SUMMARY

Natural products have been the source of many of the active ingredients of medicines. Numerous reports suggest a protective role for a diet rich in fruits and vegetables. One of the areas where plants and their constituents have had a major impact on longevity and quality of life is in the chemoprevention of cancer. Indeed, epidemiological studies have suggested that human consumption of fruits and vegetables is associated with a reduced risk of cardiovascular disease and certain types of cancers. Cancer development is a dynamic and long term process which involves many complex factors through critical steps of initiation, promotion and progression, leading to an uncontrolled growth of cancerous cells throughout the body. It is believed that dietary constituents derived from plant sources have the ability to modify the process of carcinogenesis thus relating the food stuffs, beyond their basic nutritional benefits, to disease prevention.

There are many nutritive and non-nutritive plants and natural compounds from other sources currently under investigation for their potential cancer chemopreventive effects. These include ellagic acid, some triterpenes (such as lupeol, betulinic acid, ginsenosides, oleanolic acid, etc), and ginkolide B. Various other polyphenolic antioxidants such as flavonoids, tannins, curcuminoids, galocatechins, stilbenes and anthocyanidins have also been implicated as chemopreventive agents. However, the mechanism by which these compounds inhibit proliferation and induce apoptosis in cancer cells has been the subject of considerable interest. Studies in this laboratory have centered on the anticancer mechanism of plant derived polyphenolic antioxidants. It has been shown that these antioxidants can act as prooxidants in the presence of copper ions catalyzing prooxidant DNA breakage through the generation of reactive oxygen species (ROS). Copper is an important metal ion present in chromatin, closely associated with DNA bases particularly guanine and can be mobilized by metal chelating agents. It is one of the most redox active of the various metal ions present in cells. Several reports in the literature have shown that both serum and tumor copper
levels in cancer patients are significantly elevated. Therefore, cancer cells may be more subject to electron transfer between copper ions and polyphenols than normal cells to generate ROS, leading to oxidative injuries beyond the reversible threshold.

The chemopreventive properties of gossypol and thymoquinone are well documented. However, the primary mechanism of action of these compounds has not been elucidated. In order to explore the chemopreventive activity of gossypol, in Chapter I, I have attempted to elucidate the mechanism of action of gossypol and compared it with the activity of its semi-synthetic derivative apogossypolone (ApoG2). Using fluorescence and absorption studies, it has been shown that both these compounds are able to bind to DNA. Further, both the compounds are able to bind to copper as well as reduce it. Gossypol and ApoG2 are capable of causing DNA cleavage in pBR322 plasmid DNA and Calf Thymus DNA in the presence of copper ions. Further, using alkaline single cell gel electrophoresis (comet assay) I have shown that both these compounds lead to cellular DNA breakage in lymphocytes through the same mechanism which involves the mobilization of endogenous copper ions. Moreover, it was seen that ApoG2 induced a greater degree of DNA breakage in lymphocytes as compared to gossypol, possibly due to greater permeability and greater hydroxyl radical generation.

In Chapter II, using fluorescence and absorption studies I have shown that thymoquinone is able to bind to DNA as well as copper. Further it was seen that thymoquinone also reduces copper. Using comet assay I have shown that thymoquinone is capable of causing copper mediated DNA breakage in lymphocytes. Further, using the lysed version of comet assay I have demonstrated that the DNA breakage induced by thymoquinone is inhibited by copper specific chelators neocuproine and bathocuproine (but not by compounds that bind iron or zinc). Using scavengers of reactive oxygen species, the study also shows that the cellular DNA breakage occurs through an oxidative process involving reactive oxygen species which act as proximal cleaving agents.
In chapter III, using MTT assay I have shown that these antioxidants gossypol, ApoG2 and thymoquinone cause cell death in human cancer cell lines and that such cell death is prevented to a significant extent by copper chelator neocuproine. Moreover, normal breast epithelial MCF-10A cells are refractory to such an inhibition. Further, I have shown that the supplementation of culture media of MCF-10A cells with copper leads to the upregulation of copper transporter, hCtr1 (as detected by Western Blot), indicating an increase in the uptake of copper in such cells. Increase in sensitivity of MCF-10A-Cu cells to the antioxidant-induced inhibition of cell proliferation provided further confirmation that the cytotoxic and anticancer activity gossypol, ApoG2 and thymoquinone is mediated by copper. Based on the work presented in this thesis, I would like to conclude that mobilization of nuclear copper by plant antioxidants and the consequent prooxidant action could be one of the important mechanisms for their anticancer and chemopreventive properties. Indeed such a common mechanism would better explain the anticancer effects of polyphenols with diverse chemical structures as also the preferential cytotoxicity towards cancer cells. Further they may also act as lead compounds for the synthesis and development of novel anticancer drugs with better copper chelating and ROS generating potential.