POLYSACCHARIDE KRESTIN ACTIVITY FROM *Coriolus versicolor* EXTRACT AGAINST PHAGOCYTOSIS ABILITY ON MICE INFECTED BY *Staphylococcus aureus*

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Abstract

Staphylococcus aureus is an opportunistic pathogen and cause of nosocomial infection, which has leucocidin and can reduce immunity. Because of antibiotic resistance, immunomodulator is an alternative treatment for S. aureus infection. Polysaccharide krestin (PSK) from Coriolus versicolor extract contains active β-glucan that triggers immune responses' effectiveness including phagocytosis. This research aimed to know the activity of PSK against phagocytosis ability on mice infected by S. aureus. There were six treatment groups. 100 mg/kg BW PSK was given to the mice strain Balb/C by gavage. S. aureus was infected once every two weeks. Phagocytosis ability consisted of phagocytic activity and capacity, counted on slide smears of mice intraperitoneal fluid. The results showed that PSK increased phagocytic activity in the group giving PSK after infection. Furthermore, the most effective giving PSK on phagocytic capacity was before and after infection. Based on this research, PSK increased immunity by phagocytosis ability and can be useful as an immunomodulator.

Keywords: *Polysaccharide krestin (PSK), phagocytic activity, phagocytic capacity*

1. INTRODUCTION

Staphylococcus aureus is an opportunistic pathogen that significantly caused morbidity and mortality and one of the leading causes of nosocomial infection. It has antibiotic resistance, especially Methicillin-Resistant *Staphylococcus aureus* (MRSA) which the number of death cases was more than the number of deaths due to AIDS in the USA [1]. This infection causes tissue damage and necrosis. Moreover, *S. Aureus* has exotoxin−leucocidin− against leukocytes and reduce immunity2. Naturally, all infectious diseases cause an immune response that inhibits bacterial transmission. During this time, *S. aureus* infection is only by giving antibiotics, increasing antibiotic resistance. Our body needs a particular compound to modulate the immune response as an alternative way to against *S. aureus* infection.

In Asian ccountries, mmushrooms are often used as medicine. *Coriolus versicolor* is one of the medicinal mushrooms consumed in the formed capsule, tea, and food additives [3]. Mycelium extraction results of *C. versicolor* contain active carbohydrates, polysaccharide peptide, and polysaccharide krestin (PSK). According to Cui and Chisti (2003) and Zhou *et al.* (2007), Polysaccharide krestin has many health benefits, including improving appetite and liver function, recovery from illness, immunostimulator, and immunosuppression [3, 10]. Physiological effects caused by polysaccharide krestin from *C. versicolor* were improving immune response by IL-6, IFN-γ, IgG, and activating of macrophages and T lymphocytes [3].

The active compound of PSK from *C. versicolor* is β-glucan in the fungal cell wall. It could stimulate phagocytosis and proinflammatory production to eliminate infectious agents [4]. Chan et al. (20009) also showed that β-glucan induced immunocompetent cells in clearing antigens. Dectin-I is a type II transmembrane receptor protein that binds β-1,3 and β-1,6 glucan to regulate the innate immune response. Targeted β-glucan immune cells included macrophages, neutrophils, monocytes, NK cells, and dendritic cells. So that natural and adaptive immune responses can be modulated by β-glucan and can play a role in opsonic and non-opsonic phagocytosis [4].

Furthermore, β-glucan stimulated neutrophil migration to the inflammation area and improved antimicrobe protein in acute infection rats [5].

According to Wahyuningsih et al. (2016), PSK improved immunity, significantly enhanced phagocytosis ability against *Pseudomonas aeruginosa* [6]. Another study also reported that PSK from *Coriolus versicolor* enhanced adaptive immunity due to *Neisseria gonorrhea* [7]. Nevertheless, studies of PSK activity from *C. versicolor* as an immunomodulator due to *Staphylococcus aureus* infection have not been reported yet. Therefore, this study aimed to know polysaccharide krestin activity from *Coriolus versicolor* growth in Indonesia as an immunomodulator against *Staphylococcus aureus* infection. This study only focused on innate immunity in mice, exactly on phagocytosis ability.

2. METHODS

Polysaccharide krestin was obtained from *Coriolus versicolor*. This mushroom looks like a fan; its surface looks like velvet, mushroom edges are white to yellow; The average molecular weight of krestin polysaccharides is around ± 94 kDa and contains 34-35% water-soluble carbohydrates [8,9]. The extract methods adapted from previous research. *Staphylococcus aureus* (0,25 Mc. Farland) was obtained from Balai Besar Laboratorium Kesehatan Surabaya. This research used 30 adult female mice Balb/C, 8-10 weeks, weight 30-40g. The animals were maintained in cages made of plastic at 20°C, with a 12-h light/12-h dark cycle, fed and watered by ad libitum. The mice acclimatized for a week.

The research was an experimental design and used a completely randomized design with six treatment groups (KN: Normal control; K+: Positive control, K–: Negative control; P1: PSK administration before infection; P2: PSK administration after infection; and P3: PSK administration before and after administration). PSK (100 mg/kg BW) was given at 1-7 days and 23-30 days by gavage. Mice were exposed to S. aureus (1,5 x 108 cells/mL) twice on the 8th and 22nd day. Mice were sacrificed on 31st day, and one hour before that mice were intraperitoneally injected with 0,2 mL of S. aureus suspension. 3 mL of 3% ethylenediaminetetraacetic acid (EDTA) was injected intraperitoneally as an anticoagulant and homogenized with intraperitoneal fluid. The intraperitoneal fluid was collected to be used to determine phagocytic activity and capacity.

The intraperitoneal fluid was smeared on glass slides and air-dried. Methanol was used to fix the smear for 15 minutes, then stained with Giemsa solution for 20 minutes. Phagocytic activity was determined by counting the number of phagocytes in a population of 100 phagocytes. Phagocytic capacity was determined by counting the number of bacteria in the 50 phagocytes.

Statistical analysis performed by one-way analysis variance followed by Duncan's test. $P < 0,05$ was considered statistically significant. All analysis performed using SPSS Statistic 16 software for windows. The results reported as the mean ± standard deviation of five repeats.

3. RESULT AND DISCUSSION

Phagocytic activity

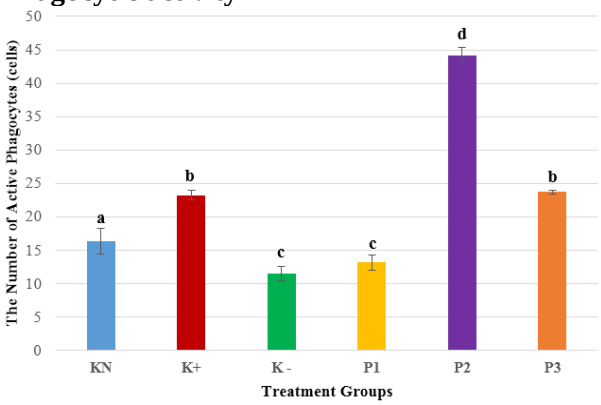

Figure 1. Effect of polysaccharide krestin on several active phagocytes (cells). KN=Normal control; K+=Positive control; K−=Negative control; P1=Administration of PSK before infection; P2=Administration of PSK after infection; P3=Administration of PS before and after infection. Different letters in each section show significant differences ($p < 0,05$).

Phagocytic activity is used to determine the number of active phagocytes in phagocytic antigen [6]. Based on the results

(Figure 1), phagocytic activity was significantly increased in the K+ group, and P2 group and P3 group compared to the normal control group and negative control group ($p < 0,05$). The highest phagocytic activity was shown by the P2 group (44,13±1,18 cells). Meanwhile, the lowest phagocytic activity was the negative control group (11,53±1,02 cells). The difference between active phagocytes and non-active phagocytes can be seen in Figure 2.

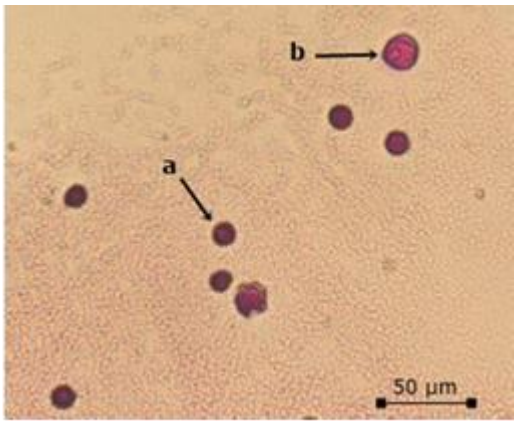

Figure 2. Non-active phagocyt (a), active phagocyt (b) with Giemsa staining

There was no enhancement of phagocytic activity in the negative control group, likely because of phagocytic cell lysis together with bacterial lysis. Neutrophils can eliminate antigens faster after the first contact, about 39 minutes for migration to tissue and lyse antigens12. Furthermore, low phagocytic activity due to protein A found in *Staphylococcus aureus* bacterial cell walls that inhibit phagocytosis [2]. Polysaccharide krestin administration without bacterial infection increased the number of immunocompetent cells. According to Jang et al. (2009), PSK administration had the potential as an immunomodulator, which activated immunocompetent cells to improve immunity [13]. The enhancing immunity was intended as prevention due to the entry of antigen.

The active compound of PSK, β-glucan, is one of the biological response modifiers and immunomodulators that restore disturbed immunity [11]. The enhancement of phagocytic activity on the P2 group was probably affected by increasing pattern recognition receptors (PRRs) signaling so that phagocytes can immediately detect antigens. Phagocytosis also increased cytokines and chemokines that stimulated other phagocytes coming to the infection site [10].

Phagocytic Capacity

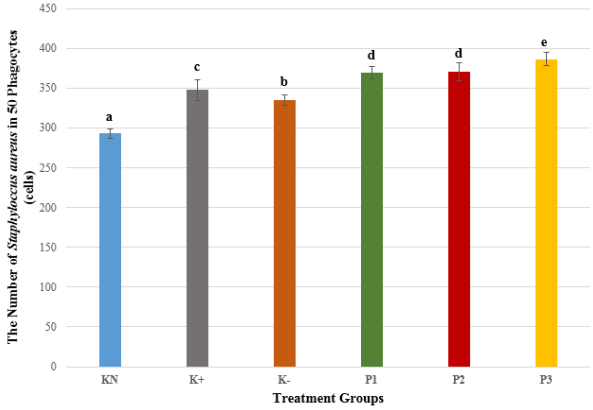

Figure 3. Effect of polysaccharide krestin on phagocytic capacity (cells). KN=Normal control; K+=Positive control; K−=Negative control; P1=Administration of PSK before infection; P2=Administration of PSK after infection; P3=Administration of PS before and after infection. Different letters in each section show significant differences ($p <0,05$).

Phagocytic is used to determine phagocytes' ability to ingest and digesting antigens that cause phagocytes to enlarge [6]. The phagocytic capacity observation counted the number of bacteria in 50 phagocytes in an intraperitoneal fluid smear [14]. According to the results (Figure 3), phagocytic capacity was significantly increased in the positive control group (K+) compared to the normal control group and negative control group ($p < 0,05$). The highest number of phagocytic capacity was shown by the P3 group (386,27±8,23 cells). All PSK treatment groups were shown enhancement in the number of phagocytic capacity. The active phagocytes, which are ingesting *Staphylococcus aureus*, could be seen in Figure 4 .

All PSK treatment groups were shown that PSK has the effect of increasing phagocytic capacity in eliminating antigens. Enhancement of phagocytic capacity due to

the effectiveness of the opsonization. Opsonin is a large molecule where is bound to the surface microbes and is recognized by phagocytic receptors. The efficiency of phagocytosis increased when the microbes bound to macromolecules called opsonin, which is recognized by phagocytic receptors [15,16]. Active compound β-glucan increased opsonin and non-opsonin phagocytosis [4]. Opsonization improved the ability of phagocytes to ingest bacteria [17]. Hence, PSK administration twice caused β-glucan more effective to improve activation and modulation of phagocytic opsonization.

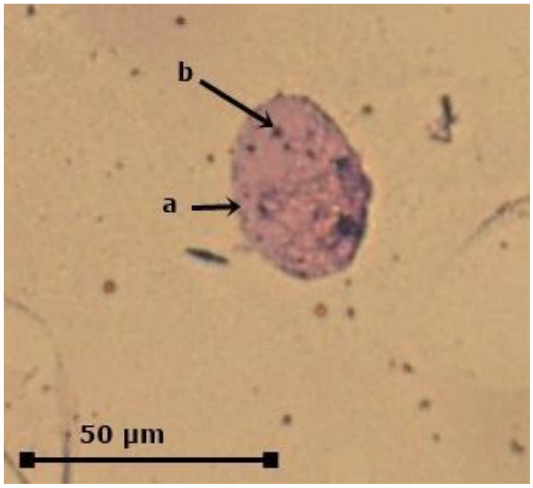

Figure 4. Phagocytes ate some *Staphylococcus aureus* bacteria with Giemsa staining (a. phagocytes; b. *Staphylococcus aureus* bacterial cells).

β-glucan has resistance to acids in the stomach so that the structure remains intact. In the intestinal wall, β-glucan binds to microfold cells (M cells) and intact it through pinocytosis, then brought to other immunocompetent cells, such as T cells, B cells, and others [18]. β-glucans degraded into fragments, then transported to the bone marrow and released to be captured by the receptor complement (CR3), located on the surface of granulocytes, monocytes, and dendritic cells [4]. Therefore, β-glucans can be used as an immunomodulator to eliminate and to stop the spread of bacteria. Phagocytosis of *Staphylococcus aureus* can be seen in Figure 6.

4. CONCLUSION

Based on this study, we conclude that polysaccharide krestin from *Coriolus versicolor* increased phagocytic activity and phagocytic capacity as a response to *Staphylococcus aureus* infection. This study suggests that PSK from *Coriolus versicolor* could act as a useful compound to modulate the immune response (imunomodulator).

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