Chapter 1

INTRODUCTION

In early 2019, a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic occurred, later introduced as CoronaVirus Disease (COVID-19). COVID-19 has accounted for 3.4% mortality rate worldwide (Hopkins, 2021). Coronavirus mainly infect the upper respiratory tract, which causes flu-like symptoms and for mild to severe affects the lung. In addition to COVID-19 itself, pneumonia is also considered as a global concern during this pandemic. This is due to the fact that pneumonia is commonly found as secondary bacterial co-infection in severe COVID-19 infection, namely COVID Pneumonia which accounts for higher morbidity and mortality rate than COVID-19 infection alone (Sharifipour et al., 2020). The rapid disease progression is supported by the occurrence of acute respiratory distress syndrome (ARDS) which progresses to fatal acute lung injury (ALI), that mostly follows the aftermath of secondary lung infection and causes death in severe cases. Reported in August 2020, the incidence of ARDS in COVID-19 with secondary bacterial pneumonia has reached 15.2%, and in severe cases, accounts for 18.1% of all infections (Manohar et al,2020; Zhang, Chen & Meng, 2020). ARDS are considered as the milder case of ALI which are indicated by over recruitment of neutrophils, severe hypoxemia, pulmonary edema, and the accumulation levels of granulocytes inside the lung tissue (Yingkun et al.,2012).

Pneumonia was classified into community-acquired pneumonia (CAP), hospital-acquired pneumonia (HAP), as well as ventilator-associated pneumonia (VAP) (Jain et al.,2021). According to Wu, Adhi & Highland (2020), it was found 27.8% of the patients infected with COVID-19 are mostly followed by secondary bacterial infection, in regard to HAP. Meanwhile, the occurrence of COVID-19 cases followed by secondary bacterial infection due to VAP is only shown in 5 to 10 cases per 1,000 hospital admission. The causative agent includes the resistant gram-positive bacteria, methicillin-resistant *S. aureus* (MRSA), which was found in 47% of patients, as well as Gram-negative

pathogen *Escherichia coli* and *Klebsiella pneumoniae* although cross-contamination also raised concern (Manohar et al, 2020; Wu, Adhi & Highland, 2020).

It was reported that pneumonia bacterial co-infection in moderate to severe cases of COVID-19 contributes to higher mortality rates worldwide (Sharifipour et al., 2020). The most prominent clinical finding is the presence of cytokine storm which is regarded as a 'red flag' in COVID Pneumonia. Research shown that the administration of immunomodulators, parallel to the first-line treatment, able to reduce the risk of cytokine storm occurrence in COVID-19 patients and improve recovery process (Choudhary, Sharma, Singh & Silakari, 2020; Burrage, Koushesh, & Sofat, 2020). Recent research from Ye, Wang & Mao (2020) reported the usage of immunomodulators to regulate inflammatory responses are considered as an effective measures to improve the prognosis of Human endemic coronavirus (HCoV) infection due to past experience in mitigating severe acute respiratory syndrome (SARS) and middle east respiratory syndrome (MERS). During these cases, immunomodulators are shown effective to control the inflammatory response in the early stage of infection and help to restore immune balance in the patient (Ye, Wang, & Mao, 2020).

Immunomodulators such as JAK/STAT inhibitors, IL-6 blockade, and corticosteroid drugs have been used during COVID pneumonia treatment to improve patient recovery (Choudhary, Sharma, Singh & Silakari, 2020). Previous studies showed it is beneficial to give immunomodulators in the early phase of infection or if the symptoms worsen due to hyperinflammation. Proinflammatory cytokines namely, IL-6, IL-1β, IFNG, TNF-α, Type I IFN was found to be elevated in the presence of cytokine storm, thus ensuring a lower antiviral response. During this phase, the use of antivirals and administration of immunomodulators can be an alternative to induce immune balance restoration by initiating apoptosis of infected cells (Feuillet, Canard, & Trautmann, 2021). Therefore, came the statement that the current need to fight this pandemic is to find far-reaching immunomodulators that are able to act as both anti-inflammatory and antibacterial to improve patient recovery.

In this experiment, three Indonesian plant extract treatment, namely sappan wood, clove,

and neem will be evaluated for their immunomodulatory activity through the relative pro-inflammatory cytokine gene fold ratio expression observation from the mice's lung using quantitative reverse transcriptase real time PCR (qRT-PCR) methods. This project aims to investigate the Indonesian plant extract activity as a proof of concept for its natural immunomodulator potential.

In this experiment, two target genes namely, IL1B and IFNG that play a key role in bacterial-host secondary pneumonia infection will be further evaluated. The immunomodulatory activity of the plant extract will be assessed after the administration of the chosen plant extracts *in vivo* for 7 days with LPS-induction 7 hour prior to mice sacrifice via intranasal to mimic ARDS in humans. The hypothesis made is that the plant extract will show immunomodulatory effect by cytokine gene expression observation from qRT-PCR results.