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Polysaccharide Krestin Activity from *Coriolus versicolor* on Antibody Titer of Mice Exposed *Staphylococcus aureus*

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Abstract – (The best know commercial polysaccharopeptide preparations of *Coriolus versicolor* are polysaccharopeptide krestin (PSK). One of the most important functions of PSK is their immunomodulatory actions. The purpose of this study was to analyze the activity of polysaccharides krestin on antibody titer in *Mus musculus* exposed to *Staphylococcus aureus*. Polysaccharide krestin was fractionated and precipitated with 90% ammonium sulphate. Polysaccharide krestin was given on the mice strain Balb/C. There was six treatment groups: (K) control, without adding PSK and without was exposure to *S. aureus*, (K +) positive control, adding PSK, (K -) negative control, exposure to *S. aureus*, (P1) adding PSK before exposure to *S. aureus*, (P2) adding PSK after exposure to *S. aureus*, and (P3) adding PSK before-after exposure to *S. aureus*. Polysaccharide krestin dose was 50 mg/kg bw administered for 7 days via gavage. Exposure to *S. aureus* done 2 times with an interval of 2 weeks via intraperitoneal. Antibody titer were measured by ELISA. Data were analyzed by descriptive. The results showed that the polysaccharide krestin increased the antibody titer on P1. Polysaccharides krestin could stimulate the immune response resulting from exposure to *S. aureus*. Polysaccharides krestin can be useful as immunomodulator).

1. INTRODUCTION

Staphylococcus aureus is the extracellular bacteria that live in humans, such as respiratory tract and cutaneae. The infection will occur when the immune response is down, for example, there are hormonal changes; illness, injury, use of steroids or drugs that affect immunity. The bacteria produce enzymes, protein A, and toxins that can protect bacteria from phagocytosis and cause hemolysis [1]. Bacteria cause the skin infections, acute inflammation by toxins, and cell death caused by pore-forming toxins [4]. The extracellular bacterial internalization by the APC as macrophages, dendritic cells, B cells in association with MHC II. CD4 + T cells respond to these associations and produce cytokines. Cytokines can stimulate the production of antibodies, induces local inflammation, increase phagocytosis, and activates macrophages [4].

Coriolus versicolor is medicinal mushroom used in Japan, China, Korea and other Asian countries. *Coriolus versicolor* has antimicrobial, antiviral, anti-tumor, and stimulatory effects on the immune system properties. It is called a biological response modifier (BRM)[2]. In vitro, yeast extract *C. vesicolor* effectively stimulate the activity of T lymphocytes, B lymphocytes, monocytes / macrophages, bone marrow cells, NK cells, and killer cells depends lymphocytes. Mushrooms also stimulate proliferation and or production of antibodies and a variety of cytokines such as IL-2, IL-6, intreferon, and TNF [6].

Polysaccharide krestin is extraction product of *Coriolus versicolor*. The active ingredient is a polysaccharide (-glucan). Polysaccharides are arranged in combination with krestin protein. The combination known as polysaccharide krestin (PSK) [2]. Powdered polysaccharide krestin contain 34 – 35 soluble carbohydrate (91 – 93% *β*–glucan), 28 – 35% protein, 7% moisture, 6 – 7% ash, and the reminders are free sugars and amino acids [3]. PSK has a physiological activity include immunopotentiating by inducing the production of interleukin-6 (IL-6), interferon and immunoglobulin-G, suppress the immune response (immunosuppression), increased appetite and improve liver function, calm the central nervous system, and increases the pain threshold [3,4]. The purpose of this study was to analyze the activity of polysaccharides krestin on antibody titer in *Mus musculus* were exposed to *S. aureus*.

2. METHODS

2.1 Production of *C. versicolor* extract, isolation, and purification of PSK

Mushrooms of C. versicolor *was washed with water, then air-dried. Mushrooms were cut into small pieces and put in oven at 40^{0}C for 24 hours. Mushrooms were mashed into a coarse powder. Coarse powder were made*

by the method extracts of Wahyuningsih [7,8]. Mushroom extract were precipitated with 90% ammonium sulphate. In this process produced rough polysaccharopeptide. Further duluted with PBS solution. The suspension was dialyzed for 24 hours. This process would produce a solution of PSK. PSK concentration was measurement with the phenol sulfuric acid assay.

2.2 Isolation and propagation of *S. aureus*

Staphylococcus aureus was purchased from Balai Besar Laboratorium Kesehatan, East Java, Indonesia. Bacteria were grown on Mc. Concey media at room temperature. Subsequently, the bacteria were grown in liquid media as much as 20 mL for 1 days. Bacteria were harvested. Liquid medium containing the bacteria were centrifuged at 3000 rpm for 10 minutes. Supernatant was discarded, while the pellet of bacteria were used to exposure the experimental animals. Bacterial pellet was dissolved in physiological saline solution until the solution contains as many as 0.25 Mc. Farland.

2.3 Treatment of PSK and exposure to *S. aureus* in experimental animals

Adding PSK with a concentration of 50 mg/kg bw conducted for 7 consecutive days by gavage. Exposure to *S. aureus* was as much as 2 times with an interval of 2 weeks via intraperitoneal. There were 6 treatment groups: control, without adding PSK and without exposure to *S. aureus* (K), the positive control, adding PSK (K+), negative control, exposure to *S. aureus* (K-), adding PSK before exposure to *S. aureus* (P1), adding PSK after exposure to *S. aureus* (P2), and adding PSK before- after exposure to *S. aureus* (P3).

2.4 Blood sampling, serum isolation, and measurement of antibody titer

After one week of the last adding PSK, heart blood of experimental animals were taken. Blood was left at room temperature for 2 hours. Further blood serum was isolated by centrifugation at 3000 rpm for 10 minutes.

Antibody titer were measured by ELISA Kit. A total of 100 µL of bacteria that had been sonicated in coating the cup plate 96 wells and was incubated at 4°C, 24 hours. Furthermore, blocking with 10% BSA solution of 200 µL and was incubated 15 minutes at room temperature. Each of the wells was added 100 µL of primary antibody. Antibodies derived from serum were diluted 2^1 to 2^8 and was incubated at room temperature for 1 hour. Then was washed with 200 µL wash solution. Each of the wells was added 100 µL of goat anti-mouse IgG conjugated with a concentration of 1 µg/ml and was incubated 1 hour at room temperature. Then was washed with 200 µL of the wash solution repeated three times. Each of the wells was added 100 µL of a substrate consisting of ABTS and peroxidase solution B in the ratio 1: 1. Each of the wells was added 100 µL stop solution. OD value of antibodies were measured by microplate reader, a wavelength of 405 nm.

2.5 Statistical analysis

Data were analyzed descriptively

3. RESULTS AND DISCUSSION

Antibody titer was a laboratory test that measures the presence and amount of antibodies in blood. The antibody level in the blood was a reflection of past exposure to an antigen or to something that the body does not recognize as belonging to itself. The body used antibodies to attack and remove foreign substances.

An antibody titer was a measurement of how much antibody an organism that recognized a particular epitope, that was expressed as the inverse of the greatest dilution that still gives a positive result. ELISA was a common means of determining antibody titers. Antibody titers resulting from exposure to *S. aureus* could be seen in Table 1 and Figure 1.

Table 1 (Antibody titer after administration of Polysaccharides krestin on mice due exposure to *S. aureus*)

Treatment	Antibody titer on dilution								Mean of antibody titer (OD value)
	2^1	2^2	2^3	2^4	2^5	2^6	2^7	2^8	
K	0.063	**0.071**	0.068	0.066	0.065	0.061	0.058	0.053	2^2 (0,071)
K+	0.075	**0.082**	0.078	0.072	0.065	0.059	0.054	0.074	2^2 (0,082)
K-	0.183	0.207	**0.167**	0.136	0.127	0.125	0.124	0.119	2^3 (0,164)
P1	0.191	0.170	0.160	**0.147**	0.112	0.097	0.086	0.077	2^4 (0,147)
P2	0.128	0.119	0.109	0.094	0.093	0.080	0.066	0.059	$< 2^1$ (0,128)
P3	0.195	0.161	**0.163**	0.126	0.108	0.077	0.089	0.078	2^3 (0,163)

Information: (K) control, (K +) positive control, (K -) negative control, (P1) PSK administration before exposure to *S. aureus*, (P2) PSK administration after exposure to *S. aureus*, and (P3) PSK administration before-after exposure to *S. aureus*.

Antibody titers in the control (K) was 2^2 (OD value = 0.071), while the antibody titer in the positive control (K+) was 2^2 (OD value = 0.082). OD values at K be the benchmark for other treatment. If other treatment OD value was more than 2 times the OD value at K (OD value > 0.142), it can be said that the antibodies recognize antigens still positive. On the negative control (K-), the antibody titer was 2^3 (OD value = 0.164). The antibody titer of P1 was 2^4 (OD value = 0.147). The antibody titer of P2 was $< 2^1$ (OD value = 0.128). The antibody titer of P3 was 2^3 (OD value = 0.163).

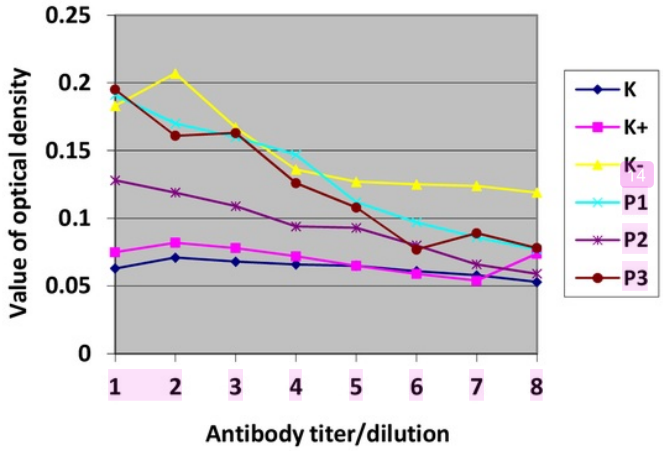

Figure 1 (Antibody titer after administration of polysaccharides krestin on mice due exposure to *S. aureus*)

Antibodies were secreted by cells of the adaptive immune system (B cells), and more specificaly, differentiated B cells called plasma cells. A control group and a positive control produced a low OD value. This happens because there was no bacterial infection, so as not to cause an immune response. If there was no exposure to the antigen, the antibody titer was low, ie the value dilution 2^2.

On the negative control group, the higher the antibody titer by diluting the value of 2^8. This shows that the antibodies were formed if there was exposure to antigen. Exposure to *S. aureus* boosted the immune response. According to [4], humoral immunity is the principle protective immune response against extracelluler bacteria, and it function to eliminate the microbes and neutralize their toxins.

An antibody is used by the immune system to identify and neutralize pathogens such as bacteria and viruses. The antibody recognizes a unique molecule of the harmful agent, called an antigen, via the variable region. An

antibody contains a paratope (analogous to a lock) that is specific for one particular epitope (similarly analogous to a key) on an antigen, allowing these two structures to bind together with precision. Using this binding mechanism, an antibody can recognize a microbe or an infected cell for attack by other parts of the immune system, or can neutralize its target directly (for example, by blocking a part of a microbe that is essential for its invasion and survival) [4].

Adding polysaccharides krestin increased the antibody titer in group P1, ie dilution 2^4. Higher antibody titers than the K- (2^3). It showed that PSK stimulated an immune response becomes even higher. The high titer antibodies caused neutralization of *S. aureus* bacteria. The antibody titer on group P2 decreased, ie dilution < 2^1. The antibody titer on group P3 was same with K-.

P1 treatment showed the highest antibody titer compared to other treatments. This proves that the PSK could be used as a preventive encourage the formation of antibodies. PSK given before exposure could enhance the immune response both specific and non-specific. According to [10], B-glucan is more effective for the prevention and treatment of diseases associated with the immune system of the body.

P2 treatment showed lower antibody titers compared to the negative control. This showed that PSK administered after exposure to further trigger the non-specific immune response, which was to increase phagocytosis by phagocytes. Phagocytic function was to kill the bacteria. When the process of phagocytosis of bacteria effectively and so many had died, then the specific immune response was reduced.

P3 treatment showed antibody titres equal to the negative control. This showed that the PSK was given before-after exposure acts to suppress specific immune response, so the antibody is not high.

In some in vivo animal studies, CV extract was observed to display a broad spectrum of antibacterial and antifungal activities against common pathogens such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococus aureus, Candida albicans*, *Klebsiella pneumoniae, Listeria monocytogenes*, and *Streptococus pneumoniae* [9].

The results showed that polysaccharide krestin given before exposure *S. aureus* increase antibody titer in mice. can stimulate the spesific immune response resulting from exposure to *S. aureus*

4. CONCLUSION

It was concluded that adding polysaccharide krestin could influence antibody titer of mice serum exposed by *S. aureus*. Adding polysaccharide krestin before exposure *S. aureus* could increase antibody titer. Polysaccharides krestin could stimulate the spesific immune response resulted from exposure to *S. aureus*.

5. REFERENCES

1. M.T. Madigan, Martinko J.M, Dunlap P.V, Clark D.P. 2008. Biology of Microorganisms 12th edition. San Francisco.
2. K.F. Cheng, dan Leung, P.C., 2008. General review of polysaccharopeptides (PSP) from *Coriolus versicolor*. *Cancer Therapy*, 6: 117-130.
3. J. Cui, dan Chisti, Y. 2003. Polysaccharopeptides of Coriolus vesicolor: Physiological Activity, Uses, and Production. Biotechnology Advances. 21: 109-122.
4. A.K.. Abbas, A.H. Litchtman and JS. Pober. 2003. *Cellular and molecular immunology,* fourth edition. W.B. Saunders Company.
5. V. Zho, H. Jiang, J. Lin, and Tang. 2007. Cytotoxic activities of Coriolus versicolor (Yunzhi) extracts on human liver cancer and breast cancer cell line. National Library of Medicen, CurrMedChem (on line). *http://www.ncbi.nim.nih.gov:80/entroz/*. 2007
6. K.K.W. Chu, S.S.S. Ho, PharmD, and A. H. L. Chow. 2002. Coriolus versicolor: A Medicinal Mushroom with Promising Immunotherapeutic Values.
7. S.P.A.Wahyuningsih, Darmanto W., Wiqoyah N. 2009. Bioaktivitas polisakarida krestin dari ekstrak jamur *Coriolus versicolor* sebagai imunomodulator respon imun akibat infeksi *Mycobacterium tuberculosis*. Lembaga Penelitian Universitas Airlangga,
8. J. Cui, K.K.T Goh, R. Archer, H. Singh. 2007. Characterisation and bioactivity of protein-bound polysaccharides from submerged-culture fermentation of *Coriolus versicolor* Wr-74 and ATCC-20545 strains. *J. Ind. Microbiol. Biotechnol.*, 34: 393–40.
9. K.K.W. Chu, SSS. Ho, PharmD, and A. H. L. Chow. 2002. Coriolus versicolor: A Medicinal Mushroom with Promising Immunotherapeutic Values. *Journal of Clinical Pharmacology,* 42:976-984.
10. P. Pietro, 2003. Composition for preventif and/or treatment of lipid metabolism disorder and allergic form. *http://freepatent online.com*. Diakses pada tanggal 24 Mei 2015.

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