CHAPTER IV

DISCUSSION

Influenza type A H1N1 virus infection is acute respiratory infectious disease that causes significant morbidity and mortality in 2009 pandemic outbreak worldwide (1, 2). World Health Organization (WHO) declared influenza pandemic on June 11, 2009 (1, 3, 5). It has spread to over 74 countries around the world and over 440,000 cases, including 5,700 deaths, have been reported up to October 25, 2009. Influenza type A H1N1 virus induces proinflammatory cytokines production, which lead to cytokine storm. This disease pathogenesis factor results in respiratory failure and lung inflammatory infiltrates (6, 7). The current tools to prevent and control influenza virus include vaccines and antiviral drugs. Despite success in the development of new antiviral agents such as oseltamivir in recent years, problems regarding these chemotherapeutic drugs have been reported: adverse effects, risk of emergence of resistant viruses, and loss of effects due to serotype variation (28-29). Therefore, the development of safe and effective anti-influenza virus is still needed.

Plants have a long evolutionary history with respect to developing resistance against viruses and are increasingly drawing attention as potential sources for development of antiviral drugs (73). Physical activities of components extracted from plants such as phytochemicals have recently been attracting attention. Many studies reported that extracts from various plants posses anti-inflammatory effects. Our study focus on phytochemicals; including xanthone from mangosteens, sesamin from

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sesame seed and *Andrographis paniculata* extract, *Moringa oleifera* extract, *Herricium erinaceus*, and *p*-hydroxycinnamaldehyde from *Alpinia galanga.* All of them have been reported to possess anti-inflammatory effect.

In the previous study, the mouse infection model has been use as a tool for studying the anti-influenza virus effect on early immune responses during influenza virus infection (74). Nevertheless, the anti-influenza virus effect of phytochemicals which are cytokine storm inhibition and increase immune response on peripheral blood mononuclear cells (PBMCs) model has never been studied. Therefore, we investigated the effect of phytochemicals from herbs on IL-1β, TNF-α, and IL-2 release from influenza type A H1N1-induced PBMCs model, which modified Brincks E.L. method (69). PBMCs suspension contains about 10% monocytes, which release IL-1β, TNF-α and 90% lymphocytes, which only 41.4% helper T lymphocytes are release IL-2 (69). Our preliminary screening has shown that xanthone from mangosteen, sesamin from sesame seed and *Andrographis paniculata* extract could reduce IL-1β and TNF-α level and increase IL-2 level in dose dependent manner. These indicated that xanthone, sesamin, and *Andrographis paniculata* extract contained anti-inflammatory activity.

IL-1β and TNF-α have important role in stimulating effects on neutrophil and macrophage functions (20). Both cytokines strongly up-regulate leukocyte adhesion molecules on the vascular endothelium, thereby mediating the first essential step for recruitment of neutrophils and/or macrophages into the respiratory tract. Many studies reported that IL-1β and TNF-α stimulate their own production or release of other cytokines such as IL-6, IL-8 and other chemokines, which lead to cytokine storm (18, 21). Moreover, macrophages are antigen presenting cells to stimulate adaptive host

defense T-cell by presenting influenza A viral peptide that production of interleukin-2 (IL-2) that send signaling to white blood cells to be ready against virus (22). IL-2 is a growth, survival, and differentiation factor for T lymphocytes, and plays a major role in regulation of T cell responses though its action on regulatory T cells (13, 15). In this study, we found that xanthone, sesamin and *Andrographis paniculata* extract, could reduce gene expression of IL-1β and TNF-α while increase IL-2. Surprisingly, our data firstly revealed the induction effects of oseltamivir (Tamiflu®) on expressions of IL-1β, TNF-α and IL-2. These may suggest the action of this drug on balancing immune system to against virus.

The effective dose at IC50 of these phytochemicals; xanthone, sesamin and *Andrographis paniculata* extract on down regulation of IL-1β mRNA level were 2.8, 2.6, 2.6 µg/ml and reduce TNF-α mRNA level are 2.7, 1.0, 1.0 µg/ml while increase IL-2 mRNA level are 5.3, 3.5, 0.7 µg/ml, which shown in the appendix in Table 2. These indicated the highest activity of *Andrographis paniculata* extract comparison with sesamin and xanthone, respectively.

Andrographolide ($C_{20}H_{30}O_5$) is the major active compound isolated from *Andrographis paniculata*, which is possessing anti-inflammatory activity (49-50). Andrographolide contains a strong anti-inflammatory effect that is active both *in vitro* and *in vivo*, and as effective as the steroid drug-dexamethasone. It appears to act primarily by inhibiting the expression of mRNA for inflammatory cytokines (50), that this effect is associated with inhibition of intracellular ERK1/2 and Akt signal transduction pathways (51). Further studies are required to investigate the releasing of the other cytokines such as IL-6, IL-8, IFN-γ and give more insight into molecular

mechanism, in which andrographolide reducing *IL-1β* and *TNF-α* and increasing *IL-2* genes though ERK1/2 and Akt signaling pathway in the conditional media.

CONCLUSION

In conclusion, xanthone, sesamin and *Andrographis paniculata* extract inhibited cytokine storm and induced immune cells in influenza type A H1N1-induced peripheral blood mononuclear cells (PBMCs). These 3 phytochemicals inhibited cytokine storm by reducing the release of IL-1β and TNF-α and enhancing the activity of immune cells via increasing of IL-2 mRNA expression and protein release. Therefore, these phytochemicals are of interest to use for further study and might be the new pharmacological agents for prevention and treatment immune induced inflammatory disorders. The effect of these phytochemicals on other cytokines using PCR array will be further investigated including their involved signaling pathways.