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Effect of Particulate Matter (PM) Exposure on Lung Histopathological Feature and IL-1β Level as Inflammatory Indicator in Rats (Rattus norvegicus)

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Background: Vehicle exhaust gases (emissions) and bushfire contain very small particles pollutants which can be affected on health. Particulate matter (PM) is a very small pollutant, can be inhaled to lower respiratory tract. Chronic PM exposure trigger an inflammatory response to release of endogenous proinflammatory mediators which cause lung tissue damage. Aim of this study to determine the effect of PM (carbon black powder) exposure on lung histopathological feature and IL-1β level in rats (Rattus norvegicus). **Method:** A true experimental study with post-test only control group design. Thirty five male rats 2-3 months old were divided into 5 groups: Control, P1 (PM 532 mg/m3 for 4 hours), P2 (PM 1064 mg/m3 for 4 hours), P3 (PM 532 mg/m3 for 8 hours), and P4 (PM 1064 mg/m3 for 8 hours). **Results:** Lung histopathological feature showed significant effect on lung's damage by Mann-Whitney test ($p < 0.05$). IL-1β levels were no significant effect among exposure rat groups by the Kruskal-Wallis test ($p > 0.05$). **Conclusion:** Particulate matter exposure cause chronic lung damage, but not accompanied by increasing IL-1β levels due to a different inflammatory response.

Keyword: particulate matter, IL-1β, lung histopathological damage

1. Introduction

Forest fires that occurred in the Kalimantan and Sumatra regions throughout 2019 have caused various health problems. Smog due to forest fires contains air pollution materials, among which particles and substances contained in it are very small organic particles, liquid droplets, PM10, CO, SO2, O3, NO2, and other materials such as aldehydes, polycyclic aromatics. hydrocarbons, benzene, toluene, styrene, metals, and dioxins which often cause health problems such as ARI [1,2].

According to WHO and UNICEF's reports on 2017, exposure to chemicals contained in smog is associated with problematic births, respiratory diseases in childhood, neurodevelopmental disorders, and cognitive function [1]. Air pollution has always been a hot topic in the world of health, air pollution causes 9 out of 10 people who breathe air containing high levels of pollutants to experience strokes,

heart disease, chronic obstructive pulmonary disease, respiratory infections, and other health problems both acutely and chronically, so that WHO determines air pollution as The Invisible Killer [3].
Pollutants that have the strongest evidence for public health problems include particulate matter (PM), ozone (O3), nitrogen dioxide (NO2) and sulfur dioxide (SO2). PM is a complex mixture of organic and inorganic substances. Particulate matter is made up of a number of components, including acids (such as nitrates and sulfates), organic chemicals, metals, soil or dust particles. Particulate matter is a particle that has a size of less than 0.1 μm to 30 μm, so that PM exposure is very dangerous for health and its very small size can allow these particles to enter into the lower respiratory tract. PM is used as the main parameter in air pollution because it can be associated with levels of other pollutants. The decrease in the rise of pollutants in the air such as carbon monoxide (CO), sulfur oxide (SO2), nitrogen oxide (NO2) can be directly proportional to PM levels. Particulate matter is also used as a target for causing health problems because it's very small size can allow entry into the lower respiratory tract [4,5].
Evidence that chronic particulate matter exposure to the inflammatory response through the formation and release of endogenous proinflammatory mediators, such as cytokines (IL-1 and IL-6, tumor necrosis factors, acute phase response molecules namely c-reactive protein and fibrinogen), activated white blood cells , platelets, and vascular-active molecules such as endothelin in the lungs can cause cell member injury characterized by an increase in malondialdehyde (MDA) compounds.

2. Method

This is a True Experimental with post-test only control group design. The purpose of this study was to determine the effect of exposure to air pollution using "Carbon black powder" on changes in the inflammatory indicator IL-1β and lung histopathology in male white rats. The inclusion criteria were male rats, body weight between 200-250 g, 2-3 months old, and healthy. The exclusion criteria in this study were weight loss of more than 10% after the adaptation period, stress, illness (before treatment), death during the treatment period.
The sample was divided into 5 groups, namely control, P1 with a dose of PM 532 mg / m3 for 4 hours, P2 with a dose of PM 1064 mg / m3 for 4 hours, P3 with a dose of PM 532 mg / m3 for 8 hours, and P4 with a dose of PM 1064 mg / m3 for 8 hours, consisting of 7 male rats for each group. Particulate Matter is used as a form of exposure to free radicals. The exposed groups of rats were put into a smoking pump measuring 50 x 40 x 20 cm with varying doses and durations for each group for 20 days. Interleukin 1β and lung histopathology was checked after termination. We used Komabiotech ELISA complete kit mouse IL-1 beta and hematoxylin eosin staining for histophatology slide staining. Data analysis used Non-parametric test Kruskal wallis after normality and homogeneity test result less then 0,05. When kruskal wallis result showed significant (P<0,05), we used mann-whitney test as post hoc to find different result between group. Our research using IBM SPSS statistics 20.

3. Result

IL-1β levels are one of the most common inflammatory indicator cytokines found in body tissues. This study looked at the effect of PM black carbon exposure on the average interleukin 1β level. The results showed that there were no significant differences between groups. Descriptive data (**Table 1**) shows the highest mean IL1 β levels in the control group and the lowest in the P3 group. The increase in IL-1β levels in the P1-P2 group descriptively had an upward trend that was higher than the P3-P4 group. The results of the Kruskal-Wallis test (**Table 2**) were not significant (P = 0.951) between treatment groups.
The effect of PM black carbon exposure on alveolar histological changes by assessing the score of the degree of lung damage in rats assessed based on the condition of the inter-alveolar septum and alveolar membrane. The descriptive test results (**Table 3**) show that there is an increasing trend in the mean score of degree of lung damage from the control group to the P4 group. The results of the Kruskal-Wallis test (**Table 4**) showed a significant result p = 0.008 (< 0.05) between treatment groups. The results of the Post Hoc test using the Mann-Whitney test (**Table 5**) showed statistically significant

differences in the effect of the control group on all treatment groups ($p < 0.05$). On the other hand, there was no significant difference between the treatment groups P1, P2, P3 and P4 ($p > 0.05$).

Table 1. Descriptive data of mean IL-1β levels between treatment groups

Group	Mean		Std. Deviation
	Statistic	Std. Error	
K	499,6803	268,62843	537,25687
P1	121,9440	6,50309	13,00618
P2	152,3325	47,17922	94,35844
P3	117,8200	23,42210	46,84420
P4	129,2738	20,07863	40,15725

Table 2. Kruskal-Wallis result test mean IL-1β S levels between treatment groups

Group	Mean±SD	Sig
K	499,6803 ± 537,25687	0,951
P1	121,9440 ± 13,00618	
P2	152,3325 ± 94,35844	
P3	117,8200 ± 46,84420	
P4	129,2738 ± 40,15725	

Table 3. Descriptive data of histological degree of lung damage scores between treatment groups

Group	Mean		Std. Deviation
	Statistic	Std. Error	
K	1,300	0,10	0,2000
P1	1,800	0,1414	0,2828
P2	2,200	0,2944	0,5888
P3	2,400	0,0577	0,4899
P4	2,900	0,1454	0,1155

Table 4. Kruskal-Wallis results test mean score of degree of lung damage between treatment groups

Group	Mean±SD	Sig
K	1,300 ± 0,2000	0,008
P1	1,800 ± 0,2828	
P2	2,200 ± 0,5888	
P3	2,400 ± 0,4899	
P4	2,900 ± 0,1155	

Asymp. Sig. > 0,05 = Normal and Homogeneous

Table 5. Mann-Whitney Post-Hoc Test

	P4	P3	P2	P1	K	Mean lung damage score
P4	-					2,900
P3	0,134	-				2,400
P2	0,137	0,655	-			2,200
P1	0,019	0,065	0,237	-		1,800
K	0,017	0,017	0,018	0,044	-	1,300

Asymp. Sig. < 0,05 = significantly

The degree of lung damage in experimental animals according to Marianti (2009) can be assessed based on the image of the alveolar membrane and interalveolar septum which is divided into 3 scores. Score 1 given if the alveolar membrane is nucleated and complete with endothelial cells reaches> 75% and relationship between alveoli> 75%. Score 2 given if the alveolar membrane is nucleated and complete with endothelial cells of about 25-75% and the relationship between the alveoli is 25-75%, and score 3 given if the alveolar membrane is nucleated and complete with endothelium cells <25% and relationship between alveoli < 25 %. According to this following **Figure 1**, K is control group, P1 group was exposed by 532 mg of carbon for 4 hours per day for 30 days, P2 group was exposed by 1064 mg of carbon for 4 hours per day for 30 days, P3 group was exposed by carbon 532 mg for 8 hours per day for 30 days, and P4 group was exposed to 1064 mg carbon for 8 hours per day for 30 days.

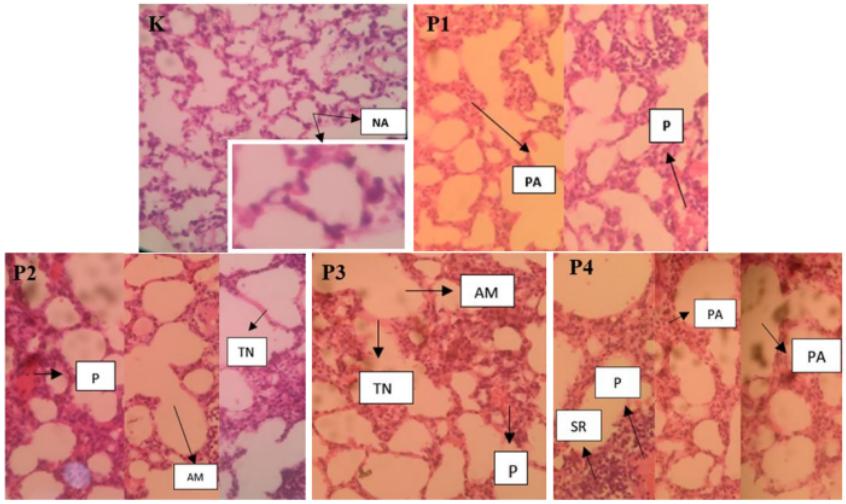

Figure 1. Histopathology of lung alveoli with a magnification of 40 x 10. HE-staining. NA: Normal Alveolus; PA: Thickening of the interalveolar septum; P: Bleeding; AM: Alveolus widens; TN: no surrounding endothelial cells; K: Congestion; SR: accumulation of inflammatory cells.

4. Discussion

4.1. IL-1β levels

IL-1β is a proinflammatory cytokine secreted by keratinocytes, endothelium, neurons, synoviocytes, fibroblasts, and immune cells as the body's defense mechanism against foreign compounds/infections

Increased levels of IL-1β due to chronic inflammation can increase the risk of certain diseases such as atherosclerosis, rheumatoid arthritis, multiple sclerosis and other diseases [6].

Descriptive data for serum IL-1β levels (**Table 1**) showed that the highest increase was found in the control group. The treatment group actually had lower levels of IL-1β than the control group. The Kruskal wallis test results (**Table 2**) also did not show any significant differences between treatment groups. This occurs when there is non-specific infection in one or all of the control group rats, causing a very high increase in the mean IL-1β levels. The low value in the treatment group could also be due to a deficiency of the NLRP3 inflamasome due to prolonged carbon exposure, judging from the low IL-1β levels in the treatment group.

The study of Dossert et al. (2008) explained that NLRP3 was linked to an increase in IL-1β. The risk of exposure to inflammation-inducing substances or compounds such as cigarettes and carbon in the lungs does not necessarily increase IL-1β levels in human monocyte THP-1 cells. On the other hand, the presence of NLRP3 was shown to affect IL-1β levels. This is evidenced by research by Eltom et al. (2014) which concluded that stimulation of inflammatory compounds such as cigarette smoke or other substances can reduce the innate immune system by reducing NLRP3 levels by increasing the proteosomal process of ubiquitin, thereby preventing the release of IL-1β and IL-18 as a defense response. Anti-microbial. IL-1β has a role that is not limited to the innate defense system but is also able to activate the release of TNF and IL-6 so as to induce TH17 cell differentiation as a cellular adaptive response. Other studies have concluded that the induction of inflammatory substances or compounds such as black carbon and cigarettes causes increased levels of IL-1β and IL-18 as a form of pathophysiological process of chronic obstructive pulmonary disease [7-11].

It can be concluded that there is a possibility that the responses generated by inflammatory compounds in the lungs differ based on cell types, model systems and kinetics. The researchers also concluded that inflammatory substances / compounds were not able to increase IL-1β except by activating the NLRP3 pathway or through other pathways so as to increase IL-1β levels.

Lung Histopathological Feature

The descriptive data of pulmonary histopathology examination (**Table 3**) showed that the control group had the lowest result compared to the treatment group, while the P4 group had the highest result compared to all treatment groups. The increase in the mean lung damage score was influenced by the duration of administration and the dose of black carbon intervention in the other treatment groups. The P1 group exposed to PM at a dose of 532 mg / m3 for 4 hours per day showed lower results than the P2 group exposed to PM with a dose of 1064 mg / m3 for 4 hours per day. Likewise with the P3 and P4 groups, the P3 group exposed to PM with a dose of 532 mg / m3 for 8 hours showed lower results than the P4 group exposed to PM with a dose of 1064 mg / m3 for 8 hours per day. The Kruskal wallis test results (**Table 4**) showed that there were significant differences between treatment groups. The Mann Whitney Post-hoc test (**Table 5**) shows a significant difference in the effect of the control and treatment groups.

This can occur because the lungs have a self-defense mechanism from foreign particles that enter the respiratory tract, namely through an inflammatory response. When inflammation occurs, the immune system releases products that can cause endothelial injury and tissue damage. A study by Tankersley et al (2008) stated that the effect of giving black carbon (400µg / m3) in 3 hours for 4 days in mice caused heart problems, increased ROS and MMP (MMP-2 and MMP-9) significantly. The study by Carter et al (2005) also mentioned an increase in ROS and reactive oxygen species in mice, while in the study of Elder et al. (2005) it was found that lung damage and inflammation detected from histopathological examination of mice showed more severe results than mice or mice. hamster [12-15].

It can be concluded that there is a real detrimental impact due to exposure to PM that is inhaled and enters the respiratory tract when seen in the histopathological picture. As well as the number of doses and length of duration given is directly proportional to the degree of lung damage caused.

5. Conclusion

There was no effect of PM black carbon exposure on IL-1β levels based on statistical test results with a significance value of P> 0.05 between the treatment groups. There is an effect of lung histopathology feature based on the average degree of lung damage with a significance value of P <0.05.

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