Saudi Journal of Biological Sciences (2017) **24**, 1172–1180

King Saud University

Saudi Journal of Biological Sciences

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Biomarkers of oxidative stress for *in vivo* assessment of toxicological effects of iron oxide nanoparticles

Utkarsh A. Reddy, P.V. Prabhakar, M. Mahboob *

Toxicology Unit, Biology Division, Indian Institute of Chemical Technology, Hyderabad 500007, Telangana, India

Received 29 July 2015; revised 21 September 2015; accepted 22 September 2015
Available online 30 September 2015

KEYWORDS

Iron oxide nanoparticles;
Subacute;
Biomarkers;
Antioxidant enzymes;
Bioaccumulation;
Histopathology

Abstract Iron oxide nanoparticles (Fe_2O_3-IONPs) have revolutionized the industry by significant economic and scientific impacts. Enormous increase in the usage of IONPs has raised concerns about their unseen adverse effects. In the current study, we investigated the effects of IONPs and its bulk on oxidative stress biomarkers, histopathology and biodistribution in rats after 28 days repeated oral treatment at 30, 300 and 1000 mg/kg body weight (b.w.). IONPs size in dry, wet forms and crystallinity was determined using TEM, DLS and XRD. The investigation of oxidative stress biomarkers demonstrated significant increase in lipid peroxidation and decrease in reduced glutathione content in the liver, kidney and the brain of the treated groups in a dose dependant manner. Further, antioxidant enzymes catalase, glutathione S transferase, glutathione peroxidase and glutathione reductase activities were significantly elevated along with significant decrease in superoxide dismutase activity in treated rat organs. ICP-OES analysis revealed dose and size dependant accumulation of IONPs in the liver followed by kidney and the brain than bulk. Moreover, accumulation of IONPs at high dose brought pathological changes only in liver. A large fraction of IONPs was eliminated in urine. Bulk material was substantially excreted in faeces than IONPs suggesting increased absorption of IONPs. In conclusion accumulated IONPs and bulk in organs trigger free radical generation, leading to the induction of oxidative stress condition in rats. The results obtained highlight the importance of toxicity assessments in evaluating the efficiency of IONPs for the safe implementation for diversified applications.

1. Introduction

Nanotechnology is the mere controlling and fabrication of materials at nano scale level, where characteristics vary with altered size, shape, density, aggregation and surface area. This technological leap leads to a revolution in the field of electronics and communications, optics, chemistry, energy and biology

* Corresponding author. Tel.: +91 40 27193135; fax: +91 40 27193227.
E-mail address: mahboobm1983@gmail.com (M. Mahboob).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjbs.2015.09.029
1319-562X

(Sanvicens and Marco, 2008). Nanotechnology is a rapidly expanding field with an estimated market of $75.8 billion by 2020 (www.reportlinker.com/april2015). IONPs have promising characteristics like biocompatibility and magnetic behaviour that makes them good agents for magnetic resonance imaging, carriers for drug delivery, magnetic hyperthermia, tissue engineering, cell separation, enzyme immobilization, protein purification and biosensing (Mahmoudi et al., 2012; Rosen et al., 2012). IONPs are also used in many analytical techniques like PCR, immunoassay, HPLC and LC MS/MS (Cao et al., 2012). Moreover, IONPs offer significant improvements in water purification and environmental remediation because of low cost technology (Baumann et al., 2014).

New approaches in nanotechnology and extensive production for numerous diversified applications of nanomaterials (NMs) might increase human exposure. A recent study on mice by Yang et al. (2015), reported size dependent bioaccumulation and transport of IONPs in the liver and spleen. Further, expression of genes associated with antioxidant defence system, transport and metabolism of iron were reported when treated with IONPs. Pregnant mice treated with multiple doses of IONPs reported biodistribution of iron in the foetal liver, placenta and increased foetal deaths (Di Bona et al., 2014). Intratracheal instillation studies of IONPs in mice reported increased cell disruption in the respiratory epithelium and affected haemostasis (Zhu et al., 2008). Initiation of microglial proliferation and pathological alterations in the brain was observed in mice treated with IONPs by intranasal route (Wang et al., 2011). IONPs induced higher levels of inflammation and immunosuppression in mice (Ban et al., 2013). This shows that IONPs can easily cross the intestinal lining and can enter into systemic circulation where it accumulates in different organs.

In vivo and *in vitro* studies of different NMs reported generation of free radicals and oxidative injury is the key mechanism to instigate adverse effects on cellular functions (Auffan et al., 2009). Overloading of cells with IONPs may trigger inimical cellular effects by initiating the fenton chemistry, which generates free radicals (Singh et al., 2009). Cells have antioxidant defence system to intercept the reactive oxygen species (ROS), however if the biological systems fail to nullify the overwhelming ROS, it will lead to oxidation of biomolecules like proteins, DNA and lipids (Prabhakar et al., 2012). Several studies demonstrated oxidative stress as an important biomarker in assessing NMs toxicity (Jain et al., 2008; Park and Park, 2009; Prabhakar et al., 2012; Negahdary et al., 2015; Arooj et al., 2015).

In the present investigation, the physicochemical properties of IONPs were determined using X-ray Diffraction (XRD), Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS). The purpose of the current study was to assess the differences in exerting toxic effects between IONPs and bulk after repeated oral treatment to Wistar rats. The liver, kidney and the brain were selected for our study, to investigate the oxidative stress induced by IONPs and its bulk using biomarkers like reduced glutathione (GSH), malondialdehyde (MDA), antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S transferase (GST). The histopathological analysis was done to detect tissue damage, if any. Biodistribution studies were undertaken to identify the potential target site of accumulation of IONPs and bulk.

2. Materials and methods

2.1. Materials

Fe_2O_3-bulk materials size of $< 5\,\mu m$ (CAT #310050, purity $\geqslant 99\%$), iron standard (CAT #43149) and all chemicals were purchased from Sigma Aldrich Inc., USA.

2.2. Synthesis and characterization of IONPs

IONPs were produced by chemical coprecipitation method following the method of Lee et al. (2004). The purity of IONPs was performed according to the method given by Yokel et al. (2009). The spectra analysis of IONPs was obtained using Siemens/D-5000 X-ray diffractometer. The size and morphology of IONPs were characterized using TEM (Hitachi H-7600). Hydrodynamic size distribution and zeta potential of IONPs were measured using Malvern Zetasizer. Probe sonicator (UP100H Heilscher, Germany) was used to disperse the NMs. Dispersion of NMs in the solution is measured by the polydispersity index (PDI).

2.3. Animals and treatment

According to the OECD (Organization for Economic Cooperation and Development) guidelines, the preferred rodent species is the rat, preferably females because of their sensitivity in comparison to males. Hence, female rats were used in our study. Institutional Animal Ethics Committee (IICT, Hyderabad) approved the present investigation. Female albino wistar rats of 8–10 weeks were procured from the National Institute of Nutrition, Hyderabad, India. Animals were acclimatized under standard laboratory conditions for 7 days prior to the start of experiments. The animals were fed on commercial pellet diet and water *ad libitum*.

The animals were randomly selected, marked and divided into following groups:

Group	Compound	Dosage
Control	Water	–
Low	Nano	30 mg/kg b.w.
Medium	Nano	300 mg/kg b.w.
High	Nano	1000 mg/kg b.w.
Low	Bulk	30 mg/kg b.w.
Medium	Bulk	300 mg/kg b.w.
High	Bulk	1000 mg/kg b.w.

The rats were treated daily with IONPs and bulk for 28 days and sacrificed on the 29th day after being anesthetized. The study and dose design were according to OECD guideline 407 (2008). High dose was chosen to illustrate the lethal effects and symptoms without mortality, whereas the low dose was selected to demonstrate no observed adverse effect levels (NOAEL). The animals were checked for toxic symptoms and mortality (if any) every day, whereas, weight gain and feed intake were recorded once in a week.

2.4. Oxidative stress biomarkers

Lipid peroxidation (LPO) in rat organs was estimated according to the method described by Wills (1969); reduced glutathione (Jollow et al., 1974); superoxide dismutase (Marklund and Marklund, 1974); catalase (Aebi, 1984); glutathione peroxidase (Paglia and Valentine, 1967); glutathione reductase (Carlberg and Mannervik, 1985); glutathione S transferase (Habig et al., 1974) and detailed procedures were described in Reddy et al. (2015). The protein estimation was done using Lowry et al. (1951) method.

2.5. Analysis of iron content in tissues

A part of the liver, kidney, and the brain from each group was collected at the end of the study. Individual urine and faecal samples were collected daily and pooled, analysed for the metal content following standard procedures as described in our earlier publication Reddy et al. (2015). ICP-OES (IRIS Intrepid II XDL, Thermo Jarrel Ash) was used to quantify Fe concentration at alternate wavelengths (232 nm and 238 nm). Quantification of Fe was done using a calibration curve.

2.6. Histopathological study

The liver, kidney and the brain collected from each rat were fixed in 10% formalin and processed following standard procedures. Detailed protocol was described in Reddy et al. (2015).

2.7. Data analysis

All the data were expressed as mean ± standard deviation (SD). Graphpad prism 5, a statistical software system was used to perform a post hoc multiple comparison test (Dunnet's test) after ANOVA. $P < 0.05$ was considered to be significant.

3. Results

3.1. Characterization

Fig. 1 summarizes the characterization of IONPs by XRD, TEM and DLS. The purity analysis by ICP-OES revealed that the purity of IONPs was >96%. This analysis was in support of XRD data obtained. Peaks obtained signify size, purity and crystallinity of IONPs and revealed similar characteristics with γ-Fe_2O_3. TEM characterization of IONPs showed spherical morphology with mean size distribution of 7.02 ± 1.58 nm. The hydrodynamic sizes of IONPs were measured using DLS in MilliQ water showed an average diameter of 155 nm with a PDI measurement of 0.526. These hydrodynamic sizes in dispersion vehicle were larger than their original size in powder form signifying nanoparticles agglomeration. Zeta potential measurements for IONPs were −29.9 mV at pH 7. For bulk material size distribution intensity was found to be beyond detection limits.

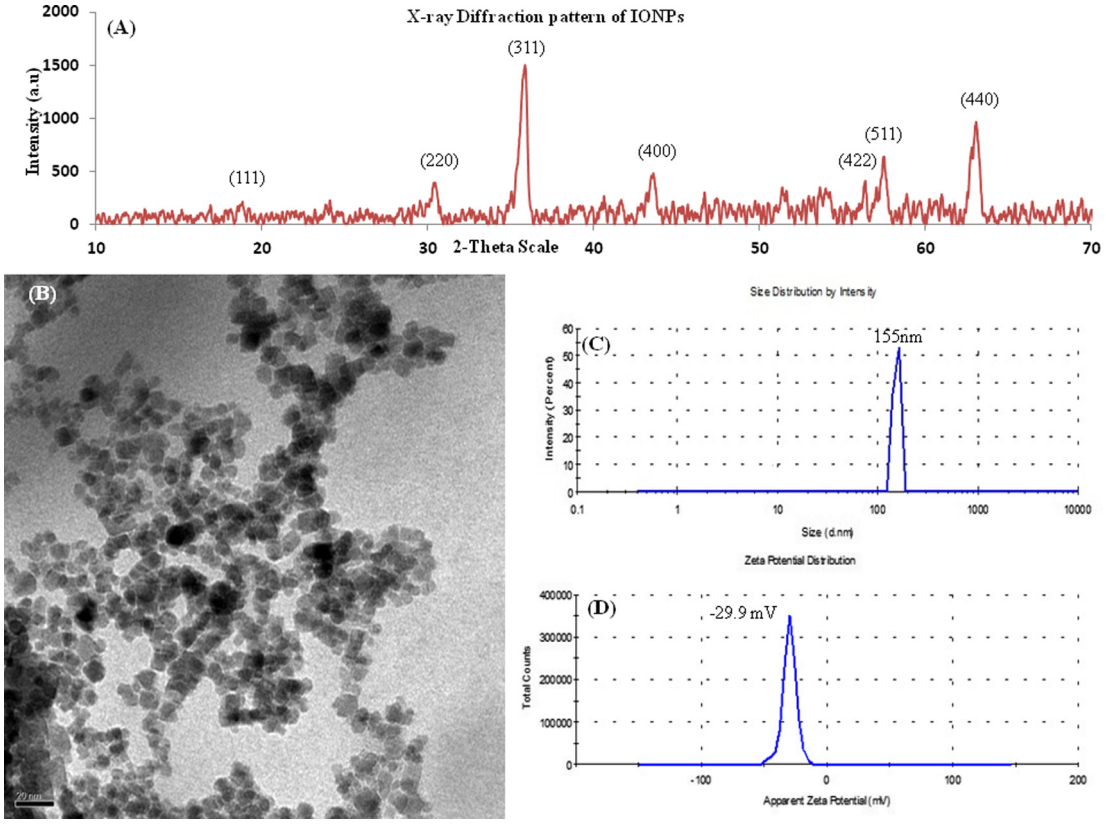

Figure 1 Characterization of IONPs by (A) XRD, (B) TEM, (C) size distribution and (D) zeta potential.

3.2. Morphology, feed intake and body weight of animals

Fig. 2 represents body weight (A) and feed intake (B) of animals treated with IONPs and bulk. Signs of toxicity and mortality were not seen in the treated rats. However, rats treated with a high dose of bulk and IONPs showed significant loss in body weight in weeks 3 and 4. Average feed intake per animal was significantly decreased in high dose IONPs treated rats in comparison to other treatment groups and control.

3.3. Oxidative stress biomarkers

The effect of IONPs and bulk on LPO in the liver, kidney and the brain is presented in Fig. 3A. IONPs and bulk treatment caused significant and a dose dependent increase in MDA levels in the liver at high and medium doses. Only high dose of IONPs and bulk increased LPO level in kidney and the brain. IONPs treated rats showed increased MDA levels than the bulk. The changes in GSH content in different tissues of treated and control rats are summarized in Fig. 3B. IONPs and bulk brought dose dependent depletion in GSH content of the liver, except low dose group of bulk material. However, IONPs at 1000 mg/kg b.w. and 300 mg/kg b.w. and bulk at 1000 mg/kg b.w. significantly decreased GSH content in kidney and the brain. Furthermore, depletion in GSH content was found high in IONPs treated rats than its bulk. High and medium dose of IONPs and bulk treatment caused significant and dose dependent inhibition of SOD in liver. However, 1000 mg/kg b.w. treatment of IONPs and bulk caused depletion in SOD activity in kidney and the brain(Fig. 4A). A significant and dose dependant elevation in activity of CAT was observed in the liver, when rats were treated with IONPs and bulk at 1000 mg/kg b.w. and 300 mg/kg b.w. Similarly statistically significant increase in CAT activity was observed in kidney and the brain after the treatment of IONPs and bulk

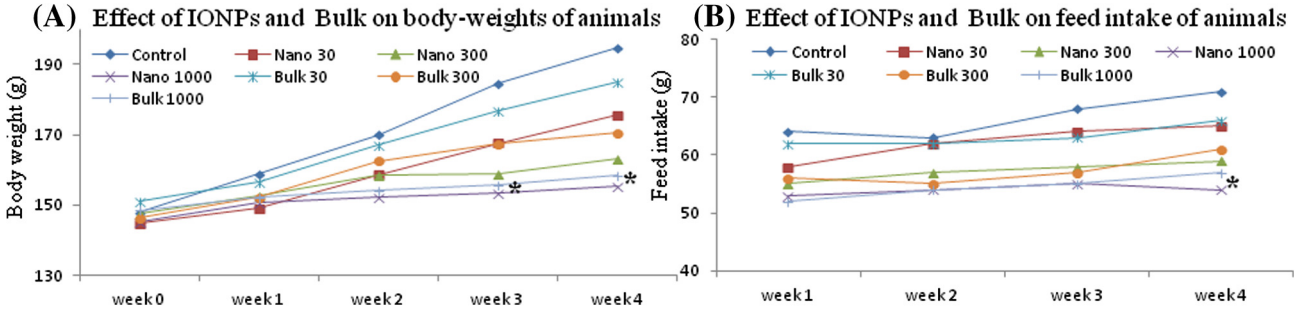

Figure 2 (A) Effect of IONPs and its bulk on body-weight profile. (B) Effect of IONPs and its bulk on feed intake profile. Each value represents the mean ± SD; $n = 5$ rats.

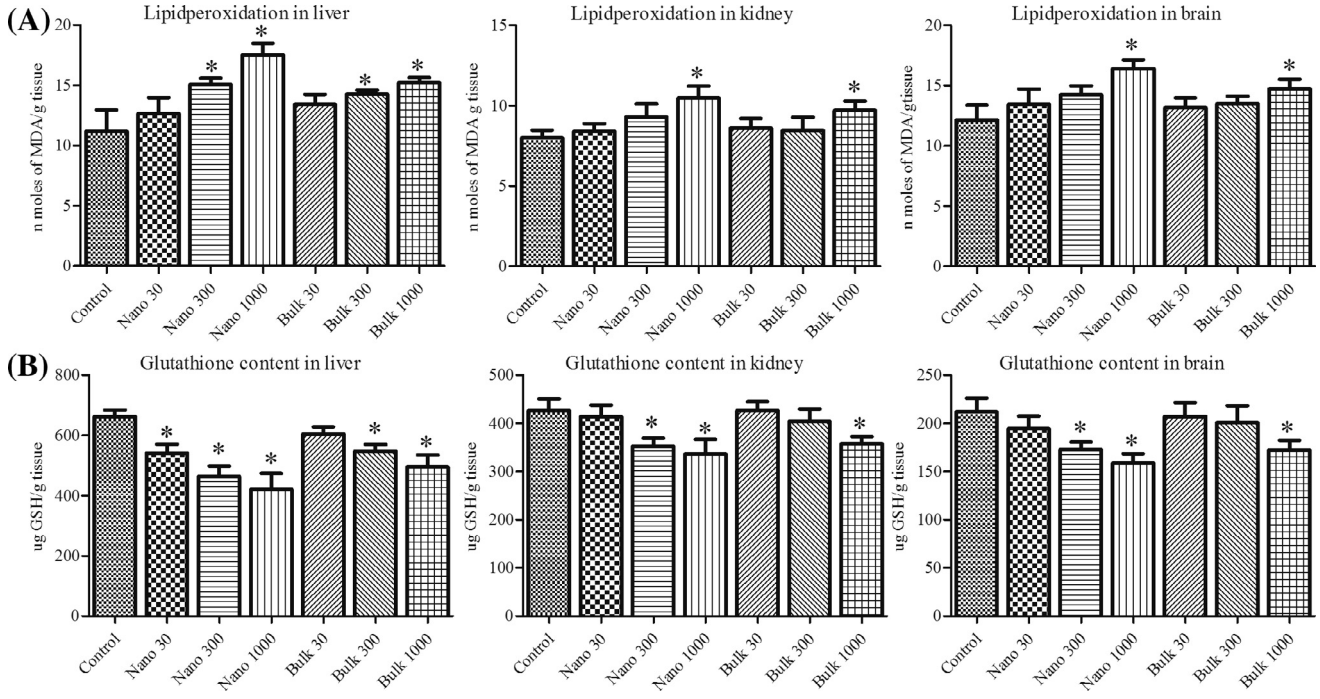

Figure 3 MDA (A) and GSH (B) levels in rats treated with IONPs and bulk for 28 days. Each value represents the mean ± SD; $n = 5$ rats. $^{*}P < 0.05$.

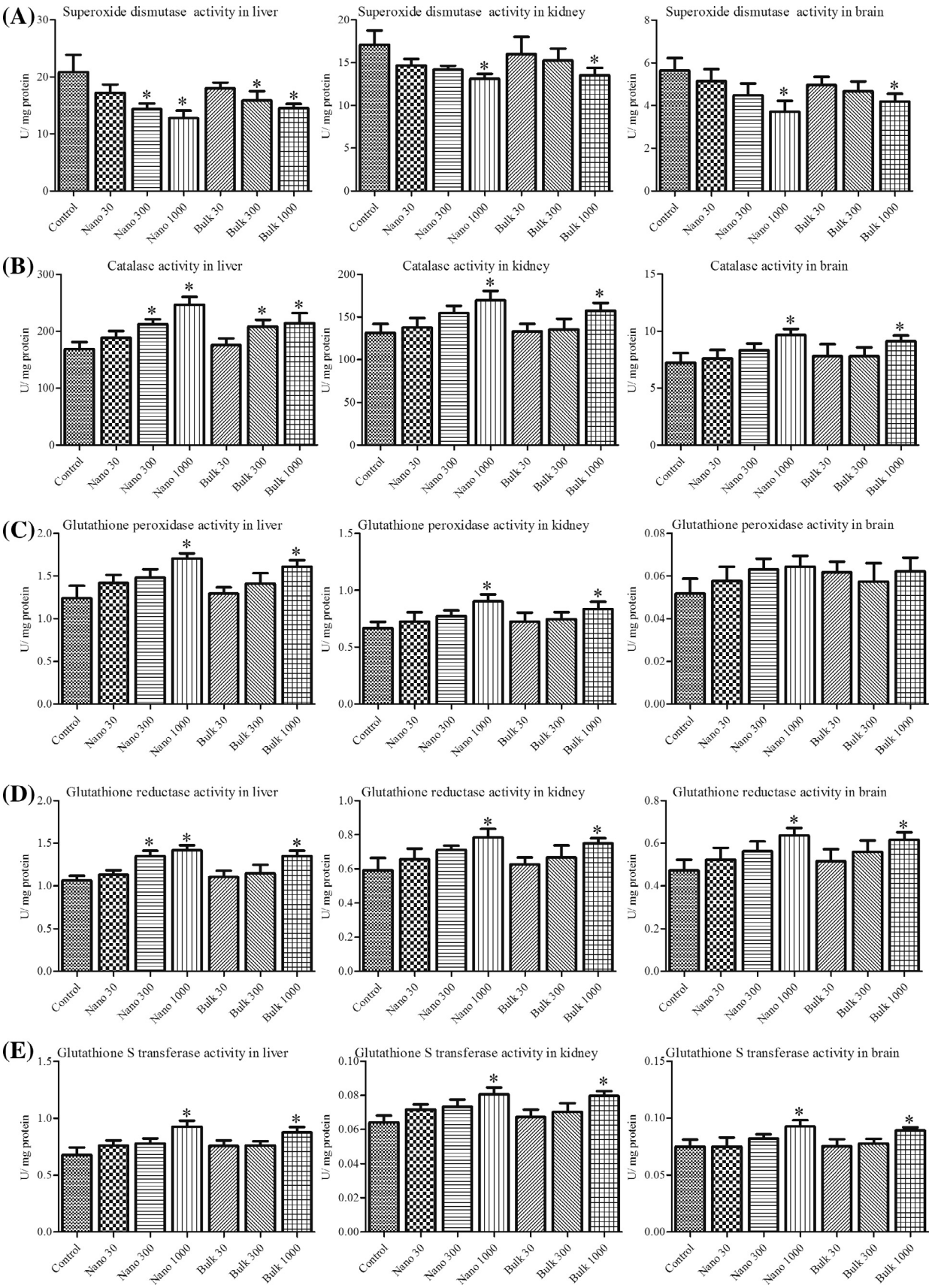

Figure 4 Effect of 28 day oral treatment of IONPs and Bulk on antioxidant enzyme activities in rat organs. Each value represents the mean ± SD; $n = 5$ rats. $^{*}P < 0.05$.

at 1000 mg/kg b.w. (Fig. 4B). IONPs and bulk treatment at 1000 mg/kg b.w. elevated GPx activity significantly only in the liver and kidney. The activity of GPx was not altered in the brain at all the doses of IONPs and bulk treatment (Fig. 4C). GR activity was significantly elevated in the liver in a dose dependent manner after IONPs treatment at 300 mg/kg b.w. and 1000 mg/kg b.w. However, bulk treatment at 1000 mg/kg b.w. increased GR activity only in the liver. Further, significant change in activity of GR was observed in kidney and the brain at mg/kg b.w. of IONPs and bulk treatment (Fig. 4D). Treatment of IONPs and bulk at 1000 mg/kg b.w. caused a substantial increase in GST activity in the liver, kidney and the brain (Fig. 4E). Antioxidant enzymes SOD, CAT, GPx, GR and GST were modulated significantly in IONPs treated rats than its bulk.

3.4. Analysis of iron content in tissues

A dose dependant increase in accumulation of Fe content was seen in the liver (188 μg/g, 224 μg/g, 303.9 μg/g), followed by kidney (58.8 μg/g, 67.4 μg/g, 88 μg/g) and the brain (28.6 μg/g, 38.3 μg/g, 45.8 μg/g) in IONPs treated rats. Similarly uptake of bulk was high in the liver (151.5 μg/g, 178.5 μg/g, 191.2 μg/g) followed by kidney (49.2 μg/g, 57.3 μg/g, 65.2 μg/g) and the brain (28 μg/g, 30.8 μg/g, 37.5 μg/g). However, accumulation of IONPs in organs was higher than bulk. Elimination of Fe through urine was found to be more significant in IONPs (65.3 μg/ml, 74.6 μg/ml, 83.6 μg/ml) treated group than bulk (51.2 μg/ml, 59.2 μg/ml, 68.3 μg/ml). In contrary, in faeces bulk (2390 μg/g, 8849.7 μg/g, 11072.4 μg/g) was eliminated more in comparison to IONPs (1116.7 μg/g, 3233.5 μg/g, 5366.9 μg/g) as shown in Fig. 5A.

3.5. Histopathological study

The histopathological changes in IOPNs and bulk treated the liver, kidney and the brain are presented in Fig. 6. Only high dose treatment of IONPs caused congested portal tract in liver whereas, normal histology was observed in the remaining groups of IONPs and bulk.

4. Discussion

Nanotoxicological studies performed *in vivo* using different routes of exposure hold greater impact in evaluating the adverse biological effects. In the present investigation, we hypothesized oxidative stress as a crucial parameter in IONPs induced toxicity in treated rats. The current study illustrates that the MDA levels were increased in tissues in a dose dependent manner in both IONPs and bulk treated rats. Elevated levels of MDA indicate the formation of hydroxyl radicals which in turn is associated with LPO of tissues. Our results illustrated a dose dependent depletion of reduced glutathione (GSH) after IONPs and bulk treatment, possibly due to enhanced utilization of GSH in both enzymatic and non-enzymatic reactions. Srinivas et al. (2012) reported elevated LPO and depleted GSH in rat lung tissue after inhalation exposure of IONPs. Similarly, oral toxicity study of aluminium oxide NMs and bulk demonstrated elevated levels of MDA and decreased GSH levels in a dose dependant manner in the liver, kidney and the brain of treated rats (Prabhakar et al., 2012). Hence, it is suggested that MDA and GSH contents are good biomarkers in assessing toxicity, which may be possibly due to free radical generation.

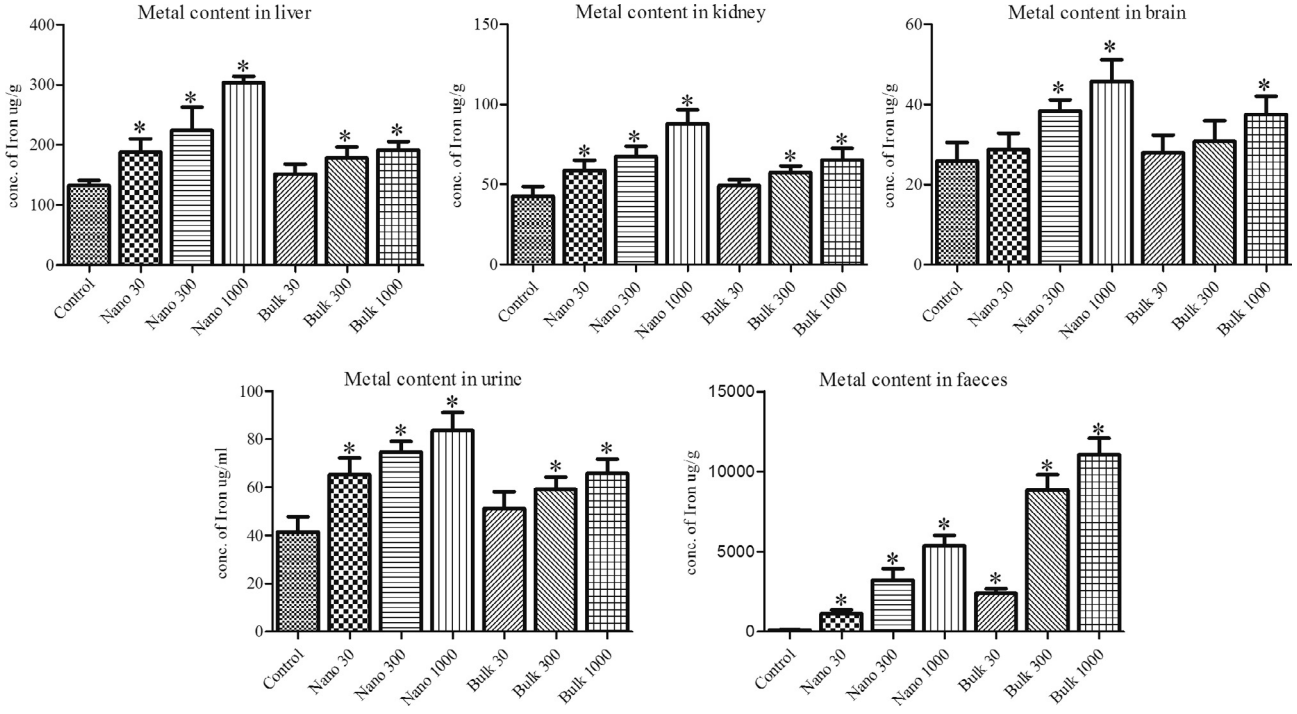

Figure 5 Metal content analysis in tissues of treated rats after 28 days repeated treatment with IONPs and bulk material. Each value represents the mean ± SD; $n = 5$ rats. $^{*}P < 0.05$.

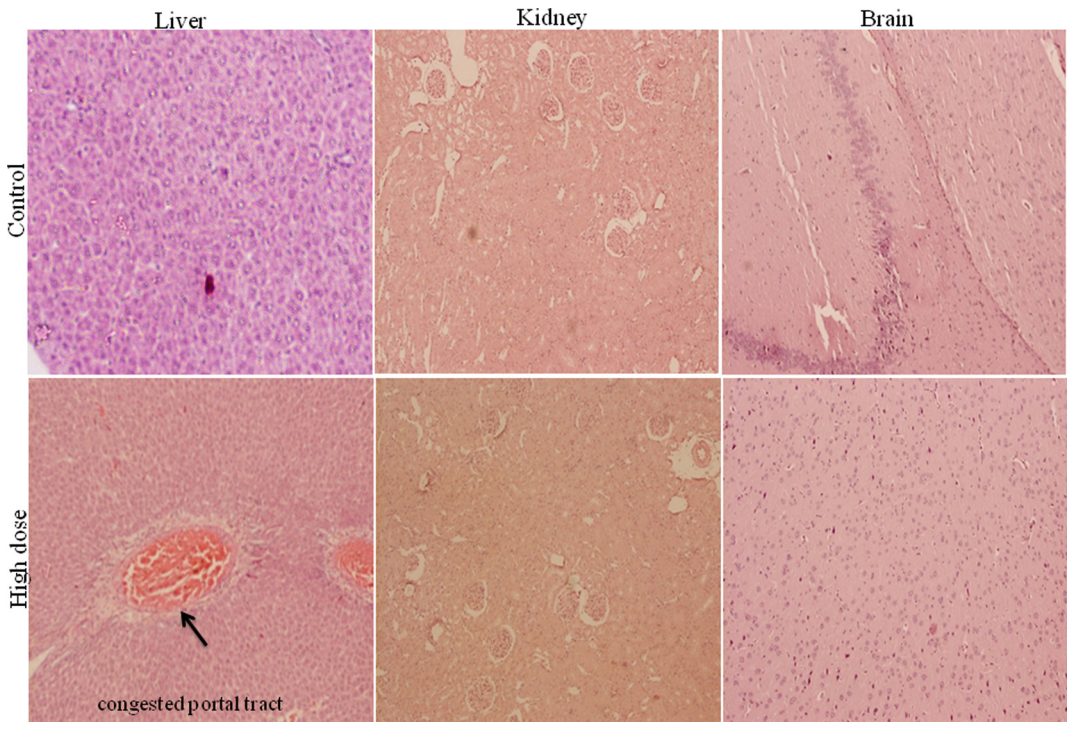

Figure 6 Photomicrographs of various organs of rats after 28 day repeated oral treatment with IONPs and bulk material. Upper panel represents normal architecture of the liver, kidney and the brain from control rats. Lower panel represents the liver, kidney and the brain from high dose group treated with IONPs, congested portal tract in the liver pointed by arrow. Observation was made at 40× magnification.

Individual antioxidant enzymes play different strategies to counter act oxidant insult. In this study, a significant increase in CAT, GR, GPx, GST and decrease in SOD activity in treated rat tissues were observed. Superoxide radicals in association with adventitious metal like Fe, triggers the oxidative damage of proteins, lipids and nucleic acids (Chevion, 1988). Current study revealed a dose dependent decrease in SOD activity in rat organs. This might probably be due to the heavy influx of superoxide radicals that in turn resulted in high levels of H_2O_2 generation, which subsequently inhibited SOD activity (Li et al., 2010b). IONPs and bulk treatment caused elevation in enzyme kinetics of CAT as a defensive mechanism against excess H_2O_2 levels. GPx initiates H_2O_2 breakdown only at low concentrations, whereas CAT needs high levels of H_2O_2 to commence its activity. An increased GR activity was found in our study, this result reflects the recycling of oxidized GSH to reduced GSH by the pro-oxidant system which involves a coupled reaction engaged by GR (Nordberg and Arnér, 2001). Elevated GST activity and depleted GSH levels suggest increased utilization of GSH in conjugation reactions as part of detoxification mechanism. Altered antioxidant status as reported in our study is supported by findings from other investigations. Prabhakar et al. (2012) reported increased enzyme activities of CAT and GST, with depleted SOD activity in rat tissues exposed to aluminium oxide NMs. Administration of TiO_2 NMs to mice for 14 days induced significant oxidative stress than its bulk (Ma et al., 2010). An altered antioxidant status revealed imbalance in oxidant and antioxidant levels in the tissues.

In the present study ICP-OES analysis revealed that IONPs and bulk material were absorbed and extensively deposited in the liver, kidney and the brain, and excreted through urine and faeces. Wang et al. (2010) reported accumulation of IONPs in major systemic organs of mice at an oral dose of 600 mg/kg b. w., which is in support of our results. In the current investigation, IONPs with a hydrodynamic size of 155 nm and with a charge of −29.9 mV were readily taken by liver for phagocytosis, which clearly indicates size and charge also influence the uptake and clearance mechanisms. Our results demonstrated that uptake of IONPs was more in the liver, kidney and the brain in a dose dependent manner. The IONPs have differential organ distribution pattern which seems to be influenced by Fe binding proteins like transferrin and ferritin, which serve as carrier of Fe to different tissues. Increased Fe levels in brain in the present investigation might be due to binding of Fe to transferrin, that triggers up regulation of Fe receptors in the brain, thus transporting Fe across the blood brain barrier (Descamps et al., 1996). Inhalation exposure study in mice demonstrated that IONPs < 50 nm reached the brain without provoking any adverse effects (Kwon et al., 2008). Higher localization of IONPs in liver might have caused histopathological changes, causing congestion in portal tract. However, histological changes were not observed in kidney and the brain of both the treated groups. A dose dependant and significant amount of IONPs was eliminated in urine. Trace amount of bulk passed through the intestinal epithelium and remaining being excreted in faeces. However, IONPs excreted in faeces were less than the bulk.

Taken together, the findings from the current investigation, confirm that IONPs accumulation in tissues triggered ROS generation, which significantly altered the antioxidant enzyme levels. The altered antioxidant status and bioaccumulation of

IONPs caused histomorphological changes, which demonstrate that increase in oxidative stress affects the cellular structure. Moreover, the results of this study can be utilized by the regulatory bodies in defining safety limits for the usage of IONPs in biomedical applications.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

The work was financially supported by Department of Biotechnology, New Delhi, India (Project No. BT/PR9998/NNT/28/84/2007). The authors Utkarsh A. Reddy and P.V. Prabhakar thanks Indian Council of Medical Research, New Delhi, India for awarding senior research fellowship.

References

Aebi, H., 1984. Catalase. In: Packer, L. (Ed.), . In: Methods in Enzymology, 105. Academic Press, Orlando, FL, pp. 121–126.

Arooj, S., Nazir, S., Nadhman, A., Ahmad, N., Muhammad, B., Ahmad, I., Mazhar, K., Abbasi, R., 2015. Novel ZnO: ag nanocomposites induce significant oxidative stress in human fibroblast malignant melanoma (Ht144) cells. Beilstein J. Nanotechnol. 6, 570–582.

Auffan, M., Rose, J., Wiesner, M.R., Bottero, J.Y., 2009. Chemical stability of metallic nanoparticles: a parameter controlling their potential cellular toxicity in vitro. Environ. Pollut. 157, 1127–1133.

Ban, M., Langonné, I., Huguet, N., Guichard, Y., Goutet, M., 2013. Iron oxide particles modulate the ovalbumin-induced Th2 immune response in mice. Toxicol. Lett. 216, 31–39.

Baumann, J., Köser, J., Arndt, D., Filser, J., 2014. The coating makes the difference: acute effects of iron oxide nanoparticles on *Daphnia magna*. Sci. Total Environ. 484, 176–184.

Cao, M., Li, Z., Wang, J., Ge, W., Yue, T., Li, R., Colvin, V.L., Yu, W.W., 2012. Food related applications of magnetic iron oxide nanoparticles: enzyme immobilization, protein purification, and food analysis. Trends Food Sci. Technol. 27, 47–56.

Carlberg, I., Mannervik, B., 1985. Glutathione reductase. Methods Enzymol. 113, 484–490.

Chevion, M., 1988. A site specific mechanism for free radical induced biological damage: the essential role of redox active transition metals. Free Radical Biol. Med. 5, 27–37.

Descamps, L., Dehouck, M.P., Torpier, G., Cecchelli, R., 1996. Receptor-mediated transcytosis of transferrin through blood-brain barrier endothelial cells. Am. J. Physiol. 270, H1149–H1158.

Di Bona, K.R., Xu, Y., Ramirez, P.A., DeLaine, J., Parker, C., Bao, Y., Rasco, J.F., 2014. Surface charge and dosage dependent potential developmental toxicity and biodistribution of iron oxide nanoparticles in pregnant CD-1 mice. Reprod. Toxicol. 50, 36–42.

Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferase. The fist enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130–7139.

Jain, T.K., Reddy, M.K., Morales, M.A., Diandra, L., Pelecky, L., Labhasetwar, V., 2008. Biodistribution, clearance, and biocompatibility of iron oxide magnetic nanoparticles in rats. Mol. Pharm. 5, 316–327.

Jollow, D.J., Mitchell, J.R., Zampaglione, N., Gillete, J.R., 1974. Bromobenzene induced liver necrosis. Protective role of glutathione and evidence for 3, 4-bromobenzeneoxide as the hepatotoxic metabolite. Pharmacology 11, 151–169.

Kwon, J.T., Hwang, S.K., Jin, H., Kim, D.S., Minai-Tehrani, A., Yoon, H.J., Choi, M., Yoon, T.J., Han, D.Y., Kang, Y.W., Yoon, B.I., Lee, J.K., Cho, M.H., 2008. Body distribution of inhaled fluorescent magnetic nanoparticles in the mice. J. Occup. Health 50, 1–6.

Lee, S.J., Jeong, J.R., Shin, S.C., Kim, J.C., Kim, J.D., 2004. Synthesis and characterization of superparamagnetic maghemite nanoparticles prepared by coprecipitation technique. J. Magn. Magn. Mater. 282, 147–150.

Li, Z.H., Li, P., Randak, T., 2010. Effect of a human pharmaceutical carbamazepine on antioxidant responses in brain of a model teleost in vitro: an efficient approach to biomonitoring. J. Appl. Toxicol. 30, 644–648.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin-phenol reagent. J. Biol. Chem. 193, 265–275.

Ma, L., Liu, J., Li, N., Wang, J., Duan, Y., Yan, J., Liu, H., Wang, H., Hong, F., 2010. Oxidative stress in the brain of mice caused by translocated nanoparticulateTiO_2 delivered to the abdominal cavity. Biomaterials 31, 99–105.

Mahmoudi, M., Hofmann, H., Rothen-Rutishauser, B., Petri-Fink, A., 2012. Assessing the in vitro and in vivo toxicity of super para magnetic iron oxide nanoparticles. Chem. Rev. 112, 2323–2338.

Marklund, S., Marklund, G., 1974. Involvement of superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem. 47, 469–474.

Negahdary, M., Chelongar, R., Zadeh, S.K., Ajdary, M., 2015. The antioxidant effects of silver, gold, and zinc oxide nanoparticles on male mice in in vivo condition. Adv. Biomed. Res. 4, 69.

Nordberg, J., Arnér, E.S., 2001. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free Radical Biol. Med. 31, 1287–1312.

OECD (2008), Test No. 407: repeated Dose 28-day Oral Toxicity Study in Rodents, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris.

Paglia, D.E., Valentine, W.N., 1967. Studies on qualitative and quantitative characterization of erythrocytes glutathione peroxidase. J. Lab. Clin. Med. 70, 158–169.

Park, E.J., Park, K., 2009. Oxidative stress and pro-inflammatory responses induced by silica nanoparticles in vivo and in vitro. Toxicol. Lett. 184, 18–25.

Prabhakar, P.V., Reddy, U.A., Singh, S.P., Balasubramanyam, A., Rahman, M.F., Indu Kumari, S., Agawane, S.B., Murty, U.S., Grover, P., Mahboob, M., 2012. Oxidative stress induced by aluminum oxide nanomaterials after acute oral treatment in wistar rats. J. Appl. Toxicol. 32, 436–445.

Reddy, U.A., Prabhakar, P.V., Rao, G.S., Rao, P.R., Sandeep, K., Rahman, M.F., Kumari, S.I., Grover, P., Khan, H.A., Mahboob, M., 2015. Biomarkers of oxidative stress in rat for assessing toxicological effects of heavy metal pollution in river water. Environ Sci. Pollut. Res. http://dx.doi.org/10.1007/s11356-015-4381-2.

Rosen, J.E., Chan, L., Shieh, D.B., Gu, F.X., 2012. Iron oxide nanoparticles for targeted cancer imaging and diagnostics. Nanomedicine 8, 275–290.

Sanvicens, N., Marco, M.P., 2008. Multifunctional nanoparticles–properties and prospects for their use in human medicine. Trends Biotechnol. 8, 425–433.

Singh, N., Manshian, B., Jenkins, G.J., Griffiths, S.M., Williams, P.M.Maffeis, T.G., Wright, C.J., Doak, S.H., 2009. NanoGenotoxicology: the DNA damaging potential of engineered nanomaterials. Biomaterials 30, 3891–3914.

Srinivas, A., Rao, P.J., Selvam, G., Goparaju, A., Murthy, P.B., Reddy, P.N., 2012. Oxidative stress and inflammatory responses of rat following acute inhalation exposure to iron oxide nanoparticles. Hum. Exp. Toxicol. 31, 1113–1131.

Wang, J., Chen, Y., Chen, B., Ding, J., Xia, G., Gao, C., Cheng, J., Jin, N., Zhou, Y., Li, X., Tang, M., Wang, X.M., 2010.

Pharmacokinetic parameters and tissue distribution of magnetic Fe_3O_4 nanoparticles in mice. Int. J. Nanomed. 21, 861–866.

Wang, Y., Wang, B., Zhu, M.T., Li, M., Wang, H.J., Wang, M., Ouyang, H., Chai, Z.F., Fenga, W.Y., Zhao, Y.L., 2011. Microglial activation, recruitment and phagocytosis as linked phenomena in ferric oxide nanoparticle exposure. Toxicol. Lett. 205, 26–37.

Wills, E.D., 1969. Lipid peroxide formation in microsomes. Relation of hydroxylation to lipid peroxide formation. Biochem. J. 113, 333–341.

Yang, L., Kuang, H., Zhang, W., Aguilar, Z.P., Xiong, Y., Lai, W., Xu, H., Wei, H., 2015. Size dependent biodistribution and toxicokinetics of iron oxide magnetic nanoparticles in mice. Nanoscale 7, 625–636.

Yokel, R.A., Florence, R.L., Unrine, J.M., Tseng, M.T., Graham, U.M.Wu, P., Grulke, E.A., Sultana, R., Hardas, S.S., Butterfield, D.A., 2009. Biodistribution and oxidative stress effects of a systemically-introduced commercial ceria engineered nanomaterial. Nanotoxicology 3, 234–248.

Zhu, M.T., Feng, W.Y., Wang, B., Wang, T.C., Gu, Y.Q., Wang, M., Wang, Y., Ouyang, H., Zhao, Y.L., Chai, Z.F., 2008. Comparative study of pulmonary responses to nano- and submicron-sized ferric oxide in rats. Toxicology 247, 102–111.