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IMMUNOSTIMULANT POTENTIAL OF OYSTER MUSHROOM *(Pleourotus ostreatus)* NUGGET

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ABSTRACT

Oyster mushrooms are widely known as having high beta glucan content. Beta glucan is a water insoluble fiber that indicates an immunomodulator with increasing the activity of NK cells in the bloodstream which provides immune effect. Oyster mushroom nuggets are processed oyster mushrooms that can be proven containing the highest beta glucans. This study aims to observe the immunostimulant potential of oyster mushroom nuggets in BALB/c mice by analyzing its white blood cell profile, spleen and liver histopathology. The research method is conducted through orbital sinus of the rat's eye using a microhematocrit to count total leukocytes, lymphocytes, and granulocytes with the Delayed Type Hypersensitivity test. The 18mg/kg body weight oyster mushroom nugget can increase lymphocytes cells 7.2 ± 1.22 10^3/µl. It is significantly different from control group. Immunostimulatory activity also was proven with the widening of the white pulp of the spleen with the growth of young lymphocytes after being given oyster mushroom nuggets of 18 mg/kg body weight.

Keywords: Beta glucan, Immunostimulant, Oyster Mushroom Nugget

INTRODUCTION

White oyster mushroom *(Pleourotus ostreatus)* contains insoluble fiber, namely Beta-glucan (β-glucan) which is widely obtained from stems and fruit bodies (Chang & Wasser, 2012). Beta-glucan indicates activity as immunomodulator, antitumor (Sari et al., 2016), antiviral (Zhang, Cui, Cheung, & Wang, 2007) and hepato-protective effects (Wasser, 2014). Consuming 200 mg of Beta-glucan for 90 days is effective to reduce symptoms of fever and flu related to the respiratory tract and can increase the activity of NK cells in the bloodstream which provides immune effect (Bergendiova K et al, 2011; Pricilla degraff et al, 2018). Beta-glucan will stimulate the immune system in the digestive tract and produce pro-inflammatory metabolites that will activate immune cells such as T cells (Pricilla degraff et al, 2018).

The highest beta glucan content in processed products will be tested with in vivo method using BALB/c mice to determine its immunostimulant potential. Immunostimulants are substances/compounds that can increase the activity of the body's immune cells (Sari et al., 2016). Nugget is a kind of food product that is very popular in the community. Beta glucan contents have been tested in some processed oyster mushrooms and processed nuggets are proven to have the highest beta glucan content among meatballs, noodles, and shredded oyster mushrooms. The beta glucan content of oyster mushroom nuggets reaches 74% per 100mg of oyster mushrooms (the data has not published yet). In this study, immunostimulant potential of oyster mushroom nuggets is observed by studying the activity of white blood cells and enlargement of the white pulp of the spleen organ.

MATERIALS AND METHODS

Material

Materials used for the experiments were fresh oyster mushrooms, chicken, beef, wheat flour, tapioca flour, bread flour, onion, garlic, onions, sugar, salt, candlenut, lemongrass, galangal, bay leaf, egg, pepper, butter, flavoring, and instant milk powder. All ingredients were bought from the traditional markets in East Surabaya. To test the potential for immunostimulants, mice (*Mus musculus*) of the BALB/c strain, aged 2 months, weighing 20-30g, were used.

Tools

The equipment for making the product was a digital scale (OHAUS), stove (kirin), panic (Maspion), knife (Maspion), shieve shaker and 80 mesh sieve, oven (kirin), Philips blender, Philips food processor, Philips rice cooker, Philips refrigerator), freezer (up to -20 °C), frying pan, fork, cutter, vortex and centrifuge.

Research Design

1. **Making oyster mushroom nuggets**
   Weigh 200 grams of oyster mushrooms as the main ingredient, blanch for 10 minutes, then drain and grind with a food processor.
   Weigh 1.5 kg of cassava as a filler. Peel, wash, and steam the cassava for 45 minutes. Prepare 1 gram of salt, 1 gram of pepper powder, 5 grams of chicken flavoring, 7 grams of minced garlic, 17 grams of chopped onions, 10 grams of instant milk powder, 20 grams of butter, and 1 egg as supporting ingredients. Mix the all ingredients, then steam the dough in aluminum foil for 30 minutes. The dough is chilled and then stored in the freezer. Egg whites and breadcrumbs is then prepared to coat the dough. The nuggets then dipped in the egg white followed by the breadcrumbs, and fried the nuggets until it becomes golden brown.

2. **Mice weight measurement**
   On mice with the age of 4 weeks, weight of the mice was measured on day 0, 6, and 14. The measuring process were performed by using a digital scale and repeated 3 times.

3. **Leukocyte profile analysis**
   The mice were acclimatized for 6 days and then the extract was induced for 14 days at a dose of 18mg/kg body weight, using the conversion of Bobovcak *et al* (2010). The weight of the mice will be measured on day 0, 6, and 14. The administering of the extract was stopped on the 15^{th} day. At that time, blood was taken from the orbital sinus of the rat's eye using a microhematocrit and placed in a tube with EDTA added. Blood cell analyzer (Medonic-M series) was used to count total leukocytes, lymphocytes, and granulocytes with the Delayed Type Hypersensitivity test (Sari, 2016). Also on the 15^{th} day, the mice were injected with intraperitoneal euthanasia, then the spleen and liver were taken for microscopic observation (organ hispathology). Spleen hispathology preparations were then observed under a microscope

4. **Histopathological analysis of the spleen**
   This analysis aims to study the activity of immune cells in the spleen organ, especially in the white pulp area. The first step is to conduct necropsy on the mice through intraperitoneal euthanasia under anesthesia by using chloroform. The spleen was then fixed and soaked in 10% formalin buffer for 24 hours. Furthermore, the spleen was stained with Hematoxylin-Eosin (HE) and observed under a microscope with a magnification of 100 µm to inspect the white pulp.

An increase in the number of leukocytes and lymphocytes of mice during the immunostimulation process with oyster mushroom nuggets indicated that the product was able to trigger the proliferation of white blood cells in the hematopoietic tissue. White blood cell is a cellular defense against pathogenic and foreign infections.

Research Stages

Immunostimulatory potency testing
Evaluation

The immunostimulant effect of betaglucan on the best processed product was studied in the BALB/c mice by referring to Bobovcak *et al.,* (2010) with a slight modification. According to Sari (2016), healthy mice was divided into 2 groups and each group contained 17 mice according to Federer's calculations in Sari (2016), with a dose conversion table in accordance with Laurence and Bacharach (1964) in Sari (2016). Parametric and non-parametric data were then obtained on organoleptic physicochemical tests and beta glucan content with two-way ANOVA and continued with Tukey test if significant (P value < 0.05). Meanwhile, non-parametric data would be tested using Kruskall Wallis. In vivo test will be executed by using t-test and all statistical data will be tested using SPSS rev 2019.

Analysis Procedure

Product with the best treatment was then tested for the betaglucans contained in vivo (Bobovcak *et al.,* 2010; Sari, 2016), hispathology of the lymph (Panjaitan *et al.,* 2007), total leukocytes, lymphocytes, and granulocytes with the Delayed Type Hypersensitivity test (Sari, 2016).

RESULTS AND DISCUSSION

Oyster mushroom (*Pleurotus ostreatus*) has been known to have special properties and benefits, including being able to increase the immune system which can act as an immunomodulator (Widyastuti *et al*., 2015). The immunomodulatory activity of beta glucan can be determined by looking at its effect on the induction of human lymphocyte proliferation. A compound is confirmed to have immunomodulator properties if it is able to induce lymphocyte proliferation. Lymphocytes are part of white blood cells and the results of increasing the number of lymphocytes are presented in Table 1.

In Figure A, the histopathology of the white pulp of five control mice with its lymphocyte cells arranged relatively dense looks normal. According to Matheos *et al*. (2013) a normal spleen is indicated with a red pulp consisting of macrophage cells, plasma cells, and blood elements, and a white pulp consisting of lymphocytes that are densely packed inside, and a central artery in the middle.

Meanwhile in Figure B, the white pulp in the spleen of mice treated with 18mg/kg oyster mushroom nuggets in ponds shows a stretch in the white pulp. This is because the germinal center in the white pulp is active and enlarges when there is immunostimulant of the immune system. This germinal center contains mature, active lymphocyte cells (Sapto, 2011). The growth area at the centrum germinativum is clearly visible with a lot of empty space and young cells. This empty space is for the growth of young lymphocytes. If the appearance of the white pulp is still loose, it means the centrum germinativum is visible. Stimulation of the white pulp will form the centrum germinativum (Underwood, JC 1999).

Table 1. The average number of leukocytes, lymphocytes, and blood granulocytes of control and treatment mice

No	Treatment of mice	Leukocytes (10^3/µl)	Granulocytes (10^3/µl)	Lymphocytes (10^3/µl)
1.	Control	6.06 ± 2.01	0.43 ± 0.11	5.48±1.89
2.	Nuggets 18mg/kg	7.80 ± 1.32	0.36 ± 0.03	7.2 ± 1.22

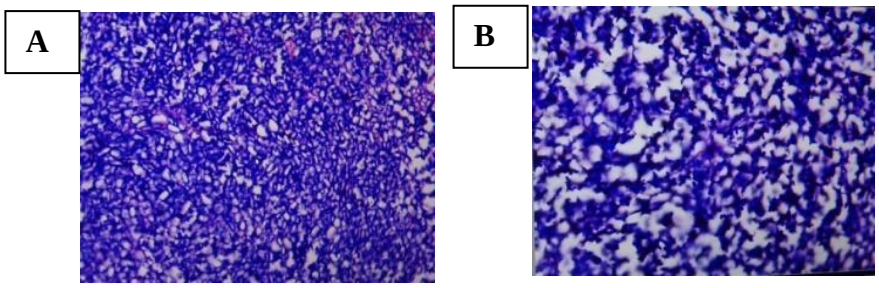

Figure 1. Histopathology of white pulp in the spleen of control mice (A) and treated mice (B)

This proves that the administration of oyster mushroom nuggets makes the white pulp stretched and widened due to the growth of new lymphocytes as the product's immunostimulant activity. The response of the spleen when receiving beta-glucan stimulation is when it enters GALT cells (gut-associated lymphoid tissue), epithelial cells (IECs) received beta-glucan directly with PPRs receptors (Peroxisome Proliferator Receptors) that bind to beta-glucans, in addition to other cells. M beta-glucan binds to the toll-like receptor (TLR2) (Lee YJ, Paik DJ, Kwon DY*, et al.,* 2017; Priscilla de Graff, 2018). PPRs have two intracellular receptors, namely RIG-I like receptor and NOD-like receptor, whereas on the plasma membrane they have receptors for beta-glucan TLRs and C-type lectin-like receptor. Dectin I is part of the PPRs receptor that will respond to beta-glucans from the mushroom and barley groups. Macrophage cells are also the cells that most frequently respond to beta-glucan from the fungus group (pullulan) (Bergendiova and Tibenska E., 2011).

CONCLUSION

Oyster mushroom nuggets can be potentially used as immunostimulants. Immunostimulatory activity has been proven with the widening white pulp of the spleen organ and the increasing leukocyte profile (lymphocytes and granulocytes) of mice (*Mus musculus*).

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