SUMMARY

Plant-derived polyphenolic compounds such as flavonoids, tannins, curcumin and the stilbene resveratrol possess a wide range of pharmacological properties, the mechanisms of which have been the subject of considerable interest. They are recognized as naturally occurring antioxidants and have been implicated as anticancer compounds. In recent years, several reports have documented that plant polyphenolics, including curcumin, resveratrol and gallocatechins such as gallic acid, epigallocatechin, epicatechin-3-gallate, and epigallocatechin-3-gallate (EGCG) induce apoptosis in various cancer cell lines. Gallocatechins are constituents of green tea, the consumption of which is considered to reduce the risk of various cancers such as those of bladder, prostate, oesphagus and stomach. Resveratrol is present in human dietary material such as peanuts, grapes, mulberries and beverages such as red wine. Of particular interest is the observation that a number of these polyphenols including epigallocatechin-3-gallate, gallic acid and resveratrol induce apoptotic cell death in various cancer cell lines but not in normal cells.

Studies in this laboratory have shown that flavonoids, tannic acid and its structural constituent gallic acid, curcumin, gallocatechins and resveratrol cause oxidative strand breakage in DNA either alone or in the presence of transition metal ions such as copper. Copper is an important metal ion present in chromatin and is closely associated with DNA bases particularly guanine. It is also one of the most redox active of the various metal ions present in 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 various xenobiotics, radiation etc. However, some data in literature suggests that antioxidant properties of the polyphenolic compounds may not fully account for their anticancer effects. Most of the plant polyphenols possess both antioxidant as well as prooxidant properties and reports from this laboratory have earlier proposed that the prooxidant action of polyphenolics may be an important mechanism of their anticancer and apoptosis inducing properties. Such a mechanism for the cytotoxic action of these compounds against cancer cells would involve mobilization of endogenous copper ions and the consequent prooxidant action.
As mentioned above resveratrol, (3,4',5-trihydroxy stilbene) a polyphenol belonging to the class of compounds known as stilbenes is considered to possess chemopreventive properties against cancer. It is recognized as a naturally occurring antioxidant but also catalyzes oxidative DNA degradation in vitro in the presence of transition metal ions such as copper. In view of this I have examined the oxidative DNA cleavage mechanism of resveratrol by comparing its properties with its structural analog piceatannol (3,3',4,5- tetrahydroxy stilbene) as well as the parent compound trans-stilbene. Using fluorescent and absorption studies I have shown that both resveratrol as well as piceatannol are able to bind as well as reduce copper ions. Further, these polyphenols are also able to bind to DNA. Both resveratrol as well as piceatannol were able to degrade calf thymus and supercoiled plasmid pBR322 DNA in the presence of copper ions. More significantly the rate of DNA breakage correlates with the efficiency of Cu(II) reduction and the rate of formations of hydroxyl radicals. Trans-stilbene which does not have any hydroxyl groups does not reduce Cu(II) and is also not a DNA cleaving agent. These results suggest that the number and position of hydroxyl groups on the stilbene molecule is important for the DNA cleavage efficiency.

In the second chapter of the thesis I have explored whether the resveratrol-Cu(II) system is capable of causing DNA degradation in cells such as lymphocytes. Using a cellular system of lymphocytes isolated from human peripheral blood and Alkaline single cell gel electrophoresis (Comet assay), I have confirmed that resveratrol-Cu(II) system is indeed capable of causing DNA degradation in cells such as lymphocytes. Also, trans-stilbene, which does not have any hydroxyl groups, is inactive in the lymphocyte system. Preincubation of lymphocytes with resveratrol indicates that it is capable of either traversing the cell membrane or binding to it. These results are in partial support of the hypothesis that anticancer properties of various plant derived polyphenols may involve mobilization of endogenous copper and the consequent prooxidant action.

In the third and final chapter of this thesis I have shown that a number of polyphenols with diverse chemical structures are capable of inducing DNA breakage in lymphocytes in the absence of added copper ions. Incubation of lymphocytes with neocuproine inhibited the DNA degradation confirming that Cu(I) is an intermediate in the DNA
cleavage reaction. Further, I have also shown that polyphenols induce generation of hydroxyl radicals in lymphocytes and neocuproine and hydroxyl radical quenchers inhibit such radical formation. These results are in further support of the hypothesis that anticancer mechanism of plant polyphenols involves mobilization of endogenous copper, possibly chromatin bound copper, and the consequent prooxidant action.

Some evidence suggests that polyphenolic compounds such as tannins and resveratrol are able to traverse cell membranes and may enter the cytoplasmic or nuclear space. Resveratrol is sufficiently hydrophobic and has been shown to be present in such tissues as heart, liver and kidney. The ability of gallotannins to enter the cell is indicated by the observation that tannic acid prevents formation of the benz-(a)-pyrene-DNA adduct by inhibiting the binding of the ultimate carcinogen to target tissue DNA rather than by altering the metabolism of benz(a)-pyrene. The question of bioavailability of polyphenols in mammalian system also needs to be addressed. Some relatively recent work with resveratrol indicates that it may have a relatively low bioavailability due to its biotransformation and rapid elimination. For example it has been reported that in the case of rabbits the half-life of resveratrol in plasma after i.v administration of 20 mg/kg b.w was about 14 minutes and the highest concentration of resveratrol in plasma was reached within the first five minutes (2.6 ± 1 μM) after receiving 20 mg res/kg.b.w orally. Nevertheless these authors further report that 5 μM resveratrol completely inhibited the growth of B-16 M murine melanoma cells. In this context we may mention that the minimum concentration of resveratrol tested by us in the presence of added copper ions for DNA breakage in lymphocytes was 10 μM. However, as mentioned the minimum concentration of resveratrol required for DNA breakage in lymphocytes is between 100-200μM. Because of higher intracellular copper levels it may be predicted that such concentrations of resveratrol for cytotoxic action against cancer cells would be considerably lower. Indeed it has been shown that ascorbate which also acts as a prooxidant in the presence of copper ions is cytotoxic to a leukemic cell line at a lower concentration than normal lymphocytes. Most studies on anticancer mechanisms of plant polyphenols invoke the induction of cell cycle arrest at the S/G2 phase transition brought about by an increase in cyclins A and E and inactivation of cdc 2. Other mechanisms
have also been proposed. Based on the work presented in this thesis I we would like to propose that mobilization of endogenous copper ions by 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.