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# Tissue-Protective Effect of Erdosteine on Multiple-Organ Injuries Induced by Fine Particulate Matter

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Data Collection B  
Statistical Analysis C  
Data Interpretation D  
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**Conflict of interest:** None declared

**Background:** Fine particulate matter (PM<sub>2.5</sub>) is the air pollutant that most threatens global public health. The purpose of this study was to observe the inflammatory and oxidative stress injury of multiple organs induced by PM<sub>2.5</sub> in rats and to explore the tissue-protective effect of erdosteine.

**Material/Methods:** We randomly divided 40 male Wistar rats into a blank control group, a saline group, a PM<sub>2.5</sub> exposure group, and an erdosteine intervention group. We assessed changes in organs tissue homogenate and biomarkers of inflammation and oxidative stress in serum and bronchoalveolar lavage fluid (BALF).

**Results:** (1) The expressions of IL-6, IL-1 $\beta$ , TNF- $\alpha$ , 8-OHdG, 4-HNE, and PCC in serum and BALF of the PM<sub>2.5</sub> exposure group increased, but decreased after treatment with erdosteine, suggesting that erdosteine treatment attenuates inflammatory and oxidative stress injury. (2) The expression of  $\gamma$ -GCS in serum and lungs in the PM<sub>2.5</sub> exposure group increased, but did not change significantly after treatment with erdosteine. This suggests that PM<sub>2.5</sub> upregulates the level of  $\gamma$ -GCS, while erdosteine does not affect this protective response. (3) The expression of T-AOC in serum, lungs, spleens, and kidneys of the PM<sub>2.5</sub> exposure group decreased, but increased after treatment with erdosteine. Our results suggest that PM<sub>2.5</sub> can cause imbalance of oxidation/anti-oxidation in multiple organs, and erdosteine can alleviate this imbalance.

**Conclusions:** PM<sub>2.5</sub> exposure can lead to inflammatory and oxidative stress damage in serum and organ tissues of rats. Erdosteine may be an effective anti-inflammatory and antioxidant that can reduce this injury.

**Keywords:** **Inflammation • Oxidative Stress • Particulate Matter • Protein Carbonylation**

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## Background

On 26 May 2015, the World Health Organization for the first time clearly defined air pollution as the world's largest single environmental health risk factor. Particulate matter (PM), especially fine particulate matter (PM<sub>2.5</sub>) in inhalable particles, has been recognized as the most harmful and representative air pollutant affecting human health among the recognized atmospheric pollutants [1]. Many studies have reported the correlation between long-term exposure to fine particulate matter and mortality. For example, an extended follow-up of the Harvard Six-Cities Study from 1974 to 2009 [2] and a study on 73 711 subjects living in California conducted by the American Cancer Society [3] showed that particulate matter was associated with all-cause mortality. In 2017, a 13-year follow-up study published in the USA, involving nearly 61 million people with health insurance (including those from small cities and rural areas), showed that long-term exposure to PM<sub>2.5</sub> increased the risk of death, and when PM<sub>2.5</sub> increased by 10 µg per cubic meter, the all-cause mortality increased by 7.3% [4].

The primary causes of PM<sub>2.5</sub> pollution are energy sector coal combustion, motor vehicle exhaust emissions, large-scale infrastructure work, and residential burning. Particulate matters consist of harmful components such as polycyclic aromatic hydrocarbons (PAHs) and heavy metals, which cause damage to multiple organs of the body [5]. Some scholars showed that heavy metals, such as lead, manganese, aluminum, and copper, carried by PM<sub>2.5</sub>, can enter the circulation after respiratory exposure and selectively accumulate in the target organs, including blood, brain, liver, and lungs, with a significant dose-effect relationship [6]. Other scholars exposed mice to PM<sub>2.5</sub> for 3 months and found that increased levels of DNA lesions were correlated with the occurrence of oxidative stress in the lungs, liver, and kidneys, in parallel with decreased global levels of 5-hmC (the currently best-characterized epigenetic marker) in lung and liver DNA [7]. In 2019, scholars studied the changes in multiple-organ DNA methylation (5-MC) caused by inhalation of PM<sub>2.5</sub> in animal experiments. They demonstrated that overall hypomethylation of DNA was observed in the lungs and heart after acute PM<sub>2.5</sub> exposure. After chronic PM<sub>2.5</sub> exposure, global DNA methylation levels were decreased in most organs, including lungs, testis, thymus, spleen, epididymal fat, hippocampus, and blood. Decreased DNA methylation levels can activate the expression of some deleterious genes, such as oncogenes and cytokines, leading to multi-organ damage in the organism [8]. In 2018, Chinese scholars showed that PM<sub>2.5</sub> exposure through long-term intratracheal instillation in Sprague-Dawley rats induced spleen damage. PM<sub>2.5</sub> triggered oxidative stress and activated ERS (endoplasmic reticulum stress) and the autophagy signaling pathway, which facilitated the spleen injury process and toxic effects in a concentration-dependent manner [9]. Other

studies have confirmed that sub-chronic exposure to PM<sub>2.5</sub> can lead to histological alterations in renal tissue, such as fibrosis, mesangial expansion, and decreased glomerular and tubular lumen volumes, and found that this was associated with early-stage renal dysfunction [10]. Human experiments have come to the same conclusion. Epidemiological studies have documented that human exposure to PM<sub>2.5</sub> is positively associated with cardiovascular disease morbidity and mortality (eg, myocardial infarction, heart failure, and rhythm disturbances), and can lead to a decline in renal function [11-14]. There are other studies on PM<sub>2.5</sub> and the occurrence and development of lung diseases [15-20]. Based on the great threat to human health caused by PM<sub>2.5</sub>, in addition to preventive measures such as controlling the environment and reducing pollution, it is urgent to find safe and effective drugs that can reduce this damage.

Erdosteine was originally developed as a mucolytic for treatment of chronic bronchitis and chronic obstructive pulmonary disease. It thins the viscosity of mucus and dissolves mucus, promotes mucociliary transport, and reduces the adhesion between mucus and the airway wall. Erdosteine plays an antioxidant role by eliminating free radicals, and also has anti-inflammatory and antibacterial effects by inhibiting the release of inflammatory mediators and reducing bacterial adhesion [21,22]. Erdosteine shows antioxidant properties and anti-inflammatory effects in many diseases. Its broad-spectrum dose (LD50: 3500-5000 mg/kg) has protective effects on many diseases [23]. Mutneja et al showed that erdosteine attenuated myocardial necrosis induced by isoproterenol (ISO) by inhibiting the MAPK and Nrf-2/HO-1 pathways [24]. In another study, Ugurel et al reported that erdosteine can protect the ovaries from torsional/resetting damage by avoiding scavenging free radicals, releasing oxidative free radicals, and by blocking pro-inflammatory processes [25]. A previous study showed that erdosteine treatment significantly decreased serum liver function. The study also found that erdosteine can improve the antioxidant capacity and stress parameters of the liver [26]. It has been recently suggested that erdosteine treatment increases serum antioxidant enzymes, thereby reducing the level of oxidative stress after ischemia-reperfusion injury in the kidneys [27]. Erdosteine also has beneficial effects on ischemia-reperfusion injury in various organs, including the lungs, brain, kidneys, intestines, ovaries, and spinal cord [28-31]. Given that inflammation and oxidative stress damage are the most important damage mechanisms of PM<sub>2.5</sub>, we speculated that erdosteine, which has anti-inflammatory and antioxidant effects, can alleviate PM<sub>2.5</sub>-induced damage to the body.

The purpose of this study was to explore the multiple-organ inflammatory and oxidative stress injury caused by sub-acute exposure to PM<sub>2.5</sub>, and to assess the effects of erdosteine treatment on levels of IL-6, IL-1β, TNF-α, 8-OHdG, 4-HNE, and

PCC in rat serum and BALF, and to measure the changes in T-AOC and  $\gamma$ -GCS in serum and in lung, heart, liver, kidney, and spleen tissue homogenate in rats. We also explored whether there is an oxidation/anti-oxidation imbalance in rats who underwent PM2.5 tracheal instillation, and studies the protective effect of erdosteine on the rat organ injury induced by PM2.5.

## Material and Methods

### Main Instruments and Reagents

Automatic microplate reader (Thermo Scientific Co., USA, Multiskan MK3), tissue homogenizer (IKN Co., Germany, CMD2000/4), desktop cryogenic high-speed centrifuge (Thermo Co., USA, Micro CL21R), optical microscope (Olympus Co., Japan, CX33), electric calorstat (Shanghai Senxin Experimental Instruments, Ltd, DGG-9070B),  $\gamma$ -GCS test kit (Nanjing Jiancheng Bioengineering Institute, A091), T-AOC test kit (Nanjing Jiancheng Bioengineering Institute, A015), rat IL-6 ELISA kit (Wuhan Liuhe Biotechnology, LH-E10103RA), rat IL-1 $\beta$  ELISA kit (Wuhan Liuhe Biotechnology, LH-E1099RA), rat TNF- $\alpha$  ELISA kit (Wuhan Liuhe Biotechnology, LH-E10183RA), rat 4-HNE ELISA kit (Shanghai Blue Gene Biotechnology, JL19014), rat 8-OHdG ELISA kit (Shanghai Blue Gene Biotechnology, XL02323) PCC test kit (Nanjing Jiancheng Bioengineering Institute, A087), and erdosteine capsules (Tantong, Xi'an Haixin Pharmaceutical).

### Preparation of PM2.5 Suspension

PM2.5 was provided by the Environmental Monitoring Center of Hebei Province. Before use, it was autoclaved, and prepared at 7.5 mg/ml concentration with sterilized water for injection, then mixed by ultrasound oscillation.

### Grouping of Experimental Animals and Methods of Administration

Forty male Wistar rats (provided by Experimental Animal Center of Hebei Medical University) were selected from March to September in 2017, weighing 190 to 220 g, and routinely raised to 250 g to start the experiment. They were divided into 4 groups, with 10 rats in each group, by random number table: the 2 control groups were the blank control group and the saline group, and the 2 experimental groups were the PM2.5 exposure group and the erdosteine intervention group. The blank control group received no intervention. The saline group was given tracheal instillation of normal saline (1 ml/kg) once a week for 4 weeks. Rats in the PM2.5 exposure group were given tracheal instillation of PM2.5 suspension 1 ml/kg (mixed as 7.5 mg/ml with the sterilized water for injection in advance) once a week for 4 weeks. The PM suspension was prepared immediately before instillation). The erdosteine intervention

group was given intragastric administration of erdosteine (500 mg/kg/day, dissolved in water for injection ahead of time) for 30 days in total; tracheal instillation was started 7 days after the administration of erdosteine, and tracheal instillation of PM2.5 suspension was given once a week (7.5 mg/ml, dissolved into 1 ml/kg of water droplets) for 4 times. During the experiment, the general state of the rats was observed and the rats were killed 24 h after the last tracheal instillation. All animals were managed and treated in accordance with the laboratory animal guidelines of Hebei Medical University. All animal experiments complied with the guidelines of the Ethics Committee of Hebei General Hospital, China.

As the exposure levels of PM2.5 determine the toxicological effects, the dosages in the study were carefully selected. Several animal publications have adopted the method of tracheal PM2.5 drip, with some scholars applied 0.2-2.7 mg/kg for tracheal drip every 3 days for 2 months, with a maximum cumulative dose of 54 mg/kg. Some scholars applied 1.8-16.2 mg/kg concentration every 3 days for 1 month, and others applied 10-40 mg/kg once a week for up to 12 times, and all of them proved that the toxic effects of PM2.5 were concentration-dependent [32,33]. Based on daily respiratory volume of rats, which is about 0.288 m<sup>3</sup> and the average concentration of PM2.5 in our city (as high as hundreds or even thousands ug/m<sup>3</sup> in winter, the average is about 600 ug/m<sup>3</sup>), the total amount of PM2.5 inhaled by rats per week was calculated. When the body weight of an adult rat is considered as 250 g, the PM2.5 exposure concentration was approximately 5 mg/kg once every 7 days. Therefore, we used 3 doses of 5 mg/kg, 7.5 mg/kg, and 10 mg/kg for the pre-experiment, and found that an intermediate dose produced significant toxic effects; no significant behavioral abnormalities were found in the animals, no significant changes in body weight were observed, and no animal deaths occurred. Therefore, we selected an intermediate dose (7.5 mg/kg) for the experiment.

### Method of Tracheal Instillation of PM2.5

After weighing, the Wistar rats were anesthetized with pentobarbital sodium (40 mg/kg) intraperitoneal injection, it was laid on their backs on a rat operating table with a 30°-40° slope, teeth and limbs were restrained, and a lamp vertically illuminated its neck. With the tongue sticking out of the mouth and pressing the tongue, we could clearly see the rat's glottis with the aid a light. We inserted a no. 12 lavage needle into the trachea, gently dripped a certain volume of PM2.5 suspension until we could hear moist rales and see bubbles coming out of the windpipe, which proves the success of tracheal drip. The lavage needle was taken out. Rats were removed from the operating table after 10 min and were kept in supine position until waking.

## Specimen Collection

The rats were killed by abdominal aorta bleeding 24 h after the last tracheal instillation (the blank control group rats were killed 4 weeks after the start of the experiment) using intraperitoneal injection of pentobarbital sodium (50 mg/kg) to anesthetize them. Blood samples were collected from the abdominal aorta, centrifuged at 3000 r/min (1509.3×g) for 10 min, and collected the serum and stored it in a freezer at -80°C for later testing.

We separated the trachea and main bronchus, clamped the right main bronchus with a hemostatic forceps, cut a small oblique opening on the trachea, then inserted a No. 16 gavage feeding needle, and fixed it with a suture. We performed bronchoalveolar lavage with 5 ml pre-cool phosphate buffer (PBS) 3 times, then BALF was obtained, centrifuged at 1500r/min (377.3×g) for 10 min at 4°C, and the supernatant was collected and stored in a low-temperature freezer at -80°C before testing.

The lung tissue of the right lower lobe of the rats without lavage was fixed with formaldehyde solution, embedded in conventional paraffin, sectioned, and stained with hematoxylin-eosin (HE). Pathological changes were observed under a light microscope, and the rest of the right lung, heart, liver, and spleen tissues were filled with liquid nitrogen and transferred to a freezer at -80°C after being completely frozen for preparing tissue homogenate.

## Preparation of Tissue Homogenate

The lung, heart, liver, kidney, and spleen tissues were accurately weighed, and the ratio of homogenization was selected as 10% (ie, homogenization was performed by adding 9-fold volume) of normal saline according to weight (g): volume (ml), and homogenized on an ice bath using a tissue homogenizer, centrifuged at 5000r/min (4193.3×g) for 15 min, and we collected the supernatant as the sample to be tested immediately.

## Index Test

IL-6, IL-1 $\beta$ , TNF- $\alpha$ , 8-OHdG, and 4-HNE in serum and BALF of rats were tested by ELISA (enzyme-linked immunosorbent assays) using plate-based assays for identification and quantification a specific protein in complex biological samples. Briefly, the capture antibody was used to target specific protein and was coated on the microplate at 2-8°C overnight. The coated microplate was washed with blocking buffer for 1 h at room temperature. Then, the standards and biological samples were injected to designated wells to bind to the capture antibody with gentle shaking at room temperature for 1 h. After that, the excess substances were removed using PBS buffer solution to wash excess unbound compounds. The detection antibody

was added to combine with the immobilized proteins, followed by washing with washing buffer. Finally, the HRP and TMB substrate solution was added to each well and the stop was injected. The obtained solution was measured, and the results were calculated using the curve-fit method. PCC was tested by using ultraviolet colorimetry and spectrophotometer according to the test kit instructions (Nanjing Institute of Bioengineering).

The contents of  $\gamma$ -GCS and T-AOC in serum and tissue homogenate of rats were measured, and  $\gamma$ -GCS and T-AOC were tested by using a chemical spectrophotometer according to the test kit instructions.

## Statistical Analysis

All data were in accordance with normal distribution (Shapiro-Wilk test of normality). The experiment results are presented as the mean $\pm$ standard deviation (mean $\pm$ SEM). All statistical analyses were conducted using SPSS 21.0 Statistics Software. Means among multiple groups were compared by one-way ANOVA followed by Student-Newman-Keuls (SNK) post hoc tests for different pairwise comparisons. A difference was considered statistically significant if  $P<0.05$ .

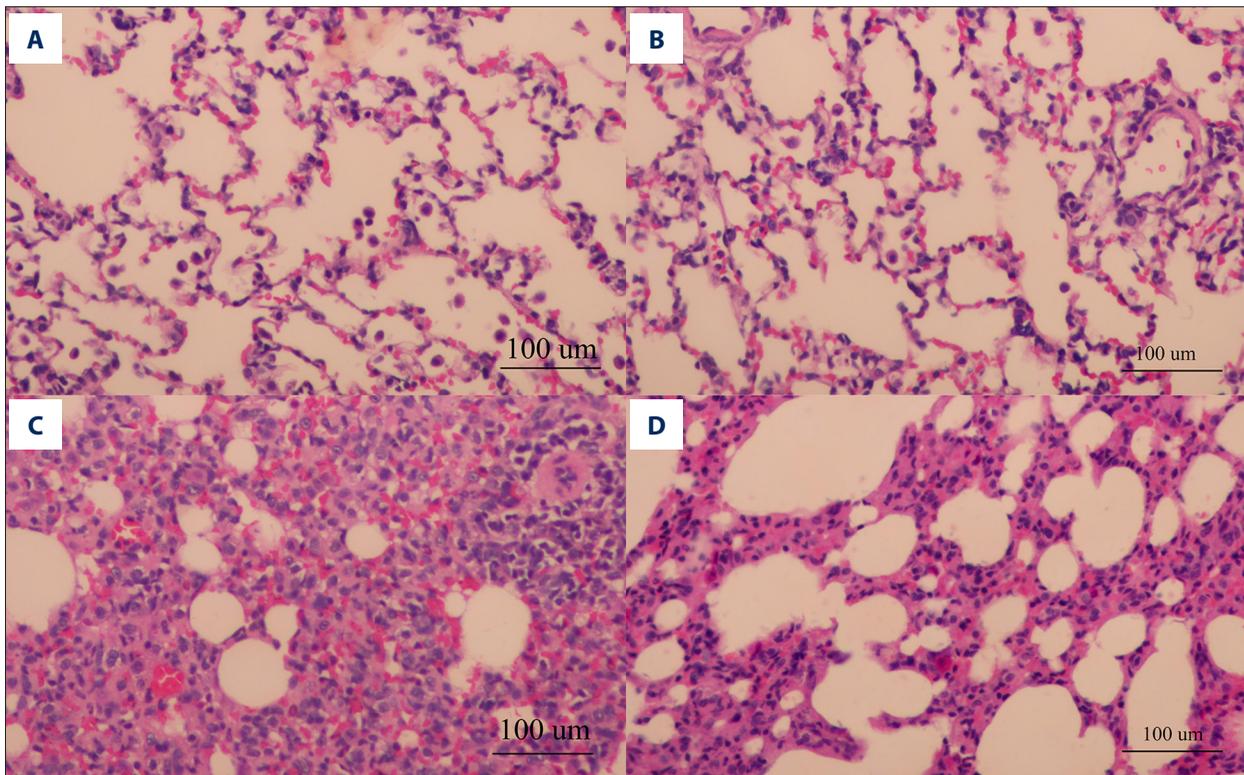
## Results

### Histopathology of Lung in Rats

There were no obvious abnormalities in pathological sections of rat lung tissues in the blank control group and the saline group under light microscopy; there were injuries in the rat lung tissues of the PM2.5 exposure group and the erdosteine intervention group with varying degrees. Infiltration of bronchiole and peripheral inflammatory cells could be seen in the PM2.5 exposure group under light microscopy, the inflammatory cells in the lumen increased, the lumen became smaller and narrower, the alveolar septa thickened, the alveolar cavity shrank, inflammatory cells exuded, peripheral inflammatory cells infiltrated, interstitial became edematous, capillary congested, small vessel wall thickened, smooth muscle became hyperplastic, and the lumen became smaller. Pathological sections of rat lung tissues in the erdosteine intervention group showed the degree of destruction was reduced (**Figure 1**).

### Index Changes in Rat Serum (see **Figure 2**, **Supplementary Table 1**)

There were statistically significant differences in IL-6, IL-1 $\beta$ , TNF- $\alpha$ , 8-OHdG, 4-HNE, and PCC in serum among the blank control group, the saline group, the PM2.5 exposure group, and the erdosteine intervention group ( $P<0.001$ ). The expressions



**Figure 1.** The pathological sections of rat lung tissues under light microscopic in each group (HE staining, 200×): the blank control group (A), the saline group (B), the PM2.5 exposure group (C), the erdosteine intervention group (D) (Adobe Illustrator CC 2019, Adobe Systems Incorporated).

of the biomarkers in the PM2.5 exposure group and the erdosteine intervention group were higher than those in the blank control group and the saline group ( $P < 0.05$ ). The expressions of IL-6, IL-1 $\beta$ , TNF- $\alpha$ , 8-OHdG, 4-HNE, and PCC in the erdosteine intervention group were lower than those in the PM2.5 exposure group ( $P < 0.05$ ). There was no statistically significant difference between the blank control group and the saline group ( $P > 0.05$ ).

#### Index Changes in Rat BALF (see Figure 3, Supplementary Table 2)

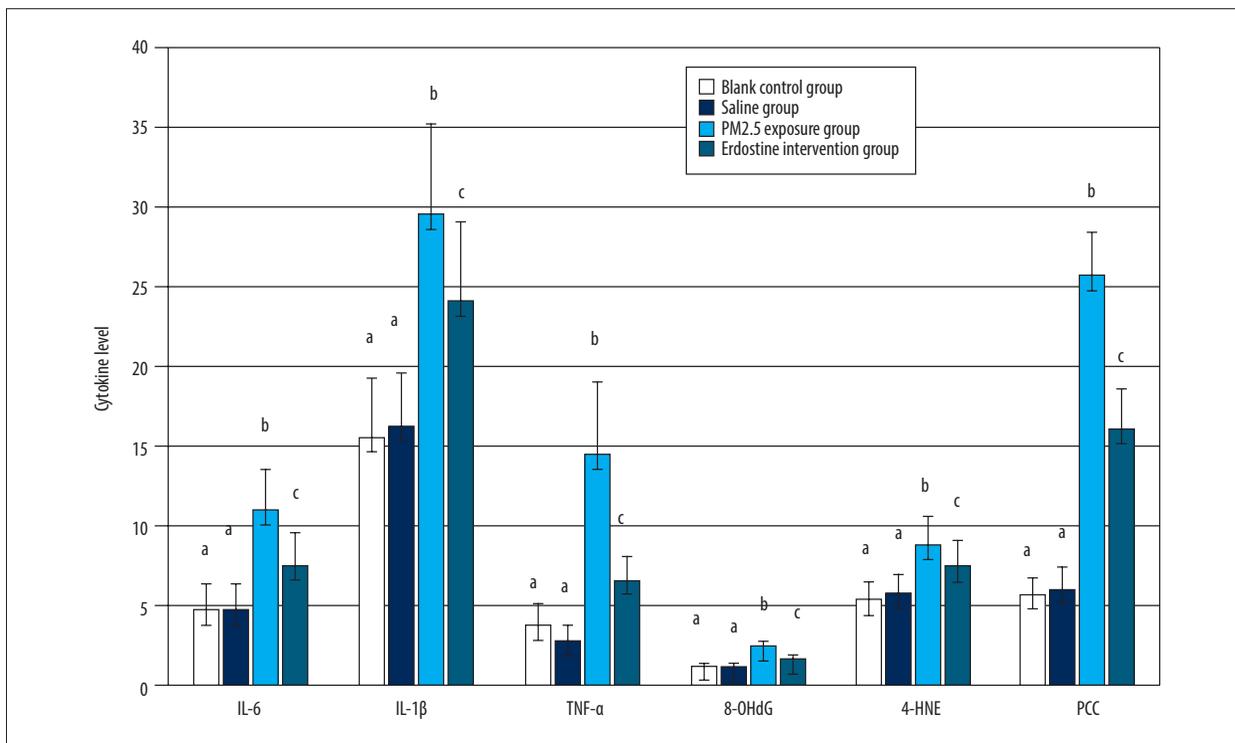
There was statistically significant difference in IL-6, IL-1 $\beta$ , TNF- $\alpha$ , 8-OHdG, 4-HNE, and PCC in BALF among the blank control group, the saline group, the PM2.5 exposure group, and the erdosteine intervention group ( $P < 0.001$ ). The expressions of the biomarkers in the PM2.5 exposure group and the erdosteine intervention group were higher than those in the blank control group and the saline group ( $P < 0.05$ ). The expressions of IL-6, IL-1 $\beta$ , TNF- $\alpha$ , 8-OHdG, 4-HNE, and PCC in the erdosteine intervention group were lower than those in the PM2.5 exposure group ( $P < 0.05$ ). There was no statistically significant difference between the blank control group and the saline group ( $P > 0.05$ ).

#### Changes of $\gamma$ -GCS in Serum and Organs (see Figure 4, Supplementary Table 3)

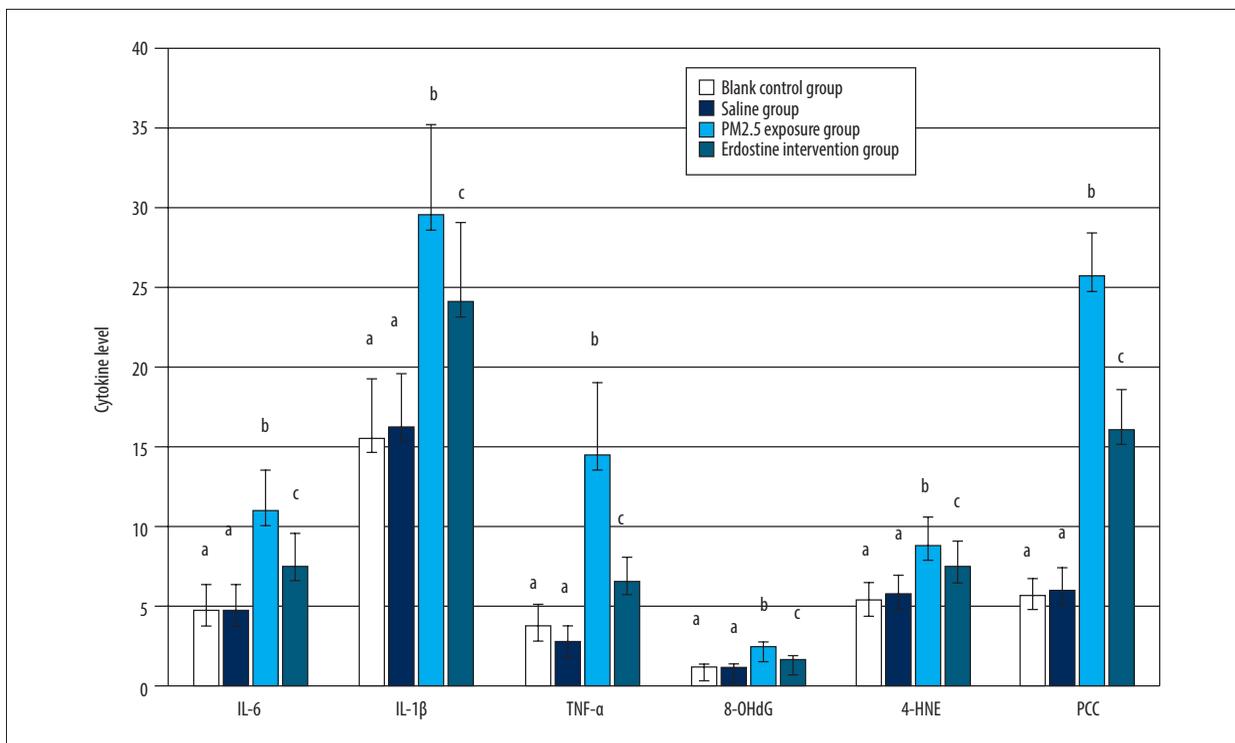
There was a statistically significant difference in  $\gamma$ -GCS in serum and lung tissues among the blank control group, the saline group, the PM2.5 exposure group, and the erdosteine intervention group ( $P < 0.05$ ); among them,  $\gamma$ -GCS expression in the PM2.5 exposure group and the erdosteine intervention group were higher than those in the blank control group and the normal saline group ( $P < 0.05$ ). There was no statistically significant difference between the blank control group and the normal saline group ( $P > 0.05$ ). There was no statistically significant difference between the PM2.5 exposure group and the erdosteine intervention group ( $P > 0.05$ ). There was no statistically significant difference in  $\gamma$ -GCS expression in the heart, liver, kidney, and spleen among the blank control group, the saline group, the PM2.5 exposure group, and the erdosteine intervention group ( $P > 0.05$ ).

#### Changes of T-AOC in Serum and Organs (see Figure 5, Supplementary Table 4)

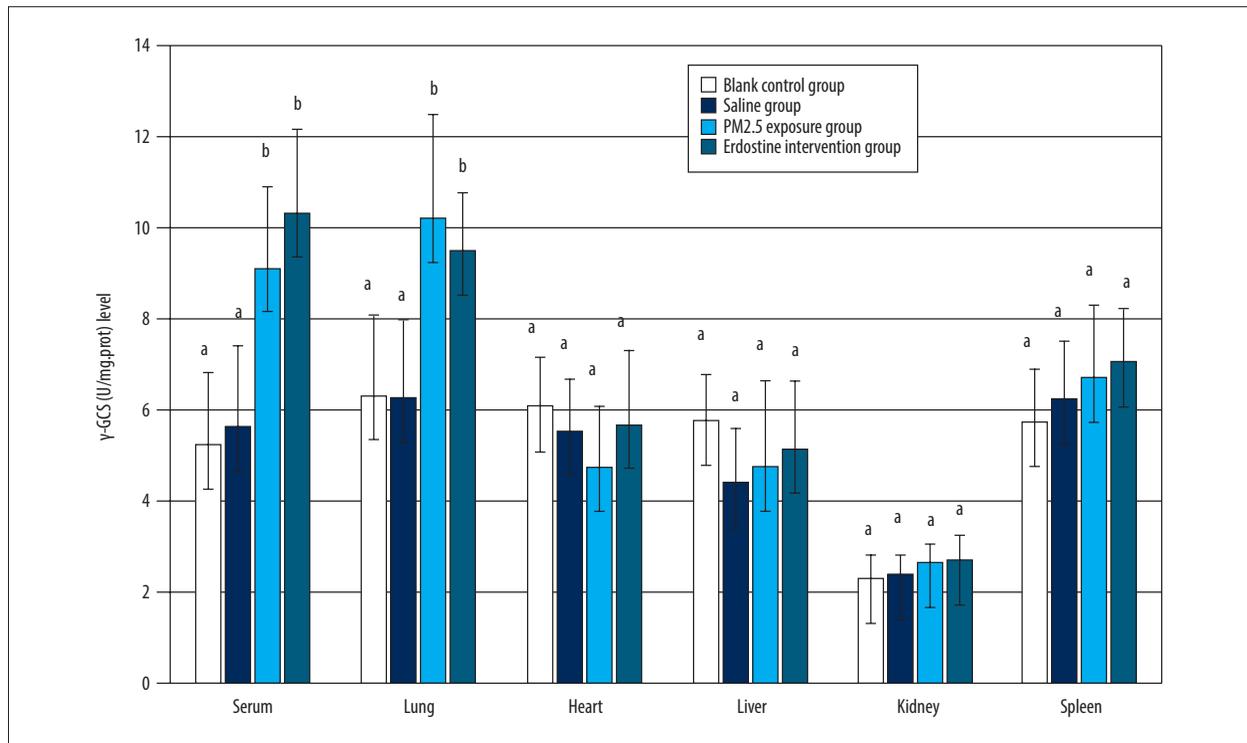
There was a statistically significant difference in T-AOC expression in serum and lung, spleen, kidney, and liver tissues among the blank control group, the saline group, the PM2.5 exposure



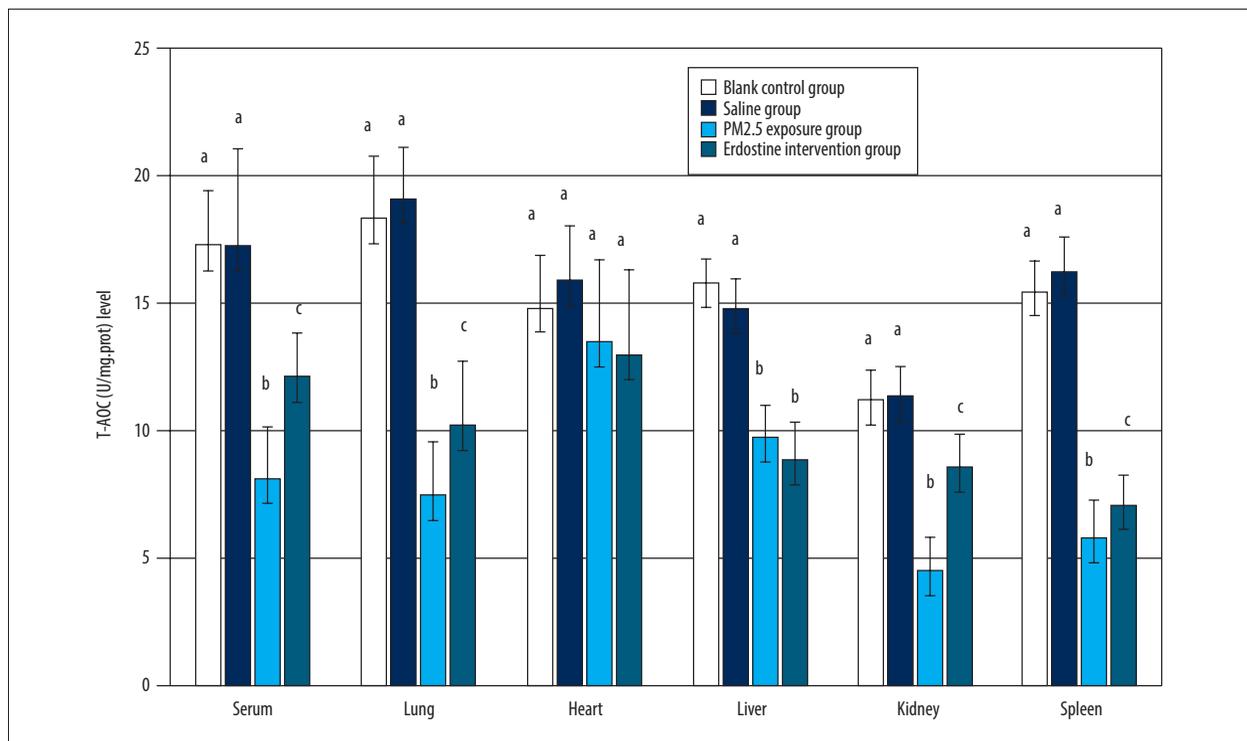
**Figure 2.** Comparisons of IL-6, IL-1β, TNF-α, 8-OHdG, 4-HNE, and PCC in rat serum of each group, as well as cytokine levels, were described by mean±standard deviation (n=10). Equal letters indicate no statistically significant differences,  $P>0.05$ ; different letters indicate statistical differences,  $P<0.05$  (Microsoft Office 2010, Microsoft Corporation).



**Figure 3.** Comparisons of IL-6, IL-1β, TNF-α, 8-OHdG, 4-HNE, and PCC in rat BALF of each group, as well as cytokine levels, were described by mean±standard deviation (n=10). Equal letters indicate no statistically significant differences,  $P>0.05$ ; different letters indicate statistical differences,  $P<0.05$  (Microsoft Office 2010, Microsoft Corporation).



**Figure 4.** Comparisons of  $\gamma$ -GCS(U/mg.prot) level in rat organs of each group (n=10). Equal letters indicate non statistical differences,  $P > 0.05$ ; different letters indicate statistical differences,  $P < 0.05$  (Microsoft Office 2010, Microsoft Corporation).



**Figure 5.** Comparisons of T-AOC(U/mg.prot) level in rat organs of each group (n=10). Equal letters indicate no statistically significant differences,  $P > 0.05$ ; different letters indicate statistical differences,  $P < 0.05$  (Microsoft Office 2010, Microsoft Corporation).

group, and the erdosteine intervention group ( $P < 0.05$ ). There was no statistically significant difference in T-AOC expression in heart tissue among the groups ( $P > 0.05$ ). Among them, the T-AOC expression in serum and lung, spleen, and kidney of the PM2.5 exposure group was lower than that of the erdosteine intervention group ( $P < 0.05$ ), while the T-AOC expression of the erdosteine intervention group was lower than in the blank control group and the saline group ( $P < 0.05$ ), and there was no statistically significant difference between the blank control group and the saline group ( $P > 0.05$ ). There was no statistically significant difference in T-AOC and liver tissue between the blank control group and the saline group ( $P > 0.05$ ), and there was no statistically significant difference between the PM2.5 exposure group and the erdosteine intervention group ( $P > 0.05$ ).

## Discussion

Currently, environmental pollution is the leading cause of premature death and disability worldwide [34,35]. PM2.5 is the air pollutant that poses the greatest threat to global public health [36]. It is estimated that environmental exposure to PM2.5 results in 4.2 million deaths and 103.1 million days of labor loss according to GBD 2015, which accounts for 7.6% of global mortality and 4.2% of total labor losses [37]. The premature death due to air pollution is caused mainly by ischemic heart disease and chronic obstructive pulmonary disease (COPD) [38], which indicates that there is a close correlation between the fine particle matters and cardiovascular and respiratory diseases. The short-term exposure to fine particle matter weakens the beneficial cardiopulmonary effects of walking in patients with chronic obstructive pulmonary disease or ischemic heart disease [39]. Air pollution exposure is closely related to deterioration of respiratory function and symptoms in patients with COPD, and even low concentrations of PM2.5 can lead to decreased lung function and increase the risks of COPD [40]. The acute effects of air pollution are reflected in more respiratory symptoms, as well as increased cardiovascular events, hospitalization rates, and mortality. The long-term effects include lung growth retardation in children and adolescents, decreased lung function in adults, cardiovascular disease and lung cancer [41]. PM2.5 exposure is associated with liver diseases, stroke, diabetes, myocardial infarction, hypertension, heart failure, anxiety, lung cancer, and other system diseases [42-47].

Particulate matters consist of harmful components such as polycyclic aromatic hydrocarbons (PAHs) and heavy metals, which mainly come from industrial emissions and traffic exhaust. It has been reported that PAHs can produce reactive oxygen species (ROS) through the redox cycle, which leads to oxidative alteration of DNA and lipids in the body. Some heavy metals, such as arsenic, copper, cobalt, and chromium,

can generate ROS through Fenton reaction. The generation of ROS can cause oxidative damage to biological macromolecules such as lipids, DNA, and proteins in the body [48-53]. Many studies have suggested that the pathological mechanism of the increase of mortality caused by PM2.5 is mainly reflected in the following aspects: (1) PM2.5 and oxidative stress; (2) PM2.5 and inflammatory injury; (3) PM2.5 and nucleic acid repair; and (4) PM2.5 and immune reaction [54]. Oxidative stress is a normal oxidation/anti-oxidation imbalance caused by oxidant excess and/or antioxidant reduction in the body, which is the common basis for multi-system and multi-organ oxidative injury, and the key to the occurrence, development, and deterioration of disease triggered by PM2.5. Recently, many studies have confirmed that the inflammation and oxidative stress injury caused by acute PM2.5 exposure can affect multiple organs throughout the body [55-57]. The antagonists against this mechanism may provide protection for the prevention and treatment of the health effects of PM2.5 air pollution.

TNF- $\alpha$  is a potent mediator of immune and inflammatory responses. IL-6 and IL-1 $\beta$  are acute cytokines that induce other cells to produce cytokines and produce acute phase reactions. The activated macrophages produce pro-inflammatory mediators, such as IL-1 $\beta$  and TNF- $\alpha$ , which are involved in a variety of respiratory symptoms. Airway hyper-responsiveness and asthma are associated with the increase of IL-1 $\beta$  and TNF- $\alpha$  level [58-60]. PM2.5 enters the alveoli and is deposited on the alveolar surface, which can lead to the expression and release of cytokines such as TNF- $\alpha$  and IL-6 in macrophages [61]. After PM2.5 treatment of human bronchial epithelial cells (BEAS-2B), with increasing exposure time and exposure dose, the genes expression of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, and other inflammatory factors was enhanced [62]. In 2015, animal experiments showed that PM2.5 caused lung congestion and inflammatory cells infiltration in rats, and increases inflammatory cell numbers in BALF. With the increase of PM2.5 concentration, the concentration of pro-inflammatory mediators, such as TNF- $\alpha$ , IL-6, IL-1 $\beta$ , in lung tissue, increased too, and accompanied by changes in oxidative stress, which may have a critical role in the susceptibility to respiratory diseases caused by PM2.5 [63]. PCC is the most common and most commonly used biomarker for evaluating oxidative injury in protein. Human diseases associated with PCC include Alzheimer's disease, chronic lung disease, chronic renal failure, diabetes, and sepsis [64-66]. 8-OHdG is a specific biomarker of DNA oxidative injury caused by a variety of endogenous and exogenous factors [67]. It is one of the main forms of oxidative damage induced by free radicals and has been widely used as a biomarker of oxidative stress and carcinogenesis [68]. Studies have shown that PM2.5 can increase the level of 8-OHdG in human urine [69]. 4-HNE is the product of oxidative injury and a marker of lipid peroxidation. PM2.5 exposure has been reported to increase 4-HNE levels in the

blood of mice [70]. All of these are classical oxidative stress biomarkers. Because inflammatory and oxidative stress injury is an important mechanism of the respiratory damage caused by PM2.5, we examined the trends of the above classical inflammatory and oxidative stress markers in serum and BALF. We observed that HE staining of rat lung tissues after PM2.5 exposure showed significant inflammatory manifestations, the inflammatory factors such as IL-6, IL-1 $\beta$ , and TNF- $\alpha$ , and the above-mentioned classical oxidative stress markers were elevated compared to normal, again demonstrating the mechanism of lung injury caused by PM2.5.

$\gamma$ -GCS is the rate-limiting enzyme in the synthesis of GSH; its activity directly affects GSH levels [71]; the expression of  $\gamma$ -GCS is increased during the oxidative stress process induced by cigarettes, and the expression levels of  $\gamma$ -GCS mRNA and its protein expression in bronchopulmonary tissues gradually increased with the prolongation of smoking. At present, there is no relevant research on whether PM2.5 affects the level of  $\gamma$ -GCS. T-AOC is the total antioxidant capacity, and its decrease means that the oxidative stress defense system of humans and animals will be compromised [73]. Therefore, we observed the changes of  $\gamma$ -GCS and T-AOC in serum and various organs after PM2.5 exposure, and found the  $\gamma$ -GCS level in serum and lung was increased, but other organs did not change much, suggesting that the lung is the primary organ in which  $\gamma$ -GCS is expressed. PM2.5 exposure upregulates GSH synthesis by upregulating  $\gamma$ -GCS level, which is a protective response against oxidative stress in the body. However, we also observed the decrease of T-AOC in serum and lung, liver, kidney, and spleen, which indicates that there is an oxidation/anti-oxidation imbalance in multiple organs, and the upregulated GSH did not affect the additional injuries of pro-inflammatory mediators and oxides, which ultimately leads to a decrease in total antioxidant capacity.

Chinese scholars have confirmed that a Chinese herbal decoction reduces oxidative stress injury in COPD rats by lowering the levels of 8-OHdG, 4-HNE, and  $\gamma$ -GCS [72]. Other studies on the toxicity of PM2.5 explored the effects after intervention. Pretreatment with selenium before tracheal instillation to PM2.5-exposed rats can decrease indicators (TNF- $\alpha$ , IL-1 $\beta$ , soluble intercellular adhesion molecule sICAM-1) of inflammatory response in rat BALF, increase the indicators of antioxidant capacity among related oxidative stress indicators, such as T-AOC, total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px), decrease malondialdehyde (MDA), a lipid peroxidation product, and decrease the cell injury biomarkers lactate dehydrogenase (LDH), total protein (TP), and alkaline phosphatase (AKP). Selenium has a protective effect on the inflammatory oxidative stress injury in lung tissue caused by PM2.5 [73]. Other scholars have found that early administration of curcumin can reduce the expression of TNF- $\alpha$  and IL-6,

increase T-AOC, and alleviate lung injury induced by PM2.5 in mice [74]. Therefore, we speculated that pretreatment with drugs with anti-inflammatory and antioxidant mechanisms can reduce the organ damage caused by PM2.5.

Erdosteine contains a closed sulfhydryl group in its molecular structure, which exerts mucolytic effects through hepatic biotransformation into active metabolites containing free sulfhydryl groups. Recent studies have shown that the metabolites of hepatic biotransformation also have free radical scavenging and anti-oxidative stress damage functions. Erdosteine prevents the accumulation of accumulated oxygen radicals and increases the antioxidant capacity of cells. The final result is a protective effect on tissues, reducing lipid peroxidation, neutrophil infiltration, and cellular noxious agent-mediated apoptosis [75]. In recent years, several studies on erdosteine supported the protective role of erdosteine in various tissue injuries mediated by oxidative stress products and inflammatory factors [76-79]. Currently, there are no international studies related to PM2.5 damage and treatment using erdosteine. We provided early treatment with erdosteine in animal experiments to observe whether it could reduce the damage caused by PM2.5 exposure, and we found that the IL-6, IL-1 $\beta$ , TNF- $\alpha$ , 8-OHdG, 4-HNE, and PCC levels in the erdosteine intervention group were lower than those of the PM2.5 exposure group, and increased the total antioxidant capacity of serum, lung, spleen, and kidney. Erdosteine can alleviate the inflammatory and oxidative stress damage caused by PM2.5 exposure, and may be an effective anti-inflammatory and antioxidant to reduce the damage caused by PM2.5 exposure. Meanwhile, we observed that the  $\gamma$ -GCS level did not change much in serum and lung tissue after erdosteine intervention, which suggested that erdosteine does not affect this protective response. Of course, in our experiment, we also found that there was little change in the expression of T-AOC in liver tissue after the intervention of erdosteine, and there was no significant difference in cardiac T-AOC among the 4 groups. We suspect that this may be related to the different expression level of T-AOC in different organs and the different concentration of erdosteine in organs, and we do not rule out the loss of some effects in the process of tissue sampling and production. We hope that this can be further assessed in future experiments.

This study provides a theoretical basis for seeking the effective clinical therapeutic drugs against oxidative stress injury caused by PM2.5. However, in this experiment, we did not further explore the mechanism of the increase of  $\gamma$ -GCS caused by PM2.5 and whether the exposure time of PM2.5 will lead to dynamic change of  $\gamma$ -GCS. Further in-depth research on erdosteine is needed, the safety and related adverse effects at different doses have not been discussed, and there is a lack of relevant data from human experiments.

## Conclusions

PM2.5 exposure can cause inflammatory and oxidative stress damage in serum and organ tissues of rats. Animal experiments have shown that early erdosteine intervention has a certain protective effect on this injury, which may be due to the downregulation of the levels of inflammatory and oxidative stress factors caused by PM2.5 exposure and the increase of T-AOC. Our results suggest that erdosteine may be an effective anti-inflammatory and antioxidant to reduce the damage caused by PM2.5.

## Declaration of Figures Authenticity

All figures submitted have been created by the authors, who confirm that the images are original with no duplication and have not been previously published in whole or in part.

## Supplementary Materials

**Supplementary Table 1.** Comparisons of cytokine level in rat serum of each group (n=10).

| Group                         | IL-6 (pg/ml)                    | IL-1 $\beta$ (pg/ml)            | TNF- $\alpha$ (pg/ml)           | 8-OHdG (ng/ml)                 | 4-HNE (ug/ml)                  | PCC (nmol/mg·prot)              |
|-------------------------------|---------------------------------|---------------------------------|---------------------------------|--------------------------------|--------------------------------|---------------------------------|
| Blank control group           | 4.725 $\pm$ 1.628 <sup>a</sup>  | 15.605 $\pm$ 3.673 <sup>a</sup> | 3.797 $\pm$ 1.337 <sup>a</sup>  | 1.238 $\pm$ 0.130 <sup>a</sup> | 5.359 $\pm$ 1.031 <sup>a</sup> | 5.679 $\pm$ 1.012 <sup>a</sup>  |
| Saline group                  | 4.980 $\pm$ 1.690 <sup>a</sup>  | 16.282 $\pm$ 3.291 <sup>a</sup> | 2.774 $\pm$ 0.923 <sup>a</sup>  | 1.190 $\pm$ 0.127 <sup>a</sup> | 5.767 $\pm$ 1.180 <sup>a</sup> | 5.990 $\pm$ 1.402 <sup>a</sup>  |
| PM2.5 exposure group          | 10.978 $\pm$ 2.572 <sup>b</sup> | 29.650 $\pm$ 5.611 <sup>b</sup> | 14.513 $\pm$ 4.451 <sup>b</sup> | 2.457 $\pm$ 0.296 <sup>b</sup> | 8.845 $\pm$ 1.695 <sup>b</sup> | 25.782 $\pm$ 2.644 <sup>b</sup> |
| Erdosteine intervention group | 7.562 $\pm$ 1.989 <sup>c</sup>  | 24.133 $\pm$ 4.911 <sup>c</sup> | 6.619 $\pm$ 1.491 <sup>c</sup>  | 1.669 $\pm$ 0.236 <sup>c</sup> | 7.416 $\pm$ 1.650 <sup>c</sup> | 16.137 $\pm$ 2.43 <sup>c</sup>  |
| F                             | 21.053                          | 22.574                          | 45.758                          | 18.277                         | 13.420                         | 231.090                         |
| P                             | <0.001                          | <0.001                          | <0.001                          | <0.001                         | <0.001                         | <0.001                          |

In the above table, equal letters indicate no statistical differences,  $P>0.05$ ; different letters indicate statistical differences,  $P<0.05$  (Microsoft Office 2010, Microsoft Corporation).

**Supplementary Table 2.** Comparisons of cytokine level in rat BALF of each group (n=10).

| Group                         | IL-6 (pg/ml)                    | IL-1 $\beta$ (pg/ml)            | TNF- $\alpha$ (pg/ml)           | 8-OHdG (ng/ml)                 | 4-HNE (ug/ml)                  | PCC (nmol/mg·prot)              |
|-------------------------------|---------------------------------|---------------------------------|---------------------------------|--------------------------------|--------------------------------|---------------------------------|
| Blank control group           | 7.547 $\pm$ 1.59 <sup>a</sup>   | 26.981 $\pm$ 7.051 <sup>a</sup> | 5.79 $\pm$ 1.01 <sup>a</sup>    | 1.299 $\pm$ 0.118 <sup>a</sup> | 5.742 $\pm$ 1.169 <sup>a</sup> | 12.084 $\pm$ 3.034 <sup>a</sup> |
| Saline group                  | 7.442 $\pm$ 1.777 <sup>a</sup>  | 25.938 $\pm$ 7.114 <sup>a</sup> | 4.42 $\pm$ 1.185 <sup>a</sup>   | 1.381 $\pm$ 0.23 <sup>a</sup>  | 6.251 $\pm$ 1.254 <sup>a</sup> | 13.974 $\pm$ 2.285 <sup>a</sup> |
| PM2.5 exposure group          | 13.167 $\pm$ 2.413 <sup>b</sup> | 54.497 $\pm$ 9.317 <sup>b</sup> | 23.773 $\pm$ 4.881 <sup>b</sup> | 2.657 $\pm$ 0.387 <sup>b</sup> | 9.747 $\pm$ 1.555 <sup>b</sup> | 34.387 $\pm$ 4.33 <sup>b</sup>  |
| Erdosteine intervention group | 10.041 $\pm$ 1.835 <sup>c</sup> | 45.718 $\pm$ 4.594 <sup>c</sup> | 9.169 $\pm$ 1.49 <sup>c</sup>   | 1.709 $\pm$ 0.23 <sup>c</sup>  | 7.071 $\pm$ 1.159 <sup>c</sup> | 18.205 $\pm$ 3.833 <sup>c</sup> |
| F                             | 19.515                          | 38.309                          | 110.883                         | 57.624                         | 32.156                         | 85.985                          |
| P                             | <0.001                          | <0.001                          | <0.001                          | <0.001                         | <0.001                         | <0.001                          |

In the above table, equal letters indicate no statistical differences,  $P>0.05$ ; different letters indicate statistical differences,  $P<0.05$  (Microsoft Office 2010, Microsoft Corporation).

**Supplementary Table 3.** Comparisons of  $\gamma$ -GCS (U/mg-prot) level in rat organs of each group (n=10).

| Group                         | Serum                     | Lung                      | Heart                    | Liver                    | Kidney                   | Spleen                   |
|-------------------------------|---------------------------|---------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Blank control group           | 5.265±1.575 <sup>a</sup>  | 6.362±1.698 <sup>a</sup>  | 6.081±1.051 <sup>a</sup> | 5.790±1.012 <sup>a</sup> | 2.299±0.518 <sup>a</sup> | 5.742±1.169 <sup>a</sup> |
| Saline group                  | 5.659±1.757 <sup>a</sup>  | 6.287±1.702 <sup>a</sup>  | 5.568±1.114 <sup>a</sup> | 4.420±1.185 <sup>a</sup> | 2.381±0.423 <sup>a</sup> | 6.251±1.254 <sup>a</sup> |
| PM2.5 exposure group          | 9.171±1.763 <sup>b</sup>  | 10.258±2.236 <sup>b</sup> | 4.775±1.317 <sup>a</sup> | 4.773±1.881 <sup>a</sup> | 2.657±0.387 <sup>a</sup> | 6.747±1.555 <sup>a</sup> |
| Erdosteine intervention group | 10.369±1.835 <sup>b</sup> | 9.541±1.258 <sup>b</sup>  | 5.718±1.594 <sup>a</sup> | 5.169±1.490 <sup>a</sup> | 2.709±0.523 <sup>a</sup> | 7.071±1.159 <sup>a</sup> |
| F                             | 21.431                    | 14.056                    | 1.832                    | 1.690                    | 1.884                    | 2.019                    |
| P                             | <0.001                    | <0.001                    | 0.159                    | 0.187                    | 0.150                    | 0.129                    |

In the above table, equal letters indicate no statistical differences,  $P>0.05$ ; different letters indicate statistical differences,  $P<0.05$  (Microsoft Office 2010, Microsoft Corporation).

**Supplementary Table 4.** Comparisons of T-AOC (U/mg-prot) level in rat organs of each group (n=10).

| Group                         | Serum                     | Lung                      | Heart                     | Liver                     | Kidney                    | Spleen                    |
|-------------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| Blank control group           | 17.246±2.157 <sup>a</sup> | 18.364±2.391 <sup>a</sup> | 14.847±2.030 <sup>a</sup> | 15.797±1.012 <sup>a</sup> | 11.248±1.139 <sup>a</sup> | 15.647±1.174 <sup>a</sup> |
| Saline group                  | 17.256±3.754 <sup>a</sup> | 19.084±2.046 <sup>a</sup> | 15.926±2.121 <sup>a</sup> | 14.821±1.147 <sup>a</sup> | 11.326±1.187 <sup>a</sup> | 16.261±1.320 <sup>a</sup> |
| PM2.5 exposure group          | 8.163±2.035 <sup>b</sup>  | 7.434±2.127 <sup>b</sup>  | 13.484±3.206 <sup>a</sup> | 9.765±1.216 <sup>b</sup>  | 4.528±1.309 <sup>b</sup>  | 5.801±1.473 <sup>b</sup>  |
| Erdosteine intervention group | 12.084±1.74 <sup>c</sup>  | 10.206±2.564 <sup>c</sup> | 12.979±3.308 <sup>a</sup> | 8.847±1.496 <sup>b</sup>  | 8.615±1.233 <sup>c</sup>  | 7.084±1.158 <sup>c</sup>  |
| F                             | 30.073                    | 64.882                    | 1.872                     | 83.310                    | 68.749                    | 183.935                   |
| P                             | <0.001                    | <0.001                    | 0.153                     | <0.001                    | <0.001                    | <0.001                    |

In the above table, equal letters indicate no statistical differences,  $P>0.05$ ; different letters indicate statistical differences,  $P<0.05$  (Microsoft Office 2010, Microsoft Corporation).

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