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Activity Red *Moringa oleifera* Leaf Extract As a Preventive Measure on the Profile of $CD4^+CD62L^+$ and $CD8^+CD62L^+$Cells in BABL/c Mice Injected *Salmonella typhimurium*

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Abstract

Moringa oleifera has both nutritional and complex phytochemical content which provide protection and recovery from infection. *Moringa oleifera* has the potential as an immunomodulator both in preventive and curative measures. *Salmonella typhimurium* can cause body immunity, especially $CD4^+CD62L^+$ and $CD8^+CD62L^+$ T cells have a deficiency so that *Salmonella typhimurium* can infect body cells. This researched aims to determine whether the administration of *Moringa oleifera* leaves extract can increase the number of T $CD4^+CD62L^+$ and $CD8^+CD62L^+$cells in mice infected by *Salmonella typhimurium*. This experiment used was a complete randomized factorial pattern design. Mice were divided into two groups, namely the control group (positive control and negative control) and the infection group, mice (given *Moringa oleifera* leaves extract dose of 14 mg/kg BW, 42 mg/kg BW, and 84 mg/kg BW) and infected by *Salmonella typhimurium*. Data analysis was confirmed with the ANOVA test followed by Tukey test ($p<0.05$). The results showed that red *Moringa oleifera* leaves extract can increase the number of $CD4^+CD62L^+$ and $CD8^+CD62L^+$ T cells in all groups of infected mice and of *Moringa oleifera* leaves extract with high doses caused immunosuppression. Red *Moringa oleifera* leaves extract can function as an immunostimulant and immunosuppression on $CD4^+CD62L^+$ and $CD8^+CD62L^+$ T cells.

Keywords: $CD4^+CD62L^+$, $CD8^+CD62L^+$, *Moringa oleifera, Salmonella typhimurium*

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1. Introduction

The activity and function of the immune system can be decreased due to various factors including the disease caused by bacteria, fungi, and viruses. An antibiotic for treatment can be used to kill bacteria, but many antibiotics are resistant. So we need another alternative to antibiotic use. Medicinal plants that have been widely

studied prove the presence of immunostimulators that function to improve non-specific immune system functions and activities such as lymphocytes, NK cells, macrophage stimulation, interferon release and interleukin [16]. *Moringa oleifera* is thought to have immunostimulatory properties because it has complex nutrients and phytochemicals. *Moringa oleifera* is known as the 'tree of life' because every part of this plant can be used as food, medicine, and industrial purposes [7]. The families of *Moringa oleifera* is Moringaceae that contain antioxidants including, saponins, alkaloids, phytosterols, tannins, phenolics, and flavonoids [11]. The presence of compounds that can enhance the immune system and are very helpful to overcome the decline in the immune system. Immunomodulators are drugs that can restore and repair the immune system whose function is disrupted or to suppress its excessive function [2].

Salmonella typhimurium is a gram-negative, rod-shaped and nonporous bacteria, has somatic antigen (O) structure, flagellar antigen (H) and Vi antigen. Vi antigen is invasive and can live intracellularly and can reduce IL-8 expenditure which plays a role in inducing polymorphonuclear (PMN) neutrophils [9]. The body's immune system that has a pivotal role in eliminating S. *typhimurium* bacteria is TCD4^{+} cells and CD8 T cells [17].

The mechanism of systemic infection of *S. typhimurium* can be described in several phases, which in turn can differentiate excess CD4^{+} T cells resulting in chronic infection. The workings of the immune system in the face of an invasion of foreign substances from outside the bodywork simultaneously, with CD4^{+} T-helper (Th) lymphocytes as the leader. In other words, animal susceptibility and resistance to microbial infections are highly dependent on lymphocyte cell activation characterized by the expression of several subsets of lymphocyte cells such as CD4^{+}and CD62L^{+}T cells [12]. So that observation of spleen organs is expected to increase the immune system of BABL/c mice after being given a red *Moringa oleifera* extract.

2. Methods

2.1. Experimental design

The type of research conducted was experimental research using 2 experimental groups which were divided into 5 parts namely the positive control group (mice injected with *Salmonella typhimurium*), negative controls (healthy mice), and the treatment group P1-P3 (red *Moringa oleifera* leaves extract dose 14, 42, 84 mg/kg BW respectively) for 21 days. On the 22nd day, mice were infected by *Salmonella tyhpimurium* at 10^{7} CFU/g

of 100 µl intraperitoneally. After the 28th day after treatment, the mice were sacrifice by cervical dislocation, then surgery and cell isolation were carried out.

2.2. Preparation of red M. oleifera leaves extract

The making of *Moringa oleifera* leaves extract was carried out using the infusion method. Moringa leaves which had been dried, blended and filtered to obtain it in the form of simplicia. The simplicia obtained was taken as much as 10 g and mixed with 100 mL of distilled water. Simplicia which has been mixed with distilled water at 85°C while being distilled at 200 rpm, and after reaching a temperature of 85°C is maintained for 15 minutes. Then filtering is done using filter paper. The extract obtained was taken by the prescribed dosage and added with 0.5% Sodium Carboxymethyl Cellulose (Na-CMC) solution to be given to mice orally with a volume of 100 µL.

2.3. Animal treatment

Mice obtained from the LPPT-UGM before treatment were adapted for seven days at the Molecular Biology Laboratory, Brawijaya University, Malang. Adapted mice were grouped according to the completely randomized design (CRD). Mice in this experiment were divided into two groups, including the control group (healthy control and sick control) and the infection group, *Salmonella typhimurium* infected group (given *Moringa oleifera* leaves extract 1 x day, for 21 days and on the 22 days infected *Salmonella thypimurium* at 10^7 CFU/g *intraperitoneal).*

2.4. Confirmation test for the presence of bacteria Salmonella typhimurium in the blood

Mice group infected with *Salmonella typhimurium*, on the 23 days. The confirmation test was conducted to determine the success of *Salmonella typhimurium* in infected mice. The confirmation test is done by taking the blood of mice by cutting the tail. The blood that has been taken is then poured plate and catalase test. The pour plate test is carried out using xylose lysine deoxycholate jelly (jelly XLD) while the catalase test uses hydrogen peroxide [5].

2.5. The splenocyte isolation

After the 28 days post-treatment, mice were sacrificed with neck dislocations, then surgery for spleen organ harvesting. Spleen organs that have been obtained are crushed, filtered, and suspended with phosphate buffered saline (PBS). The homogenate was centrifuged at 2500 rpm at 40°C for 5 minutes. The pellets were resuspended with 1 mL PBS. The homogenate obtained was transferred to a new microcentrifuge tube, and a 500 µL PBS was added. Then centrifuged at 2500 rpm with a temperature of 40 °C for 5 minutes and after that, the pellet is taken.

2.6. Analysis flow cytometry

Analysis flow cytometry was performed to detect cell populations expressing $CD4^+$ and $CD62L^+$. $CD8^+$ and $CD62L^+$. In this study, cells isolated from spleen were incubated with anti $CD4^+$ $CD8^+$and $CD62L^+$ anti-mouse. Then the connection is made with the computer and flow cytometry is set in acquiring state and parameter settings will be analyzed. After incubation, the sample was added with 500 ml of PBS and transferred to cuvette flow cytometry. Furthermore, the acquire and flow cytometer will be chosen to calculate the total cell count and the number of cells detected by the antibody label. The results obtained were then processed with BD cellquest Pro^{TM}.

2.7. Data analysis

After obtaining data from all treatments carried out, the results obtained were tested for normality. The design of the experiment was conducted using a completely randomized design. Furthermore, the data obtained was tested by ANOVA with SPSS 16.0 for Windows, then if a significant result was obtained then proceed with the Tukey test.

3. Results and Discussion

The immune response that plays a role in eliminating *Salmonella typhimurium* is a specific immune response represented by activated macrophages, which can increase killing cells (NK) which will produce cytokines [10]. Activated macrophages will secrete cytokines such as IL-11β, IL- 6 in which this interleukin can be used by T cell to differentiate into Th 17 cell [14], and ultimately induce T cell proliferation. According to Rifa'i (2011), $CD4^+CD62L^+$or called L-selectin is an adhesion molecule that has a function

as an attachment or rolling on vascular endothelial cells, based on the analysis of $CD4^+CD62L^{+\cdot}$T cell flow cytometry. After administration of red *Moringa oleifera* leaves extract at a dose of 24 mg / kg BW the relative cell number of $CD4^+CD62L^+$ (31.60%) and a dose of 42 mg/kg BW is (35.81%). There was a significant increase in naïve $CD4^+$ cells (P> 0.05) in mice infected with *S.typhimurium* compared with positive control, whereas at a dose of 84 mg/ Kg BW is (3.48%). In this experiment, we showed an increase of naïve CD4 T cells but not too high when compared to positive control. $CD4^+CD62L^-$ cell (T helper) is a type of T cell subset that has been activated due to antigen attacks in the body. Activation that occurs causes double positive cells ($CD4^+CD62L^+$) to differentiate characterized by loss of $CD62L^+$ (L selectin) expression so that it is called single positive T cell (mature). $CD62L^+$ is expressed in lymphocytes and is involved in regulating cell trade by binding to ligands on the vascular endothelium, and $CD62L^+$ cell is effector memory cells that migrate to the inflammatory area and are rapidly stimulated by bacteria to produce cytokines, such as IFN-γ, IL-4, and IL-5 [8].

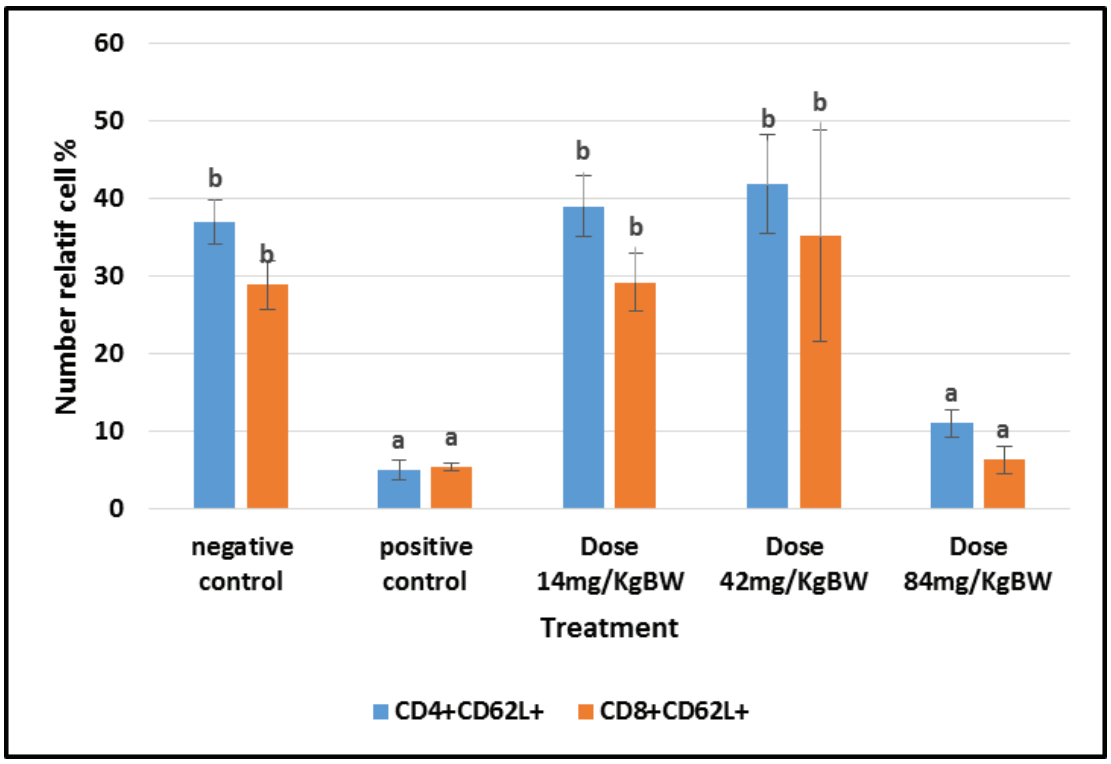

Figure 1: Relative number of CD4+$CD62L^+$ T cell after treatment of *Salmonella typhimurium* infection and adding of red *Moringa oleifera* leaves extract.

CD4+ T cell coordinate immune responses by producing cytokines that play a role in cell communication to stimulate other cell activation [13]. Immunostimulator activity is seen by increasing the number of $CD4^+CD62L^+$ T cell. This is due to the presence

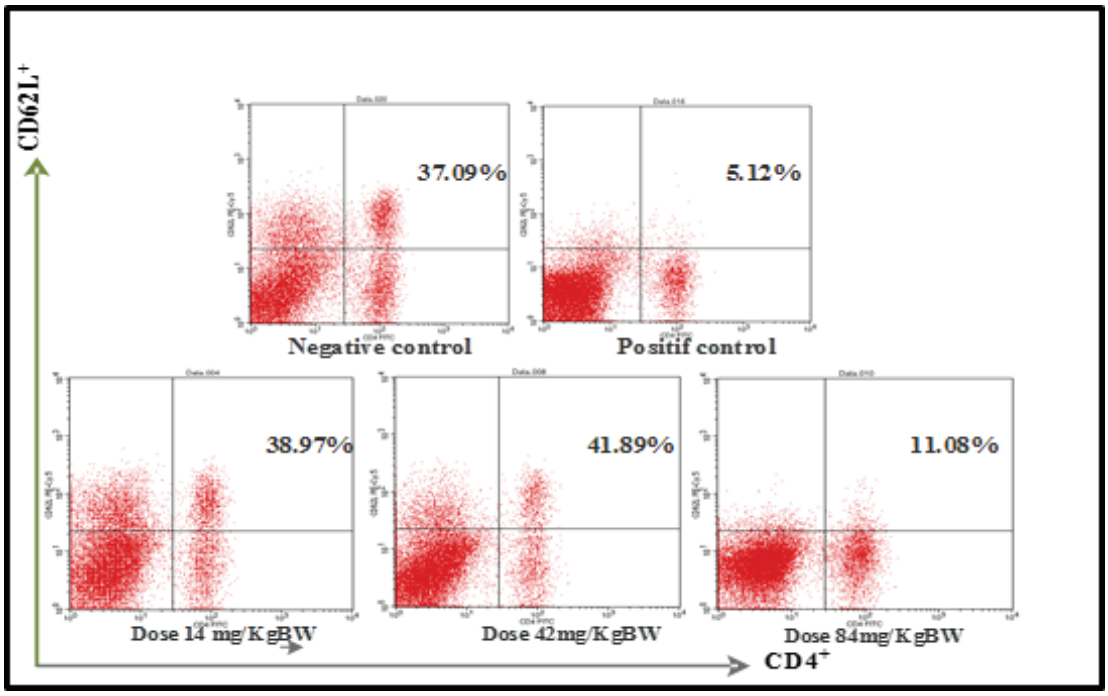

Figure 2: Profile of $CD4^+CD62L^+$T cell expression on spleen after treatment of *Salmonella typhimurium* infection and adding of red *Moringa oleifera* leaves extract.

of active substances in the form of saponins and flavonoids in the ethanol extract of *Moringa oleifera* leaves which play a role in increasing the number of naïve CD4 T cell in the spleen. Cell proliferation can be caused by an exogenous stimulus in the form of active plant compounds.

The functional $CD8^+CD62L^+$·T cell produces cytokines and granzymes that kill pathogen-infected cells and control the infection. $CD8^+$ T cell produces cytokines IFN-γ and IL-2. These cytokines contribute to the efficient elimination of infectious pathogens by activating macrophages and natural killer (NK) cells [4].

According to Hefni (2013), the active compound found in red *Moringa oleifera* acts as mitogen activated protein kinase (MAPK), will stimulate the proliferation of T and B cell through increased IL-2 expression. Cytokine synthesis is initiated by gene transcription and occurs because of a stimulus, in this case, the stimulus comes from the active compound in the extract. IL-2 is one of the earliest immune responses of T helper cells. These cells will also bind to antigen presenting cells (APC), which is generally macrophages after phagocytosis, resulting in an increasingly large immune response [3]. $CD4^+CD62L^+$T cell is included in the immune system which is mediated by cell and function to recognize foreign antigens, which has been presented on the cell surface by antigen presenting cells (APC). The antigen can come from a pathogenic

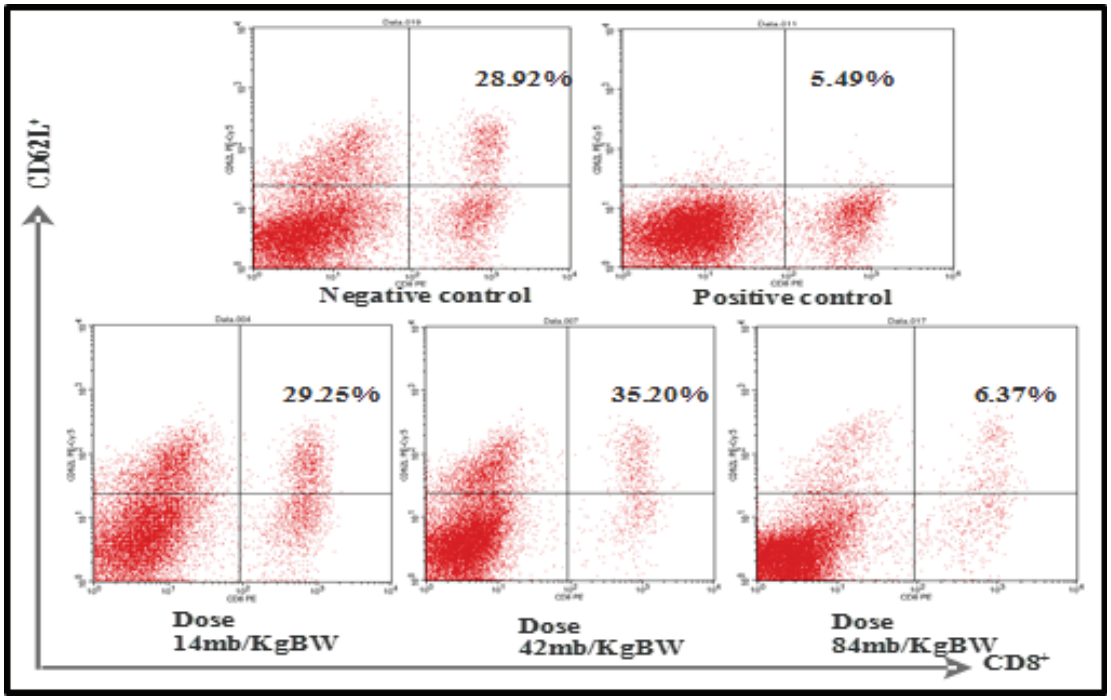

Figure 3: Profile of $CD8^{+}CD62L^{+}$T cell expression on spleen after treatment of *Salmonella typhimurium* infection and adding of red *Moringa oleifera* leaves extract.

virus or intracellular bacteria that replicate inside cells [13]. Besides, have functioned as immunostimulants, *Moringa oleifera* leaves extract can function as an immunosuppressant. This can be seen in the administration of high doses of *Moringa oleifera* leaves extract causing a lower number of $CD4^{+}$ T cell compared to low doses. These results were reported by Sudha *et al*., (2010) and Biswas *et al*., (2012) that low doses of *Moringa oleifera* leave extract are more effective than high doses.

4. Conclusions

Based on the research conducted can be concluded that:

1. The addition of ethanol extract of red Moringa *oleifera* leaves in Salmonella *typhimurium* infected mice was able to provide immunostimulatory effects from a dose of 14 mg / KgBW and 42 mg / KgBW.

2. The Dose of 14mg/KgBW and 42 mg/KgBW given ethanol extract of red *Moringa oleifera* can increase the proliferation of T $CD4^{+}CD62L^{+}$ and $CD8^{+}CD62L^{+}$ cells

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