Borneo Journal of Pharmacy
Vol 3 Issue 2 May 2020
Page 52 – 57

http://journal.umpalangkaraya.ac.id/index.php/bjop/article/view/1318
DOI: https://doi.org/10.33084/bjop.v3i2.1318
e-ISSN: 2621-4814

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

Adryan Fristiohady [1]
Wahyuni [1]
Fadhliyah Malik [1]
Muhammad Ilyas Yusuf [1]
Wa Ode Salma [2]
Rini Hamsidi [3]
Fredy Talebong [1]
Yuliansyah [1]
La Ode Muhammad Julian Purnama [1]
Saripuddin [1]
Sahidin [1*]

[1]Department of Pharmacy, Universitas Halu Oleo, Kendari, Southeast Sulawesi, Indonesia

[2]Department of Nutrition Science, Universitas Halu Oleo, Kendari, Southeast Sulawesi, Indonesia

[3]Department of Health, Universitas Airlangga, Surabaya, East Java, Indonesia

[*]email: sahidin02@uho.ac.id

Keywords:
Etlingera elatior (Jack) R.M. Smith
Immunomodulator
Plasma IL-1β
Plasma IL-6

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). Results 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.

Received: March 24th 2020
Accepted: April 16th 2020
Published: May 21st 2020

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/IPH.1.01/If.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 (*Phylantus 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 intra peritoneal 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 intra peritoneally. *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**.

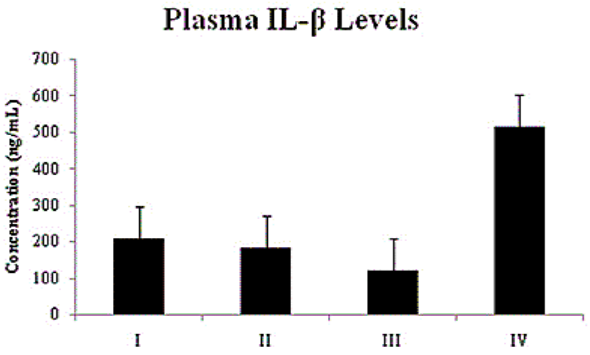

Figure 1. Measurement of Plasma IL-1β levels. Group I as negative control, group II as positive control, group III as treatment group concentration of 300 mg/kg BW and Group IV as treatment group concentration of 400 mg/kg BW

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**.

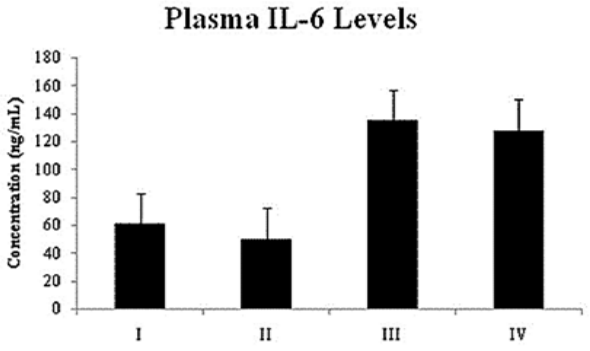

Figure 2. Measurement of Plasma IL-6 levels. Group I as negative control, group II as positive control, group III as treatment group concentration of 300 mg/kg BW and Group IV as treatment group concentration of 400 mg/kg BW

Increased levels of IL-1β and Il-6 in the negative control group may have been caused by bacterial infections that triggered an immune response in the rat's body. When an infection occurs, the immune system will respond by sending phagocytic cells to the site of infection by removing two types of cytokines consisting of IL-1β and IL-6. Both IL-1β and IL-6 levels are normally expressed in low amounts and increase during the infection process (Lopez-Castejon & Brough, 2011; Sahoo *et al*., 2011).

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.

ACKNOWLEDGMENT

The authors thank the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia for a research grant scheme/Penelitian Dasar Unggulan Perguruan Tinggi 2018 (No. 519a/UN29.20/PPM/2019) and Adryan Fristiohady was supported by Penelitian

Dasar Internal Universitas Halu Oleo 2019 (No.1584b/UN29.20/PPM/2019) for financial support.

REFERENCES

Abbas, A.K., Andrew, H.L., & Shiv, P. (2016). *Basic Immunology Functions and Disorders of the Immune System Fifth Edition*. Amsterdam, Netherlands: Elsevier.

Atri, C., Guerfali, F.Z., & Laouini, D. (2018). Role of Human Macrophage Polarization in Inflammation during Infectious Diseases. *International Journal of Molecular Sciences, 19*(6), 1801. doi:10.3390/ijms19061801

Bascones-Martinez, A., Mattila, R., Gomez-Font, R., & Meurman, J.H. (2014). Immunomodulatory drugs: Oral and systemic adverse effects. *Medicina Oral Patologia Oral y Cirugia Bucal, 19*(1), 24-31. doi:10.4317/medoral.19087

Besung, I. N. K., Astawa, N. M., Suata, K., & Suwiti, K. (2016). Hubungan Antara Aktivasi Makrofag Dengan Kadar Interleukin-6 Dan Antibodi Terhadap Salmonella Typhi Pada Mencit (Relationship between the Macrophage Activity with Interleukin-6 Levels and Titers of Antibodies against Salmonella typhi). *Jurnal Kedokteran Hewan, 10*(1), 1–4. doi:10.21157/j.ked.hewan.v10i1.3359

Catanzaro, M., Corsini, E., Rosini, M., Racchi, M., & Lanni, C. (2018). Immunomodulators Inspired by Nature: A Review on Curcumin and Echinacea. *Molecules, 23*(11), 2778. doi:10.3390/molecules23112778

Chaplin, D.D. (2010). Overview of the Immune Response. *Journal of Allergy and Clinical Immunology, 125*(2 Suppl 2), 3-23. doi:10.1016/j.jaci.2009.12.980

Duque, G.A., & Descoteaux, A. (2014). Macrophage cytokines: Involvement in immunity and infectious diseases. *Frontiers in Immunology, 5*, 491. doi:10.3389/fimmu.2014.00491

Farida, S. & Maruzy, A. (2016). Kecombrang (Etlingera elatior): Sebuah Tinjauan Penggunaan Secara Tradisional, Fitokimia Dan Aktivitas Farmakologinya. *Jurnal Tumbuhan Obat Indonesia, 9*(1), 19-28. doi:10.22435/toi.v9i1.6389.19-28

Fristiohady, A., Zubaydah, W.O.S., Wahyuni, W., Mirda, M., Saripuddin, S., Andriani, R., Purnama, L.O.M.J., & Sahidin, S. (2019). Immunomodulator Activity of Effervescent Granule of Wualae Fruit (Etlingera elatior (Jack) R.M. Smith) Based on Specific Phagocytic Activity. *Borneo Journal of Pharmacy, 2*(2), 35–40. doi:10.33084/bjop.v2i2.868

Ginwala, R., Bhavsar, R., Chigbu, D.G.I., Jain, P., & Khan, Z.K. (2019). Potential Role of Flavonoids in Treating Chronic Inflammatory Diseases with a Special Focus on the Anti-Inflammatory Activity of Apigenin. *Antioxidants, 8*(2), 35. doi:10.3390/antiox8020035

Handayani, V., Ahmad, A.R., & Sudir, M. (2014). Uji Aktivitas Antioksidan Ekstrak Metanol Bunga dan Daun Patikala (Etlingera elatior (Jack) R.M.Sm) Menggunakan Metode DPPH. *Pharmaceutical Sciences and Research, 1*(2), 86–93. doi:10.7454/psr.v1i2.3321

Hariyanti, Sunaryo, H., & Nurlaily, S. (2015). Efek Imunomodulator Fraksi Etanol Dari Ekstrak Etanol 70% Kulit Buah Manggis (Garcinia mangostana L.) Berdasarkan Peningkatan Aktivitas Dan Kapasitas Fagositosis Sel Makrofag Peritoneum Mencit Secara In Vitro. *PHARMACY: Jurnal Farmasi Indonesia (Pharmaceutical Journal of Indonesia), 12*(1), 58-69.

Juwita, T., Puspitasari, I.M., & Levita, J. (2018). Torch Ginger (Etlingera elatior): A Review on its Botanical Aspects, Phytoconstituents and Pharmacological Activities. *Pakistan Journal of Biological Sciences, 21*(4), 151-165. doi:10.3923/pjbs.2018.151.165

Kany, S., Vollrath, J.T., & Relja, B. (2019). Cytokines in Inflammatory Disease. *International Journal of Molecular Sciences, 20*(23), 6008. doi:10.3390/ijms20236008

Lachumy, S.J.T., Sasidharan, S., Sumathy, V., & Zakaria, Z. (2010). Pharmacological activity, phytochemical analysis and toxicity of methanol extract of Etlingera elatior (torch ginger) flowers. *Asian Pacific Journal of Tropical Medicine, 3*(10), 769-774. doi:10.1016/S1995-7645(10)60185-X

Lopez-Castejon, G. & Brough, D. (2011). Understanding the mechanism of IL-1β secretion. *Cytokine and Growth Factor Reviews, 22*(4), 189–195. doi:10.1016/j.cytogfr.2011.10.001

Panche, A.N., Diwan, A.D., & Chandra, S.R. (2016). Flavonoids: an overview. *Journal of Nutritional Science, 5*, 47. doi:10.1017/jns.2016.41

Sahidin, Salsabila, S., Wahyuni, Fristiohady, A., & Imran. (2019). Potensi Antibakteri Ekstrak Metanol dan Senyawa Aromatik dari Buah Wualae (Etlingera elatior). *Jurnal Kimia Valensi, 5*(1), 1-7. doi:10.15408/jkv.v5i1.8658

Sahoo, M., Ceballos-Olvera, I., Del Barrio, L., & Re, F. (2011). Role of the inflammasome, IL-1β, and IL-18 in bacterial infections. *The Scientific World Journal, 11*, 2037–2050. doi:10.1100/2011/212680

Syarif, R.A., Sari, F., & Ahmad, A.R. (2015). Torch ginger (Etlingera elatior Jack.) rhizomes as phenolic sources. *Jurnal Fitofarmaka Indonesia, 2*(2), 102–106. doi:10.33096/jffi.v2i2.178

Wahyuni, Malaka, M.H., Fristiohady, A., Yusuf, M.I., & Sahidin. (2017). Potensi Imunomodulator Ekstrak Etanol Buah Kecombrang (Etlingera elatior (Jack) R.M. Smith) Terhadap Aktivitas Fagositosis Makrofag Mencit Jantan Galur Balb/C. *Pharmacon, 6*(3), 350–355.