Iron Oxide Nanoparticles Induced Oxidative Damage in Peripheral Blood Cells of Rat

Usha Singh Gaharwar, Paulraj R*

School of Environmental Sciences, Jawaharlal Nehru University, New Delhi, India
Email: *paulrajr@hotmail.com

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Abstract

Nanotechnology is a rapidly growing field that has elicited much concern due to a variety of applications in different fields such as industry, medicine, and cosmetics. These developments increase the concern among the general population. Hence, there is an urgent need to explore the possible human health effects of these nanomaterials. The present study is aimed to evaluate the cytotoxic and genotoxic effects of iron oxide nanoparticles (IONPs) in-vivo. In order to study the toxic effects, Wistar rats were administered intravenously with various doses of IONPs (Fe2O3) through caudal vein once in a week for 28 days, and various biochemical assays such as antioxidant enzymes activity (SOD, CAT, and GSH), lipid peroxidation, DNA damage and hematological parameters were evaluated. Genotoxicity was evaluated by comet assay and oxidative stress was measured by antioxidant enzymes. The results reveal that IONPs alter hematological factor such as RBC counts, WBC counts, neutrophils, monocytes and hemoglobin. A dose-dependent inhibition (p < 0.05) of antioxidant enzymes was found, and meanwhile the level of MDA elevated significantly (p < 0.05) in IONPs treated groups in dose-dependent manner; however comet assay results indicate that IONPs did not induce any significant DNA damage. The present study concluded that IONP affects inflammatory response, which induces the oxidative stress and may adversely affect the cellular function.

Keywords

Oxidative Stress, Iron Oxide Nanoparticles, Antioxidant Enzymes, Genotoxicity, Peripheral Blood Cells

1. Introduction

In the last few decades, scientific research has been mainly focused on nanotechnology and nanoscience. Nano-

*Corresponding author.

technology and nanoscience studies have received much consideration in the last decade. Nanotechnology, which is one of the new technologies, can be defined as the science and engineering involved in the design, synthesis, characterization, and application of materials and devices whose size varies from 1 to 100 nano-meters (nm) [1] [2]. Metal and metal oxide nanomaterials (NMs) are used in cosmetics and skin care products, abrasives, polishers and as drug delivery systems. Increasing applications have raised the concern about the toxicity of these nanomaterials toward the human health. Currently, the most commercially important NMs are simple metal oxides, such as iron oxide (Fe₃O₄, Fe₂O₃), aluminium oxide (Al₂O₃), silica dioxide (SiO₂), titanium dioxide (TiO₂) and zinc oxide (ZnO). Iron (II, III) oxide nanoparticles (IONPs) have attracted much consideration not only because of their magnetic properties, but also due to their abundant potential in several biomedical and in-vivo clinical applications, such as magnetic resonance imaging (MRI), contrast enhancement, tissue repair, as drug delivery, and magnetic hyperthermia cancer treatment [3]. However, possible risk due to ever increasing application of Fe₂O₃ is not fully elucidated. Previous studies reported that Fe₂O₃ and Fe₃O₄ induce DNA damage in A549 cells [4] [5]. Similarly, Bhattacharya et al. [6] reported that Fe₂O₃ nanoparticles induced DNA-breakage in IMR-90 and BEAS-2B cells.

Earlier in-vivo studies show that Fe₂O₃ induces inflammation in the lungs, liver and kidney after two weeks post-treatment [7]. Many studies have revealed that IONPs induce dose-dependent oxidative stress and reduce cell viability [7]-[10]. ROS was found to be elevated in the cells treated with IONPs as compared to control [11] and production of ROS was correlated with concentration of nanoparticles in cells. Many studies suggest that iron oxide nanoparticles produce free radical species which is found to significantly reduce the levels of cellular antioxidants that lead to oxidative damage to intracellular proteins and DNA and increased lipid peroxidation (LPO) [12]-[15]. It has been demonstrated that IONPs and other surface coated IONPs showed significant DNA damage [9]. However, the immunological effects of intravenously administered nanoparticles have not been extensively studied. Although the intravenous exposure of IONPs is rare in human, but increasing application of these NPs in biomedical field, intravenous injection has become a choice for administration of the IONPs. Since, iron oxide nanoparticles are being used widely in different biomedical applications (drug delivery, hyperthermia, MRI), as a result these nanoparticles may enter into the blood stream and may interact with blood cells and induce oxidative stress. Hence, present investigation is aimed to evaluate the time- and dose-dependent effects of IONPs (Fe₂O₃) on immune system and oxidative stress in response to genotoxicity.

2. Material and Method

2.1. Chemicals

Iron oxide nano-powder (Fe₃O₄) purchased from Intelligent Materials Pvt. Ltd. (USA). 5,5-Dithiobis-(2-nitrobenzoic acid) (DTNB), reduced glutathione (GSH), 2-thio-barbituric acid, ethylene diamine tetraacetic acid (EDTA), bovin serum albumin, were purchased from Sigma-Aldrich. All other chemicals used were of the highest purity available from local chemical company’s commercial sources.

2.2. Characterization of Nanoparticles

Fe₂O₃ nanoparticles used in the present study were procured from Intelligent Materials Pvt. Ltd. (USA, CAS: 1309-37-1). Nanoparticles were in powder form and further characterized with different methods (TEM, SEM-EDX and XRD). Morphology and size of Iron oxide (Fe₂O₃) nanoparticles were evaluated by Transmission electron microscopy (JEOL-JEM-2100F) and scanning electron microscopy (ZEISS, GERMANY EV-40) at an accelerating voltage of 200 kV and 15 kV, respectively. Elemental composition analysis was done using an energy dispersive X-ray analyzer (EDX-200). The aqueous suspension was drop casted on a carbon coated copper grid, and the grid was air dried at room temperature (25°C) before loading into microscope. The amorphous nature of iron oxide nano-powder was determined by X-ray diffraction (XRD) at room temperature. The XRD patterns (Bragg peaks) were recorded with a PANalytical, Netherland X’pert PRO diffractometer using a solid state detector with a monochromatized Cu Ka1 (kCu = 1.54 A⁻) radiation source at 45 kV.

2.3. Animals

Healthy male Wistar rats (6 - 8 week old) were obtained from Animal house, JNU, New Delhi, India for this study. Before initiation of the study, animals were allowed to acclimatize to animal room conditions for a week
period. Animals were kept in an experimental room in polypropylene cages under controlled environmental conditions of temperature (21°C ± 3°C) and humidity (50 ± 10). All animals were kept in an animal house at 12 hours day/night cycle for 28 days and fed food pellet and water. All animal were kept in stress-free, hygienic, and animal-friendly conditions. After acclimatisation, the animals were selected randomly and divided into four groups and each group consist 6 animals. All experimental protocol was followed as per the guideline and approval of Animal Ethics Committee, Jawaharlal Nehru University, New Delhi.

2.4. Experimental Design
Freshly prepared suspensions of IONPs were ultra-sonicated for 15 min and mixed thoroughly prior to use. Group one was treated as a control injected (iv) with saline and other three groups were treated as experimental group. Fe$_2$O$_3$ NPs were intravenously injected in rat’s tail vein with different doses such as 7.5, 15 and 30 mg/kg/bw once in a week up to 28 days. Tail vein was cleaned with xylene before injection.

2.5. Sample Collection
Blood (1 ml) was withdrawn from the retro-orbital sinus with a heparin-coated capillary and collected in tube with 20 mg/ml EDTA anticoagulant. Blood samples were collected regularly at weekly intervals from control and treated animals. Before taking the blood, animals were anesthetized with 0.3 ml/250mg ketamine/xylazine. Approximately 500 µl blood was used for comet assay and haematological assay and remaining 500 µl blood was used for the oxidative stress parameters. Blood sample (500 µl) was centrifuge at 2200 g for 15 min and collected serum was stored at −20°C for further analysis. The pellet, containing erythrocytes, washed thrice with phosphate buffer saline (0.9% NaCl in 0.01 M phosphate buffer, pH 7.4) and centrifuged at 2000 rpm for 15 min at 4°C. Hemolysate was prepared by mixing 1.9 ml of cold distilled water to 0.1 ml of packed cell volume (PCV) suspension. Hemolysates were then stored at −20°C for analysis of antioxidant assays. Erythrocyte hemolysates were prepared according to the method of Dodge et al. [16].

2.6. Hematological Analysis
Automated haematological analyser (KX-21, Sysmex, Transasia, India) was used to analyse haematological and immunological parameters of blood such as White blood cells (WBC), Neutrophils, Monocyte, Red blood cell (RBC), haemoglobin (Hb), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC).

2.7. Measurement of Oxidative Stress

2.7.1. Thiobarbituric Acid Reactive Substances (TBARS) Measurements
Lipid peroxidation in hemolysate was evaluated spectrophotometrically by measuring thiobarbituric acid reactive substances (TBARS) level according to Varshney and Kale [17] and is expressed in terms of malondialdehyde (MDA), which is the end product of the reaction. In brief, 400 µl sample was mixed with 1.6 ml of Tris-KCl (0.15 M KCl + 10 mM Tris-HCl, pH 7.4) buffer and then 0.5 ml of 30% TCA and 0.5 ml of TBA was added to this mixture. Then the sample was heated for 45 min at 80°C in a water bath, after that sample was allow to cool in ice and then centrifuged at room temperature for 10 min at 3000 rpm. Supernatant was taken for the absorbance and it was measured at 531.8 nm with a spectrophotometer (Shimadzu spectrophotometer UV4100).

2.7.2. Reduced Glutathione (GSH) Level
Reduced glutathione level was measured with the method described by Ellman [18] and modified by Jollow et al. [19]. It is based on the yellow colour formation when available sulphhydryl groups react with DTNB. In brief, reaction mixture contain 0.2 ml sample and 3 ml of 4% sulphosalicylic acid and tubes were centrifuged at 2500 g for 15 min. Collected supernatant (0.2 ml) was mixed with 0.4 ml of 10 mM DTNB and 1 ml phosphate buffer (0.1 M, pH 7.4). Finally, absorbance was taken at 412 nm. Total GSH content was expressed as n mol GSH/mg protein.

2.7.3. Superoxide Dismutase (SOD) Activity
The activity of superoxide dismutase (SOD) was assayed according to the method of Kakkar et al. [20]. Chilled
ethanol (0.25 ml) and chloroform (0.15 ml) was added to 1 ml hemolysate. The assay mixture contained 0.1 ml of supernatant, 1.2 ml of sodium pyrophosphate buffer (pH 8.3, 0.052 M), 0.2 ml of phenazinemethosulphate (186 μm), 0.3 ml of nitro blue tetrazolium (300 μm), 0.2 ml of NADH (750 μm). The reaction was stopped by the addition of 0.1 ml of glacial acetic acid after incubation at 37°C for 90 sec, followed by the addition of 4.0 ml of n butanol. The mixture was incubated for 10 min, centrifuged then butanol layer was separated. The colour intensity of the chromogen in butanol layer was measured at 560 nm against n-butanol and one unit of the enzyme activity is defined as enzyme concentration required to inhibit the optical density at 560 nm of chromogen production, and expressed as Units/min /mg protein.

2.7.4. Catalase (CAT) Activity
Catalase (CAT) activity was measured by the method of Aebi [21]. The principle of the method was based on the determination of the rate constant (k) or the rate of hydrogen peroxide decomposition at 240 nm. The Reaction mixture contain sample, sodium phosphate buffer (0.1 M, pH 7.4), 1% triton X-100 (final concentration) and 500 mM H₂O₂ in phosphate buffer. The absorbance of H₂O₂ was recorded at 240 nm for 60 sec in the spectrophotometer (Shimadzu spectrophotometer UV4100). Catalase activity was estimated from the slope of the H₂O₂ absorbance curve and normalized to protein concentration. Catalase activity was expressed as nKat per mg protein (One Kat is defined as 1 mole of H₂O₂ consumed per second per mg protein).

2.8. Protein Estimation
The total protein concentration was spectro-photometrically estimated by the Bradford method [22] using bovine serum albumin as the standards.

2.9. Comet Assay
The alkaline comet assay was performed to analysed DNA damage, according to Paulraj and Behari [23]. Cell viability was assessed by trypan blue exclusion assay. All the steps were performed under dim light to prevent the occurrence of background DNA damage. Peripheral blood sample was collected and slides were prepared in triplicate per sample per experiment. Blood samples (10 μl) were mixed with 0.2 ml of 0.5% low melting point agarose (LMPA) suspended in phosphate buffered saline (PBS). Cells in LMPA were then subjected to slide which were precoated with 0.8% agarose and immediately covered with coverglass (24 mm × 50 mm) and refrigerated for 5 min to gel. After removing cover glass from the slide, slides were immersed in ice-cold alkaline lysing solution (2.5 M NaCl, 10 mM Tris, 100 mM EDTA, 10% dimethyl sulphoxide, 1% triton X-100, final pH 10.0) for overnight at 4°C. The slides were then incubated for 20 min in ice-cold electrophoresis solution (0.3 M NaOH, 1 mM EDTA, pH 13) to unwind the DNA, then allowed to electrophoresis at 25 V: 300 mA (1.25 V/cm) for 25 min. After electrophoresis, the slides were subjected to neutralization buffer (0.5 M Tris-HCl, pH 7.4) for 15 minutes then slides were stained with 20 μg/ml EtBr (ethidium bromide) dye. Images (50 cells/ slide randomly) were acquired using fluorescence microscope (Carl Zieiss, Deutschland, Germany) attached with digital camera (TK1280, JVC, Yokohama, Japan). Tail moment and olive tail moment (OTM) of 50 randomly selected cells were measured from each slide by using Comet Score software.

2.10. Statistical Analysis
In this study, the results are presented as means ± standard deviation (SD) made in six replicate. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Duncan Multiple Range Test and Dunnett test. Significance was ascribed at p < 0.05. All analyses were conducted using sigma plot 11 statistical package (Systat Software, San Jose, CA).

3. Result
3.1. Characterization of Fe₂O₃ NPs
In present study, SEM-EDX observation of Fe₂O₃ NPs (Figure 1(a), Figure 1(b)) showed spherical morphologies and EDX analysis revealed that Fe₂O₃ NPs sample was devoid of any impurity. TEM images (Figure 2(a)) show that the average size of Fe₂O₃ NPs was ~30 - 35 nm diameters and spherical size. XRD data was found (Figure 2(b)) to indicate that particles consist of Fe₂O₃.
Figure 1. (a) SEM image of iron oxide nanoparticles; (b) Energy-dispersive X-ray profile, sharp peak of iron and oxygen peak ($\text{Fe}_2\text{O}_3$ in gold grid).
3.2. Hematological Changes

Hematological parameter has been shown in Figures 3(a)-(g). In RBC (Figure 3(a)) no significant changes (p < 0.05) were observed in first week in any group meanwhile 15 mg/kg and 30 mg/kg groups showed significant changes in 2nd and 3rd week as compared to control while only time dependent changes have been seen in last week in treated groups and Hemoglobin conc. was found to decrease significantly (p < 0.05) (Figure 3(b)) as compared to control group. Mean corpuscular hemoglobin (MCH) (Figure 3(c)) was found to reduce significantly (p < 0.05) in 15 and 30 mg/kg doses as compared to control. A significant increase (p < 0.05) was observed in Mean corpuscular Hb conc. (MCHC) (Figure 3(d)) as compared to control in a first week of treatment while slight changes were observed in later weeks. In WBC count (Figure 3(e)), significant increase (p < 0.05) has been observed in 7.5 mg/kg group as compared to control in 2nd week however no significant changes were
Figure 3. Hematology analysis for the Wistar rats treated with different doses of iron oxide nanoparticle and saline. (a)-(g) These results show Mean and standard deviation of RBC (a), Hemoglobin (b), Mean corpuscular Hb (c) and Mean corpuscular Hb conc. (d), WBC (e), Monocyte (f) and Neutrophils (g). Error bars represent standard deviation. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Duncan Multiple Range Test. Significance was ascribed at p < 0.05. The inter-group statistical significant differences have been marked by letters: a (vs. control), b (vs. 7.5 mg/kg dose of Fe2O3), c (vs. 15 mg/kg dose of Fe2O3), and inter-week significant differences were marked by symbol: # (vs. 1st week), * (vs. 2nd week), $ (vs. 3rd week).
found in other weeks meanwhile 15 mg/kg and 30 mg/kg showed significant increase (p < 0.05) in first 3 week but later on the changes were neutralized. There was a significant (p < 0.05) increase in neutrophils and monocytes (Figure 3(g), Figure 3(f)) level in initial weeks but no changes were measured at the end of the experiment.

3.3. Effect of Fe$_2$O$_3$ NP on Anti-Oxidative System

The effect of Fe$_2$O$_3$ NPs on lipid peroxidation has been shown in Figure 4(a). It has been observed that lipid peroxidation level was not significantly changed in first week of treatment but later on a significant (p < 0.05) elevated level of TBARS was found as compared to control group. Results indicate that Fe$_2$O$_3$ NPs induce the lipid peroxidation in dose and time dependent manner. Effect of Fe$_2$O$_3$ NPs in GSH level is shown in Figure 4(b). A significant increase has been found in the 7.5 mg/kg dose (p < 0.05) in first week only but on later weeks there was no significant changes were observed as compared to control. Whereas in 15 mg/kg treated group showed a significant reduction (p < 0.05) in GSH level in first 2 weeks but later on period effects were unchanged while the higher dose (30 mg/kg) of Fe$_2$O$_3$ NPs significantly reduced the GSH level in whole exposure period. It indicated that higher doses of Fe$_2$O$_3$ NPs reduced the GSH level in a dose and time dependent manner. SOD activity was significantly decreased (p < 0.05) in a time dependent manner in higher dose (30 mg/kg) of Fe$_2$O$_3$ NPs treated groups whereas in 7.5 mg/kg and 15 mg/kg treated groups, SOD activity was reduced significantly (p < 0.05) in later period but in initial period changes were insignificant (Figure 4(c)). Effect of Fe$_2$O$_3$ NPs in CAT enzyme activity is shown in Figure 4(d). The CAT level was slightly elevated in first week of

![Figure 4](image_url)

**Figure 4.** Oxidative stress marker and antioxidant parameter analysis. (a) TBARS level; (b) GSH level; (c) SOD activity; (d) CAT activity. Data represented are mean ±SD of experiments made in six replicate. Statistically significant was ascribed at (p < 0.05). The inter-group statistical significant differences have been marked by letters: a (vs. control), b (vs. 7.5 mg/kg dose of Fe$_2$O$_3$), c (vs. 15 mg/kg dose of Fe$_2$O$_3$), and inter-week significant differences were marked by symbol: # (vs. 1st week), * (vs. 2nd week), $ (vs. 3rd week).
treatment in 7.5 mg/kg and 15 mg/kg groups although the changes were insignificant, but later on a significant reduction (p < 0.05) in CAT activity was observed as compared to the control group whereas 30 mg/kg treatment group was found to show a significant decrease in CAT activity in whole exposure period. These results indicate that the Fe2O3 NPs inhibit CAT activity in a time and dose dependent manner.

3.4. DNA Damage

Comet assay analysis was done to measure geno-toxic effect of Fe2O3 NPs (Figures 5(a)-(c)). Cell viability was assessed by Trypan blue assay. The cell viability was >90% in all groups. Outcome of the treated and control blood cells are represented with tail moment and olive tail moment. Tail moment and olive tail moment of 50 randomly selected comets are presented through quantile box plots. Rats exposed with 7.5, 15 and 30 mg/kg doses of Fe2O3 NPs did not show any significant changes in tail moment and olive tail moment as compared to control group. Tail moments were 4.04 ± 0.95 µm, 4.4 ± 1.27 µm, 4.75 ± 2.0 µm 5.2 ± 3.01 µm in control, 7.5 mg/kg, 15 mg/kg and 30 mg/kg of Fe2O3 NPs treated groups, respectively. Olive tail moments were 2.88 ± 1.12 µm, 3.25 ± 0.88 µm, 3.33 ± 1.12 µm, 3.5 ± 1.12 µm in control, 7.5 mg/kg, 15 mg/kg and 30 mg/kg groups, respectively. The results indicate that Fe2O3 NPs did not induce DNA damage.

4. Discussion

Nanotechnology has become a crucial word of public attention, since wide application of nanotechnology in various fields. In spite of the beneficial aspect of nanotechnology, society cannot ignore its other aspect which is...
toxicity, as nanoparticles may lead to significant toxic effects on human and environment either intentionally or accidently. As we know due to small size nanoparticles can move freely or cross the physiological barriers and have the ability to enter, translocate, generate oxidative stress and can cause significant damage to the cell or tissue [24]. Nanoparticles have large surface to size ration that enable them to penetrate into the human body via different route such as inhalation, ingestion, dermal and injection and may generate toxicity. Hence, the concern about the nanoparticles toxicity to human and environment has been increased since it remains mostly unexplored. The present study will provide the first evidence of the oxidative stress and genotoxicity of iron oxide nanoparticles by intravenous route in peripheral blood cells.

Super-paramagnetic iron oxide nanoparticles (SPIONs) have been extensively used in potential biomedical applications such as magnetic drug targeting, enhanced resolution magnetic resonance imaging, transfection, cell and tissue targeting and tissue repair [25]-[28]. When nanoparticles enter in to the bloodstream, they immediately encounter by the plasma proteins and immune cells. The uptake of nanoparticle may occur through various pathways like hemolysis, thrombogenicity and complement activation. These pathways include numerous activities like reduced number of blood cells, anti-mitotic properties, stimulation of oxidative stress and reduction in cellular antioxidants and increasing the number of cells involved in the immune processes. Present study reveals that IONPs can induce an inflammatory response and modulate the activity of the immune system and alter hematological factors such as significant changes in blood cell counts, increased white blood cells (monocytes, and neutrophils) and decreased number of red blood cells after the administration of different doses of IONPs. Our results are line with Rezaei and Zarchi [29], which shows that TiO2 NPs cause significant changes in blood cells such as increased white blood cells (lymphocytes, monocytes, eosinophils and basophils).

Red blood cells are derived from hemopoietic stem cells in bone marrow. After maturation or erythropoiesis, red cells enucleate and enter in to the circulatory system. Thus, the variation in red blood cells can be related to the dysfunction of hematopoietic system. Reduction in red blood cells are found at the high dose in groups (15 and 30 mg/kg). In 1989 Machiedo et al. [30] demonstrated that free radicals produced by nanoparticles may be the main cause for destruction of red blood cells. Similar results have been found in previous study when mice were exposed with TiO2 NPs [31]. It is predicted that high concentration of iron oxide nanoparticles, reduce number of blood cells, enhance oxidative stress, reduction of cellular antioxidants and involvement of immune cells in the immune processes.

Toxic effect of nanoparticles can be elucidated by the estimation of oxidative stress [10] [15] [32]-[35]. Nanoparticles are found to produce free radicals and to induce oxidative stress and thus can disturb the antioxidant defence system in the animal [36]. Our data suggest that ROS-mediated oxidative stress may be a crucial mechanistic paradigm to explain the toxic effects of IONPs. In the case of IONPs treated cells, cellular oxidative stress was manifested by reduced GSH, SOD and catalase level and increased lipid peroxidation. Moreover, free radicals also resulted in the production of malonaldehyde, an indication of lipid peroxidation. The increased levels of ROS may induce cell membrane damage and thus result an increased lipid peroxidation and later cell death [37]. Our results are in line of previous studies that magnetite nanoparticles found to induce lipid peroxidation at 20 µg/ml conc. in lung’s epithelial cell and human bone marrow mesenchymal stromal cells (hBMSCs) [9] [38]. Our previous study reported that intra-peritoneal injection of magnetic iron oxide nanoparticles (MIONPs) increase the production of oxidative stress in dose dependent and time dependent manner in rat kidney and brain cells [39].

Reactive oxygen species such as superoxide anion (O$_2^-$), hydroxyl radical (HO$^\cdot$) and hydrogen peroxide (H$_2$O$_2$) is responsible to stimulate a range of cellular and physiological actions including inflammation, DNA damage and apoptosis [34] [40]. Genotoxic chemical induce DNA strand break and this can be standard marker for DNA damage [41]. Present study shows that IONPs did not induce DNA damage even at higher doses. This is may be because these nanoparticles were not able to induce sufficient oxidative stress, which may react with DNA and can cause DNA damage. Similarly Hong et al. [42] reported that SPION did not show any DNA damage in L-929 fibroblast cell. Likewise, bare iron oxide nanoparticles and surface modified iron oxide nanoparticles showed lower toxicity in A549 cell [4] [43]. On the other hand some studies showed that Fe$_3$O$_4$ nanoparticles significantly induced DNA damage in BEAS-2B [44].

5. Conclusion

Iron oxide nanoparticles alter the hematological parameters in dose- and time-dependent manner. Similarly,
Fe₂O₃ nanoparticles may induce oxidative stress, which may reduce antioxidant potential of the blood cells. However there was no significant DNA damage in cells, indicating that NPs did not cause any significant genotoxic alteration in peripheral blood cells. Thus it may be further concluded that Fe₂O₃ NPs of higher dose alter inflammatory system which may adversely affect the cellular function.

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