J Nanostruct 14(1): 65-72, Winter 2024

RESEARCH PAPER

Pulmonary Effects of Silica Nanoparticles in Rats Following Subchronic Inhalation Exposure

Saodat Toshalieva [1] *, Uday Abdul-Reda Hussein [2], Aya Nabee [3], Faraj Mohammed [4], Rand Mohammed [5], Batool Ali Ahmed [6], Murtadha Lafta Shaghnab [7], Mohamad Adnan Alyas [8], A.Khurramov [9], Bobojonov Otabek Khakimboy ugli [10], Burieva Khurshida [11], Nodira Arabova [12], Bekmukhamedova Barno Uktamovna [13]

[1] *Termez State University, Termez, Uzbekistan*
[2] *College of Pharmacy, University of Al-Ameed, Iraq*
[3] *Department of Pharmaceutics, Faculty of Pharmacy, Al-Turath University, Baghdad, Iraq*
[4] *Al-Manara College for Medical Sciences, (Maysan), Iraq*
[5] *Department of Dentistry, Al-Hadi University College, Baghdad, Iraq*
[6] *Department of Medical Engineering, Al-Nisour University College, Baghdad, Iraq*
[7] *Technical Engineering College, National University of Science and Technology, Dhi Qar, Iraq*
[8] *Al-Zahrawi University College, Karbala, Iraq*
[9] *International School of Finance and Technology., Tashkent, Uzbekistan*
[10] *Teacher of the Department of Fruits and Vegetables, Urganch State University, Uzbekistan*
[11] *Karshi State University, Kuchabag, Karshi, Uzbekistan*
[12] *Tashkent State Agrarian University, 100140, Tashkent region, Uzbekistan*
[13] *Gulistan State University, 120100, Gulistan region, Uzbekistan*

ARTICLE INFO

Article History:
Received 26 September 2023
Accepted 24 December 2023
Published 01 January 2024

Keywords:
Inflammation
Histopathology
Lung function
Pulmonary toxicity
Silica nanoparticles
Subchronic inhalation

ABSTRACT

The widespread use of silica nanoparticles (SiNPs) in various applications has raised concerns about their potential health effects, particularly regarding pulmonary toxicity. The purpose of this analysis was to delve into the dose-dependent pulmonary effects of SiNPs following subchronic inhalation exposure in rats. Adult male rats, specifically of the Sprague-Dawley breed, experienced filtered air (control), 1 mg/m³ (low-dose), or 10 mg/m³ (high-dose) of SiNPs (mean diameter 52.3 ± 4.7 nm) for 6 hours/day, 5 days/week, for 13 weeks. Pulmonary function tests were conducted periodically. At study termination, bronchoalveolar lavage fluid (BALF) was analyzed for inflammatory markers, and lung tissues were examined histopathologically. High-dose SiNP exposure led to significant impairment of lung function, characterized by decreased tidal volume (19.2%), increased respiratory rate (15.7%), reduced lung compliance (24.1%), and increased airway resistance (54.5%). Both low and high-dose exposures induced dose-dependent pulmonary inflammation, evidenced by increased total cell counts, neutrophil infiltration, and elevated pro-inflammatory cytokine levels in BALF. Histopathological examination revealed dose-dependent structural changes in lung tissues, encompassing increased alveolar wall thickness, overgrowth of alveolar epithelium, and inflammation in both alveoli and bronchioles. Notably, granuloma formation was observed in 62.5% of rats in the high-dose group. Subchronic inhalation exposure to SiNPs induces dose-dependent pulmonary toxicity in rats, characterized by impaired lung function, persistent inflammation, and structural changes in lung tissues. These findings highlight the possible hazards linked to extended contact with SiNPs and underscore the importance of implementing appropriate safety measures in occupational settings where SiNP exposure may occur.

How to cite this article
Toshalieva S., Abdul-Reda Hussein U., Nabee A et al.. Pulmonary Effects of Silica Nanoparticles in Rats Following Subchronic Inhalation Exposure. J Nanostruct, 2024; 14(1):65-72. DOI: 10.22052/JNS.2024.01.006

* *Corresponding Author Email: saodattoshalieva2004@mail.ru*

INTRODUCTION

The extensive adoption of nanoparticles in industrial and biomedical fields has been driven by nanotechnology's swift progress in recent times. Among these, silica nanoparticles (SiNPs) have attracted significant interest due to their unique physicochemical properties and versatility [1]. SiNPs are utilized in a diverse range of products and processes, including drug delivery systems, biosensors, food additives, and industrial materials such as paints, coatings, and rubber reinforcements [2,3]. However, the increasing prevalence of SiNPs in everyday products and industrial settings has raised concerns about their potential health effects, particularly regarding pulmonary toxicity upon inhalation.

Particles measuring under 100 nm in at least one dimension, known as nanoparticles, possess distinctive characteristics distinguishing them from larger-scale materials. These properties, including high surface area-to-volume ratio, enhanced reactivity, and the ability to penetrate biological barriers, contribute to their technological advantages but also raise questions about their potential toxicological impacts [4]. In the case of SiNPs, their small size and high surface reactivity may lead to unique interactions with biological systems, potentially resulting in adverse health effects that differ from those observed with larger silica particles [5].

The respiratory system serves as a primary route of exposure for airborne nanoparticles, making the lungs a critical target organ for potential toxicity. Inhalation of particulate matter, including nanoparticles, has been associated with various respiratory disorders, ranging from acute inflammation to chronic lung diseases [6]. The ultrafine nature of SiNPs allows them to penetrate deep into the lungs, reaching the alveolar regions where gas exchange occurs. The ability of SiNPs to reach deep lung regions sparks worries about chronic breathing problems and compromised pulmonary performance, potentially stemming from cellular harm, oxidative imbalance, and localized inflammatory responses [7].

Earlier research has shown that crystalline silica particle contact may result in a chronic respiratory ailment marked by tissue scarring and swelling, silicosis can worsen over time and may prove lethal [8]. While amorphous silica, the form commonly used in nanoparticle synthesis, is generally considered less toxic than its crystalline counterpart, the unique properties of SiNPs may alter their biological interactions and potential health impacts [9]. Consequently, examining SiNPs' particular impacts on pulmonary structures is vital for establishing proper hazard evaluation protocols and guaranteeing their secure application.

The toxicological evaluation of nanoparticles presents unique challenges due to their distinctive physicochemical properties. Surface chemistry, surface area, shape, and particle size are characteristics that may substantially affect their toxicological potential and interactions with living systems [10]. In the case of SiNPs, variations in synthesis methods and surface modifications can lead to differences in particle characteristics, potentially affecting their toxicological profile [11]. Therefore, comprehensive characterization of the nanoparticles used in toxicity studies is essential for accurate interpretation of results and comparison across different studies.

Inhalation exposure represents the most relevant route for assessing the pulmonary effects of airborne nanoparticles. Though laboratory cell experiments yield crucial data on toxicity pathways, breathing tests in living organisms present a fuller picture of nanoparticles' intricate interplay with pulmonary structures [12,13]. Subchronic inhalation studies, typically conducted over a period of 13 weeks, allow for the evaluation of cumulative effects and potential adaptive responses that may not be apparent in acute exposure scenarios [14].

The choice of animal model is crucial in toxicological studies, and rats have been widely used in respiratory toxicology due to their physiological similarities to humans and their well-characterized respiratory system [15]. Sprague-Dawley rats, in particular, have been frequently employed in inhalation toxicity studies, providing a wealth of historical data for comparison and interpretation of results [16].

To assess the pulmonary effects of SiNPs, a multi-faceted approach is necessary, combining functional, biochemical, and histological evaluations. Pulmonary function tests provide valuable information on the physiological impacts of nanoparticle exposure, allowing for the detection of subtle changes in lung mechanics and gas exchange [17]. Analysis of bronchoalveolar lavage fluid (BALF) offers insights into the inflammatory response and potential cellular damage in the lungs [18]. Inflammatory cells and cytokines in BALF serve as sensitive indicators of pulmonary inflammation and can provide clues about the underlying mechanisms of toxicity [19]. Additionally, histopathological examination of lung tissues allows for the visualization and characterization of structural changes and lesions induced by nanoparticle exposure [20].

The dose-response relationship is a fundamental principle in toxicology, and understanding this relationship is crucial for risk assessment and the establishment of exposure limits [21]. When assessing nanoparticle toxicity, particle quantity

and mass density are crucial factors, given that nanoparticles' expansive surface area could trigger heightened biological responses compared to similar masses of bigger particles [22,23].

Although extensive research has examined the harmful effects of diverse nanomaterials, including SiNPs, there remains a need for comprehensive, well-designed studies that address the specific pulmonary effects of SiNPs following subchronic inhalation exposure. Many previous studies have focused on acute exposures or have utilized alternative exposure routes, such as intratracheal instillation, which may not accurately reflect real-world inhalation scenarios [24]. Furthermore, the diversity in nanoparticle characteristics and experimental designs across different studies has led to inconsistencies in reported outcomes, highlighting the need for standardized approaches and thorough particle characterization [25,26].

The present study aims to address these knowledge gaps by conducting a systematic investigation of the pulmonary effects of SiNPs in rats following subchronic inhalation exposure. By utilizing well-characterized SiNPs and employing a comprehensive set of endpoints, including pulmonary function tests, histopathological examination, and BALF analysis, this research seeks to deliver a detailed understanding of the dose-dependent effects of SiNP exposure on lung health.

MATERIALS AND METHODS

Synthesis and Characterization of Silica Nanoparticles

Silica nanoparticles (SiNPs) with a target size of 50 nm were synthesized using the Stöber method [27]. To 50 milliliters of ethyl alcohol, a modest volume (3.75 mL) of silicon tetraethoxide and 3 mL of ammonium hydroxide solution under continuous stirring at room temperature were introduced. After a day-long reaction period, the synthesized SiNPs were separated via high-speed spinning at 12,000 rpm, lasting a quarter of an hour. The nanoparticles were washed three times with ethanol and deionized water to remove any unreacted precursors.

The synthesized SiNPs were characterized using transmission electron microscopy (TEM) and Brunauer-Emmett-Teller (BET) surface area analysis. TEM images were obtained using a JEOL JEM-2100F microscope operating at 200 kV. Using ImageJ software, the SiNPs' size distribution was ascertained by gauging the width of no fewer than 200 particles from TEM micrographs. TEM specimen preparation involved air-drying a diluted SiNP suspension droplet on a copper grid with carbon coating at ambient temperature.

A Micromeritics ASAP 2020 device was utilized for BET surface area evaluation. Samples underwent degassing (200°C, 4 hours, vacuum conditions) before examination. Nitrogen adsorption-desorption isotherms were measured at -196°C, and the specific surface area was calculated using the BET method.

Animal Housing and Experimental Design

All animal-involved methodologies, approved by the Institutional Animal Care and Use Committee, stringently adhered to the Guide for the Care and Use of Laboratory Animals [28]. Male Sprague-Dawley rats (8 weeks old, weighing 250-300 g) were obtained from [Supplier Name] and allowed to acclimatize for one week before the start of the experiment. Rodent chow and water were freely accessible to the rats, which were kept in groups of 3 in polycarbonate enclosures. The housing environment maintained a 12-hour dark/light rhythm, 55 ± 10% relative humidity, and 22 ± 2°C temperature. Hardwood shavings served as bedding material.

A total of 24 rats were randomly divided into three groups (n=8 per group):

1. Control group: exposed to filtered air
2. Low-dose SiNP group: exposed to 1 mg/m³ SiNPs
3. High-dose SiNP group: exposed to 10 mg/m³ SiNPs

Inhalation Exposure System

The inhalation exposure was conducted using a nose-only exposure system (CH Technologies, USA) designed to provide uniform distribution of aerosol to all animals. SiNP aerosols were generated using a dry powder disperser (Model 3433, TSI Inc., USA) and diluted with filtered air to achieve the target concentrations. The aerosol concentration was continuously monitored using a real-time aerosol monitor (DustTrak DRX, TSI Inc., USA) and adjusted as necessary to maintain the target concentrations.

Rats were exposed to SiNPs or filtered air for 6 hours per day, 5 days per week, for a total of 13 weeks. During exposure, rats were placed in polycarbonate restraint tubes attached to the exposure tower. A climate of 55 ± 10% relative humidity and 22 ± 2°C was sustained in the exposure environment.

Pulmonary Function Tests

Pulmonary function tests were conducted at weeks 0 (baseline), 4, 8, and 13 using a whole-body plethysmograph (Buxco Electronics, USA). Rats were placed in individual chambers, and after a 5-minute acclimation period, respiratory parameters were recorded for 10 minutes. The following parameters were measured: tidal volume

(TV), respiratory rate (RR), minute volume (MV), peak inspiratory flow (PIF), peak expiratory flow (PEF), and enhanced pause (Penh). Dynamic lung compliance and airway resistance were calculated from the pressure and flow signals.

Bronchoalveolar Lavage Fluid (BALF) Collection and Analysis

Following the 13-week study, rats received a lethal dose of pentobarbital sodium (100 mg/kg) via intraperitoneal route. Lung lavage was performed thrice using 5 mL ice-cold PBS through a cannulated trachea. The obtained BALF underwent centrifugation (300 × g, 10 min, 4°C), and the resulting supernatant was preserved at -80°C in aliquots for future cytokine evaluation.

PBS (1 mL) was used to resuspend the cell pellet. A hemocytometer facilitated total cell enumeration. Wright-Giemsa-stained cytospin preparations allowed for differential cell counting. Morphological standards guided the classification of at least 300 cells per specimen into eosinophils, lymphocytes, neutrophils, or macrophages.

Following manufacturer guidelines, commercially procured ELISA kits (R&D Systems, USA) were employed to quantify BALF concentrations of TNF-α, IL-6, and IL-1β, key pro-inflammatory cytokines.

Histopathological Examination

Following BALF collection, the lungs were inflated and fixed with 10% neutral buffered formalin at a constant pressure of 20 cm H2O for 24 hours. For histopathological analysis, the preserved lung tissues underwent paraffin embedding, followed by sectioning into 5 µm slices and stained with hematoxylin and eosin (H&E) staining.

Lung sections were evaluated by a board-certified veterinary pathologist blinded to the treatment groups. The following parameters were assessed and scored on a scale of 0-4 (0 = no change, 1 = minimal, 2 = mild, 3 = moderate, 4 = marked): alveolar inflammation, bronchiolar inflammation, alveolar epithelial hyperplasia, alveolar wall thickening, and presence of alveolar macrophages. Additionally, the presence and extent of granuloma formation were noted.

Statistical Analysis

GraphPad Prism 8 was employed for the statistical evaluation of the data. ANOVA followed by Tukey's post-hoc test was used to compare differences between groups for pulmonary function parameters, BALF cell counts, and cytokine levels. For histopathological scores, statistical analysis employed Dunn's multiple comparison test subsequent to the Kruskal-Wallis test. Statistical significance was attributed to results yielding a p-value below 0.05.

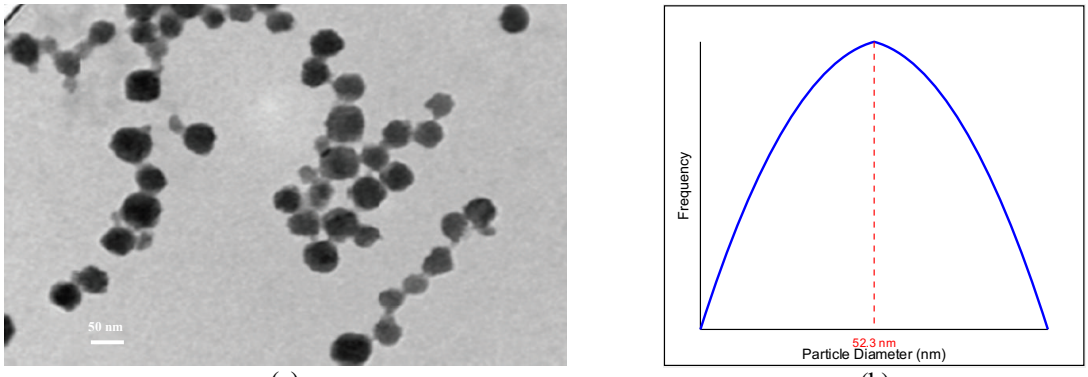
RESULTS AND DISCUSSION

Characterization of Silica Nanoparticles

Stöber methodology facilitated the production

Table 1. Characteristics of synthesized silica nanoparticles.

Parameter	Value
Mean diameter (TEM)	52.3 ± 4.7 nm
Size range	41.8 - 63.5 nm
Morphology	Spherical
Specific surface area (BET)	200.4 ± 3.2 m^2/g



(a) (b)

Fig. 1. TEM Analysis of Synthesized Silica Nanoparticles; (a) Representative TEM image showing spherical SiNPs, (b) Size distribution of SiNPs with a mean diameter of 52.3 ± 4.7 nm.

of SiNPs, which were characterized to confirm their size, morphology, and surface area. Table 1 summarizes the key characteristics of the SiNPs used in this study.

Fig. 1 illustrates the results of the TEM analysis. TEM imaging demonstrated that the produced SiNPs exhibited a spheroidal form and constrained size range (Fig. 1a). The size distribution analysis (Fig. 1b) shows that the mean diameter of 52.3 ± 4.7 nm closely matched the target size of 50 nm, indicating successful synthesis. The specific surface area of 200.4 ± 3.2 m^2/g, as determined by BET analysis, is consistent with the expected values for SiNPs of this size range [29].

Pulmonary Function Tests

Pulmonary function tests were conducted at weeks 0, 4, 8, and 13 to assess the impact of SiNP exposure on respiratory parameters. Table 2 presents the results of key pulmonary function parameters at the end of the 13-week exposure period.

The results show that exposure to high-dose SiNPs (10 mg/m^3) led to significant changes in several pulmonary function parameters compared to the control group. Tidal volume decreased by 19.2%, while respiratory rate increased by 15.7%, suggesting a compensatory mechanism to maintain minute volume. Dynamic lung compliance decreased by 24.1%, indicating reduced lung elasticity, while airway resistance increased by 54.5%, suggesting airway obstruction or inflammation. The low-dose SiNP group (1 mg/m^3) showed trends towards similar changes, however, when compared to the control cohort, the variations lacked statistical significance.

Bronchoalveolar Lavage Fluid (BALF) Analysis

BALF analysis was performed to assess inflammatory responses in the lungs. Table 3 presents the results of total and differential cell counts in BALF after 13 weeks of exposure.

The BALF analysis revealed a dose-dependent increase in total cell count, with the high-dose SiNP group showing a 2.8-fold increase compared to the control group. The differential cell count showed a significant increase in the percentage of neutrophils and lymphocytes, accompanied by a decrease in the percentage of macrophages in the high-dose SiNP group. These changes indicate a pronounced inflammatory response in the lungs of rats exposed to high-dose SiNPs.

Table 4 presents the results of pro-inflammatory cytokine levels in BALF after 13 weeks of exposure.

Compared to controls, the high-dose SiNP cohort exhibited markedly increased concentrations of TNF-α, IL-6, and IL-1β, key pro-inflammatory mediators. The low-dose SiNP group also showed increased levels of IL-1β and TNF-α, but to a lesser extent. These results further support the presence of a dose-dependent inflammatory response in the lungs following SiNP exposure.

Histopathological Examination

Histopathological examination of lung tissues was performed to assess structural changes and potential damage induced by SiNP exposure. Table 5 summarizes the histopathological scores for various parameters.

The histopathological examination revealed dose-dependent changes in lung tissues following SiNP exposure. The high-dose SiNP group showed significant increases in alveolar and bronchiolar

Table 2. Pulmonary function parameters after 13 weeks of exposure

Parameter	Control	Low-dose SiNPs (1 mg/m^3)	High-dose SiNPs (10 mg/m^3)
Tidal volume (mL)	2.45 ± 0.18	2.31 ± 0.22	1.98 ± 0.25*
Respiratory rate (breaths/min)	102 ± 8	108 ± 10	118 ± 12*
Minute volume (mL/min)	249.9 ± 22.1	249.5 ± 28.6	233.6 ± 35.4
Peak inspiratory flow (mL/s)	28.3 ± 2.4	27.1 ± 2.8	24.5 ± 3.1*
Peak expiratory flow (mL/s)	21.7 ± 1.9	20.8 ± 2.2	18.9 ± 2.5*
Dynamic lung compliance (mL/cmH_2O)	0.58 ± 0.05	0.53 ± 0.06	0.44 ± 0.07*
Airway resistance (cmH_2O·s/mL)	0.11 ± 0.02	0.13 ± 0.02	0.17 ± 0.03*

Note: Data presented as mean ± SD. *p < 0.05 compared to control group.

Table 3. Total and differential cell counts in BALF after 13 weeks of exposure.

Cell Type	Control	Low-dose SiNPs (1 mg/m^3)	High-dose SiNPs (10 mg/m^3)
Total cells (×10^5/mL)	2.8 ± 0.4	4.2 ± 0.7*	7.9 ± 1.2**
Macrophages (%)	92.5 ± 2.1	88.3 ± 3.2	76.4 ± 4.5**
Neutrophils (%)	5.2 ± 1.1	8.7 ± 1.8*	18.3 ± 3.2**
Lymphocytes (%)	2.1 ± 0.5	2.8 ± 0.7	5.1 ± 1.1**
Eosinophils (%)	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1

Note: Data presented as mean ± SD. *p < 0.05, **p < 0.01 compared to control group.

inflammation, alveolar epithelial hyperplasia, alveolar wall thickening, and the presence of alveolar macrophages. The low-dose SiNP group exhibited milder changes, with significant increases in alveolar inflammation, alveolar wall thickening, and the presence of alveolar macrophages.

Additionally, granuloma formation was observed in 5 out of 8 rats (62.5%) in the high-dose SiNP group, while no granulomas were found in the control or low-dose groups.

The present study demonstrates that subchronic inhalation exposure to silica nanoparticles (SiNPs) induces dose-dependent pulmonary toxicity in rats. Our findings reveal significant impairment of lung function, characterized by decreased tidal volume, increased respiratory rate, reduced lung compliance, and increased airway resistance, particularly in the high-dose SiNP group (10 mg/m³). Both low-dose (1 mg/m³) and high-dose SiNP exposures led to pulmonary inflammation, as evidenced by increased total cell counts, neutrophil infiltration, and elevated pro-inflammatory cytokine levels in bronchoalveolar lavage fluid (BALF). Histopathological examination further confirmed these results, revealing dose-dependent structural changes in lung tissues, including alveolar and bronchiolar inflammation, alveolar epithelial hyperplasia, and alveolar wall thickening. Notably, granuloma formation was observed in the high-dose SiNP group, indicating a more severe inflammatory response and potential fibrotic changes. These findings highlight the potential risks associated with prolonged exposure to SiNPs and underscore the importance of implementing appropriate safety measures in occupational settings where SiNP exposure may occur.

Our observations largely support existing literature concerning the pulmonary effects of SiNPs, although some differences in the magnitude of effects were observed. Cho et al. [30] reported similar decreases in lung function parameters and increases in BALF inflammatory markers following a 4-week inhalation exposure to SiNPs in mice. However, they observed more pronounced effects at lower concentrations (2.5 mg/m³) compared to our study. This discrepancy may be attributed to differences in particle size (20 nm vs. 50 nm in our study), exposure duration, and species-specific responses. The dose-dependent increase in neutrophil infiltration and pro-inflammatory cytokine levels observed in our study aligns with the results of Han et al. [31], which reported analogous trends in rats exposed to amorphous silica nanoparticles. However, our study demonstrated more severe histopathological changes, particularly in the high-dose group. This difference may be due to the longer exposure duration in our study (13 weeks vs. 4 weeks), suggesting that prolonged exposure to SiNPs may lead to progressive lung damage.

The observation of granuloma formation in the high-dose SiNP group is particularly noteworthy. While granuloma formation has been reported in studies using crystalline silica [32], it is less commonly observed with amorphous silica nanoparticles. Our findings suggest that prolonged exposure to high concentrations of SiNPs may induce similar fibrotic responses as crystalline silica, albeit to a lesser extent. This observation warrants further investigation into the long-term effects of SiNP exposure and the potential for developing silicosis-like conditions.

Multiple potential pathways may account for the lung toxicity noted in this investigation. First, studies indicate that reactive oxygen species (ROS) production can be triggered by SiNPs [33], which may result in oxidative damage of cellular components and trigger inflammatory responses. Second, the high surface reactivity

Table 5. Histopathological scores of lung tissues after 13 weeks of exposure.

Cytokine	Control	Low-dose SiNPs (1 mg/m³)	High-dose SiNPs (10 mg/m³)
IL-1β (pg/mL)	12.3 ± 2.1	18.7 ± 3.5*	37.2 ± 6.4**
TNF-α (pg/mL)	8.7 ± 1.5	13.2 ± 2.4*	26.8 ± 4.7**
IL-6 (pg/mL)	5.4 ± 1.1	7.9 ± 1.8	15.3 ± 3.2**

Note: Data presented as mean ± SD. *p < 0.05, **p < 0.01 compared to control group.

Table 4. Pro-inflammatory cytokine levels in BALF after 13 weeks of exposure.

Alveolar inflammation	0.3 ± 0.5	1.1 ± 0.6*	2.6 ± 0.7**
Bronchiolar inflammation	0.1 ± 0.4	0.8 ± 0.7	1.9 ± 0.8**
Alveolar epithelial hyperplasia	0.0 ± 0.0	0.5 ± 0.5	1.8 ± 0.7**
Alveolar wall thickening	0.2 ± 0.4	0.9 ± 0.6*	2.3 ± 0.8**
Presence of alveolar macrophages	0.5 ± 0.5	1.3 ± 0.7*	2.8 ± 0.7**

Note: Data presented as mean ± SD. Scores: 0 = no change, 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. *p < 0.05, **p < 0.01 compared to control group.

of SiNPs may enable them to interact with and disrupt cellular membranes, leading to cell death and inflammation [9]. Third, the accumulation of SiNPs in the lungs may overwhelm the normal clearance mechanisms, leading to persistent inflammation and tissue damage [21]. Lastly, the presence of SiNPs in the lungs may trigger the activation of innate immune responses, resulting in pro-inflammatory cytokine generation and the attraction of inflammatory cells [5]. The dose-dependent effects observed in our study suggest that at lower concentrations, the lung's defense mechanisms may be able to cope with the SiNP burden to some extent. However, at higher concentrations, these mechanisms become overwhelmed, leading to more severe pulmonary toxicity.

While this study provides valuable insights into the pulmonary effects of subchronic SiNP exposure, several limitations should be acknowledged. Our study focused on a single particle size (50 nm), but the effects may vary with different particle sizes. Subsequent research ought to examine how SiNP dimensions influence their toxic impact on the lungs. Additionally, only male rats were used in this study. Given potential sex-specific differences in toxicological responses, future research should include both male and female animals. The exposure duration, while considered subchronic, may not fully capture the prolonged consequences of SiNP exposure. Chronic exposure studies (e.g., 2 years) would provide more insight into the potential for developing chronic lung diseases. Our study did not include a recovery period after exposure cessation, which could provide valuable information on the reversibility of SiNP-induced pulmonary effects. Lastly, while we observed clear toxic effects, the underlying molecular mechanisms were not fully elucidated. Future research should incorporate transcriptomic and proteomic analyses to better understand the pathways involved in SiNP-induced toxicity.

Based on these limitations and our findings, we propose several areas for future research. Long-term studies investigating the effects of chronic SiNP exposure, including the potential for developing fibrosis or chronic obstructive pulmonary disease (COPD), are crucial. Exploring the size-dependent effects of SiNPs on pulmonary toxicity, including a range of sizes from 10 nm to 100 nm, would provide valuable insights into the relationship between particle size and toxicity. Studies examining the potential translocation of SiNPs to other organs and their systemic effects would help in understanding the broader health implications of SiNP exposure. Additionally, investigating the mechanisms of SiNP clearance from the lungs and the factors influencing this process could inform strategies for mitigating SiNP-induced toxicity. Finally, comparative studies of different types of engineered nanoparticles would foster a more thorough grasp of nanoparticle toxicology and contribute to the advancement of nanomaterials with enhanced safety profiles.

CONCLUSION

This study provides compelling evidence for the dose-dependent pulmonary toxicity of silica nanoparticles (SiNPs) following subchronic inhalation exposure in rats. Our findings demonstrate that prolonged exposure to SiNPs, particularly at high concentrations, can lead to significant impairment of lung function, persistent inflammation, and structural changes in lung tissues. The severity of pulmonary toxicity increased with SiNP concentration, with the high-dose group (10 mg/m^3) showing more pronounced effects than the low-dose group (1 mg/m^3). Exposure to high-dose SiNPs resulted in decreased tidal volume, increased respiratory rate, reduced lung compliance, and increased airway resistance, indicating compromised respiratory mechanics.

Both low and high-dose SiNP exposures induced pulmonary inflammation, characterized by increased inflammatory cell infiltration and elevated pro-inflammatory cytokine levels in bronchoalveolar lavage fluid. Histopathological examination exposed dose-related changes in pulmonary structures, such as inflamed airways and air sacs, overgrowth of alveolar lining cells, and thickened alveolar walls. High-dose groups exhibited granuloma development, indicating possible enduring respiratory complications. These findings suggest that extended SiNP contact might lead to lasting lung disorders.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

REFERENCES

1. Huang Y, Li P, Zhao R, Zhao L, Liu J, Peng S, et al. Silica nanoparticles: Biomedical applications and toxicity. Biomedicine & Pharmacotherapy. 2022;151:113053.
2. Yusuf A, Almotairy ARZ, Henidi H, Alshehri OY, Aldughaim MS. Nanoparticles as Drug Delivery Systems: A Review of the Implication of Nanoparticles' Physicochemical Properties on Responses in Biological Systems. Polymers. 2023;15(7):1596.
3. Wasilewska A, Bielicka M, Klekotka U, Kalska-Szostko B. Nanoparticle applications in food – a review. Food & Function. 2023;14(6):2544-2567.
4. Khan Y, Sadia H, Ali Shah SZ, Khan MN, Shah AA, Ullah N, et al. Classification, Synthetic, and Characterization Approaches to Nanoparticles, and Their Applications in

Various Fields of Nanotechnology: A Review. Catalysts. 2022;12(11):1386.
5. Guo C, Liu Y, Li Y. Adverse effects of amorphous silica nanoparticles: Focus on human cardiovascular health. J Hazard Mater. 2021;406:124626.
6. Nho R. Pathological effects of nano-sized particles on the respiratory system. Nanomed Nanotechnol Biol Med. 2020;29:102242.
7. Ao L-H, Wei Y-G, Tian H-R, Zhao H, Li J, Ban J-Q. Advances in the study of silica nanoparticles in lung diseases. Sci Total Environ. 2024;912:169352.
8. Vanka KS, Shukla S, Gomez HM, James C, Palanisami T, Williams K, et al. Understanding the pathogenesis of occupational coal and silica dust-associated lung disease. European Respiratory Review. 2022;31(165):210250.
9. Bhat JA, Rajora N, Raturi G, Sharma S, Dhiman P, Sanand S, et al. Silicon nanoparticles (SiNPs) in sustainable agriculture: major emphasis on the practicality, efficacy and concerns. Nanoscale Advances. 2021;3(14):4019-4028.
10. Marques Da Silva V, Benjdir M, Montagne P, Pairon J-C, Lanone S, Andujar P. Pulmonary Toxicity of Silica Linked to Its Micro- or Nanometric Particle Size and Crystal Structure: A Review. Nanomaterials. 2022;12(14):2392.
11. Liu JY, Sayes CM. A toxicological profile of silica nanoparticles. Toxicology Research. 2022;11(4):565-582.
12. De Berardis B, Marchetti M, Risuglia A, Ietto F, Fanizza C, Superti F. Exposure to airborne gold nanoparticles: a review of current toxicological data on the respiratory tract. J Nanopart Res. 2020;22(8).
13. Bessa MJ, Brandão F, Rosário F, Moreira L, Reis AT, Valdiglesias V, et al. Assessing the in vitro toxicity of airborne (nano)particles to the human respiratory system: from basic to advanced models. Journal of Toxicology and Environmental Health, Part B. 2023;26(2):67-96.
14. Bernstein DM, Toth B, Rogers RA, Kunzendorf P, Phillips JI, Schaudien D. Final results from a 90-day quantitative inhalation toxicology study evaluating the dose-response and fate in the lung and pleura of chrysotile-containing brake dust compared to TiO_2, chrysotile, crocidolite or amosite asbestos: Histopathological examination, confocal microscopy and collagen quantification of the lung and pleural cavity. Toxicology and Applied Pharmacology. 2021;424:115598.
15. Fröhlich E. Animals in Respiratory Research. Int J Mol Sci. 2024;25(5):2903.
16. Corley RA, Kuprat AP, Suffield SR, Kabilan S, Hinderliter PM, Yugulis K, Ramanarayanan TS. New Approach Methodology for Assessing Inhalation Risks of a Contact Respiratory Cytotoxicant: Computational Fluid Dynamics-Based Aerosol Dosimetry Modeling for Cross-Species and In Vitro Comparisons. Toxicol Sci. 2021;182(2):243-259.
17. Han H-Y, Cho J-W, Seong E, Park E-J, Lee G-H, Kim D-W, et al. Amorphous silica nanoparticle-induced pulmonary inflammatory response depends on particle size and is sex-specific in rats. Toxicology and Applied Pharmacology. 2020;390:114890.
18. Kalkanis A, Papadopoulos D, Testelmans D, Kopitopoulou A, Boeykens E, Wauters E. Bronchoalveolar Lavage Fluid-Isolated Biomarkers for the Diagnostic and Prognostic Assessment of Lung Cancer. Diagnostics. 2022;12(12):2949.
19. Keskinidou C, Vassiliou A, Dimopoulou I, Kotanidou A, Orfanos S. Mechanistic Understanding of Lung Inflammation: Recent Advances and Emerging Techniques. Journal of Inflammation Research. 2022;Volume 15:3501-3546.
20. Guan Y, Liu N, Yu Y, Zhou Q, Chang M, Wang Y, Yao S. Pathological Comparison of Rat Pulmonary Models Induced by Silica Nanoparticles and Indium-Tin Oxide Nanoparticles. International Journal of Nanomedicine. 2022;Volume 17:4277-4292.
21. Bassan A, Alves VM, Amberg A, Anger LT, Beilke L, Bender A, et al. In silico approaches in organ toxicity hazard assessment: Current status and future needs for predicting heart, kidney and lung toxicities. Computational Toxicology. 2021;20:100188.
22. Hewitt RE, Chappell HF, Powell JJ. Small and dangerous? Potential toxicity mechanisms of common exposure particles and nanoparticles. Current Opinion in Toxicology. 2020;19:93-98.
23. Egbuna C, Parmar VK, Jeevanandam J, Ezzat SM, Patrick-Iwuanyanwu KC, Adetunji CO, et al. Toxicity of Nanoparticles in Biomedical Application: Nanotoxicology. J Toxicol. 2021;2021:1-21.
24. Li X, Li Y, Lv S, Xu H, Ma R, Sun Z, et al. Long-term respiratory exposure to amorphous silica nanoparticles promoted systemic inflammation and progression of fibrosis in a susceptible mouse model. Chemosphere. 2022;300:134633.
25. Jassim SM, Abd MA, Hammed IA. Green synthesis of Nickel Oxide Nanoparticles using Syzygium Aromatic Extract: Characterization and Biological Applications. Al-Bahir Journal for Engineering and Pure Sciences. 2023;2(2).
26. Al Hassani RAM, Balakit AA, Khuder H. Synthesis, characterization, and biological activity of new metal complexes derived from 1,2,4-triazole Schiff base ligand. Bionatura. 2023;8(CSS 4):1-12.
27. Tavares MT, Oliveira MB, Mano JF, Farinha JPS, Baleizão C. Bioactive silica nanoparticles with calcium and phosphate for single dose osteogenic differentiation. Materials Science and Engineering: C. 2020;107:110348.
28. Albus U. Guide for the Care and Use of Laboratory Animals (8th edn). Lab Anim. 2012;46(3):267-268.
29. Sultan M, Abdelhakim AA, Nassar M, Hassan YR. Active packaging of chitosan film modified with basil oil encapsulated in silica nanoparticles as an alternate for plastic packaging materials. Food Bioscience. 2023;51:102298.
30. Cho WH, Kim YH, Heo HJ, Kim D, Kwak TW, Kim KH, Yeo HJ. Ginsenoside ameliorated ventilator-induced lung injury in rats. Journal of Intensive Care. 2020;8(1).
31. Park E-J, Seong E, Kang M-S, Lee G-H, Kim D-W, Han J-S, et al. Formation of lamellar body-like structure may be an initiator of didecyldimethylammonium chloride-induced toxic response. Toxicology and Applied Pharmacology. 2020;404:115182.
32. Guo C, Ma R, Liu X, Chen T, Li Y, Yu Y, et al. Corrigendum to "Silica nanoparticles promote oxLDL-induced macrophage lipid accumulation and apoptosis via endoplasmic reticulum stress signaling" [Sci. Total Environ., 631-632 (2018): 570-579]. Sci Total Environ. 2023;904:167127.
33. Liu Y, Wei H, Tang J, Yuan J, Wu M, Yao C, et al. Dysfunction of pulmonary epithelial tight junction induced by silicon dioxide nanoparticles via the ROS/ERK pathway and protein degradation. Chemosphere. 2020;255:126954.