ORIGINAL ARTICLE

Number of Peritoneal Macrophages Cells in *Salmonella typhi*-Induced Mice After *Spirulina platensis* Administration

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ABSTRACT

Introduction: *Salmonella typhi* is a facultative intracellular bacterium causing typhoid fever, characterized by symptoms of headache, dizziness, muscle aches, anorexia, nausea, vomiting, and diarrhea. An alternative treatment used to treat the infections caused by *Salmonella typhi* other than using antibiotics is increasing the body's immune system. *Spirulina platensis* is a bluish-green microalgae with several benefits, one of which is an immunomodulator. The aim of the study was to determine the number of peritoneal macrophages of mice induced by *Salmonella typhi* after the administration of *Spirulina platensis*. **Material and methods:** The research method used is Experimental Laboratory with Post Test Only-Control Group Design. The treatment was conducted using 24 mice divided into 4 groups. Variations in the doses of Spirulina plantesis given are 400 mg/KgBW and 800 mg/KgBW. Peritoneal macrophage cells in this study were examined using a Haemocytometer. **Result:** There is an influence on peritoneal macrophages of mice induced by *Salmonella typhi* after the addition of *Spirulina platensis*, with lower numbers of macrophages in treatment groups compared to the positive control group. **Conclusion:** *Spirulina platensis* can affect the number of peritoneal macrophages in mice induced by *Salmonella typhi*.

Keywords: *Salmonella typhi,* Peritoneal macrophage, *Spirulina platensis*, Immunomodulator

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INTRODUCTION

Typhoid fever is an endemic disease and is one of the critical public health problems in Indonesia. Typhoid fever is closely related to the quality of personal hygiene and environmental sanitation. This disease is characterized by fever symptoms that last for more than one week, resulting in digestive disorders that can reduce a person's level of consciousness (1). Based on data from the World Health Organization (WHO), it is estimated that the incidence rate of typhoid fever cases worldwide is around 11-21 million cases with around 128,000- 161,000 deaths in a year, with the majority of cases occurring in South Asia, Southeast Asia, and sub-Saharan Africa (2). The incidence rate of typhoid fever in Indonesia is still high at 358/per 100,000 rural residents and 810/per 100,000 urban residents in a year (3). *Salmonella sp.* is a bacteria that causes salmonellosis or typhoid fever which is transmitted through the consumption of food and drink contaminated with feces from typhoid patients (4). Patients with typhoid fever are usually treated with antibiotics. However, recently the use of antibiotics before patients come to health services has become widespread, resulting in several large outbreaks caused by multidrug-resistant *Salmonella typhi* in Asia (2). Based on these data, one alternative that can be done to treat infections caused by these bacteria is to increase the patient's immune system, namely by increasing the number of macrophages using certain compounds or materials as immunomodulators. Immunomodulators are substances that can modulate the function of the immune system and increase the number of macrophages thereby increasing the activity of the immune system. One of the natural ingredients that can be used as an immunomodulator is *Spirulina platensis*. Spirulina is a bluish-green-microalgae, which can be used as an immunomodulator as it contains

phycocyanin and β-carotene which are useful as anti-allergy and improve the immune system (5-6). Until now there has not been much research on *Spirulina platensis,* so the researchers wish to conduct research on *Spirulina platensis* as an immunomodulator on the number of macrophage cells in mice induced by *Salmonella typhi* bacteria.

METHODS

Sample

Spirulina platensis as the test material used in this study was obtained from Sukoharjo, Jogjakarta. The type of *Salmonella typhi* bacteria culture was obtained from the Laboratory of Gastroenteritis Institute of Tropical Disease (ITD) Universitas Airlangga Surabaya. The bacterial suspension was taken from *Salmonella typhi* bacteria culture on Nutrient Agar Slant (NAS).

Research method

The research method used in this research is experimental laboratory research with Post Test Only-Control Group Design. The treatment of experimental animals and isolation of macrophage cells from the peritoneal cavity of mice was carried out at the Professor Nidom Foundation Animal Diagnostic Laboratory. The process of examining the number of peritoneal macrophages in mice was carried out at the TB Laboratory at the Institute of Tropical Disease (ITD), Airlangga University, Surabaya. This research was conducted from March to April 2020.

Preparation of animal study

The population of this study was male mice with the balb/c strain aged ± 2 months. Samples were taken from the population randomly as many as 24 individuals and then grouped into 4 groups to be used in the study. Each group was replicated 6 times, thus the number of samples in this study was 24 samples. The test material used in this study was *Spirulina platensis* at a dose of 400 mg/kg BW and 800 mg/kg BW. The material used to check the number of macrophages was a suspension of macrophages, made with a mixture of Rosewell Park Memorial Institute (RPMI) and Fetal Bovine Serum (FBS) medium, Trypan Blue 0.08%. The instruments used were scalpel, 10 cc syringe, falcon tube, centrifuge, micropipette, yellow tip, hemocytometer, and microscope.

Salmonella typhi-induced methods

The experimental animals were first adapted for 7 days and then grouped into 4 groups, namely the negative control group, positive control group, first treatment group (P1), and second treatment group (P2). In the negative control group, the mice were not induced by *Salmonella typhi* and were not given any *Spirulina platensis*. In the positive control group, each mouse was induced by *Salmonella typhi* orally. In the P1 group, *Spirulina platensis* was given at a dose of 400 mg/kg BW for seven days then mice were induced by suspension of *Salmonella typhi* bacteria orally on the 8h day, then continued with S*pirulina platensis* at a dose of 400 mg/kg BW each day for seven days. Mice in the P2 group were given *Spirulina platensis* equivalent to a dose of 800 mg/kg BW for seven days then induced by suspension of *Salmonella typhi* bacteria orally on the 8th day, then continued with *Spirulina platensis* at a dose of 800 mg/kg BW each day for seven days. Furthermore, the peritoneal macrophages of mice were isolated and the number of macrophages was examined.

Examination of peritoneal macrophages

The examination of peritoneal macrophages number was carried out firstly by isolating macrophages in the peritoneal cavity, by inserting RPMI medium into the peritoneal cavity of mice, and then shaking it for ± 3 minutes, so that macrophages attached to the walls of the peritoneal cavity could be suspended into the medium. The suspension is then aspirated using a syringe and placed into a falcon tube. After that, the suspension was centrifuged at 1200 rpm for 10 minutes, then the supernatant formed was discarded and 3 mL of RPMI medium containing 10% FBS was added to form a ready-to-use suspension. The procedure for counting macrophages was carried out by pipetting 100 µL of macrophage cell suspension and 100 µL of 0.08% trypan blue into a test tube. Once homogeneous, the mixture was put a few drops into the hemocytometer and the number of macrophages was counted in the five counting chambers in the middlebox. The results of the number of cells expressed in macrophages/mm^3 area. The calculation formula is as follows:

$$\text{Volume of Counting Chamber (VCC)} = 5 \times \left(\frac{1}{5} \times \frac{1}{5} \times 0.1\right)$$

$$= \frac{1}{50}$$

Dilution Concentration (DC) = 1 (the ratio between dye and cell suspension is 1:1)

$$\text{Number of macrophage cells / mm}^3 = \frac{1}{\text{VCC}} \times \frac{1}{\text{DC}} \times \text{Number of cells in Counting Chamber}$$

$$= \frac{1}{1/50} \times \frac{1}{1} \times \text{Number of cells in Counting Chamber}$$

$$= 50 \times \text{Number of cells in Counting Chamber}$$

Statistical analysis

The data obtained were statistically tested with the help of the IBM SPSS Statistics 21 Program. A nonparametric test was conducted using the Kolmogorov-Smirnov test and homogeneity of variance was tested using the Levene Test. Statistical data was then analyzed using the Kruskal-Wallis method to determine the effect of the peritoneal macrophages number in mice induced by *Salmonella typhi* after administration of *Spirulina platensis*. Obtained results of the mean and standard

deviation of each group are shown in Table I.

RESULTS

Obtained data based on the study results are shown in Table I. The average number of peritoneal macrophages of mice in the negative control group (NCG), namely the group without treatment, was 1891.67 cells/mm^3, which was lower than the positive control group (PCG), where the mice were induced by *Salmonella typhi* without *Spirulina platensis*, with the average number 7783.33 cells/mm^3. The average number of mice peritoneal macrophages in treatment group 1 (dose of *Spirulina platensis* 400 mg/kg BW) was 2925 cells/mm^3 and the P2 group (dose of *Spirulina platensis* 800 mg/kg BW) was 1375 cells/mm^3. The results obtained lower numbers of macrophages in both treatment groups compared to the PCG. The Kruskal-Wallis test showed a significance value of p=0.01 (p< 0.05).

Table I: Value of *Spirulina platensis* immunomodulatory test results on the number of peritoneal macrophages in mice induced by *Salmonella typhi*

Groups	Mean SD	*p-value*
Negative Control	1891.67 ± 474.78	p= 0.01*
Positive Control	7783.33 ± 1742.03	
P1	2925 ± 1089.38	
P2	1375 ± 567.23	

Noted: Negative Control: not induced *Salmonella typhi*; Positive Control: induced *Salmonella typhi*; P1: induced *Salmonella typhi* + *Spirulina platensis* dose of 400 mg/KgBW; P2:. induced *Salmonella typhi* + *Spirulina platensis* dose of 800 mg/KgBW; α = 0.05 (p < 0.05).

DISCUSSION

A previous study by Ahsan et al stated that *Spirulina platensis* extract had antimicrobial activity against *Salmonella typhi*, which was observed in vitro, and an inhibition zone with a diameter of 9.5-16.0 mm was formed in the media (6-7). The positive control group was a group of mice that were induced only by *Salmonella typhi*. This caused the number of macrophages in the positive control group to be higher than in the negative control group that was not induced by *Salmonella typhi.* When an infection occurs, macrophages act as innate immunity which is the first form of defense. This causes the number of macrophage cells to increase to fight infections caused by *Salmonella typhi* (7).

Salmonella typhi has Pathogen-Associated Molecular Patterns (PAMPs) receptors that will be recognized by Pattern Recognition Receptors (PRR) on macrophages expressed on their cellular membranes. Salmonella contains several PAMPs, including lipopolysaccharide (LPS) (8). Macrophages activated by microbial products can act as professional phagocytes and Antigen Presenting Cells (APCs). Macrophage cells play an important role in the process of phagocytosis and also as APCs that initiate and direct immunity towards the cellular immune system mediated by T-cells in infections caused by intracellular bacteria (9). *Salmonella typhi* antigens stimulate T-lymphocytes to secrete Macrophage Activating Factor (MAF) substances that affect morphological changes in macrophages and result in very active metabolism, more actively killing and digesting bacteria (8,10-11). Therefore, in the positive control group, the number of macrophages was found higher to fight the infection originating from the induction of *Salmonella typhi* in mice.

In the first treatment group, where mice were given *Spirulina platensis* at a dose of 400 mg/kg BW and induced by *Salmonella typhi,* there was a decrease in the number of macrophages compared to the positive control group. Immune cells, especially macrophages, theoretically will be more active against infection in this group, compared to the positive control group without *Spirulina platensis* treatment, because *Spirulina platensis* acts as an immunomodulator that could increase the immunity of mice against *Salmonella typhi* bacterial infection. This is related to research from Shokri which states that *Spirulina platensis* can act as an immunomodulator by knowing the levels of Interleukin (IL)-4, IL-10, IL-17, Tumor Necrosis Factor (TNF)-α, and Interferon (IFN)-γ in mice that have been given *Spirulina platensis* (12). Immunomodulators are substances that can stimulate, suppress, or restore the activity of the immune system, both the specific and non-specific immune systems (13). *Spirulina platensis* acts as an immunomodulator in increasing innate immunity.

Spirulina platensis contains protein, carbohydrates, fat, zeaxanthin, vitamins, zinc, and chlorophyll. The presence of proteins contained in *Spirulina platensis* allows cells to survive and remain able to proliferate so that the number of cells will increase (14). A study by Seyidoglu also stated the presence of high protein content, amino acids, vitamins, β-carotenes, pigments, and polysaccharides in *Spirulina platensis* has an increasing effect on the production of antibodies and cytokines (15). Polysaccharides in these microalgae affect macrophages and T and B cell proliferation, so it is said that *Spirulina platensis* can improve the body's immune system (16). The decrease in the number of mice peritoneal macrophages also occurred in the treatment group with *Spirulina platensis* at a dose of 800 mg/kg BW. This indicates that the administration of *Spirulina platensis* can overcome the infection that occurred in mice caused by *Salmonella typhi*. Thus, it is known that the decrease in the number of peritoneal macrophages in mice is influenced by the dose of *Spirulina platensis* given to mice. The higher the number of doses given to mice, the number of peritoneal macrophages in mice will also decrease.

CONCLUSION

The conclusion of this study was *Spirulina platensis* can affect the number of peritoneal macrophages in mice induced by *Salmonella typhi*. Suggestions for further research are to explore the number of *Spirulina platensis* doses given and determine the expression of receptors found in macrophages after being given *Spirulina platensis*.

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