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The Improvement of TNF- α and Inflamation Cell on the Lungs Hypersensitivity Reaction becauses of Wood Dust Exposure

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ABSTRACT

This research aimed to analyze the effect of wood dust on TNF-α on Mice blood (MusMusculus) BALB / C and to analyze the influence of wood dust on the number of inflammatory cells, ie macrophage, lymphocyte, neutrophil and mast cell in lung tissue of mice BALB / C, as well as explain the mechanism of lungs hypersensitivity reaction due to wood furniture dust exposure. The experimental laboratory research was conducted, and mice was become the animal experiment. The research design used was Randomized the post-test only control group design. Exposure was performed 4 hours a day with 2 hours length of exposure in the morning with 1 hour rest period in 6 times a week for 4 weeks at exposure space in a glass-shaped cube with a size of 20x20x20 cm^3. Concentrations of wood furniture dust exposed were 0.50 mg / m^3, 0.75 mg / m^3, 1.00 mg / m^3. The findings showed that: (1) wood furniture dust exposure increased between experimental group compared with control group in mice (Musmusculus) BALB / C; (2) wood furniture dust exposure increased TNF, in lung tissue between experimental group compared with control group in mice (Musmusculus) BALB / C; and (3) wood furniture dust exposure increased mast cells, neutrophils, lymphocytes, and macrophages in lung tissue between experimental group compared with control group in mice (Musmusculus) BALB / C.

Keywords: Hypersensitivity pneumonitis (HP), TNF-α, Inflamation cell, Dust, Paddy Milling

INTRODUCTION

Background

The main problem in working health of wooden furniture craftsmen is respiratory disorder. The results of preliminary research conducted by researchers on the content of wood dust and respiration complaint from wooden furniture craftsmen in Pasuruan, involving 100 craftsmen indicated that almost all craftsmen had respiratory complaints that were 92 craftsmen, and only 8 craftsmen who did not have respiratory problem. From 92 craftsmen who had respiratory complaint, it was revealed that craftsmen who had respiratory complaints with light rate were 46 craftsmen (50.0%); moderate complaints were 28 craftsmen (30.4%); and heavy complaints 18 craftsmen (19.6%). The measurement results of wood dust content in five wooden furniture craftsmen showed high dust level, i.e. 3.2212 mg / m^3, 3.4230 mg / m^3, 4.731 mg / m^3, 3.4121 mg / m^3, and 4.3723 mg / m^3 when it was compared with the Threshold Value (NAB) of only 1.00 mg / m^3 [(1)].

The effect of wood furniture dust not only has caused a lung physiology abnormality in the exposed craftsmen, but also has leaded to hypersensitivity reaction that harmed the craftsmen's lung tissue, and ultimately threatened the craftsmen's life. Based on the investigation conducted by researchers in preliminary study of wood furniture dust, it was stated that wood furniture dust was organic dust containing microbiological organisms, such as bacteria and fungi spores, and there was low-molecular agents. Moreover, diseases caused by hypersensitivity reaction of wood dust include hypersensitivity pneumonitis, occupational asthma, hypersensitive pneumonitis (extrinsic allergic alveolitis), granuloma, interstitial, bronchiolar, and alveolar-filling lung diseases[(2),(3)].

Hypersensitivity pneumonitis is an inflammatory reaction because of an inadequate immune response to an inhaled antigen that can cause shortness of breath, certain lung damage, interstitial seepage, due to the accumulation of large amounts of activated lymphocytes in the lungs[(2)]. Hypersensitive pneumonitis (HP) is also known as extrinsic allergic alveolitis, consisting of inflammation, a granulomatous spectrum, interstitial bronchiolar, and alveolar-filling lung disease, resulting from persistent inhalation of organic antigens or particles or chemicals of low molecular weight[(3-5)].

Hypersensitivity reaction is a pathologic immune reaction, caused by excessive immune responses that cause damage to body tissues. Hypersensitivity reactions can also be generated as an uncontrolled reaction to foreign antigens such as microbes and non-infected environmental antigen. In hypersensitivity reactions, it is divided into four types of hypersensitivity based on the velocity and immune mechanism that occur, namely type I (allergic), type II (cytotoxic), III (immune complex), and IV (DTH)[(6-7)].

The primary elements of immune response in type I hypersensitivity reactions are Th2. IgE, mast cells, eosinophils, while the usually released mediators are histamine, bradykinin, TNF-α, IL-4, and IL-1. The main elements of immune response in type II are NK, IgM, IgG, complementary cells. The main elements of immune response in type III are immune complex, IgM, IgG, complement (C2a, C3a), neutrophils, TNF-α, IL-1, IFN-γ and TGF-ß. And The main elements of immune response in type IV are macrophage, CD4, CD8, meutophil, TNF-α, IL-1, IFN-γ and TGF-ß[(6-7)] .

TNF and IL-1 are the two cytokines that play an important role in the inflammatory response produced by macrophage[(8)].The main producer of TNF-α is mononuclear phagocyte, the other cell that can produce this cytokine although it is in small amounts, among others, are fibroblasts, mas cells. In most cases, the production of TNF-α is highly dependent on the stimulus (higly stimulus-dependent), and only can be observed in the state of infection or injury (Wallach, 1999). The main cytokine that can increase TNF-α production is interferon-γ is interferon γ (INF-γ) by activated T lymphocytes via Th1; besides, TNF-α itself can stimulate its production[(9)].

The results of the researcher's initial observation on the process of making furniture were the process of making wooden furniture that was through five main processes, namely the process of sawmilling, the preparation of raw materials, the process of component preparation, the assembly and bending process, and the finishing process. All processes have risks to the hypersensitivity reaction of the craftsmen although the greatest risk that can lead to hypersensitivity reactions is in the process of sawmills and the final process that produces high dust wood waste.

Activities undertaken on finishing were sanding furniture surfaces, paving holes and joints, bleaching furniture with H_20_2, sanding sealer, painting with wood stain or other dye, gloss with melamic clear. In the last part, it generated a lot of wood dust, chemicals and dyes that are available in the air, such as H_20_2, sanding sealer, melamic clear, and wood stain that evaporate and fly in the air, especially in spraying using sprayer that will be easily inhaled by craftsmen and may result in hypersensitivity reactions in the lung.

Purpose

The objective of this study was to analyse the increase of THF-α and inflammatory cell (i.e neurophil cell, macrophage cell, and lymphocyte cell) due to exposure of wood furniture dust to mice blood (*MusMusculus)* BALB/C.

METHODS

The research was conduct on June-August 2016 in Pasuruan Regency, East Java- Indonesia. The experimental laboratory research was conducted, and mice was become the animal experiment. The research design used was Randomized the post-test only control group design. Exposure was performed 4 hours a day with 2 hours length of exposure in the morning with 1 hour rest period in 6 times a week for 4 weeks at exposure space in a glass-shaped cube with a size of 20x20x20 cm^3. Concentrations of wood furniture dust exposed were 0.50 mg / m^3, 0.75 mg / m^3, 1.00 mg / m^3.

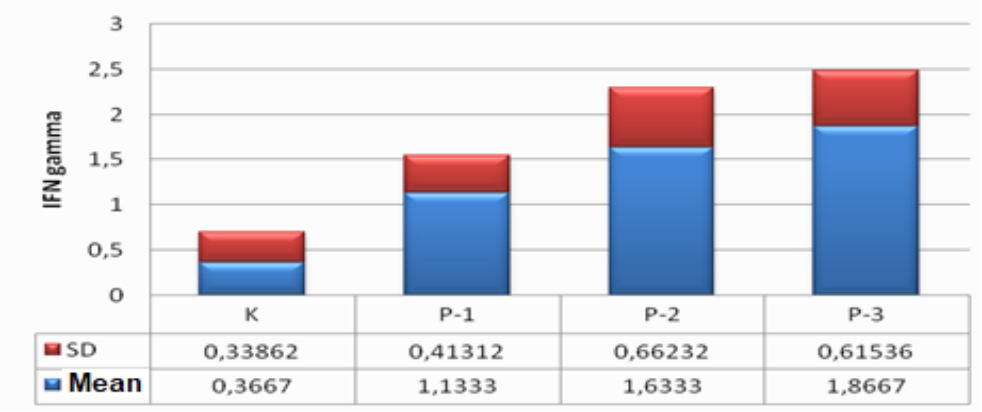
The research variables were independent variables, dependent variables, connecting variables and control variables. The independent variable was wood furniture dust while the dependent variables were pulmonary hypersensitivity, TNF, mast cells, neutrophils, lymphocytes, and macrophages and histopathologic features of the mice whereas control variables were strains, weight, and age.

Data analysis used in this research was: First, data analysis was begun with normality test and homogeneity test of variance for TNF, mast cell, neutrophil, lymphocyte, and macrophage, and histopathologic picture of lung of mice; Second, the assessment to test the hypotheses 1, 2, 3, 4 and 5 use the F test in ONE WAY ANOVA to test the differences of each variable. The test result was significant when $p < 0.05$; Third, the data processing that was obtained from the research, analyzed by using a computer device.

RESULTS

TNF-α in Mice Lung Tissue (*Mus musculus)* BALB/C

TNF-α examinationwas done by using Imunnohistokimia method, average and SD IFN-γ are shown like the table below:



	K	P-1	P-2	P-3
SD	0,33862	0,41312	0,66232	0,61536
Mean	0,3667	1,1333	1,6333	1,8667

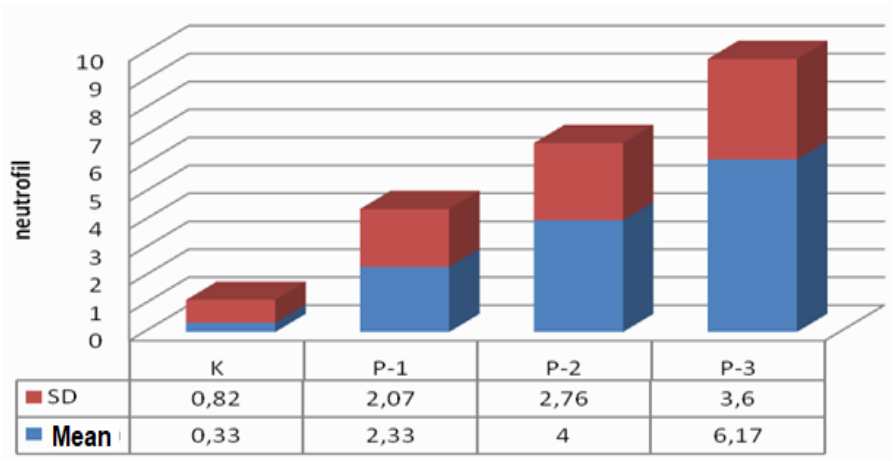
Figure 2. Average and SD levels of TNF-α table in Mice Lung Tissue (*Mus Musculus*) BALB/C

From the table above, it is shown that there is TNF-α increase between the control and treatment group. The K average TNF-αis 0.3667±0.33862, then the P-1 average IFN-γ is 1.1333±0.41312, the P-2 average TNF-αis 1.6333±0.66232, while the P-3 average IL-4 is 1.8667±0.61536.

The result of the F test is shown that the mast cell when it is examined differently by giving wood furniture dust treatment, it is significant, with the F value = 18.425 & p=0.000 (<α = 0.05), which meant that giving wood furniture dust treatment differently is significant towards the increase of TNF-α.

Neutrophil Cells on Mice Lung Tissue (Musmusculus) BALB / C

Neutrophil examination was performed by histopathologic staining of Hematoxilin-Eosin (HE) by Indirect method, the result of examination showed that there was an increase of neutrophil between the control and the treatment. The average neutrophil rate was 0.33 ± 0.82, then the average P-1 neutrophil was 2.33 ± 2.07. For P-2 the average neutrophil was 4 ± 2.76, while the average P-3 neutrophil was 6.17 ± 3.6.



	K	P-1	P-2	P-3
SD	0,82	2,07	2,76	3,6
Mean	0,33	2,33	4	6,17

Figure 3. Average Chart and Neutrophil SD in Mice Lung Tissue (MusMusculus) BALB / C

The result of F test showed that neutrophil when tested was different with the dust treatment of wood furniture was significant, with the value of F = 11.287 & with p = 0.000 <α = 0.05, which means that the dust treatment of wood furniture differs significantly to the increase of neutrophil

Macrophage Cells in Mice Lung Tissue (Musmusculus) BALB / C

The macrophages examination was done by histopathologic staining of hematoxylin-Eosin (HE) by indirect method, the result of the examination showed that there was an increase of macrophages between the control and the treatment. The average macrophage was 14.67 ± 1.21, then P-1 macrophage was 22 ± 10.84. For P-2 the average of macrophages was 49.5 ± 23.79, whereas P-3 average of macrophages was 80.5 ± 52.41.

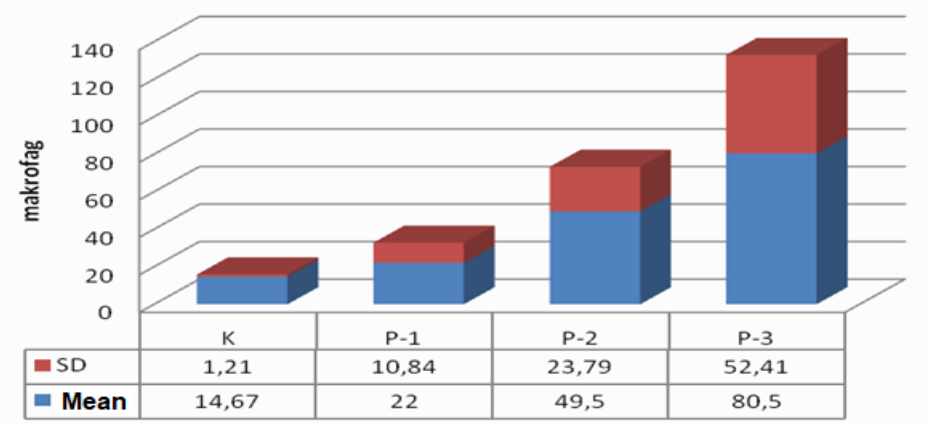


Figure 4. Average Chart and SD Macrophages on Mice Lung Tissue (Musmusculus) BALB / C

The result of F test showed that macrophages when tested were different with the treatment of wood furniture dust were significant, with the value of F = 11.090 & with an p = 0.000 <α = 0.05, which means that the dust treatment of wood furniture differs significantly to the increase of macrophages.

Lymphocyte Cells in Mice Lung Tissue (Mus musculus) BALB / C

Lymphocyte examination was performed by histopathologic staining of hematoxylin-Eosin (HE) with indirect method. The result showed that there was an increase of lymphocyte between control and treatment. The K average of lymphocyte was 3.67 ± 2.25, and the P-1 average of lymphocyte was 20.33 ± 13.66. The P-2 average of lymphocyte was 30.33 ± 19.42, while the P-3 average of lymphocyte was 38.83 ± 33.35.

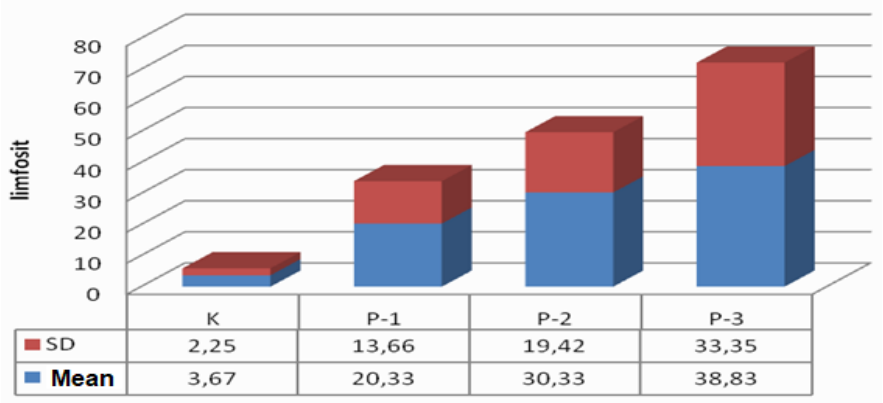


Figure 5. Average Chart and Lymphocyte SD in MiceLungTissue (Mus Musculus) BALB/C

The result of F test showed that ethical lymphocyte tested differently with the treatment of wood furniture dust was significant, with the value of F = 6.875 & with p = 0.002 <α = 0.05, which meant that the dust treatment of wood furniture differed significantly to the increase of lymphocyte.

DISCUSSION

Dust is referring to solid particle that flutters in the air coming from organic and mineral materials that occur in the process of grinding, sawing, splitting, or disintegration of the material[10]. Various factors have an effect on the occurrence of disease or disruption of airway caused by dust. Those factors are dust factor which

includes particle size, shape, concentration, solubility, chemical properties, and long exposure. Individual factors include pulmonary defense mechanism, anatomy and airway physiology and logical immuno factors[11].

From the kind of dust, it can also be grouped into organic dust (cotton dust, dust, tobacco and so on). mineral dust (a complex compound: SiO_2, SiO_3, charcoal etc.) and metal dust (dust which contains of metal elements: Pb, Hg, Cd, Arsen, etc.). In terms of character, the dust material consists of physical dust (soil dust, stones, minerals, and fiber), chemicals (organic and inorganic minerals), biological (virus, bacteria, and cyst) and radioactive dust.

According to WHO in 1996, the size of harmful particle dust is 0.1-5 or 10 micron in size[12]. The size of dust which is harmful ranges from 0.1 to 10 microns[1]. Dust which is sized between 5-10 microns inhaled will be retarded and buried in the upper airway; which are between 3-5 microns hold and accumulated in the middle airway. Dust particles of 1-3 microns are called the most respirable dust, which is the most dangerous because it is suspended and buried from the terminal bionkiolus to the alveoli. Dust which is less than 1 micron in size is not easily settled in the alveoli, dust which measures between 0.1-0.5 microns diffuses with Brownian motion in and out of the alveoli; if it hits the alveoli it can be buried there[10-12].

Wood dust is a dust produced by the process of sawing, shrinking and sanding which can cause air pollution in the workplace and be harmful to the labour. To anticipate the negative effects of exposure to wood dust in the workplace, it is necessary to prevent and protect the safety and health of the labour. One of the prevention efforts is to determine the threshold value of chemical substances in the air of the workplace into Indonesian National Standard (SNI) so that entrepreneurs can control the working environment of their company by referring to this Standard. This standard contains the time weighted average of chemicals in the workplace air, where there will be labours that can be exposed to daily chemicals for no more than 8 hours per day or 40 hours per week, as well as ways to determine the Boundary Threshold Value for workplace air containing more than one chemical substance[13-15].

The Threshold value is a dangerous factor standard in the workplace as a control guide so that the labour can still deal with it without causing illness or health problems in daily work for no more than 8 hours a day or 40 hours a week. The use of this NAB is as a recommendation on the practice of higene companies in conducting the management of the working environment as an effort to prevent its impact on health (SE.01 / Men / 1997). For hardwood dust like mahogany dust and teak wood, it has been established by the Ministry of Labor in the Circular of the Minister of Manpower No: SE. 01 / Men / 1997 on Threshold Value of Chemical Factor in Air Working Environment about 1 mg / m^3. The threshold value indicates the degree to which humans can react physiologically to a substance[1].

The danger of wood dust for health is that the dust is particulate matter if it enters into human respiratory organs it can cause disease at work especially in the form of respiratory system disorder characterized by excessive mucus expenditure which cause the main symptom of prolonged cough with phlegm. Common disorders that often occur are coughing, shortness of breath, general fatigue and decreased body weight[13].

Workers who exposed to dust continuously at age of 15 to 25 years will experience a decrease in working ability, ages 25 to 35 years of productive cough and decrease in VEP 1 (Force Expiratory Volume 1 second (FEV 1) expression volume, ages of 45 to 55 years are congested and hypoxaemia, ages of 55 to 65 years of corpulmonale up to respiratory failure and death. These can be detected by spirometer examination[14-15].

The results of this study revealed that there was an increase in CD-8 between the control and the treatment group, and based on the results of the different test, it was significant. Increased in the amount of CD8 in hypersensitivity pneumonitis can be explained that first, sensitized CD8 through Class II MHC activates plasma cells, where plasma cells then produce antibodies, among them IgG, which will form immune complexes[16]. Second, CD8 can directly damage the target cells[7]. Third, CD8 will also release IFN-γ necessary for the development of hypersensitivity pneumonitis (HP) disease.) Increased exposure will result in modulated severity of pneumonitis hypersensitive (HP)[17].

Naive T cells which exposed to MHC-bound antigens presenting APC or specific cytokine stimuli will develop into T cell subunits of CD4+ and CD8+ with different effector functions. The mature T-cells that leave the thymus but have not been exposed to the antigen are called naïve T cells. These cells can enter the circulation, enter and settle in lymphoid organs such as lymph nodes for many years.

The increase in TNF-α between control and treatment in this study can be explained because TNF's primary function is a mediator of inflammatory responses to inflammatory infections and other stimuli. TNF plays a role in nonspecific immunity with a primary source with TNF-activated mononuclear phagocytes[8].

The results of inflammatory cell examination performed by HE staining showed that there was an increase in inflammatory cells between the control and the treatment group and based on the basis of different test results, it was significant. The difference of inflammatory cells between control and treatment can be understood, because once the detected antigen is immediately captured by alveolar macrophages and presented by APC to T lymphocytes, which then activate plasma cells to produce antibodies and form immune complexes. This immune complex then activates complement and alveolar macrophages, alveolar macrophages activated and releases various chemokines and cytokines. One of the chemokines released is regulated on activation. T cells expressed

and secreted (RANTES)[16]. The complement itself will activate the mast cells[8]. RANTES chemotactic factor is what attracts neutrophils to the alveoli and increases T lymphocytes continuesly until lymphocytic alveolitis occurs. Humoral, cellular and cytokine responses in unity cause progressive inflammation until granulomatous formation occurs in the pulmonary parenchyma. Continuous exposure to antigens will activate neutrophils, fibroblasts and result in deposition of collagen and fibrosis[16].

Inflammation is a limited protection mechanism against trauma or microbial invasion by a devastating reaction, diluting or limiting harmful substances and damaging tissues. Inflammation is necessary for the body to defend itself from various dangers that disrupt the balance but can also repair structural damage as well as impaired tissue function. Inflammation is characterized by fluid transfer, plasma proteins and leukocytes from circulation to tissues in response to hazards[8].

Excessive and sustained cytokine production in response to bacterial lipopoliskharadida (LPS) or superantigen is the characteristic of a deadly systemic inflammatory response. The spread of these bacteria induces a wave of production of pro-inflammatory cytokines such as TNFα, IL-1, IL-6, IL-8, which activates more immune cells and recruits them into the area of infection. These excessive amounts of pro-inflammatory cytokines can damage the vascular wall and result in organ dysfunction. Both LPS and superantigen induce signal transduction to the mononuclear and T cell phagocyte nuclei[18].

There are two kinds of inflammation, namely acute inflammation and chronic inflammation. It happens because acute inflammation can be a strange thing that enters the body, invasion of microorganisms, trauma, harmful chemicals, physical factors and allergies. The main features of acute inflammation are redness, heat, edema / swelling and pain. If swelling is severe, the function of the affected app will be disrupted. Histologic appears of edem fluid and tissue infiltration by leukocytes. Plasma factors such as immunoglobulins, complement, coagulation-fibrinolotic contact activation systems and inflammatory cells interact with each other and contribute to inflammation[8].

Chronic inflammation follows an acute response of monocyte influx, eosinophils. When the condition becomes controlled, neutrophils are deployed again and degenerate. Next it will be deployed mononuclear cells. At this stage, monocytes, macrophages, lymphocytes and plasma cells are given a pathological picture of chronic inflammation[8]. Chronic inflammation can originate in acute inflammation if the destroyer is sedentary, but more often it is that the inflammatory response is chronic inflammatory response from the beginning. In contrast to widespread vascular changes or damage and neutrophil infiltration seen in acute inflammation, chronic inflammation characterizes tissue infiltration with mononuclear cells such as macrophages, lymphocytes, and plasma cells accompanied by tissue destruction. Macrophages are key players of a chronic inflammatory response. This is due to the large number of bioactive products or mediators that it releases. This mediator is part of a very strong body defense system against invasion of foreign objects and tissue damage. Adverse macrophages are that continuous macrophage activity can result in sustained tissue damage[18].

CONCLUSION

The above results can be concluded, first timber wood dust exposure increases TNF between study groups compared with control group in BALB / C mice (Musmusculus). Second, wood furniture dust exposure increased mast cells, neutrophils, lymphocytes, and macrophages in lung tissue between study groups compared with control group in BALB / C mice (*Musmusculus*).

The suggestion of this research is that it can be used to base the efforts of prevention, therapy and or diagnosis of HP disease earlier so that it can improve lung quality. Considering that mice are more susceptible to exposure to rice milling dust, future research needs to be repeated with other experimental animals that have characteristic adjustments to rice milling dust similar to humans, such as monkeys and pigs. The results should be repeated with longer pajan time so that lung damage can be more visible, especially the occurrence of granulomatous lung. Future research should be conducted in-depth examination of the content of compounds, bacteria and fungi in rice grinding dust as the main antigen in hypersensitive pneumonitis (HP).

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