Level of Cytokine Interleukin-6 and Interleukin 1-β on Infectious Rat Model Treated with *Etlingera elatior* (Jack) R.M. Smith Fruit Extract as Immunomodulator

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Abstract

*Etlingera elatior* (Jack) R.M Smith or locally in Southeast Sulawesi known as wualae fruit has activity as an immunomodulator by increasing phagocytosis activity. Prior studies have been conducted to observe the effect of *E. elatior* as an immunomodulator, thus further study is needed to observe the production of cytokines such as IL-1β and IL-6 which are responsible for the immune responses. *Etlingera elatior* fruit macerated with 96% ethanol for three days and produced a total of ±74.6 g concentrated extract. Experimental animals used were divided into four groups (n=4) and treated orally once per day for seven days as follows: group I (0.5% Na-CMC); group II (Stimuno®); group III (*E. elatior* fruit extract concentration of 300 mg/kg BW); and group IV (*E. elatior* fruit extract concentration of 400 mg/kg BW). On the eight-day, animals were infected with *Staphylococcus aureus* intraperitoneally and left for an hour. Thereafter, blood was collected and assayed using ELISA Kit (Elabscience rat IL-1β and Elabscience rat IL-6). Result demonstrated that group IV increased levels of IL-1β and group III and IV increased level of IL-6 (p<0.05). Increased levels of IL-1β and IL-6 are associated with phagocytosis in the immune response. In conclusion, *E. elatior* fruit extract at concentration of 300 and 400 mg/kg BW increases levels of IL-1β and IL-6.

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INTRODUCTION

*Etlingera elatior* (Jack) R.M. Smith is one of the spices native to Indonesia which belongs the Zingiberaceae family. It has been used traditionally used as medicines and flavor enhancers (Farida & Maruzy, 2016; Syarif et al., 2015). *Etlingera elatior* fruit is known as wualae by people in the Konawe region of Southeast Sulawesi as spices. Besides that, it empirically used as traditional medicine as immunomodulator in recovery of typhoid fever in the
North Kolaka district of Southeast Sulawesi (Sahidin et al., 2019; Wahyuni et al., 2017). Immunomodulator is drugs that modify response immune system increasing the production of serum antibodies, namely immunostimulators, thus enhance immune responses against infectious diseases, tumours and immunodeficiency (primary or secondary); and by decreasing production of serum antibodies, namely immunosuppressive, thus reduce the immune responses against autoimmune disease or transplanted organs (Catanzaro et al., 2018; Bascones-Martinez et al., 2014).

Previous studies reported that E. elatior methanol extract contains flavonoids, phenolics, alkaloids, and saponins. Secondary metabolite compounds that may have immunomodulatory activity are flavonoids (Juwita et al., 2018; Lachumy et al., 2010; Handayani et al., 2014). Flavonoids can increase the activity of lymphocyte proliferation thus prevents invasion of viruses, bacteria, and other microbes (Panche et al., 2016). Further studies demonstrated that phagocytic activity of macrophages increase after administration of E. elatior (Fristiohady et al., 2019; Wahyuni et al., 2017). Increased activation of macrophage and lymphocytes stimulate secretion of cytokines such as interleukin (IL)-1β and IL-6 (Kany et al., 2019). They play important role in inflammatory reactions and induces antibody production (Besung et al., 2016; Duque & Descoteaux, 2014).

There is no study have been reported about the effect of E. elatior fruit from Southeast Sulawesi as immunomodulator by increasing production of cytokines such as IL-1β and IL-6. Thus, this study aims to investigate the effect of cytokines secretes in model rats infected with Staphylococcus aureus as immunomodulator using E. elatior as local plants from Southeast Sulawesi.

MATERIALS AND METHODS

Material

Material used in the study were E. elatior Fruit (in local name, wualae), male Wistar rats, inocula S. aureus ATCC 25923, Rat IL-6 ELISA Kit (Elabscience®), 96% ethanol (Mercks® (technical grade)), 70% ethanol (Mercks® (technical grade)), methanol (Mercks® (technical grade)), ether (Mercks® (technical grade)), aqua pro injection (Otsu-NS®), 0.5% Na-CMC (Mercks®), 0.9% NaCl (Otsu-NS®), nutrient agar (Merck®), and commercial Phylantus niruri extract (Stimuno®).

Sample determination

Determination of E. elatior plants was conducted to ensure the validity of the samples used in the study. Determination was conducted by observing the morphological characteristics of E. elatior plants based on references and proven at the Researches Center For Biology, Indonesian Institute of Sciences/Lembaga Ilmu Pengetahuan Indonesia, Cibinong, West Java (No. 355/1PH.1.01/I/07/II/2017).

Sample collection, preparation, and extraction

Etlingera elatior fruit sample as amount 20 kg was collected in Kalu-kaluku Village, Kodeoha Subdistrict, North Kolaka Regency, Southeast Sulawesi. Collected samples were then continued by wet sorting, separated from the fruit stalks, washed with running water, then cut into smaller sizes, and dried in the sun. After sample was dried, dry sorting were conducted and then powdered with a weight of 2.1 kg obtained.

The powder sample was then macerated with 96% ethanol for 3 x 24 hours with a sample : solvent ratio of 1 : 2. Every 24 hours the solvent was filtered and replaced with a new solvent to obtain the filtrate for the first to the third day. All filtrates were then collected and concentrated using a rotary vacuum evaporator at 50ºC.
The total amount of concentrated extract produced was ±74.6 g with a yield of 3.5%.

**Bacteria**

*Staphylococcus aureus* was planted into slanted agar and incubated for 24 hours in an incubator at 37°C. Inocula is then suspended with 0.9% NaCl until a turbidity equal to the McFarland 0.5 standard is obtained.

**Experimental animals**

A total of 32 male Wistar rats used were acclimatized for seven days. The acclimatization temperature is 23-25°C in a light/dark cycle for 12/12 hours and is fed with chow pellet diet and access to ad libitum water. All experiments involving these animals were carried out with the approval of the Animal Ethics Committee from Universitas Halu Oleo (No.2739/UN29.20/PPM/2018).

**Experimental design**

Experimental animals were divided into four groups (n = 4) and treated orally once every day for seven days. The division of groups is done based on different treatments as follows:

1. Group I: negative control (0.5% Na CMC)
2. Group II: positive control (*Phyllanthus niruri* extract/Stimuno®)
3. Group III: *E. elatior* fruit extract with concentration of 300 mg/kg BW
4. Group IV: *E. elatior* fruit extract with concentration of 400 mg/kg BW

On the eighth day, each animal was infected with 0.5 mL *S. aureus* suspension intraperitoneal and left for one hour. Animal blood was then collected with cardiac puncture as much as 3 mL and put in a test tube containing EDTA. The tube was then centrifuged for 15 minutes at 3000 rpm and a temperature of 25°C. Blood plasma was then tested using the ELISA Kit (Elabscience® rat IL-1β and Elabscience® rat IL-6).

**RESULTS AND DISCUSSION**

Administration of extracts in experimental animals is carried out for seven consecutive days orally once per day, which aims to stimulate the immune system of each group of experimental animals. On the eighth day, *S. aureus* inocula is injected into animals intraperitoneally. *Staphylococcus aureus* is gram-positive bacteria that can cause infections both in humans and animals. They do not produce protein A, which is an antiphagocyte protein, causing *S. aureus* unable to avoid phagocytosis of peritoneal macrophages (Hariyanti et al., 2015; Wahyuni et al., 2017). After being injected with a bacterial inocula, all the experimental groups were left for an hour to make the innate immune system work. The innate immune system can be active within the range of 0-12 hours after infection (Abbas et al., 2016).

Macrophages and neutrophils, including the first line of defense in the immune system. Macrophages are able to fight off infections for about an hour before the immune mechanism is mobilized. On this basis, macrophage taking is done one hour after bacterial induction, so it can be seen the extent of the ability of macrophages to conquer bacterial invasion (Chaplin, 2010). Macrophages enter the site of infection are increased and the phagocytic ability of antigens also increases. In addition, levels of IL-1β and IL-6 also increased (Besung et al., 2016).

Measurement of plasma IL-1β shows that the highest IL-1β levels is the group IV (concentration of 400 mg/kg BW) (p <0.05) compared to group III (concentration of 300 mg/kg BW) and group II (positive control). The average level of IL-1β in Group II was higher than the concentration of group III (p <0.05). Group I (negative control group) had lower levels of plasma IL-1β value compared to group II but not significant (p >0.05). These results are shown in Figure 1.
While in measurement of Plasma IL-6, both groups III and IV had higher levels of plasma IL-6 compared to group I (p <0.05). Both groups also demonstrated better activity by providing a significant difference to group II (p <0.05). These results are presented in Figure 2.

Increased levels of IL-1β at concentration of 400 mg/kg BW and IL-6 at concentration of 300 and 400 mg/kg BW are thought to be caused by the presence of flavonoid compounds in E. elatior fruit extracts. According to previous research by Wahyuni et al. (2017), phytochemical screening of E. elatior fruit showed the presence of flavonoid compounds. Flavonoid compounds are known to have the ability to enhance the immunomodulatory system by increasing the effectiveness of the proliferation of lymphokines produced by T cells, thereby stimulating phagocytic cells to respond to phagocytosis. Flavonoids also accelerate the proliferation and differentiation of macrophages, so that macrophage migration increases (Ginwala et al., 2019).

Increased migration of macrophages to stimuli causes the number of macrophages in the peritoneum to increase. Accelerated macrophage migration occurs due to increased levels of IL-1β and IL-6 produced by monocytes and macrophage cells (Atri et al., 2018). In addition, an increase in the number of T lymphocytes triggers macrophage activation so that bacterial phagocytosis will increase (Abbas et al., 2016).

CONCLUSION

_Etlingera elatior_ fruit extract increase levels of plasma IL-1β and IL-6 in male Wistar rats after administration concentration of 300 and 400 mg/kg BW. This increased level is associated with an immune response.

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