Chapter 1

Introduction

Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs. *Sphaeranthus indicus* Linn belongs to the family Asteraceae, an annual spreading, aromatic herb, strongly scented, 30 – 60 cm tall, found abundantly all over India, ascending to an altitude of 1500m, especially as a weed that grows plentifully in rice fields. Leaves ovate – oblong narrowed at the base, dentate and serrate. Flowers composed heads, globose, violet inflorescence. Stems are glandular hairy with toothed wings. The entire plant of *S. indicus* is reportedly used in the Indian system of traditional medicine as a remedy for various ailments, being used as a tonic, laxative, digestive, in the treatment of tuberculosis, diseases of the spleen, asthma, bronchitis, elephantiasis, pain of the uterus and vagina, anemia, leucoderma, epilepsy and hepatitis. The Sanskrit word mungi literally means that which cuts off or wards off. In ancient scriptures it is mentioned to be one of the best herbs, used as medhya rasayana – intellect promoting herb. No detailed study has been conducted on the anticancer activity of *S. indicus*. Therefore, the present study was carried out to explore the anticancer efficacy of *S. indicus*.

1.1 Cancer

Cancer is a condition characteristic by the uncontrolled growth and spread of abnormal cells, causing their massive aggregation producing either tumors or dispersal in the vascular system such as blood and lymph. Cancer is growing
public health concern worldwide with new incidence of six million cases every year. Cancer development depends on things such as family history (genetics), personal habits, mutation and the environment. Genetic factors by them probably account for only a small fraction of cancer. The factors such as tobacco, smoke, natural and manmade radiation, chemical agents, viral infections, food borne toxins, consumption of alcoholic beverages, polluted air, contaminated water and mutation contribute to the production of gene expression, which finally leads to the formation of cancer.

1.2 Classification of Cancer

There are five major histological classifications:

**Carcinoma**  These cancers grow from epithelial tissue, which is tissue that makes up the outer and inner lining of the body. Carcinomas account for as much as 90 percent of all cancers.

**Sarcoma**  These cancers develop in supportive and structural body tissues, like bones, muscles, tendons and cartilage.

**Myeloma**  These cancers develop in the plasma cells of the bone marrow. These are cells that produce some of the proteins that circulate in the bloodstream.

**Leukemia**  These cancers are also called liquid cancers or blood cancers. They start in the bone marrow. They cause an overproduction of white blood cells that do not reach their mature form, but they can also cause cancerous growth of red blood cells.
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**Lymphoma** These cancers originate in the organs and tissues of the lymphatic system, including lymph nodes, spleen, or tonsils. Since lymph vessels circulate all through the body, a lymphoma may develop in the lymph system of any organ, such as the stomach, breast, or brain.

1.3 **Hepatocellular Carcinoma (HCC)**

Hepatocellular carcinoma is one of the most common malignant tumors world wide, ranking fourth in annual mortality rates. An estimated 564,000 new cases of HCC are diagnosed each year, with the highest incidence in Eastern and South Eastern Asia, some of the western Pacific islands and sub-Saharan Africa. Men are affected four to eight times more often than women and the incidence generally increases with increasing age, although there is a definite shift towards a younger age distribution in black African and ethnic Chinese populations. Toxic industrial chemicals, air and water pollutants, Hepatitis B virus (HBV), Hepatitis C virus (HCV) infections, food additives and the fungal toxins produced by the fungi, *Aspergillus flavus* and *Aspergillus parasiticus* have also been linked to HCC. Chronic hepatitis and life style-induced oxidative stress are also the major factors associated with hepatic cancer.

Liver is the vital organ of metabolism and excretion. About 20,000 deaths were found every year due to liver disorders. Since liver is the major site in the body that metabolizes ingested material and therefore it is more susceptible to carcinogenic insult. Moreover, due to high tolerance of liver, HCC is seldom detected at the early stage and once detected treatment faces a poor prognosis in most cases (Jeena et al., 1999). The increase in the use of synthetic chemicals in cancer therapy had lead to many side effects and undesirable hazards at their effective dose and substantial number of tumors are resistant to
currently available drugs. The main disadvantage of anti-cancer drugs are their cytotoxicity which not only destroy the cancer cell but also all other normal cells of the body. Many a time, the mortality becomes more due to the chemotherapeutic agents than to the disease itself. It is detected in the later stages in many patients, and the current treatment modalities fail to keep the disease under control. There is no really effective treatment for hepatocellular carcinoma and so it stands high in global cause of mortality.

Many formulation containing herbal extracts are sold in the Indian market for liver disorders. Management of liver disorders by a simple and precise herbal drug is still an intriguing problem. Plants and plant-derived compounds have been found to be effective against hepatic cancer in animal models and through a few clinical studies. The antiviral and free-radical scavenging activities of the plant-derived constituents in many cases have proven to be beneficial. S. indicus, a traditional Indian medicinal plant is commonly used to nourish and improve the liver conditions. This plant has already been reported to possess strong antioxidant, hepatoprotective and immunomodulatory activity. Therefore the present investigation has been designed to investigate the effect of leaves and flower heads of S.indicus on human liver cancer cell lines in- vitro and diethylnitrosamine (DEN) induced hepatocellular carcinoma in mice in- vivo. it will be certainly helpful in chemoprevention and therapy.

1.4 Aims and Objectives

The main objectives of the present investigation is to find out a new anticancer drug from plant origin, having minimal toxicity towards normal cells but very effective against cancer cells.
1.5 **Characterization of the plant extracts**

The present investigation is undertake,

- To analyze the phytochemical compounds present in the plant extracts by qualitative and quantitative method.
- To determine Pharmacognostical characteristics of the plant material such as ash values, extractive values fluorescence analysis.
- To detect the possible bioactive compounds present in the active fraction of the plant extract by GC-MS analysis.
- To detect the specific compound present in the active fraction of the plant extract by HPTLC finger printing.

1.6 **In vitro anticancer activity**

- To observe the cytotoxicity activity of *S. indicus* plant extract on human liver cancer cell lines (HepG2) by MTT assay.
- To find out the anticancer activity of *S. indicus* plant extraction human liver cancer cell lines (HepG2) by short term tryphan blue dye exclusion method.

1.7 **In vivo anticancer activity**

- To determine acute toxicity of the plant extracts (LD50 values)
- To carryout experimental induction of cancer in mice.
- To determine biochemical parameters in control and experimental mice.
- To assess the marker enzymes such as ALT, AST, LDH, 5’ND and γGTP in serum and liver tissues.
- To estimate the antioxidant enzymes such as CAT, SOD and GPX in liver tissues.
To estimate lipid peroxidation (LPO) in liver tissues.

To estimate alpha fetoprotein (AFP) in serum and liver tissues.

To estimate ATPase enzymes ($\text{Na}^+\text{K}^+$ ATPase, $\text{Ca}^{2+}$ ATPase and $\text{Mg}^{2+}$ ATPase) in liver tissues.

To determine hematological parameters in control and experimental mice.

To estimate the level of haemoglobin in blood.

To determine total RBC in blood.

To determine total WBC in blood.

To examine the histopathology of liver sections in control and experimental mice.