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RESEARCH ARTICLE

Effect of subacute toxicity of mosquito coil smoke inhalation on lung histology and blood hematology parameters in Wistar rats

Mulyati Sri Rahayu [1], Sri Wahyuni [2]

[1]Department of Anatomical Pathology, Faculty of Medicine, Universitas Malikussaleh, Aceh Utara, Indonesia

[2]Department of Biochemistry, Faculty of Medicine, Universitas Malikussaleh, Aceh Utara, Indonesia

Correspondence:
Mulyati Sri Rahayu
Faculty of Medicine, Universitas Malikussaleh, Cot Teungku Nie, Reuelet, Muata Batu, Aceh Utara, Aceh Province – 24355, Indonesia

Email: mulyati.srirahayu@unimal.ac.id

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Abstract

Mosquito coils are made from incense products and are designed to repel insects using pyrethrins and pyrethroids as their main active ingredients. However, the use of these coils poses significant public health and ecological risks due to the emitted toxic fumes. These fumes can have long-term damaging effects on humans and the environment. This study aimed to investigate the effects of various durations of subacute exposure to mosquito coil smoke (MCS) on lung histology and blood hematology in white male Wistar rats (*Rattus norvegicus*). Rats were divided into four groups (n = 6): control; P1, P2, and P3, exposed to MCS for 5, 7, and 9 h per day, respectively, for 21 days. Hematological blood analyses (red cell count (RBC), hemoglobin (Hb), and hematocrit (Hct) were performed, and lung histopathology was evaluated using Manja Roenigk's criteria. The group exposed to MCS for 9 h/day showed a significant decrease in mean body weight (185.00 ± 9.59) ($p = 0.008$) compared with the control group. All treated groups had normal Hb and Hct levels and RBC indices, with no significant differences compared with the control group. The 9-hour exposure group had significantly lower lung weights than the control group, and all treatment groups showed significant differences in lung weight. Histopathological analysis revealed significant loss of alveolar septal integrity, leading to alveolar lumen rupture and development of emphysema. This study provides clear evidence of emphysema due to subacute allethrin-based MCS inhalation, although it did not affect hematological parameters in rats.

1. INTRODUCTION

Mosquitoes transmit diseases such as malaria, dengue, Zika virus, chikungunya, and yellow fever, and their bites cause itching, discomfort, and unattractiveness (1). Mosquito coils are frequently used to control mosquito populations in homes (2). Approximately 29.5% of the Indonesian population uses mosquito coils (3).

Factors such as affordability and accessibility for low-income populations have contributed to the widespread use of mosquito coils in developing countries. Mosquito coils (MCs) serve as a means of insecticide dispersal through combustion, providing a limited safeguard against life-threatening mosquito-vectored illnesses. However, inhalation of these insecticidal compounds not only targets the intended pests, but also introduces substantial health risks due to associated toxicities. The prevalence of MC usage continues to rise, especially in developing countries; however, this phenomenon has not received sufficient scrutiny from governmental bodies or the public (1). The general population annually uses these coils for extended periods. However, prolonged smoke exposure from these coils can have cumulative health impacts. Many developing nations continue to incorporate mosquito coil smoke into their daily lives despite the potential health risks (4).

Mosquito coils are created by combining granulated insecticide paste with fillers, such as sawdust, to form a coiled mixture that serves as an insect repellent (1). Pyrethrins and pyrethroids, making up 3-4% of the coil mass, are the primary

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active ingredients that provide several hours of protection against mosquito bites (5). Despite their protective function, mosquito coils pose significant public health and ecological risks because of the harmful fumes they emit (6). Research shows that burning one mosquito coil can release particulate matter equivalent to burning 75-137 cigarettes and formaldehyde, similar to burning 51 cigarettes (7). Mosquito coil combustion of generates elevated indoor levels of PM2.5 and CO, which are associated with potential respiratory health risks (8). This combustion increases indoor levels of PM2.5 and CO, which are associated with respiratory health risks (8). The hazards of mosquito coils are attributed to their emissions during burning, whereas their efficacy in repelling mosquitoes is related to their benefits (9).

Burning mosquito coils indoors generates smoke that can effectively control mosquitoes. Despite the fact that mosquito coil smoke may have many potential adverse health effects, large populations in developing countries still use mosquito coils in their daily lives. This practice is currently used in numerous households in Indonesia. Because coil consumers usually use mosquito coils for at least several months every year, the cumulative effects of long-term exposure to the coil smoke may also be a concern. Epidemiological studies have shown that epidemiological studies have shown that children and their parents exposed to mosquito coil smoke are more susceptible to the released chemicals during sleeping quarters. The long-term exposure to mosquito coil smoke can induce chronic bronchitis, asthma, and persistent wheezing in children and adults (10,11).

The use of mosquito repellent products releases chemicals into the air, raising concerns about their impact on people who inhale these compounds (12). When assessing the health implications of inhaled vaporizer fumes containing allerthrin and mosquito coil smoke, special attention is given to the respiratory system (13). Mosquito coils emit smoke containing sub-micrometer particles, volatile and semi-volatile organic compounds, gaseous pollutants, polyaromatic hydrocarbons, and insecticides (14,15). The highest concentration of allethrin is typically detected 30-35 minutes after using a mosquito coil and decreases over six hours. Smoke exposure at night can result in the inhalation of tiny particles, metal fumes, and free radicals that reach the alveolar region of the lungs and irritate the upper respiratory tract (15).

The Wistar rat model is frequently used in toxicological studies to investigate the effects of inhaled substances on pulmonary histology, because these rats exhibit physiological and anatomical similarities to humans (16). Studies have demonstrated that the inhalation of fumes produced by mosquito coils can result in numerous adverse health consequences, particularly focus on respiratory complications and pulmonary damage. Specifically, these findings establish a link between exposure to mosquito coil emissions and the onset of chronic respiratory disorders, including asthma and chronic obstructive pulmonary disease (COPD) (17). Moreover, histopathological changes detected in the pulmonary tissues of experimental subjects exposed to mosquito coil emissions suggest that such smoke triggers inflammatory processes and morphological alterations in the lungs, potentially leading to respiratory function impaired (18). In particular, bronchiolar epithelial cells degeneration and bronchial walls thickening have been reported, leading to impaired gas exchange and reduced oxygen-carrying capacity in the blood (18). Furthermore, the presence of toxic compounds in smoke, such as allethrin, is associated with oxidative stress and cellular injury, potentially exacerbating lung damage (19).

Exposure to harmful particles or free radicals can cause immediate and long-term respiratory issues by damaging the epithelial cells of the respiratory tract, particularly the alveolar epithelium, or by inducing inflammatory responses in lung tissue (20). Inhaled mosquito coil smoke deposits particles in the lungs and bloodstream. The active substances and combustion gases in mosquito coils cause oxidative stress, affecting various tissues, organs, and cells, including erythrocytes, leading to cell lysis and free hemoglobin (21). Prolonged exposure to allethrin and other particles from mosquito coils alters hemoglobin levels and other biochemical parameters. This noxious synthetic chemical is responsible for cellular, tissue, and organ injury owing to its action on membrane phospholipids, resulting in membrane fluidity and tissue damage (22). Despite their widespread use for mosquito protection in tropical countries, such as Indonesia, further studies are necessary to assess the short-term exposure to subacute toxicity. Previous studies on the effects of acute deltamethrin exposure in adult and developing Sprague-Dawley rats on acoustic shock response in relation to brain and plasma deltamethrin concentrations for 5, 7, and 9 h/day, each for 21 days. However, the subacute effects of mosquito coil smoke exposure on both lung histopathology and systemic hematological parameters, especially under varying exposure durations using Wistar rats as the model organism, remains unclear. The existing literature mostly focuses on either acute exposure or separate physiological parameters, lacking an integrated approach. The current study compared pulmonary histology and blood hematology parameters at 5, 7, and 9 h/day for 21 days. Spatial repellents are chemicals that volatized and prevent human-vector contact by disrupting the host-seeking behavior. Some of the widest-used spatial repellent products are coils, which usually contain an insecticide that is burned to produce a repellent smoke and can last around 9 h (23–25). Therefore, this study aimed to examine the effects of different durations of subacute exposure to mosquito coil smoke on lung histology and blood hematology in male white Wistar rats (*Rattus norvegicus*). The subacute toxicity test aims to obtain information on the toxic effects of haematology that are not detected in the acute toxicity test. In addition, the toxic effects on haematology and lung histology after repeated different durations of subacute exposure within 21-day period were determined.

2. MATERIALS AND METHODS

2.1. Chemicals

The TR mosquito coil brand, containing 0.3% d-allethrin (0.3 %), was purchased from the market and used in this study.

2.2. Animals and Experimental Design

Animal studies were carried out in the Laboratory of Animal Experiments and Anatomical Pathology, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia. A true experiment with a post-test-only control design involving male Wistar rats was obtained from the Animal House of the Faculty of Veterinary Medicine of Universitas Syiah Kuala, Indonesia. The inclusion criteria were that the male rats were healthy and weighed between 150 and 200 grams on average. The rats were individually housed in a well-ventilated room with carefully controlled temperature and lighting conditions to maintain a constant environment (23±0.5°C and 12-h light/dark cycle). The animals were fed standard rat food (Brailler-I pellet (BRI) ad libitum) and water and underwent a 7-day acclimatization period in their cages.

Twenty-four adults male Wistar rats were randomly divided into four groups, each comprising six rats: negative control groups, experimental groups 1, 2, and 3. The negative control group (C) was not exposed to mosquito coils. Groups P1, P2, and P3 were the experimental groups that were exposed to mosquito coil smoke. After 7 days of acclimatization, the animals were randomly divided into four groups: control groups 1, 2, and 3, each comprising six rats. The negative control group (C) was not exposed to mosquito coils. Groups P1, P2, and P3 were the experimental groups that were exposed to mosquito coil smoke. Throughout the experiment, rats were housed in designated cages measuring 60 × 50 × 20 cm, each equipped with two ventilation holes of 4 cm diameter, allowing whole-body inhalation of mosquito coil smoke (MCS). The mosquito coil was ignited and strategically placed in the center of the room, with the animal cages arranged at equal distances around it to provide uniform exposure for all rats. Groups P1, P2, and P3 were exposed to mosquito coil smoke for 5, 7, and 9 h/day, respectively, for 21 days (26). Some of the widest-used spatial repellent products are coils, which usually contain an insecticide that is burned to produce a repellent smoke and can last around 9 h (24). Animal randomization methods during the allocation to groups was simple randomization type used a function such as Rand in spreadsheet software such as MS Excel.

2.3. Hematological Examination

The experiment lasted for 21 days, after which the animals were euthanized using chloroform anesthesia. The surgical intervention was commenced in the abdominal region and progressed toward the thoracic cavity until the heart was exposed. Blood was extracted directly from the heart using a 3 mL syringe as promptly as possible. A needle was inserted into the heart, and blood was slowly withdrawn until the syringe contained 3 mL of blood. The blood was then transferred into a vacutainer tube containing ethylenediaminetetraacetic acid (EDTA) by slowly introducing it along the tube wall, followed by gentle agitation to ensure uniform mixing with EDTA anticoagulant. Blood samples were analyzed within 24 h using a fully automated hematological cell counter (XN1000A, Sysmex Corp., Kobe, Japan). Sysmex conducted quality control using specific calibrators, including XN Cal and SCS-1000, specifically designed for their analyzers. These calibrators, with established values, are regularly used in the Laboratorium Riset Banda Aceh to ensure the traceability of measurements to international standards, such as ISO 17511, and. The parameters measured were the red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb) concentration, packed cell volume (PCV%), mean cell volume (MCV), and platelet count. The mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated.

2.4. Histopathological Examination of the Lung

The lungs were carefully dissected and weighed using an electronic scale. The lungs were then rinsed with normal saline and fixed in 10% buffered neutral formalin for fixation. Tissue processing was performed using an automated tissue processor, followed by paraffin embedding. Serial paraffin sections (3–4 µm thick) were acquired and stained with standard Hematoxylin and Eosin (H&E) for histopathological analysis. The slides were examined under a light microscope (CX33, Olympus, Tokyo, Japan) at 10X and 40X magnifications. Photomicrographs were obtained using Cellsen imaging software (Olympus, Tokyo, Japan). Lung histology was evaluated in five fields of view for each lung, and the average scores were used to assess changes in lung histopathology based on the Manja Roenigk's histopathology. Herdiani et al. (27) adapted the Manja Roenigk score (Table 1) to assess lung histopathology, including the damage of the alveolar membrane, alveolar lumen, and interalveolar connection. The total scores for the three parameters were averaged. Therefore, the mean results of the degree of lung damage were classified as follows: degree 1, mild damage; degree 2, moderate damage; degree 3, severe damage.

Pulmonary histopathological assessments were performed using a double-blind protocol and were subsequently validated by two independent pathologists. Inter-rater agreement was assessed to ensure scoring reliability, and the final dataset comprised the average scores derived from the double-blind evaluations of lung tissue damage.

Table 1. Histopathology of Manja Roenigk

The alveolar membrane score	1: Intact alveolar membrane containing complete cell nuclei with endothelial cells >75% normal 2: Intact alveolar membrane containing complete cell nuclei with normal endothelial cells 25-75% 3: Intact alveolar membrane containing complete cell nuclei with endothelial cells <25% normal
The alveolar lumen score	1: Round shape with proportional size >75% normal 2: Round shape with proportional size of 25–75% normal 3: Round shape with proportional size <25% normal
Inter-alveolar connecting score	1: Density >75% normal 2: Density 25 - 75% normal 3: Density <25% normal

2.5. Ethical Approval

The Ethics Research Commission of the Faculty of Medicine, Universitas Malikussaleh, approved all experimental procedures in this study (reference number: 72/KEPK/FKUNIMAL-RSUCM/2023, July 12, 2023).

2.6. Statistical Analysis

GraphPad Prism software version 10.2.2 (GraphPad Software, LLC, California, USA) was used for data analysis and visualization. A Kruskal-Wallis test was used to perform qualitative analysis, followed by Dunn's test for multiple comparisons. The level of statistical significance was set at $p < 0.05$. The Kruskal-Wallis test is a nonparametric test for ordinal scale data (lung histology, lung color grading) and ratio scale data that non-normally distributed data (hematology parameters).

3. RESULTS AND DISCUSSION

This study aimed to explore the effects of different durations of subacute exposure to mosquito coil smoke on lung histology and blood hematology in white male Wistar rats.

3.1. Mean Body Weight

Figure 1 shows the effects of inhaled mosquito coil smoke on the mean body weight of negative control and MCS-treated rats. A significant decrease in mean body weight was observed in all groups exposed to MCS compared to the control group ($p = 0.019$). Specifically, groups P1 (196.50 ± 14.71; $p = 0.2578$) and P2 (188.80 ± 23.71; $p = 0.0626$) exhibited a reduction in mean body weight; however, this reduction was not statistically significant compared with the control group (217.70 ± 14.84). In contrast, Group P3 exhibited a significant decrease in mean body weight (185.00 ± 9.59) compared with the control group ($p = 0.008$).

Our findings align with previous research indicating that MCS inhalation leads to substantial changes in the mean body weight of rats exposed to smoke from local and commercial mosquito repellents compared with the control group (28). The longer the exposure (9 h in P3), the greater the impact on weight loss. It is important to show the cumulative toxicity effect. Our findings are consistent with those of previous studies, which reported that allerthin vapors cause weight loss in animal models. Allerthin vapors, which are released through coil smoke, reduce erythrocytes' oxygen-carrying capacity, leading to decreased metabolism and energy output, which indicates body weakening (29).

The body weight of an organism indicates its general metabolic condition and its ability to maintain regular growth and development. The potential hypothesis for weight reduction in this study may involve reduced food absorption or use due to gastrointestinal disruptions resulting from altered hydrolytic enzyme function in the small intestine following pyrethroids exposure (30). Ingestion of a single dose of pyrethroid-based pesticides leads to significant changes in intestinal enzyme activity, which can cause significant disruptions in the absorption of food components (30). Several factors, such as a decrease in appetite, specific organ-related toxicities (including those affecting the gastrointestinal, endocrine, and central nervous systems), and other stressors, such as transitioning to a new colony and experiencing anxiety due to unfamiliar surroundings, colud contribute to a reduction in weight gain in the exposed group (28,29).

3.2. Hematological Parameters

As shown in Table 2, all treatment groups exhibited a decrease in RBC count compared with the control group; however, these differences in RBC counts were not statistically significant ($p = 0.2424$). Regarding Hb and Hct levels and RBC indices, all treated groups exhibited normal values, and no significant differences were detected between any of the groups and the control group. The hematological values of all treatment groups were within normal limits and showed a non-significant decrease compared with those of the control group ($p>0.05$).

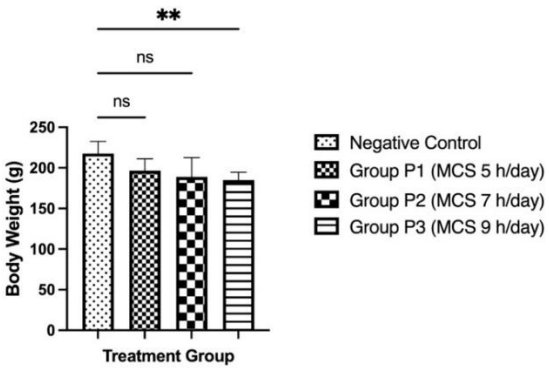

Figure 1. Effect of mosquito coil smoke on body weight in the control and treated groups (Group C: negative control; Group P1: mosquito coil smoke 5 h/day; Group P2: mosquito coil smoke 7 h/day; and Group P3: mosquito coil smoke 9 h/day). Values are presented as the mean±SD (n = 6). **p < 0.05.

Table 2. Hematology parameters in the control and treated rats

Parameters	Control	Group P1 (MCS 5 h/day)	Group P2 (MCS 7 h/day)	Group P3 (MCS 9 h/day)	*p*-value
RBC (10^6/µL)	7.27±4.10	6.98±0.51	6.77±0.79	6.44±0.86	0.2424
Hb (g/dl)	14.33±0.52	13.97±0.66	13.68±0.6	13.30±0.89	0.2354
Hct (%)	39.70±1.94	39.05±1.90	38.20±1.72	37.92±0.73	0.1792
MCV (fl)	54.62±1.54	56.26±5.69	56.94±5.02	59.79±8.36	0.625
MCH (pg)	19.74±0.86	20.06±1.22	20.39±1.77	20.83±1.78	0.796
MCHC (g/dL)	36.14±1.27	35.82±0.48	35.82±0.48	35.08±2.37	0.838

Values are mean ± SD (n = 6). Data were analyzed using Kruskal–Wallis. RBC: red blood cell; Hb: hemoglobin; Hct: hematocrit; MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration.

In Wistar rats, the normal range for the RBC count is 7.2 to 9.6 million/µL of blood, and the normal Hb level ranges from 11.1 - 18 g/dL (31). In this study, insignificant changes in RBC count, Hb levels, and Hct were observed in both treated groups compared with those in the control groups. Hematological parameters, including erythrocyte count and Hb levels, decreased in the control group, although the difference was not statistically significant. The Hb level, Hct, MCV, MCH, and MCHC remained within the normal range in male Wistar rats. Erythrocyte values may differ from reference ranges due to environmental conditions, cage and feed methods, maintenance practices, and climatic conditions during the treatment (27). This finding is consistent with a recent study by Anyabolu et al. (32) who reported that rats exposed to inhaled MCS did not exhibit signs of anemia. MCV, MCH, and MCHC are the three primary red blood cell indices that aid in measuring the average size and hemoglobin composition of red blood cells. In this study, we did not observe any significant changes in these indices between the control and treatment groups. Similarly, rats exposed to mosquito coil smoke for 6 h per day for 4 weeks did not show any changes in their total RBC count, Hb levels, Hct, MCV, MCH, or MCHC levels (33). This observation can be attributed to the relatively short exposure duration in our study. In contrast, our results are inconsistent with those of previous studies, which revealed that MCS exposure significantly increased rat hematological parameters (21,34,35).

Erythrocytes are produced in the bone marrow and are released into the bloodstream as they mature. An insufficient number of RBCs may result in anemia, fatigue, and weakness. In this study, the RBC count decreased. The control group did not exhibit any change in the RBC count, whereas the experimental groups demonstrated a slight decrease in the RBC count; however, these results were not statistically significant ($p>0.05$).

Idowu et al. (36) suggested that an increase in EPO stimulates the bone marrow to produce red blood cells that counteract hypoxia. The normal hematological parameters in this study can be attributed to the elevated levels of EPO in the exposed groups. Prolonged exposure to antimosquito coil smoke in rats is associated with decreased hematological parameters (36,37). The smoke from mosquito coils poses a threat to red blood cells by damaging their membrane structure and increasing their susceptibility to breakage. The lipid bilayer of the erythrocyte membrane contains lipids, such as cholesterol and phospholipids, which are crucial for maintaining structural integrity. However, the presence of fatty peroxides in erythrocytes disrupts lipids, decreasing their proportion and ultimately affecting the structural integrity of the cellular membrane. This leads to an increase in cellular permeability, allowing extracellular molecules, such as Na^+, Ca^{2+}, and H_2O, to enter the cell, causing cell swelling and compromising the cell membrane integrity. This could result in the red blood cells lysis and the subsequent release of hemoglobin. Prolonged hemolysis can decrease hemoglobin levels, leading to anemia (38,39).

3.3. Change in the Rat Lung

The weight of the rat lungs was significant different between the treatment and control group (p = 0.029). The lung weight was significantly lower in group P3 (9 h/day) that in the control group (p = 0.009) (Figure 2). Regarding the classification of lung color, the lung color of the control group appeared pink without bleeding. Group P3 (9 h/day) exhibited greater color changes than the control group and group P1 (5 h/day), with the latter showing pulmonary hemorrhage with pus (Figure 3).

A previous study reported reduced organ weights, including the lungs, in experimental groups exposed to coil smoke for an extended duration of 8 weeks (28). However, inconsistent findings have been reported in which exposure to liquid vaporizers and mosquito coil emissions did not significantly change the lung weight of mice (28,40). Alletrin has the potential to induce damage and inflammation in the lung tissue, thereby altering the structure of organ over time. Rat lung weight variations are the result of alveolar collapse (41). Organ weight is as an optimal parameter for detecting toxicity because a decrease in organ weight indicates structural changes in the organ. Prolonged exposure to anti-mosquito coil smoke leads to reduced organ weights in rats (39). Long-term exposure causes a greater accumulation of free radicals within the tissue, leading to oxidative stress and lipid peroxidation. This process involves the oxidation of unsaturated long-chain lipid acids within the cell membranes, which triggers alveolar collapse (42).

In terms of lung color classification, our results are consistent with those of a previous study, which also revealed variations in the lung samples across the test groups. Moreover, the group exposed to allethrin-based mosquito coils for 8 h every day, 6 days a week, for 8 weeks demonstrated the most significant variation, exhibiting a range of colors from reddish-brown to grayish (28). Rats exposed to allethrin through the fumes of mosquito coils exhibited substantial changes in lung coloration, which may indicate potential tissue damage. Red discoloration in the lungs is believed to be caused by damaged alveoli containing trapped or accumulated blood debris. In contrast, the black spots observed in the lungs may be attributed to blood vessels rupture or oxygen-deprived lung tissue (43). The observed color changes in the lung samples could be indicative of various underlying pathological processes that occur in response to allethrin exposure. These changes may include inflammation, oxidative stress, or blood flow alterations, which could lead to long-term respiratory complications.

This study provides compelling evidence of the harmful effects of short-term allethrin-based mosquito coil smoke (MCS) inhalation on lung tissue, specifically leading to emphysema. Histological analysis revealed extensive damage to the alveolar septa, resulting in alveolar lumen rupture and emphysema development. This damage is primarily attributed to the generation of reactive oxygen species (ROS) and subsequent oxidative stress induced by MCS exposure. This study highlights the critical role of ROS in triggering various cellular stress responses, which can affect multiple signaling pathways associated with carcinogenesis, apoptosis, necrosis, and inflammatory processes. Further examination of MCS composition revealed the presence of free radicals and volatile organic compounds, which are potent contributors to oxidative DNA damage and tissue injury. These harmful MCS components can lead to significant alterations in the histological structure of the pulmonary tissues, ultimately resulting in severe organ damage. This study emphasizes the importance of understanding the mechanisms by which MCS exposure, even for short durations, can lead to profound changes in lung tissue architecture and function (34,44).

The accumulation of free radicals alters the functionality of alveolar macrophages, affecting their ability to effectively remove inhaled particles and increasing their susceptibility to pulmonary infections and inflammation. Macroscopic evidence of lung inflammation at the pathological level indicates tissue damage such as alveolar collapse. The collapse of alveoli results in reduced lung surface area and shrinkage. Obstructive elements, such as mucus clots, manifest as white areas in the pulmonary parenchyma. The recruitment of polymorphonuclear leukocytes and monocytes in the respiratory tract due to of epithelial injury caused by the toxic substance of coil smoke may be the cause of emphysema (39,44).

In addition, thickening of the alveolar septa caused by a buildup of allethrin causes the alveoli to burst and the formation of air sacs, which increases the surface area of the lungs to increase, and the level of oxygen reaching the bloodstream to decrease. These air pockets result in reduced lung area and capacity, and the air inhalation capacity of the lungs becomes smaller. As a result, oxygen levels reach the blood flow pressure, and acetylcholine accumulates. Accumulation of acetylcholine stimulates bronchial contraction, resulting in alveolar walls degeneration, which causes air buildup in one place (28,32).

Conversely, MCS exposure results in extensive infiltration of inflammatory cells around the alveolar septa through disruption of the interalveolar septa. The compromised alveoli subsequently coalesced, forming large air spaces, particularly in the lung's peripheral region. Histological examination of the lung tissue revealed substantial destruction of the alveolar septa and bronchial epithelial wall, leading to a reduction in alveoli and capillary surface area. Thus, this study provides compelling evidence for severe emphysema induced by prolonged allethrin-based MC smoke inhalation. Furthermore, the recruited inflammatory cells and proteolytic enzymes interact with anti-proteases (collagen and elastin) in the alveolar region, inactivating them, resulting in the degradation of alveolar septa and ultimately emphysema development. The involvement of neutrophils in the degradation of elastin fibers, leading to the subsequent development

of emphysema, has been substantiated. The accumulation of inflammatory cells likely induces the deposition of extracellular matrix in the subepithelial region and contributes to the thickening of the bronchial epithelial wall. Fibroblast activation by inflammatory cells, along with subsequent thickening of the bronchial epithelial wall and alveolar septa, is considered a manifestation of airway resistance and remodeling in response to the ongoing destruction of the alveolar septa (39,41).

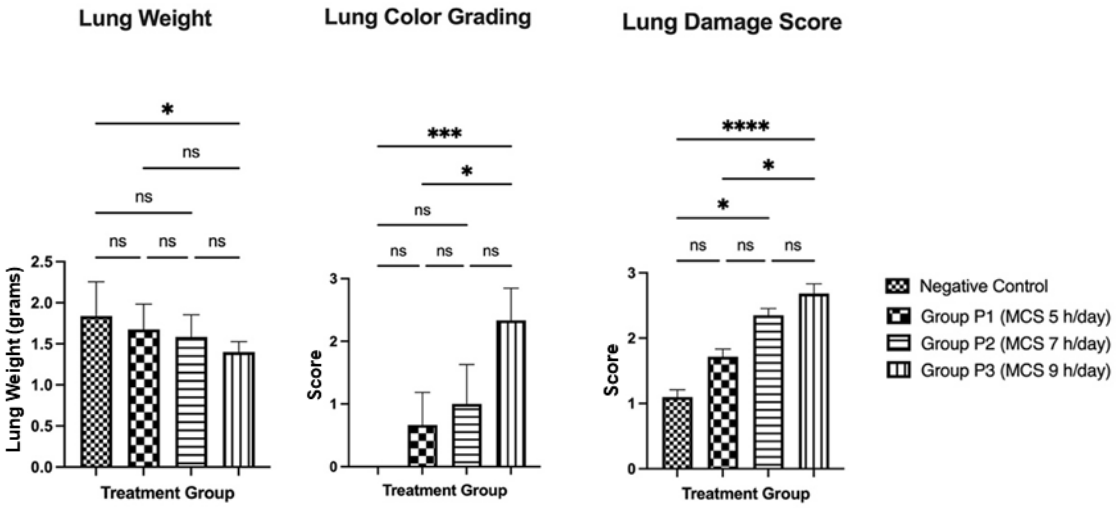

Figure 2. Effect of mosquito coil smoke (MCS) exposure on lung weight, lung color classification, lung damage score, and lung histopathology in different experimental groups. Each bar represents the mean ± SD (n = 6). (****p<0.0001, ***p<0.001, **p<0.05, *p<0.05).

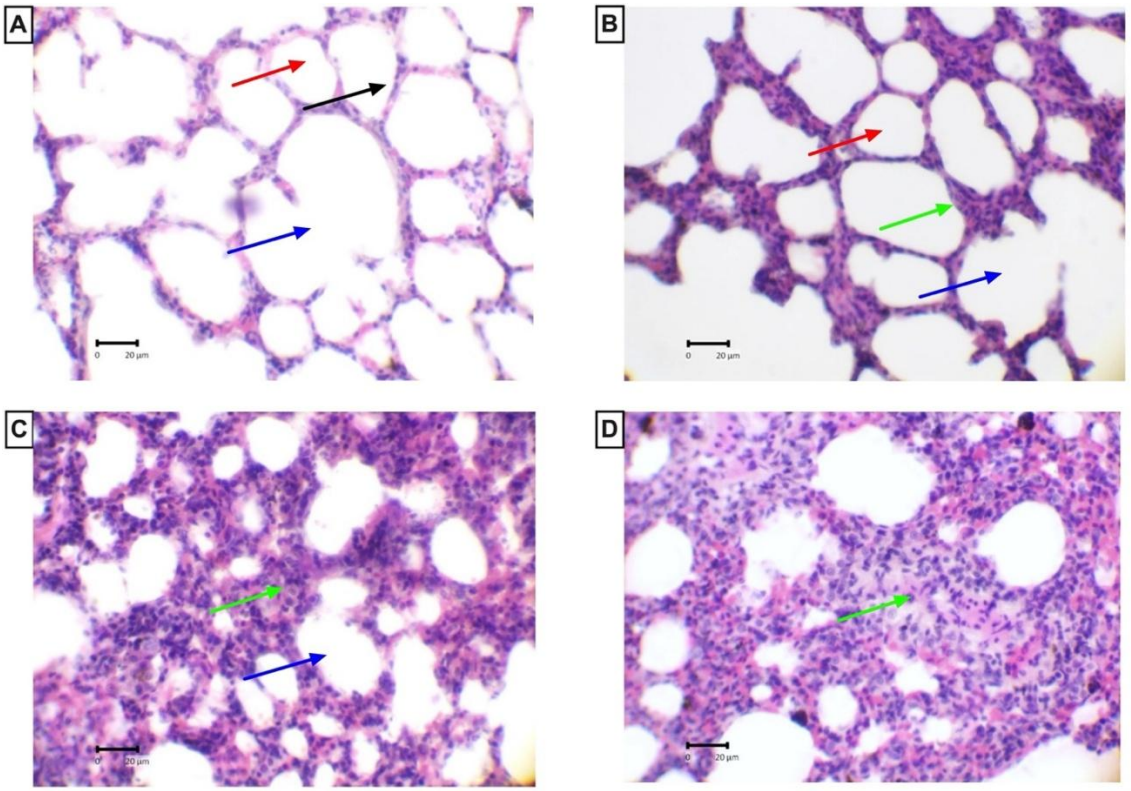

Figure 3. Effect of exposure to mosquito coil smoke (MCS) on lung histopathology in different experimental groups. Each bar represents mean ± SD (n = 6). Arrows in photomicrographs of lung histopathology (40x): black, intact alveolar membrane, fully nucleated with endothelial cells; red, rounded alveolar lumen but not thickened; green, thickened alveolar membrane; blue, ruptured alveolar lumen, and emphysema. A: negative control; B: Group 1: mosquito coil smoke for 5 h/day; C: Group 2: mosquito coil smoke for 7 h/day; D: Group 3: mosquito coil smoke for 9 h/day.

An increase in reactive oxygen species (ROS) levels leads to an inflammatory response in the pulmonary system. This inflammatory cascade initiates the recruitment of immune cells into the interstitial space, which promotes edema (45). Activation of immune cells subsequently causes injury to endothelial cells within the pulmonary alveoli, resulting in a decrease in the number of healthy endothelial cells, ultimately leading to pulmonary blood vessels rupture (39,44).

These findings indicated that inhaling mosquito coil (local brand) smoke causes toxic effects in a time-dependent manner on lung experimental mice models. Moreover, burning one mosquito coil releases the same amount of particulate matter (0.3% d-allethrin) as burning 75-137 cigarettes. Also, the emission of formaldehyde from burning one coil can be as high as that released from burning 51 cigarettes. Furthermore, long-term exposure to mosquito coil smoke (MCS) might induce chronic bronchitis, asthma, and persistent wheeze (11,46).

4. CONCLUSIONS

Subacute exposure to allethrin-based MCS has a significant effect on lung histology in Wistar rats. This study provides clear evidence of emphysema resulting from subacute inhalation of mosquito coil smoke. The lung weight and change in lung color analyses demonstrated the severity of tissue damage in the smoke exposed group. The decline in mean body weight trend was significant with longer exposure (9 hours/day) in the rat model upon MCS inhalation. However, the hematological parameters of the rats were not affected by subacute mosquito coil smoke inhalation.

However, further investigation is required to study potential biological mechanisms such as oxidative stress and inflammation due to Reactive Oxygen Species (ROS), the mechanism of its toxicity, and the reversibility or irreversibility of these harmful effects on the experimental model system.

Author contributions: MSR: Conceptualization, Methodology, Investigation, Data Curation, Writing-Original draft preparation. SW: Formal analysis, Visualization, Writing- Reviewing, and Editing.

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Ethics statement: All experimental procedures in this study were approved by the Ethics Research Commission of the Faculty of Medicine, Universitas Malikussaleh (reference number: 72/KEPK/FKUNIMAL-RSUCM/2023; July 12, 2023).

Conflict of interest: The authors declare that they have no competing interests.

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