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TEA CONSUMPTION ON OXIDATIVE DAMAGE AND CANCER

The ancient use of tea as a beverage has been practised by mankind since anywhere between 500-5000 years ago[1]. Owing primarily due to their untoward effects, general apathy towards synthetic chemical agents in modern medicinal care has featured throughout the world during the last two decades. This resulted in an intense global search for plant extracts and their constituents for health care. Simultaneously information from scientific world on life-style and impact of diet on human health have been increasingly emerging.

Tea stood mythologically as a beverage with positive health effects in Far-East. The traditional use of tea prompted scientific research employing modern research methodology in China, Japan, and US on the influence of tea consumption on human health, since mid-sixties. As a result, by the end eighties, green tea became known for its health benefits internationally. By this time, sufficient data were generated for claiming that consumption of green tea has preventive effects especially on occurrence of cancer and cardiovascular disorders. Concurrently, increasing emphasis has been given on events at the cellular level by green tea *per se* and its constituents which revealed their diverse pharmacotherapeutic efficacy, most importantly protection against oxidative damage.

Unlike green tea, scientific information on biological activities of therapeutic interest of black tea had been very scanty till 1990. However, on the recommendation of the Food and Agricultural Organisation (FAO) studies were initiated to evaluate the protective effect of green tea and black tea both on human health in general. This led to a systematic research on black tea and its constituents unfolding several beneficial effects of black tea consumption related to human health.

India is the largest black tea producing country in the world. In India a collaborative research programme was launched between Tea Research Assocation (TRA) and the Indian Institute of Chemical Biology (IICB) in 1990s with the primary objective to evaluate the pharmacotherapeutics of black tea in totality *ie.* as is consumed. The present write-up is an attempt to highlight the two most promising health benefits of tea consumption documented during the last decade in India and across the world.

The processing of black tea consists of four steps *viz* withering, rolling, fermentation and drying. Withering of tea leaves is necessary to physically condition the fresh tea leaves making them amenable to subsequent processing. In addition, loss of moisture and a number of important biochemical changes take place during

withering. Leaves are subjected to rolling in which the cell structures are disrupted and leaves are macerated. During this stage, enzymes polyphenol oxidase present in tea leaves are brought into intimate contact with substrate *ie.* catechins. The chemical and biochemical reactions initiated during rolling are allowed to continue and completed during the next stage of black tea processing, referred to as fermentation. It was previously believed that the changes occuring during this stage are caused by microorganisms. It is now well known that the principal reaction is oxidation of catechins by enzyme polyphenol oxidase which results in the formation of two types of dimeric and polymeric products, namely theaflavins (orange coloured) and thearubigins (brown coloured) respectively. Fermentation step is followed by drying or firing which is necessary for ceasation of enzyme activity and reduceing moisture content of the fermented product.

Chemical Composition

The main difference between black tea and green tea manufacture is heat inactivation of enzymes in the flush. In the case of green tea, steaming of leaves is the first step, by which polyphenol oxidase and other enzymes are inactivated and thus retaining its green colour. The steamed leaves are then rolled and subsequently dried to get the final product.

Dietary research on the impact of foods and beverages on human health has been globally dominant in the last decade. Flavonoids, a group of phenolic compounds occurring abundantly in vegetables, fruits, and green plants, attracted special attention as they showed high antioxidant property. The antioxidants are known to prevent cellular damage caused by reactive oxygen species. Catechins are highly potent flavonoids present in tea and serve perhaps as the best dietary source of natural antioxidants. United States Department of Agriculture (USDA) is currently engaged in precisely quantifying various flavonoids in fruits using modern analytical techniques. Approximately 30% of the tea solids in a typical infusion is composed of flavonoids, whereas less than 5% of the water soluble solid extract of tea is formed by flavonols like, quercetin, kaempferol, myricetin and their glycosides. Of the catechins, epigallocatechin gallate (EGCG) is present maximally (more than 10% of dry weight) in green tea. It is also found in black grapes, red wines, apples and chocolates[2, 3]. Methylxanthines, mainly in the form of caffeine is present in tea in a proportion of one-third of its content in coffee. In addition, tea contains theanine, an important amino acid[4]. Interflavanoid linkages resulting in formation of proanthocyanidines is an area of recent interest in chemistry of tea[5,6].

The flavonoid content of plants, vegetables and fruits varies with plant variety and environmental conditions. Light is required for the synthesis of flavonoids and they are generally found in the outer portion of plants, vegetables and fruits like skin of the fruit, the outer leaves of the vegetables (cabbage and lettuce), or in the leaves as in the case of tea. Since these portions of fruits and vegetables are often discarded before human consumption, it has relevance while estimating or recommending dietary intakes. Flavonoids are not found to any appreciable degree in root vegetables and to a lesser extent when cultivated in glass houses as compared to grown in outdoors.

Flavonoids, phenolic acids and non-flavonoid polyphenols found in plants are classified in Fig.1.

From the chemical point of view, the accepted definition of flavonoids is dibenz pyrans and pyrones and their derivatives, *ie*. any compound with a C6-C3-C6 ring structure, including those which are oxidized and those attached to sugar molecules (glycosides), as long as the derivatives retain the ring structure (Fig.2).

The fresh tea leaves contain four major catechins as colourless water soluble compounds – epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG). Most of the green tea catechins, during the manufacture of black tea, are oxidized and converted into orange or brown products known as theaflavins (TF) and thearubigins (TR). These compounds retain the basic C6-C3-C6 structure and are thus still classified as flavonoids. Theaflavins consist of two catechin molecules joined together, and account for about 10% of the converted catechins, whereas the thearubigins are more complex flavonoid molecules, whose structural chemistry are still unknown, and may account for up to 70% of flavonoids in black tea

Oxidative Damage

Currently, intensive search is conducted to obtain active antioxidant compounds from non-nutritive components of plant origin including tea, spices and herbs. Antioxidants protect the body against the damaging

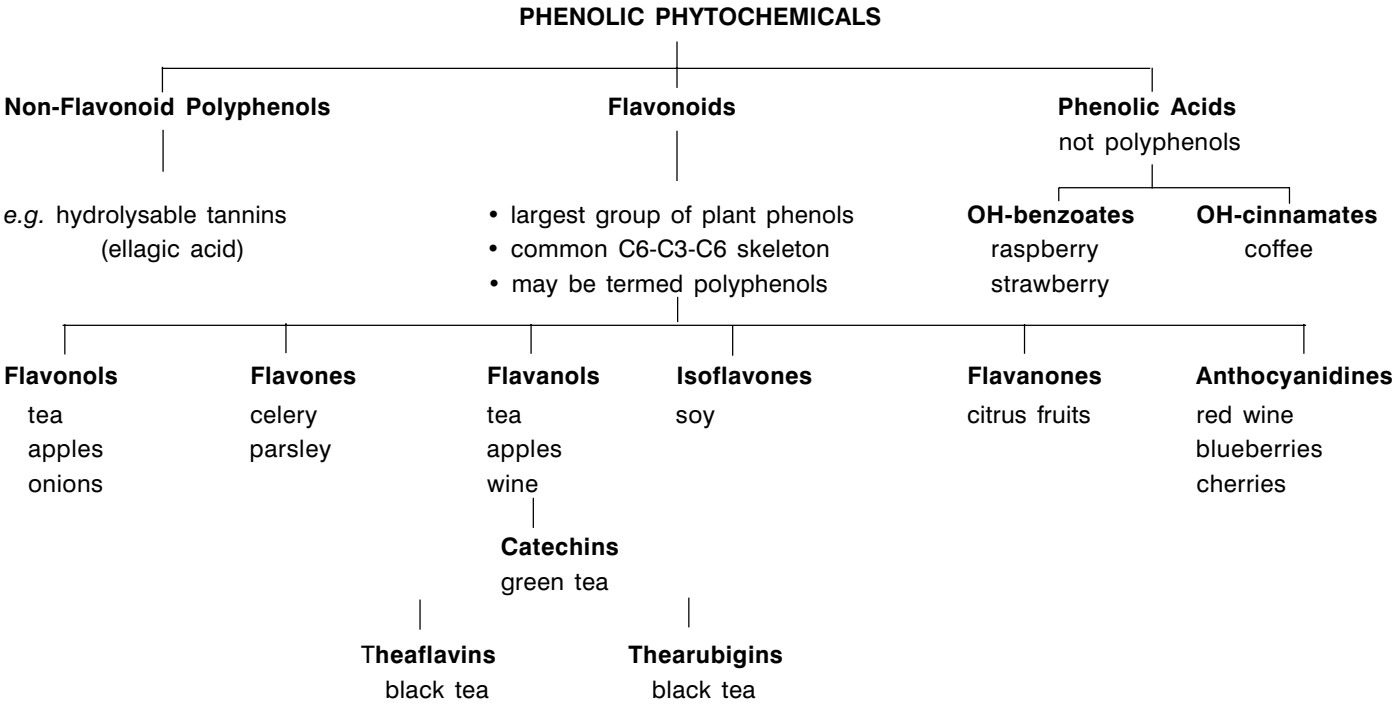

Fig.1. Phenolic Phyotchemicals

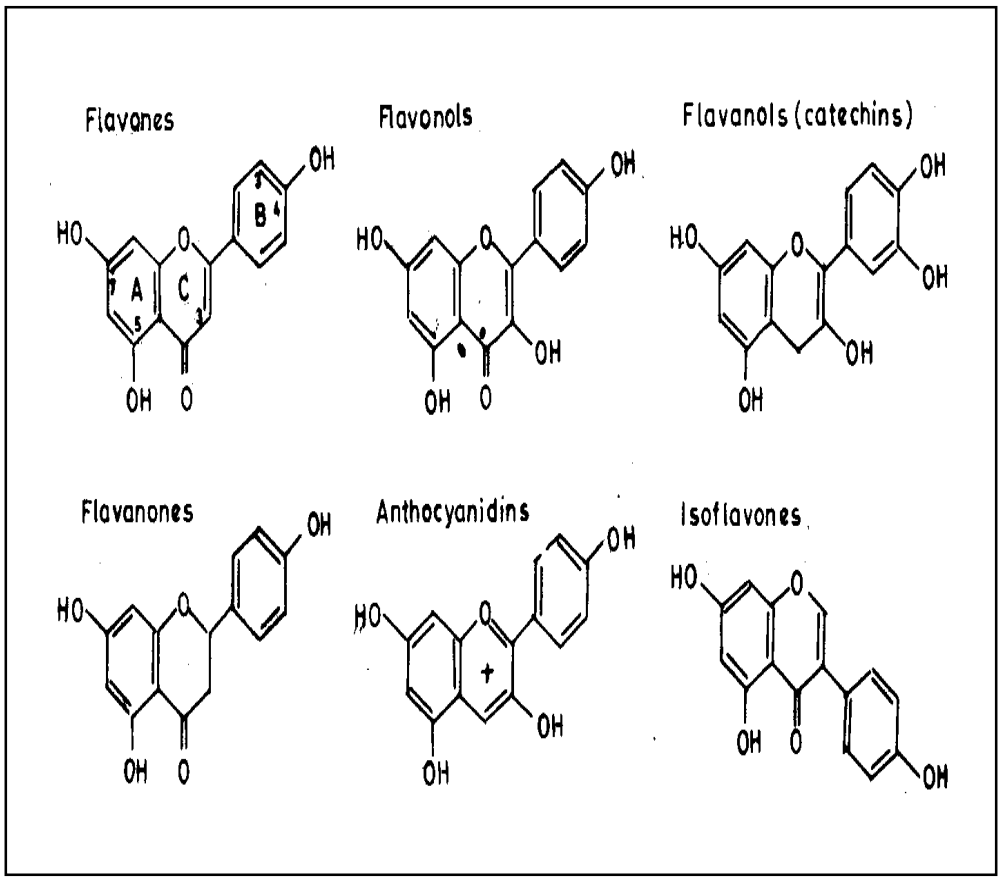

Fig.2. Chemical structure of phenolic compounds

effects of free radicals generated in the body during conversion of glucose and fat to energy, exercise and by the action of sunlight on the skin. Free radicals generated by metabolic activities are unstable molecules which can freely interact with electron donors to equilibrate its charge. Oxidative stress induced by over-production of reactive oxygen species (ROS) like hydroxyl radical (-OH), ferryl ion ($Fe^{2+}O$) or $Cu(OH)_2$ disrupt cellular function, whereas superoxide radical anion (O_2^-), peroxyl radical (ROO^+), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), hypochlorus acid (HOCl), nitric oxide (NO^+), peroxylnitrite ($ONOO^-$) and acetyl radicals (RO^+) exhibit lower reactivity[7,8]. ROS can interact in themselves as well as with other molecules to form more or less reactive molecules. Overproduction of

free radicals also occurs with smoking, environmental pollution, chronic inflammation, external action of toxic substances, microbial attacks and ozone[8-12]. Aging process and radiation damage have been particularly attributed to protein oxidation by ROS. Cellular defence mechanism to oxidative damage is activated endogenously by glutathione and other enzymes which convert the oxidized molecules to their reduced form. Oxidative damage to DNA promotes mutations and thus enhancing the risk of carcinogenesis. Further, the risk of atherosclerosis, diabetes and immunodeficiency has been linked to oxidation of lipids and proteins[13].

Our body contains its own antioxidant system, made up of enzymes like catalase, superoxide dismutase and metal-binding proteins. The endogenous defense mechanism against oxidative damage is complemented by antioxidants like vitamin C, E, carotenoids and flavonoids, mainly found in vegetables, fruits and tea. These antioxidants from nutrients contribute to the overall protection of cell integrity and the immune function[8, 14-19]. Of these, ascorbic acid and tocopherol are the most effective protectors against a wide range of oxidizing molecules. The optimal activity of the enzymatic system against oxidation is also dependent upon adequate mineral nutrition like selenium, copper and zinc[8, 15]. The protection of cell membrane and DNA integrity is offered by carotenoids including lycopenes[20]. An imbalance between free radical production and protection by antioxidants leads to oxidative damage in proteins, lipids and DNA. Amongst the exogenous antioxidants, polyphenolic compounds especially flavonoids present only in plants, fruits, vegetables and herbs are potent phytochemicals in the diet protecting the body from oxidative damage. A spectrum of biological activities has been attributed to flavonoids in plants, the content of which varies with plant-variety and environmental conditions.

Tea as Powerful Antioxidant

Flavonoids found in tea shows 20 times more powerful antioxidant activity than vitamin C[21, 22] in the lipoprotein oxidation model. Methods for evaluating antioxidant properties ex-vivo are fairly well established and the lipoprotein oxidation model simulates the oxidation of low-density lipoproteins that promotes atherosclerosis. The antioxidant efficacy of polyphenols, as evaluated by a battery of tests, indicates that they can inhibit lipid hydroperoxide formation[23-25]. Scavenging property of polyphenols has been demonstrated against a spectrum of offensive oxidants, like superoxide radicals[26,27], free radicals[21,27] and peroxinitrite[7,19,28]. Polyphenols exhibit modifying influence on the protein phosphorylation process[29], and following the catalytic activities of many enzymes especially the oxidative ones[7]. They are able to prevent metal-catalyzed free radical formation caused by copper and iron chelate[2]. An important property of flavonoids is that they do not affect b-carotene, vitamin C and E, which attribute for the endogenous antioxidant protection system of the body[30]. Production of offending N-nitroso compounds is a consequence of interaction of nitrogen-containing compounds with nitrosating agents which is prevented by polyphenols[19]. Flavonoids in presence of some metals or in high concentration can also act as pro-oxidants and inhibit P-450-catalysed activity[7], a property also shared by ascorbic acid and a-tocopherol[31-33].

Many pharmacotherapeutic properties of both green and black tea have been attributed to polyphenols and their gallates present in tea, though direct demonstration of such an action in many cases has been missing. Different polyphenol molecules exhibit scavenging activity that are linked to the number of o-dihydroxy and o-hydroxyketo groups, C_2-C_3 double bonds, concentration, solubility, the affinity of the active group to the oxidant and the stability of the final product[32, 34]. However, flavonoid glycoside molecules like rutin, and the glycoside of quercetin, are less potent as antioxdants[21]. The intracellular antioxidant efficacy of flavonoid glycosides depends on their capacity to react against ROS. The recycling potential of the cell also plays an important role in the antioxidant status of the flavonoid glycosides[35].

Green tea has received much attention as chemopreventor for protection, promotion and progression of various forms of tumour and EGCG with five phenolic hydroxy groups is claimed to be the most potent tea antioxidant[36, 37]. EGCG has been shown to act as an inhibitor of urokinase, the enzyme crucial for tumour growth[38]. Owing to higher content of polymerized polyphenols, black tea has been generally neglected and assumed to be of lesser value. This assumption has proven to be wrong as black tea extract has now been demonstrated to be a better protecting agent as compared to free catechins against various types of oxidative stress[39]. Further, it was shown that theaflavin was the most powerful in abrogating NO production, as

well as the most important constituent in down-regulating synthesis of nitric oxide synthease (i NOS)[40].

Inflammatory bowel disease is characterised by oxidative and nitrosative stress and NO-related treatments serve as a promising pharmacological approach in the treatment of these disorders[41,42]. In accordance, the protective effects of thearubigin, the major constituent of polyphenol in black tea, were uniquely demonstrated on 2, 4, 6-trinitrobenzene sulphonic acid (TNBS)-induced colitis in mice associated with overproduction of NO[43].

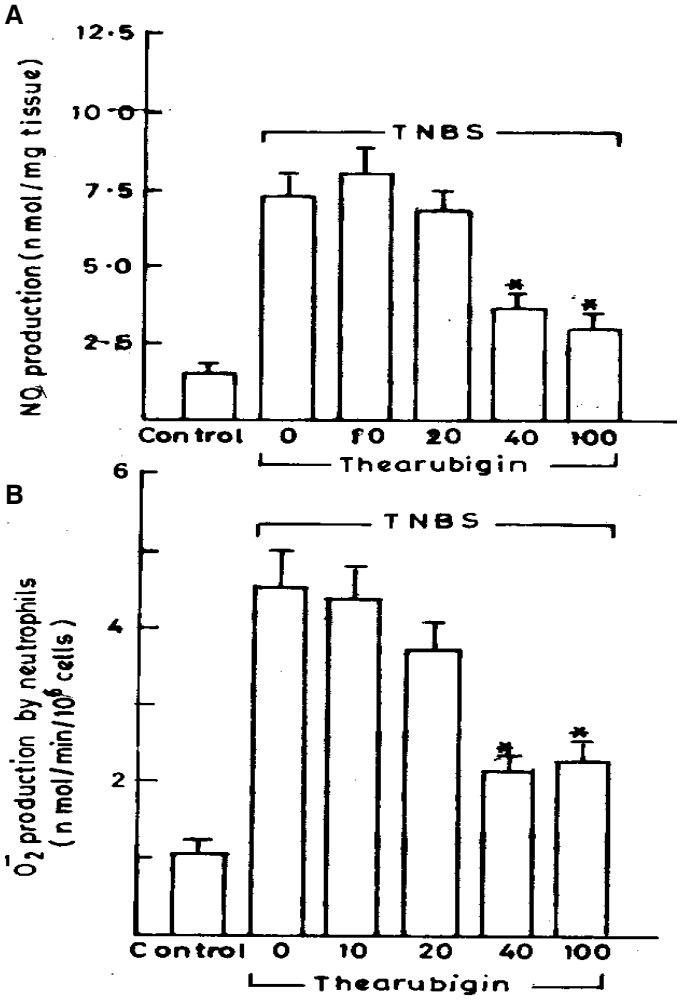

Fig.3. Effects of thearubigin on NO and O_2^- production. (A) NO production in colonic tissue and (B) O_2^- production in neutrophils.
Values are mean ± SD of 10 rats for each group.
***p <0.01 *vs.* TNBS**
Source: Reference 43

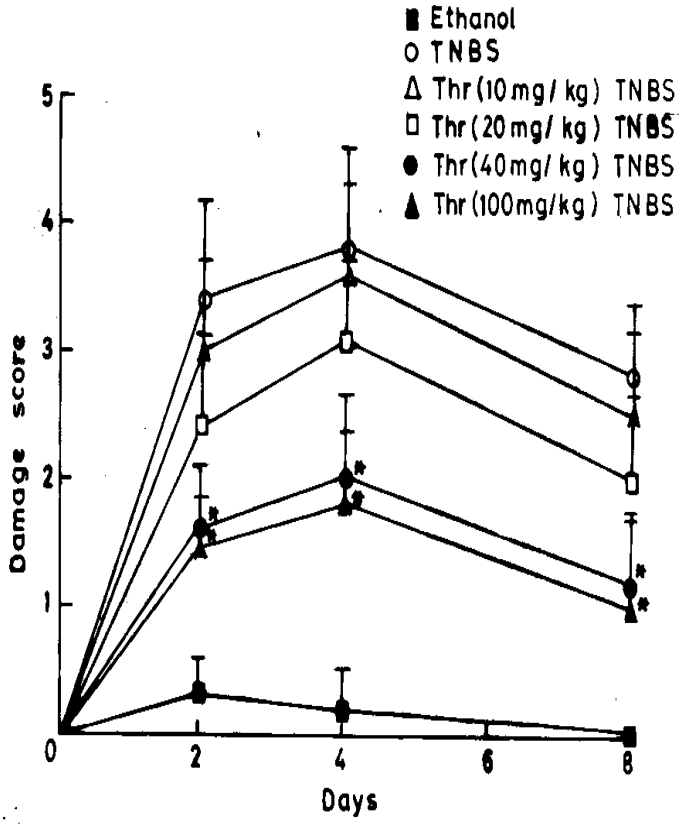

Fig.4. Effect of thearubigin pre-treatment on macroscopic damage score of colonic tissue after induction of colitis by TNBS. Colonic damage was scored on a scale of 0 (normal) to 5 (severe) by two independent observers.
Values are mean ± SD of 10 rats for each group.
***p <0.001 *vs.* TNBS**
Source : Reference 43

Offending effects of ROS on cellular membranes and intracellular structures affect cellular metabolism and contribute to onset of various disorders including cancer, cardiovascular and metabolic diseases. Positive indications that flavonoids fraction in human *in vivo* system have emerged as tea consumption is shown to reduce oxidative damage and lipids in the human body. Employing multiple biomarkers it has been found that green tea consumption decreases damage to DNA among smokers and non-smokers. The decreased level of oxidative damage was correlated with decreased levels of free radicals found in urine[44,45].

Tea Flavonoids on Cancer

Occurrence of cancer is associated with the natural loss of the cells in any tissue or organ and thus undergoing unregulated growth. Accumulation of reactive oxygen

species in cells damages the defence mechanisms of the body including the DNA structure and enzymatic balance triggering the process of cancer pathogenesis[46]. Thus antioxidants present in tea, fruits and vegetables contribute towards prevention and progress of cancer. The three main stages of cancer are : initiation, promotion and progression. Studies have revealed that black and green tea are effective at all the three stages. In general, there are more consistent findings for the cancer preventive effects of green tea than for black tea, probably due to insufficient data on black tea intakes and also because of small range studies of black tea intakes.

Interaction of tea flavonoids with procarcinogens plays a prominent role for the beneficial effects of tea against cancer initiation. Cancer of the colon, breast and pancreas is associated with formation of heterocyclic amines, the genotoxic carcinogens from cooked food and meat that can be prevented by tea polyphenols[47]. Phase I enzymes are known to induce tumour formation by activating procarcinogens which modify genomic DNA and black tea polyphenols probably inhibit cytochrome P450 dependent bioactivation of the carcinogen[48]. Reports on potent antimutagenic effects of green tea and its constituents are available in the literature, but most of the commonly consumed teas (green, black and oolong) are shown to possess similar antimutagenic efficacy[49-55]. This leads to a general conclusion that development of cancer is prevented by tea consumption through antimutagenic protection paralleling to their antioxidant efficacy. EGCG has been most extensively studied against mutation and ROS-scavenging property and is perhaps the most potent antimutagenic agent protecting DNA scissions and non-enzymatic interception of superoxide anions. ECG emerges as the most potent enzymatic scavenger amongst the green tea polyphenols[26,56]. Antimutagenic property of black tea and its constituents has been widely documented in many reports, especially in Salmonella strains[57]

The effects of specific tea ploypheno ls (polyphenol 60 and polyphenol 100 from green tea and polyphenon B containing mixture of polyphenols from black tea) were examined against a number of genotoxic carcinogens in Salmonella strains TA98, TA100 and TA1535. All of these polyphenols sharply decreased mutagenicity of a number of aryl and heterocyclic amines of aflatoxin B1, 1,2-dibromomethane, 2-nitropropane, involving an induced rat lever S9 fraction[58]. TF, the black tea polyphenol, was found to inhibit DNA single strand cleavage and mutagenicity induced by hydrogen peroxide[59] and prevent the cytochrome P450 dependent bioactivation of carcinogens[60,48]. Tea tannin and its metabolites act as comutagens and antimutagens, both based on inhibition and promotion of DNA excision repair property[61]. Black tea extract and its two active polyphenols, TF and TR, possess considerable antimutagenic effects in Ames Salmonella assays, the TF being 4 to 5 times more potent than TR[57](Table I and II).

Black tea and its two polyphenols (TF and TR) were investigated against chemically induced genetic damage as measured by chromosome aberrations and sister chromatid exchanges in mice[62]. The study appears to be a novel one on the anticlastrogenic effect of black tea (Table III).

Interestingly, a synergistic interaction between catechins plays a role in cancer prevention. At a time when EC was inactive, it was found to markedly enhance the apoptosis induced by EGCG on the lung cancer cell line[63]. Conjugation and subsequent inactivation of carcinogens by EGCG mediates through inhibition of phase I enzymes and activation of phase II enzymes[64,65]. Thus it appears that tea polyphenols can interact with enzymatic defence mechanisms in cells and contribute to cancer prevention at the early stage of carcinogenesis.

There is increasing evidence to support that flavonoids present in green and black tea can exert a protective effect on cancer promotion. These flavonoids help to maintain normal cell growth by blocking the activation of an oncogene AP1 (activator protein), maintaining cell-cell communication and increasing apoptosis of malfunctioning cells. The factors NF-κB (nuclear factor kappa B) and AP1 are redox-regulated components of the signal transduction cascade and, thus, sensitive to the oxidant/antioxidant status of the cell[46]. EGCG and theaflavins, the prominent flavonoids from green and black tea respectively, were investigated employing the JB6 mouse epidermal cell line on ultra violet (UV) radiation induced AP1-dependent transcriptional activation. The study revealed a concentration-dependent inhibition of UV induced AP1 activation and, when compared, theaflavins were found to be more potent than EGCG[66]. Further, EGCG and theaflavins were examined for inhibitory effects on 12-o-tetradeconylphorbol-13-acetate (TPA) induced protein kinase C (PKC) and transcription activator protein-1 binding activities in N1H 3T3 cells. It was shown that the theaflavin-3-3'-digallate (TF-3) was the most potent

Table I. Antimutagenic effects of thearubigins in Salmonella strains TA97a, TA98, TA100 and TA 102 in preincubation tests with or without S9 activation.

Theaflavins (100 ml/plate)	(Mean ± SD) - S9	Thearubigins (100 ml/plate)	(Mean ± SD) + S9
TA97a			
NPD (20 mg/plate)	1100 ± 81	2-AF (10 mg/plate)	1144 ± 12
NPD + 125	1028 ± 52	2-AF + 125	1070 ± 38[a]
NPD + 250	1020 ± 59[a]	2-AF + 250	815 ± 30[b]
NPD + 500	825 ± 58[b]	2-AF + 500	707 ± 48[b]
NPD + 1000	760 ± 51[b]	2-AF + 1000	494 ± 29[b]
TA98			
NPD (20 mg/plate)	1133 ± 52	2-AF (10 mg/plate)	1273 ± 40
NPD + 125	1097 ± 69	2-AF + 125	947 ± 34[b]
NPD + 250	960 ± 43[b]	2-AF + 250	832 ± 49[b]
NPD + 500	886 ± 11[b]	2-AF + 500	438 ± 40[b]
NPD + 1000	696 ± 59[b]	2-AF + 1000	103 ± 25[b]
TA100			
SA (1.5 mg/plate)	1153 ± 95	2-AF (10 mg/plate)	1228 ± 30
SA + 125	1088 ± 28	2-AF + 125	1083 ± 64[b]
SA + 250	997 ± 14[b]	2-AF + 250	993 ± 56[b]
SA + 500	922 ± 66[b]	2-AF + 500	817 ± 41[b]
SA + 1000	871 ± 46[b]	2-AF + 1000	512 ± 20[b]
TA102			
CH (100 mg/plate)	1238 ± 71	DN (30 mg/plate)	1210 ± 65
CH + 125	1128 ± 50[a]	DN + 125	1110 ± 43[b]
CH + 250	1061 ± 63[b]	DN + 250	1050 ± 50[b]
CH + 500	979 ± 33[b]	DN + 500	855 ± 49[b]
CH + 1000	900 ± 60[b]	DN + 1000	673 ± 38[b]

Mean and ± SD of four plates. Results of each concentration were compared with the solvent control by Dunnett's test. *p<0.0.05.
NPD - 4-nitro-0-phenylenediamine; SA - sodium azide; 2-AF - 2-aminofluorene; CH - Cumine hydroperoxide; DN - danthron.
-S9, without S9 activation; +S9 activaiton.
Source : Reference 57.

inhibitor of TPA-mediated tumour promotion[67]. Initiation of skin carcinogenesis by AP1 is effectively blocked by EGCG and theaflavin-3-3'-digallate[67-70].

In earlier studies, topical application or ingestion of green tea polyphenols or EGCG were shown to inhibit tumour initiation and promotion by chemical carcinogens and UV light[71-74], a property which was shared by black tea[74-77]. The heterocyclic amine 2-amino-1-methyl-6-phenyl imidazo(4,5-b) pyridine (Ph1P), formed during cooking of proteinaceous animal food, can induce lymphoma, colon and prostrate tumour in male and mammary tumours in female Fischer 344 rats by DNA-adduct formation in various organs including the target organs. It is reported that both green and black tea exhibit potential chemopreventive property against Ph1P induced tumourogenesis in Fischer rats[78]. Important information on the mechanism of action of tea polyphenols has been obtained in liposomal studies by examining the influence of EGCG and ECG on protein kinase activator, an enzyme involved in the cell activation process and promotion of tumour. While EGCG blocks the interactions between proteins and ligands[79], both EGCG and ECG inhibit the gap junctional intercellular communication caused by tumour promoters[47].

The arrest of progression of various cancers by green and black tea and their constituents has been adequately reported. Of these, the most widely investigated anticancer effects of tea are on skin, lung and digestive tract. In mice, the conversion of benign skin papillomas to malignant ones was significantly inhibited by topical application of a green tea polyphenolic fraction[80]. Interestingly, so far in a single study, complete regression has been reported in 4% of 346 papilloma-bearing mice[74]. Some overlapping mechanisms, as discussed earlier, have been shown to play possible role in prevention of progress of skin cancer by polyphenols of green tea[72,74] and black tea[72,74,76,77]. Conversion of mice skin papillomas from benign to malignant stage is significantly decreased

Table II. Antimutagenic effects of theaflavins in Salmonella strains TA97a, TA98, TA100 and TA 102 in preincubation tests with or without S9 activation.

Theaflavins (100 ml/plate)	(Mean ± SD[a]) - S9	Theaflavins (100 ml/plate)	(Mean ± SD[a]) + S9
TA97a			
NPD (20 ml/plate)	1100 ± 810	2-AF (10 ml/plate)	1144 ± 12
NPD + 25	1000 ± 54	2-AF + 25	864 ± 23[b]
NPD + 50	1020 ± 62	2-AF + 50	719 ± 85[b]
NPD + 100	894 ± 26[b]	2-AF + 100	685 ± 53[b]
NPD + 200	848 ± 49[b]	2-AF + 200	501 ± 13[b]
TA98			
NPD (20 ml/plate)	1133 ± 52	2-AF (10 ml/plate)	1273 ± 40
NPD + 25	987 ± 50[b]	2-AF + 25	1110 ± 47[b]
NPD + 50	998 ± 43[b]	2-AF + 50	1041 ± 49[b]
NPD + 100	841 ± 38[b]	2-AF + 100	741 ± 65[b]
NPD + 200	747 ± 53[b]	2-AF + 200	455 ± 31[b]
TA100			
SA (1.5 ml/plate)	1153 ± 95	2-AF (10 ml/plate)	1228 ± 30
SA + 25	1043 ± 45	2-AF + 25	980 ± 76[b]
SA + 50	1011 ± 70[a]	2-AF + 50	796 ± 52[b]
SA + 100	964 ± 56[b]	2-AF + 100	672 ± 61[b]
SA + 200	829 ± 63[b]	2-AF + 200	498 ± 48[b]
TA102			
CH (100 ml/plate)	1238 ± 71	DN (30 ml/plate)	1210 ± 65
CH + 25	1079 ± 69[a]	DN + 25	1084 ± 28[a]
CH + 50	1026 ± 80[a]	DN + 50	1041 ± 72[b]
CH + 100	936 ± 103[b]	DN + 100	873 ± 64[b]
CH + 200	909 ± 41[b]	DN + 200	761 ± 55[b]

Mean and ± SD of four plates. Results of each concentration were compared with the solvent control by Dunnettt's test. *$p<0.05$.
NPD - 4-nitro-o-phenylenediamine; SA - sodium azide; 2-AF - 2-aminofluorene; CH - Cumine hydroperoxide; DN - danthron.
-S9 - without S9 activation; +S9, with S9 activation.
Source: Reference 57.

by green tea polyphenols[80]. Black tea, when fed to tumour bearing mice, can afford protection against different stages of skin cancer through acceleration of apoptosis and modulation of DNA synthesis[74,77]. An inflammatory response is an early step to development of mouse skin cancer induced by chemical carcinogen which is inhibited by black tea polyphenols[75]. More recently, both black and green tea extracts have been reported to inhibit the inflammatory response and solid tumour growth by 3-methylchloranthrene in mice[81] .

Several cancers such as cancer in the lung, oral cavity and oesophagus are associated with cigarette smoking and tobacco use[82] A series of studies in animal models, especially in mice and rats, employing the appropriate carcinogens, mainly nitrosamine and in particular 4-(methylnitrosoamino)-1-(3-pyridyl)-butanone (NNK) found in tobacco have revealed that green and black tea or the corresponding polyphenols decrease the incidence of these cancers through inhibition of oxidative reaction caused by the carcinogens[83,84]. Formation of nitrosamines, the carcinogens also found in tobacco, can be prevented by phenolics of green tea[19,64,85]. Pretreatment with black or green tea, decaffeinated tea and EGCG reduces the number of lung tumours induced by chemical carcinogens[71,86]. Studies with tea and its constituents (black and green) on spontaneously developing and induction of tobacco-specific nitrosamine (NNK) lung tumour show parallel results[84, 87, 88]. Of all the tea flavonoids, EGCG, theaflavin-3-3'-digallate and ECG exhibit maximum anticarcinogenic efficacy[87]. Further, enhancement of 8-hydroxydeoxyguanosine level in mouse lung DNA is also suppressed by green tea[84].

In short, it can be said that initiation of skin cancer is prevented by green and black tea and their polyphenols[72-77,89]. The promotion of skin cancer is inhibited by green tea[74,80], black tea[74-77] and EGCG[74], and its progression is reduced by green tea and EGCG,[72,74] like black tea and its polyphenols[72, 74-77]. Although adequate studies on initiation of lung cancer vis-à-vis tea consumption are not available, its promotion

Table III. Anticlastogenic effects of black tea (World blend) and its polyphenols TF and TR against CP and DMBA induced chromosome aberrations in bone marrow cells of mice.

Treatment (mg/kg)	Gaps	Aberrations/cell[b] Chromatid Type	Chromosome Type	Aberrant cells% (Mean ± SD)[c]	Mitotic indices (Men ± SD)[c]
Distilled water	9	0.01	0.00	1.00 ± 0.71	2.57 ± 0.13
5% tea	12	0.002	0.00	0.25 ± 0.43	2.62 ± 0.30
10% tea	12	0.002	0.002	0.50 ± 0.87	2.44 ± 0.33
20% tea	11	0.010	0.0025	1.25 ± 0.43	2.28 ± 0.38
CP (20 mg/kg)	58	0.540	0.095	30.75 ± 1.48	2.01 ± 0.38
5% tea + CP	61	0.550	0.0575	24.25 ± 3.77*	1.60 ± 0.46
10% tea + CP	32	0.475	0.0275	21.50 ± 3.57**	1.60 ± 0.61
20% tea + CP	26	0.222	0.032	18.00 ± 3.67**	1.95 ± 0.44
[d]TF (40 mg/kg) + CP	31	0.45	0.02	19.75 ± 3.77**	2.47 ± 0.54
[d]TR (160 mg/kg) + CP	44	0.23	0.03	18.00 ± 3.08**	2.55 ± 0.71
DMBA (50 mg/kg)	90	0.175	0.025	20.75 ± 1.92	1.22 ± 0.34
5% tea + DMBA	95	0.232	0.0275	16.00 ± 2.12*	1.25 ± 0.68
10% tea + DMBA	69	0.173	0.025	15.00 ± 2.74**	1.12 ± 0.35
20% tea + DMBA	53	0.10	0.008	9.00 ± 1.22**	1.12 ± 0.39
[d]TF (40 mg/kg) + DMBA	33	0.16	0.02	13.50 ± 2.69**	1.26 ± 0.09
[d]TR (160 mg/kg) + DMBA	43	0.16	0.015	11.50 ± 1.50**	1.65 ± 0.58

[a] Total chromatid and chromosome gaps at each dose were recorded but not included as aberrations/cell.

[b] Total number of aberration (chromatid or chromosome type)/total number of cells scored per dose group. Results are of 4 animals (100 cells/animal).

[c] Results at eah dose were compared with that of control using Dunnett's multiple comparison with control. * $lp<0.05$, ** $p<0.01$.

[d] Results at each dose were compared with that of positive control using paired student's 't' test.

Source: Reference 62.

and progression is inhibited by green tea[84, 86, 88, 90] and black tea[86, 88]. Similar reports are also available on varied types of other cancers in which initiation[91,92], promotion[93,94] and progression[95] are shown to be prevented by both green and black tea and their polyphenols in animal models.

In several situations, like oesophageal tumour, results are equivocal[71,95,96]. Similar anomaly exists for colon cancer with controversial and contradictory reports[93,96], but indications are rather strong favouring the protective role of green tea and its polyphenolic fractions against colon cancer, especially when induced by azoxymethane[96-98]. At a time when protective effect of tea has been shown on liver cancer[71, 86, 96, 98], results appear to be ambiguous in cases of mammary gland and prostate cancer[71, 99, 100] except for EGCG[91,92].

Clinical and Epidemiological Studies

Human population studies to corroborate the general outcome of the in- vivo and ex-vivo animal studies that tea may be involved in reducing cancer risk are indeed more equivocal. Studies with humans are far more complex to carry out, quite understandably due to heredity, diversity in food habits, environment, age and gender, *etc.* Most case-control or cohort studies were not specifically designed to examine the relationship between tea consumption and cancer incidence, and some studies are without adjustment of cancer risk factors like smoking. Some anomalies in results have also arisen from the fact that the temperature of the beverage was not always controlled for[71,101-103]. Consumption of very hot beverages may increase the risk of cancer, especially of the pharynx and oesophagus.

Stomach cancer, prevalent in the orient, as well as in northern and eastern Europe, is related to the elevated intake of salted and self-preserved food[104,105]. The rate is particularly high in individuals who carry the bacterium, Helicobacter pylori, that enhances cell cycling in gastric mucosal damage[106]. Bacterial, viral and parasitic infestations are also known to cause gastric cancer[26]. The antimicrobial property of tea and its polyphenols[107], and their ability to inhibit the growth of *Helicobacter pylori*[108,109] have been implicated to play a role in prevention of human gastric cancer. There is reasonable evidence that the carcinogens associated with high incidence of cancer seen in the western world, namely cancer of breast, colon, prostate and pancreas, originate from frequent and excessive intake of cooked meat containing heterocyclic amines, a novel class of carcinogen[110]. The heterocyclic amines require a 2-step metabolic activation to DNA-reactive genotoxicants, including oxidative modification of DNA. These reactions are inhibited by black and/or green tea and their polyphenols[60,111]. Epidemiological, especially cohort studies indicate a protective effect against various human cancers like stomach, colon, pancreatic, oesophageal and urinary bladder[103]. Metastatic cancer in human can be reproduced by the growth of transplantable cancers in synergistic systems and some studies demonstrate that intake of tea lowers the uptake, growth and progression of cancers[72,112]. Decreased numbers of auxiliary lymph node metastatsis in premenopausal patients and increased expression of progesterone and oestrogen receptors in postmenopausal patients were found to be linked with increased tea consumption in Japanese studies. The investigators also reported decreased recurrence of breast cancer and increased survival time of cured subjects[113,114]. Another Japanese study has shown that consumption of green tea reduces the risk of total cancer related with a reduction of cancer incidence to age[115].

Studies on human cancer development have been highly supplemented by identification of biomarkers as measurable biological criteria from easily available clinical samples reflecting early onset of cancer[116]. The gambit of biomarkers for early detection of cancer encompasses a variety of indicators like change in the ROS status, enhanced expression of oncogene-related genes like *c-Jun* and/or *c-Fas*, extent of DNA damage in lymphocytes, activation of signal transduction cascade and selective activity of target enzymes[65]. A good evidence of the protective effect of tea on oral cancer has emerged from a Chinese study with diagnosed oral leukoplakias, wherein micronuclei from exfoliated cells of buccal mucosa, lymphocytes and chromosome aberration in lymphocytes were employed as biomarkers. Oral and topical administration of tea were shown to reduce the number of micronuclei and DNA aberrations with decrease in the number and volume of lesions[117]. Employing biomarker techniques, green tea consumption has been reported to reduce the oxidative stress parameters in smokers as compared to nonsmoker volunteers[44]. However, highly rigid control and critical evaluation are necessary in studies with biomarkers for valid conclusion due to their sensitivity to phenotypic and genotypic polymorphism[116].

Conclusions

An important question is emerging on the basis of widely reported anticancer property of tea as to whether or not its consumption contributes in chemotherapy. Efficacy of chemotherapy has been reported to increase by oral administration of green tea in mice bearing implanted Ehrlich ascites carcinomas with dexorubiein[118]. A positive synergistic effect has been found when green tea and phytic acid are combined in suppression of azomethane-induced aberrant crypt foci in rat colon[97]. Further, at a time when tea had no significant effect on development of mammary tumours in mice it was completely inhibited by a combination of 50% effective dose of tamoxifen and green tea[119]. Theamine present in tea at a proportion of 1% on dry weight basis could inhibit hepatic metastasis in mice with ovarian carcinoma and augment the anti-tumour efficacy of doxorubicin[120]. The anticancer efficacy of tea as documented against varied types of cancer makes a strong case for its supportive role in chemoprevention of cancer.

References

1. Gutman, R.L. and Ryu B-H. Rediscovering tea. An exploration of the scientific literature. *Herbal Gram 37*: 33, 1996.

2. Rice-Evans, C.A., Miller, N.J. and Paganga, G. Antioxidant properties of phenolic compounds. *Trends Plant Sci 2*: 152, 1997.

3. Arts, I.C., Hollman, C.H. and Kromhout, D. Chocolate as a source of tea flavonoids*. Lancet 354:* 488, 1999.

4. Dufresne, C.J. and Farnworth, E.R. A review of latest research findings on the health promotion properties of tea. *J Nutr Biochem 12*: *404*, 2001.

5. Subramanian, N., Venkatesh, P., Ganguli, S. and Sinker, V.P. Role of polyphenol oxidase and peroxidase in generation of black tea theaflavins. *J Agr Food Chem 47:* 2571, 1999.

6. Lopez-Serrano and Barcelo, A.R. Reversed phase and size exclusion chromatography as useful tools in the resolution of peroxidase-mediate (+) -catechin oxidation products. *J Chromatogr A919*: 267, 2001.

7. de Groot, H. and Rauen, U. Tissue injury by reactive oxygen species and the protective effects of flavonoids. *Fundam Clin Pharmacol 12*: 249, 1998.

8. Diplock, A.T., Charleux, J.L., Crozier-Willy, G., Kok, F.J., Rice-Evans, C., Roberfroid, M., Stahl, W. and Vina-Ribes, J. Functional food Science and defence against reactive oxidative species. *Br J Nutr 80*: S 77, 1998.

9. Jackson, M.J., McArdle, A. and McArdle, F. Antioxidant micronutrient and gene expression. *Proc Nutr Soc 57*: 301, 1998.

10. Kanter, M. Free radicals, exercise and antioxidant supplementation. *Proc Nutr Soc 57*: 9, 1998.

11. Hillbom, M. Oxidants, antioxidants, alcohol and stroke. *Front Bio Sci 4:* 67, 1999.

12. Goldfarb, A.H. Nutritional antioxidants as therapeutic and preventive modalities in exercise-induced muscle damage. *Can J Appl Physiol 24*: 249, 1999.

13. Vendemiale, G., Grattagliano. E. and Altomare, E. An update on the role of free radicals and antioxidant defense in human disease. *Int J Lab Res 29:* 49, 1999.

14. Meydani, M., Lipman, R.D., Han, S.N., Wu, D., Beharka, A., Martin, K.R., Bronson, R., Cao, G., Smith, D. and Meydani, S.N. The effect of long-term dietary supplementation with antioxidants. *Ann N Y Acad Sci 854:* 352, 1998.

15. Beck,M.A. The influence of antioxidant nutrients on viral infection, *Nutr Rev 56:* S140, 1998.

16. Bradly, J. and Xu, X. Diet, age and the immune system. *Nutr Rev 54:* S43, 1996

17. Grimble, R.F. Nutritional modulation of cytokine biology. *Nutr Rev 14:* 634, 1998.

18. Hugues, D.A.: Effect of dietary antioxidants on the immune function of middle-aged adults. *Proc Nutr Soc 58:* 79, 1999.

19. Lampe, J.W. Health effects of vegetables and fruit: Assessing mechanisms of action in human experimental studies. *Am J Clin Nutr 70:* (Suppl.) 475S, 1999.

20. Nguyen, M.L. and Schwartz, S.J. Lycopene : Chemical and biological properties. *Food Technol 53:* 38, 1999.

21. Vinson, J.A., Dabbagh, Y., Serry, M. and Jang, J. Plant flavonoids, especially tea flavonols, are powerful antioxidants using an *in vitro* oxidation model for heart disease. *J Agric Food Chem 43*: 2800, 1995.

22. Craig, W.J. Health promoting properties of common herbs. *Am J Clin Nutr 70*: 491S, 1999.

23. Hirayama, O., Takagi, M., Hukumoto, K. and Katoh, S. Evaluation of antioxidant activity by chemiluminescence. *Anal Biochem 247:* 237, 1997.

24. Kaneko, T., Matsuo, N. and Baba, N. Inhibition of linoleic acid hydroperoxide-induced toxicity in cultured human umbilical vein endhothelial cells by catechins. *Chem Biol Interact 114*: 109, 1998.

25. Lin, A.M.Y., Chyi, B.Y., Wu, L.Y., Hwang, L.S. and Ho, L.T. The antioxidative property of green tea against iron-induced oxidative stress in rat brain. *Chin J Physiol 41:* 189, 1998.

26. Pillai, S.P., Mitscher, L.A., Menon, S.R., Pillai, C.A. and Shankel, D.M. Antimutagenic/antioxidant activity of green tea components and related compounds. *J Environ Pathol Oncol 18:* 147, 1999.

27. Chen, C.W. and Ho, C.T. Antioxidant properties of polyphenols extracted from green and black teas. *J Food Lipids 2:* 35, 1995.

28. Chung, H.Y., Yokozawa, T., Soung, D.Y., Kye, I.S., No, J.K. and Baek, B.S. Peroxynitrite-scavenging activity of green tea tannin. *J Agric Food Chem 46:* 4484, 1998.

29. Middleton, E. Effect of plant flavonoids on immune and inflammatory cell function. *Adv Exp Med Biol 439:* 175, 1998.

30. Pietta, P. and Simonetti, P. Dietary flavonoids and interaction with endogenous antioxidants. *Biochem Mol Biol Int 44:* 1069, 1998.

31. Cao, G., Sofic, E. and Prior, R. Antioxidant capacity of tea and common vegetables. *J Agric Food Chem 44:* 3426, 1996.

32. Salah, N., Miller, N.J., Paganga, G., Tijburg, L., Bolwell, G.P. and Rice-Evans, C. Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. *Arch Biochem Biophys 322:* 339, 1995.

33. Sergediene, E., Jonsson, K., Szymusiak, H., Tyrakowska, B., Rietjens, Y.M.C.M. and Cenas, N. Prooxidant toxicity of polyphenolic antioxidants to HL-60 cells: description of quantitative structure- activity relationship. *FEBS Lett 462:* 392, 1999.

34. Guo, Q., Zhao, B., Shen, S., Hou, J. and Hu, J.X.W. ERS study on the structured-antioxidant activity relationship of tea catechins and their epimers. *Biochem Biophys Acta 1427:* 13, 1999.

35. Kagan, V.E. and Tyurina, Y.Y. Recycling and redox cycling of phenolic antioxidants. *Ann N Y Acad Sci 854:* 425, 1998.

36. Shraki, M., Hara, Y., Osawa, T., Kumon, H., Nakauma, T. and Kawakishi, S. Antioxidative and antimutagenic effect of theaflavins from black tea. *Mutat Res 323:* 29, 1994.

37. Ho, C.T., Chen, Q., Shi, H. and Rosen, R.T. Antioxidative effect of polyphenol extract prepared from various Chinese teas. *Prev Med 21:* 520, 1992.

38. Lin, Y.L., Juan, I.M., Chen, Y.L., Liang, Y.C. and Lin, J.K. Composition of polyphenols in fresh tea leaves and association of their oxygen- radical-absorbing capacity with antiproliferative actions in fibroblast cells. *J Agric Food Chem 44:* 1387, 1996.

39. Halder, J. and Bhaduri, A. Protective role of black tea against oxidative damage of human red blood cells. *Biochem Biophys Res Commun 244:* 903, 1998.

40. Sarkar, A. and Bhaduri, A. Black tea is a powerful chemopreventor of reactive oxygen and nitrogen species: Comparison with its individual catechin constituents and green tea. *Biochem Biophys Res Commun 284:* 173, 2001.

41. Rachmilewitz, D., Karmeli, F., Okon, E. and Bursztyn, M. Experimental colitis is ameliorated by inhibition of nitric oxide synthase activity. *Gut 37*: 247, 1995.

42. Neilly, P. J., Gardiner, K. R. and Rowlands, B. J. Experimental colitis is ameliorated by inhibition of nitric oxide synthase activity. *Gut 38*: 475, 1996.

43. Maity, S., Ukil, A., Karmakar, S., Datta, N., Chaudhuri, T., Vedasiromoni, J.R., Ganguly, D.K. and Das, P.K. Thearubigin, the major polyphenol of black tea ameliorates mucosal injury in trinitrobenzene sulfonic acid–induced colitis. *Eur J Pharmacol* 470: 103, 2003.

44. Klaunig, J.E. Xu, Y., Han, C., Kamendulis, L.M., Chen, J., Heiser, C., Gordon, M.S. and Mohler, E.R. The effect of tea consumption in oxidative stress in smokers and non-smokers. *Proc Soc Exp Biol Med 220:* 249, 1999.

45. Benzie, I.F., Szeto, Y.T., Strain, J.J. and Tomlinson, B. Consumption of green tea causes rapid increase in plasma antioxidant power in humans. *Nutr Cancer 34:* 83, 1999.

46. Mates, J.M. and Sanchez-Jimenez, F.M. Role of reactive oxygen species in apoptosis : implications for cancer therapy. *Int J Biochem Cell biol 32:* 157, 2000.

47. Katiyar, S. and Mukhtar, H. Tea in chemoprevention of Cancer : Epidemiologic and experimental studies (Review). *Int J Oncol 8:* 221, 1996.

48. Catteral, F., Copeland, E., Clifford, M.N. and Ioannides, C. Contribution of theaflavins to the antimutagenicity of black tea: Their mechanism of action. *Mutagenesis 13:* 631, 1998.

49. Yamada, J. and Tomita, Y. Antimutagenic activity of water extracts of black tea and oolong tea. *Biosci Biotech Biochem 12 1:* 2197, 1994.

50. Yen, G.C. and Chen, H.Y. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J Agric Food Chem 43:* 27, 1995.

51. Kuroda, Y. and Hara, Y. Antimutagenic and anticarcinogenic activity of tea polyphenols. *Mutat Res 436:* 69, 1999.

52. Surono, I.S. and Hosono, A. Bacterial mutagenicity of terasi and antimutagenicity of Indonesian Jasmine tea against terasi. *Int J Food Microbiol 32:* 49, 1996.

53. Bu-Abbas, A., Copeland, E., Clifford, M.N., Walker, R. and Ioannides, C. Fractionation of green tea extracts : Correlation of antimutagenic effect with flavanol content. *J Sci Agric 75:* 453, 1997.

54. Hour, T.C., Liang, Y.C., Chu, I.S. and Lin, J.K. Inhibition of eleven mutagens by various tea extracts, (-) epigallocatechin –3- gallate, gallic acid and caffeine. *Food Chem Toxicol 37:* 569, 1999.

55. Steele, V.E., Kelloff, G.J., Balentine, D., Boone, C.W., Mehta, R., Bagheri, D., Sigman, C.C., Zhu, S. and Sharma, S. Comparative chemopreventive mechanisms of green tea, black tea and selected polyphenol extracts measured by *in vitro* bioassays. *Carcinogenesis 21:* 63, 2000.

56. Yoshioka, H., Akai, G., Yoshinaga, K., Hasegawa, K. and Yoshioka H. Protective effect of a green tea percolate and its main constituents against gamma ray induced scission of DNA. *Biosci Biotech Biochem* 60**:** 117, 1996.

57. Gupta, S., Chaudhuri, T., Seth, P., Ganguly, D.K. and Giri, A.K. Antimutagenic effects of black tea (World Blend) and its two active polyphenols, theaflavins and thearubigins in Salmonella assays. *Phytother Res 16:* 655, 2002.

58. Weisburger, J.H., Hara, Y., Dolan, L., Luo, FQ., Pittman, B. and Zang, E. Tea polyphenols as inhibitors of mutagenicity of major classes of carcinogens. *Mutat Res 371:* 57, 1996.

59. Shiraki, M., Hara, Y., Osawa, T., Kumon, H., Nakayama, T. and Kawakishi, S. Antioxidative and antimutagenic effect of theaflavins from black tea. *Mutat Res 323:* 29, 1994.

60. Apostolides, Z., Balentine, D.A., Harbowy, M.E., Hara, Y. and Weisburger, J.H. Inhibition of PhIP mutagenicity by catechins and by theaflavins and gallate esters. *Mutat Res 371:* 167, 1997.

61. Imanishi, H., Sasaki, Y.F., Ohta, T., Watanabe, M., Kato, T. and Shirasu, Y. Tea tannin components modify the induction of sister chromatid exchanges and chromosome aberrations in mutagen-treated cultured mammalian cells and mice. *Mutat Res 259:* 79, 1991.

62. Gupta, S., Chaudhuri, T., Ganguly, D.K. and Giri, A.K. Anticlastogenic effects of black tea (World Blend) and its two active polyphenols theaflavins and thearubigins *in vivo* in Swiss albino mice. *Life Sci 69*: 2735, 2001.

63. Fujiki, H. Two stages of cancer prevention with green tea. *J Cancer Res Clin Oncol 125:* 589, 1999.

64. Gordon, M.H. Dietary antioxidants in disease prevention. *Natural Prod Rep 13:* 265, 1996.

65. Lin, J.K., Liang, Y.C. and Lin-shiau, S.Y. Cancer chemoprevention by tea polyphenols through mitotic signal transduction blockade. *Biochem Pharmacol 58:* 911, 1992.

66. Nomura, M., Ma, W.Y., Yang, C.S., Bowden, G.T., Miyamoto, K. and Dong, Z. Inhibition of ultraviolet B induced AP-1 activation by theaflavins from black tea. *Mol Carcinog 28:* 148, 2000.

67. Chen, Y.C., Liang, Y.C., Lin-shiau, S.Y., Ho, C.T. and Lin, J.K. Inhibition of TPA induced protein kinase C and transcription activator protein1 binding activities by theaflavin-3, 3'- digallate from black tea in NIH3T3 cells. *J Agric Food Chem 47:* 1416, 1999.

68. McNamee, D. Olives, tea and tamoxifen—new and not so new ways to prevent cancer. *Lancet 355 :* 729, 2000.

69. McCarty, M.F. Polyphenol-mediated inhibition of AP-1 transactivating activity may slow cancer growth impeding angiogenesis and tumor invasiveness. *Med Hypotheses 50:* 511, 1998.

70. Chung, J.Y., Huang, C., Meng, X., Dong, Z. and Yang, C. Inhibition of activator protein 1 activity and cell growth by purified green tea and black tea polyphenols in H-*ras*-transformed cells: Structure-activity relationship and mechanisms involved. *Cancer Res 59:* 4610, 1999.

71. Yang, C.S. and Wang, Z.Y. Tea and cancer: Review. *J Natl Cancer Inst 85:* 1038, 1993.

72. Wang, Z.Y., Huang, M.T., Chang, R., Ma, W., Ferraro, T., Reuth, K.R., Yang, C.S. and Conney, A.H. Inhibitory effect of green tea and the growth of established skin papillomas in mice. *Cancer Res 52:* 6657, 1992.

73. Wang, Z.Y., Huang, M.T., Ferraro, T., Wong, C.Q., Lou, Y.R., Reuth, K., Iatropoulos, M., Yang, C.S. and Conney, A.H. Inhibitory effect of green tea in the drinking water on tumorigenesis by ultraviolet light and 12-0-tetradecanoylphorbol-13-acetate in the skin of SKH-1 mice. *Cancer Res 52:* 1162, 1992.

74. Conney, A.H., Lu, Y-P., Lou, Y-R., Xie, J-G. and Huang, M.T. Inhibitory effect of green tea and black tea on tumour growth. *Proc Soc Exper Biol Med 220:* 229, 1999.

75. Katiyar, S.K. and Mukhtar, H. Inhibition of phorbol ester tumor promoter 12-o-tetradecanoylphorbol -13-acetate-caused inflammatory responses in SENCAR mouse skin by black tea polyphenols. *Carcinogenesis 18:* 1991, 1997.

76. Wang, Z.Y., Huang, M.T., Lou, Y.R., Xie, J.G., Reuhl, K.R., Newmark, H.L., Ho, C.T., Yang, C.S. and Conney, A.H. Inhibitory effect of black tea, green tea, decaffeinated black tea, and decaffeinated green tea on ultraviolet B light-induced skin carcinogenesis in 7,12-dimethylbenz[a]anthracene-initiated SKH-1 mice. *Cancer Res 54:* 3428, 1994.

77. Lu, Y.P., Lou, Y.R., Xie, J.G,. Yen, P., Huang, M.T. and Conney, A.H. Inhibitory effect of black tea on the growth of established skin tumours in mice : Effect on tumour size, apoptosis, mitosis, bromodeoxyuridine incorporation into DNA. *Carcinogenesis 18:* 2163, 1997.

78. Schut, H.A. and Yoa, A.R. Tea is a potential chemopreventive agent in (Ph1P) carcinogenesis: Effect of green tea and black tea on Ph1P-DNA adduct formation in female F-34 rats. *Nutr and cancer 36:* 52, 2000.

79. Fujiki, H., Suganuma, M., Okabe, S., Sueoka, E., Suga, K., Imai, K., Nakachi, K. and Kimura, S. Mechanistic findings of green tea as cancer preventive for humans. *Proc Soc Exp Biol Med 220:* 225, 1999.

80. Katiyar, S.K., Agarwal, R. and Mukhtar, H. Protection against malignant conversion of chemically induced benign skin papillomas squamous cell carcinomas in SENCAR mice by a polyphenolic fraction isolated from green tea. *Cancer Res 53:* 5409, 1993.

81. Das, M., Sur, P., Gomes, A., Vedasiromoni, J.R. and Ganguly, D.K. Inhibition of tumour growth and inflammation by consumption of tea. *Phytother Res 16:* S40, 2002.

82. Wynder, E.L. and Hoffman, D.H. Smoking and lung cancer: Scientific challenges and opportunities. *Cancer Res 54:* 5284, 1994.

83. Young, C.S., Chen, L., Lee, M.J. and Landau, J.M. Effects of tea on carcinogenesis in animal model and humans. *Adv Exp Med Biol 401:* 51, 1996.

84. Xu, Y., Ho, C.T., Amin, S.G., Han, C. and Chung, F.L. Inhibition of tobacco specific nitrosomine induced lung tumerogenesis in A/J mice by green tea and its major polyphenol and oxidants. *Cancer Res 52:* 3875, 1992.

85. Hecht, S.S. and Hoffmann, D. Tobacco-specific nitrosamines, an important group of carcinogens in tobacco and tobacco smoke. *Carcinogenesis 9:* 875, 1998.

86. Cao, J., Xu, Y., Chen, J. and Klaunig, J.E. Chemopreventive effects of green and black tea on pulmonary and hepatic carcinogenesis. *Fund Appl Toxicol 29:* 244, 1996.

87. Yang, C.S., Kim, S., Yang, Y.H., Lee, M.J., Liao, J., Chung, J.Y. and Ho, C.T. Inhibition of carcinogenesis by tea: Bioavailability of tea polyphenols and mechanism of action. *Proc Soc Exp Biol Med 220:* 213, 1999.

88. Landau, J.M., Wang, Z.Y., Ding, W. and Yang, C.S. Inhibition of spontaneous formation of lung tumors and rhabdomyosarcomas in A/J mice by black and green tea. *Carcinogenesis. 19:* 501, 1998.

89. Huang, M.T., Xie, J.G., Wang, Z.Y., Ho, C.T., Lou, Y.R., Wang, C.X., Hard, G.C., and Conney, A.H. Effects of tea, decaffeinated tea, and caffeine on UVB light-induced complete carcinogenesis in SKH-1 mice:demonstration of caffeine as a biologically important constituent of tea. *Cancer Res 57:* 2623, 1997.

90. Liu, Q., Wang, Y., Crist, K.A., Wang, Z.Y., Lou, Y.R., Huang, M.T., Conney, A.H. and You, M. Molecular epidemiology and cancer prevention. Effect of green tea on p53 mutation distribution in ultraviolet B radiation-induced mouse skin tumors. *Carcinogenesis 19:* 1257, 1998.

91. Liao, S., Umekita, Y., Guo, J., Kokontis, J.M. and Hiipakka, R.A. Growth inhibition and regression of human prostate and breast tumours in athymic mice by tea epigallocatechin gallate. *Cancer lett 96:* 239, 1995.

92. Kao, Y.H., Hipkka, R.A. and Liao, S. Modulation of endocrine systems and food intake by green tea epigallocatechin gallate. *Endocrinology 141:* 980, 2000.

93. Wiesburger, J.H., Rivenson, A., Reihardt, J., Aliaga, C., Braley, J., Pittman, B. and Zang, E. Effect of black tea on azoxymethane-induced colon cancer. *Carcinogenesis 19:* 229, 1998.

94. Dashwood, R., Xu, M., Hernaez, J.F., Hasaniya, N., Youn, K. and Razzuk, A. Cancer chemopreventive mechanisms of tea against heterocyclic amine mutagens from cooked meat. *Proc Soc Exp Biol Med 220:* 239, 1999.

95. Steele, V.E., Bagheri, D., Balentine, D.A., Boone, C.W., Mehta, R., Morse, M.A., Sharma, S., Sigman, C.C., Stoner, G.D., Wargovich, M.J., Weisburger, J.H., Zhu, S. and Kellof, G.J. Preclinical efficacy studies of green and black tea extracts. *Proc Soc Exp Biol Med 220:* 210, 1990.

96. Dreosti, I.E., Wargovich, M.J. and Yang, C.S. Inhibition of carcinogenesis by tea: The evidence from experimental studies. *Crit Rev Food Sci Nutr 37:* 761, 1997.

97. Challa, A., Rao D.R. and Reddy, B.S. Interactive suppression of aberrant crypt foci induced by azoxymethane in rat colon by phytic acid and green tea. *Carcinogenesis 18,* 2023, 1997.

98. Xu, M., Baily, A.C., Hernaez, J.F., Taoka, C.R., Schut, H.A.J. and Dashwood, R.H. Protection by green tea, black tea, and indole-3-carbinol against 2-amino-3-methylimidazo [4,5-f]quinoline-induced DNA adducts and colonic aberrant crypts in the F344 rat. *Carcinogenesis 17:* 1429, 1996.

99. Rogers, A.E., Hafer, L.J., Iskander, Y.S. and Yang, S. Black tea and mammary gland carcinogenesis by 7, 12-dimethylbenz[a]anthracene in rats fed control or high fat diets. *Carcinogenesis 19:* 1269, 1998.

100. Hirose, M., Mizoguchi, Y., Yaono, M., Tanaka, H., Yamaguchi, T. and Shirai, T. Effects of green tea catechins on the progression or late promotion stage of mammary gland carcinogenesis in female Sprague-Dawley rats pretreated with 7,12-dimethylbenz(a)anthracene. *Cancer Lett 112:* 141, 1997.

101. Kohlmeier, L., Wetering, K.G.C., Steck, S. and Kok, F.J. Tea and Cancer prevention: An evaluation of the epidemiological literature. *Nutr Cancer 27:* 1, 1997.

102. Blot, W.J., McLaughlin, J.K. and Chow, W.H. Cancer rates among drinkers of black tea. *Crit Rev Sci Nutr* 739, 1998.

103. Bushman, J.L. Green tea and cancer: A review of literature. *Nutr Cancer 31:* 151, 1998..

104. Correa, P. Human gastric carcinogenesis: A multifunctional process. First American Cancer Society Award Lecture on Cancer Epidemiology and prevention. *Cancer Res 52:* 6735, 1992.

105. Chen, W., Weisburger, J.H., Fiala, E.S., Spratt, T.E., Carmella, S.G., Chen, D. and Hecht, S.S. Gastric carcinogenesis: 2-chloro-4-methylthiobutanoic acid, a novel mutagen in salted, pickled Sanma hiraki fish or similarly treated methionine. *Chem Res Toxicol 9:* 58, 1996.

106. Hwang, H., Dwyer, J. and Russell, R.M. *Helicobacter pylori* infection, food preservation and gastric cancer risk: Are there new roles for preventive factors? *Nutr Rev 52:* 75, 1994.

107. Weisburger, J.H. Tea and health : The underlying mechanisms. *Proc Soc Exp Biol Med 220:* 271, 1999.

108. Diker, K.S. and Hascelik, G. The bacterial activity of tea against *Helicobactor pylori. Lett Appl Microbiol* 299, 1994.

109. Ernst, P. The role of inflammation in the pathogenesis of gastric cancer. *Aliment Pharmacol Ther 13* (Suppl 1):13, 1999.

110. Weisburger, J.H., Nagao, M., Wakabayashi, K. and Oguri, A. Prevention of heterocyclic amine formation by tea and tea polyphenols. *Cancer Lett 83:* 143, 1994.

111. Xu, M., Bailey, A.C., Hernaez, J.F., Taoka, C.R., Schut, H.A.C. and Dashwood, R.H. Protection by green tea, black tea and indole-3-carbinol against 2-amino-3-methylimidazo[4,5]quinoline induced DNA adducts and colonic aberrant crypts in the F344 rat. *Carcinogenesis 17:* 1429, 1996.

112. Katiyar, S.K., Mohan, R.R., Agarwal, R. and Mukhtar, H. Protection against induction of mouse skin papilomas with low and high risk of conversion of malignancy by green tea polyphenols. *Carcinogenesis 18:* 497, 1997.

113. Suganuma, M., Okabe, S., Sueoka, N., Matsuyama, S., Imai, K., Nakachi, K. and Fujiki, H. Green tea and cancer chemoprevention. *Mutat Res 428:* 339, 1999.

114. Nakachi, K., Suemasu, K., Suga, K., Takeo, T., Imai, K. and Higashi, Y. Influence of drinking green tea on breast cancer malignancy among Japanease patients. *Jpn J Cancer Res 89:* 254, 1998.

115. Imai, K., Suga, K. and Nakachi, K. Cancer-preventive effects of drinking green tea among a Japanese population. *Prev Med 26:* 769, 1997.

116. Collins, A.R. Molecular epidemiology in cancer research. *Mol Aspects Med 19:* 359, 1998.

117. Li, N., Sun, Z., Han, Z.C. and Chen ,J. The chemopreventive effects of human oral precancerous mucosa lesions. *Proc Soc Exp Biol Med 220:* 218, 1999.

118.Mukhtar, H. and Ahmed, N. Mechanism of cancer chemopreventive activity of green tea. *Proc Soc Exp Biol Med 220:* 234, 1999.

119.Fujiki, H., Suganuma, M., Okabe, S., Komori, A., Sueoka, E., Sueoka, N., Kosu, T. and Sakai, Y. Japanese green tea as a cancer preventive in human. *Nutr Rev 54:* S67, 1996.

120.Sugiyama, T. and Sadzuka, Y. Combination of theanine with doxorubicin inhibits hepatic metastasis of M5076 ovarian sarcoma. *Clin Cancer Res 5:* 413, 1999.

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