REVIEW

Tea and Cancer Chemoprevention: A Comprehensive Review

Yogeshwer Shukla

Abstract

Dietary components that are capable of inhibiting the growth of cancer cells without affecting the growth of normal cells are receiving considerable attention in developing novel cancer-preventive approaches. Tea, made from young leaves and leaf buds of the tea plant, '*Camellia sinensis*', and the world's second most consumed beverage, has received a great deal of attention both from the general public and the scientific community because tea polyphenols are strong antioxidants, and tea preparations have inhibitory activity against tumorigenesis. Besides this, the wide spread consumption of tea throughout the world evoked the interest of the scientific community in the possibility of its use in cancer prevention. There are three main types of tea, all coming from the tea plant viz. black tea (fermented,) green tea (unfermented), or oolong tea (semi-fermented), classified based on the methods of brewing and processing. Inhibition of tumorigenesis by green or black tea preparations has been demonstrated in various animal models in different organs. Various epidemiological studies substantiate the correlation between tea consumption and cancer prevention; however, they have not yielded clear conclusions pertaining to the protective effects of tea consumption against cancer development in humans. Many mechanisms have been proposed for the inhibition of carcinogenesis by tea, including the modulation of signal transduction pathways (including growth factor-mediated, mitogen-activated protein kinase (MAPK)-dependent, and ubiquitin/proteasome degradation pathways) that lead to the inhibition of cell proliferation and transformation; induction of apoptosis of preneoplastic and neoplastic cells, and inhibition of tumor invasion as well as angiogenesis. These mechanisms need to be evaluated, verified and corroborated in animal models and humans in order to gain more understanding on the effects of tea consumption on human cancer. Because the causative factors are different for different populations, tea consumption may affect carcinogenesis only in selected situations rather than having the general effect on all cancers. Although, on the basis of many epidemiological observations and numerous laboratory studies, it can be concluded that tea consumption is likely to have beneficial effects in reducing cancer risk in different populations, yet there is a need to define the population that could benefit from tea consumption. After careful evaluation of additional studies, it may be possible to recommend consumption of tea polyphenols by humans. Although considerable accumulating information provides a compelling body of evidence for the preventive potential of tea against cancer, naturally occurring tea polyphenols have yet to be evaluated in clinical intervention in human trials.

Key Words: Tea - cancer prevention - animal models - epidemiological studies - clinical trials

Asian Pacific J Cancer Prev, **8**, 155-166

Introduction

Tea, the world's second most consumed beverage, is made from young leaves and leaf buds of the tea plant, "*Camellia sinensis*"--a species of evergreen shrub of Theaceae family. There are three main types of tea, all coming from the tea plant viz. black tea (fermented,) green tea (unfermented), or oolong tea (semi-fermented), classified based on the methods of brewing and processing. On an average about 2 million metric tons of tea is produced worldwide. About 20% of the total tea manufactured is green tea, which is mainly consumed in Asian and Middle East countries; 78% is black tea which is predominantly consumed in Western and some Asian countries: the other 2% is oolong tea, which is produced and consumed in southeastern China (Katiyar et al., 1992a).

Green and black teas have similar chemical make-ups, the primary difference between the two being in the chemical changes that take place during their production. Among the components, polyphenols are most important, as they constitute approximately 36 per cent of the dry weight of tea. Other components of fresh green tea leaf include methylxanthines (including caffeine), proteins and amino acids, carbohydrates, lipids, vitamins (including B-vitamins) and minerals. Tea also contains fluoride, traces of vitamins A, K, C, β-carotene and B vitamins (among them folate). Green tea contains polyphenols,

Environmental Carcinogenesis Division, Industrial Toxicology Research Centre, Mahatma Gandhi Marg, Lucknow 226001,India
Fax No. (+91) 522-2628227 Phone: (+91) 522-2506755 Email: yogeshwer_shukla@hotmail.com ys.yshukla@gmail.com

including flavonols, flavondiols, flavonoids and phenolic acid.

According to Weisburger "tea, which is the second most consumed beverage in the world next to water, has great potential to help reduce the incidence of major diseases worldwide, especially when combined with a healthy lifestyle," (Weisburger, 1997). The antioxidant properties of tea can be attributed to polyphenols (Katiyar et al., 1997), which neutralize free radicals that can damage the body's cells leading to disease.

Tea and Cancer Prevention

Perhaps the most surprising result of recent research is the discovery that tea may serve to protect against skin cancer. In a recent study by several researchers at Rutgers, it was revealed that tea consumption inhibited the formation of tumors associated with the exposure to UVB sunrays. Where UVB tumors already existed, tea drinking slowed their growth and in some cases, actually decreased their size (Wang et al., 1992b). In yet another study, researchers applied green tea compounds directly to the skin and then exposed it to ultraviolet rays. It was seen that the skin protected by tea compounds suffered significantly less damage than unprotected skin (Katiyar, 2003).Besides skin cancer tea may also work against the development of oral cancer. In China, patients with precancerous mouth lesions -- a warning sign in the growth of oral cancer -- were treated with a combination of black and green tea. Those treated with the tea compounds showed a significant decrease in abnormal cell growth (Lee, 2004). Lesions present at the beginning of the study actually shrank in size and no new lesions appeared. Tea administration also appears to slow the growth of lung cancer in mice and rats exposed to carcinogens from tobacco. Researchers speculate that the heavy smoking populations in Great Britain may be offset to some degree by an equally heavy consumption of tea. Studies have also revealed an association between heavy tea drinking and a reduced risk of digestive and urinary tract cancers.

Tea Consumption, Composition and Chemistry

Consumption

Tea plant is the native of Southeast Asia and is grown in about 30 countries but is consumed worldwide, with a per capita consumption of approximately 0.12 liter per year. Although the largest total consumption of tea is registered in India (540,000 metric ton, 620 g per capita), Ireland has the largest per capita consumption of tea (3220 g).

Composition

The composition of tealeaf varies with climate, season, horticulture practices, variety of the plant, and age of the leaf, i.e. the position of leaf on the harvested shoot. There are three main types of tea – green, black and oolong tea. These come from the same plant, but are treated differently: green tea is heated soon after picking and is not subjected to further processing; black tea, on the other hand, is dried and then exposed to the air before it is heated i.e. oxidized. Consequently, green and black teas differ noticeably in appearance, taste and chemical composition. Table 1 shows principal polyphenolic components present in typical green and black tea beverages, but variation may be considerable. Oolong tea composition in general falls between that of green and black tea.

Table 1. Polyphenolic Composition of Green and Black Tea (%w/w)

Constituents	Green Tea	Black Tea
Catechins	30-42	3-10
Flavanols	5-10	6-8
Other flavonoids	2-4	-
Theagallin	2-3	-
Gallic acid	0.5	-
Quinic acid	2.0	-
Theanine	4-6	-
Methylxanthines	7-9	8-11
Theaflavins	-	3-6
Thearubigins	-	12-18

Green Tea: During manufacturing of green tea, preservation of the intact green leaf is of utmost importance. Green teas are not fully fermented like black teas, or partially fermented as oolongs. Instead, the tea leaves are plucked, steamed or pan fried, (which removes the fermentation enzymes), rolled, and then dried. This process yields a chemical composition in green tea similar to the fresh tealeaf. Green teas are generally produced in two different varieties, white tea and yellow tea, the latter being less fermented because of a process known as wilting. Green tea has a high content of vitamins and minerals including ascorbic acid (vitamin C), which is present in amounts comparable to a lemon and several B vitamins, which are water-soluble and quickly released into a cup of tea. Five cups of green tea a day will provide 5-10% of the daily requirement of riboflavin, niacin, folic acid, and pantothenic acid. The same five cups of green tea also provide approximately 5% of the daily requirement of magnesium, 25% of potassium, and 45% of the requirement for manganese. Green tea is also rich in fluoride content. A single cup of green tea provides approximately 0.1 mg of fluoride, which is higher than in fluorinated water.

Black tea : In the process of manufacturing the black tea, the harvested leaves are allowed to wither. Known as 'withering', this process softens up the tea leaves. Next the leaves are rolled (crushed). After the leaves have been crushed, they often bunch together in balls and must be unrolled so as to allow the entire surface of leaf to be exposed to air for an even fermentation. Once the fermentation process has come to end, the leaves are then Fired (exposed to hot air and temperatures). The firing process actually causes the end of the fermentation cycle by killing the bacteria and enzymes. Once fired the black tea is then ready for consumption.

Oolong tea : Being an intermediate between black and green tea – oolong tea is partially fermented. The leaves are partially withered, then allowed to ferment immediately. The leaves are then fired, rolled, and then allowed to partially ferment again. Oolong tea extracts

contain catechins at a level of 8 to 20% of the total dry matter. The fermentation process results in the oxidation of simple polyphenols, giving oolong tea its characteristic color and flavors (Harbowy and Balentine, 1997).

Chemistry

Steaming or drying fresh tea leaves at elevated temperatures makes commercial green tea. Its chemical composition is similar to that of fresh tealeaves. Green tea polyphenols may account for up to 30% of the dry weight (Balentine 1997). Most of the green tea polyphenols are flavonols, commonly known as catechins. Some major green tea catechins are (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), (-)-epicatechin (EC). (+)-gallocatechin, and(+)-catechin (Graham, 1992).

Caffeine, theobromine, and theophylline, the principal alkaloids, account for about 4% of the dry weight. In addition, there are phenolic acids such gallic acids and characteristic amino acids such as theanine. A cup (200 mL) of green tea (Gun Powder, Hangzhou, China) contains about 142 mg EGCG, 65 mg EGC, 28 mg ECG, 17 mg EC, and 76 mg caffeine.

During the manufacture of black tea, the monomeric flavan-3-ols undergo polyphenol oxidase-dependent oxidative polymerization leading to the formation of bisflavanols, thea-flavins, thearubigins, and other oligomers in a process commonly known as "oxidation". Theaflavins (about 1%-2% of the total dry matter of black tea), including theaflavin, theaflavin-3-O-gallate, theaflavin-3'-O-gallate, and theaflavin-3, 3'-O-digallate, possess benzotropolone rings with dihydroxy or trihydroxy substitution systems, which give the characteristic color and taste of black tea. About 10%-20% of the dry weight of black tea is due to thearubigens, which are more extensively oxidized and polymerized, have a wide range of molecular weights, and are less well characterized. Oolong tea, a partially oxidized tea, contains monomeric catechins, theaflavins, and thearubigins along with some characteristic components, like epigallocatechin esters, theasinensins, dimeric catechins, and dimeric proanthocyanidins. The flavanols are easily oxidized to the corresponding O-quinones. During enzyme oxidation or non-enzyme oxidation, including autoxidation or coupled oxidation, tea flavanols may undergo oxidative condensation via either C-O or C-C bond formation in oxidative polymerization reactions. In addition, tea polyphenols effectively interact with reactive oxygen species. Tea polyphenols also have high complex formation affinity with metals, alkaloids, and biologic macromolecules such as lipids, carbohydrates, proteins, and nucleic acids.

Sometimes tea constituents are described using the term "tannins". In industrial and botanic literatures, tannins are characterized as plant materials that give a blue color with ferric salts and produce leather from hides. Monomeric flavanols, the major components in green tea, are precursors of condensed tannins. It would be more appropriate to use the term "tea polyphenols" or "tea flavanols" because they are quite distinct from commercial tannins and tannic acid.

Tea and Cancer Chemoprevention: Epidemiological Studies

The wide spread consumption of tea throughout the world evoked the interest of the scientific community in the possibility of its use in cancer prevention. Epidemiological studies suggest that drinking tea offers protection against various cancers in humans, but evidence is contradictory. Some problems with studies are cultural variables, including diet, that make it difficult to determine the absolute effects of tea. Although experimental studies have shown consistently that tea preparations and tea polyphenols may inhibit the induction of a variety of cancers, yet, epidemiological studies of tea consumption and cancer are limited and the results are inconclusive. Accumulating more epidemiological data is required to draw a conclusion.

Cohort studies of tea drinkers and case control studies of specific cancers show mixed results. Differences in tea drinking habits are likely to vary between populations and could contribute to the inconsistencies found between studies comparing tea consumption and cancer risk. This section provides a general overview of the pertinent epidemiological studies on tea consumption and cancer prevention at different sites. It is worthwhile mentioning that a few studies have shown either no effect or enhanced effect of tea consumption on susceptibility of developing cancer.

Cancer of the esophagus

Research shows that tea may be a promising weapon in the fight against cancers of the stomach, bladder, esophagus, and prostate. In Linzhou, which is a high-incidence area for esophageal cancer in northern China, it was seen that tea consumption is very rare. In a follow-up study, decaffeinated green tea was not shown to have any beneficial effects in alleviating esophageal precancerous lesions and abnormal cell proliferation patterns (Wang et al. 2002). Thus, the epidemiological studies conducted in China revealed that most of the areas with higher esophageal cancer mortality rates are the northern provinces, where tea is neither produced nor frequently consumed. Another case control study, (Gao et al., 1994) showed that consumption of green tea reduces the risk of esophageal cancer. The daily consumption of tea was found to be associated with a surprising figure of over 50% lower risk of cancer.

Cancer of the nasopharynx and stomach

In three case control studies (Henderson et al., 1976; Lin et al., 1973; Shanmugaratnam et al., 1978), no correlation between tea consumption and nasopharyngeal cancer was observed. Since stomach cancer is the second most common form of cancer worldwide, extensive research has gone into searching for cures and treatments thereof. One such study, conducted in China (Setiawan, 2001), was aimed at investigating the effect of green tea consumption on chronic gastritis and the risk of stomach cancer. The sample included 133 stomach cancer cases, 166 chronic gastritis cases and 433 healthy controls.

Results elucidated an inverse association between green tea consumption and both the diseases. Furthermore, dose-response relationships were observed, and years of green tea consumption proved to be more effective in combating both stomach cancer and chronic gastritis. In yet another case control study in Huaian City of Jiangsu Province, China, 153 cases of stomach cancer patients and 223 population-based controls were analyzed and was found that tea consumption reduced the risk of stomach cancers (Gao et al., 2002).

Cancer of the bladder, kidney and urinary tract

The Iowa Women's Health Study, which surveyed about 35,369 postmenopausal women, showed those who drank two or more cups of black tea per day had a decreased risk of digestive and urinary tract cancers (Zheng et al., 1995). In a cohort study, positive correlation was found between tea consumption and kidney cancer (Simon et al., 1975).The association between usual adult tea consumption and risk of bladder and kidney cancers in another population was analyzed, which included 1452 bladder cancer cases, 406 kidney cancers cases, and 2434 controls. For bladder cancer, the age and sex adjusted odds ratios were: 0.9 for <1.0 cup/day, 1.0 for 1.0-2.6 cups/day, and 0.9 for >2.6 cups/day. People consuming >5 cups/day (>90th percentile) showed a considerably decreased risk (Bianchi et al., 2000). Another case control study in Minneapolis-St. Paul area (McLaughlin et al., 1984) indicated that heavy tea drinking (>3 cups/day) had a positive association with renal cell carcinoma in women but not in men.

Cancer of colon, rectum and uterus

Several studies (Armstrong et al., 1976; D'Avaanzo et al., 1992; Goodman et al., 1986; Nomura et al., 1991; Yu et al., 1986) elucidated that the consumption of tea decreased the risk of rectal cancer while several other studies (Dales et al., 1979; Miller et al., 1983; Morgan and Jain, 1974; Phillips and Snowdon, 1985; Inoue et al., 1998) revealed that negative correlation existed between the two. However, there exists an inconsistency in the findings and further more studies are warranted to draw conclusive results.

Cancer of the prostate

Epidemiological studies seeking an association between green tea and prostate cancer should be undertaken to establish the validity of cell culture and animal data pertaining to prostate cancer patients. Various studies show that people who consumed tea have lower risk of prostate cancer (Heilbrun et al., 1986; Jain et al., 1998). In a cohort study employing 7833 men of Japanese ancestry living in Hawaii, a weaker significant negative association between black tea intake (more than one cup per day) and prostate cancer incidence was observed. Another case-control study conducted in three different geographical areas of Canada (Jain et al., 1998) suggested a decreased risk of prostate cancer with intake of more than two cups of tea per day. To substantiate the findings, in concordance with chemoprevention, a population at a higher risk for prostate cancer development should be taken into consideration.

Cancer of the liver, lung, breast and pancreas

A recent Japanese study showed that 472 women with breast cancer who regularly drank green tea prior to diagnosis had less severe cases than women who did not consume green tea (Nakachi et al., 1998). Another study, including 8,000 women, revealed that drinking 10 cups or more of green tea daily delayed cancer onset. Stage I and II breast cancer patients who consumed 5 or more cups/day experienced lower recurrence with longer disease-free period compared to occasional tea drinkers (Fujiki, 1999). This suggests that consumption of green tea could play a role both in cancer prevention and as an adjunct to chemotherapy. In Japan, a large-scale study was carried out from 1990 to 1995 including 1,706 histologically diagnosed cases of digestive tract cancers and a total of 21,128 non-cancer outpatients. It elucidated that high intake of green tea (7 or more cups/day) reduced the odd ratio of stomach cancer to 0.69 (95% confidence interval) (Inoue et al., 1998).

To speculate the association between black tea consumption and the subsequent risk of stomach, colorectal, lung and breast cancers in the Netherlands Cohort Study on Diet and Cancer was conducted. The findings showed that the risk among tea drinkers in each consumption category was similar to that among nondrinkers (Goldbohm et al., 1996). Therefore, the contemplation that the intake of black tea protects against four of the major cancers in humans cannot be substantiated. However, the study conducted on cohort of 33,976 white Iowa women with 9 years of follow-up showed that tea consumption is not related to the incidences of cancer (Harnack et al., 1997). Hence, on the basis of available data from epidemiological studies and animal testing, it can be concluded that tea consumption has preventive effects against the risk of cancer .

Tea and Cancer- Laboratory Studies

Tea has received a great deal of attention both from the general public and the scientific community because tea polyphenols are strong antioxidants, and tea preparations have inhibitory activity against tumorigenesis. The bioavailability and biotransformation of tea polyphenols, however, are key factors limiting these activities *in vivo*. The inhibition of tumorigenesis by green or black tea preparations has been demonstrated in animal models on different sites such as skin, lung, oral cavity, esophagus, forestomach, stomach, small intestine, colon, pancreas, and mammary gland. Epidemiological studies, however, have not yielded clear conclusions pertaining to the protective effects of tea consumption against cancer development in humans. The discrepancies between the results from humans and animal models could be due to: (1) the much higher doses of tea used in animals in comparison to human consumption, (2) the differences in causative factors of cancer between humans and animals,

and (3) confounding factors limiting the potential of epidemiological studies to detect an effect. It is possible that tea may be only effective against specific types of cancer caused by certain etiological factors. Many mechanisms have been proposed for the inhibition of carcinogenesis by tea, including the modulation of signal transduction pathways that lead to the inhibition of cell proliferation and transformation; induction of apoptosis of preneoplastic and neoplastic cells, and inhibition of tumor invasion as well as angiogenesis. These mechanisms need to be evaluated, verified and corroborated in animal models and humans in order to gain more understanding on the effects of tea consumption on human cancer (Yang et al., 2002)

Prevention of skin tumorigenesis

Various experimental studies all over the world have demonstrated the inhibitory effects of tea against tumorigenesis in experimental models (Shukla et al., 2000; Katiyar et al., 1992a; Katiyar and Mukhtar, 1996; Mukhtar et al., 1994; Shanmugaratnam et al., 1978). Studies revealed that topical application of polyphenols isolated from green tea (GTP) to BALB/C (Wang et al., 1989) as well as oral feeding of GTP to BALB/C, CD-1 and SENCAR mice, result in significant prevention against the occurrence of skin tumors.

The potential of various tea components (polyphenols) has also been tested as cancer chemopreventive agents in a two-stage skin carcinogenesis study (DMBA/TPA) which exhibited significant reduction in skin tumor occurrence (Wang et al., 1989). Consistent with the above finding, topical application of EGCG prior to DMBA/TPA application offered significant protection against skin tumor formation in SENCAR mouse skin. In this study EGCG was found to reduce 30% carcinogen metabolite binding to epidermal DNA, suggesting that EGCG may be inhibiting the metabolism of the precarcinogen. Oral feeding of GTP (0.05%) in drinking water for 50 days prior to DMBA-TPA treatment resulted in significant prevention both in terms of tumor incidence and tumor multiplicity (Katiyar et al., 1992b).

Prevention of skin tumor promotion

Studies on the two-stage skin tumorigenesis protocol (Agarwal and Mukhtar, 1991; Katiyar and Mukhtar, 1996; Katiyar et al., 1992c) have shown that GTP offers significant prevention against DMBA-initiated and TPA-induced skin tumor promotion in SENCAR mouse. The pretreatment of GTP substantially lowered the frequency of tumor body burden, exhibited by a decrease in the total number of tumors per group, number of tumors per animal, tumor volume per mouse, and average tumor size, as compared with the animals that did not receive GTP. On similar lines, other studies have shown that topical application of GTP inhibits tumor promotion mediated by TPA and skin tumor promoters such as teleocidin and okadaic acid (Huang et al., 1992; Yoshizawa et al., 1987). Yoshizawa, (1996) has also shown the inhibitory effect of EGCG in a dose dependent manner prior to okadaic acid treatment.

Prevention of UVB radiation-induced photo-carcinogenesis

Ultraviolet b (UVB) radiation (280 to 320 nm) present in the solar spectrum is the major risk factor for skin cancer in humans (Elmets, 1991). Studies (Wang et al., 1992b) showed that the infusion of green tea extracts (1.25%, w/v) in water, used as a sole source of drinking fluid to mice offered substantial prevention against UVB radiation-induced intensity of red color and area of skin lesions, as well as UVB radiation-induced tumor initiation and tumor promotion. In another study (Wang et al., 1994) it was shown that black tea consumption by SKH-1 hairless mice reduced the tumor formation, which was initiated by DMBA and followed by multiple UVB exposure. These studies suggest that consumption of tea as a sole source of drinking fluid may reduce the risk of some forms of human cancers induced by solar UV radiation, while Gensler et al., (1996) assessed that the induction of skin tumors by UV radiation was significantly reduced by topical treatment but not by oral administration of EGCG through the mechanism distinct from inhibition of photoimmunosuppression.

Chatterjee et al., (1996) showed that a novel 32P postlabelling method was employed to detect UVB-induced DNA lesions in the epidermis and its prevention by topical application of GTP. Huang et al., (1997) showed an inhibitory effect of post -administered green and black tea on UVB-induced complete carcinogenesis, but the decaffeinated teas were either inactive at moderate dose levels or they enhanced the tumorigenic effect of UVB at high dose levels. However, the oral administration of caffeine was found to have an inhibitory effect on UVB-induced complete carcinogenesis. The study concluded that caffeine was a biologically important constituent of tea.

Effect on the regression and growth of established skin tumors

There is a compelling body of evidence suggesting that green tea possesses chemotherapeutic effects. The oral administration of black tea has been shown to modulate apoptosis, mitotic index, and incorporation of bromodeoxy uridine into DNA of tumors. The treatments also prevent the proliferation and enhanced apoptosis in malignant and nonmalignant tumors (Lu et al., 1997). Chemotherapy typically has side effects, and anything that can decrease the amount of drug administered while maintaining a tumor-inhibiting effect would most likely improve the quality of life for cancer patients. It was seen that when green tea and doxorubicin - a cancer treatment drug that prevents the development, growth and proliferation of malignant cells - were given together to mice with cancer, the inhibitory effect of doxorubicin was enhanced 2.5-fold (Sujiyama et al., 2004)

Prevention of intestinal and colon carcinogenesis

Orner et al (2002) compared the inhibitory effects of white and green teas with sulindac, a nonsteroidal anti-inflammatory agent, in two different mouse models of

intestinal tumorigenesis. In the Apc(min) mouse, white and green teas given at human-relevant concentrations and sulindac each suppressed polyp formation by approximately 50%. Moreover, the combination of white tea plus sulindac was more effective than either treatment alone. Expression of a beta-catenin/Tcf reporter was inhibited by EGCG in the transfected cells, and the beta-catenin/Tcf target genes cyclin D1 and c-jun were downregulated in vivo by tea plus sulindac treatment. Collectively, the data supports a chemopreventive role of tea and sulindac against intermediate and late stages of colon cancer, via effects on the beta-catenin/Tcf signaling pathway.

Prevention of fore stomach and lung tumorigenesis

Oral administration of green tea infusion to female A/J mice during the carcinogen treatment period, the N-nitrosodiethylamine (NDEA)-treatment period, or the post-NDEA period, induced fore stomach tumorigenesis (Wang et al., 1992a, 1994). On the contrary, Katiyar and Mukhtar (1993) have shown the cancer chemopreventive effects of chronic administration of green tea in several animal tumor models. They demonstrated that oral administration of a polyphenolic fraction of green tea (GTP) prior to challenge with carcinogen, afforded significant protection against both diethylnitrosamine (DEN)- and benzo(a)pyrene [B(a)P]-induced fore stomach and lung tumorigenesis in A/J mice. In the fore stomach tumorigenesis protocol, GTP afforded 71% and 66% protection against DEN- and B(a)P-induced tumor multiplicity respectively. In the case of lung tumorigenesis protocol, however, the protective effects of GTP were 41% and 39%, respectively. Histological examination of forestomach tumors showed significantly lesser number of squamous cell carcinoma formation in GTP-fed groups of mice as compared to carcinogen alone-treated controls. When pulmonary tumors were examined histologically, no adenocarcinomas were observed in GTP-fed groups as compared to 15% mice with adenocarcinomas in DEN and B(a)P alone-treated controls. The results of this study suggest that limited doses of GTP administration by gavage 30 min prior to carcinogen challenge may offer protection against carcinogen-induced tumorigenesis in internal body organs.

Prevention of esophageal tumorigenesis

Li et al (2003) determined the effects of hot water on N-nitrosomethylbenzylamine (NMBA)-induced rat esophageal tumorigenesis model. F344 rats were given one treatment of hot water and NMBA, or a combination treatment of NMBA plus hot water, or/and EGCG. The results revealed that the number of tumors and incidence of carcinomas were significantly increased in groups supplemented with hot water as compared with the group, which received NMBA injections only. Study further confirmed that the drinking of hot beverages increased the risk of esophageal carcinogenesis, and drinking hot tea will abolish the inhibitory effects of EGCG on this disease.

Wang et al (1995) investigated the effects of green tea and black tea, when given either during or after N-nitrosomethylbenzylamine (NMBzA) treatment, on esophageal tumorigenesis in male Sprague-Dawley rats. In the groups of rats receiving 0.6% of decaffeinated green tea (DGT) or decaffeinated black tea (DBT) (6 mg tea solids/ml) as the sole source of drinking fluid during the NMBzA-treatment period, esophageal tumor incidence and multiplicity were reduced by approximately 70%. When the tea preparations were given after the NMBzA treatment period, the esophageal papilloma incidence and multiplicity were reduced by approximately 50%. The volume per tumor was much smaller in rats that received black tea after the carcinogen treatment period. In a second experiment, the groups of rats receiving 0.9% regular green tea (RGT) or DGT after the NMBzA treatment period, showed decreased tumor multiplicity by > 55%. The volume per tumor was reduced by approximately 60% in the rats receiving 0.9% RGT. Histological analysis indicated that both the incidence and multiplicity of esophageal carcinoma was decreased by either RGT or DGT. The above results indicate that both green tea and black tea can inhibit the tumorigenic action of NMBzA during the period of carcinogen treatment and also inhibit the subsequent molecular events important for esophageal tumorigenesis.

Prevention against lung tumorigenesis

Chung et al., (1998) have examined the effect of black tea and caffeine on lung tumorigenesis in F344 rats induced by the nicotine-derived carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in a 2-year bioassay. The animals were examined for tumors in target organs, including lung, liver, nasal cavity, and other major organs. Significant reduction of the total lung tumor (adenomas, adenocarcinomas, and adenosquamous carcinomas) incidence was observed from 47% to 19%. The tumor incidence in the nasal cavity, however, was not affected by either black tea or caffeine at any of the concentrations tested. The most unexpected finding was the remarkable reduction of the lung tumor incidence, from 47% to 10%, in the group treated with 680-ppm caffeine, a concentration equivalent to that found in 2% tea. This study demonstrated for the first time in a 2-year lifetime bioassay that black tea protects against lung tumorigenesis in F344 rats, and this effect appears to be attributed, to a significant extent, to caffeine as an active ingredient of tea.

Prevention of prostate carcinogenesis

Schut et al., (2000) examined the chemopreventive properties of green tea and black tea in 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) carcinogenesis by evaluating their effects on PhIP-DNA adduct formation in the female F-344 rat. Compared with animals on regular drinking water, PhIP-DNA adduct formation was inhibited in small intestine, colon, liver, and mammary epithelial cells (MECs) of animals receiving green tea or black tea as the sole source of drinking fluid. Green tea inhibited adduct formation in colon, liver, and MECs in small intestine. Black tea inhibited adduct formation in liver, colon and in small intestine; it had no effect on MEC adducts. Neither green tea nor black tea had an effect on

adduct levels in pancreas, lungs, white blood cells, heart, kidneys, spleen, cecum, or stomach. It was hence evident by the above data that green and black tea are potential chemopreventive agents in PhIP-induced tumorigenesis in the F-344 rat. Black tea extracts have also been reported to modulate the oxidative stress induced by testosterone in prostate tissue of rat (Siddiqui et al., 2004).

Prevention of mammary carcinogenesis

The cancer-preventive effects of green tea and its main constituent (-)-EGCG, are widely supported by results from epidemiological, cell culture, animal and clinical studies in the recent decade. *In vitro* cell culture studies show that tea polyphenols potently induce apoptotic cell death and cell cycle arrest in tumor cells but not in their normal cell counterparts. Green tea polyphenols affect several signal transduction pathways, including growth factor-mediated, the mitogen-activated protein kinase (MAPK)-dependent, and ubiquitin/proteasome degradation pathways (Chen et al., 2004). Various animal studies have revealed that treatment by green tea inhibits tumor incidence and multiplicity in different organ sites such as skin, lung, liver, stomach, mammary gland and colon. Phase I and II clinical trials were carried out recently to explore the anticancer effects of green tea in patients with cancer. At this time, more mechanistic research, animal studies, and clinical trials are mandatory to further evaluate the role of green tea in cancer prevention.

Telomerase is elevated in >90% of breast carcinomas and therefore has received much attention as a target for breast cancer therapy and cancer diagnostic research. Studies have shown that (-)-EGCG from green tea imparts a growth inhibitory effect on cancer cells. Mittal et al., (2004) showed that treatment of EGCG dose-dependently inhibited (20-100%) the reproductive or colony forming potential, and also decreased cell viability at different time points studied (approximately 80% inhibition) in human breast carcinoma MCF-7 cells but had no adverse effect on the growth of normal mammary cells. In order to identify the possible mechanism of decreased cell viability and induction of apoptosis in breast carcinoma cells by EGCG, the studies showed that treatment of MCF-7 cells with EGCG dose-dependently inhibited telomerase activity (40-55%), and also inhibited the mRNA expression (40-55%) of hTERT, a catalytic subunit of telomerase. Results indicate that EGCG down-regulates telomerase in human breast carcinoma MCF-7 cells, leading to the suppression of cell viability and induction of apoptosis, thus providing the molecular basis for the development of EGCG as a novel chemopreventive and pharmacologically safe agent against breast cancer.

Prevention of colon carcinogenesis

Although the emerging data strongly indicates significant anti-tumorigenic benefits from green tea catechins yet the potential molecular mechanisms involved remain obscure. In HT-29 human colon cancer cells, the effects of quercetin (flavonoid) on proliferation, apoptosis, and differentiation were assessed as these processes are known to be disregulated during cancer development (Wenzel, 2004). Quercetin inhibited the proliferation of HT-29 cells with an IC(50)-value of 81.2 +/- 6.6 mM. Cell differentiation based on surface expression of alkaline phosphatase was enhanced 4-fold and the activity of the pro-apoptotic effector caspase-3 increased 3-fold. Henceforth, it can be concluded that quercetin alters the levels of a variety of proteins involved in growth, differentiation, and apoptosis of colon cancer cells. Their identification as molecular targets of quercetin may explain the anti-cancer activities of this flavonoid.

Modulating effects of green tea

Ichikawa et al (2004) propounded that Interleukin-12 (IL-12) which is a heterodimeric cytokine comprising p40 and p35 subunits, plays an essential role in the regulation of the differentiation of Th1 cells. Green tea polyphenols exhibit potent anti-oxidative activities and anti-inflammatory effects by modulating cytokine production. The investigation showed that catechins effect IL-12-p40 production in murine macrophages induced by bacterial lipopolysaccharide (LPS). Of the various catechins, (-)-EGCG was the most potent inhibitor, followed by (-)-gallocatechin gallate (GCG) and (-)-epicatechin gallate (ECG), suggesting that gallate-containing catechins, particularly EGCG, inhibits LPS-induced IL-12-p40 production in murine macrophages by inhibiting p38-MAPK while enhancing p44/p42 ERK, leading to the inhibition of IkappaBalpha degradation and NF-kappaB activation.

Suppression of the growth of implanted tumor cells

Various studies reported that oral, subcutaneous or intraperitoneal administration of EGCG or green tea polyphenols to mice resulted in a statistically significant suppression of the growth of implanted tumor cells such as sarcoma 180 or 3-methylcholanthrene (3-MC) induced carcinoma cells (Hara, 1989, Yang et al., 1992). Oral administration of the green tea infusion to Swiss albino mice started one week prior to intraperitoneal injection of Ehrlich ascites carcinoma cells and continued for an additional 8 day period resulted in 41% inhibition of carcinoma growth and prolonged the survival time by about 55% (Yi, 1984).

Molecular Mechanisms of Cancer Chemoprevention by Tea Polyphenols

The inhibitory effects of the tea against various site-specific carcinogenesis have been attributed to the biologic activities of the tea polyphenols and their antioxidative properties.

Inhibition of mutagenicity and genotoxicity

There is growing interest in the potential health benefits of tea, and a recent report described the potent antimutagenic activity of white tea in comparison with green tea against several heterocyclic amines, including PhIP (Santana-Rios, 2001). Antimutagenic action of green and black tea extracts and its polyphenols against several food xenobiotics such as aflatoxin B1 has been reported. The property of antimutagenesis has been related to different components of tea extracts like isolated

theaflavins (Shiraki et al., 1994) and other polyphenolic flavonoids (Rice-Evans et al., 1995). In cultured cells, the frequencies of mitomycin C or UV light induced sister chromatids exchange and also the chromosomal aberrations were suppressed by treatment with tea polyphenols in the presence of liver metabolizing enzymes. In the Ames test black tea effectively reduced the mutagenic activity of structurally diverse chemical carcinogens (Bu-Abbas et al., 1994).

Theaflavins are catechin dimers that have been shown to antagonize the carcinogenicity of nitrosamines in mice (Shukla et al., 2002) and to possess antimutagenic activity (Apostolides et al., 1997). Tea polyphenols inhibit the activation of mutagens and carcinogens by interacting with microsomal protein and impairing electron transfer (Hernaez et al., 1998). The epicatechin derivatives inhibit cytochrome P-450 dependent monooxygenase, NADPH cytochrome reductase that reduces the transformation of promutagen to mutagen (Wang et al., 1988; Hernaez et al., 1998). The gallic acid moiety of theaflavins is known to inhibit DNA single strand breaks and scavenge electrophilic free radicals to produce antioxidative and antimutagenic effects (Shiraki et al., 1994). Antimutagenic effects of aqueous black tea extract (ATE) and black tea polyphenols (BTP) were evaluated in the Ames test using Salmonella typhimurium tester strains TA 98 and TA 100. The antimutagenic activity of BTP was found to be higher than that of ATE, which may be attributable to the higher amount of polyphenolic ingredients. Hence the study revealed that black tea has a protective efficacy in suppressing B[a]P and CP induced mutagenicity in a microbial test system (Taneja et al 2003, Shukla et al, 2003).

Inhibition of biochemical markers of tumor promotion

A number of studies report that the consumption of green tea inhibits tumor promotion as assessed by inhibition of the biochemical markers of tumor promotion (Katiyar and Mukhtar, 1996).

Modulation of metabolizing/detoxification enzymes.

Sugiyama et al., (2004) showed that theanine, a specific glutamate derivative in green tea, decreased doxorubicin (DOX)-induced adverse reactions such as the induction of the lipid peroxide level and the reduction of glutathione peroxidase activity in normal tissues; involved in oxidative damage. UDP-glucuronosyltransferase (UDP-GT) activities towards p-nitrophenol were markedly increased (51.8% or 1.5-fold) in rats that consumed tea compared with the control animals on water. Therefore, a major mechanism of tea as a chemopreventive agent is induction of the microsomal detoxification enzyme, UDP-glucuronosyltransferase (Embola et al., 2002).

Trapping of activated forms of carcinogens

The structure of flavanols provides strong nucleophillic centers at position 6 and 8. This property enables flavanols to react with electrophilic carcinogens. An initial step of carcinogenesis is the metabolic activation of chemical carcinogens by the P-450-dependent biotransformation reaction.

Antioxidative effects

The ability of green tea components to function as antimutagens/antioxidants has been well established, and their role in cancer prevention is supported by numerous epidemiological studies. In Ames tests, employing hydrogen peroxide as a mutagen, EGCG offered the highest level of protection of all antioxidants tested. Measurement of protection against DNA scissions produced results that again showed that EGCG produced the strongest protective effects (Pillai et al., 1999). In scavenging assays using a xanthine-xanthine oxidase (enzymatic system), ECG showed the highest scavenging potential. In a nonenzymatic (phenazine methosulfate-NADH) oxidizing system, EGCG once again showed the strongest effects.

Tea polyphenols act as antioxidants *in vitro* by scavenging reactive oxygen and nitrogen species and chelating redox-active transition metal ions. They may also function indirectly as antioxidants through (1) inhibition of the redox-sensitive transcription factors, nuclear factor-kappaB and activator protein-1; (2) inhibition of "pro-oxidant" enzymes, such as inducible nitric oxide synthase, lipoxygenases, cyclooxygenases and xanthine oxidase; and (3) induction of phase II and antioxidant enzymes, such as glutathione S-transferases and superoxide dismutases. The fact that catechins are rapidly and extensively metabolized emphasizes the importance of demonstrating their antioxidant activity *in vivo*. Animal studies offer a unique opportunity to assess the contribution of the antioxidant properties of tea and tea polyphenols to the physiological effects of tea administration in different models of oxidative stress. Most promising are the consistent findings in animal models of skin, lung, colon, liver and pancreatic cancer that tea and tea polyphenol administration inhibit carcinogen-induced increases in the oxidized DNA base, 8-hydroxy-2'-deoxyguanosine. In animal models of atherosclerosis, green and black tea administration has resulted in modest improvements in the resistance of lipoproteins to *ex vivo* oxidation, although limited data suggest that green tea or green tea catechins inhibit atherogenesis (Frei, 2003). To determine whether tea polyphenols act as effective antioxidants *in vivo*, future studies in animals and humans should employ sensitive and specific biomarkers of oxidative damage to lipids, proteins and DNA.

Green tea and apoptosis

Only a limited number of chemopreventive agents are known to induce apoptosis (Jiang et al., 1996). In recent years apoptosis has become a challenging subject in biomedical research, and the life span of both normal and cancer cells within a living system is regarded to be affected by apoptosis. EGCG was found to suppress cell growth and induce apoptosis largely through mitochondrial depolarization, activation of caspase-3 and cleavage of DNA fragmentation factor-45 in human endothelial ECV 304 cells. The induction of apoptosis by EGCG was confirmed by cleaved and condensed nuclear chromatin and DNA hypoploidy (Yoo et al., 2002). The results suggest that EGCG may exert at least part of its anticancer effect by inhibiting angiogenesis through

inducing endothelial apoptosis.

Prevention against cardiovascular disease

Recent evidence indicates that EGCG, the major catechin from green tea leaves, lowers the risk of cardiovascular diseases such as atherosclerosis and hypertension. However, a precise mechanism for this biologic function has not yet been clearly delineated. Angiotensin II (Ang II) stimulates vascular smooth muscle cell (VSMC) hypertrophy, which is a critical event in the development of atherosclerosis, hypertension, and angioplasty-induced restenosis. Various studies showed that EGCG inhibits Ang II-stimulated VSMC hypertrophy, as determined by [3H] leucine incorporation into VSMC. Since MAPK families are involved in cell growth (Zheng et al., 2004). EGCG pretreatment did not exhibit any significant changes in Ang II-stimulated activation of ERK and p38-MAPK. EGCG only inhibited Ang II-stimulated activation of JNK. However, EGCG suppressed Ang II-induced c-jun mRNA expression. In contrast, EGC, a structural analogue of EGCG, did not inhibit the JNK activity or c-jun mRNA expression. In addition, a specific JNK inhibitor, SP600125, dose-dependently suppressed Ang II-stimulated VSMC hypertrophy. These results suggest that the effect of EGCG on Ang II-induced VSMC hypertrophy is due to specific inhibition of the JNK signaling pathway at both transcriptional and posttranslational levels, which may underlie its beneficial effect on the cardiovascular diseases.

Conclusions and Future Directions for Research

The available epidemiological information, though not sufficient, indicates that dietary substances cause one third of all cancers. Thus dietary habits are crucial for the development of cancer and modulating the life style including the diet can reduce the incidence of cancer . On the basis of many epidemiological observations and numerous laboratory studies, it can be concluded that tea consumption is likely to have beneficial effects in reducing cancer risk in different populations. Additional studies on the chemical properties and biological activities of the tea and tea components are warranted to provide a sound background for examining the effects of tea on health. Black tea, the major form of tea, is consumed in Western countries. However, the chemistry, biological activities, and chemopreventive properties of black tea, especially the polyphenols present in it are poorly understood. Considerable attention has to be focused on this area and extensive research is needed to consolidate the proposed benefits.

Although a great deal of information has been accumulated on the effect of tea on cancer, a clear understanding of the mechanism by which tea components may affect the genesis, growth, and progression of specific cancers is essential. The two important factors are the selection of relevant experimental models and the ability to correlate results obtained *in vitro* to situations in whole animals and in humans. The dose response is the key issue in such studies. However, the bioavailability of tea polyphenols following tea consumption by the human population, studies on the absorption, distribution, and metabolism of green and black tea polyphenols in animals and humans are of utmost importance. In this regard, a method has been developed for the analysis of plasma and urinary tea polyphenols in human subjects (Lee et al., 1995). This methodology may prove valuable for studying the effects of tea consumption, bioavailability of tea polyphenols and an association of the two with human cancer.

Definite deleterious or beneficial effects of tea consumption have to come from studies with human populations. Because the causative factors are different for different populations, tea consumption may affect carcinogenesis only in selected situations rather than having the general effect on all cancers. Thus there is a need to define the population that could benefit from tea consumption. Such intervention studies in various populations may provide useful information on the protective effects of tea polyphenols on cancer of specific organs or in specific populations. After careful evaluation of additional studies, it may be possible to recommend consumption of tea polyphenols by humans. Such agents do not necessarily have to be consumed by tea drinking; rather they can be supplemented in other food items, such as cosmetic products, consumer items, in vitamin supplements and other products. This approach involves the production of "designer items" for consumption by human population.

Thus in future epidemiological studies, it is crucial to consider the etiologic factors of the specific cancer and to collect more specific information on the qualitative and the quantitative aspects of tea consumption. Information on the composition of the tea preparations consumed and the availability of biomarkers for tea consumption would allow future epidemiologic studies to be more quantitative. Better-designed case-control and cohort studies are needed to address the issue of whether tea consumption enhances or inhibits the development of specific cancers in specific populations. A phase I clinical trial has suggested that green tea is well tolerated and can be safely administered without any side effects over a period of 6 months (Pisters et al., 2001) Another recent phase II clinical trial, however, concluded that green tea carries only a limited antineoplastic activity in patients with androgen-dependent prostate cancer (Jatoi et al., 2003). Thus the ideal chemopreventive study should be conducted in a population with high risk of prostate cancer development. Although, considerable accumulating information provides compelling body of evidence for the preventive potential of tea against cancer; yet the naturally occurring tea polyphenols should be evaluated in clinical intervention in human trials.

References

Agarwal R, Mukhtar H (1991). Cutaneous chemical carcinogenesis, in Pharmacology of Skin. Ed., CRC Press, Boca Raton, FL , pp 371-87.

Apostolides Z, Balentine DA, Harbowy ME, et al (1997). Inhibition of PhIP mutagenicity by catechins, and by theaflavins and gallate esters. *Mutat Res*, **389**, 167-72.

Armstrong B, Garrod A, Doll RA (1976). Retrospective study of renal cancer with special reference to coffee and animal protein consumption. *Br J Cancer,* **33**, 127- 36.

Balentine DA, Wiseman SA (1997). The chemistry of flavonoids. *Crit Rev Food Sci Nutr*, **37**, 693-704.

Bianchi GD, Cerhan JR, Parker AS, et al (1994). Selective induction of rat hepatic CYP1 and CYP4 proteins and of peroxisomal proliferation of green tea. *Carcinogenesis,* **15**, 2575-9.

Chatterjee ML, Agarwal R, Mukhtar H (1996).Ultraviolet B radiation-induced DNA lesions in mouse epidermis: an assessment using a novel 32P-postlabelling technique. *Biochem Biophys Res Commun* , **229** , 590-5.

Chen D, Daniel KG, Kuhn DJ, et al (2004). Green tea and tea polyphenols in cancer prevention. *Front Biosci,* **9**, 2618-31.

Chung FL, Wang M, Rivenson A, et al (1998). Inhibition of lung carcinogenesis by black tea in Fischer rats treated with a tobacco-specific carcinogen: caffeine as an important constituent. *Cancer Res,* **51**, 4096-101.

Claude J, Kunze E, frentzel-Beyme R, et al (1986). Life-style and occupational risk factors in cancer of the lower urinary tract. *Am J Epidemiol*,**124**, 578-89.

D'Avanzo B, La Vecchia C, Franceschi S, et al (1992). Coffee consumption and bladder cancer risk. *Eur J Cancer,* **28**, 1480-4.

Dales LG, Friedman GD, Ury HK, et al (1979). A case-control study of relationship of diet and other traits to colorectal cancer in American blacks. *Am J Epidemiol,* **109**, 132-44.

Elmets CA (1991). Cutaneous photocarcinogenesis, in Pharmacology of the Skin, Mukhtar H, Ed., CRC Press, Boca Raton, FL, pp 389-416.

Embola CW, Sohn OS, Fiala ES, et al (2002). Induction of UDP-glucuronosyltransferase 1 (UDP-GT1) gene complex by green tea in male F344 rats. *Food Chem Toxicol,* **40**, 841-4.

Frei B, Higdon JV (2003). Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. *J Nutr,* **133**, 3275-84.

Fujiki H (1999). Two stages of Oncol. cancer prevention with green tea . *J Cancer Res Clin,* **125**, 589-97.

Gao CM, Takezaki T, Wu JZ, et al (2002). Glutathione-S-transferases M1 (GSTM1) and GSTT1 genotype, smoking, consumption of alcohol and tea and risk of esophageal and stomach cancers: a case-control study of a high-incidence area in Jiangsu Province,China. *Cancer Lett,* **188**, 95-102.

Gao YT, McLaughlin JK, Blot WJ, et al (1994). Reduced risk of esophageal cancer associated with green tea consumption. *J Natl Cancer Inst,* **86**, 855-8.

Gensler HL, Timmermann BN, Valcic S, et al (1996). Prevention of photocarcinogenesis by topical administration of pure epigallocatechin gallate isolated from green tea. *Nutr Cancer*, **26**, 325-35.

Goldbohm RA, Hertog MG, Brants HA, et al (1996). Consumption of black tea and cancer risk: a prospective cohort study. *J Natl Cancer Inst,* **88**, 93-100.

Goodman MT, Morgenstern H, Wynder EL (1986). A case-control study of factors affecting the development of renal cell cancer. *Am J Epidemiol* , **124**, 926-41.

Graham HN (1992). Green tea, composition, consumption and polyphenols chemistry. *Prev Med* , **21**, 334-50.

Hara Y, Matsuzaki S, Nakamura K (1989). Antiumor activity of green tea catechin. *J Jpn Soc Nutr Food Sci* (in Japanese) **42**, 39-45.

Harbowy ME, Balentine DA (1997). Tea chemistry. *Critical Rev Plan Sci* ,**16**, 415-80.

Harnack LJ, Anderson KE, Zheng W, et al (1997). Smoking, alcohol, coffee, and tea intake and incidence of cancer of the exocrine pancreas: the Iowa Women's Health Study. *Cancer Epidemiol Biomarkers Prev*, **6**, 1081-6.

Heilbrun LK, Nomura A, Stemmermann GN (1986). Black tea consumption and cancer risk: a prospective study. *Br J Cncer,* **54**, 677-83.

Henderson BE, Louie E, Soo Hoo Jing J, et al (1976). Risk factors associated with nasopharyngeal carcinoma. *New Eng J Med*, **295**, 1101-6.

Hernaez J, Xu M, Dashwood R (1998). Antimutagenic activity of tea towards 2-hydroxyamino-3-methylimidazo (4,5) quinoline: effect of tea concentration and brew time on electrophillic scavenging. *Mutat Res* , **402**, 299-306.

Huang MT, Ho CT, Wang ZY, et al (1992). Inhibitory effects of topical application of a green tea polyphenol fraction on tumor initiation and promotion in mouse skin. *Carcinogenesis,* **13**,947- 54.

Huang MT, Xie JG, Wang ZY, et al (1997) 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** , 2623-9.

Ichikawa D, Matsui A, Imai M, et al (2004). Effect of Various Catechins on the IL-12p40 Production by Murine Peritoneal Macrophages and a Macrophage Cell Line, J774.1. *Biol Pharm Bull,* **9**, 1353-8.

Inoue M, Tajima K (1998) Tea and coffee consumption and risk of digestive tract cancers: data from comparative case-referent study in Japan. *Cancer Causes Control* ,**9**, 209-16.

Jain MG, Hislop GT, Howe GR, et al (1998). Alcohol and other beverage use and prostate cancer risk among Canadian men. *Int J Cancer* ,**78**, 707-11.

Jatoi A, Ellison N, Burch AP, et al (2003). A phase II trail of green tea in the treatment of patients with androgen independent metastatic prostate carcinoma. *Cancer*, **97**, 1441-6.

Jiang MC, Yang-Yen HF, Yen JJY, et al (1996). Curcumin induces apotosis in immortalized NIH 3T3 and malignant cancer cell lines. *Nutr Cancer,* **26**, 111-20.

Katiyar SK (2003). Skin photoprotection by green tea: antioxidant and immunomodulatory effects. *Curr Drug Targets Immune Endocr Metabol Disord* , **3**, 234-42.

Katiyar SK, Agarwal R, Mukhtar H (1993). Protective effects of against N-nitrosodiethylamine and benzo(a)pyrene-induces forestomach and lung tumorigenesis in A/J mice by green tea. *Carcinogenesis* ,**14**, 849-55.

Katiyar SK, Agarwal R, Mukhtatr H (1992a). Green tea in chemoprevention of cancer. *Comprehensive Therapy,* **18**, 3-8.

Katiyar SK, Agarwal R, Wang ZY, et al (1992b). Epigallocatechin-3-gallate in Camellia sinensis leaves from Himalayan region of Sikkim: inhibitory effects against biochemical events and tumor initiation in SENCAR mouse skin. *Nutr Cancer,* **18**, 73-83.

Katiyar SK, Agarwal R, Wood GS, et al (1992c). Inhibition of 12-O-tetradecanoylphorbol-13-acetate-caused tumor promotion in 7,12-dimethylbenz[a]anthracene-initiated SENCAR mouse skin by a polyphenolic fraction isolated from green tea. *Cancer Res,* **52**, 6890-7.

Katiyar SK, Mukhtar H (1996). Tea in chemoprevention of cancer: epidemiologic and experimental studies (review). *Int J Oncol* , **8**, 221-38.

Katiyar SK, Mukhtar H. (1997) Tea antioxidants in cancer chemoprevention. *J Cell Biochem Suppl*, **27**, 59-67.

Lee MJ, Lambert MJ, Yang CS (2004). Delivery of tea polyphenols to the oral cavity by green tea leaves and black

tea extract. *Cancer Epidemiol Biomarkers Prev,* **3**,132-7.
Lee MJ, Wang ZY, Li H, (1995). Analysis of plasma and urinary tea polyphenols in human subjects. *Cancer Epidemol Biomarkers Prev* , **4**, 393-9.
Li ZG, Shimada Y, Sato F, et al (2003). Promotory effects of hot water on N-nitromethylbenzylamine-induced esophageal tumorigenesis in F344 rats. *Oncol Rep,* **10**, 421- 6.
Lin TM, Chen KP, Lin CC, et al (1973). Retrospective study on nasopharyengeal carcinomas. *J Natl Cancer Inst* , **51**, 1403-8.
Lu YP, Lou YR, Xie JG, et al (1997) Inhibitory effect of black tea on the growth of established skin tumors in mice: effects on tumor size, apoptosis, mitosis and bromodeoxyuridine incorporation into DNA.*Carcinogenesis*, **18** , 2163-9.
McLaughlin JK, Mandel JS, Blot WJ (1984). A population-based case-control study of renal cell carcinoma. *J Natl Cancer Inst* ,**72**, 275-84.
Miller AB, Howe GR, Jain M, et al (1983). Food items and food groups as risk factors in a case-control study of diet and colo-rectal cancer. *Int J Cancer* , **32**, 155- 66.
Mittal A, Pate MS, Wylie RC, et al (2004). EGCG down-regulates telomerase in human breast carcinoma MCF-7 cells, leading to suppression of cell viability and induction of apoptosis. *Int J Oncol* , **24**, 703-10.
Morgan RW, Jain MG (1974). Bladder cancer: smoking, beverages and artificial sweeteners. *Can Medical Assoc J,* **111**, 1067-70.
Mukhtar H, Katiyar SK, Agarwal R (1994). Green tea and skin: anticarcinogenic effects. *J Investigative Dermatol,* **102**, 3-7.
Nakachi K, Suemasu K, Higashi Y, et al (1998). Influence of drinking tea on breast cancer malignancy among Japanese patients. *Jpn J Cancer Res,* **89**, 254-61.
Nomura AM, Kolonel LN, Hankin JH et al (1991). Dietary factors in cancer of the lower urinary tract. *Int J Cancer* , **48**, 199-205.
Orner GA, Dashwood WM, Blum CA, et al (2002). Response of Apc(min) and A33 (delta N beta-cat) mutant mice to treatment with tea, sulindac, and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine(PhIP).*Mutat Res,* **30**, 506-7.
Phillips RL, Snowdon DA (1985). Dietary relationship with fatal colorectal cancer among Seventh-Day Adventists. *J Natl Cancer Inst,* **74**, 307-37.
Pillai SP, Mitscher LA, Menon SR, et al (1999). Antimutagenic/antioxidant activity of green tea components and related compounds. *J Environ Pathol Toxicol Oncol,* **18**,147-58.
Pisters KM, Newman RA, Coldman B, et al (2001) Phase I trial of oral green tea extract in adult patients with solid tumors. *J Clin Oncol,* **19** , 1830-8.
Rice-Evans CA, Miller NJ, Bolwell PG, et al (1995). The relative antioxidant activities of plant derived polyphenolic flavonoids. *Free Radical Res,* **22,** 375- 88.
Santana-Rios G, Orner GA, Amantana A, et al (2001). Potent antimutagenic activity of white tea in comparison with green tea in the Salmonella assay. *Mutat Res,* **495**, 61-74.
Schut HA, Yao R (2000). Tea as a potential chemopreventive agent in PhIP carcinogenesis: effects of green tea and black tea on PhIP-DNA adduct formation in female F-344 rats. *Nutr Cancer,* **36**,52- 8.
Setiawan VW, Zhang ZF, Yu GP, et al (2001). Protective effect of green tea on the risks of chronic gastritis and stomach cancer. *Int J Cancer*, **92**, 600- 4.
Shanmugaratnam K, Tye CY, Goh EH, et al (1978). Etiological factors on naso-pharyngeal carcinoma: a hospital-based, retrospective, case control, questionnaire study, in Nasopharyngeal Carcinoma, Etiology and Control, Ito, Y. Ed.,IARC, Lyon, pp 199-212.
Shiraki M, Hara Y, Osawa T, et al (1994). Antioxidative and antimutagenic effects of theaflavins from black tea. *Mutat Res*, **323**, 29-34.
Shukla Y, Javed S (2000). Effect of black tea extract on transplantable and solid tumors in Swiss albino mice. *Biomed Env Sci* , **10**, 213- 8.
Shukla Y, Taneja P (2002). Antimutagenic effects of black tea on pulmonary tumors in Swiss albino mice. *Cancer Lett* ,**176**, 137- 41.
Shukla Y, Arora A, Taneja P (2003). Antigenotoxic potential of certain dietary constituents. *Terato Carcino Mutagen,* **1**, 323-35.
Siddiqui IA, Afaq F, Adhami VM, et al (2004). Antioxidants of the beverage tea in promotion of human health. *Antioxid Redox Signal,* **6**, 571-82.
Simon D, Yen S , Cole P (1975).Coffee drinking and cancer of the lower urinary tract. *J Natl Cancer Inst*, 54, 587-91
Sugiyama T, Sadzuka Y (2004). Theanine, a specific glutamate derivative in green tea, reduces the adverse reactions of doxorubicin by changing the glutathione level. *Cancer Lett,* **212**,177-84.
Taneja P, Arora A, Shukla Y (2003). Antimutagenic effects of black tea in the Salmonella typhimurium reverse mutation assay. *Asian Pac J Cancer Prev* , **4**, 193- 8.
Tea (2000). Consumption and risk of bladder and kidney cancers in a population-based case-control study. *Am J Epidemiol*, **151**, 377-83.
Wang LD, Zhou Q, Feng CW, et al (2002). Intervention and follow up on human esophageal precancerous lesions in Henan, Northern China, a high incidence area for esophageal cancer. *Gan To Kagaku Ryoho*, **29**, 159-72.
Wang ZY, Agarwal R, Khan WA, et al (1992a) Protection against benzo(a)pyrene and N-nitrsodimethylamine-induced lung and forestomach tumorigenesis in A/J mice by water extracts of green tea and licorice. *Carcinogenesis* , **13**, 1491- 4.
Wang ZY, Das M, Bickers DR, et al (1988). Interaction of epicatechins derived from green tea with rat hepatic cytochrome P-450. *Drug Metab Dispo*, **16**, 98-103.
Wang ZY, Huang MT, Ferraro T, et al (1992b). Inhibitory effect of green tea in drinking water in tumorigenesis by ultraviolet light and 12-O-tetradecanoylphorbol-13-acetate in the skin of SKH-1 mice. *Cancer Res*, **52**, 1162- 71.
Wang ZY, Huang MT, Lou YR, et al (1994). 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- 35.
Wang ZY, Jhan WA, Bickers DR, et al (1989). Protection against polycyclic hydrocarbon induced skin tumor initiation in mice by green tea polyphenols. *Carcinogenesis* , **10**, 411- 5.
Wang ZY, Wang LD (1995) Inhibition of N-nitrosomethyl benzylamine induced esophageal tumorigenesis in rat by green and black tea. *Carcinogenesis* , **16**, 2143-8.
Weisburger JH (1997). Tea and health a historical prespective. *Cancer Lett*, **114**, 315- 7.
Wenzel U, Herzog A, Kuntz S, et al (2004). Protein expression profiling identifies molecular targets of quercetin as a major dietary flavonoid in human colon cancer cells. *Proteomics,* **4**, 2160-74.
Yang CS, Maliakal P, Meng X (2002). Inhibition of carcinogenesis by tea. *Ann Rev Pharmacol Toxicol* , **42**, 25-54.
Yang YS, Wang QD, Zhou YZ (1992). Effects of Chinese green tea on the immune function of mice bearing tumor and their antitumor activity. Chung Hua Liu Hsing Ping Hsueh tsa Chih. *Chinese J Cancer Res* , **26**, 5-7.
Yi ZX, Yi CS, Li ZY (1984). Effects of green tea on transplanted

tumors. *Tumor*, **4**,128-34 (in Chinese).
Yoo HG, Shin BA, Park JC, et al (2002).Induction of apoptosis by the green tea flavonol (-)-epigallocatechin-3-gallate in human endothelial ECV 304 cells. *Anticancer Res* , **22**, 3373-8.
Yoshizawa S (1996). (-)Epigallactocatechin gallate, the main constituent of Japanese green tea, inhibits tumor promotion of okadaic acid. *Fukuoka Igaku Zasshi* , **87**, 215-21.
Yoshizawa S, Horiuchi T, Fujiki H, et al (1987). Antitumor promoting activity of (-)epigallactocatechin gallate, the main constituent of "tannin" in green tea. *Phytother Res* , **1**, 44-7.
Yu MC, Mack TM, Hanisch R, et al (1986). Cigarette smoking, obesity, diuretic use, and coffee consumption as risk factor for renal cell carcinoma. *J Natl Cancer Inst*, **77**, 351- 6.
Zheng W, Doyle TJ, Kushi LH, et al (1996). Tea consumption and cancer incidence in a prospective cohort study of postmenopausal women. *Am J Epidemiol*, **144**, 175-82.
Zheng Y, Song HJ, Kim CH, et al (2004). Inhibitory effect of epigallocatechin 3-O-gallate on vascular smooth muscle cell hypertrophy induced by angiotensin II. *J Cardiovasc Pharmacol*, **43**, 200- 8.