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Elephantopus scaber and Sauropus androgynus Regulate Macrophages and B Lymphocyte Cells during Salmonella typhi Infection

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Abstract—Macrophages and B lymphocyte play an important role as the first cell type to encounter bacterial pathogen and as a mediator that initiate the adaptive immune response. Those types of cell can die in many ways such as apoptosis, necrosis, pyroptosis and autophagy during the host cell-pathogen recognition. This study aimed to investigate the ability of *E. scaber* and *S. androgynus* formula in regulating macrophage and B lymphocyte cells during bacterial infection. The pregnant mice were randomly divided into seven experimental groups: T1 (control), T2 (*S. typhi* infection), T3 (*S. typhi*, *E. scaber* 100%), T4 (*S. typhi*, *E. scaber* 75% and *S. androgynus* 25%), T5 (*S. typhi*, *E. scaber* 50% and *S. androgynus* 50%), T6 (*S. typhi*, *E. scaber* 25% and *S. androgynus* 75%), and T7 (*S. typhi*, *S. androgynus* 100%). Flowcytometry analysis was performed on day 18. S. typhi infection decrease the formation of macrophage and B lymphocyte cells in bone marrow and induce cell death in PBMC. We clearly proved that *E. scaber* 75% and *S. androgynus* 25% formula was able to ameliorate the formation of macrophage and B lymphocyte cells in BM. While *E. scaber* 25% and *S. androgynus* 75% formula increased the relative number of macrophage and B lymphocyte cells in PBMC.

Key words—Hematopoiesis; Immunomodulator; Infection; Katuk; Pregnant; Tapak Liman.

INTRODUCTION

Indonesia is a mega-biodiversity country, including diversity of plants as material for herbal medicine. Ethanol extracts from all parts of E. scaber plant in the phytochemical analysis showed that this plant contains a number of secondary metabolites include epiphrieelinol, lupeol, stigmasterol, triacontan-1-ol, dotriacontan-1-ol, lupeol acetate, deoxyelephantopin, isodeoxyelephantopin, lactone, flavonoids, polyphenols lutein-7 and glucoside [1]. Ethanol extracts of this plants has extensive bioactivity as the treatment of wounds in mice, antimicrobial activity, and dysuria treatment. Compounds identified as an anti-cancer is sesquiterma lactones and compounds identified as an anti-tumor is deoxyelephantopin [2]. Water extract of E. scaber can be used as an anti-inflammatory in a rat model of acute and chronic arthritis [3]. Indonesia there is also many *Sauropus androgynus* (katuk). Chemical compounds contained in *S. androgynous* among others Saponin, Flavonoid, and Tanin. Saponin is triterpenoid glycosides or steroid glycosides compounds which are active compounds like soap and can be detected by their ability to form a foam and hemolyzed red blood cells. Saponin has activity as an antifungal agent and inhibits the growth of cancer cells [4]. Polyphenols consisting of tannins, flavonoids, and phenolic acids are the most prominent component in relation to antimicrobial activity [5]. Saponins and flavonoids have long been known to have efficacy as an immunomodulator that can modulate the immune system, especially in increasing the proliferation of immune cells [6].

Pregnant women have a higher risk of infectious diseases because a pregnant woman has a unique immunological condition as pregnancy [7]. Our recent study proved that the decreasing of immune system occur in pregnant mice which infected by *Salmonella typhi* [8]. Before bacterial pathogen cause an infection, they will contact with the first body's defense system such as skin, respiratory and gastro-intestinal system then recognized with many kinds of the host cell such as an epithelial cell, PMN cell, and antigen-presenting cells (APC). APC such as macrophages and B lymphocyte play an important role as the first cell type to encounter bacterial pathogen and as a mediator that initiate the adaptive immune response. B lymphocyte cells are capable of mounting responses to a bewildering range of potentially pathogenic antigens through the production of high-affinity antibodies and the establishment of immunological memory. When macrophages activated by microbial products, they acquire microbicidal competence that usually leads to effective immunity [9]. Nevertheless, some of the bacterial pathogens have evolved strategies to interrupt macrophage activation and to modulate host responses. Furthermore, during the host cell-pathogen recognition, macrophages and can B lymphocyte cells die in many ways such as apoptosis, necrosis, pyroptosis and autophagy [10]. Thus, this study aims to investigate the efficacy of the combination of *E. scaber* and *S. androgynous* against the expression level of macrophage and B lymphocyte cells during *S. typhi* infection in a pregnant condition.

MATERIAL AND METHOD

a. Experimental animals

The experimental animal that used in this study was 8 weeks old of female BALB/c mice, pathogen free, and 5 day old of pregnancy. They were given free access to food and drinking mineral water ad libitum. The pregnant mice were randomly divided into seven experimental groups of 5 mice each: T1 (control), T2 (S. typhi infection), T3 (S. typhi, E. scaber 100%), T4 (S. typhi, E. scaber 75% and S. androgynus 25%), T5 (S. typhi, E. scaber 50% and S. androgynus 50%), T6 (S. typhi, E. scaber 25% and S. androgynus 75%), and T7 (S. typhi, S. androgynus 100%). All the animals were treated for 18 days and still allowed free access to food and drinking mineral water.

b. Preparation of *Elephantopus scaber* and *Sauropus androgynus* leaf ethanol extract

Fresh leaves of Elephantopus scaber and *Sauropus androgynus* were obtained from Balai Materia Medica Batu, Malang, Indonesia. The powdered of each leaf was macerated in 70% of ethanol for 24 h in room temperature then dried by vacuum pump evaporator. The initial dose of E. scaber was 200 mg/kg BW and S. androgynus was 150 mg/kg BW.

c. Preparation of *S. typhi*

The isolate of *Salmonella typhi* was obtained from Microbiology laboratory, Faculty of Medicine, Brawijaya University, Malang, Indonesia. Typhoid fever was induced by intraperitoneal injections of S. typhi bacteria as much as 107 CFU in 0.5 mL solvent.

d. Bone marrow cell and PBMC isolation

Bone Marrow (BM) was isolated and washed twice with PBS. Cells in BM were isolated from femoral bone. PBMC were taken using a pipette capillary hematocrit through the orbital veins. Homogenates of cells were centrifuged at a speed of 2500 rpm, at 10o C, for 5 minutes and the pellet was resuspended in 1 ml of PBS.

e. FACS analysis

Cell suspensions were put in microtube then centrifuged with the speed of 2500 rpm at 10o C for 5 minutes. Supernatant was discarded and the pellets were stained with antibodies. Cell was stained by FITC-

conjugated rat anti-mouse CD68 and PE-conjugated rat anti-mouse B220, then incubated for 20 minutes at 10o C then added by 500 µl of PBS. Each sample was transferred into a flowcytometry cuvette and then analyzed by flowcytometer.

f. Data analysis

Data were analyzed by BD Cell Quest PRO™ software then tabulated and analyzed statistically. The statistical analysis used a parametric one-way ANOVA analysis with the significance of 0.05% and was followed by Tukey test. The application for statistical analysis was SPSS version 16 for Windows.

RESULT AND DISCUSSION

a. Macrophage cells

Macrophages are located in all body tissues, where they are important in detecting, ingesting, and processing foreign material, dead cells, and other debris. Macrophages play an important role in the innate and adaptive immune responses to pathogens and are important mediators of inflammatory processes [11]. *S. typhi* infection was cause macrophage death which was indicated by the decrease in the number of macrophages both in BM and PBMC significantly compared to control (Fig. 1 and Fig. 2). After internalization, Salmonella is initially enclosed in compact phagosomes. Salmonella is capable of persisting in the relatively mild phagosome uncoupled from the normal endocytic route and live inside the macrophage by subverting the formation of phagolysosome thus inhibiting digestion by lysosomal action, which provides an environment for the pathogen to hide from the immune system and replicate. Within a certain period of time, phagosomes are destroyed through known or unknown bacterial factors, allowing microbe access to the cytosol where the cytoplasmic bacteria replicate [10]. *Salmonella* of a particular growth phase (transition from the exponential to the stationary) is reported to induce 90% of the macrophages apoptosis within 30 min [12]. In the innate immune system, *Salmonella* evades the oxygen killing mechanisms of macrophages by disrupting NADPH oxidase and iNOS trafficking to the SCV.

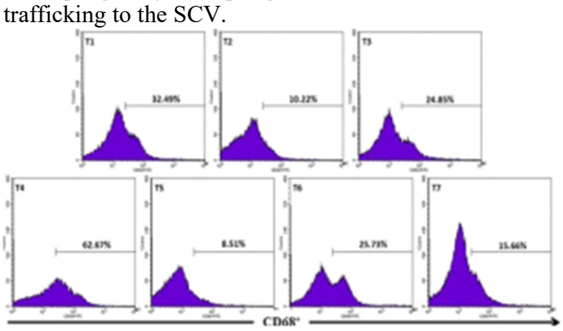

Figure 1. *Effect of different formulation of ethanol extract of E. scaber and S. androgynus leaves on the relative number of macrophage cells (CD68$^+$) from BM. T1, control; T2, S. typhi infection; T3, S. typhi infection with 100% of E. scaber; T4, S. typhi infection with 75% of E. scaber and 25% of S. androgynus; T5, S. typhi infection with 50% of E. scaber and 50% of S. androgynus; T6: S. typhi infection with 25% of E. scaber and 75% S. androgynus; T7: S. typhi infection with 100% of S. androgynus. Values are mean of three replicate determinations with $p < 0.05$.*

As shown in Figure 1, formulation of E. scaber and *S. androgynus* especially in T3, T4, T6, T7 increased the relative number of macrophage cell in BM during *S. typhi* infection. However, T4 (75% *E. scaber* and 25% *S. androgynus*) gave the greatest effect among other formula. Figure 2 showed that only T6 (25% *E. scaber* and 75% *S. androgynus*) that could ameliorate the number of macrophage cell in PBMC during S. typhi infection.

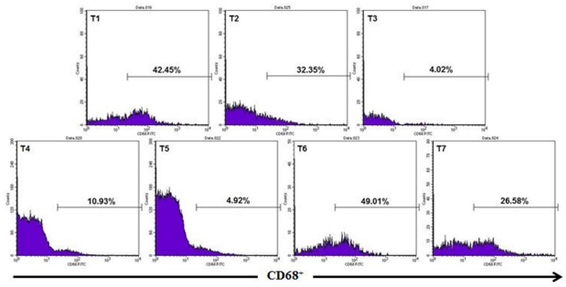

Figure 2. *Effect of different formulation of ethanol extract of E. scaber and S. androgynus leaves on the relative number of macrophage cells (CD68$^+$) from PBMC. T1, control; T2, S. typhi infection; T3, S. typhi infection with 100% of E. scaber; T4, S. typhi infection with 75% of E. scaber and 25% of S. androgynus; T5, S. typhi infection with 50% of E. scaber and 50% of S. androgynus; T6: S. typhi infection with 25% of E. scaber and 75% S. androgynus; T7: S. typhi infection with 100% of S. androgynus. Values are mean of three replicate determinations with $p < 0.05$.*

b. Lymphocyte Cell

B cells have long been knwn as simple antibody producer and as a player in both adaptive and innate immune responses. Many types of bacteria, viruses and parasites have evolved the ability to manipulate B cell functions to modulate immune responses [13]. This study proved that *S. typhi* infection successfully suppressed the relative number of B lymphocyte cell. As shown in Figure 3, the infection of *S. typhi* dramatically reduced B lymphocyte cell number in BM. It also occurred in PBMC (Fig. 4). Furthermore, pathogens affected B cells indirectly and directly [13]. They attack the innate immune cells and alter the cytokine environment. They also impair the B lymphocyte cell-mediated immune responses.

E. scaber and *S. androgynus* with formula T3 and T4 were increased the relative number of B lymphocyte cell in BM during S. typhi infection. Nevertheless, T4 (75% *E. scaber* and 25% *S. androgynus*) given the higher increased of B lymphocyte number (Fig. 3). As shown in Figure 4, only T6 (25% *E. scaber* and 75% *S. androgynus*) that could improve the relative number of B lymphocyte cell in PBMC, during *S. typhi* infection.

This study demonstrates that the higher concentration of *E. scaber* when combined with lower concentration of *S. androgynus*, may play a role in a hematopoietic cell in BM. The deoxyelephantopin contained in *E. scaber* can activate PPARγ (peroxisome proliferator-activated receptor gamma) [14]. PPARγ1 is abundantly expressed hematopoietic cells. During differentiation, the progeny of hematopoietic cells progresses through various intermediate maturational stages, generating multi-potential progenitors and lineage-committed progenitors, prior to reaching maturity. Bone marrow (BM) is the major site of hematopoiesis in humans and, under normal conditions [15]. PPARγ play a role during the elicitation of immune responses and may be involved in changes in energy states required during activation and development of many cell types involved, and has additional immunologically relevant effects in erythroid, myeloid, monocytic, T and B lymphocytic, stromal, and endothelial cell function [16].

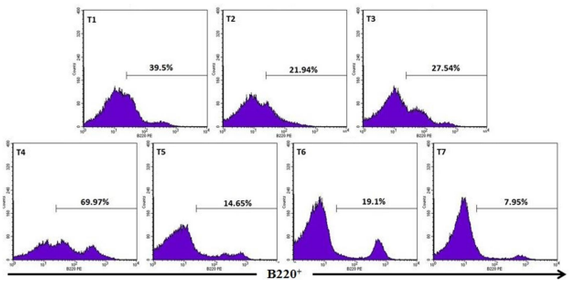

Figure 3. *Effect of different formulation of ethanol extract of E. scaber and S. androgynus leaves on the relative number of B lymphocyte cells (B220^{+}) from BM. T1, control; T2, S. typhi infection; T3, S. typhi infection with 100% of E. scaber; T4, S. typhi infection with 75% of E. scaber and 25% of S. androgynus; T5, S. typhi infection with 50% of E. scaber and 50% of S. androgynus; T6: S. typhi infection with 25% of E. scaber and 75% S. androgynus; T7: S. typhi infection with 100% of S. androgynus. Values are mean of three replicate determinations with $p < 0.05$.*

In the other hand, the higher concentration of *S. androgynus* when combined with lower concentration of *E. scaber* may play a role in protecting cells from damage due to bacterial infection or stimulate proliferation of cells that have been activated in PBMC due to bacterial infection. In traditional medicine, *S. androgynus* is reported have beneficial effects as an antioxidant, anti cancerous, antifungal and anti septic. Methanol extract has exhibited significant antibacterial activity against six bacterial strains including *Salmonella* [17]. The antibacterial activity of the leaf extract is due to the presence of multivitamins and peptides, glycosides, alkaloids, saponins, terpenoids, flavonoids etc. This antibacterial activity will help to reduced the number of *S. typhi* in the host, especially in the bloodstream, directly. If the number of bacteria is reduced, it would reduce the cell death in the bloodstream mainly macrophages and B lymphocyte cells that interact directly with bacteria during infection. *S. androgynus* have the immunomodulatory effect that can modulate the expression level of the immunocompetent cell including macrophage and B lymphocyte cells [18]. However the combination of those two plants given the better results to ameliorate the immunity condition than given individually.

CONCLUSION

E. scaber 75% and *S. androgynus* 25% formula was able to ameliorate the formation of macrophage and B lymphocyte cells in BM. While *E. scaber* 25% and *S. androgynus* 75% formula was increase the relative number of macrophage and B lymphocyte cells in PBMC.

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