## CONCLUSION:

Nowadays, the use of nanoparticles has become an emerging field in cancer therapy. Different metal oxide based nanoparticles are known for their anti-cancer activity. CuONPs is very cost effective and easily available. Several studies indicate that green CuONPs has potent anticancer activity. The transition metal elements like Cu have an interesting d orbital configuration by which they can easily change the oxidation state. Changes in oxidation state release electrons from metals and showed redox mediated oxidative stress in cancer cells.

During green synthesis, several bio-active components of plant materials remain attached to the surface of CuONPs and give stability to the NPs formation. But the chemical CuONPs were synthesized by chemical reactions and at last by thermal decomposition method. High surface to volume ratio of NPs contribute a major role in their physico-chemical properties. Several techniques were used to characterize both the CuONPs. Then the toxicity differences were evaluated between them. In this study it is found that the green CuONPs is less toxic than the chemical CuONPs. However the green CuONPs was significantly toxic against treated lymphocytes compared to the control group i.e normal lymphocytes. Anticancer activity of green CuONPs against MCF-7 and HeLa cells was better than the chemical CuONPs, may be due to the contribution of bio-active components of leaf extracts. The solid tumor was developed in Balb/c mice by 4T1 cells. Green CuONPs was able to reduce the tumor size significantly.

The main aim of this study was, to find a potent anti-cancer drug with minimal toxicity. To reduce toxicity, the surface of CuONPs was coated with CS to minimize the toxicity. The double layer coated CuONPs@CS specifically killed the cancer cells with minimal toxic effect in lymphocytes. This happens possibly due to pH responsive nature of CS. Cu ions from CuONPs@CS internalized inside the cancer cells in an acidic condition which helped to trigger apoptotic pathway in cancer cells.

The immunomodulatory activitiy of the nano conjugate was observed through the activation of macrophages. Cu ions internalized inside the macrophages and induced the macrophages to release pro-inflammatory cytokines like TNF-α, IL-12, IFN-γ. These are the indirect indication of macrophages activation. Activated macrophages killed the cancer cells at a ratio of 1:10 for 48hrs treatment. IgG response in mice serum indicated the activation of humoral immunity and

CD+4 activation indicated cellular immunity. Both humoral and cellular immunity contribute towards anti-cancer therapy, where CuONPs@CS acts as an adjuvant.

Finally, the conjugate was coated with folic acid (CuONPs@CS@FA) for better targeting to the cancer cells. Ultimately the cancer cells were significantly and selectively killed with a minimal toxic effect on lymphocytes. The ultimate conjugated NPs showed apoptotic event inside the cancer cells by the generation of ROS due to the release of Cu ions from the conjugate. So, the current conjugate CuONPs@CS@FA can be used as a potential anti-cancer drug as well as an immunostimulant in the near future. However more studies including in vivo, clinical and formulation studies are needed to conclusively confirm the safety, efficacy and pharamceutical properties of these CuONPs@CS@FA.