5. Discussion

When a pathogen invades the human body, it will trigger an inflammatory response, and the pathogen will be recognized and destroyed. Inflammation also plays an important role in restoring tissue structure and function to normal. In order to initiate pathogenesis, neutrophils and macrophages will be recruited to the site of inflammation (Cunha et al., 2008; Williams et al., 2011; Zhang & Wang, 2014). However, the over-activation of macrophages and neutrophils may occur due to the presence of inflammatory stimuli such as gram-negative bacterial endotoxin lipopolysaccharides (LPS). This phenomenon induces cells to produce inflammatory mediators such as nitric oxide (NO), and may cause inflammatory diseases (Czura, Friedman, & Tracey, 2003; Muralidharan & Mandrekar, 2013).

The activation of macrophages and neutrophils plays an important role in the process of inflammation, so they can be used as biomarkers of inflammation. In the early stages of inflammation, neutrophils will accumulate and interact with resident cells, and local inflammatory mediators will amplify the inflammatory response by releasing more pro-inflammatory cytokines and mediators. This phenomenon will further increase the number of neutrophils (Cunha et al., 2008; Zhang & Wang, 2014).

In addition, pro-inflammatory cytokines help to up-regulate the expression of adhesion molecules, thereby increasing the recruitment of neutrophils in inflamed tissues (Williams et al., 2011;Mitroulis et al., 2015) and prolonging the survival of neutrophils (Dibbert et al., 1999; Sikora, 2002). The activation of neutrophils also produces more oxygen free radicals at an increased rate, and releases granzymes during the inflammatory cascade induced by LPS (Matthay & Zimmerman, 2005; Tan et al., 2016).

To evaluate protective effect of jaboticaba against LPS-induced inflammation, three parameters inclding NO production, macrophago recruitment, and neutrophils level were determined using zebrafish model. NO concentrations in zebrafish embryos were determined by Griess stain. To investigate the recruitment of inflammatory cells, neutral red and sudan black staining were used to detect the levels of macrophage and neutrophils, respectively.

In this study, LPS-induced inflammation is confirmed by evidence of increased NO production and recruitment of macrophages and neutrophils. Jaboticaba extracts, both peel extract or seed extract, had shown potential anti-inflammatory effects. Different

extract solvents (95% ethanol, 50% ethanol and water extract) could extract anti-inflammatory components.

The results of individual size, total number and total size of neutrophils were inconsistent. In particular, the individual size and total number of neutrophils of zebrafish vary greatly among the groups. Generally speaking, treatment with jaboticaba extracts could reduce the individual size and total number of neutrophils, indicating that jaboticaba extracts had a potential anti-inflammatory effect on neutrophils. Further study may be needed to understand which bio-compound has a key anti-inflammatory effect and the detailed mechanism of how it affects neutrophils.

Jaboticaba is also known to be rich of ellagic acid (Abe et al., 2012) and this compound has significant role in suppresing inflammation (Lee et al., 2013; Guan et al., 2017). According to the research of Lee et al. (2013), ellagic acid (0.3 and 1.0 μM) and strawberry extracts rich in ellagic acid could attenuate expression of iNOS, TNF-α and IL-1β in LPS-treated RAW 264.7 macrophages . As for the mechanism, it seems that strawberry extract rich in ellagic acid could inhibit the NF-κB pathway, thereby inhibiting LPS-induced inflammation (Lee et al., 2013).

Guan et al. (2017) reported that ellagic acid pretreatment resulted in a significant reduction in inflammatory cells, including neutrophils, and also reduced levels of TNF-α, IL-6, and IL-8. The anti-inflammatory effect of ellagic acid on acute lung injury caused by LPS might be through inhibiting the activation of the NF-κB pathway. We suspect that a similar mechanism may occur with the effect of jaboticaba as an anti-inflammatory agent.

Blackberry contains abundant amount of anthocyanin, which also shows anti-inflammatory effects on macrophages. RAW 264.7 macrophages treated with blueberry-blackberry dealcoholized fermented beverage for one hour and LPS-induced inflammation could be reduced by the evidence in reduction of nitric oxide and TNF-α (Garcia-Diaz et al., 2015). The author proposed that the anthocyanin in the test beverage was responsible for anti-inflammatory ability.

From the results shown in this study, jaboticaba has potential anti-inflammatory effect in dose-dependent manner. Between jaboticaba peel and seed, the first tends to have better effect and extraction with 95% ethanol is shown to have more favorable result. According to Lenquiste et al., (2015), extraction with ethanol is supposed to yield better

results compared to water extraction. Possible reason for this is due to the large content and composition variation of bioactive compounds in plants, most of which are more soluble with ethanol rather than water (Jaakola & Hohtola, 2010; Kumazawa, Hamasaka, & Nakayama, 2004).

However, zebrafish larvae which has been treated with jaboticaba showed attenuation of NO in a dose-dependent manner, indicating anti-inflammatory potential of jaboticaba. The same result is also reported by da Silva-Maia et al., (2019).

6. Conclusion

From the results shown in this study, jaboticaba has potential anti-inflammatory effect in dose-dependent manner. Between jaboticaba peel and seed, the first tends to have better effect and extraction with 95% ethanol is shown to have more favorable result. Further research is needed to evaluate the bioactive compounds in jaboticaba that may reduce inflammation. In addition, it is necessary to understand the detailed mechanism of how jaboticaba affects pro-inflammatory mediators and cytokines.