A Histological Study on the Acute Effect of Zinc Oxide Nanoparticles Administered by Different Routes on Albino Rat Lung

Heba Abdel Latif Mohammed, Nagwa M. El Shakaa, Nevine Bahaa, Asmaa A. Abo Zeid
Department of Histology and Cell Biology, Faculty of Medicine, Ain Shams University, Cairo, Egypt

Abstract

Introduction and Aim of the Work: Zinc oxide nanoparticles (ZnO NPs) are considered the most frequently utilized NPs, so the potential for human exposure has increased tremendously. Hence, the study is aimed to compare the histopathological effects of high and low doses of ZnO NPs administered intranasally or intravenously on lung tissue of adult rat’s male albino. Materials and Methods: Thirty-five male Wistar rats were divided into Group I; control group, Group II (intranasal administered group) was subdivided into Subgroup IIA and IIB, in which the animals were injected with 4 and 30 mg/kg of ZnO NPs, respectively. Group III (intravenous administered group) was subdivided into two subgroups with the same doses as Group II. Blood samples were collected after 24 h for estimating serum level of lactate dehydrogenase. Rat lungs were processed for histological, immunohistochemical, and ultrastructural analysis. Results: ZnO NPs caused thickening of interalveolar septa. Extravasated red blood cells were noticed in the alveolar lumen and in some bronchioles. Many dilated blood vessels exhibited focal disruption and focal thickening of their wall. Collagenous fibers were deposited in the interalveolar septa and the walls of bronchi. Tumor necrosis factor-alpha immune reactivity was significantly increased. These findings increased on dose increase, mainly in the intranasal administered group when compared with the intravenous group. Conclusion: ZnO NPs administration caused toxic effects on the histological structure of albino rat lung. These effects were route and dose-dependent, being more obvious after intranasal administration.

Keywords: Lung, rat, tumor necrosis factor-alpha, zinc oxide nanoparticles

INTRODUCTION

The nanoparticles (NPs) are engineered compounds with size ranging between 1 and 100 nm. The large surface area: volume ratio of NPs led to an alteration in their biological activity compared to the parent bulk materials. By modifying their size, surface properties, and shape NPs can be used in an application-specific manner. Zinc oxide NPs (ZnO NPs) are considered one of the commonly utilized NPs. They enter in many industries and biomedicines such as personal hygiene products, textiles, electronics, and cosmetics. Furthermore, they are important component of sunscreens and moisturizers, food industries and in packaging. Furthermore, their anticancer effect was documented.

These ZnO NPs could access the body through skin topical application, lung inhalation, food consumption, and intravenous injection. It was reported that the major route for entry of ZnO NPs is the respiratory system. Nevertheless, the possibility of intravenous injection of ZnO NPS has been elevated as a carrier for different chemotherapeutic. Due to their wide surface area and small size, they could easily pass the physiological barriers and be widely distributed in circulation. It is, therefore, necessary to assess their toxic effect to avoid their potential adverse effect.

This study was designed to compare potential histopathological effects of high and low doses of ZnO NPs, either administered...
intranasally or intravenously on the histological structure of the rat lung.

**Materials and Methods**

**Preparation of zinc oxide nanoparticles**

ZnO NPs were bought from “NanoTech Egypt for Photo-Electronics,” October 6, city, Giza, as white powder, of size = 20 ± 10 nm (surface area 15–25 m²/g and purity >99%). They were dissolved in 0.4 ml phosphate-buffered saline (PBS) with 5% bovine serum albumin (BSA). The ZnO NPs suspension was then sonicated for 10 min.¹³

**Characterization of zinc oxide nanoparticles**

Transmission electron microscope (TEM) (JEM-1200 EX II Electron Microscope, Tokyo, Japan) was used in evaluating the size, shape, and aggregation state of ZnO NPs. The NPs were grinded and diluted in PBS, 5% bovine serum was added to form a suspension which was deposited on copper grids coated with carbon. The grids were left to dry at ambient temperature before examination by the TEM.¹⁴

**Animals**

Thirty-five adult male Wistar rats of average 250 g were bought from and housed in Medical Ain Shams Research Institution, Faculty of Medicine, Ain Shams University, Cairo, Egypt. All animal procedures were performed in compliance with the general guidelines for the care and use of laboratory animals and approved by the animal ethical committee at the Faculty of Medicine, Ain Shams University. The animals were kept in plastic cages and mesh wire protections and were given rat water and chow ad libitum. The animals were exposed to 12 h of artificial light and 12 h of darkness throughout the experiment. All procedures were performed in compliance with the animal ethical committee at the Faculty of Medicine, Ain Shams University.

**Experimental groups**

In the present study, the rats were separated into three main groups:

- **Group I** (control group): It included 15 rats, which were further subdivided into Subgroup IA: (n = 5), which were left without interference. Subgroup IB: (n = 5) which received single intranasal administration of 0.4 ml PBS with 5% BSA. Subgroup IC: (n = 5) which received single intravenous injection of 0.4 ml PBS with 5% BSA.

- **Group II** (intranasal administered group): It included 10 rats, which were further subdivided equally into: Subgroup IIA (low-dose intranasal subgroup): (n = 5) which received 4 mg/kg ZnO NPs intranasally as a single dose, dissolved in 0.4 ml PBS with 5% BSA.¹⁵
  - Subgroup IIB (High-dose intranasal subgroup): (n = 5), which received 30 mg/kg ZnO NPs intravenously as a single dose in tail vein, dissolved in 0.4 ml PBS with 5% BSA.¹⁶

  The experimental animals of all groups were sacrificed one day after ZnO NPs administration by cervical dislocation after ether inhalation. Just before sacrificing the animals, blood samples were aspirated from the aorta. The lungs were then dissected out and the bodies of the dead animals were disposed by using the incinerator. The lung specimens were processed for histological and ultrastructural examination.

**Biochemical analysis of serum level of lactate dehydrogenase**

Blood specimens were taken from the aorta and sent to the clinical pathology department, Faculty of Medicine, Ain Shams University. The samples were centrifuged to separate the serum fractions, kept in clean bottles and frozen at −20°C till processed. The released lactate dehydrogenase (LDH) was determined with commercially available LDH-Kits (CytoTox 96 Kit).¹⁷

**Histological study**

The right lung of each animal was fixed in 10% neutral buffered formaldehyde, dehydrated, cleared and impregnated in soft paraffin, then embedded in paraffin blocks. Serial sections were taken of –5 μm thick and processed for staining by hematoxylin and eosin stain and Masson’s trichrome stain.¹⁸

**Immunohistochemical study**

Immunohistochemical staining for the inflammatory marker tumor necrosis factor-alpha (TNF-α) was done. It was purchased from LABVISION USA as monoclonal mouse anti-human TNF-α antibody (dilution 1:500) product number: (MAB510). The site of antibody binding was visualized by 3,3’-diaminobenzidine staining, which appeared as dark-brown cytoplasmic discoloration. Negative control slide was prepared in which PBS was used instead of the primary antibody.¹⁹

**Transmission electron microscopic study**

The left lungs of each rat in all subgroups were divided into 1 mm³ pieces and fixed in 1.5% glutaraldehyde to be further processed into epoxy resin-filled capsules. Ultrathin sections of 60–80 nm thick were cut, mounted on copper grids, and stained with the metal stains uranyl acetate and lead citrate to be examined and photographed by TEM (JEM-1200 EX II Electron Microscope, Tokyo, Japan) in the regional center for mycology and biotechnology, Ain-Shams University.

**Morphometric study and statistical analysis**

- Statistical analysis for the serum level of LDH of all rats in each subgroup was done
- Five nonoverlapping different fields were studied in all sections of the lung of all rats in each subgroup to measure the following parameters:
  - Mean area percentage of collagen fibers in Masson’s trichrome-stained sections (×40)
RESULTS

Serum lactate dehydrogenase level

Serum LDH level was significantly increased in all examined subgroups; IIA, IIB, IIIA, and IIIB ($P < 0.05$) as compared to the control group.

Comparing the low-dose subgroups, a statistically significant decrease of serum LDH level was detected on intravenous ZnO NPs administration in subgroup IIIA as compared to the intranasal administration in subgroup IIA ($P < 0.05$). Likewise, the high-dose subgroup showed a significant decrease of the serum LDH level after intravenous ZnO NPs administration in subgroup IIIB as compared to the intranasal administration in subgroup IIB ($P < 0.05$).

Comparing both subgroups of the intranasally administered ZnO NPs Group (II), a significant increase of serum LDH level was detected in the subgroup IIB as compared to the low-dose subgroup IIA ($P < 0.05$). Same finding was recorded when comparing subgroups of the intravenously administered ZnO NPs Group (III), as a significant increase of serum LDH level was detected in subgroup IIIB in comparison with subgroup IIIA ($P < 0.05$) [Table 1].

Characterization of zinc oxide nanoparticles

Examination of NPs using TEM revealed aggregates of spheroid tiny electron-dense particles of a relatively similar size range of 15.3–34.8 nm [Figure 1].

H and E-stained sections

No structural differences were observed in all subgroups of the control animals of Group (I). The lung appeared having sponge-like architecture. The alveolar wall was lined by the alveolar epithelium: Type I and II pneumocytes. Type I pneumocytes formed most of the alveolar epithelium, appearing flat with flattened nuclei. Type II pneumocytes were scattered cuboidal cells having round nuclei and were bulging into the alveolar lumina. Thin interalveolar septa were separating alveolar spaces. Simple cubical or low columnar epithelium was seen lined the bronchioles with visible bulging dome-shaped club cells in between [Figure 2a].

In subgroup IIA, few focal areas of apparently thickened interalveolar septa were seen infiltrated with mononuclear cells. Many dilated blood vessels exhibited focal disruption and focal thickening of their tunica media together with mononuclear cellular infiltration. Furthermore, acidophilic hyaline exudate was seen filling the blood vessels lumina [Figure 2b].

On examination of subgroup IIB, apparent thickening of many interalveolar septa was demonstrated in comparison with Group I and to subgroup IIA. These thickened septa, as well as the bronchiolar wall, were seen heavily infiltrated by mononuclear cells. Apparent thickening of most of the blood vessels’ wall was observed as compared to Group I and to subgroup IIA. Focal disruption of the blood vessels’ endothelium and muscle fibers of the tunica media was noticed. Some of these blood vessels were seen congested [Figure 2c and d].

On the other hand, subgroup IIIA showed minimal thickening of some interalveolar septa in a few focal parts could be detected. Mild infiltration of few interalveolar septa with few mononuclear cells together with homogenous acidophilic exudate could be seen [Figure 2e] (you mean as compared to control).

Table 1: Serum lactate dehydrogenase level in different groups

| Subgroup      | Serum LDH level |
|---------------|-----------------|
| Group I       | 193.25±29.14    |
| Subgroup IIA  | 903.5±71.57$^*$ |
| Subgroup IIB  | 1406±128.41$^{**}$ |
| Subgroup IIIA | 497.75±58.66$^{***}$ |
| Subgroup IIIB | 1152.5±28.41$^{***}$ |

Values are presented as mean±SD. $^*$Compared to Group I statistically significant (Control group) ($P<0.05$), $^{**}$Compared to Group IIA statistically significant (low-dose intranasal subgroup) ($P<0.05$), $^{***}$Compared to Group IIIB statistically significant (high-dose intranasal subgroup) ($P<0.05$), $^{***}$Compared to Group IIIA statistically significant (low-dose intravenous). LDH: Lactate dehydrogenase, SD: Standard deviation.
In subgroup IIIB, exfoliated epithelial cells were seen occupying the lumen of many bronchioles, with the appearance of many club cells with pyknotic nuclei. In contrast to subgroup IIIB, some bronchioles demonstrated extravasated red blood cells (RBCs) and acidophilic exudate in their lumen. Cytoplasmic vacuolations were also seen in the smooth muscle fibers of the bronchiolar wall in some areas. Mononuclear cellular infiltration of the bronchiolar walls [Figure 2f].

Masson’s trichrome stained sections (histogram 1)
Examination of Group I sections revealed the presence of few collagen fibers in the interalveolar septa and in the walls of bronchioles [Figure 3a]. The mean area percentage of collagen fibers was measured as 1.15 ± 0.59.

Subgroup IIA showed few collagenous fibers deposition in the interstitial tissue between the alveoli and in the adventitia of the bronchioles [Figure 3b]. The mean area % of collagen fiber was significantly increased \((P < 0.05)\) as compared to Group I, measuring 8.36 ± 2.5.

Moreover, subgroup IIB demonstrated an apparent increase in the content of collagen fibers in the interalveolar septa as compared to Group I and subgroup IIA [Figure 3c]. The mean area % of collagen fiber was significantly increased \((P < 0.05)\) as compared to Group I and subgroup IIA, measuring 21.08 ± 4.97.

On the other hand, subgroup IIIA exhibited minimal collagenous fibers deposition in the interstitium in between
alveoli and the walls of the bronchioles [Figure 3d]. The mean area % of collagen fiber was detected as 4.67 ± 1.41, which was significantly decreased as compared to subgroup IIA.

Subgroup IIIB revealed an apparent mild increase in the content of collagen fibers in the walls of bronchi and in the interalveolar septa in focal areas as compared to those of the control group. Nevertheless, they were apparently less than that of subgroup (IIB). Some collagen fibers were seen, extending to alveolar spaces [Figure 3e]. The mean area percentage of collagen fibers was 18.26 ± 4.24. That was significantly decreased as compared to subgroup IIB. Although, it was significantly increased as compared to Group I and subgroup IIIA [Figure 3f].

**Immunohistochemical analysis of tumor necrosis factor-alpha (histogram 2)**
The control group showed a minimal positive immune reaction in cells of the interalveolar septa [Figure 4a]. The mean number of TNF-α-positive cells was 2.2 ± 1.3.

Subgroup IIA showed mild positive cytoplasmic immune reaction in mononuclear cells infiltrating the interalveolar septa [Figure 4b]. The mean number of TNF-α-positive cells was 10.4 ± 1.82, which was significantly increased as compared to Group I.

Subgroup IIB demonstrated apparent increase in the TNF-α immune reactivity as compared to that of Group I and subgroup IIA. This was reflected by the presence of numerous immune positive mononuclear cells exhibiting brownish cytoplasmic granules [Figure 4c]. The mean number of TNF-α-positive cells was 24.8 ± 3.35, which was significantly increased (P < 0.05) as compared to Group I and subgroup IIA.

On the other hand, subgroup IIIA revealed less brownish cytoplasmic immune reaction in the alveolar macrophages as compared to those of the subgroup IIA [Figure 4d]. The mean number of TNF-α positive cells was significantly decreased as compared to subgroup IIIA, measuring 5.2 ± 1.3.

In addition, subgroup IIIB demonstrated moderate positive brown immune cytoplastic reaction, mainly in the mononuclear cells infiltrating the interalveolar septa. This was apparently increased when compared to that of the control group and both subgroups IIA and IIIA, however, apparently less than that of subgroup IIB [Figure 4e]. The mean number of TNF-α positive cells was 16.6 ± 2.3. This value was significantly decreased as compared to subgroup IIB. Yet, it was significantly increased as compared to Group I and subgroup IIIA [Figure 4f].

**Ultrastructural examination of lung**
Group I showed type I pneumocytes with their flat, slightly elongated euchromatic nuclei and few organelles in their attenuated cytoplasm. Type II pneumocytes could be easily recognized with their large euchromatic nuclei and characteristic rounded lamellar bodies. Lysosomes could be seen inside their cytoplasm, and short microvilli were observed protruding from their surface [Figure 5].

Subgroup IIA showed type II pneumocytes with few dissolved lamellar bodies. Alveolar macrophages were seen with the large eccentric hyperchromatic nucleus and many lysosomes. Moderately electron-dense material was noticed in the interalveolar septum [Figure 6].

Subgroup IIB showed multiple cytoplasmic vacuolation of partially dissolved lamellar bodies of pneumocyte type II. Extravasated RBCs were seen obliterating the capillaries in the lung interstitium. Alveolar macrophages were noticed in the alveolar space and in the septa in between alveoli. They appeared with large eccentric hyperchromatic nucleus and many lysosomes [Figure 7a and b].
In subgroup IIIA, few pneumocyte type II appeared with dissolved lamellar bodies. There was the infiltration of the interstitium with eosinophils with their bilobed nuclei and oval granules with electron dense core [Figure 8].

Subgroup IIIB showed the appearance of some type I and type II pneumocytes having nuclei with irregular nuclear envelopes. Irregular chromatin distribution was seen in the nuclei of pneumocyte type II, together with partial dissolved lamellar bodies [Figure 9a]. Interalveolar septa showed infiltration of the interstitium with eosinophils exhibiting cytoplasmic specific granules with electron-dense core. Macrophages were also seen with many lysosomes and phagocytic vacuoles [Figure 9b].

**DISCUSSION**

The current study evaluated the histological and ultrastructural changes in rat lung induced by intranasal and intravenous injection of ZnO NPs in rats. Wide variation in NPs toxicity was reported, and not only depends on their size and dose but also on the route of administration and duration of exposure. Hence, in the present study, low and high doses of ZnO NPs (4 and 30 mg/kg, respectively) were used once intranasally and intravenously, aiming to detect the acute consequence of increasing dose ZnO NPs administration by dissimilar routes on the rat lung structure.

Examination of ZnO NPs by TEM in the present study showed their size ranging between 15 nm and 34 nm. The smaller-sized NPs exhibited greater particle solubility than the particles of larger surface area. The small size of NPs might be a cause of their high surface area. Consequently, this might increase their binding to serum proteins and promote their surface receptors
α free radical’s production causes oxidative stress resulting in apoptosis. An imbalance between antioxidant defense and expression of genes that are involved in inflammation and mitochondrial function and may also cause variations in the systems in which there is an increase in the oxidant ones that persuade an imbalance between oxidant and antioxidant systems.

Going with these findings, ZnO NPs were reported to detect as compared to Group I. An apparent thickening of most of the blood vessels and alveolar septa was shown in the current study mainly in the intranasal extravasation of RBCs in lung alveolar spaces and alveolar connective tissues and stimulate the proliferation of myofibroblasts. These cells began to form and deposit immature collagen fibers within the lung interstitium.

In the present study, the LDH levels were increased in all subgroups compared to Group I. However, there was a significant increase in the high-dose subgroups IIB and IIIB as compared with the low-dose subgroups IIA and IIIA. ZnO NPs was reported to cause elevation of the serum level of LDH in the rat after repeated oral administration. It was also reported to be significantly increased in mice after exposure to ZnO NPs by 24 h, proposing that ZnO NPs caused acute lung cytotoxicity. The acute increase in the level of LDH proposed that cell death was taking place in the lungs as early as 24 h after ZnO NP exposure.

In the present study, the interalveolar septa with apparent thickening by inflammatory cells infiltration were demonstrated in all experimental subgroups compared to Group I. However, there was an increase thickening after high-dose administration in subgroups IIB and IIIB as compared with low dose in subgroups IIA and IIIA (intranasal and intravenous routes, respectively). This thickening led to the narrowing of some air spaces and compensatory widening of others. Moreover, extravasation of RBCs in lung alveolar spaces and alveolar septa was shown in the current study mainly in the intranasal and in the high-dose subgroup (IIIB) of the intravenous group. Apparent thickening of most of the blood vessels’ and focal disruption of the blood vessels’ endothelium wall was detected as compared to Group I.

Going with these findings, ZnO NPs were reported to persuade an imbalance between oxidant and antioxidant systems in which there is an increase in the oxidant ones that cause damage. The reactive oxygen species can disturb mitochondrial function and may also cause variations in the expression of genes that are involved in inflammation and apoptosis. An imbalance between antioxidant defense and free radical’s production causes oxidative stress resulting in cell damage, and it is also very important in the pathogenesis of some diseases.

In the present study, exfoliated epithelial cells were seen in high-dose intravenous subgroup (IIIB) occupying the lumen of many bronchioles. The epithelial shedding could be a consequence of some toxic inflammatory mediators. Furthermore, the increased epithelial fragility and shedding might be due to the weak attachment of the epithelial cells to the basement membrane.

In the present experiment, macrophages were observed by TEM. After NPs engulfing by macrophages, they induce an inflammatory reaction and stimulate the production of interleukin (IL)-1β, IL-18, and TNF-α. This can explain the presence of numerous mononuclear cells, including eosinophils and macrophages which were noticed by TEM in both subgroups of the intranasal group, and in the high-dose intravenous subgroup (IIIB) of the present work. Eotaxin and IL-13 were reported to be secreted in rats exposed to ZnO NPs and were suggested to be key mediators of eosinophil recruitment. IL-13 was stated to be tangled in the regulation of eosinophil infiltration and immunoglobulin E synthesis. Therefore, IL-13 activated by ZnO NPs might play a vital role in the development of the histopathological effects that demonstrated upon ZnO NPs exposure.

In the present study, examination of Masson’s trichrome-stained sections showed the presence of collagen fibers in the alveolar and bronchial walls. There was a significant increase in the area percentage of collagen fibers in all experimental subgroups as compared to Group I. However, there was a significant increase after high-dose administration in subgroups IIB and IIIB as compared with low dose in subgroups. TNF-α was documented to increase the manufacture of transforming growth factor-β1, which was the major stimulant of fibroblasts to secrete collagen fibers. Meanwhile, IL-1 β was also reported to increase the expression of platelet-derived growth factor (PDGF) and its receptors on the fibroblasts of the lungs. Therefore, the synchronized secretion of PDGF and its receptors might play a role in attracting fibroblasts from the interalveolar connective tissues and stimulate the proliferation of myofibroblasts. These cells began to form and deposit immature collagen fibers within the lung interstitium.

Immunohistochemical stained sections for TNF-α in the present work revealed a significant increase in the number of immune positive inflammatory cells infiltrating the interalveolar septa. This was detected in all experimental subgroups as compared with that of Group I. Meanwhile, they were significantly increased after high-dose administration in subgroups IIB and IIIB as compared with low dose in subgroups. TNF-α production was increased in bronchoalveolar lavage samples of mice after 10 and 20 μg ZnO NPs exposures for the 24-h follow-up experiment. The TNF-α is considered an early marker of inflammation. It is produced by many cells, such as activated macrophages, endothelial cells, and epithelial cells, and it causes recruitment and activation of neutrophils.
Similarly, intratracheal instillation of ZnO NP in rat increased inflammatory cells and many cytokine levels as TNF-α and IL-6 in the bronchoalveolar fluid at 24 h after administration of ZnO NP. Consequently, TNF-α was suggested to cause ZnO NP-induced acute inflammatory reactions with enrollment of neutrophils, monocytes, and macrophages.\(^{[31]}\) Hence, it can be suggested that ZnO NPs might cause activation of the immune cells to secrete cytokines that result in inflammation. The ZnO NPs-induced TNF-α expression was previously demonstrated to be mediated through the ROS-ERK-Egr-1 pathway.\(^{[32]}\) Remarkably, toll-like receptors (TLRs) related to the innate immune response have also been concerned in ZnO NP-induced expression of the proinflammatory cytokines such as TNF-α.\(^{[33]}\) Furthermore, inflammatory reactions causing upregulation of IL-1β, IL-6, and TNF-α strongly depend on mitogen-activated protein kinase (MAPK) signaling mediated by TLR6.\(^{[34]}\)

### Conclusion

It was concluded that the ZnO NPs administration caused toxic effects on the histological structure of the male albino rat lung. These effects were route and dose-dependent, being more obvious after intranasal administration.

### Recommendation

It is recommended to limit the dose of ZnO NPs to the level that just meets the need of their usage in the medical field. Further studies on the outcome of ZnO NPs on other organs are needed. Finally, it is recommended to do further evaluation of the toxicity of ZnO NPs using diverse sizes, duration, and possible reversible effects after their cessation.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

### References

1. Kim CS, Le ND, Xing Y, Yan B, Tonga GY, Kim C, et al. The role of surface functionality in nanoparticle exocytosis. Adv Healthe Mater 2014;3:1200-2.
2. Hoseinzadeh E, Makhdoumi P, Taha P, Hossini H, Stelling J, Kamal MA, et al. A Review on nano-antimicrobials: Metal nanoparticles, methods and mechanisms. Curr Drug Metab 2017;18:120-8.
3. Xi C, Zhou J, Du S, Peng S. Autophagy upregulation promotes macrophages to escape mesoporous silica nanoparticle (MSN)-induced NF-kB-dependent inflammation. Inflamm Res 2016; 65:325-41.
4. Kumari L, Li W. Synthesis, structure and optical properties of zinc oxide hexagonal microprisms. Crystal Res Technol 2010;45:311-5.
5. Husein A. Natural Product-Based Fabrication of Zinc-Oxide Nanoparticles and Their Applications. Nanomaterials and Plant Potential. Springer Nature Switzerland AG, Cham; 2019. p. 193-219.
6. Zare M, Namratha K, Byrrapa K, Surendra DM, Yallappa S, Hungund B. Surfactant assisted solvothermal synthesis of ZnO nanoparticles and study of their antimicrobial and antioxidant properties. J Materials Sci Technol 2018;34:1035-43.
7. Rasmussen JW, Martinez E, Louka P, Wingett DG. Zinc oxide nanoparticles for selective destruction of tumor cells and potential for drug delivery applications. Expert Opin Drug Deliv 2010;7:1063-77.
8. Lin D, Xing B. Phytoxicity of nanoparticles: Inhibition of seed germination and root growth. Environ Pollut 2007;150:243-50.
9. Choi SJ, Choy JH. Biokinetics of zinc oxide nanoparticles: Toxicokinetics, biological fates, and protein interaction. Int J Nanomedicine 2014;9 Suppl 2:261-9.
10. Al-Ajmi MF, Hussain A, Ahmed F. Novel synthesis of ZnO nanoparticles and their enhanced anticancer activity: Role of ZnO as a drug carrier. Ceramics Int 2016;42:4462-9.
11. Wu T, Tang M. Review of the effects of manufactured nanoparticles on mammalian target organs. J Appl Toxicol 2018;38:25-40.
12. De Mattes V. Exposure to inorganic nanoparticles: Routes of entry, immune response, bio distribution and in vitro/in vivo toxicity evaluation. Toxics 2017;5:4-29.
13. Chuang HC, Chen HT, Chang CN, Yan YH, Yuan TH, Wang JS, et al. Cardiopulmonary toxicity of pulmonary exposure to occupationally relevant zinc oxide nanoparticles. Nanotoxicology 2014;8:593-604.
14. Wang D, Li H, Liu Z, Zhou J, Zhang T. Acute toxicological effects of zinc oxide nanoparticles in mice after intratracheal instillation. Int J Occup Environ Health 2017;23:11-9.
15. Morimoto Y, Izumi H, Yoshira T, Tomonaga T, Oyabu T, Myojo T, et al. Evaluation of pulmonary toxicity of zinc oxide nanoparticles following inhalation and intratracheal instillation. Int J Mol Sci 2016;17:1241.
16. Choi J, Kim H, Kim P, Jo E, Kim HM, Lee MY, et al. Toxicity of zinc oxide nanoparticles in rats treated by two different routes: Single intravenous injection and single oral administration. J Toxicol Environ Health A 2015;78:226-43.
17. Wachsmann P, Lamprecht A. Polymeric nanoparticles for the selective therapy of inflammatory bowel disease. Methods Enzymol 2012;508:377-97.
18. Suvarna SK, Layton C, Bancroft JD. Bancroft’s Theory and Practice of Histological Techniques. 7th ed. New York, London and Madrid: Churchill Livingstone; UK, El Sevier; 2013. p. 408, 500.
19. Bancroft JD, Gamble MN. Theory and Practice of Histological Techniques. 6th ed. New York, London and Madrid: Churchill Livingstone; 2007.
20. Lee JH, Kim YS, Song KS, Ryu HR, Sung JH, Park JD, et al. Biopersistence of silver nanoparticles in tissues from Sprague-Dawley rats. Part Fibre Toxicol 2013;10:36.
21. Xie G, Sun J, Zhong G, Shi L, Zhang D. Biodistribution and toxicity of intravenously administered silica nanoparticles in mice. Arch Toxicol 2010;84:183-90.
22. Shokouhian A, Soheili S, Moradhaseli S, Fazli L, Ardestani MS, Ghorbani M. Toxicity of zinc oxide nanoparticles in lung tissue after repeated oral administration. Am J Pharmacol Toxicol 2013;8:148.
23. Chuang HC, Chuang KJ, Chen JK, Hua HE, Shen YL, Liao WN, et al. Pulmonary pathobiology induced by zinc oxide nanoparticles in mice: A 24-hour and 28-day follow-up study. Toxicol Appl Pharmacol 2017;327:13-22.
24. Heng BC, Zhao X, Xiong S, Ng KW, Boey FY, Loo JS. Toxicity of zinc oxide (ZnO) nanoparticles on human bronchial epithelial cells (BEAS-2B) is accentuated by oxidative stress. Food Chem Toxicol 2010;48:1762-6.
25. De Berardis B, Civitelli G, Condello M, Lista P, Pozzi R, Arancia G, et al. Exposure to ZnO nanoparticles induces oxidative stress and cytokotoxicity in human colon cancer cells. Toxicol Appl Pharmacol 2010;246:116-27.
26. Vandebriel RJ, De Jong WH. A review of mammalian toxicity of ZnO nanoparticles. Nanotechnol Sci Appl 2012;5:61-71.
27. Nakayama M. Macrophage recognition of crystals and nanoparticles. Front Immunol 2018;9:103.
28. Fulkerson PC, Fischetti CA, Rothenberg ME. Eosinophils and CCR3 regulate interleukin-13 transgene-induced pulmonary remodeling. Am J Pathol 2006;169:2171-27.
29. Byrne JD, Baugh JA. The significance of nanoparticles in particle-induced pulmonary fibrosis. Megill J Med 2008;11:43-50.
30. Pereda J, Sabater L, Aparisi L, Escobar J, Sandoval J, Viña J. Interaction between cytokines and oxidative stress in acute pancreatitis. Curr Med Chem 2006;13:2775-87.
31. Sayes CM, Reed KL, Warheit DB. Assessing toxicity of fine and nanoparticles: Comparing in vitro measurements to in vivo pulmonary
Mohammed, et al.: Effect of zinc oxide nanoparticles on rat lung

toxicity profiles. Toxicol Sci 2007;97:163-80.
32. Jeong SH, Kim HJ, Ryu HJ, Ryu WJ, Park YH, Bae HC, et al. ZnO nanoparticles induce TNF-α expression via ROS-ERK-Egr-1 pathway in human keratinocytes. J Dermatol Sci 2013;72:263-73.
33. Chang H, Ho CC, Yang CS, Chang WH, Tsai MH, Tsai HT, et al. Involvement of MyD88 in zinc oxide nanoparticle-induced lung inflammation. Exp Toxicol Pathol 2013;65:887-96.
34. Roy R, Singh SK, Das M, Tripathi A, Dwivedi PD. Toll-like receptor 6 mediated inflammatory and functional responses of zinc oxide nanoparticles primed macrophages. Immunology 2014;142:453-64.