

Ethanollic Extract of *Xestospongia Sp.* Induces CD⁴⁺ and CD¹⁴ Cells Levels on Wistar Male Rat Infected with *Staphylococcus aureus*

Adryan Fristiohady¹, Wahyuni¹, Muhammad H. Malaka¹, Dewiyanti Madu¹, Dayatriana Muthalib¹, Dian Munasari¹, La Ode M.J Purnama¹, Baru Sadarun², Muhammad Ilyas Y^{1,3}, Idin Sahidin¹

¹Faculty of Pharmacy, Universitas Halu Oleo, Kendari, Southeast Sulawesi, Indonesia

²Faculty of Fisheries and Marine Sciences, Universitas Halu Oleo, Kendari, Southeast Sulawesi, Indonesia

³Department of Health Analyst, Polytechnic Bina Husada, Kendari, Southeast Sulawesi, Indonesia

Abstract

Immunomodulator is a substance that increases or suppresses the immune response through the certain mechanism. The marine sponge *Xestospongia sp.* has immunomodulatory activity by increasing phagocytic activity. In addition, the phagocytic activity is affected by CD⁴⁺ and CD¹⁴ cells levels. Thus, this study aims to investigate the effect of *Xestospongia sp.* extract toward CD⁴⁺ and CD¹⁴ cells level in model rat. Animals were divided into 4 groups (n=5) and treated for 7 days, as follow: Group I (Ethanollic extract of *Xestospongia sp.* dose of 300 mg/KgBW); Group II (Ethanollic extract of *Xestospongia sp.* dose of 400 mg/KgBW); Group III (*Phylantus niruri* extract); and Group IV (0.5% Na CMC). On day 8, animals were intraperitoneally infected to *Staphylococcus aureus* and the blood was collected by cardiac puncture and assayed with ELISA kit CD4 (elabscience®) and ELISA kit CD14 (elabscience®). Ethanollic extract of *Xestospongia sp.* provided high levels of both CD⁴⁺ and CD¹⁴ cells (group II) compared to baseline (group IV) (p<0.05). Group I provided similar activity to group III (p>0.05) and group II provided significant activity with higher levels of CD4+ and CD14 cells compared to group III (p<0.05). In conclusion, both doses of *Xestospongia sp.* extract showed immunomodulatory activity by increasing CD⁴⁺ and CD¹⁴ cells levels, yet dose of 400 mg/KgBw provides the higher immunomodulatory activity.

Keywords: *Xestospongia sp.*; immunomodulatory; CD⁴⁺ Cells; CD¹⁴ Cells

Introduction

Immune systems act by body survival and protection against antigen that arise due to various materials from the environment.¹ Body's immune systems are able to identify and eliminate microorganisms that potentially harm to the body to prevent infections causing organs damage.²

WHO (World Health Organization) stated that immune system disorder is the biggest

problem in the worldwide. Immune system disorders are divided into primary and secondary disorders that caused by environmental factors, drugs and radiation³ resulted in high sensitivity to infection, thus, immunomodulators can be used to increase the immune system as an alternative to control against infection.

Immunomodulator is a substance that changes activity of immune system, either

Corresponding author: Idin Sahidin. Faculty of Pharmacy, Universitas Halu Oleo, Kendari, Southeast Sulawesi, Indonesia.
Email: sahidin02@uho.ac.id

Received: 17 April 2020. Revised: 16 June 2020. Published: 1 August 2020.

by increasing or suppressing the immune response through certain mechanism. Their works in two ways, the stimulatory effect (increasing the immune response) or vice versa, the effect of suppression effect (suppress the immune response).^{1,2,4}

The use of terrestrial products such as marine sponge can be utilized in discovering novel drug such as immunomodulator agents. Marine sponges exhibit abundant biopharmacological activity such as antibacterial, antiviral, antifungal, antimalarial, antitumor, immunomodulator, and cardiovascular activity.⁵ *Xestospongia sp.* reported has activity as immunomodulator by stimulating phagocytosis examined by its specific phagocytic activity.⁶

Immune system involves phagocytic cells such as macrophages that play important role in the body's immune system against pathogens. One of the main roles of macrophages is phagocytosis, which aims to eliminate antigens, damaged or dead cells, and pathogenic bacteria such as CD⁴⁺ and CD¹⁴ cells. CD⁴⁺ cells are antigen expressing cells in the subset of thymocytes and T cell inflammatory cells (about 2/3 peripheral T cells), monocytes, and macrophages.^{7,8} CD⁴⁺ cells are type of white blood cells or lymphocytes which are important as part of the immune system. CD⁴ or CD⁴⁺ cells are referred to as T helper or T4 cells that activate Th1 cells thus activate macrophages in eliminating pathogens.⁹

In other hand, CD¹⁴ is Pattern Recognition Receptors (PRR) which enhance nonspecific immune responses to infections by increasing the sensitivity of immune cells to components of bacteria including lipopolysaccharide (LPS), lipoprotein and lipoteichoic acid (LTA). CD¹⁴ delivers the bacterial component to various TLR (Toll-Like Receptors) on the

surface membrane and induces immune cell activation.¹¹ Binding bacteria by the CD¹⁴ receptor activate phagocytosis.¹²

Thus, this study aims to examine the activity of ethanolic extract of *Xestospongia sp.* to phagocytosis activity observed from the increase in CD⁴⁺ and CD¹⁴ levels of rats models-treated with extracts and induced with *Staphylococcus aureus*.

Methods

Sample Preparation and Extraction

Sample collected from Soropia, Konawe Regency, Southeast Sulawesi at the depth of 3 m. Sample collected (5.3 Kg) was sorted and cleaned under running water. The sample was determined in Faculty of Fisheries and Marine Sciences of Halu Oleo University with document number 008b/UN29.112.1.1/pp/2018. Then, sample was chopped into pieces. The sample was macerated for 3 days with 96% ethanol. Filtrate collected was then concentrated and obtained 205.28 g of the concentrated extract (3.87%).

Animals

Animals were acclimatized for 7 days under controlled temperature (20-21°C), relative humidity maintained (45-70%), 12 h light/dark cycle, and free access to food and water ad libitum. The animals were conducted in accordance with ethical clearance issued by Komisi Etik Penelitian Kesehatan of Halu Oleo University (No: 2739/UN29.20/PPM/2018)

Bacteria

Inocula *Staphylococcus aureus* was suspended in 0.9% NaCl equivalent with 0.5 Mc Farland turbidity (1.5x10⁸ Colony Forming Unit (CFU)/mL).

Immunomodulatory Activity in vivo

Animals used in the study were divided into

4 groups (n=5), which were treated for 7 days with 5 mL of treatment orally in one daily dose, as follow:

Group I : ethanolic extract of *Xestospongia sp.* dose of 300 mg/KgBw

Group II : ethanolic extract of *Xestospongia sp.* dose of 400 mg/KgBw

Group III : as positive control, *Phylantus niruri* extract (Stimuno®) dose of 1.064 mg

Group IV : as a control, 0.5% Na CMC (used as baseline)

On day eight, each animal was infected intraperitoneally to 0.5 mL of *Staphylococcus aureus*, and left for 1 hour. After that, 3 mL of blood was collected intra cardial and put in EDTA-tube. Collected blood was centrifugated 3000 rpm for 15 minutes. Blood assayed with ELISA kit CD⁴ (elabscience®) and ELISA kit CD¹⁴ (elabscience®).

Data Analysis

Data was statistical analysis by using SPSS with ANOVA (Analysis of Variance) one-way test. p<0.05 value considered as significant in increased levels of CD⁴ and CD¹⁴.

Results and Discussion

Immunomodulator is a substance that enhance immune response with various mechanisms. Both CD⁴ and CD¹⁴ cells are activators of macrophages that involved in phagocytosis activity. The CD⁴⁺ cells expressed on surface of lymphocytes and the CD¹⁴ cells are expressed on surface of macrophages membranes. The CD⁴⁺ cells stimulating Th1 cell, thus activating macrophages to eliminate pathogens. In other hand, macrophages are expressing CD¹⁴ on the surface of membrane, named mCD¹⁴. As the cell is activated, the mCD¹⁴ decay into a membrane-free form (dissolved CD¹⁴, sCD¹⁴). The sCD¹⁴ is a marker of activation of macrophages and monocytes, thus the more macrophages and monocytes are activated, the higher dissolved CD¹⁴ levels in the circulation.¹³⁻¹⁵ Rats induced with ethanolic extract of *Xestospongia sp.* and positive control (*Phylantus niruri* extract), extract of *Xestospongia sp.* demonstrated expected results in increasing CD⁴⁺ and CD¹⁴ cell levels.

According to Figure 1, all groups was significant to group IV as normal control

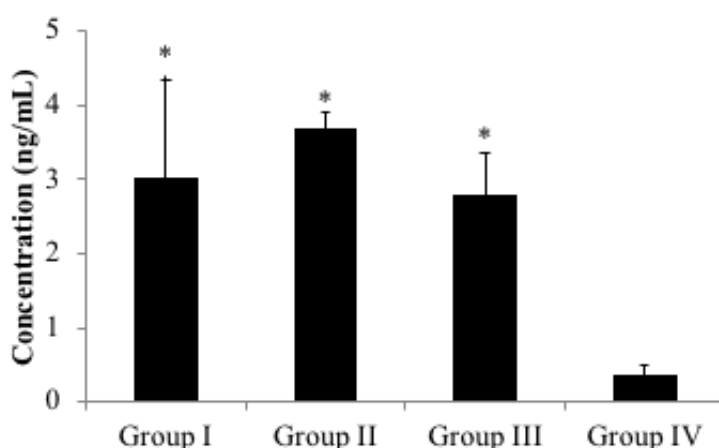


Figure 1. Increasing levels of CD4 in administration of ethanolic extract of *Xestospongia sp.* (n=5, Group I: of *Xestospongia sp.* dose of 300 mg/KgBw; Group II: *Xestospongia sp.* dose of 400 mg/KgBw; Group III: *Phylantus niruri* extract (Stimuno®); Group IV: 0.5% Na CMC)

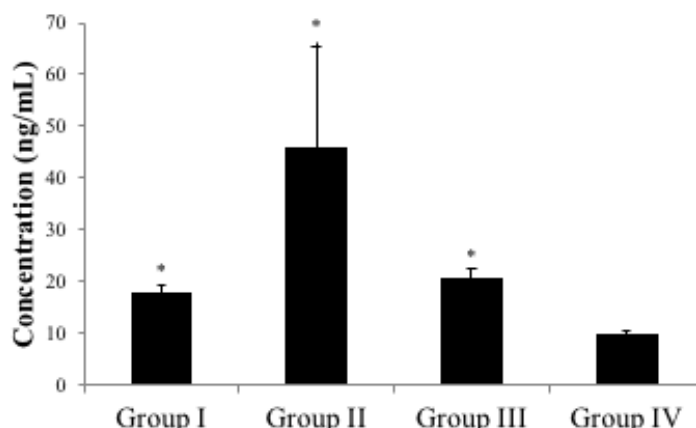


Figure 2. Increasing levels of CD14 in administration of ethanolic extract of *Xestospongia* sp. (n=5, Group I: of *Xestospongia* sp. dose of 300 mg/KgBw; Group II: *Xestospongia* sp. dose of 400 mg/KgBw; Group III: *Phyllanthus niruri* extract (Stimuno®); Group IV: 0.5% Na CMC)

($p < 0.05$) in increased CD^{4+} levels. Group II (dose of 400 mg/KgBw) with 3.695 ± 0.20 ng/mL provided higher differences compared other groups, as well as significance to group I (dose of 300 mg/KgBW) with 3.029 ± 1.31 ng/mL, and group III as positive control (*Phyllanthus niruri* extract) with 2.796 ± 0.56 ng/mL ($p < 0.05$). Group I and Group III had similar activity in increasing CD^4 levels.

According with results conducted at Figure 2, group II with 45.85 ± 19.69 ng/mL provided high increasing levels of CD^{14} compared to group I with 18.03 ± 1.13 ng/mL, although both group was significant to group IV with 9.93 ± 0.74 ng/mL as normal control ($p < 0.05$). Group II was significant to group III as positive control ($p < 0.05$) with 20.76 ± 1.80 ng/mL, meanwhile the group I was not significant with group III ($p > 0.05$).

Previous study showed that ethanolic extract of *Xestospongia* sp. has activity as immunomodulator by increasing phagocytic activity of macrophages. Flavonoids contained in extract are suspected responsible in increasing phagocytic activity by activating Th1 cell assisted with T cell helper (CD^{4+}

cell).⁶ The high levels of CD^{4+} cell as well CD^{14} cells are supporting previous study that ethanolic extract of *Xestospongia* sp. increases phagocytic activity possibly affected by flavonoids contain in it characterized by increased levels of CD^{4+} and CD^{14} cells, although the exact mechanisms are not well understood.¹⁶

Conclusion

Ethanolic extract of *Xestospongia* sp. might have immunomodulatory activity by increasing CD^{4+} and CD^{14} levels in rats which is responsible for enhancing immune system. Dose of 300 mg/KgBw is considered the lowest effective dose by knowing the immunomodulatory activity of ethanolic extract of *Xestospongia* sp. These results can be formulated into dosage form and used as immunomodulator.

Acknowledgment

We would like to thanks to the Ministry of Education of The Republic of Indonesia

Funding

This research was supported financially by Hibah Penelitian Dasar Scheme 2019 with

Contract no: 519a/UN29.20/PPM/2019.

Conflict of Interest

None declared

References

1. Bratawidjaja K, Rengganis. *Imunologi Dasar*. Jakarta: Badan Penerbit Fakultas Ilmu Kedokteran Universitas Indonesia; 2014.
2. Indrisari M, Habibie, Rahimah S. Uji Efek Ekstrak Etanol Daun Jarak Pagar (*Jatropha curcas* L) Terhadap Titer Immunoglobulin M (IgM) dan Immunoglobulin G (IgG) pada Tikus Putih Jantan (*Rattus norvegicus*). *Jurnal Farmasi Fik Uinam*. 2017;5(4):244–50.
3. WHO (World Health Organization). World Health Statistic 2014. Switzerland: WHO; 2014.
4. Fitria L, Nurrahman Marwayana O. Potensi Propolis Sebagai Imunomodulator Pada Tikus (*Rattus norvegicus* Berkenhout, 1769) Galur Wistar yang diinduksi Penisilin-G. *Biogenesis Jurnal Ilmiah Biologi*. 2015;3(2):124–31.
5. Anjum K, Abbas SQ, Shah SAA, Akhter N, Batool S, Hassan SSU. Marine sponges as a drug treasure. *Biomolecules and Therapeutics*. 2016;24(4):347–62.
6. Fristiody A, Wahyuni W, Malik F, Leorita M, Yusuf MI, Febriansyah H, et al. Efek Imunomodulator Ekstrak Etanol Spons *Xestospongia* Sp. Terhadap Aktivitas Fagositosis Makrofag Pada Mencit Jantan Galur Balb/C. *Jurnal Mandala Pharmacon Indonesia*. 2019;5(01):15–30.
7. Haniastuti T. (Reducing of Phagocytosis Activity of Mouse Macrophage Cell After. *Dentika Dental Journal*. 2009;14(1):11–4.
8. Roffico R, Djati MS. Efektivitas Pemberian Ekstrak Ethanol Daun *Polyscias obtusa* dan *Elephantopus scaber* terhadap Modulasi Sel T CD4⁺ dan CD8⁺ pada Mencit Bunting BALB/c. *Biotropika Journal of Tropical Biology*. 2014;2(3):174–80.
9. Rifa'i M. *Imunologi dan Alergi-Hipersensitivitas*. Malang: UB-Press; 2013.
10. Kelley S, Lukk T, Nair S, Tapping R. The crystal structure of human soluble CD14 reveals a bent solenoid with a hydrophobic amino-terminal pocket. *Journal of Immunology*. 2013;190(3):1304–11.
11. Kwak MS, Lim M, Lee YJ, Lee HS, Kim YH, Youn JH, et al. HMGB1 Binds to Lipoteichoic Acid and Enhances TNF- α and IL-6 Production through HMGB1-Mediated Transfer of Lipoteichoic Acid to CD14 and TLR2. *Journal of Innate Immunology*. 2015;7(4):405–16.
12. Rosnizar R, Maulida S, Eriani K. Potensi ekstrak daun flamboyan [*Delonix regia* (Boj. Ex Hook.) Raf] terhadap peningkatan aktivitas dan kapasitas makrofag. *Jurnal Bioleuser*. 2017;1(3):104–15.
13. De Voeght A, Martens H, Renard C, Vaira D, Debruche M, Simonet J, et al. Exploring the link between innate immune activation and thymic function by measuring sCD14 and TRECs in HIV patients living in Belgium. *PLoS One*. 2017;12(10):1–11.
14. Shive C, Jiang W, Anthony D, Lederman M. Soluble CD14 is a nonspecific marker of monocyte activation. *AIDS* [Internet]. 2015;29(10):1263–5. Available from: [file:///C:/Users/Carla Carolina/Desktop/Artigos para acrescentar na qualificação/The impact of birth weight on cardiovascular disease risk in the.pdf](file:///C:/Users/Carla%20Carolina/Desktop/Artigos%20para%20acrescentar%20na%20qualifica%C3%A7%C3%A3o/The%20impact%20of%20birth%20weight%20on%20cardiovascular%20disease%20risk%20in%20the.pdf)
15. Neu C, Sedlag A, Bayer C, Forster S, Peter Crauwels, Jan-Hendrik Niess. CD14-Dependent Monocyte Isolation Enhances Phagocytosis of *Listeria monocytogenes* by Proinflammatory, GM-CSF-Derived Macrophages. *PLoS One*. 2013;8(6).
16. Hosseinzade A, Sadeghi O, Biregani AN, Regulatory CD, Through C. Immunomodulatory Effects of Flavonoids : Possible Induction of T CD4 + Regulatory

Cells Through Suppression of mTOR
Pathway Signaling Activity. *Frontiers in
Immunology*. 2019;10(January):1–12.