Antibacterial Activity of *Dactylicapnos scandens* Extract against *Mycoplasma pneumoniae*-induced Pneumonia in Mice: An *In vitro* and *In vivo* study

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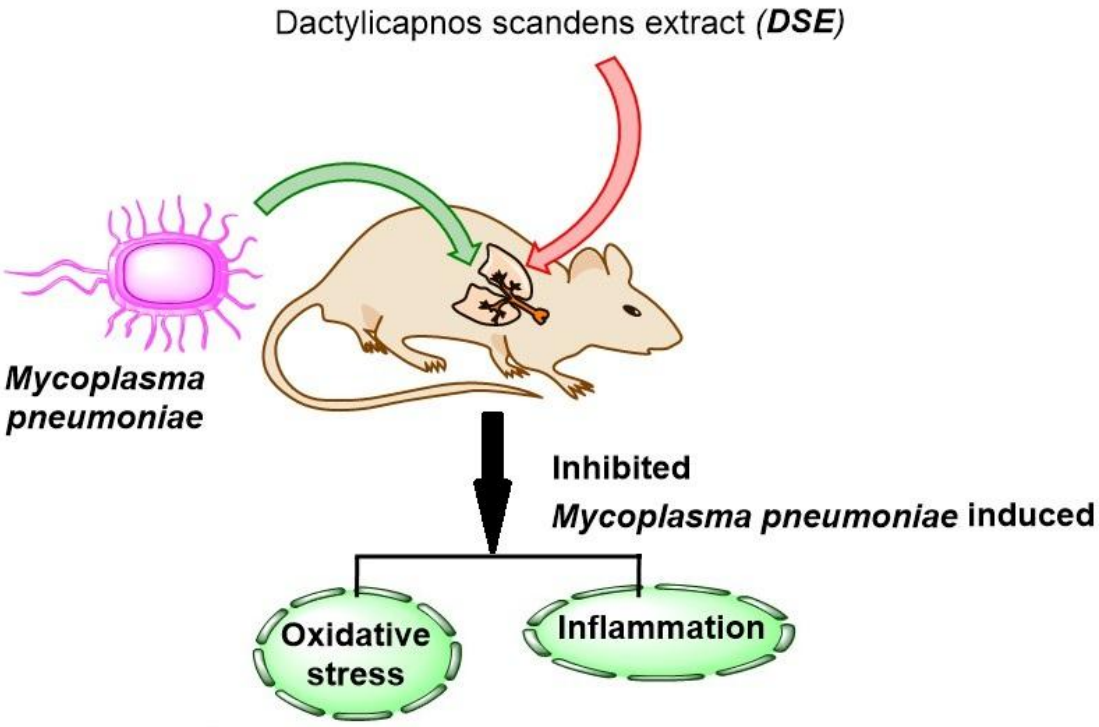
GRAPHICAL ABSTRACT



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This study analyzed the antibacterial potential of *Dactylicapnos scandens* extract (DSE) against *Mycoplasma pneumoniae (MP)*-induced pneumonia in mice. The DSE was tested for *in-vitro* antibacterial activity against MP and pharmacological activity in MP-induced pneumonia in mice. DSE showed significant *in-vitro* antibacterial activity against MP. It improved the level of nitric oxide, and myeloperoxidase, including the lung weight index near to control in a dose-dependent manner in experimental animals. The levels of glutathione and superoxide dismutase were found significantly increased in DSE treated rats with a reduction in malondialdehyde activity as compared to pneumonia-induced mice in a dose dependent manner. The level of pro-inflammatory cytokines, such as interleukin-6, Interleukin-1 beta, and tumour necrosis factor alpha, and total cells and DNA content were also found reduced in DSE treated group as compared to disease control mice. This study demonstrated potent antibacterial activity of *Dactylicapnos scandens* extract against *Mycoplasma pneumoniae* infection.

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Keywords: Pneumonia; MIC; Oxidative stress; Cytokines

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INTRODUCTION

Even with the considerable gains that have been made in preventing infections and the antimicrobial arsenal, pneumonia continues to be the cause of an enormous number of fatalities (Thomas 2016). According to the World Health Organisation (WHO), infections of the lower part of the respiratory system are among the top infectious causes of mortality in the entire globe, accounting for around three million fatalities annually (Waites and Talkington 2004; Grijalva 2009).

Because *Mycoplasma pneumoniae* (M.P.) does not possess a membrane that surrounds its cells, it is insensitive to beta-lactams as well as any other antibiotic that targets the cell wall (Pereyre *et al.* 2016; Zhan *et al.* 2022). According to the findings of studies, it also possesses resistance to various modern antibiotics. Therefore, it is worthwhile to investigate the possibility of developing novel antibacterial medicines that are effective against M.P. infection (Bébéar *et al.* 2011; Guo *et al.* 2019; Rothstein *et al.* 2022).

Plants that have been cultivated for their medicinal properties have been utilized

from the earliest days of civilization to cure a wide variety of disorders, including infectious diseases, and they continue to play an important part in the practice of this branch of medicine (Ali Alsarhan *et al.* 2021; Nwozo *et al.* 2023; Al-Rajhi *et al*. 2024).

In the past few decades, there has been a shift regarding an increased openness within conventional medical practice toward investigating and creating new kinds of antibacterial medicines that originate from botanicals (Silva and Fernandes Júnior 2010). This shift might be associated with the actuality that although pharmaceutical firms typically generate a few novel antibiotics annually, the overall amount of freshly discovered medicines in the laboratory or clinical trial pipelines has started decreasing during the last two decades (Hutchings *et al.* 2019; Cook and Wright 2022). This might also be a contributing factor in the increasing prevalence of antibiotic-resistant bacteria. As a consequence of this, the pharmaceutical industry has shown an active interest in the likelihood of using medications derived from plants as antibacterial agents. Furthermore, there seems to be an increase in the general population's knowledge regarding the improper as well as excessive consumption of antimicrobial agents. Thus, plant-based medicines are attracting a lot of attention from customers due to the fact that they have been regarded to be simultaneously secure and efficient (Butler *et al.* 2014).

The dried root of *Dactylicapnos scandens* (D. Don) Hutch. (*D. scandens*), a renowned ethnomedicine of the Bai nationality, has been traditionally utilized for its antipyretic, anti-inflammatory, and analgesic properties in Yunnan region, P. R. China (Wang *et al.* 2018, 2020). Contemporary pharmacology indicates that the total alkaloids of *D. scandens* exhibit both peripheral and central analgesic properties, distinct from the analgesic effects of morphine and nonsteroidal anti-inflammatory drugs, implying their action on pain-related receptors beyond opioid receptors. Excellent antioxidant properties have been shown (Niwano *et al.* 2011). Until now, no study has enumerated the antibacterial potential of this plant. The present study assessed the antibacterial activity of *Dactylicapnos scandens* extract (DSE) against *Mycoplasma pneumoniae*-induced pneumonia in mice. This experimental model is widely used to study the pathogenesis, immune response, and potential therapeutic interventions for MP infections in humans. While mice do not naturally acquire MP infection as humans do, they can be experimentally infected to mimic aspects of human disease, including airway inflammation, cytokine responses, and lung pathology (Cao *et al*. 2021; Sun *et al.* 2022). This model provides valuable insights into disease progression and host-pathogen interactions, helping researchers evaluate the efficacy of antimicrobial agents and immunomodulatory therapies (Yang *et al*. 2020).

EXPERIMENTAL

Plant Collection and Extract Preparation

Dactylicapnos scandens (D. Don) Hutch. has a sturdy rootstock that is either simple or branching and resembles a carrot. Its climbers are perennial herbaceous plants, 1 to 5 meters in height, slender, sulcate, branching, and leafy all the way through. The leaves are reflexed three times on a zigzag stem, with a petiole measuring between 0.5 and 3 cm. The leaf blade is glaucous on the abaxial side and dark green on the adaxial side, and it has one pair of primary pinnae that are typically displaced (alternate). The rachis ends in a thin branched cirrose tendril. Leaves are compound and leaflets are ovate, measuring between 5 and 30 mm in diameter, with a small hooked mucro. The inflorescence is a raceme of 1

to 5 cm in length, with 6 to 14 flowers, and a nutant; a peduncle measuring 2 to 8 cm; bracts that are narrowly oblanceolate and measuring 3 to 6 mm in diameter, with a margin that is either completely or somewhat dentate, and an apex that is acute. The pedicel is slender, measuring 10 to 20 mm, and it grows to a length of 30 mm in fruit. The sepals are ovate-lanceolate in shape, measuring around 3 to 4 × 2 mm, and the flower is complete. The plant was collected from the outskirts of the Heilongjiang Provincial Hospital, China and was identified by botanist Dr. Wei Yang. The voucher specimen No. HPH002024045 was deposited in the Heilongjiang Provincial Hospital, Heilongjiang, China. Approximately 500 g of the whole plant was air dried for 15 days and pulverized to coarse powder. The raw powder form of the plant is extracted thrice with distilled water, filtered, and concentrated with a rotary evaporator (Buchi, R-200 Switzerland) to obtain the raw extract powder.

Mycoplasma pneumoniae (MP) Culture

The *Mycoplasma pneumoniae* FH strain of Eaton Agent (ATCC15531) was obtained from ATCC, USA and was cultured as previously described (Yu *et al*. 2020).

In-vitro MIC Determination

The MIC value was calculated so that the smallest concentration of DSE necessary to inactivate a given bacterial strain could be identified. Using the broth dilution method (Wiegand *et al*. 2008), the MIC for DSE was calculated. A 10-µL bacterial culture produced overnight was used to inoculate 990 µL of new LB broth, which was then incubated at 37 °C, 120 rpm, and for 4 h. The bacterial culture was cultured at 37 °C and 120 rpm for 24 hours before various concentrations of DSE in a serially diluted manner (0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8, 25.6, 51.2, and 102.4 mg/mL) were added to the mixture. The MIC values were then calculated by measuring the optical density (OD) of each bacterial culture at 600 nm with a UV-Vis spectrophotometer.

Animals

BALB/c mice aged 4 to 6 weeks were obtained from the Institutional animal house. They were strictly housed in hygienic rooms in polypropylene cages under ambient light and humidity. They were fed with a laboratory diet and water *ad libitum*, and the acclimatization was 7 days prior to the start of the experiment. The animal experiment was approved by the Animal Experiment Ethics Committee of Heilongjiang Provincial Hospital, China and all the experiments were conducted in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals.

Induction of M.P. Pneumonia in Mice

A total of 40 mice were randomly divided into five groups, where n=8/group. After randomization, the groups were categorized as follows:

Group 1: Normal Control (Non-treated)
Group 2: Disease Control (100 µl of M.P.)
Group 3: DSE (M.P. + 10 mg/kg b.w.)
Group 4: DSE (M.P. + 20 mg/kg b.w.)
Group 5: DSE (M.P. + 30 mg/kg b.w.)
Group 6: Azithromycin (M.P. + 10 mg/kg b.w)

The entire group except Group 1 received M.P. inoculum for the induction of M.P.

infection in the nostrils of mice once daily for two days. After induction of M.P., mice received the dose of test drugs as stated above for the next three consecutive days. In the normal control group, all of the mice received the same amount of sterile water. At the completion of the study, all animals had been administered halothane anesthesia and then killed *via* cervical decapitation (Yu *et al.* 2020).

Estimation of Myeloperoxidase (MPO) and Nitric Oxide (NO) Levels

The lung tissue supernatant of mice was used for the estimation of MPO and NO using the commercial kits according to the protocol supplied (Nanjing Jiancheng Bioengineering Institute, China) (Min *et al.* 2021).

Estimation of Oxidative Stress Biomarkers

The levels of malondialdehyde (MDA), glutathione (GSH), and superoxide dismutase (SOD) in the lung tissues of mice was determined using the commercial kits according to the protocol supplied (Lu *et al.* 2022) (Nanjing Jiancheng Bioengineering Institute, China).

Estimation of Pro-inflammatory Cytokines

The levels of IL-1β, IL-6, and TNF-α, in the lung tissues of mice were determined using the commercial kits according to the protocol supplied (Yu and Cheng 2023) (Nanjing Jiancheng Bioengineering Institute, China).

Statistical Analysis

The results underwent analysis using the GraphPad Prism application and were subsequently presented as means ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) was used, and p-values of 0.05 or lower were considered statistically important.

RESULTS AND DISCUSSION

In vitro Antibacterial Activity

As shown in Table 1, the DSE showed excellent antibacterial activity against *Mycoplasma pneumoniae*. This antibacterial activity of DSE was found equipotent to the Azithromycin as standard.

Table 1. Antibacterial Activity

Compound	MIC (in mg/mL)
DSE	1.6
Azithromycin (Standard)	1.6

In vivo Antibacterial Activity in Mice

The effect of DSE was analyzed relative to various parameters in the M.P.-induced pneumonia mice. Following the introduction of M.P., the effects of DSE on MPO activity, NO production, and lung weight index were investigated. Neutrophil hemoprotein MPO is widely distributed and has been linked to critical roles in modulating host defense (Tiruppathi *et al.* 2004). According to the findings of several studies, infection with M.P. is associated with increased inflammation as well as increased tissue destruction. Due to

this, the levels of MPO and NO are significantly greater in the lung tissues of individuals affected by M.P. (Chapman *et al.* 2010). As a consequence, MPO and NO play an important part as biomarkers for M.P. infection. As shown in Fig. 1, the M.P.-infected mice showed an increased level of MPO activity and NO level as compared to non-treated control. The administration of sesamol led to a slight increase in activity. However, the groups treated with DSE showed a dose-dependent reduction in the elevated level of MPO and NO near normal. The DSE also increased the lung weight index in the MP-induced pneumonia-infected mice. These effects were found more pronounced in Group 3 treated animals.

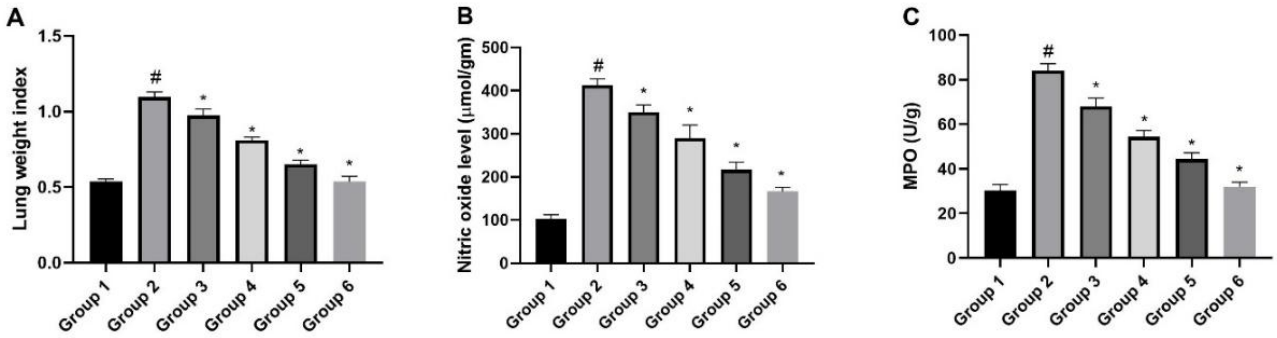

Fig. 1. Effect of DSE on the lung weight, NO concentration, and MPO activity in the pneumonia-induced mice. Data (n = 3) were represented as the mean ± S.E.M. The level of significance was indicated by $^{\#}P < 0.01$ vs. Group 1, $^{\#}P < 0.05$ vs. Group 2.

Increased synthesis of inflammatory regulators in the airway system as a result of oxidative stress is a key component of the host's immune response to invading pathogens (Chung and Adcock 2008). The inflammatory responses are closely linked to oxidative stress. Increased synthesis of inflammatory regulators exacerbates oxidant accumulation and attracts inflammatory cells (Domej *et al.* 2014). When oxidative stress levels rise, lung cells generate a cocktail of deleterious byproducts, including reactive oxygen species (ROS) such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH\bullet$), along with reactive nitrogen species (RNS) such as nitric oxide ($NO\bullet$) and peroxynitrite ($ONOO^-$). These reactive molecules trigger lipid peroxidation, generating malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4-HNE), which damage cell membranes. Additionally, protein oxidation leads to carbonylated proteins, while DNA damage results in 8-hydroxy-2'-deoxyguanosine (8-OHdG) and strand breaks. These oxidative byproducts contribute to lung inflammation and apoptosis. More specifically, the phenomenon of oxidative stress arises as a consequence of the host's immune response to invading pathogens, leading to an increase in the synthesis of regulators of lung inflammation (Xu *et al.* 2006; Odeh and Simecka 2016; Lin *et al.* 2018). Significantly greater amounts of oxidative damage have been identified among individuals having pneumonia compared to normal controls (Kuwano *et al.* 2003; Carr *et al.* 2020; Vollbracht and Kraft 2022). Therefore, this study defines the effect of DSE on the various mediators of oxidative stress, such as MDA activity, GSH content, and SOD activity (Fig. 2).

The results suggested that DSE caused significant improvement in the level of MDA, GSH, and SOD as compared to pneumonia-induced mice. The DSE also showed a reduction in the elevated level of total cells and DNA content in the lung tissue of mice (Fig. 3).

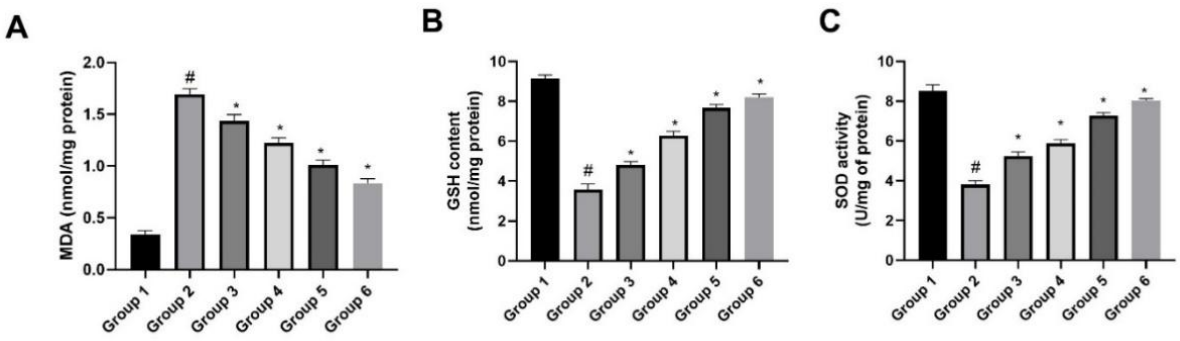

Fig. 2. Effect of DSE on oxidative stress. Data (n = 3) were represented as the mean ± S.E.M. The level of significance was indicated by $^{\#}P < 0.01$ vs. Group 1, $^{\#}P < 0.05$ vs. Group 2.

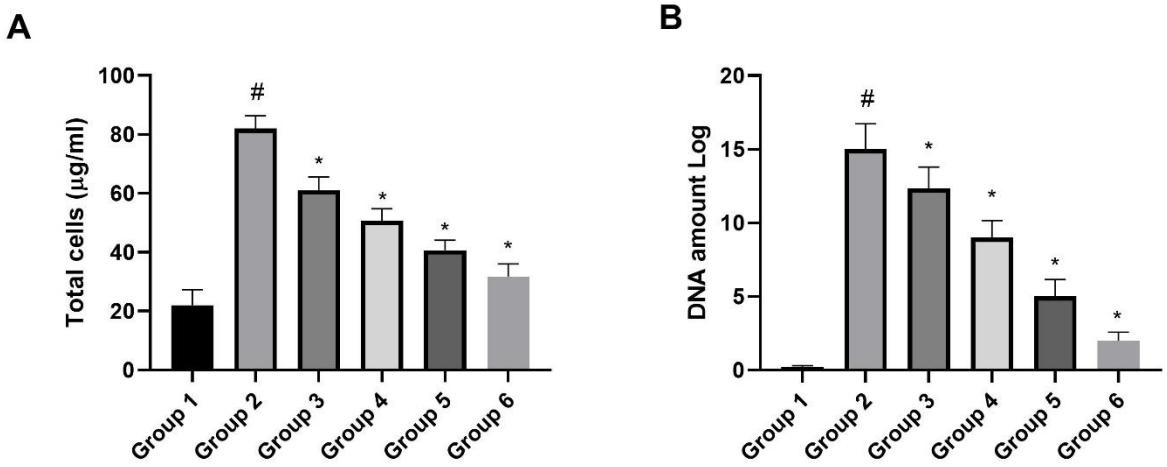

Fig. 3. Effect of SEAS on total cells and DNA content. Data (n = 3) were represented as the mean ± S.E.M. The level of significance was indicated by $^{\#}P < 0.01$ vs. Group 1, $^{\#}P < 0.05$ vs. Group 2.

Throughout the course of the infection of *M. pneumoniae*, there are triggered responses to inflammation and the production of inflammatory biomarkers such as IL-6, IL-1β, and TNF-α play a crucial part (Lee *et al.* 2021; Yin *et al.* 2021; Liu *et al.* 2022). Previous research has shown that increased levels of TNF-α, IL-1β, and IL-6 are expressed in the lungs of experimental animals infected with *M. pneumoniae* (Chu *et al.* 2004; Xu *et al.* 2016; Shi *et al.* 2023). This study demonstrated that DSE causes a dose-dependent reduction of TNF-α, IL-1β, and IL-6 as compared to M.P.-infected mice (Fig. 4). Thus, DSE could be a significant anti-oxidant and anti-inflammatory action.

The therapeutic effects of DSE observed in this study are likely due to specific bioactive compounds present within the extract. These components may contribute to its antibacterial, antioxidant, and anti-inflammatory properties, potentially acting through distinct molecular pathways. However, additional research is necessary to acquire a complete comprehension of the mechanisms that explain how DSE can combat pneumonia and exhibit its curative benefits. Future research should focus on isolating and characterizing these active compounds to determine their precise mechanisms of action. Advanced analytical techniques such as high-performance liquid chromatography (HPLC), Gas chromatography–mass spectrometry (GC-MS), mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy can help identify key phytochemicals.

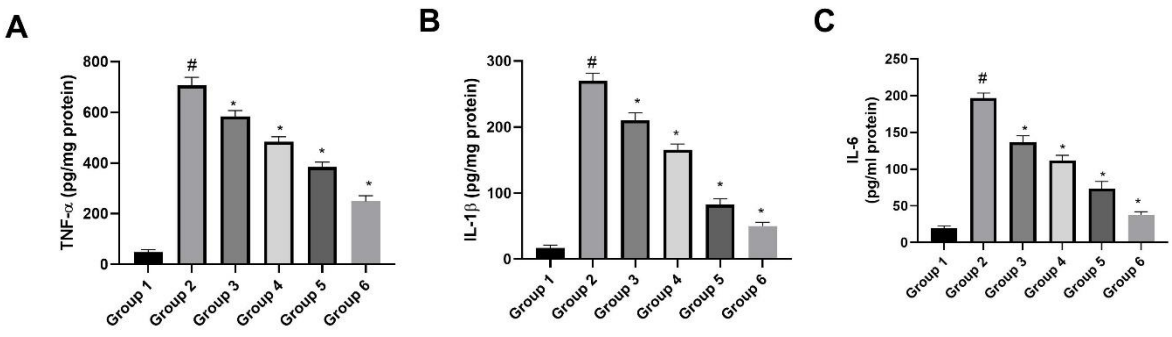

Fig. 4. Effect of DSE on pro-inflammatory cytokines. Data (n = 3) were represented as the mean ± S.E.M. The level of significance was indicated by $^{\#}P < 0.01$ vs. Group 1, $^{\#}P < 0.05$ vs. Group 2.

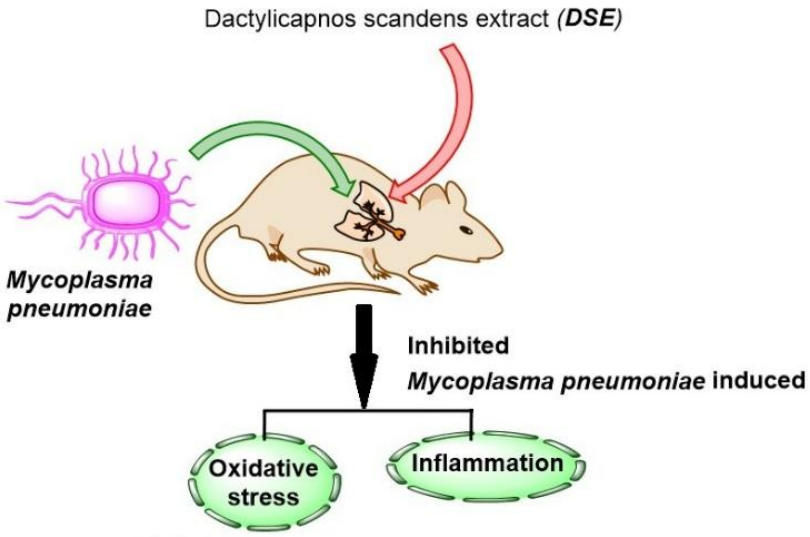

Fig. 5. Summary of DSE action against *Mycoplasma pneumoniae*-induced pneumonia

CONCLUSIONS

1. The results of this study documented the potent antibacterial activity of *Dactylicapnos scandens* extract (DSE) against *Mycoplasma pneumoniae* (MP) infection. It is the first report of its kind to exploit the antibacterial potential of DSE.
2. The DSE showed potent anti-oxidant and anti-inflammatory activity in MP-infected mice. It also showed potent inhibition of generation of pro-inflammatory cytokines in lung tissues of MP-infected mice, Fig. 5.
3. The pharmacological effect of DSE was observed to be comparable to Azithromycin as standard.
4. The conclusion drawn from this is that DSE has the potential to be an effective therapeutic agent in the treatment of pneumonia.

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