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Control of Occupational Health and Safety (OHS) to Prevent Disruption of Circadian Rhythm Resulting Mice Alveolar Damages Caused by Night Shift Coal dust Exposure

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ABSTRACT

Coal mines have welfare impacts as well as adverse impacts to humans. The process of coal mining raises dust inhaled by workers and the surrounding community. In a time and a certain amount of respirable dust can cause tissue damage. Lung tissue damage caused by coal dust increases the production of reactive oxygen species (ROS), such an effect on proinflammatory cytokines tumor necrosis factor-α (TNF-α) and lower antioxidant. Damage to the lungs structure caused by coal dust exposure could be observed through the measurement of pulmonary alveolar wall thickness. To prevent lung damage required occupational Health and Safety (OHS) Management such as administrative controls, engineering controls, especially Personal Protective Equipment (PPE) controls with the use of masks to minimize inhalation of fine coal dust. Prevention can also be done with the substance of free-radical scavengers and treat inflammation, which prevent the thickening of the alveolar wall. One substance is melatonin, which functions as an antioxidant and anti-inflammatory. Melatonin is a natural hormone found in vegetables, animals, humans and other organisms. This study aimed to investigate the effect of using PPE OHS control and melatonin in maintaining lung function by measuring coal dust exposure levels malondealdehid (MDA), the activity of superoksid dismutase (SOD), TNF-α, and pulmonary alveolar wall thickness. In this study used rat rattus norvegicus animal wistar strain. The research method is Randomized Posttest Control Group Design, sample number 36 wistar strain male mice were divided into three groups, negative control, exposure and exposure during the night. Each exposure group will be further divided into four experimental groups: positive control, melatonin dose 1 (0.5 mg / kg / hr), melatonin dose 2 (1mg/kg/hr) and melatonin dose 3 (2mg/kg/hr) . By giving melatonin treatment for 6 days, and then exposed to coal dust for 3 days for 30 minutes. The results exposure to coal dust turns day and night affect all samples. Hasi research shows that exposure to higher night. 1.395 + 1.1273 MDA with TBA test, the levels of SOD 44.286 + 9.8179 with NBT, and the levels of TNF-α58, 830 + 7.270 by ELISA. The thickness of the alveolar wall 20.417 +3.487 Olympus BX51 microscope with a magnification of 400x as much as 3 field . From these results it can be concluded that melatonin may reduce levels of MDA, SOD activity, levels of TNF-α, and wall thickness. Application of K3 is expected to minimize the occurrence of occupational diseases thus increasing productivity

Key words: *Coal dust, Melatonin, Mask, SOD, MDA, alveolar wall thickness*

INTRODUCTION

Coal is a natural resource that can not be renewed. Coal as a primary energy suppliers, the second largest after the oil is equal to 34.4%. Indonesia is largest coal exporter after Australia. (Miranti,2008). Combustion coal in mining area produce substances that harmful to lungs because they contain sulfur dioxide, carbon dioxide, particulates and nitrogen oxides. Sulfur dioxide can cause respiratory damage to the lungs (Miller, 1990).

Humans inhale coal dust and can cause coal workers' pneumoconiosis (CWP), silicosis, bronchitis and emphysema. Mechanisms of ROS generation occurs through the particles intrinsic properties and Fe content in coal dust (non-cellular mechanism), inflammation and increased arachidonic acid metabolism in the cell membrane. (Fixman et al 2007).

Miner working in shift pattern day and night shift . Shift work pattern can change circadian rhythms associated with melatonin hormone. Shift work have risks of cardiovascular disease, metabolic disorder risk such as obesity, increased lipid contents, impaired glucose balance and decreased contents of antioxidants and other metabolic syndrome (Knutsson A, 2003).

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Problems miners who worked night shift exposed to coal dust and suppressed melatonin release, because awake at night and illuminated by lamps. In the daytime melatonin levels are low and exposed to coal dust. Implementation of OSH management in the company

Problem solving is the implementation of OSH management as a way to minimize the incidence of occupational diseases. Conducted administrative control such as the use of Personal Protective Equipment masks to minimize coal dust inhalation while working. Melatonin as an antioxidant and anti-inflammatory to cope with oxidative stress caused by exposure to coal dust and circadian rhythm disruption. (Kuempel ED, et al.1995).

Research objective is to determine the effect of OSH control in using PPE and melatonin in maintaining lung function that exposed to coal dust by measuring contents malondealdehyde MDA), activity of super oxide dismutase (SOD), TNF-α, and pulmonary alveolar wall thickness.

RESEARCH METHODS

Experimental design was Randomized Posttest Control Group Design. Total population of 72 individuals (36 +36 during the night) wistar strain male mice aged 4-6 months, weighing mice 300-500g , were divided into three groups, negative control, day exposure and night exposure. Each exposure group will be further divided into four experimental groups, positive control group, first dose of melatonin (0.5 mg/kg/day), doses 2 (1 mg/kg/day) dose 3 (2mg / kg/day).

How to Work and Data Collection

Melatonin administration.

Melatonin administration (dissolved in distilled water) using intra gastric feeding tube every day for 6 days according dose of the treatment.

Coal Dust Exposure

Exposure to coal dust carried out for 3 days at 09.00 (day exposure) and 21.00 (night exposure) using a set of exposure (Figure 1.) Each exposure 15 grams coal dust (≤ 50 μ) and mice put into set of exposure that covered by gauze as PPE mask for 15 minutes.

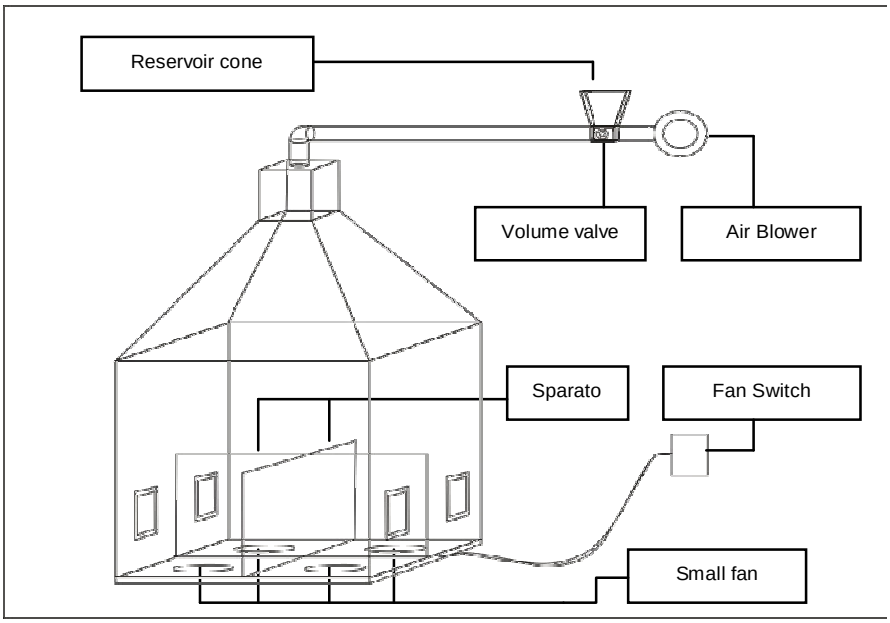

Figure 1 . Schematic set exposure

Taking BAL and lung tissue of mice.

After exposure for 3 days, 16-36 hours later prepared sample is taken. BAL fluid filled into the tube and seal tightly. Then centrifuge at 3500 rpm for 10 min at 4 ° C. Supernatant was used for the sample

(MDA, SOD and TNF-α) After BAL fluid taken, lung tissue was fixed in 10% formalin, and then made histopathology preparations with HE staining.

Examination of BAL

MDA was measured by the TBA test. The principle of this method is the reaction of MDA with thiobarbituric acid in acidic conditions and heat. This process produces a pink subsequently measured with a spectrophotometer at a wavelength of 532 nm.

SOD BAL examination

Measurement of SOD activity based on the inhibition of SOD working through reduction of NBT by xanthine-xanthine oxidase system (Ukeda et.al in Bhavsar, Patel and Lau-Cam, 2010)

Examination of BAL TNF-α using antigen antibody reaction was then measured using ELISA reader at λ = 492 nm

Alveolus wall thickness measurement.

Rat alveolar wall thickness was measured using Olympus BX51 microscope with a magnification of 400x as much as 3 field of view in each preparation (Leigh R. et al., 2002).

1. Results of MDA BAL measurement

Table 1. Measurement results contents of MDA BAL mice exposed coal dust during the day and night

Group of mice	Contents MDA BAL mice exposed coal dust during the day x ± SD (nanogr/cc)	Contents MDA BAL mice exposed coal dust during night x ± SD (nanogr/cc)
Control (-)	1,799 ± 1,7139 a	1,799 ± 0,7139 a
Control (+)	1, 926 ± 0,8169 a	1,394 ± 0,3375 a
M 1	1,416 ± 0,8244 a	1,277 ± 0,3002 a
M 2	1,054 ± 0,1886 a	0,713 ± 0,7529 a
M 3	**1,395 ± 1,1273 a**	**0,692 ± 0,400 a**

Mean of MDA BAL contents in mice exposed to coal dust during the day at each reserach groups are presented in graphical in Figure 2

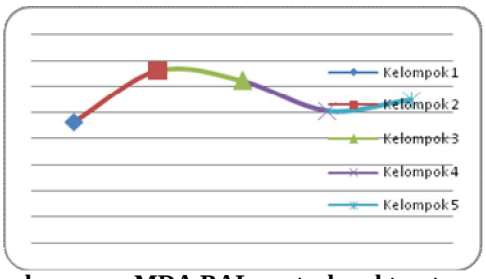

Figure 2. Graph average MDA BAL control and treatment group mice (exposure to coal dust during the day)

Description:
Group 1: no treatment
Group 2: exposed to coal dust
Group 3: melatonin 0.5 mg / kg / day + exposed to coal dust
Group 4: melatonin 1 mg / kg / day + exposed to coal dust
Group 5: melatonin 2 mg / kg / day + exposed to coal dust

Contents of MDA BAL coal dust exposure day and night did not differ between control and treatment ($p>0.05$)

2. The results of SOD BAL activity measurements

Table 2. Measurement results of SOD BAL activity mice exposed coal dust during the day and night

Group of mice	SOD BAL activities mice during the day x ± SD (nanogr/cc)	SOD BAL activities mice during night x ± SD (nanogr/cc)
Control (-)	55,662 ± 27,2371 a	38,162 ± 9,9505 a
Control (+)	125,115 ± 27,634 b	181,261 ± 49,8408 b
M 1	79,307 ± 47,788 bc	72,068 ± 22,4756 a
M 2	66,443 ± 47,788 c	77,198 ± 30,9924 a
M 3	**37,878 ± 12,455 c**	**44,286 ± 9,8179 a**

Description :
Control (-): no treatment
Control (+): exposed to coal dust
M1: melatonin 0.5 mg / kg / day + exposed to coal dust
M2: melatonin 1 mg / kg / day + exposed to coal dust
M3: melatonin 2 mg / kg / day + exposed to coal dust

Results of mice SOD BAL measurement after administration of melatonin and exposure of coal dust. It sows increased SOD BAL compared to the negative control (P <0.05), and decreased after the administration of melatonin (p <0.05)

3. TNF-α BAL for mice

Table 3 . Measurement results of TNF-α BAL mice exposed coal dust during the day and night

Group of mice	Contents of TNF- α BAL mice at day x ± SD (ng/ml)	Contents of TNF- α BAL mice at night x ± SD (ng/ml)
control (-)	52,274 ± 17,383	52,274 ± 17,383
control (+)	704,418 ± 283,736	734,575± 283,736
M 1	654,175 ± 128,572	275,390± 33,386
M 2	666,841 ± 133,271	59,042 ± 6,812
M 3	**766,843 ± 103,485**	**58,830 ± 7,270**

Description :
Control (-): no treatment
Control (+): exposed to coal dust
M1: melatonin 0.5 mg / kg / day + exposed to coal dust
M2: melatonin 1 mg / kg / day + exposed to coal dust
M3: melatonin 2 mg / kg / day + exposed to coal dust

From the table 3 that TNF-α levels tended to decreased when a higher dose of melatonin at night exposure group, whereas for the group during the exposure there was no significant difference (p> 0.05) even tend to rise.

4. The results of mice pulmonary alveolar wall thickness measurements

Table 4. The results of measurements alveolar wall thickness mice were exposed to coal dust during the day and night
(Histopathology preparations stains with HE 400x magnification)

Group of mice	Average alveolar wall thickness (day) x ± SD (µm)	Average alveolar wall thickness (nifht) x ± SD (µm)
Control (-)	25.638±4.674	18.82±3.1036
Control (+)	40,142± 4,336	48,57± 7,2092
M 1	35,606± 3,847	25,31± 3,5040
M 2	24,279±2,226	21,14±3,2405
M 3	**20,417±3,487**	**22,28±4,0836**

Description :
Control (-): no treatment
Control (+): exposed to coal dust
M1: melatonin 0.5 mg / kg / day + exposed to coal dust
M2: melatonin 1 mg / kg / day + exposed to coal dust
M3: melatonin 2 mg / kg / day + exposed to coal dust

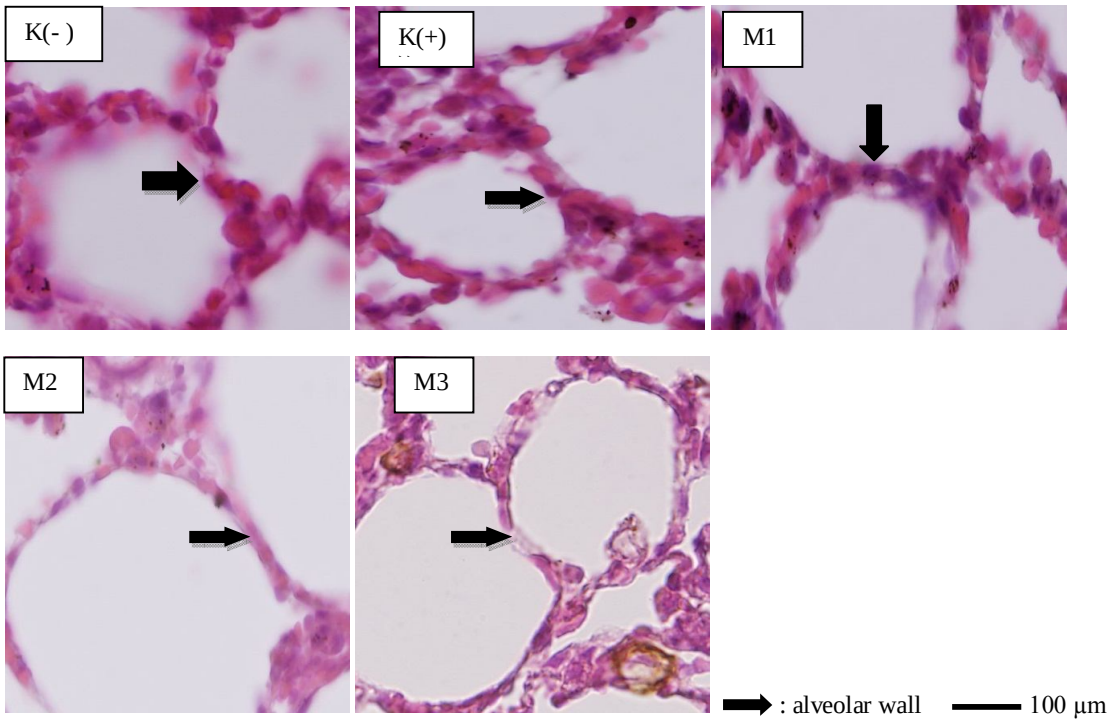

Figure 3. Results of alveolar observations at control group mice exposure to coal during the day and after the administration of melatonin

DISCUSSION

The ability of the lung antioxidant in eliminating ROS if exceeded will occur lipid peroxidation and cell damage (Weiss, Lo Buglio, 1982; Halliwell, 1999). MDA results showed no significant difference. It is caused by exposure to coal is subacute. (Armutcu, et al. 2007) Oxidative stress markers (MDA, XO and NO) increased significantly in mice exposed to coal for 1 and 2 weeks (Qomariyatus sholihah, 2011)

Antioxidants administration such as melatonin results were not statistically significantly different, the trend of a decrease MDA , BAL in mice, presumably because of Melatonin as an antioxidant eliminate free radicals such as H2O2, •OH, peroxinitrite anion (ONOO-), singlet oxygen (1O2), O2• - and peroxyl radical (LOO•) (Halliwell, 1999). As well as eliminating melatonin in oxidative damage to both lipids and the hydrophilic (Zapico, 2007).

SOD BAL activity results positive control rat increased significantly ($p < 0.05$) in both coal exposure day and night. After administration melatonin actually decreased SOD activity approaching negative control (see table 5.3 and 5.4). Increased contents of mice SOD after being exposed coal BAL showed that increasing free radicals (FR) due to exposure to coal will increase SOD to ward FR. SOD is an enzyme of the most powerful natural antioxidants, which will send a signal to other cells in order to produce more SOD, as well as activate and mobilize all the forces of defense systems, including secondary antioxidant (pineal, J. Res. 2003). After administering melatonin, SOD decreased compared to the positive control.

The results of measurements of TNF-α in this study shows the negative control obtained average contents of TNF-α: 52.274 + 17.383 ng / ml, because the mice always contained endogenous ROS produced by mitochondria, lysosomes, etc., contents of TNF-α to increase the positive control higher than the negative control in both groups during the exposure is equal to 704.418 ± 283.736 ng / ml and the exposure of the night in the amount of 734.575 + 283.73 ng / ml.

Elevated contents of tumor necrosis factor (TNF)-α, interleukin (IL)-1,(IL)-6 and intercellular cell adhesion molecule (ICAM)-1 has been found in experimental models of lung and airway fibrosis (Sime et al , 1998; Yucesoy, 2008) (Qomariyatus sholihah, 2011)

Melatonin administration may reduce contents of TNF-α, but not significant, even as he added a dose, contents of TNF-α trend is increasing even in the treatment of 3 with a dose of 2 mg / kg / day on average exceed the positive control that is equal to 766.843 ng / ml. This can be caused by melatonin dosage is less precise, because the endogenous melatonin during the day was suppressed , consequently the effects are not optimal.

The results of thickness measurements of the alveolar wall mice in each group in this study can be seen in table 4 and figure 3.

Rat alveolar wall thickness at day coal dust exposure increased at positive control group compared to the negative control group was significantly ($p < 0.05$). That coal dust can cause lung sensitization in which one of the indications that rat alveolar wall thickening.

Thickness of the alveolar wall thinning in a row M1, M2, and M3 are all significantly different when compared to the positive control group but not significantly different than the negative control group. This indicates that the thickness of the alveolar walls in the rat M1, M2, and M3 back to normal. Reduction of rat alveolar wall thickening in these groups are most likely a result of the administration of melatonin which acts as a free radicals binder in coal dust. It has been shown that melatonin is very strong free radical binding that able to dispose of H2O2, OH radical, peroxynitrite anion (ONOO ¬), singlet oxygen (1O¬2), O2 radicals and peroxyl radical (LOO *). The ability of melatonin as a protective antioxidative and detoxification of free radicals is very high especially for hydroxyl radicals (Tan et al., 2003). Melatonin is specifically reacts with hydroxyl radicals and nitric oxide via the release of electrons / hydrogen atoms at low and high reactivity (Agozzino et al., 2003). It shows melatonin's antioxidative broad spectrum performance. The ability of melatonin to neutralize radicals is a very significant advantage considering the lifetime of these radicals are very long and stable so that the effect and the damage can widespread (Hardeland and Poeggeler, 2005).

This will reduce the occurrence of alveolar hyper reactivity , which one of manifestation is the thickening of the alveolar wall. Thus, alveolar wall thickening can be reduced. This means that in the rat M1, M2, and M3, melatonin proved to reduce alveoli walls tickening in mice subacute exposed to coal dust .

CONCLUSION

1. Subacute exposure to coal dust day and night significantly increase contents of malondealdehid (MDA), activity of superoxide dismutase (SOD), the contents of TNF-α, alveolar wall thickness in broncho alveolar lavage (BAL) at mice
2. Body's reaction to sub-acute coal dust exposure, especially inflammatory reaction followed by oxidative stress reaction . Melatonin administration can overcome the short term inflammatory conditions.
3. The use of PPE mask actually can minimize the risk of occupational diseases caused by coal dust. Implementation of OSH management can increase productivity and reduce incident of occupational diseases. .

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