RESEARCH ARTICLE

Investigation of hematotoxic effect of nano ZnO, nano Fe_3O_4 and nano SiO_2 in vitro

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ABSTRACT

Objective(s): Evaluation of nanomaterials interaction with blood ingredients is a part of preclinical risk assessment of newly-synthesized materials, especially for nano-sized pharmaceuticals which are intravenously administrated. The red blood cells (RBCs) are susceptible to oxidative stress damage. This study was designed to evaluate induced oxidative hematotoxic effect of nano ZnO, Fe_3O_4, and nano SiO_2 on human red blood cells ***in vitro***.

Methods: Blood samples were collected from healthy male volunteers. RBCs were exposed to different concentrations (50, 100, 250mg/ml) of nano ZnO, nano Fe_3O_4, and nano SiO_2 at 4°C for 24hours. Lipid peroxidation and intracellular Glutathione (GSH) level were studied as the biomarkers of oxidative stress.

Results: The results showed that the lipid peroxidation had significantly increased. However, after exposure to nanoparticles, the GSH level of RBCs considerably decreased compared to the controls ($p<0.05$).

Conclusions: This study suggests that nanoparticles could induce oxidative stress that eventually leads to cell toxicity and hemolysis of RBCs at certain doses. This study provides important experimental information for safety assessment and pharmacokinetics of high-doses intravenous administration of nanoparticles for design of clinical trials.

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INTRODUCTION

Nanomaterials are defined as a new class of materials having unique physical and chemical properties. There is great interest in applying nanomaterials in different fields of medicine and industry. The potential benefit of nanomaterials in medicinal devices or drug delivery proposes new methods in diagnosis, medication, and treatment of complicated diseases such as cancers. Any nanomaterials applied as pharmaceuticals or medical devices must be assessed for potential risk for consumers before approval for prescription. Evaluation of nanomaterials interaction with blood ingredients is a part of preclinical risk assessment of newly -synthesized materials, especially for nano-sized pharmaceuticals which are intravenously administrated [1]. Several studies have shown that the unique structure and size of the nanoparticles could lead to oxidative injury and cell toxicity in different cell cultures and also in blood cells [2-3]. Formation of reactive oxygen and nitrogen species (RONS) at levels higher than the body's antioxidant capacity has been known as the oxidative stress. Reactive oxygen and nitrogen species are generated in the body as the result of normal metabolism

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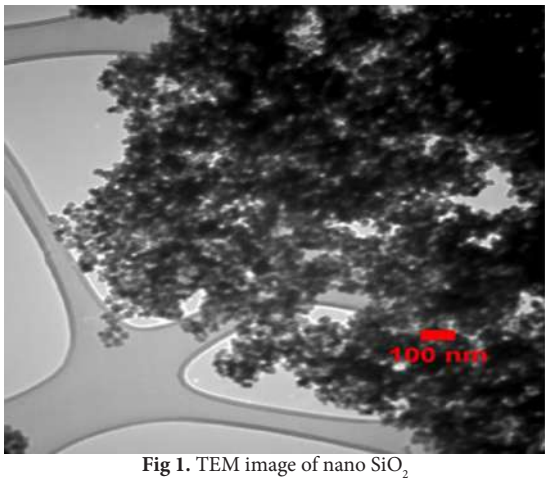

Fig 1. TEM image of nano SiO_2

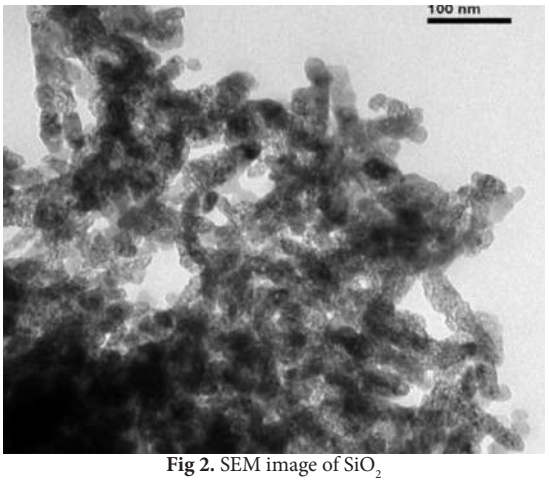

Fig 2. SEM image of SiO_2

and either as the result of exposure to a variety of chemicals, drugs, and pollutants. Nucleic acids, proteins, lipids are cellular macromolecules that may be harmed by RONS. Oxidative stress also involves in pathogenesis of different diseases including cancer, neurodegenerative diseases, atherosclerosis, kidney and liver damage, rheumatoid arthritis, immunological disorder, and aging [4-5].

Lipid peroxidation of polyunsaturated fatty acids leads to production of malondialdehyde (MDA) that is a useful biomarker of oxidative stress. MDA is commonly measured by the thiobarbituric acid-reactive-substances (TBARS) assay. Small thiols including tripeptide glutathione (GSH) are considered as the protective sulfhydryl antioxidants and radical scavengers. Glutathione protects sensitive thiol groups (-SH) of proteins against oxidative stress [6].

The RBCs are intrinsically susceptible to oxidative stress damage because of exposing to high oxygen pressure and the presence of polyunsaturated fatty acid in their cell membrane. Furthermore, RBCs do not have endoplasmic reticulum and nucleus to replace damaged proteins [7]. Some studies have investigated the hematotoxic effects of nanoparticles on the blood cells in vivo and in vitro [8-13].

Nano Fe_3O_4, nano ZnO, and nano SiO_2 are well-known nanoparticles that have promising biomedical applications and their oxidative stress effects have been reported in different studies [9, 14-15]. Zinc nanoparticles are used in cosmetics ointment, sunscreens, food additive, antimicrobial, fungicide, UV protection coatings, and as a catalyst [15]. SiO_2 nanoparticles have found extensive applications in chemical mechanical polishing and as the additives to drugs, cosmetics, printer toners, varnishes, and food. In recent years, the use of SiO_2 nanoparticles has been extended to biomedical and biotechnological fields [16]. Fe_3O_4 nanoparticles have been used as the contrast agents in Magnetic resonance imaging (MRI) angiography and perfusion imaging [17].

In this study, the induced oxidative hematotoxic effects of different doses of nano ZnO, nano Fe_3O_4, and nano SiO_2 have been studied on human RBC. .

MATERIAL AND METHODS

Nano particles characterization

Silicon Dioxide (SiO_2) Nanopowder (99+%, 20-30 nm, amorphous) was purchased from Nanosany Corporation, Iranian Nanomaterials Pioneers Company, Mashhad, Iran. Figure 1 and Figure 2 represent transmission electron microscope (TEM) and scanning electron microscope (SEM) images of nano SiO_2.

Iron Oxide (Fe_3O_4) Nanopowder (99+%, 20-40 nm, spherical) was purchased from Nanosany Corporation, Iranian Nanomaterials Pioneers Company, Mashhad, Iran. Figure 3 represent the TEM image of nano Fe_3O_4.

The nano-zinc metal powder (99.99+%, 20-40 nm) was purchased from the Intelligent Materials Pvt. Ltd., Nanoshel LLC, Wilmington, DE, USA. Figure 4 represent transmission electron the TEM image of nano ZnO.

All the other chemicals used in this experiment were analytical grade with the highest purity .

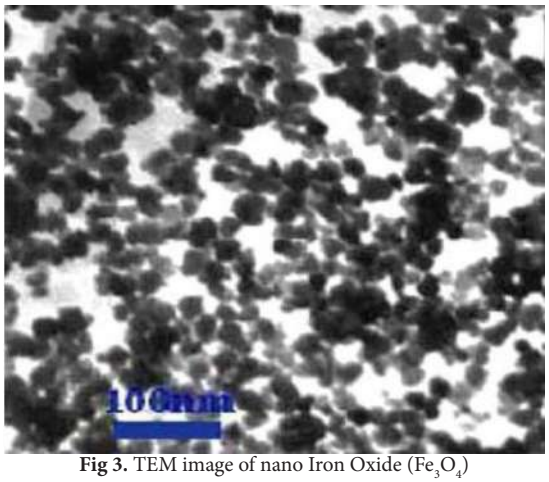

Fig 3. TEM image of nano Iron Oxide (Fe_3O_4)

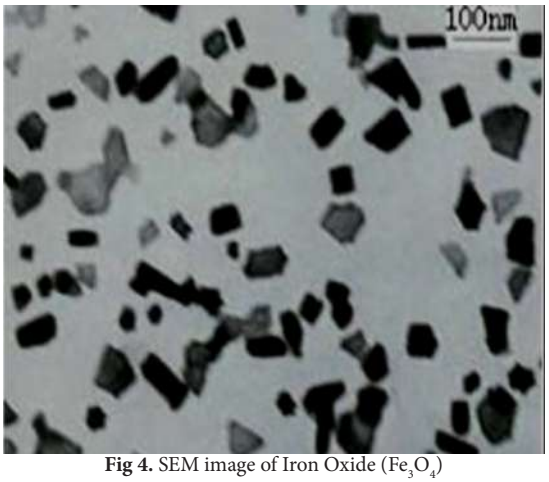

Fig 4. SEM image of Iron Oxide (Fe_3O_4)

Blood samples preparation

Blood samples were collected from healthy male volunteers in heparinized tubes and centrifuged at 3000 rpm for 25 min. Plasma and the buffy coats of blood were discarded. RBCs were washed three times with phosphate-buffered normal saline and then exposed to different concentrations (50, 100, 250 mg/ml) of nano ZnO, nano Fe_3O_4, and nano SiO_2 before incubation at 4C° for 24h. Each experiment was performed in triplicate.

Lipid peroxidation assay

Lipid peroxidation in human erythrocytes was quantified by measuring the formation of TBARS. Erythrocytes were mixed with trichloroacetic acid 20% (1:1). Samples were centrifuged (600g×10min) and then thiobarbituric acid 15% was added to the supernatants. Finally, the samples were heated at temperature 100°c for15 min and the absorbance of the supernatant was measured at 532 nm. The quantities of TBARS were presented as the percentage of TBARS production over the control.

Quantification of intracellular GSH levels

Cellular level of reduced GSH was determined using the GSH colorimetric assay kit. This method is based on a chemical reaction between GSH and 5, 50-dithiobis (2-nitrobenzoic acid) (DTNB) that results in generating glutathione disulfide (GSSG) and-nitro-5-thiobenzoicacid, a yellow colored product. Thus, GSH concentration in a sample solution can be determined by the absorbance measurement at 412 nm [18].

Statistical analysis

One-way analysis of variance (ANOVA) was used to compare the results. P value greater than 0.05 was considered insignificant.

Figure 5 indicates schematic of the quantification of oxidative stress induction assay.

RESULTS AND DISCUSSION

Effect of Zn, Fe_3O_4 and SiO_2 nanoparticles on lipid peroxidation

TBARS assay is a sensitive and simple method for detecting lipid peroxidation which is commonly used as an important biomarker of oxidative stress. As showed in table 1, the percentage of lipid peroxidation biomarker, malondialdehyde level, was significantly increased in treated red blood compared to the control groups after incubation with different concentrations of all nanoparticles

Table 1. TBARS level of RBCs after 24 h exposure to ZnO, Fe_2O_3 and SiO_2 nanoparticles

Concentration (mg/ml)	TBARS (% of control)		
	Nano ZnO	Nano Fe_2O_3	Nano SiO_2
0 mg/ml (control)	100	100	100
50 mg/ml	151±9.3*	178±6.4*	187±15.1*
100 mg/ml	157±7.4*	182±4.3*	152±3.6*
250 mg/ml	183± 11*, **	190±9.4*	171±15.1*

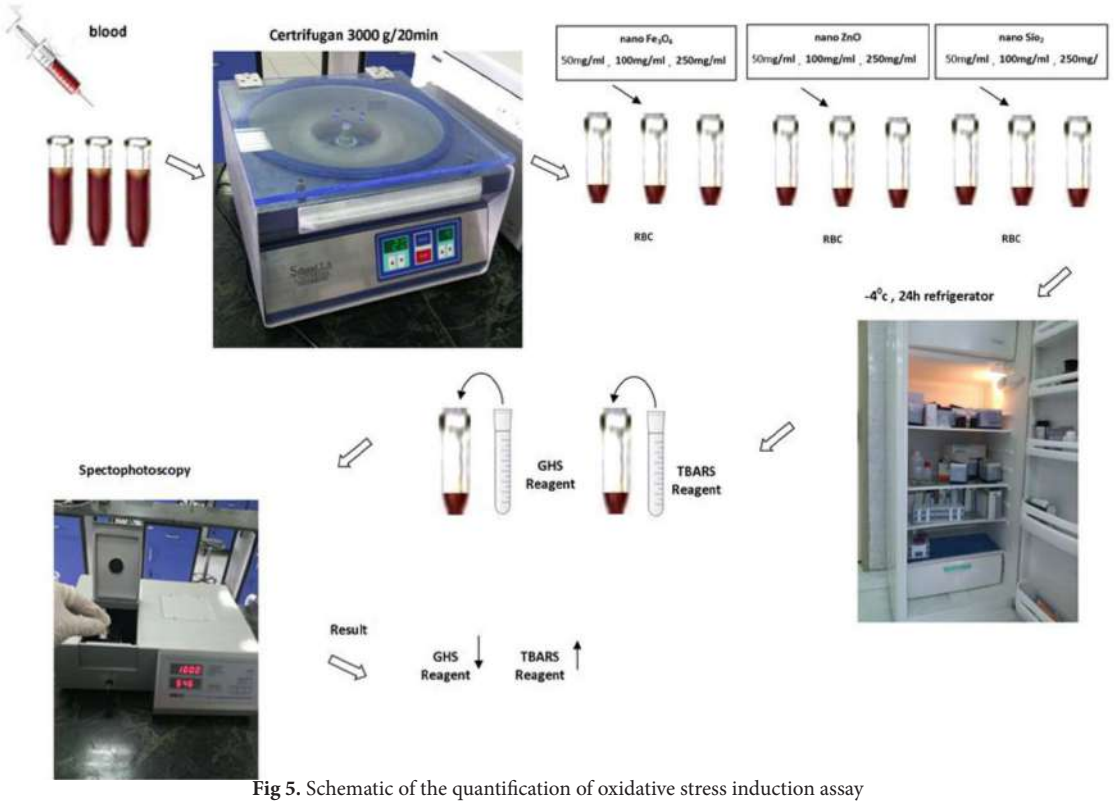

Fig 5. Schematic of the quantification of oxidative stress induction assay

(p<005) and at higher doses of nano ZnO and Fe_3O_4 nanoparticles more significant effects were seen (p<005). Furthermore, exposure to the different doses of Fe_3O_4 nanoparticles has led to high lipid peroxidation level compared to controls. However, dose dependency of lipid peroxidation was not significant. In SiO_2-treatedt groups, significant increase in lipid peroxidation level was showed (p <0.05).

Nanoparticle-free RBCs were used as the control groups. The significant data are indicated by* versus control p <0.05. ** shows significant data compared to other doses of that nanoparticle p <0.05.

Effect of ZnO, Fe_3O_4, and SiO_2 nanoparticles on intracellular GSH levels

GSH is a special sulfhydryl-contained molecule in cells that maintains homeostasis of cellular oxidation–reduction. Alterations in GSH homeostasis can be considered as the indication of functional damage to the cells. As can be seen in Table 2, compared to the controls, , the level of GSH in RBCs significantly decreased after exposure to nanoparticles in all treated groups (p <0.05).

Nanoparticle-free RBCs were used as the control groups. The significant data are indicated by * (p <0.05) versus control.

The data showed a significant decrease in GSH

Table 1. GSH levels of RBCs after 24h exposure to ZnO, Fe_3O_4, SiO_2 nanoparticles

Concentration (mg/ml)	GSH (% of control)		
	Nano zinc oxide	Nano Fe_3O_4	Nano SiO_2
0 mg/ml (control)	100	100	100
50 mg/ml	49±7.2*	32±4.4*	13±3.1*
100 mg/ml	43±8.8*	18±9.1*	48±15.7*
250 mg/ml	17±3.9*	10±2.6*	29± 9.5*

level in nanoparticles-exposed RBCs. There was reverse relation between MDA and GSH levels in our experiment.

The purpose of present study was to investigate induced oxidative damage in RBCs and the possible mechanisms of hematotoxicity of nano ZnO, nano Fe_3O_4, and nano SiO_2. Increased membrane lipid peroxidation (TBARS level) and decreased level of GSH are indicators of elevated oxidative stress. Many studies have shown that nanoparticles could induce oxidative stress and cause cell toxicity in cell cultures and animal models [19-24].

Fe_3O_4 nanoparticles were used as contrast agents in MRI and imaging. Iron is known as the oxidative stress progenitor via the Fenton reaction and eventually causes cell toxicity at high doses [25]. Previous studies have indicated that the Fe_3O_4 nanoparticles could induce oxidative stress, cell membrane damage, and cytotoxicity in a dose-dependent manner [10, 26-29]. Our study showed that in presence of iron oxide nanoparticles, the level of TBARS has increased while the level of GSH has decreased and the Fe_3O_4 . Nano SiO_2 have applied in food industry and also as drug additives and cosmetics. It was shown that cristalobalite nanoparticles induce oxidative stress and cell toxicity in different types of cell lines [30-31]. Our study revealed that SiO_2 could induce oxidative stress and cytotoxic effects in RBCs. However, the oxidative stress indicators, TBARS and GSH, did not show a dose dependent relationship. Nano ZnO particles were used in biomedical imaging, drug and gene delivery, and food/cosmetics industries. Some in vitro and in vivo studies revealed that oxidative stress has a principle role in nano ZnO cytotoxicity. Our study suggested that oxidative stress is a mechanism of RBCs cytotoxicity. Our experiment demonstrated significant increase in TBARS and reduced GSH levels, especially in higher doses [32-36]. Our results and previous studies have shown the pro-oxidant effects of certain doses of nanoparticles in RBCs which should be considered in people exposed to nanoparticles via pharmaceuticals especially through systemic administration routs. In population who are susceptible to oxidative stress, such as sickle cell anemic patients, anemic persons, and those who are suffering from chronic disease like diabetes, the nanoparticles should be prescribed carefully [37-38].

CONCLUSIONS

In conclusion, this study suggested that nanoparticles could induce oxidative stress, cell toxicity, and RBC hemolysis at certain doses. This study provides important experimental information for the safety assessment and pharmacokinetics of high doses intravenous administration of nanoparticles and for the design of clinical trials.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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