1 Introduction

1.1 Cancer

Cancer is the most dreadful and deadly disease with its increasing influence in the twenty first century. It is characterized by the uncontrolled growth of cells in the body which has the ability to invade into nearby tissue and migrate to distant sites as well. Cancer affects different parts of the body and different kinds of tissue. The burden of cancer may be described in terms of incidence, prevalence, and mortality (Kinzler and Vogelstein, 2002).

1.1.1 Epidemiology

The World Health Organization (WHO) proclaimed cancer as one of four leading threats to human health and development along with cardiovascular diseases, chronic respiratory diseases and diabetes (WHO, 2008). Around 12.66 million people were diagnosed with cancer across the world in 2008, of which 7.56 million people died of the disease (Ferlay and Steliarova-Foucher, 2010). The American Cancer Society with other resources has estimated the annual incidence for 2012 to be 40,000 cases or more in USA alone. Canadian Cancer Statistics 2011 stated that about 177,800 new cases of cancer and 75,000 deaths from cancer in Canada.

In India, more than 556, 000 cancer deaths were estimated in 2010 for people of all ages, and among them 71.1% occurred in people aged between 30–69 years. Cancer deaths accounted for 8.0% of the 2.5 million total male deaths and 12.3% of the 1.6 million total female deaths at age 30–69 years. In 2010, at all ages, the rates of cancer deaths were about 59 per 100 000 for men and about 52 per 100 000 for women. Rajesh et al., (2012) have reported that the rates of cancer deaths per 100 000 rose sharply with age and at age 30–69 years were about 98 for men and 95 for women.

1.1.2 Classification

Cancers are classified by the type of cell that the tumor cell resembles and is therefore presumed to be the origin of the tumor. The types of cancer are carcinoma, leukemia, lymphoma, myeloma, sarcoma and mesothelioma (Parthasarathy, 2011)
1.1.3 Causes

Many factors are known to increase the risk of cancer. Some important factors are tobacco (Peto, et al., 2012), excessive body weight (Parkin and Boyd, 2011), unhealthy diet and alcohol consumption (Parkin and Boyd, 2011), Certain chemicals and other substances (Kuper et al., 2002), ionizing radiation (Anand et al., 2008), lack of physical activity (Parkin, 2011), occupations (Baan et al., 2009; Straif et al., 2009), sunlight and sunbeds (Parkin et al., 2011), certain hormones (Doll and Fau, 2003), cancer genes (Andrew et al., 2004) and viruses (Parkin, 2011).

1.2 Symptoms

General symptoms of cancer are unintentional weight loss, fever, chronic cough, persistent fatigue, bowel changes, pain and skin changes.

1.2.1 Treatment

Treatment for cancer involves chemotherapy, surgery and radiotherapy.

1.2.1.1 Chemotherapy

Chemotherapy is the treatment of cancer with cytotoxic drugs that can destroy cancer cells. The cytotoxic drugs affect rapidly dividing cells in general, in contrast to targeted therapy. Most forms of chemotherapy target all rapidly dividing cells and are not specific to cancer cells, although some degree of specificity may come from the inability of many cancer cells to repair DNA damage, while normal cells generally can (Takimoto and Calvo, 2008).

Chemotherapy can cause permanent changes or damage to the heart, lungs, nerves, kidneys, reproductive or other organs and certain types of chemotherapy may have delayed effects, such as a second cancer, that show up many years later.

1.2.1.1 Cytotoxic Drugs

Cytotoxic drugs include any drug that inhibits or prevents the function of cells. The most common forms of cytotoxic drugs are known as antineoplastic, and sometimes these terms are used reversibly (Thomas et al., 2006). However, cytotoxic drugs also affect the growth of other quick dividing cells in the body such as hair follicles and the lining of the digestive system. As a result of the treatment, many normal cells are damaged along with the cancer cells.
1.2.1.2 Side Effects

The side effects of chemotherapy can be unpleasant, but they must be measured against the treatment's ability to destroy cancer. The short term side effects of cytotoxic drugs are loss of appetite, nausea, vomiting, stomatitis, malaise, flu like feeling, fever, cystitis, haematuria, constipation and diarrhoea. The long term side effects are cardiac toxicity, pulmonary toxicity, haematologic impairment, immunologic impairment myelo suppression, skin reactions, liver toxicity, nephrotoxicity, neurotoxicity, and premature menopause.

1.3 Molecular Mechanism of Cancer

Several lines of evidence indicate that tumorogenesis in humans is multistep process and these steps reflect genetic alterations that drive the progressive transformation of normal cell into highly malignant derivatives. Tumor cells are invariably altered at multiple sites having suffered disruption through lesion as subtle as point mutations and as obvious as changes in chromosome complement (Kinzler and Vogelstein, 1996).

Cancer cell genotypes are manifestation of six essential alterations in cell physiology that collectively dictate malignant growth. They are (1) Self sufficiency in growth signals, (2) Insensitive growth inhibitory signals (antigrowth signals), (3) Evasion of programmed cell death, (4) Limitless replicative potentials, (5) Sustained tissue angiogenesis and (6) Tissue invasion and metastasis. (Douglas and Robert, 2000).

1.3.1 Self Sufficiency in Growth Signals

Many of the oncogenes in the cancer catalog act by mimicking normal growth signal in one way or other. There are three molecular strategies to achieve acquired growth signal (GS) autonomy. They are alterations in extracellular growth signals, transcellular transducers of these signals and intra cellular circuits that translate these signals into action. The most complex mechanisms of acquired GS autonomy is derived from alterations of components of downstream cytoplasmic circuitry that receives and processes the signals emitted by ligand activated GF receptors and integrins, in which SOS-Ras-Raf- MAPK cascade plays a central role.
1.3.2 Insensitivity to Antigrowth Signals

Normal cells response to antigrowth signals is associated with cell cycle clock, specifically the components governing the transit of the cell through G1 phase of its growth cycle. Cells monitor their external environment during this period and on the basis of sensed signals, decide whether to proliferate, to be quiescent, or to enter into promitotic state. Antiproliferative signals are funneled through the retinoblastoma protein (pRb) and its close relatives p107, and p130. When in a hypophosphorylated state pRb blocks proliferation by sequestering and altering the function of E2F transcription factor that controls the expression of genes essential for progression from G1 to S phase (Weinberg, 1995). Distribution of the pRb pathway liberates E2Fs and thus allows cell proliferation, rendering cells insensitive to antigrowth factors that result in the uncontrolled proliferation of the cells.

1.3.3 Evading Apoptosis

Resistance to apoptosis can be acquired by cancer cells through a variety of strategies. The most commonly occurring strategy is loss of proapoptotic regulator p53 tumor suppressor gene. Mutation in the p53 gene results in the functional inactivation of its product p53 protein which ultimately results in the inactivation of apoptotic pathway. More than 50% of human cancers are outcome of this mutation of p53 gene.

1.3.4 Limitless Replicative Potentials

Three acquired capabilities viz., growth signal autonomy, insensitive to antigrowth signals and resistance to apoptosis, all lead to deregulated proliferation programme resulting in a vast cell population that constitute macroscopic tumors. All type of mammalian cells carries an intrinsic cell autonomous program that limits their multiplication. But in some cell population might have progressed through a certain number of doublings a process termed “senescence”. All type of tumor lose senescence state and has critical state characterized by massive cell proliferation, karyotypic disarray associated chromosomal alterations and have the ability to multiply without limit (Wright et al., 1989).
1.3.5 Sustained Angiogenesis

Tumors appear to activate the angiogenic switch by changing the balance of angiogenesis inducers and inhibitors (Hanahan and Folkman, 1996). One common strategy for shifting the balance involves altered gene transcription. Many tumors evidence increased expression of VEGF and FGF compared to their normal tissue. Expression of endogenous inhibitors such as thrombospondin-1 is down regulated in few tumors (Singh et al., 1995).

1.3.6 Tissue Invasion and Metastasis

Invasion and metastasis are exceedingly complex process and their genetic and biochemical determinants remain incompletely understood. At the mechanistic level, they are closely allied process which justifies their association with one another as one general capability of cancer cell. Both utilize similar operation strategies involving changes in the physical coupling of cells to their microenvironment and activation of extra cellular proteases. The activation of extracellular proteases and the altered binding specificities of cadherins, and integrins are clearly central to the acquisition of invasiveness and metastatic ability. But the regulatory circuits and molecular mechanism that govern these shifts remains unclear.

1.4 Remedies

1.4.1 Ayurvedic Remedies

Ayurveda, the traditional Indian system of medicine, is practised in the Indian subcontinent since 2000 BC. It is a more scientifically verifiable and classifiable medical system. The two most important aims of Ayurveda are (i) to maintain the health of healthy people and (ii) to cure the diseases of sick people. The Ayurvedic remedies, both preventive and therapeutic, are mostly made of plants and when compared with their synthetic counterparts are either nontoxic or less toxic (Arora, 2010).

The treatment by Ayurveda is almost based on herbs. The Indian sages in the ancient times used herbs and mixed them (with other herb) to formulate medicines that could cure many deadly diseases. Some of them proved to be elixirs (Valiathan, 2006). Some herbs are used as general tonic to clean, nourish and rebuild on a cellular level, while others have affinity for a specific system or organ of the body (Murthy, 2005).
1.4.2 Herbal Medicine

Herbal medicine differs from the allopathic view of conventional Western medicine, which often seeks to address the symptoms of disease rather than the underlying cause (Tapsell, 2006). A synthetic drug may effectively stall allergy symptoms, but certain herbs act as adaptogens and immune modulators to enable the body to better manage stress and immune response (Thatte and Dhahanukar, 2001). Most of the herbs are free of side effects. Herbs have medicinal value which provides rational means for the treatment of many internal diseases, which are otherwise considered incurable in other systems of medicine (Skolnick, 1991).

The World Health Organization (WHO) reported that 80% of Asian and African countries presently use herbal medicine for their primary health care. The studies of US and Europe have shown that use of herbals is less in clinical settings, but has increased more in recent years as scientific evidence for its effectiveness (Edgar et al., 2002). Studies in medicinal science also suggested that Ayurvedic herbs can treat deadly diseases like cancer, AIDS and autoimmune disorders (Pandey, 2002). It is also found that herbal treatment is an effective alternative to allopathic medicines. The use of herbs to treat disease is almost universal among non industrialized societies, and is often more affordable than purchasing expensive modern pharmaceuticals (Meskin, 2002).

1.4.3 Phytochemicals

Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals (Chopra and Doiphode, 2002). Many of these phytochemicals have beneficial effects on long term health when consumed by humans, and can be used to effectively treat human diseases (Russo et al., 2001).

1.4.4 Phytocompounds for Cancer

Several phytocompounds have been isolated from plant sources and a number of them are used for treatment of dreadful diseases such as TB, cancer, autoimmune disorders. Pettit et al., (1995) isolated Combretastatin A from bark of *Combretum caffrum* (Combretaceae) which is active against colon, lung and leukemia cancers. Similarly, Vinca alkaloids, vinblastine and vincristine isolated from *Catharanthus roseus* (Apocynaceae) acted as anticancer agents. The vinblastine and vincristine are used in combined form with other
cancer chemotherapeutic drugs for a variety of cancers like leukemias, lymphomas, advanced testicular cancer, breast and lung cancers, and Kaposi’s sarcoma. (Cragg and Newman, 2005)

The paclitaxel from the bark of the *Taxus brevifolia* (Taxaceae) is another evidence of the success in natural product drug discovery while *Taxus baccata* was used in the Indian Ayurvedic medicine for the treatment ovarian, advanced breast, small and nonsmall lung cancer (Rowinsky *et al.*, 1992). Similarly, Elliptinium isolated from *Bleekeria vitensis* is marketed in France for the treatment of breast cancer. Betulinic acid, a pentacyclic triterpene, is a common secondary metabolite of plants, primarily from *Betula* species (Betulaceae) (Cichewitz and Kouzi, 2004) which acted as selective Cytotoxic drug against human melanoma cell lines (Balunas and Kinghorn, 2005). Silvestrol was isolated from the fruits of *Aglaila sylvestre* (Meliaceae) (Hwang *et al.*, 2004) and exhibited cytotoxicity against lung and breast cancer cell lines.

1.4.5 Nanotechnology

Nanotechnology is an enormously powerful technology, which holds a huge promise for the design and development of many types of novel products with its potential medical applications on early disease detection, treatment, and prevention. One of the most active applications in nanotechnology is the use of nanoparticles in medicine. It exhibits new or improved properties based on specific characteristics such as size, distribution and morphology (Chandran *et al.*, 2006). Nanoparticles, because of their small size, have distinct properties compared to the bulk form of the same material, thus offering many new developments in the fields of biosensors, biomedicine, and bio nanotechnology (Barnali, 2011).

1.4.5.1 Silver Nanoparticles

The Silver Nanoparticles (AgNPs) have emerged as an arch product from the field of Nanotechnology. The applications of AgNPs are developing a benign effect in all forms of human life (David *et al.*, 2005). Synthesis of AgNPs has impressed through their diverse properties like catalysis, magnetic and optical polarizability (Shiraishi and Toshima, 2000), electrical conductivity (Chang and Yen, 1995), and Surface Enhanced Raman Scattering (SERS) (Matejka *et al.*, 1992). AgNPs also have some potential activities in medicine like biosensing (Han *et al.*, 2001), antimicrobial activity (Sharverdi *et al.*, 2007), biological labeling (Nicewarner-Peña, 2001), detection of genetic disorders (Taton *et al.*, 2000; Cao *et
al., 2002), drug delivery (Joshi et al., 2006), gene therapy and DNA sequencing (Sandhu et al., 2002). The potential benefits of AgNPs in biomedical and industrial applications for human health and environment are now accepted.

1.4.5.2 Green Synthesis of AgNPs

Among the synthetic methods, chemical and physical methods are used to prepare the pure, well defined AgNPs successfully, but these are quite expensive and potentially biological hazards and toxic to the environment (Sastry et al., 2004). An alternative to chemical and physical methods, biological organisms such as microorganisms or plant extract is used for the production of nanoparticles in an eco-friendly manner (Mohanpuria et al., 2008).

Jose-Yacaman and his co-workers first reported the formation of gold nanoparticles (Gardea-Torresdey et al., 2002) and silver nanoparticles by living plants (Gardea-Torresdey et al., 2003). AgNPs was synthesized from leaf extracts of Camellia sinensis (Nestor et al., 2008), Cinnamomum camphora (Huang et al., 2007), Aloe vera (Chandran et al., 2006), Cymbopogon flexuosus (Shankar et al., 2005), Tamarindus indica (Ankamwar et al., 2005a), Azadirachta Indica (Shiv Shankar et al., 2004), Pelargonium graveolens (Shankar et al., 2003) and fruit extract of Emblica officinalis (Ankamwar et al., 2005b), Medicago sativa (Gardea-Torresdey et al., 2003) sprouts and germinating seeds extracts of Brassica juncea (Haverkamp et al., 2007), etc.,

1.4.5.3 Silver Nanoparticles for Cancer

Silver nanoparticles (AgNPs) are also very popular for their antimicrobial potential against several other bacteria and viruses. AgNPs have been shown to possess intrinsic cytotoxic activity (Kim et al., 2007; Baker et al., 2005). The earlier reports have shown that AgNPs inhibit bovine retinal endothelial cells (Kalishwaralal et al., 2009), Pliss lymphosarcoma cells (Sheikpranbabu et al., 2009). The silver nanoparticles have shown that antitumor activity against ascitic tumors and the DLA cell lines (Sriram et al., 2010) and Ehrlich’s ascite carcinoma (EAC) cell line (Vadivel and Suja, 2012).
1.5 **Aim and Objectives**

The aim of the present study is to determine the effects of both leaf extracts of *E. indica* and green synthesized AgNPs from *E. indica* on HT29, a Human colon colorectal adenocarcinoma, under *in vitro* condition and Ehrlich Ascites Carcinoma (EAC), a colorectal cancer, under *in vitro* and *in vivo* conditions.

**Objectives**

- To identify and authenticate the selected plant, *Elaeagnus indica* Servett.
- To determine physicochemical properties of *E. indica*.
- To analyse the phytochemical properties of various leaf extracts.
- To evaluate antioxidant activity of various extracts.
- To synthesise and characterise the silver nanoparticles from the leaf using AgNO₃.
- To evaluate the antitumor potential of both leaf extract and AgNPs *in vitro* methods against Ehrlish ascites carcinoma (EAC) cell lines and Human Colon rectal cancer cell lines (HT29 Cell line).
- To find out the antitumor potential of both leaf extract and AgNPs by *in vivo* studies against Ehrlish ascites carcinoma cells in Swiss albino mice.
- To isolate and characterize the marker compounds using sophisticated instruments such as UV-Vis spectroscopy, FTIR, GC-MS and NMR.
- To predict the interaction of selected plant compounds with proteins responsible for cancer through *in silico* method.