

## Bone Marrow Cells Lymphocyte Activity of Pregnant Mice with Therapy of *E. scaber* and *S. androgynus* Post Infection *Salmonella typhimurium*

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### Abstract

Pregnancy can cause immune system changes. It is characterized by a decrease in the activity of immunocompetent cells. The use of antibiotics was intended to combat pathogenic microorganisms, but antibiotics have negative effects on pregnant women. The use of antibiotics can be replaced with extracts of *Elephantopus scaber* and *Sauropus androgynus* because both plants have chemical compounds that act as immunomodulators. This study was aimed to determine the activity of lymphocytes B220<sup>+</sup>, TER119<sup>+</sup>, and GR-1<sup>+</sup> on bone marrow pregnant mice given the combination of extracts of *Elephantopus scaber* and *Sauropus androgynus* after infected with *Salmonella typhimurium*. This research uses seven treatment groups name: (K-) 0.05% NaCMC without bacterial infection; (K+) 0.05% NaCMC infected by bacteria; (P1) *E. scaber* 200 mg.kg<sup>-1</sup>BW infected by bacteria; (P2) *E. scaber* 150 mg.kg<sup>-1</sup>BW and *S. androgynus* 37.5 mg.kg<sup>-1</sup>BW infected by bacteria; (P3) *E. scaber* 100 mg.kg<sup>-1</sup>BW and *S. androgynus* 75 mg.kg<sup>-1</sup>BW infected by bacteria; (P4) *E. scaber* 50 mg.kg<sup>-1</sup>BW and *S. androgynus* 112.5 mg.kg<sup>-1</sup>BW infected by bacteria; and (P5) *S. androgynus* 150 mg.kg<sup>-1</sup>BW infected bacteria. The initial dose of *E. scaber* and *S. androgynus* was 50 mg.kg<sup>-1</sup>. Each treatment has three repetitions, surgery performed on day 12<sup>th</sup> and 18<sup>th</sup>. Lymphocyte cells isolated from bone marrow, the obtained results were analyzed by flowcytometry and statistical analysis using SPSS 16.0 one-way ANOVA, Tukey test and path. Based on the results from ANOVA tables, the formulations on mice that can restore their normal conditions with B220<sup>+</sup> cells is *E. scaber* 150 mg.kg<sup>-1</sup>BW and *S. androgynus* 37.5 mg.kg<sup>-1</sup>BW, TER119<sup>+</sup> cells is *S. androgynus* 150 mg.kg<sup>-1</sup>BW, while the GR-1<sup>+</sup> cells affected by the surgery. Those three dose formulations can be used to obtain the optimum value which can increase the number of lymphocytes and not harmful to the developing fetus.

**Keywords:** Bone marrow, *Elephantopus scaber*, Lymphocyte, Pregnant mice, *Sauropus androgynus*

### INTRODUCTION

Pregnancy in women will affect the physiological condition of the body. In the state, pregnant women will experience changes in their immune system, which is intended to facilitate embryo implantation, placental development, fetal tolerance initiated, as well as the defense of the maternal immune system [1]. Changes in the pregnant woman's immune system will lower the immune system [2], thus with such a situation pregnant women susceptible to disease [3].

One change that affects the immune system of pregnant women is *Salmonella* [4]. Previous study analyzed 200 blood samples and found that 129 women have positive samples of typhoid fever [5]. The ability of the bacteria causing typhoid fever because it has a Vi antigen capable of reducing the expenditure of IL-8, which has the role of neutrophils inducers [6].

Intracellular facultative bacteria live and reproduce in varies of cell types, including macrophage which leads prominent in immunity. Cellular immunity mechanism is the most appropriate mechanism to do elimination, where antibody in blood circulation unable to reach progress antigens in cells [7]. Infection in pregnant women will affect the activity of immunocompetent cells that play a role in controlling the entry of viruses, bacteria and other pathogens. Influence exerted by pathogenic microorganisms will lead to symptoms of toxicity. Delays in handling would result in perforation, perionitis, loss of consciousness and death [2]. The infection will also cause damage to erythrocytes and through lysis [8]. Infection will result in inflammatory cells and affect the polymerase and differentiation of B cells thus activity of erythrocyte disturbed [9].

The synthetic compounds administered to pregnant women to prevent immune deficiencies. However, this compound causes destructive ends effects that harm the mother and the fetus [2]. Therefore the need to look for

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alternatives with systemic effects found herbal medicine [10].

*E. scaber* and *S. androgynus* has been widely studied as a natural immunomodulator [11,12], but it is not known how the effect when both plants use together. Therefore, this study was aimed to determine the influence of *E. scaber* and *S. androgynus* on the activities of B220<sup>+</sup> cells, TER119<sup>+</sup>, and GR1<sup>+</sup> on pregnant mice infected by *S. typhimurium*.

## MATERIALS AND METHODS

### Design of Experiments

This experimental research was conducted by using seven experimental groups, i.e. two control groups and five treatment groups. Surgery performed on the 12<sup>th</sup> and 18<sup>th</sup> after the animal was assumed to be pregnant by comparing the results of observation on treatment and control, as well as between the treatment groups.

Total of 42 Balb/C mice were divided into seven treatment groups, each group consisted of three replications. Mice got the same standard treatment of eat and drink ad libitum. An object of experimentation is pregnant female mice strains balb/c obtained from PT. Galaxy science Jember. Bacteria *S. typhimurium* was injected intraperitoneal. We used strains 444-D from the collection of the Laboratory of Microbiology, Faculty of Medicine, University of Brawijaya.

### Extract Preparation

Simplicia obtained from medika materia, Batu. The extract was made by maceration in 70% ethanol solution of 1:3 for 1x24 hours with occasional stirring. After 24 hours, the results were filtered using a Buchner funnel to get the plsntd extract, then evaporated at evaporator tube rotating speed of 200rpm at a temperature of 40°C waterbath. The condensed filtrate was weighed and prepared by dissolving treatment with 0.05% NaCMC and administered orally to the mice.

### Treatment

Pregnant mice were given the extract of *E. scaber* and *S. androgynus* referred to the design (Table 1). The initial dose of *E. scaber* and *S. androgynus* was 50 mg.kg<sup>-1</sup>BW. Each treatment contained three repetitions.

Later on 5<sup>th</sup> day after administration of the extract, the mice infected with the bacterium *S. typhimurium* concentration of 10<sup>7</sup> cfu.mL<sup>-1</sup>. Administration of extracts combination was

continued until surgery on the 12<sup>th</sup> and 18<sup>th</sup> day. Extracts were given by sonde daily before meal.

**Table 1.** Experiment design of mice groups

Mice Groups	Bacterial Infection	Therapy (mg.kg <sup>-1</sup> BW)	
		<i>E. scaber</i>	<i>S. androgynus</i>
K -	-	-	-
K +	infected	-	-
P1	infected	200	-
P2	infected	150	37.5
P3	infected	100	75
P4	infected	50	112.5
P5	infected	-	150

**Note:** Control (K) were given 0.05% NaCMC

### Isolation of Lymphocytes

Isolated bone marrow flushed with PBS using a syringe and placed in polypropylene tubes, whereas the isolation of peripheral blood samples were taken from the heart inserted into propylene and added PBS and centrifuged 2500 rpm for 5 min at 4°C. Pellet resuspended in 1 mL of PBS to be taken 30μL then put in a microtube containing 1 mL of PBS for re centrifuged at 1500 rpm temperature of 10°C for 5 min. Pellets then added PBS containing FITC monoclonal antibody anti-B220, anti-TER-PE 119 and PE-anti GR-1 for 50μL and incubated for 20-30 min prior to analysis using flowcytometry.

### Analysis of flowcytometry

The cell suspension was transferred into a cuvet flowcytometer, added 500 mL of PBS and homogenized. The setting of flowcytometer has been on acquired by the computer. After all the instruments were ready, cuvet mounted on the nozzle BD FACS Calibur TM Bioscience flowcytometry. Data from flowcytometer was subsequently processed with software of BD CellQuest PRO™ and displayed as a histogram.

### Data Analysis

The results of the relative number of B220<sup>+</sup> cells, TER119<sup>+</sup> and GR-1<sup>+</sup> were tested its normality with SPSS 16.0. Furthermore, the data were tested by ANOVA, and if there is significance then proceed to the Tukey test.

## RESULT AND DISCUSSION

### B220<sup>+</sup> Cells

Flowcytometry analysis of bone marrow showed the average relative number of B220<sup>+</sup> cells which was significantly different (P<0.05) to the B220<sup>+</sup> cell activation. The results ANOVA showed that the administration of 70% ethanol extract of the leaves of *E. scaber* and leaves of *S.*

*androgynus* were potential to increase the relative amount of B220<sup>+</sup> cells. Figure 1 is the expression generated from flowcytometry. It described the increase in the relative number of B220<sup>+</sup> cells. The real difference indicated that P2 treatment (ES 150 mg.kg<sup>-1</sup>: SA 37.5 mg.kg<sup>-1</sup>) with significance value of p < 0.05.

The existence of foreign objects that enter the body will be received by surface receptors [13,14]. Incoming antigens will trigger non-specific immune system by forming immuno-complex through the production of antibodies and some cytokines [15]. Other research found that flavonoid compound and the injection of *S. typhimurium* increased the proliferation of lymphocytes [9]. Thus its existence has immunostimulatory effects to stimulate the production of IL-2 [16].

Increased B220<sup>+</sup> cells showed an increase in plasma cell populations. It because the B220<sup>+</sup> cells are a subset of CD45R isoform predominantly expressed on all B-lymphocytes and plasma cells regulate the development. Increased plasma cells showed that the 70% ethanol extract of *E. scaber* and *S. androgynus* have immunostimulatory effects. Middleton [16] revealed the presence of flavonoids can inhibit the activity of MAPK (mitogen-Activated Protein Kinase) that causes posporilasi various transcription factor protein needed for protein synthesis. The induction of MAPK protein would activate the

transcription factor NF-kB is a transcription factor in the proliferation and differentiation of B220<sup>+</sup> cells through regulation of cytokines [17].

#### TER119<sup>+</sup> cells

The mean relative number of TER-119<sup>+</sup> cells was analyzed by flowcytometry (Fig. 2.) There is a decrease compared to the control. SPSS analysis showed that the relative number of TER119<sup>+</sup> cells is significantly different for the P5 treatment (SA 150mg.kg<sup>-1</sup>BW).

TER-119<sup>+</sup> cells are antigen expressed on erythrocytes. Viability of erythrocytes in normal mice is 42-56 days. Otherwise, 3-4 days for erythropoiesis, anemia, hypoxia and inflammation will lead to faster and release eritropoesis erythrocytes in peripheral area [18]. Decreased expression of TER-119<sup>+</sup> cells caused by an infection of *S. typhimurium*. Sudoyo [8] described the *Salmonella* bacteria have the ability to live well in the gastrointestinal tract and other organs, thus causing an inflammatory reaction. Inflammatory process will affect the activity of erythrocyte during oxidation will cause damage to hemoglobin. Erythrocytes will be separated from the cytoplasm, whereas macrophage cells will phagocytose abnormal erythrocyte [19]. The effect of extract gives a stable condition or equal to normal treatment. It is because pregnant mice require adequate nutrition, thus proliferation and differentiation does not work faster.

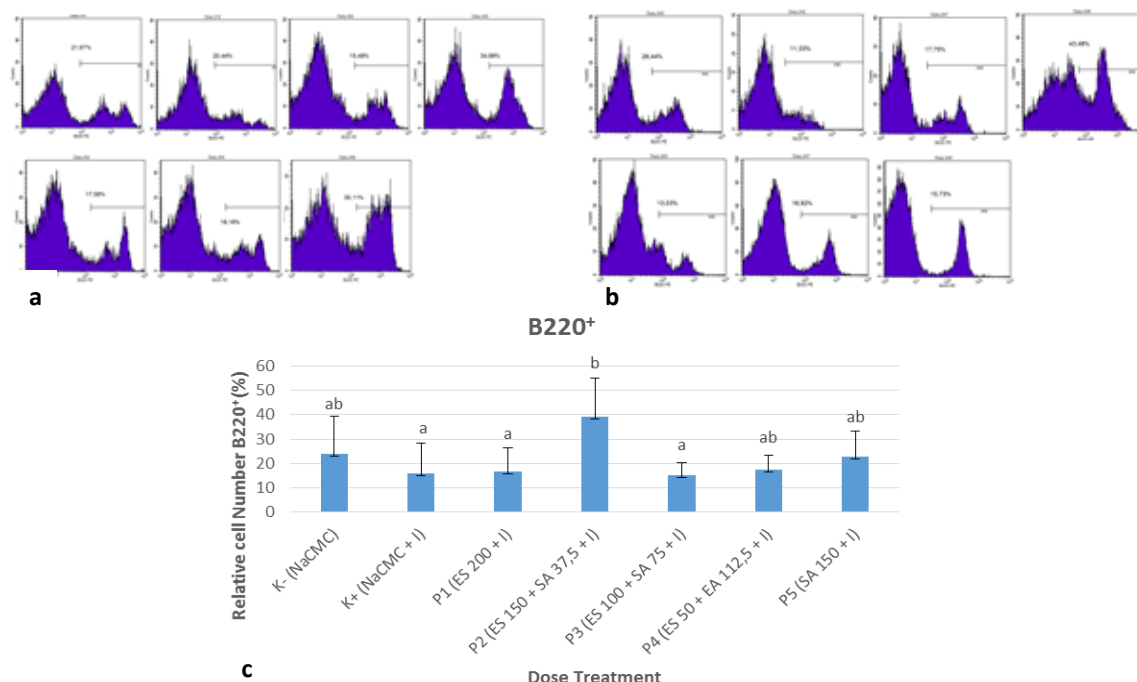
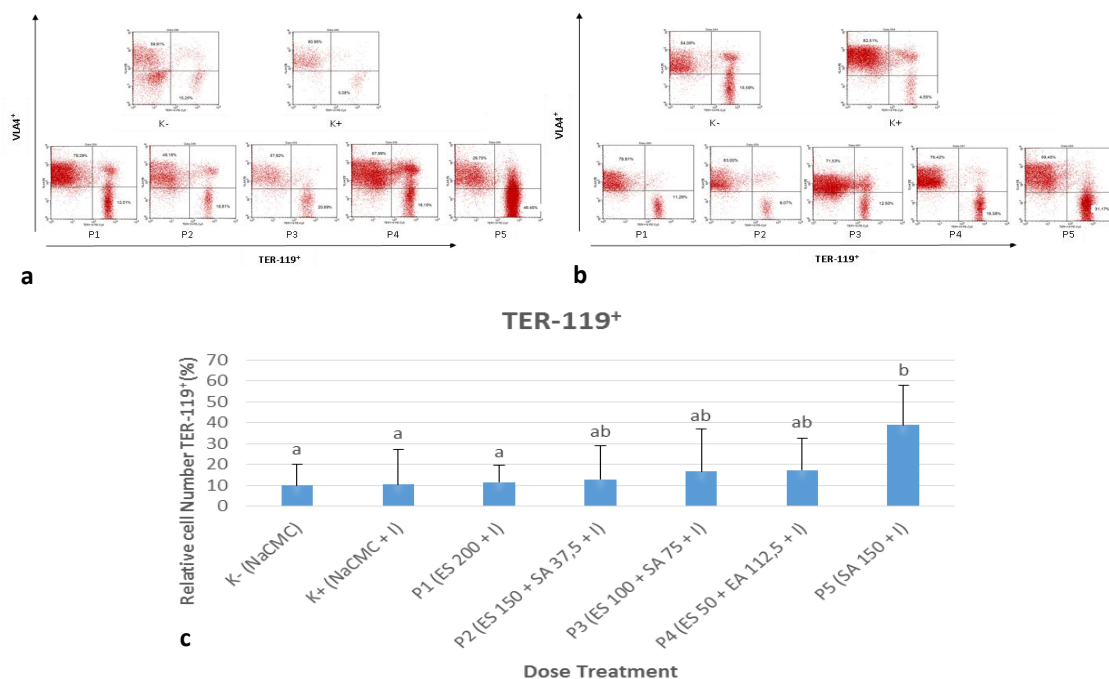


Figure 1. Expression of B220<sup>+</sup> cells

Description: ES= *Elephantopus scaber*, SA= *Sauropus androgynus*, I= infected by *Salmonella typhimurium*  
a. day surgery to 12<sup>th</sup>; b. day surgery to 18<sup>th</sup>; c. mean relative number of B220<sup>+</sup> cells (%)



**Figure 2.** Expression of TER119<sup>+</sup> cells

**Description:** ES= *Elephantopus scaber*, SA= *Sauropus androgynus*, I= infected by *Salmonella typhimurium*  
a. day surgery to 12<sup>th</sup>; b. day surgery to 18<sup>th</sup>; c. mean relative number of TER119<sup>+</sup> cells (%)

### GR1<sup>+</sup> cells

Expression of GR-1<sup>+</sup> cell proliferation showed in Figure 3. Infection of *S. typhimurium* triggered cell deficiency GR-1<sup>+</sup> to phagocytose cells that have been damaged or abnormal. ANOVA results showed that the 70% ethanol extract of the leaves of *E. scaber* and *S. androgynus* can increase the GR-1<sup>+</sup> molecule but not significant ( $p > 0.05$ ). Calculation continued to post hoc analysis with Tukey test. It showed that the treatment group had a significant difference to the control. Negative control group had an average increased compared to the positive control group. According to previous research, the increase in the number of neutrophils and IFN $\gamma$  cells in animals affected by an infection with *Salmonella* bacteria than uninfected animals [20]. Other study stated that in Balb/C mice were given *Salmonella* infection increased the number of leukocytes including neutrophils and IFN $\gamma$  significantly compared with the group treated with the standard feed [21].

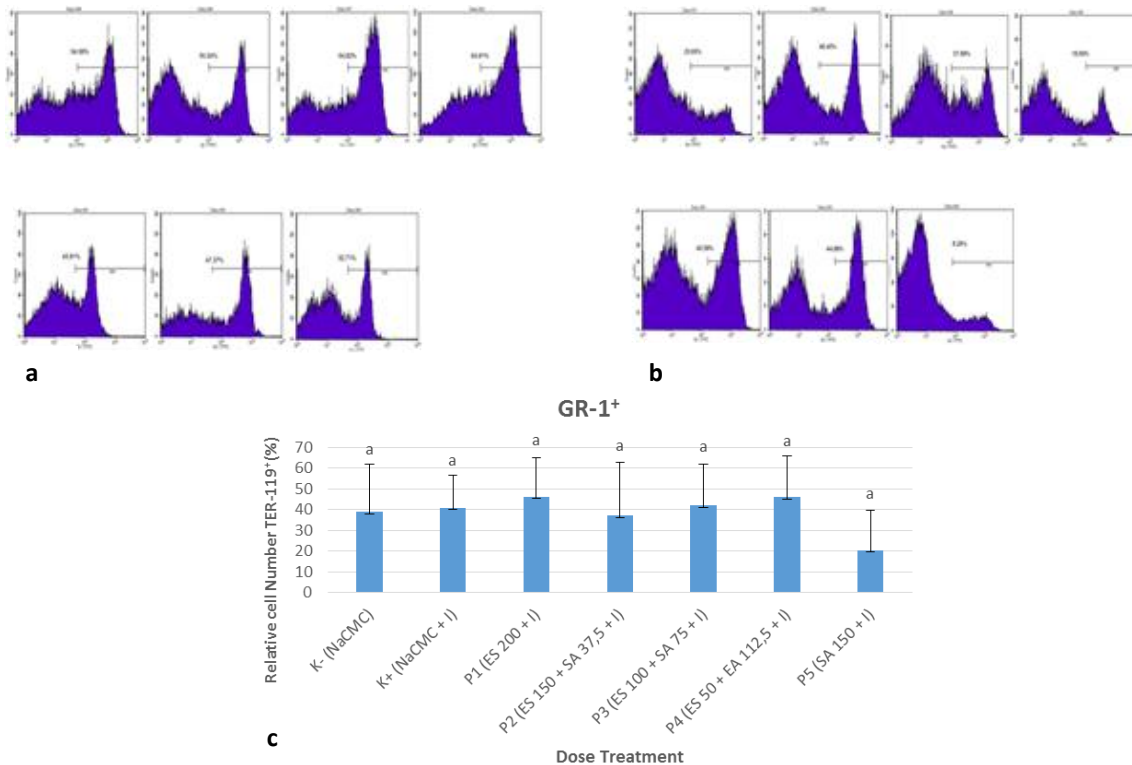
Microorganisms will spur the immune system of the body's defense nonspecific starts with the way the body's defenses destroy bacteria and more complex through antibody production or manufacture of various cytokines [15]. Neutrophils (GR-1<sup>+</sup>) work on non-specific immune response pathway. The cytokine IFN $\gamma$  will act as immunomodulator through the regulation of

gene expression to signal transduction. Neutrophil cells will express the receptor molecule with 1000 fast and stable molecule binds IFN $\gamma$  [22]. After binding, there will be internalization, thus decreasing surface receptors. IFN $\gamma$  regulates expression on number of genes including complement receptor regulator, B lymphocyte stimulator, dendrites chemotactic factor, chemokine receptors, neutrophil chemotactic factors and proinflammatory cytokines.

### Path Analysis

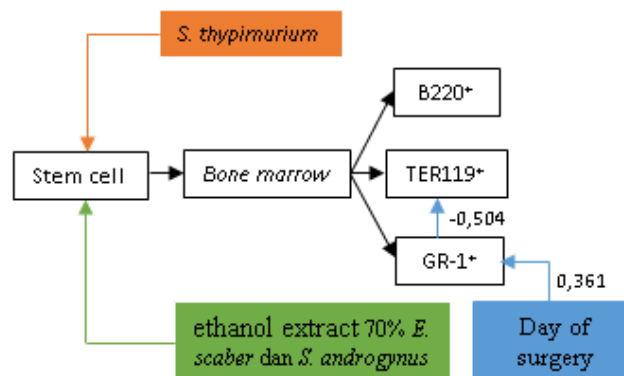
Path diagram was made based on the results on statistical analysis of the correlation (Fig. 4). Proliferation and differentiation of B lymphocyte cells are affected by the *S. typhimurium* infection and extract combinations. The antigen will be responded by expressing cell surface receptors, i.e. B220<sup>+</sup>, TER119<sup>+</sup> and Gr-1<sup>+</sup>. Day surgery affects the relative amount of macrophage cells as much as 13.03% and the relative number of cells suppresses TER119<sup>+</sup> to 18.19%.

Infection of *S. typhimurium* will increase the number of cells of neutrophils and cytokines IFN $\gamma$  [20]. Infection also affects the activity of B cells. The cells that are not growing will be phagocytosed by macrophages thus the number will be reduced. The existence of the extract will maintain the number of B lymphocyte cells in proliferation and differentiation [19].



**Figure 3.** Expression of GR1<sup>+</sup> cells

**Description:** ES= *Elephantopus scaber*, SA= *Sauropus androgynus*, I= infected by *Salmonella typhimurium*  
a. day surgery to 12<sup>th</sup>; b. day surgery to 18<sup>th</sup>; c. mean relative number of GR1<sup>+</sup> cells (%)



**Figure 4.** Development of B220<sup>+</sup> cells, TER119<sup>+</sup>, GR-1<sup>+</sup> on the organ affected Bone marrow by *S. typhimurium* infection and extract combinations of *E. scaber* and *S. androgynus* and day surgery

## CONCLUSION

The combination of *E. scaber* and *S. androgynus* for *S. typhimurium* infected mice can restore the mice into their normal conditions. The best combination for B220<sup>+</sup> cells is P2 treatment (150 mg.kg<sup>-1</sup>BW *E. scaber* and 37.5 mg.kg<sup>-1</sup>BW *S. androgynus*). While the best dose for increasing TER119<sup>+</sup> cells is P5 treatment (*S. androgynus* 150 mg.kg<sup>-1</sup>BW, while the GR-1<sup>+</sup> cells affected by the surgery. These dose formulations can be used to optimize the number of lymphocytes and not harmful for the developing fetus.

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