

Bioactivity of Combination *Elephantopus scaber* and *Sauropus androgynus* on the Level of B220 cells of Lymph Node in Pregnant Typhoid BALB/c Mice

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Abstract

Elephantopus scaber and *Sauropus androgynus* are herbal remedies that contain flavonoids as immunomodulatory agents. This study was aimed to observe the changes in the relative number of B cells in lymph node tissue of pregnant typhoid mice. Mice were divided into seven groups K-, K+, *E. scaber* 200 mg.kg⁻¹ BW, *E. scaber* 150 mg.kg⁻¹ BW + *S. androgynus* 37.5 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, dan *S. androgynus* 150 mg.kg⁻¹ BW. Mice were dissected on the 12th and 18th day after herbal treatment. Data was analyzed using one way ANOVA ($p < 0.05$) and Duncan. The result show that *E. scaber* combination of 200 mg.kg⁻¹ BW and *E. scaber* 150 mg.kg⁻¹ BW + *S. androgynus* 37.5 mg.kg⁻¹ BW can increase the number of B220 cells ($p < 0,05$) on pregnant mice typhoid model.

Keywords: B220, *Elephantopus scaber*, pregnant, *Sauropus androgynus*, typhoid fever

INTRODUCTION

Salmonella typhi bacteria can cause typhoid fever [1]. Total 20,000 patients recorded in 2003 died of typhoid fever in Indonesia [2]. The prevalence of typhoid increases each year [3]. *Salmonella* bacteria is classified as facultative intracellular bacteria, making it difficult for immune system to recognize antigens.

In early primary infection, *Salmonella* is controlled by the innate immune system. After seven days, the adaptive immune system will start to work [4]. First time with a CD4-T cells activation by binding CD4-T cells, antigen-specific, and MHC class II presented by APC (Antigen Presenting Cell) on lymph nodes. The activated CD4-T cells would secrete cytokines IL-2 (Interleukin-2) which stimulates proliferation of CD8-T cell, B cell, and CD4-T cells itself [5]. CD8-T cells are active will lysis infected cells that recognized by specific antigens presented by MHC class I [6]. CD8-T cells activation occurred along with the decrease of CD62L-T cells [7]. Whereas B cells will produce antibodies that can recognize specific antigens in infected cells and in cooperation with NK (Natural Killer) for lysis of the cells [6].

Previous research showed that 65.4% of pregnant women exposed to typhoid fever [8]. In pregnant women, the disease can cause

miscarriage because of excessively NK cell lysis. The role of hormones of pregnancy against the maternal immune system is different between pregnant and nonpregnant woman [9].

Antibiotic that cure typhoid fever can be teratogens [10]. It is require a safer alternative to minimize these risks is by using herbs. Plants that have the potential as a medicinal herb is *Elephantopus scaber* and *Sauropus androgynus*. Both herbs contain flavonoids and saponins as immunomodulatory [11,12,13]. However, it is not known if the two combined herbal will serve as an immunosuppressant or immunostimulant agent, especially in the case of typhoid fever in pregnancy. This study aims to determine the effect of the day pregnancy and combination of extract *E. scaber* and *S. androgynus* on the relative number of B cells in pregnant mice model of typhoid fever.

MATERIALS AND METHODS

Ethanol Extract Preparation of *E. scaber* and *S. androgynus*

Preparation of ethanol extract of *E. scaber* and *S. androgynus* based on previous research [14]. Leaf powder *E. scaber* and *S. androgynus* obtained from the Material Medica, Batu, East Java. The powder as much as 27.6 g from each plant was macerated in a glass jar with 70% ethanol at room temperature and dark for 24 hours. The ethanol extract was filtered and added another 70% ethanol for 24 hours, it was performed 2 to 3 times. The material is filtered

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and then evaporated at 50°C using a vacuum pump evaporator.

Bacteria

Bacterial isolates of *S. typhimurium* (444-D) was obtained from the Microbiology Laboratory, Faculty of Medicine, University of Brawijaya, Malang. The number of bacteria that is injected as much as 10^7 CFU.mL⁻¹.

Animals and Treatment

Mus musculus BALB/c pregnant females aged 8 weeks was obtained from PT. Galaxy Science, Jember, East Java. Mice were randomly divided into seven groups with each group consisting of 6 mice (Table 1). The combination of the extract given by gavage from day 1st of pregnancy. Later in the day 5th infected intraperitoneally with *S. typhimurium*, while given the extract until day 12th and 18th of pregnancy, and mice dissected for lymph node organs.

Table 1. Treatment Group

Treatment group	Infection	Extract Powder (mg.kg ⁻¹ BW)	
		<i>E. scaber</i>	<i>S. androgynus</i>
1 (K-)	-	-	-
2 (K+)	+	-	-
3	+	200	-
4	+	150	37.5
5	+	100	75
6	+	50	112.5
7	+	-	150

Note: Group 1 and 2 use NaCMC 0.05%, Infection = 10^7 CFU.mL⁻¹ *S. typhimurium*

Lymphocytes Isolation and Flowcytometry Analysis

After treatment, mice were dislocated and lymphocytes were isolated from lymph node to determine the relative quantity of B220. The lymph nodes were crushed and filtered using a wire. The cell suspension was transferred into a 15 ml centrifuge tube and centrifuged 2500 rpm for 5 minutes 4°C. Pellets was added 50 µL of monoclonal antibody anti B220⁺ (BioLegend & No Cat. 109813) with concentration 0.01 mg.mL⁻¹. After transferred into a cuvet, cell suspensions was analysed using flow cytometry (BD Bioscience FACSCaliburTM).

Data analysis

This study used a factorial completely randomized design (factorial CRD), with the first factor extract was dose combinations and the second factor was the day of pregnancy. Data

was analyzed by one-way ANOVA with p=0.05 followed by Duncan as further testing.

RESULT AND DISCUSSION

The results showed that the extract treatment significantly increase the relative number of B220 cells in lymph node pregnant mice model of typhoid fever. Furthermore, based on Duncan test, on the 12th day of pregnancy there is a difference in the mean number of cells B220 relatively significant among pregnant infected, with normal pregnant group *E. scaber* extract 200 mg.kg⁻¹ BW as well as combinations of *E. scaber* 150 mg.kg⁻¹ BW and *S. androgynus* 37.5 mg.kg⁻¹ BW (Fig. 1).

The relative number of cells B220 on the 12th day indicates the infections in pregnant mice are lower than without infection. It shows that when infection occurs, the immune system of pregnant mice decreased. This is because the cytokines of lymphocytes decreased during pregnancy when activated monocytes and granulocytes [15]. Monocytes and granulocytes are necessary for the first defense in the *S. typhimurium* infection for the first 7 days as the innate immune system [4]. The number of CD4 and CD8-T cells in pregnant mice infected with *S. typhimurium* was significantly decreased compared with non-pregnant mice [9]. Other study also found the decrease number of CD4-T cells in infected pregnant mice, although the difference was not significant [16].

However, number of CD4 and CD8 T cells in the pregnant condition maintained to be low by hormones that increase during pregnancy, e.g. progesterone, estrogen, and prolactin. Another immune system is maintained low NK cell [17]. The defense mechanism through a reduction in cytokine production, such as IFN γ , in this case progesterone is immunosuppressive [18].

The *E. scaber* extract 200 mg.kg⁻¹ BW as well as combinations of *E. scaber* 150 mg.kg⁻¹ BW and *S. androgynus* 37.5 mg.kg⁻¹ BW showed immunostimulatory activity. The activity can be viewed through an increase in the number of B220 cells compared to infected pregnant mice. The increase was due to the flavonoids in *E. scaber* were able to increase production of the cytokine IL-2, IL-4, and IL-1 which stimulate the proliferation and differentiation of T cells and B cells [19]. Increasing number of B220 cells that occurs is not significantly different from the conditions of pregnant mice without infection. This shows that the extract on both combination stabilizes the immune system such as required in pregnant mice to maintain pregnancy.

But if the extract is carried out continuously, it resulting decline in the immune system. This can be seen by administration of a combination of extracts was not significantly different from infected pregnant mice. Based on the average number of cells B220, it can be concluded that

the optimum dose to stabilize the immune system required for pregnancy is the *E. scaber* extract 200 mg.kg⁻¹ BW as well as combinations of *E. scaber* 150 mg.kg⁻¹ BW and *S. androgynus* 37.5 mg.kg⁻¹ BW 12th day of pregnancy.

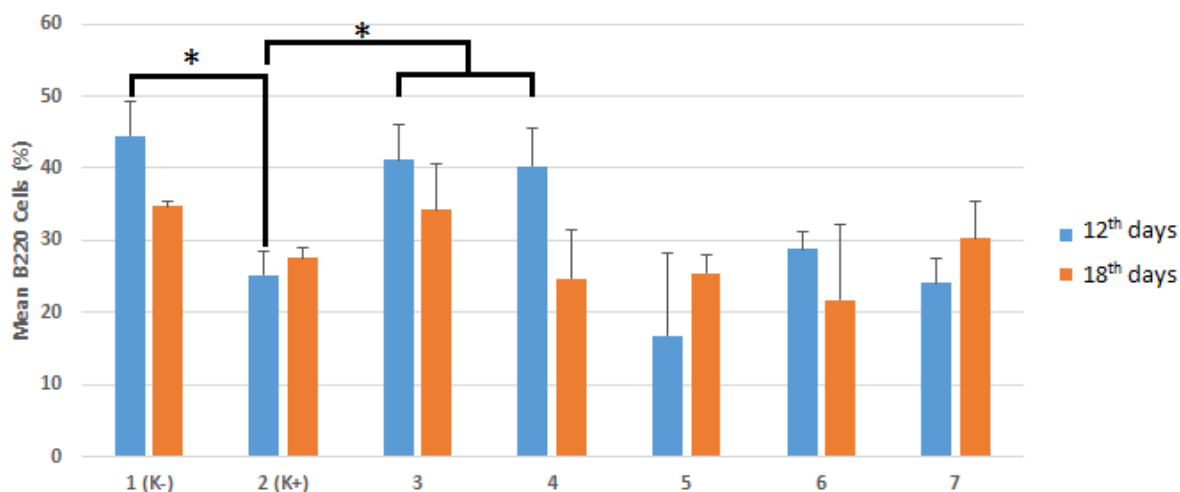


Figure 1. The relative number B220 cells after administration of a combination of extracts of *E. scaber* and *S. androgynus* (mg.kg⁻¹ BW). An asterisk (*) indicates that on the 12th day, the positive control was significantly different with negative control, giving a combination of *E. scaber* extract 200 mg.kg⁻¹ BW, as well as *E. scaber* 150 mg.kg⁻¹ BW

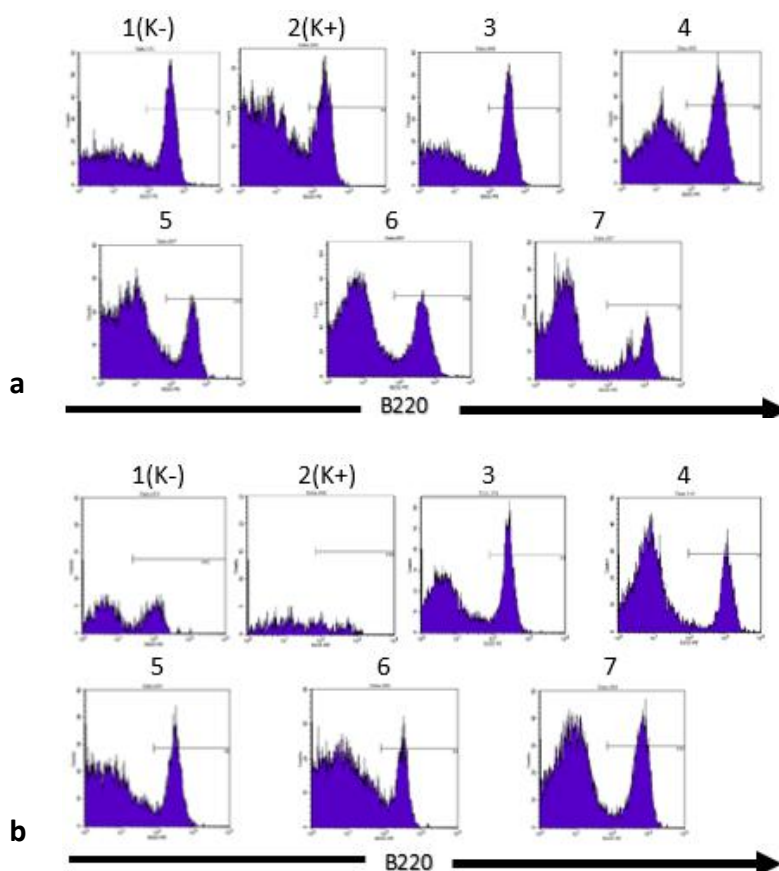


Figure 2. Percentage of relative cell number B220 after giving treatment. (a) 12th day of pregnancy; (b) 18th day of pregnancy.

CONCLUSION

The ethanolic extracts combination of *E. scaber* and *S. androgynus* showed a significant effect on the number of B220 cells with optimum doses of the administration of a combination of extracts of *E. scaber* 200 mg.kg⁻¹ BW, followed by *E. scaber* 150 mg.kg⁻¹ BW + *S. androgynus* 37.5 mg.kg⁻¹ BW.

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