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Research

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Effect of nanocurcumin (*Curcuma longa*) on lung histopathology in rats exposed to carbon black

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Abstract

Background Exposure to carbon black can induce respiratory toxicity, leading to tissue remodeling, inflammation, and fibrosis. Curcumin, a compound from *Curcuma longa*, has been shown to exert protective effects on lung tissue by inhibiting cell proliferation and reducing alveolar epithelial thickening and inflammatory responses.

Objective This study aimed to evaluate the effect of nanocurcumin administration on alveolar septal thickness and fibrosis in the lungs of rats (*Rattus norvegicus*) exposed to carbon black.

Methods Thirty female rats were divided into five groups: the negative control group (K-) received distilled water without carbon black exposure; the positive control group (K+) received distilled water and was exposed to carbon black at a dose of 1064 mg/m³ for 6 hours; and treatment groups P1, P2, and P3 received nanocurcumin at doses of 50, 100, and 200 mg/kg body weight, respectively, along with carbon black exposure at a dose of 1064 mg/m³ for 6 hours/day over 30 days.

Results Alveolar septal thickness and fibrosis in the P1, P2, and P3 groups showed significant reductions ($P < 0.05$) compared to the K+ group. Notably, groups P2 and P3 exhibited similar alveolar septal thickness to the K- group, with statistically lower ($P < 0.05$) fibrosis levels than P1 but not significantly different from K- ($P > 0.05$).

Conclusion Nanocurcumin administration demonstrates potential in mitigating alveolar septal thickening and fibrosis in rat lungs subjected to carbon black exposure, suggesting a protective role against pulmonary damage from particulate matter.

Keywords alveolar septal thickening | carbon black exposure | *Curcuma longa* | fibrosis | nanocurcumin

Introduction

In recent years, monitoring the concentration of carbon black in the environment has drawn global attention due to its potential health risks (Peralta *et al.*, 2019). Carbon black, primarily found in particulate matter (PM2.5) in the atmosphere, serves as a prominent indicator of airborne carbon particles. Unlike other carbon particles, carbon black has a well-defined and homogeneous chemical composition, making it a representative model for studying the impacts of carbon-based air pollution (Liu *et al.*, 2021; Ma *et al.*, 2017).

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The respiratory system is one of the primary targets of airborne pollutants, including PM2.5, which can trigger oxidative stress and inflammatory responses. Such exposure stimulates both innate and adaptive immune responses, leading to potential health risks in both experimental animals and humans (Leikauf *et al.*, 2020). The mechanisms of carbon black toxicity are multifaceted. First, exposure induces alveolar epithelial cell dysfunction, characterized by the increased reactive oxygen species (ROS) production, epithelial hyperplasia, cell death, and apoptosis in pulmonary tissues. Second, it triggers systemic immune responses that may lead to tissue remodeling and fibrosis in the respiratory system (Hussain *et al.*, 2010).

Indonesia, known for its biodiversity, is rich in plants with antioxidant potential, such as turmeric (*Curcuma longa*). Antioxidants are bioactive compounds that can prevent, delay, or reduce oxidative damage caused by ROS, thereby protecting cellular structures (Kurnijasanti *et al.*, 2015; Sandhiutami *et al.*, 2022). The primary bioactive component of turmeric is curcuminoids, particularly curcumin, which has been shown to have substantial antioxidant and anti-inflammatory properties (Itokawa *et al.*, 2008; Babu *et al.*, 2013). Curcumin specifically can inhibit epithelial thickening and proliferation in alveolar tissues, reduce inflammatory responses, alleviate airway inflammation, prevent emphysema, and counteract ischemic complications (Yuan *et al.*, 2018).

Despite its therapeutic potential, curcumin has limitations, particularly in terms of low intestinal absorption (Pan *et al.*, 1999). To overcome this limitation, curcumin has been developed into nanoparticle formulations, which enhance its solubility, absorption, and bioavailability, thus optimizing its therapeutic effects (Shimatsu *et al.*, 2012). The antioxidant properties of nanocurcumin are anticipated to mitigate the negative impacts of inflammation and ROS in tissues exposed to carbon black. Therefore, this study investigates the therapeutic effects of nanocurcumin on carbon black-induced lung damage in white rats (*Rattus norvegicus*), with a focus on alveolar septal thickening and fibrosis.

Methods

Study design

This study has received ethical clearance from the Animal Ethics Committee of the Faculty of Veterinary Medicine, Universitas Airlangga (No. 2.KEH.171.11.2023). It was conducted as a randomized complete block design (RCBD) experimental study with 30 female white rats (*Rattus norvegicus*), aged 12 weeks, weighing approximately 100–150 g. The experimental rats were allocated into five groups: a negative control group (K-) receiving only distilled water, a positive control group (K+) exposed to carbon black and given distilled water, and three treatment groups (P1, P2, and P3), which received nanocurcumin at doses of 50, 100, and 200 mg/kg BW, respectively. The study was carried out at the Faculty of Veterinary Medicine, Universitas Airlangga, from February to June 2024.

Nanocurcumin preparation

Nanocurcumin was synthesized using an ionic gelation technique to produce curcumin nanoparticles. The formulation followed a chitosan: tripolyphosphate (TPP): curcumin ratio of 5:1:1, as described by Proboningrat *et al.* (2019). Chitosan solution (0.2%) was used as a cationic agent, while TPP solution (0.082%) functioned as an anionic agent. Curcumin was derived from Merck Curcumin Isolate (77% purity). Doses of curcumin for each treatment group were: P1 = 0.0375 g (1.25%), P2 = 0.075 g (2.5%), and P3 = 0.15 g (5%). Curcumin dissolved in 3 mL of 96% ethanol was mixed with chitosan dissolved in 60 mL of 1% acetic acid, stirred at 1000 rpm for 30 minutes, followed by a gradual addition of TPP (dissolved in 5 mL distilled water). The final solution was stirred for an additional 3 hours at 1000 rpm, resulting in nanoparticles with an average particle size of 22.03 nm as measured by a particle size analyzer (PSA).

Nanocurcumin administration

Nanocurcumin was administered orally as a preventive intervention, given daily to treatment groups P1, P2, and P3 at doses of 50, 100, and 200 mg/kg BW, respectively, for 30 days (Ambarsari *et al*., 2019).

Carbon black exposure

The carbon black particles (Vulcan N330) were administered via inhalation using a carbon black inhalation chamber, following the protocol by Juliprihanto (2012). Experimental rats in the positive control and treatment groups were exposed to a carbon black at a concentration of 1064 mg/m³ for 6 hours daily over 30 days, with each chamber receiving an exposure dose of 11.4 mg/m³.

Sample preparation and HE staining

After 30 days of carbon black exposure, the experimental rats were anesthetized with Ketamine and Xylazine and subsequently euthanized for necropsy. The lung tissues were collected, trimmed to 1 cm × 1 cm × 1 cm, and fixed in 10% Neutral Buffered Formalin (NBF). The samples underwent standard dehydration and clearing protocols, with sequential treatments in 70%, 80%, 90%, 96%, and absolute ethanol, followed by toluene and embedding in paraffin wax. Sections were cut to 4–5 µm thickness using a microtome and stained with Hematoxylin and Eosin (HE) for histological examination.

Histopathological evaluation

Histopathological examination of alveolar septal thickening and fibrosis was conducted using a Nikon Eclipse E-100 microscope with 400x magnification. Images were captured using an OptiLab camera and analyzed with Image Raster software. Histopathological features were evaluated across five random fields per slide using a modified scoring system (Azzahra *et al.*, 2018) as outlined in **Table 1**. Scoring reliability was confirmed with inter-rater validation, yielding a Kolmogorov-Smirnov test p-value of 0.730 and a significance value of $p = 0.661$ ($p > 0.05$), indicating consistency between evaluators.

Table 1 Modified histopathological scoring for rat lung (adapted from Azzahra *et al*. 2018)

Lesion type	Score	Description
Alveolar septal thickening	0	No thickening observed
	1	Thickening in <33% of the field
	2	Thickening in 34–66% of the field
	3	Thickening in 67–100% of the field
Fibrosis	0	No fibrosis observed
	1	Fibrosis in <33% of the field
	2	Fibrosis in 34–66% of the field
	3	Fibrosis in 67–100% of the field

Data Analysis

Histopathological data on alveolar thickening and fibrosis were analyzed using SPSS version 2023. The Kruskal-Wallis test was performed for overall comparisons between groups, followed by post-hoc Mann-Whitney tests to compare control and treatment groups (Dahlan, 2013).

Results

Alveolar septal thickening

The mean rank values for alveolar septal thickening, shown in **Table 2**, indicate the lowest mean rank in the negative control group (K-) at 6.80, while the positive control group (K+) had the highest mean rank value of 23.00. The descending order of mean rank values for alveolar septal thickening across groups was K+ (23.00), P1 (17.00), P2 (11.20), P3 (7.00), and K- (6.80) (**Figure 1**). The Mann-Whitney test results indicated a statistically significant increase in septal thickening ($P < 0.05$) between K+ and the other groups (K-, P1, P2, P3). Significant differences ($P < 0.05$) were also observed between group P1 and groups P2 and P3, whereas no significant difference ($P > 0.05$) was detected between K- and groups P2 and P3.

Table 2 Mean rank scores for alveolar septal thickening and alveolar fibrosis in rat lungs treated with nanocurcumin followed by carbon black exposure

Group	Alveolar septal thickening score (mean rank)	Alveolar fibrosis score (mean rank)
K-	6.80a	3.00a
K+	23.00b	22.90b
P1	17.00c	17.70c
P2	11.20a	12.40a
P3	7.00a	9.00a

Different superscripts (a, b, c) within the same column denote statistically significant differences ($P < 0.05$).

Alveolar fibrosis

For alveolar fibrosis, the mean rank scores, detailed in **Table 2**, show the lowest value in the negative control (K-) at 3.00, and the highest in the positive control (K+) at 22.90. The descending order of mean rank values for alveolar fibrosis across groups was K+ (22.90), P1 (17.70), P2 (12.40), P3 (9.00), and K- (3.00). The Mann-Whitney test revealed a statistically significant increase in alveolar fibrosis ($P < 0.05$) in the K+ group compared to the other groups (K-, P1, P2, P3). Notable differences in fibrosis ($P < 0.05$) were also present between group P1 and groups P2 and P3, while no significant differences ($P > 0.05$) were observed between K- and groups P2 and P3 (**Figure 2**).

Discussion

The results showed distinct histopathological changes in the lungs of rats exposed to carbon black, with noticeable thickening of alveolar septa and alveolar fibrosis compared to the unexposed control group. This observation aligns with the findings of Delzell (2013), which highlight that carbon black particles in contact with pulmonary epithelial cells can induce the release of various pro-inflammatory factors, including cytokines, that draw immune cells to the site, contributing to tissue damage and inflammation. Such inflammatory responses often result in structural changes in lung tissue, as evidenced by the significant septal thickening and fibrosis observed in the positive control group (K+).

All groups treated with nanocurcumin, despite also being exposed to carbon black, showed these histopathological markers (septa thickening and fibrosis) to a lesser extent than the untreated positive control group. This effect can likely be attributed to the anti-inflammatory and antioxidant properties of curcumin (Rong *et al.*, 2012). The polyphenolic compounds in curcumin react with reactive species, countering oxidative stress and protecting cells from further damage. Nanocurcumin's anti-inflammatory action is mediated through the inhibition of Nuclear Factor-Kappa Beta (NF-kB) by modulating the PPAR-γ-NF-kB signaling pathway. This modulation, described by Li *et al.* (2019), decreases the expression of inflammatory cells and pro-inflammatory mediators via NF-kB inhibition, helping to prevent further lung tissue remodeling, as also suggested by research on respiratory tissue remodeling (Di Stefano *et al.*, 2002).

The groups treated with different doses of nanocurcumin were able to demonstrate this protective effect to varying extents. The lowest dose (50 mg/kg BW) provided limited efficacy in preventing alveolar septal

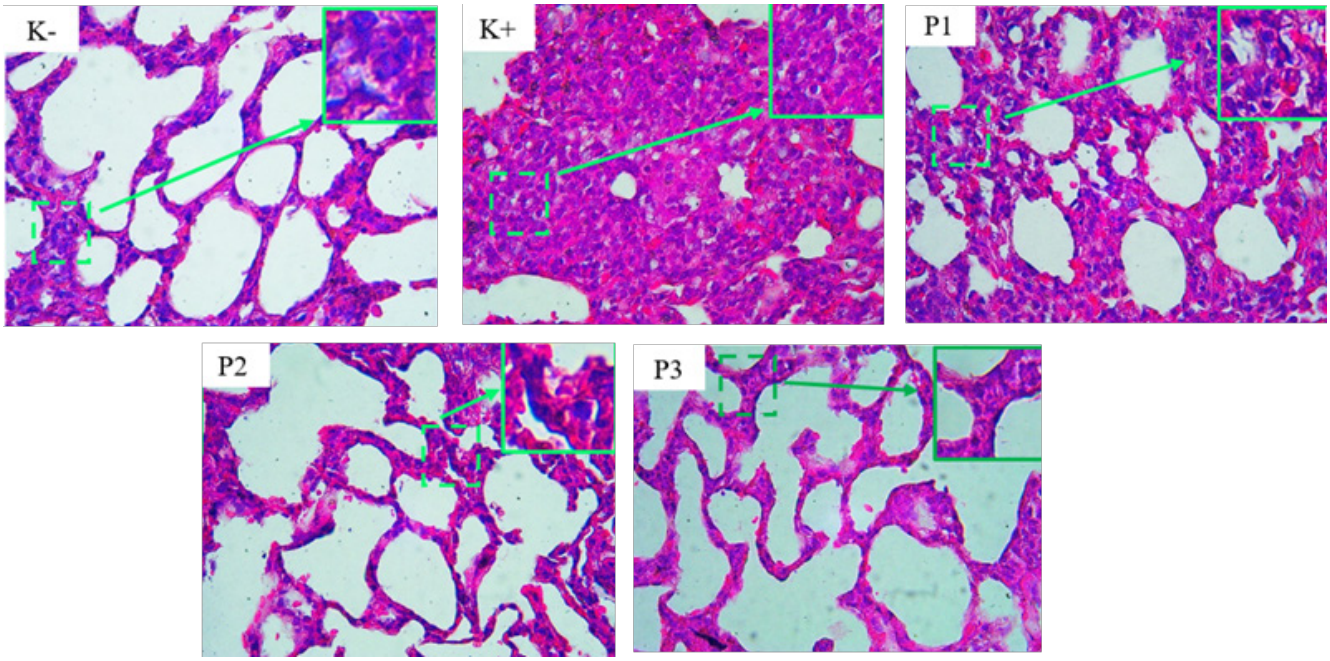

Figure 1 Histopathological sections of alveolar septal thickening in rat lung tissues (400× magnification, H&E staining). *K-* displays normal septal thickness with typical blue nuclei, while *K+* shows the absence of alveolar septa due to extensive thickening and fusion. *P1* exhibits thickened alveolar septa, *P2* shows a thinner septal structure, and *P3* reveals septal thickness between *P2* and *P1*. Green dashed boxes highlight areas of septal thickening.

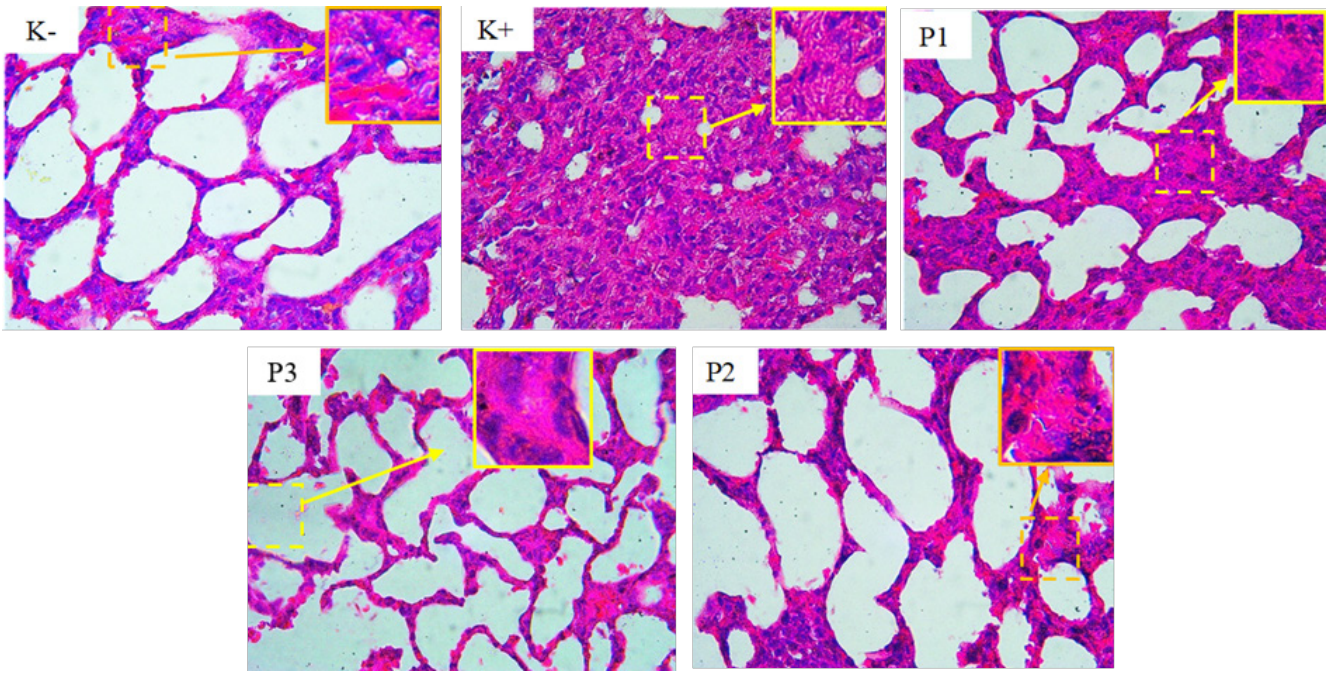

Figure 2 Histopathological images of alveolar fibrosis in rat lung tissues (400× magnification, H&E staining). *K-* shows normal alveolar fibrosis with clearly defined blue nuclei, whereas *K+* displays extensive fibrosis with obliterated alveolar septa. In *P1*, fibrosis appears prominent in alveolar interstitial tissue; *P2* presents moderate fibrosis, and *P3* shows minimal fibrosis. Yellow dashed boxes mark fibrosis regions.

thickening and fibrosis. In contrast, groups receiving 100 mg/kg BW and 200 mg/kg BW nanocurcumin doses displayed reduced septal thickening and fibrosis, nearing normal lung histology. Statistical analyses indicate that the 200 mg/kg BW dose was most effective in mitigating these structural changes, suggesting it as the optimal dose for preventive efficacy in this study.

Conclusion

In summary, the study concludes that oral administration of nanocurcumin as a preventive measure can effectively reduce alveolar septal thickening and alveolar fibrosis in rats exposed to carbon black. The optimal preventive dose identified was 200 mg/kg BW, which provided the

most significant histopathological improvements in lung tissue structure, indicating nanocurcumin's potential for therapeutic application in oxidative stress-induced pulmonary conditions.

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Conflicts of interest All authors declare no conflicts of interest regarding this study.

Author contributions ERAR conducted the experiments, performed data analysis, and drafted the manuscript. IM, EPH, WW, RK, and HP designed the study and contributed to manuscript preparation.

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