Scope of Work
SCOPE OF THE WORK PRESENTED

In recent years a number of reports have documented the chemopreventive effect of green tea consumption on various types of cancers such as those of bladder, prostate, esophagus and stomach (Wei et al., 1999). However, such chemopreventive effect is not attributed to the consumption of black tea to the same extent. Gallotannins such as tannic acid, gallic acid, EGC, ECG and EGCG induce apoptosis in various cancer cell lines (Inoue et al., 1994; Ahmad et al., 1997). Of particular interest is the observation that EGCG (a polyphenol constituent of green tea) and gallic acid induce apoptosis in cancer cell lines but not in normal cells (Inoue et al., 1994; Ahmad et al., 1997). This property is also shared by a number of other plant derived polyphenolic compounds such as curcumin and resveratrol (Clement et al., 1998; Piwocka et al., 1999).

Various pharmacological properties of plants derived polyphenolic compounds are considered to be the effect of their antioxidant action. However, as mentioned above studies in our laboratory have confirmed that these compounds are themselves capable of oxidative DNA damage, particularly in the presence of transition metal ions (Khan and Hadi, 1998). Several lines of evidence in the literature strongly suggest that it is the prooxidant action of plant derived polyphenolics rather than their antioxidant activity that may be an important mechanism for their anticancer and apoptosis.
inducing properties (Piwocka et al., 1999; Mukhtar et al., 1988; Kane et al., 1993). Thus a mechanism for the cytotoxic action of polyphenols against cancer cells that involves mobilization of endogenous copper and the consequent prooxidant action has been proposed (Hadi et al., 2000). In partial support of this idea, in part I of the thesis, I have shown that the water extract of green tea is considerably more efficient than black tea extract in DNA cleavage in the presence of copper ions. Green tea extract also shows a higher rate of Cu (II) reduction and consequent hydroxyl radical production. Further, I have examined the biological activity of the reaction and have shown that green tea–Cu(II) mediated reaction cause inactivation of bacteriophage T₄ through a mechanism similar to DNA breakage.

Gallic acid, a naturally occurring plant phenol with antioxidative properties, was found to induce cell death in promyelocytic leukemia HL-60RG cells. Morphological and biochemical studies indicated that the gallic acid induced cell death is apoptosis. Structure activity analysis suggests that gallic acid induced apoptosis in HL-60RG cells depends on its distinctive feature derived from the structure but not on its antioxidative activity. Gallic acid shows selective cytotoxicity to tumor cells compared with normal cells (Inoue et al., 1994). The effect of gallic acid was significantly reduced by blocking the free hydroxyl group with acetyl, methyl, ethyl, n-propyl or isoamyl group. These data suggest the involvement of pro-oxidative action of gallic acid in the induction of apoptosis. Gallic acid is considered to trigger apoptosis by
means of reactive oxygen species such as hydrogen peroxide, superoxide anion in addition to Ca\(^{2+}\)-ion and calmodulin dependent enzymes. Gallic acid is a common structural constituent of EGC, EGCG, the most active constituents of green tea therefore in order to identify the structural features of these compounds I have compared in part II, the prooxidant DNA cleavage properties and antioxidant properties of gallic acid and green tea extract.

Tannic acid [gallotannic acid, gallotannin or penta-(m-digalloyl-glucose)] is a principal of tannin, derived from Chinese nutgalls (Galle rhois) belonging to Anacardiaceae and constitutes penta to octa-galloyl glucose. The term “tannin” is ordinarily used as a synonym for tannic acid. Tannic acid has numerous industrial, pharmacological and food additive applications. It is used as an additive in medicinal products for humans, including those used for treatment of burns, diarrhea, chemical antidotes in poisoning and as local astringent (Hirono, 1987). It is also used as a flavour enhancer and processing aid in alcoholic beverages (United States Food and Drug Administration, Rockville, 1988). Tannic acid is also used as a clarifying agent in the brewing and wine industries (IARC monograph, 1976) and for colour stabilization of orange fruit juice (Maccarone et al., 1987). When applied topically, injected or added to diet or drinking water, tannic acid has been shown to decrease the risk of tumorigenecity in the skin and other organs (Mukhtar et al., 1988; Athar et al., 1989). Tannic acid and several gallic acid derivatives strongly inhibit the activity of 12-O-
tetradecanoylphorbol-13-acetate (TPA) induced ornithine decarboxylase (ODC) (a biochemical marker of stage 2 promotion) in mouse epidermis in vivo (Gali et al., 1991), suggesting that hydrolyzable tannins inhibit the 2\textsuperscript{nd} stage of tumor promotion phase of skin tumorigenesis. Tannins have high reducing power and form complexes with various metal ions and cofactors, chelate iron and inhibit the iron-catalyzed reactions generating free radicals (Gali et al., 1992). Numerous studies have also demonstrated that tannic acid could induce the DNA excision repair system in bacterial and mammalian cells ((Kuroda, 1988; Shimoi et al., 1985).

Ellagic acid, another polyphenol has been shown to have an anticarcinogenic effect against an array of nitrosamines, polycyclic aromatic hydrocarbons and fungal toxins, for example skin tumorigenesis induced by 20-methylcholanthracene in mice (Mukhtar et al., 1986), N-nitrosobenzylmethylamine-induced tumors in rat oesophagus (Daniel and Stoner, 1991), N-2-fluorenylacetamide-induced liver carcinogenesis in the rat (Tanaka et al., 1988) and neoplasia caused by benzo(\(\alpha\))pyrene in mice (Lesca 1983). Mutagenicity of aflatoxin B\(_1\) (Mandal et al., 1987) and N-methyl-N-nitrosourea (Dixit and Gold, 1986) was also decreased by ellagic acid. In order to understand the chemical basis of the various biological properties of tannic acid, I have studied in part III the structure – activity relationship between tannic acid, gallic acid and ellagic acid using the DNA cleavage assay and Cu (II) reduction. The structures of these compounds are given in Figure 2.