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Effect on Polysaccharide Krestin from *Coriolus versicolor* Extract on Phagocytic Activity and Capacity of *Mus musculus* Exposed by *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa is an opportunistic bacterium that causes infections in human. The wall cell of its bacteria contains lipopolysaccharide as virulency factors to protect it from human immunity. Lipopolysaccharide can inhibit phagocytosis in the body. Polysaccharide krestin (PSK) from *Coriolus versicolor* extract contains β-glucan that can increase phagocytic activity and capacity. This research aimed to identify the effect on polysaccharide krestin from *C. versicolor* extract on phagocytic activity and capacity of mice exposed by *P. aeruginosa*. The design of this research was experimental design. There were six treatment groups. The phagocytic activity and capacity were counted on slide smears of mice peritoneal fluid. The data was analyzed by using one way ANOVA. The results of the phagocytic activity and capacity showed that PSK was added before exposure (P1) or after exposure (P2) or before-after exposure (P3) had potential to increase phagocytic activity and capacity. The conclusion of the research was that adding polysaccharide krestin either before exposure or after exposure or both of them could increase phagocytic activity and capacity. The benefits of this research to development of science are expected to reduce human infection and to utilize natural ingredients as immunomodulator.

How to Cite

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INTRODUCTION

Pseudomonas aeruginosa is an opportunistic bacterium that causes infections in human, such as urinary tract infections, respiratory tract infections, gastrointestinal infections and dermatitis (Hauser et al., 2005). Furtherrmore, *P. aeruginosa* is an extracellular bacteria which has lipopolysaccharide (LPS) that one of virulence factors protects it from human immunity (Todar, 2004).

Lipopolysaccharide (LPS) can activate complement alternative way without antibody. One of the results of activation complemen affects on bacteria opsonization and increases phagocytosis (Munasir, 2001). Receptor of LPS bacteria is found in macrophage or monocyt (Rifa'i, 2009).

Extracellular bacteria is phagocyted and presented by Antigen Presenting Cell (APC). Antigen Presenting Cell will activate Th naive cell and produce IL-23 and IL-1. Th naive cell differentiate becomes Th17 which secretes IL-17, IL-22, and IL-21 that eliminates extracellular bacteria (D'Elios, 2011).

Pseudomonas aeruginosa infection happens because of decreasing immunity. The material that serves to improve or suppress the immune system is called immunomodulator. It is increasing immunity that can eliminate *P. aeruginosa*. Immunomodulator on *P.aeruginosa* infection will stimulate immune response by producing antibody, activating and proliferating T cell and macrophage, and activating NK cell that can eliminate infection cell and kill bacteria on its cell (Abbas et al., 2012).

One of the immunomodulator agents is polysaccharide krestin from *Coriolus versicolor* extract. According to Wahyuningsih and Win (2010), Polysaccharide krestin can increase a number of immonocompetent cells. The active molecule of PSK is β-glucan. Beta-glucan not only can activate phagocyt cell, but also has a role on antigen opsonization (Rifa'i, 2009).

Giving PSK before exposure *P. aeruginosa* can be preventive that increases immune response when exposure phatogen. Polysaccharide krestin can use be curative to increase immune respon such of activating phagocyt (LeBlanc, 2006). The objective of this research was to know the effect on Plysaccharide krestin from *Coriolus versicolor* extract on phagocytic activity and capacity of mice exposed by *Pseudomonas aeruginosa*.

METHODS

The research was conducted in the Molecular Genetics Laboratory, Department of Biology, Faculty of Science and Technology, Airlangga University, Mulyorejo street, Surabaya, East Java, Indonesia.

Polysaccharide krestin was obtained from *Coriolus versicolor* extract method, adapted from previous research, *Pseudomonas aeruginosa* (0.25 Mc. Farland) was attained from Balai Besar Laboratorium Kesehatan, Surabaya, East Java, Indonesia. Calculation of phagocytic activity and capacity used smear method with Giemsa dye.

This research used 30 adult female mice (*Mus musculus*) strain Balb/C, aged between 8 – 10 weeks, weighing between 30 – 40 g. The mice were obtained from Faculty of Pharmacy, Airlangga University and Veterinary center Surabaya (Surabaya, Indonesia). The mice went to aclimatization for a week. Completely randomized design was used with six groups and five repetition, namely control (K), positive control (K+), negative control (K-). It is treated with polysaccharide krestin before being exposed by *P. aeruginosa* (P1), by polysaccharide krestin after being exposed by *P. aeruginosa* (P2) and by polysaccharide krestin before-after being exposed by *P. aeruginosa* (P3). The mice were exposed to *P. aeruginosa* (1.5 x 10^8 cell/mL) twice intraperitoneally within two weeks gap from the first to the second exposure. 50 mg/kg BW of polysaccharide krestin was added by gavage in seven days. This research design is according to Wahyuningsih et al. (2016).

The mice were exposed to *P. aeruginosa* 0.2 ml intraperitoneally an hour before anesthetized by cloroform. Then, 3 ml EDTA liquid was injected intraperitoneally and it was homogenized with intraperitoneal liquid. Phagocyt was taken from cavum peritoneum. Intraperitoneal liquid was smeared on object glass and was dried. This smear was given methanol for fixation and colored with giemsa dye. The smear of phagocyt observed under microscope with 1000x magnification. According to Kusmardi (2007) phagocytic activity calculates active phagocyt cell from 100 cells and phagocytic capacity calculates a number of bacteria in active phagocyt cell.

All values were analyzed statistically using one way analysis of variances (ANOVA) to determine whether there were differences in the average of variable among treatments. If there were significant differences, Duncan test (α=0.05) was taken to determine the best treatment among the treatment groups.

RESULT AND DISCUSSION

Phagocytic activity

Phagocytic activity is a parameter which is used to determine how many phagocytes are capable of antigen phagocytosis. Observations on phagocytic activity were determined by the number of active phagocytes from 100 phagocytes which were observed on smear slide of intraperitoneal fluid (Kusmardi et al., 2007). According to thr result of the research (Figure 1.), the lowest average of the number of active phagocytes is on K group as control group (14.47±3.25 cells) and the highest average of the number active phagocytes is on P3 group which was given PSK before and after exposure to *P. aeruginosa* (26.47±2.36 cells).

This research data was not qualified to use one way analysis of variances (ANOVA). Because of that, this research data was analyzed using Brown-Forsyth test. According to Brown-Forsyth test, giving PSK affects phagocytic activity (α <0.05). The Games-Howell test as post hoc test showed differences among treatment groups (Figure 1).

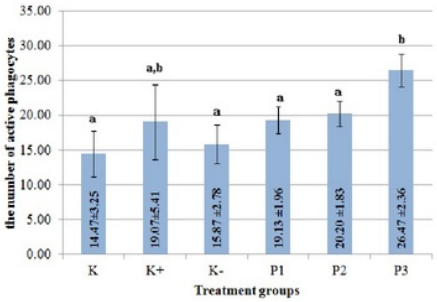

Figure 1. The average of phagocytic activity. Note: different letters in each section show significant differences.

Pseudomonas aeruginosa is an extracellular bacterium that has lipopolysaccharide (LPS) on the cell wall. LPS is one of pathogen-associated molecular patterns (PAMPs), which will be recognized by macrophages by LPS-binding protein (LBP), CD14 and TLR-4. Macrophages will phagocyt *P. aeruginosa*. The phagocytosis process results in fragment antigen of *P. aeruginosa* which will be presented by MHC class II and recognized by T CD4 + cells. The T CD4 + cells are activated and differentiated into active Th1 cells. It is Th1 cells that secrete IFN-γ and activate other macrophages to accelerate the elimination of antigens (Abbas, 2012).

Accroding to Figure 1, the average of K group is almost the same as the K+ group, because the two groups were not given bacteria exposure, thus the number of active phagocytes is low. As mentioned before, that LPS of *P. aeruginosa* causes activation of macrophages. The average of K- group is similar to the average of K and K+ group, because the bacteria did defense with phagocytes three times the exposure of *P. aeruginosa* (day 8, day 22, and 1 hour before collecting the intraperitoneal fluid). This is supported by Todar (2004), *P. aeruginosa* has alginate form of viscous gel that surrounds the bacterium to protect the bacteria from immunity.

The average of giving PSK groups, before exposure to *P. aeruginosa* (P1) and after exposure to *P. aeruginosa* (P2) showed that the average is not significantly different to controls, because the number of phagocytes is activated only by antigen. β-glucan in PSK does not really affect the activation of phagocytes in P1 and P2 group. The average of P3 group is the highest phagocytic activity and there is a significant difference to the other four groups (K, K, P1 and P2). This indicates that giving PSK before and after exposure to *P. aeruginosa* affects on phagocytic activity which will eliminate the bacteria. This is supported by Jang et al. (2009) that PSK has potential as a stimulator that can activate immunocompetent cells to increase the immune system.

Giving PSK is able to induce IFN-γ production that will stimulate macrophage activation. Increased levels of IFN-γ will also increase the number of active macrophages. According to Chui and Chisti (2003), polysaccharide krestin can cause physiological effect to increase immune response by inducing the secretion of IL-6, interferon, Ig-G, macrophages, and T lymphocytes. In addition, the activation of macrophages will cause the secretion of cytokines, such of TNF, IL-1, and chemokines. Those cytokins will lead to diapedesis cells of neutrophils to the infection area where the phagocytosis *P. aeruginosa* is (Abbas et al., 2012).

Polysaccharide Krestin is an immunomodulator that can stimulate the increase of immunocompetent cells. According to Chi-Fung et al. (2009), after β-glucan enters the body orally, a specific chain of β-glucan which is 1,3-β-glycosidic cannot be digested by the digestive system. Therefore the β-glucan structure will be intact. According to Hong et al. (2004) β-glucan which enters the body orally has resistance to acids, thus when it goes into gaster, the structure is not going to change, β-glucan in the intestines will contact with the macrophages on the wall of intestines

assisted by M cells (microfold), an unspecialized cell in the ileum. M cells will take β-glucan then macrophages will phagocyte β-glucan by Dectin-1 with the toll-like receptor 2/6 (TLR-2/6). Furthermore, β-glucan which is phagocyted by macrophages would degrade into fragments, and then is transported to the bone marrow where fragments of β-glucan of degradation will be released. The fragments will be captured by the receptor located on the surface of phagocytic cells such as neutrophils, macrophages, dendritic so that the phagocytic cells activated.

Phagocytic capacity

Phagocytic capacity is the ability of phagocytes to ingest and digest antigens and causing phagocytes become bigger than ever. According Handojo (2003), the phagocytic capacity calculate the number of bacteria *P. aeruginosa* within phagocyted by 50 active phagocytes. 50 active phagocytes observed on some of the view in a smear slide of intraperitoneal fluid. According result of research (Figure 2), the lowest average of the number of phagocyted bacteria is on K group (294.8±3.56) and the highest average of the number of phagocyted bacteria is on P3 group, which was given PSK before and after exposure to *P. aeruginosa* (426.8±12.15).

One way analysis of variances (ANOVA) statistical analysis showed that there was significant difference in the average of variable among treatments ($\alpha<0.05$). According to Duncan test, it resulted in Figure 2 (different letters showed significant differences). No significant differences were observed between K- group and P1 group. There was no significant differences between P1 group and P2 group. There were significant differences between K group and the others, between K+ group and the others, and P3 group and others. Figure 2 showed that the present of PSK after exposure (P2) and before-after exposure (P3) increased the number of bacteria in active phagocytes.

The increase in phagocytic capacity caused by the effectiveness of opsonization phagocyt cells. Opsonin is a molecule that binds the surfaces of microbes and can be recognized by the cell surface receptor of phagocytes such as macrophages and neutrophils thus improving the efficiency of phagocytosis (Baratawidjaja, 2006). One of the opsonins was recognized by Fcγ-R on phagocytes is IgG.

According to Abbas (2012), the efficiency of phagocytosis increase when microbes or antigens bind to macromolecules called opsonin. IgG structure has two distinct parts function. Two sections are Fab (fragment of antigen binding) that serves to bind antigen specifically and to recognize antigenic determinants on the antigen, the other part is called the Fc (Fragment crystallizable) that serves as an effector that can be recognized by cell-surface receptors Fc-R , IgG will bind to antigens on the Fab and the Fc portion of IgG will be recognized by phagocytic cells.

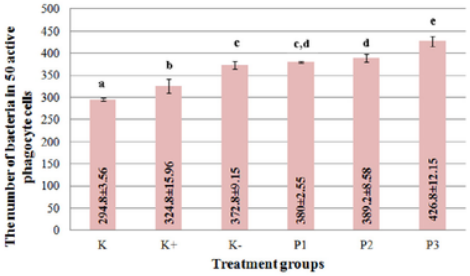

Figure 2. The average of the number of bacteria in 50 active phagocyt cells. Note: different letters in each section show significant differences.

The Fc portion will be recognized through Fc receptor-R on the surface of phagocytic cells which then gives a signal to the phagocytic cells to enhance the phagocytosis of these antigens. Phagocytic cells, such as macrophages are activated and digest the antigen (Baratawidjaja, 2006).

According to Koendhori (2008), the process of opsonization improve the ability of bacteria attachment by phagocytes. Based on Figure 2, the control group (K) showed the lowest average phagocytic capacity and was significantly different from the other treatment groups, because the K group was not given PSK and not exposed to bacteria. There is no opsonization process that can increase the number of bacteria which was phagocyted.

In the group of K and K +, although not given exposure to the bacteria *P. aeruginosa* on day 8 and the 22, group K and K + continued to show the process of phagocytosis, because of an hour before the intraperitoneal fluid collection, experimental animals have been given exposure to *P. aeruginosa*. Giving exposure to one hour before intraperitoneal fluid collection aims to stimulate the process of phagocytosis. According Ridconi et al. (2011), giving exposure to bacteria carried on for the intraperitoneal has many macrophages.

On the negative control group (K) values mean higher phagocytic capacity and differ significantly from the K and K+. This is due to the increase in bacterial opsonization. Increased opsonization caused the formation of specific immune responses, in particular antibody. Accor-

ding to Abbas (2012), the primary antibody in the immune response may develop within 5-10 days after exposure to the bacteria is given. K- group was given exposure 2 times (day 8 th and 22) so that antibodies formed then more than a group of K and K + were only given one hour prior to exposure to the fluid collection intraperintoneal.

In giving PSK groups (P1, P2, and P3) shows the average value of greater capacity than the control groups, group P1 did not differ significantly from the K- groups, but the average value of the group phagocytic capacity P1 is higher than group K-. It can be proved that the administration of PSK before being given exposure to bacteria can be used as a preventive by stimulating the body's immune response. The treatment group showed that an average value of capacity P2 was higher than P1 group. This suggests that the immune response increased after PSK exerted on individuals exposed to the bacteria. This shows that the PSK can be used as a curative or treatment.

The most effective time to give PSK is given before and after exposure to *P. aeruginosa*. This was evidenced by the average of the highest phagocytic capacity. The time of giving PSK before and after exposure to the bacteria was shown to increase phagocytic capacity. Giving PSK twice caused active compounds β-glucan to be more effective and more effectively enhance antibody modulates phagocytosis opsonization process. Therefore, the process of bacterial attachment easier. With the increasing opsonization process, the number of bacteria which was phagocyted would also increase.

CONCLUSION

It was concluded that giving polysaccharide krestin could influence phagocytic activity and capacity of intraperitonneal fluid exposed by *P. aeruginosa*. Giving polysaccharide krestin before and after exposure (P3) could increase phagocytic activity. Giving polysaccharide krestin after exposure (P2) and before-after exposure (P3) could increase phagocytic capacity. Based on the evidences, giving polysaccharide krestin before and after *Pseudomonas aeruginosa* exposure could increase both of phagocytic activity and capacity.

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