Summary

Plant-derived polyphenolic compounds such as flavonoids, tannins, curcuminoi
dals, stilbenes and the hydroxycinnamate phenolic acids (caffeic acid and p-
coumaric acid) 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 also been implicated as anticancer compounds. In recent years, several reports have documented that plant polyphenolics including curcumin, caffeic acid, 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 known to reduce the risk of various cancers such as those of bladder, prostate, esophagus and stomach. Caffeic acid is found in fruits, vegetables, grains and coffee is a major source. Of particular interest is the observation that a number of these polyphenols including epigallocatechin-3-gallate, gallic acid and resveratrol induce apoptosis 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 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.

Caffeic acid is a polyphenol possessing a catecholic dihydroxy moiety and it belongs to a class of compounds known as hydroxycinnamates. As mentioned in "Introduction" of the present study caffeic acid possesses chemopreventive properties against cancer. It is recognized as a naturally occurring antioxidant but also catalyses oxidative DNA degradation \textit{in vitro} in the presence of transition metal ions such as copper. In view of this in the first chapter of the thesis I have compared the oxidative DNA cleavage mechanism of caffeic acid with its structural analogue $p$-coumaric acid which is also its parent compound and also to $o$-coumaric acid. All three are able to cleave calf-thymus DNA in the presence of copper ions. However, among all the three phenols only caffeic acid could cleave supercoiled plasmid pBR322 DNA in the presence of copper ions at the concentrations tested. More significantly the rate of DNA breakage correlates with the efficiency of Cu(II) reduction and the rate of formation of hydroxyl radicals. $P$-coumaric acid and $o$-coumaric acid cleave DNA but less efficiently as compared to caffeic acid suggesting that the number and position of hydroxyl groups on the cinnamate molecule are important factors in determining the DNA cleavage efficiency. In fluorescence and absorption studies it is shown that all the three phenols are able to bind as well as reduce copper ions. Further these phenols are also able to bind DNA.

In the second chapter of the thesis an attempt has been made to explore whether the caffeic acid-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), it was confirmed that caffeic acid-Cu(II) system is indeed capable of causing DNA breakage in cells such as lymphocytes. Also, $p$-coumaric acid possessing a single hydroxyl group is less efficient in this system. Preincubation of lymphocytes with caffeic acid 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 the thesis it is shown that a number of polyphenols with diverse chemical structures including caffeic acid are capable of inducing DNA breakage in lymphocytes in the absence of added copper ions. Incubation of lymphocytes with neocuproine inhibited the DNA breakage confirming that Cu(I) is an intermediate in the DNA cleavage pathway. Further, it is 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 may involve mobilization of endogenous copper possibly chromatin bound copper.

The question of bioavailability of polyphenols also needs to be addressed. Some evidence suggests that polyphenolic compounds such as tannins, resveratrol and caffeic acid are able to traverse cell membranes and may enter the cytoplasmic space. Caffeic acid is sufficiently hydrophobic and has been shown to be present in human plasma. The ability of gallotannins to enter the cell is indicated by the observation that tannic acid prevents formation of 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. It has been reported that 0.8% caffeic acid in the diet is associated with plasma concentrations of up to about 5.52 micromolars. Dietary caffeic acid is readily absorbed by humans and rat where it circulates in plasma at micromolar concentrations. Oral administration of caffeic acid has been reported to inhibit subcutaneous tumor growth in mice. In a relatively recent work caffeic acid has been shown to inhibit hepatoma growth and metastasis achieving complete regression. In this context it should be noted that in the present study the minimum concentration of caffeic acid tested in the presence of copper ions for DNA breakage in lymphocytes is 10 μM. However the minimum concentration of caffeic acid alone required for DNA breakage in lymphocytes is between 200-400 μM. Because of higher intracellular copper levels in cancer cells it may be predicted that such concentrations of caffeic acid required for the
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cytotoxic action against such 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 cdc2. Other mechanisms have also been proposed. Based on the work presented in this thesis it is proposed that mobilization of endogenous copper ions 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 the polyphenols with diverse chemical structures as also the preferential cytotoxicity towards cancer cells.