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Potential of *Syzygium aromaticum* (Clove) Leaf Extract on Immune Proliferation Response in Balb/c Mice Infected with *Salmonella typhimurium*

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Abstract

Background and Objective: *Syzygium aromaticum* is an immunomodulator with the main active compound eugenol which can stimulate the function of lymphocyte proliferation and macrophage production. Lymphocytes play an important role for body protection against infection. This study aims to analyze the effect of *S. aromaticum* extract to increase proliferation of lymphocytes, lymphoblasts and macrophage production in Balb/c mice infected with *Salmonella typhimurium*. **Methodology:** Mice strain Balb/c were divided into 4 groups: treatment group infected with *S. typhimurium* and control group (without treatment) to tested the activity of peritoneal macrophages. The treatment group was administrated by *S. aromaticum* leaf extract (15 mg, 75 mg, 150 mg/kgbw) for 12 days. **Results:** *S. aromaticum* leaf extract increased the proliferation activity ($p < 0.001$) at dose of 15, 75, and 150 mg/kgbw, increased lymphocytes at dose 150 mg/kgbw ($p < 0.05$), increased the activity of macrophage ROI secretion at dose of 150 mg/kgbw ($p < 0.05$), and increased in lymphoblast ($p < 0.05$). **Conclusion:** Administration of *S. aromaticum* leaf extract increased the proliferation activity of lymphocytes, lymphoblasts and ROI secretion of macrophagesin Balb/c mice infected with *S. typhimurium*.

Keywords

Syzygium aromaticum, Lymphocytes, Lymphoblast, Macrophage, *Salmonella typhimurium*

1. Introduction

Clove (*Syzygium aromaticum*) is Indonesian native species (Maluku) which belongs to Myrtaceae family, ordo Myrtales [1] and is one of herbal plant that has long been used in Middle Eastern and Asian countries [2]. Cloves are used as a traditional medicine in the treatment of various diseases, as well as flavoring. Typical cloves aroma is produced by eugenol compounds, which are the main compounds of cloves (72% - 90%). Eugenol also has antiseptic and anesthetic properties [1]. Analysis of clove leaf extract from Bangladesh with GC-MS method showed that the extract consisted of 74.28% eugenol, 5.78% eucalyptol, 3.85% caryophyllene, 2.43% α-cardinol and 2.08% limonene [3].

The content of clove compounds has chemical properties and pharmacological effects that function as anesthetics, antimicrobials, antiseptics, antioxidants, immunomodulators, and immunostimulants [2] [4]. Phenolic compounds of clove are also responsible for antioxidant activity and flavonoid compounds act as antidotes to free radicals [5]. The content of clove compounds can induce specific and non-specific immunity and activate cellular components of the immune system, such as phagocytic function without affecting both humoral and cellular immunity [6]. The human body has defense mechanism against the intervention of exogenous or endogenous antigens, especially those that are detrimental to the body. This mechanism could be natural and adaptive [7].

Natural defense mechanisms include NK cells, lymphoblasts and lymphocytes. In addition to being multi effector, these cells also act as an immunoregulator that could increase the activity of the defense system through Th1 or Th2 cells in the adaptive immune response [8]. The main target of these cells is the body cells that are infected with viruses, bacteria and cancer cells [9]. Furthermore, macrophages and dendritics are also involved in natural immunity activities through phagocytic activity. Macrophages in the submucosal tissue are the first cells exposed by pathogens that successfully penetrate the physical barrier in the form of epithelium tissue [10]. Microbes are detected through macrophage surface receptors, then phagocytosed and degraded, and then stimulated neutrophils that will lead to the site of infection to strengthen the immune response against microbial infection [11].

Natural immune response is a front line defense against pathogenic threats [10]. Any failure in the natural immune response will be taken over by the adaptive immune response (macrophages and dendritic cells). Macrophages and dendritic cells have the ability to present antigens to TCD 4^+ lymphocytes through degradation of antigens into peptide fragments with MHC class II,

LFA3, ICAM-1, CD22 and B7 molecules [12]. This activity is the beginning of a cellular immune response in an adaptive defense system [8]. If there is an infection by microbes, stimulated macrophages or dendtritic cells respond by synthesizing IL-12, the Interleukin 12 then activates NK cells to produce IFNγ, then IFNγ activates native TCD 4^+ for differentiation into Th1 cells which could produce various cytokines such as IL-2, IFNγ and TNFβ [13]. The role of $TCD8^+$ cells in the immune response to viruses and tumors is quite prominent. Cells $TCD8^+$ recognize antigen peptide fragments along with class I MHC molecules and the LFA3, ICAM-1 and CD22 molecular co-stimulators that continue to destroy target cells [14]. When activated NK cells produce interferon γ (IFNγ), this cytokine stimulates phagocytic activity of macrophages in destroying target cells [8]. Activity NK cell in killing tumor cells could be triggered by IL-2 through the response of IL-2α and IL-2$\beta\gamma$ expressed on the membrane. NK cells even though in the inactive phase still express IL-2 receptors, especially IL-2$\beta\gamma$ [15].

Natural and adaptive immune responses could occur in the form of humoral and cellular reactions (cell-mediated imunity). Cellular immune response, among others, is caused by infection with intracellular microorganisms [16]. Both types of natural and adaptive responses do not have clear limits, and could even be interrelated where the initial adaptive immune response is supported by natural responses [10]. The body's reaction to infection is carried out through cellular and humoral mechanisms. The content of clove compounds has been known to have immunomodulatory pharmacological activity, anti-bacterial, and anti-inflammatory [2] [17] [18]. Clove potential as the body's defense system has not been widely reported. Pior research is still limited to the treatment of toothache, prevention of inflammation, and the source of antioxidants [19] [20].

Therefore, this study was aims to analyse the effect of clove leaf extract on proliferation activity of lymphocytes and phagocytosis of macrophages in mice infected with *Salmonella typhimirium*. Induction of immune response of mice in this study using *S. typhimurium* as a model that has been widely used to study innate immune response. *S. typhimurium* has the main virulence factor in the form of lipopolysaccharide (LPS) which can stimulate cellular immune responses through phagocytosis by macrophages activation [21]. *S. typhimurium* infection was studied in mice because systematic infections believed to be compatible with typhoid fever in humans. The *S. typhimurium* strain efficiently infects mice so that these animals are often used for the study of infection pathogenesis and immune responses to bacteria.

2. Material and Methods

This study was conducted at the Faculty of Biology UGM at Laboratory of FALITMA UGM, from March to June 2018. Leaves of *S. aromaticum* var. siputik were obtained from the plantation of Negeri Lima, Maluku, Indonesia. The chemicals used for extraction are n-hexane pro analysis. Leave extract of *S. aro-*

maticum var. siputik was tested for chemical compounds by GC-MS method at Organic Chemistry Laboratory of FMIPA UGM. About 0.5 µL extract was injected into GC-MS QP2010S SHIMADZU, the column temperature was programmed to 120˚C - 310˚C with an increase about 10˚C per minute, with the carrier gas is helium, pressure 13.7 kPa, and ionizing detector EI (electron impact). The level of compound is determined based on the standard peak area compared to the sample peak area. *S. typhimirium* were obtained from Medical Laboratory Technology Solo (Indonesia), and Balb/c mice were obtained from Laboratory of LPPT UGM. Light microscopes (XSZ-107 BN, Japan) and a microscope with a camera (Olympus BX 51, Japan) were used to observe the histology and spectropometer (Perkin elmer comom 44 ILL, USA) was used to calculate the bacteria concentration.

Twenty of male mice Balb/c weighing 20 - 25 grams, 6 week was divided into 4 groups, each group consisted of 5 male mice. The control group was administrated with sterile distilled water and the treatment group was administrated with clove leaf extract for 12 days. P1 treatment with dose 15 mg/kgbw, P2 treatment with dose 75 mg/kgbw, P3 treatment with dose 150 mg/kgbw. Infection in mice was carried of *S. Typhimurium* in 0.2 ml PBS 0.01 M pH 7.4 was administered intraperitoneally with a dose of 10^8 per mice before administrating with clove leaf extract. After the inoculation of bacterial mice was killed, splenocytes and peritoneal macrophages were taken to examine lymphocyte proliferation activity, phagocytosis and ROI secretion.

Calculation of lymphocyte was followed Farizal (2012) and Ulfa (2017) with minor modification. By using a petri dish containing 1.5 ml of RPMI, the spleen was crushed until smooth. Then washed with 10 ml PBS then centrifuged at a speed of 10,000 rpm for 5 minutes, with a temperature of 40˚C, the supernatant was discarded and 2 ml PBS was added. The lymphocyte was calculated by a counting chamber (Neubouer Improve) by dripping the spleen fluid. Calculation of the number of lymphoblasts by using a drop of a spleen substrate is taken and a smear preparation is made on a glass of preparation, fixation with absolute methanol and dried, then painted with giemsa. Lymphoblasts are identified as large cells with nucleus and nucleolus, chromatin was not solid (light purple) and cytoplasm is still visible. Whereas lymphocyte cell size was smaller with a solid chromatin round core (dark purple) there was no nucleolus and cytoplasm is almost invisible. Examination of macrophages was carried out by the NBT reduction method based on the number of latex particles which were successfully phagocytosed by every 100 cells of macrophages. The latex particles that are calculated are particles that are in the cytoplasm. While reactive oxygen intennediate (ROI) secretion is a blue formulation (ROI) which is expressed in the cytoplasm of macrophages which is in phagocytic activity. Macrophages that express blue formations are counted in every 100 cells of macrophages.

Macrophages are stimulated with PMA, which secretes superoxid ($O2^-$) anions which will oxidize NBT. Macrophage suspension (PEC) at microplate 24 wells that had been given a round coverslip, were incubated inincubator CO_2 5%

at 37˚C for 30 minutes, then added 1 ml/well medium complete and incubated for 2 hours. Then add 500 µL of NBT solution containing 125 ng/ml of PMA. Cells were washed with PBS 3 times, and then dried at room temperature. Fixed with absolute methanol for 2 - 3 minutes, and stained with Giemsa [22] [23].

Data were analyzed with SPSS-20 program using Shapiro Wilk evaluation to determine the normality of the data as a requirement of One Way Anova then continued with LSD.

Ethical Clearance

The methods used in this study were approved by the Ethical Commission of the Integrated Research and Evaluation Laboratory of Gadjah Mada University, No. 00051/04/LPPT/IV/2017.

3. Results and Discussion

The results of GC-MS analysis showed that leaf eextract of *S. aromaticum* var. siputik (**Figure 1**) consisted of 4 compounds: peak 1) 80.15% eugenol ($C_{10}H_{12}O_2$) with molecular weight 164, peak 2) 13.44% β-caryophyllene ($C_{15}H_{24}$) with molecular weight 204, peak 3) 1.50% α-humulene ($C_{15}H_{24}$) with molecular weight of 204, and peak 4) 4.90% caryophyllene oxide ($C_{15}H_{24}$) with molecular weight 220.

Eugenol compound is an essential oil component derived from phenylpropene [24]. This compound is known for its pharmacological properties, such as analgesics, local anesthetics, anti-inflammatory, antimicrobial, antioxidant, and antitumor [25]. Prior research using leaf clove extract from India with GC-MS method of acetone solvent showed that the extract consisted of 80.19% eugenol compound, 7.91% eugenyl acetate, 3.79% caryophyllene, 2.26% tetrahydro-3-methyl, and 1.54% methylhidrazone [25]. Clove compound tested in different countries used gas chromatography showed different content of compound. In Madagascar, clove leaf extract was found consisted of 91.81% - 96.65% eugenol, 1.66% - 4.48% β-caryophyllene, 0.22% - 0.79% α-humulene, 0.37% - 2.53% eugenyl acetate, 0.14% - 0.6% caryophyllene oxide. In Zanzibar, clove leaf extract was found consisted of 87.52% - 89.47% eugenol, 7.19% - 9.70% β-caryophyllene, 0.75% - 1.08% α-humulene, 0.55% - 0.88% eugenyl acetate, and 0.25% - 0.68% caryophyllene oxide [1].

3.1. Weight of Spleens

After clove leaf extract administrated in the treatment group, mice were dissected to determine the weight of the spleen as an indicator of increased of proliferation. Data from spleen weight analysis shown in **Table 1**.

The results showed that mean value of spleen weight of treatment group was higher than the control. There was a significant difference ($p < 0.001$) between treatment and control group at doses of 15, 75, and 150 mg/kgbw. Cellular immune response will be activated to eliminate the infections by intra-cellular bacteria such as *S. typhimurium*, including the presence of a proliferative

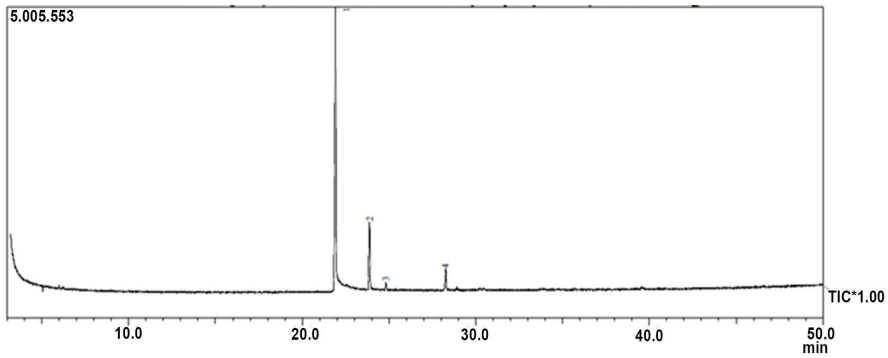

Figure 1. Histogram of *S. aromaticum* var. siputik leaf extract (Peak 1: eugenol, peak 2: *β*-caryophyllene, peak 3: *α*-humulene, peak 4: caryophyllene oxide).

Table 1. Spleen weight and LSD test.

Spleen weight	Dose	Average ± st deviation
	Control	0.126 ± 0.015
	15 mg	0.556 ± 0.049
	75 mg	0.516 ± 0.015
	150 mg	0.510 ± 0.015
LSD	Dose	Sig.
Control	15 mg	0.000*
	75 mg	0.000*
	150 mg	0.000*

*Significant.

response, and lymphocyte activation [26]. Microscopic proliferation and lymphocyte response is seen by the difference in the addition of large and heavy size of the spleen [22] [23]. In this study mice infected with *S. typhimurium* then adminstrated with clove leaf extract for 12 days and there was an increase in spleen compared to mice that were not administrate of clove leaf extract (control group).

Clove leaf extract with eugenol content could increase lymphocyte proliferation. Eugenol was a major compound in cloves that could modulate the immune response including anti-inflammatory effects [27]. Each immune system has effector cells that have the ability to lyse cells infected by pathogens or activate other cells in the immune system [28]. In natural immunity, effector cells are played by neutrophil leukocytes, macrophages, dendritics and NK cells [29]. Leukocytes and neutrophils could lyse microbes, in the neutrophil cytoplasm there are specific granules [16]. Macrophage cells could do phagocytosis efficiently, whereas NK cells have cytotoxic activity against cells infected with viruses and tumor cells. Dendritic cells are involved in the natural immune response because they could phagocytosis against microorganisms. Moreover, dendritic cells could combine the activity of natural and adaptive immune response [30].

3.2. Lymphocytes

Lymphocytes are mediators of the adaptive immune response [31]. Lymphocytes are divided into B lymphocytes and T lymphocytes. B lymphocytes are produced and undergo maturation in the bone marrow, and originate from multipotent stem cells. B lymphocytes function in producing antibodies and have a surface receptor (Fcy-R) from IgG, if stimulated by an antigen, they will experience proliferation and differentiation that develops into plasma then produces intracellular antibodies [16]. Activation of B lymphocytes is initiated by the occurrence of complex bonds between specific antigens with receptor complexes consisting of molecular immunoglobulin Igα membranes, Igβ, and supporting receptors (CD19, CD21, CD18) [9]. T lymphocytes derived from bone marrow then undergo maturation in the thymus. T lymphocytes have $\alpha\beta$ hetrodimer receptors. When exposed to antigens bound to MHC molecules, T lymphocytes will be presented by APC cells or stimulated by specific cytokines, then differentiate into $TCD4^+$ and $TCD8^+$ lymphocyte subsets, each has a different effector function [32]. Lymphocyte were calculated from spleen fluid which is crushed until smooth and then observed under a microscope. Lymphocyte cells were calculated using a counting chamber with smaller and dark purple characteristics [23]. Number of lymphocyte is shown in **Table 2**.

Statistical analysis showed that there are significant differences between control and treatment group with dose 150 mg/kgbw, while there is no significant difference between 15 mg and 75 mg doses. This explains that the administration of clove leaf extract at dose of 150 mg/kgbw increased the number of lymphocytes better than 15 mg and 75 mg/kgbw doses. This is because 150 mg/kgbw dose of clove leaf extract contains more eugenol compound that act as immunomodulators in increasing lymphocyte cell proliferation through IL-2 production [33]. IL-2 has a role in activating T lymphocytes to proliferate. Proliferation of T lymphocytes is stimulated by an antigen that is regulated by the bond between IL-2 and its receptor [23]. In addition, lymphocyte proliferation will affect $CD4^+$ cells, then cause Th1 cells to be activated. Th1 cells that are activated will affect the Specific Macrophage Activating Factor which could activate macrophages [31].

Table 2. Number of lymphocyte and LSD test.

Number of Lymphocyte	Dose	Average ± st deviation
	Control	101.61 ± 8.853
	15 mg	109.67 ± 9.292
	75 mg	112.00 ± 2.00
	150 mg	142.33 ± 20.404
LSD	Dose	Sig.
Control	15 mg	0.833
	75 mg	0.21
	150 mg	0.022*

*Significant.

Adaptive immune response is a form of immunity mediated by lymphocyte mediators as the main driving motor after stimulation from antigen exposure as an infectious agent [16]. Adaptive and natural immune responses support each other to improve the effectiveness of responses to foreign antigens. In adaptive responses where antigens that stimulate T cells will also activate macrophages, inorder to doing phagocytic activity efficiently [11]. Activities that occur in the adaptive immune response could indirectly affect the natural immune response [8].

3.3. Lymphoblasts

Calculation of lymphoblasts from crushed spleen fluid was then observed under a microscope and lymphoblasts were calculated using a counting chamber with features of larger and light purple cells. Lymphoblast exposure shown in **Table 3**.

Statistical analysis showed that there are significant differences between control and treatment group with dose 150 mg/kgbw. The microscopic analysis result showed that lymphocytes and lymphoblasts present as round cell shape and appear clustered with small cores. Lymphocytes and lymphoblasts were calculated using a hemocytometer with 400x magnification. Existing lymphocytes were thought to be a mixture of T cells and B cells. Visually, it is difficult to distinguish between T cells and B cells because the similarity of the morphology of the two cells and is present in the blood circulation and through the body tissues [34]. Lymphocytes appeared as small dark purple cells, while lymphoblasts appeared as larger light purple celss [22]. Differences in lymphocytes and lymphoblast cells shown in **Figure 2**.

Administrate of clove leaf extract for 12 days in mice infected with *S. typhimurium* in this study increased the number of lymphocytes at 150 mg/kgbw dose, while lymphoblasts have not been proven significantly. This is due to the stimulation of clove leaf extract compounds such as eugenol which was the main effector molecule in the modulator of increasing lomfocytes [21]. Eugenol was a major component of the role of immunomodulators and anti-inflammatory agents [19].

3.4. Macrophages

Macrophages are belong to mononuclear phagocytic system group. The mononucleate phagocytic system consisted of cells from the same bloodline and has the ability of phagocytic activity. Mononuclear phagocyte cells had an important role in natural and adaptive immune responses [8]. These cells were made in the bone marrow and then migrated into the blood circulation as monocytes, then mature and become active after existed in the tissues as macrophages. Macrophages are one of 3 types of phagocyte cells in the immune system that are widely distributed in tissues and have an important role in the natural immune responses [27]. Macrophages play a role both in natural and adaptive immune responses, in the natural immune response clearing pathogens

Table 3. Number of lymphoblasts and LSD tes.

Number of lymphoblasts	Dose	Mean ± deviation
	Control	102.11 ± 10.012
	15 mg	117.33 ± 11.015
	75 mg	123.33 ± 10.408
	150 mg	150.00 ± 13.229
LSD	Dose	Sig.
Control	15 mg	0.31
	75 mg	0.550
	150 mg	0.017*

*Significant.

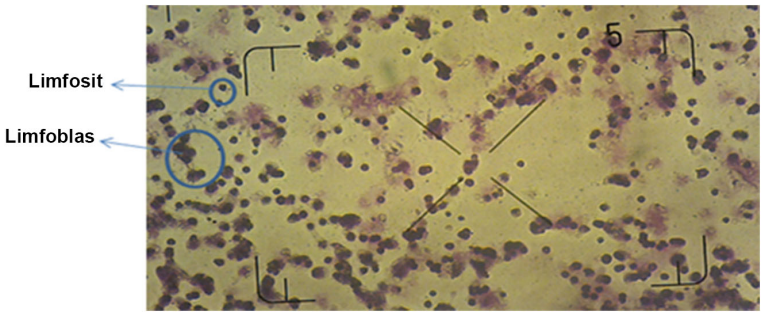

Figure 2. Lymphocytes and lymphoblasts (400× magnification).

through phagocytosis, and in the adaptive immune response acts as APC (antigen presenting cells) which is presenting antigens to T lymphocytes resulting in stimulation of T cells [30]. The introduction of foreign antigens by T lymphocytes was a very important initial process for a series of activities in the adaptive immune response [32]. Examination of macrophages in this study was carried out by reducing NBT. Macrophages are stimulated with PMA which secrete superoxide ($O2^{-}$) anions which will oxidize NBT. The number of macrophages shown in **Table 4**.

The average number of latex particles that can be phagocytosis by every 100 macrophages showed that the macrophage activity of treatment group (treated with clove leaf extract) was stronger than that the control group (without extract treatment). In the control group, the average latex which can be phagocytosis by every 100 macrophages was 4.21, whereas in the treatment of clove leaf extract it was higher at dose 150 mg/kgbw (17.00), followed by dose 75 mg/kgbw (9.80), dose 15 mg/kgbw (5.20). Phagocytic activity in the treatment of clove leaf extract showed gradual increase in phagostosis activity at dose 150 mg /kgbw.

Statistical analysis result showed that there is significant in between treatment with dose 150 mg/kgbw, while there are no significant differences in dose 15 and 75 mg/kgbw. This showed that the administration of clove leaf extract for 12 days at dose 150 mg/kgbw in mice infected with *S. typhimurium* increased the number of macrophages better than 15 and 75 mg/kgbw doses. *S. typhimurium*

Table 4. Number of macrophages and LSD test.

Number of macrophages	Dose	Average ± st deviation
	Control	4.21 ± 0.24
	15 mg	5.20 ± 0.44
	75 mg	9.80 ± 1.78
	150 mg	17.00 ± 3.00
LSD	Dose	Sig.
Control	15 mg	0.120
	75 mg	0.072
	150 mg	0.017*

*Significant.

infection in mice in the control and treatment groups was given together with a dose of 0.2 ml/mice before being administrate with clove leaf extract. ROI production of macrophages was strongly influenced by microbial infections because the secretory products are stimulated by pathogens and are useful as ammunition for the destruction of pathogens [32]. Macrophage and phagocytosis ROI secretion was the main defense of macrophages in the immune response against bacterial infections [19].

The mechanism of the macrophage immune response to bacterial infection begins with signal transduction from bacterial proteoglycoulds through the TLR-2 membrane receptor and the protein adapter MyD 88, then recruitment of several protein adapters, kinases protein, activation of gene transcription factors, and gene expression [10]. Basal level of macrophage phagocytosis against bacterial infection was very dependent on the activation of Rac-1 enzyme and the posphatidylinositol-3 kinase enzyme (PI3K). Rac-1 and PI3K kinase enzymes were stimulated by signals derived from bacterial proteoglycould components that were transduced through TLR-2 and MyD-88 receptors, and activated the enzymes [8].

In this study, macrophage ROI secretion in the treatment group with 150 mg/kgbw dose of clove extract showed better secretion compared to 75 mg/kgbw dose. This showed that the number of doses of clove leaf extract was very influential on the addition of the number of macrophages. The dose concentration was strongly related to the active compounds contained in clove leaf extract in increasing macrophage activity. Macrophages are one of the cells that play an important role in the immune response, both in phagocytosis and as antigen presenting cells (APC). Macrophages as phagocytic cells have two defense mechanisms that is oxidative and non-oxidative processes [33]. Macrophages are also able to secrete IL-12 which helps differentiate $CD4^+$ T cells into Th1. Th1 cells and NK cells will secrete IFN γ as activating factors for macrophages while increasing MHC II expression on the APC surface [13]. Th2 plays a role in humoral immunity through antibodies in the opsonization process [6]. Macrophages stimulated by external bacteria will became active to formed epi-

thelial-like cells called epitheloid, and can diffuse to form large cells with many nucleid [27]. Macrophages that are actively conducting phagocytic activity morphologically are characterized by relatively larger sizes, clustered with plasma membrane extensions. Macrophage cells are shown in **Figure 3**.

The results of observations on the ROI secretion activity of macrophages in a number of macrophages that showed blue NBT reduction formations calculated from every 100 macrophages showed that the treatment of clove leaf extract increased the ROI secretion activity of macrophages. Mice macrophages in the group treated with clove leaf extract showed an increase in ROI secretion activity at dose 150 mg/kgbw ($p < 0.05$) while at doses of 15 and 75 mg/kgbw there was a decrease.

The role of macrophages in the immune system was very large. Macrophages that are in the central nervous system are called microglia, in liver sinusoids called kuffer cells, in pulmo called alveolar macrophages, in bone tissue called osteoclasts. Macrophages after being activated by microbes will produce cytokines which could induce an inflammatory reaction to microbes [19]. Cytokines secreted by macrophages include TNF, IL-1, IL-12 and chemokines. Activation of macrophages begin with signal stimulation through cell membrane receptors [26]. Macrophages recognized pathogens through membrane receptors that are able to distinguish between antigens expressed by microbes and body cells. Macrophage receptors on membranes that could detect microorganisms is called manose receptors. The function of the receptor is to recognize several components of bacteria, including carbohydrate bacterial. Phagocytic activity sequentially begins with cell migration to the site of infection, then detection of microbes and eventually phagocytosis to microbes lysis [35].

Cloves with eugenol compounds can increase the proliferation and production of macrophages, and act as a modulator of lymphocyte proliferation. Increased activity of mice macrophages given by the clove leaf extract is due to stimulation of the substance in the compound extract. Clove leaf extract compounds activates macrophage tyrosine which is transduced via SRPβ [30]. Active tyrosine induces phosphorylation of DAP 12, syk and SLP-76 so that the MEK-MAPK-myosin kinase is accelerated and cascade MEK-MAPK-myosin occurs. This cascade has a very important role in the mechanism of phagocytosis

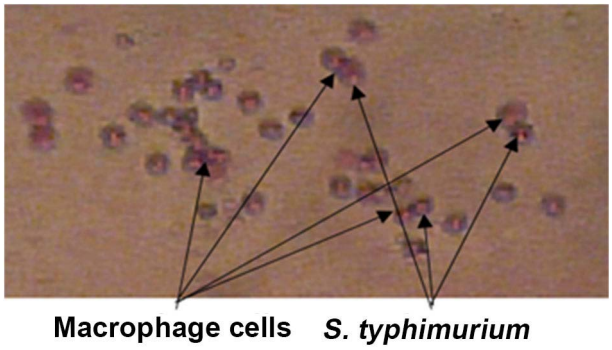

Figure 3. Cells of macrophages.

[33]. Research on the production of Th1/Th2 cytokines in mice administrate clove flower extract reported that there was an increase in the production of Th1 cytokines (IFNγ and IL-2) and Th2 (IL-4 and IL-10) [27]. The active compound of clove leaf extract can act as an immunomodulator against Th1 for synthesis of IFN-γ [33].

The results of prior studies on the administration of clove essential oil for 35 days in *Oreochromis niloticus* were proved to be beneficial in improving the physiological status, through increased lysozyme activity, immunoglobulins, and blood serum proteins [32]. Furthermore, addition of clove powder could improve the immune status of quail [26] and increased primary and secondary immune responses [30]. The main clove compounds, eugenol, could change the structure of DNA through the formation of eugenol-DNA chimera which is known to inhibit *Listeria monocytogenes* and the main enzyme activity (isocitrate dehydrogenase, citric synthase and α-ketoglutarate dehydrogenase) in the TCA pathway to improve respiratory metabolism [19]. This study proves that the phagocytic activity of macrophages on latex particles shows increased activity in line with the variation in the increase in dosage given. Secretion of ROI and phagocytosis are the main strengths of macrophages in the immune response to *S. typhimurium* infection. Macrophages with active phagosity activity are characterized by relatively larger sizes, clustered with plasma membrane extensions (**Figure 2**). The content of clove compounds such as Eugenol, β-caryophyllene and eugenol acetate can act as cell growth factors, increase lymphocyte proliferation when immune response occurs and increase the production of macrophage ROI which is a major factor against extracellular bacteria.

Clove leaf extract containing eugenol, β-caryophyllene, caryophyllene oxide and α-humulene can increase the proliferation of activity of lymphocytes, lymphoblasts and ROI secretion of macrophages. Infected mice will increase IFN-γ levels because there are immunogens that activate the immune system [8]. Immunogen is displayed by antigen precenting cell including macrophages that interact with T lymphocyte cells. T cell interactions will produce IFN-γ cytokines which will then activate macrophages [32]. This mechanism occurs in all experimental groups so that T lymphocytes in all groups undergo proliferation and produce IFN-γ. The proliferation of lymphocyte cells causes the percentage of lymphoblasts in the spleen to also increase.

4. Conclusion

Administration of clove leaf extract for 12 days in Balb/c mice infected with *S. typhimurium*, at dose 15, 75 and 150 mg/kgbw increased the weight of the spleen as an indication of increased proliferation, and significantly increase the number of lymphocytes, lymphoblasts and ROI secretion of macrophage in dose 150 mg/kgbw.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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