Procyanidinere therapy of pulmonary fibrosis induced by CdSe nanorods with pulmonary instillation

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Abstract
Background: The CdSe nanorod as a one-dimensional nanostructure has an excellent performance in photoelectric conversion, biological labeling and environmental protection. Thus, it is crucial to investigate its potential adverse health effects at an early stage.

Methods: Sprague-Dawley (SD) male rats were exposed to 15 mg/kg CdSe and 200 mg/kg procyanidine (OPC) for 30, 60 and 90 days. Lung tissues were collected on days 30, 60 and 90. Oxidation damages, histopathological analysis, transmission electron microscopy and hydroxyproline level were measured.

Results: The lung tissue would be the main target organ after CdSe nanorods entering into biological bodies. Pulmonary instillation of CdSe nanorods could decrease vitality of T-SOD and T-AOC in lung tissues of a rat, increase MDA and hydroxyproline levels and lipid peroxidation products, induce mitochondrial cristae broken and vacuolization, cause inflammatory responses, and finally induce pulmonary fibrosis. The oral administration of procyanidinere could significantly increase the content of antioxidant enzymes, scavenge free radicals, reduce the lipid peroxidation and have protective effects on CdSe nanorods-induced pulmonary fibrosis.

Conclusions: CdSe nanorods could induce an extensive inflammatory response, elevate ROS, induce pulmonary fibrosis and reveal time accumulation appearance. OPC has a protective effect on lung injury induced by CdSe nanorods, which might be related to anti-oxidative and anti-inflammatory properties.

Background
Due to their small scale of compositive units, considerable specific surface area and high surface reactivity [1], nanomaterials exhibit unique physicochemical, mechanical, electrical, and thermal properties that can be applied in environmental protection, electronic information and energy storage [2, 3]. Compared with their bulk counterparts, nanomaterials generally enter into living organisms more easily, in particular, lung tissues and more toxic [4–7]. They might enter into cells through free penetration or receptor-mediated endocytosis, and actively interact with cellular components, such as lipids, proteins and genomic DNA [8–12]. For example, a number of toxicological studies using rats
have shown that exposure to nanomaterials such as carbon nanotubes, nickel, and TiO$_2$ nanoparticles induced greater lung inflammatory potency and cytotoxic effects than larger-size particles at equivalent mass concentrations [6, 7, 12-14].

One-dimensional (1D) nanostructures have attracted much attention in the past decade owing to their unique optical and electrical properties, and they are good candidates as the building blocks of functional nanodevices such as field-effect transistors [15-17], photodetectors [18], light-emitting diodes [19], and photovoltaic devices [20]. CdSe nanoparticles have been widely applied in photoelectric conversion, biomedical imaging and drug delivery [21, 22]. CdSe nanorods as one-dimensional nanostructures have the excellent performance in photoelectric conversion as they could provide natural channel for directional electron transport, and the rods’ structure made the transfer resistance smaller and the transmission speed faster [23]. Just as many other nanomaterials [24], CdSe nanoparticles have been demonstrated to be toxic to mammalian cells and tissues [25-27].

To date, most nanomaterial toxicity studies have focused on quantum dots. There are many reports showing that in vitro quantum dots might lead to reactive oxygen species (ROS) accumulation [28-30], inactivation of protein functions [31], and cause pulmonary fibrosis in lung tissues [32]. However, only a few studies have addressed the toxicity of rod-like nanoparticles [33-35]. Although there have been reports of cytotoxicity induced by some metallic nanoparticles [9], there is no comprehensive study of the toxicity of CdSe nanorods to lung tissues and lung cells. Accompany with the application of CdSe nanorods, the lung tissue would be the main target organ after CdSe nanorods entering into biological bodies. Through respiratory tract route, CdSe nanorods would deposit in the bronchial epithelium, pulmonary interstitial and alveolar walls. For a foreign matter to lung tissues, the defense and removal function of macrophages could be activated, but it is difficult for macrophages to identify such small granule. Their function is weaken [36], So CdSe nanorods might be up-taken by cells, penetrate across the barrier into circulation, and migrate to the liver and other organs [37-39].

For many years, although many efforts have been made in fighting pulmonary fibrosis [40, 41], and various drugs, such as anti-inflammatory [42], immunosuppressive [43] and anti-fibrotic agents [44]
have been clinically used for the treatment of pulmonary fibrosis, there is only very limited success case. As such, it is desirable to search new therapeutic strategies.

Procyanidine (OPC) has a special molecular structure of biological flavonoids derived from grape seeds, and it has been internationally recognized as the most effective natural antioxidant which can clean up free radicals in biological bodies [45]. Thus, it had been reported to exert antibacterial, antiviral, anticarcinogenic, antimutagenic, anti-inflammatory, antiallergic, and vasodilatory actions [45–47]. Most physiological benefits have been attributed to its antioxidant and free radical scavenging properties [45, 48]. OPC exhibited dramatic scavenging ability towards biochemically generated superoxide anion, hydroxyl and peroxyl radicals [49–51]. Furthermore, OPC has been shown to modulate expression of apoptotic related genes, reduce generation of free radicals and increase activity of antioxidant enzymes in various types of animal tissues and cells, implying that it is a promising cytoprotective agent against a range of exogenous toxic stimuli [52].

In this work, we investigated the potential effect of synthesized CdSe nanorods on lung tissues of SD rat and A549 cells for the first time, examined the effects of oxidative stress, and explored possible toxicity mechanisms of CdSe nanorods in the lung tissue of rats. Considering the antioxidant property of OPC, we explored its use in vivo and in vitro against adverse effects caused by CdSe nanorods.

Materials And Methods
Reagents
All chemical reagents were purchased from Beijing Chemical Reagent Ltd., China, and used without further purification. OPC purchased from Beijing Chemical is a standardized water–ethanol extract from grape seeds. The extract was supplied in the form of standardized 95%. Total antioxidant capacity (T-AOC), total superoxide dismutase (T-SOD) and malondialdehyde (MDA) assay kits were purchased from the Nanjing Jiancheng Bioengineering Institute, China.

Preparation and characterization of CdSe nanoparticles
For the synthesis of CdSe nanorods, 0.266 g Cd(CH$_3$COO)$_2$·2H$_2$O and 0.345 g sodium selenite were added into 50 mL capacity Teflon-lined stainless autoclave, followed by the addition of 20 mL distilled water and 20 mL ethanediamine. The mixture was stirred using magnetic stirrer to a homogeneous system. The autoclave was placed in an oven at 150°C for 12 h. After completion of duel time,
autoclave is allowed to cool to room temperature. The black product was collected and washed with distilled water and ethanol for 6 times respectively, and dried at room temperature for further use. The general morphology of the products was characterized by transmission electron microscopy. The crystal structure and composition of the sample was characterized by powder X-ray diffraction.

Animal administration and sampling
The main purpose was to estimate the toxicity to respiratory systems with CdSe nanorods and not concerned about gender differences. Hence, adult specific-pathogen-free (SPF) Sprague-Dawley (SD) male rats were employed in this study, SD rats (8-9 weeks old at the start of the study, 200-250 g/rat, the Center for Experimental Animals of Hebei United University) were used. For housing of animals, plastic cages filled with hardwood bedding were placed within an air-conditioned (23 ± 2°C) animal room and with relative humidity ranging from 30 to 70%. A 12 h light/dark cycle was maintained throughout the study, except during the exposure, with free access to standard laboratory rats’ diet and tap water. After acclimation for 1 week, 72 male SD rats were randomly divided by weight into 9 groups (control groups, CdSe groups and OPC groups administrate for 30, 60 and 90 days, respectively) with eight rats per group. Control groups received pulmonary instillation of saline based on body weight for three groups for evaluation on days 30, 60 and 90. CdSe groups received pulmonary instillation of 15 mg/kg CdSe nanorods dispersed with saline water per week. OPC groups with 24 rats, a dose of 200 mg/kg OPC was gavaged though the whole experiment process, and meanwhile received pulmonary instillation of 15 mg/kg CdSe nanorods dispersed with saline water per week, respectively, rats of corresponding group were sacrificed on days 30, 60 and 90, the lung samples were collected and stored at -80°C.

Cell culture
The human lung carcinoma A549 cell line was obtained from the School of Public Health, Hebei United university. A549 cells were cultured in RPMI-1640 medium containing 100 µg/ml of penicillin, 100 µg/ml of streptomycin, and 10% heat-inactivated fetal bovine serum (FBS) at 37°C in a humidified atmosphere of 5% CO₂. Upon reaching 80-90% confluence, the cells were trypsinized, harvested, and seeded into a new cell culture dish. The control group was treated with vehicle and RPMI-1640
medium, the CdSe group was treated with CdSe nanorods (at a final concentration is 20 µg/ml), vehicle and RPMI-1640 medium, while the OPC group was treated with CdSe nanorods and OPC (at a final concentration is 20 and 40 µg/ml, respectively), vehicle and RPMI-1640 medium, 3 groups above were treated for 24 h. A549 cells were used to determine the oxidative damage effects of CdSe nanorods and the protective effect of OPC.

Determination of oxidation damages in lung tissues and A549 cells
About 1.0 g of frozen lung tissues in 9 mL of homogenization buffers (0.9% sodium chloride) were homogenized on ice by a homogenizer (VCX130, Sonics & Materials, Inc), working for 5 seconds each time, pause for 10 seconds, repeatedly for 4 times. The homogenate was centrifuged at 4,000 rpm for 15 min at 4 °C and the supernatant was used for analysis. The T-SOD, T-AOC and MDA dynamics were measured using T-SOD, T-AOC and MDA assay kits for A549 cells and homogenate of lung tissues according to the instruction, respectively.

Histopathological analysis of Lung tissues
The 8 rats per group were sacrificed under sodium pentobarbital anesthesia on days 30, 60 and 90, respectively. Selected lung tissues were fixed in paraformaldehyde and embedded in paraffin and then cut into 4 µm slices which mounted on glass microscope slides. For the hematoxylin and eosin (H&E) staining, thin-sections were stained with haematoxylin-eosin. For the Masson’s trichrome staining, strict accordance with the instructions of the Masson staining kit, sealed and finally examined by a light microscopy.

Transmission electron microscopy
Animals designated for transmission electron microscopic examination (TEM) were sacrificed, opening the thorax of animals followed by perfusion of 5% buffered glutardialdehyde (GAH) as fixation solution. The tissue samples of the lungs were refixed with 2% buffered osmium tetraoxide aqueous solution. The fixed tissue embedded in epoxy resin, from appropriate locations ultrathin sections (50 nm) were obtained and examined by TEM.

Determination of hydroxyproline level in lung tissues
On days 30, 60 and 90 after CdSe and OPC administration, tissue samples were weighed and cut into pieces, and 1 ml HCl (6 M) was added into the grinding test tube, which was capped and hydrolyzed in
boiling water for 5 h. The pH of the solution was adjusted between 6.0 and 6.8. A total of 1 ml diluted solution supernatant was obtained for determination. This experiment included the following three groups: blank, standard, and detected sample tubes. Distilled water (1 ml) was added to the supernatant, as well as 5 µg/ml standard solution and analysis solution. The supernatant was analyzed (acid hydrolysis, Nanjing Jiancheng Co., Ltd., China) at 550 nm with a spectrophotometer. The blank tube solution was used as zero control [53].

Statistical evaluation
Data are expressed as mean ± SD (for histology). For in vitro methods, 6 independent experiments were performed, unless specified otherwise. For in vivo techniques, 4 (histopathology and transmission electron microscopy) rats per treatment group are used. Data were analyzed using SPSS version 15 for windows. Treatment-related differences were evaluated by one way analysis of variance (ANOVA) or the nonparametric Mann-Whitney U-test (Bio-plex assay and histopathological scoring). Multiple comparisons were assessed by the Anova post hoc analysis according to the Tukey’s method or the LSD method.

Results
Effects of CdSe nanorods in lung tissues after pulmonary instillation
TEM and XRD images of the as-prepared CdSe nanorods are shown in Fig. 1. Black-colored CdS nanorods (NRs) have diameters ranging from 40 to 60 nm with lengths of 150 to 300 nm (Fig. 1a). The powder XRD pattern is shown in Fig. 1b correspond to the hexagonal phase of CdS (a = 4.299 Å, c = 7.01 Å, JCPDS: 08-0459). The histological changes of SD rat lung tissue sections with Hematoxylin–Eosin (HE) stained were observed under light microscopy [54]. The representative images are presented in Fig. 1. The HE staining revealed that the lung structures of control groups those administrated with the physiological saline were found to be the normal morphology, and alveolar walls composed with single epithelial cells. The normal alveolar cells with the equilibrium size are seen in the alveolar corner (Fig. 1a). There were also slight interstitial inflammation found after pulmonary instillation of physiological saline for 60 and 90 days (Fig. 1b, c), appear as the normal phenomenon.

Compared with the control groups, CdSe nanorods treated groups produced significant adverse
Effects. Numerous black spots were observed under light microscopy. These black spots are corresponding to the CdSe nanorods-positive sites (right arrows of Fig. 1d, e, f). The instillation of CdSe nanorods induced an extensive inflammatory response including alveolar walls widening, alveolar mucosa with swelling, and congestion (down arrows of Fig. 1d). As a result, development of lung fibrosis, inflammatory cell infiltration, and injury were observed in CdSe nanorods treated SD rats on day 30 (Fig. 1d). Followed by the pulmonary instillation of CdSe nanorods on days 60 and 90, black spots, corresponding to the CdSe nanorods-positive sites were frequently observed under microscope. Macrophages were also frequently observed in some of the alveolar walls. Inside the alveolar cavities, the alveolar wall thickness with typical fibrotic changes with time (down arrows of Fig. 1e, f).

Importantly, part of the lung tissue degeneration changed. The typical nodular histiocyte hyperplasia (up arrows of Fig. 1e, f) formed with CdSe nanorods are fibroblast cells, collagen and macrophages [55], providing an encapsulation of the fibrous materials, also known for other particulate/fibrous materials [33] (quartz, asbestos), were obviously found on days 60 and 90 when compared with healthy lung tissues (control groups).

**Oxidative stress and pulmonary fibrosis level in lung tissues**

Nanoparticles mediated tissue damages and cell death is attributed to the production of ROS leading oxidative stress caused by nanoparticles small size and large surface area to volume ratio [38, 56]. T-SOD is an important superoxide dismutase in biological bodies and widely distributed in all kinds of organisms [57]. It also convert harmful superoxide free radicals to hydrogen peroxide in cells. In presence of catalase and peroxidase, hydrogen peroxide broke down to harmless water molecule. Hence, protect and/or recover cells from free radical damages [58, 59]. T-AOC not only accurately reflects the oxidation state, but also evaluate the activity of oxygen free radicals indirectly [60]. The increase of free radicals could reduce the body T-AOC vigor. MDA is an end-product formed from peroxide decomposition of unsaturated fatty acid. The content of MDA directly reflects the strength and speed of body lipid peroxidation. The MDA indirectly illustrate the severity of damaged tissues or cells. MDA is often considered as an important indicator to estimate the damage induced by oxidative stress [25, 61, 62].
To evaluate oxidative stress caused by pulmonary instillation of CdSe nanorods, the activity of T-SOD and T-AOC, and MDA content levels in lung tissues of rats were measured. Results were shown in Fig. 2. Compared with the control groups, the activity of T-SOD and T-AOC in lung tissues of rats treated with CdSe was gradually lower with continually instillation than those of control groups, while the MDA levels were significantly higher compared to control groups. CdSe nanorods stimulated the lung tissue to produce abundant of superoxide anion radical, and enhance the peroxide reaction. In order to determine the degree of CdSe nanorods-induced pulmonary fibrosis, the Masson’s trichrome stain of lung tissue slices were evaluated. The representative results are shown in Fig. 3. The normal lung cells in the control groups were dyed red color, while filament collagen fiber dyed blue color which is mingled in the normal lung cells, belong to the normal phenomenon. Pulmonary fibrosis increased significantly in SD rats pulmonary after instillation of CdSe nanorods on days 30 and became more serious on days 60 and 90, along with collagen deposition, also increased with the progression of instillation. In addition, the CdSe nanorods treatment significantly increased the hydroxyproline content of the lung compared with the control groups (Fig. 3g). The hydroxyproline level is an indicator to measure collagen deposition offibrosis [63]. We found that the hydroxyproline level significantly increased on days 30, and slightly increased on days 60 and 90 in case of CdSe nanorods-treated lungs of rats compared with the control groups, which is according to the result obtained from the Masson’s trichrome stain. These results demonstrated that pulmonary instillation of CdSe nanorods could induce pulmonary fibrosis.

OPC therapy

Lung fibrosis caused by pulmonary instillation of CdSe nanorods, and OPC treatment in vivo significantly mitigated the extent of inflammation (Fig. 4). The histological morphological determination demonstrated that gavages with OPC for 30, 60 and 90 days along with alveolar walls widening, alveolar mucosa with swelling, inflammatory cell infiltration, and macrophages were seen in some of the alveolar walls compared with the control groups. However, OPC treatment groups could significantly prevented the CdSe nanorods-induced inflammation and fibrosis [53], when compared with the CdSe nanorods groups for 30, 60 and 90 days, respectively. Lung tissues had less nodular
histiocyte hyperplasia, lung fibrosis is significantly mitigated, the number of fibrocytes was also significantly decreased, the thickness of alveolar walls was thicker compared with the control groups and thinner compared with CdSe nanorods groups.

Similar to other toxicity indicators discussed about lung tissues, T-SOD, T-AOC and MDA levels indicated a greater effect for the rats gavaged with the OPC solution (Fig. S2). The activity of T-SOD and T-AOC had slightly decrease and the MDA levels slightly increased when compared with the control groups after having gavaged with the OPC solution. However, the activity of T-SOD and T-AOC in the OPC groups were significantly higher, and the MDA level was significantly lower, when compared with that in the CdSe groups. The findings indicated that those gavaged with the OPC solution could prevent cytotoxicity mediated by free radical and lipid peroxidation, and protect low density lipoproteins from oxidation, while CdSe nanorods stimulated lung tissues to produce abundant of superoxide anion radical.

The Masson staining (Fig. 5a, b, and c) showed that the collagen deposition in the OPC group also gradually increased with the progression of pulmonary fibrosis when compared with the control groups, but the collagen level decreased sharply in the OPC intervention groups when compared with CdSe groups, which is further supported by the hydroxyproline level and the collagen level analysis in lungs (Fig. 5d). Hydroxyproline levels decreased in the OPC-administrated lung tissues compared with those in the CdSe nanorods-treated lungs on days 30, 60 and 90, respectively. The hydroxyproline level of OPC-administrated lung tissues on days 90 (5.95 ± 0.43 µg/mg pro) is equivalent with the level of CdSe nanorods-treated lung tissues on days 30 (5.92 ± 0.41 µg/mg pro). The protective effect of oral OPC is obvious at the preliminary stage of pulmonary fibrosis induced by CdSe nanorods, and the observed effect is persistent with the progress of pulmonary fibrosis until 90 days.

Ultrastructure of alveolar macrophage and oxidative stress of A549 cells
As the TEM images of ultrathin sections of alveolar macrophage in lung tissues, we observed that the control group had normal mitochondria in alveolar macrophage. Numerous phagolysosomes in the CdSe group, mitochondrial cristae were broken, swelling (up arrows in Fig. 6b), vacuolization (right arrows in Fig. 6b), and aggregated CdSe nanorods mainly located within secondary lysosome (down
arrows in Fig. 6b) of alveolar macrophages. In the OPC group, part of mitochondria had normal (left arrow in Fig. 6c) could observed, while less mitochondria became swelling and vacuolization (up arrows in Fig. 6c) compared with the CdSe group were observed.

Undoubtedly, OPC has a protective effect on lung injury induced by CdSe nanorods, in particular, has obvious properties of antioxidation and free radical scavenging. Oral administration of OPC could significantly prevent pulmonary fibrosis induced by CdSe nanorods in lung tissues. However, whether CdSe nanorods could change the permeability of cells under the protection of OPC is not clear. Thus, we employed the A549 cells for in vitro study. The oxidative stress assessment of A549 cells shown that the activity of T-SOD and T-AOC in A549 cells of the CdSe group was obviously lower than that of the control group, while the MDA levels were significantly higher than those in the control group.

The OPC group had a dramatic difference compare to the CdSe group, the activity of T-SOD and T-AOC also slightly decreased and the MDA levels increased when compared with the control group (Fig. S3). Contracted with lung tissues, the activity of T-SOD and T-AOC and the MDA levels of A549 cells in the OPC group just had a marginal difference with the control group, due to the elimination of free radicals by OPC, decrease in the excess ROS production, and contraction with in vivo pathway. OPC is likely to play more effective role in vitro experiment, just for only part of OPC could absorb within SD rats through gavage.

The electron microscopic images in the transmission mode showed the appearance of lipid containing vesicles, nanoparticles internalized in the fat droplet. The three groups did not observe obvious organelle lesions. The CdSe nanords mainly aggregated in the fat droplet. The control group had transparent and clear fat droplets (Fig. 7a), while CdSe and OPC groups had semitransparent fat droplets. CdSe nanords mainly aggregated at fat droplets (Fig. 7b, c), and two groups had not obvious difference, thus suggesting that OPC could eliminate free radicals, decrease the excess ROS production, but have no effect with permeability of cell and organelle.

Discussion
Biological effect and safety of nanoparticles have attracted widespread attention with the continuous development of nanoparticle technology in recent years, when considering the novel type property of
CdSe nanorods and their foreseen widespread application, led us to investigate its potential adverse health effects at an early stage [33]. Various metal selenide nanoparticles have shown to induce tissue damage and cell death after internalization. Some researches considered that metal selenide nanoparticles could dissolution in culture medium which causes release of ions and induced cell death. The present study shows that CdSe nanorods are capable to produce ROS in lung tissues after pulmonary instillation for 30 days, and the continuous accumulation reveals a time dependent manner up to 60 and 90 days, CdSe nanorods decreased vitality of T-SOD and T-AOC in lung tissues of a rat, and increased the MDA level, and lipid peroxidation products, indicating that the CdSe nanorods induced oxidative damage in lung tissues of a rat.

To observe the evidence of pathological damaging impact on lung tissues of a rat by CdSe nanorods, the HE staining was employed to observe changes in lung tissue structures of a rat. The space between pulmonary alveoli increased and infiltration of inflammatory cells such as macrophage was more obvious in the CdSe nanorods groups, including extensive inflammatory responses such as alveolar walls widening, alveolar mucosa with swelling, congestion as a prelude to the development of lung fibrosis, and lung tissue fiber deposition. Particularly in late stage for 60 and 90 days, the damage was more serious. However, there was no obvious tissue inflammation and interstitial fiber deposition in the control groups.

The Masson’s trichrome staining and hydroxyproline levels were evaluated. Collagen deposition indicates pulmonary fibrosis and the hydroxyproline levels significantly increased in the SD rats which intermit tented CdSe nanorods on days 30 and became more serious on days 60 and 90, the results demonstrated that pulmonary instillation of CdSe nanorods could induce pulmonary fibrosis in short time. Formation of pulmonary fibrosis is mainly attributed to increased fibrosis factors [64, 65] and anti-fibrosis factor deficiency that cause abnormality of extracellular matrix during pulmonary fibrosis process [66, 67]. Shvedova et al. (2009) [68] demonstrated that carbon nanotubes could induce pulmonary fibrosis by oxidation damage. Van Berlo et al. (2014) [33] demonstrated that MWCNT induce inflammatory cell influx, a markedly increased granuloma formation and induce clear fibrotic responses. These lesions are characterized by the concentration of one-dimensional nanostructures,
higher cellular density, accumulation of immune cells, especially macrophages, the nodular histiocyte hyperplasia and fibroblastic proliferation increased.

The toxicity of CdSe nanoparticles has been demonstrated to be associated with ROS generation, mitochondrial dysfunction, and autophagy-related cell death [69, 70]. The pathogenesis of CdSe nanorods-induced pulmonary fibrosis is probably related to combination effects of types of cells including pneumocytes, inflammatory cells, endothelial cells, fibroblasts, and mitochondrial disruption [71]. In the present study, the electron microscopic images in the transmission mode could show that pulmonary instillation of CdSe nanorods induced mitochondrial cristae broken, and vacuolization. OPC has been reported to possess a variety of potent properties including antioxidant, anti-inflammation, radical scavenging, renal protecting, and antitumor activities [72–75]. Many physiological benefits of OPC have been attributed to its antioxidant as well as free radical scavenging properties. It is also found that OPC could inhibit lipid peroxidation and modulate the activity of regulatory enzymes, and has dramatic scavenging ability towards biochemically generated superoxide anion, hydroxyl and peroxyl radicals. The oral administration of OPC could significantly prevent those negative changes induced by CdSe nanorods in lung tissues and had a protective effects on CdSe nanorods-induced pulmonary fibrosis. Through the assessment of oxidative stress and malonaldehyde in lung tissues, both in vivo and in vitro experiments, the OPC solution could significantly increase the content of antioxidant enzymes, scavenged free radicals, and reduced the lipid peroxidation. As for the histopathological change of lung tissues, OPC significantly improved inflammatory changes of lung tissues, including slowing down the alveolar walls swelling and alveolar mucosa congestion, improving scores of lung alveolitis and fibrosis, and lowering the hydroxyproline content that directly correlated with the collagen deposition. Above results suggested that oral administration of OPC could benefit not only the early inflammatory stage, but also the later stages of the CdSe nanorods-induced pulmonary fibrosis. Once safety accidents caused by CdSe nanorods or other one-dimensional nanomaterials inhalation happened, oral OPC could be an effective therapy to treat pulmonary fibrosis.

Conclusions
In conclusion, the present study indicates that one-dimensional CdSe nanorods could induce an extensive inflammatory response, elevate ROS, induce pulmonary fibrosis and reveal time accumulation appearance. OPC has a protective effect on lung injury induced by CdSe nanorods, which might be related to anti-oxidative and anti-inflammatory properties. OPC appears to be an effective therapy against pulmonary fibrosis, although the mechanisms remain need to be further explored.

Abbreviations
T-SOD: total superoxide dismutase; T-AOC: total antioxidant capacity; MDA: malondialdehyde; ROS: reactive oxygen species; OPC: procyanidine; SPF: specific-pathogen-free; SD: Sprague-Dawley; FBS: fetal bovine serum; HE: Hematoxylin–Eosin; TEM: transmission electron microscopic; GAH: glutardialdehyde; XRD: X-ray diffraction; MWCNT: multi-walled carbon nanotube.

Declarations

Ethical Approval and Consent to participate
The study did not include human data. The animal study protocol was approved by the Ethics Committee of Nankai University and was in accordance with the guidelines for the Care and Use of Laboratory Animals in Nankai University.

Consent for publication
Not applicable.

Availability of supporting data
Fig. S1-S3 are available online with the main text.

Competing interests
The authors declare that they have no competing interests.

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Authors’ contributions
QZ designed experiments and wrote the manuscript; ZY and QL performed experiments; RZ analyzed
data; LL designed experiments.

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Figures
The alteration of lung tissue pathological morphology of SD rats induced by CdSe nanorods. Control groups that pulmonary instillation with saline for 30 days (a), 60 days (b) and 90 days (c). Pulmonary instillation CdSe nanorods for 30 days (d), 60 days (e) and 90 days (f). Blackspots were CdSe nanorods-positive sites (right arrows), the lung tissues from CdSe nanorods-exposed rats showed alveolar mucosa with swelling (down arrows), nodular histiocyte hyperplasia (up arrows) and injury.
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Figure 2

The bar graph shows SOD and T-AOC activity (a, b) and MDA content (c) in rats’ lung tissue measured using microplate reader represented as mean ± SD of control groups and instillation CdSe nanorods for 30, 60 and 90 days (*p<0.05, as compared with normal control groups).
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Control groups that pulmonary instillation with saline for 30 days (a), 60 days (b) and 90 days (c). Pulmonary instillation CdSe nanorods for 30 days (d), 60 days (e) and 90 days (f). Blackspots were CdSe nanorods-positive sites (right arrows), the lung tissues from CdSe nanorods-exposed rats showed alveolar mucosa with swelling (down arrows), nodular histiocyte hyperplasia (up arrows) and injury.
Masson trichrome staining to assess the pulmonary fibrosis. The collagen deposition increased significantly in lung interstitium at 30(d), 60(e) and 90(f) days in CdSe nanorods-induced lung tissues compared with that in control groups(a,b and c). Detection of hydroxyproline content in lung tissue(g), similar to the changes in collagen level, hydroxyproline level was also enhanced in CdSe nanorods-induced lungs on days 30, 60 and 90. Data are shown as mean ± SD (*p<0.05, as compared with normal control groups).
The bar graph shows SOD and T-AOC activity (a, b) and MDA content (c) in rats’ lung tissue measured using microplate reader represented as mean ± SD of control groups and instillation CdSe nanorods for 30, 60 and 90 days (*p<0.05, as compared with normal control groups).

The alteration of lung tissue pathological morphology of SD rats after the repair strategy with OPC. (a) Oral administration of OPC for 30 days. (b) Oral administration of OPC for 60 days. (c) Oral administration of OPC for 90 days. Black spots are CdSe nanorods-positive sites (right arrows), the lung tissues from OPC groups showed alveolar mucosa with slightly swelling (down arrows), relatively few nodular histiocyte hyperplasia (up arrows).
Figure 4

The bar graph shows SOD and T-AOC activity (a, b) and MDA content (c) in rats’ lung tissue measured using microplate reader represented as mean ± SD of control groups and instillation CdSe nanorods for 30, 60 and 90 days (*p<0.05, as compared with normal control groups).
Masson trichrome stain in assess the pulmonary fibrosis of OPC effect. The collagen level decreased sharply in OPC intervention groups compared with CdSe groups at 30(a), 60(b) and 90(c) days, respectively. OPC decreased the hydroxyproline levels effectively in OPC-treated pulmonary fibrosis lung tissues compared with CdSe groups(d). (*p<0.05, as compared with normal control groups, #P<0.05, as compared with CdSe groups).
Masson trichrome staining to assess the pulmonary fibrosis. The collagen deposition increased significantly in lung interstitium at 30(d), 60(e) and 90(f) days in CdSe nanorods-induced lung tissues compared with that in control groups(a,b and c). Detection of hydroxyproline content in lung tissue(g), similar to the changes in collagen level, hydroxyproline level was also enhanced in CdSe nanorods-induced lungs on days 30, 60 and 90. Data are shown as mean ± SD (*p<0.05, as compared with normal control groups).
Figure 6

TEM images of ultrathin sections made with alveolar macrophage in the lung tissue. (a) Control group with normal mitochondria (left arrow). (b) CdSe group, mitochondrial cristae broken, swelling (up arrows) and vacuolization (right arrows), numerous secondary lysosome containing aggregated CdSe nanorods (down arrows) were observed. (c) OPC group, mitochondria became slightly swelling, less vacuolization (up arrows) were observed, secondary lysosome (down arrows) and normal mitochondria (left arrow) were also observed.
Masson trichrome staining to assess the pulmonary fibrosis. The collagen deposition increased significantly in lung interstitium at 30(d), 60(e) and 90(f) days in CdSe nanorods-induced lung tissues compared with that in control groups(a,b and c). Detection of hydroxyproline content in lung tissue(g), similar to the changes in collagen level, hydroxyproline level was also enhanced in CdSe nanorods-induced lungs on days 30, 60 and 90. Data are shown as mean ± SD (*p<0.05, as compared with normal control groups).
Figure 7

Representative photos of the ultrathin sections made with A549 cells after 24 h treatment. (a) control group. (b) 20 μg/ml of CdSe nanorods. (c) 20 μg/ml of CdSe nanorods and 20 μg/ml of OPC. The illustration in b and c are magnified fat droplet, the arrows in b and c are aggregated CdSe nanorods.

Figure 7

The alteration of lung tissue pathological morphology of SD rats after the repair strategy with OPC. (a) Oral administration of OPC for 30 days. (b) Oral administration of OPC for 60 days. (c) Oral administration of OPC for 90 days. Black spots are CdSe nanorods-positive sites (right arrows), the lung tissues from OPC groups showed alveolar mucosa with slightly swelling (down arrows), relatively few nodular histiocyte hyperplasia (up arrows).
The alteration of lung tissue pathological morphology of SD rats after the repair strategy with OPC. (a) Oral administration of OPC for 30 days. (b) Oral administration of OPC for 60 days. (c) Oral administration of OPC for 90 days. Black spots are CdSe nanorods-positive sites (right arrows), the lung tissues from OPC groups showed alveolar mucosa with slightly swelling (down arrows), relatively few nodular histiocyte hyperplasia (up arrows).
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Figure 12

TEM images of ultrathin sections made with alveolar macrophage in the lung tissue. (a) Control group with normal mitochondria (left arrow). (b) CdSe group, mitochondrial cristae broken, swelling (up arrows) and vacuolization (right arrows), numerous secondary lysosome containing aggregated CdSe nanorods (down arrows) were observed. (c) OPC group, mitochondria became slightly swelling, less vacuolization (up arrows) were observed, secondary lysosome (down arrows) and normal mitochondria (left arrow) were also observed.

Figure 13

Representative photos of the ultrathin sections made with A549 cells after 24 h treatment. (a) control group. (b) 20 μg/ml of CdSe nanorods. (c) 20 μg/ml of CdSe nanorods and 20 μg/ml of OPC. The illustration in b and c are magnified fat droplet, the arrows in b and c are aggregated CdSe nanorods.
Figure 14

Representative photos of the ultrathin sections made with A549 cells after 24 h treatment. (a) control group. (b) 20 μg/ml of CdSe nanorods. (c) 20 μg/ml of CdSe nanorods and 20 μg/ml of OPC. The illustration in b and c are magnified fat droplet, the arrows in b and c are aggregated CdSe nanorods.

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