Jurnal Biosains Pascasarjana Vol. 26 (2024)

EFFECT OF *Clitoria ternatea* INFUSION ON THE NEUTROPHIL CELLS AND LYMPHOCYTE CELLS IN A MODEL OF *Salmonella typhimurium INFECTION*

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Abstrak

Penyakit Salmonelosis disebabkan oleh infeksi bakteri Salmonella sp. yang menyebabkan terjadinya disregulasi imunitas dan kerusakan pada sel epitel pencernaan. Sehingga perlu adanya peningkatan imunitas untuk melawan bakteri Salmonella sp. Tujuan penelitian ini adalah untuk mengetahui pengaruh infusa bunga telang terhadap jumlah sel Nuetrofil dan sel Lomfosit terhadap infeksi Salmonella typhimurium. Penelitian dibagi menjadi 6 kelompok, negatif, positif (normal saline), Stimuno (0,26ml/20gBB), terapi 1 (0,25 ml/20gBB), terapi 2 (0,5 ml/20gBB), terapi 3 (1 ml/20gBB). Pembuatan infusa, Infeksi Salmonella typhimurium, terapi infusa, pewarnaan giemsa, dan analisa data dengan p-value 95%. Berdasarkan hasil penelitian yang telah dilakukan bahwa terjadi perbedaan jumlah sel neutrofil antara kelompok negatif terhadap kelompok positif (0,00<0,05), stimuno terhadap kelompok positif (0,02<0,05), terapi 1 terhadap stimuno (0,62>0,05), terapi 2 terhadap stimuno (0,04<0,05), dan terapi 3 terhadap stimuno (0,04<0,05). Sedangkan jumlah sel limfosit pada uji anova berbeda signifikan (0,00<0,05), kemudian dilanjutkan dengan uji tuckey bahwa terjadi perbedaan jumlah limfosit kelompok negatif terhadap kelompok positif (0,40>0,05), kelompok positif terhadap stimuno (0,95>0,05), stimuno terhadap terapi 1 (0,97>0,05), stimuno terhadpa terapi 2 (0,14>0,05), dan stimuno terhadap terapi 3 (0,00<0,05). Kesimpulan dari penelitian ini bahwa infusa bunga telang dari dosis rendah sampai tinggi berpengaruh terhadap peningkatan jumlah sel neutrofil dan sel limfosit.

***Kata Kunci**: Infusa bunga Telang, limfosit, neutrofil.*

Abstract

Salmonellosis is caused by infection with the bacterium Salmonella sp. which causes immune dysregulation and damage to digestive epithelial cells. So there is a need to increase immunity to fight Salmonella sp. The purpose of this study is to determine the effect of butterfly pea flower infusion on the number of Nuetrophophil cells and Lomfosyte cells on Salmonella typhimurium infection. The study was divided into 6 groups, negative, positive, Stimuno (0.26ml/20gBB), therapy 1 (0.25 ml/20gBB), therapy 2 (0.5 ml/20gBB), therapy 3 (1 ml/20gBB). Infusion making, Bacterial infection, infusa therapy, Giemsa staining, and 95% p-value data analysis. The results showed that there was a significant difference in the number of neutrophil cells in each group (p<0.05). Meanwhile, the number of lymphocytes after the anova test showed a significant increase in the number of lymphocytes (0.00<0.05). The conclusion of this study is that the infusion of butterfly pea flowers (*Telang*) from low to high doses has an effect on increasing the number of neutrophil cells and lymphocyte cells.

***Keywords**: Infusion of Butterfly Pea flower, lymphocytes, neutrophils.*

1. INTRODUCTION

Salmonellosis is a disease caused by infection *Salmonella sp.* serovar *enterica*. *Salmonella typhimurium infection* causes clinical symptoms of fever, loss of appetite, diarrhea, and bacteriosis (Khan & Shamim, 2022). *Salmonella typhimurium* is also resistant to antibiotics (Jahan et al., 2022). *Salmonella typhimurium* is a model

bacterium to determine the biological mechanism of *Salmonella infection*.

Salmonella typhimurium has three types of secretion to invade interocyte cells, among them T3SS1 encoded by SPI1, Resistance to complement killing (Rck) and PhoP activated gene N (PagN). SPI1 directly binds to cells affecting the stimulation of the GTPase signaling pathway, inducing massive actin polymerization, and membrane remodeling that facilitates bacteria to enter the cell. In addition, Rck and PagN proteins bind to EGFR and proteoglycans/β1 integrins thereby influencing the activation of the GTPase signaling pathway, actin polymerization, and membrane remodeling (Ménard et al., 2022).

Salmonella located inside epithelial cells will express *Salmonella* pathogenicity Island 2 (SPI-2) which role as virulence factors. SPI2 effector SseI (also called srfH) binds to an IQ motif containing GTPase activating protein 1 (IQGAP1) which role in systemic infections (bacterimia) (Li, 2022).

Salmonella typhimurium *bacteria* are recognized by immunity receptors that are derived from TLR4, TLR5, NOD1, and NOD2. TLR4 recognizes LPS, TLR5 recognizes bacterial flagella, NOD1 recognizes peptides containing diaminopimelic acid, NOD2 recognizes muramyl dipeptides found in the peptidoglycan layer (Patel & McCormick, 2014). The introduction will induce immunity to the intestine, which consists of neutrophils, macrophages, cell dendritics, and lymphocytes (F. Huang, 2021).

The role *Salmonella* is lowering or inhibiting the innate immune response, making it easier for salmonella bacteria to continue to develop and increase infection in host cells (Zaldívar-López et al., 2023) (T. Huang et al., 2020). In addition, *Salmonella typhimurium* infection may degrade CD4 T lymphocytes at positive control (Destiawan, 2022).

Telang flower infusion (*Clitoria ternatea*) contains polyphenols, anthocyanins, anthocyanidins, flavanols, fatty acids, phytosterols, tocol, and phenols which function as antioxidants by lowering ROS levels so that there is a decrease in cell damage and an increase in cell repair, and anti-inflammatory by regulating the immune system to suppress the action of the transcription factor NFκB-CD68 which can reduce pro-inflammatory cytokine levels, thereby lowering ROS levels and lowering cell damage (Multisona et al., 2023).

The largest component of telang flowers is anthocyanins derived from delphinidin, especially anthocyanins such as A1-A3, B1-B4, C1 and D1-D3 (Thilavech et al., 2021). In addition, the protein found in the telang flower (*Clitoria ternatea*), namely cyclotides, plays a role in antibacterial and immunostimulant activity (Nguyen et al., 2016). Based on this background, the study aims to determine the effect of telang flower infusion on the neutrophil cells and lymphocyte cells in the *Salmonella typhimurium bacterial infection model*.

2. RESEARCH METHOD

This study uses several implementation methods, including the method of making infusions, grouping mice, *Salmonella typhimurium bacterial infection*, therapy, examination of neutrophil cells and lymphocytes using giemsa staining, and data analysis.

2.1 Telang Flower Infusion

The flowers are separated from the petals and then dried in the oven at a temperature of 40-45^{o}C for 24 hours. Simplisia is weighed as much as 100 grams, 100 ml of sterile aquades solvent is added in an erlenmeyer tube. The solution is then homogenized and heated using a hotplate and magnetic stirrer at a temperature of 75-90oC for 15 minutes, then continued with the filtration stage using Whatman filter paper (Aini et al., 2023), then divided into 3 doses consisting of 0.25ml/20gBB, 0.5ml/20gBB, 1ml/20gBB.

2.2 Group of mice

The mice (Mus musculus) used were 24 *balb/c strains* weighing 20-25 grams,

then acclimatized in the laboratory of experimental animals, Faculty of Health Sciences, dr. Soebandi University for 7 days. Then, the mice were divided into 6 groups, namely the negative group, the positive group (mouse infection+normal saline), the stimuno group (mouse infection+stimuno 0.26/20gBB), the therapy group 1 (mouse infection+dose 0.25ml/20gBB), the therapy group 2 (mouse infection+dose 0.5ml/20gBB), and the therapy group 3 (mouse infection+dose 1ml/20gBB).

2.3 Salmonella typhimurium *Bacterial Infection*

Infection induction using *Salmonella typhimurium* bacteria with a concentration of $1.5x10^8$ cfu was then injected intraperitoneally 0.2 ml, mice were evaluated after 3 days post-infection to determine the presence of bacteriumemia.

2.4 Telang Flower Infusion Therapy

oral therapy (p.o) for 7 days at doses of 0.25ml/20gBB, 0.5ml/20gBB, and 1ml/20gBB).

2.5 Giemsa Staining

Giemsa staining is used to determine the morphology and number of leukocyte cells, especially neutrophils and lymphocytes.

The giemsa staining method is to place a drop of blood on a glass object, then do a smear of blood and let it dry, then add methanol and wait for it to dry. Giemsa dye is added thoroughly and let it sit for 20 minutes, then washed using running water and observed using a microscope (Muflihah et al., 2024).

2.6 Counting Neutrophil Cells and Leukocytes

Neutrophil cells and lymphocyte cells are counted in 100 fields of view.

2.7 Statistical Analysis

Statistical analysis on Neutrophil cell parameters using the Independent *T Test* with a significant value of 95% ($p<0.05$) due to abnormal homogeneity test. Statistical analysis of lymphocyte cell parameters using Anova followed by *the Tuckey* test with a significance value of 95% ($p<0.05$).

2.8 Research Ethics

This research has been examined by the University ethics commission dr. Soebandi with the number 439/KEPK/UDS/VI/2024.

3. RESULTS AND DISCUSSION

Based on the results of the research that has been conducted, it shows that there are differences in several treatment groups in both neutrophil cells and lymphocytes. In neutrophil cell parameters, there was a significant difference between negative and positive groups (0.00<0.05), stimuno (0.00<0.05), therapy 1 (0.00<0.05), therapy 2 (0.00<0.05), and therapy 3 (0.00<0.05). In the positive group, there was a significant difference with stimuno (0.02<0.05), therapy 1 (0.00<0.05), therapy 2 (0.00<0.05), and therapy 3 (0.01<0.05) **(Figure 1)**. In the stimuno group, there was a difference with the negative group (0.00<0.005), positive (0.02), Therapy 2 (0.04<0.05) and therapy 3 (0.04<0.05), but there was no significant difference from therapy 1 (0.62>0.05). In 1 therapy, there was no significant difference between stimuno (0.62>0.05). In therapy 2 there was a difference in negative, positive, stimuno, therapy 1, and therapy 2, and there was no difference in therapy group 3 (0.25>0.05), and in therapy group 3 there was a significant difference in negative, positive, stimuno, therapy 1, but there was no significant difference with therapy group 2 (0.25>0.05) **(Figure 1).**

Anova analysis with a significance value of 95% (0.05) in the examination of lymphocyte cells showed a significant difference (0.00<0.05) in each group, then continued with the *tuckey test, the* tuckey *analysis* showed that in the negative group there was a significant difference with therapy 2 (0.00<0.05), therapy 3 (0.00<0.05), then in the positive group there was a significant difference in therapy group 3 (0.00<0.05) **(Figure 2)**.

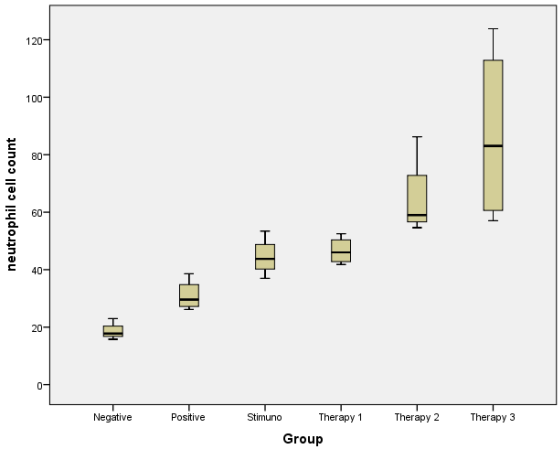

Figure 1. Graph of differences in neutrophil count from each group.

There was a significant difference in the stimuno group in therapy group 3 (0.00<0.05). Therapy group 1 was significantly different from therapy group 2 (0.03<0.05), and therapy group 3 (0.00<0.05). Therapy group 2 was significantly different from the negative (0.00<0.05), positive (0.02<0.05) and therapy groups 1 (0.03<0.05). Therapy group 3 was significantly different from the negative (0.00<0.05), positive (0.00<0.05), stimuno (0.02<0.05), and therapy groups 1 (0.00<0.05) **(Figure 2)**.

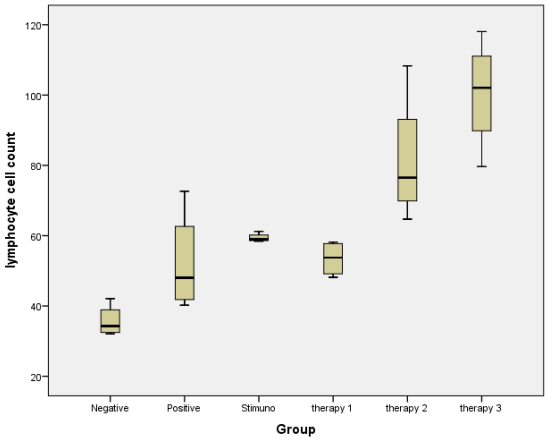

Figure 2. Graph of differences in the number of lymphocytes from each group.

The ratio of the total number of neutrophil cells and lymphocytes in each group is shown in the figure below.

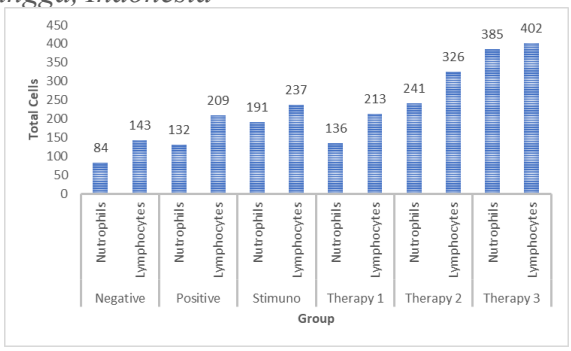

Figure 3. Difference in cell count between neutrophil cells and lymphocyte cells.

Based on the figure above, it shows the difference in the number of neutrophil cells and lymphocyte cells, where the ratio of the number of neutrophil cells to lymphocyte cells, is higher than that of neutrophil cells, this is because on the 7th day neutrophils decrease and lymphocytes will increase, so the ratio of the number of lymphocyte cells is higher than that of neutrophil cells.

Salmonella typhimurium is a gram-negative bacterium that causes gastroenteritis. *Salmonella typhimurium* has lipoproteins, curl amyloid fibrils, lipopolysaccharide (LPS), flagellin, and CpG DNA, the structure of which is called pathogen-associated molecular patterns (PAMPs). PAMPs are recognized by the immune system to clear the bacteria *Salmonella typhimurium* (Wang et al., 2020).

PAMPs will be recognized by macrophages in the tissue through pattern recognition receptors (PRRs), yang terdiri dari Toll-like receptor 2 (TLR2) (Deepinder et al., 2021), Toll-like receptor 5 (TLR5) (F. Huang, 2021), Toll-like receptor 9 (TLR9) (L. et al., 2021), NOD-like receptors (NLRs) (Wang et al., 2020). The interaction inducing an increase in the pro-inflammatory cytokines IL-1β, TNF-α, IL-6 (Tuxpan-Pérez et al., 2022).

Based on the results of the study and the graph above **(Figure 1)** shows that there is a significant difference in the number of neutrophil cells between negative and positive, this is because in the positive group there is an infection *of Salmonella*

typhimurium bacteria in the gastrointestinal tract so that it triggers an increase in pro-inflammatory cytokines IL-1β, TNF-α, IL-6. IL-1β and IL-6 play a role in inducing the proliferation of hematopoietic stem cells (HSCs) in the bone marrow thereby increasing the production of neutrophils in the bone marrow (Jahandideh et al., 2020).

In addition, lymphocyte cells in the positive group were different but not significant **(Figure 2),** this is because on the 7th day the production of lymphocyte cells has not increased significantly. Increased lymphocyte production occurs on the 7th to 14th day after infection (Moonen et al., 2018). Based on the ratio **(Figure 3)** the number of neutrophils is lower than that of lymphocytes, this is because on the 3rd day after infection, neutrophils will decrease, this is because the level of pro-inflammatory cytokines has begun to decline (Feng et al., 2023). Lowering pro-inflammatory cytokines will increase the number of lymphocyte cells on days 7 to 14. An increase in pro-inflammatory cytokines will decrease the number of lymphocyte cell proliferation (Fathi and Rezaei, 2020).

The stimuno group, in neutrophil cells, has a significant difference ($p<0.05$) with the negative group and the positive group. Stimuno can increase the proliferation of neutrophil cells after an infection. The lymphocyte cells, stimuno has a difference but not significant ($p>0.05$) between the negative group and the positive group, where Stimuno plays a role in increasing the proliferation of lymphocyte cells after infection. Stimuno has 25mg of *Phyllantus niruri* every 5 ml. *Phyllantus niruri* may increase the proliferation of lymphocytes in the spleen (Obaro-Onozeyi et al., 2021), the role of *Phyllantus niruri* acts as an immunomodulator by increasing the proliferation of PBMC cells (Hidayat R and Hayati L, 2020), and stimulates neutrophil cells (Dahanayake et al., 2020).

The use of telang flower infusion plays a role in increasing immunity cells, especially neutrophil cells and lymphocyte cells after *Salmonella typhimurium* infection. In therapy groups 1, therapy 2, and therapy 3, the larger the dose used, the higher the number of neutrophil cells, this increase was significant in each therapy group ($p<0.05$). This also occurred in the number of lymphocyte cells, therapy group 1, therapy 2, therapy 3, experienced an increase in lymphocyte cells, but there was an increase in cognitive ($p<0.05$) between therapy group 1 and therapy group 2, but a non-significant increase ($p>0.05$) between group 2 and group 3.

Telang flower (*Clitoria ternatea L*) contains bioactive compounds, namely alkaloids, tannins, glycosides, resins, steroids, saponins, flavonoids and phenols (Multisona et al., 2023). The flavonoid content in the telang flower is 35.73 + 0.978 mg/g dry weight, which is the 2nd largest content after the total phenolic which is 102.37 + 1.063 mg/g dry weight (Tuan Putra et al., 2021). Flavonoids act as antioxidants and have the ability to modulate proteins in cell proliferation and cell death pathways. Quercetin, which is included in flavonoids, plays a role in improving immune function and regulating the expression of the pro-inflammatory cytokine, IL-6 (Manoharan et al., 2024). Then, another content owned by the infusion of telang flowers is flavonol. Flavonols play a role in increasing the proliferation of lymphocyte cells. Flavonols found in aronia can stimulate the proliferation of lymphocytes in the white pulp of the spleen organs (Bushmeleva et al., 2024). Based on the above research, the content of metabolite compounds contained in the infusion of telang flowers can increase the number of neutrophil cells and lymphocyte cells.

4. CONCLUSIONS

Based on the research conducted, the infusion of telang flowers at doses of 0.25ml/20gBB, 0.5ml/20gBB, and 1m/20gBB has an effect on increasing the number of neutrophil cells and lymphocyte cells. The highest increase in neutrophil cell count and lymphocyte is a dose of 1ml/20gBB. But this increase cannot explain whether the cells are active against Salmonella typhimurium or not. Further

research is needed on receptors that interact with *Salmonella typhimurium* bacteria to find out whether the cells are active against *Salmonella typhimurium* or not.

ACKNOWLEDGEMENT

Thank you to the Ministry of Education, Culture, Research, and Technology (Kemendikbudristek) for the Beginner Lecturer Research grant, so that with this grant we can develop from previous research.

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