Chapter 6

Summary

Cancer has become an important topic in medicine since it is a major cause of death in both the developed and developing countries and it is now only secondary to that of myocardial infarction. A great majority of human cancer (about 80 – 90%) are attributed to environmental factors. Modern surgery has significantly reduced the cancer mortality, the use of additional treatment such as radiotherapy and chemotherapy has resulted in no more than 5% reduction in the number of deaths. Since the increase in the use of synthetic chemicals in cancer therapy has lead to many side effects and undesirable hazards. Therefore there is a continuing search for better control and preventive methods in order to reduce cancer mortality and related side effects. Many investigation are now being carried out to discover naturally occurring compounds which can suppress or prevent the process of carcinogenesis. Investigation have therefore been initiated to determine the ability of the plant *Sphaeranthus indicus* methonolic and aqueous leaves and flower heads extracts on human liver cancer cell lines *in vitro* and *in vivo* DEN – induced hepatocarcinogenesis in mice.

Methanolic and aqueous extracts of *S. indicus* leaves and flowers were standardized by evaluating pharmacognostical characteristics, phytochemical analysis was carried out to detect the chemical constituents in the plant extracts, HPTLC and GC-MS profiles to find out the chemical constituents present in the bioactive fractions. A higher content of total ash, acid insoluble ash, water soluble ash were found in *S. indicus* flower than leaves. The percentage yield of water soluble extractive value of leaves and flower were found to be slightly higher than the percentage yield of alcohol soluble extractive values.
Phytochemical screening reveals that both methanolic and aqueous extracts of leaves contained higher percentage of secondary metabolites such as alkaloid, flavanoids, saponins, tannins, steroids, reducing sugars and phenolic compounds than other plant parts.

The GC-MS profile on methanolic leaves extract showed the presence of sesquiterpene lactones, sesquiterpene alcohol. The HPTLC finger printing on methanolic leaves extracts of *S. indicus* contain sesquiterpene glycoside (Sphaeranthanolide) with the $R_f$ value of 0.34. These compounds exhibit anticancer potential and it was found to be an immunostimulant.

The methanolic and aqueous extracts of *S. indicus* leaves and flower heads could exist a strong cytotoxicity on human hepatocellular carcinoma (HePG2) cells in-vitro as assessed by MTT and trypan blue exclusion assays. When comparing the cytotoxic potential of these two extracts, the methanolic extracts of leaves showed a marginally (though significantly, $p < 0.05$) higher cytotoxic activity than the aqueous extracts. The $IC_{50}$ value of methanolic and aqueous leaves extracts were found to be 15 µg/ml.

The therapeutic value of any drug depends not only on its clinical efficacy, but also in its lack of toxic side effects. The plant extract does not produce any significant toxic effects it is the relatively safe to use for therapeutic purposes.

Diethylnitrosamine (DEN) is a major environmental carcinogen suggested to increase the generation of reactive oxygen species (ROS) resulting in oxidative stress and cellular injury. Since liver is the main site of DEN
metabolism, the production of ROS in the liver may be responsible for its carcinogenic effects.

In this study, injection of DEN to mice leads to a marked elevation in the levels of serum AST, ALT and ALP which is indicative of hepatocellular damage, as previously reported. Serum AST, ALT and ALP are biomarkers in the diagnosis of hepatic damage because they are released into the circulation after cellular damages. Due to its ability to reduce free radical-induced oxidative damage in the liver, the methanolic leaves extract has been shown to decrease liver enzymes in serum prevent liver damage.

In the present study, there was significant increase in the level of lipid peroxidation in the liver of mice treated with DEN. The observed reduction in the level of lipid peroxidation in plant extracts mice was presumably due to its ability to scavenge the hydroxyl and peroxide radicals.

It has been reported that administration of plant extract significantly decreased lipid peroxidation and increased endogeneous antioxidant enzymes, such as SOD, CAT and GPX in liver tissues. A significant decrease in the activities of glutathione metabolizing enzymes. These altered parameters were found to be normal in methanolic leaves extract treated mice.

The membrane bond ATPase in liver of DEN induced mice was found to be decreased. All these altered parameters were found to be normal in plant extracts treated mice.

The concentration of α-fetoprotein (AFP) is often be extremely increased in DEN induced mice. In the present study, the observed increase in the
concentration of AFP in hepatoma bearing mice were brought back to near normal in methanolic and aqueous leaves extracts treated mice. This clearly indicates the anticarcinomic effect of plant extracts.

Histopathological observation were found to support the findings of serum tumour markers analysis. The mice in control groups showed normal histological liver architecture having cells with granulated cytoplasm and small uniform nuclei. On the other hand, the DEN induced group showed significant loss of lower architecture, whereas in the group treated with methanolic and aqueous leaves extract showed normal hepatocyte architecture with well defined aggregation of hepatic and portal veins. The potential beneficial effect of the plant extracts on the liver tissue were also visible evident from histological findings.

Histological examination also showed substantial improvement in the overall tissue architecture in the liver of plant extracts treated mice. Results obtained in the present study would be useful for the preparation of a standardized pharmaceutical product that may be used in the future for clinical therapy of hepatocellular carcinoma.