Ind. J. Pharm. Edu. Res., 2025; 59(3):1100-1108.
https://www.ijper.org

Original Article

Monotropein Attenuates Nuclear Factor Kappa B Activity there by Ameliorates *Mycoplasma pneumoniae* Triggered Pneumonia in Rodent Model

Jiaying Fan[1], Lin Zhao[2,*]

[1]Xi'an TCM Hospital of Encephalopathy, Xi'an, CHINA.
[2]Department of Integrated TCM and Western Medicine, Xi'an TUS-Children's Hospital, Xi'an, CHINA.

ABSTRACT

Background: Pneumonia is a prevalent acute respiratory illness that impacts the alveoli and the distal airways and if left untreated, it presents a significant health challenge. It is linked to elevated rates of illness and death across all age groups worldwide. The treatment of pneumonia has become increasingly difficult due to antibiotic resistance, which complicates management strategies and leads to poorer patient outcomes. The rise of multi-drug resistant pathogens requires the use of stronger and often more toxic antibiotics, heightening the risk of adverse effects and further health complications. **Materials and Methods:** In this research, our objective was to investigate the efficacy of the phytochemical monotropein in mitigating bacterial pneumonia triggered by *Mycoplasma pneumoniae* in a rodent model. Swiss albino mice with pneumonia were administered monotropein and their antimicrobial and anti-inflammatory responses were assessed. The pathogen clearance capability of monotropein was evaluated by measuring lung weight index, nitric oxide and myeloperoxidase levels. Moreover, we quantified antioxidant levels to determine monotropein's ability to scavenge oxidative stress in the context of pneumonia infection. To assess the anti-inflammatory effects of monotropein, we examined the levels of inflammatory cytokines, corroborated by total cell counts in Bronchoalveolar Lavage Fluid (BALF) and lung tissue DNA content. Additionally, NF-kB levels were quantified to assess monotropein's capacity to enhance the host defense mechanisms against pneumonia. Histopathological analyses of the lung tissue were performed to validate the beneficial effects of monotropein on *Mycoplasma pneumoniae*-induced pneumonia. **Results:** The findings of our study demonstrate that monotropein treatment markedly decreased levels of nitric oxide and myeloperoxidase, effectively preventing pulmonary edema in mice exposed to *Mycoplasma pneumoniae*. Furthermore, it inhibited oxidative damage and DNA harm in the pneumonic mice. The treatment also led to a considerable reduction in inflammatory cytokines and NF-kB levels, supporting the anti-inflammatory properties of monotropein against pneumonia. Histopathological evaluations confirmed the beneficial effects of monotropein in the pneumonia-induced rodent model. **Conclusion:** Overall, our findings indicate that monotropein is a powerful antioxidant and anti-inflammatory substance that effectively mitigates pneumonia infection. These promising results suggest that monotropein warrants further investigation for potential development as a compound for the management of pneumonia.

Keywords: Bacterial Pneumonia, Inflammation, Monotropein, *Mycoplasma pneumoniae*, NFkB, Phytochemical Drug.

Correspondence:
Dr. Lin Zhao
Department of Integrated TCM and Western Medicine, Xi'an TUS-Children's Hospital, Xi'an-710000, CHINA.
Email: zd85932411@sina.com

Received: 25-10-2024;
Revised: 06-12-2024;
Accepted: 18-03-2025.

INTRODUCTION

Pneumonia remains a major global health concern, contributing to considerable morbidity and mortality, particularly among vulnerable populations such as children and the elderly.[1] The two primary forms of pneumonia are community-acquired pneumonia and hospital-acquired pneumonia. Community-acquired pneumonia is prevalent in the general population and leads to substantial rates of morbidity and mortality.[2] According to a study published in The Lancet, pneumonia is one of the primary contributors of death worldwide, with an expected 2.5 million fatalities each year, highlighting its status as a major public health challenge.[3] According to the World Health Organization, pneumonia is responsible for 15% of all deaths among children under the age of five, underscoring the urgent need for preventative and therapeutic interventions.[4] Factors such as

DOI: 10.5530/ijper.20251173

Copyright Information :
Copyright Author (s) 2025 Distributed under Creative Commons CC-BY 4.0

Publishing Partner : Manuscript Technomedia. [www.mstechnomedia.com]

antibiotic resistance, the emergence of new pathogens and the lack of access to healthcare exacerbate the situation.[5]

Pneumonia is an infection of the alveoli that arises when the body's defense system fails to eliminate infectious agent from the lower airways and alveolar spaces.[6] In response, leukocytes produce cytokines and local inflammatory mediators, which can further damage lung tissue. This localized inflammation triggers a systemic inflammatory response, resulting in common clinical features such as fever, chills and fatigue. As white blood cells accumulate and fluid builds up, pus forms within the lung parenchyma, leading to decreased alveolar compliance.[7] Consequently, these pathological changes increase the effort required for breathing, exacerbating conditions like hypoxemia and tachypnea.[8] Pneumonia can impact individuals of all ages and health statuses. Patients with coexisting conditions that impair mucociliary clearance and the cough reflex are more prone for developing Community-Acquired Pneumonia (CAP). Additionally, lifestyle factors such as smoking heighten this risk, as do certain medical conditions that predispose individuals to aspiration, including esophageal disorders, alcoholism and neuromuscular diseases.[9,10]

The treatment of bacterial pneumonia primarily relies on antibiotics, which are chosen based on the identified pathogen and local antibiotic resistance patterns. Commonly prescribed antibiotics include beta-lactams, macrolides and fluoroquinolones. Appropriate antibiotic therapy can significantly reduce morbidity and mortality associated with pneumonia.[11] However, these medications can also lead to various side effects. Gastrointestinal issues, including nausea, diarrhea and abdominal discomfort, are frequently reported due to alterations in the gut microbiome.[12] Allergic reactions may occur, ranging from mild rashes to severe anaphylactic responses, tendonitis and tendon rupture, as well as neuropsychiatric symptoms including confusion and hallucinations.[13] Therefore, careful selection of antibiotics and monitoring for potential side effects are crucial to optimize treatment outcomes for patients with bacterial pneumonia.

Phytochemicals have garnered attention for their possible in function in therapy of pneumonia due to their diverse pharmacological properties, including anti-inflammatory, antimicrobial and immunomodulatory effects.[14] Monotropein, a prominent iridoid glycoside isolated from Morinda officinalis, is recognized for its significant therapeutic potential.[15] Monotropein is present in Morinda officinalis and other edible plants, such as Pyrola decorate and several species of Vaccinium.[16] Research indicates that monotropein exhibits diverse pharmacological effects, including antioxidant, anti-osteoporosis, anti-inflammatory, anti-arthritic and anti-apoptotic activities,[17] underscoring its potential for various therapeutic applications. Hence, we aimed to explore the ameliorative potency of monotropein against bacterial pneumonia.

MATERIALS AND METHODS

Animals and Grouping

Male Swiss albino mice weighing above 27±5 g of body weight was kept at 21°C with 55% humidity, maintained on a 12 hr light/dark cycle and had unrestricted access to food and water. The animals were assigned into four groups each consists of six animals. Group I consisted of normal control mice. Group II included mice that were administered a 100 µL culture of *M. pneumoniae* via nasal drops for 2 days to induce pneumonia. Group III consisted of mice injected with *M. pneumoniae* and then treated with 25 mg/kg of Monotropein for three days. Group IV comprised pneumonia-induced mice that received 100 mg/kg of the standard drug azithromycin for three days.

At the conclusion of the treatment period, blood samples were obtained via cardiac puncture using EDTA-containing plasma separation tubes (BD Biosciences) following anesthesia with 40 mg/kg of sodium pentobarbital. The mice were then euthanized with an intraperitoneal injection of 120 mg/kg sodium pentobarbital and immediately necropsied. Bronchoalveolar Lavage Fluid (BALF) and lung tissues were collected from the mice and stored at -80°C for subsequent experiments.

Assessment of Lung Weight Index

The animals were weighed using electronic balance and then subjected to euthanization. The lung tissue was then carefully excised by gently detaching the lungs from the surrounding organs. Excess tissue such as the trachea and major blood vessels was removed, as these could impact the lung weight. The presence of all the lobes was confirmed before measuring the weight. The lung tissue was then placed on the electronic balance, which was set to 0 and the weight was measured. The lung weight index was calculated using the formula

$$\text{Lung weight index} = \text{Lung weight (mg)} / \text{Total body weight (mg)} \times 100$$

Assessment of immune response

The immune response induced by *M. pneumoniae* and the attenuating effect of monotropein were assessed via quantifying the levels of myeloperoxidase activity and nitric oxide levels. Since both MPO and NO are essential in modulating the inflammatory response, pathogen clearance and tissue damage during pneumonia. The levels of MPO and NO was quantified using the Mouse Myeloperoxidase ELISA Kit and Nitric Oxide Colorimetric Detection Kit respectively procured from Invitrogen. The final absorbance of MPO and NO was read at 450 and 540 nm respectively. The concentrations of test samples were read using the standard curve.

Assessment of oxidative insult

The oxidative insult caused by *M. pneumoniae* and the inhibitory effect of monotropein were assessed via measuring the lipid peroxidation and antioxidant levels in the lung tissue of the experimental animals. The lung tissue homogenate was prepared with Tris buffer (pH 7.4) and the supernatant was obtained upon centrifugation at 5000xg for 15 min at 4°C. MDA levels were measured with[18] protocol. The antioxidants GSH and SOD were quantified using the protocols prescribed by[19] and[20] respectively. MDA levels are determined using the Thiobarbituric Acid Reactive Substances (TBARS) assay, where the reaction with thiobarbituric acid produces a colored complex measured spectrophotometrically. GSH is quantified using the Ellman's assay, where the reduction of 5,5'-Dithiobis (2-Nitrobenzoic acid) (DTNB) is monitored spectrophotometrically. SOD activity is assessed by the xanthine oxidase method, measuring the inhibition of superoxide radical generation, involving the reduction of Nitroblue Tetrazolium (NBT).

Assessment of BALF total cells count

Bronchoalveolar Lavage Fluid (BALF) was harvested by injecting 30 mL aliquots of saline into the right middle lobe of the experimental mice. The BALF was subjected to centrifugation at 6000 rpm for 5 min and pellets were collected for counting the total number of cells. Using hemocytometer the total cells were counted under an optical microscope.

Quantification of *Mycoplasma pneumoniae* DNA content

The lung tissues of the experimental mice were subjected to DNA extraction using the Qiagen DNeasy Blood and Tissue kit. The experiment was performed as per the guidelines provided in the kit manual. The extracted DNA was subjected to PCR analysis with specific primer targeting Mycoplasma pneumonia gene to quantify the DNA content. The results were compared with standard curve to measure the DNA content of experimental mice.[21]

Assessment of inflammatory cytokines in BALF

The levels of cytokines IL-6, TGF-β, IL-1, IL-8, TNF-α inducing inflammation were quantified in BALF of experimental mice using the ELISA kits procured from Abcam. Test samples and standard solutions were added to the specific antibody coated wells and allowed to bind for 1-2 hr at room temperature, followed by washing with wash buffer. Detection antibody, conjugated to an HRP enzyme was then added and incubated for an additional hour. The excess antibody, was eliminate through washing and a substrate solution was added. The reaction was then terminated after an hour and the absorbance was measured at 450 nm using a microplate reader. The results were analyzed against a standard curve to determine the concentration of the target molecule in the sample.

Quantification of NF-κβ

The NF-κβ levels in the lungs of pneumonia induced untreated and monotropein treated mice were quantified using the ELISA kit procured from MyBioSource. The experiments were performed as per the guidelines provided in the kit manual. NF-κβ concentration in the test samples were detected using the standard curve plotted with absorbance of known concentrations.

Lung histopathological examination

Lung tissue section excised from control and experimental mice were fixed in a phosphate-buffered formalin solution for a 24 hr, to preserve cellular structure. After fixation, the tissues were processed through a series of dehydration steps using graded alcohol solutions and cleared in xylene. The lung tissues were then infiltrated with paraffin wax and molded into paraffin blocks. The blocks were then sectioned into 5-micron thick slices, using a microtome. The tissue sections were then mounted on glass slides and subsequently subjected to deparaffinization and rehydration. The processed lung tissue sections were then stained with hematoxylin and eosin stains and viewed under a light microscope for pathological changes.

Statistical Analysis

The results were statistically analyzed using SPSS software, with findings reported as the mean±Standard Deviation (SD) of triplicate measurements. One-way ANOVA accompanied by Student Newmann Keul's *post hoc* test was used to evaluate the data and a *p*-value of less than 0.05 was considered statistically significant.

RESULTS

Pathogen clearance effect of monotropein on pneumonia triggered mice

Pulmonary edema and increased vascular permeability, which are common features of pneumonia were assessed with lung weight index in the experimental mice (Figure 1A). The untreated pneumonia triggered mice exhibited 0.9±0.00004 lung weight index which is significantly higher compared to the control (0.4±0.00001), monotropein treated (0.75±0.00002) and azithromycin treated (0.65±0.00001) mice triggered with pneumonia. NO and MPO are integral to the immune response in pneumonia, balancing effective pathogen clearance with the risk of inflammatory collateral damage. Monotropein effectively decreased the levels of both NO and MPO in pneumonia triggered mice to 275±0.006 µmol/g and 78±0.00 U/g respectively which is significantly higher as 340±0.004 µmol/g and 93±0.001 U/g respectively in pneumonia triggered untreated mice. Control and azithromycin treated mice shown 110 0.002 µmol/g, 145 0.001 µmol/g of nitric oxide and 45±0.006 U/g, 70±0.007 U/g of myeloperoxidase respectively.

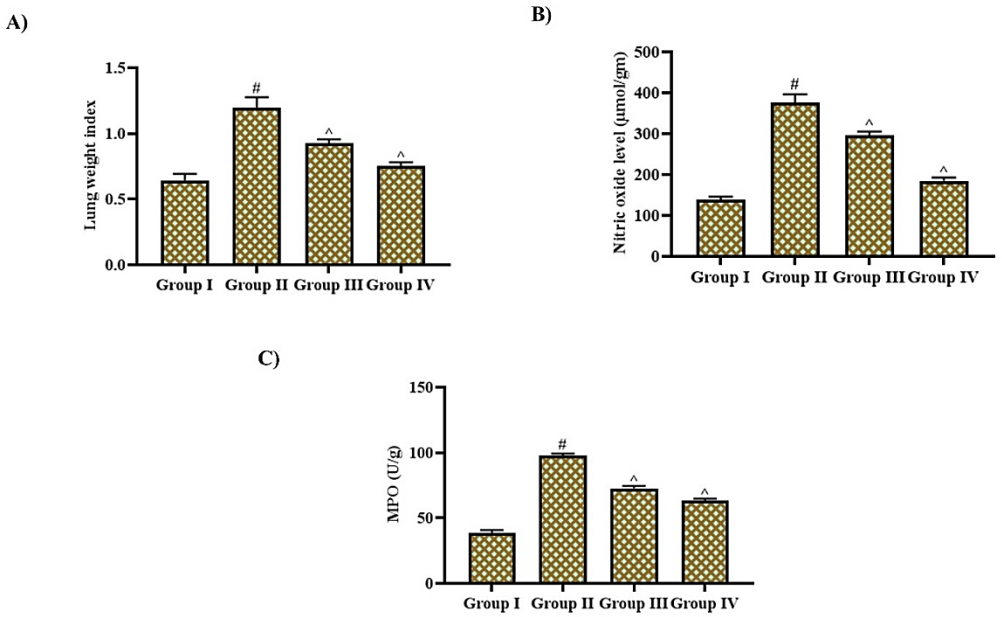

Figure 1: Pathogen clearance effect of monotropein on pneumonia triggered mice. A) Lung weight index B) Nitric oxide C) Myeloperoxidase levels in the pneumonia triggered untreated and monotropein treated mice. The results are expressed as the mean±Standard Deviation (SD) from three independent experiments. For statistical analysis, one-way ANOVA followed by Student's Newmann Keul's *post hoc* test was employed. # indicate a significant difference from the control group, while ## indicate a significant difference compared to the pneumonia group. *p*-value of less than 0.05 was considered statistically significant.

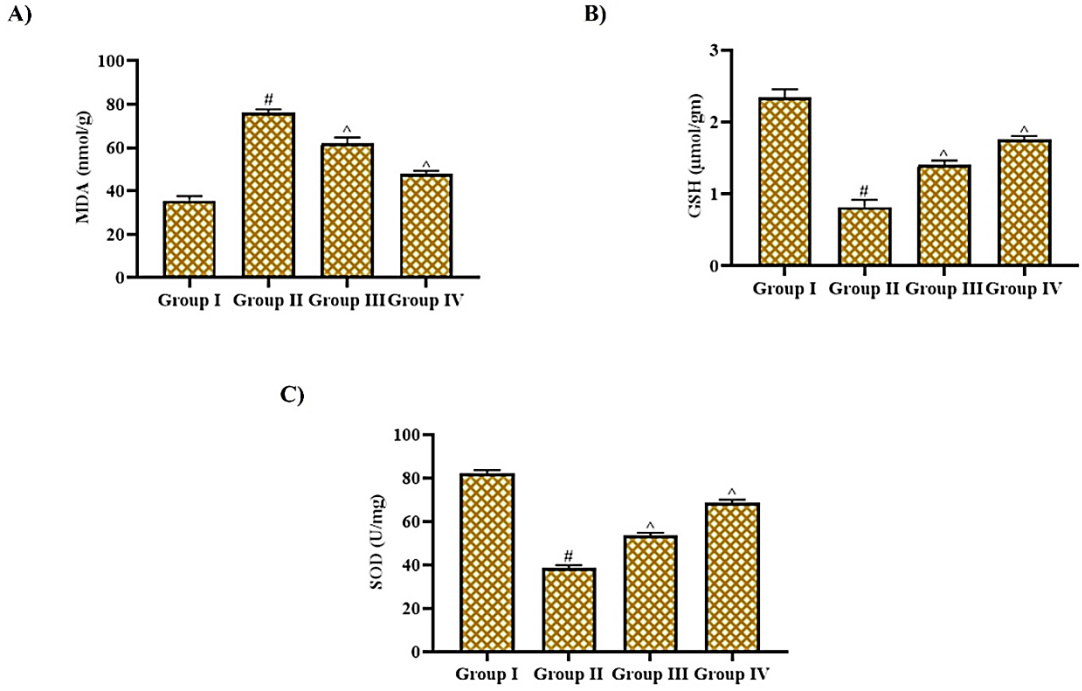

Figure 2: Attenuating effect of monotropein against oxidative insult induced in pneumonia triggered mice. A) Malondialdehyde levels B) Reduced glutathione C) Superoxide dismutase activity in the pneumonia triggered untreated and monotropein treated mice. The results are expressed as the mean±Standard Deviation (SD) from three independent experiments. For statistical analysis, one-way ANOVA followed by Student's Newmann Keul's *post hoc* test was employed. # indicate a significant difference from the control group, while ## indicate a significant difference compared to the pneumonia group. *p*-value of less than 0.05 was considered statistically significant.

Attenuating effect of monotropein against oxidative insult induced in pneumonia triggered mice

The levels of antioxidants and the lipid peroxidation induced due to the exposure of *Mycoplasma pneumoniae* and the attenuating effect of monotropein in the experimental mice were illustrated in Figure 2. Exposure to *Mycoplasma pneumoniae* had considerably elevated the lipid peroxidation which is exhibited with 70±0.003 nmol/g of MDA whereas the control mice shown only 34±0.002 nmol/g of MDA. Treatment with both monotropein and azithromycin decreased the MDA levels to 58±0.005 nmol/g and 48±0.004 nmol/g respectively. Treatment with monotropein and azithromycin increased the antioxidant GSH and SOD levels to 0.9±0.0001 µmol/g, 1.3±0.0004 µmol/g and 42±0.003 U/mg, 61±0.006 U/mg whereas the pneumonia triggered untreated mice exhibited only 0.75±0.0002 µmol/g of GSH and 32±0.002 U/mg of SOD. The control mice shown increased levels of GSH (2.2±0.0003 µmol/g) and SOD (76±0.004 U/g) compared to the other experimental mice.

Inhibitory effect of monotropein against inflammatory cell infiltration in pneumonia triggered mice

Figure 3A illustrated the count of total cells present in the BALF of control, pneumonia triggered untreated, monotropein treated and azithromycin treated mice. Exposure to *Mycoplasma pneumoniae* significantly induced the infiltration of inflammatory cells which exhibited with 58 ±0.0006 µg/mL of cells in pneumonia triggered untreated mice whereas the treatment with monotropein and azithromycin treatment inhibited immune cell infiltration and the total cell counted were only 46±0.0005 µg/mL and 36±0.0004 mg/mL respectively.

The pathological lung tissue damage induced by *Mycoplasma pneumoniae* and the ameliorating effect of monotropein was assessed via quantifying the DNA content of lung tissue of the experiment mice (Figure 3B). Exposure to *Mycoplasma pneumoniae* had significantly enhanced the DNA content whereas treatment with monotropein had attenuated the levels

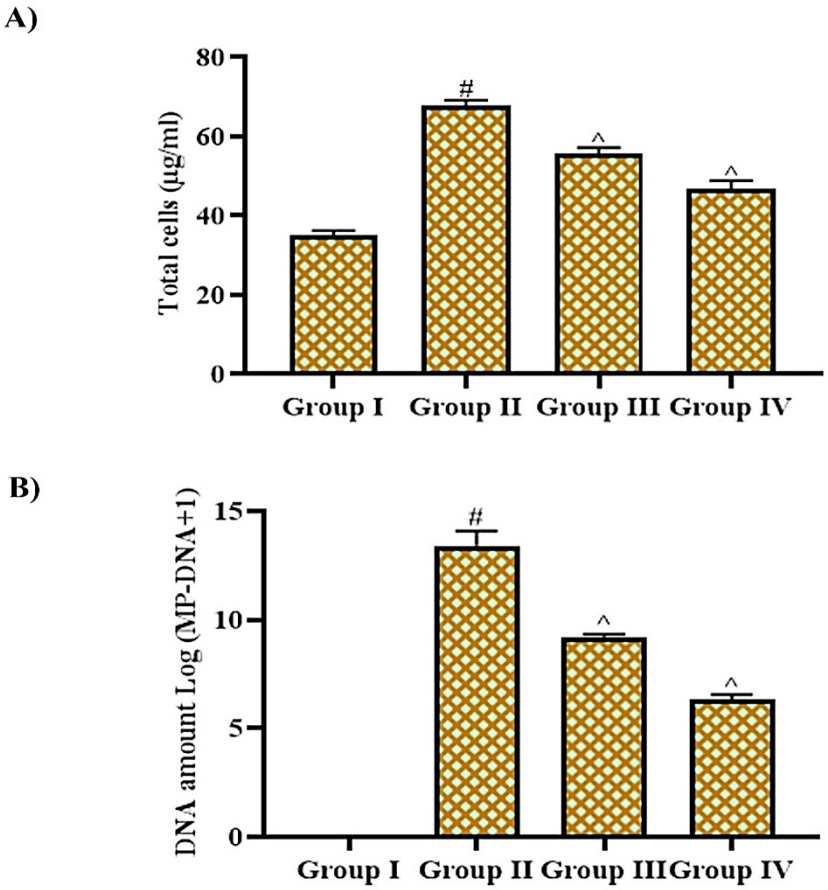

Figure 3: Inhibitory effect of monotropein against inflammatory cell infiltration in pneumonia triggered mice. A) Total cell count in the BALF fluid B) DNA content in the lung tissue of the pneumonia triggered untreated and monotropein treated mice. The results are expressed as the mean±Standard Deviation (SD) from three independent experiments. For statistical analysis, one-way ANOVA followed by Student's Newmann Keul's *post hoc* test was employed. # indicate a significant difference from the control group, while ## indicate a significant difference compared to the pneumonia group. *p*-value of less than 0.05 was considered statistically significant.

of DNA content. The DNA content in control and azithromycin treated mice were significantly decreased compared to the other experimental mice.

Mitigating effect of monotropein against the inflammatory response induced in pneumonia triggered mice

Inflammatory response induced by the *Mycoplasma pneumoniae* exposure and the attenuating of monotropein against it were measured by quantifying the levels inflammatory stimulating cytokines. *Mycoplasma pneumoniae* exposure had elevated the levels of inflammatory stimulating cytokines IL-6, TGF, IL-8, IL-1 and TNF-α compared to the control mice. Both monotropein and the standard drug azithromycin treatment had considerably reduced the levels of inflammatory stimulating cytokines compared to the untreated pneumonia triggered mice (Figure 4).

Antimicrobial activity of monotropein against *Mycoplasma pneumoniae* induced pneumonia in mice

Figure 5 depicts the levels of NF-kB which facilitates the activation and survival of immune cells which are critical for combating bacterial infections causing pneumonia. The mice exposed to *Mycoplasma pneumoniae* alone exhibited increased level of NFkB 56±0.006 units whereas it is considerably reduced to 38 0.05 units with monotropein treatment and 35 0.06 units with azithromycin treatment. The control mice exhibited 32 0.004 units of NFkB.

Ameliorative effect of monotropein against *Mycoplasma pneumoniae* induced pneumonia in mice

The lungs of control mice showed intact alveolar architecture without any evidence of lesions or inflammation (Figure 6A). However, mice with pneumonia exhibited significant inflammatory cell infiltration, thickened alveolar walls and constricted bronchial passages (Figure 6B). After treatment with monotropein (6C) and azithromycin (6D), these pathological features were greatly improved, with a marked decrease in hyperemia, bronchial congestion and the number of inflammatory cells present in the lung tissue.

DISCUSSION

Mycoplasma pneumoniae is a leading pathogen responsible for chronic respiratory tract diseases and pneumonia, particularly affecting children and adolescents, who are most susceptible to these infections.[22] While *M. pneumoniae* infections are typically mild and self-limiting, they can escalate into severe or life-threatening conditions in immunocompromised patients. This atypical bacterium accounts for approximately 40% of Community-Acquired Pneumonia (CAP) in children older than 5 and is associated with significant morbidity and mortality due to lower respiratory tract infections.[23] *M. pneumoniae* respiratory infections can exacerbate asthma, presenting symptoms such as cough, respiratory distress, wheezing and chest pain, attributed to cytokine release.[24] Furthermore, *M. pneumoniae* is linked to various extrapulmonary manifestations, including meningoencephalitis, myocarditis and mucocutaneous

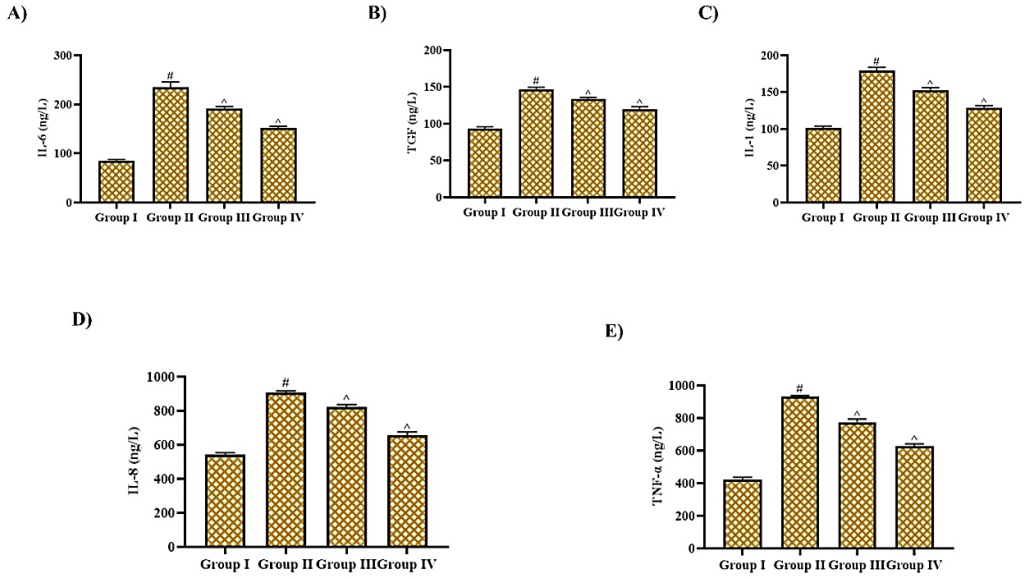

Figure 4: Mitigating effect of monotropein against the inflammatory response induced in pneumonia triggered mice. Inflammatory stimulating cytokines A) IL-6, B) TGF, C) IL-8, D) IL-1, E) TNF-α levels in the pneumonia triggered untreated and monotropein treated mice. The results are expressed as the mean±Standard Deviation (SD) from three independent experiments. For statistical analysis, one-way ANOVA followed by Student's Newmann Keul's *post hoc* test was employed. # indicate a significant difference from the control group, while ## indicate a significant difference compared to the pneumonia group. *p*-value of less than 0.05 was considered statistically significant.

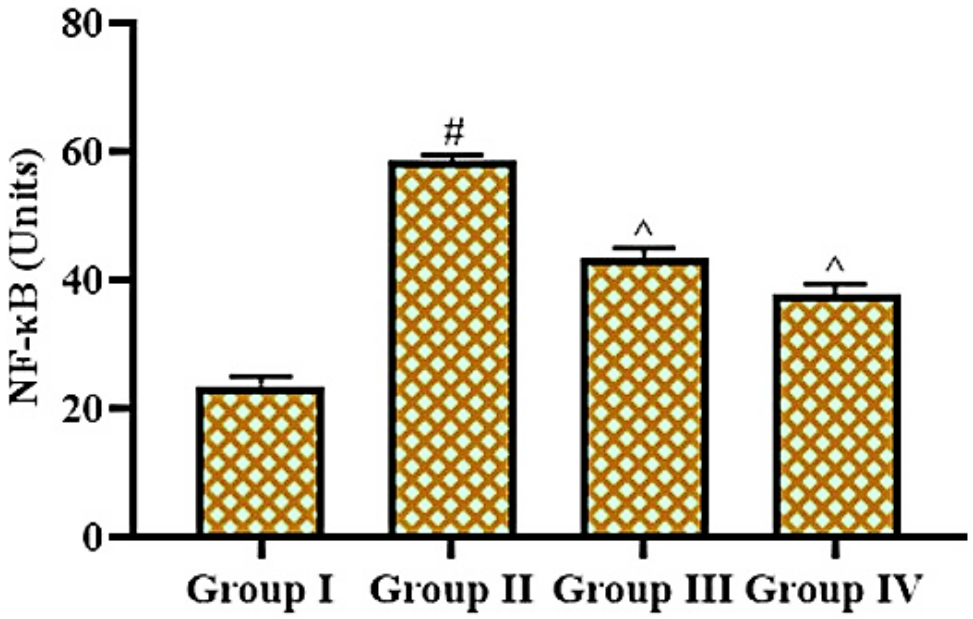

Figure 5: Antimicrobial activity of monotropein against Mycoplasma pneumoniae induced pneumonia in mice. Nuclear Factor kappa B levels in the pneumonia triggered untreated and monotropein treated mice. The results are expressed as the mean±Standard Deviation (SD) from three independent experiments. For statistical analysis, one-way ANOVA followed by Student's Newmann Keul's *post hoc* test was employed. # indicate a significant difference from the control group, while ## indicate a significant difference compared to the pneumonia group. *p*-value of less than 0.05 was considered statistically significant.

conditions like Stevens-Johnson syndrome.[25,26] Traditional antibiotics like tetracyclines and fluoroquinolones are effective against this infection but have significant side effects, making macrolides the preferred treatment in recent years.[27] However, the widespread use of macrolides has led to high resistance rates in China complicating treatment options and contributing to rising mortality rates. Patients infected with macrolide-resistant strains experience longer febrile periods and extended hospital stays, highlighting the impact of antibiotic resistance on clinical outcomes.[28] Hence in this study we analyzed the efficacy of phytochemical monotropein against the *Mycoplasma pneumoniae* triggered pneumonia in rodent model.

Elevated levels of Myeloperoxidase (MPO) and Nitric Oxide (NO) are critical factors contributing to lung tissue damage in pneumonia. Myeloperoxidase, an enzyme produced by activated neutrophils, plays a significant role in the inflammatory response. Increased MPO activity has been associated with tissue injury through the generation of Reactive Oxygen Species (ROS), which can exacerbate lung inflammation and damage bronchial epithelial cells.[29] Elevated MPO levels have been identified as a biomarker for severe pneumonia, linking its presence to heightened inflammation and oxidative stress in lung tissues.[30] In parallel, nitric oxide produced during inflammatory responses has a dual role; while it is essential for pathogen clearance, excessive NO can lead to cellular toxicity and further lung injury. Research indicates that overproduction of NO, particularly in the context of pneumonia, can contribute to oxidative stress and the formation of peroxynitrite, a reactive nitrogen species that damages lung tissue and exacerbates inflammation.[31] Therefore, both MPO and NO play pivotal roles in the pathogenesis of pneumonia, where their elevated levels underline the severity of lung tissue damage and highlight potential targets for therapeutic intervention. In our study monotropein treatment significantly inhibited the nitric oxide and myeloperoxidase levels in pneumonia-triggered mice.

During instances of oxidative stress or persistent inflammation, Myeloperoxidase (MPO) is secreted outside of cells, resulting in increased concentrations of MPO-derived Hypochlorous acid (HOCl) in the extracellular environment. The strong reactivity of HOCl with biological molecules leads to the oxidative modification of DNA, RNA, proteins and lipids,[32] consequently causing tissue damage and disrupting normal biological functions.[33] This accumulation of MPO and its oxidant, HOCl, can contribute to the development of lung diseases by triggering oxidative stress and promoting inflammatory responses. In children with cystic fibrosis, substantial amounts of HOCl have been detected, which can oxidize reduced Glutathione (GSH) in the airways, leading to damage of the lung epithelium.[34,35] These research correlates with our results, exposure to *Mycoplasma pneumoniae* had significantly elevated the MPO level and increased MDA levels, DNA content was observed. Whereas treatment with monotropein had attenuated the MPO activity

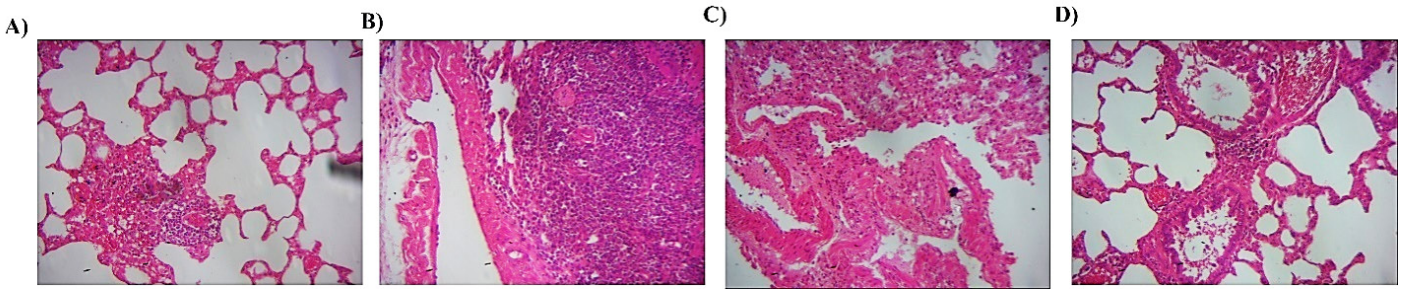

Figure 6: Ameliorative effect of monotropein against Mycoplasma pneumoniae induced pneumonia in mice. Representative images of Haematoxylin and Eosin stained lung tissue sections. A) Control B) Pneumonia triggered untreated mice C) Pneumonia triggered monotropein treated mice D) Pneumonia triggered azithromycin treated mice.

thereby increased the levels of antioxidant and prevent lung tissue damage.

Innate immune cells, such as macrophages, dendritic cells and neutrophils, are crucial components of the immune response and play significant roles in inflammation. These cells express Pattern-Recognition Receptors (PRRs) that identify microbial elements known as Pathogen-Associated Molecular Patterns (PAMPs) as well as Damage-Associated Molecular Patterns (DAMPs) released from necrotic and damaged tissues.[36] A key outcome of PRR activation is the stimulation of the NF-κB signaling pathway, which drives the transcription of pro-inflammatory cytokines, chemokines and other mediators across various innate immune cell types.[37,38] These mediators directly initiate inflammation and indirectly facilitate the differentiation of inflammatory T cells. While NF-κB signaling is normally tightly regulated to promote acute inflammation and subsequent resolution of injury, dysregulation can occur in various inflammatory diseases, leading to chronic inflammation and tissue damage.[39,40]

In Acute Lung Injury (ALI), NF-κB-driven inflammation and coagulation may reinforce each other, establishing a cycle of persistent pro-inflammatory signaling. Given its central role in disease progression, NF-κB has become a promising target for therapeutic intervention, resulting in the development of small molecule inhibitors aimed at blocking its activity.[41,42] Therefore we analyzed the NFkB inhibitory property of monotropein in pneumonia triggered mice. Monotropein potentially attenuated NFkB activity thereby prevented the inflammatory stimulating cytokines IL-6, IL-1, IL-8, TGF and TNF-α levels. It also inhibited the innate immune response which is evidenced with our decreased total cell count in BALF of monotropein treated pneumonia induced mice. Histopathological analysis of lung tissue confirms the ameliorative potency of monotropein which attenuated the NFkB activity thereby prevented the oxidative stress, inflammation induced damage in lung tissue.

CONCLUSION

In conclusion, our study highlights the promising curative process of monotropein in the treatment of pneumonia induced by *Mycoplasma pneumoniae*. The compound demonstrated significant antimicrobial and anti-inflammatory effects, as evidenced by reduced levels of nitric oxide and myeloperoxidase, oxidative stress inhibition and decreased inflammatory cytokines in a mouse model of pneumonia. Histopathological analyses further affirmed the protective role of monotropein against lung tissue damage. Given the increasing challenge of antibiotic-resistant pneumonia, monotropein emerges as a viable alternative or adjunctive therapy, offering a multifaceted approach to managing this critical infection. Upcoming investigations should focus on elucidating the underlying mechanisms of monotropein's action and exploring its efficacy in human clinical trials to ascertain its potential as a novel therapeutic agent for pneumonia treatment.

ACKNOWLEDGEMENT

This work was supported by Xi'an TUS-Children's Hospital, Xi'an, 710000, China.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

NF-κβ: Nuclear Factor kappa B; **BALF:** Bronchoalveolar lavage fluid; **CAP:** Community-acquired pneumonia; **NBT:** Nitroblue tetrazolium; **MPO:** Myeloperoxidase; **NO:** Nitric oxide; **HOCl:** Hypochlorous acid; **GSH:** Glutathione; **PRR:** Pattern-recognition receptors; **DAMP:** Damage-associated molecular patterns; **PAMP:** Pathogen-associated molecular pattern.

SUMMARY

Pneumonia remains a major global health concern, contributing to considerable morbidity and mortality, particularly among vulnerable populations such as children and the elderly. Monotropein, a prominent iridoid glycoside isolated from

Morinda officinalis, is recognized for its significant therapeutic potential. Monotropein potentially attenuated NFkB activity thereby prevented the inflammatory stimulating cytokines IL-6, IL-1, IL-8, TGF and TNF-α levels. The compound demonstrated significant antimicrobial and anti-inflammatory effects, as evidenced by reduced levels of nitric oxide and myeloperoxidase, oxidative stress inhibition and decreased inflammatory cytokines in a mouse model of pneumonia.

REFERENCES

1. Mackenzie G. The definition and classification of pneumonia. Pneumonia (Nathan). 2016;8(1):14. doi: 10.1186/s41479-016-0012-z, PMID 28702293.
2. Alshammari MK, Alotaibi MA, AlOtaibi AS, Alosaime HT, Aljuaid MA, Alshehri BM, *et al.* Prevalence and etiology of community- and hospital-acquired pneumonia in Saudi Arabia and their antimicrobial susceptibility patterns: A systematic review. Medicina (Kaunas). 2023;59(4):760. doi: 10.3390/medicina59040760, PMID 37109718.
3. McAllister DA, Liu L, Shi T, Chu Y, Reed C, Burrows J, *et al.* Global, regional and national estimates of pneumonia morbidity and mortality in children younger than 5 years between 2000 and 2015: a systematic analysis. Lancet Glob Health. 2019;7(1):e47-57. doi: 10.1016/S2214-109X(18)30408-X, PMID 30497986.
4. WHO. Pneumonia in children; 2022. Available from: https://www.who.int/news-room/fact-sheets/detail/pneumonia.
5. Torres A, Cilloniz C, Niederman MS, Menéndez R, Chalmers JD, Wunderink RG, *et al.* Pneumonia. Nat Rev Dis Primers. 2021;7(1):25. doi: 10.1038/s41572-021-00259-0, PMID 33833230.
6. Kaysin A, Viera AJ. Community-acquired pneumonia in adults: diagnosis and management. Am Fam Physician. 2016;94(9):698-706. PMID 27929242.
7. Sattar SB, Nguyen AD, Sharma S. Bacterial pneumonia. In: Treasure Island, (FL): StatPearls Publishing; Updated 2024 Feb 26. StatPearls [Internet]. p. 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK513321/.
8. Cilloniz C, Martin-Loeches I, Garcia-Vidal C, San Jose A, Torres A. Microbial etiology of pneumonia: epidemiology, diagnosis and resistance patterns. Int J Mol Sci. 2016;17(12):2120. doi: 10.3390/ijms17122120, PMID 27999274.
9. Gupta D, Agarwal R, Aggarwal AN, Singh N, Mishra N, Khilnani GC, *et al.* Guidelines for diagnosis and management of community- and hospital-acquired pneumonia in adults: joint ICS/NCCP (I) [recommendations]. Lung India. 2012; 29 Suppl 2:S27-62. doi: 10.4103/0970-2113.99248, PMID 23019384.
10. Rider AC, Frazee BW. Community-acquired pneumonia. Emerg Med Clin North Am. 2018;36(4):665-83. doi: 10.1016/j.emc.2018.07.001, PMID 30296998.
11. Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, Dean NC, *et al.* Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis. 2007; 44 Suppl 2:S27-72. doi: 10.1086/511159, PMID 17278083.
12. Coleman JJ, Pontefract SK. Adverse drug reactions. Clin Med (Lond). 2016;16(5):481-5. doi: 10.7861/clinmedicine.16-5-481, PMID 27697815.
13. Baggio D, Ananda-Rajah MR. Fluoroquinolone antibiotics and adverse events. Aust Prescr. 2021;44(5):161-4. doi: 10.18773/austprescr.2021.035, PMID 34728881.
14. Thebti A, Meddeb A, Ben Salem I, Bakary C, Ayari S, Rezgui F, *et al.* Antimicrobial activities and mode of flavonoid actions. Antibiotics (Basel). 2023;12(2):225. doi: 10.3390/antibiotics12020225, PMID 36830135.
15. Wu M, Lai H, Peng W, Zhou X, Zhu L, Tu H, *et al.* Monotropein: A comprehensive review of biosynthesis, physicochemical properties, pharmacokinetics and pharmacology. Front Pharmacol. 2023;14:1109940. doi: 10.3389/fphar.2023.1109940, PMID 36937894.
16. Heffels P, Müller L, Schieber A, Weber F. Profiling of iridoid glycosides in Vaccinium species by UHPLC-MS. Food Res Int. 2017;100(3):462-8. doi: 10.1016/j.foodres.2016.11.018, PMID 28964369.
17. Shi Y, Liu XY, Jiang YP, Zhang JB, Zhang QY, Wang NN, *et al.* Monotropein attenuates oxidative stress via Akt/mTOR-mediated autophagy in osteoblast cells. Biomed Pharmacother. 2020;121:109566. doi: 10.1016/j.biopha.2019.109566, PMID 31698268.
18. Sinha K, Ranjan B. A simple method for the determination of malondialdehyde (MDA) in biological samples: validation and clinical applications. Mol Biol Rep. 2013;40(1):467-73.
19. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82(1):70-7. doi: 10.1016/0003-9861(59)90090-6, PMID 13650640.
20. Nishikimi M, Appaji N, Yagi K. Determination of superoxide dismutase activity by a novel spectrophotometric method. Biochem Biophys Res Commun. 1972;46(2):849-54. doi: 10.1016/s0006-291x(72)80218-3, PMID 4400444.
21. Razin S, Yogev D, Naot Y. Molecular biology and pathogenicity of mycoplasmas. Microbiol Mol Biol Rev. 1998;62(4):1094-156. doi: 10.1128/MMBR.62.4.1094-1156.1998, PMID 9841667.
22. Kumar S. Mycoplasma pneumoniae: A significant but underrated pathogen in paediatric community-acquired lower respiratory tract infections. Indian J Med Res. 2018;147(1):23-31. doi: 10.4103/ijmr.IJMR_1582_16, PMID 29749357.
23. Kutty PK, Jain S, Taylor TH, Bramley AM, Diaz MH, Ampofo K, *et al.* Mycoplasma pneumoniae among children hospitalized with community-acquired pneumonia. Clin Infect Dis. 2019;68(1):5-12. doi: 10.1093/cid/ciy419, PMID 29788037.
24. Waites KB, Xiao L, Liu Y, Balish MF, Atkinson TP. Mycoplasma pneumoniae from the respiratory tract and Beyond. Clin Microbiol Rev. 2017;30(3):747-809. doi: 10.1128/CMR.00114-16, PMID 28539503.
25. Jiang Z, Li S, Zhu C, Zhou R, Leung PH. Mycoplasma pneumoniae infections: pathogenesis and vaccine development. Pathogens. 2021;10(2):119. doi: 10.3390/pathogens10020119, PMID 33503845.
26. Meyer Sauteur PM, Goetschel P, Lautenschlager S. Mycoplasma pneumoniae and mucositis--part of the Stevens-Johnson syndrome spectrum. J Dtsch Dermatol Ges. 2012;10(10):740-6. doi: 10.1111/j.1610-0387.2012.07951.x, PMID 22672205.
27. Spuesens EB, Meyer Sauteur PM, Vink C, van Rossum AM. Mycoplasma pneumoniae infections--does treatment help? J Infect. 2014; 69 Suppl 1:S42-6. doi: 10.1016/j.jinf.2014.07.017, PMID 25267596.
28. Cao B, Qu JX, Yin YD, Eldere JV. Overview of antimicrobial options for Mycoplasma pneumoniae pneumonia: focus on macrolide resistance. Clin Respir J. 2017;11(4):419-29. doi: 10.1111/crj.12379, PMID 26365811.
29. Arnhold J. The dual role of myeloperoxidase in immune response. Int J Mol Sci. 2020;21(21):8057. doi: 10.3390/ijms21218057, PMID 33137905.
30. Lin W, Chen H, Chen X, Guo C. The roles of neutrophil-derived myeloperoxidase (MPO) in diseases: the new progress. Antioxidants (Basel). 2024;13(1):132. doi: 10.3390/antiox13010132, PMID 38275657.
31. Papi S, Ahmadizar F, Hasanvand A. The role of nitric oxide in inflammation and oxidative stress. Immunopathol Persa. 2019;5(1):e08. doi: 10.15171/ipp.2019.08.
32. Winterbourn CC, van den Berg JJ, Roitman E, Kuypers FA. Chlorohydrin formation from unsaturated fatty acids reacted with hypochlorous acid. Arch Biochem Biophys. 1992;296(2):547-55. doi: 10.1016/0003-9861(92)90609-z, PMID 1321589.
33. Karakas M, Koenig W. Myeloperoxidase production by macrophage and risk of atherosclerosis. Curr Atheroscler Rep. 2012;14(3):277-83. doi: 10.1007/s11883-012-0242-3, PMID 22407325.
34. Magon NJ, Turner R, Gearry RB, Hampton MB, Sly PD, Kettle AJ. Oxidation of calprotectin by hypochlorous acid prevents chelation of essential metal ions and allows bacterial growth: relevance to infections in cystic fibrosis. Free Radic Biol Med. 2015;86:133-44. doi: 10.1016/j.freeradbiomed.2015.05.022, PMID 26006104.
35. Dickerhof N, Pearson JF, Hoskin TS, Berry LJ, Turner R, Sly PD, *et al.* Oxidative stress in early cystic fibrosis lung disease is exacerbated by airway glutathione deficiency. Free Radic Biol Med. 2017;113:236-43. doi: 10.1016/j.freeradbiomed.2017.09.028, PMID 28982600.
36. Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. Signal Transduct Target Ther. 2017;2:17023. doi: 10.1038/sigtrans.2017.23, PMID 29158945.
37. Newton K, Dixit VM. Signaling in innate immunity and inflammation. Cold Spring Harb Perspect Biol. 2012;4(3):a006049. doi: 10.1101/cshperspect.a006049, PMID 22296764.
38. Mogensen TH. Pathogen recognition and inflammatory signaling in innate immune defenses. Clin Microbiol Rev. 2009;22(2):240-73. doi: 10.1128/CMR.00046-08, PMID 19366914.
39. Hayden MS, Ghosh S. NF-kappaB in immunobiology. Cell Res. 2011;21(2):223-44. doi: 10.1038/cr.2011.13, PMID 21243012.
40. Sun SC, Chang JH, Jin J. Regulation of nuclear factor-kappaB in autoimmunity. Trends Immunol. 2013;34(6):282-9. doi: 10.1016/j.it.2013.01.004, PMID 23434408.
41. Ramadass V, Vaiyapuri T, Tergaonkar V. Small molecule NF-κB pathway inhibitors in clinic. Int J Mol Sci. 2020;21(14):5164. doi: 10.3390/ijms21145164, PMID 32708302.
42. Millar MW, Fazal F, Rahman A. Therapeutic targeting of NF-κB in acute lung injury: A double-edged sword. Cells. 2022;11(20):3317. doi: 10.3390/cells11203317, PMID 36291185.

Cite this article: Fan J, Zhao L. Monotropein Attenuates Nuclear Factor Kappa B Activity there by Ameliorates *Mycoplasma pneumoniae* Triggered Pneumonia in Rodent Model. Indian J of Pharmaceutical Education and Research. 2025;59(3):1100-8.