ORIGINAL ARTICLE

Inhalation Toxicity of Coal Fly Ash in Mice Models

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Abstract

Background: Indonesia is one of world's largest producers of coal. Coal fly ash (CFA), a product of the coal combustion process, consists of various minerals and causes toxic effects by inhalation. This study aimed to analyze the toxic effects of CFA inhalation in mice models by examining oxidative stress and inflammation markers in the blood.

Methods: A true experimental with post-test control group design was used in this study. Twenty-four mice were randomly divided into three groups including control (P1), CFA inhalation for 24 hours (P2), and CFA inhalation for 30 days (P3). Mice in the P2 and P3 groups were exposed to CFA measuring 0.075 mm with doses equal to 12.5 mg/m3 for 1 hour/day. Malondialdehyde (MDA) and the percentage of blood eosinophils were examined as parameters of toxic effects. One way analysis of variance (ANOVA) test was used to compare the toxic effects between groups.

Results: MDA were significantly increased in between groups ($p<0.05$). The percentage of blood eosinophils in P1 was significantly differenced to P2 and P3 ($p=0.04$ and $p=0.14$, respectively), however there was no different than P2 and P3 ($p = 0.891$).

Conclusions: CFA inhalation induces toxic effects through increased oxidative stress and inflammation in mice models. This may indicates health hazards after CFA inhalation.

Keywords: Coal fly ash, Inhalation, Mice, Malondialdehyde, Eosinophil

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INTRODUCTION

Coal is one of the most important sources of energy, and make up nearly 40% of the fuel in power plants worldwide (1). Indonesia is the fifth largest coal producing country in the world with a total production of 470.8 tons in 2014 and 392 tons in 2015 (2). Asam-asam power station is a coal fuel power plant in South Kalimantan, Indonesia. The coal combustion process produces ash as a pollutant, consisting of 80% fly ash and 20% bottom ash. Air pollution produced by coal combustion is a larger health risk to society than emissions in water or soil. Health impacts due to coal-fired air pollution include respiratory and cardiovascular system disorders, cancer, growth and development disruption (3,4). Coal fly ash (CFA) inhalation causes toxic effects that are dependents on components, quantity, and frequency. CFA exposure to high intensity in a short time is associated with severe toxicity in the body (5,6).

Asam-asam CFA contains various minerals, including relatively high silica (74.2%), iron (14.4%), alumina (5.7%), magnesium (2.03%), and others (5). CFA inhalation induces cell damage due to free radical exposure and increased lipid peroxidation reactions (7). Lipid peroxidation is a complex chain reaction of polyunsaturated fatty acid (PUFA) and reactive oxygen species (ROS) leading to the formation of hydrogen peroxide which causes the decomposition of aldehyde. Malondialdehyde (MDA), 4-hydroxy-2-noneal (HNE), F2-isoprostanes, and other active compounds are the secondary products of lipid peroxidation reaction. MDA is highly reactive compound and easily penetrates into the tissue, inducing cell disruption. Therefore, MDA is widely used as a biomarker of oxidative stress (8,9).

The toxic effects of CFA inhalation can also be seen through abnormalities in blood tests (6). Hematological abnormalities are directly correlated to inflammatory response due to lung tissue damage (10). Blood eosinophil count is one of the inflammatory parameters commonly used by clinicians. Eosinophils on the peripheral blood smear are characterized by round cells with two lobes, purple chromatin and eosinophilic granules. Eosinophilia is associated with decreased lung function and has been linked to airway degeneration (6, 11).

Our study aimed to analyze the toxic effects of CFA inhalation in mice models. In this case, MDA levels were an oxidative stress parameter and peripheral blood eosinophil counts were an indicator of inflammatory response.

METHODS

This true experimental study with post-test control group design was conducted in January-March 2020 at Lambung

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Mangkurat University, Banjarmasin, Indonesia. A total of 24 male mice (*Mus musculus*), aged 2-3 months old, and weighing 20-30 grams were used in our study. Twenty-four mice were randomly divided into three groups, namely control (P1), 24-hours CFA inhalation (P2) as an acute exposure, and 30-days CFA inhalation (P3) as a sub-chronic exposure. We used the CFA from Asam-asam power plant in Jorong District Tanah Laut Regency, South Kalimantan Province, which consists of silica (74.2%), iron (14.4%), alumina (5.7%), magnesium (2.03%), and others (5). We obtained the CFA from the power station's coal-burning, which was stacked in landfills and filtered using a sieve number 200 to get a particle size of ≤0.075 mm.

Mice were exposed to aerosolized CFA or/and normal saline fluid using a special tool designed by the Department of Pharmacology, Lambung Mangkurat University. The device provides an ambient environment for CFA or/and normal saline fluid to be inhaled into the airways. Normal saline fluid inhalation was given for 30 days in the P1group. Mice in P2 were given CFA at the dosage of 12.5 mg /m^3 for 1 hour in the first day and normal saline fluid inhalation in the days 2- 30. Mice in P3 were given CFA at the dosage of 12.5 mg/m^3 for 1 hour/day for 30 days (13).

At the end of the investigation, a peripheral blood smear was done by drawing blood from the lateral veins of mice tails. Peripheral blood eosinophil count was done under a binocular microscope XSZ 107BN (Yazumi, Japan) by counting white blood cells to a total of 100. Results were expressed as a percentage (%). Eosinophilia was defined as more than 3%. Then, the animals in all groups were anesthetized and MDA levels were determined as thiobarbituric acid reactive substance (TBARS) method using Elabscience assay kit (Catalog no. 5 E-BC-K025-M, USA) (14). Results were expressed as µM.

Data were expressed as mean±standard because the data was normally distributed and the data variance was homogeneous using Saphiro Wilk test. Comparison data between groups were analyzed using one-way analysis of variance (ANOVA) with SPSS version 17.0 software (IBM SPSS). A p-value less than 0.05 was defined as statistically significant. Our research had been approved by the Ethical Committee of Medical Research Medical Faculty of Lambung Mangkurat University, Banjarmasin, Indonesia (008/KEPK-FK UNLAM/EC/I/2020).

RESULTS

Figure 1 shows MDA levels in mean±standard deviation of each group (185.7±1.9 µM for P1, 193.0±1.5 µM for P2, and 201.8±1.1 µM for P3). Mice exposed to CFA had significantly higher MDA levels than control mice (p=0.000, respectively). MDA levels were significantly increased in 30-days CFA inhalation compared to 24-hours CFA inhalation or without CFA inhalation (p=0.000, respectively).

Figure 2 summarizes the percentage of eosinophil counts in peripheral blood smear in each group, namely 2.5±1.9 (P1), 7.4±2.8 (P2), and 8.2±4.2 (P3). Eosinophil counts in the control group were significantly different to both CFA inhalation groups (p=0.04 for P2 and p=0.014 for P3). However, the percentage of eosinophils was not different between CFA inhalation groups (p=0.891).

DISCUSSION

CFA is a material produced during the coal combustion process in a power plant. CFA contains both inorganic and organic minerals with its main components being silica (SiO_2), alumina (Al_2O_3), iron oxide (Fe_2O_3), and calcium oxide (CaO), along with carbon, magnesium, and sulfur (5,15). CFA inhalation causes health hazards, depending on its chemical content, size, and concentration in the air. CFA particles inhaled and precipitated in the lungs induce alveolar macrophages and epithelial cells activation, resulting in the appeareance of inflammatory mediators, reactive oxygen species (ROS), various enzymes, cytokines, and growth factors (6,16,17).

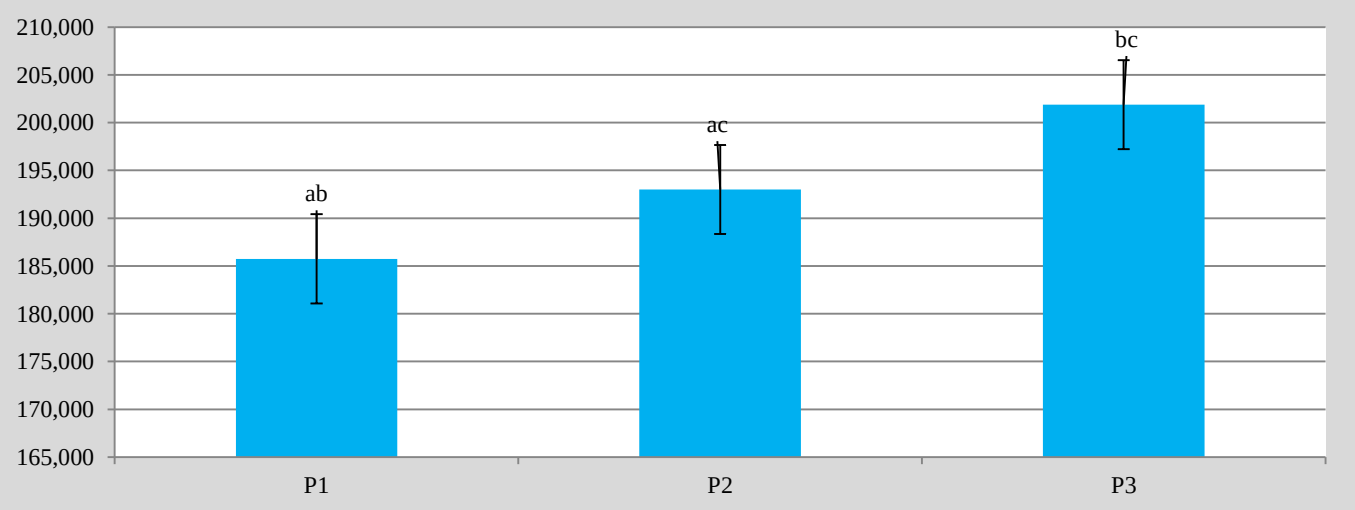

Figure 1. Malondialdehyde levels with thiobarbituric acid reactive substance method were measured after investigation. Values are presented mean±standard deviation. P1 as control group. P2 as 24-hours CFA inhalation group. P3 as 30-days CFA inhalation group. ap=0.000; comparison between P1 to P2. bp=0.000; comparison between P1 to P3. cp=0.000; comparison between P2 to P3.

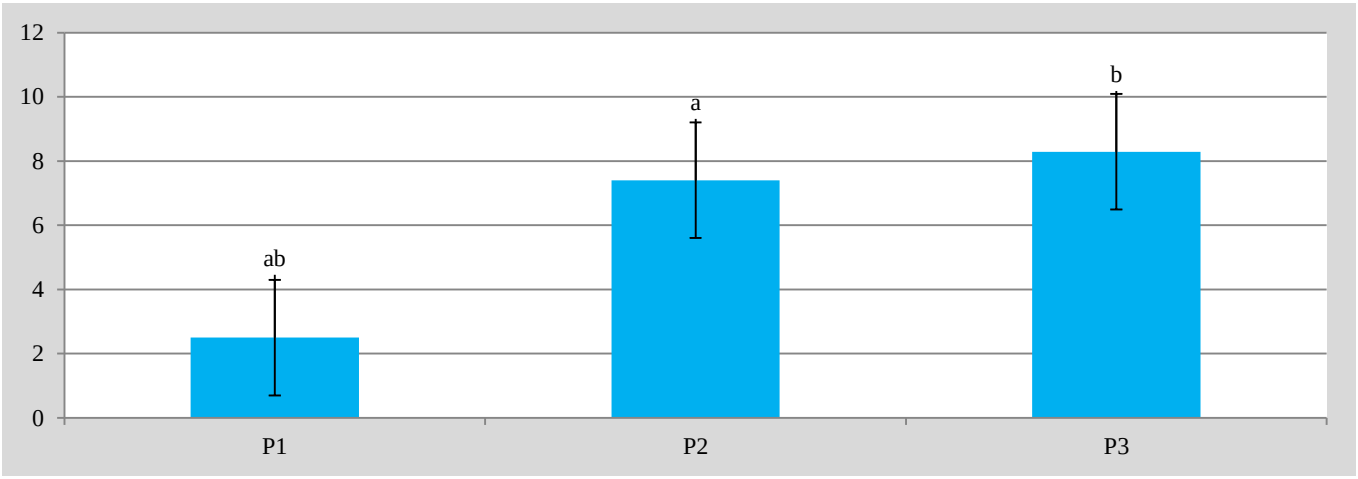

Figure 2. The percentage of eosinophil blood counts were measured after investigation. Values are presented mean±standard deviation. P1 as control group. P2 as 24-hours CFA inhalation group. P3 as 30-days CFA inhalation group. ap=0.04; comparison between P1 to P2. bp=0.014; comparison between P1 to P3.

Respiratory tract epithelial cells function as structural barriers for inhaled particlesand also produce inflammatory mediators. These processes cause increased epithelial cells permeability, which is also induced by oxidative stress, so that CFA particles can penetrate the alveoli toward the circulatory system (17,18). Thiobarbituric acid malondialdehyde, a product of lipid peroxidation, is used as a parameter of oxidative stress (7,10,13,18). We found significant increased MDA level in both CFA inhalation groups compared to the control group. In a previous study, in which coal dust was inhaled, TBARS level was significantly increased in a nondiabetic rat group compared to the control group (18). However, another study found no significant increase in MDA level between diabetic rats with/without different doses of coal dust exposure (13). Our study also found a significant increase in oxidative stress between acute (24-hours) and sub-chronic (30-days) CFA exposure. A study from Korea found significant differences in MDA levels in rat plasma exposed to high concentrations of industrial incineration fly ash for 30 days (19). Increased oxidative stress is related to the dose and length of exposure to free radicals (20-23).

One of the main explanations for the toxicity of CFA is through the generation of ROS which causes cell membrane damage due to lipid peroxidation, protein oxidation, and DNA damage. ROS formation occurs directly or indirectly. The surface load or metal transition triggers Fenton reaction, which is a direct process. The release of inflammatory mediators during particle phagocytosis and inflammation is an indirect process (24).

CFA inhalation also induces inflammatory mediators, like elevated blood eosinophil count. Hematological examination can also show decreased hemoglobin, which is associated with toxic effects inducing anemia conditions. However, eosinophil examination has been more specifically used as a parameter of toxic effects in CFA exposure (6). Similarly, we found a significant increase in eosinophil percentage in peripheral blood between CFA exposure and non-CFA exposure.The percentage of eosinophil counts in sub- chronic CFA inhalation was higher than acute CFA inhalation, however there was no difference statistically. Although a study found an increase in eosinophil count between different doses of fly ash inhalation from industrial waste incinerators, eosinophil blood counts between groups were not considered to be associated with fly ash exposure (19). A Nigeria study also found no significant differences in hematological parameters including eosinophil count in rabbits instilled with various doses of CFA (25). An other study showed no difference in oxidative stress and hematological inflammatory parameters due to differences in the composition and quantity of CFA used in each study (11).

We conclude that MDA levels and eosinophil blood counts can be used as inhalation toxicity parameters of CFA in mice models. These toxicity markers indicate a health hazard after CFA inhalation. Prolonged exposure causes CFA particles to settle more in the lungs, leading to more sever toxic effects. Limitation of our study included that we couldn not examine the histopathology of respiratory tract due to the semi-lockdown and pandemic situation.

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