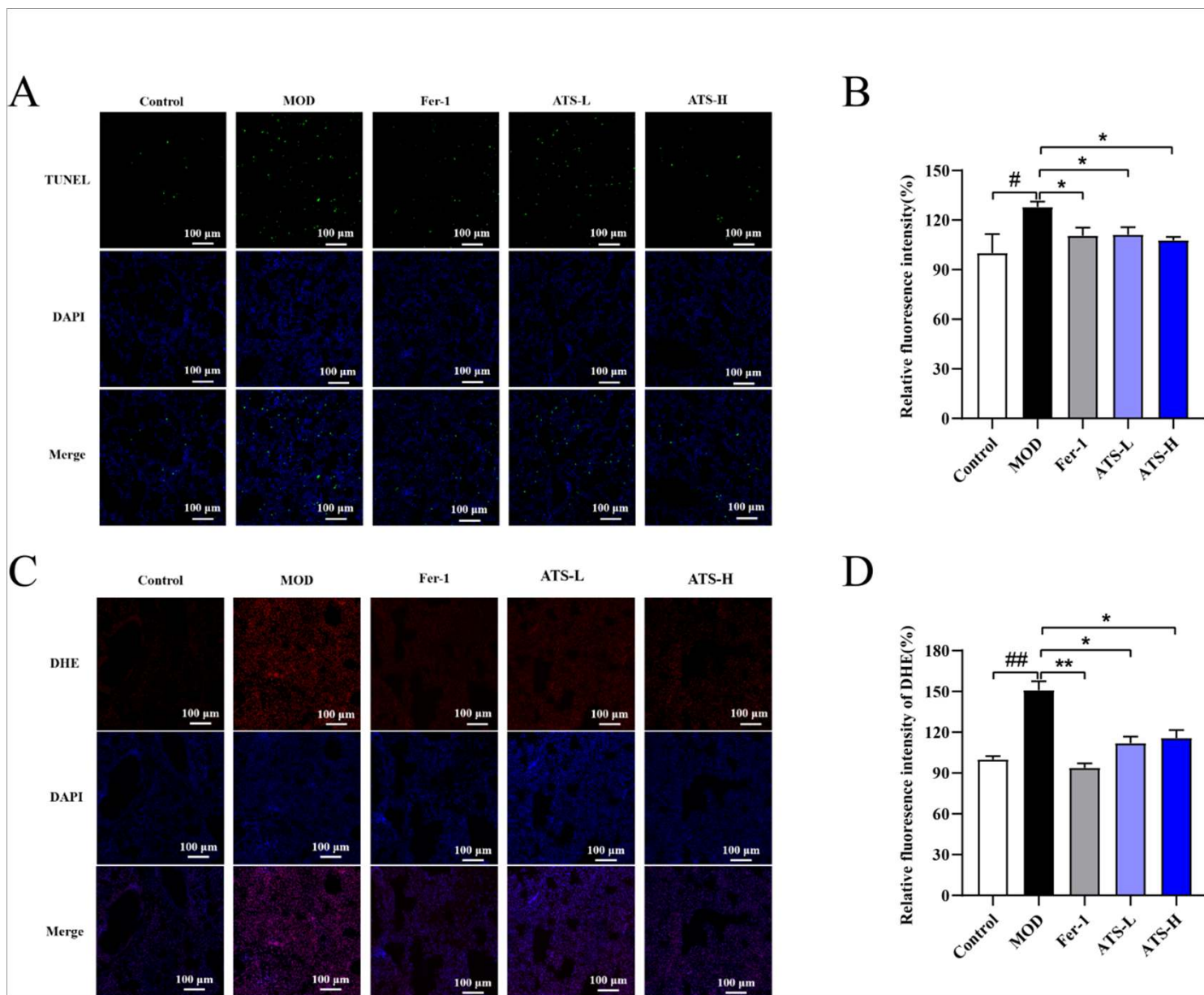


Figure 6

A: Effect of ATS on apoptosis in lung tissue of ALI mice induced by LPS; B: Quantification results of relative fluorescence intensity; C: Effect of ATS on ROS generation in lung tissue of ALI mice induced by LPS D: Relative fluorescence Intensity quantification results; n=3; compared with normal control group, $\#P<0.05$; compared with LPS modeling group, $*P<0.05$)

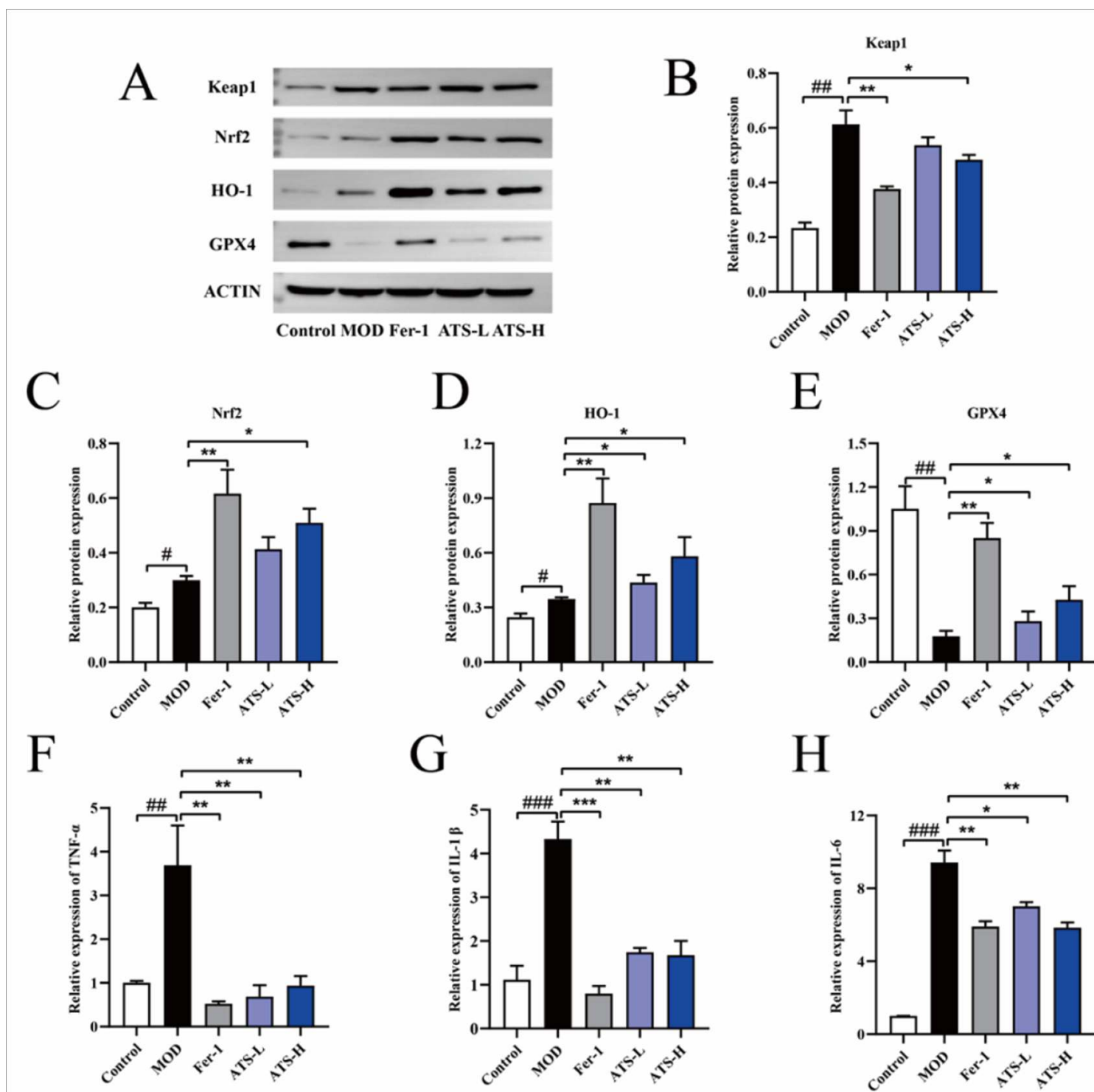


Figure 7

The effect of ATS on the mRNA expression of inflammatory factors and the protein level of Keap1/Nrf2/GPX4/HO-1 in the lung tissue of ALI mice induced by LPS: A: Western blotting of Keap1/Nrf2/GPX4/HO-1; B~E: Protein expression levels of Keap1, Nrf2, GPX4, and HO-1; n=3; compared with normal control group, ## $P<0.01$, # $P<0.05$; compared with LPS modeling group, ** $P<0.01$, * $P<0.05$; F: Relative expression of TNF- α mRNA; G: Relative expression of IL-1 β mRNA ; H: relative expression of IL-6

mRNA; n=6; compared with normal control group, ### $P<0.001$, ## $P<0.01$; compared with LPS model group, *** $P<0.001$, ** $P<0.01$, * $P<0.05$.

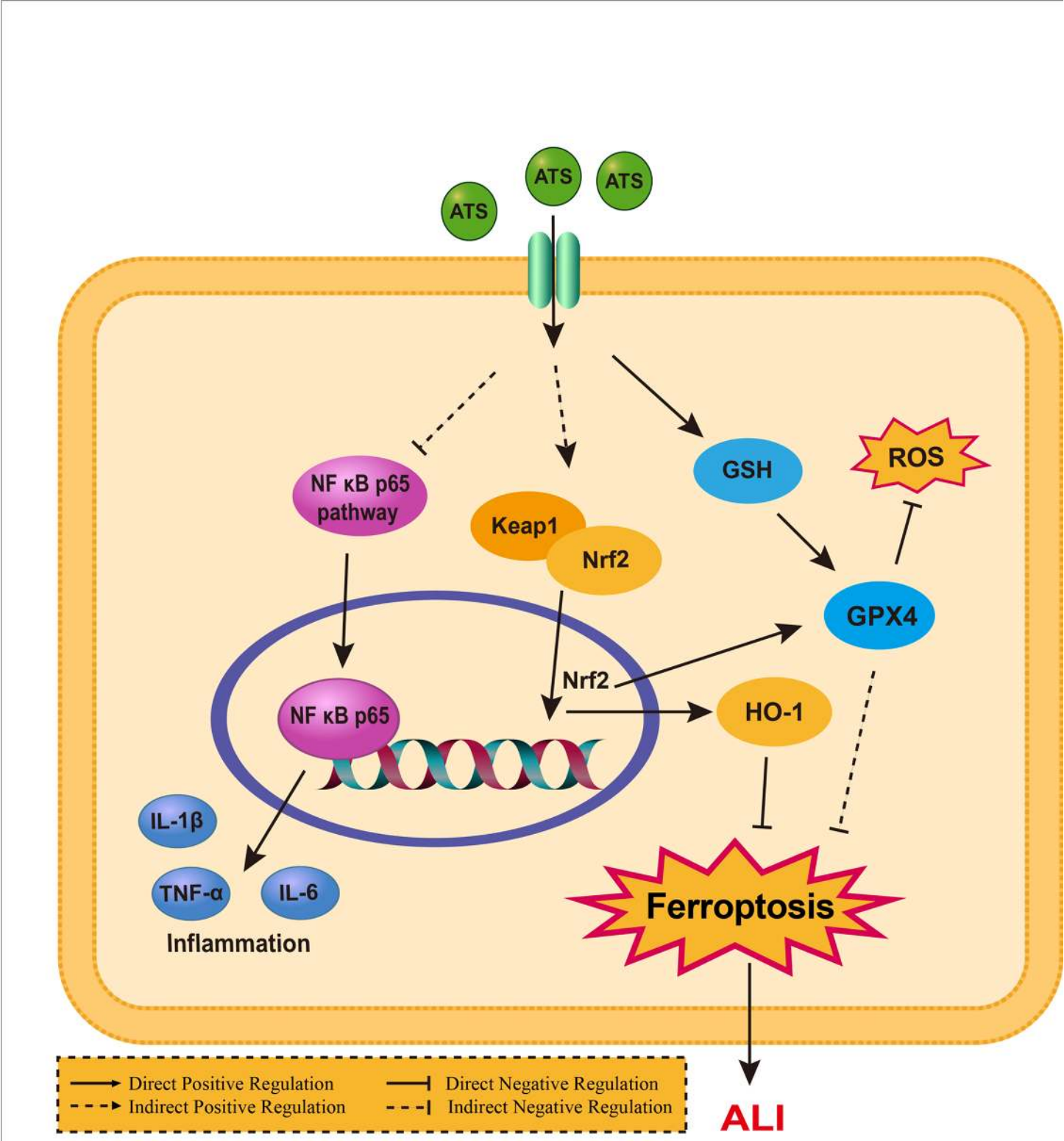


Figure 8

Preliminary speculation on the signaling pathways involved in the mechanism of ATS on ALI.

Supplementary Files

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