

## IMMUNOSTIMULATORY EFFECT OF VITAMIN C ON MICE INDUCED BY *STAPHYLOCOCCUS AUREUS*

Iffa Afiqa Khairani<sup>1</sup>, Elisa Nurma Riana<sup>1\*</sup>, Gres Maretta<sup>1</sup>, Najla Nur Azizah<sup>1</sup>,  
Isnaini Anggraini Putri<sup>1</sup>, Berkat Afni Maruli<sup>1</sup>, Salsa Pratiwi Mulyadi<sup>1</sup>, Antonius<sup>1</sup>

<sup>1</sup>Program Studi Biologi ITERA

Jl. Terusan Ryacudu, Way Huwi, Kec. Jati Agung, Kabupaten Lampung Selatan, Lampung 35365

\*Email: elisa.riana@bi.itera.ac.id

### Abstract:

Several compounds that can boost immune activity (immunostimulatory) include vitamin E, curcumin, flavonoids, and vitamin C. In this study, ascorbic acid or vitamin C, was used as one method to enhance the immune system. This study aims to determine the immunostimulatory effect of vitamin C on the phagocytosis activity of macrophages in mice induced by *Staphylococcus aureus*. The mice were divided into two groups: the control group, which received 0.3 ml of distilled water orally, and the treatment group, which received 0.036 mg/g BW of vitamin C orally for 14 days. On the 15th day, each mouse was intraperitoneally induced with 0.5 mL of *Staphylococcus aureus* bacterial suspension and left for two hours. The phagocytic activity value represents the percentage of active macrophage cells among all macrophages observed. The result showed that the value of % phagocytic activity in the control group was  $21.43\% \pm 7.92$ , whereas the % phagocytic activity in the treatment group was  $48.31\% \pm 22.27$ . The group of mice given vitamin C 0.036 mg/g BW orally showed increased phagocytic activity compared to the control group mice. This shows that vitamin C has potential as an immunostimulator.

*Keywords: immunostimulator, vitamin C, mice, macrophage, phagocytic activity*

### Introduction

Exposure to pathogenic microorganisms can lead to various diseases, particularly those related to infectious diseases. A compromised immune system can result in reduced immunity. The immune system is the body's response to defense against foreign objects, whether cellular or molecular, that enter the body. It becomes active when the body is attacked by external antigens, such as viruses, toxins, and poisons, which can damage cells, tissues, and organs. Based on its response, the immune system is divided into two categories: the specific immune system, which recognizes antigens specifically, and the nonspecific immune system, which serves as the body's primary defense against microorganisms. The non-specific immune system comprises cells that play crucial roles, such as macrophages. Macrophages function to phagocytose or digest antigens that enter the body (Puspitaningrum et al., 2017).

The process of antigen phagocytosis can be carried out by several cells, one of them is macrophages. Macrophages are widely distributed in the body and play a vital role in the inflammatory process as the initial response to foreign objects or microbes entering the body. They phagocytose

pathogens, dead cells, and extracellular matrix components. One way to enhance the phagocytic activity of macrophage cells is through the use of immunomodulatory substances (Akrom et al., 2015).

Immunomodulators are compounds that can dynamically alter or modify the activity of the body's immune system. They can work in two ways to enhance the biological response of the immune system. Immunostimulation involves stimulating the immune system to respond to antigen exposure. On the other hand, immunosuppression suppresses the immune system's reaction to antigens. Increasing immunostimulants is a method used to improve an imbalanced immune system by administering immunomodulatory compounds. Several types of bioactive compounds in plants have the potential to enhance the immune system in the body (Setiarto & Widhyastuti, 2022).

Several compounds that can boost immune activity (immunostimulatory) include vitamin E, curcumin, flavonoids, and vitamin C. In this study, ascorbic acid, or vitamin C, was used as one method to enhance the immune system. Vitamin C can accumulate in neutrophils, thereby increasing phagocytosis and chemotaxis. It was easily oxidized in the blood to form ascorbate and dehydroascorbate

molecules. Vitamin C, as an immunomodulator, can stimulate neutrophil movement, neutrophil phagocytosis, and apoptosis (Alquraishi et al., 2021; Dzakhirah, 2021).

This study aims to determine the immunostimulatory effect of vitamin C on the phagocytosis activity of macrophages in mice induced by *Staphylococcus aureus*.

### Material and Methods

The materials used in this research included 1 ml syringe, digital scales, analytical scales, aluminum foil, light microscope, object glass, dissecting set, mice cages, female mice (*Mus musculus*), *Staphylococcus aureus*, ascorbic acid (vitamin C), distilled water, Giemsa staining solution, methanol, phosphate buffered saline (PBS) pH 7.8, and immersion oil.

#### *In vivo testing*

The mice are maintained by regularly providing them with food and water. The cleanliness of the mice cages is maintained by regularly removing the husks. The mice were divided into two groups (three mice in each group). The control group, which received 0.3 ml of distilled water orally, and the treatment group, which received 0.036 mg/g BW of vitamin C orally for 14 days.

#### *Immunostimulatory activity test by observing macrophage phagocytosis*

*Staphylococcus aureus* bacteria were suspended in a tube containing 2 mL of 0.9% NaCl solution until turbidity was achieved, matching the standard turbidity of McFarlan solution 0.5 (equivalent to  $1.5 \times 10^8$  cfu/ml).

On the 15th day, each mouse was intraperitoneally induced with 0.5 mL of *Staphylococcus aureus* bacterial suspension and left for two hours. The mice then anesthetized and dissected to collect the peritoneal fluid. If a small amount of peritoneal fluid was found in the abdominal cavity, 1-2 mL of sterile phosphate buffered saline (PBS) pH 7.8 solution was added, gently shaken, and then the peritoneal fluid was drawn with a 1 ml syringe. The peritoneal fluid was smeared on a glass slide and fixed with methanol for

5 minutes, followed by staining with 10% Giemsa stain. After 20 minutes, it was rinsed with running water. Once the preparation was dry, it was observed under a microscope using immersion oil with a magnification 10x - 1000x (Yusuf et al., 2020).

While observing, macrophages were differentiated between active and passive. Active macrophage cells exhibit an amoeboid (irregular) shape and relatively larger nuclear sizes, whereas inactive cells (passive macrophage) appear round with smaller nuclei. Immunostimulatory activity was determined by calculating the phagocytic activity of mice peritoneal macrophage cells. The phagocytic activity value represents the percentage of active macrophage cells among all macrophages observed.

$$\% \text{ Phagocytic Activity} = \frac{\text{number of active macrophages}}{\text{total macrophages observed}}$$

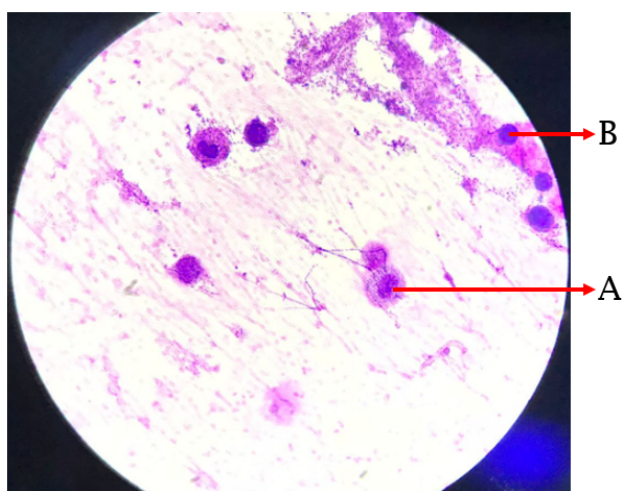
### Results and Discussion

The immunostimulatory properties of vitamin C were tested by counting the phagocytic activity of macrophages in mice. An immune response is triggered when *Staphylococcus aureus* bacteria invade the body as an antigen. According to Hariyanti et al. (2015), *Staphylococcus aureus* bacteria lack protein A, an antiphagocytic protein, resulting in their vulnerability to phagocytosis by peritoneal macrophages. After the injection with the bacterial suspension, all treatment groups were left for two hours. This duration allows the non-specific immune system to activate, given that it typically functions within 0 to 12 hours post-infection (Wahyuni et al., 2019). This response is initiated by an increase in phagocytic cells moving towards the site of infection. Cells such as macrophages and neutrophils migrate towards the source and engulf foreign cells through phagocytosis. Within macrophages, the phagocytosis process involves several enzymes, with lysozyme being the most dominant. The outcome of phagocytosis is the production of protein fragments that are subsequently presented to T cells to facilitate the process of antibody formation (Besung et al., 2016).

If there is an insufficient amount of peritoneal fluid in the mice, a phosphate buffered saline (PBS) solution was added into the mice's peritoneal cavity. This procedure is intended to facilitate the

detachment of macrophage cells from the mice's organs, simplifying the collection of the mouse's peritoneal fluid (Wahyuni et al., 2019).

Based on the results of observations of macrophages under a microscope at 1000x magnification, increased macrophage activity is characterized by an increase in the shape and size of the macrophages. Macrophages were differentiated between active and passive. Active macrophage cells exhibit an amoeboid (irregular) shape and relatively larger nuclear sizes, whereas inactive cells (passive macrophage) appear round with smaller nuclei (Fristiohady et al., 2019; Yusuf et al., 2020). Additionally, active macrophages are identifiable when one or more *Staphylococcus aureus* bacteria are observed being phagocytosed and contained within these active macrophages. *Staphylococcus aureus* is classified as an antigen from the group of gram-positive bacteria with round shape (cocci) and exhibits a clearer affinity with Giemsa staining (Jusuf et al., 2023). This can be seen in Figure 1., active macrophages are indicated by the arrow labeled 'A,' while passive macrophages are indicated by the arrow labeled 'B'.



**Figure 1.** Slide of peritoneal fluid sample showing active macrophages (A) and passive macrophages (B); Giemsa stain, magnification 1000x.

Observations of the phagocytic activity of mice's macrophages were examined by observing smears of mice peritoneal fluid that had been stained with Giemsa. The result showed that the value of % phagocytic activity in the control group was 21.43%  $\pm$  7.92, whereas the % phagocytic activity in the

treatment group (0.036 mg/g BW of vitamin C) was 48.31%  $\pm$  22.27 (Table 1).

**Table 1. The effect of vitamin C on phagocytosis activity**

Groups	% Phagocytic Activity $\pm$ STDEV
Control	21.43 $\pm$ 7.92
0.036 mg/g BW of vitamin C	48.31 $\pm$ 22.27

Based on the data above, it can be seen that vitamin C at 0.036 mg/g BW given orally for 14 days can increase the phagocytic activity of macrophage cells in female mice induced by *Staphylococcus aureus* bacteria, compared to the control group that was only given distilled water.

Vitamin C also known as ascorbic acid is a strong antioxidant and can be used to improve the body's immune system and is useful in treating infections. Vitamin C also enhances the immune system so that it can prevent various infectious diseases. Increasing the dose of vitamin C results in a significant increase in macrophage phagocytosis. Various studies report that vitamin C can increase the body's immune response. Giving vitamin C doses in graded doses will cause an increase in intraperitoneal vitamin C levels. The level of intraperitoneal vitamin C has a positive correlation with the level of macrophage phagocytic activity (Widjaja et al., 2012; Samudra et al., 2018; Kumari et al., 2020).

Vitamin C is also recognized for its involvement in the cellular function of both the innate and adaptive immune systems. Acting as an antioxidant, it has the capability to trap reactive oxygen species (ROS), thereby protecting biomolecules such as proteins, lipids and nucleotides from oxidative damage and dysfunction (Fatimah & Gozali, 2021). In addition to its role as an antioxidant, vitamin C plays a significant part in its immunomodulatory effects as a cofactor in various biosynthetic regulatory enzymes and genes. Vitamin C stimulates neutrophil chemotaxis to the site of infection, increasing phagocytosis, ROS release and

killing microbes. Simultaneously, vitamin C protects the tissue from excessive damage by increasing neutrophil apoptosis, clearance by macrophages, and reducing neutrophil necrosis. Vitamin C has the potential to protect the body from infection because of its role in body health (Carr & Maggini, 2017).

### Conclusion

The group of mice given vitamin C 0.036 mg/g BW orally showed increased phagocytic activity compared to the control group mice. This shows that vitamin C has potential as an immunostimulator.

### Conflict of Interest

The authors have no conflicts of interest to declare.

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