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Effect of *Tinospora cordifolia* Extract on Neutrophils, TNFα, and IFNγ Percentage in Balb/c Mice Infected with *Salmonella typhimurium*

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Abstract: Herbal medicine is still consumed by 80% populations in developing countries for primary health care. *Tinospora cordifolia* extracts are widely used as a system of traditional medicine for the treatment. *T. cordifolia* is a plant species that has a function as a natural immunomodulation. It increases the activity and function some components of nonspecific and specific immunity. The aim of the research is to know the effects of *T. cordifolia* extract on the immunity system and the regulation of neutrophil, TNFα, and IFNγ percentage. This study was conducted in Microbiological and Biomedical Laboratories, Faculty of Medicine, University of Brawijaya. There was 5 treatment groups namely the negative control group (C-), the positive control group mice (C+), and the mice group were injected with *S. typhimurium* and treated with 0.225(D1), 0.375(D2), 0.75(D3) mg/day *T. cordifolia* extract. The result showed that the highest average of neutrophil was in D3 treatment, and the lowest average was in D1 treatment. The highest average of TNFα and IFNγ were in C(+) treatment and the lowest average were in C(-) treatment. The levels of TNFα decreased in proportion to increase the dose administered on rats treated with *T. cordifolia* extract. The increasing doses of therapy were not accompanied by elevated levels of IFNγ. The conclusions are **t**he percentage of neutrophil in the treatment group was higher than the control group, the percentage of IFNγ is increasing of the treatment group than the control group, and there is increasing of the treatment group than the control group.
Keywords: IFNγ, neutrophil, *Salmonella typhimurium*, *Tinospora cordifolia* extract, TNFα.

Introduction

Several efforts have been done by the human body to protect against the infection of foreign objects. Naturally, a human has good immunity for the defense system. Many types of pathogens can cause the infection such as bacteria, viruses, fungi, protozoa and parasites. In developing countries, one of emerging disease is typhoid fever. The use of antibiotics can reduce the incidence of complications. Although the incidence is relapsed typhoid fever and it resistant the antibiotics increasingly in some endemic countries. The severity of infection in typhoid fever is determined by the relationship between host and microbes. *Salmonella typhimurium* caused typhoid fever as a germ stem move, gram negative and facultative intracellular. According to WHO, antibiotic has been used by several developing countries widely as an optimal treatment which can be absorbed well by human body[1-3].

Research has shown that several medicinal plants inhibit the growth of bacterial pathogens. One of them is *Tinospora cordifolia.* It is a plant species that has a function as a natural immunomodulation. It grows

well in tropical areas especially Indonesia. This study shows that the extract of *T. cordifolia* inhibits have grown the bacteria significantly. In addition, it has medicinal value to improve its application. The use of antibiotics has side effects for human, so it is necessary to find out the new antimicrobial substance. Medicinal plants are good alternative of chemical antibiotics[4].

In this study, infection of *S. typhimurium* is used as a model of intracellular infection that spurs immunity. The infection of *S. typhimurium* is caused by the growth of bacteria, such as macrophages that produce cytokines. So, the innate and adaptive immunity of cellular systems become active. The extract of *T. cordifolia* can increase the activity and function some components of nonspecific and specific immunity. The effects of nonspecific immunity response are increasing of phagocytosis and chemotaxis macrophages, neutrophil, and NK cell. Cellular immunities increase proliferation of T lymphocytes by increasing the secretion of TNFα, IFNγ, and IL-4, and decrease the secretion of IL-2 and IL-10[5].

The study of *T. cordifolia* is aimed particularly at preventing and treating infected diseases. It is in line with the previous researchers who seek the advantages *T. cordifolia. S. typhimurium* is a companion for increasing cellular immunity[6-8]. The aim of the research is to know the effects of *T. cordifolia* extract on the immunity system and the regulation of neutrophils, TNFα, and IFNγ percentage.

Materials and Methods

This study was conducted over three months in 2015 at Microbiological and Biomedical Laboratories, Faculty of Medicine, University of Brawijaya. This study was an experimental research uses the post-test, only the control group uses experimental animals as the object of research. Measurement variable parameters are used to measure neutrophils, TNFα, and IFNγ percentage. This research used male mice strain Balb/c were 6-8 weeks old, body weight 20-30 grams, healthy, no anatomical abnormalities, and had undergone adaptation for a week. There were 5 treatment groups and each group consisted of 7 animals. First, the negative control group mice were not injected with *S. typhimurium* and were not treated with *T. cordifolia* extract (C-). Second, the positive control group mice were injected with *S. typhoidrium* for one day (C+). Third, mice group were injected with *S. typhimurium* and treated with 0,225 mg /day *T. cordifolia* extract (D1). Fourth, mice group were injected with *S. typhimurium* and treated with 0,375 mg/day *T. cordifolia* extract (D2). Fifth, mice group were injected with *S. typhimurium* and treated with 0.75 mg / day *T. cordifolia* extract (D3).

There are several operational definitions of the research variables as follows:

1. *T. cordifolia* extract is a solution of water extract from *T. cordifolia* given to the treatment group Balb/c mice (infected with *S. typhimurium*) with1 x doses of 0.225 mg / each day and 1 x 0.375 mg/each day, and 1 x 0. 750 mg / each day.
2. The content of TNFα and IFN γ in the blood serum of mice. Blood sampling was done by intravenously in mice. Venous blood was centrifuged. Serum was taken and read in Enzyme Linked Immuno-Sorbent Assay (Elisa) Reader.
3. The neutrophil percentage was the percentage of neutrophil within the number leukocytes in the blood of Balb/c mice (infected with *S. typhimurium*) calculated on blood clots with Giemsa staining using light microscopy in clinical laboratories. The scale used is a numerical scale of neutrophil percentage.

Data analysis was performed in two stages that include Univariate analysis and Bivariate analysis. The hypothesis test was conducted using Analysis of Variance (ANOVA or Kruskal-Wallis) with α 0.05. If ANOVA test was significant, it can be continued with Duncan Multi Range Test (DMRT)[9-11].

Result and Discussion

The effect of *T. cordifolia* on Neutrophil

The immune system became active when *S. typhimurium* is injected into the mice. Neutrophils play an important role dealing with bacterial infections, especially as cell migration and phagocytosis[12]. Neutrophils are the first immune cells taken from the blood stream leading to the inflammation area. Mechanism of bacterial

elimination process begins with neutrophils contact. The bacteria are engulfed by neutrophils into the phagocytic vacuole called the phagosome. Intercellular granules will join the phagosome then issue to form phagolysosome[13].

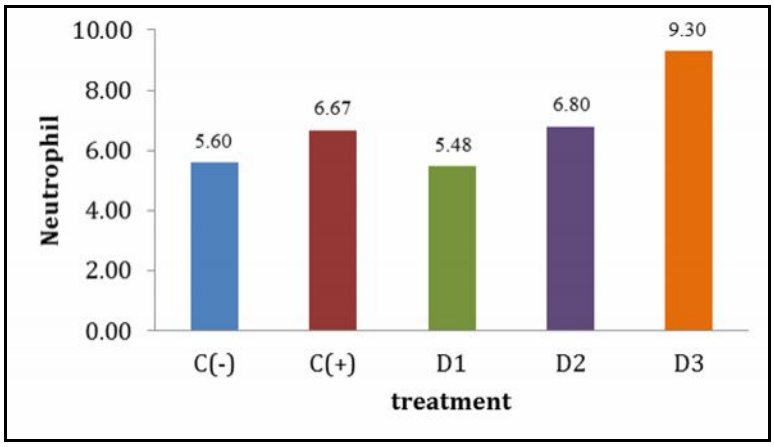

Figure 1. Average of Neutrophil; C (-): control group with normal saline, C (+): S.typhimurium only, D1: S. typhimurium with 0.0225 mg)/day T. cordifolia extract, D2: S.typhimurium with 0.375 mg/day T. cordifolia extract, and D3: S. typhimurium with 0.75 mg/day T. cordifolia extract.

Based on Figure 1 showed that the average of neutrophil was different each other for each treatment. The adding dose proportional was increasing the number of neutrophil in the blood. The increasing of the number or percentage Neutrophils showed the increasing activity of neutrophil. The highest average of Neutrophil was in D3 treatment at 9.30, and the lowest average was in D1 treatment at 5.48. The increasing of the number or percentage neutrophil showed the increasing activity of neutrophil. In the control group C(+) increased the number of neutrophil by an average of 19.29% compared to the healthy group. This data explained that it was occurred immune response by neutrophil that was caused by bacterial LPS injection. The increasing the average number of neutrophil was found in the treatment group (D2 and D3) respectively by 21.43% and 66.07%. This value indicated that the active component in water extract of *T. cordifolia*, namely (1, 4) -α-D-glucan that was given stimulate a signal to activate the immune response of neutrophil. This study showed that *T. cordifolia* was able as immune stimulator optimal therapeutic dose range 0.375-0.75 mg / day.

The effect of *T. cordifolia* on TNFα

The role of *TNF-alpha* in the mice that injected with bacterial LPS, it suppresses *S. typhirium* infection and increase the contact hypersensitivity response[14]. TNFα has a role in infection and inflammation caused by bacterial invasion. It is as active and transmembrane protein molecule from the stimulus response of macrophages[15].

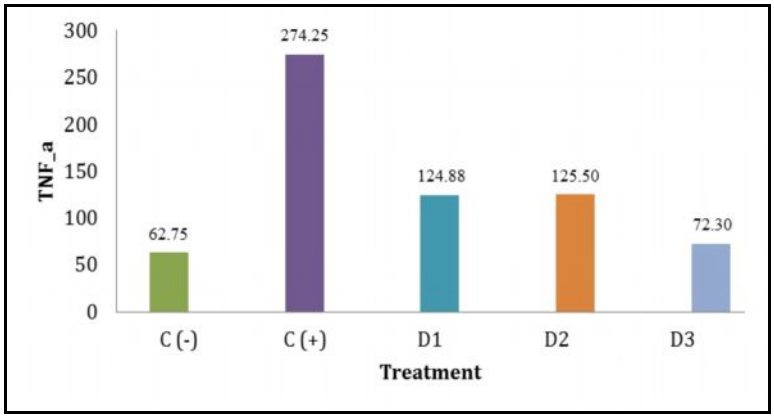

Figure 2. Average of TNFα; C (-): control group with normal saline, C (+): S. typhimurium only, D1: S. typhimurium with 0.0225 mg)/day T. cordifolia extract, D2: S. typhimurium with 0.375 mg/day T. cordifolia extract, and D3: S. typhimurium with 0.75 mg/day T. cordifolia extract.

Based on Figure 2 showed that the average of TNFα for each treatment was different each other. The highest average of TNFα was in C(+) treatment at 274.25 and the lowest average was in C(-) treatment at 62.75. The increasing TNFα in the control group of mice caused by TNFα receptor activity against the toxic effects of bacterial LPS macrophages through the receptor complex involves the presence of TLR4 / Md 2 / CD14[16]. In addition, TNFα increases cell resistance to apoptosis by modulating the expression of some proteins[17]. Therefore, the increasing levels of TNFα significantly by 77.12% of the normal mice.

The levels of TNFα decreased in proportion to increase the dose administered to rats treated with *T. cordifolia* extract. The decreasing average levels of TNFα therapy mice respectively at the dose of 0225; 0.375 and 0.750 mg /day compared to the positive control were 54.4%; 54.24% and 73.64%. The results showed that the flavonoids and alkaloids contained in the water *T. cordifolia* extract have the potential to inhibit ROS through activation of the transcription factor NF-kB. It binds to the promoter of the gene kB on the side and will do a series of transcription to express inflammatory proteins, including IL-6, TNFα, and IL-1[18]. The higher antioxidant activity will be followed by a decrease in ROS. It reduced activation of NF-kB followed by the decreasing TNFα. In this study, the decreasing levels of TNFα in the treatment of mice with the optimal dose of 0.75 mg/day, with an average yield of TNFα levels approach normal mice.

Results of the study regarding the potential activity of *T. cordifolia* extract stimulate immune respone on Balb/c mice that injected with LPS. At a dose of 125ug/mL, *T. cordifolia* extract increases the levels of IL-6 is more significant than the dose of 200ug/mL[19]. The increasing is more influence on the dose of antioxidant activity than the increasing levels of pro-inflammatory cytokines. Therefore, it is necessary to conduct further research on the therapeutic use of *T. cordifolia* with a combination of other herbal ingredients or substances that can enhance stimulation of pro-inflammatory cytokines.

The effect of *T. cordifolia* on IFNγ

Cellular immunity is a continuation of natural immunity. Bacterial infection induces NK cells to produce IFNγ. It plays an important role in vivo in enhancing the initial response effectors in the immune response system and it increases the ability of macrophages to act as antigen presenting cells. In addition, it enhances the ability of macrophage phagocytosis and induces transcription the gene by encoding the enzyme that forms reactive oxygen in a radical generating system[20].

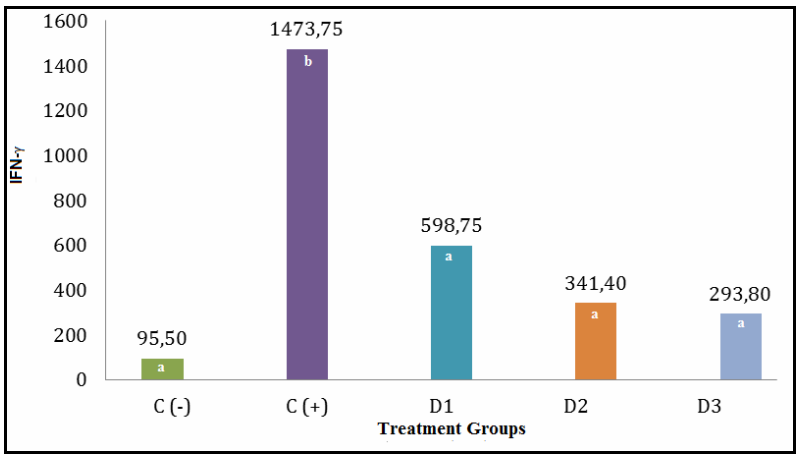

Figure 3. Average of IFN gamma; C (-): control group with normal saline, C (+): S. typhimurium only, D1: S. typhimurium with 0.0225 mg)/day T. cordifolia extract, D2: S. typhimurium with 0.375 mg/day T. cordifolia extract, and D3: S. typhimurium with 0.75 mg/day T. cordifolia extract.

Based on Figure 3 showed that the average of IFNγ was different each other. The highest average of IFNγ was in C(+) treatment at 437.75 and the lowest average was in C(-) treatment at 95.50. The increasing doses of therapy were not accompanied by elevated levels of IFNγ. Decline in average levels of IFNγ on mice respectively at the dose of 0225; 0.375 and 0.750 mg / day compared to the positive control mice amounted to 59.37%; 76.83% and 80.01%. It was possible to the stimulation of the T cell competition from *T. cordifolia* by conducting an immune response against bacterial invasion. The increasing activity by *T. cordifolia* will lead the

reducing levels of IFNγ thus the stimulating bacteria were not able to stimulate Th1 response compared Th2. This response means the immune response took place in the extracellular than intracellular infection.

In this study, the results of IFNγ analysis was similar results obtained with TNFα. Treatment in mice using *T. cordifolia* extract that increases the immune response. The development of bacterial invasion will increase the levels of IFNγ in inflammatory cells. Furthermore, *T. cordifolia* extract on therapy of inflammatory cells will activate the immune system as indicated by increased levels of IFN-γ. The higher levels of IFNγ increase the numbers of neutrophils that change the form into inflammatory cells. *T. cordifolia* will activate T cells, and T cells would undergo cell polarization Th1 that will be accompanied by the release of cytokines IFN-gamma and IL-2. Proinflammatory cytokines can be instrumental in stimulating macrophage phagocytosis and activities in the process of bacteria elimination. Besides, T cells also activated other immune cells like B cells to produce antibodies against extracellular bacteria.

The result in this study was similar to the previous study that regarding the potential *T. cordifolia* and immune activity in high-dose cisplatin administration in Balb/c mice were injected with fewer bacteria *L. donovani*[21]. *T. cordifolia* therapy with 100mg/kg dose stimulates a Th2 response. It was higher than Th1, otherwise the mixture *T. cordifolia* and cisplatin therapy selective Th1 response was higher than Th2. The increasing Th1 response is followed by the levels of TNF-gamma and IL-2 high although Th2 enhancement will increase the levels of IL-4 and IL-10. This meant that it took a combination therapy using a drug or other ions, as well determination of optimal therapeutic dose to stimulate an immune response synergy each other.

Conclusion

According to the result, it can be concluded that the use of *T. cordifolia* extract has a role in therapeutic. The percentage of neutrophil in the treatment group is higher than the control group. The percentage of IFN gamma does not increase, although there is increasing of the treatment group better than the control group. There is increasing of the treatment group better than the control group. Since the decreasing levels of TNF-alpha in the treatment of mice has an optimal dose of 0.75 mg/day.

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