

Bioactivity of *Sauropus androgynus* and *Elephantopus scaber* to CD4⁺IL2⁺ and CD4⁺IL4⁺ T Cells Modulation in Balb/c Pregnant Mice Model of Typhoid

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Abstract

Pregnant woman have higher risk to get infection, because pregnancy decreasing the cell T activity. *Sauropus androgynus* and *Elephantopus scaber* has substance like saponin and flavonoid which has been well known as natural immunomodulator, particularly to increase amount of immunocompetent cell. This research is important to recognize effective supplement supply for immunomodulator of *S. Androgynus* and *E. Scaber* to increase mice's (*Mus musculus* Balb/c) immune system. This research conducted in seven treatments by 3 repetitions for each treatment by using pregnant mice which has been infected by bacteria *Salmonella typhimurium* (dose 10⁷ CFU.mL⁻¹). Bacteria are injected to mice intraperitoneal in day 5th after giving combination of extract *E. scaber* and *S. androgynus*. The dose of *E. scaber* and *S. androgynus* combination are 200; 150:37.5; 100:75; 50: 112.5; 150. Five group of treatment were infected by *S. typhimurium*. Two other groups were the control, namely negative control which was only given NaCMC 0.05% without infection and positive control which was given NaCMC 0.05% and infected by *S. typhimurium*. After being injected, treatment was redone till the day of surgery. The surgery was executed in day 12th and 18th of pregnancy. Data were analyzed using ANOVA ($p < 0.05$) and Duncan test. Result indicated that extract of *S. androgynus* and *E. scaber* could increase amount of immune system in pregnant mice. This was indicated from significant increasing in amount of cell T CD4⁺IL2⁺ and CD4⁺IL4⁺ in pregnant mice which has been infected by *S. thypimurium*. Formula of extract *S. androgynus* and *E. scaber* which could return immune condition was approached condition of healthy pregnant mice such as *E. scaber* 200 mg.kg⁻¹ BW; *E. scaber* 100 mg.kg⁻¹ BW and *S. androgynus* 75 mg.kg⁻¹ BW; *E. scaber* 50 mg.kg⁻¹ BW and *S. androgynus* 112.5 mg.kg⁻¹ BW; and *S. androgynus* 150 mg.kg⁻¹ BW, respectively.

Keywords: CD4⁺IL2⁺, CD4⁺IL4⁺, *E. scaber*, Immunomodulator, *S. androgynus*, *S. typhimurium*

INTRODUCTION

Pregnant woman who has been infected by *Salmonella* has more decrease amount of innate immune system such as dendrite, neutrofil, and NK cell than non-pregnant woman [1]. Effect of decreased T cell in pregnant woman is they susceptible to illness of infection, like urine canal, bacterial infection like *Salmonella* impact of typhoid fever [2,3].

Indonesia has 25,000-30,000 species of plants, and 80% type of plants in the world and 90% from type of plants in Asia [4]. Indonesian people known and used plants as medicine in healing of illness. In body, herbal medicine has systemic effect not like synthetic active medicine [5]. One of herbal medicine in Indonesia is *Sauropus androgynus* and *Elephantopus scaber*. Both plants have substance such as saponin and flavonoid which well known as natural immunomodulator [6,7].

Saponin and flavonoid is active compound that can increase immunity response especially in increasing amount of immune competent cells such as macrophage, T cell and B cell. Plant extract which has saponin and flavonoid substance can increase the amount of CD4⁺ T cell [7,8,9]. Proliferation of CD4⁺ T cell will increase IL-4 production to activate IgG to phagocytosis bacteria. CD8⁺ T cell will be activated in 14th day after infected which has function as CTL that will kill the infected cell, then cell becomes lysis [10]. Besides that, *E. scaber* is effective to stimulate hematopoiesis process [7,11]. But it has not been recognized that both of leaf when being combined, wheter synergistic and antagonistic as immunomodulator agent.

Activation of lymphocyte cell is expressed by many subset of lymphocyte cell like T cell of CD4⁺ IL-2⁺ and CD4⁺ IL-4⁺. Therefore, this research was aimed to recognize the effective suplement formulation of *E. scaber* and *S. androgynus* towards system immune enhancement in pregnant mice (*Mus musculus*) BALB/c by the observation in spleen.

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MATERIALS AND METHODS

Extraction of *S. Androgynus* and *E. Scaber*

Powder leaf of *S. androgynus* and *E. Scaber* were macerated by using ethanol 70% for 24 hours. The material is filtered and alcohol replaced and it was soaked till the color of alcohol showed that the compound had been extracted completely. All of the result of filtering was combined and steamed to release the substance of ethanol in extract on temperature 50°C inside the water bath by using vacuum pump evaporator. Result of evaporation was thick extract or paste.

Isolat *S. typhimurium* for injection

Isolates of *S. typhimurium* (444-D) was obtained from the Laboratory of Microbiology, Faculty of Medicine, University of Barawijaya, Malang. Amount of injected bacteria was 10^7 CFU.mL⁻¹ as many as 0.5 mL.

Treatment

The combination of the extract were given until day 12th and 18th by gavage after acclimation. It is conducted every day in 5 days of pregnancy, and then it is injected *S. typhimurium*. The bacteria were injected to mice intraperitoneal in day 5th. The following (Table 1) is dose of of *S. androgynus* and *E. scaber* given extract.

Table 1. *S. androgynus* and *E. scaber* Extract Combination

Group	NaCMC 0.05 %	Infec tion	Extract (mg.kg ⁻¹ BW)	
			<i>E. scaber</i>	<i>S. androgynus</i>
1	+	-	-	-
2	+	+	-	-
3	-	+	200	-
4	-	+	150	37.5
5	-	+	100	75
6	-	+	50	112.5
7	-	+	-	150

Note: Infection= 10^7 CFU.mL⁻¹ of *S. typhimurium*

Flowcytometry of Cell Lymphocyte Isolation

Lymphocyte cell whose population was counted will be isolated from spleen of surgery mice, then it is added PBS 10 mL and saved in ice box. The cell suspension centrifuged 2500 rpm for 5 minutes 4°C. Pellet was resuspended with 1 mL of PBS to be taken 200 mL then put into microtube and PBS was added to 1 mL of PBS. Then centrifuged at 2500 rpm for 5 minutes 4°C. Pellets then added with monoclonal antibody anti CD4⁺IL2⁺ and anti CD4⁺IL4⁺, each by 50 µL

with a concentration of 0.01 mg.mL⁻¹. Then put into a microtube and added 100 µL cytofix-cytoferm (BioLegend No. cat 420801) the pipetted and incubated for 20 minutes 4°C. It is added washperm (BioLegend No. cat 421002) as much as 500 µL. After being centrifuged 2500 rpm for 5 minutes 4°C and next procedure using antibody PE-conjugated anti IL-2⁺ and anti IL-4⁺ as much 50 µL by concentration 0.01 mg.mL⁻¹ and incubated for 20 minutes. And it's added 300 µL PBS and resuspension using micropipette. Then transferred into a cuvet and attached to the nozzle BD Bioscience FACSCalibur™ flow cytometry.

Data analysis

Parametric one way ANOVA by significance degree $p < 0.05$ was used and then its significant result continued by Duncan test. This research used a factorial completely randomized design (factorial CRD). The first factor was dose combinations and the second one was the day of pregnancy.

RESULT AND DISCUSSION

Relative Number of CD4⁺IL-2⁺ T cell

Relative number of CD4⁺IL2⁺ T cell in this research used to recognize the treatment effect of *E. scaber* and *S. androgynus* formulation towards the quantity enhancement of CD4⁺IL2⁺ T cell (Figure 1). The result of flow cytometry that conducted in mice after *S. typhimurium* infected showed the difference on relative number of lymphocyte cell, i.e. cell CD4⁺IL-2⁺. Highest amount of CD4⁺IL-2⁺ cell was found in pregnant mice which has been infected and given extract *E. scaber* 50 mg.kg⁻¹ BW and *S. androgynus* 112.5 mg.kg⁻¹ BW (1.71%). The result of ANOVA shows that relative number of CD4⁺IL-2⁺ cell after infection of *S. typhimurium* is significantly different ($p < 0.05$).

While the infected mice Salmonella typhimurium and given extract *E. scaber* 200 mg.kg⁻¹ BW, *E. scaber* 100 mg.kg⁻¹ BW + *S. androgynus* 75 mg.kg⁻¹ BW, *E. scaber* 50 mg.kg⁻¹ BW + *S. androgynus* 112.5 mg.kg⁻¹ BW, *S. androgynus* 150 mg.kg⁻¹ BW is not significantly different with healthy mice. The significant difference with normal mice was only found in pregnant mice which was given extract *E. scaber* 150 mg.kg⁻¹ BW and *S. androgynus* 37.5 mg.kg⁻¹ BW and infected by *S. typhimurium*.

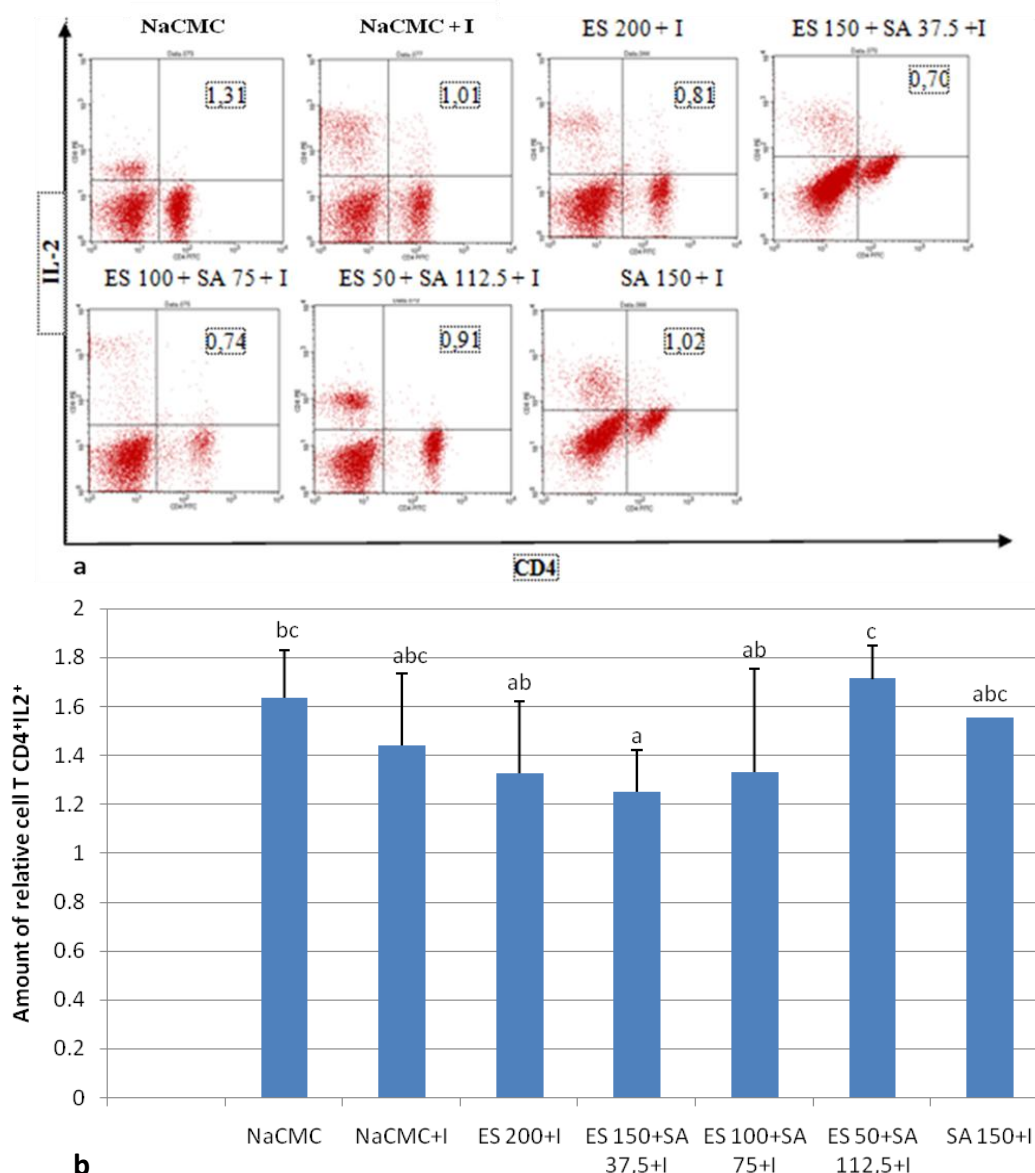


Figure 1. Profile of Average Relative Number on CD4⁺IL2⁺ T cell. a. Flowcytometry analysis, b. statistic analysis
Description: NaCMC 5%, I: Infection of *S. typhimurium*, ES: *E. scaber*, SA: *S. androgynus*

Extract combination of *E. Scaber* and *S. androgynus* can enhance immune system. It can increase proliferation sitocin IL-2. Both plants are known having flavonoid content and saponin that can be immunomodulator [8,9,12]. Saponin can enhance body's immune by inducing proliferation immune cells [7]. Pregnant mice that were infected by *S. typhimurium* in spleen can trigger proliferation of CD4⁺ T cell which secretes cytokine IL-2. This occurred because of spleen act as erythrocyte storage and contains leukocyte specialized to phagocytose macrophage will filter antigen from blood. This organ helps body to identify and kill pathogen bacteria.

Along with the phases of pregnancy, the cytokine IL-2 increased. Accumulation of bacteria in placenta slowed the bloodstream that brings nutrition into fetus and has bad impact for the fetus. Infected placenta has significant enhancement on inflammation cell, except NK cell. Suppression system immune in pregnant phase is connected with hormonal condition. Concentration of progesterone hormone that enhanced in pregnant phase has impact to slow activation of T lymphocyte to the stimulation antigen. Progesterone can reduce cell which mediate activity of NK cell (*Natural Killers*) and activity of cytotoxic T cell.

Expression of reseptor progesterone will cause NK cell and lymphocyte in periphery blood enhance in pregnant phase, but this enhance-ment is not significant [13]. Enhancement of progesterone in its receptor induces secretions protein which is called *progesterone induced protein blocking factor* that has impact to slow the activity of cytolytic NK cell and lymphocyte directly [14].

Relative Number of CD4⁺IL-4⁺ T cell

The analysis of ANOVA shows that the amount of relative CD4⁺IL4⁺ T cell has significant difference ($p < 0.05$). In all infected pregnant *M. musculus* and given treatment of *E. scaber* and *S. androgynus*, the relative number of CD4⁺IL4⁺ T

cell is not significantly different than *M. musculus* normal pregnant (Figure 2).

This results show that steroid saponin is contained naturally in *E. Scaber* that can enhance imunity response Th2 cell connected with regulator T cell enhancement which is mediated by secretion cytokine. The enhancement of cytokine IL-2 production will also enhance cell TCD4⁺ in producing IL-4. The enhancement of IL-4 production can enhance IgG in fagociting bactery and proliferation cell T CD8 can be CTL that will lysis infected cell by *S. typhimurium* [10]. Other study also explained that vaccinated pregnant mice by *S. typhimurium* showed increase in IL-4 production compared with non vaccinated pregnant mice [15].

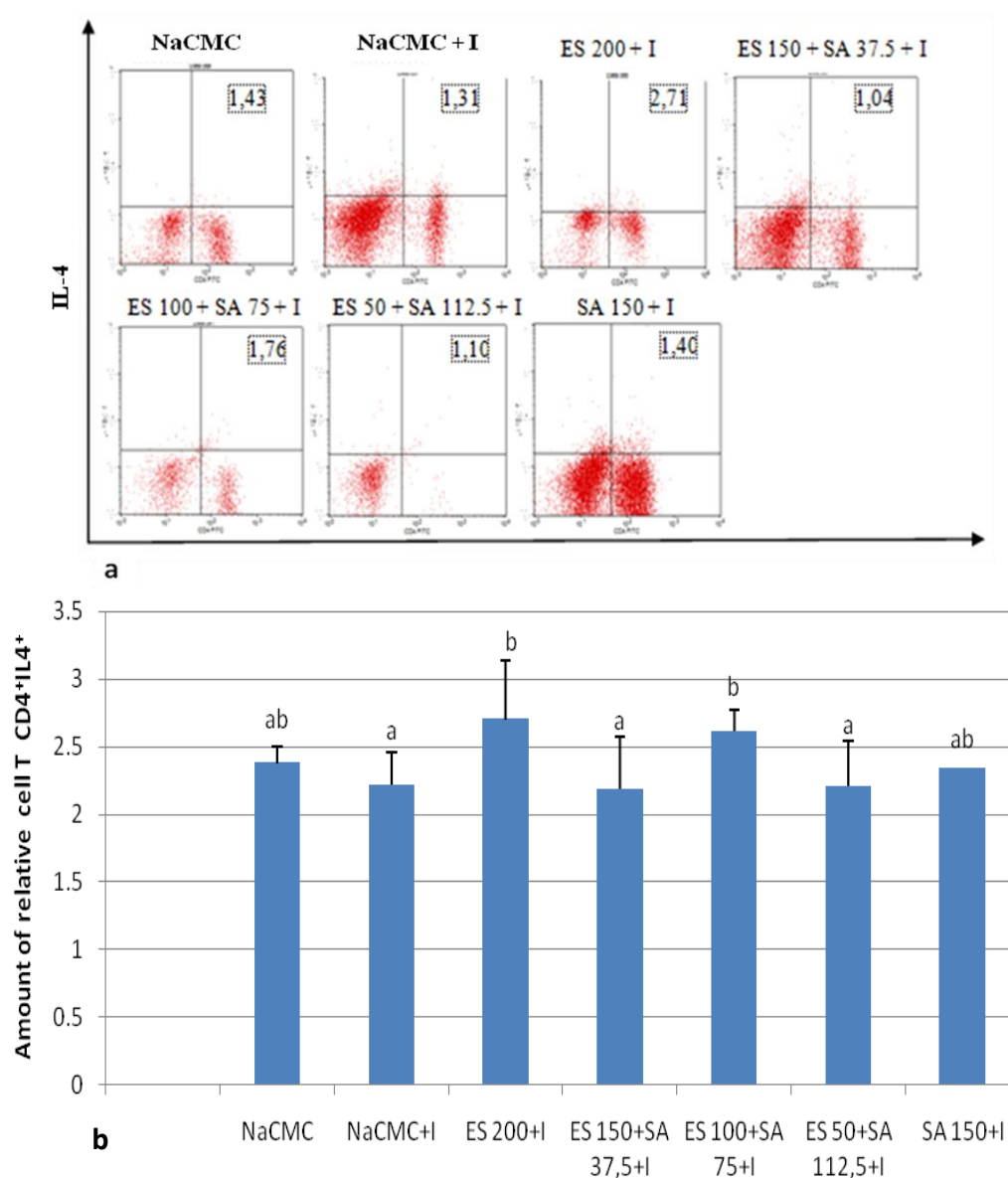


Figure 2. Profile of Average Relative Number on CD4⁺IL4⁺ T cell. a. Flowcytometry analysis, b. statistic analysis
Description: NACMC 5%, I: Infection of *S. typhimurium*, ES: *E. scaber*, SA: *S. androgynus*

CONCLUSION

Administered extract of *S. androgynus* and *E. scaber* formulation can enhance the immune system in pregnant mice. It is indicated from the significant enhancement in average amount of CD4⁺IL2⁺ and CD4⁺IL4⁺ T cell in pregnant mice which were infected by *S. thypimurium*. Four combinations which were supposed to be the optimum dose that can enhance immune condition of pregnant mice and cannot cause the abortion are respectively as follows: *E. scaber* 200 mg.kg⁻¹ BW; *E. scaber* 100 mg.kg⁻¹ BW and *S. androgynus* 75 mg.kg⁻¹ BW; *E. scaber* 50 mg.kg⁻¹ BW and *S. androgynus* 112.5 mg.kg⁻¹ BW; and *S. androgynus* 150 mg.kg⁻¹ BW.

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