CHAPTER VII

Summary and Conclusion
Dietary factors are known to influence several biological processes. They also contain several biologically active components which could inhibit the action of toxic chemicals including chemical carcinogens (1, 5). In addition to the nutritional factors, non-nutrients in the diet such as, indoles, aromatic isothiocyanates, flavones etc. have also been, attributed these protective properties. Most of these are either antioxidants or stimulate the activity of detoxifying enzymes in the host (5, 6).

In the present work, certain food additives were studied for their action on the adverse effects caused by certain xenobiotics. Food additives used are turmeric (Curcuma longa), garlic, (Allium sativum), asafoetida (Ferula asafoetida), curcumin (diferuloyl methane), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and ellagic acid.

The first part of the work included the studies on aflatoxin B₁-induced toxicity in animals and the inhibitory effect of food additives on its toxic action. Aflatoxin B₁ (AFB₁), a fungal metabolite,
naturally occur as a food contaminant and has been shown to be carcinogenic in several animal species and in human beings (67, 69, 91, 92). Using Ames test all the food additives studied were found to be protective against the mutagenicity of AFB₁ towards Salmonella typhimurium tester strains TA 98 and TA 100. These food additives were also found to inhibit the production of aflatoxin by Aspergillus parasiticus, when they were added to the medium containing the fungus. Among these food additives, ellagic acid, BHA and aqueous extract of garlic inhibited the growth of the fungus at a concentration of 10 mg/ml.

The dietary intake of different food additives during the administration of AFB₁ was found to reduce the hepatotoxicity induced by this toxin in ducklings as seen from the pathological and biochemical analysis. Pathological observations of the liver of ducklings treated with the food additives showed that fatty changes, necrosis and degeneration of the hepatocytes were very mild, as compared to the control group. Turmeric and its active ingredient curcumin were found to be the
most effective. Both of them produced almost complete inhibition of all the toxicities including the biliary hyperplasia, which was found to be the most severe manifestation of aflatoxin induced liver injury in ducklings.

The effect of food additives on the carcinogenic potential of AFB1 was studied in rats. Dietary administration of turmeric, garlic, ellagic acid and curcumin significantly inhibited the development of hepatocellular altered foci which were quantified using GGT as a marker. Turmeric and curcumin were found to be more effective compared to the other food additives. Since the altered foci are considered as the precursors of hepatocellular neoplasm, the inhibitory effect of food additives on this initial stage of carcinogenesis suggest their role as chemopreventive agents against AFB1-induced carcinogenesis.

Curcumin, ellagic acid, BHA and BHT were shown to inhibit the action of lipoxygenase on linoleic acid. Lipoxygenase, one of the important enzymes in the arachidonic acid metabolism, is found to be
enhanced during chronic inflammatory response. The tissue injury produced during the inflammatory reaction is thought to be mediated by the reactive oxygen species produced during the arachidonic acid metabolism (156). Curcumin, ellagic acid, BHA and BHT were also found to inhibit the production of superoxide radical and lipid peroxides in vitro, in a dose-dependent manner. The inhibition produced by these food additives on lipoygenase as well as on the free radical formation suggest their possible protective action towards the tissue injury produced during chronic inflammatory response.

Curcumin, the active ingredient in turmeric, was further studied for its effect on the fibrosis induced by certain xenobiotics including carbon tetrachloride (CCl₄), paraquat and ethanol as well as on cotton pellet induced granuloma formation. CCl₄, paraquat and ethanol are known to produce free radicals in vivo which cause tissue damages mainly through the peroxidation of membrane lipids. The extent of fibrosis formed was measured both by histological examination and by estimating
the hydroxyproline content in the fibrotic tissues, which is a measure of collagen accumulation. The administration of curcumin and ellagic acid significantly inhibited the cotton pellet induced granuloma formation in rats, as seen from the decrease in the weight of granuloma and the hydroxyproline content.

Administration of curcumin significantly inhibited the fibrosis induced by CCl₄ in rats. The histological examination of the liver from the animals treated with curcumin showed a less degree of fibrosis compared to that of the cirrhotic control animals.

Serum lipid peroxide level was significantly also low in the animals treated with curcumin than in the animals, treated with carbon tetrachloride alone.

Similarly, curcumin was also found to inhibit the fibrosis induced by paraquat and ethanol in rats. The amount of hydroxyproline and lipid peroxide were low in the curcumin treated animals, compared to that of the control animals in these conditions.
Studies were also conducted to determine the effect of curcumin in modifying the lipid profile. Curcumin produced an inhibitory effect on cholesterol synthesis by L929 lung fibroblast cells in vitro. The incorporation of $^{14}$C acetate into cholesterol by these cells were inhibited by curcumin in a dose-dependent manner. Dietary administration of curcumin significantly reduced the total cholesterol levels in hypercholesterolemic rats. At the same time HDL-cholesterol level was elevated by the administration of curcumin. Similar effects were also observed in a group of healthy human volunteers who received 500 mg of curcumin for a period of one week. An increase in the HDL-cholesterol by curcumin suggests its role in the extrahepatic mobilisation of cholesterol, thereby enhancing its excretion.

In conclusion, the food additives used in the present study, including some spices and antioxidants, were found to have protective effect against the toxicities induced by certain xenobiotics. Since human population is exposed to
a variety of environmental toxicants in day to day life, it is important to develop a preventive strategy against the adverse effects of these toxicants. Dietary modification has been considered as a powerful and cost-effective method to achieve this goal. Most of the dietary materials are non-toxic and inclusion of these food materials in our daily diet, can considerably reduce the expression of toxicity by the xenobiotics.

Although the exact mechanism of action of these food additives is unclear, most of their action may be attributed to their antioxidant properties.