Chapter 1

Introduction
Cancer, derived from the Greek word “Karkinos” (meaning “crab”), is a group of more than 100 diseases that develop across time and involve uncontrolled division of the body’s cells. In simple terms, cancer is wild growth of cells, where they reproduce themselves unrestrained. It is a disease characterized by loss of the regulatory mechanisms that control cell growth and differentiation required for homeostasis in complex multicellular organisms (Meyers, 2007). Cancer cells normally have no function like the healthy cells, but they spread at the expense of the healthy cells by growing into the healthy tissues and destroying it. Although cancer can develop in virtually any of the body’s tissues, and each type of cancer has its unique features, the basic processes that produce cancer are quite similar in all forms of the disease.

“Tumors destroy man in a unique and appalling way, as flesh of his own flesh which has somehow been rendered proliferative, rampant, predatory, and ungovernable . . . Yet, despite more than 70 years of experimental study, they remain the least understood . . . What can be the why for these happenings?” —Peyton Rous, in his acceptance lecture for the Nobel Prize in Physiology or Medicine (1966)

Global cancer trends: Even though it kills fewer individuals than heart disease, cancer has long ranked as Public Enemy No. 1 in the view of the general public and many health professionals. Estimates of the worldwide incidence and mortality from 27 cancers in 2008 have been prepared for 182 countries as part of the GLOBOCAN series published by the International Agency for Research on Cancer (IARC, 2008). The global patterns for the eight most common cancer results for 20 world regions suggests an overall estimate of 12.7 million new cancer cases and 7.6 million cancer deaths occurring in 2008, with 2.9 million in economically developed countries and 4.7 million in economically developing countries. The most commonly diagnosed cancers worldwide are lung (1.61 million, 12.7% of the total), breast (1.38 million, 10.9%) and colorectal cancers (1.23 million, 9.7%). Projections from GLOBOCAN 2008 also suggest that this rising trend in incidence and prevalence is likely to remain over the next couple of decades with the number of new cases of cancer diagnosed every year around the world set to increase by 69 % reaching up to 21 million by
2030. Statistics also indicate that one in three people in the developed world will get the disease and one in four will die as a result.

**Indian scenario:** More than half of the cancer cases in the world originate from the developing countries (Bobba and Khan, 2003; Mudur, 2005). In India, cancer has become one of the ten leading causes of death. It is estimated that there are nearly 2 – 2.5 million cancer cases at any given point of time with an annual incidence and mortality rate of about 0.95 million and 0.6 million respectively (GLOBOCAN, Figure 1.1).

**Figure 1.1.** The gender-wise incidence and mortality rates for the fifteen leading cancer types in Indian population (Ferlay et al., 2008).

**Cancer causes:** Cancers are generally believed to be of multi-factorial origin. According to National Cancer Institute, several factors may contribute to the process of cancer development which include, aging, tobacco/alcohol consumption, exposure to radiation (sunlight and other ionizing radiation), certain chemicals (like asbestos, aflatoxin etc.), hormones (like estrogen and diethylstilbestrol), several kinds of
viruses (human papilloma virus, hepatitis B and hepatitis C viruses, Human Immunodeficiency Virus, helicobacter pylori, Epstein-Barr virus etc.). Apart from these, poor lifestyle (including poor diet quality, low fruit and vegetable intake, being overweight and obese, physical inactivity etc.) as well as family history of cancer are also known to be risk factors in cancer (Danaei et al., 2005). The contribution of some of these risk factors to all cancer deaths worldwide is shown in Figure 1.2.

![Figure 1.2. Contribution of selected risk factors to all cancer deaths, worldwide, in high-income countries, and in low- and middle-income countries (Danaei et al., 2005)](image)

**Figure 1.2.** Contribution of selected risk factors to all cancer deaths, worldwide, in high-income countries, and in low- and middle-income countries (Danaei et al., 2005)

**Treatment of cancer**

One of, if not the most, crucial decisions to be made following diagnosis of cancer is the assessment the most appropriate treatment modality. The best treatment approach for the effective cancer cure is based on several factors such as type of cancer, its stage, whether or not there is evidence of metastasis, the age and health of patient, and in some cases, the presence of certain genetic mutations (DeVita et al., 2005).

The three most commonly employed treatment modalities for cancer include,

1. Surgery - for the treatment of localized disease aimed to either remove the tumor or to reduce to smaller mass/volume;
2. Radiation - for the treatment of localized disease; and
Apart from these traditional cancer treatment methods, hormone therapy, immunotherapy, gene therapy, therapies with antiangiogenic agents are also being tested for their anticancer potential. However, a combination of one or more treatment methods is often used when combating the disease to eliminate as much, if not all, of the cancer as possible and to reduce the likelihood of a future recurrence.

**Surgery:** It is the oldest and the most commonly used treatment approach in cancer, and is considered to be an effective and quick method to bring down large volume of cancer cells in a single attempt, offering the largest number of cures. During earlier days, the entire organ carrying the tumor was surgically removed as in the case of radical mastectomy. However, owing to the advancement in the surgical techniques, the present day surgery involves surgical removal of only the tumor tissue (lumpectomy) and not the organ. In recent years, enormous advancement in the surgical methodologies have resulted in techniques that are less invasive, less expensive and have enhanced the ability to not only remove a tumor but to do so with fewer side effects or complications. Precisely focused, high-energy beams of light are used in laser surgery to target, superheat, and essentially vaporize cancerous cells while sparing nearby healthy ones. On the opposite end of the spectrum is cryosurgery, which kills malignant cells by exposing them either to liquid nitrogen that freezes them to a temperature of $-195.5^\circ\text{C} (-320^\circ\text{F})$, or to an ultra-cold metal probe. However, surgery is often combined with other treatment modalities such as radiotherapy and/or chemotherapy for preventing the recurrence and/or metastasis.

**Radiotherapy and importance of radiosensitizers:** Since the discovery of X-rays by Roentgen way back in 1895, radiotherapy has been one of the mainstays in the treatment of cancer, with about half of all cancer patients receiving some type of radiation therapy during the course of their illness (Barton *et al.*, 2006; Desai *et al.*, 2007). Although this mode of therapy is often effective, its success is far from assured. Radiotherapy failure has often been attributed to the inability to destroy all of the tumor cells within the treatment fields. Theoretically, all cancers could be controlled locally if a sufficiently high radiation dose could be delivered to a treatment volume that encompassed all of the tumor cells. In practice, however, the administration of a radiation dose high enough to eradicate all of the tumor cells...
would pose an unacceptably high risk of causing severe normal tissue toxicities. The presence of hypoxic cells (that are known to be three times more radioresistant than the normoxic cells) within the solid tumor mass brings about tumor radioresistance, which is another major biological determinant in the failure of radiotherapy (Hall, 2000). This resistance of tumor cells to radiation has led researchers to develop chemical or pharmacological agents (radiosensitizers) that selectively sensitize the tumor cells to radiation while sparing normal cells. As a result of such research efforts, the number of radiosensitizers has increased significantly in the last two decades. These radiosensitizers may be grouped into the following major classes:

1. Halogenated pyrimidines – are compounds that are very similar to the DNA precursors except that their methyl group is substituted with halogens. The differential radiosensitization is based on the premise that tumor cells cycle faster than the normal cells and therefore incorporate more of the drug than the surrounding normal cells. Radiosensitizers of this class include 5-bromodeoxyuridine (BrdU), 5-iododeoxyuridine (IdU) etc.

2. Hypoxic cell sensitizers – Agents that enhance the radiation induced damage in cells deficient in molecular oxygen but have no effect on normally aerated cells. In this case a differential effect is based on the premise that hypoxic cells occur only in tumors and not in normal tissues. These compounds are known to mimic the effect of oxygen by fixing the free-radical induced damage. Radiosensitizers of this class include nitroimidazoles such as metronidazole, misonidazole, etanidazole, doranidazole, nimorazole etc. (Hall, 2000).

3. Chemotherapeutic agents as radiosensitizers – In solid adult tumors, owing to its limited biological efficacy, chemotherapeutic agents are seldom used as a sole curative treatment modality. It is, however, used more often in combination with other curative treatments such as radiotherapy and/or surgery, at least for locally advanced diseases (Joiner and van der Kogel, 2009). Such a combination may result in enhancement of the radiation effect which may either be additive or synergistic in nature. Several of conventional chemotherapeutic drugs are known to sensitize cells to ionizing radiation. The
mechanisms involved in the radiosensitization by these chemotherapeutic drugs are diverse and only partially understood. For example, radiation appears to increase the cellular uptake of platinum drugs (Yang et al., 1995) and increase the number of toxic platinum intermediates (Richmond, 1984). The involvement of other mechanisms such as alterations in radiation induced DNA damage repair and cell-cycle checkpoint functions (DeVita et al., 2005) are also reported for platinum analogues. On the other hand, hydroxyurea, a cytotoxic agent is known to selectively kill the cells in the most radioresistant synthetic (S) phase of the cell cycle (Sinclair, 1967). Conversely, paclitaxel is thought to synchronize tumor cells in G2/M phase, which is a relatively radiosensitive phase. Other examples of chemotherapeutic agents that cooperate with radiation-induced cell killing include camptothecin compounds (topotecan, CPT-11), etoposide, doxorubicin, and dactinomycin. Based on the encouraging results from these preliminary studies, several randomized phase-III trials for many relevant cancer sites were conducted and they provide strong evidence that supports the use of combined modality approaches involving the combination of ionizing radiation with cytostatic drugs. This holds true especially for glioblastoma multiforme (Stupp et al., 2005), head and neck cancers including nasopharyngeal cancer and laryngeal cancer (Brizel et al., 1998; Forastiere et al., 2003), esophageal cancer (Minsky et al., 2002), colorectal- and anal cancer (Bartelink et al., 1997; Sauer et al., 2004), cervical cancer (Green et al., 2001), as well as lung cancer (Schaake-Koning et al., 1992). However, the need for novel compounds with better anticancer and radiosensitizing potential still persists.

4. Hyperthermia - The term ‘hyperthermia’ refers to various techniques of heat application administered as an adjunct to already established strategies (especially radiotherapy and chemotherapy) in the treatment of cancer patients (Hildebrandt et al., 2002). The major reason for the increasing interest in hyperthermia seen in the mid seventies was due to the observation that hypoxic cells appeared to be more sensitive to hyperthermia than normal oxygenated cells (Schulman and Hall, 1974; Overgaard, 1977; Overgaard et al., 1991; Lee et al., 2010). Although some studies have shown beneficial
effect of combination of hyperthermia and radiotherapy (Overgaard et al., 1995; van der Zee et al., 2000; Jones et al., 2005), other studies show no beneficial effect of such a combination (Vasanthan et al., 2005; Mitsumori et al., 2007; De Haas-Kock et al., 2009). The scarcity of data on the large scale randomized clinical trials makes it extremely difficult to draw any kind of conclusions on the definitive role of hyperthermia as an adjunct to radiotherapy (Lutgens et al., 2010) as well as necessitates more multicentric clinical trials for establishing its potential in cancer therapy.

Therefore methods that sensitize tumor cells (radiosensitizer) while sparing normal tissues could potentially lead to greater success with radiation as a therapy (Muschel et al., 1998). Although a large number of compounds have been evaluated for their radiosensitizing potential with substantial breakthroughs, the lack of US-FDA approved radiosensitizers on the market necessitates the search for novel compounds with better radiosensitizing potential.

Chemotherapy and importance of drug targeting: The use of cytotoxic drugs, those that inhibit the growth of or kill cells, to treat cancer is referred to as antineoplastic drug therapy or, more commonly, as chemotherapy. Approximately five decades of systemic drug discovery and development have established a respectable armamentarium of useful chemotherapeutic agents as well as a number of important successes in the treatment and management of human cancer. Many of these chemotherapeutic agents are also known to possess potent radiosensitizing properties. These drugs may either be used singly or as a part of multimodality therapy, along with surgery and/or radiotherapy, to achieve and maintain remission. Unfortunately, more than 50% of all cancer patients either do not respond to initial therapy or experience relapse after an initial response to treatment and ultimately die from progressive metastatic disease. Thus, an ongoing commitment to the design and discovery of new anticancer agents with radiosensitizing potential is critically important (Grever et al., 1992; Kaufman and Chabner, 2001).

Despite the discovery of large variety of chemotherapeutic agents, the treatment of most human solid tumors remains largely palliative (Li et al., 1999). The
central problem of cancer chemotherapy is the severe toxic side effects of anticancer drugs on healthy tissues (Longo and Chabner, 2001; DeVita et al., 2005; Pastorino et al., 2007). Apart from that, many compounds with potent anticancer properties are found to have extremely short biological half lives rendering them clinically inactive (Loadman et al., 2002). One of the approaches often employed to overcome the limitations of the anticancer chemotherapies is by the use of various targeted drug delivery approaches that provide selective, and sufficiently high, localization of “active” drug at the tumor site, thereby improving the efficacy and reducing the toxicity of the entrapped drug (Ferrari, 2005; Davis et al., 2008; Lammers et al., 2008). Targeting is especially important in circumstances where a localized tumor is removed by surgery and chemotherapy is prescribed as follow-up preventive against potential metastases. Also, these targeted drug delivery systems are reported to reduce the risk of anticancer drug resistance (Mamot et al., 2003). Nanotechnology is attracting increasing attention in the biomedical community, owing to unique prospects for targeted delivery in imaging, therapy, and drug delivery (Phillips, 1999; Boerman et al., 2000). Cancer nanotechnology is expected to transform current treatment systems by providing more efficient cancer diagnostics and therapeutics (Ting et al., 2010). Today, nanocarriers are used in detecting cancer at an early stage, delivering anticancer drugs specifically to malignant cells, and determining if these drugs are killing malignant cells (Bawarski et al., 2008).

Over the past few decades, there has been considerable interest in developing biocompatible, biodegradable carriers as effective drug delivery devices. Among the various particulate drug carriers, liposomes have gained most attention (Harrington et al., 2000a; Harrington et al., 2000b). From the biomedical viewpoint, liposomes are biocompatible/biodegradable, cause very little or no antigenic, pyrogenic, allergic and toxic reactions; they protect the host from any undesirable effects of the encapsulated drug, at the same time protecting the entrapped drugs from the inactivation under physiological conditions; and, last but not least, liposomes are capable of delivering their content inside many cells (Torchilin, 2008). From the perspective of a formulation scientist, liposomes are considered to be versatile as they can encapsulate both hydrophilic as well as hydrophobic drugs. Also, they as regarded as being very
flexible in that their surfaces can be easily modified with a variety of functional moieties such as polyethylene glycol (PEG) and targeting ligands (Sofou, 2007). It is therefore important to develop novel nano-carrier technologies that can be used for targeted drug delivery to tumors and thereby improve the therapeutic index of the carried drugs.

Present knowledge

It is long-established that plants possess diverse principles, which are of immense nutritional and medicinal value. Quinones represent one such broad category of quinoid compounds, which are widely distributed in nature. Many quinones have been associated with various biological activities, including anticancer activity (Babula et al., 2007; Babula et al., 2009). Although, there are many clinically important anticancer agents containing quinone nucleus such as anthracycline, mitoxantrones and saintopin, many other quinones are still being tested for their anticancer activity (Kim et al., 2006). Plumbagin (5-hydroxy-2-methyl-1, 4-napthoquinone), is one such compound derived from the roots of *Plumbago zeylanica*, is among the most extensively studied quinones. Previous studies have given sufficient insight into the anticancer and radiosensitizing properties of plumbagin (Naresh et al., 1996; Prasad et al., 1996; Devi et al., 1998; Tiwari et al., 2002; Srinivas et al., 2004). Besides, other naturally occurring napthoquinones such as lapachone, shikonin, menadione etc. are also known to possess promising cytotoxic/anticancer and radiosensitizing properties (Taper et al., 1996; Wu et al., 2004a; Wu et al., 2004b; Woo et al., 2006).

Juglone (5-hydroxy-1, 4-Napthoquinone), a structural analogue of plumbagin, is a pigment that occurs as a natural product in the roots, leaves, nut-hulls, bark and wood of black walnut (*Juglans nigra* L.), European walnut (*Juglans regia* L.) and butternut (*Juglans cinerea* L.) (Family: Juglandaceae) (Duke and Ayensu, 1985). The herbal preparations of walnut have been extensively used in folk medicine for the treatment of acne, inflammatory diseases, ringworm, bacterial, viral, fungal infections including cancer (Duke and Ayensu, 1985; Blumenthal, 1998). Bhargava and Westfall (Bhargava and Westfall, 1968) reported that herbal preparation of walnut suppresses
the growth of spontaneous mammary adenocarcinoma in Swiss albino mice. Juglone is known to be the principal component of walnut, with other constituents such as alpha-hydrojuglone (1,4,5-trihydroxynaphthalene) and its glycoside beta-hydrojuglone, along with caffeic acid, ellagic acid, hyperin, and kaempferol. A review of existing literature suggests that apart from a few very recent papers, the *in vitro* cytotoxic potential of juglone was not studied (Cenas et al., 2006; Fila et al., 2008; Ji et al., 2011). Further, the existing literature also reveals that the *in vivo* anticancer potential of juglone is very ambiguous with some papers showing juglone to possess potent antitumor properties (Okada et al., 1967; Sugie et al., 1998; Ji et al., 2009) and others showing juglone to promote the DMBA induced carcinogenesis (Van Duuren et al., 1978; Monks et al., 1990). In addition, there are no previous reports about the radiosensitizing potential of juglone per se. Moreover, although it is a known fact that quinones exert significant toxicities to normal cells, no previous attempts have been made to formulate juglone using targeted drug delivery platforms with an aim of improving the anticancer potential as well as reducing the normal tissue toxicity associated with juglone.

Therefore, in the present study, an attempt was made to evaluate the *in vitro* cytotoxic potential of juglone against cell lines of murine and human origin and to elucidate its underlying mechanisms. Given that the structure of juglone resembles that of plumbagin, this investigation was also aimed to evaluate the radiosensitizing potential of juglone against a chemo- and radio-resistant B16F1 melanoma cell using both *in vitro* as well as *in vivo* tumor models. In addition, the present investigation was intended to formulate juglone as sterically stabilized liposomal forms aiming enhanced antitumor efficacy and reducing the toxicity associated with juglone.
REFERENCES


Ji Y, Qu Z, Zou X, Cui L, Hu G. Studies on Inhibition of Juglone on Sarcoma 180 in Mice. 3rd International Conference on Bioinformatics and Biomedical Engineering Beijing: IEEE; 2009. p. 1 - 4


Chapter 1


