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Effect of Telang Flower Infusion on Lymphocyte Cells and Neutrophil Cells (Segment And Band) in Microplastic-Induced Rats

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ABSTRACT

Background: Microplastics causing tissue damage and increasing the number of lymphocyte cells and neutrophils (segment and band). Telang flower infusion contains flavonoids as antioxidants that can as immunomodulatory. The purpose of this study was to determine the effect of telang flower infusion on the number of lymphocyte cells, band and segment neutrophils. **Methods:** Methode begins with the manufacture of telang flower infusion, the manufacture of microplastics (heating bottled drinking water at a temperature of 65°C), the analysis of the size of microplastics using electron microscopes, the analysis of microplastic types using the FTIR method, the grouping of negative control, positive control, and treatment with a dose of 4 ml/250g BW, exposure to microplastics, blood smear, giemsa staining for lymphocyte and neutrophil cell analysis, and statistical test using One-Way ANOVA ($p<0.05$) with Tuckey HSD ($p<0.05$). **Results:** The results showed that the average number of negative control band neutrophil cells (3.50±4.50), positive control (3.17±3.81), and treatment (5.33±3.61) and the One-Way ANOVA analysis showed values ($p=0.81>p=0.05$), the average segment neutrophil cells of the negative control (23.67±6.80), the positive control (21.33±5.82), the treatment (24.00±8.19), and the One-Way ANOVA analysis showed values ($p=0.776>p=0.05$), negative control lymphocyte cells (68.50±14.84), positive control (72.17±4.99), and treatment (64.00±12.10). The One-Way ANOVA analysis showed a value of ($p=0.481>p=0.05$). **Conclusions:** It can be concluded that telang flower infusion has a tendency to act as an immunomodulator at the biological level characterized by a decrease in lymphocyte cells, an increase in band neutrophil cells and segment nuetrophic cells.

Keyword: Microplastics; Lymphocyte cells; Segment neutrophil cells; Band neutrophil cells; Telang flower.

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INTRODUCTION:

Plastic has content that cannot be degraded by the environment or digestion, plastic has been used since 1950, which causes major problems in the environment. Plastic can be found in seas, rivers, lakes, and dirty environments (Hirt & Body-Malapel, 2020). Microplastics are small particle polymers of <5mm in size that are not easily degraded in the environment and in the bodies of living things (Yang et al., 2023). Microplastics have a diameter of <5mm, and can accumulate in the environment and in the bodies of living things (Liu et al., 2019). The groups that include

microplastics are polypropylene (PP-MP), Polyvinyl chloride (PVS-MP), and Polyester (PES-MP) (Poosrisom et al., 2025). In addition, secondary microplastics are produced by physical, chemical, UV radiation, weathering, and mechanical weathering, so that they can spread more widely, especially to living things (Lee et al., 2025).

Exposure to microplastics, especially in living things, over a long period of time can lead to pathophysiological conditions in some body responses. Microplastics can be detected in the placenta, blood vessels, heart, and lungs, as well as the digestive tract. Exposure to microplastics can induce oxidative stress, cell damage, these two conditions can induce the emergence of an inflammatory response in the form of the production of cytokines IL-6, TNF-α, IL-8, and also IL-1. This effect can lead to failure in some tissues, and lead to fibrosis. Exposure to microplastics causes changes in the heart, where the accumulation of microplastics in the heart is caused by the consumption of foods containing microplastics, which later microplastics will enter the blood vessels and move to the heart, some microplastim that can be identified in the heart are terephthalate (PET), polyvinyl chloride (PVC), and polymethyl methacrylate (Lee et al., 2025).

Microplastic exposure in the digestive tract is the largest exposure compared to microplastic exposure in other organs. Exposure to this leads to oxidative stress, an inflammatory response, and an imbalance of the immune system, which can lead to inflammation of the colon and inflammatory bowel disease (Lee et al., 2025).

Microplastics cause changes in immune regulation. In the liver organs, microplastics can induce and increase the infiltration of NK cells and pro-inflammatory macrophage cells into non-parenchymal lymphatic liver cells; however, microplastics can lower B lymphocyte cells in tissues. In addition, microplastics can also cause an increase in several cytokines, including IFN-γ, TNF-α, IL-1β, IL-6, and IL-33, but decrease the cytokines IL-4, IL-5, IL-10, IL-18, and TGF-β1. This regulation through the activation of NF-κB (Zhao et al., 2021). Microplastics in the body's tissues can cause activation of neutrophil cells. Neutrophils bind strongly to microplastics and carry out the endocytosis process that leads to an increase in inflammatory cytokines. Microplastics localized in tissues can cause an immune response and interfere with the function of neutrophil dai. The results of neutrophil RNA sequencing showed that neutrophils exposed to microplastics showed increased cell death (Park et al., 2024).

Research conducted by (Sun et al., 2021) showed that a dose of 0.5 mg of microplastics can reduce the number of white blood cells, one of which is neutrophils, and inhibit the growth factors of colony-forming unit granulocytes (CFU-G), colony-forming unit–macrophage (CFU-M), and colony-forming unit-granulocytes and monocytes (CFU-GM). In addition, exposure to microplastics can cause hematotoxicity, affect gene expression, and disrupt the molecular and biological pathways of the bone marrow (Sun et al., 2021). Inhibition of growth factors reduces white blood cell production in the spinal cord; this reduction can impair the immune system's response to infection. Telang flower (*Clitoria ternatea*) is a plant that is often found in the community. Telang flowers are used as a drink, one of which is a tea drink. Clitoria ternatea serves as a valuable source of diverse phytochemicals, including polyphenols, anthocyanins, anthocyanidins, flavanols, and other bioactive compounds such as fatty acids, phytosterols, tocols, and phenols, which may contribute to its biological activities. (Multisona et al., 2023). which acts as an antioxidant. The antioxidant content in telang flowers can prevent cell damage, one of which is in white blood cells in the bone marrow.

Research on the effects of telang flowers as an antioxidant has been widely conducted, but it has not focused on microplastics. One of them is telang flower, which is generally used to treat metabolic diseases. Previous research on microplastics has focused only on biochemical

and cytokine effects; research on cellular aspects, particularly lymphocytes and neutrophils, remains rare. This study combines telang flower infusion with microplastic exposure to provide a comprehensive approach related to the use of telang flower infusion on microplastic exposure. The purpose of this study was to determine the effect of telang flower infusion on the number of lymphocyte and neutrophil cells (segments and bands) in microplastic-induced rat.

METHODS:

Telang Flower Infusion

Telang flowers were dried in an oven at 40–45 °C for 24 hours. Subsequently, 100 g of the dried material was weighed and mixed with 1000 mL of sterile distilled water in an Erlenmeyer. The prepared mixture underwent homogenization and was heated under continuous magnetic stirring on a hotplate at 75–90 °C for 15 minutes. After 15 minutes, the homogenate results are filtered using Whatman filter paper (Aini et all., 2023).

Microplastic

Manufacture of modified microplastics from research (Supriyo & Noviana, 2023). The drinking water sample is put into a bottled water bottle, then heated at 60^{o}C for 4 hours.

Microplastic Analysis

Drinking water that has been heated for 4 hours, then filtered using a hydrophilic PTFE filter membrane with a pore of 0.22 µm. The filter paper with microplastics is then dried in a desiccant for 24 hours. The filter paper is then observed under a Hitachi TM 3000 brand electron microscope with a magnification of 1mm to 20µm (Supriyo & Noviana, 2023).

Treatment Grouping

The rats used were 18 wistar strains weighing 200-250 grams, then assimilated in the experimental animal laboratory of the Faculty of Health Sciences, University dr. Soebandi for 14 days. After the acclimatization process, the rats were divided into 3 groups, namely negative control (rats without being given microplastics), positive control (rats plus microplastics), treatment control (rat induced by microplastics and treated using 100% telang flower infusion). Microplastic exposure was given daily for 30 days, then after 30 days, the treated rat were given telang flower infusion therapy, for positive control, the rat were given placebo.

Microplastic Induction

Microplastic exposure was carried out for 30 days through oral per-section with a volume of 4 ml/250g BW/day in positive group rats and treated.

Telang Flower Infusion Therapy

The administration of telang flower therapy was carried out on the 31st day after microplastic induction. Telang flower infusion therapy with a dose of 4 ml/250g BW for 7 days in the treatment group.

Blood Smears and Giemsa Staining

The making of blood smears is carried out by smearing the tail, the tail of the rat is cut at the end and then dripped into a glass object, then a blood smear is carried out. Next, giemsa coloring is carried out. Giemsa staining is carried out after the blood smear has dried, the dried blood smear is then dripped with methanol thoroughly and then waited for it to dry. After drying, then add the dyeing of the giemsa thoroughly or cover all layers of blood smear then let it sit for 20 minutes. After 20 minutes, wash with running water and observe with a light microscope (Muflihah, 2024).

Neuthropil cells and Lymphocytes cells Examination Analysis

Neutrophil and lymphocyte counts were performed by reading sections of the counting area of a stained blood smear at magnification 1000x. Observations were made on fairly thin sections with an even distribution of leukocytes. Furthermore, counts were performed by

observing the number of cells in each field of view, up to 100 leukocytes, using a Schilling hemogram.

Data Analysis

Data analysis used One-Way ANOVA with a significance value of 95% (P<0.05) then continued with the Tuckey HSD test (p<0,05).

Research Ethics

This research protocol was reviewed and approved by the Ethics Commission of University dr. Soebandi, as indicated by ethical clearance number 1313/KEPK/UDS/VII/2025.

RESULTS:

Microplastic Identification

Based on microplastic examination and analysis, it is shown that there are microplastic particles in bottled drinking water that is heated to a temperature of 60°C for 4 hours, **(Figure 1).**

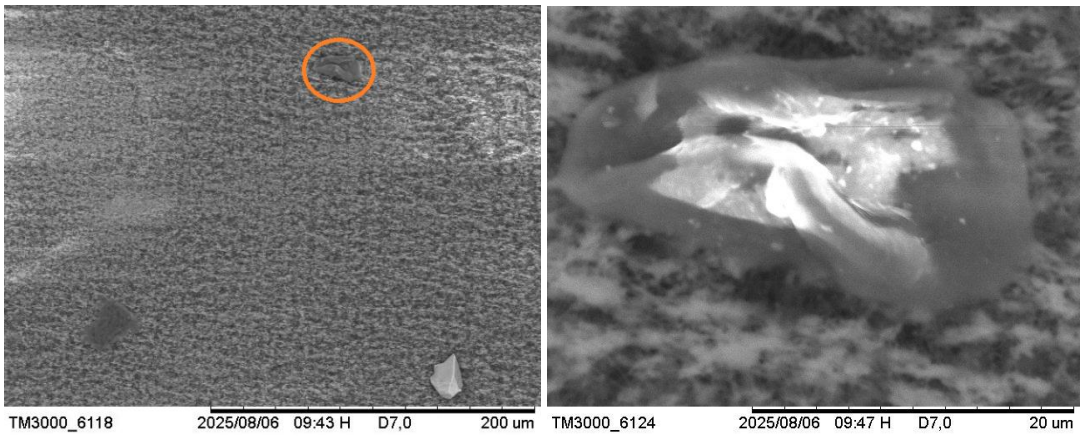

Figure 1. Description of Microplastics in bottled drinking water heated within 4 hours with a temperature of 60°C. 200µm magnification (left) and 20µm magnification (right).

The microplastic found in this examination is Polyethylene Terephthalate (PET). PET plastic material used to make packaging bottles. PET has an irregular shape that is commonly found in personal items, especially plastic bottled drinking water (Prapanchan et al., 2023).

Analysis of Lymphocytes and Neutrophil Cells

In this study, after microplastic induction and therapy using telang flower infusion, it was shown that there was a change in segment and bands/band neutrophils cells and lymphocyte cells **(Figures 2, 4, and 6).**

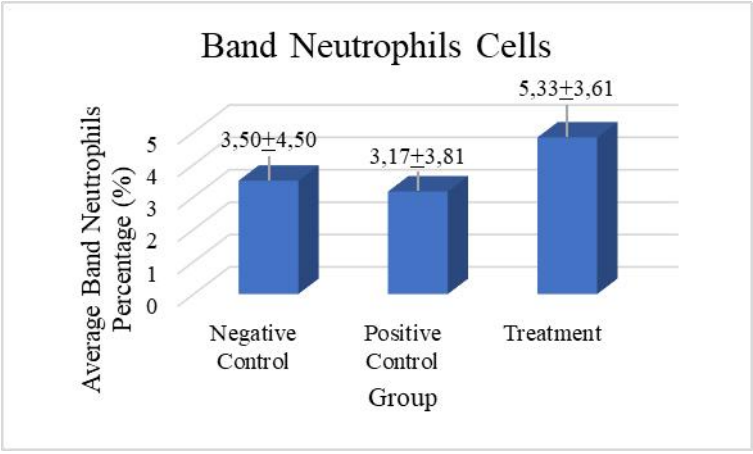

Figure 2. The effect of microplastic induction and infusion therapy of telang flowers is based on the number of Band Neutrophils

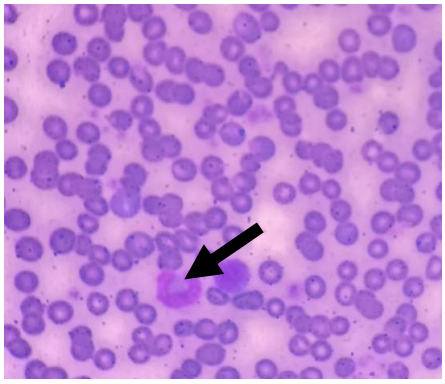

Figure 3. neutrophil Band cells (Black Arrow). It has a core shaped like the letter 'C' and is not segment.

Based on the image above, it shows that in the positive control there was a decrease in band neutrophil cells with an average number of band neutrophils of 3.17±3.81 compared to the negative control, which was 3.50±4.50. Then, after therapy, it showed an increase in segment neutrophil cells, which was 5,33±3.61. Based on statistical analysis using one-way ANOVA, it showed that there was no significant difference between the groups, because the significance value of the test was (p=0,81>p=0.05)

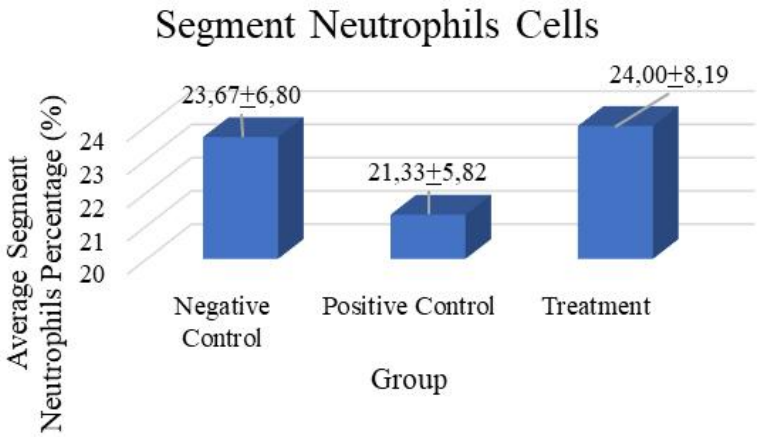

Figure 4. Effect of microplastic induction and infusion therapy of telang flower in the number of neutrophils segment

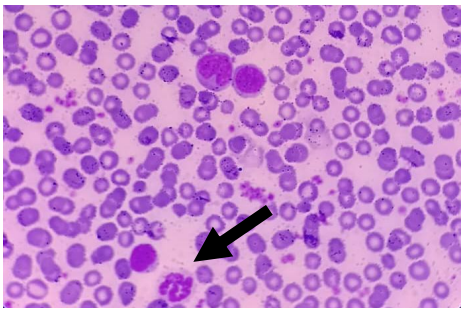

Figure 5. Segment neutrophil cells (black arrows). It has 2-5 lobes with chromatin thread connections.

Based on the image above, it shows that in the positive control, there was a decrease in segment neutrophil cells with an average number of segment neutrophils of 21.33±5.82 compared to the negative control of 23.67±6.80. Then, after therapy, it showed an increase in segment neutrophil cells, which was 24.00±8.19. Based on statistical analysis using one-way ANOVA, it showed that there was no significant difference between groups, because the significance value of the test was (p=0,776>p=0.05).

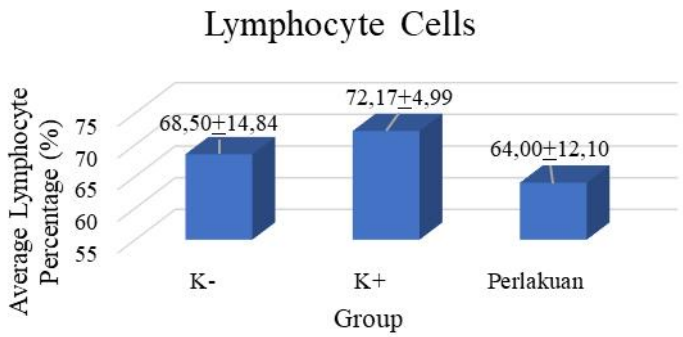

Figure 6. Effect of microplastic induction and infusion therapy of telang flower on lymphocyte count

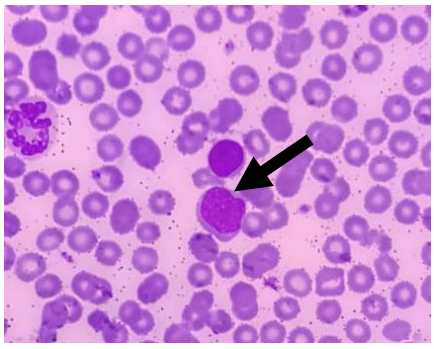

Figure 7. Lymphocyte cells (black arrows). Lymphocyte cells have the characteristic of being thick almost covering their cytoplasm.

Based on the image above, it shows that there was an increase in lymphocyte cells after 30 days of mycoplastic induction, with an average value of lymphocyte cell count of 72.17±4.99 when compared to the negative control of 68.50±14.84. However, after therapy, there was a decrease in lymphocyte cells with an average value of lymphocyte cell count of 64.00±12.10. Based on statistical analysis, One-Way ANOVA showed a significance value of the treatment of (p=0,481>p=0.05), which indicates that there was no significant difference between groups.

DISCUSSION:

Microplastics are small particles produced from household products, one of which is plastic bottles and is not easily degraded both in the environment and in the body (Tamargo et al., 2022)**.** Various types of microplastics in the environment consist of Polymethyl methacrylate (PMMA), PE, Polyamide (PA), Polyethylene terephthalate (PET), PU, PP and Polystyrene (PS). High-density polyethylene (HDPE), and Low-density polyethylene (LDPE) (Prapanchan et al., 2023) (Zheng et al., 2020).

Microplastics in the environment can cause environmental pollution while in the human body can cause tissue damage, imbalance in ROS production, vascular damage. Tissue damage releases cell components consisting of

heat shock protein (HSP), high mobility group box 1 (HMGB-1), and genetic material called Damage-associated molecular patterns (DAMPs). DAMPs will be induce an increase in segment and band/band neutrophil cells (Persiani et al., 2023). This is because DAMPs recognized by phagocyte cells through TLR-4 receptors cause increased production of cytokines IL-6 and granulocyte colony-stimulating factor (G-CSF). Increased IL-6 and G-CSF affect increased proliferation and differentiation of nuetrophils in the bone marrow (Devlies et al., 2021). This increased proliferation and differentiation causes not only segmental neutrophil cells to be in the blood circulation, but band neutrophil cells are also in the blood circulation.

However, on 30 day after microplastic exposure, there was a decrease in the mean neutrophil segment compared to negative controls (p=0,776>p=0,05) (**Figure 4**) and band nuetrophil cells compared negative controls (p=0,81>p=0,05) **(Figure 2)**, Although, statistically one-way ANOVA the decrease is not significant, because on day 30, neutrophil cells may not trigger an acute inflammatory response resulting in a decrease, neutrophil cells only live in the bloodstream for 5.4 days (Rosales, 2020). Biologically, the decrease in segment and band neutrophils is the adaptation of neutrophil cells to exposure to microplastics. Mechanistically, decrease in segment and band neutrophil cells due to microplastics can lead to genotoxicity and mutagenicity that affect the development of neutrophils. In addition, microplastics can also bind to neutrophils, inducing neutrophil dysfunction and death through TLR signaling pathways (Park et al., 2024), With this death, it can reduce neutrophil cells in the blood vessels (Amran et al., 2022).

Increase in lymphocyte cells in positive control compared to negative control (p=0,481>p=0,05) **(Figure 6)**, showed a tendency for adaptive immune system biological activity, but the statistical increase in One-Way ANOVA did not make a significant difference. Biologically, the increase in the average lymphocyte cells occurs after the 14th day, due to the activation of lymphocyte cells through the presentation of dendritic cells, thus inducing the proliferation and differentiation of lymphocyte cells to migrate to damaged tissues through blood vessels (R. Wang et al., 2024). Mechanistically, the increase in lymphocyte cells is caused by the DAMPs produced by damaged tissue are recognized by dendritic cells through TLR-4 receptors, then dendritic cells present DAMPs to lymphocyte cells present in the lymph nodes, resulting in activation and increase in lymphocyte cells to proliferate and differentiate into CD4+/CD8+ lymphocyte cells, then lymphocyte cells migrate to damaged tissues through blood vessels (Mehrani et al., 2025). However, on 30 day, the response of T lymphocyte cells is likely to undergo a balanced phase or an adaptation phase, namely by activating regulatory T lymphocyte cells and lowering porinflammatory cytokines to reach a balanced phase (Wang et al., 2025). So that in these conditions there was no significant difference in the positive control group compared to the negative control.

Telang flower infusion contains secondary metabolites in the form of alkaloids, steroids, flavonoids, glycosides, and tannins. These compounds play a role in antioxidants, namely repairing damaged tissues and suppressing pro-inflammatory cytokines, so that it can prevent chronic inflammation caused by lymphocyte cells (Ahmed et al., 2024) however, statistically there was no significant difference between the treatment group, the positive group, and the negative group (p>0.05) in band neutrophil cells, segment neutrophils, and lymphocytes. However, the changes that occur reflect biological activity. The bioactive compounds of telang flower have the potential to act as immunomodulators, which can affect the number of lymphocyte cells and leukocytes such as neutrophils. Based on the data above **(Figures 2,4, and 6)** Bioactive compounds flavonoids and anthocyanins play a role in suppressing oxidative stress and pro-inflammatory cell production so that they can

decrease lymphocyte cells, then the compound content can synthesize the activation and proliferation of inet immune cells, such as neutrophils (Putri et al., 2025)

Insignificant results between groups may be due to too small effects, too few samples, or the presence of an adaptation mechanism to chronic conditions i.e. by day 30. So that later there will be a need for further research on increasing the number of samples, and increasing the dose used so that it has an optimal effect as an immunomodulator.

CONCLUSIONS:

Based on the research that has been carried out, telang flower infusion with a dose of 4ml/250g BW has a tendency to act as an immunomodulator at the biological level, because statistically One-Way ANOVA this difference is not significant ($p>0.05$), which is characterized by a decrease in lymphocyte cells, an increase in band neutrophil cells and segment nuetrophil cells in mice exposed to microplastics for 30 days.

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