SUMMARY

The current study demonstrates the synthesis of two different types of Copper Oxide nanoparticles by chemical method and green synthesis method. Both the synthesized nanoparticles were characterized by several techniques to confirm the successful formation of nanoparticles. After that the anti-oxidant activity of both these CuONPs was evaluated using ascorbic acid as a control. The results indicated higher anti-oxidant property of green synthesized CuONPs than the chemically synthesized CuONPs. GC analysis was performed to know the contribution of bio-active compounds responsible for the anti-oxidant property of green synthesized CuONPs.

The cytotoxicity difference between these two CuONPs was performed in detail. From the study, it was found that the IC_{50} value of green CuONPs and chemical CuONPs against lymphocytes were 42.46µg/ml and 26.76µg/ml respectively. Above this dose, both forms of CuONPs showed severe toxicity in lymphocytes. Also above 500µg/Kg Body Weight *in vivo*, green CuONPs was toxic. However, the chemical CuONPs showed more prominent toxicity from initial dose of 100µg/Kg Body Weight. This study clearly indicated the toxic effect of both these NPs. But between these two forms of CuONPs, green CuONPs was found less toxic compared to the chemical CuONPs. Green CuONPs deposited highly in the spleen, whereas the chemical CuONPs were broadly deposited in the liver, heart, kidney and intestine. Measurements of cell viability, Hemolysis assay, Reactive Oxygen Species (ROS) generation, biochemical estimation, apoptotic study in lymphocytes after 24hrs and measurements of body and organ weight, serum chemistry evaluation, cytokines level, protein expressions and histopathology of Balb/c mice after 15 days indicated significant toxicity difference between the chemical CuONPs and green CuONPs. Current observations proved that the NPs physiochemical properties influenced toxicity and biodistribution profiles in *in vitro* and *in vivo*.

On the basis of toxicity, green CuONPs was selected for further study. Anticancer efficacy of green synthesized CuONPs was analyzed against breast cancer (MCF-7) and cervical cancer (HeLa) cells. The NPs were able to kill both cancer cells significantly compared to the control group. Cu ions from the green CuONPs are purported to work by invasion through the leaky membrane of cancer cells. The Cu ions were responsible for ROS production inside the cancer

cells, which disrupted the redox balance of cancer cells and subsequently TNF-α triggered the activation of cascades of Caspases.

The toxicity is the major concern for any anti-cancer therapy. To overcome this problem, a cationic biopolymer Chitosan was coated on to the surface of green CuONPs. This double layered effect worked as a double edged sword. At first, CS protected the normal lymphocytes due to its pH responsive nature. Subsequently, CS released more Cu ions inside the cancer cell microenvironment as the cancer cells are acidic by nature. After the coating, the cell viability of lymphocytes increased and the IC_{50} value became 69.12µg/ml. After the coating, the nano-conjugate was able to attack selectively the cancer cells without significant toxicity against lymphocytes and Balb/c mice. From this study, 50µg/ml dose in lymphocytes and 1000µg/Kg Body Weight dose in mice model were selected as a biologically safe dose.

The immunofluorescence study indicated that, both ROS mediated mitochondrial and non-mitochondrial pathway were responsible for nanoconjugate mediated apoptosis of cancer cells. The *in vivo* study also showed an interesting result, that the CuONPs@CS reduced the tumor weight after 30 days of treatment.

The CuONPs@CS acted as a smart immunotherapeutic tool by activating the immune cells. Specific cancer antigen associated CuONPs@CS boosted up the immunoregulation and helped in the activation of T cells. However, CuONPs@CS without cancer antigen also helped to activate Th1 and Th2 subset of cells and played a role as an adjuvant. Activated macrophages destroyed the cancer cells by modulating the tumor microenvironment and at the same time antigen associated conjugated NPs showed better anticancer activity due to macrophage activation, which means the NPs may act as an antigen delivery vehicle in the system. Th1 response was observed by the proliferation in CD4+ cell population. IgG estimation in the study indirectly indicated the activation of both Th1 and Th2 cells although the Th1 response was more pronounced. However, in the presence of antigen specific IgG in the serum of mice indicated the activation of both Th1 and Th2 cells.

Adjuvants in immunology are often used to modify the effects of a vaccine by stimulating the immune system vigorously, and thus providing increased immunity to a particular disease. By activating the immune cells, CuONPs@CS conjugate showed promising immunotherapy against

cancer. In this study CuONPs@CS conjugate appeared to play a role as a strong immunostimulant and as well as an adjuvant.

In the last part of the study, the main aim was to deliver these nano conjugates to the cancer cells without any severe toxicity. For the targeted delivery of these NPs, the surface of the particle was again coated with folic acid. As the folate receptor expressions on the cancer cell surface were high, the CuONPs@CS@FA internalized through receptor mediated endocytosis pathway. After internalization, the Cu ions released and provoked the cancer cell apoptosis. Caspase 3 mediated extrinsic and intrinsic pathways were involved in apoptosis. Collectively it was elucidated that activation of p38, MAPK by ROS generation is responsible for apoptosis.

Double layered coating on CuONPs helps to reduce the toxicity, as well as provide a platform to act as an immunostimulant and adjuvant. The CS coated green CuONPs effectively killed the cancer cells in a dose dependent manner. Finally CuONPs@CS@FA reached the cancer cell microenvironment with the help of folate receptor to destroy the cancer cells *in vitro* and *in vivo* model.