Scope of the work presented.
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As mentioned in the previous section, there is evidence in literature suggesting that antioxidant activity of such plant derived polyphenols may not fully account for their chemopreventive effects. Therefore, it is likely that other mechanisms may be responsible for the varied pharmacological properties. Most antioxidants of plant origin are redox (reduction-oxidation) agents, protecting against ROS in some cases and promoting radical generation in others (Herbert, 1996). Studies in this laboratory have shown that plant polyphenols behave as prooxidants in the presence of copper ions catalyzing DNA breakage through the generation of ROS (Ahmad et al., 1992; Bhat & Hadi, 1994; Ahsan & Hadi, 1998; Ahmad et al., 2000a; Azam et al., 2004; Ahmad et al., 2005). Copper is a major metal ion present in the nucleus and is also implicated in tumorigenesis and angiogenesis (Chevion, 1988). Oxidative DNA breakage by these compounds correlates with their apoptosis inducing capacity. Further, properties of polyphenols, such as binding and cleavage of DNA and the generation of ROS in the presence of transition metal ions are similar to those of some known anticancer drugs (Ehrenfeld et al., 1987). It is also known that serum, tissue and cellular copper levels are significantly elevated in a number of malignancies (Linder, 1991; Gupte and Mumper, 2008). Induction of apoptosis by several polyphenols has been shown in various human cancer cell lines and animal models (Lee et al., 2002; Sarkar and Li, 2003; Whitsett et al., 2006; Moiseeva et al., 2007; Noda et al., 2007; Scarlatti et al., 2007). Interestingly, some studies have indicated that apoptosis induction by polyphenols and other anticancer agents is independent of caspases and mitochondria (Piwocka et al., 1999; Leist and Jaattela, 2001) and is accompanied by an increase in the intracellular levels of ROS (Yoshino et al., 2004; Heiss et al., 2007; Noda et al., 2007).
Thus, there is significant data in literature that points to the prooxidant rather than the antioxidant property of polyphenols as the mechanism of their anticancer properties. Taking into consideration our own observations and those of others this laboratory has proposed a mechanism according to which plant polyphenols mobilize endogenous copper in cancer cells leading to cytotoxic action through the generation of ROS (Hadi et al., 2000; Hadi et al., 2007). In confirmation of this hypothesis, the following milestones have been achieved, (i) an in vitro reaction between polyphenols, Cu(II) and DNA leading to DNA cleavage has been characterised (Ahmad et al., 2000a; Azam et al., 2004); (ii) as a further step it has been shown that polyphenol-Cu(II) system is indeed capable of causing DNA degradation in a cellular system (Azmi et al., 2005); (iii) it has also been shown that polyphenols are capable of mobilizing endogenous copper ions from cells leading to cellular DNA breakage (Azmi et al. 2006); (iv) further, in the above oxidative cellular DNA breakage nuclear copper has been demonstrated to be mobilized (Shamim et al. 2008); (v) recently, isoflavone genistein induced growth inhibition in breast cancer cell lines has been shown to be inhibited by copper chelator to a significant extent whereas iron and zinc chelators proved to be relatively ineffective (Ullah et al. 2011).

Based on the above hypothesis, in the work presented here, I have attempted to further elucidate and confirm the mechanism of action of plant derived polyphenolic compounds which may be responsible for their anticancer effects. The studies have been carried out using several different types of polyphenols such as the flavones apigenin, luteolin and chrysin; the catechin EGCG; the isoflavone genistein; and the stilbene resveratrol. The work has been divided into three chapters. In the first chapter, absorption and fluorescence studies have been employed to explore the DNA binding and copper reducing abilities of the flavones - apigenin, luteolin and chrysin. Further, using single cell gel electrophoresis (comet assay), the ability of these
flavones to induce DNA breakage in intact as well as permeabilized cells has also been tested. The second chapter deals with studies done on rats by orally administering them with copper and measuring the copper levels in the isolated lymphocytes of such rats. Subsequently, isolated rat lymphocytes have been subjected to treatment with polyphenols in vitro and cellular DNA breakage has been analyzed (Khan et al., 2011). Finally, in the third chapter, the effect of different metal specific sequestering agents and the scavengers of ROS such as SOD, catalase and thiourea on the polyphenol-induced antiproliferation and apoptotic cell death in breast, prostate and pancreatic cancer cell lines has been studied.