Summary
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. Consistent with this observation are the epidemiological findings associating high soy consumption with lower incidences of breast, prostate and colon cancer in Asian countries particularly in Japan. It has been suggested that the isoflavone constituents provide at least part of the protective effect of soy food. Genistein, a predominant isoflavone present in soy has been shown to have potent anticancer properties both in vitro and in vivo. The 4'-O- methyl derivative of genistein, biochanin A which is a major isoflavone constituent in red clover (Trifolium pretense) has also been reported to possess cytotoxic properties against cancer cells. Various other polyphenolic compounds such as flavonoids, tannins, curcuminoids, gallocatechins, stilbenes and anthocyanidins have 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. In recent years several reports have documented that plant polyphenolics including genistein (from soybean) induce apoptosis in various cancer cell lines. Of particular interest is the observation that a number of these polyphenols including EGCG, gallic, resveratrol and genistein induce apoptotic cell death in various cell lines but not in normal cells. Most of the pharmacological properties of plant polyphenols are considered to reflect their ability to scavenge endogenously generated oxygen radicals or those free radicals formed by xenobiotics, radiation etc. However, some data in the literature suggests that antioxidant properties of the polyphenolic compounds may not fully account for their chemopreventive effects. Most of the plant polyphenols possess both antioxidant as well as
prooxidant properties and we have proposed that the endogenous copper dependent prooxidant cytotoxic action of polyphenolics rather than antioxidant effect may be an important mechanism of their anticancer and apoptosis inducing properties. 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 reactive oxygen species, leading to oxidative injuries beyond the reversible threshold.

The chemopreventive properties of genistein are well documented. In order to explore the chemical basis of the chemopreventive activity of genistein, in this thesis I have attempted to elucidate the mechanism of action of isoflavone genistein and also studied the structure-activity relationship between genistein and its methylated structural analog biochanin A. In chapter I, using fluorescence and absorption studies it has been shown that both the isoflavones are able to bind as well reduce copper ions. Further, they are able to bind to DNA as well. Isoflavones are also capable of degrading supercoiled plasmid pBR322, calf thymus and cellular DNA in the presence of copper ions. These results suggest that Isoflavone-Cu (II) system for DNA breakage is physiologically feasible and could be of biological significance.

In chapter II, using a cellular system of lymphocytes isolated from human peripheral blood and alkaline single cell gel electrophoresis (Comet Assay). I have confirmed that isoflavone genistein and biochanin A are capable of mobilizing endogenous copper ions from lymphocytes, which in turn leads to the degradation of cellular DNA. Further using lysed version of Comet assay it has been demonstrated that the DNA breakage induced by isoflavones involves nuclear copper as such DNA degradation is inhibited by copper chelaters (neocuproine / bathocuproine) but not by compounds that specifically bind iron.
and zinc (desferrioxamine mesylate and histidine respectively). 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 a permeabilized cellular system, the relative DNA breakage efficiencies of genistein and and its methylated structural analogue biochanin A has been compared with their relative antioxidant potential. It is shown that both genistein and its methylated derivative biochanin A are able to mobilize nuclear copper in permeabilized cellular system leading to degradation of cellular DNA. However the relative rate of DNA breakage was greater in the case of genistein. The antioxidant activity of the two isoflavones against tert-butylhydroperoxide (TBHP) induced oxidative breakage in lymphocytes demonstrated genistein to be more effective than biochanin A in providing protection against oxidative stress induced by TBHP. It would therefore appear that the structural features of isoflavones that are important for antioxidant properties are also the ones that contribute to their prooxidant action through a mechanism that involves redox cycling of chromatin-bound nuclear copper.

In chapter IV, genistein has been shown to cause cell death in human breast cancer cells and that such cell death is prevented to a significant extent by copper chelator neocuproine. Based on the work presented in this thesis, I would like to conclude that mobilization of nuclear copper by plant polyphenols 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.