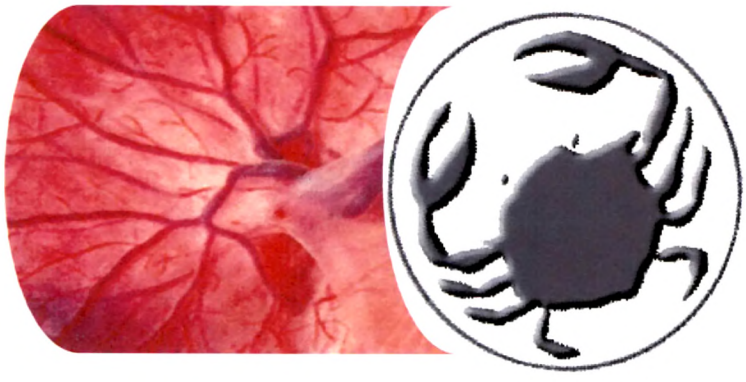
Chapter 1



INTRODUCTION

1.1 INTRODUCTION

Cancer is one of the leading causes of death in the world. By 2050, the global burden is expected to grow to 27 million new cancer cases and 17.5 million cancer deaths. (Global Cancer Facts & Figures, American Cancer Society, Atlanta, Georgia.)

Current cancer therapy usually involves chemotherapy, surgical removal of tumors, and radiation therapy. But this complex approach is far from being satisfactory.

Though advances in the treatment and diagnosis of cancer have improved lifespan and quality of cancer patients, there is still scope for the improvement in the survival rate. Treatment related toxicity, development of drug resistance; inadequate target drug delivery and recurrences of disease are the major reasons behind the morbidity and mortality. (Srinivasan M and Harris A L, 2002)

The advanced research in cancer treatment is exploring the avenues in the form of either new agent against cancer or new ways of delivering the anticancer agents.

With the revolutionary discoveries in the molecular biology and better understanding of biology of cancer, the specific targets can be identified in tumor cells, the function of which are necessary prerequisites for the replication of the same. (Eckhardt , 2002)

The study of cancer at molecular level better enables us to design drugs to halt their proliferation and spread. This new approach of targeting the mechanisms by which cancer cells prosper has been called, appropriately, a mechanism-based approach.

Such one mechanism which can be targeted is the tumor angiogenesis. Tumor growth and metastasis is angiogenesis dependant. The solid tumor needs to initiate angiogenesis to create their own blood supply. The proliferation of endothelial cells (ECs) is the key feature of angiogenesis.

Tumor angiogenesis inhibition may severely limit the tumor growth as well as metastasis. This concept of cancer antiangiogenic therapy was first proposed by Judah Folkman in his work in 1971. (Folkman, 1971) Since then tumor vasculature has emerged as a potential target in cancer therapy. The objective of antiangiogenic therapy is to interfere with the angiogenic switch mechanisms and prevent tumor cells from developing a viable blood supply. More than 75 compounds are under clinical trial for their antiangiogenic properties. (Furness et al., 2005)

Antiangiogenic therapy has been shown to increase the efficacy of classical chemotherapeutic agents in anticancer treatment. (Gasparini, 1999) Antiangiogenics alone

have not found to induce the tumour regression but the new therapeutic strategy is to combine them with conventional chemotherapeutic agent. Combination therapy have proven to be more beneficial as compared to conventional cytotoxic chemotherapy as it offers fewer toxic side effects and reduced drug resistance. (Gasparini et al., 2005; Yance and Sagar, 2006).

Nanoparticulate delivery is one of the most important areas explored in recent research and development of drug deliver science. The use of colloidal carriers for drug offers enhanced delivery of chemotherapeutic agent. Encapsulating within or binding drug to the colloidal delivery served many purposes like to accumulate preferentially in tumor tissue, to circulate longer, reduced systemic toxicity, controlled release and in some cases bypass certain cellular mechanisms which leads to drug resistance.

This thesis describes our efforts to study the delivery of anticancer-antiangiogenic combination (Etoposide + Quercetin Dihydrate) therapy by incorporating into PLGA (50:50) nanoparticles individually and also to investigate the antitumour effect in B16F10 melanoma animal model. We also attempted to establish the *in vitro* drug release profiles and *in vitro* cell cytotoxicity parameter. Radiolabelling of drugs and nanoparticles has been performed to study their biodistribution and tumor retention.

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1.2 OBJECTIVES OF THE WORK

The objectives of this investigation are:

- To prepare and optimize colloidal delivery system (Polymeric Nanoparticles) by Nanoprecipitation (Solvent Diffusion) Method.
- To characterize the delivery systems for its parameters like particle size, particle size distribution, zeta potential, drug entrapment efficiency, shape and morphology by TEM.
- To assess the *in vitro* drug release.
- To assess the *in vitro* stability of the formulations.
- To label the drug delivery system and plain drugs with radioisotope of technetium (^{99m}Tc) and to study the biodistribution of the labeled complexes..
- To assess and compare the in vitro cytotoxicity of drugs and the formulations on cancer cell line.
- To perform the pharmacokinetic study by administering radiolabeled formulations and drugs to the animals.
- To assess and compare the in vivo therapeutic efficacy of the formulations and of the plain drugs by using tumor induced mice.

1.3 MATERIALS

PLGA 502H, (lactide/glycolide ratio 50:50, inherent viscosity 0.22dl/g) - Boehringer Ingelheim, (Germany).

Etoposide - Cadilla Pharma, Ahmedabad, (India).

Quercetin Dihydrate (extrapure grade) - Sisco Research Laboratories Pvt. Ltd. Mumbai, (India).

Poloxamer-407 (Lutrol F-127) - Sun Pharma Advanced Research Company Ltd (SPARC), Vadodara, (India).

Trehalose - Hi Media (India)

Lung adenocarcinoma cell line A549- National Centre for Cell Science (NCCS), Pune, (India).

Fetal bovine serum (FBS) - Hi Media, (India)

(MTT) 3-[4,5-dimethylthiazolyl-2-yl]-2,5-diphenyltetrazolium bromide - Hi Media, (India)

(DMEM) Dulbecco's modified Eagle's medium - Hi Media, (India)

Plastic wares for cell line studies - Hi Media, (India)

Acetone (HPLC grade) - S. D. Fine Chemicals, (India).

Acetonitrile (HPLC grade) - S. D. Fine Chemicals, (India).

Dimethyl sulphoxide (HPLC grade) - S. D. Fine Chemicals, (India).

Sodium pertecnetate – obtained by elution from molybdenum-99 through solvent extraction (BRIT) Board of Radiation and Isotope Tecnology, (India)

Stannous chloride (Analytical grade) – Qualigens, Mumbai, (India).

Potassium dihydrogen phosphate (Analytical grade)

Sodium hydroxide and all other reagents and solvents used were of analytical grade.

Distilled water used was filtered through 0.22μm filter Millipore, (India).