SYNOPSIS

Introduction

The applications of nano particles (size < 100 nm) (NPs) across the various fields are expanding rapidly because of their unique physicochemical features compared to their bulk analogues. According to the market survey, the production of metal oxide NPs was estimated to rise from 2, 70, 041 tons in 2012 to 16, 63, 168 tons by 2020. On the other hand it has been reported that the novel properties of these NPs could cause unpredictable outcomes due to their interactions with biological systems. This increasing presence of NPs in the ecosystem urges us to study their potential toxic impact on health and to understand as well as assess their adverse effects on environment. Among the known metal oxides, nickel oxide (NiO) NPs have attracted wide scientific interest due to their use in solar cells, lithium batteries, LEDs, resistive memory chips, and sensors. The applications of NiO-NPs as chemical catalysts, conduction paste, magnetic materials, and in ceramics are worth mentioning. They are also used in films (electrochromic), electronics, devices for storing energy, fuel cells, inks for printing, and for wastewater treatment. Hence, in the current investigation the toxicity of NiO-NPs was evaluated using *in vitro*, *in vivo* and eco (*Allium cepa*) toxicity systems.

Statement of problem

The rapid rise in manufacturing and use of NiO-NPs raises a concern of intentional and unintended delivery into the environment with possible adverse implications for human and environmental health. The lack of toxicological data on NiO-NPs makes it difficult to determine the risk associated with them. Thus, there is an urgent need to develop rapid, accurate and efficient testing strategies to assess toxic effects of these particles on humans. Characterization of materials is an essential part to understand their behaviour in biological systems prior to toxicological testing. Therefore in this study, characterization of NiO-NPs was performed to provide the basis for understanding the properties of NPs that determine their biological effects. We have investigated whether NiO-NPs exposure can cause environmental and human health risk, with a special focus on genotoxic, biochemical, histological and biodistribution patterns. *In vitro* (human peripheral blood lymphocytes), *in vivo* (acute and 28 day repeated oral exposure in albino Wistar rats) and plant bio systems (*Allium cepa*) were tested with NiO-NPs for toxicity. The data generated in this study may be useful in predicting the possible health hazards that may arise from the introduction of NiO-NPs into the environment and give an estimate of human health risk.

Methodology used & sample results

Characterization of NiO-NPs

The NiO-NPs were characterized by Transmission electron microscopy (TEM), Dynamic Light Scattering (DLS), Laser Doppler Velocimetry (LDV) and Brunauer–Emmett–Teller (BET) analysis. The size obtained by TEM for NPs was 15.6 (± 7.6) nm and showed cubic crystalline lattice structure by X-ray Diffraction (XRD) analysis. The mean hydrodynamic diameter and zeta potential (ζ) in Milli- Q water and RPMI 1640 media was measured by DLS and was found to be 189.9 (±17.1) and 285.9 (±19.6) nm, whereas the zeta potential of NiO-NPs was 36.3 and 24.16 mV, respectively. Electrophoretic mobility of the NiO-NPs in Milli- Q and RPMI 1640 medium was 2.85 (±0.3) and 1.4 (±0.4) μm cm/s V, respectively when studied by LDV. The specific surface area was found to be 115.9 (±5.3) $m^2 g^{-1}$ by BET analysis.

Toxicity assessment of NiO NPs on human peripheral blood lymphocytes

The *in vitro* parameters investigated were cytotoxicity, genotoxicity and oxidative damage in human peripheral blood lymphocytes. The cytotoxicity studies were conducted with 0.1, 1, 5, 10, 20, 50 and 100 μg ml^{-1} concentrations of NiO-NPs. The result of trypan blue dye exclusion and MTT assays revealed that NiO-NPs reduced cell viability in a dose dependent manner. The IC50 for NiO-NPs was determined as 23.58 μg ml^{-1} after 24 h of exposure. Genotoxicity studies performed with 12.5, 25 and 50 μg ml^{-1} concentrations of NiO-NPs revealed a significant dose dependent increased in DNA damage and micronucleus formation. The oxidative stress analysis for ROS by DCFDA dye, and increased levels of MDA by lipid peroxidation assays were noted upon increasing the concentration of NiO-NPs. Morphological assessment of the lymphocytes upon exposure to NiO-NPs showed that the mechanism of toxicity was apoptosis. Thus, the preliminary mechanism of NiO-NPs for cytotoxicity on lymphocytes was assumed to be oxidative stress-mediated apoptosis and DNA damage.

Toxicity assessment of NiO-NPs by using in vivo studies (acute and 28 day repeated oral dose)

In vivo study of NiO-NPs is essential because animal systems are extremely complicated and the interaction of the NPs with biological systems could lead to novel biodistribution, clearance, immune response, and metabolism patterns. *In vivo* examination was carried out for genotoxicity, biochemical, oxidative stress, hematological, histopathology parameters and biodistribution patterns after acute and 28 day repeated oral dose exposures in Wistar rats. The genotoxicity evaluation was performed by using comet, MNT and CA assays. The antioxidant

enzyme status and lipid peroxidation profile were determined to examine the ROS generation and oxidative stress levels.

The results of comet assay revealed significant ($P < 0.001$) DNA damage at 500 mg kg^{-1} bw dose in the PBL, kidney and liver cells of female rats at the 24-h sampling time. The result of MNT and CAs assays was in agreement with the comet assay data after single oral dose. The % tail DNA values of PBL, liver and bone marrow cells of male and female rats showed dose dependent increment and significant at the higher dose (200 mg kg^{-1}) treatment after 28 days repeated oral exposure. The frequency of MN-PCEs and CAs in bone marrow cells of rats revealed clastogenic events and supported well the comet assay results of repeated oral dose study. These results provided the preliminary evidence that the NiO-NPs are capable of inducing genotoxicity when administered through the oral route after acute and repeated exposure regimens. Histological analysis showed hepatic damage and alterations in the tissue architecture of kidneys in the female rats exposed to acute doses of NiO-NPs. On the other hand, histological studies performed in rats after 28 days repeated oral dose exposure depicted mild to severe alterations in the vital organs including brain. The observed histological alterations might be attributed to the distribution profile of these NiO-NPs, which indicated that a significant quantity of Ni content was distributed to all the visceral organs. NiO-NPs induced substantial biochemical perturbations and imbalance in the antioxidant enzyme profiles in a dose dependent manner in both acute and repeated oral dose toxicity. Activation of the hepatotoxicity marker enzymes, aminotransferases, was recorded in serum and liver, whereas inhibition was observed in kidney. The activity of antioxidant enzymes was also altered by NiO-NPs in a dose-dependent manner and found to be significant at the higher doses of exposure. Lipid peroxidation profile in rats treated with acute and repeated oral doses of NiO-NPs provided further evidence for the NiO-NPs capability for inducing ROS and oxidative stress. Biodistribution analysis helps in understanding the absorption, distribution, metabolism and excretion rates (ADME kinetics) along with the organ specific accumulation after acute and 28 days of repeated oral treatment. Biodistribution of NiO-NPs revealed a maximum accumulation of Ni in the liver tissue at the 24-h sampling time. Moreover, the results of 28 days study showed significant accumulation of Ni in all tissues and clearance via urine and feces (elimination kinetics) which was noted to be dose dependent. Overall, the results obtained from the *in vivo* investigations revealed NiO-NPs produced significant increase in DNA damage, histopathological changes, alterations in

biochemical enzyme profiles and induced oxidative stress along with distribution of Ni content in vital organs. The low-dose (125 mg kg^{-1}) treated rats in acute oral dose toxicity study were asymptomatic, which gives an important clue about the NOAEL. Furthermore, the results of the present study indicated that the NOAEL concentration for NiO-NPs is <50 mg kg^{-1}, when exposed repeatedly via the oral route in rats.

Toxicity assessment by using A. cepa (plant) bio-system as eco-toxicity indicator

The optical and fluorescent microscopy results gave a comprehensive view on the impact of the NiO-NPs on *A. cepa* root tip cells. A variety of chromosomal aberrations (CAs), like laggard, clumped, disturbed, sticky anaphase, chromosomal break, bridge and c-mitosis were found upon interaction of root tip cells with various concentrations (5, 10, 20 and 40 μg ml^{-1}) of NiO particles. Results indicated a dose dependent increase in CAs, decrease in mitotic index (MI) and the effect was more significant ($P<0.05$). The results were directly proportional to the concentration of NiO NPs treatment to the *A. cepa* root bio-system. The genotoxic effect on root tip cells was evaluated using comet assay in isolated nuclei showed an increased % tail DNA with a notable decrease in % head DNA. After exposure of NiO-NPs, there was a significant increase in production of superoxide ($O_2^{\cdot-}$), hydroxyl ($^{\cdot}OH$) and hydrogen peroxide (H_2O_2) when examined in root elongation zone of *A. cepa* with histochemical staining using Nitro blue tetrazolium (NBT) and 3,3′-Diaminobenzidine (DAB), whereas, no alteration was observed in the maturation zone. Oxidative stress analysis revealed increased levels of $O_2^{\cdot-}$, H_2O_2, $^{\cdot}OH$ radicals and MDA upon increasing the concentration of NiO-NPs. The elemental analysis by using ICPAES showed a concentration and time dependent increase in the internalization of Ni into the root tip cells.

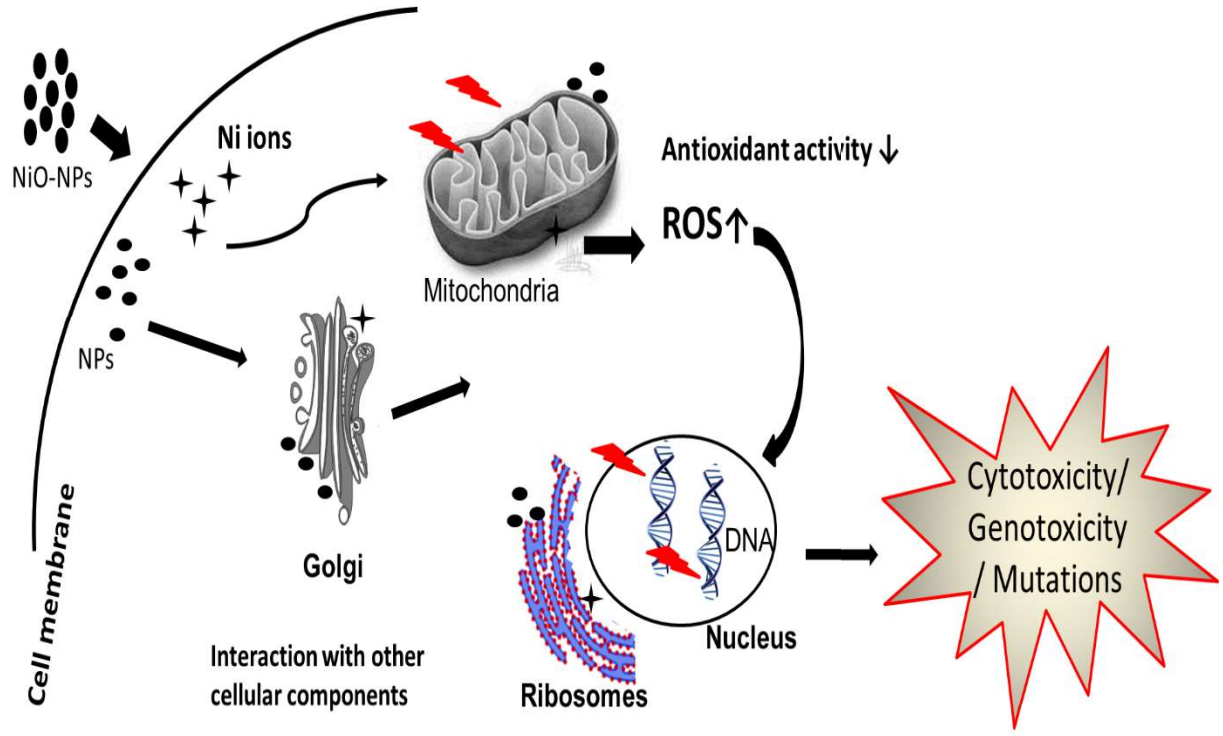
Conclusions

In this study, a range of *in vitro, in vivo* and plant model approaches have been applied to assess the possible toxic behaviour of NiO-NPs. The result of *in vitro* studies revealed NiO NPs reduced cell viability, produced oxidative stress in dose and time dependent manner. The present investigation revealed acute and 28 day repeated exposure to NiO-NPs beyond the threshold levels might lead to serious consequences including DNA damage and ROS production. The toxicity (genotoxicity, biochemical, histopathology and biodistribution) was validated by standard protocols. The blood chemistry and lipid profiles are evidence for the toxic effect of NiO-NPs. The induced DNA damage may be attributed to increased oxidative stress which

correlated with histological alteration and presence of particles in the various tissues. The ROS induced oxidative stress may be the most frequently observed mechanism of DNA damage after NiO-NPs exposure. The NiO-NPs treated *A. cepa* root tip cells indicated a dose-dependent increase in DNA damage, cytological changes and decrease in MI compared to controls. The interaction of root cells with NPs resulted in the generation of ROS, which was found to be a strongly dependent on the dose.

From this investigation it can be concluded that in comparison to controls, NiO-NPs induced significant changes in the target biochemical as well as genotoxic parameters in a dose and time dependent but not gender dependent manner. The smaller size, large surface area and may be the ions of NPs could have contributed to the obtained toxic effects. The data from the current investigation suggested a moderate toxicity risk with nano NiO. Therefore NiO particles can be considered as chemical substances that induce toxicity at high doses of exposure. However, more studies are needed to understand the exact mechanism of toxicity by extending these studies further using toxico-proteomics and metabolomics approaches. This knowledge may help to develop new approaches for the design of safe NP based consumer and medical products.

Possible Mechanism of Toxicity of NiO-NPs



Publications from the thesis work

1. **Naresh Dumala**, Bhanuramya Mangalampalli, Paramjit Grover. "In vitro genotoxicity assessment of nickel (II) oxide nanoparticles on lymphocytes of human peripheral blood" ***Journal of Applied Toxicology*** (2019): DOI:10.1002/jat.3784. (***IF: 2.909).***
2. **Naresh Dumala,** Bhanuramya Mangalampalli, Srinivas Chinde, Srinivas Indu Kumari, M. Mahboob, M. F. Rahman, and Paramjit Grover. "Genotoxicity study of nickel oxide nanoparticles in female Wistar rats after acute oral exposure." ***Mutagenesis*** (2017) 32 (4): 417-427. (***IF: 2.84).***
3. **Naresh Dumala,** Bhanuramya Mangalampalli, Sarika Srinivas Kalyan Kamal, and Paramjit Grover. "Biochemical alterations induced by nickel oxide nanoparticles in female Wistar albino rats after acute oral exposure." ***Biomarkers*** (2018) 23 (1): 33-43. (***IF: 1.976***).
4. **Naresh Dumala**, Bhanuramya Mangalampalli, Sarika Srinivas Kalyan Kamal, Paramjit Grover. "Repeated oral dose toxicity study of nickel oxide nanoparticles in Wistar rats: a histological and biochemical perspective" ***Journal of Applied Toxicology*** (2019): DOI:10.1002/jat.3790. ***(IF: 2.909)***.