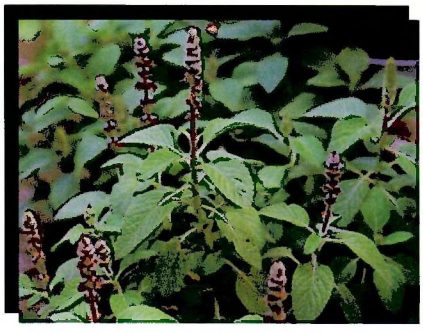

Introduction

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Cancer remains a major health problem in both the developed and the developing countries. Cancer is set to become the leading cause of death in the world, followed by heart disease and then stroke (Boyle and Lewin, 2008). About 7.6 million people died from cancer in the world during 2007 (American Cancer Society, 2007). By 2020, the number of cancer patients is likely to grow by 15 million and the death due to cancer is also likely to increase (Jimbo *et al.*, 2006). In spite of the spectacular advances made by medical sciences during the present century, treatment of cancer remains an enigma.

The development of more effective drugs for treating patients with cancer has been a major human endeavor over the past 50 years and the 21st century now promises some dramatic new directions. While improvements in surgery and radio therapy have had a major impact on cancer treatment, the concept of systemic chemotherapy specific for cancer cells, free of major side effects remains the critical goal for the future. However, most cancers can be cured and successfully treated if detected early and most often failure of cancer therapy is due to a delay in the diagnosis (John, 2008).

One of the major drawbacks of the current cancer therapeutic practices such as chemotherapy and radiation therapy is the suppression of the body's immune system. Side effects produced by both of these conventional therapies of cancer are nausea, vomiting, mucosal ulceration, alopecia, pulmonary fibrosis, cardiac and hepatic toxicity etc. Drugs that could alleviate these side effects will be useful in cancer therapy (Guruvayoorappan and Kuttan, 2007).

Alternative systems of medicine like ayurvedha, siddha, unani and other tribal folklore medicines have significantly contributed to the health care of the

population of India. Today these systems are not only complementary but also competitive in the treatment of various diseases (Radha *et al.*, 2008). At present, the scientific community is interested in elucidating the role of several therapeutic modalities, currently considered as elements of complementary and alternative medicine, on the control of certain diseases. Plant derived natural products such as terpenoids and steroids have received considerable attention in recent years due to their diverse pharmacological activity (Sivakumar *et al.*, 2008).

Since chemotherapy and radiation cause severe toxicity, herbal plants have become popular throughout the world nowadays, and are also used as a therapy for tumors or cancers. More than 50% of all modern drugs in clinical use are natural products, many of which have been recognized to have the ability to induce apoptosis in various types of tumor cells (Rosangkima and Prasad, 2004). It has been well recognized that allopathic drugs are not without danger as they exhibit severe toxicity in normal tissues. Therefore worldwide research is going on to investigate effective antitumor agents from different sources. Recent pharmacological researches are driven by the urgency to evolve suitable chemotherapeutic agents for the treatment of tumors (benign and malignant) free of toxic effects. The indigenous systems of medicine in India have in use several medicinal plants which have versatile antitumor properties that need detailed research for the development of antitumor herbal drugs. The contribution of ethnomedicinal plants in discovering new drugs has been enormous for treating diseases like cancer, hypertension and diabetes (Madhuri and Pandey, 2009).

India has a rich heritage of medicinal plants and natural herbal products. These rich herbal resources offer a unique opportunity for specific evaluation for health applications. The use of plant drugs is increasing in many of the developing countries because modern life-saving drugs are beyond the reach of

the common man and have high toxic potential. Currently there is renewed interest in developing drugs from plant for cancer therapy. Many indigenous plants are being tested against a spectrum of experimental tumors with the perceived advantage of local availability, low cost and less side effects. Many plant derived products exhibit potent antitumor activity against several cancer cell lines (Madhuri and Pandey, 2008). Plants with potent antitumor, hepatoprotective, and antioxidant properties were also reported by Gupta *et al.* (2004).

Oxygen is essential for survival. However, its univalent reduction generates several harmful reactive oxygen species (ROS), inevitable to living cells and highly associated with wide range of pathogenesis such as diabetes, liver damage, inflammation, aging, neurological disorder and cancer. In spite of the comprehensive network of cellular defensive antioxidants, many ROS still escape surveillance inflicting serious anomalies favoring such disease states. Antioxidant principles from natural resources are multifaceted in their multitude and magnitude of activities and provide enormous scope for correcting the imbalance through regular diet. Therefore, in recent years, the interest is also centered on antioxidants derived from herbal medicines. Phytoantioxidants, which are commonly available (less toxic medicinal components) are believed to reduce the threat of wide range of ROS. The World Health Organization estimates show that 80% of the earth's inhabitants rely on traditional herbal medicines for their primary health care (Tripathi and Kamat, 2007).

Interest in the search for natural antioxidants has increased over the past few years as the reactive oxygen species (ROS) production and oxidative stress have been shown to play a vital role in a nubmer of disorders (Finkel and Halbrook, 2000). Various reactive oxygen species include the hydroxyl radical, superoxide anion, hydrogen peroxide, singlet oxygen, nitric oxide, hypochlorite

and various lipid peroxides. These species cause cellular damage by reacting with various biomolecules such as membrane lipids, nucleic acids, proteins and enzymes. This damage is the major contributor for many disorders like cancer, hepatic ailments, cardiovascular diseases, cataracts, immune system decline, diabetes mellitus, inflammation, renal failure, brain dysfunction and the process of aging. Antioxidants are the agents that are capable of effectively neutralizing the deleterious effects of free radicals. Natural antioxidants that are present in the body are catalase, superoxide dismutase, glutathione peroxidase, while synthetic antioxidants like butylated hydroxyl toluene and butylated hydroxyl anisole are suspected to be carcinogenic and hence are no more in use. Therefore the need for the search of antioxidants from natural origin has been greatly felt in the recent years (Lavhale and Misra, 2007).

Cells have developed antioxidant mechanisms to quench the free radicals but when the generation of free radicals exceeds the scavenging capacity of the cell, the excess free radicals seek stability through electron pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells, resulting in the induction of lipid peroxidation which leads to cancer, atherosclerosis, cardiovascular diseases, ageing and inflammatory diseases (Hazra *et al.,* 2008). The free radicals are known to be scavenged by synthetic antioxidants, but due to their adverse side effects leading to carcinogenicity; the search for effective and natural antioxidants has become crucial (Adeolu *et al.*, 2009). The chemopreventive properties of plant anticarcinogen are due to antilipid peroxidative action, modulating carcinogen detoxification or by improving the antioxidant defense system (Dongre *et al.*, 2008).

Humankind has for centuries used many species of medicinal plant to treat several diseases and these are also known to produce a wealth of phytochemicals. In recent years, secondary plant metabolites with previously unknown

pharmacological activities have been extensively used as the source of medicinal agents. In order to make these remedies acceptable, there is a need to scientifically evaluate and identify the active principles (Bhagat *et al.*, 2010). Plants can, therefore, be described as the major source of medicine, not only as isolated active principles to be dispensed in standardized dosage forms but also as crude drugs for the population of developing countries. The World Health Organization has stressed the need to promote the indigenous system of medicine among the rural population of the Third World Countries (Arjun *et al.*, 2009).

The National Cancer Institute has collected about 35,000 plant samples from 20 countries and has screened around 114,000 extracts for anticancer activity (Shoeb *et al.*, 2005). Natural products or natural product derivatives comprised 14 of the top 35 drugs in 2000 based on worldwide records. Of the 92 anticancer drugs commercially available, 60% are of natural origin. In this instance, natural origin is defined as natural products, derivatives of natural products or synthetic pharmaceuticals based on natural product models (Shoeb, 2006). However the quality control of herbal medicine remains a challenge owing to the fact that there is a high variability in the active constituents involved. Hence the World Health Organization has approved fingerprint technique or standardized extract for quality assurance of herbal medicines (Mustaffa *et al.*, 2010).

Kumar and Kuttan (2005) have reported about plant derived extracts containing antioxidant principles with cytotoxicity towards tumor cells and antitumor activity in experimental animals. Plants with potent antitumor, hepatoprotective and antioxidant properties were also reported by Natesan *et al.* (2010). Antitumor activity of these antioxidants is either through the induction of apoptosis or by the inhibition of neovascularization. The implication of free radicals in tumors is well documented. Vinca alkaloids, epiphyllotoxins, taxanes

and camptothecins are the four main classes of anticancer agents from the plants currently in clinical use and natural products isolated from the medicinal plants can remain as essential components in the search for new medicines (Balunas and Kinghorn, 2005). Anticancer drug is one area of modern therapeutics which has drawn profoundly on compounds of nature both for use as such and as platforms for developing more efficacious molecules. Even at present, there is considerable activity in the search for new leads and scaffolds (Sukh dev, 2010).

Tumor growth normally affects various haematological parameters and the anticancer activity is generally assessed by the restoration of the changes in these parameters (Radha and Sivakumar, 2008). Plant-derived natural products such as flavanoids, terpenes, alkaloids and so on have received considerable attention in recent years due to their diverse pharmacological properties including cytotoxic and cancer chemopreventive effects (Gupta *et al.*, 2004).

Many biological assays require the measurement of surviving and/or proliferating mammalian cells. This can be achieved by several methods, e.g., counting cells that include/exclude a dye during cell proliferation. The cell viability assay is often used to determine cytotoxicity following exposure to toxic substances. MTT (3-[4,5-dimethylthiozole-2-yl]-2,5-diphenyl tetrazolium bromide) assay is the most sensitive cytotoxic assay showing statistically significant difference between the treated cells and controls.

The pressing need for additional research related to traditional herbal remedies is directed towards the identification of active principles. Without the knowledge of active principles, it is difficult to standardize a product and its dose. There is a need to establish a procedure for identifying, analysing and determining the concentration of active components in herbs (Inamder *et al*, 2008).

The first step in the discovery of new natural product drugs from the plants is the selection of the plant species to be investigated (Adou *et al.*, 2010). Plants belonging to the family Lamiaceae such as Rosemary, Sage, Thyme and Coleus are being extensively used in traditional medicinal practices in India. The genus Coleus has 300 species of which *C.amboinicus, C.forskohlii, C.spicatus* and *C.aromaticus* are naturally occurring species (Mohammed *et al.*, 2007). Many Coleus species such as *C.aromaticus, C.barbatus, C.blumei* and *C.scutellaroides, C.parvifolius* were reported for their constituents and biological activities viz., cytotoxicity, antimicrobial and antioxidant activities (Yuenuongsawad, 2005).

Coleus forskohlii (Lamiacea) is an important plant in Indian Ayurvedic medicine. The roots of the plant produce the labdane diterpenoid, forskolin. It is distributed over the subtropical warm temperature climatic zones on the mountains of India, Nepal, Myanmer, Srilanka, Thailand, and Africa. In India, it is reported to be distributed in the dry hills of Western Uttarpradesh, Gujarat, parts of Orissa, Western ghats and in kitchen gardens in Northern Karnataka (Belgaum and Dharward districts) with its carrots like tubers, which are used as condiments in the preparation of pickles (Reddy *et al.*, 2004) *Coleus forskohlii* is a superior species which can be used as a potent medicinal herb for novel bioactive compounds with high free radical scavenging activity (Rasineni *et al.*, 2008).

Forskolin *is* purified from the roots of the wild or the cultivated *Coleus forskohlii* plant. This is the only plant derived compound known to directly stimulate the enzyme, adenylate cyclase, and in turn, stimulates cellular cyclic AMP, which boosts metabolism (Mohammed *et al., 2007*).

Forskolin from *Coleus forskohlii* has been identified as a potent activator of adenylate cyclase leading to an increase in the levels of cAMP affecting

cardiac muscle contraction, blood and intraocular pressure, cancer, eczema, rheumatism and obesity (Duke and Mary, 2002). Many metastasizing tumor cell lines induce platelet aggregation both *in vivo* and *in vitro*. Upon aggregation, platelets release substances that promote tumor growth. Studies have demonstrated forskolin's ability to block platelet adenylate cyclase and increase intracellular cAMP. Forskolin has been used to treat hypertension, congestive heart failure, eczema and respiratory disorders.

The present study has been carried out to evaluate the antioxidant status and antitumor activity of roots of *Coleus forskohlii* against DLA tumor bearing mice.

The main objectives of the study are

1. To investigate the antitumor activity of different extracts of *Coleus forskohlii* roots against standard drug in mice with DLA tumor.

2. To evaluate hematological profile and cytotoxic activity of methanolic extract of roots of *Coleus forskohlii* against standard drug in mice with DLA tumor.

3. To screen the phytochemicals and find out the active constituents responsible for the antioxidant and antitumor activity.