DOI: 10.4149/BLL_2015_071

Bratisl Lek Listy 2015; 116 (6)
373 – 378

EXPERIMENTAL STUDY

Toxic effects of the Fe_2O_3 nanoparticles on the liver and lung tissue

Sadeghi L[1], Yousefi Babadi V[2], Espanani HR[3]

Department of Biochemistry, Faculty of Biological Science, Tarbiat Modares University, Tehran, Iran.
v.yoosefi@gmail.com

Abstract
Iron oxide nanoparticles are magnetic nanoparticles which have widespread application in MRI and heat therapy of cancer as contrast elements. They are also used effectively for drug and gene delivery because of effective penetrating to the cells and tissues. However, these features cause Fe_2O_3 nanoparticles have toxic effects that are not completely understood yet. In this study, effects of iron oxide nanoparticles on lung tissue in adult male Wistar rats were studied. We used pulmonary inhalation method for nanoparticle administration and used ether as a helper. Our results showed administered nanoparticles penetrated to the circulation and rapidly reached to liver and created serious inflammation in lung and liver tissues. This study used two different nanoparticle doses (20 and 40 mg/kg) and two exposing numbers (7 and 14 times). Results showed significant enhancement of free radicals and reduction of the GSH in lung tissue. Histological studies showed nanoparticle treatment of rats caused pulmonary emphysema, interstitial hyperemia and inflammation in lungs. By increasing the administrated dose lung tissue showed all of the mentioned symptoms with increased intensity. Nanoparticle exposition causes presence of neutrophils, lymphocytes and eosinophils in the lung tissue that confirmed there is a serious pathologic condition. Hepatic cells injuries cause penetration of the hepatic enzymes in to the blood serum *(Tab. 2, Fig. 4, Ref. 32)*. Text in PDF *www.elis.sk.*
Key words: reactive oxygen species, liver cell necrosis, inflammation, pulmonary administration, immune system.

Introduction

Nanoparticles, based on physical and chemical properties and special shape, size and surface area to volume ratio, are unique for biological, medical and industrial applications. One of the most useful features of this is high surface area of nanoparticles that causes its widespread applications in medical science and production of nano based drugs (1).

Nanotechnology has an enormous potential in the field of human imaging and early recognition of disease, with the tailoring of specific nano-agents for molecular imaging in the context of Magnetic Resonance Imaging, ultrasound, optical imaging, and X-ray imaging. Magnetic nanoparticle also can be used widely in drug delivery, gene delivery and targeting (2). But for the mentioned application magnetic nanoparticles should be biocompatible and biodegradable. Nanoparticles with a size between 10–100 nm should be used for biological purposes because nanoparticles that are smaller than 10 nm are excreted by the kidneys and particles with larger size than 200 nm don't pass through the cell membrane easily and they can induce immune system as a foreign elements and be removed from the body (3).

[1]Department of Biochemistry, Faculty of Biological Science. Tarbiat Modares University, Tehran, Iran, [2]Department of Physiology, Payam Noor university of Iran, Iran, and [3]Department of Biology, Faculty of Sciences, Payam Noor University of Isfahan, Isfahan, Iran

Address for correspondence: H.R. Espanani, MSC, Physiology Department of Biology, Faculty of Sciences, Payam Noor University of Iran, Employee Social Security, Isfahan, Iran.
Phone: +98381.2223586, Fax: +98381.2223587

Published literature showed material at the nano size has relatively greater toxicity than large sizes materials, because nanoparticles are highly reactive and cause oxidative stress in humans and animals. Nanoparticles can pass through cell membrane easily and even pass through blood-brain barrier and blood-testes barrier (4), so they can affect all organs of the body (5). Between all of the organs lung is the primary target of nanoparticles (NPs), but NPs can enter the circulation and migrate to various organs and tissues (including the brain, heart, kidneys), where they can build up and injure organs that are sensitive to oxidative stress (5).

Biomedical applications of iron oxide magnetic nanoparticles are wider than others due to biocompatibility, high stability and ease of use. Magnetic nanoparticles such as Fe_3O_4 and Fe_2O_3 have more applications in drug delivery (6, 7). Because of widespread application of these particles in various industries human exposition to them increased, so investigation of nanoparticle role in cell growth and survival has more importance (8). Human skin, lungs and digestive system are the most common entry routes for nanoparticles and its pathogenicity (9).The airborne nanoparticles have high mobility and can be inhaled into the respiratory system easily (10).

One of the most common damaging effects of nanoparticles are rise of reactive oxygen species (ROS) that refer to oxidative stress in human and animal tissues. It's confirmed that almost all of the studied nanoparticle produce reactive oxygen species and it is the main mechanism for nanoparticle toxicity that can lead to inflammation and apoptosis (11).

Slow and direct injection of the test material into the lungs through the trachea is used as an alternative to the normal ambient

conditions in many studies. In this method we can ensure that all of the nanoparticles that entered the lung are absorbed completely (12). Therefore in this study lung is the first organ that is exposed to the NPs but liver as an internal organ is away from these. This study aimed at showing that NPs reach all parts of the body, for this we chose lung and liver as goal tissues.

We investigated the effects of administrated gamma Fe_2O_3 nanoparticles at different doses (20 and 40 mg/kg) and different injection numbers (7 times injection in 14 day and 14 times in 28 day) on rats lung and liver tissues. We measured GSH and ROS amounts as a representative of tissue oxidative status, appearance of lung tissue assessed as a supplementary data. Liver enzymes (ALT, AST and ALP) were measured to assess liver health condition.

Material and methods

Nano iron oxide (Fe_2O_3) particles prepared from Pishgamane Nano Company (Iran-Mashhad). XRD results showed nano Fe_2O_3 is in crystalline phase with a size of 20 nm. Purification of nano Fe_2O_3 was determined as 99.5 % by ICP-MS. Toxicological assessment of manmade nanomaterials requires information about the route (inhalation, oral, dermal) of exposure, as well as their complete physicochemical characterization of them in order to provide thorough information as summarized in Table 1.

This study was conducted on experimental animals and we used adult male wistar rats weighing 250–300 g obtained from the animal house. Animals whit average age of 3–5.2 months were selected. Testing was carried out at a temperature of 28–32 degree of centigrade at day duration of 12 hours and 12 hours of dark light. Municipal tap water was used and adjusted to drinking water and eating animals for food by rats (feed compression) that the company prepared feed was buying. Nanoparticle administration was done by inhalation with anesthetic ether. Experimental animals were randomly divided into six groups (8 rats in each group) as follows: first control group fed by usual water and food. Second control group that referred to placebo, administrated by 1 ml of distillated water every other day by inhalation for equivalency of shock that was caused by inhalation of ether. In groups 3 and 4 1 ml Fe_2O_3 nanoparticles were injected in 20 and 40 mg/kg doses respectively, administration was repeated every other day. This continued until 7 injections in 14 days. In other groups from 5th and 6th 1 ml Fe_2O_3 nanoparticles were inhaled in 20 and 40 mg/kg doses, respectively, administration was repeated every other day by inhalation. This continued until 14 injections in 28 days.

Tab. 1. Physical parameters of nano Fe_2O_3 used in the present study. These features are more important in chemical and biological properties of Fe_2O_3 nanoparticle.

Color	Brown
Morphology	Spherical
Crystalline phase	gamma
Specific surface area	40–80 M^2/g
Size	20 nm
Purity	99.5 %

Administration technique

One of the important techniques of exposing the animals to nanoparticle is pulmonary administration or inhalation. Pulmonary administration is widely used to evaluate the different materials effects in the body (13). This method was also used for evaluation of toxicity potential of materials in air routes. In this method, penetration into the respiratory tract depends on the substance dose, particle shape, size and species of animals. In this method particles are inhaled into the lungs without passing through the upper respiratory passages, so some of the defense mechanisms related to the upper respiratory tract are removed (12, 14). One of the advantages of these methods is receiving the whole administrated dose without any losses (14). It can be ensured that the administrated dose is the dose received by the host animal. Fluidity of administrated material is more important in this way due to possibility of choking.

Reactive oxygen species measurement

Measurement of ROS was based on the methods of Wang and Joseph with minor modification (15). Lung tissue homogenates were prepared and diluted in ice-cold buffer to obtain a concentration of 5 mg tissue/ml. The homogenates were then pipetted into 24-well plates (0.45 ml/well) and allowed to warm to room temperature (21 °C) for 5 min. Then, 5 ml of DCFH-DA (10 mM final concentration) was added to each well and the plates were pre incubated for 15 min at room temperature to allow the DCFH-DA to be incorporated into any membrane-bound vesicles and the diacetate group was cleaved by esterases. After pre-incubation, 50 ml of the appropriate concentration of Fe^{2+} was added to the wells. After 30 min (at 37 °C), the conversion of DCFH to the fluorescent product DCF was measured using a Perkin Elmer luminescence spectrometer LS 55 with excitation at 485 nm and emission at 530 nm. Excitation and emission slit were both set at 5 nm. Background fluorescence (conversion of DCFH to DCF in the absence of homogenate) was corrected by the inclusion of parallel blanks. The interference of NPs alone was subtracted from the results. ROS production was quantified from a DCF standard curve and results expressed in pmol DCF formed/mg protein/min. Protein concentration did not differ between different groups and was determined by Bradford method in homogenates (16).

Measurement of glutathione

Total glutathione (GSH) was measured spectrophotometrically in tissues as previously described (17). GSH was measured by adding the standard or sample to 100 µL of a 1:1 mixture of 3 units/mL glutathione reductase with 0.67 mg/mL 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB). The reaction was initiated by the addition of 20 µL of 0.67 mg/mL NADPH and the increase in absorbance at 412 nm was monitored. Values measured in tissues were normalized to protein content. The limit for detection of GSH was 0.2 µM.

Enzymes measurement

One day after the end of experiments blood samples of all animals were prepared from retro orbital eye veins. Samples were centrifuged at 3000 rpm for 15 minutes. After separating the serum

from the clot by Smplr, serums were freezed at a temperature of –20 °C and stored, then Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were assayed by Reitman and Frankel method (18).

Histological studies

For histological studies, one day after last injection, rats were anesthetized by diethyl ether and their lung and liver were removed and fixed by 10 % natural formalin buffer. After tissue processing, the samples were blocked in cylindrical paraffin blockers and then stained by hematoxilin–eosin (19). It should be noted that the sample's diameter is 5–6 μm. Stained tissue samples were studied by light microscope.

Results

Oxidative stress analysis (ROS and GSH)

One of the confirmed damaging effects of nanoparticles in the body is oxidative stress. It is assumed that reducing the size of a particle increases the ratio of surface area to mass. Because the reactive portion of the particle is on the surface, so increasing the relative surface area will increase the reactivity of a given amount of material. Reactive oxygen species (ROS) can be produced in the whole body and hurt all kinds of cells and result in inflammation and apoptosis in all of the organs. Therefore we tried to assess this parameter in lungs of treated animals by a standard method. ROS concentration in the lowest dose (20 mg/kg and 7 times administration) is 93.21 ± 4.8 pmol/mg protein that shows 3 fold increases versus control. The ROS assay showed free radicasl increased dose dependently. Therefore the ROS amount at 40 mg/kg, 14 times was 305.25 ± 5.0 that was the highest amount of measured ROS and is statistically significant. This is more than 10 fold increase compared to control. Increasing of ROS is dose dependent but is not linear probably due to aggregation of nanoparticles in high doses that reduced its toxicity. It is interesting to note that inhalation of ether and distillated water caused an increase by 0.16 fold of ROS in placebo rats (Fig. 1).

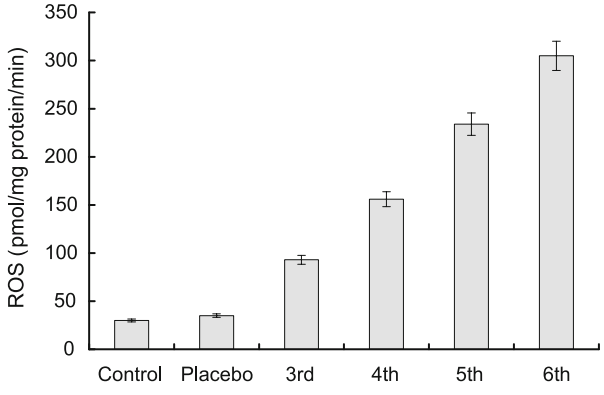

Fig. 1. Measurement of ROS concentration in control and treated groups.

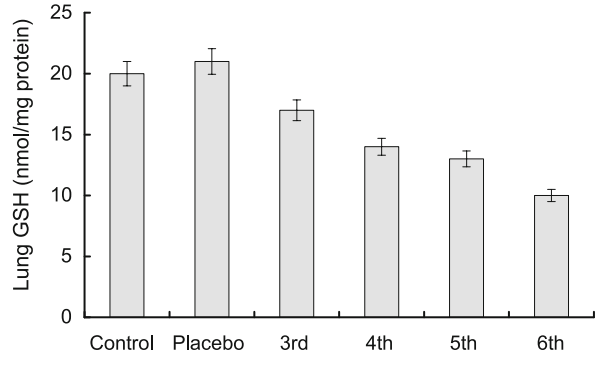

Fig. 2. Measurement of GSH concentration in control and treated groups.

Tab. 2. Measurement of the hepatic enzymes. Results showed by treating rats by iron oxide nanoparticles that ALT increased and this enhancement is dose dependent. But AST and ALP did not change significantly.

Groups	ALT (u/l)	AST (u/l)	ALP (u/l)
Control	48.00±3.31	134.12±7.98	318.87±6.90
Placebo	53.00±3.37	193.50±8.21	320.62±6.80
1st (7 times, 20 mg/kg dose)	32.62±3.56	225.37±10.35	247.00±5.21
2nd (7 times, 40 mg/kg dose)	41.00±4.89	229.00±10.65	269.62±5.20
3rd (14 times, 20 mg/kg dose)	78.25±4.76*	258.50±10.38*	383.62±6.51
4th (14 times, 40 mg/kg dose)	70.87±4.06*	271.12±9.40*	269.02±5.92

Asterisks * indicate significant variances at $p < 0.05$ for each test group compared to the control group

GSH is one of the most important barriers against oxidative damage. Note that reduced glutathione (GSH) represents reducing power or status of the cells or tissues. GSH is a tripeptide comprised of glutamate, cysteine, and glycine and is synthesized and utilized in every organ throughout the body therefore assessing the GSH amount can be used for oxidant agent concentration and oxidant damaging measurements in the body. Our results showed decreasing GSH concentration in the presence of nanoparticles. This indicated weakness of the antioxidant barrier against the nanoparticle. Reduction of the GSH concentration is dose dependent and more reduced with high doses, for example in 40 mg/kg and 14 times treatment was GSH reduced more than 2 fold compared to control (Fig. 2).

Hepatic enzymes concentration

Increase of liver enzymes activity in the serum refers to liver damage. Increased activity of liver enzymes Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) in blood serum is because of leakage from the hepatic cells cytosol into the blood stream, this leakage is increased by liver damage (20). So AST and ALT are good indicators for liver function.

Statistical studies and comparison of the hepatic enzymes (ALT, ALP and AST) in animals that were treated by iron oxide nanoparticles and controls were done. Results are shown in Table 2. Asterisks indicate significant differences at $p < 0.05$ for each test group compared with control. Results show there is no significant difference between experimental and control groups in the ALP activity (Tab. 2). AST concentration increased only in 2 last groups that received 14 times NPs so were more exposed to the NPs. But ALT enzyme

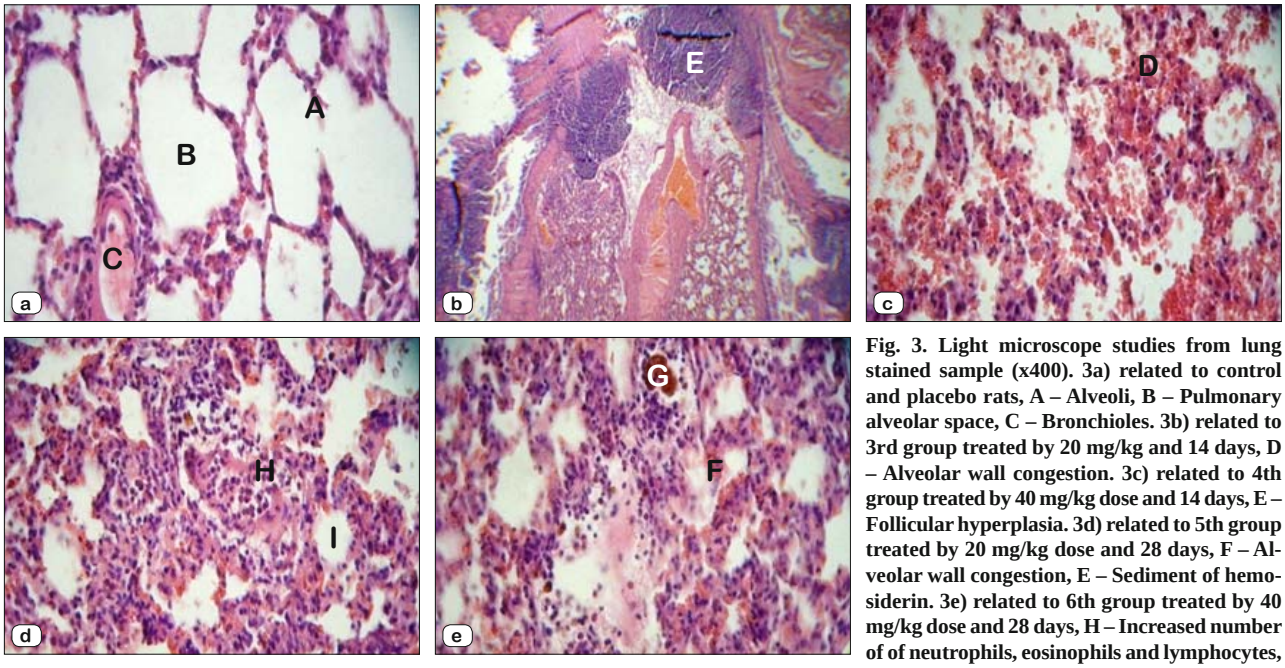

Fig. 3. Light microscope studies from lung stained sample (x400). 3a) related to control and placebo rats, A – Alveoli, B – Pulmonary alveolar space, C – Bronchioles. 3b) related to 3rd group treated by 20 mg/kg and 14 days, D – Alveolar wall congestion. 3c) related to 4th group treated by 40 mg/kg dose and 14 days, E – Follicular hyperplasia. 3d) related to 5th group treated by 20 mg/kg dose and 28 days, F – Alveolar wall congestion, E – Sediment of hemosiderin. 3e) related to 6th group treated by 40 mg/kg dose and 28 days, H – Increased number of of neutrophils, eosinophils and lymphocytes, I – Alveolar degeneration, fatty degeneration.

activity increased in group receiving the highest dose rather than control (Tab. 2). Note that ALT activity increased in all treatment groups that received NPs but this was more enhanced at higher doses. Therefor it is possible that ALT and AST enzymes activity in serum are dose dependent. It has been demonstrated that nanoparticle properties and biological activities are size dependent and more affected by size alteration, so this result may be repeated in other studies.

Histological studies

1 day after last injection and after the endof the administration time course, rats were killed and lung and liver separated. Tissue samples were treated by formalin for fixation and stained by hematoxilin- eosin method and then studied by light microscope.

Histological studies showed hyperemia in alveolar wall capillaries with focal hemorrhage in this area (Fig. 3). In rats that were treated by 40 mg/kg dose in 14 day hyperemia was accompanied by bleeding in the lining air spaces and deposition of hemosiderin in parenchyma tissue of lung (Fig. 3). Histological changes observed after 7 administrations suggest that lung and liver cells exposed to NPs have been irritated and the inflammation increased with increased duration to 28 days (14 administrations). Inflammation intensity depends on the dose and administration duration.

Increase of the experiment time duration to 28 days increased bleeding in the lining air spaces and hyperplasia of lymphoid follicles in lung. By increasing both the time duration and dose that occurred in 6th group (28 day and 40 mg/kg) bleeding in air space, losing of alveols, changing of fat shapes, interstitial hyperemia and emphysema were observed in lung tissue, were presence of neutrophils, lymphocytes and eosinophils was also observed. Presence of these immune cells indicates an abnormal and unhealthy condition. Therefore we don't observe this variation in control and placebo groups who healthy and untreated by nanoparticle.

Histological studies of rats liver that were treated by nano iron oxide showed inflammation in liver (presence of lymphocytes in the liver tissue), interstitial congestion, fatty degeneration around the central vein and hepatocytes necrosis in all the hreated rats in 4 experimental groups compared to control and placebo (Fig. 4).

Dissection of rats also showed accumulation of aggregate nanoparticles in the pancreas, kidney, liver, and testes especially at high doses. This accumulation changed nanoparticle biological features and inhibited the infiltration of blood vessels and reduced the oxidative damage.

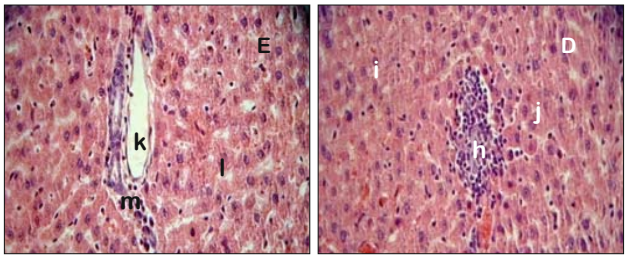

Fig. 4. Tissue sections of rat liver that were prepared by hematoxylin staining method (*400). Cellular necrosis (h and m) at higher doses of Fe_2O_3 NPs in 5th and 6th group that received NPs 14 times.

Discussion

Nanoparticles have very specific chemical and physical characteristics of size, shape and high surface area to volume ratio that facilitate its medical and biological applications. This material is distributed in the whole body rapidly after injection by circulation

and reaches to all of the organs and tissues (21). Before its application as medicine, effects of NPs on environment, biocompatibility and its toxic effects on human and animals should be assessed. These particles because of small size have high surface area and they are highly reactive, it is one important reason for their toxicity (22). Fe_2O_3 NPs due to optical, electrical and catalytic properties, have very important applications in various industries including industrial targeting, carrier of gene delivery, bioremediation, air and water filtration and cancer treatment (23). Therefor because of widespread use of Fe_2O_3 NPs humans and animals are exposed to this material (24). In 1990, the Journal of Aerosol Science published two of the first cornerstone papers on a higher than expected effect on lung inflammation patterns in rats exposed to NPs, effects that could not be predicted by just taking the chemical composition and the inhaled amounts of the particles into account (25, 26). After that different studies have been done by different administration methods and different sources of NPs that have various physicochemical properties that cause alternative effects on living systems. The aim of our study was to investigate the Fe_2O_3 NPs exposing effects in lung tissues such as antioxidant barrier (GSH and ROS concentration) and lung tissue appearance. We also investigated toxic effects of Fe_2O_3 NPs in the liver. Nanomaterials that are released into the environment through natural or artificial processes generally enter the body through the respiratory pathways (27) and the lung considered as the main entrance of the nanoparticles to the body (28) so lung is the first organ that is exposed to these materials. Lung cancer is one of the deleterious effects of exposing to NPs because they create mutation in cells by damaging the DNA that results in uncontrolled cell proliferation (12). The present study showed lung tissue and lung cells are engaged by NPs because they try to remove particles and prevent them to cross the blood circulation. By increasing the nanoparticle dose or exposing time, lung injuries increased and lung was unable to refin all of the NPs that is due to special shape, size and ability to cross the filter. Previous studies confirmed that NPs can pass through cell membrane easily and even pass through blood-brain barrier and blood-testes barrier (4), so it can affect all organs of the body in similar way as liver (29). NPs can enter the bloodstream and reach to the organs (including the brain, heart, kidneys) rapidly by blood circulation (5) and by production of free radicals they can create a serious biological response. Our results showed hepatic damages and penetration of the liver enzymes to the blood serum. Later experiment showed NPs by 20 nm size rather than small particles whit 250 nm size cause more frequently infection in lung tissue. Our results are in agreement with others and after 7 expositions to Fe_2O_3 NPs by 20 nm size there was significant inflammation in lung tissue. This inflammation depends on NPs surface area and surface properties. Mechanisms that are used by nanoparticles for inflammation are direct effects on alveolar macrophages causing phagocytic and cytoskeletal variations, all of these are caused by free radicals such as OH and H_2O_2 (30). The same results were obtained from the liver tissue studies (necrosis in liver cells). Results showed that in the presence of the NPs ROS concentration increased and this increase and and resulting tissue damage are does dependent. Therefore with higher doses and more frequent administrations in the 6th group ROS concentration increased. Note that GSH is consumed for neutralization of the ROS so reduction of the GSH concentration in presence of the NPs is predictable. Devaluation of the GSH such as growth of the ROS is dose dependent but like that is not linear because of NPs aggregation at higher doses. This aggregation reduces the toxic effects of NPs in higher doses.

Lung injection of Fe_2O_3 increased capillary permeability and blood coagulation time was also prolonged (31). In this study by extending the exposing time (which occurred in 5th and 6th group) bleeding in the lining of the air spaces and lung lymph follicle hyperplasia (Fig. 4) and necrotic cells in liver were observed. By increasing the administered dose lung and liver tissues showed all of the mentioned symptoms by increasing intensity. All events that occurred due to nanoparticle exposition cause presence of neutrophils, lymphocytes and eosinophils in the lung tissue (Fig. 4) that showed invasive behavior of nanoparticles and produced free radicals can induce immune system.

Particle–cell interactions occur especially in the lungs where there is a rich pool of ROS producers like the inflammatory phagocytes, neutrophils and macrophages. Overproduction of ROS activates cytokines and up-regulates interleukin (IL), kinases and tumor necrosis factor-α (TNF-α) as an indicator of pro-inflammatory signaling processes as a counter reaction to oxidative stress (32). Miura et al reported that the expressions of ho-1 and mt-2A, well-known oxidative stress related genes, were up-regulated by nano-silver treatment. These results indicated that apoptosis induction by silver nanoparticles (Ag NPs) may be created by ROS generation (11).

Note that inhalation of Fe_2O_3 only affected lung tissue and other organs such as spleen, liver and kidney did not show such symptoms (28), but this was opposed by this study. Previous experiments showed improvement of body weight by administration of the Fe_2O_3 NPs but weight of lungs significantly decreased. Wang and coworkers in 2010 administrated Fe_2O_3 through nose to rats and reached the same results (Pulmonary emphysema, interstitial hyperemia and inflammation in lung tissue) (30). Our results punctuated previous results and confirmed that size of administered nanoparticle, dose, routes of exposure and exposing time are more important factors that affected the mentioned pathologic symptoms.

Particles larger than 100 nm remain in intercellular space and can't pass through the cell membrane and enter in to circulation or remain attached to the vessel wall. Therefore particles with average size between 5 to 20 nm can be effectively used as a carrier to gene delivery and drug delivery. Results approved nanoparticle that were used in this study are able to pass through the lung cells membrane and enter the circulation so that they can be effectively used as a carrier for genes and drugs.

References

1. Jani P, Halberd GW, Langridge J, Florence AT. Nanoparticle uptake by the gastrointestinal mucosa: quantitation and particle dependency. J Pharm Pharmacol 1990; 42: 821–826.

2. Indira TK, Lakshmi PK. Magnetic Nanoparticles – Review. Int J Pharm 2010; 3: 1035–1042.

3. Gupta AK, Gupta M. Synthesis and Surfaceengineeringof iron oxide nanoparticles and their biomedical applications. Biomater 2005; 26: 3995–4021.

4. Mc Auliffe ME, Perry MG. Are nanoparticles potential male reproductive toxicant? A literature review. Nanotoxicol 2007; 1: 204–210.

5. Yousefi Babadi V, Najafi L, Najafi A, Gholami H, Beigi Zarji ME, Golzadeh J, Amraie E, Shirband A. Evaluation of iron oxide nanoparticles effects on tissue and enzymes of liver in rats. J Pharma Biomed Sci 2012; 23: 1–5.

6. Liang XJ, Chen C, Zhao Y, Jia L, Liang XJ, Chen C, Zhao Y, Jia L, Wang PC. Biopharmaceutics and Therapeutic Potential of Engineered Nanomaterials. Curr Drug Metab 2008; 9: 1–13.

7. Espanani HR, Fazilati M, Sadeghi L, Yousefi Babadi V, Bakhshiani S. Amraie E. Investigation the Zinc Oxide Nanoparticle's Effect on Sex Hormones and Cholesterol in Rat. Int Res J Biological Sci 2013; 2: 1–6.

8. Zhu MT, Feng WY, Wang Y, Wang B, Wang M, Ouyang H, Zhao YL. Chai ZH. Particokinetics and Extrapulmonary Translocation of IntratracheallyInstilled Ferric Oxide Nanoparticles in Rats and the Potential HealthRisk Assessment. Toxicolo Sci 2009; 107: 342–351.

9. Buzea C, Ivan I, Pacheco Blandino II, Robbie K. Nanomaterials and nanoparticles: Sources and toxicity. Biointerphases 2007; 2: 17–172.

10. Yah CS, Simate GS, Iyuke SE. Nanoparticles toxicity and their routes of exposures. Pak J Pharm Sci 2012; 25: 477–491.

11. Miura N, Shinohara Y. Cytotoxic Effect and Apoptosis Induction by Silver Nanoparticles in Hela Cells. Biochem Biophysi Res Com 2009; 390: 733–737.

12. Driscoll K, Costa D, Hatch G, Henderson R. Intracheal Instillation as an Exposure Technique for Respiratory Tract Toxicity: Uses and Limitation. Toxicolo Sci 2000; 55: 24–35.

13. Borm PJ, Kreyline W. Toxicological hazards of inhaled nanoparticles potential implications durg delivery. J Nanosci Nanotechnol 2004; 4: 521–531.

14. Roursgaard M. Inflammatory effects of nanoparticles in lungs of mice, PhD thesis, Faculty of Health Sciences, University of Copenhagen 2009; 4: 1–8.

15. Wang H, Joseph JA. Quantifying cellular oxidative stress by Dichlorofluorescein assay using microplate reader. Free Radical Bio Med 1999; 27 (5–6): 612–616.

16. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem 1976; 72: 248–254.

17. Rahman I, Kode A, Biswas SK. Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. Nat Protoc 2006; 1 (6): 3159–3165.

18. Hyder MZ. Comparative Levels of ALT, AST, ALP and GGT in Liver associated Diseases. Am J Clin Pathol 1957; 28: 56–62.

19. Dhandapani S., Subramanian VR, Rajagopal S, Namasivayam N. Hypolipidemic Effects of Cuminum Cyminuml on Alloxan-induced diabetic rats. Pharmacol Res 2002; 46: 251–255.

20. Concepción Navarro M, Pilar Montilla M, Martín A, Jiménez J, Pilar Utrilla M. Free Rradical Scarenger and Antihepatotoxic Activity of Rosmarinus. Plant Med 1993; 59: 312–314.

21. Berry CC, Charles S, Wells S, Dalby MJ, Curtis AS. The influnence of transferrin stabilized magnetic nanoparticles on human dermal fibroblasts in culture. Int. J. Pharm 2004; 269: 211–225.

22. Yousefi Babadi V, Amraeai E, Salehh H, Sadeghi L, Najafi L, Fazilati M. Evaluation of Iron Oxide nanoparticles effects on tissue and Enzymes of Thyroid in Rats. Int Res J Biol Sci 2013; 2: 67–69.

23. Mital, GS, Manoj, TA. Review of Tio2 nanoparticles. Chinese Sci Bull 2011; 56: 1639–1657.

24. Rezaei-Zarchi S, Rad S, Soezi M, Negahdary M, Amrollahi F, Mazaheri GR, Borna H. Investigation of The effect of Fe2O3 nanoparticles on the blood cells and liver enzymes inmale rat. Adv Environ Biol 2012; 6: 334–342.

25. Ferin J, Oberdorster G, Penney DP, Soderholm SC, Gelein R, Piper HC. Increased pulmonary toxicity of ultrafine particles? I. Particle clearance, translocation, morphology. J Aerosol Sci 1990; 21: 381–384.

26. Oberdörster G, Ferin J, Finkelstein G, Wade P, Corson N. Increased pulmonary toxicity of ultrafine particles? II. Lung lavage studies. J Aerosol Sci 1990; 21: 384–387.

27. Park EJ, Cho WS, Jeong j, Yi JH, Choi k, kim Y, Park K. Induction of inflammatory responses in mice treated with Crium oxide Nanoparticles by intratra chealinstillation. J Health Sci 2010; 56: 387–396.

28. Szalasy B, Ogden JA, Strawbridge RR, ZE Pierce R, Hoopes PJ. Iron 0xide nanoparticles and their toxicological effects: in vivo and invitro studies 2009; 1: 7181–7181.

29. YousefiBabadi V, Amraeai E, Salehh H, Sadeghi L, Najafi L, Fazilati M. Evaluation of Iron Oxide nanoparticles effects on tissue and Enzymes of Thyroid in Rats. Int Res J Biological Sci 2013; 2: 67–69.

30. Dodd N, Jha AN. Titanium dioxide induced cell damage: A Proposed of the carboxyl radical. Mutat Res 2008; 660: 79–82.

31. Wang L, Ding W, Zhang, F. Acute Toxicity of Ferric Oxide and Zinc Oxide Nanoparticles in Rats. J Nanosci Nanotechnol 2010; 10: 8617–8624.

32. Jia'en Li J, Muralikrishnan S, Ng CT, Yung LYL, Bay BH. Nanoparticle-Induced Pulmonary Toxicity. Exp Biol Med 2010; 235: 1025–1033.

Received June 21, 2014.
Accepted July 9, 2014.