

***Forsythia suspensa* Ethanolic Extract Alleviates *Mycoplasma pneumoniae*-induced Lung Inflammation via Inhibition of NF- κ B Signaling**

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Abstract

Background/Objectives: *Mycoplasma pneumoniae* is a significant contributor to respiratory tract infections, notably community-acquired pneumonia (CAP), bronchitis, and exacerbations of chronic lung diseases. Its epidemiology varies across regions and time periods, and while often presenting as a mild illness known as “walking pneumonia,” it can be severe in high-risk populations, with mortality reaching 30% in some cases. The increasing emergence of antibiotic-resistant *M. pneumoniae* strains presents a significant challenge, highlighting the need for deeper insights into its pathogenicity to develop more effective treatment strategies.

Materials and Methods: BALB/c mice were challenged with *M. pneumoniae* and administered with an ethanolic extract of *Forsythia suspensa* ethanolic extract (FSEE). Survival rate and pulmonary index inhibitory rate were assessed. Nitric oxide, myeloperoxidase, C-reactive protein, and immunoglobulin M levels were measured. Total cell counts and *M. pneumoniae* deoxyribonucleic acid (DNA) content were quantified. Anti-inflammatory potential was evaluated through cytokine measurements. Regulatory effects on inflammation-associated signaling pathways were examined by assessing extracellular signal-regulated protein kinases 1/2 (ERK1/2), c-Jun N-terminal protein kinase 1/2 (JNK1/2), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) expression levels.

Results: FSEE treatment significantly improved survival rates and reduced pulmonary index scores. It effectively attenuated excessive immune responses induced by *M. pneumoniae* infection, leading to a marked reduction in total cell count and *M. pneumoniae* DNA content. Pro-inflammatory cytokines and NF- κ B levels were downregulated, while JNK1/2 expression was notably enhanced following FSEE treatment.

Conclusion: These findings indicate that FSEE mitigates *M. pneumoniae*-induced lung inflammation by modulating immune responses and inflammatory mediators, suggesting its potential as a therapeutic agent for *M. pneumoniae*-associated pneumonia.

Keywords

Pneumonia, *Mycoplasma pneumoniae*, inflammation, phytomedicine, ERK/JNK/NF- κ B pathway

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Introduction

Community-acquired pneumonia (CAP) stands as a foremost global pandemic concern, representing the leading infectious cause of death worldwide despite advancements in prevention and treatment (Alshahwan et al., 2019; Ferreira-Coimbra et al., 2020; Regunath & Oba, 2024). It places a significant burden on healthcare systems due to high hospitalization rates, which can reach up to 50% of cases (Almirall et al., 2017). While CAP affects all age groups, young children, the elderly, and immunocompromised individuals face particularly poor outcomes (Chen et al., 2021). The incidence among adults can

reach as high as 14 cases per 1,000, with mortality rates reaching 0.7/1,000 people per year (Wahl et al., 2020). Globally, CAP accounts for approximately 4 million deaths annually,

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contributing to 7% of the total annual mortality rate (Bai & Li, 2021). Mortality rates can escalate to 10% among inpatients and surpass 30% in those requiring critical care and respiratory support (Chaudhry et al., 2016; Morley et al., 2017).

Mycoplasma pneumoniae frequently leads to CAP, especially among younger individuals, including children and teenagers, accounting for a substantial percentage of pediatric pneumonia cases (10%–40%) (Atkinson & Waites, 2014; Rueda et al., 2022). While often causing mild, self-limiting respiratory infections, *M. pneumoniae* can also lead to severe complications, including refractory pneumonia requiring hospitalization, especially in school-aged children, where it is related to lobar pneumonia and complications like pleural effusion (Upadhyay & Singh, 2024; Wood et al., 2017). It is recognized as a factor in exacerbating chronic lung diseases, and though often termed “walking pneumonia” due to its milder presentation, mortality can reach up to 30% in some populations, particularly the elderly (Miyashita, 2022; Poddighe, 2018). Pathogenesis involves multiple mechanisms, including direct damage to cells, inflammation, and nutrient depletion (He et al., 2016; Hu et al., 2022). Notably, macrolide resistance may complicate treatment (Li et al., 2024; Meyer Sauter et al., 2014).

The primary treatments for *M. pneumoniae* infections involve antibiotics like macrolides, tetracyclines, and fluoroquinolones, which target protein or deoxyribonucleic acid (DNA) synthesis as a result of the bacterium's absence of a cell wall, rendering beta-lactams and glycopeptides ineffective (Wang et al., 2024). Macrolides, particularly azithromycin, are often preferred for their *in vitro* activity (Waites et al., 2017). However, these antibiotics can cause significant side effects, including gastrointestinal issues, hepatotoxicity, and photosensitivity, especially with prolonged use (Chinese Experts Cystic Fibrosis Consensus Committee et al., 2023; Lenz et al., 2021). In individuals with pre-existing lung conditions, several factors raise significant concerns: the possibility of adverse drug interactions, the buildup of toxic effects from medications, and the increased susceptibility to antibiotic-related issues such as *Clostridioides difficile* infections and disturbances to the delicate balance of microorganisms in the respiratory system (Lessa et al., 2015). To manage symptoms, slow disease advancement, and reduce the recurrence of infections, long-term approaches might involve a combination of antibiotics alongside bronchodilators and corticosteroids. However, extended antibiotic use elevates the risk of both antibiotic resistance and the development of unwanted side effects (Guo, Jin, et al., 2024; Kim et al., 2022).

With growing interest in plant-derived treatments, researchers are exploring phytochemicals for their potential in combating various diseases, including lung disorders (Patel & Sharma, 2021). The World Health Organization (WHO) reports that approximately 88% of countries utilize herbal remedies within traditional medicine practices. Furthermore, natural products constitute more than 40% of pharmaceutical formulations and provide essential healthcare for 70%–95%

of the population in developing nations (Singh et al., 2010; WHO, 2023). Since herbal medicines are rich in alkaloids, flavonoids, terpenoids, and other compounds, they can serve as alternatives or complements to conventional antibiotics. Previous studies also reported that the pharmaceutical properties of medicinal plants like *Glycyrrhiza glabra*, *Hyssopus officinalis*, *Allium sativum*, and so on possess a traditional application in addressing respiratory conditions such as pneumonia, cough, and asthma (Hanifah et al., 2023).

Forsythia suspensa is one such herbal medicinal plant, a member of the *Forsythia* genus within the Oleaceae family (Wang et al., 2018), which is commonly utilized in Traditional Chinese Medicine to address conditions such as fever, the common cold caused by viruses, gonorrhea, and ulcers (Han et al., 2021; Li & Chen, 2005). *F. suspensa* is also used clinically for various inflammation-related ailments, including wind–heat-induced colds, scabies, ulcers, and mastitis (Xia et al., 2016). In this research, we elucidate the potency *F. suspensa* extract in ameliorating *M. pneumoniae*-induced pneumonia in a mouse model.

Materials and Methods

Chemicals

The primary chemicals and reagents utilized in this study were procured from Sigma–Aldrich, USA. The kits for the biochemical marker estimations were obtained from Thermo Fisher Scientific, USA; Elabscience, USA; NovaTeinBio, USA; MyBiosource, USA; and Cusabio, USA, respectively.

F. suspensa Extract Preparation

F. suspensa samples were ground into coarse powder and mixed with a tenfold volume of 75% ethanol, followed by ultrasonication for 1 h. After ultrasonication, the mixture was allowed to sit for 24 h and then filtered four times using Whatman filter paper. The resulting filtrate was lyophilized and reconstituted to create a stock solution at a concentration of 1,000 mg/mL, which was subsequently diluted with distilled water.

Cultivation of *Mycoplasma Pneumoniae* (MP)

The MP, FH strain (ATCC 15531) was cultivated in a modified mycoplasma medium consisting of PPLO broth, horse serum, and 25% yeast extract. This medium was supplemented with penicillin G at a concentration of 200 U/mL, thallium acetate at 0.025%, glucose at 1%, and phenol red at 0.002%, adjusted to a pH of 7.8. The culture was incubated at 37°C in an atmosphere of 5% CO₂ for 7 days. The concentration of MP was assessed using the color-change unit (CCU) method, resulting in a determination of 10⁸ CCU/mL (Yoon et al., 2017).

Experimental Animals

Six-week-old male BALB/c mice, weighing approximately 20–25 g, were kept in an environmentally controlled animal housing facility (temperature: 20°C ± 2°C, humidity: 50% ± 10%). The research adhered to the International Standards for the care and use of animals.

Treatment Regimen

The mice were allocated into four groups: the normal control group, the MP-challenged group, the MP-challenged group treated with *F. suspensa* extract, and the MP-challenged group treated with the standard drug azithromycin. All groups, except for the normal control group, were intranasally infected with 100 µL of the pathogen per mouse over two consecutive days. In the groups receiving *F. suspensa* extract and azithromycin, the MP-infected mice were administered 200 mg/kg of *F. suspensa* extract and 100 mg/kg of azithromycin, respectively, for 3 days, starting on the day of infection. Mice in the control group were given an equivalent volume of distilled water. On treatment day 4, the animals were euthanized, and blood samples and lung tissue were collected for further analysis.

Assessment Pulmonary Index

The body weight (BW) as well as the lung weight (LW) of the experimental animals were recorded. This data was used to calculate the pulmonary index (PI) and the inhibitory rate of pulmonary indices (IRPI). The formulas used were as follows:

$$PI = (LW/BW) \times 100$$

$$IRPI = (LW \text{ of MP challenged} - LW \text{ of MP challenged} + \text{Drug treated}) / (LW \text{ of MP challenged} - LW \text{ of control}) \times 100$$

Assessment of Survival Percentage

The experimental animals were observed daily for 10 days following MP infection, monitoring for any signs of morbidity and mortality. The percentage of survival was calculated using the formula

$$\text{Survival percentage} = \text{No. of animals survived} / \text{Total no. of animals} \times 100$$

Assessment of MP-induced Immune Response

Total nitric oxide levels in the experimental animals were detected using the Nitric oxide assay kit procured from Thermo Fisher Scientific, USA. The conversion of nitrate to nitrite by nitrate reductase was measured with the Griess reaction at 540 nm. Myeloperoxidase levels were quantified using the myeloperoxidase assay kit procured from Elabscience, USA. The reduction of hydrogen peroxide by myeloperoxidase was quantified with o-dianisidine at 460 nm.

Quantification of C-reactive Protein (CRP) and MP-immunoglobulin M (IgM) Levels

Sensitive markers of pneumonia, CRP and the levels of MP-induced IgM antibodies were quantified in the experimental animals using the enzyme-linked immunosorbent assay (ELISA) technique. CRP levels were quantified with Sigma–Aldrich ELISA kit and the IgM levels were quantified with the Mouse MP IgM ELISA kit procured from NovaTeinBio, USA. The assay was performed as per the guidelines provided in the kit manuals.

Measurement of Bronchoalveolar Lavage Fluid (BALF) Total Cell Count

To obtain BALF from the experimental and control groups, 30 mL aliquots of saline were administered into the right middle lobe of each mouse. Following the instillation, BALF was collected, and the procedure was repeated thrice. Collected BALF was subsequently processed by centrifugation at 4,500 rpm for 10 min to separate the cell pellets from the lavage fluid. The total cell count in the resulting samples was determined using a hemocytometer in conjunction with an optical microscope.

Quantification of MP-DNA

Lung tissue homogenates were prepared from the experimental mice using the homogenizing buffer and subsequently incubated in DNA extraction buffer at 37°C for 10 min. Following incubation, the mixture was centrifuged at 13,000 rpm for 5 min at 4°C to facilitate DNA extraction from the samples. The concentration of MP-DNA in the DNA samples was quantified using the polymerase chain reaction technique.

Assessment of Inflammation

The levels of inflammatory stimulators triggered by MP infection and the attenuating potency of *F. suspensa* extract were assessed by quantifying the levels of interleukins (IL), tumor necrosis factor (TNF)-α, and transforming growth factor (TGF)-β levels in the experimental animals. ELISA kits procured from Abcam, USA, were utilized to quantify IL-1, IL-6, and IL-8. TNF-α and TGF-β were quantified using the ELISA kits procured from MyBiosource, USA. The guidelines provided in the manual of the kit were followed to perform the experiments, and the concentration of inflammatory stimulators was measured with the standard curve plot.

Assessment of NF-κB Signaling

Extracellular signal-regulated protein kinases 1/2 (ERK1/2), c-Jun N-terminal protein kinase 1/2 (JNK1/2), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) levels in the experimental animals were measured to determine

the role of *F. suspensa* extract against *M. pneumoniae*-triggered NF- κ B signaling. ERK1/2, JNK1/2, and NF- κ B ELISA kits obtained from Cusabio, USA, were utilized for the quantification. The final absorbance of the test samples and standards was measured at 450 nm using an ELISA microplate reader.

Statistical Analysis

All data were subjected to statistical analysis using SPSS version 18.0. The results are expressed as triplicate measurements' mean $\pm$ standard deviation (SD). Group comparisons were performed using one-way analysis of variance (ANOVA), followed by Dunnett's test to evaluate differences between the groups. A threshold of $p < .05$ was established to determine statistical significance.

Results

Forsythia suspensa Ethanolic Extract (FSEE) Attenuated Pulmonary Index in MP-challenged Animals

Figure 1 illustrates the pulmonary index and the inhibitory rate of the pulmonary index measured in the experimental animals. Infection with *M. pneumoniae* increased the levels of pulmonary index to 1.7 ± 0.00006 , which was only 0.6 ± 0.00003 in the normal control rats. Treatment with FSEE and the standard drug azithromycin significantly reduced the pulmonary index to 1.3 ± 0.00004 and 0.8 ± 0.00004 , respectively. The inhibitory rate of the pulmonary index was significantly enhanced with treatment with FSEE and azithromycin in the mycoplasma-infected animals.

FSEE Enhanced the Survival Rate of MP-challenged Animals

The number of days the mice infected with mycoplasma survived and the experimental animals' survival percentage were

noted, and the results were tabulated in Figure 2. The survival percentage and the days were severely reduced in the untreated mycoplasma-infected animals; only $38 \pm 0.3\%$ of animals survived for 4.8 ± 0.02 days. Whereas treatment with FSEE and azithromycin increased the survival percentage of animals to $72\% \pm 0.4\%$ and $87\% \pm 0.5\%$, respectively, the survival days were extended to 8.1 ± 0.04 and 9.6 ± 0.05 days, respectively.

FSEE Inhibited Uncontrolled Immune Response Triggered in MP-challenged Animals

The attenuating effect of FSEE against immune response triggered by mycoplasma infection was measured via quantifying the sensitive markers NO and MPO levels in the experimental animals (Figure 3). The control animals exhibited 127 ± 0.04 $\mu\text{mol/g}$ of nitric oxide and 27 ± 0.5 U/g of MPO, whereas with mycoplasma infection, both the NO and MPO levels were enhanced to 325 ± 0.02 $\mu\text{mol/g}$ and 44 ± 0.7 U/g, respectively. Treatment with FSEE and azithromycin decreased the NO levels to 295 ± 0.02 $\mu\text{mol/g}$ and 210 ± 0.02 $\mu\text{mol/g}$ and MPO levels 38 ± 0.4 U/g and 32 ± 0.06 U/g, respectively.

FSEE Suppressed CRP and MP-IgM Levels in MP-challenged Animals

CRP and MP IgM levels were quantified in the MP-challenged animals treated with FSEE, and the results are depicted in Figure 4. Treatment with FSEE and azithromycin reduced the CRP levels to 38 ± 0.004 and 28 ± 0.005 , and the MP-IgM levels were decreased to 152 ± 0.02 and 148 ± 0.01 , respectively, whereas the animals challenged with MP alone exhibited 59 ± 0.006 of CRP and 162 ± 0.03 of MP-IgM. Control animals exhibited 21 ± 0.003 of CRP and 141 ± 0.04 of MP-IgM levels.

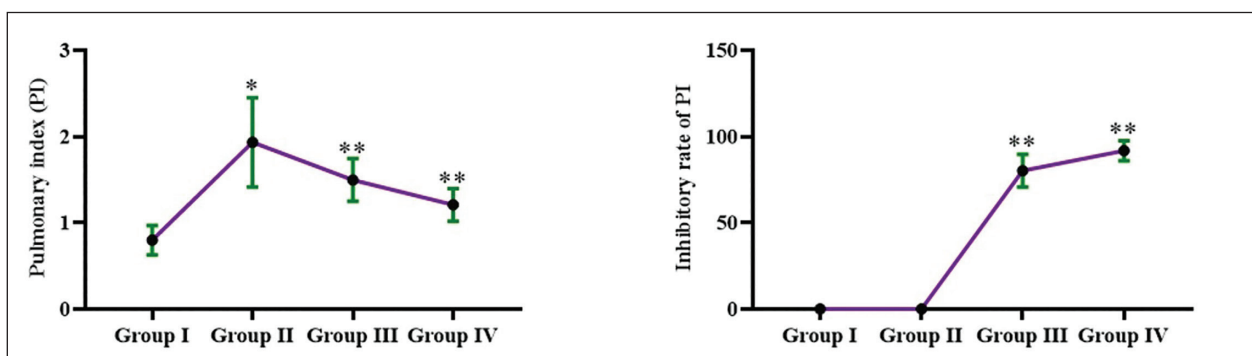


Figure 1. *Forsythia suspensa* Ethanolic Extract (FSEE) Attenuated the Pulmonary Index in *Mycoplasma pneumoniae* (MP)-challenged Animals.

Note: Pulmonary index and inhibitory rate of pulmonary index were measured in control, MP challenged, MP challenged + FSEE treated, and MP challenged + azithromycin-treated animals. One-way analysis of variance, followed by Dunnett's test, was conducted to evaluate differences between the groups. A threshold of $p < .05$ was established to determine statistical significance.

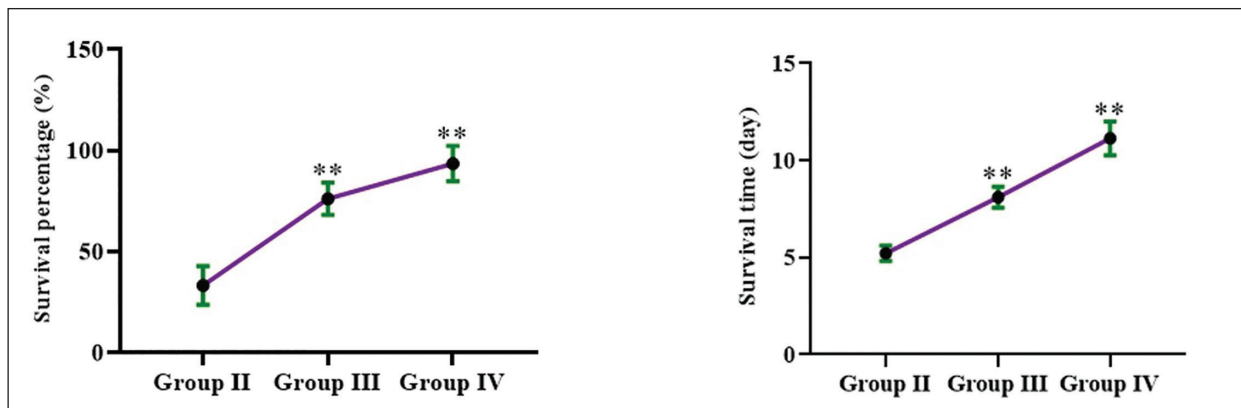


Figure 2. *Forsythia suspensa* Ethanolic Extract (FSEE) Enhanced the Survival Rate of *Mycoplasma pneumoniae* (MP)-challenged Animals.

Note: Survival percentage and survival days were measured in control, MP challenged, MP challenged + FSEE treated and MP challenged + azithromycin-treated animals. One-way analysis of variance, followed by Dunnett's test, was conducted to evaluate differences between the groups. A threshold of $p < .05$ was established to determine statistical significance.

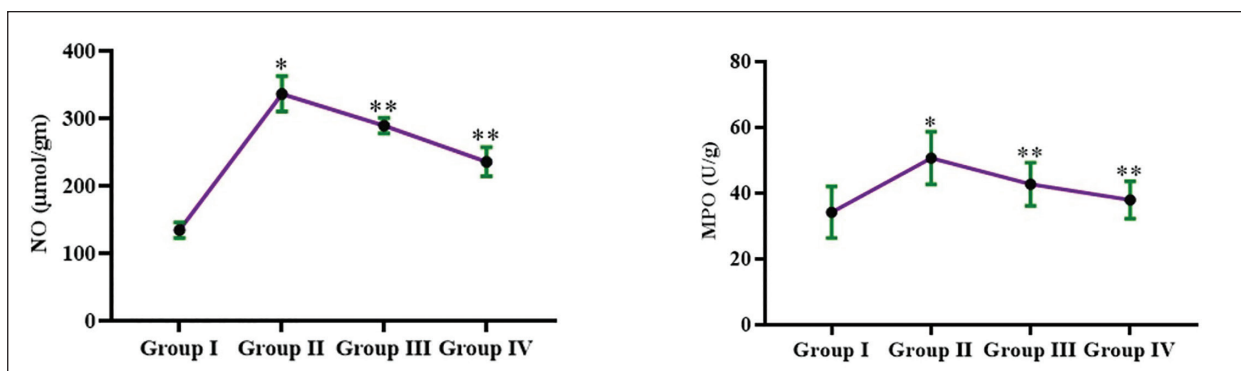


Figure 3. *Forsythia suspensa* Ethanolic Extract (FSEE) Inhibited the Uncontrolled Immune Response Triggered in *Mycoplasma pneumoniae* (MP)-challenged Animals.

Note: Nitric oxide and myeloperoxidase levels were quantified in control, MP challenged, MP challenged + FSEE treated, and MP challenged + azithromycin-treated animals. One-way analysis of variance, followed by Dunnett's test, was conducted to evaluate differences between the groups. A threshold of $p < .05$ was established to determine statistical significance.

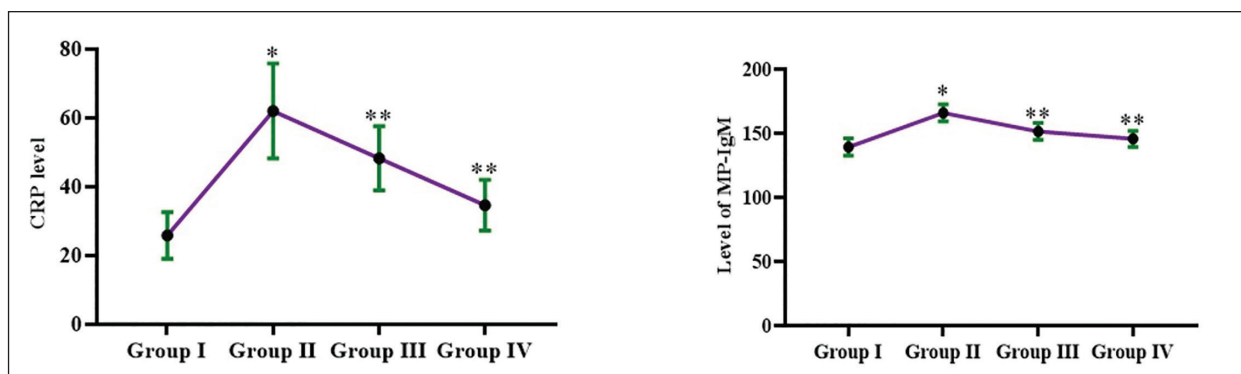


Figure 4. *Forsythia suspensa* Extract Suppressed C-reactive Protein (CRP) and *Mycoplasma pneumoniae* (MP)-immunoglobulin M (IgM) Levels in MP-challenged Animals.

Note: C-reactive protein and *Mycoplasma pneumoniae* IgM levels were quantified in control, MP challenged, MP challenged + FSEE treated, and MP challenged + azithromycin-treated animals. One-way analysis of variance, followed by Dunnett's test, was conducted to evaluate differences between the groups. A threshold of $p < .05$ was established to determine statistical significance.

FSEE Reduced the Total Cell Count in the BALF of MP-challenged Animals

Figure 5A depicts the total cell count in the BALF of MP-challenged and FSEE-treated animals. Treatment with FSEE decreased the total cell count in BALF to 37 ± 0.003 $\mu\text{g/mL}$, whereas it is 48 ± 0.005 $\mu\text{g/mL}$ in mycoplasma alone challenged animals. The control and azithromycin-treated animals exhibited 29 ± 0.003 and 34 ± 0.004 $\mu\text{g/mL}$, respectively.

FSEE Reduced MP-DNA Content in MP-challenged Animals

Figure 5B depicts the MP-DNA content in MP-challenged untreated and FSEE-treated animals. FSEE treatment decreased the MP-DNA content to 6.3 ± 0.0006 , whereas the MP challenged, untreated animals exhibited 7.8 ± 0.0006 of MP-DNA content. Treatment with the standard drug azithromycin reduced the MP-DNA content levels to 5.8 ± 0.0005 .

FSEE Attenuated Inflammation in MP-challenged Animals

Inflammatory stimulatory triggered by mycoplasma infection and the attenuating effect of FSEE in experimental animals were measured, and the results are illustrated in Figure 6. Infection with mycoplasma elevated the IL levels in experimental animals. IL-1, IL-6, and IL-8 levels were significantly enhanced with mycoplasma infection. Treatment with both FSEE and azithromycin decreased the levels of ILs in MP-challenged mice. IL-1, IL-6, and IL-8 levels were significantly decreased in FSEE and azithromycin-treated animals

compared to the mycoplasma-challenged untreated animals. TNF- α and TGF- β levels were also increased with MP infection compared to TGF- β ; the TNF- α levels were significantly increased. FSEE and azithromycin-treated animals showed significantly reduced levels of TNF- α and TGF- β compared to the MP-challenged untreated animals.

FSEE Inhibited NF- κ B Signaling in MP-challenged Animals

Figure 7 represents the ERK1/2, JNK 1/2, and NF- κ B levels in the MP challenged, untreated, and FSEE-treated animals. ERK1/2 and NF- κ B were elevated to 4.6 ± 0.00008 and 4.4 ± 0.00007 units, respectively, compared to the control animals, which exhibited 2.1 ± 0.00003 units of ERK1/2 and 2.1 ± 0.00004 units of NF- κ B. FSEE and azithromycin treatment reduced the levels of ERK1/2 to 3.4 ± 0.00004 and 2.7 ± 0.00008 units, and NF- κ B to 3.8 ± 0.00006 and 3.2 ± 0.00002 units, respectively. JNK 1/2 levels were significantly elevated with FSEE and azithromycin treatment to 0.8 ± 0.00006 , 1.3 ± 0.00002 units compared to the MP-challenged untreated animals, which showed 0.7 ± 0.00005 units. Control animals exhibited 1.4 ± 0.00003 units of JNK1/2.

Discussion

M. pneumoniae infection contributes to 10%–40% of CAP within the pediatric age group (Kutty et al., 2019). While typically manifesting as a transient condition characterized by mild respiratory manifestations, including a distinctive paroxysmal, non-productive cough with limited mucoid or mucopurulent expectoration, a subset of cases (0.5%–2.0%) progresses to severe pneumonia. This condition is associated

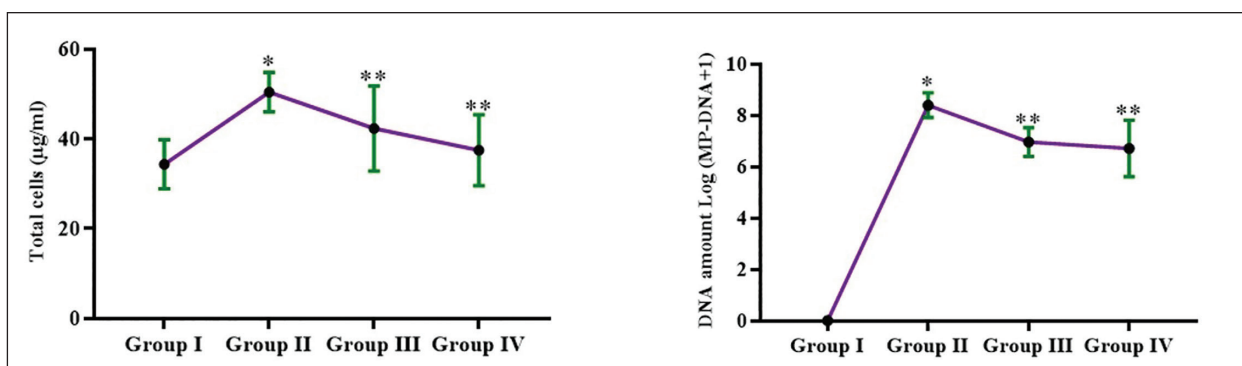


Figure 5. *Forsythia suspensa* Ethanolic Extract (FSEE) Reduced Total Cell Count and *Mycoplasma pneumoniae* (MP)-deoxyribonucleic Acid (DNA) Content of MP-challenged Animals.

Note: Total cell count in BALF and MP-DNA content were quantified in control, MP challenged, MP challenged + FSEE treated, and MP challenged + azithromycin-treated animals. One-way analysis of variance, followed by Dunnett's test, was conducted to evaluate differences between the groups. A threshold of $p < .05$ was established to determine statistical significance.

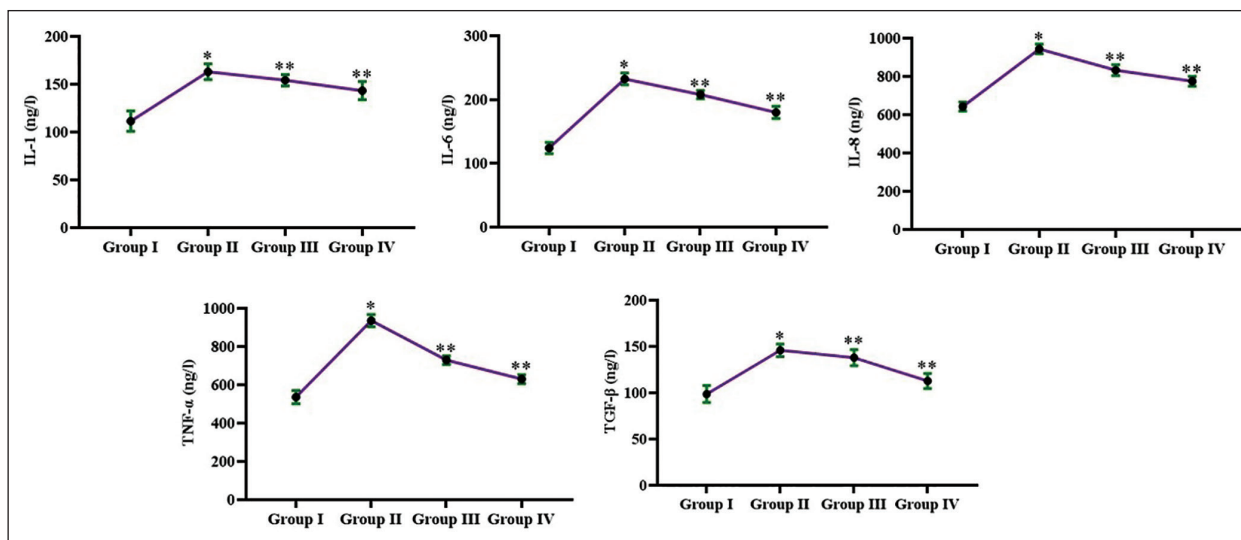


Figure 6. *Forsythia suspensa* Ethanolic Extract (FSEE) Attenuated Inflammation in *Mycoplasma pneumoniae* (MP)-challenged Animals.

Note: Inflammatory stimulants—interleukins (IL)-1, IL-6, IL-8, tumor necrosis factor- α , and transforming growth factor- β —were quantified in control, MP challenged, MP challenged + FSEE treated and MP challenged + azithromycin-treated animals. One-way analysis of variance, followed by Dunnett's test, was conducted to evaluate differences between the groups. A threshold of $p < .05$ was established to determine statistical significance.

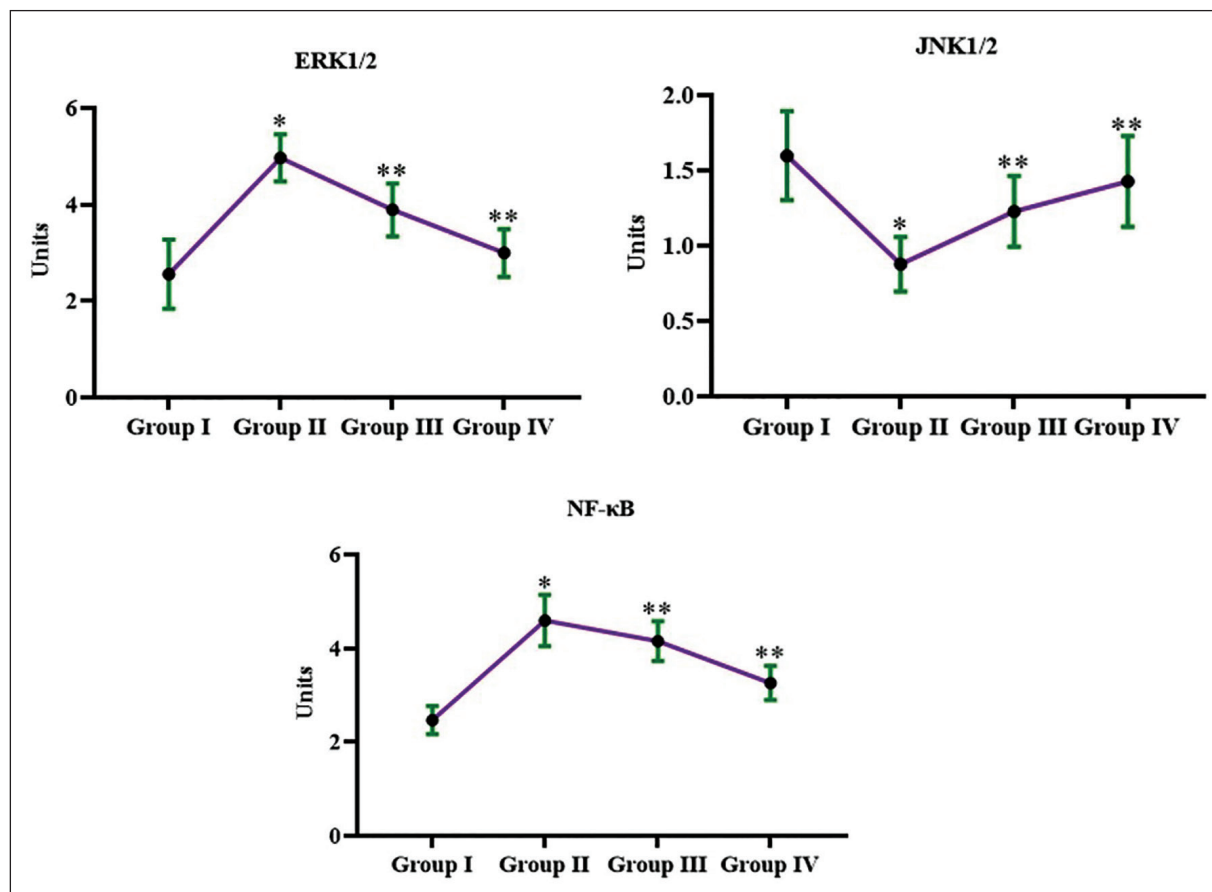


Figure 7. *Forsythia suspensa* Ethanolic Extract (FSEE) Inhibited Nuclear Factor Kappa-light-chain-enhancer of Activated B Cells (NF- κ B) Signaling in *Mycoplasma pneumoniae* (MP)-challenged Animals.

Note: Extracellular signal-regulated protein kinases 1/2, c-Jun N-terminal protein kinase 1/2, and nuclear factor kappa-light-chain-enhancer of activated B cells were quantified in control, MP challenged, MP challenged + FSEE treated, and MP challenged + azithromycin-treated animals. One-way analysis of variance, followed by Dunnett's test, was conducted to evaluate differences between the groups. A threshold of $p < .05$ was established to determine statistical significance.

with significant morbidity, including severe breathing difficulties and a related critical lung condition, posing a threat to patient survival (Izumikawa, 2016; Lee et al., 2021). The pathogenesis of *M. pneumoniae* involves complex interactions between bacterial virulence factors, host immune responses, and subsequent respiratory tissue damage (Esposito et al., 2021). The organism employs specialized adhesions and cytoadherence proteins to facilitate attachment to and invasion of respiratory epithelial cells, leading to cellular injury and disease progression (Chen et al., 2020; Guo, Gu, et al., 2024).

The absence of a cell wall contributes to *M. pneumoniae* exhibiting resistance to antibiotics that inhibit cell wall synthesis, but it remains susceptible to quinolones, tetracyclines, and macrolides (Ding et al., 2024). Macrolides are typically the initial management strategy for *M. pneumoniae* pneumonia in children (Pereyre et al., 2012). However, the emergence and increasing prevalence of macrolide-resistant *M. pneumoniae* strains limit treatment options and potentially require the use of less effective or more toxic agents, especially in the therapeutic strategies for chronic lung conditions (Jiang et al., 2024; Yin et al., 2017). This resistance, along with the complications associated with macrolide use, necessitates exploring alternative treatment strategies (Dou et al., 2020). In the present study, we elucidated the potency of *F. suspensa* extract in ameliorating *M. pneumoniae*-induced pneumonia. Over 200 phytochemicals have been reported from *F. suspensa*, and few have been proven to exhibit anti-inflammatory properties (Feng et al., 2018). Consequently, *F. suspensa* is frequently employed in the clinical practice of traditional Chinese medicine to treat diverse inflammation-related conditions (Xia et al., 2016).

FSEE administration had significantly reduced the pulmonary index and increased the inhibitory rate of pulmonary index in the MP-challenged mice. The survival percentage and survival rate were also enhanced with FSEE treatment, which signifies that FSEE had rendered a potent ameliorating effect against the *M. pneumoniae*-induced lung inflammation. Myeloperoxidase, a protein highly expressed in neutrophils and monocytes, is critical for immune surveillance and host defense (Huang et al., 2025). Stored within granules, this hemeprotein is released from activated leukocytes into extracellular and phagolysosomal spaces. Utilizing hydrogen peroxide produced during the respiratory burst, MPO generates cytotoxic oxidants and diffusible radical species (Fan et al., 2024). Recent findings indicate that inflammatory-immune cells, particularly through heme peroxidases like MPO, can catalyze the oxidation of nitric oxide into more reactive nitrogen species, thereby promoting protein nitration (Zhao et al., 2021). While nitric oxide typically functions as a signaling molecule under normal physiological conditions, its production can become excessive during pathological states such as atherosclerosis, asthma, and other inflammatory conditions (Wolak et al., 2024). Infection with *M. pneumoniae* in murine model considerably enhanced the levels of both nitric oxide and myeloperoxidase. In contrast, treatment with FSEE

suppressed the immune response triggered by pneumonia infection, as evidenced with decreased levels of nitric oxide and myeloperoxidase.

Medical concerns include the impact of *M. pneumoniae* infections on extrapulmonary systems in children. MP adheres to respiratory cilia via the P1 protein, replicates within the respiratory epithelium, and triggers the release of pro-inflammatory cytokines like IL-6 and CRP, resulting in acute cellular inflammation and airway damage (Atkinson & Waites, 2014). The most common and reliable serological method for diagnosing severe MP infection is the detection of MP-specific IgM titers. Studies indicate that MP load and MP-specific IgM titers correlate with pathological features in MP pneumonia (Poddighe, 2018). Notably, MP-specific IgM has been linked to extrapulmonary symptoms, potentially indicating heightened immune activation in affected patients (Choo et al., 2022). FSEE administration in *M. pneumoniae* challenged animals attenuated the levels of CRP and MP-IgM antibodies, proving its efficacy against inhibiting extrapulmonary infection induced by *M. pneumoniae*. The decrease in total cell counts and MP-DNA content in FSEE-treated mice further confirmed the alleviating potency of FSEE against pneumonia infection.

M. pneumoniae infection primarily affects the airway epithelium, leading to epithelial damage and the release of inflammatory cytokines. As the initial barrier against environmental pathogens, the airway epithelium relies on various signaling mechanisms to regulate immune responses (Chang et al., 2022). One key pathway involved is the activation of NF- κ B following MP infection. NF- κ B, a crucial regulator of inflammation, is usually bound to its inhibitor, I- κ B, within the cytoplasm. When MP lipoproteins interact with toll-like receptors, I- κ B undergoes phosphorylation and degradation, allowing NF- κ B to translocate into the nucleus and stimulate the pro-inflammatory genes contributing to airway inflammation (Yang et al., 2024).

Studies have shown that the NF- κ B signaling pathway is vital in regulating inflammatory responses within lung tissues (Guo, Gu, et al., 2024). Certain cytokines, including TNF- α , IL-1 β , IL-8, and IL-6, contribute to the overactivation of NF- κ B (Chen et al., 2015, 2022; Shi et al., 2017). IL-6, a major pro-inflammatory cytokine, is produced by Th2 cells, fibroblasts, and macrophages and is known to amplify inflammatory responses. Elevated IL-6 levels have been observed in children with CAP (Odeh & Simecka, 2016). TNF- α and IL-1 β are primarily released during the initial phase of inflammation, where they increase vascular endothelial permeability and stimulate the production and release of additional cytokines (Jiang et al., 2016). Additionally, IL-8 is primarily produced by the airway epithelium and is associated with several lung diseases due to its elevated levels in inflammatory conditions (Russell et al., 2024). Treatment with FSEE in *M. pneumoniae* challenged animals exhibited significantly decreased levels of NF- κ B, IL-1, IL-6, IL-8, and TNF- α . FSEE treatment inhibited NF- κ B-dependent lung inflammation in *M. pneumoniae* challenged animals.

The sustained NF- κ B activation in bronchial epithelial cells, mediated by ERK1/2 activity, has been linked with chronic pulmonary inflammation. This phenomenon is observed in patients with a homozygous Z mutation in alpha1-anti-trypsin, a genetic condition that leads to early-onset emphysema (van't Wout et al., 2014). In individuals with respiratory diseases like pneumonia, increased levels of TGF- β 1 were detected in bronchial tissue samples (Wang et al., 2025). In our study, *M. pneumoniae* infection increased the NF- κ B activation, which may be mediated due to ERK1/2, which is significantly increased with pneumonia infection. It also increased the levels of TGF- β 1, which is reported to be increased in the bronchial samples of asthmatic patients. FSEE treatment attenuated the ERK1/2, TGF- β 1, and increased the JNK1/2 levels in the mycoplasma challenged animals, which eventually prevented pulmonary inflammation in *M. pneumoniae*-infected animals.

Conclusion

In summary, FSEE exhibits significant therapeutic potential against *M. pneumoniae*-induced pneumonia by improving survival rates and mitigating lung inflammation. Its effectiveness is attributed to its ability to regulate immune responses, lower pro-inflammatory cytokine levels, and reduce bacterial load. By downregulating NF- κ B, enhancing JNK1/2 expression, and suppressing excessive immune activation, FSEE emerges as a promising candidate for treating *M. pneumoniae*-associated pneumonia. Further in-depth molecular studies are necessary to fully understand the therapeutic potential of FSEE against *M. pneumoniae*-induced pneumonia.

Abbreviations

ANOVA: Analysis of variance; BALF: Bronchoalveolar lavage fluid; BW: Body weight; CCU: Color-change units; CO₂: Carbon dioxide; CRP: C-reactive protein; DNA: Deoxyribonucleic acid; ELISA: Enzyme-linked immunosorbent assay; ERK1/2: Extracellular signal-regulated protein kinases 1/2; FSEE: *Forsythia suspensa* ethanolic extract; IgM: Immunoglobulin M; IL: Interleukin; IRPI: Inhibitory rate of pulmonary indices; JNK1/2: c-Jun N-terminal protein kinase 1/2; LW: Lung weight; MP: *Mycoplasma pneumoniae*; MPO: Myeloperoxidase; NF- κ B: Nuclear factor kappa-light-chain-enhancer of activated B cells; NO: Nitric oxide; PI: Pulmonary index; PPLO: Pleuropneumonia-like organisms; SD: Standard deviation; TCM: Traditional Chinese medicine; TGF- β : Transforming growth factor-beta; TNF- α : Tumor necrosis factor-alpha; USA: United States of America.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval and Informed Consent

This work was approved by the Institutional Ethical Committee Xi'an Children's Hospital, Xi'an, Shaanxi 710000, China (Ethical committee number: 202210).

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References

- Almirall, J., Serra-Prat, M., Bolibar, I., & Balasso, V. (2017, July 25). Risk factors for community-acquired pneumonia in adults: A systematic review of observational studies. *Respiration; International Review of Thoracic Diseases*, 94(3), 299–311. <https://doi.org/10.1159/000479089>
- Alshahwan, S. I., Alsowailmi, G., Alsahli, A., Alotaibi, A., Alshaikh, M., Almajed, M., Omair, A., & Almodaimegh, H. (2019, January–February). The prevalence of complications of pneumonia among adults admitted to a tertiary care center in Riyadh from 2010–2017. *Annals of Saudi Medicine*, 39(1), 29–36. <https://doi.org/10.5144/0256-4947.2019.29>
- Atkinson, T. P., & Waites, K. B. (2014). *Mycoplasma pneumoniae* infections in childhood. *The Pediatric Infectious Disease Journal*, 33(1), 92–94. <https://doi.org/10.1097/INF.0000000000000171>
- Bai, F., & Li, X. (2021, June). Comparing several treatments with antibiotics for community-acquired pneumonia: A systematic review and meta-analysis of randomized controlled trials. *Iranian Journal of Public Health*, 50(6), 1108–1119. <https://doi.org/10.18502/ijph.v50i6.6410>
- Chang, Q., Chen, H.-L., Wu, N.-S., Gao, Y.-M., Yu, R., & Zhu, W.-M. (2022, June 6). Prediction model for severe *Mycoplasma pneumoniae* pneumonia in pediatric patients by admission laboratory indicators. *Journal of Tropical Pediatrics*, 68(4), Article fmac059. <https://doi.org/10.1093/tropej/fmac059>
- Chaudhry, R., Ghosh, A., & Chandolia, A. (2016, January–March). Pathogenesis of *Mycoplasma pneumoniae*: An update. *Indian Journal of Medical Microbiology*, 34(1), 7–16. <https://doi.org/10.4103/0255-0857.174112>
- Chen, L., Han, X., Li, Y., Zhang, C., & Xing, X. (2021). The severity and risk factors for mortality in immunocompromised adult patients hospitalized with influenza-related pneumonia. *Annals of Clinical Microbiology and Antimicrobials*, 20(1), 55. <https://doi.org/10.1186/s12941-021-00462-7>
- Chen, L., Yan, X., Yan, Q., Fan, J., Huang, H., Shi, X., Han, L., Han, T., & Zhu, H. (2015). The modified Jiu Wei Qiang Huo decoction alleviated severe lung injury induced by H1N1 influenza virus by regulating the NF- κ B pathway in mice. *Evidence-based Complementary and Alternative Medicine: eCAM*, 2015(1), Article 790739. <https://doi.org/10.1155/2015/790739>
- Chen, Y.-C., Hsu, W.-Y., & Chang, T.-H. (2020, July). Macrolide-resistant *Mycoplasma pneumoniae* infections in pediatric community-acquired pneumonia. *Emerging Infectious Diseases*, 26(7), 1382–1391. <https://doi.org/10.3201/eid2607.200017>
- Chen, Y., Dong, S., Tian, L., Chen, H., Chen, J., & He, C. (2022, July 19). Combination of azithromycin and methylprednisolone alleviates *Mycoplasma pneumoniae* induced pneumonia by regulating miR-499a-5p/STAT3 axis. *Experimental and Therapeutic Medicine*, 24(3), 578. <https://doi.org/10.3892/etm.2022.11515>

- Chinese Experts Cystic Fibrosis Consensus Committee, Chinese Alliance for Rare Lung Diseases, & Chinese Alliance for Rare Diseases, Bronchiectasis-China. (2023, April 12). Chinese experts consensus statement: Diagnosis and treatment of cystic fibrosis (2023). *Zhonghua Jie He He Hu Xi Za Zhi*, 46(4), 352–372. <https://doi.org/10.3760/cma.j.cn112147-20221214-00971>
- Choo, S., Kim, S.-H., & Lee, E. (2022, May 16). Clinical significance of *Mycoplasma pneumoniae* specific IgM titer in children hospitalized with *Mycoplasma pneumoniae* pneumonia. *BMC Infectious Diseases*, 22(1), 470. <https://doi.org/10.1186/s12879-022-07456-6>
- Ding, G., Zhang, X., Vinturache, A., van Rossum, A. M. C., Yin, Y., Zhang, Y. (2024, July). Challenges in the treatment of pediatric *Mycoplasma pneumoniae* pneumonia. *European Journal of Pediatrics*, 183(7), 3001–3011. <https://doi.org/10.1007/s00431-024-05519-1>
- Dou, H. W., Tian, X. J., Xin, L., Wei, R., Zhou, W., Wang, H., Qin, X. G., Shao, J. Y., Xu, B. P., Ge, L. X., Shi, D. W. (2020, December 20). *Mycoplasma pneumoniae* macrolide resistance and MLVA typing in children in Beijing, China, in 2016: Is it relevant? *Biomedical and Environmental Sciences*, 33(12), 916–924. <https://doi.org/10.3967/bes2020.125>
- Esposito, S., Argentiero, A., Gramegna, A., & Principi, N. (2021, June 13). *Mycoplasma pneumoniae*: A pathogen with unsolved therapeutic problems. *Expert Opinion on Pharmacotherapy*, 22(9), 1193–1202. <https://doi.org/10.1080/14656566.2021.1882420>
- Fan, L., Xu, N., Guo, Y., & Li, L. (2024, October 4). Enhanced insights into the neutrophil-driven immune mechanisms during *Mycoplasma pneumoniae* infection. *Heliyon*, 10(21), Article e38950. <https://doi.org/10.1016/j.heliyon.2024.e38950>
- Feng, Z., Gao, X., Han, Y., Wang, F., Zhou, S., & Jiang, Y. (2018). Study progress of *Forsythia suspensa*. *Modern Agricultural Science and Technology*, 12, 60–62.
- Ferreira-Coimbra, J., Sarda, C., & Rello, J. (2020, April). Burden of community-acquired pneumonia and unmet clinical needs. *Advances in Therapy*, 37(4), 1302–1318. <https://doi.org/10.1007/s12325-020-01248-7>
- Guo, Q., Jin, Y., Chen, X., Ye, X., Shen, X., Lin, M., Zeng, C., Zhou, T., & Zhang, J. (2024, March 4). NF- κ B in biology and targeted therapy: New insights and translational implications. *Signal Transduction and Targeted Therapy*, 9(1), 53. <https://doi.org/10.1038/s41392-024-01757-9>
- Guo, Z. Q., Gu, S. Y., Tian, Z. H., & Du, B. Y. (2024, September 25). A comprehensive review of *Mycoplasma pneumoniae* infection in chronic lung diseases: Recent advances in understanding asthma, COPD, and bronchiectasis. *Frontiers in Medicine*, 11, Article 1437731. <https://doi.org/10.3389/fmed.2024.1437731>
- Han, Z., Guo, J., Meng, F., Liao, H., Deng, Y., Huang, Y., Lei, X., Liang, C., Han, R., & Yang, W. (2021, June 18). Genetic toxicology and safety pharmacological evaluation of forsythin. *Evidence-based Complementary and Alternative Medicine: eCAM*, 2021, Article 6610793. <https://doi.org/10.1155/2021/6610793>
- Hanifah, W., Andriani, Y., Zin, N. A. M., Ryandini, D., Abdul Majid, F. A., Subhan, E., & Muhammad, T. S. T. (2023). Selected coastal plants as potential treatment for pneumonia disease: Determination of their phytochemicals and antibacterial activity against some pneumonia bacteria. *Oriental Journal of Chemistry*, 39(6), 1479–1494. <https://doi.org/10.13005/ojc/390608>
- He, J., Liu, M., Ye, Z., Tan, T., Liu, X., You, X., Zeng, Y., & Wu, Y. (2016, November). Insights into the pathogenesis of *Mycoplasma pneumoniae*. *Molecular Medicine Reports*, 14(5), 4030–4036. <https://doi.org/10.3892/mmr.2016.5765>
- Hu, J., Ye, Y., Chen, X., Xiong, L., Xie, W., & Liu, P. (2022, December 2). Insight into the pathogenic mechanism of *Mycoplasma pneumoniae*. *Current Microbiology*, 80(1), 14. <https://doi.org/10.1007/s00284-022-03103-0>
- Huang, X., Qin, H., Zhang, R., Jia, X., Zhao, D., & Liu, F. (2025, March 8). Neutrophils are involved in the development and outcomes of plastic bronchitis associated with *Mycoplasma pneumoniae* pneumonia. *Respiratory Research*, 26(1), 92. <https://doi.org/10.1186/s12931-025-03167-z>
- Izumikawa, K. (2016, June 1). Clinical features of severe or fatal *Mycoplasma pneumoniae* pneumonia. *Frontiers in Microbiology*, 7, 800. <https://doi.org/10.3389/fmicb.2016.00800>
- Jiang, Y. H., Yu, J. E., Guo, A. H., Li, X., Lin, Y., Jiang, Z. Y., & Xiao, Z. (2016, April). Ameliorative effects of Qingfei Tongluo formula on experimental mycoplasmal pneumonia in mice. *Journal of Natural Medicines*, 70(2), 145–151. <https://doi.org/10.1007/s11418-015-0944-2>
- Jiang, C., Bao, S., Shen, W., & Wang, C. (2024, September 30). Predictive value of immune-related parameters in severe *Mycoplasma pneumoniae* pneumonia in children. *Translational Pediatrics*, 13(9), 1521–1528. <https://doi.org/10.21037/tp-24-172>
- Kim, K., Jung, S., Kim, M., Park, S., Yang, H.-J., & Lee, E. (2022, July 1). Global trends in the proportion of macrolide-resistant *Mycoplasma pneumoniae* infections: A systematic review and meta-analysis. *JAMA Network Open*, 5(7), Article e2220949. <https://doi.org/10.1001/jamanetworkopen.2022.20949>
- Kutty, P. K., Jain, S., Taylor, T. H., Bramley, A. M., Diaz, M. H., Ampofo, K., Arnold, S. R., Williams, D. J., Edwards, K. M., McCullers, J. A., Pavia, A. T., Winchell, J. M., Schrag, S. J., & Hicks, L. A. (2019, January 1). *Mycoplasma pneumoniae* among children hospitalized with community-acquired pneumonia. *Clinical Infectious Diseases*, 68(1), 5–12. <https://doi.org/10.1093/cid/ciy419>
- Lee, K.-L., Lee, C.-M., Yang, T.-L., Yen, T.-Y., Chang, L.-Y., Chen, J.-M., Lee, P.-I., Huang, L.-M., & Lu, C.-Y. (2021, January). Severe *Mycoplasma pneumoniae* pneumonia requiring intensive care in children, 2010–2019. *Journal of the Formosan Medical Association*, 120(1 Pt. 1), 281–291. <https://doi.org/10.1016/j.jfma.2020.08.018>
- Lenz, K. D., Klosterman, K. E., Mukundan, H., & Kubicek-Sutherland, J. Z. (2021, May 12). Macrolides: From toxins to therapeutics. *Toxins*, 13(5), 347. <https://doi.org/10.3390/toxins13050347>
- Lessa, F. C., Mu, Y., Bamberg, W. M., Beldavs, Z. G., Dumyati, G. K., Dunn, J. R., Farley, M. M., Holzbauer, S. M., Meek, J. I., Phipps, E. C., Wilson, L. E., Winston, L. G., Cohen, J. A., Limbago, B. M., Fridkin, S. K., Gerding, D. N., & McDonald, L. C. (2015, February 26). Burden of *Clostridium difficile* infection in the United States. *The New England Journal of Medicine*, 372(9), 825–834. <https://doi.org/10.1056/NEJMoa1408913>
- Li, H., Li, S., Yang, H., Chen, Z., & Zhou, Z. (2024, June). Resurgence of *Mycoplasma pneumoniae* by macrolide-resistant epidemic clones in China. *The Lancet. Microbe*, 5(6), e515. [https://doi.org/10.1016/S2666-5247\(23\)00405-6](https://doi.org/10.1016/S2666-5247(23)00405-6)
- Li, H.-B., & Chen, F. (2005, August 12). Preparative isolation and purification of phillyrin from the medicinal plant *Forsythia suspensa* by high-speed counter-current chromatography.

- Journal of Chromatography A*, 1083(1–2), 102–105. <https://doi.org/10.1016/j.chroma.2005.06.025>
- Meyer Sauter, P. M., Jacobs, B. C., Spuesens, E. B. M., Jacobs, E., Nadal, D., Vink, C., & van Rossum, A. M. C. (2014, June 12). Antibody responses to *Mycoplasma pneumoniae*: Role in pathogenesis and diagnosis of encephalitis? *PLoS Pathogens*, 10(6), Article e1003983. <https://doi.org/10.1371/journal.ppat.1003983>
- Miyashita, N. (2022, January). Atypical pneumonia: Pathophysiology, diagnosis, and treatment. *Respiratory Investigation*, 60(1), 56–67. <https://doi.org/10.1016/j.resinv.2021.09.009>
- Morley, D., Torres, A., Cillóniz, C., & Martin-Loeches, I. (2017). Predictors of treatment failure and clinical stability in patients with community acquired pneumonia. *Annals of Translational Medicine*, 5(22), 443. <https://doi.org/10.21037/atm.2017.06.54>
- Odeh, A. N., & Simecka, J. W. (2016, May 13). Regulatory CD4+ CD25+ T cells dampen inflammatory disease in murine mycoplasma pneumonia and promote IL-17 and IFN- γ responses. *PLoS One*, 11(5), Article e0155648. <https://doi.org/10.1371/journal.pone.0155648>
- Patel, M., & Sharma, B. (2021). Recent advances of the medicinal plant compound for combating pneumonia disease: A review. *Journal of Medical Research and Surgery*, 2(6), 1–5. <https://doi.org/10.52916/jmrs214063>
- Pereyre, S., Charron, A., Hidalgo-Grass, C., Touati, A., Moses, A. E., Nir-Paz, R., & Bébéar, C. (2012, June 6). The spread of *Mycoplasma pneumoniae* is polyclonal in both an endemic setting in France and in an epidemic setting in Israel. *PLoS One*, 7(6), Article e38585. <https://doi.org/10.1371/journal.pone.0038585>
- Poddighe, D. (2018). Extra-pulmonary diseases related to *Mycoplasma pneumoniae* in children: Recent insights into the pathogenesis. *Current Opinion in Rheumatology*, 30(4), 380–387. <https://doi.org/10.1097/BOR.0000000000000494>
- Regunath, H., & Oba, Y. (2024, January 26). *Community-acquired pneumonia*. StatPearls (p. 2025). StatPearls Publishing. <https://www.ncbi.nlm.nih.gov/books/NBK430749/>
- Rueda, Z. V., Aguilar, Y., Maya, M. A., López, L., Restrepo, A., Garcés, C., Morales, O., Roya-Pabón, C., Trujillo, M., Arango, C., Copete, Á. R., Vera, C., Giraldo, M. R., Herrera, M., & Vélez, L. A. (2022, March 31). Etiology and the challenge of diagnostic testing of community-acquired pneumonia in children and adolescents. *BMC Pediatrics*, 22(1), 169. <https://doi.org/10.1186/s12887-022-03235-z>
- Russell, R. J., Boulet, L.-P., Brightling, C. E., Pavord, I. D., Porsbjerg, C., Dorscheid, D., & Sverrild, A. (2024, April 4). The airway epithelium: An orchestrator of inflammation, a key structural barrier and a therapeutic target in severe asthma. *The European Respiratory Journal*, 63(4), Article 2301397. <https://doi.org/10.1183/13993003.01397-2023>
- Shi, S., Liu, X., & Li, H. (2017). Downregulation of caspase 3 alleviates *Mycoplasma pneumoniae* induced apoptosis in alveolar epithelial cells. *Molecular Medicine Reports*, 16(6), 9601–9606. <https://doi.org/10.3892/mmr.2017.7782>
- Singh, P., Shukla, R., Kumar, A., Prakash, B., Singh, S., & Dubey, N. K. (2010, September). Effect of *Citrus reticulata* and *Cymbopogon citratus* essential oils on *Aspergillus flavus* growth and aflatoxin production on *Asparagus racemosus*. *Mycopathologia*, 170(3), 195–202. <https://doi.org/10.1007/s11046-010-9311-8>
- Upadhyay, P., & Singh, V. (2024, April 22). *Mycoplasma pneumoniae* outbreak in 2023: Post-pandemic resurgence of an atypical bacterial pathogen. *Cureus*, 16(4), Article e58757. <https://doi.org/10.7759/cureus.58757>
- van 't Wout, E. F. A., Dickens, J. A., van Schadewijk, A., Haq, I., Kwok, H. F., Ordóñez, A., Murphy, G., Stolk, J., Lomas, D. A., Hiemstra, P. S., & Marciniak, S. J. (2014, February 15). Increased ERK signalling promotes inflammatory signalling in primary airway epithelial cells expressing Z α 1-antitrypsin. *Human Molecular Genetics*, 23(4), 929–941. <https://doi.org/10.1093/hmg/ddt487>
- Wahl, B., Knoll, M. D., Shet, A., Gupta, M., Kumar, R., Liu, L., Chu, Y., Sauer, M., O'Brien, K. L., Santosham, M., Black, R. E., Campbell, H., Nair, H., & McAllister, D. A. (2020). National, regional and state level pneumonia and severe pneumonia morbidity in children in India: Modelled estimates for 2000 and 2015. *The Lancet. Child and Adolescent Health*, 4(9), 678–687. [https://doi.org/10.1016/S2352-4642\(20\)30129-2](https://doi.org/10.1016/S2352-4642(20)30129-2)
- Waites, K. B., Xiao, L., Liu, Y., Balish, M. F., Atkinson, T. P. (2017, July). *Mycoplasma pneumoniae* from the respiratory tract and beyond. *Clinical Microbiology Reviews*, 30(3), 747–809. <https://doi.org/10.1128/CMR.00114-16>
- Wang, Z., Xia, Q., Liu, X., Liu, W., Huang, W., Mei, X., Luo, J., Shan, M., Lin, R., Zou, D., & Ma, Z. (2018). Phytochemistry, pharmacology, quality control and future research of *Forsythia suspensa* (Thunb.) Vahl: A review. *Journal of Ethnopharmacology*, 210, 318–339. <https://doi.org/10.1016/j.jep.2017.08.040>
- Wang, Y.-S., Zhou, Y.-L., Bai, G.-N., Li, S.-X., Xu, D., Chen, L.-N., Chen, X., Dong, X.-Y., Fu, H.-M., Fu, Z., Hao, C.-L., Hong, J.-G., Liu, E.-M., Liu, H.-M., Lu, X.-X., Luo, Z.-X., Tang, L.-F., Tian, M., Yin, Y., ... Chen, Z.-M. (2024, September). Expert consensus on the diagnosis and treatment of macrolide-resistant *Mycoplasma pneumoniae* pneumonia in children. *World Journal of Pediatrics*, 20(9), 901–914. <https://doi.org/10.1007/s12519-024-00831-0>
- Wang, P., Yao, J., Li, Y., Zhang, Z., Zhang, R., Lu, S., Sun, M., & Huang, X. (2025). Immune responses in children with secondary infection of *Mycoplasma pneumoniae* after COVID-19: Focus on eosinophils and IgE. *BMC Infectious Diseases*, 25(1), 134. <https://doi.org/10.1186/s12879-025-10534-0>
- Wolak, T., Dicker, D., Shifer, Y., Grossman, A., Rokach, A., Shitrit, M., & Tal, A. (2024, July 26). A safety evaluation of intermittent high-dose inhaled nitric oxide in viral pneumonia due to COVID-19: A randomised clinical study. *Scientific Reports*, 14(1), Article 17201. <https://doi.org/10.1038/s41598-024-68055-w>
- Wood, P. R., Kampschmidt, J. C., Dube, P. H., Cagle, M. P., Chaparro, P., Ketchum, N. S., Kannan, T. R., Singh, H., Peters, J. I., Baseman, J. B., & Brooks, E. G. (2017, August 1). *Mycoplasma pneumoniae* and health outcomes in children with asthma. *Annals of Allergy, Asthma and Immunology*, 119(2), 146.e2–152.e2. <https://doi.org/10.1016/j.anaai.2017.05.022>
- World Health Organization. (2023). *Catalysing ancient wisdom and modern science for the health of people and the planet*. <https://www.who.int/initiatives/who-global-centre-for-traditional-medicine>
- Xia, W., Dong, C. M., Yang, C. F., & Chen, H. (2016). Advances in chemical constituents and pharmacology of *Forsythia suspensa*. *Modern Chinese Medicine*, 18, 1670–1674.
- Yang, Z., Zhou, J., Su, N., Zhang, Z., Chen, J., Liu, P., & Ling, P. (2024, March 22). Insights into the defensive roles of lncRNAs during *Mycoplasma pneumoniae* infection. *Frontiers in Microbiology*, 15, Article 1330660. <https://doi.org/10.3389/fmicb.2024.1330660>

- Yin, Y.-D., Wang, R., Zhuo, C., Wang, H., Wang, M.-G., Xie, C.-M., She, D.-Y., Yuan, X., Wang, R.-T., Cao, B., & Liu, Y.-N. (2017, October). Macrolide-resistant *Mycoplasma pneumoniae* prevalence and clinical aspects in adult patients with community-acquired pneumonia in China: A prospective multicenter surveillance study. *Journal of Thoracic Disease*, 9(10), 3774–3781. <https://doi.org/10.21037/jtd.2017.09.75>
- Yoon, I. A., Hong, K. B., Lee, H. J., Yun, K. W., Park, J. Y., Choi, Y. H., Kim, W. S., Lee, H., Eun, B. W., Ahn, Y. M., Cho, E. Y., Cho, H. J., & Choi, E. H. (2017, June 7). Radiologic findings as a determinant and no effect of macrolide resistance on clinical course of *Mycoplasma pneumoniae* pneumonia. *BMC Infectious Diseases*, 17(1), 402. <https://doi.org/10.1186/s12879-017-2500-z>
- Zhao, Q., Zhang, T., Zhu, B., Bi, Y., Jiang, S.-W., Zhu, Y., Zhao, D., & Liu, F. (2021). Increasing age affected polymorphonuclear neutrophils in prognosis of *Mycoplasma pneumoniae* pneumonia. *Journal of Inflammation Research*, 14, 3933–3943. <https://doi.org/10.2147/JIR.S321656>