

MEKANISME PNEUMONITIS HIPERSENSITIF (HP) AKIBAT PAJANAN DEBU PENGGILINGAN PADI PADA MENCIT (*MUS MUSCULUS*) BALB/C

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HYPERSENSITIVITY PNEUMONITIS

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ABSTRACT

Mechanism of hypersensitivity Pneumonitis (HP) as a result from the exposure of dust from paddy milling on mice (*Mus musculus*) BALB/C

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The purpose of this research was to analyze the immune response mechanism of Hypersensitivity Pneumonitis (HP) as a result from the exposure of dust from paddy milling on mice (*Mus musculus*)BALB/C.

The research done was a laboratory experimental research with mice (*MusMusculus*) as experimental animal. The research design used was *the post only control group design* using mice (*MusMusculus*) Balb/c as experimental animal. Mice (*Musmusculus*) Balb/c were exposed to dust from paddy milling for four (4) hours/day and it was done for thirty (30) days with the exposed concentrations respectively were 0.50 mg/m³, 0.75 mg/m³, 1.00 mg/m³.The research variables were free variable, dependent variable, and control variable. Independent variable was dust from paddy milling, dependent variables were Hypersensitivity Pneumonitis (HP), IgE, IL-4, CD8, IFN- γ , inflammatory cells, and histopathological picture of mice lung, while control variables were strain, body weight and age of mice (*MusMusculus*) Balb/c.

The research result showed that there was an increase of IgE, yet statistically there was no significant difference; there was an increase on IL-4, CD-8, IFN- γ , inflammatory cells and lung histopathology and statistically there was a significant difference between the study and control on mice BALB/C.

The conclusion of the research was that the immune response mechanism of Hypersensitivity Pneumonitis (HP) as a result from the exposure of dust from paddy milling on mice (*Mus musculus*)BALB/C. It could be concluded that dust from paddy milling inhaled repeatedly passed into the alveoli and then it was caught by alveolar macrophages which then generated the increase of IL-4 and CD-8. After that, IL-4 generated the increase of IgE which afterward attracted mast cells while CD8 expressed IFN- γ which then activated alveolar macrophages and attracted a number of neutrophil and mast cell which subsequently induced inflammation. The inflammation occurred would develop into other tissue damage and led to Hypersensitivity Pneumonitis (HP).

It was suggested that: it be better to repeat with longer exposure time so that the damage in lung be seen more clearly especially the occurrence of granuloma in lung.

Keywords: Hypersensitivity Pneumonitis (HP), dust from paddy milling

SUMMARY

Mechanism of hypersensitivity Pneumonitis (HP) as a result from the exposure of dust from paddy milling on mice (*Mus musculus*) BALB/C

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Hypersensitivity Pneumonitis (HP) is an inflammation reaction as a result of an inappropriate immune response towards antigen inhaled which can cause dyspnea (shortness of breath), certain damage in lung, and interstitial infiltration due to the accumulation of a number of T lymphocytes activated in lung (Cormier *and* Schuyler, 2006). Three main categories from antigen causing Hypersensitivity Pneumonitis (HP) are microbiology organism / germs (bacteria, fungi and their components), animal protein, and low molecular weight chemicals (Bourke *et al.*, 2001; Rose 2005). Geographical, social and working factor determine a special type of Hypersensitivity Pneumonitis (HP) found all around the world (Banham, 1986; Nakazawa *and* Tochogi, 1989; Sharma, 1989; Bourke *et al.*, 2001).

The result of the preliminary research on three paddy milling industries in Lamongan showed that the environment did not support the safety and health of the workers. Firstly, the dust level from the three paddy millings respectively are 2.1414 mg/m³, 4.4362 mg/m³, and 6.2421 mg/m³. Secondly, the working environment was hot, at about 31.463 °C. Thirdly, no one wore a mask as a self protection device. The result of the interview towards 88 workers revealed that all workers experienced subjective respiratory disorder from mild: 53 workers (60.2%), moderate: 20 workers (22.7%), to extreme: 15 workers (17%).

From the result of the preliminary research mentioned above, a thought that dust from paddy milling not only cause lung function abnormalities but also cause Hypersensitivity Pneumonitis (HP) on workers concerning that dust from paddy milling is organic dust containing microbiology organisms such as bacteria and fungal spores that can act as Hypersensitivity Pneumonitis (HP) agent emerged. Besides that, up till now there is no similar research and the mechanism on how Hypersensitivity Pneumonitis (HP) can occur is still not revealed clearly. The purpose of this research was to analyze the influence of dust from paddy milling on Hypersensitivity Pneumonitis (HP) occurrence on mice (*Mus musculus*) BALB/C as well as to reveal the mechanism of immune responses on HP as a result from the exposure of dust from paddy milling on mice BALB/C.

The research done in laboratory experimental research with mice (*Mus Musculus*) BALB/C as an experimental animal. The research design used was Randomized the post test only control group design. The reason to use this design was because all characteristic among population units were equal or homogeneous, in this case mice (*Mus musculus*) Balb/c population with the same characteristics and for both treatment group and control group got the same period of time that was four (4) hours/day for thirty (30) days. Exposure came from dust from paddy milling in which the exposed concentrations respectively were 0.50 mg/m³, 0.75 mg/m³, 1.00 mg/m³. The research variables were free variable, dependent variable, intervening variable, and control variable. Independent variable was dust from paddy milling, dependent variable was Hypersensitivity Pneumonitis (HP), IgE, IL-4, CD8, IFN- γ , inflammatory cells, and histopathological picture of mice lung, while control variables were strain, body weight and age. There was several data analyses used in the research of the influence of organic dust from paddy milling on Hypersensitivity Pneumonitis (HP). First, data analysis was initiated by performing normality varian test and homogeneity varian test for data of IgE, IL-4, CD8, IFN- γ level, and histopathological picture of mice lung. Second, the assessment to test hypothesis 1, 2, 3, 4, and 5 using F-test in ANOVA for differential test on each variable. Path analysis was used to see the mechanism pattern on how Hypersensitivity Pneumonitis (HP) occurred. The test result was meaningful if p was less

than 0.05 (< 0.05). Third, data processing obtained from the research was analyzed using computer statistical package. The research result of the exposure of dust from paddy milling on mice balb/c with 0.5 mg/m³, 0.75 mg/m³, and 1.0 mg/m³ concentration for four (4) hours/day for thirty (30) days based on F-test in *One Way Anova* showed that there was no difference in dust exposure on the increase of IgE, and there was a difference in dust exposure from paddy milling on IL-4, CD-8, IFN- γ , mast cell, neutrophil, lymphocyte, macrophage and histopathology showing that dust variable had significant influence on the increase of IL-4, CD-8, IFN- γ , mast cell, neutrophil, lymphocyte, macrophage and lung histopathological damage so that it can generate Hypersensitivity Pneumonitis (HP).

The conclusion of the research was that the immune response mechanism of Hypersensitivity Pneumonitis (HP) as a result from the exposure of dust from paddy milling on mice (*Mus musculus*)BALB/C could be explained more detail based on the result of the research. It could be concluded that dust from paddy milling inhaled repeatedly passed into the alveoli and then it was caught by alveolar macrophages which then generated the increase of IL-4 and CD-8. After that, IL-4 generated the increase of IgE which afterward attracted mast cells while CD8 expressed IFN- γ which then activated alveolar macrophages and attracted a number of neutrophil and mast cell which subsequently induced inflammation. The inflammation occurred would develop into other tissue damage and led to Hypersensitivity Pneumonitis (HP). It was suggested that : First, the research result be used to underlie a preventive action and an early therapy on Hypersensitivity Pneumonitis (HP) to increase the quality of lung. Second, the research result be used by central government and local government in making strict rule on giving the license for paddy milling industries as well as assure that there be no lung disorder on paddy milling workers. Third, in the future the research be repeated using another experimental animal which has quite similar characteristic to human regarding the adaptation on paddy milling dust. Forth, it be better to repeat the research using level of paddy milling dust bigger than 1 mg/m³ with longer exposure time so that the damage in lung be seen more clearly especially the occurrence of granuloma in lung.

RINGKASAN

Pneumonitis Hipersensitif (HP) Akibat Paparan Debu Penggilingan Padi pada Mencit (*Mus musculus*) BALB/C **Isa Ma'rufi**

Pneumonitis hipersensitif (HP) merupakan reaksi radang sebagai akibat respons imun terhadap antigen yang dihirup yang dapat menyebabkan sesak nafas, kerusakan parenkim paru, infiltrasi jaringan interstisial, karena akumulasi sel T limfosit dalam jumlah besar (Cormier dan Schuyler, 2006). Tiga macam antigen yang menyebabkan HP adalah organisme mikrobiologi (bakteria, jamur dan komponennya), protein hewan, dan bahan kimia dengan berat molekul rendah (Bourke *et al.*, 2001; Rose dan Lara, 2010). Faktor geografis, sosial dan pekerjaan menentukan tipe khusus HP yang ditemukan di seluruh dunia (Banham, 1986; Nakazawa dan Tochigi, 1989; Sharma, 1989; Bourke *et al.*, 2001).

Hasil penelitian pendahuluan peneliti di tiga industri penggilingan padi di Kabupaten Lamongan, menunjukkan bahwa lingkungan kerja sangat tidak menunjang bagi keselamatan dan kesehatan para pekerja. Pertama, kadar debu hasil penggilingan padi di tiga industri penggilingan padi yang diukur menunjukkan kadar debu yang cukup tinggi, yaitu 2,1414 mg/m³, 4,4362 mg/m³, dan 6,2421 mg/m³ jika dibandingkan dengan Nilai Ambang Batas

(NAB) yang hanya 3,00 mg/m³. Kedua, lingkungan kerja yang panas, yaitu rerata 31,463 °C. Ketiga, tidak satupun pekerja memakai masker sebagai Alat Pelindung Diri (APD). Keempat, dari hasil wawancara terhadap 88 pekerja terungkap bahwa semua pekerja mengalami gangguan pernafasan, dengan tingkat gangguan pernafasan ringan 53 pekerja (60,2%), sedang 20 pekerja (22,7%), serta berat 15 pekerja (17%).

Dari hasil penelitian pendahuluan tersebut muncul pemikiran bahwa debu penggilingan padi diduga tidak hanya menimbulkan kelainan faal paru saja, tetapi dapat menimbulkan terjadinya pneumonitis hipersensitif (HP) pada pekerja, mengingat berdasarkan pemeriksaan yang dilakukan peneliti pada penelitian pendahuluan terhadap debu penggilingan padi, bahwa debu penggilingan padi merupakan debu organik yang mengandung organisme mikrobiologi, seperti bakteri dan spora jamur, serta terdapatnya agen molekuler berbobot rendah. Di samping itu, sampai saat ini belum pernah ada penelitian yang sejenis serta mekanisme terjadinya pneumonitis hipersensitif (HP) belum terungkap dengan jelas. Tujuan umum dari penelitian ini adalah mengungkap respons imun pneumonitis hipersensitif (HP) akibat pajanan debu penggilingan padi pada mencit (*Mus musculus*) BALB/C. Penelitian yang dilakukan adalah penelitian eksperimental laboratoris dengan hewan coba mencit, rancangan penelitian yang digunakan adalah *Randomized the post test only control group design*. Pemajanan dilakukan 4 jam sehari dengan lama pajanan 2 jam pagi dengan waktu istirahat 1 jam selama 6 kali seminggu selama 4 minggu pada ruang pemajanan dalam kotak pemajanan yang berbentuk kubus dari kaca dengan ukuran 20x20x20 cm³. Konsentrasi debu penggilingan padi yang dipajan adalah 0,50 mg/m³, 0,75 mg/m³, 1,00 mg/m³. Variabel penelitian adalah variabel bebas, variabel tergantung, variabel penghubung dan variabel kendali. Variabel bebas adalah debu penggilingan padi, sedangkan variabel tergantung adalah pneumonitis hipersensitif, IgE, IL-4, CD8, IFN-gamma (IFN- γ), sel mast, neutrofil, limfosit, dan makrofag serta gambaran histopatologi paru mencit, sedangkan variabel kendali adalah strain, berat badan, dan umur. Analisis data yang digunakan dalam penelitian mekanisme pneumonitis hipersensitif (HP) akibat pajanan debu penggilingan padi pada mencit (*Mus musculus*) BALB/C ini adalah: Pertama, analisis data diawali dengan melakukan uji normalitas dan uji homogenitas varians untuk data kadar IgE, IL-4, CD8, IFN- γ , sel mast, neutrofil, limfosit, dan makrofag, serta gambaran histopatologis paru mencit. Kedua, penilaian untuk menguji hipotesis 1,2,3,4, dan 5 menggunakan uji F dalam ONE WAY ANOVA untuk uji perbedaan masing-masing variabel, disamping itu dilakukan analisis jalur, untuk melihat model mekanisme respons imun kejadian pneumonitis hipersensitif. Hasil pengujian bermakna bila $p < 0,05$. Ketiga, pengolahan data yang diperoleh dari penelitian, dilakukan analisis dengan menggunakan perangkat komputer.

Hasil penelitian terungkap bahwa, pertama pajanan debu penggilingan padi dapat meningkatkan IgE serum darah antara kelompok studi dibandingkan dengan kelompok kontrol pada mencit (*Mus musculus*) BALB/C, tetapi hasil uji statistik tidak ada perbedaan bermakna antara kedua kelompok. Kedua, pajanan debu penggilingan padi dapat meningkatkan IL-4, CD-8, dan IFN- γ pada jaringan paru antara kelompok studi dibandingkan dengan kelompok kontrol pada mencit (*Mus musculus*) BALB/C, dan hasil uji statistik ada perbedaan bermakna antara kedua kelompok. Ketiga, pajanan debu penggilingan padi dapat meningkatkan sel mast, neutrofil, limfosit, dan makrofag pada jaringan paru antara kelompok studi dibandingkan dengan kelompok kontrol pada mencit (*Mus musculus*) BALB/C, dan hasil uji statistik ada perbedaan bermakna antara kedua kelompok.

Keempat, pajanan debu penggilingan padi dapat meningkatkan lesi pada histopatologi jaringan paru antara kelompok studi dibandingkan dengan kelompok kontrol pada mencit (*Mus musculus*) BALB/C, dan hasil uji statistik ada perbedaan bermakna antara kedua kelompok. Kelima, pajanan debu penggilingan padi meningkatkan kejadian pneumonitis

hipersensitif (HP) antara kelompok studi dibandingkan dengan kelompok kontrol pada mencit (*Mus musculus*) BALB/C, dan secara statistik ada perbedaan bermakna antara kedua kelompok.

Keenam, mekanisme respons imun kejadian pneumonitis hipersensitif (HP) akibat pajanan debu penggilingan padi pada mencit (*Mus musculus*) BALB/C berdasarkan hasil penelitian ini dapat dijabarkan lebih detil, bahwa debu penggilingan padi yang terinhalasi secara berulang sampai ke alveoli kemudian ditangkap oleh makrofag alveolar dan memicu peningkatan IL-4 dan CD8. IL-4 kemudian memicu peningkatan IgE, dan IgE menarik sel mast, sedangkan CD8 mengespresikan IFN- γ , yang selanjutnya IFN- γ mengaktifkan makrofag dan menarik sejumlah besar neutrofil dan sel mast yang kemudian menginduksi inflamasi. Inflamasi yang terjadi akan berkembang ke bentuk kerusakan jaringan paru dan menjadi penyakit pneumonitis hipersensitif (HP).

Disarankan, pertama perlu diinformasikan upaya pencegahan, diagnosis dan terapi penyakit HP lebih dini sehingga dapat meningkatkan kualitas paru. Kedua, hasil penelitian ini dapat membantu instansi pemerintah yang terkait dalam membuat aturan yang ketat terhadap perijinan industri penggilingan padi dalam upaya untuk mencegah kejadian kelainan paru akibat kerja pada pekerja penggilingan padi. Ketiga, mengingat mencit lebih rentan terhadap pajanan debu penggilingan padi, maka kedepan penelitian perlu diulang dengan hewan coba lain yang mempunyai karakteristik penyesuaian dengan debu penggilingan padi yang hampir sama dengan manusia, seperti pada monyet dan babi.

Keempat, perlu dilakukan penelitian lanjutan dengan waktu pajan yang lebih lama sehingga kerusakan paru dapat lebih tampak terutama untuk pembentukan granuloma di parenkim paru. Kelima, penelitian lanjutan dapat dilakukan dengan melibatkan pekerja penggilingan padi untuk memastikan pengaruh debu penggilingan padi terhadap pneumonitis hipersensitif (HP) pada pekerja penggilingan padi. Keenam, perlu dilakukan penelitian untuk mencari bahan aktif dari debu penggilingan padi sebagai antigen utama pada pneumonitis hipersensitif (HP).