

The Immunomodulatory Effect of *Elephantopus scaber* and *Sauropus androgynus* Extract to Cellular Immune Response in Pregnant *Mus musculus* Infected by *Salmonella typhimurium*

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Abstract

Pregnancy has a high risk and is more susceptible to infectious diseases. *E. scaber* and *S. androgynus* contains saponins and flavonoids as an immunomodulatory that can increase the body's immunity. The purpose of this study is to determine the immunomodulatory effects of *E. scaber* and *S. androgynus* leaf extract against cellular immune response in pregnant mice infected *S. typhimurium*. This research used seven treatment groups including negative control (K-) mice without injected *S. typhimurium*, positive control (K+) mice were injected *S. typhimurium* and extract treatment with 5 variant doses and dissected on the 12th and 18th days. Lymphocytes was isolated from the blood, then relative number of CD4⁺ and CD8⁺ were analyzed with flow cytometry and data continued with ANOVA. Statistical analysis showed that different extract dose and duration of treatment had a significant effect on the number of CD4⁺ and CD8⁺ T cells. *E. scaber* and *S. androgynus* act as immunomodulatory effect and treatment with combination of extracts *E. scaber* and *S. androgynus* 50 mg.kg⁻¹ BW + 112.5 mg.kg⁻¹ BW respectively and treatment with extract of *S. androgynus* 150 mg.kg⁻¹ BW are the optimum treatment which can restore immune system conditions such as normal pregnancy without infection.

Keywords: Cellular Immune, *E. scaber*, Immunomodulatory, *S. androgynus*, *S. typhimurium*.

INTRODUCTION

Typhoid fever is a disease with serious threat in developing countries because it can cause death; which is caused by *S. typhimurium* bacteria [1,2]. In humans, the infection is caused by a decline activity of immune system thus the immune system is incapable to kill the bacteria causing the bacteria to survive, thrive, invade and damage the body's cells [3].

Pregnancy is more vulnerable and have a high risk of infection because in pregnant condition, immunological conditions is unique [4]. Previous research indicated that typhoid fever because of *Salmonella* in pregnancy can cause abortion [5].

Typhoid fever is usually treated with antibiotics and synthetic antibacterial, but either of these medications provides teratogenic effects to fetus. Thus the fetus may be at risk of mental or physical disability [6]. One alternative to solve the problem is using herbs, which is harmless compare to synthetic drugs [7].

E. scaber and *S. androgynus* contains saponins and flavonoids known as a natural

immunomodulatory that can enhance the immune system [8]. Flavonoid compounds can improve the activity of IL-2 and lymphocyte proliferation. Lymphocyte proliferation active Th1 cells macrophage activation through cytokines IFN- γ produced by CD4⁺ T cells and lysis of infected cells by CD8⁺ T cells [9,10]. Based on this background, this study aims to determine the immunomodulatory effects of *E. scaber* and *S. androgynus* leaf extract against cellular immune response in pregnant mice infected by *S. Typhimurium*, based on CD4⁺ T cells and CD8⁺ T cells in the blood.

MATERIALS AND METHODS

Treatment Group

Pregnant mice were obtained from PT. Galaxy Science Jember divided into seven groups (Table 1). Mice were infected with *S. typhimurium* on day 5 after the extract and the extract was continued until dissected on the 12th and 18th day.

Isolation of Lymphocytes Cells

Obtained blood put into propylene tubes that contained 10 ml of RBC lysis and then centrifuged 300 rpm, for 5 minutes 10⁰C to obtain pellets. Then added RBC lysis again about 5 mL and then recentrifuged again. The pellet was added 1 ml

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PBS and then centrifuged 300 rpm for 2 minutes 10°C . Pellets can be added as much as 1 ml PBS then resuspended and divided into 3 microtubes, each 300 μL then centrifuged at 2500 rpm, at temperature of 4°C for 5 minutes.

Table 1. Treatment Group

Group	Extract ($\text{mg.kg}^{-1}\text{ BW}$)		Infection (10^7 CFU.mL^{-1})
	<i>E.scaber</i>	<i>S.androgynus</i>	
K-	-	-	-
K+	-	-	✓
P ₁	200	-	✓
P ₂	150	37.5	✓
P ₃	100	75	✓
P ₄	50	112.5	✓
P ₅	-	150	✓

Flowcytometry Analysis

Pellets are added to the monoclonal antibody anti-mouse CD4 monoclonal antibody (BioLegend No. Cat. 100 531). The concentration is 0.01 mg.mL^{-1} and 50 μL phycoerythrin (PE)-conjugated anti-mouse CD8 (BioLegend, No Cat. 100 708) with a concentration of 0.01 mg.mL^{-1} . Then it was incubated for 20 minutes in the ice box, then added 300 μL PBS and resuspended. Later it

transferred to the cuvet for flowcytometry analysis.

Data Analysis

This study used a completely randomized design factorial pattern. Data from the flow cytometry analyzed statistically with one-way ANOVA with a significance level of $p < 0.05$ using SPSS, then followed by Tukey's test.

RESULT AND DISCUSSION

Result showed that the relative number of CD4^{+} and CD8^{+} T cells at day 12 in pregnant mice without infection *S. typhimurium* lower than infected pregnant mice by the *S. typhimurium* (Figure 1a). On the 18th day of pregnant also showed the same result that the relative number of CD4^{+} T cells and CD8^{+} normal pregnant mice lower than infected pregnant mice. It was significantly different (Figure 1b). The increase is due to antigen enters to the body. It can enhance the immune response for the production of immunocompetent cells and increasing the proliferation and differentiation of T cells to antigen elimination which infect the body [11].

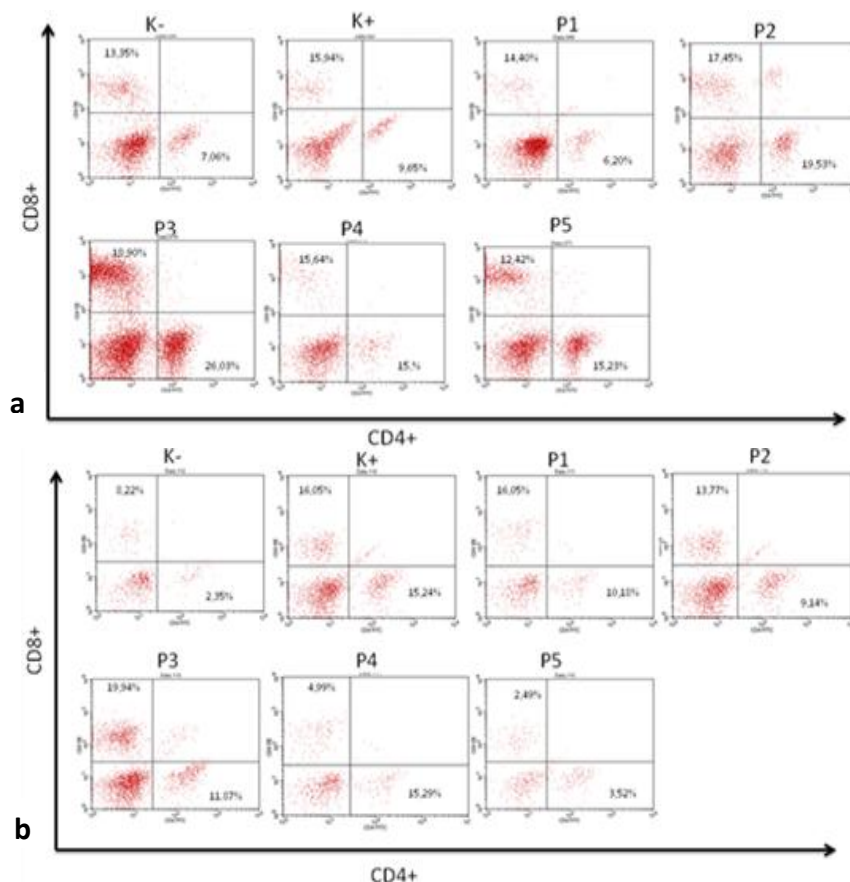


Figure 1. Profile of relative number CD4^{+} and CD8^{+} T cells. (a) 12th day of pregnancy; (b) 18th day pregnancy

The presence of *Salmonella* infection would induce Th1 cell responses and after infection, then CD4⁺ T cells are activated to increase the number of CD4⁺ T cells and cytokines [12]. CD4⁺ T cells contribute to activate macrophages, whereas the CD8⁺ T cells called cytotoxic T cells kill cells containing microbes in the cytoplasm resulting in a reservoir of infection [13].

The treatment of *E. scaber* and *S. androgynus* extract on day 12 showed a lack of regulation of immune system. It was observed through the number of CD4⁺ T cells were significantly different compared with the positive control (infected pregnant mice without treatment). CD4⁺ cell number at treatment *E. scaber* extract 200 mg.kg⁻¹ BW has 19.53% and *E. scaber* extract 100 mg.kg⁻¹ BW and *S. androgynus* 75 mg.kg⁻¹ BW

has 26.03% (Figure 2). The relative number of CD8⁺ T cells on the 12th and 18th day of dissection show that treatment with *E. scaber* extract 100 mg.kg⁻¹ BW and *S. androgynus* 75 mg.kg⁻¹ BW has 18.90% and 19.94% (Figure 3). This increase is predicted because of the content of the two plants in the form of saponins and flavonoids. Both of these compounds contribute in cell proliferation that is able to induce synthesis the proto-oncogene c-fos and c-myc. The role of proto-oncogene on cell proliferation is increasing the mitogen signal transduction through the increased expression of cytokines IL-2 [14]. Flavonoids can increase IL-2 activity and lymphocytes proliferation [9]. IL-2 can trigger the CD8⁺ activation to CD8⁺ produce perforin and granzyme that will destroy infected cells [15].

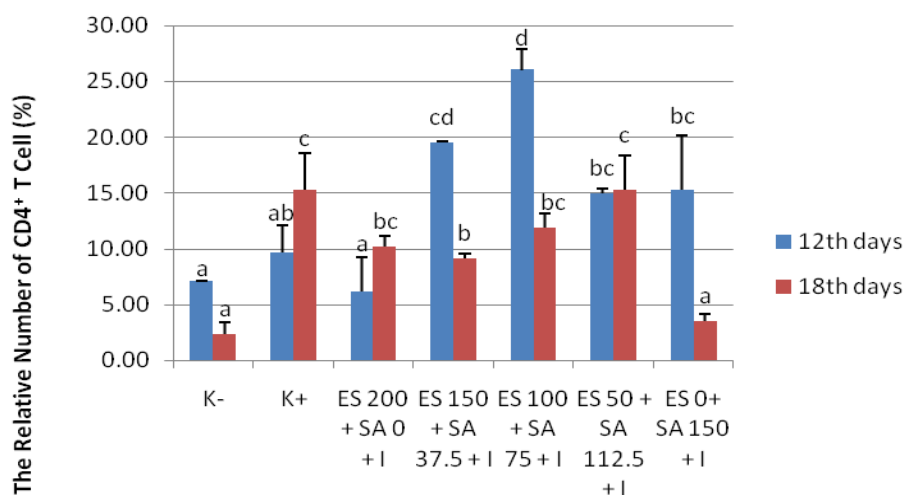


Figure 2. The relative number of CD4⁺ T cells. The dose and duration of administration of herbs affect the number of CD4⁺ T cells. Different notation indicates a significant difference (P < 0.05).

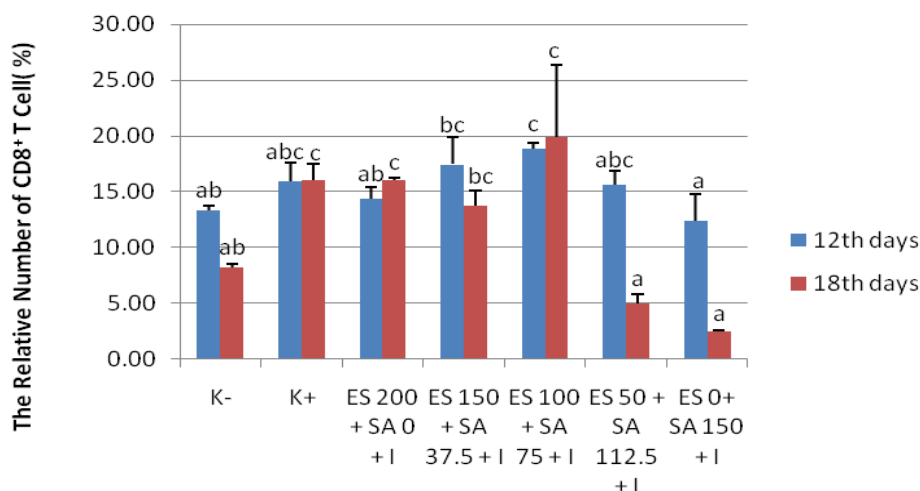


Figure 3. The relative number of CD8⁺ T cells. The dose and duration of administration of herbs affect the number of CD8⁺ T cells. Different notation indicates a significant difference (P < 0.05).

The relative number of CD4⁺ T cells at the 18th day of dissection in treatment *E. scaber* extract 150 mg.kg⁻¹ BW has 9.14% and treatment *S. androgynus* extract 150 mg.kg⁻¹ BW has 3.52% significantly decreased compared with a positive control (infected pregnant mice without treatment). The relative number of CD8⁺ T cells also showed the same result the treatment with extract *E. scaber* 50 mg.kg⁻¹ BW and *S. androgynus* 112.5 mg.kg⁻¹ BW has 4.99% and extract of *S. androgynus* has 2.49%. It significantly decreased compared with a positive control. Saponins and flavonoids in addition act as immunostimulatory also serves as immunosupresor that suppresses the immune response. Both of these compounds are amphiphilic that can increase the level of Cyclin-Dependent-Kinase (CDK) inhibitor in the form of protein P27^{KIP} that play a role in the regulation of cell proliferation in phase G0/G1 by inhibiting compound G1 Cyclin-CDK resulting in cell cycle does not continue and the cessation of cell proliferation [16].

In pregnancy, the function of humoral and cellular immune suppression that occurred suppression of Th1 and Tc cells which will reduce the secretion of IL-2, IFN- γ and TNF- β . Suppression of Th1 response is needed to sustain a pregnancy [17]. In addition, pregnancy hormones such as progesterone, estrogen and prolactin are also affects the immune system that is able to minimize the effects of peripheral NK cells [18]. Treatment with combination of extracts *E. scaber* 50 mg.kg⁻¹ BW and *S. androgynus* 112.5 mg.kg⁻¹ BW and treatment with extract of *S. androgynus* 150 mg.kg⁻¹ BW are the optimum treatment which can restore immune system conditions such as normal pregnancy without infection.

CONCLUSION

E. scaber and *S. androgynus* extract showed a significant difference to the number of CD4⁺ and CD8⁺ T cell in infected pregnant mice by *S. typhimurium*. Optimal treatment to help balance the immune system in pregnancy treated with a combination of extracts *E. scaber* 50 mg.kg⁻¹ BW and *S. androgynus* 112.5 mg.kg⁻¹ BW and treatment P5 with *S. androgynus* extract 150 mg.kg⁻¹ BW.

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