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Herbal Supplement Formula of *Elephantopus scaber* and *Sauropus androgynus* Promotes IL-2 Cytokine Production of CD4+ T Cells in Pregnant Mice with Typhoid Fever

Abstract: Production of IL-2 by CD4+ T cells is shown to be suppressed in pregnant women infected by bacteria such as *Salmonella typhi*. *Elephantopus scaber* and *Sauropus androgynus* may be used as herbal supplement to ameliorate this condition. This study aimed to investigate the efficacy of *E. scaber* and *S. androgynus* formulation to promote lymphoid proliferation and CD4 cytokine productions. The pregnant mice were randomly divided into seven experimental groups: K- (control), K+ (with *S. typhi*), P1 (*S. typhi* + *E. scaber* 100%), P2 (*S. typhi* + *E. scaber* 75% and *S. androgynus* 25%), P3 (*S. typhi* + *E. scaber* 50% and *S. androgynus* 50%), P4 (*S. typhi* + *E. scaber* 25% and *S. androgynus* 75%), and P5 (*S. typhi* + *S. androgynus* 100%). FACS analysis was done on day 18. Typhoid fever caused decreasing IL-2 and IFN-γ and in contrast increasing IL-4 production. In this experiment we clearly showed that typhoid fever decreased the amount of CD4 T cells but rather increased the amount of B cells. Formulation of *E. scaber* and *S. andogynus* was able to ameliorate the condition by increasing IL-2, IFN-γ, CD4 T cells and decreasing both IL-4 cytokine production and the amount of B cells.

Keywords: Katuk; Immunomodulator; Pregnancy; *S. typhi*; tapak liman

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1 Introduction

Elephantopus scaber (tapak liman) is one of the traditional herbal medicines used to treat certain diseases. Ethanol extract of the leaves can stimulate wound healing activity in mice is characterized by decreasing chronic inflammatory cells, reduced swelling and increased collagenation [1]. Stigmasterol serves to stimulate naïve CD4+ T cells to differentiate into Th1 or Th2 cells. In addition, *Sauropus androgynus* (katuk) is also often used as an herbal medicine. Chemical compounds contained in *S. androgynous* are saponin, flavonoid, and tanin. Flavonoid is a polyphenolic compound known to have properties as a free-radical, hydrolysis and oxidative enzyme inhibitor, and works as an anti-inflammatory agent [2]. 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 [3].

Pregnant women have changes in the immune system response due to the implantation of an embryo which is basically non-self antigen. Immunological recognition of pregnancy is vital for the maintenance of pregnancy, and inadequate recognition of fetal antigens may cause abortion [4]. These changes make pregnant women more susceptible to pathogen infection. A recent case of typhoid fever in the first trimester of pregnancy demonstrated that *Salmonella enterica* serotype *typhi* (*S. typhi*) could be one of the other pathogens on the TORCH list of pathogens [5]. Fetal loss occurred at 16 weeks with *S. typhi* found in the fetus at autopsy [6].

However some pathogenic bacteria have the ability to suppress the production of IL-2 cytokine by CD4+ T cells. This strategy is done to prevent rapid T cells proliferation so that the response of the immune system to bacterial invasion is weakened. IL-2 exerts its effects on many cell types in the immune system, especially the T lymphocyte cells [7]. IL-2

also acts to stimulate NK cells [8]. The major activities of IL-2 cytokine in CD4+ T cells are to induce development of antigen-specific clones via both proliferative and anti-apoptotic mechanisms, increase the production of other cytokines such as IFN-γ and decrease cytokines that are produced by Th2 such as IL-4, IL-5, IL-6, and IL-10 [9].

Until now, herbal medicines have been chosen as a safer treatment option during pregnancy because they have no teratogenic effects like antibiotics. As described before, *E. scaber* and *S. androgynus* have the potential as therapeutic herbal supplements during pregnancy. The combination of both supplements is suspected to lead to a synergistic effect in stimulating the immune system through increased secretion of IL-2 by CD4$^+$ T cells, which is a growth factor for immune cells. Our recent finding demonstrated that *E. scaber* combined with *Polyscias obtusa* significantly increased immune activity, especially CD4$^+$ T cells, CD8$^+$ T cells, and TER119$^+$ [10]. Based on that, in this study we want to investigate the effectiveness of the formulation of *E. scaber* when combined with *S. androgynus* on the secretion of IL-2 by CD4$^+$ T cells and its effect on the secretion of the other cytokines such as IFN-γ and IL-4, and lymphocyte cell proliferation.

2 Material and Methods

2.1 Plant material

Fresh leaves of *E.scaber* and *S.androgynus* were collected from Balai Materia Medica Batu, Malang, Indonesia during August 2014. The leaves were identified by the Taxonomist of Balai Materia Medica Batu, Malang, Indonesia. *Elephantopus scaber* and *Sauropus androgynus* fresh leaves were air-dried at room temperature. 1 kg of the air-dried leaves of the plant was milled into powder. The powder of each leaf was macerated in 70% ethanol at room temperature for 24 h. The ethanol extract soluble of each plant was concentrated under reduced pressure at 50°C in a vacuum pump evaporator. Crude plant ethanol extract of each plant was weighed in accordance with predetermined formula and dissolved in mineral water for use on each day of experiments. The initial dose of *E. scaber* was 200 mg/kg BW (body weight) and *S. androgynus* was 150 mg/kg BW.

2.2 Animals

Experimental animals in this study were 8 weeks old female Balb/C mice, pathogen free, and 5 days pregnant from Gadjah Mada University, Indonesia. The animals were housed in a pathogen free chamber under standard laboratory conditions of temperature, humidity, and light. The animals were given free access to food (standard mice pellets) and mineral water *ad libitum*. The pregnant mice were randomly divided into seven experimental groups of 5 mice each: Group K- (without *S. typhi* infection or pregnant and healthy mice as a control), Group K+ (*S. typhi* infection of pregnant mice with typhoid fever), Group P1 (*S. typhi* infection + 100% of *E. scaber*), Group P2 (*S. typhi* infection + 75% of *E. scaber* and 25% of *S. androgynus*), Group P3 (*S. typhi* infection + 50% of *E. scaber* and 50% of *S. androgynus*), Group P4 (*S. typhi* infection + 25% of *E. scaber* and 75% *S. androgynus*), and Group P5 (*S. typhi* infection + 100% of *S. androgynus*). All the animals were treated for 18 days and still allowed free access to food and mineral water.

2.3 Induction of experimental typhoid fever

Typhoid fever was induced by intraperitoneal injections of *S. typhi* bacteria at a dose of 10^7 CFU in 0.5 mL solvent. The isolate of *S. typhi* was collected from Microbiology laboratory, Faculty of Medicine, Brawijaya University, Malang, Indonesia.

2.4 Cell isolation

Spleen was isolated and washed twice with PBS. Cells were isolated from spleen by crushing spleen in PBS. Homogenates of cells were centrifuged at a speed of 2500 rpm, at 10°C, for 5 minutes. Supernatant was discarded while the pellet was being resuspended in 1 ml of PBS.

2.5 Flow cytometry analysis

Spleen cell suspensions were divided into two microtubes A and B with as much as 100 µl to each microtube. Both A and B microtubes were centrifuged with the speed of 2500 rpm at 10°C for 5 minutes. Supernatant was discarded and the pellets were stained with antibodies. The 2 types of dye combinations used were: dye A: FITC-conjugated rat anti-mouse CD4, PE-conjugated rat anti-mouse IFN-γ, and PE/Cy5-conjugated rat anti-mouse IL-2; dye B: FITC-conjugated rat anti-mouse CD4, PE-conjugated rat anti-mouse B220, and PE/Cy5-conjugated rat anti-mouse IL-4. Cells were stained with extracellular antibodies first and then incubated for 20 minutes at 10°C. Subsequently, the cells added with a fixative solution cytofix/cytoperm

as much as 50 μl and incubated for 20 minutes at 10°C. Residual of fixative solution was removed by washing solution washperm as much as 500 μl and then centrifuged at a speed of 2500 rpm at 10°C for 5 minutes. Supernatant was discarded while the pellets were stained with intracellular antibodies (IFN-γ, IL-2 or IL-4) then incubated for 20 minutes at 10°C. The incubated cells were added with 500 μl of PBS. Each sample was transferred into a flow cytometry cuvette and then analyzed by flow cytometer.

2.6 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 a significance level of $p < 0.05\%$, followed by the Tukey's test. Statistical analyses were done by SPSS version 16 for Windows.

3 Result

3.1 Intracellular expression level of IL-2 cytokine that produced by CD4+ T cells

As shown in figure 1, typhoid fever induced by *S. typhi* intraperitoneal caused a significant decrease of IL-2

produced by CD4+ T cells (25.77%) ($p < 0.05$) compared to the control (60.58%). Oral administration of *E. scaber* and *S. androgynus* extract with formulas (P1-P5) for 18 days significantly increased the number of the cytokine IL-2 produced by CD4+ T cells ($p < 0.05$) as compared to K+ group. The highest increase occurred in P5 treatment with the formula of 100% *S. androgynus* and the lowest in P3 treatment with the formula 50% of *E. scaber* and 50% of *S. androgynus*. However, the five treatments of extract all indicated that the number of IL-2 was not significantly different ($p < 0.05$) than control (Figure 1). To confirm the changes of IL-2 cytokine we also checked other parameters that were affected by the presence of the IL-2 cytokine, which will be discussed in the next section.

## 3.2 The relatives number of CD4+ T lymphocytes cells and intracellular IFN-γ cytokine

CD4+ T cells and IFN-γ cytokine are the two parameters that have a positive correlation with the presence of IL-2 cytokine. This study proved that changes in the number of IL-2 cytokine gave a concrete impact on CD4+ T cells and IFN-γ cytokine. Figure 2 showed that the decrease of IL-2 in K+ impacts the number of CD4+ T cells significantly ($p < 0.05$) compared to those healthy mice (K-). The relative number of CD4+ T cells increased significantly ($p < 0.05$) in all treatments (P1 to P5) compared to K+ treatment. The P2 and P3 treatments indicated the number of CD4+ T cells
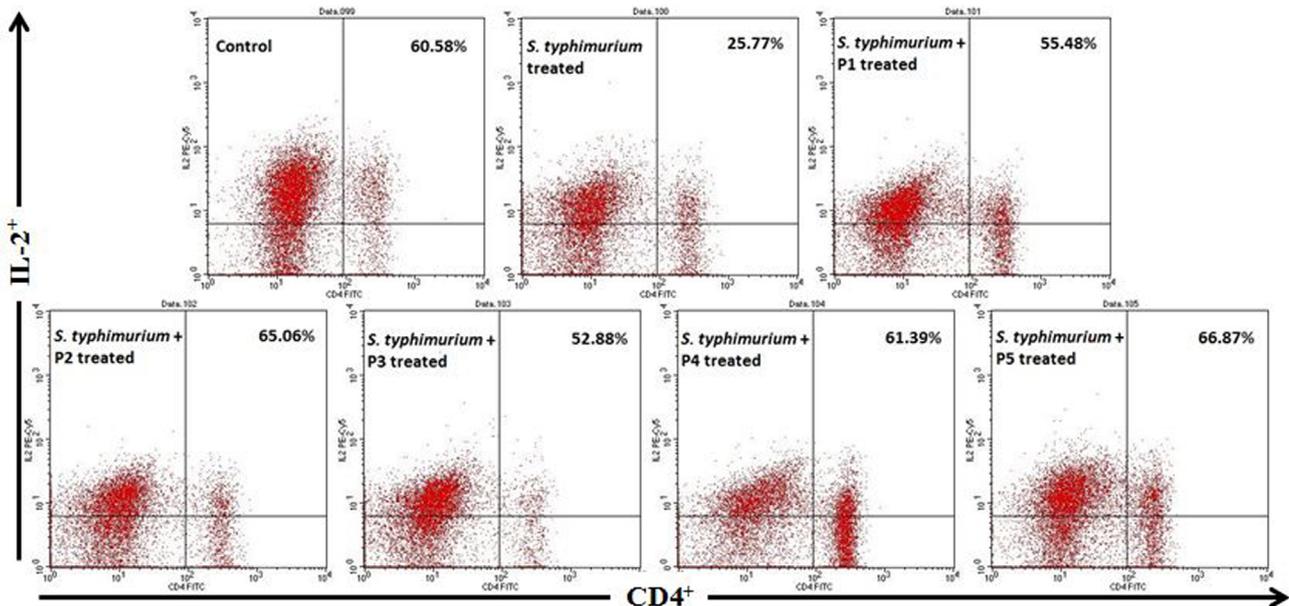
Figure 1 The intracellular expression levels of IL-2 cytokine produced by CD4+ T cells in all treatments. P1 = 100% *E. scaber*; P2 = 75% *E. scaber* : 25% *S. androgynus*; P3 = 50% *E. scaber* : 50% *S. androgynus*; P4 = 25% *E. scaber* : 75% *S. androgynus*; P5 = 100% *S. androgynus*. On day 18, mice were dissected, splenocyte cells were stained by intracellular staining then analyzed by flowcytometry. Data were mean ± SD values of 5 mice in each group with *p* value < 0.05.
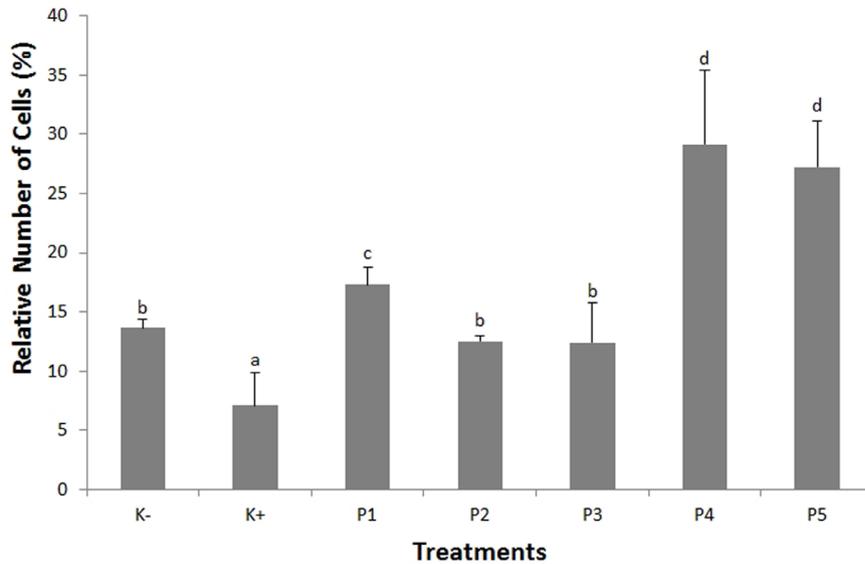
Figure 2 The relative number of CD4+ T lymphocyte cells in all treatments. P1 = 100% *E. scaber*; P2 = 75% *E. scaber* : 25% *S. androgynus*; P3 = 50% *E. scaber* : 50% *S. androgynus*; P4 = 25% *E. scaber* : 75% *S. androgynus*; P5 = 100% *S. androgynus*. On day 18, mice were dissected, splenocyte cells were stained by extracellular staining then analyzed by flowcytometry. Data presented is mean ± SD values (0.78, 2.74, 1.44, 0.48, 3.35, 6.23, 3.96) of 5 mice in each group with *p* value < 0.05. Notation letters printed on each bar showed the significant difference between the investigated groups.
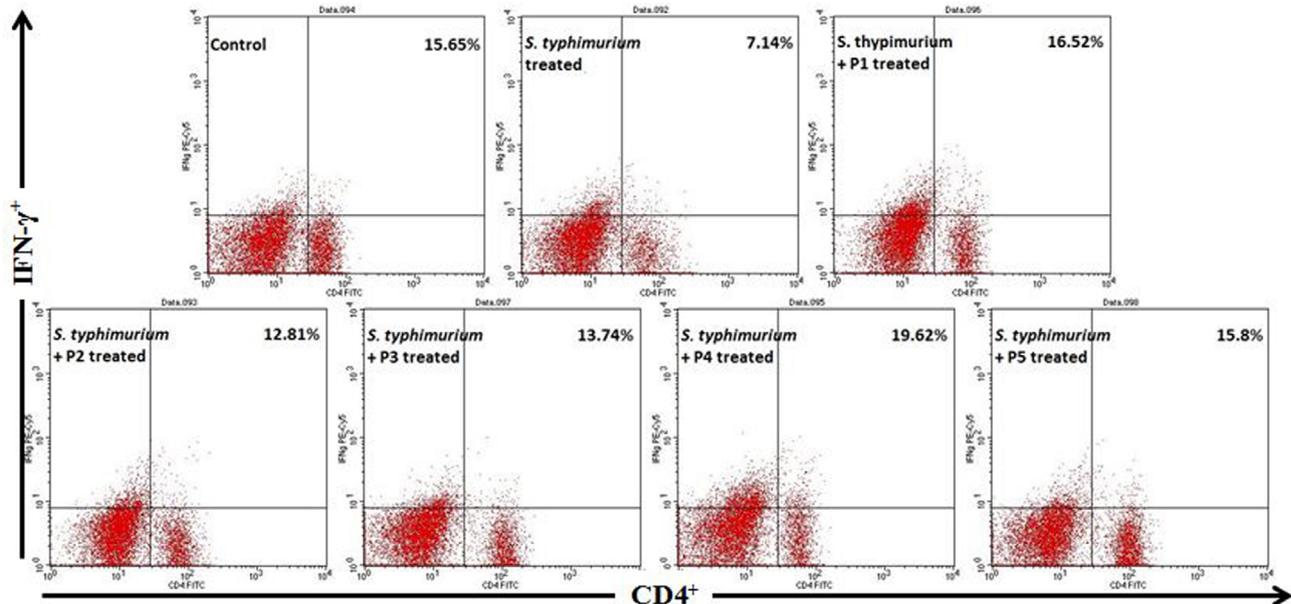
Figure 3 The intracellular expression levels of IFN-γ cytokine produced by CD4+ T cells in all treatments. P1 = 100% *E. scaber*; P2 = 75% *E. scaber* : 25% *S. androgynus*; P3 = 50% *E. scaber* : 50% *S. androgynus*; P4 = 25% *E. scaber* : 75% *S. androgynus*; P5 = 100% *S. androgynus*. On day 18, mice were dissected, splenocyte cells were stained by intracellular staining and then analyzed by flowcytometry. Data presented is mean ± SD values of 5 mice in each group with *p* value < 0.05.

which were not significantly different (*p* > 0.05) in the healthy mice group (K-), while P1, P4, and P5 treatments were significantly different (*p* < 0.05).

Furthermore, figure 3 showed that IFN-γ cytokine had a change similar to the IL-2 cytokine. In the typhoid fever group without extract administration (K+), the IFN-γ cytokine decreased significantly (*p* < 0.05) compared to the control group or healthy mice. Oral administration of the extract with formulation P1 to P5 all showed an increase in the number of IFN-γ cytokine significantly different

($p < 0.05$) with K+ group. P1, P3, P4 and P5 groups indicated the number of IFN-γ cytokine was not significantly different ($p > 0.05$) with the control group (healthy mice), whereas P2 was significantly different ($p < 0.05$).

3.3 The intracellular expression level of IL-4 cytokine produced by CD4+ T and the relative number of B lymphocyte cells

Another case with the CD4+ T lymphocytes and IFN-γ cytokine, IL-4 cytokine and B lymphocytes cells (B220+) are the two parameters which have a negative correlation with the IL-2 cytokine. This study proved that during typhoid fever the expression level of IL-4 cytokine produced by CD4+ T cells increased significantly ($p < 0.05$) compared to control. In the combination of *E. scaber* and *S. androgynus* leaves extract (P1 until P5) the number of IL-4 cytokine decreased significantly ($p < 0.05$) compared with the K+ group (Figure 4).

IL-4 is a cytokine that plays a role in the activation of B lymphocyte cells (B220+), particularly in the differentiation of IgG1 and IgE synthesis which plays a role in allergic responses. Figure 5 showed that *S. typhi* infection caused a significant increase of B220+ cells ($p < 0.05$) compared to control. In P1 to P5 treatment, the relative number of B220+ cells decreased significantly ($p < 0.05$) when compared to the K+ group.

4 Discussion

A unique immune condition during pregnancy can cause pregnant women to be more susceptible to infection including *Salmonella* infections. Our finding proved that typhoid fever caused by *S. typhi* infections during pregnancy led to a decrease of IL-2 cytokine that normally plays an important role in the immune system. In general, *Salmonella* has many strategies to evade both the innate and adaptive immune system. In the innate immune system, *Salmonella* evades the oxygen killing mechanisms of macrophages by disrupting NADPH oxidase and iNOS trafficking to the SCV. *Salmonella* also alters chemokine receptor expression on dendritic cells. As we know, dendritic cells (DCs) are one of the expert antigen presenting cells and necessary for naïve T cell activation [11,12]. In the adaptive immune system, the strategy used to prevent T cell activation is reduction of *Salmonella* antigens such as flagellin after the initial steps of bacterial entry [13,14]. This failure of T cell activation causes the least amount of IL-2 cytokine secretion because IL-2 will be secreted by the activated CD4+ T cells.

CD4+ T cells (T *helper* cells) consist of two distinct subsets termed Th1 and Th2. It is defined by their cytokine secretion. Th1 cells produced and secreted IL-2, IFN-γ, and TNF-β cytokines. While Th2 cells produce and secrete IL-4, IL-5, IL-6, IL-10 and IL-13 cytokines. Th1 and Th2 work alternately. Cytokines produced by Th1 will have an impact on the
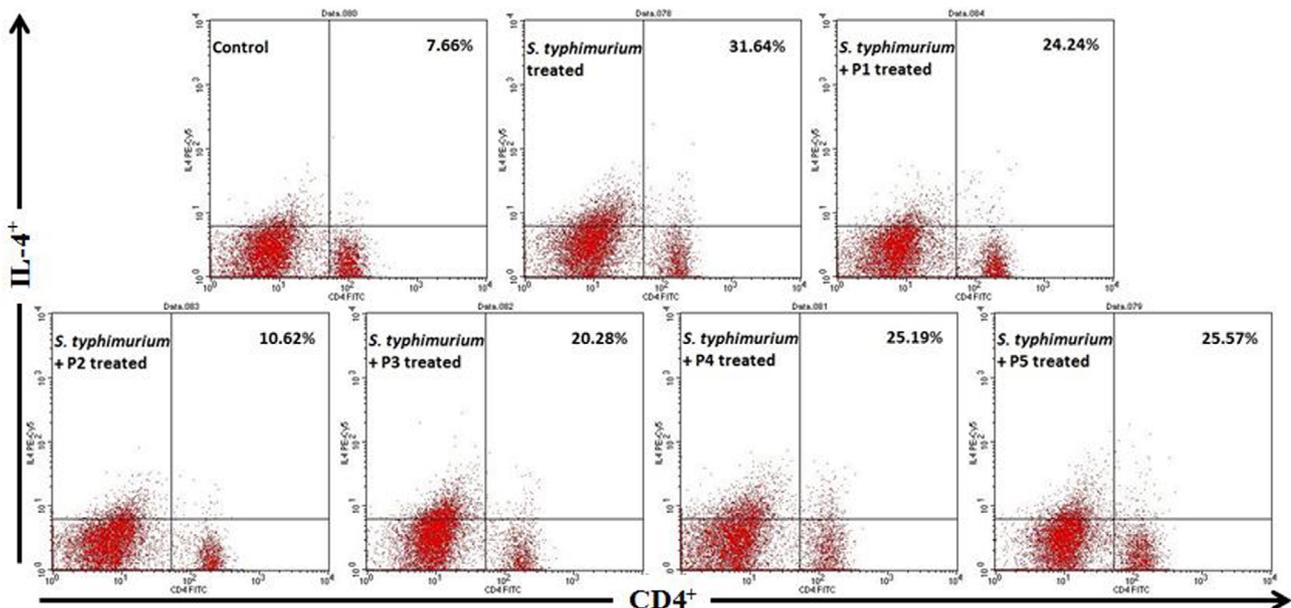
Figure 4 The intracellular expression levels of IL-4 cytokine produced by CD4+ T cells in all treatments. P1 = 100% *E. scaber*; P2 = 75% *E. scaber* : 25% *S. androgynus*; P3 = 50% *E. scaber* : 50% *S. androgynus*; P4 = 25% *E. scaber* : 75% *S. androgynus*; P5 = 100% *S. androgynus*. On day 18, mice were dissected, splenocyte cells were stained by intracellular staining and then analyzed by flowcytometry. Data were mean ± SD values of 5 mice in each group with *p* value < 0.05.

proliferation of Th1 and increase its cytokine production, which inhibits proliferation of Th2 including its activity in producing cytokines. That's why in typhoid fever when IL-2 is decreased, CD4$^+$ T cells and IFN-γ is also decreased, while IL-4 cytokine and B220$^+$ cells are increased.

Herbal medicine has long been used as the treatment during infection in pregnancy because the use of antibiotics can lead to teratogenic effects. In this study, we investigated the possible effectiveness of formulation of *E. scaber* and *S. androgynus* in modulating the immune system during pregnancy especially in promoting IL-2 secreted by CD4+ T cells. This study demonstrated that all of the formulations that we made have a significant impact in increasing the number of IL-2 cytokine, CD4$^+$ T cells, and IFN-γ cytokine during typhoid fever in pregnancy. As described before, *E. scaber* contains triterpenoids lupeol, sesquiterpene lactones (deoxyelephantopin, isodeoxyelephantopin, 17.19-dihydrodeoxyelephantopin, scabertopin isoscabertopin and elescaberin), fatty acid esters (ethyl hexadecanoate, ethyl-9, 12-octadecadienoate, ethyl- (Z) -9-octadecenoate and ethyl octadecanoate), stigmasterol, stigmasterol glucoside, alkaloids, aurones (one type of flavonoid), chalcones and phenolic compounds in small quantities [15]. *Sauropus androgynus* contains tannins, saponins, flavonoids and alkaloids papaverine which shows potential as a natural medicine. Some of these compounds have long been known as immunomodulators, especially flavonoids and saponin.

Flavonoid and saponin can modulate the immune system, especially in increasing the proliferation of immune cells [3]. High levels of flavonoids and alkaloids in *E. scaber* enable this plant to function as an anti-oxidant that is capable of controlling the activity of nitrix oxide (NO) [16]. Furthermore, flavonoids have the ability to trigger the activity of MAPK that can stimulate an increase of IL-2 [17].

IL-2 is a growth factor for immunocompetent cells which can increase the concentration of cyclin D2, E, and A, that plays an important role in the cell cycle. After passing through S phase, cyclin A binding will bind to Cdk1 to regulate cell transition from S to G2 [18]. Cyclin A-Cdk1 complex causes chromatin condensation that is needed for cell division [19]. This causes immunocompetent cells, especially CD4$^+$ T cells, to proliferate actively. Formulation P4 (25% *E. scaber* : 75% *S. androgynus*) and P5 (100% *S. androgynus*) results in the highest increase of CD4$^+$ T cells. Nevertheless, it was not a strong enough effect to shown significant difference from controls. It remains possible that the higher number of cells as compared to controls may be due to reactive cells. Formulation P2 (75% *E. scaber* : 25% *S. androgynus*) and P3 (50% *E. scaber* : 50% *S. androgynus*) produced the optimal effect on CD4$^+$ T cells proliferation despite not showing significant difference from controls.

CD4$^+$ T cells once activated by IL-2 will both proliferate and produce IFN-γ, thus the number of IFN-γ cytokines will increase. Moreover, IFN-γ cytokines will also stimulate T
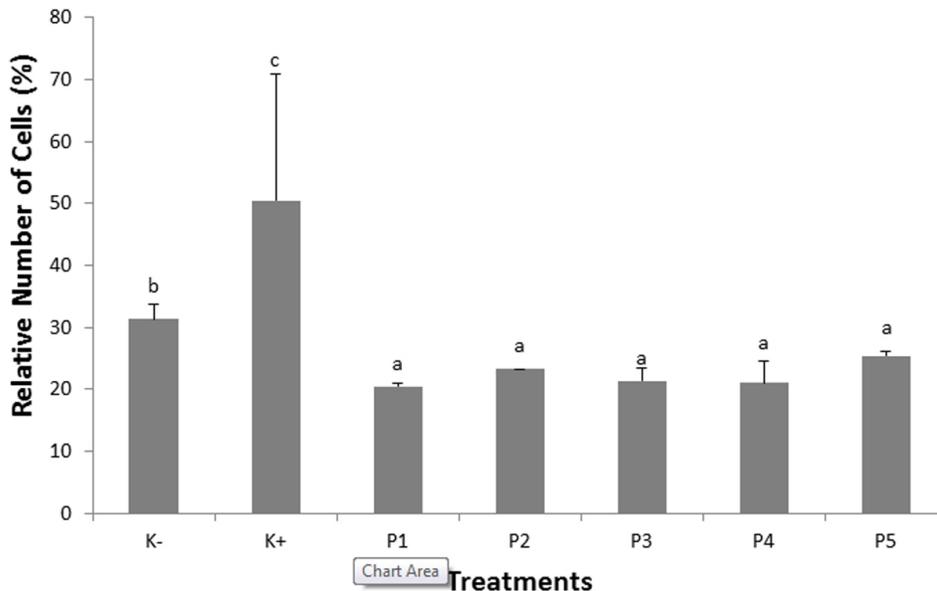
Figure 5 The relatives number of B220$^+$ T lymphocyte cells in all treatments. P1 = 100% *E. scaber*; P2 = 75% *E. scaber* : 25% *S. androgynus*; P3 = 50% *E. scaber* : 50% *S. androgynus*; P4 = 25% *E. scaber* : 75% *S. androgynus*; P5 = 100% *S. androgynus*. On day 18, mice were dissected, splenocyte cells were stained by extracellular staining then analyzed by flowcytometry. Data presented is mean ± SD values (2.39, 20.45, 0.63, 0, 2.08, 3.42, 0.75) of 5 mice in each group with *p* value < 0.05. Notation letters printed on each bar showed the significant difference between the investigated groups.

cells to differentiate into CD4^{+} T cells. IFN-γ can stimulate the up-regulation of MHC-II expression so that more naïve T cells differentiate into CD4^{+} T cells [20, 21]. IFN-γ cytokines will both stimulate macrophages to control bacterial, in this case *S. typhi*, replication via the iNOS response and production of complement-fixing and -opsonizing antibodies [22]. This innate activation of T cells plays a key role in strengthening the effector function in cytokines production at sites of infection, especially when the pathogen can inhibit antigen presentation [23]. Moreover, IFN-γ cytokines can stimulate phagocytosis [24,25], the oxidative burst [26], and intracellular killing of microbes [27,28]. Patients with defects in the IFN-γ, IL-12, or IL-12-receptor genes are unable to produce IL-12 and/or IFN-γ and as a result have developed severe infections caused by *Salmonella* [29,30]. The increase of IFN-γ cytokines is not only caused by IL-2 but also saponin contained in both *E. scaber* and *S. androgynus*. Saponin has the ability to increase IFN-γ cytokines [31]. The optimum formulations of *E. scaber* and *S. androgynus* were P1 (100% *E. scaber*), P3 (50% *E. scaber* : 50% *S. androgynus*), P4 (25% *E. scaber* : 75% *S. androgynus*), and P5 (100% *S. androgynus*) which did not show significant difference compared to healthy pregnant mice.

Naïve T cells and Th1 cells absolutely require IL-2 for activation and proliferation [32]. However, Th2 cells are perfectly capable of proliferating without IL-2 if IL-4 is present, which is convenient for them since they secrete large quantities of IL-4 [33,32]. IL-4 cytokine can inhibit the secretion of IL-12 and IFN-γ [34,35,36]. IL-4 also inhibits phagocytosis and intracellular killing [37,38], and suppresses inflammatory cytokine production [39]. In the *E. scaber* and *S. androgynus* treatment, the number of IL-4 cytokine and B220[+] decrease. It is caused by the increase of IFN-γ cytokine. The IFN-γ cytokine secreted by Th1 cells directly suppresses IL-4 secretion and thus inhibits differentiation of naive Th0 cells into Th2 cells [40-42]. In typhoid fever, the relative number of B220^{+} cells increase because IL-4 can activate B cell proliferation. Furthermore, IL-4 also provides optimal support for antibody production, and class-switching, and promotes both mast cell growth and eosinophil differentiation and activation resulting in humoral or allergic responses [43-45].

5 Conclusion

Based on our study, we conclude that the formulation of *E. scaber* and *S. androgynus* was able to modulate the immune system via promoting IL-2 cytokines in

pregnancy during typhoid fever. This condition made the relative number of CD4^{+} T cells and the production of IFN-γ cytokines increase while the expression level of IL-4^{+} cytokine and B220^{+} lymphocyte cells decreased. The formulation of *E. scaber* and *S. androgynus* that gave the optimum effect in all investigated parameters was P3 (50% *E. scaber* : 50% *S. androgynus*) which was not significantly different compared to healthy pregnant mice.

Conflicts of interest: Authors declare that there are no conflicts of interest.

References

[1] Singh S.D.J., Wound healing activity of the leaf extracts and deoxyelephantopi isolate from *Elephantopus scaber* Lin, Phytochemistry, 2005, 37, 238-242

[2] Van Melderen L., Molecular interaction of the CcdB poison with its bacterial target, the DNA gyrase, Int. J. Med. Microbiol, 2002, 291 (6-7), 537-544

[3] Dwijayanti D.R., Rifa'i M., *Gynura procumbens* ethanolic extract promotes lymphocyte activation and regulatory T cell generation in vitro, J. Trop. Life Sci., 2015, 5(1), 14-19

[4] Chen S-J., Liu Y-L., Sytwu H-K., Immunologic regulation in pregnancy: from mechanism to therapeutic strategy for immunomodulation, Clin. Dev. Immunol., 2012, 2012, 1-10

[5] Vigliani M.B., Bakardjiev A.I, First trimester typhoid fever with vertical transmission of *Salmonella typhi*, an intracellular organism, Case Reports Med., 2013, 2013,1-5

[6] Givens M.D., Marley M.S., Infectious causes of embryonic and fetal mortality. Theriogenology, 2008, 70 (3), 270–285

[7] Gaffen S.L., Liu K.D., Overview of interleukin-2 function, production and clinical applications, Cytokine, 2004, 28 (3), 109-123

[8] Vuletić A.M., Jovanić I.P., Jurišić V.B., Milovanović Z.M., Nikolić S.S., Tanić N.T., et al, In-vitro activation of natural killer cells from regional lymph nodes of melanoma patients with interleukin-2 and interleukin-15, Melanoma Res., 2015, 25 (1), 22-34

[9] Lenardo M., Chan F.K-M., Hornung F., McFarland H., Siegel R., Wang J., et al., Mature T lymphocyte apoptosis-immune regulation in a dynamic and unpredictable environment, Annu. Rev. Immunol., 1999, 17, 221-253

[10] Djati M.S., Dwijayanti, D.R., Rifa'i, M, Synergetic modulation of T lymphocyte and TER 119+ Cell using combination of *Elephantopus scaber* and *Polyscias obtusa* extract in pregnant mice after *Salmonela typhi* infection, Int. J. Pharmn. Bi. Sci., 2015, 6 (4B), 1228-1234

[11] Banchereau J., Briere F., Caux C., Davoust J., Lebecque S., Liu Y.J., et al., Immunobiology of dendritic cells, Annu. Rev. Immunol., 2000, 18, 767-811

[12] Hugues S., Boissonnas A., Amigorena S., Fetler L., The dynamics of dendritic cell-T cell interactions in priming and tolerance, Curr. Opin. Immunol., 2006, 18(4), 491-495

[13] Cummings L.A., Barrett S. L., Wilkerson W.D., Fellnerova I., Cookson B.T., FliC-specific CD4+ T cell responses are restricted by bacterial regulation of antigen expression, J. Immunol., 2005, 174, 7929-7938

[14] Alaniz R. C., Cummings L.A., Bergman M.A., Rassoulian-Barrett S.L, Cookson B.T., *Salmonella typhi* coordinately regulates FliC location and reduces dendritic cell activation and antigen presentation to CD4+ T cells, J. Immunol., 2006, 177, 3983-3993

[15] Liang Q.L., Min Z.D., Tang Y.P.A., New elemanolide sesqui-terpene lactone from *Elephantopus scaber*, J. Asian Nat. Prod. Res., 2008, 10, 403-407

[16] Subramanian A., Kumar A.S., Suja S.K., Sudarshan M., Chakraborty A., Antioxidant and free radical scavenging activity of annular and seasonal ayurvedic medical plants *Elephantopus scaber* L. and *Biophytum sensitivum* DC, Int. J. Pharm. Biol. Chem. Sci., 2014, 3 (1), 6-17

[17] Middleton E., Kandaswami C., Theoharides T.C., The effects of plant Flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer, Pharmacology, 2000, 52, 673-751

[18] Dhulipala V.C., Welshons W.V., Reddy C.S., Cell cycle proteins in normal and chemically induced abnormal secondary palate development: a review, Hum. Exp. Toxicol., 2006, 25, 675-682

[19] Lapenna S., Giordano A., Cell Cycle Kinases as Therapeutic Targets for Cancer, Nat. Rev. Drug Discov., 2009, 8 (7), 547-566

[20] Lee Y.H., Ishida Y., Rifa'i M., Shi Z., Isobe K., Suzuki H., Essential role of CD8+CD122+ regulatory T cells in the recovery from experimental autoimmune encephalomyelitis, Immunology, 2008, 180, 825-832

[21] Shi Z., Rifa'i M., Lee Y.H., Shiku H., Isobe K., Suzuki H., Importance of CD80/CD86–CD28 interactions in the recognition of target cells by CD8+CD122+ regulatory T cells, Immunology, 2008, 124, 121- 128

[22] Nauciel C., Espinasse-Maes F., Role of gamma interferon and tumor necrosis factor alpha in resistance to *Salmonella typhi* infection, Infect. Immun., 1992, 60, 450-454

[23] Cheminay C., Mohlenbrink A., Hensel M., Intracellular *Salmonella* inhibit antigen presentation by dendritic cells, J. Immunol., 2005, 174, 2892-2899

[24] Szulc B., Piasecki E., Effects of interferons, interferon inducers and growth factors on phagocytosis measured by quantitative determination of synthetic compound ingested by mouse bone marrow–derived macrophages, Arch. Immunol. Ther. Exp. (Warszawa), 1988, 36, 537–45

[25] Livingston D.H., Appel S.H., Sonnenfeld G., Malangoni,M.A., The effect of tumor necrosis factor–alpha and interferon-gamma on neutrophil function, J. Surg. Res., 1989, 46 (4), 322–326

[26] Newburger P.E., Ezekowitz R.A., Whitney C., Wright J., Orkin S.H., Induction of phagocyte cytochrome b heavy chain gene expression by interferon gamma, Proc. Natl. Acad. Sci. USA, 1988, 85 (14), 5215–5219

[27] Diamond R.D., Lyman C.A., Wysong D.R., Disparate effects of interferongamma and tumor necrosis factor–alpha on early neutrophil respiratory burst and fungicidal responses to *Candida albicans* hyphae *in vitro*, J. Clin. Invest., 1991, 87 (2), 711–720

[28] Dellacasagrande J., Capo C., Raoult D., Mege J.L., IFN-gamma-mediated control of *Coxiella burnetii* survival in monocytes: the role of cell apoptosis and TNF, J. Immunol, 1999, 162 (4), 2259–2265

[29] Altare F., Lammas D., Revy P., Jouanguy E., Döffinger R., Lamhamedi S., et al., Inherited interleukin 12 deficiency in a child with bacille Calmette-Gue´rin and *Salmonella enteritidis* disseminated infection, J. Clin. Invest., 1998, 102 (12), 2035–2040

[30] De Jong R., Altare F., Haagen I.A., Elferink D.G., van Breda V.P.J., Kabel P.J., et al., Severe mycobacterial and salmonella infections in interleukin-12 receptor–deficient patients, Science, 1998, 280,1435–1438

[31] Cheeke P.R. Actual and potential aplication of *Yucca schidigere* and *Quillaja saponaria* Saponin in human and animal nutrition, Anim. Sci., 2000, 77, 1-10

[32] Kurt-Jones E.A., Hamberg S., Ohara J., Paul. W.E., Abbas A.K., Heterogeneity of helper/inducer T lymphocytes. I. Lymphokine production and lymphokine responsiveness, J. Exp. Med., 1987, 166, 1774–1787

[33] Fernandez-Botran R., Sanders V.M., Oliver K.G., Chen Y.W., Krammer P.H, Uhr J.W., et al., Interleukin 4 mediates autocrine growth of helper T cells after antigenic stimulation, Proc. Natl. Acad. Sci. USA, 1986, 83 (24), 9689–9693

[34] D'Andrea A., Aste-Amezaga M., Valiante N.M., Ma X., Kubin M., Trinchieri G., Interleukin 10 (IL-10) inhibits human lymphocyte interferon gamma production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells, J. Exp. Med. 1993, 178 (3), 1041–1048

[35] Ohmori Y., Hamilton T.A., IL-4-induced STAT6 suppresses IFN gamma-stimulated STAT1-dependent transcription in mouse macrophages, J. Immunol., 1997, 159, 5474–5482

[36] Ito S., Ansari P., Sakatsume M., Dickensheets H., Vazquez N., Donnelly R.P., et al., Interleukin-10 inhibits expression of both interferon alpha- and interferon gamma– induced genes by suppressing tyrosine phosphorylation of STAT1, Blood, 1999, 93 (5), 1456–1463

[37] Oswald I.P., Gazzinelli R.T., Sher A., James S.L., IL-10 synergizes with IL-4 and transforming growth factor-b to inhibit macrophage cytotoxic activity, J. Immunol, 1992, 148 (11), 3578–3582

[38] Cenci E., Romani L., Mencacci A., Spaccapelo R., Schiaffella E., Puccetti P., Interleukin-4 and interleukin-10 inhibit nitric oxide–dependent macrophage killing of *Candida albicans*, Eur. J. Immunol, 1993, 23 (5), 1034–1038

[39] De Waal M.R., Figdor C.G., Huijbens R., Mohan-Peterson S., Bennett B., Culpepper J.,et al., Effects of IL-13 on phenotype, cytokine production, and cytotoxic function of human monocytes: comparison with IL-4 and modulation by IFN-gamma or IL-10, J. Immunol, 1993, 151 (11), 6370–6381

[40] Gajewski T.F., Fitch F.W., Anti-proliferative effect of IFN-gamma in immune regulation. I. IFN-gamma inhibits the proliferation of Th2 but not Th1 murine helper T lymphocyte clones, J. Immunol., 1988, 140 (12), 4245–4252

[41] Gajewski T.F., Goldwasser E., Fitch F.W., Anti-proliferative effect of IFNgamma in immune regulation. II. IFN-gamma inhibits the proliferation of murine bone marrow cells stimulated with IL-3,

IL-4, or granulocyte-macrophage colony-stimulating factor, J. Immunol., 1998, 141 (8), 2635–2642

[42] Demeure C.E., Wu C.Y., Shu U., Schneider P.V., Heusser C., Yssel H., et al., *In vitro* maturation of human neonatal CD4 T lymphocytes. II. Cytokines present at priming modulate the development of lymphokine production, J. Immunol, 1994, 152 (10), 4775–4782

[43] Lundgren M., Persson U., Larsson P., Magnusson C., Smith C.I., Hammarström L., et al., Interleukin 4 induces synthesis

of IgE and IgG4 in human B cells, Eur. J. Immunol, 1989, 19 (7), 1311–1315

[44] Punnonen J., de Vries J.E., IL-13 induces proliferation, Ig isotype switching, and Ig synthesis by immature human fetal B cells, J. Immunol., 1994, 152, 1094–1102

[45] Lai Y.H., Mosmann T.R., Mouse IL-13 enhances antibody production in vivo and acts directly on B cells in vitro to increase survival and hence antibody production, J. Immunol., 1999, 162 (1), 78–87