Tapak liman (*Elephantopus scaber* L) Extract Induced $CD4^+$ and $CD8^+$ Differentiation from Hematopoietic Stem Cell/Progenitor Cell Proliferation of Mice (*Mus musculus*)

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Abstract

Tapak liman (*Elephantopus scaber* L) is one of the traditional medicinal plants, which are containing several active compounds that potentially affecting hematopoietic. The aims of this study are to elucidate the effect of leaf extract of Tapak Liman on hematopoietic in mice BALB/c, by observation on relative number of cells expressing CD4/CD8, CD4/CD62L, and TER119/B220 in the spleen; TER119/B220, TER119/VLA-4 and TER119/CD34 in bone marrow, after given the leaf extract for 2 weeks. This experiment was used 12 female mice, which is divided on 3group treated with leaf extract of Tapak Liman (P_1= 0.5 g. g bw^{-1}.day^{-1}, P_2= 1.0 g. g bw^{-1}.day^{-1} and P_3 = 2.0 g. g bw^{-1}.day^{-1}) and control. The relative numbers of cells expressing surface molecules were analyzed by flowcytometry and quantitative data were tested using one way ANOVA. The result showed the leaf extract of Tapak Liman has no significant effect on erythrocyte proliferation, on the other hand it have significant effect on both proliferation and differentiation of B lymphocytes ($B220^+$) in the bone marrow (p=0.044) and increase the expression of $CD4^+$, $CD8^+$molecule in B cells (p=0.026) and erytroid ($TER119^+VLA\text{-}4^+$) in spleen and bone marrow. The administration of 1 g. g bw^{-1}.day^{-1} of the extract (P_2) stimulate proliferation of lymphocytes and erythrocytes lineage ($TER119^+VLA\text{-}4^+$), in spleen and bone marrow.

Keywords: $CD4^+$, $CD8^+$, *Elephantopus scaber*, erythrocyte, hematopoiesis.

INTRODUCTION

Tapak liman (*Elephantopus scaber* L) is one of the traditional medicinal plants that has been known for centuries, especially among pharmacologists in China. Tapak liman contain epifrieelinol, lupeol, stigmasterol, triacontan-1-ol, dotriacontan-1-ol, lupeol acetate, deoxyelephantopin, isodeoxyelephantopin, flavonoid, polyphenol luteolin-7, and glucoside. Tapak liman is used as a traditional medicine in the form of fresh, dried, even extracted and put into capsules. Types of diseases that can be treated with Tapak Liman include various inflammatory diseases such as inflammation of the tonsils, influenza, sore throat, eye inflammation, kidney inflammation, acute and chronic inflammation of the uterus or vaginal discharge. Additionally, Tapak Liman also serves as a laxative of urine, decrease fever, clear phlegm, antioxidants, and blood booster. It is also useful to overcome the chicken pox and anemia [1].

Anemia is a disease in deficiency of red cell volume and hemoglobin levels (Hb) thus the body will have hypoxia as a result of reduced oxygen-carrying capacity capabilities [2]. Anemia can be caused by autoimmune diseases such as hemolytic and aplastic anemia due to delays in the process of erythropoiesis [3]. Until now, the treatment of anemia that caused by erythropoiesis barriers solely depends on the provision of artificial hematopoietin commonly is called the ESA (*Erythropoiesis Stimulating Agent*), a synthetic compound that can stimulate the production of blood cells. However, the ESA has harmful side effects to the user's health such as cardiovascular complications [4] and retinopathy [5]. It provides an opportunity to investigate and search for compounds that could stimulate and affect *erythropoiesis* as a safe anemia drug ingredient, with no side effects, and keep the immune system in which a chemical compound has the potential to induce the process of proliferation and differentiation of *erythropoiesis* and *lymphopoiesis*.

Based on the above description, the research focused on the effects of Tapak Liman (*E. scaber* L) leaf extract on hematopoiesis in female mice (*Mus muscullus*) BALB/c. Expected chemical substances contained in extracts of Tapak Liman

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(*E. scaber* L) leaves could potentially induce and influence the process of hematopoiesis differentiation and proliferation, especially on *lymphopoiesis* and *erythropoiesis*.

MATERIALS AND METHODS

Preparation of Leaves Extract and Treatment

The extraction method of Tapak Liman leaves was conducted by using sterilized aquades solvent [6,7]. The dried leaves of Tapak Liman weighed 13.8 g and mashed by mortar into fine powder. Then it dissolved with 100 mL sterilized aquades, stirred in 50°C for a night and then filtered with filter paper. The results of the extract were calcutaed to measure the final concentration, and the extract solution is ready to be tested.

Experiment animal used in this study is mice (*Mus musculus*) which randomly sampled from an affordable population with criteria included: female mice strain BALB/c, 6 weeks old and healthy (actively move and intact fur). The administration of Tapak Liman (*E. scaber* L) leaves extraxt was given orally. Previously, body weigh of the mice was monitored for two weeks. Each mice group has the same standard feed and drink. The main dependent variable that measured is the relative number of the cell in hematopoiesis phase with following parameters. The assessment was only conducted post test, by comparing the results of observations in the treatment group and control, as well as between the treatment groups.

1. The number of cell population which expressed CD4/CD8, CD4/CD62L, TER119/ B220 in the spleen
2. The relative number of cell which expressed TER119/B220, TER119/VLA-4, dan TER119/ CD34 in the bone marrow

Spleen isolation was done by cut part way of the spleen (dark red) with surgical scissors. The spleen then washed with PBS and cleaned of attached fat. Homogenates were filtered and collected in a sterile microtube and stored at a temperature of 4°C.

The bone marrow was obtained from the femur bone of the left and right legs of mice. Muscles are separated from the femur, both ends of the joints were cut with surgical scissors [8].

Bone marrow was isolated by flushing method by injecting PBS with 1 ml syringe (26½ needle) at one end of the bone. The results of the injection are accommodated in a sterile petri dish, filtered and stored in a sterile microtube, and then stored at a temperature of 4°C.

Flowcytometry

Flowcytometry method used homogenat from spleen and bone marrow which is centrifuged at 3200 rpm, 4°C for 2 minutes [9]. Supernantant removed and pelet resuspensed in 1 mL of sterilized PBS and then homogenized. Homogenat were taken 100-200 μL by using micropipet and inserted into new steril microtube which covered with aluminium foil. Homogenat recentrfuged in 3200 rpm, 4°C for 2 minutes, then supernatant removed and microtube put into the ice box.

Pelet in the microtube was added by antibody BD Bioscience™ DC4 FITC *anti mouse conjugated* and PE-CD8, DC4 FITC *anti mouse conjugated* dan PE-CD62L, as well as BD Bioscience™ *antimouse* TER-199*/Erytrocyt Cell* FITC *conjugated* and PE- B220, for pelet which isolated from spleen. While pelet from bone marrow was added by antibody BD Bioscience™ *antimouse* TER-199*/ Erytrocyt Cell* FITC *conjugated* PE- B220, TER-199*/ Erytrocyt Cell* FITC *conjugated* CD49d/(VLA-4), and *antimouse* TER-199*/ Erytrocyt Cell* FITC *conjugated* PE-CD34 and then incubated for 15 minutes.

Next step is connected the computer and *flowcytometry* in the condition *Acquiring* and setting program on computer according expected parameters, include setting instrument (*Detector, Treshold,* and *Compensation*) on the number of cells which will be analysed (*Acquisition* and *storage*), label of antibody and laser excitation power, simple name and determine the *grated area* (R_1) on the plot of histogram. *Setting* plot on *Aquiring mode*, according to the label of antibody on the axis of Y and X (FITC or PE) and *grating area* (G_1=R_1). Flowcytometry was ensured in the set of *Low-Run*. After the instrument ready, pelet whaich has been added with antibody put into the kuvet of flowcytometry by micropipet, then added 1000 μL of steril PBS and homogenized by *pipetting*. Kuvet attached into *nozzle* BD Bioscience FACS Calibur™ flowcytometry and pressed *acquire* panel on the *aquisition control.* Data from flowcytometry was processed with *software* BD CellQuest Pro™ and displayed in the form of a histogram.

Data Analysis

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Quantitative data include the relative number of progenitor and precursor cells in the development of lymphoid and erythroid of bone marrow and spleen which obtained from flowcytometer. Data were statistically analysed by normality test and variant homogenity test. Data which has been distributed with homogeneous variance, test by one way ANOVA with α= 0.05. If p value $>$ 0.05 then there is no significant differences, whereas if the p value $<$ 0.05 then there is significant differences between treatments. Last, the different group was determined by *post hoc-test* with uji Turkey HSD (*High Significant Difference*). Data analysis was processed by using program SPSS 13 for Windows.

RESULTS

Profile of Cell Express CD4 and CD8 Molecules in Spleen

CD4 is an expressed antigen by inflammation T cells, monocyte cells, and macrophage cells with function as co-receptor molecules of MHC Class II, and receptor for HIV. Otherwise, CD8 is an expressed antigen by a subset of thymocytes, cytotoxic T cells, and has functioned as co-receptor of MHC Class I [10].

Flowcytometer analysis found that in the spleen (Fig. 1), there are no significant differences between cells that expressed $CD8^+CD4$, $CD4^+CD8^-$, and $CD4^+CD8^+$ on each group, compared to the control. It indicates that the tretament with Tapak Liman on doses 1, 2 and 3 was not stimulated the *hematopoietic stem cell* (HSC) to differentiate spesifically into cells of $CD4^+CD8^-$, $CD4^-CD8^+$, and $CD4^+CD8^+$ (p>0.05).

The number of cell population of $CD4^+CD8^-$ and cell of $CD4^-CD8^+$ on treatment P_2 (treatment 1g.g $bw^{-1}.day^{-1}$) increased, compared to control (Fig. 1). This can be explained that the P_2 increased the total number of cells isolated from spleen. We assumed that it happened due to several factors. First, the concentration of leaves extract of Tapak Liman at dose P_2 increase the lymphocytes proliferation; second, population of P_2 exposured to the disease; Last, the population of P_2 has a bigger spleen (splenomegaly), i.e. the spleen ability to get and keep the erythrocytes will be increased. Splenomegaly caused the decrease of erythrocytes, leukocytes, and trombocytes in the blood circulation.

Figure 1 describes the number of population T cell $CD4^+$ and $CD8^+$ increased on P_2 (1 g.g $bw.^{-1}$), for 7.92 x 10^6 calls and 8.712 x 10^6 cells, consecutively. Otherwise, the number of T cell $CD4^+$ and $CD8^+$ tend to decrease on treatment P_3. It is expexted du to the *negative feedback* from the administration of Tapak Liman leaves extract. Low dose treatment will stimulate the proliferation of T cell, while contrastly the high doses will prevent the proliferation of T cell. It is caused by the abundant compound of lupeol and flavanoid in the extract of Tapak Liman as anti inflammation. The phenomenon is in accordance with the previous study which mentioned that the effect of herbal medicine will stimulate or conversely suppress the degree of immunity [11].

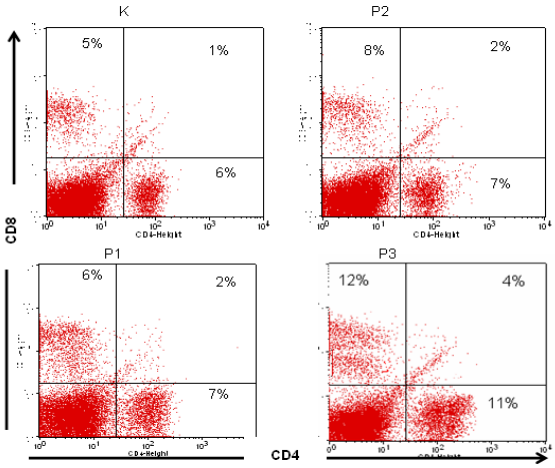

Figure 1. Profile of T Cell CD4 and CD8; the number of cell expression $CD4^+CD8^-$, $CD4^+CD8^+$ and $CD4^+CD8^+$ in spleen. **Description:** Control (K), Treatment 1 (P_1: 0.5 g.g $bw.^{-1}$), Treatment 2 (P_2: 1.0 g.g $bw.^{-1}$), Treatment 3 (P_3: 2.0 g.g $bw.^{-1}$).

Profile of Cell Express CD4 and CD62L Molecules in Spleen

CD62L is an antigen expressed by B cell, T cell, monocyte and *Natural Killer Cell* (NK cell). It bind CD34 which functioned as leukocyte adhesion molecule (LAM/L-selectin), GlyCAM, and has roles in the interaction of *rolling* with endotel [3].

The *flowcytometer* analysis (Fig. 2) showed that the treatment group has an average percentage of cell that expresses $CD4^+CD62L^-$ and $CD4^+CD62L^+$ cells with no significant differences for all treatment (p>0.05). Whereas the average percentage of cell that express $CD4^-CD62L^+$ (B cell) increase on the treatment P_1 and P_2, each for 6% and 3% respectively, and tend to decrease on treatment P_3 for 5%, compared to control.

Statistical analysis showed a significant effect (p<0.05) towards the increased percentage of CD62L on treatment P_2 (1 g.g $bw^{-1}.day^{-1}$). It indicates that the flavonoid compound has an increasing effect in the production of IL-2, thus increase the B lymphocytes.

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The number of cell population that express $CD4^+CD62L^-$, $CD4^-CD62L^+$, and $CD4^+CD62L^+$ on treatment (Fig. 2) showed that the highest number of cell population expression $CD4^+CD62L^-$ and $CD4^-CD62L^+$ is in treatment P_2 for 7.128 x 10^6 cells and 7.524 x 10^6 cells respectively, compared to other treatment and control. It indicates that there is increasing in the cell number that express $CD4^+CD62L^-$ ($CD4^+$ T Cell) and $CD4^-CD62L^+$. With the increased doses of Tapak Liman leaves extract usage, but tend to decreased cell number found in the treatment group of mice P_3. The number of cells that express the highest $CD4^+CD62L^+$ in P_3 (2.270667 x 10^6 cells) compared to control.

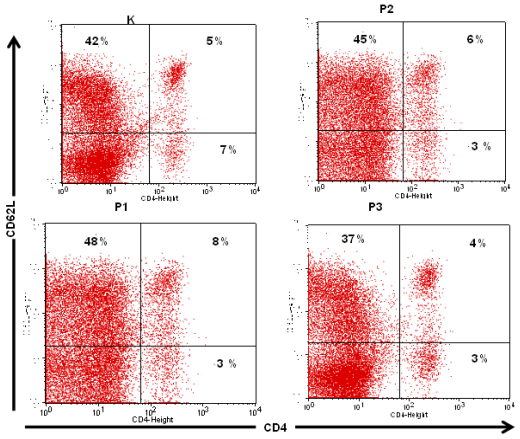

Figure 2. Profile of CD4 and CD62L cells; relative cell number of $CD4^+CD62L^-$, $CD4^-62L^+$, and $CD4^+CD62L^+$ in spleen. **Description:** Control (K), Treatment 1 (P_1: 0.5 g.g bw.$^{-1}$), Treatment 2 (P_2: 1.0 g.g bw.$^{-1}$), Treatment 3 (P_3: 2.0 g.g bw.$^{-1}$).

Profile of Cell Expres TER119 and B220 in Spleen

B220 or CD45R antigen is expressed by B cell, a subset of T cell (naive T cell), and monocyte. It functioned as isoform on A exon-contained CD45 [10]. *Flowcytometer* analysis (Fig. 3) showed that the given treatment was not affected the percentage cell number that expresses $TER119^+B220^-$ and $TER119^+B220^+$. Otherwise, cells that express $TER119^-B220^+$ tend to increase on treatment P_2 (treatment 1 g.g bw.$^{-1}$.day^{-1}) for 9% compared to control and decreased for 6% compared to treatment P_3 (2 g.g bw.$^{-1}$.day^{-1}).

Statistical anaylsis showed that the percentage of $TER119^+B220^-$, $TER119^-B220^+$ and $TER119^+B220^+$ were not significantly different ($p>0.05$). Whereas the cell population number of $TER119^+$ and $B220^+$ tend to increase on P_2 (Fig. 3), compared to other treatment and control. It indicates that flavonoid compound has effects in increasing the production of IL-2, thus increase the proliferation of B lymphocytes.

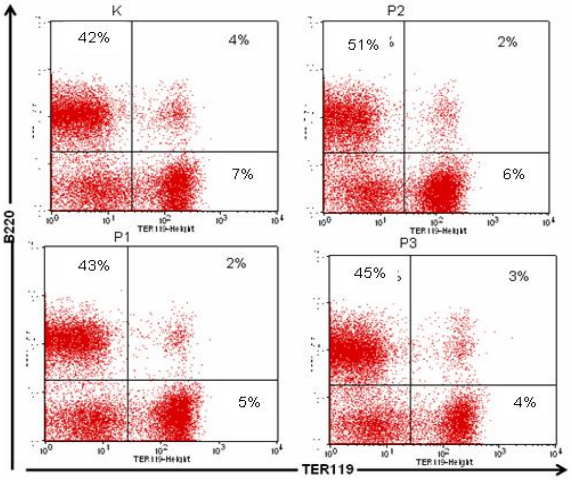

Figure 3. Profile of TER119 and B220 cells; relative cell number of $TER119^+$ $B220^-$, $TER119^-B220^+$ and $TER119^+B220^+$ in the spleen. **Description:** Control (K), Treatment 1 (P_1: 0.5 g.g bw.$^{-1}$), Treatment 2 (P_2: 1.0 g.gbw.$^{-1}$), Treatment 3 (P_3: 2.0 g.g bw.$^{-1}$).

Profile of Cell Express TER119 and B220 Molecules in Bone Marrow

Bone marrow is the primary or central lymphoid organs where the process of hematopoiesis occurred. It includes the *lymphopoeisis* for maturation, differentiation and proliferation of T cell and B cell into lymphocytes that recognize the antigen. Bone marrow also where the *erythropoiesis* (the red blood cells production) take places.

In this study, isolated cells from the bone marrow which added with antibody *antimouse* TER-119*/Erytrocyt Cell* conjugated to FITC *danantimouse* B220 which conjugated to PE (BD Bioscience©. TER-119 is a marker on the erythroid development in *erythropoiesis* from *proerythro-blast* to mature erythrocyes. Otherwise, B220 (CD45) is a molecular marker of B cell and all hematopoietic cells which function for phosphatase thyroxine, increase signal through the antigen receptor of B and T cells.

Flowcytometer analysis (Fig. 4) showed the difference on the average percentage of $B220^+$ (CD45) between treatment group compared to control. Percentage of cells that expressed $TER119^-B220^+$ tend to increase on treatment P_1 for 9%, while on the treatment P_2, the population is significantly increased by 16% compared to control. Otherwise, the number of cell population $TER119^+B220^-$ on each individual is not significantky different, similar to $TER119^+B220^+$. Refer

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to these data, the administration of doses on the Tapak Liman leaves extract indicates the increasing number of B cell population ($B220^+/CD45^+$).

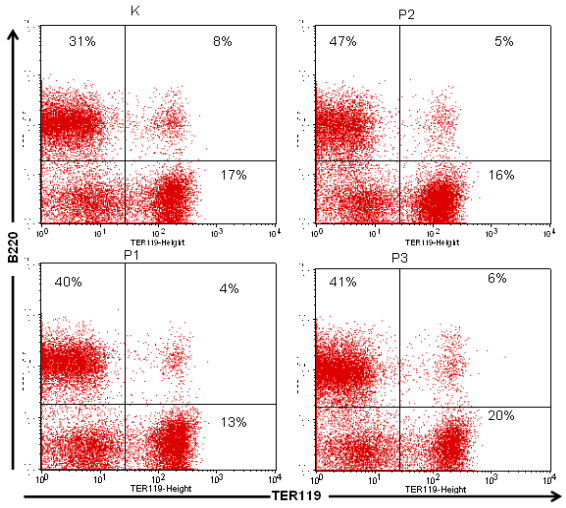

Figure 4. Profile of TER119 and B220 cells; relative cell number of $TER119^+B220^-$, $TER119^-B220^+$ and $TER119^+$ $B220^+$ in bone marrow. **Description:** Control (K), Treatment 1 (P_1: 0.5 g.g $bw.^{-1}$), Treatment 2 (P_2: 1.0 g.g $bw.^{-1}$), Treatment 3 (P_3: 2.0 $g.gbw.^{-1}$).

The highest number of cell population $TER119^-B220^+$ found in P_2 (24.546666 x 10^6 cells) compared to other treatments. It showed that proliferation and differentiation of *stem cell hematopoietic* occurred which tend to the production of B cell ($B220^+/CD45^+$). The increasing of B cell was assumed due to the flavonoid and lupeol compound which contained in Tapak Liman.

Profile of Cell Express the TER119 and VLA-4 Molecules in Bone Marrow

VLA-4 (*Very Late Integrin Antigen-4*) or CD49d is an antigen expressed by B cell, thymocytes, granulocyte and dendritic cell. It functioned as integrin a4, connected to CD29 (Leukocytes), bind fibronectin, MadCAM-1 and VCAM-1 (*Vacular Adhesion Molecule-1*).

The number of cells that expressed the $TER119^-VLA\text{-}4^+$ and $TER119^+VLA\text{-}4^+$ analysed with *flowcytometer* presented in Figure 5. Percentage of the cells that expressed $TER119^-VLA\text{-}4^+$ was decreased for 11% on treatment P_2, followed by the increase of cell percentage of $TER11^+VLA\text{-}4^+$ for 12%, compared to control.

Based on Figure 5, the highest cell population of $TER119^+VLA\text{-}4^+$ is in treatment P_2, i.e. 7.536 x 10^6 cells compared to control. The increased expression of $TER119^+VLA\text{-}4^+$ on P_2 group treatment was assumed due to the iron content in Tapak Liman which functioned to stimulate the progenitor hematopoietc cell to proliferate and differentiate into the precursor of erythroid and lymphoid into $TER119^+VLA\text{-}4^+$, which express nucleated erythrocyte cell, and other cells. Besides that, the increasing expression of $TER119^-VLA\text{-}4^+$ caused by the adhesion molecules interact between cells with the extracellular matrix which is needed I the activation of T cell for leukocytes maturation. The increasing of cell number expressed surface molecules of $TER119^+$ and $VLA\text{-}4^+$. It indicates that the leaves extract of Tapak Liman 1 g.g $bw.^{-1}day^{-1}$ adequate to stimulate the proliferation of hematopoietic stem cell on the lane of lymphoid and erythroid. It is explained that protein VLA on the surface of T cell function to channel the activation signal through the T cell receptor. On the normal condition leukocyte only attach to the endotel cell, but due to the infalmmation stimulus, adhesion between leukocyte and endotel enhanced [3].

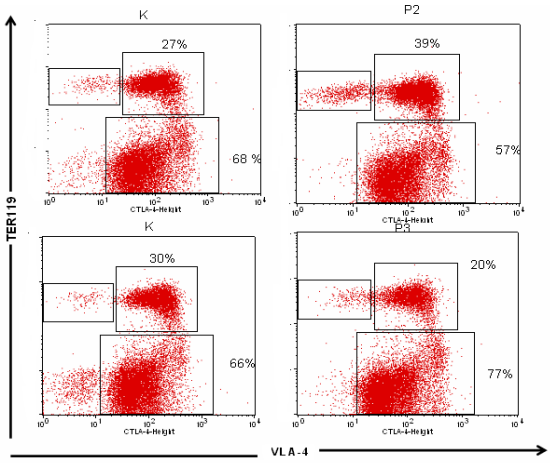

Figure 5. Profile of TER119 and LA-4 cells; relative cell number of $TER119^-VLA^+$ and $ER119^+VLA\text{-}4^+$ cells bone marrow. **Description:** Control (K), Treatment 1 (P_1: 0.5 g.g $bw.^{-1}$), Treatment 2 (P_2: 1.0 $g.gbw.^{-1}$), Treatment 3 (P_3: 2.0 g.g $bw.^{-1}$).

Profile of Cell Express the TER119 and CD34 Molecules in Bone Marrow

Cluster of diferentiation 34 (CD34) is an antigen expressed by the hematopoietic precursor cell and capillary endothelial cells function as Ligand CD62L (L-selectin). Based on the analysis of flowcytometer towards the average cell percentage of $TER119^-CD34^+$, $TER119^+CD34^-$, and $CD34^+TER119^+$ in the bone marrow presented in Figure 6.

The expression of cell percentage number on $TER119^-D34^+$ was decreased compared to con-

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trol, as well as the percentage of $TER119^{-}D34^{+}$ cells. While the cell that expresses $CD34^{+}TER119^{+}$ increased for 4% in treatment P_1 and conversely tend to decrease on treatment P_2 and P_3, compared to control.

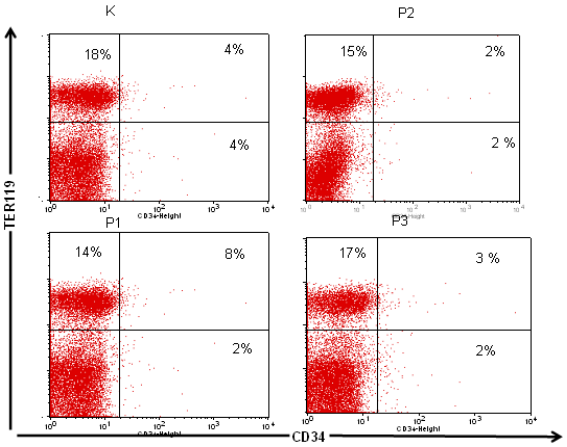

Figure 6. Profile of TER119 and CD34 cells; relative cell number of $TER119^{+}CD34^{+}$, $TER119^{-}CD34^{+}$ and $TER119^{+}$ $CD34^{+}$ in bone marrow. **Description:** Control (K), Treatment 1 (P_1: 0.5 g.g bw.$^{-1}$), Treatment 2 (P_2: 1.0 g.gbw.$^{-1}$), Treatment 3 (P_3: 2.0 g.g bw.$^{-1}$).

Based on the absolute number of cell, the number of $TER119^{+}CD34^{-}$cell population was not increased after being treated with leaves extract of Tapak Liman. However, it tends to increase on treatment P_2, compared to other two treatments. Otherwise, the number of $TER119^{-}CD34^{+}$ and $CD34^{+}TER119^{+}$ cell population tend to increase in treatment P_1 compared to control (Fig. 6).

Statistical analysis showed no significant difference ($p>0.05$) between treatments. It is assumed due to several factors: 1. Method for extraction of active compound was not compatible to obtain sufficient active compound from the leaves of Tapak Liman *(E. scaber* L); 2. time of samples isolation was not simultaneously.

DISCUSSION

Based on the study, mice group that given with leaves extract of Tapak Liman (*E. scaber* L) for two weeks compared to the control group, showed no significant effects towards the *eryhtropoiesis* on female BALB/c mice. It is most likely caused by several factors, e.g. extraction method of the active compound has not appropriate to obtain the active substance from the leaves of Tapak Liman (*E. scaber* L), and the different time of extraction. However, the study indicates a significant effect on the production of B lymphocytes in the bone marrow and the increasing number of B cell ($CD62L^{+}$) in the spleen.

The mice group that given the leaves extract of Tapak Liman with doses 1 g.g bw.$^{-1}$day^{-1} (P_2) showed the significant effect ($p=0.026$) towards the increasing percentage of cell $CD62L^{+}$ in the spleen and $B220^{+}$ ($p=0.044$) in the bone marrow; compared to the dose 0.5 and 2 g.g bw.$^{-1}$day^{-1}, as well as the control group for two weeks. It indicates that there was increased proliferation and differentiation of the hematopoietic cell into lymphocytes, i.e. T cell of $CD4^{+}$, $CD8^{+}$, and $B220^{+}$. It is caused by the flavonoid compound that potentialy stimulate the acivity of IL-2, thus increase the proliferation of lymphocyte cell.

Base on the number of cell population from all of the treatment groups, it is shown that the cell population tends to increase on the addition of Tapak Liman leaves extract dose 1 g.g bw.$^{-1}$day^{-1} (P_2) compared to the control group. Otherwise, the cell population tends to decrease on the treatment dose 2 g.g bw.$^{-1}$day^{-1} (P_3). It is explained that the flavonoid compound, besides has the effect of immunostimulant, it also has the effect of immunosuppressant [12]. Moreover, the cytotoxic effects of compounds in Tapak Liman which serves as an immunosuppressant allow the resistance progenitor cells in the lymphoid and erythroid pathway to proliferate and differentiate into lymphocytes (*lymphopoiesis*) and erythrocytes (*erythropoiesis*).

Based on the estimation of cell that expresses the $TER119^{+}VLA\text{-}4^{+}$ which identified as the pathway of the development of erythrocyte (erythroid). The increasing cell percentage of $TER11^{+}VLA\text{-}4^{+}$ for 12% occurred on treatment P_2, compared to control. The increased expression of $TER119^{-}VLA\text{-}4^{+}$ is caused by the adhesion molecules interact with the extracellular matrix which is needed in the activation of T cell, in the maturation of leukocyte. Furthermore, the increased expression of $TER119^{+}VLA\text{-}4^{+}$ assumed due to the iron content in Tapak Liman which function to stimulate the progenitor hematopoietic cell to proliferate and differentiate into a precursor of erythroid ($TER119^{+}VLA\text{-}4^{+}$). It expresses the nucleated erythrocyte cell and other cells. There is an increasing number of cell expressed by the surface molecules $TER119^{+}$ and $VLA\text{-}4^{+}$. It indicates that the leaves extract Tapak Liman with dose 1 g.g bw.$^{-1}$day^{-1} to stimulate the proliferation of hematopoietic stem cell on the path of lymphoid and erythroid.

CONCLUSION

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The administration of 1 g. g $bw^{-1}.day^{-1}$ of the extract (P_2) stimulate proliferation of lymphocytes and erythrocytes lineage ($TER119^+VLA\text{-}4^+$), in spleen and bone marrow.

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