CHAPTER X

SUMMARY AND CONCLUSION

Cancer is the most dreadful disease of mankind. Despite the great human achievements in science, technology and medicine, a definite cure for cancer is yet to be found. The treatment modalities available even today are surgery, chemotherapy and radiotherapy, though immunotherapy, hormone therapy, laser therapy, photodynamic therapy etc. are being tried to a limited extend. For tumors, which are resectable and diagnosed at an early stage, surgery is the best suited. Chemotherapy and radiotherapy are useful at all stages, though these cause several undesirable side effects and toxicities to the normal tissues.

Apart from this, tumor display resistance to the treatments because of the peculiar microenvironment. The only way to overcome these difficulties is to deliver the drugs specifically to the tumor cells at effective concentrations. Targeted drug delivery using nanoparticle platforms offers a convenient and versatile approach in this direction. The present thesis is an attempt to develop an enabling technology to annihilate tumors by specifically targeting cytotoxic and chemotherapeutic agents using nanoparticles by utilizing the special characteristics of the tumor microenvironment and that of nanoparticles.

The major studies carried out and the results obtained from them are summarized below:-

1. Iron-oxide nanoparticles were synthesized via co-precipitation method and surface-coated with PVP and POES.
2. These NPs were characterized by XRD and TEM
3. The surface-coated magnetic NPs were complexed with cytotoxic alkaloid BBN, hypoxic cell sensitizer SAN, antineoplastic agent DOX, BBN-SAN and DOX-SAN to get complexes of NP-BBN, NP-SAN, NP-DOX, NP-BBN-SAN and NP-DOX-SAN.
4. These NP-drug complexes were characterized by XRD and TEM (to analyse approximate size and shape), FTIR (to verify the presence of added drugs) and nano-size analyser (for hydrodynamic size).
5. Mice-bearing transplanted DLA solid tumors on hind limbs were divided into different groups and orally administered with these complexes. The diameter of the solid tumors was monitored. Mice were sacrificed, tumors and other tissues were analysed for biochemical, molecular and morphological parameters.

6. NP-BBN complexes could be targeted to tumors with the aid of an external magnetic field to achieve tumor control. The treatment was effective in reducing tumor volume due to the induction of apoptosis through intrinsic pathway by the magnetic NPs directed delivery of BBN to tumors.
7. SAN has been reported to accumulate in hypoxic solid tumors. Before using it as a targeting agent, molecular studies conducted under both *in vitro* and *in vivo* conditions revealed SAN down regulated *hif-1α* and its target genes – *egfr* and *vegf*.
8. The cytotoxic potential of NP-BBN-SAN complexes in murine tumor cells was evaluated and the molecular mechanisms were investigated. The studies revealed that SAN enhanced the cytotoxic potential of NP-BBN complexes and the underlying mechanism of this enhancement was found to be due to apoptosis.
9. Studies on tissue distribution of NP-BBN and NP-BBN-SAN complexes by chemical and spectroscopic methods revealed that the later accumulated faster in tumor tissues, suggesting that complexing with SAN facilitated its faster accumulation in the tumor and this was further confirmed by tumor imaging. The administration of NP-BBN-SAN complexes to animals bearing tumor cells in the peritoneal cavity, proposed the anti-angiogeneic potential of the complexes.
10. Complexing with SAN enhanced the therapeutic efficacy of NP-BBN complexes. Administration of these complexes reduced tumor volume in tumor-bearing mice. Molecular studies on the tumor from these animals revealed the down regulation of *hif-1α*, *vegf*, *egfr* and *akt* as well as up-regulation of *caspase 8* and *tnf-α*. The results suggested that the extrinsic pathway of apoptosis could be the underlying mechanism of tumor regression brought about by the complexes.
11. The NP-BBN complexes could be specifically targeted to tumor by the use of an external magnetic field (physical targeting) as well as SAN (chemo-directed targeting) followed by the administration in tumor-bearing animals. In both cases there was regression of the tumor. Molecular analysis of the tumors, following these treatments, suggested apoptosis – both intrinsic and extrinsic – as the mechanism of tumor regression. However, it was revealed that there was a predominance of intrinsic pathway in the external magnetic field directed targeting, while the extrinsic pathway was more predominant in SAN directed targeting. The use of physical targeting, by creating an external magnetic field, is limited only to

peripheral tumors, while SAN directed chemo-targeting is more versatile as it is applicable to both peripheral and deep-seated tumors.

12. The effectiveness of SAN directed chemical targeting was also further confirmed by the administration of NP-DOX-SAN complexes in tumor-bearing mice. The tumor volume was found reduced upon the administration of the complexes. Molecular analyses of tumor tissues revealed apoptosis as the underlying mechanism of tumor regression. Morphology of normal tissues and serum parameters further envisages the therapeutic efficacy of the treatment.

Thus, the thesis presents the results of strategies developed for specific targeting of cytotoxic drug BBN (both physical and chemical methods) and antineoplastic agent DOX (chemical method). This specific targeting of cytotoxic drugs and antineoplastic agent using NPs either by physical method or by chemical method, to tumors can circumvent the systemic toxicities and side effects, and result in the eradication of the tumors. However, the chemo-directed therapeutic strategy has to try in other tumor models before taking up clinical trials.