

COMPARISON OF LUNG DAMAGES DUE TO PETROL AND DIESEL CAR SMOKE EXPOSURES: HISTOLOGICAL STUDY

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ABSTRACT: Motor vehicles have significantly recognized to have a role in air pollution. The emissions regarding particles and gasses have identified to affect human health depending on the concentration, constituents, kind of vehicles, and fuels. This study investigated the impacts of the petrol and diesel car smoke on mice lung. The aims were to observe the mice lung injury caused by the smoke exposures emitted by petrol and diesel car. The lung injury was identified by using the histological images. The mice were exposed to the smoke with a particular concentration conducted by introducing the smoke into the chamber with the dimension of 30x20x20 cm³ as long as 40 seconds. The mice exposures were carried out daily for eight days. The lung injury was observed using a Binocular BX-51 Computer Microscope with the 400x magnification to the histological images. We identified the healthy cells and damage cells to determine the lung damage showed by the destructive index. The results showed that the mice exposed to diesel cars have higher index rather than those with the petrol car exposures.

Keywords: Smoke exposures, Petrol cars, Diesel cars, Lung damage

1. INTRODUCTION

The traffic pollutants have significantly contributed to the air quality and adverse on health effects. The car emissions contained some dangerous compounds have triggered health problems [1] based on the type of the pollutant itself [2]. There are two kinds of the fuel used to run the engine such as diesel and gasoline. The emissions are in the form of Both types of engine produced compounds that are in the form of gasses [3] and particles [4]. The emission compounds consist of polycyclic aromatic hydrocarbon [5], nitro polycyclic aromatic hydrocarbon [6], black carbon [7], NO_x [8], particulate matter in various size (PM_{2.5}, PM₁₀, PM_{2.5}, PM_{1.0}, and PM_{0.1}) [4] in the different concentration depended on their composition, blended, driving methods, [9], engine type [10], and compression ratio [11].

Fine particles (PM_{2.5}) and ultrafine particles (PM_{0.1}) are commonly found in the car smoke [12]. The problem of these particles is due to their size, made them possible to be in their airborne and inhaled by the human [13]. The result is a health problem [2] that has reported mostly in respiratory diseases because of the particle emissions [14]. The concentration of fine particle and ultrafine particles was found high in the area close to Highway [15]. This resulted in the increase of the risk for the people living near the highway that had to be exposed daily [16]. Based on the previous research about the effect of the vehicles smoke for the traffic assistance that increased the chance of developing cancer [17].

In general, the vehicle smoke exposures have identified to affect a lung damage. Previous studies showed that particle exposures have resulted in the respiratory inflammation response [18], alveolar enlargement [19], oxidative stress [20], and alveolar lavage fluids [21]. The allergy response was the most reported cases [22], [23]. The other circumstances were influenza-like illness [24], alveolar emphysema [25], lung cancer [26], and chronic obstructive pulmonary disease (COPD) [27]. The impacts of the vehicle emissions on lung, there is less information available, especially for the emissions from the car using a different fuel. In this study, we tried to observe the effects of the car smoke exposures on lung damage to better understanding the impacts of the emissions emitted by the car operate using diesel and petrol fuel.

2. METHODS

2.1 General Method

Three different cars were used as the samples based on their availability in Indonesia. The car sample contains four different cars that operated by using two different engine. Two cars were operated using petroleum specifically with 90 octane and 92 octane fuel type and the others were fueled by a diesel of the 48 Cetane and 51 Cetane. To characterize the effect of petrol and diesel car emission to the lungs, we prepared 4 groups of the mice. The mice were chosen by their similar characteristic in the response of the lungs to reveal the effect of the smoke on the human respiratory

system [28]. The first group acted as a control that we named the control group. The second group was exposed to 90 octane petrol car smoke as petrol group, the third group exposed by 92 octane petrol car smoke, the third group was exposed by 48 cetane diesel car smoke, and the fourth group was exposed by 51 cetane diesel car smoke. Each of the group excepted the control group was experienced for the 100-seconds exposures with the dose concentration that was set up by introducing the smoke into a chamber as long as 40 seconds. The lung of the mice was taken after the 8 exposure days and observed using a Binocular BX-51 Computer Microscope with the 400x magnification. Each histological images of the lung were identified to determine the damage.

To know the concentration of the ultrafine particles and the fine particles in the smoke, we measured the concentration using a *P-Track type 8525* and a *Kanomax model 2443* dust monitor. The smoke of the car was collected by the airbag; then the smoke was injected into the chamber with the dimension of 30x20x20 cm³ with the rate of 2 m/s for 40 seconds. The total concentration of the particles in the smoke was calculated by summing of the measured particle concentrations.

2.2 Mice preparation

The mice were treated by following the standard of humane animal care and the guidelines by the Ethics Committee of Experimental Animal of the University of Brawijaya. The mice were kept in the quarantine chamber to isolate from any external pollutant source to avoid the contamination in the standard light (12:12 dark-light) and standard RH-temperature conditions [29]. To reduce the mice stress during exposures, the acclimation processes were done for 3 days by putting the mice into the exposure chamber for 100 seconds.

2.3 Histological Analysis

The mice were treated by following the standard of humane animal care and the guidelines by the Ethics Committee of Experimental Animal of the University of Brawijaya. The mice were kept in the quarantine chamber to isolate from any external pollutant source to avoid the contamination in the standard light (12:12 dark-light) and standard RH-temperature conditions [29]. To reduce the mice stress during exposures, the acclimation processes were done for 3 days by putting the mice into the exposure chamber for 100 seconds.

The histological damage analysis was conducted by observing the lung sample images by using the microscope with the magnification of

400 times and were identified by a counting method. The lung injuries were found as alveolar geometrical damage [30], loss of alveolar wall [31], hemorrhage [32], and the inflammation responses [33]. The destructive index [34] was used to analyze the damage level by following the eq. (1)

$$D_e = \frac{D_c}{T_c} \quad (1)$$

With D_e is dea structive index, D_c is the damage cell counted, T_c is total cells counted by adding the damage cell with the normal once [35].

3. RESULT AND DISCUSSION

The total concentration that exposed to the mice were measured at different levels for all cars sample. In the car that using petrol with 90 octane fuel, the concentration of ultrafine particulate was measured $10.32 \pm 0.56 \times 10^{20}$ particles/m³ while in the octane 92 measured $14.88 \pm 1.54 \times 10^{20}$ particles/m³. In the exposure of diesel fuel group, the UFPs measured in 60.00×10^{20} particles/m³ while in 51cetane group measured 17.76×10^{20} particles/m³. In the concentration of fine particulate, 90 octane petroleum group measured in 15.04 ± 1.72 mg/m³ while in 92 group was measured in 1.54 ± 0.09 mg/m³. The concentrations were used as the dose concentrations exposed to the mice once a day for eight days. Table 1 shows the detail of the measured particle concentrations.

Table 1 Total concentrations of ultrafine and fine particles

Fuel type	Octane/Cetane	UFPs x 10 ²⁰ pts/m ³		FPx10 ⁻³ mg/m ³	
		Mean	std	Mean	std
Petrol	Octane 90	10.32	0.56	15.04	1.72
Petrol	Octane 92	14.88	0.24	1.54	0.09
Diesel	Cetane 48	60.00	3.20	162.06	6.90
Diesel	Cetane 51	17.76	1.30	22.00	0.42

The measurement result presented in table 1 indicates that the engine type affects the concentration of ultrafine and fine particles in the smoke [36]. This data are supported by the fact that the properties of the fuel that used in the combustion system emitting the significant different amount of particles [37], [38]. The diesel engine vehicles had a more significant contribution

in the particulate matter concentration than the petrol once in the road.

3.1 Lung damage Identification

The lung damages identified in the histological images are found similarly. The alveolus damages (blue dots) that are the geometrical changes both the shape and size, the inflammation (red circle), and loss of alveolus wall (yellow circle) may as the result of the oxidative response caused by the interaction between the tissues and particles [39]. As the result of the interaction, The reactive oxygen series (ROS) is released [40]. ROS affects the loss of alveolar septum [41] and triggers the pro-inflammatory cytokine [42].

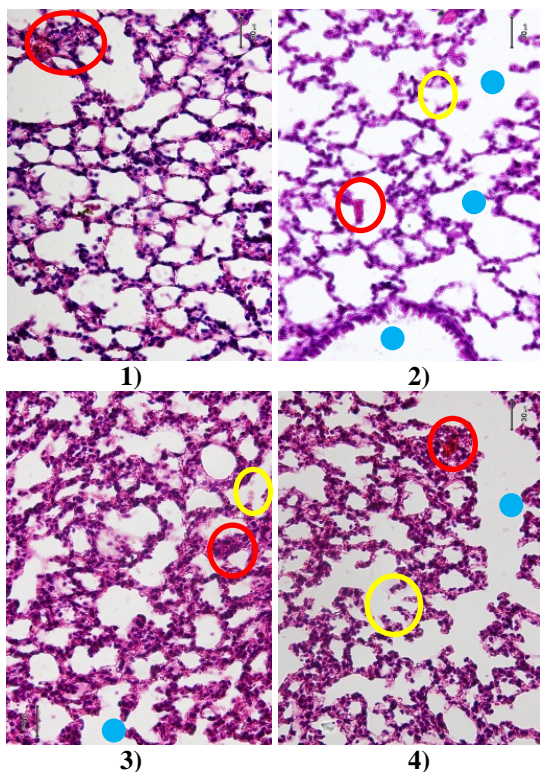


Fig.1 The mice lung condition after exposure to various types of smoke from the different engine. (1) Control; (2) Petrol; (3) Cetane 51 diesel; (4) Cetane 48 diesel.

The development of emphysema in the lung is caused by the work of pro-inflammation cytokine and anti-inflammation cytokine [43]. The pro-inflammation triggers the development of alveolar damage [44]. Meanwhile, the anti-inflammation stops the damage but it can not repair the alveolus geometrical shape [45].

In Fig 1, the alveolar damage can be seen as the irregular shape of alveolar and the enlargement of the area [19], and the inflammation that is believed to trigger the emphysema [46]. The depleted alveolar septum is also found by identifying the loosing of the alveolar wall. The hemorrhage is also obtained by the appearance of the erythrocytes in particular area.

3.2 Damage analysis

The damages presented in the lung were observed in the different level is shown in Figure 2. The highest destructive index of 0.47 is obtained for the mice exposed by the cetane 48 fuel car. The mice exposures with the car smoke fueled by the Octane 92 and Cetane 51 cause the lung damage with the destructive index of 0.32. The mice lung damage with the lowest destructive index of 0.30 is found for the exposure with the octane 90 fuel car smoke.

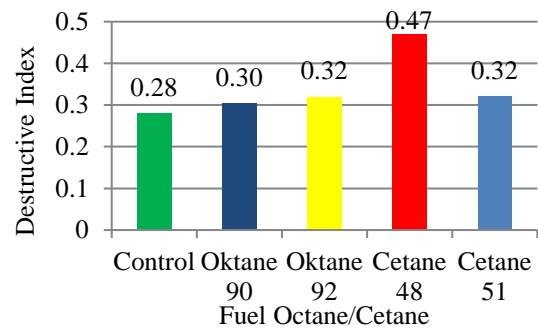


Fig. 2 The destructive index of the lung damage caused by the car exposures.

The difference in the lung damage between the control and the exposed samples indicates that the car smoke surely damages the lung tissues. The smoke exposures from the petrol engine car give relatively similar the mice lung. Meanwhile, the large difference in the mice lung damage is caused by the smoke exposure from the diesel car. The engine technology affects the particle production [47]. The relationship between the particle concentration and the lung destruction index is presented in Figure 3.

The FPs and UFPs concentration was measured differently in the smoke with different fuel type. Their effect on the lungs is present in figure 3 that shows the graph of the correlation between the destructive index and the concentration of particles by using R^2 value. The R^2 value that higher than 0.8 is shown the strong correlation [48] between the concentration of the particle and the destructive index in the lung. Further, the UFPs value, that was calculated in 0.9972, was found higher than FPs in 0.8966. This result showed that the

destructive index of the lung is more affected by the UFPs than the FPs. The identical behavior of the destructive index graph with the UFPs concentration has supported this result. The other research about the behavior of FPs and UFPs in epidemiological term has also supported this argument [49].

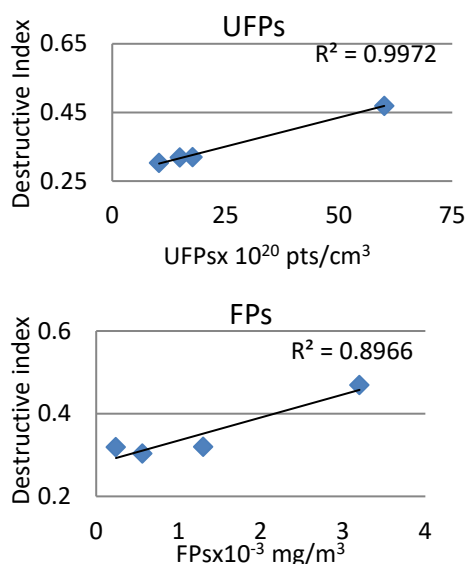


Fig 3. The relationship between the particle concentration and the lung destructive index.

4. CONCLUSION

The car smoke exposures influence the damage of the mice lung. The car operating with a different fuel produces the smoke with the different particle concentration. The diesel fuel cars produced the large particle concentration rather than the petrol car did. The diesel-car smoke exposures to mice cause lung damage.

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