SUMMARY

SUMMARY

Nanoparticles (>100nm) are found in nature in volcanic dust, natural water and soil and are called as **Natural nanoparticles,** whereas the artificially produced particles by physical, chemical or microbial processes are called as **Engineered nanomaterials.** Engineered nanoparticles are produced within the range of 1-100 nm (10^{-9}m) in length or diameter for commercial uses. Nanoparticles posses increased structural integrity as well as unique mechanical, optical, chemical, electrical and magnetic properties because of which they have gained enormous attention and are currently being widely used in modern technology and products such as cosmetics, electronics, food packaging, medical devices, catalysts and water treatment technologies. Although the applications and benefits of these engineered nanomaterials are extensive there is a severe lack of information concerning their implications on human health due to occupational exposure and on environment. Since each engineered nanoparticle is unique with respect to its properties and toxicity, it becomes imperative to investigate their toxicity in living systems.

Hence, this study mainly focuses on the adverse effects of metal oxide ENPs (Al_2O_3, Fe_2O_3, MgO, TiO_2 and ZnO) to understand the possible mechanism of toxicity in chicken embryo and chicken embryonic fibroblasts. Toxicity of NPs reported is mainly due to its structure, solubility, shape and surface/mass ratio therefore, particle characterization is a crucial step because the shape, size, surface area, surface charge, monodispersity affects the physicochemical properties and biological functions of ENPs. Dynamic light scattering was used to determine the size of the particles in suspension (Milli Q water), the results demonstrated that the particles were stable and monodispersed. Hemolysis testing is a type of acute toxicity screening assay mainly used to evaluate the hemocompatibility of the materials when in contact with blood. In this study, hemocompatibility of metal oxide nanoparticles was evaluated with chicken blood in which red blood cells treated with MgO and ZnO NPs caused hemolysis whereas, Al_2O_3, Fe_2O_3, TiO_2 NPs did not induce hemolysis. Chicken embryonic fibroblasts were isolated from 10-12 day old embryo of *Gallus gallus domesticus* and treated with the above mentioned nanoparticles to study their impact on the cells. ZnO NPs resulted to be the most toxic with 50% cell death at 10 µg/ml. Al_2O_3 NPs accounted for 50% cell death at 500 µg/ml and the other nanoaprticles (Fe_2O_3, MgO, TiO_2) resulted in 50% cell death upto the highest dose (1000 µg/ml). In ZnO NPs, zinc is known to leach out and cause toxicity and the same was observed in our study. Hence, further studies were conducted on ZnO NPs, which induced toxicity in chicken embryonic fibroblast by distortion of cellular morphology, LDH leakage, mitochondrial dysfunction, increased intracellular oxidative stress and nuclear condensation. ZnO NPs also triggered apoptosis as evidenced by changes in p53, BAX, BCL2 and CASP3 genes.

In the second part of the study, the aim was to evaluate the impact of the same metal oxide nanoparticles on an avian embryo (*Gallus gallus domesticus*). HET-CAM analysis, employed to quantitatively assess the biological response of nanometals to chorioallantoic membrane, revealed Fe_2O_3 NPs induced moderate hemorrhage to the vasculature of CAM. Further, the metal oxide nanoparticles dosed in the airspace of fertilized eggs and incubated until 19^{th} day of development caused an increase in the mortality of embryos and caused a decrement in their body lengths and weights without having an impact on the organ weights. The results showed that metal oxide nanoparticles could cross the inner shell membrane and the chorioallantoic membrane and effect the development of the embryo. Malformations were visible in TiO_2 nanoparticles treated embryos in the form of omphalocele and flexed limbs whereas; Fe_2O_3, ZnO, MgO and Al_2O_3 nanoparticles did not affect the embryonic development. Figure 7.1 summarizes the first half of the study.

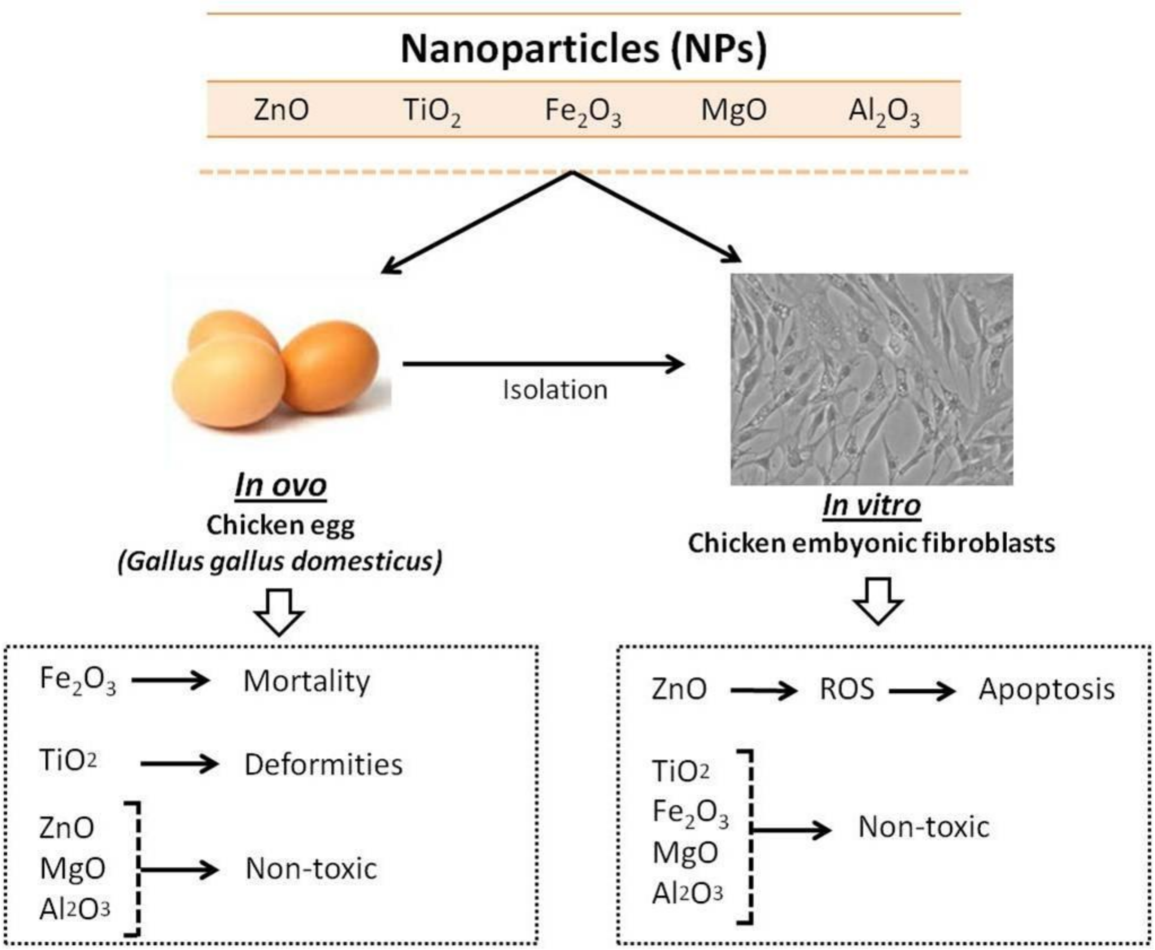

FIGURE 7.1: Summary of screening of metal oxide nanoparticles for possible toxicity *in ovo* and in *in vitro*

In an avian egg, albumen is of crucial importance in regulating whole-body protein synthesis in chicken embryos during incubation. Nanoparticles are known to interact with proteins and form a protein corona resulting in a change in its physicochemical properties. Hence, we aim to assess the changes in physicochemical parameters such as density, viscosity, surface tension, friccohesity, activation energy and molecular radii of nanoparticles (Fe_2O_3 and TiO_2 NPs) after dispersion in saline, water and egg albumen with Mansingh Survismeter. Stronger interactions of Fe_2O_3 NPs with albumen resulted in an increase in viscosity of albumen. Alterations in limiting density, viscosity, friccohesity and molecular radii of Fe_2O_3 NPs + egg albumen implied towards its binding with albumen. Higher dose of Fe_2O_3 NPs (200µg/ml) recorded a significant increment in surface tension of Fe_2O_3 NPs + saline immobilizing the interacting molecules with stronger binding forces and forming a spherical shaped corona with a smaller surface area and a stronger attractive force that could cross *in ovo* barriers resulting in 100% mortality at 200 µg/ml dose possibly due to Fe^{2+} ions overload. Also, these interactions possibly allowed Fe_2O_3 NPs to traverse the biological barriers within embryo and reach the brain and result in the observed neuronal loss which is attributable to production of Fe^{2+} ions by Fe_2O_3 NPs.

Immersion is used in toxicology as it tends to mimic the exposure in nature and allows us to assess the threat of chemicals to avian embryos. As the above results showed Fe_2O_3 NPs crossing biological barriers, eggs were immersed in Fe_2O_3 nanosuspension to assess the ability of Fe_2O_3 NPs to cross barriers (egg shell, shell membrane and chorioallantoic membrane) in an avian egg. Egg immersion studies showed similar results where morphometry of viable embryos revealed significant decrement in body weight and crown rump length at 100 and 200 µg/ml doses indicating Fe_2O_3 NPs could cross *in ovo* biological barriers viz. egg shell, shell membrane and chorioallantoic membrane. The CAM and the egg albumen are responsible for the uptake of nanoparticles into the embryo. These results indicate the toxic effect of Fe_2O_3 NPs irrespective of the method of dosing.

Changes in the physicochemical indices indicated stronger intermolecular forces of attraction between TiO_2 NPs and egg albumen. Dose dependent increase in density of albumen was observed following addition of TiO_2 NPs, but relatively lowest density observed at 10 µg/ml hints at effective dispersion of TiO_2 NPs in egg albumen. Also, increment in molecular radii of TiO_2 NPs-egg albumen implies towards formation of nanoparticle agglomerates at higher doses due to which they do not impact the chicken embryo and cause toxicity at the lower doses only. TiO_2 NPs were found to interact with egg albumen as evidenced by changes in their proteinic secondary structure. The malformations (omphalocele and flexed limbs) caused could be because of the interactions of TiO_2 NPs with egg albumen allowing TiO_2 NPs to traverse the biological barriers (shell membrane and CAM) within chicken egg as confirmed by ICP-OES studies and affect the growth and development of embryos. Wnt signaling plays an important role in the ventral body wall formation. Downregulation of genes involved in Wnt signaling (CTNNB1, PITX2, LEF1, WNT11, PRKCA, CaMK2D, ROCK1 and ROCK2) leads to the distruption of somite myogenesis which interfers with the movement of the lateral body wall folds. Several genetically-modified animals that have been reported to display abnormalities in the body wall region had accompanying limb deformities. In our study, significantly lowered expression of HOXD13 (regulator of limb pattern in vertebrate development) in TiO_2 NPs treated embryos corroborate with the observed omphalocele. TiO_2 NPs are known to cause damage by producing free radicals where the radical becomes affixed to or within the cell membranes and hence may be causing omphalocele by disrupting lateral plate mesoderm, somite myogenesis and inducing apoptosis in the somites. Figure 7.2 summarizes the possible mechanism of TiO_2 NPs and Fe_2O_3 NPs induced toxicity to chicken embryo.

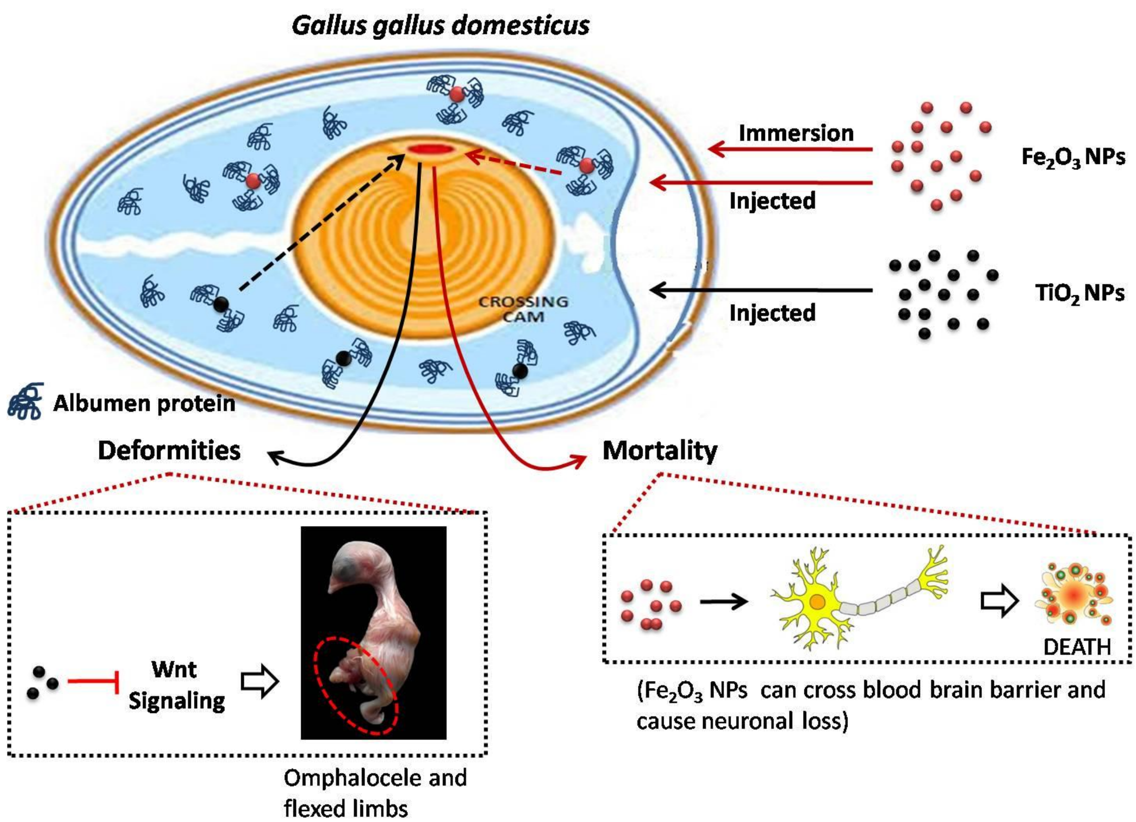

FIGURE 7.1: Possible mechanism of TiO_2 and Fe_2O_3 NPs induced toxicity to chicken embryo.

SIGNIFICANCE OF THE STUDY

The data reported in this thesis assumes significance as it helps in better understanding the mechanism of nanoparticle-egg albumen interaction and the toxic effects of widely used Titanium dioxide (TiO_2) nanoparticles in chicken embryo. Hence, caution should be exercised in their handling as the exposure of TiO_2 NPs results in mortality and deformities (omphalocele and flexed limbs) in chicken embryos and exposure to Fe_2O_3 NPs leads to neuronal loss in the embryos and mortality at the higher doses. This study also sets a new bench mark for toxicity assessment of commercially used nanometals.