Nanostructured materials are attracting a great deal of attention in recent years because of their potential for achieving a specific process and selectivity, especially in biological and pharmaceutical applications. Over the past few years, NMs whose structures exhibited significantly novel and improved physical, chemical and biological properties, due to their nanoscale size and hence have elicited much interest as nano silver (Ag). The goal of the present study was to carry out the acute toxicity and toxicokinetic studies of different sized Ag NM. Another important goal of the current study was to evaluate the genotoxicity of nanosize Ag after single as well as repeated dose treatment. Before initiating any toxicological study on NMs characterisation is essential. Therefore in the present study TEM, SEM, DLS and LDV were used to characterise the Ag NMs. The characterization of Ag-25nm and Ag-35nm showed spherical morphologies and mean size distribution < 100nm that correlated with the manufacturer specified sizes 25nm and 35nm. However Ag-25nm and Ag-35nm average diameter in solutions revealed agglomeration. This could be due to physicochemical interactions between the NMs. Hence in order to diminish the agglomeration, the solutions were constantly re-suspended prior to use in the toxicity studies. The acute treatment of Ag NM caused no mortality or toxic symptoms, nor showed any significant differences in the body weight gain and feed efficiency in treated rats when compared to control rats. Since, no treatment related effects were noted in the animals, these results suggest that the NM were not toxic

to the rats at the acute levels by oral route as the LD_{50} for all the NM was found to be more than 2000mg/kg, categorizing them in class 5 (relatively low acute toxicity hazard).

Histopathological examination of the tissues of treated animals showed significant lesions. However the tissue damage was most in livers of Ag-25nm treated animals and least in animals dosed with Ag-bulk. The histopahological effects were also dose dependent.

A short term (14 day) oral toxicokinetic study was also conducted to evaluate the oral toxicity of Ag-25nm, Ag-35nm and Ag NO_3. The doses for the two NMs were 36, 72, 144 mg/kg/bw/day and 6, 12, 24 mg/kg/bw/day for the Ag NO_3 the bulk material corresponding to low, medium and high doses. No significant changes in clinical signs, body weight and food consumption was observed. Significant differences between treated and control animals were found in the biodistribution studies after 14 day repeated study. Specifically Ag deposition with liver, kidney, heart and brain was observed. The Ag accumulation showed a significant enhancement as follows liver < kidney < heart < brain. The Ag content increased in the various tissues in a size and dose dependent manner. Histopathological evaluation of liver, kidney, heart and brain tissues from treated with Ag-25nm, Ag-35nm and Ag-bulk for 14 days with various doses showed significant abnormalities substantiating the results obtained in briodistribution studies with significantly elevated alterations displayed by the liver followed by kidney, heart and brain. The histological changes were size and dose dependent. Significant changes were observed in biochemical parameters revealing the oxidative stress condition of the treated

animals. The results of the biochemical assays showed that Ag-25nm produced the maximum effect whereas Ag-bulk showed least alteration.

The acute genotoxicity study revealed a significant increase in mean MN in rat's bone marrow and peripheral blood treated with different doses of Ag-25nm and Ag-35nm indicating size and dose dependent genotoxicity at their respective time intervals in comparison to the Ag-bulk and control. Likewise the CA assay in bone marrow cells of treated rats at 18 and 24h elucidated size and dose dependent genotoxicity. Furthermore, the comet assay data in rat's peripheral blood at respective sampling times revealed size, dose and time dependent DNA damages compared with the Ag-bulk and control. With the increase in time of exposure, the DNA damage was significantly reduced probably due to the action of DNA repair system.

Genotoxicity study with Ag-25nm, Ag-35nm and Ag-bulk revealed a significant increase in mean frequency of MN in peripheral blood and bone marrow and total CA in rat's bone marrow after 14 days of repeated dosing indicating size and dose dependent genotoxicity vis-à-vis the Ag-bulk and control. Likewise, the comet assay data showed sufficient increases in comet tail length to elicit a genotoxic response.

Taken together, the present findings suggested that Ag NMs were able to cause toxicity effects *in vivo*. Based on this study it could be hypothesized that the size and dose of the Ag NMs may be the reason of the significant toxicity. Therefore considering the ever increasing commercial application Ag NMs, these Ag NMs should be used cautiously.