Toxicity of iron oxide nanoparticles, on antioxidant enzymes and free radicals in male rats.

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Abstract

The present study Wistar male rats were used. Rats were divided into 2 equal groups, 10 rats each. Group 1 served as control, group 2 was administered orally with Fe_2O_3NPs. Animals were treated with the doses every day for 90 days. Results showed significant (P<0.05) decrease in the antioxidant enzymes (GPX, GST, CAT and SOD) and Reduced Glutathione (GSH) and Total Antioxidant Capacity (TAC), while significant (P<0.05) increase in Thiobarbituric Acid-Reactive Substances (TBARS) and Nitric Oxide (NO) in plasma and testes of rats treated with Fe_2O_3NPs, compared to control group.

Keywords: Iron oxide nanoparticles, Antioxidant enzymes, Free radicals.

Introduction

Nanoparticles are particles with one or more dimensions at the nanoscale .They possess various new properties and their industrial use creates new opportunities, but they also present new risks and uncertainties. The biological effects of NPs are determined by various factors including particle size, shape and ability to interact with the surrounding tissue [1]. Metal nanoparticles have widespread applications. Of these, iron oxide NPs (Fe_2O_3NPs) is the most prominent. Fe_2O_3NPs is found in the environment as particulate matter originating from air pollution and volcanic eruptions. Fe_2O_3NPs particles can also be generated by traffic, industry and power station emissions. In addition, they are purposely chemically synthesised for a wide variety of applications [2]. The most concerning element is their ability to infiltrate groundwater and soil, which are the greatest avenues of exposure. Ingested Fe_2O_3NPs can be translocated into the bloodstream and distributed throughout vital organs such as the heart, liver, kidney, brain and lungs [3]. Raising concerns about their acute and chronic toxic effects. There is a compelling body of evidence that addresses the toxicological effects of Fe_2O_3NPs on animal cells and tissues [4]. NPs may cause inflammation, cytokine production, cytoskeletal changes, altered vesicular trafficking, oxidative stress, apoptosis and changes in gene expression and cell signaling [5]. Toxicity is further heightened by the metallic nature of Fe_2O_3NPs [6]. Furthermore, Fe_2O_3NPs release free iron, which induces Reactive Oxygen Species (ROS) production through the Fenton reaction process [7]. The toxicities of Fe_2O_3NPs is well documented; however, therefore the present study aimed to address this issue [8]. Rats were subchronically exposed to Fe_2O_3NPs, for 90 days and the effects on different organs were assessed. The present study Wistar male rats were used. Rats were divided into 2 equal groups, 10 rats each. Group 1 served as control, group 2 was administered orally with Fe_2O_3NPs. Animals were treated with the doses every day for 90 days. Results showed significant (P<0.05) decrease in the antioxidant enzymes (GPX, GST, CAT and SOD) and Reduced Glutathione (GSH) and Total Antioxidant Capacity (TAC), while significant (P<0.05) increase in Thiobarbituric Acid-Reactive Substances (TBARS) and Nitric Oxide (NO) in plasma and testes of rats treated with Fe_2O_3NPs, compared to control group.

Materials and Methods

Tested compounds and doses

Fe_2O_3NPs (spherical; 50 nm particle size; 50-245 m2/g surface area) were purchased from Sigma-Aldrich (Merck KGaA). Fe_2O_3NPs were dispersed in distilled water by sonication for 30sec to form suspensions before use at doses of 5mg/ml and 50mg/ml, respectively. The hydrodynamic size distribution of each NP in suspension was determined by dynamic light scattering using a Zetasizer Nano ZS (Malvern Instruments, Ltd). Szalay and Sharma were consulted to deter- mine the appropriate dosage of Fe_2O_3NPs (5 mg/kg/day).In vivo study and experimental groups [9,10]. Forty adult male Wistar rats weighing 160-170 g at 5-6 months of age were used in the present study. Animals were provided by the Faculty of Medicine of Alexandria University. Rats had free access to tap water and a basal diet, which were provided ad libitum.

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Following two weeks of acclimation, animals were divided into 2 equal groups (n=10): Group 1 served as the control, group 2 was administered with Fe_2O_3NPs (5 mg/kg) by oral gavage. The rats were dosed every day for 90 days with this time period selected to cover two reproductive cycles of spermatogonia to study the reproductive toxicity (unpublished data). No signs of stress were observed in rats from any of the experimental groups during the study period. All experimental procedures, animal handling, sampling, and scarification were performed in accordance with the Guide for the Care and Use of Laboratory Animals, 8th edition (National Research Council 2011) and were approved by the Research Ethical Committee of the Medical Research Institute of Alexandria University. All efforts were made to minimize the rats suffering during the experimental period

Blood sample collection and tissue preparation

On day 90 of the experimental period, all animals were sacrificed by cervical dislocation under anaesthesia (ketamine 100 mg/kg and xylazine 10 mg/kg intraperitoneally) in accordance with the literature [11]. The final body weight was <200 g. There were no signs of toxicity at the stated doses of anaesthetic agents. Blood samples were collected in test tubes containing heparin as an anticoagulant and placed immediately on ice. The blood samples were centrifuged at 860 x g for 20 min to separate the plasma. The plasma was kept at 80˚C until the experimental parameters were measured and analyzed. The hearts and lungs were immediately removed and washed with chilled saline solution (0.9%). The Griess reaction was supplemented with the reduction of nitrate to nitrite by nicotinamideadenine dinucleotide phosphate dependent nitrate reductase. In brief, the first step required the diazotisation of sulphanilic acid with nitrite ions followed by the coupling of this product with diamine, resulting in a measurable pink metabolite, which was analysed at a wavelength of 540 nm.

Antioxidant determination

The total Antioxidant Capacity (TAC) and the activities of Superoxide Dismutase (SOD), Glutathione Peroxidase (GPX), Glutathione S-transferase (GST) and Catalase (CAT) in the tissue homogenates were measured using colorimetric kits (Bio-Diagnostic, Ltd.). Reduced Glutathione (GSH) content was assessed after protein precipitation using a metaphosphoric acid reagent. The assay was based on the oxidation of GSH by 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to yield Glutathione Disulphide (GSSG) and 5-thio-2-nitrobenzoic acid (TNB). The rate of TNB formation was assessed at 412 nm and was proportional to the GSH amount present in the sample [12]. The rate of formation of TNB was monitored by recording the change in the absorbance at 412 nm. The total GSH content in the samples was determined from a GSH standard curve. The results were subsequently expressed as nmol/g tissue by dividing the concentration of GSH in the sample by the total weight (g).

Statistical analysis

Results are expressed as mean ± standard error. All statistical analysis was carried out using the general linear model (SAS Institute, Inc). Multiple comparisons were performed using one-way analysis of variance followed by Duncan's new multiple range test. To test for interactions between the individual treatments when given in combination, a factorial design test was used. $P<0.05$ was considered to indicate statistical significance.

Results

Levels of p53, TNF-α, and IL-6 increase in tissue following exposure to NPs. p53 controls the cell cycle to suppress the production of tumours, and acts as genome guard and as an inducer of apoptosis. TNF-α and IL-6 have important roles in tissue injury and oxidative stress. The results indicated that p53, TNF-α and IL-6 levels were significantly higher in tissues of rats exposed to Fe_2O_3NPs caused the production of a significantly higher level of p53 in tissues (Figure 1A), and TNF-α in tissues (Figure 1B) compared to the rats exposed to Fe_2O_3NPs compared to the rats exposed to Fe_2O_3NPs. Regarding IL-6 levels (Figure 1C), there was no significant difference in the tissues of rats exposed to Fe_2O_3NPs. The rats co-exposed to both NPs demonstrated significantly higher p53, TNF-α, and IL-6 levels compared with the control rats or rats exposed to individual NPs (Figure 1).

**P<0.05 vs. control group; #P<0.05 vs. Fe $_2$O$_3$ NP-exposed group; $P<0.05 vs. using Factorial Design. Fe2O3NP, iron oxide nanoparticle; TNF-α, tumour necrosis factor- α; IL, interleukin.*

aP<0.05 vs. control group; bP<0.05 vs. Fe2O3NP-exposed group; cP<0.05 vs.TBARS, thiobarbituric acid-reactive substances; NOx, nitric oxide end products; GSH, glutathione; SOD, superoxide dismutase; CAT, catalase; GST, glutathione S-transferase; GPx, glutathione peroxidase; TAC, total antioxidant capacity; Fe_2O_3NPs, iron oxide nanoparticles. to catastrophic cell damage. It has a short half-life, so upon analysis, it is typically identified as total nitrite and nitrate as these are both NOx end products. The male rats exposed to Fe_2O_3NPs demonstrated significantly higher tissues levels of thiobarbituric acid-reactive substances (TBARS) and NOx compared with the control rats.

Antioxidant levels decrease following exposure to NPs

The antioxidant parameters detected in the present study included GSH, which represents 90% of the reducing power of the cell, and antioxidant enzymes SOD, CAT, GPX, and GST, and TAC. Exposure to Fe_2O_3NPs caused a significant decline antioxidant parameters compared with the levels found in control rats (Table 1). When comparing rats exposed to Fe_2O_3NPs the only significant difference was a significantly lower level of TAC in tissues of rats.

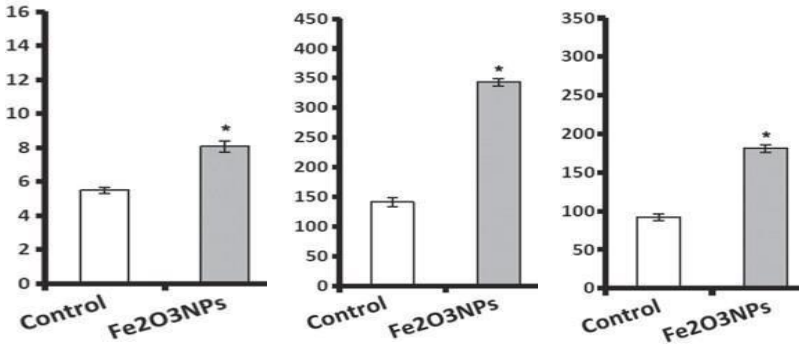

Figure 1. *Levels of inflammatory cytokines in control, Fe2O3NP-exposed group.*

(A) *Levels of p53(ng / mg protein) in rat tissues.*

(B) *TNF-α(ng / mg protein) levels in rat tissues.*

(C) *Levels of IL-6(ng / mg protein) in rat tissues.*

Table 1. *Rat tissues levels of TBARS, NOx, GSH, SOD, CAT, GST, GPx and TAC of male rats exposed to Fe_2O_3NPs.*

Experimental groups		
Parameter	**Control**	**Fe_2O_3NPs**
TBARS (nmol/g tissue) NO (µmol/g tissue) GSH (nmol/g tissue) SOD (mU/mg protein)	59.9±1.2 28.24±0.74 5.0±0.20 38.3±2.2	87.5±3.4[a,b] 40.78±2.5[a,b] 4.0±0.06[a] 25.4±1.7[a]
CAT (U/mg protein)	55.0±2.94	38.0±1.88[a]
GST (U/mg protein)	0.46±0.03	0.32±0.02[a]
GPX (mU/mg protein)	26.6±1.81	17.1±1.29[a]
TAC (µmol/g tissue)	21.50±0.10	17.08±0.16[a]

Discussion

The risk of human exposure to various NPs that may cause synergistic toxicities on tissues. The tissues are of particular concern as they face the most interaction with NPs in medical processes and through general environmental exposure. The present study confirmed the tissues toxicity of both Fe_2O_3NPs also demonstrated their synergistic and additive effects in inducing these toxicities. The present study confirmed the proinflammatory effects of Fe_2O_3NPs. For example, significantly higher levels of TNF-α and IL-6 in tissues were detected. In accordance with these results, Zhu et al [13] reported that intravascular Fe_2O_3NPs induces endothelial system inflammation and dysfunction via either direct interaction with the endothelial monolayer or indirectly by releasing free iron, thus impacting the endothelial cells and provoking oxidative stress responses. Fe_2O_3NPs have evident effects as they cause specific pathomorphological damage in rat tissues [14]. NPs are known to up regulate the transcription of various proinflammatory genes, including TNF-α, IL-1, IL-6, and IL-8, by activating NF-κB signalling. These sequential molecular and cellular events are known to cause oxidative stress, followed by severe cellular genotoxicity and then programmed cell death through activation of the JNK, p53 and NF-κB pathways. Fe_2O_3NP induced inflammatory responses in murine tissues [15,16]. Indices of oxidative damage on tissues suffer oxidative stress damage not only as a result of increased ROS generation but also due to the impairment of the antioxidant mechanism caused by NP exposure. The present study demonstrated that Fe_2O_3NPs significantly hampered the main antioxidant processes in tissues including levels of antioxidant enzymes SOD, GST, GPx, and CAT, TAC, and the GSH system. Therefore, a growing body of evidence, including the present findings, confirm the pro-oxidant effects of Fe_2O_3NPs [17,18]. Besides their general pro-oxidant and Proinflammatory effects, Fe_2O_3NPs also induced specific metabolic alterations that may have important roles as atherogenic factors. Reducing the risk of cardiovascular disease [19]. In summary, the present study determined the adverse effects of long-term exposure to Fe_2O_3 NPs in tissues toxicity, induction of inflammation, free radical generation, and inhibition of antioxidant mechanisms. In addition, NPs caused alterations in tissues. Furthermore, the results evidently demonstrated that simultaneous exposure to Fe_2O_3 NPs resulted in greater ramifications to the tissues compared to the effect of individual NPs.

References

1. Yousef MI, Abuzreda AA, Kamel MA. Cardiotoxicity and lung toxicity in male rats induced by long-term exposure to iron oxide and silver nanoparticles. Exp Ther Med. 2019;18(6):4329-39.
2. Karlsson HL, Gustafsson J, Cronholm P, et al. Size-dependent toxicity of metal oxide particles—a comparison between nano-and micrometer size. Toxicol Lett. 2009;188(2):112-8.
3. Senapati S, Ahmad A, Khan MI, et al. Extracellular biosynthesis of bimetallic Au–Ag alloy nanoparticles. Small. 2005;1(5):517-20.
4. Sharma VK, Yngard RA, Lin Y. Silver nanoparticles: green synthesis and their antimicrobial activities. Adv Colloid Interface Sci. 2009;145(1-2):83-96.

Citation: *Abuzreda A. Toxicity of iron oxide nanoparticles, on antioxidant enzymes and free radicals in male rats. Mater Sci Nanotechnol. 2023;7(2):140*

5. The University of Iowa. Office of animal resources: Institutional animal care and use committee, IACUC Guidelines: Anesthesia. 2018.

6. Yousef MI, Abuzreda AA, Kamel MA. Neurotoxicity and inflammation induced by individual and combined exposure to iron oxide nanoparticles and silver nanoparticles. J Taibah Univ Sci. 2019;13(1):570-8.

7. Foldbjerg R, Olesen P, Hougaard M, et al. PVP-coated silver nanoparticles and silver ions induce reactive oxygen species, apoptosis and necrosis in THP-1 monocytes. Toxicology letters. 2009;190(2):156-62.

8. Patil US, Adireddy S, Jaiswal A, Mandava S, Lee BR, Chrisey DB. In vitro/in vivo toxicity evaluation and quantification of iron oxide nanoparticles. Int J Mol Sci. 2015;16(10):24417-50.

9. Voinov MA, Pagan JO, Morrison E, et al. Surface-mediated production of hydroxyl radicals as a mechanism of iron oxide nanoparticle biotoxicity. J American Chem Soc. 2011;133(1):35-41.

10. Lee JH, Ju JE, Kim BI, et al. Rod-shaped iron oxide nanoparticles are more toxic than sphere-shaped nanoparticles to murine macrophage cells. Environ Toxicol Chem. 2014;33(12):2759-66.

11. Alarifi S, Ali D, Alkahtani S. Nanoalumina induces apoptosis by impairing antioxidant enzyme systems in human hepatocarcinoma cells. Int J Nanomed. 2015;10:3751.

12. Park EJ, Park K. Oxidative stress and pro-inflammatory responses induced by silica nanoparticles in vivo and in vitro. Toxicol Lett. 2009;184(1):18-25..

13. Mosa IF, Abd HH, Abuzreda A, et al. Bio-evaluation of the role of chitosan and curcumin nanoparticles in ameliorating genotoxicity and inflammatory responses in rats' gastric tissue followed hydroxyapatite nanoparticles' oral uptake. Toxicol Res. 2020;9(4):493-508.

14. Pujalte I, Passagne I, Brouillaud B, et al. Cytotoxicity and oxidative stress induced by different metallic nanoparticles on human kidney cells. Particle and fibre toxicology. 2011;8:1-6.

15. EL-Sebeay A, Kishk AM, Abuzreda AA. The Ability of Blackberry Juice Compared to Anthocyanin and Gallic Acid to Reduce the Harmful Effects of Acrylamide in Rats' Kidneys. J High Inst Public Health. 2020;50(1):32-8.

16. Nel A, Xia T, Madler L, et al. Toxic potential of materials at the nanolevel. Sci. 2006;311(5761):622-7.

17. Mosa IF, Abd HH, Abuzreda A, et al. Chitosan and curcumin nanoformulations against potential cardiac risks associated with hydroxyapatite nanoparticles in Wistar male rats. Int J Biomater. 2021.

18. Nemmar A, Beegam S, Yuvaraju P, et al. Ultrasmall superparamagnetic iron oxide nanoparticles acutely promote thrombosis and cardiac oxidative stress and DNA damage in mice. Part Fibre Toxicol. 2015;13(1):1-1.

19. Ibrahim AF, Hafez LM, Yousif AB. Protective Role of Polyphenols (Anthocyanin, Gallic Acid) and Blackberry Juice against Acrylamide Reproductive Toxicity in Male Rats. 2020.

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