CHAPTER 1
CHAPTER 1: INTRODUCTION

1.1 General Introduction

The Indian Cancer Society defines cancer as the abnormal growth of one cell or a group of cells. If it is not destroyed or removed, it can spread very rapidly and eventually lead to death. Normal body cells grow, divide and die in an orderly fashion. In adults, this process takes place only in connection with normal growth process or to replace worn out or dying cells and to repair injuries. Cancer cells are different from the normal cells since they do not stop dividing or die. Instead, they outlive the normal cells and continue to form new abnormal cells.

Cancer is not just one disease but rather a group of diseases, all of which cause cells in the body to change and grow out of control. Cancers are classified either according to the kind of fluid or tissue from which they originate, or according to the location in the body where they first developed. In addition, some cancers are of mixed types. Among all cancer types, lung and prostate cancers are the leading causes of death due to cancer.

Human lung cancer can be divided into non-small-cell lung carcinoma (NSCLC) and small-cell lung carcinoma (SCLC) based on histopathological features. About 80% of human lung cancers are NSCLC, and they are subdivided broadly into adenocarcinoma, squamous cell carcinoma (SCC), and large-cell carcinoma, of which adenocarcinoma is the most prevalent and appears to be increasing in frequency, especially in women and nonsmokers (Husain and Kumar, 2005). Lung cancer is currently the most frequently diagnosed major cancer in the world and the most common cause of the cancer mortality worldwide.

Prostate cancer is the most commonly diagnosed cancer and the second leading cause of cancer-related deaths among American males. The incidence of this disease is highest among Caucasian and African-American males, with reported incidences of 140/100,000 and 222/100,000, respectively (U.S. Department of Health and Human Services [DHHS], 2006). The incidence is lowest among the Asian Americans and Hispanic Americans.

Current treatment options for prostate and lung cancer include watchful waiting or active surveillance, radical prostatectomy, external beam radiation therapy (EBRT), and brachytherapy and chemotherapy. Potent curative procedures are normally offered to men with a life expectancy of at least 10 years (Johansson et al., 1997). In some patients, adjuvant therapy such as androgen deprivation has been shown to improve survival when given during, and for 3 years after, radiation therapy (Bolla et al., 1997, 2002), and there is increasing evidence that hormone
therapy for prostate cancer patients with an intermediate or poor prognosis delays disease progression (de Koning et al., 2002; Wirth and Hakenberg, 2002). Chemotherapeutic drugs have a very narrow therapeutic window and often lead to cyto-toxicity, neuro-toxicity, myelo-suppression, etc. Patients experiencing these toxicities and the pain from the disease, and knowing the statistical fact that conventional therapies offer little hope at the advanced stage of disease, the patients are desperate to use complementary and alternative medicines with the hope of boosting the immune system, relieving pain, and controlling side effects related to disease or treatment. Only a minority of patients include CAM in the treatment plan with curative intent since the fundamental problem with CAM therapies is a dearth of evidence-base.

There are several complementary and alternative medicines now in practice, but many of them too may produce side effects. The search for a safer alternative system of medicine takes us to look for geographic areas in the world where the cancer incidence is comparatively lesser than in the Western population, and where people continue to practice the respective traditional medicinal systems. In Asia there are unique systems of traditional medicines such as Ayurveda and Siddha in India, Chinese in China and Unani in several parts of Asia.

Since the beginning of human civilization, ethno-botanicals such as herbs have been valued for both culinary and medicinal properties (Wasser and Weis, 1999; Mahady, 2001). The base assumption in herbal medicine is that plants contain natural substances that can promote health and alleviate illness. There is also a general, but erroneous, assumption that natural substances and traditional remedies are non-toxic (Mahady, 2001).

During the last decade, the use of traditional medicines has expanded globally, and over-the-counter supplements have become very popular. Furthermore, this trend of increased usage of traditional remedies gets accelerated when conventional medicine is ineffective in the treatment of disease (Eisenberg et al., 1993, 1998). Hence, it is crucial that, along with the growing interest of the population in herbs and medicinal mushrooms, scientific research should be conducted in order to evaluate and investigate the benefits and possible deleterious effects of ethno-botanicals.

Ayurveda and Siddha are time-tested systems of natural health care that comprehensively address the patient from a holistic perspective. These medicines have been widely used in India as a system of primary health care for thousands of years. Research over the last 100 years has shown encouraging results for Ayurvedic and Siddha treatment of various ailments, including
cancer. These systems of medicine are unique in that the approach is holistic and the medicines are directed not only to the immediate causative factor of the disease, but also towards protecting the whole body. Composition, dose, duration, modality of administration, precautions, etc., have been clearly laid down. Common spices are utilized, as well as herbs, herbal mixtures, and special preparations known as Rasayanas. Thus, it becomes possible that the drugs in this system of medicine would be the potential CAM’s.

CAM is a group of diverse medical and health care systems, practices, and products that are not presently considered to be part of conventional medicine. Conventional medicine is medicine as practiced by holders of M.D. (medical doctor) or D.O. (doctor of osteopathy) degrees and by their allied health professionals, such as physical therapists, psychologists, and registered nurses. Some health care providers practice both CAM and conventional medicine. While some scientific evidence exists regarding some CAM therapies, for most there are key questions that are yet to be answered through well-designed scientific studies—questions such as whether these therapies are safe and whether they work for the diseases or medical conditions for which they are used.

The list of what is considered to be CAM changes continually, as those therapies that are proven to be safe and effective become adopted into conventional health care and as new approaches to health care emerge.

Towards finding a potential CAM for cancer in the two Indian systems of medicine, Ayurveda and Siddha, literature on treatment modalities for cancer was searched. Research has been conducted worldwide on Ayurveda and Siddha. Additional research that uses current scientific technologies is needed to further validate the therapeutic efficacy of the herbal medicines. Lack of evidence in terms of modern science is a major hurdle in introducing/practicing these efficacious drugs. Strong concerns over the safety and toxicities amidst of several fake claims about dietary and herbal supplements boosted by the marketers exist, there is attempt for scientific validation of drugs that are available from Indian traditional medicinal systems like Ayurveda and Siddha.

Rasagenthi lehyam (RL), an Indian traditional medicine, prescribed by Siddha practitioners for cancer, is a formulation containing 38 different botanicals and 8 inorganic compounds. RL is claimed to cure prostate, lung and other cancers. An interview was conducted on 96 patients taking this therapy and they told that RL controlled the disease and comforted
them without any side effects. Many patients reported completed remission after treatment. In the context of the search for CAM modalities for cancers in general, and prostate and lung cancers in particular, RL was subjected for detailed study.

The scientific exploration of this lehyam was carried out with a hope that a potent extract of the extract, a combination of different extracts or the compound(s) present in it could be broadly used as complementary or alternative therapy for incurable cancers, or for palliative care for the cancer patients, and specifically to find its role in different cancer treatment strategies, including in “cell proliferation”, “angiogenesis”, “hormonal regulation of cell differentiation”, “drug resistance”, “cell cycle arrest”, “DNA repair”, “effect on growth factors”, and “carcinogen metabolism”, or effect on molecular targets like pro-survival / pro-apoptotic factors such as NF-kB, AP-1, Bcl-2, Bax, cox-2 or tumor suppressor genes like p53, etc.

1.2 Brief Review of Literature

Lung cancer is the most common cancer throughout the world, particularly in the United States (Parkin et al., 2005) and it accounts for 14% of all cancers and 28% of all cancer-related deaths worldwide (Murphy, 2000, Ries et al., 2000). Chemotherapy is the standard treatment for lung cancer patients, but in spite of its ability to improve the symptoms and the quality of life of the patients with lung cancer, only a minimal increase in survival rate can be achieved (ten Bokkel Huinink, Bergman et al., 1999; Sandler et al., 2000).

Prostate cancer (PCa) is the second most common cancer among males (Greenlee et al., 2000). Every year 220,000 new cases are diagnosed in the United States alone, and these numbers are projected to increase as the aging population expands (Jemal et al., 2003). Although there has been significant improvement in early detection and treatment of PCa, the mortality rate of the disease still remains high (Scardino, 1994; Walsh, 1994). In the recent years, studies have shown the role of oncogenes and tumor suppressor genes in the development and treatment of PCa. However, a better understanding of prostate tumor biology is critical for developing more effective ways for preventing and combating the disease.

Several treatment modalities such as androgen deprivation, radiation therapy, and cryoablation exist for primary PCa, but there is currently no effective treatment for patients presenting advanced or metastatic disease. Approximately 80-90% of PCa cases are dependent on androgen at initial diagnosis, and endocrine therapy of PCa is directed toward the reduction of serum androgens and inhibition of androgen receptor (AR) (Denis and Griffiths, 2000).
However, androgen ablation therapy ultimately fails, which is clinically more aggressive and difficult to treat the disease (Heinlein and Chang, 2004). The major limitation is that it offers only temporary relief; the cancer eventually reappears in an androgen-independent form, characterized by aggressive growth and invasion to distal organs (Grossmann et al., 2001; Gelmann, 2002). It has been reported that current chemotherapies induce low response rates (8.7%) in hormone-resistant PCa (Yagoda and Petrylak, 1993). In addition, chemotherapy also causes myelosuppression, immunosuppression and other toxic effects such as nephrotoxicity and neurotoxicity in normal cells (Vayalil et al., 2002). These realities fuel the need for continuing research into promising treatment strategies for PCa.

Apoptosis is regulated by various genes such as $p53$, $Bcl2$ and $Bax$ (White, 1996; Stuanton and Gaffney, 1998) and in this, $p53$ gene has come to the forefront of cancer treatment because it is commonly mutated and its functions are inhibited in many cancer types (Vogelstein and Kinzler, 1992; Tanaka et al., 2000). The $p53$ tumor suppressor protein is a transcription factor that regulates several genes, especially those involved in the cell cycle, DNA repair and apoptosis (Levine 1997). The $p53$ protein can also activate the expression of $Bax$ and mediate $Bcl-2$ suppression, leading to cellular apoptosis (Miyashita et al., 1994). Regulation of apoptosis is a complex process and involves a number of cellular genes, including $Bcl-2$ (Fisher et al., 1993) and $Bcl-2$ related family members such as $Bcl-xL$, $Bcl-xs$, $Bad$ and $Bax$ (Boise et al., 1993). $Bcl-2$ and $Bcl-xL$ exert their antiapoptotic effect, at least in part by binding to $Bax$ and related pro-apoptotic proteins. The members of $Bcl-2$ family regulate the initiation of mitochondrial apoptotic pathway. The major function of $Bcl-2$ is to inhibit apoptosis and to prolong cell survival. Over-expression of $Bcl-2$ protein is associated with enhanced oncogenic potential and poor response in lung cancer treatment (Groeger et al., 2004). Apoptosis proceeds through caspase activation cascades, known as the extrinsic and intrinsic pathways. The extrinsic pathway-induced apoptosis is mediated by receptors (Malinin et al., 1997), which activate initiator caspase-8 or -10 signaling that leads to activation of executioner caspases such as caspase -3, -6, -7 and -9. Steps in the intrinsic pathway, which is induced by stress, radiation and chemotherapeutic drugs, include cytochrome-c release from mitochondria, caspase-8 activation and then activation of effector caspases, particularly caspase-3 (Green and Reed, 1998).

Several studies show that radiation induces pro-survival factors such as increased NFκB activity and $Bcl-2$ up-regulation in PC-3 cells. Ionizing radiation induces NFκB activation
(Hallahan et al., 1989; Van Antwerp et al., 1996) and it plays an important role in inhibiting TNF-α or chemotherapy-induced apoptosis (Wang et al., 1996; Plummer et al., 1999). TNF-α is also a potent inducer of NF-κB activity (Hallahan et al., 1989; Van Antwerp et al., 1996). The multiplicity of mechanisms of NF-κB activation and its role in inhibition of antiapoptotic function is more complex. The antiapoptotic target genes for NF-κB include Bcl-2 (Tamatani et al., 1999), Bcl-xL (Dixon et al., 1997; Tamatani et al., 1999) and Bcl-2 homologue A1/Bfl-1 (Wang et al., 1999). It has been reported that ectopic over-expression of Bcl-2 in prostate cancer cells showed enhanced radiation resistance and inhibition of apoptosis in prostate cancer cells and other tumor cell types (Hockenbery et al., 1990; Sentman et al., 1991). Induction of prosurvival and antiapoptotic genes strongly suggests that PC-3 cells harbor a tight regulatory loop that inhibits the cell killing effects of ionizing radiation.

Par-4 is induced exclusively by apoptotic agents (Sells et al., 1994). Par-4 function is essential for the induction of apoptosis by a broad range of agents, implying that endogenous Par-4 must be activated in response to these apoptotic agents (El-Guendy et al., 2003). Two potential modes of Par-4 activation are, (a) up-regulation of Par-4 above the necessary thresholds to override inhibition by Akt, translocate Par-4 to the nucleus, and induce apoptosis (Sells et al., 1997), and (b) inhibition of Akt to release Par-4 for apoptosis (Goswami et al., 2005).

Both androgen and its cognate receptor [androgen receptor (AR)] are recognized risk factors in the development of PCa (Heinlein and Chang, 2004). These observations are further corroborated by genetic evidence from transgenic mouse models, suggesting that increased AR signaling in the prostate is linked to an increase in precancerous lesions. Accordingly, the most effective treatment for early-stage PCa includes suppression of AR function either by blocking androgen signaling with the anti-androgens bicalutamide (Culig et al., 1994) or flutamide or by inhibiting the conversion of testosterone to the potent androgen dihydrotestosterone with finasteride. As prostate cancer cells are dependent on AR signaling for survival and growth, either the removal of androgen or blocking dihydrotestosterone synthesis leads to the induction of apoptosis in clinical prostate cancer as well as in cell culture and animal models of prostate cancer (Thompson et al., 2003; Isaacs and Isaacs, 2004). However, 30% of these patients show relapse of the disease within 3 years as a result of the emergence of androgen-independent prostate cancer cells, which are either AR-positive or AR-negative. The molecular mechanisms
dictating the progression from androgen dependence to androgen independence are unclear due to the lack of suitable experimental models and molecular markers (Hara et al., 2003).

In general, however, AR can be activated despite androgen blockade therapy in AR-positive prostate cancer (Ahmed et al., 2001). This is attributed to an increased sensitivity of AR to low concentrations of androgen due to mutations in AR, AR partner-protein interactions, or post-translational modifications; these underlying factors function to promote androgen depletion-independent signaling (An et al., 2004). AR function is integrally linked to prostate cancer progression. Initially, androgen independence was believed to be a consequence of AR loss, but it is now apparent that AR is expressed in hormone-refractory prostate cancer. There is a growing consensus, therefore, that the lack of responsiveness to anti-androgen therapy is a function of either enhanced sensitivity of AR to androgen levels (owing to mutations) or post-translational alterations in AR (Roy-Burman et al., 2005). Accordingly, both androgen-responsive and androgen-refractory prostate cancer cells have been identified in clinical specimens as well as in cell lines derived and established from advanced prostate cancer, as exemplified by the AR-negative PC-3 and DU145 cells and the LNCaP and CWR22Rv-1 (AR mutant) cells.

Several risk factors have been associated with the development of cancer. These may include intake of dietary fat, age, race, ethnic origin, androgen levels and receptor expression. It is in general agreement that the mortality rates of cancer in Asian countries is at least 10 times lower than in the West (Geller, 1995; Thompson et al., 1995). For example, Caucasian and African-American men in the United States have a PCa incidence that is 5-50 times greater than that of Japanese men residing in Japan, and the incidence of PCa in Japanese immigrants to the United States is four times that of their native Japanese counterparts. African Americans are about 33% more likely to die of cancer than are whites and more than twice likely to die of cancer as are Asian-Islander, American-Indians and Hispanics (Izevbigie, 2003). This marked racial and cultural disparity suggests that dietary factors may affect cancer growth (Nelson and Montgomery, 2003). Rates of lung cancer incidence are highest in North America and Europe and the rates are moderate in Australia/New Zealand and parts of East Asia (Parkin et al., 1999).

Many epidemiological studies show that the people who consume a diet rich in vegetables and fruits have a lower incidence of cancer. Vegetables, fruits, and whole grains contain a wide variety of phytochemicals that have the potential to interfere with the
development of cancer (Zeegers et al., 2001). Over 60% of anticancer drugs available in the market are of natural origin. Natural products are lead molecules for many of the drugs that are currently in use (Cragg et al., 1997).

Many naturally occurring agents have shown chemopreventive and chemotherapeutic potentials in a variety of bioassay systems and animal models. Extracts of medicinal plants are believed to contain different chemopreventive or chemotherapeutic compounds, which possess more than one mechanism of actions. Epidemiological studies have suggested that certain dietary components are associated with lower cancer risk (Gerber, 2001). Plant-derived phytoestrogens (or isoflavones) are also selective estrogen receptor modulators (SERMs) because they show mixed estrogen agonist-antagonist activities. Phytoestrogens are found in particularly high doses in soy beans, which contain genistein, and daidzein; which have been associated with reduced risk of breast cancer (Brownson et al., 2002; Shang and Brown, 2002). Since phytochemicals are safe at levels found in the diet, they are of interest to those investigating the role of chemoprevention in lowering cancer risk. The diet contains many types of phytochemicals, including vitamins and other compounds, that cannot be synthesized by humans or animals (Kim et al., 2004).

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical conditions; however, the scientific basis regarding their modes of action is limited. Extracts of medicinal plants are believed to contain different chemopreventive or chemotherapeutic compounds, which possess more than one mechanism of action. The induction of apoptosis is known to be an efficient strategy for cancer therapy. Several studies have demonstrated that extracts from herbal medicines or mixtures have anti-cancer potential (Agarwal et al., 2002; Hu et al., 2002) in vitro and in vivo (Kao et al., 2001; Bonham et al., 2002). Recently, several dietary phytochemicals that play a significant role in the anti-carcinogenic process have been identified.

1. 3 Herbal Medicines for the Treatment of Cancer

PCA has been identified as a 'nutritional disease' (Moyad, 1999a; Hirsch, 2000), because there is a relatively uniform rate of latent, sub-clinical PCA throughout the world and clinical PCA shows distinct geographical variation (Franks, 1973). Most initial studies of the value of nutritional supplements in the prevention of PCA have simply observed these differences but have not explained them. Withanoloids extracted from Datura metel exhibit cytotoxic effects
against A549 (lung), BGC-823 (gastric), and K562 (leukemia) cancer cell lines (Pan et al., 2007). Lycopene, one of active ingredient of tomato, a non-vitamin-A carotenoid, has potent antioxidant activity. An epidemiological survey suggested that the lycopene intake was related to a lower risk of PCa (Muir et al., 1991). A recent study on lycopene showed a significant reduction of oxidative damage in patients undergoing prostatectomy for localized PCa (Chen et al., 2001).

An active component of a traditional Chinese herbal medicine, Danggui Longhui Wan, Indirubin, has been shown to inhibit the growth of prostate and breast cancer cells (Nam et al., 2005). The extract of Hibiscus syriacus induces apoptosis by activating p53 and AIF in human lung cancer cells (Cheng et al., 2008). The active ingredient baicalin from Chinese medicine was found to inhibit prostaglandin E2 production (Kyo et al., 1998; Nakahata et al., 1998), reduction of high blood pressure and relaxation of arterial smooth muscle cells (Chen et al., 1999). This drug has been shown to be relatively non-toxic when given orally, but intramuscular injection caused fever and muscle aches (Huang, 1999). Baicalin exhibited a concentration-dependent growth inhibitory effect on several human PCa cells, which are different in their sensitivities to androgen (Chan et al., 2000).

There have been several in vitro studies showing that an extract from a Japanese medicinal herbal preparation demonstrated some anti-tumor effects on several human hepatoma, pancreatic and a cholangiocarcinoma cancer lines (Okita et al., 1993; Motoo and Sawabu, 1994; Yano et al., 1994). Two new flavonoids - 3'-formyl-4',6'-dihydroxy-2'-methoxy-5'-methylchalcone (FMC) and (2S)-8-formyl-5-hydroxy-7-methoxy-6-methylflavanone (FMF) - isolated from the buds of Cleistocalyx operculatus showed broad-spectrum anticancer activity against five human cancer cell lines, SMMC-7721 (liver cancer), 8898 (pancreatic cancer), K562 (chronic leukemia), HeLa (tumor of cervix uteri) and 95-D (high metastatic lung carcinoma) (Ye et al., 2007). In the recent past, an herbal preparation, named PC-SPES, has been sold in the US as a dietary supplement for PCa patients. This preparation, which contains extract from the S. baicalensis Georgi, has been claimed to be cytotoxic against PC-3 and LNCaP cells by the induction of apoptosis (Halicka et al., 1997). In animal studies, this herbal preparation inhibited the growth and metastasis of a Dunning rat PCa cell line, MAT-LyLu, grown in Copenhagen rats (Tiwari et al., 1999). In a recent clinical study, PC-SPES was shown to decrease the serum levels of testosterone and prostate-specific antigen in patients with hormone-sensitive PCa (DiPaola et
al., 1998). However, multicentric case controlled studies have revealed PC-SPES to be highly estrogenic and of little therapeutic use and, hence, removed from the market (Cordell, 2002).

Selenium, a trace element found in many foods, including meat, fish, eggs, dairy products and grains (Combs and Combs, 1984). Epidemiological studies suggested an inverse correlation between selenium intake and PCa incidence and mortality (Menter et al., 2000). Patients in this trial who received 200 μg/day of selenium had a remarkable 65% reduction in the incidence of PCa. Selenium has been shown to inhibit experimental carcinogenesis by various mechanisms (Platz and Helzlsouer, 2001). In addition to chemopreventive and chemotherapeutic role of selenite, it also prevents cellular DNA damage induced in rat hepatoma (Thirunavukkarasu et al., 2008). Selenium activates p53 and p38 pathways and induces caspase-independent cell death in cervical cancer cells (Rudolf et al., 2008)

In Asian countries, green tea is a popular beverage and some studies indicate a lower incidence of prostate carcinoma in tea drinkers (Jain et al., 1998). Active compounds for anti-tumor effect in tea are mainly tea phenols (Yang et al., 1997). Most studies have evaluated green-tea preparations that contain at least four active polyphenolic compounds including epicatechin, epigallocatechin, epicatechin-3-gallate and epigallocatechin-3-gallate (EGCG). In bioassays, EGCG comprises 10–60% of the polyphenol constituents in green-tea infusions and has been shown to arrest LNCaP and DU145 PCa cells and induce apoptosis (Gupta et al., 2003). Green-tea preparations, when administered orally to TRAMP mice for 24 weeks, achieved a 40% reduction in localized tumor development at 20 and 30 weeks. Administration of green tea alone through oral route, completely suppressed metastatic spread to lymph nodes, liver, lungs and bone, and improved survival by 70% compared to control animals (Gupta et al., 2001). Epigallocatechin gallate (EGCG) stabilizes p27kip1 in estrogen- stimulated MCF-7 breast cancer cells through downregulation of the Skp2 protein (Huang et al., 2008). Combination of polyphenon E (PPE, a standardized green tea polyphenol preparation) and atorvastatin (trade name Lipitor) synergistically inhibited 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumorigenesis in mice and the growth of lung cancer cells H1299 and H460 (Lu et al., 2008). EGCG significantly suppressed oral cancer cell-invasive ability by decreasing the number of invasive foci as well as invasion depth in three-dimensional collagen invasion model (Kato et al., 2008). A phase I study of green-tea extract in the treatment of patients with various tumors recently reported no significant responses using the maximally tolerated oral dose of 1
g/m² three times a day, although 10 of 49 patients had stable disease for six months or longer (Pisters et al., 2001). A more recent study showed that i.p. administration of EGCG can modulate the endocrine system of rats by lowering several circulating hormones, reducing body weight, the size of prostate and other organs (Gupta et al., 1999).

Other promising herbals are soy products, which have shown to exhibit the broad range of mechanisms for chemopreventive and anti-neoplastic activities. A flavonol-rich diet may decrease the risk of advanced adenoma recurrence (Bobe et al., 2008). More recently, investigators have begun to study about genistein and daidzein that are isoflavones found in soy. In cell-culture studies, genistein inhibited the growth of PCa cells (Moyad, 1999b, 2001). Genistein induces cell apoptosis in MDA-MB-231 breast cancer cells via the mitogen-activated protein kinase pathway (Li et al., 2008). Genistein induces apoptosis in ovarian cancer cells (Thasni et al., 2008). Soy isoflavones inhibit 5α-reductase, the enzyme that is responsible for conversion of testosterone to dihydrotestosterone (Evans et al., 1995). Genistein also inhibits various tyrosine kinases, as well as DNA topoisomerases (Markovits et al., 1989). Administration of genistein to TRAMP mice for 25 weeks suppressed the development of a histologically defined subset of aggressive prostate tumors in a dose-dependent manner (Markovits et al., 1989).

These findings suggest that the herbal medicines are a rich source for the development of molecular target-specific drugs (Engel and Straus, 2002). In the last several decades, a tremendous amount of effort has been invested to isolate individual compounds from traditional herbal medicinal mixtures and to determine their chemical structures (Yan et al., 1999). Many of these natural products have been screened for their anticancer activity in cancer cells in vitro and in vivo models.

1.4 Complementary and Alternative Medicine

Alternative medicines have gained importance over the past decade, fueled in part by the public's desire to participate in their own health-care and the perception that the allopathic system of medicine has failed to find a reliable and definitive cure for cancer, despite almost three decades of virtual war against cancer. Thus, the patients, in their desire to keep themselves alive, go for unconventional medical therapies (UMTs). The use of unconventional/non-traditional therapies in the general population has increased dramatically in the past decade (Jones et al., 2002; Surh, 2002).
As medical care becomes more complex, technical and expensive, more cancer patients turn to complementary and alternative medicine (Addis and Corrin, 1985). CAM is a group of medical and health systems, practices, and products that are not presently considered to be part of conventional medicine. While some scientific evidence exists regarding some CAM therapies, for most there are key questions that are yet to be answered through well-designed scientific studies—questions such as whether they are safe and whether they work for the disease or medical conditions for which they are used. Complementary medicine is used together with conventional medicine. Alternative medicine is used in place of conventional medicine. The two are together known as CAM. Interestingly, higher levels of education and income are associated with greater use of CAM. Among the patients who seek CAM treatment, 90% believe that it will help them live longer and improve their quality of life, 60% believe that it will relieve symptoms and 47% expect it to cure cancer (Surh, 2002). About 80% of patients suffering from prostate cancer receive some form of CAM (Smith and Mills, 2001). Some patients with advanced disease, after conventional treatment has failed, turn to CAM with the hope of keeping the disease under control, thereby extending their survival and improving their quality of life. Patients of high socioeconomic level and those who are clinically disease-free after radical treatment are most likely to turn to CAM, among which herbal medicine is the most commonly adopted by prostate cancer patients (Kao and Devine, 2000).

1.5 Rasagenthi Lehyam

RL, the Siddha medicine investigated in this study, as indicated, possesses several medicinal properties including immuno-modulatory, anti-oxidant, and anti-tumor, as has been shown in \textit{in vitro} and \textit{in vivo} models (Details are furnished in the Chapter 2). Some of the herbal ingredients in it that follow have been shown to be effective therapeutically.

\textbf{Zingiber officinale:}

Ginger is the rhizome of \textit{Z. officinale}. The oleoresin from ginger contains [6]-gingerol, which has a wide variety of pharmacological and physiological activities (Surh \textit{et al.}, 1998). A number of reports support the use of this compound as an antioxidant and as a suppressor of prostaglandin biosynthesis (Flynn \textit{et al.}, 1986; Kiuchi \textit{et al.}, 1992, Surh \textit{et al.}, 1998). Gingerol strongly inhibits TPA-induced epidermal ODC activity and TNF-\textalpha production in mouse skin (Park \textit{et al.}, 1998).
**Curcuma longa:**

Curcumin is the major secondary chemical of rhizome of turmeric (*Curcuma longa*), which is used as a spice to give a specific flavor and yellow color in Asian food. Recent reports have shown that curcumin treatment suppresses both constitutive NF-κB and AP-1 in DU-145 cells (Chen *et al.*, 1997). Curcumin also abolishes the TNF-α induced NF-κB activity in LNCaP cells. Curcumin was found to inhibit TNF-α induced NF-κB activation in human myelomonoblastic leukemia cells and phorbol ester-induced c-Jun/AP-1 activation in mouse fibroblast cells (Jeswal, 1998; Waffo-Teguo *et al.*, 2001). The molecular mechanism of NFκB inhibition by curcumin is unclear, but appears to involve inhibition of IκB degradation (Huang *et al.*, 1991; Bierhaus *et al.*, 1997; Pendurthi *et al.*, 1997). Inhibitory effects of curcumin on NF-κB activation have been documented in prostate cancer cells (Mukhopadhyay *et al.*, 2001), mouse fibroblast cells (Huang *et al.*, 1991), human leukemia cells (Singh and Aggarwal, 1995) and human colon epithelial cells (Plummer *et al.*, 1999). Curcumin inhibits NFκB activation by inhibiting IκB-α phosphorylation that is necessary to export NFκB from cytosol to nucleus and to activate its target genes.

It has been shown that curcumin induces apoptosis either by mitochondria-dependent or mitochondria-independent mechanism, depending on the cell types. Curcumin-induced mitochondria-independent apoptosis has been shown in breast cancer cell lines (Mehta *et al.*, 1997), basal cell carcinomas (Jee *et al.*, 1998) and T-Jurkat cells (Piwocka *et al.*, 1999). In conclusion, curcumin, a major active component of turmeric, has been reported to induce growth inhibition and apoptosis in many cancer cell types.

**Semecarpus anacardium:**

*S. anacardium* nut contains flavonoids, phenolic compounds, minerals, vitamins and amino acids. An experimental study in the rat, using aflatoxin B-induced carcinogenesis as the model, has shown that *S. anacardium* nut extract affords anticancer activity by inducing both phase I and phase II biotransformation enzymes (Premalatha, 2000; Premalatha and Sachdanandam, 2000). In mammary carcinoma-bearing rats there was a significant rise in glycolytic enzyme activities and a simultaneous fall in gluconeogenic enzyme activities; administration of *S. anacardium* nut milk extract returned these enzyme activities to their respective control levels (Sujatha and Sachdanandam, 2002).
Terminalia chebula:

The methanolic extract of fruit of *T. chebula* inhibited the growth in several malignant cell lines including human (MCF-7) and mouse (S115) breast cancer, an osteosarcoma (HOS-1), a PCa (Alcock *et al.*, 2002), and a non-tumorogenic immortalized human prostate cell lines. The extract decreased cell viability, inhibited cell proliferation and induced cell death in a dose-dependent manner (Saleem *et al.*, 2002). At lower concentrations the extract induced some apoptosis but at higher concentrations necrosis was the major mechanism of death. Methanolic extract and its isolated compounds like gallic acid, chebulagic acid and chenbulinic acid showed moderate *in vitro* cytotoxicity in a variety of tumor cell lines (Pettit *et al.*, 1996).

*Nigella sativa*:

Several reports have ascribed an anti-carcinogenesis role to the seed of *N. sativa*, a natural food additive in India and the rest of Asia. Topical application of *N. sativa* extract inhibited two-stage inhibition/promotion of induced skin carcinogenesis in mouse. Oral treatment of the extract delayed the onset of papilloma formation. *IP* administration of the extract 30 days after subcutaneous administration of methylcholanthrene (MCA) restricted tumor incidence to 33% (Salomi *et al.*, 1992). *In vitro* cytotoxicity studies using the active principle of *N. sativa* seed, containing certain fatty acids, showed 50% cytotoxicity to EAC, Dalton’s lymphoma ascites and sarcoma 180 cells. The cell growth of KB cells in thymidine incorporation studies indicated the possible action at DNA level (Salomi *et al.*, 1992).

*Vitis vinifera*:

Grape, *Vitis vinifera*, is prescribed as a cardio-tonic and for other disorders (Paul *et al.*, 1999). These phenolic compounds are known as antioxidants and cancer chemopreventive agents for hepato-renal carcinogenesis in mouse (Jeswal, 1998). A potential cancer chemopreventive activity of astringin, quite different from resveratrol, has also been demonstrated (Waffo-Teguo *et al.*, 2001). A more recent study has found grape pomace extracts to be potent in inhibition of lipid peroxidation, hydroxyl radical scavenging activity and oxidation of human low density lipoprotein (Chidambara Murthy *et al.*, 2002).

*Withania somnifera*:

The total alcoholic extract of *W. somnifera* produced a dose-dependent increase in the complete regression at the primary site and induced significant growth delay in uncured tumors with respect to transplantable mouse tumor sarcoma-180 grown as solid tumor in BAL B/c
mouse (Umadevi, 1996). More interestingly, a radio-sensitizing effect, over and above the anti-tumor effect, has been attributed to Withaferin. A combination of the alcohol extract of \textit{W. somnifera} with radiation and/or hyperthermia enhanced the complete tumor regression above that produced by single modality treatments, and with all the three modalities the anti-tumor response increased to 100\% with 80\% complete regression (Umadevi, 1996; Bhattacharya \textit{et al.}, 1997, 2000). It was also shown that Withaferin-A is responsible for the radio-sensitizing effect (Umadevi \textit{et al.}, 1993). Another study showed that Withaferin-A treatment one hour before irradiation enhanced the tumor cell killing \textit{in vitro} (Umadevi, 1996). The radio-sensitizing effect of Withaferin-A was reported later on several cell lines \textit{in vitro} (Sharada \textit{et al.}, 1996; Ganasoundari \textit{et al.}, 1997; Umadevi \textit{et al.}, 2000) and \textit{in vivo} (Umadevi \textit{et al.}, 1995, 1996, 2000).

1.6 Hypothesis and Objectives

Hypothesis:

1. Drugs in the Indian systems of medicines are efficacious, which can be proved scientifically adopting modern methods and tools.
2. Thus tested, drugs in Indian medicines or preparations there from can be prescribed as CAM.
3. Individual compounds can be isolated from complex formulations and subjected to reverse pharmacology for projection as prescription drugs.

Objectives:

1. To scientifically validate a drug in an Indian system of medicine adopting the modern methods and tools, for application in prostate and lung cancers.
2. To find the most efficacious extracts of this medicine for application as a CAM for these cancers.
3. To subject the drug to reverse pharmacology approach by fractionation of the efficacious extract, identification of the major compounds and testing some of these compounds for treatment against these cancers so as to recommend the compounds as main line therapies.

Thus, the thesis is novel in approaching a classical Indian medicine for application in the modern context so as to popularize and earn credibility for the Indian medicines and to discover newer drugs for cancer from these medicines. It is a "classical-modern" approach or "East-West" fusion. It is also an aspect of "Reverse Pharmacology".