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RESEARCH ARTICLE

Mitigation of cadmium-induced lung injury by *Nigella sativa* oil

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Abstract Induction of oxidative stress and inflammation are considered the primary mechanism of cadmium (Cd) toxicity. *Nigella sativa* (NS) seeds and their oil (NSO) have been reported to possess antioxidant and anti-inflammatory potential. This study was conducted to assess the protective effect of NSO on Cd-induced lung damage in rat. Forty adult male Wistar rats were divided equally into 4 groups. Animals in groups I, II, and III received 1 ml of isotonic saline intraperitoneally (IP), 2 mg/kg of cadmium chloride (CdCl$_2$) dissolved in isotonic saline IP, and 1 ml/kg of NSO by gastric gavage, respectively. Group IV rats received NSO an hour prior to CdCl$_2$ administration via the same routes and doses as previously described. All animals were treated for 28 days. At the end of the study, animals were sacrificed; lungs were harvested for histopathological studies using light and electron microscopy. Saline-treated and NSO-treated rats showed normal lung parenchyma. However, CdCl$_2$-treated rats showed massive degenerative changes in alveolar epithelial lining, disrupted interalveolar septa, and hemolytic debris in alveoli. Rats treated with both NSO and CdCl$_2$ (group IV) showed amelioration of most Cd-induced lung damage with minimal histopathological changes in lung architecture. This study elucidates the protective effects of NSO on Cd-induced lung injury in rats and highlights the possibility of using NSO as a protective agent in individuals at high risk of Cd-induced lung toxicity.

Keywords Cadmium toxicity · Histopathological changes · Lung · *Nigella sativa* oil

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Introduction

Cadmium (Cd) is an environmental contaminant that represents a significant health hazard to humans. Because Cd is a natural element found in earth's crust, mining and industrial activities are associated with high emissions of this metal into the environment. Exposure to Cd from food may be sufficiently high and represents a human health hazard because of its widespread low-grade contamination of agricultural soil in many areas of the world, and it is easily taken up by crops such as rice, wheat, and potatoes. Other important sources of Cd exposure include garbage incineration and tobacco smoking (Akesson et al. 2014).

Cadmium accumulates and induces toxic effects in several organs. Liver is the primary site of metabolism and accumulation in case of Cd acute toxicity, whereas kidneys are the main organs affected by chronic exposure to Cd (Kukongviriyapan et al. 2014). Other adverse health outcomes including neurotoxicity, hypertension, type 2 diabetes mellitus, and cancers have been reported in populations with chronic low-dose cadmium exposure (Cao et al. 2009; Colacino et al. 2014).

Cadmium-induced tissue injury and cell death may be related to systemic inflammation and/or induction of oxidative stress (OS) with the production of reactive oxygen species (ROS). Chronic exposure to Cd does not only enhance ROS generation but also depletes antioxidant levels, resulting in a state of oxidant/antioxidant imbalance (Kukongviriyapan et al. 2014). Cadmium-induced generation of ROS is both time- and concentration-dependent. Additionally, a decrease in enzymes involved in cellular redox reactions such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) has been frequently reported (Chen et al. 2008; Wang and Du 2013).

Nigella sativa (NS) seeds and their oil (NSO) have been used for centuries as traditional remedies worldwide. *N. sativa* has been reported to possess many beneficial applications such as diuretic, antihypertensive, antidiabetic, anticancer, immunomodulatory, analgesic, antimicrobial, spasmolytic, bronchodilator, gastroprotective, hepatoprotective, antioxidant, and anti-inflammatory properties (Ahmad et al. 2013). Bioactive molecules such as thymoquinone, tocopherols, phytosterols, and polyunsaturated fatty acids are the major active chemical components responsible for the therapeutic properties of NS (Al-Ali et al. 2008; Butt and Sultan 2010).

The benefit of chelation therapy in Cd intoxication is unproven. Multiple chelating agents have been investigated in animal models with inconsistent results. There is no evidence that chelation of poisoned animals improves long-term outcomes. Moreover, administration of chelating agents may be associated with a number of adverse effects including trace elements imbalance (Goldfrank et al. 2006). The use of medicinal plants for the prevention and/or treatment of heavy metals intoxication has attracted a great deal of recent attention as a result of their low toxicity and costs (Entok et al. 2014). Cadmium-induced OS and inflammation may justify amelioration of these effects using plant products with antioxidant and anti-inflammatory properties such as NS. Hence, this study was conducted to investigate the potential mitigating effect of NSO on Cd-induced lung injury in adult male Wistar rats.

Materials and methods

Chemicals

Cadmium chloride ($CdCl_2$) (analytical grade) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used in the study were of the highest purity available and were obtained from local commercial sources.

Preparation of *N. sativa* oil

The dried seeds of NS were purchased from the local market and were identified and authenticated in the Department of Pharmacognosy, Faculty of Pharmacy, Tanta University, Tanta, Egypt. *N. sativa* seeds were crushed and cold macerated in petroleum ether (40–60 °C) for 3 days. *N. sativa* oil was obtained by filtration of the collected extract after evaporation of petroleum ether. *N. sativa* oil yield was 17.5 % v/w with reference to dried seeds. The extracted NSO was preserved in screw-capped dark tubes at −20 °C until used (Mohamadin et al. 2010).

Compliance with ethical standards

The study protocol was approved by the research ethics committee of the Faculty of Medicine, Tanta University. Forty adult male Wistar rats weighing 200–240 g were used in this study. Animals were obtained from the animal house of the Faculty of Medicine, Tanta University, Tanta, Egypt. They were housed under standard conditions of temperature (23 ± 2 °C) and lighting (12 h light/dark cycles) and were allowed free access to food and drinking water.

Experimental design

Animals were randomly divided into four equal groups (ten rats in each group). Group I rats served as a control and received 1 ml of isotonic saline intraperitoneally (IP). Rats of group II received 2 mg/kg body weight of $CdCl_2$ dissolved in isotonic saline IP (Koyuturk et al. 2007), whereas group III rats were given NSO (1 ml/kg) by gastric gavage (Mohamadin et al. 2010). Group IV rats received NSO an hour prior to $CdCl_2$ administration via the same routes and doses as previously described. All animals were treated for 28 days.

Preparation of the specimens

At the end of the treatment period, the overnight-fasted rats were anesthetized by diethyl ether. Animals were then sacrificed by cervical dislocation, dissected, and their lungs were removed. Specimens were taken from the lungs of each animal and divided into two pieces. One piece was fixed in 10 % formol saline, paraffin-embedded, sectioned at 5 μm, and prepared for light microscopic study (Olympus BX-50) using hematoxylin and eosin (H&E) stain (Bancroft and Stevens 1996). The other one was immersed in phosphate-buffered glutaraldehyde solution and stained with toluidine blue stain for semi-thin sections or uranyl acetate and lead citrate stains for

ultra-structural examination using JEOL JEM electron microscope at 80 kV (Hayat 1978).

Results

(A) Light microscopy

Group I (control rats)

The lung parenchyma appeared well-formed and included respiratory bronchioles, alveolar ducts, alveolar sacs, and alveoli lined by type I (flat squamous cells with dark, elongated, central nuclei and scanty cytoplasm) and type II (cuboidal cells with central, round nuclei) pneumocytes. The adjacent alveoli were separated by thin interalveolar septa lined on both sides by alveolar epithelium and contained many capillaries.

Group II (CdCl₂-treated rats)

There were wide areas of lung collapse. Many alveoli showed widespread pulmonary hemorrhage and most of the capillaries appeared markedly congested. Infiltration of the alveoli with inflammatory cells was also observed. The lining epithelium of the alveoli and the alveolar-capillary membrane were apparently normal (Fig. 1).

Group III (NSO-treated rats)

No structural changes were seen in the lungs after administration of NSO. The lungs appeared normal with many cup-shaped alveoli and alveolar sacs. Pulmonary alveoli appeared as small, thin-walled pouches separated by thin interalveolar septa containing extensive capillary bed. The alveoli were lined by flat type I, and cuboidal type II pneumocytes. The interalveolar septum and the alveolar-capillary membrane also appeared normal (Fig. 2).

Group IV (rats treated with CdCl₂ and NSO)

Most of the alveoli and alveolar sacs appeared normal. Small areas of collapse and alveolar hemorrhage were present. The interalveolar septum appeared normal with normal capillaries. The lining epithelium of the alveoli as well as the alveolar-capillary membrane appeared normal as compared with the control group (Fig. 3).

(B) Electron microscopy

Group I (control rats)

The alveoli appeared as cup-shaped spaces that were lined by two types of epithelial cells, the flat type I and the cuboidal type II pneumocytes. The rounded alveolar macrophages with their characteristic pseudopodia were also seen in the wall of the alveoli. The alveoli were separated from each other by a thin, three-layered interalveolar septum.

Group II (CdCl₂-treated rats)

Many alveoli were collapsed with aggregation of their abnormal lining epithelium and collagen precipitation. There was widening of the interstitial space together with

Fig. 1 A photomicrograph of a section in the lung of CdCl₂-treated rat showing the alveoli (*A*) with widespread alveolar hemorrhage (*H*) and many congested capillaries (*C*). There is also areas of lung collapse (*Cp*) and infiltration of the lung parenchyma with inflammatory cells (*arrow*) (H&E ×400)
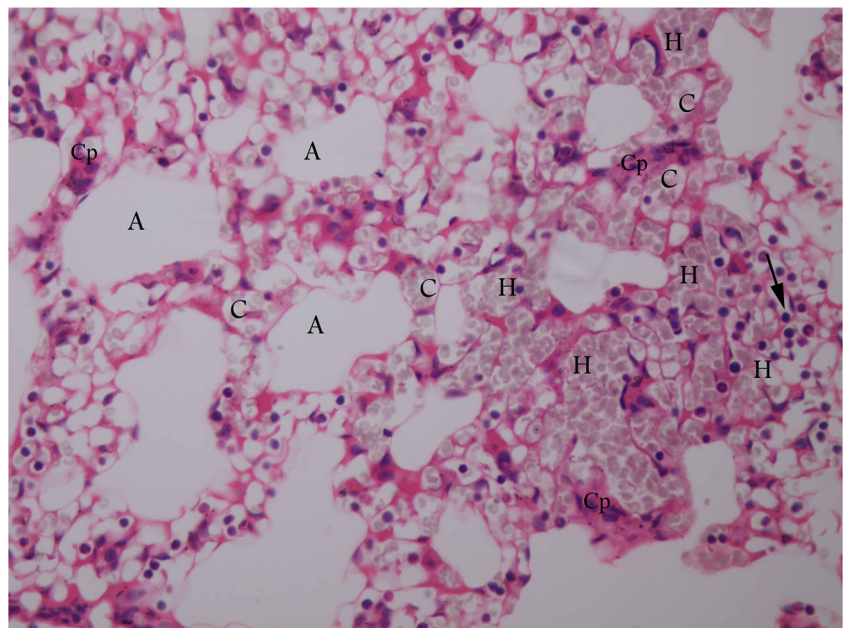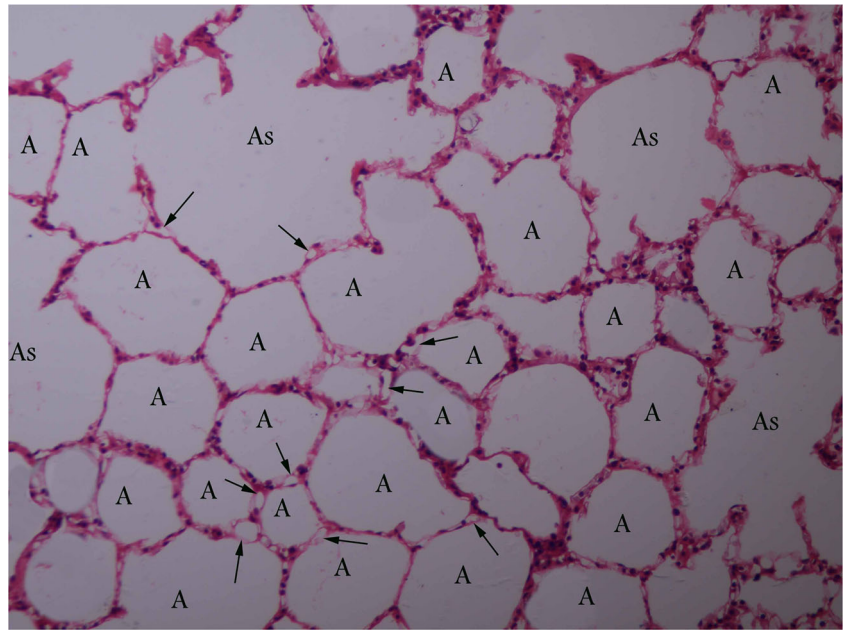
Fig. 2 A photomicrograph of a section in the lung of NSO-treated rat showing normal cup-shaped alveoli (*A*) and alveolar sacs (*As*). The thin interalveolar septum contains many capillaries (*arrow*) (H&E ×400)

alveolar infiltration by eosinophilic granulocytes. Free RBCs were seen in some alveoli. Type I pneumocytes showed irregular nuclear membrane. Similarly, many type II pneumocytes appeared abnormal with small, pyknotic, irregular nuclei and condensation of the chromatin near the nuclear membrane. The lamellar bodies appeared empty. In addition, some alveolar macrophages showed small, dark, pyknotic nuclei. The interalveolar septum became thick in certain parts of its course (Figs. 4, 5, and 6).

Group III (NSO-treated rats)

The alveoli appeared normal. Its wall was lined by types I and II pneumocytes as well as by alveolar macrophages. The squamous type I pneumocyte had large surface area, elongated dense nucleus, and attenuated cytoplasm. The cuboidal type II pneumocyte was seen bulging into the alveolar lumen and its luminal surface was covered by microvilli. It had central, round euchromatic nucleus. Its cytoplasm showed the characteristic electron-dense lamellar bodies. Alveolar macrophage

Fig. 3 A photomicrograph of a section in the lung of a rat treated with CdCl₂ and NSO showing normal interalveolar septum containing normal capillaries (*arrow*) in between normal alveoli (*A*). Small areas of lung collapse (*Cp*) and intra-alveolar hemorrhage (*H*) are also seen (H&E ×400)
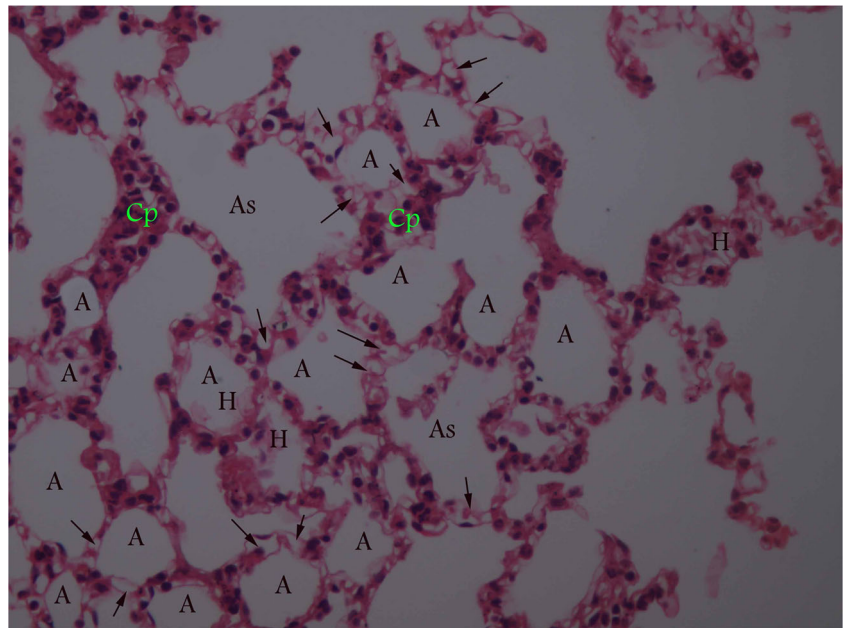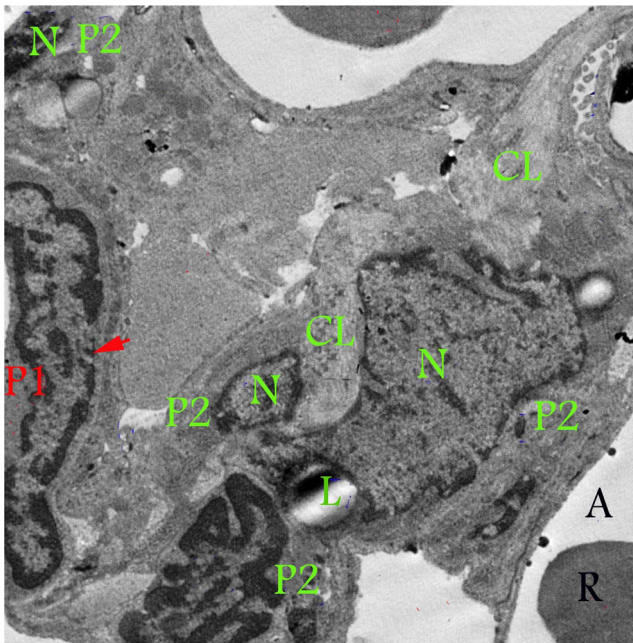
Fig. 4 An electron micrograph of an ultra-thin section from the lung of CdCl$_2$-treated rat showing a collapsed alveolus with aggregation of its lining epithelium and collagen precipitation (*CL*). Type II pneumocytes (*P2*) have small, dark, pyknotic nuclei with chromatin aggregation on the nuclear membrane (*N*) and partially empty lamellar bodies (*L*). Type I pneumocyte (*P1*) also showed irregular nuclear membrane (*arrow head*). There is also free RBCs in the alveoli (*R*) (EM ×2500)

was seen projecting into the lumen of the alveoli. It had a central nucleus with deeply indented nuclear membrane. Its
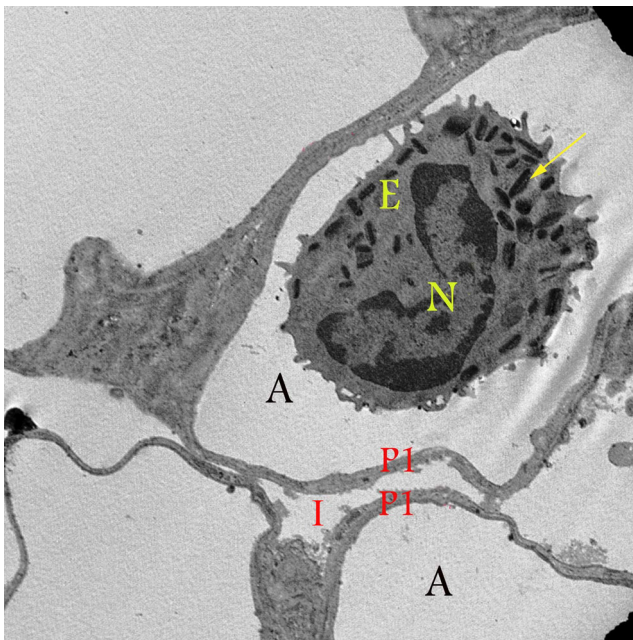

Fig. 5 An electron micrograph of an ultra-thin section from the lung of CdCl$_2$-treated rat showing infiltration of an alveolus (*A*) by an eosinophilic granulocyte (*E*). The latter has characteristic discus-shaped, electron-dense granules (*arrow*) and bi-lobed nucleus (*N*). There is also increase in the space (*I*) between two opposing type I pneumocytes (*P1*) (EM ×2000)
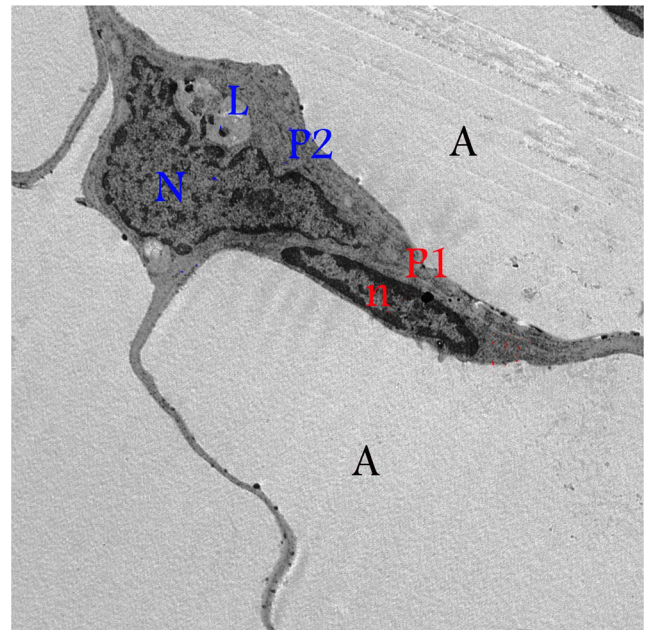

Fig. 6 An electron micrograph of an ultra-thin section from the lung of a rat treated with CdCl$_2$ and NSO showing normal alveolus (*A*) with its lining epithelium. Type I pneumocyte (*P1*) appears squamous with elongated dense nucleus (*n*) and attenuated cytoplasm. The more cuboidal type II pneumocyte (*P2*) has oval euchromatic nucleus (*N*) and cytoplasmic lamellar bodies (*L*) (EM ×2000)

surface appeared irregular because of its pseudopodia. The interalveolar septum also appeared normal and was formed of three layers.

Group IV (rats treated with CdCl$_2$ and NSO)

Type I pneumocyte appeared normal with dense, elongated nucleus and attenuated cytoplasm. Similarly, most of the type II pneumocytes appeared normal with cuboidal nuclei and intact lamellar bodies. Alveolar macrophages showed central nuclei, indented nuclear membranes and surface pseudopodia. The three-layered interalveolar septum was thin as compared with the control group (Fig. 7).

Discussion

Humans are susceptible to Cd toxicity inevitably through environmental and/or occupational sources. Even relatively low Cd exposure creates an evident health risk as it has a very long half-life, and it is stored in the human body mainly liver and kidneys (Kukongviriyapan et al. 2014; Rogalska et al. 2011).

The present study revealed structural changes in the lungs of cadmium-intoxicated rats, including wide areas of lung collapse, widespread pulmonary hemorrhage, markedly congested capillaries, and inflammatory cellular infiltration of the alveoli. Electron microscopy revealed many type II pneumocytes with small, pyknotic, irregular nuclei and
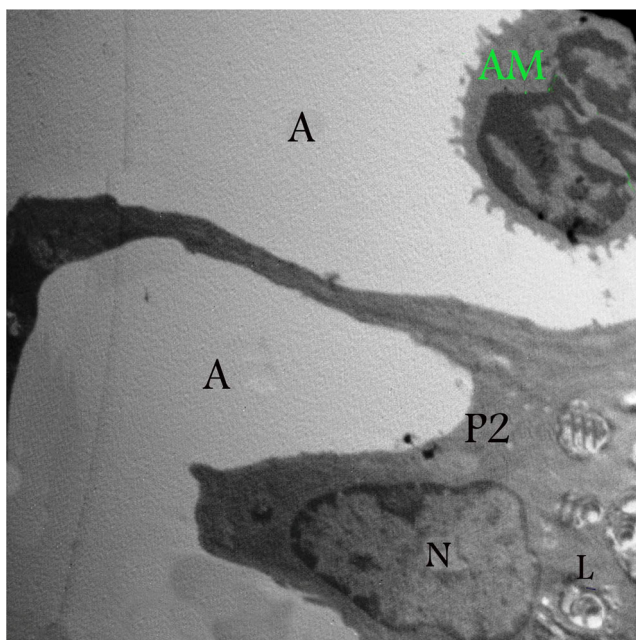
Fig. 7 An electron micrograph of an ultra-thin section from the lung of a rat treated with CdCl$_2$ and NSO showing one normal alveolar macrophage (*AM*) and type II pneumocyte (*P2*) with its oval nucleus (*N*) and lamellar bodies (*L*) (EM ×2000)

condensed chromatin near the nuclear membrane. Some of the type I pneumocytes showed irregular nuclear membrane, and some alveolar macrophages showed small, dark, pyknotic nuclei. There was thickening of the interalveolar septum with collagen precipitation and widening of the interstitial space most probably due to interstitial edema. These findings indicate a significant damaging effect of Cd on lung parenchyma. In this study, using systemic Cd-toxicity rather than the pulmonary routes of exposure showed lung injuries similar to that attained by intratracheal, inhalational, and intrathoracic Cd administration. This may be explained by the extensive blood supply of the lungs. In the blood, Cd is stored in RBCs and in combination with plasma proteins (Oh et al. 2014).

The diffuse areas of collapse seen may be due to damage of the surfactant-containing lamellar bodies. In addition, the mediators that may be secreted by the infiltrating inflammatory cells might cause bronchoconstriction and augment lung collapse (Lag et al. 2010; Ovalle et al. 2008). Interstitial edema, collagen precipitation, and thickening of the alveolar wall may result also from inflammatory mediators. Cadmium could produce pulmonary fibrosis through stimulation of abnormal proliferation of fibroblasts and excessive deposition of extracellular matrix components (Damiano et al. 1990; Kumar and Lykke 1995).

The molecular mechanisms underlying cadmium-induced lung injury are not fully understood, and several theories had been proposed. The presence of inflammatory cellular infiltrates in the lungs suggests inflammation as a possible mechanism for Cd-induced lung injury. Cadmium is a known activator of the transcription factors involved in the synthesis of various inflammatory mediators. Cadmium causes up-regulation of the mediators and markers of inflammation, such as nuclear factor kappa-B (NF-κB), interleukin (IL)-6, tumor necrosis factor-alpha (TNF-α), prostaglandin E$_2$, and C-reactive protein (CRP) and seems to have pro-inflammatory properties. Several research studies highlighted the role of immune-mediated inflammatory process in Cd-induced lung injury (Colacino et al. 2014; Lag et al. 2010; Olszowski et al. 2012).

Population studies have shown the association between Cd exposure and high levels of systemic inflammation and OS markers such as CRP and gamma glutamyltransferase (Lee et al. 2006; Lin et al. 2009). Al-Johany and Haffor (2009) reported a significant increase in free radical production in the lungs of Cd-exposed animals. Tissues exposed to OS undergo extensive cellular damage due to oxy-radical-induced DNA-breakage, lipid peroxidation, and extensive protein damage (Metodiewa and Koska 2000). Cadmium replaces iron in various proteins and thus increases the concentration of free iron. The latter is a redox-active element that directly enhances the production of ROS. In addition, Cd inhibits several antioxidant enzymes and shows a high affinity for thiol-containing compounds, including glutathione, causing depletion of this important cellular antioxidant (Cuypers et al. 2010; Djukic-Cosic et al. 2008).

The present study showed no significant structural changes in the lungs of NSO-treated animals. The safety of NSO was reported in several studies, which declared a wide safety margin for NSO (Ahmad et al. 2013; Salem 2005). Concomitant administration of Cd and NSO showed significant improvement in Cd-induced lung injury, where most of the alveoli, alveolar epithelium, interalveolar septum, capillaries, and alveolar-capillary membrane appeared normal except for limited areas of collapse and alveolar hemorrhage. This protective effect of NSO may be attributed to the antioxidant as well as the anti-inflammatory properties of this herbal plant. Cadmium-induced tissue damage is attributed, at least in part, to the cadmium-induced inflammation and/or induction of the OS. Hence, many authors have postulated that the use of antioxidants or anti-inflammatory agents should be an effective protective approach in Cd toxicity. Fometerol, a beta 2 agonist with bronchodilator and anti-inflammatory properties, was found to protect against Cd-induced lung damage in rats (Zhang et al. 2010). Furthermore, selenium and zinc, which are essential elements for some antioxidant enzymes, were found to ameliorate Cd-induced lipid peroxidation in experimental animals (Jihen El et al. 2008).

The anti-inflammatory and antioxidant properties of NS have been reported in several in vitro, in vivo, and clinical studies. Both NS crude oil and pure thymoquinone reduced eicosanoid generation in rat peritoneal leukocytes due to inhibition of different pathways of arachidonate metabolism. Both

substances, particularly thymoquinone, also inhibited non-enzymatic peroxidation in ox brain phospholipid liposomes (Houghton et al. 1995). In addition, thymoquinone supplementation in KK1 cells suppressed inflammatory pathways and ROS induction (Arif et al. 2016). In animal studies, the anti-inflammatory effect of the NS aqueous extract and NS fixed oil were comparable to that of 100 mg/kg aspirin (Al-Ghamdi 2001) and dexamethasone (Abdel-Aziz et al. 2014). The therapeutic significance of NS in treating hand eczema was investigated in a clinical trial with an effect comparable to that of betamethasone (Yousefi et al. 2013). More recently, NS was anticipated as a beneficial adjuvant therapy that could improve inflammation and reduce OS in patients with rheumatoid arthritis (Hadi et al. 2016).

The cytoprotective effects of NS and its major active ingredient, thymoquinone, are mainly mediated through anti-inflammatory and antioxidant activities. The potent anti-inflammatory effect could be attributed to suppression of mRNA expression of multiple proinflammatory mediators such as TNF-α, IL-1β, IL-8, and monocyte chemoattractant protein-1. Thymoquinone has been proved to modulate a number of factors that are critically involved in the NF-κB and p38 mitogen-activated protein kinase signaling pathways. It has been reported to block TNF-α-induced activation of NF-κB and to suppress the translocation of NF-κB to the nucleus (Chehl et al. 2009; Vaillancourt et al. 2011). The antioxidant activity could be related to ability to increase blood and tissue glutathione, to reduce ROS and lipid-peroxides production, and to improve the activity of several antioxidant enzymes (Rastogi et al. 2010; Sankaranarayanan and Pari 2011; Sultan et al. 2015). Furthermore, NS may have a potential role in enhancing Cd elimination given that it has been reported to reduce Cd blood levels to below the detection limit in Cd-intoxicated mice (Massadeh et al. 2007).

Herbs have attracted a great deal of recent attention as a potential therapy of metal-induced toxicity because of their safety and low costs. To the best of our knowledge, NSO has not been investigated as a protective therapy in Cd-induced pulmonary toxicity. Testing this hypothesis has shown that treatment of Cd-intoxicated rats with NSO alleviated most pathological structural changes of the lung parenchyma. The potent cytoprotective effects shown in the current study may be attributed to the anti-inflammatory and antioxidant activities of NSO. Both the nourishing and medicinal benefits could make this herbal remedy a promising therapeutic option. However, further experimental and clinical studies are necessary to elucidate the exact mechanisms of its favorable effects and to emphasize its clinical application.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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