Name: SHWETA AGARWAL

Supervisor: Dr. SADAF FATIMA

Department: DEPARTMENT OF BIOTECHNOLOGY

TITLE OF THESIS: "To evaluate the anti-oxidant activities induced by Biopolymeric Nanoparticles"

ABSTRACT

Nanotechnology based drug delivery systems are in current use to increase the therapeutic potential of the delivered drug to treat cancer. In the present study, Doxorubicin, a broad-spectrum anti-neoplastic drug, was entrapped in chitosan nanoparticles (CHNP) and the anti-oxidant potential of this system was assessed for cancer therapeutics. Chitosan nanoparticles synthesized by ionic gelation method (size ~115.4 nm; charge ~19.5 mV ± 1.0). TEM and FTIR was also performed for further characterization. Doxorubicin (DOX) was physically entrapped in nanoparticles having ~48% Entrapment Efficiency with a biphasic release pattern. Cytotoxicity assays and cellular uptake of the free drug was observed on various cancerous and non-cancerous cell lines. DNA damage caused by the free drug and entrapped drug was also asessed by performing DNA fragmentation and Comet assay. To asess the anti-oxidant potential, various biochemical assays were performed. Systemic administration of the Doxorubicin loaded chitosan nanoparticles (DLCHNP) were performed for evaluation of pharmacokinetics, bio-distribution, and tumour regression studies. The nanoparticle based drug delivery system developed in this work have shown great potentials for sustained drug delivery.

METHODS

Ionic Gelation Method was used to prepare nanoparticles. Characterization was done by DLS, Zeta Sizer, TEM, FTIR techniques. DOX was physically entrapped in the nanoparticles. *In vitro* release profiles of the free drug was observed at pH 7.4 & pH 5.8. *In vitro* cytotoxicity evaluation (MTT assay) and cellular uptake was performed on HEK, EAC, SiHa, MCF-7 cell lines. Oxidative stress was determined using Lipid peroxidation (LPO), Nitric oxide (NO) reduced Glutathione (GSH), Glutathione-*S*-transferase (GST), Glutathione Peroxidase (GPx), Glutathione reductase (GR), oxidized glutathione (GSSG) and Superoxide dismutase (SOD) biochemical assays. DNA Fragmentation assay and Single cell gel

electrophoresis was performed in SiHa cells. Immunocytochemistry was also performed to assess the expression of p21 and cyt. c. *In vivo* pharmacokinetics and bio-distribution studies were performed in animal rat model. Tumour regression studies in various experimental groups were performed in tumour mice model. Histopathological examinations of Liver, Kidney and lung tissues of tumour bearing mice model were done by H&E staining method.

RESULTS

Chitosan nanoparticles (CHNP) were successfully prepared with high entrapment efficiency. MTT assay depicted that the efficacy of the drug was not compromised after encapsulation. Enhanced killing of cancerous cells was observed with DLCHNP as evidenced by DNA fragmentation, comet assay and higher expression of p21 and Cyt c. An imbalance in oxidant-antioxidant levels with decreased anti-oxidants levels was achieved by DLCHNP thereby creating oxidative stress in transformed cells, specifically, leading to their death. We further extended our studies in *in vivo* models. Prolonged circulation in the blood coupled with high accumulation in other organs was observed with DLCHNP as compared to the free drug. Tumour burden was substantially reduced in DLCHNP treated group as compared to free drug and *void* nanoparticles. Similar results were obtained with histopathological data that indicated reduced stress in liver, kidney and lung tissues when treated with DLCHNP as compared to DOX *per se*. Both the *in vitro* and *in vivo* data revealed that our drug delivery system is potent in inhibiting the growth of cancerous cells and may be used as an important tool in cancer therapy.