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Immunomodulatory Activity of *Callyspongia* sp. Extract Towards Interferon-gamma (IFN-γ) and Tumor Necrosis Factor-Alpha (TNF-α) Levels in *Staphylococcus aureus* – Induced Wistar Male Rats

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Abstract: The IFN-γ and TNF-α are cytokines that involved in the phagocytic activity, and *Callypsongia* sp. increases phagocytic activity; thus, this study aims to investigate the effect of *Callspongia* sp. extract toward IFN-γ and TNF-α levels in *Staphylococcus aureus*-induced Wistar male rats. The animals were divided into four groups (n=5) and treated orally for 7 d as follows: Group I (extract dose of 300 mg/kgBW); Group II (extract dose of 400 mg/kgBW); Group III (*Phylantii* extract); and Group IV (0.5% NaCMC). On day 8, animals were infected with *Staphylococcus aureus* intraperitoneally and left for 1h. Blood was collected and assayed with ELISA Kit for IFN-γ and TNF-α. Data collected were then statistically analyzed using SPSS. According to results obtained, the *Callyspongia* sp. extract effect in both IFN-γ and TNF-α is significantly different from Group IV as the negative control (p<0.05). *Callyspongia* sp. extract provided similar potency between Group I and Group III (p>0.05), yet Group II provided higher activity in increasing IFN-γ levels. In contrast, *Callyspongia* sp. provided similar activity between Group I, Group II, and Group III to increase TNF-α levels (p>0.05). Therefore, we concluded that *Callyspongia* sp. extract increases both INF-γ and TNF-α, responsible for the phagocytic activity.

Keywords: *Callyspongia* sp.; Immunomodulatory; IFN-γ; TNF-α.

1. Introduction

Infection is infectious diseases that spread directly or indirectly from one person to another, or animals to humans, caused by pathogenic microorganisms such as bacteria, viruses, parasites, and/ or fungi [1]. The immune system is required to fight infections. The immune system consists of innate and adaptive immune systems. The innate immune system provides the first line of defense against pathogens, which cytokines play a vital role in trigger the inflammatory response, produce and elevate the body temperature, and activate NK (natural killer) cells, as well macrophages. The immune systems are the potent defense against pathogenic microorganisms and mainly composed of lymphocytes and macrophages. As our body cannot defend itself, the immunomodulatory agent is required [2,3].

One of immunomodulatory activity is increasing phagocytic activity. Macrophages are playing a role in the immune system that eliminating the pathogens. Macrophages are activated by cytokines that are involved, namely interferon-gamma (IFN-γ). The macrophages stimulate the Tumor Necrosis Factor-Alpha (TNF-α) secretions; thus, phagocytic activity by macrophages increases [4-6].

Immunomodulators are clinically used to stimulate the immune system or slow the progress of diseases caused by the immune system itself. Immunomodulatory agents can cause undesirable effects such as microscopic gastrointestinal bleeding, decreased platelet levels, respiratory depression, increased uric acid levels, urticaria, agranulocytosis, and are toxic to the liver and digestive tract disorders. Therefore, natural immunomodulators are an option to reduce the undesirable effect. One of the natural products that can be utilized as an immunomodulator is marine sponge *Callyspongia* sp. [7].

Callyspongia sp. is one of the marine sponges mainly found in Indonesia. The sponge contains steroids, alkaloids, and terpenoids, potentially utilized as raw material for drug discoveries. *Callyspongia* sp. is beneficial for anticancer, antioxidant, antifungal, antibacterial, and exhibiting cytotoxic activity [8-10]. The previous study showed that *Callypsongia* sp. ethanol extract exhibits phagocytic activity of macrophages in *Staphylococcus aureus* induced-Wistar male rats at the dose of 300 and 400 mg/kg BW [11]. There have been no reports of studies of the effects of *Callyspongia* sp. extract as an immunomodulatory agent toward IFN-γ and TNF-α levels of rats. Thus, this study aims is the more exploring immunomodulatory activity of *Callyspongia* sp. extract toward interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) levels in *S. aureus* induced-Wistar male rats.

2. Materials and Methods

2.1. Materials.

Materials used in this study were marine sponge *Callyspongia* sp, Wistar male rats, *Staphylococcus aureus* (ATCC 25923), 96% ethanol (Merck®), distilled water, 0.5% Na-CMC (Lansida®), *nutrient agar* (KGaA®), ether (Merck®), 0.9% NaCl (Widatra®), Mc Farland 0,5, pellet chow dan commercially Phylantii extract (Stimuno® (48J0603)).

2.2. Instruments.

The instrument used in this study were rotary evaporator (Buchi®), autoclave (Hirayama®), Erlenmeyer flask (Pyrex®), analytical balance (Precisa®), graduated cylinder (Pyrex®), beaker glass (Pyrex®), oven (Gallenkamp Civilab-Australia), incubator (Glorya Medica), laminar airflow/LAF (Biobase®), water bath (DSB), Bunsen (Pyrex®), pipette (Pyrex®), volumetric pipette (Pyrex®), stirring rod (Pyrex®), evaporating dish (Pyrex®), electromantle (Cambium Furni Industry), Eppendorf tube, surgical blade, magnetic stirrer, hot plate, cage, ELISA kit Rat IFN-γ (elabscience®), and ELISA kit Rat TNF-α (Aviva Systems Biology®).

2.3. Sample collection, preparation, and extraction.

Marine sponge *Callyspongia* sp. was collected in Bintang Samudra Marine Edu-Park, located in Konawe Regency, Southeast Sulawesi. A total of 5.2 Kg of the sample was collected. The sample was then sorted to remove from impurities and chopped into pieces to broaden the

sample's contact with the solvent. The chopped sample was then immersed in ice water to reduce the salt content in the sample, followed by storing in a sealed jar for further steps.

Sample (5.2 Kg) was macerated in 96% ethanol for 3 x 24 h (1:2) following by filtrated and solvent replacement each 24 h, thus obtained filtrate I, filtrate II, and Filtrate III. The filtrates were mixed up and concentrated with a rotary vacuum evaporator (50ºC) and obtained a total of 94.65 g concentrated extract (1.82%).

2.4. Inocula preparation.

Staphylococcus aureus inocula were planted in slant agar (Nutrient Agar) and incubated at 35-37ºC. The inocula were then suspended in 0.9% NaCl until the turbidity was equivalent to 0.5 McFarland.

2.5. Animals.

Animals used in the study were Wistar male rats, which were obtained at animal farms in Surabaya, Indonesia. Animals were acclimatized for 7 d in the standard environment with temperature 25±1ºC, humidity 55±5%, and 12:12h light: dark cycle. The animals accessed the food and water *ad libitum.*

2.6. Experimental design.

Animals were divided into four groups (n=5), and treated orally for 7 d, as follow:
Group I: treatment group of *Callyspongia* sp. extract dose of 300 mg/kg BW;
Group II: treatment group of *Callyspongia* sp. extract dose of 400 mg/kg BW;
Group III: positive control, administered with *Phylantii* extract commercial (Stimuno®);
Group IV: 0.5% Na CMC;

On day 8, animals were induced with *Staphylococcus aureus* intraperitoneally and sit out for 1 h at which the blood was collected by cardiac puncture. The blood was put in an EDTA-contained Eppendorf tube and centrifuged for 15 m at 3000 rpm. The plasma was assayed with ELISA Kit IFN-γ and ELISA kit TNF-α.

2.7. Statistical analysis.

Data collected were processed with SPSS® (*Statistical Product and Service Solution*) version 24. Data were analyzed with one-way Analysis of Variance (ANOVA). Data were continued with LSD (Least Significant Difference) if they were normally distributed and were continued with Kruskal-Wallis if they were not normally distributed. p>0.05 indicated no significant difference, and p<0.05 were indicating provide a significant difference.

3. Results and Discussion

An Immunomodulatory is a drug or substance that restores the immune system imbalance by stimulating and improving the immune system function. Immunomodulatory consists of 3 mechanisms, which are as an immunostimulant, immune restoration, and immunosuppression. Sponge *Callyspongia* sp. is one of marine biodiversity that acts as an immunomodulator, individually as an immunostimulant. One of the immunostimulant activity is increasing phagocytic activity by increasing plasma TNF-α and IFN-γ secretion [11-14].

According to Figure 1, the plasma IFN-γ levels at Group I-IV were 455,265 pg/mL; 384,319 pg/mL; 353,486 pg/mL; and 160,314 pg/mL, respectively. The plasma IFN-γ levels of each group were also increasing, expect the group IV as the negative control (p<0.05). The plasma IFN-γ levels of group I was higher than group II and group III (p<0.05).

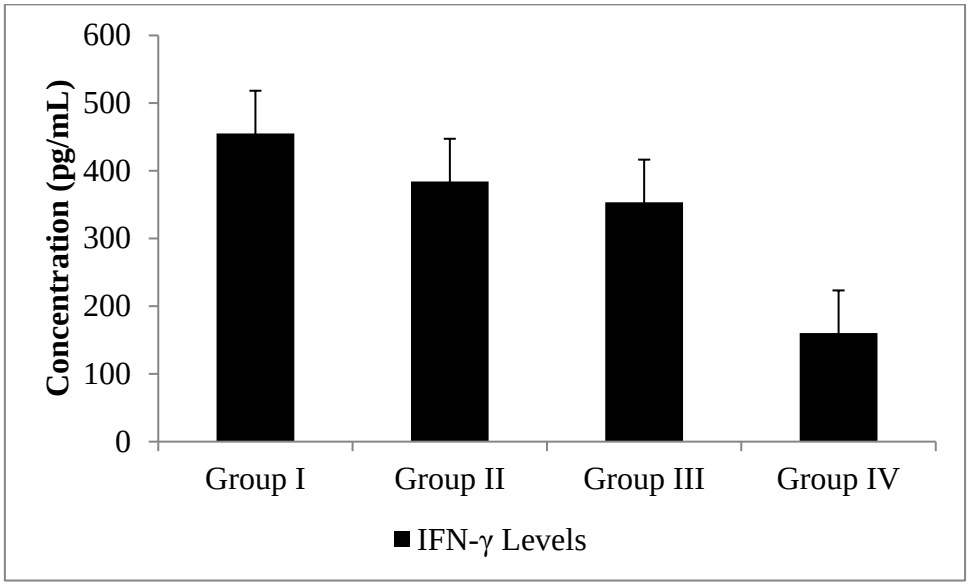

Figure 1. The IFN-γ levels of rats.

Although group II (*Callyspongia* sp. extract dose of 400 mg/kg BW) was having lower plasma IFN-γ levels than the group I (*Callyspongia* sp. extract dose of 300 mg/kg BW), it was still similar levels with group III as the positive control (p>0.05). It concluded that *Callyspongia* sp. dose of 300 mg/kg BW has better activity than a dose of 400 mg/kg BW and *Phylantii* extract in increasing plasma IFN-γ levels in rats.

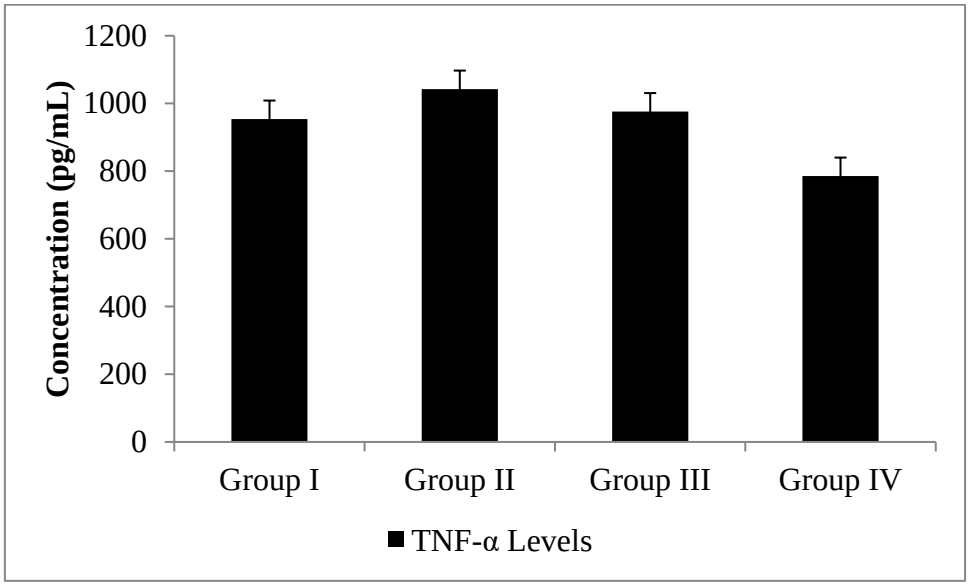

Figure 2. TNF-α levels of rats.

A similar activity is also exhibited by the plasma IFN-γ levels (Figure 2). The plasma TNF-α levels for Group I-IV were 954 pg/mL; 1042 pg/mL; 976 pg/mL; and 785,5 pg/mL, respectively. The plasma TNF-α levels of each group were increasing, expect the group IV as the negative control (p<0.05). The plasma TNF-α levels of both Group I (*Callyspongia* sp. extract dose of 300 mg/kg BW) and Group II (*Callyspongia* sp. extract dose of 400 mg/kg BW)

were not significantly differenced to Group III as the positive control (p>0.05). It concluded that both *Callyspongia* sp. extract dose of 300 and 400 mg/kg BW have similar potency in increasing plasma TNF-α level in rats to the positive control, which is commercial *Phylantii* extract (Stimuno®).

LTAs (lipoteichoic acids) of *Staphylococcus aureus* triggers the activation of macrophages and B lymphocytes. LTA, along with TNF-α and IFN-γ from other cells, initiates the signal transduction cascade, thus inducing the proinflammatory macrophage response [15]. It leads to the production of additional cytokines, chemokines, and activation of proinflammatory genes such as iNOS (Inducible nitric oxide synthase), thereby the pathogens eliminated [7,16]. The IFN-γ is a mediator in the innate and adaptive immune cells. It promotes B-cell differentiation toward Ig-G production and activates phagocytosis through activation of macrophages by LTA-induction. Macrophages produce the TNF-α in the acute phase of inflammation. LTA of *Staphylococcus aureus* induces the inflammation in this study. The TNF-α mediates the infection's clearance through the recruitment of neutrophils and macrophages to the site of the infection [17-20].

Alkaloids contained in *Callyspongia* sp. extract are suspected of playing a vital role in its immunomodulatory activity. Alkaloids increase the number and activity of macrophage phagocytosis production by increasing cytokines secretions such as TNF-α and IFN-γ [21,22]. Steroids recognize and bind to immune cells, just like toll-like receptor ligands that. Thus, activate the NF-κB as cytokines transcription factors that increasing the secretion of IFN-γ levels to extra cells [23-27]. Besides, Saponins enhance the immune system by regulating the activity of lymphocyte cell proliferation. Saponins also affect the production of TNF-α, which will react with antigens from *S. aureus* bacteria [28-30].

4. Conclusions

Callyspongia sp. extract dose of 300 mg/kg BW and 400 mg/kg BW were effective as immunomodulatory agents by increasing IFN-γ and TNF-α levels compared with negative controls (0.5% NaCMC) in Wistar male rats induced by *Staphylococcus aureus*. IFN-γ and TNF-α are cytokines involved in the innate immune system, namely, phagocytic activity.

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Conflicts of Interest

The authors declare no conflict of interest.

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