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The Effect of Zingiber officinale var. rubrum Extract on IL-6 Cytokine Levels in Pneumonia-Induced by Streptococcus pneumoniae on Rattus norvegicus

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ABSTRACT

Background: Pneumonia remains a leading cause of death from infectious diseases, often associated with elevated levels of proinflammatory cytokines such as Interleukin-6 (IL-6). Zingiber officinale var. rubrum (red ginger) contains bioactive compounds with potential anti-inflammatory effects. **Objectives:** To evaluate the effect of red ginger extract on IL-6 expression in the lung tissue of Wistar rats induced with Streptococcus pneumoniae, either as monotherapy or in combination with azithromycin. **Methods:** An experimental study was conducted using 30 male Wistar rats divided into five groups (n = 6 each): K1 (negative control), K2 (positive control), K3 (red ginger extract 400 mg/kgBW), K4 (azithromycin 11 mg/kgBW), and K5 (combination of red ginger extract and azithromycin). Pneumonia was induced intratracheally using a suspension of S. pneumoniae. Treatments were administered once daily for seven days. IL-6 expression in lung tissue was analyzed using immunohistochemistry and evaluated semi-quantitatively based on staining intensity and distribution. **Results:** The Kruskal-Wallis test showed a significant difference in IL-6 expression among groups ($p < 0.05$). Post hoc analysis revealed that K4 and K5 had significantly lower IL-6 expression than K2. No significant difference was found between K4 and K5, nor between K3 and K2, indicating that red ginger extract alone was not effective in significantly reducing IL-6 expression. **Conclusion:** Azithromycin, both alone and in combination with red ginger extract, effectively reduced IL-6 expression in rats with pneumonia. Red ginger extract alone did not show a significant anti-inflammatory effect in this model.
Keywords: Azithromycin, IL-6, Pneumonia, *Rattus norvegicus*, *Zingiber officinale var. rubrum*

ABSTRAK

Latar Belakang: Pneumonia masih menjadi salah satu penyebab utama kematian akibat penyakit infeksi, sering dikaitkan dengan peningkatan kadar sitokin proinflamasi seperti Interleukin-6 (IL-6). Zingiber officinale var. rubrum (jahe merah) mengandung senyawa bioaktif yang berpotensi memiliki efek antiinflamasi. **Tujuan:** Mengetahui pengaruh ekstrak jahe merah terhadap ekspresi IL-6 pada jaringan paru tikus Wistar yang diinduksi Streptococcus pneumoniae, baik sebagai monoterapi maupun dalam kombinasi dengan azitromisin. **Metode:** Penelitian eksperimental dilakukan menggunakan 30 ekor tikus Wistar jantan yang dibagi menjadi lima kelompok (n = 6 setiap kelompok), yaitu K1 (kontrol negatif), K2 (kontrol positif), K3 (ekstrak jahe merah 400 mg/kgBB), K4 (azitromisin 11 mg/kgBB), dan K5 (kombinasi ekstrak jahe merah dan azitromisin). Pneumonia diinduksi secara intratrakeal menggunakan suspensi S. pneumoniae. Perlakuan diberikan satu kali sehari selama tujuh hari. Ekspresi

IL-6 pada jaringan paru dianalisis menggunakan pemeriksaan imunohistokimia dan dinilai secara semi-kuantitatif berdasarkan intensitas serta distribusi pewarnaan. **Hasil:** Uji Kruskal-Wallis menunjukkan adanya perbedaan bermakna ekspresi IL-6 antar kelompok ($p < 0,05$). Analisis post hoc menunjukkan bahwa kelompok K4 dan K5 memiliki ekspresi IL-6 yang secara signifikan lebih rendah dibandingkan kelompok K2. Tidak ditemukan perbedaan bermakna antara K4 dan K5 maupun antara K3 dan K2, yang menunjukkan bahwa ekstrak jahe merah saja tidak efektif dalam menurunkan ekspresi IL-6 secara signifikan. **Kesimpulan:** Azitromisin, baik sebagai monoterapi maupun dalam kombinasi dengan ekstrak jahe merah, efektif menurunkan ekspresi IL-6 pada tikus dengan pneumonia. Ekstrak jahe merah sebagai monoterapi tidak menunjukkan efek antiinflamasi yang signifikan pada model penelitian ini.
Kata kunci: Azitromisin, IL-6, Pneumonia, *Rattus norvegicus*, *Zingiber officinale var. rubrum*

1. Introduction

Pneumonia is an acute respiratory tract infection that primarily affects the alveoli and distal airways. The etiology of pneumonia is diverse, encompassing various microorganisms such as bacteria, viruses, and fungi, depending on risk factors and the patient's immunological condition. The prevalence of pneumonia varies significantly across geographic regions, influenced by factors such as environmental hygiene, nutritional status, access to healthcare services, and community vaccination coverage.[1] Pneumonia remains a major global health problem, causing a high burden of morbidity and mortality, especially among children and the elderly.[2,3] According to the World Health Organization (WHO), pneumonia is the third leading cause of death globally and one of the most common infectious diseases.[4] The estimated global incidence of pneumonia ranges from 1.5 to 14 cases per 1,000 people per year, with higher rates among the elderly.[5]

The immune response to pneumonia involves several proinflammatory cytokines, including interleukin-6 (IL-6), which plays a role in cell differentiation, chemotaxis, and inflammation regulation. Uncontrolled inflammatory processes are key contributors to the pathophysiology of acute respiratory distress syndrome (ARDS), and persistent inflammation is strongly correlated with poor prognosis. Studies show that IL-6 levels correlate with disease severity scores such as APACHE II and CURB-65, and are linked to early mortality in patients with community-acquired pneumonia. Moreover, elevated IL-6 levels have also been detected in patients with antibiotic treatment failure.[4,6]

Pneumonia treatment guidelines recommend empirical therapy based on disease severity and the presence of risk factors for specific resistant pathogens.[7,8] One of the first-line therapies for pneumonia is the macrolide class, with azithromycin being commonly used. Several reasons support the use of azithromycin for pneumonia: it has a broad spectrum of activity covering gram-positive bacteria, some gram-negative bacteria, and atypical organisms that frequently cause pneumonia. In addition, azithromycin has excellent lung tissue penetration and remains at effective concentrations in lung tissue for several days even after the last dose.[9] In more severe cases or in cases where first- and second-line therapy fails, alternatives such as levofloxacin, linezolid, clindamycin, or vancomycin may be used.

Although these treatment methods are effective, antimicrobial therapy can be compromised by the emergence of organisms resistant to commonly used antibiotics. Streptococcus pneumoniae has developed resistance to various antibiotics including penicillin, macrolides, fluoroquinolones, and sulfamethoxazole-trimethoprim.[10] This resistance highlights the urgent need for novel or adjunctive therapies that can improve treatment outcomes for community-acquired pneumonia.

Indonesia possesses the second-highest biodiversity in the world, after the Amazon rainforest. Nearly 80% of the herbal plants used as traditional medicine in Southeast Asia can be found in Indonesia. With such rich medicinal plant resources, Indonesians widely consume herbal remedies, known locally as jamu.[11]

Zingiber officinale (ginger) is a herbal plant native to Asia. It is believed that China was the first country to cultivate Z. officinale, which then spread to India, Southeast Asia, West Africa, and the Caribbean. Ginger has long been used in traditional Chinese medicine to treat various ailments including heart disorders, gastrointestinal disturbances, diarrhea, and nausea.[12] The active compounds in ginger include essential oils, phenolics, flavonoids, carbohydrates, proteins, saponins, steroids, and terpenoids.[13] Ginger has been

reported to have antioxidant, immunomodulatory, anti-inflammatory, anti-rheumatic, hepatoprotective, and antimicrobial effects. It also exhibits antimicrobial activity against both gram-positive and gram-negative bacteria.[11,12]

A previous study reported that serum levels of proinflammatory cytokines such as IL-1, IL-6, and TNF-α were significantly reduced on days 5 and 10 in groups treated with ginger extract. This reduction was attributed to the inhibition of the cyclooxygenase-2 (COX-2) pathway by the extract.[14]

This study aims to investigate the effect of Zingiber officinale var. rubrum extract on IL-6 levels in a rat (Rattus norvegicus) model of pneumonia induced by Streptococcus pneumoniae.

2. Method

This study employed a post-test-only control group experimental design and was conducted at the Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara. Ethical approval for this study was obtained from the Ethics Committee of the Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara (No. 0624/KEPH-FMIPA/2024).

Thirty healthy male Wistar rats (Rattus norvegicus), weighing approximately 150–200 g, were acclimatized and randomly allocated into five groups (n = 6 per group): K1, negative control (non-infected and untreated); K2, positive control (induced with Streptococcus pneumoniae without therapy); K3, pneumonia-induced rats treated with red ginger (Zingiber officinale var. rubrum) extract at 400 mg/kgBW/day; K4, pneumonia-induced rats treated with azithromycin at 11 mg/kgBW/day; and K5, pneumonia-induced rats treated with a combination of red ginger extract (400 mg/kgBW/day) and azithromycin (11 mg/kgBW/day). Pneumonia was induced intratracheally using a suspension of Streptococcus pneumoniae. All treatments were administered once daily for seven consecutive days. On day 8, lung tissues were harvested for immunohistochemical (IHC) analysis of IL-6 expression. IL-6 staining intensity and distribution were evaluated semi-quantitatively, and the data were analyzed using the Kruskal–Wallis test followed by pairwise post hoc comparisons.

3. Result and Discussion

The Kruskal-Wallis test revealed a statistically significant difference in IL-6 expression among the groups ($p < 0.001$). Post hoc analysis demonstrated that Group K4 (azithromycin 11 mg/kgBW) and Group K5 (Zingiber officinale var. rubrum extract combination with azithromycin) exhibited significantly lower IL-6 expression compared to the positive control Group K2 ($p < 0.05$). However, no significant difference was observed between Group K4 and K5 ($p > 0.05$), indicating that the combination therapy did not produce an additive anti-inflammatory effect. Moreover, Group K3 (Zingiber officinale var. rubrum extract only) did not show a significant reduction in IL-6 expression compared to K2, suggesting limited efficacy as monotherapy.

Scoring from the IHC analysis showed the highest IL-6 expression in K2 (infected, untreated group), while the lowest was observed in the negative control group (K1). Representative IHC images supported the quantitative results, displaying reduced brown DAB staining in K4 and K5 compared to K2.

Differences in IL-6 Expression Between Groups

Table 1. Differences in IL-6 Expression Between Groups

Experimental Groups		Mean Rank	p-*value*
K1	K2	3.50 vs 9.50	0.002
	K3	3.50 vs 9.50	0.002
	K4	6.50 vs 6.50	1.000
	K5	6.50 vs 6.50	1.000
K2	K3	6.50 vs 6.50	1.000
	K4	9.50 vs 3.50	0.002
	K5	9.50 vs 3.50	0.002
K3	K4	9.50 vs 3.50	0.002
K4	K5	6.50 vs 6.50	1.000

Immunohistochemical Evaluation of IL-6 Cytokine Expression in the Lung Tissue of *Rattus norvegicus*

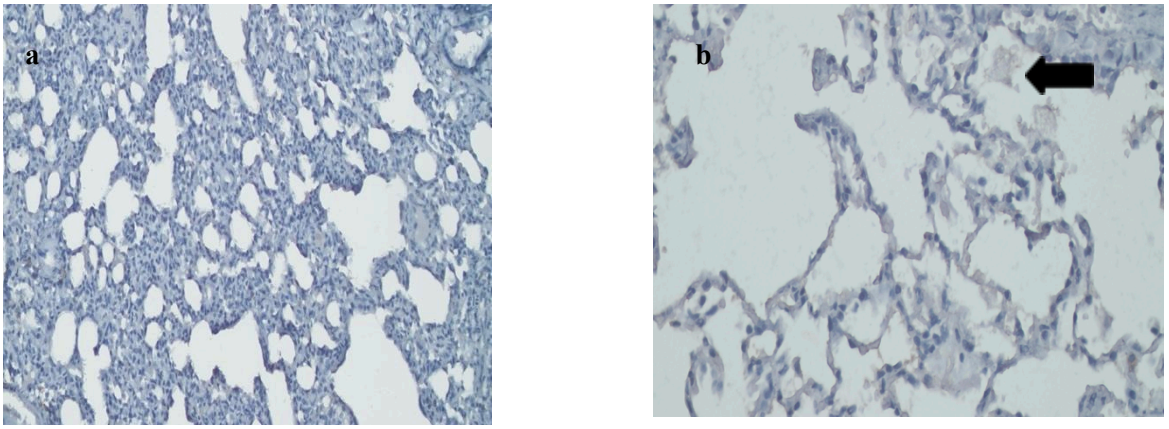

Figure 1. Representative immunohistochemical staining of IL-6 expression in lung tissue. (a) K1 (negative control) at 200× magnification and (b) K2 (positive control) at 400× magnification. Black arrows indicate IL-6-positive cells showing brown DAB staining. K2 demonstrates increased IL-6 expression compared with K1.

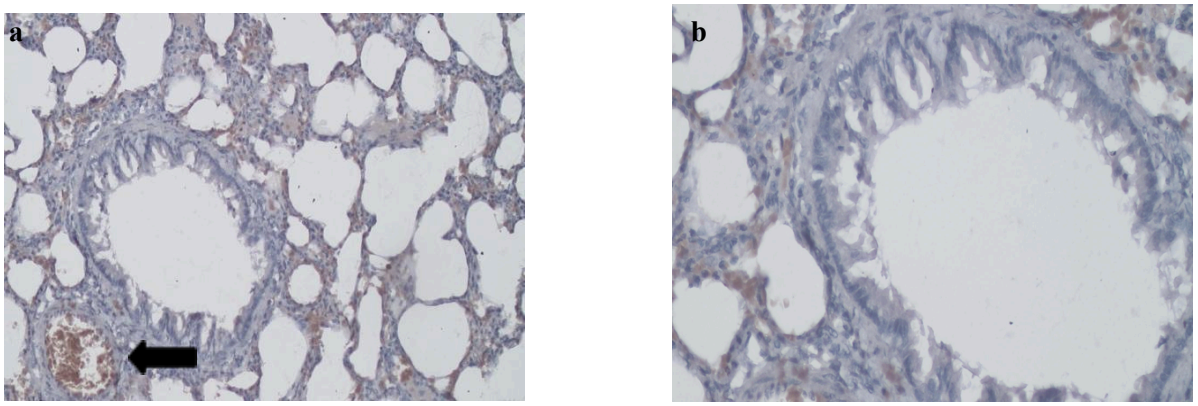

Figure 2. Representative immunohistochemical staining of IL-6 expression in lung tissue from K3 (red ginger extract group). (a) 200× magnification and (b) 400× magnification. Black arrows indicate IL-6-positive cells showing brown DAB staining in alveolar epithelial cells.

4. Conclusion

Interleukin-6 (IL-6) is a proinflammatory cytokine that plays an important role in the pathogenesis of pneumonia. Elevated IL-6 levels are associated with severe inflammation, lung tissue damage, an increased risk of acute respiratory distress syndrome (ARDS), and higher mortality rates. Therefore, reducing IL-6 levels is expected to help decrease the severity of inflammation and improve outcomes in patients with pneumonia. In this study, the combination of red ginger extract (Zingiber officinale var. rubrum) and azithromycin significantly reduced IL-6 expression compared to the positive control group. These findings are supported by previous studies demonstrating that red ginger possesses anti-inflammatory effects through the reduction of several inflammatory markers, including IL-6, TNF-α, CRP, and procalcitonin.[14,15,16,17] The absence of a significant reduction in IL-6 expression in the red ginger monotherapy group (K3) may indicate that the anti-inflammatory activity of red ginger alone was insufficient to overcome the intense inflammatory response triggered by Streptococcus pneumoniae infection in this model. In contrast, the significant reduction observed in the azithromycin-treated groups (K4 and K5) is likely related to the antimicrobial effect of azithromycin, which decreases bacterial burden and consequently reduces the stimulation of pro-inflammatory cytokine production. Red ginger may contribute through immunomodulatory and anti-inflammatory mechanisms, including suppression of NF-κB signaling and modulation of cytokine release; however, these effects appear to be supportive rather than directly antibacterial. Therefore, the beneficial effect observed in the combination group is more plausibly attributed to bacterial eradication by azithromycin, while red ginger may serve as an adjunctive agent that helps regulate the host inflammatory response.

In this study, red ginger administered as monotherapy did not show a significant reduction in IL-6 expression compared to the positive control group. The absence of a significant reduction in IL-6 expression in the red ginger monotherapy group may be explained by the different mechanisms of action between red ginger and azithromycin. Azithromycin directly reduces the bacterial burden by inhibiting bacterial protein synthesis, thereby eliminating the primary stimulus for IL-6 production and attenuating the inflammatory response.[9] In contrast, red ginger primarily exerts immunomodulatory and anti-inflammatory effects through inhibition of the NF-κB signaling pathway and suppression of pro-inflammatory cytokine production rather than through direct antibacterial activity.[18-21] Therefore, modulation of the inflammatory response alone may not be sufficient to significantly reduce IL-6 expression in the presence of an ongoing bacterial infection. This mechanism may also explain why the combination of red ginger and azithromycin did not produce a significantly greater reduction in IL-6 expression than azithromycin alone. Once bacterial eradication had been achieved by azithromycin, the additional immunomodulatory effect of red ginger was insufficient to generate a measurable additive effect under the present experimental conditions.[17, 25-29]

Several previous studies have also reported inconsistent findings regarding the effect of red ginger on reducing IL-6 levels in pneumonia and bacterial infections.[17,25,26,27-29] These differences may be influenced by variations in dosage, duration of administration, animal or human models used, and the complexity of the inflammatory pathways involved. In this study, a dose of 400 mg/kgBW/day was used based on previous studies that demonstrated significant anti-inflammatory effects in animal models.[30] Pharmacologically, red ginger still has potential as an adjuvant therapy for pneumonia to help reduce inflammation.17 However, further studies with stricter designs, larger sample sizes, and wider variations in dosage and duration of administration are still needed to confirm its effectiveness in reducing IL-6 levels in pneumonia.[31,32]

5. Recommendations

Red ginger extract was ineffective as monotherapy for reducing IL-6 expression in rats with Streptococcus pneumoniae-induced pneumonia. In contrast, azithromycin, administered either alone or in combination with red ginger extract, significantly reduced IL-6 expression, while the combination provided no additional benefit over azithromycin alone.

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