Chapter 7

Summary and Conclusion
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PART I

In the present study we evaluated the Pharmacological properties of *H. adakodien* root extract. This includes antioxidant, anti-inflammatory and protective effects of *H. adakodien* root extract. The anticancer properties and the mechanism of anticancer properties were also studied.

The 70% methanolic extract of *H. adakodien* could inhibit the generation of superoxide radical, Hydroxyl radical, Nitric oxide radical and lipid peroxidation *in vitro*. (Table 3.1, 3.2, 3.3, 3.4). The role of oxygen derived free radicals, such as hydroxyl radical and super oxide radical, in the inflammatory process is well known. It is also generally assumed that most of the antioxidants possess anti inflammatory effect. The 70% methanolic extract of *H. adakodien* effectively inhibit the inflammatory effect induced by formalin and carrageanen. (Table 3.5, 3.6).

The hepatic damage caused by CCl₄ is due to the lipid peroxidation in the microsomal membrane. There is abundant evidence that a significant amount of lipid peroxidation taken place in the endoplasmic reticules of the liver following exposure to CCl₄. Antioxidants are well known for their capacity to inhibit lipid peroxidation in biological membranes initiated by a variety of means (chemically, enzymatically and photo chemically). The protective effect of antioxidants on the hepatotoxic effects of CCl₄ could be a consequence of their inhibition of lipid peroxidation associated with the metabolism of the halocarbon.

The biochemical parameters such as GOT, GPT, ALP, total protein and Albumin both in serum and Liver were carried out to find out the hepatoprotective activity of the extract. The levels will be higher in Liver damaged animals. In the present study the levels of GOT, GPT, ALP. (Table 3.7, 3.8, 3.9). Total protein and Albumin were found coming
closer to the normal level as compared to the control group in a dose dependent manner. The histopathological studies also confirmed the ability of the extract against induced hepatic damage. The liver section of control animals shows cellular degeneration, hydropic changes more around the central veins, fatty changes wide spread hepatocellular receives etc. all these symptoms were found to be minimal in treated groups animals.

The use of chemotherapeutic drugs (Cyclophosphamide) cause side effects such as myelo suppression, hepatotoxicity, nephrotoxicity etc. Administration of 70% methanolic extract of *H. adakodien* (250 mg/kg body wt and 500 mg/kg body wt) enhanced the total count and haemoglobin level in a dose dependent manner. In treated groups the level of GSH, GPX and SOD were increased and came closer to normal level and the decrease in lipid peroxidation was also detected. The present result confirmed that the *H. adakodien* root extract can be used as a chemoprotective agent. (Table 3.11, 3.12, 3.13).

The plant derived extracts containing antioxidant principles showed cytotoxicity towards tumour cells and anti tumor activity in experimental animals. Hence we decided to evaluate cytotoxic effect of *H. adakodien* in tumor cells. The crude extract as well as different fractions were analysed for the preliminary cytotoxic test, and it was found that the acetone fraction of *H. adakodien* have the maximum cytotoxicity (515μg/ml). (table 4.1).

Hence the acetone fraction was used for cytotoxicity as well as anti tumor studies. The extract found to inhibit the proliferation of DLA and EAC cells at 5 hr. Culture. Oral administration of the acetone fraction shown anti tumor activity in DLA experiment. Oral administration was also found to reduce the ascites tumor in mice and in EAC experiment. Cytotoxic evaluation of *H. adakodien* was done using MDA MB 231 and MCF-7 at 48hrs through MTT assay. For 50% growth inhibition 150μg/ml *H. adakodien* AF was required for both MDA MB 231 and MCF-7 cells (Fig. 5.3).
It is well known that the antitumor activity of the antioxidants is possibly through induction of apoptosis. Even though the acetone fraction of *H. adakodien* root extract induces cell death, there are no studies on the mechanism of cell death induced by the *H. adakodien* root extract so we studied the cell death mechanism in human breast cancer cells. It was found that *H. adakodien* root extract effectively inhibit the cell proliferation of human breast cancer cells. (MDA MB 231 and MCF – 7).

We have also investigated whether the cell death induced by *H. adakodien* fraction occurs through apoptosis. The results indicate that the *H. adakodien* root extract acetone fraction induced inhibition of cell growth involves apoptotic cell death.(Fig.5.1, 5.2, 5.4). There fore our results suggest that the induced cell death leads to the anti tumor effect and it is through the induction of apoptosis.

The preliminary screening of crude extract as well as the acetone fraction of *H. adakodien* root extract shows the presence of flavanoids. There is much evidence that flavanoids have important effects on various biological properties including protective effects through several mechanism such as antioxidant effects. Further investigations are necessary for the isolation of active principle and to elucidate the mechanism of action.
PART II

Natural products have played a significant role in drug discovery. Successful new and innovative approaches are required for the scaling up of synthesis of these compounds by cultured cell. Many technological advances have been shown to produce higher amounts of the products than the intact plants from which they are derived.

In the present study we evaluated the enhanced production of Camptothecin by different biological elicitors like chitin, chitosan and a fungus sacharromyces cervicæae. Chitin and chitosan enhanced the production of CPT at 5ml/l concentration. But 10ml/l concentration they inhibit the growth of the cultures thereby decreased the amount of Camptothecin. S.cervicæae gave maximum CPT production at 10ml/l.

The time of application of elicitor is crucial for the yield of secondary metabolites. In the present study with chitin, chitosan and S.cervicæae the yield of CPT decreased at the 7th day cultures.

Permeabilization allow cultures for the controlled release of products in to the medium. Permeabilization with DMSO at different concentration did not allow any significant release of CPT in to medium. But the application increased the CPT production to 0.086 % at 24th hr.