INTRODUCTION
1. INTRODUCTION

1.1 General Introduction and Need for the Study

It is reported that there are approximately 12.6 million new cancer cases and 7.6 million cancer deaths Worldwide (Ferlay et al., 2010). The most commonly diagnosed cancers Worldwide are lung (12.7% of the total), breast (10.9%) and colorectal cancers (9.7%). Lung cancer is the most common cause of cancer-related death in men and women. In the year 2010, it is estimated that nearly 1.35 million new cases and 1.18 million deaths with lung cancer occurred Worldwide. There is approximately 63,000 new lung cancer cases reported each year in India, as per the estimates compared to the rates in other parts of the World (Ferlay et al., 2010; Ganesh et al., 2011). Lung cancer is ranked as the leading cancer in Bhopal, Chennai, Delhi, Kolkatta and Mumbai, besides north-eastern registries (Ganesh et al., 2011). The most common symptoms are shortness of breath, cough and weight loss (Minna and Schiller, 2008). The main types of lung cancer are small cell lung carcinoma and non-small cell lung carcinoma. Lung cancer can be seen on chest radiograph and computed tomography (CT scan). The diagnosis is confirmed with biopsy test. This is usually performed by bronchoscopy or CT-guided biopsy. Treatment and prognosis depends upon the histological type of cancer, the stage i.e. degree of spread and the patient's performance status. Possible treatments include surgery, chemotherapy and radiotherapy (Minna and Schiller, 2008).

All trans retinoic acid (ATRA) is an active metabolite of vitamin A and comes under the family retinoid. Retinoids through their cognate nuclear receptors exert potent effects on cell growth, differentiation and apoptosis. They have a significant promise for cancer therapy and chemoprevention. Differentiation therapy with ATRA has marked a major advance and become the first choice drug in the treatment of acute promyelocytic leukemia (APL) (Lengfelder et al., 2005; Di et al., 2005), where the
APL cells are extremely sensitive to ATRA, which induces differentiation into mature granulocytes and results in cell apoptosis. Treatment of APL patients with ATRA in addition to other chemotherapy yields a high rate of complete remission and long term survival. Therefore, ATRA had the distinction of being the first “differentiation therapy” for cancer (Laura et al., 2000). Conversions of 13-cis-retinoic acid and 9-cis-retinoic acid to ATRA are very rapid. Currently, two distinct families of retinoid responsive nuclear receptors have been identified and characterized: retinoic acid receptors (RARs) and retinoid X receptors (RXRs), each of which include three isoforms α, β and γ.

Retinol is ingested in a precursor form, the most usable form of vitamin A. Animal sources (liver and eggs) contain retinyl esters, whereas plants (carrots, spinach) contain pro-vitamin A carotenoids. Retinol is converted to a group of active metabolite called retinoids in living system. ATRA is one such natural product derived by irreversible oxidation of retinol, the parent compound for all natural retinoids. Though ATRA is the immediate ligand for the nuclear receptors, it is not sufficient to maintain vision and reproduction, which require retinol or its retinal derivative, neither of which may be derived by reduction of ATRA. It is however, necessary for the maintenance of epithelial differentiation and hence plays a fundamental role in differentiation therapy and in chemoprevention of epithelial carcinogenesis (Hansen et al., 2000). ATRA is considered as an anti-cancer chemotherapy drug (Gropper et al., 2009). ATRA is increasingly included in anti tumor therapeutic schemes for the treatment of various tumor diseases such as Kaposi’s sarcoma, head and neck squamous cell carcinoma, ovarian carcinoma, bladder cancer and neuroblastoma (Choi et al., 2003). ATRA has shown anti-angiogenic effects in several systems, inhibiting the proliferation in vascular smooth muscle cells (VSMCs) and anti-inflammatory in Rheumatoid arthritis (Lee et al., 2004). ATRA has been shown to exert anti-cancer activities in a number of cancer cells and tissues too (Otsuki et al., 2003; Arce et al., 2005).
ATRA and its other active derivatives are potent modulators of cell growth, differentiation and apoptosis in a variety of cell types (Crowe et al., 2003; Gumireddy et al., 2003). They can also be used as chemotherapeutic and chemopreventive agents in a variety of malignancies such as leukemias, uterine leiomyomas as well as colon, gastric, solid tumor and breast cancers (Czeczuga-Semeniuk et al., 2004; Liu et al., 2004). Retinoic acid (RA) has also been suggested to be efficacious in treating lung cancer (Dahl et al., 2000; Chang et al., 2004). Anticancer activity of ATRA is achieved by binding to retinoic acid receptors present in the nucleus of cancer cells, leading to the induction of cell growth inhibition, differentiation or apoptosis (Fang et al., 2002). Angiogenesis is physiologically important in normal growth, development, wound healing and reproduction, whereas pathological angiogenesis contributes to tumor growth, exacerbation of diabetic retinopathy and various inflammations (Akiko et al., 2007). Several studies have shown that the ATRA and its derivatives could modulate angiogenesis (Blebea et al., 2002; Arsenou et al., 2005). Several research studies have shown that the ATRA and its derivatives could modulate antioxidants in vivo and thereby prevent cancer development or progression (Lee et al., 2009). Moreover, ATRA is a metabolite form of vitamin A which may have the good antioxidant property. This study was therefore intended to explore the effectiveness of ATRA as a potent antioxidant in cancer condition. Retinoids are required for the maintenance of the immune system, as they are important immunomodulators (Gullu and Francine, 2002). It was found by researcher that the vitamin A levels are consistently elevated in healthy centenarians, suggesting a protective role in immune system maintenance by retinoids (Polidori et al., 2007). The immunomodulatory activity of vitamin A metabolites, including RA, has been extensively documented in several inflammatory and autoimmune diseases (Daniel et al., 2007). ATRA is also having several immunological effects beside its anticancer activity (Carratù et al., 2012). ATRA is used to treat a number of haematological malignancies as it causes haematopoietic cells to differentiate and prolongs the lifespan of cells (Orlandi et al., 2003).
RA has its own effects on cellular proliferation and differentiation in various cancers including melanoma. It has a capability to inhibit cell growth in malignant cells by induction of growth arrest in the G0/G1 phase of the cell cycle and by inducing apoptosis. Retinoids are effective chemo preventive agents against skin, head and neck, breast, liver and other forms of cancers too (Hansen et al., 2000). Synthetic analogues of retinoids, have been used in numerous oncology studies and impressive data have been generated in adult leukemia studies. Retinoids have been shown to suppress carcinogenesis in various epithelial tissues in experimental animal model systems by inhibition of tumor promotion to a preneoplastic or neoplastic cell (Lotan, 1992). The activity of ATRA is mediated by regulation of a variety of forms of gene expression through ATRA-dependent activation of retinoic acid receptors (RAR) and retinoid X receptors (RXR) in the nucleus of cancer cells, leading to the growth inhibition, differentiation, and apoptosis of cancer cells (Freemantle et al., 2003). The cytotoxic effect of ATRA on cell growth and induction of apoptosis is mediated via specifically binding and activating retinoic acid receptors, such as RARα, RARβ and RARγ (Zhang et al., 2003).

However, ATRA is used as a chemo therapeutic drug or supplementary drug in pre-clinical studies as well as used clinically to treat other solid cancers such as lung cancer, breast cancer and liver cancer (Liu et al., 2008), but their remission rate is less. One possible reason may be that it could not reach the target site in sufficient concentration for longer time which is essential for complete remission as it was achieved in the case of APL. Decreased level of ATRA in blood circulation after ATRA treatment and the poor aqueous solubility of ATRA are the major two drawbacks for its administration. ATRA can be loaded in carriers such as solid nano particles, cyclodextrins, liposome etc. in order to improve its aqueous solubility, enhancing its concentration and half life in the blood circulation (Ozpolat et al., 2003; Choi et al., 2003). It is also necessary to develop a drug delivery system for ATRA incorporation such as carriers, with enhanced localization in the target site and
sustained drug release (Labhasetwar et al., 1997). Meanwhile many researchers have developed DSPC and cholesterol combined microparticles and nanoparticles that encapsulate water insoluble, low molecular weight pharmaceutical agents, which are employed for clinical use. Liposomes can target a specific lesion after intravenous administration and are less likely to burst at an early stage of administration so that they can gradually release the agent at the site of the lesion over prolonged period of time. The delivery of ATRA by emulsions can reduce the elimination of ATRA from the blood circulation and referentially accumulate in the liver after intravenous injection. The retention of ATRA in the liver could suppress the progression of liver metastasis in mice injected with colon carcinoma cells (Chansri et al., 2006). So, encapsulation of ATRA may overcome this problem and can be considered as a good multi drug approach or in combinational therapy.

Overall, lung cancer represents the most common cancer globally as well as in India. ATRA has been used as first choice drug for acute promyelocytic leukemia (APL). Because of its poor solubility, lower half life and lower reach ability to the site of cancer from blood circulation, ATRA is still considered as one of the supplementary drug for solid cancers including lung cancer. Hence based on these studies ATRA can be encapsulated in liposome which can enhance its activity against solid cancers specially lung cancer along with cancer associated oxidative stress. In addition, in the tumor microenvironment, inflammation contributes to proliferation and also the functionality of immune system is critical to the pathophysiologic mechanisms of cancers. Hence, the anti-inflammatory and immunomodulation effects of encapsulated ATRA are also studied. This study will help to understand in detail about the encapsulated ATRA and its role on experimental lung cancer induced by B16F10 cells in C57BL/6 mice. It is expected that this study on encapsulated ATRA by modulating the activity of ATRA may soon provide a novel approach for prevention and treatment for the lung cancer patients.