Tapak Liman (*Elephantopus scaber L*) as Imunostimulator and Its Effect on Lymphocyte Differentiation in Mice BALB/C

Marmi Kelik
Faculty of Languages and Science, University of Wijaya Kusuma Surabaya

ABSTRACT

Tapak liman (*Elephantopus scaber, L*) is one of the plants that have medicinal properties and diverse roles and efforts of maintenance, improvement and restoration of health and disease treatment. The purpose of this study was to determine the effect of extracts of Tapak Liman (*Elephantopus scaber* L) as imuno stimulator to the development of lymphocytes in mice BALB / C The procedure of this study was to test extracts aquades in vivo with various treatments (control, treatment of 0.5 g / kg, 1.0 g/ kg, 2.0 g / kg) in mice BALB / C healthy for 2 weeks.

After the treatment carried out analysis of the percentage and number of cells that express $CD4^+$, $CD8^+$ and $CD4^+$ $CD8^+$ in thymus organ, using flowcytometry. Analysis of data using one-way ANOVA followed Tukey's test with SPSS. From the analysis showed that the extract of Tapak liman at various doses showed no significant effect on the percentage expression of $CD4^+$ $CD8^+$ and $CD4^+$ $CD8^+$ in thymus organs. While the analysis of the number of cells, extracts of Tapak liman show its effect on the number of cells that express $CD4^+$, $CD8^+$ and $CD4^+$ $CD8^+$ in thymus organs. Concentration of 1.0g/kg of mice showed a good effect on the increase in T helper cells ($CD4^+$), cytotoxic T cells ($CD8^+$) and Prothymosit cells ($CD4^+$ $CD8^+$).

Keyword: TapakLiman, Imunostimulator, Lymphocyte

Introduction

Elephantopus scaber Linn. is a small herb, which grows in the wild throughout the tropical regions of the world. The major phytochemical constituents of the plant are elephantopin, triterpenes, stigmasterol, epofriedelinol and lupeol (Rastogi and Mehrotra, 1990; Kritikar and Basu, 1991). The plant has been used in the Indian system of medicine as analgesic, diuretic, astringent and antiemetic. The leaves of the plant were known to be used for bronchitis, small pox and diarrhea and as a brain tonic (Sankar *et al*., 2001). Recently, it has been shown to possess anti-inflammatory and anti tumour activity in animal models (Reico, 1989) and also found to have antibacterial activity against a few standard bacterial strains (Avani and Neeta, 2005).

The genus *Elephantopus* consists of approximately 30 species distributed in the Neotropicsand the Old World, and its lectotype species, *E.scaber*, occurs in all tropical regions (Cabrera and Klein, 1980; Chen, 1985; Cao and But, 1999). InSouthern China, Hong Kong and Taiwan, the whole plant of *E. scaber*, a perennial herb (10D 50 cm in height), is well known as a folk medicine widely used in the treatment of nephritis, edema, dampness, pain in the chest, fever and cough of pneumonia, scabies, and arthralgia due to wounding (Peer and Metzger, 1980; Hsu, 1986; Tsai andLin, 1999). In Brazil, the infusion and the decoction of the whole plant are used to stimulate diuresis,reduce fever, and eliminate bladder stones (Cabrera and Klein, 1980; Poli *et al.*, 1992). It has also been popular as a medicinal herb in many countries of Southeast Asia, Latin America and Africa for a long time (Hammer and Johns, 1993; Cao *et al*., 1997).

Since the 1970's, aSince the 1970's, a number of chemical constituents and pharmacological evaluations of *E. Scaber* have been reported. For example, Kurokawa *et al.* (1970) and Govindachari *et al.* (1972) reported elephantopin, deoxyelephantopin, and isodeoxyelephantopin in this species; De Silva *et al.* (1982) found that both alcohol and chloroform extracts of *E. scaber* contain cytotoxic germacranolide-type sesquiterpene lactones; Poli *et al.* (1992) tested the aqueous and hydroalcoholic extracts of whole plants for acute toxicity, analgesic, antipyretic, anti-inflammatory, cardiovascular, diuretic, and constipating activities; Hammer and Johns (1993) reported that the plant extract of *E. scaber* was subjected to bioassays; Lin *et al.* (1995) and Tsai and Lin (1999) evaluated the hepatoprotective and anti-inflammatory effects of the Taiwanese folk medicine "Teng-Khia-U", derived from three plant species including *E. scaber*, and But *et al.* (1997) described the isolation and structure characterization of three germacranolide sesquiterpene lactones from *E. scaber.*

The aqueous extracts of roots and leaves from *E. scaber* portrayed excellent hypoglycemic effect indiabetic rats by lowering the blood glucose level and serum insulin level. A decrease in the elevated levels of glycosylated hemoglobin, liver glycogen, triglycerides and serum cholesterol in alloxan-induced hyperglycemic rats was also reported by Daisy et al. (2007).

Besides hypoglycemic activity in mice models, aqueous extract of *E. scaber* also showed significant antiinflammatory effect in both experimental acute and chronic arthritis rat models. The aqueous extract from the whole plant significantly inhibited the development of pad swelling in the acute experimental arthritis rats at a dose of 300 mg/kg while higher concentration of the extract(500 mg/kg) was required to inhibit the development of chronic joint swelling in the chronic inflammatory model (Tsai and Lin, 1999).

During thymocyte development, immature thymocytes that express both CD4 and CD8 genes must choose either a helper $CD4^+$ or cytotoxic $CD8^+$ T-cell fate. Over the past two years, there have been some important advances regarding T-cell lineage choice, including the identification of transcription factors required for CD4 gene silencing by CD8-lineage cells (RUNX3) or for $CD4^+$ T-cell differentiation (GATA3), and a better understanding of how T-cell receptor (TCR) signalling correlates CD4/CD8-lineage differentiation to MHC specificity. This review summarizes these recent advances and highlights potential links between TCR signals and nuclear effectors of lineage differentiation (Bosselut R, 2004)

The purpose of this study was to determine the effect of extracts of Tapak Liman (*Elephantopus scaber* L) as imunostimulator to the development of lymphocytes in mice BALB/C.

Materials and Methods

The type this experiment with the design of the post test only control group design. Using the 4 groups, 1 group control and 3 treatment groups, with simple randomization. Assessment is performed only when the post test, comparing the results of observations on the treatment and control groups, and among treatment groups. The samples taken at random (random) of the population reached the inclusion criteria as follows: murinestra in BALB/C female, age 6 weeks, and healthy. 12 mice BALB/C as are divided into 4 groups, each group consists of three mice. Each group of mice is given the same standard food and drink ad libitum.

Four groups of mice are given: Control(K): aquades without extract of Tapak Liman leaf Treatment1 (P1): Tapak Liman leaf extract 0.5 grams/kg body weight/day Treatment2 (P2): Tapak Liman leaf extract 1.0 g/kg body weight/day Treatment3 (P3): Tapak Liman leaf extract 2.0g/kg body weight/day.

The effect ofextractson the development of lymphocytes in thymus can be determined by measuring the percentage and number of lymphocytes expressing the express $CD4^{+,}$ $CD8^{+}$and $CD4^{+}$ $CD8^{+}$ in thymus organ using flowcytometry on the 16th day, after the treatments of Tapak Liman extract at various doses. The data obtained in the form of percentage and number of cells that express the CD will be analyzed statistically by calculating the standard deviation in each treatment and one-way ANOVA analysis to determine the effect of treatment on the development of lymphocytes. If there are real differences in each treatment will be followed by Tukey test to determined ifferences in each treatment. Analysis using SPSS. Significance with a P value<0.05.

Results and Discussion

Thymus is the primary lymphoid organs, bone marrow as a producer of T cell precursors are derivatives of progenitor cells after differentiation in the thymus to form a functional T cells, and after 4 stages of maturation involves a variety of protein expression and T cell receptor (TCR) as an end to the circulation cell T peripherals (Keer, 1998).

Transitional stages of thymocyte maturation can be characterized on phenotip cells with the TCR-CD3 complex, the presence of CD4 and CD8 coreceptor (Kuper et al., 2002).

Based on the results of flowcytometry analysis (Figure 1) Note that the percentage of CD4 + expression on the control has an average value of 13:04%, in the treatment of 0.5 g / kg had a mean value 10.67%, in the treatment of 1.0 g / kg had an average of 9.85%. While on treatment of 2.0 g / kg had an average of 10.81%. Based on the results of statistical analysis using one-way ANOVA (Appendix 8) found that the expression of CD4 +, with a significance value of 0864 (> 0.05). For the expression of CD8 + with a significance value 0676 (> 0.05), whereas for the expression of CD4 + CD8 + has a significance value 0.496 (> 0.05). It could be argued that there is no real influence liman extract of tread on all expressions of concentration versus percentage of CD4 +, CD8 +, CD4 + CD8 + in thymus organs.

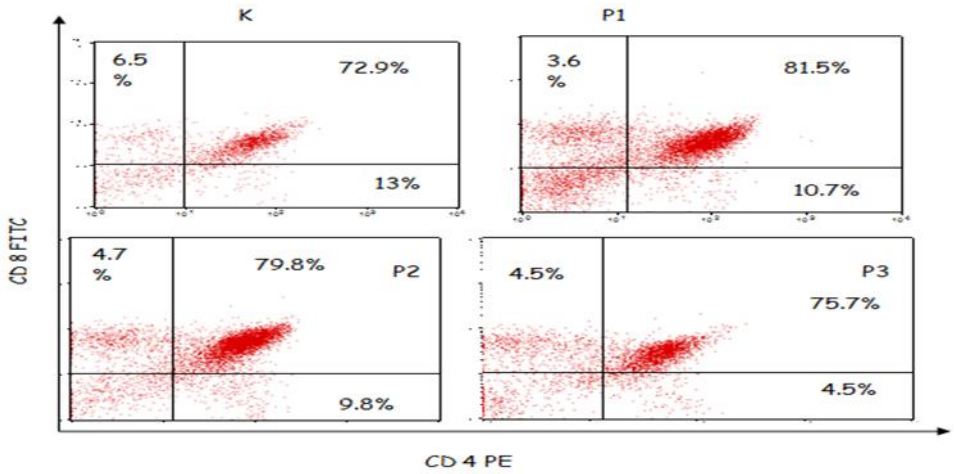

Figure 1. Cell Profile $CD4^{+}$, $CD8^{+}$ and $CD4^{+}$ $CD8^{+}$: Prosentage cell exspression $CD4^{+}$, $CD8^{+}$, $CD4^{+}$ $CD8^{+}$ on thymus. Control (K), (P1:0.5 gr/kgbw), (P2:1.0 gr/kg bw), (P3:2.0 gr/kg bw).

Total cells (Figure. 2) shows that the concentration of 1.0 g/kg increased the number of $CD4^{+}$ cells (1615946) when compared to the control and the concentration of 0.5g/kg (976 610). Concentration of 2.0 g/kg would reduce the number of cells that express $CD4^{+}$ (1438173) but still better than in the control and treatment of 0.5 g/kg. Likewise, the $CD8^{+}$

cells that express at a concentration of 1.0 g/kg caused the number of $CD8^+$cells at high (764 786) when compared to controls, 0.5 g/ kg, 2.0g/ kg. Concentration of 2.0 shows thedecline inthe number of cytotoxic cells but still higher when compared with the concentration of 0.5g/kg and control. So it can be said that the concentration of 2.0g/kg gave the best effect on the number of cytotoxic cells in mouse thymus organs. Total cells that express $CD4^+$ $CD8^+$ (prothymocyte) showed that consentration1.0g/kg showed the best effect when compared with control (7395966) and the treatment of 0.5g/kg (7,455,115), and 2.0 g treatment/kg. Showed that the concentration of 1.0g/kg increased the number of cells prothymocyte.

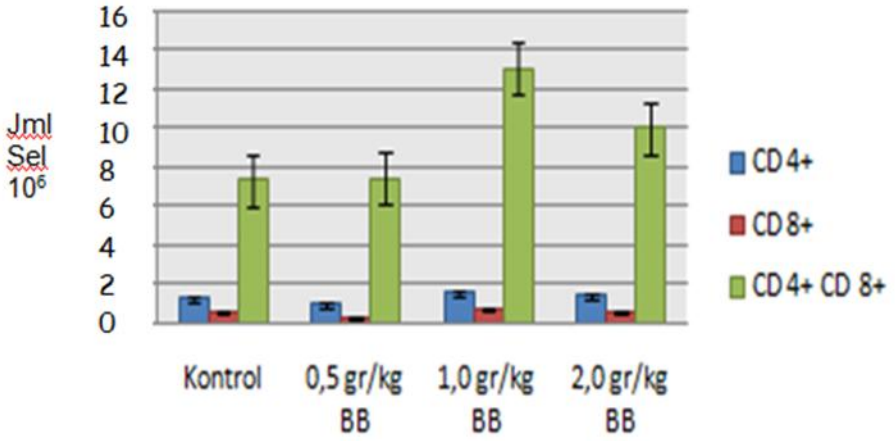

Figure 2. Total cell $CD4^+$, $CD8^+$,$CD4^+CD8^+$ on Thymus at Control, 0.5 gr/kg bw, 1.0 gr/kg bw, and 2.0 gr/kg bw.

Lymphocyte progenitor cells derived from bone marrow, some of which are relatively non-lymphocyte differentiation have migrated to the thymus and reproduce themselves, here is to obtain properties of lymphocytes T lymphocytes At first thymosit not express CD4 and CD8, cells then develop into double positive ($CD4^+$ $CD8^+$) and eventually mature into single positive ($CD4^+$ or $CD8^+$) can then log back into the blood stream, back into the bone marrow or lymphoid organs and peripheral can live several months or years (Schwarz and Bandola, 2008).

Conclusion

Extract of Tapak liman at various doses showed no significant effect on the percentage expression of $CD4^+$ $CD8^+$ and $CD4^+$ $CD8^+$ in thymus organs and $CD4^+$ $CD8^+$ in thymus organs. While the analysis of the number of cells, extracts of Tapak liman show its effect on the number of cells that express $CD4^+$, $CD8^+$ and $CD4^+$ $CD8^+$ in thymus organs. Concentration of 1.0g / kg of mice showed a good effect on the increase in T helper cells ($CD4^+$), cytotoxic T cells ($CD8^+$) and Prothymocyte cells ($CD4^+$ $CD8^+$).

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