JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Structure–Activity Relationships of Tea Compounds against Human Cancer Cells

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The content of the biologically active amino acid theanine in 15 commercial black, green, specialty, and herbal tea leaves was determined as the 2,4-dinitrophenyltheanine derivative (DNP-theanine) by a validated HPLC method. To define relative anticarcinogenic potencies of tea compounds and teas, nine green tea catechins, three black tea theaflavins, and theanine as well as aqueous and 80% ethanol/water extracts of the same tea leaves were evaluated for their ability to induce cell death in human cancer and normal cells using a tetrazolium microculture (MTT) assay. Compared to untreated controls, most catechins, theaflavins, theanine, and all tea extracts reduced the numbers of the following human cancer cell lines: breast (MCF-7), colon (HT-29), hepatoma (liver) (HepG2), and prostate (PC-3) as well as normal human liver cells (Chang). The growth of normal human lung (HEL299) cells was not inhibited. The destruction of cancer cells was also observed visually by reverse phase microscopy. Statistical analysis of the data showed that (a) the anticarcinogenic effects of tea compounds and of tea leaf extracts varied widely and were concentration dependent over the ranges from 50 to 400 μg/mL of tea compound and from 50 to 400 μg/g of tea solids; (b) the different cancer cells varied in their susceptibilities to destruction; (c) 80% ethanol/water extracts with higher levels of flavonoids determined by HPLC were in most cases more active than the corresponding water extracts; and (d) flavonoid levels of the teas did not directly correlate with anticarcinogenic activities. The findings extend related observations on the anticarcinogenic potential of tea ingredients and suggest that consumers may benefit more by drinking both green and black teas.

KEYWORDS: HPLC; theanine; catechins; theaflavins; teas; cancer cells; growth inhibition; structure–activity relationships; dietary significance

INTRODUCTION

In recent publications we reported that significantly greater quantities of individual and total flavonoids (catechins and theaflavins) were extracted from tea leaves (*Camellia sinensis*) with 80% ethanol/water at 60 °C for 15 min than with boiled water for 5 min (*1*, *2*). The latter conditions are widely used in the home to prepare tea infusions. The distribution of the individual catechins and theaflavins in individual teas extracted by the two solvents also varied. In a related study, we evaluated the antimicrobial activities of a series of tea catechins and theaflavins as well as tea infusions against the foodborne pathogen *Bacillus cereus* (*3*). Because antibacterial potencies of individual tea flavonoids and teas varied widely, to further define structure–biological activity relationships of tea compounds, we compared in the present study the anticarcinogenic activities against human cancer cells of the same tea compounds as well as tea leaf extracts with the aid of the microculture tetrazolium (MTT) assay we previously used to assess the inhibition of growth of cancer cells by potato glycoalkaloids (*4*, *5*).

Commercial teas are usually classified into three major categories: unfermented green, containing catechins; fully fermented black, containing theaflavins and polymeric thearubigins; and semifermented oolong, containing both catechins and theaflavins. Tea catechins can exist as two geometrical isomers depending on the stereochemical configuration of the 3′,4′-dihydroxyphenyl and hydroxyl groups at the 2- and 3-positions of the C-ring: *trans*-catechins and *cis*-epicatechins (**Figure 1**). Each of the isomers, in turn, exists as two optical

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10.1021/jf062276h CCC: $37.00
Published on Web 12/16/2006

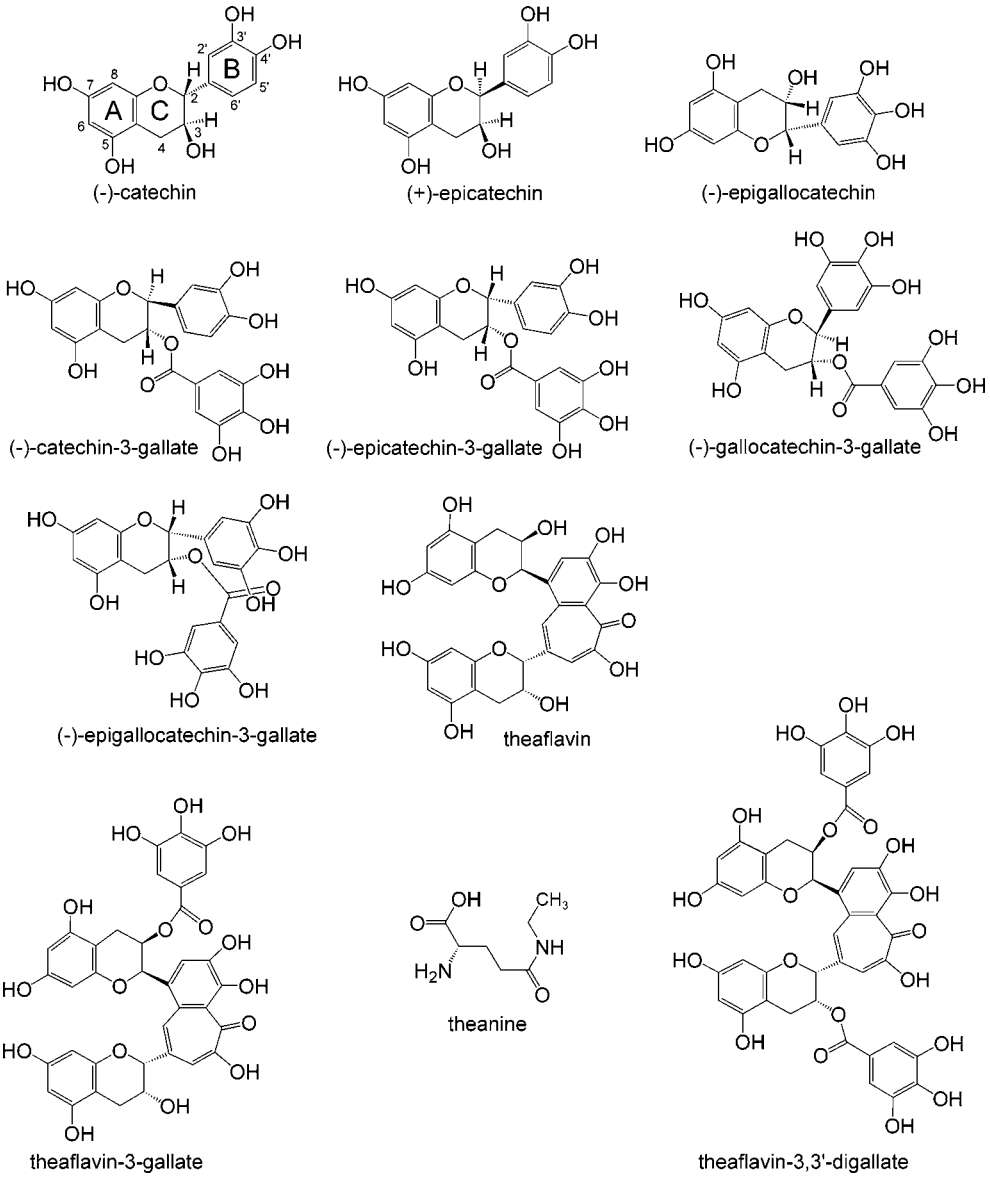

Figure 1. Structures of catechins, theaflavins, and theanine evaluated in this study.

isomers: (+)-catechin and (−)-catechin and (+)-epicatechin and (−)-epicatechin, respectively. (−)-Catechin can be modified by esterification with gallic acid to form (−)-catechin-3-gallate, epicatechin-3-gallate, (−)-epigallocatechin-3-gallate, and (−)-gallocatechin-3-gallate, respectively. Theaflavins are formed by enzyme-catalyzed oxidative dimerization of catechins (*6*−*9*).

The main objectives of the present study were (a) to validate an HPLC method for the analysis of theanine in teas; (b) to delineate the activities of 13 tea compounds against breast, colon, liver, and prostate human cancer cells compared to their effects against normal human liver and lung cells; and (c) to compare effects against cancer cells of aqueous and aqueous/ethanol extracts of 15 commercial teas with known flavonoid and theanine contents. The results obtained may make it possible to better relate the structures of active tea compounds to their health-promoting functions and thus allow the consumer to select tea brands and dietary tea supplements with the optimal content of beneficial compounds.

MATERIALS AND METHODS

Materials. Human breast (MCF-7), colon (HT-29), hepatoma (liver) (HepG2), and prostate (PC-3) cancer cells and normal human liver (Chang) and lung (HEL299) cells were obtained from American Type Culture Collection (ATCC, Rockville, MD) and from the Korean Cell Line Bank (KCLB, Seoul, South Korea), respectively. HT-29, MCF-7, and PC-3 cells were maintained in an RPMI 1640 medium supplemented with 10% of fetal bovine serum, 50 units/mL of penicillin, and 50 mg/mL of streptomycin, at 37 °C in a 5% CO_2 incubator. The other cells were maintained in an MEM medium supplemented with 10% of fetal bovine serum, 50 units/mL of penicillin, and 50 mg/mL of streptomycin, at 37 °C in a 5% CO_2 incubator. Cell culture reagents were obtained from GibcoBRL (Life Technologies, Cergy-Pontoise, France). Each sample was dissolved in DMSO (2 mg/200 μL) and stored at −4 °C.

Acetonitrile, a distilled-in-glass chromatography solvent (Burdick & Jackson, Muskegon, MI), tea catechins, and 1-fluoro-2,4-dinitrobenzene (FDNB) were obtained from Sigma-Aldrich (St. Louis, MO); theaflavins were obtained from Wako Chemical Co. (Osaka, Japan); L-theanine was purchased from LKT Laboratories (St. Paul, MN). Teas were purchased as tea bags in local markets and from the Stash Tea Co. (Portland, OR). Solvents were filtered through a 0.45 μM membrane (Millipore, Bedford, MA) and degassed in an ultrasonic bath.

Catechin and Theaflavin Contents of Commercial Tea Leaves. The procedures used for the extraction and analysis of teas by HPLC have been described previously (*1*, *2*). Each extract (1 mL) was placed into a minivial (3 mL) and evaporated at 40 °C under reduced pressure. Solutions of these samples in DMSO were used in studies on the

reduction of viabilities of normal and cancer cells with the aid of the MTT assay as described below.

Theanine Content of Tea Leaves. Theanine was extracted by two different solvents: 80% ethanol/water and water. Each tea leaf sample (0.33−1.003 g) was placed into a 20 mL volumetric flask to which was added 18 mL of 80% ethanol/water or water. The ethanol sample was heated at 60 °C for 15 min and then sonicated for 5 min. The water sample was treated with boiled water for 5 min. The volume of the cooled samples was then adjusted to 20 mL with added 80% ethanol or water. The suspension was then centrifuged at 18000*g* for 10 min at 1 °C. The supernatant was used for analysis of DNP-theanine. The 80% ethanol or water extract (50 μL) in a test tube was evaporated under reduced pressure at 30 °C. FDNB (0.1 mL) and 1% $NaHCO_3$ (2 mL) were then added to the residue, and the mixtures was allowed to stand in the dark for 3 h at 40 °C. Excess FDNB was removed with ethyl acetate (2 mL × 5). This was followed by the addition of 0.5 N HCl (0.5 mL). The resulting DNP derivative was extracted with ethyl acetate (1 mL × 5) and dried at 30 °C. Two milliliters of 80% ethanol was then added to the residue. After centrifugation (15000*g* × 2 min), this solution (10 μL) was used for HPLC.

HPLC was carried out on a Hitachi liquid chromatograph model 665-II equipped with an autosampler (model 655A-40). The DNP derivatives in teas were separated using a stainless steel column (250 mm × 4.0 mm i.d.) packed with Inertsil ODS-3v (5 μm particle diameter) (GL Sciences, Tokyo, Japan). A binary gradient elution system was used consisting of acetonitrile (A) and distilled water containing 0.5% formic acid (B). Separation was achieved by programming the mobile phase as follows: 0−40.0 min, 27% A, 73% B; 40.1−50.0 min, 80% A, 20% B; 50.1−65.0 min, 27% A, 73% B. Column temperature was maintained with a Shimadzu column oven CTO-10vp (Shimadzu, Tokyo, Japan). The flow rate was 1 mL/min at 30 °C. UV detection was set at 254 nm. Samples (10 μL) were injected directly into the column. Separate analyses, each in triplicate, were carried out with three separate extracts prepared from three samples.

A calibration curve of DNP-theanine was obtained by plotting the content obtained against the amount injected using the Hitachi Chromato-integrator model D-2500. DNP-theanine was identified by comparing the retention times of standard DNP-theanine and spiked samples. Recovery of spiked DNP-theanine was calculated by using the following equation: % recovery = (concentration of spiked sample)/(concentration of endogenous theanine + spike) × 100.

MTT Assay for Growth Inhibition of Cells. The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay that differentiates dead from living cells was adapted from the literature (*4*, *5*, *10*). The decrease in OD in the assay measures the extent of decrease in the number of cancer cells calculated by using the following formula: death of cancer cells (%) = (OD with test substance)/(OD of control) × 100.

Microscopy of Untreated and Treated Cancer Cells. The procedure was adapted from the literature (*11*). The cancer cells in the microplate reader (1×10^6 cells/well) were treated for 48 h with the tea extracts. The 96-well plates were each washed with 500 μL of cold phosphate-buffered saline, and the cells were then fixed for ∼40 min with 200 μL of cold trichloroacetic acid. Next, the cells were washed three times with water and dried at room temperature. The cells were then stained in each well with 250 μL of 0.2% of sulforhodamine B in a 1% acetic acid and photographed at ×400 magnification with the aid of a Leica microscope (Heidelberg, Germany).

Statistical Methods. The optical densities obtained with the MTT assay were transformed when necessary prior to analyses of variance to stabilize the variance among compounds and concentrations. One-way analyses of variance (ANOVA) were used along with Dunnett's one-tailed test for decreases from the control ($p > 0.05$). For the experiments with multiple compounds, the analyses were run separately for each concentration and the control was included for each run. Anticarcinogenic activities of 80% ethanol/water extracts of teas significantly different from corresponding water extracts ($p < 0.05$) were determined using the Bonferroni adjustment for multiplicity (*12*). An asymptotic, nonlinear model to fit the percent cell deaths (kill) of the cells versus concentration was used to determine IC_{50} values (micromolar concentration of test substance that inhibited 50% of the initial number of cells). Missing IC_{50} values are the result of failure to attain a maximum 50% response or lack of fit of the model.

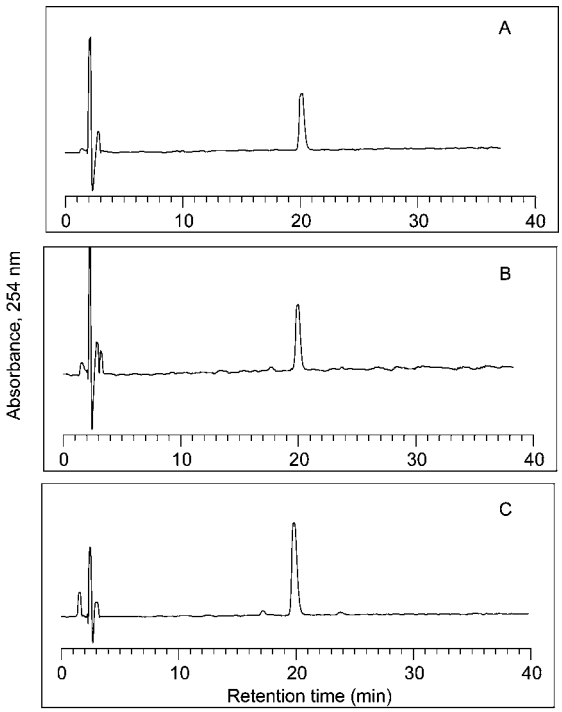

Figure 2. HPLC chromatogram of standard DNP-theanine (**A**), DNP-theanine from teas (**B**), and DNP-theanine in spiked teas (**C**). Conditions: column, Inertsil ODS-3v (5 μm, 4.0 × 250 mm); column temperature, 30 °C; mobile phase, acetonitrile/0.5% formic acid (gradient mode); detector, UV at 254 nm; chart speed, 2.5 mm/min.

RESULTS AND DISCUSSION

Catechins and Theaflavin Content of Teas. We previously compared the following conditions for the extraction and analysis by HPLC of catechins, theaflavins, and purine alkaloids in 77 commercial teas and 15 green tea-containing dietary supplements: 80% ethanol/water at 60 °C for 15 min and boiled water for 5 min (*1*, *2*). The following ranges of concentrations of flavonoids (catechins plus theaflavins) in the tea leaves extracted with 80% ethanol were observed: in 32 black teas, 19.8−115.1 mg/g; in 24 green teas, 12.3−136.3 mg/g; in 14 specialty teas, 4.9−118.5 mg/g; in 7 herbal teas, 0−46.0 mg/g. Significantly greater amounts of flavonoids were extracted from the tea leaves with aqueous ethanol than with boiled water. Levels of tea catechins in 10 capsules sold as dietary supplements ranged from 42 to 380 mg/g. The catechin content of four green tea extracts sold as powders ranged from 96 to 696 mg/g. The results make it possible to maximize the extraction of tea compounds to better relate the content of flavonoids and alkaloids of teas and dietary supplements to their health-promoting effects. For this study, we selected 15 of these teas with a known wide range of catechin and theaflavin contents for evaluation of their potential to inhibit human cancer cells.

Theanine Content of Teas. Teas also contain the biologically active amino acid theanine (**Figure 1**). Reported methods for determining the theanine content of tea leaves include HPLC of the phenylthiocabamoyl (*13*), *o*-phthalaldehyde (*14*, *15*), and 9-fluorenylmethoxycarbonylglycine (*16*) derivatives; electrophoresis of the 2,4-dinitrofluorobenzene derivative (*17*, *18*); and analysis without derivatization by anion-exchange chromatography (*19*), HPLC−mass spectrometry (*20*), micellar electro-

Table 1. Theanine Content of Teas Extracted either with 80% Ethanol/Water at 60 °C for 15 min and Sonicated for 5 min or with Boiled Water for 5 min

tea	theanine (mg/g ± SD; $n = 3$)	
	80% ethanol	water
black teas		
Darjeeling Summer	8.1 ± 0.6	9.3 ± 0.8
Darjeeling Spring	9.5 ± 0.6	7.7 ± 0.1
Breakfast Blend organic	9.9 ± 0.6	7.5 ± 0.0
English Breakfast Black	14.6 ± 0.1	13.7 ± 0.6
green teas		
Darjeeling Green organic	7.9 ± 0.3	6.1 ± 0.2
Susi Bar Mild Green	9.9 ± 0.5	8.1 ± 0.6
Jasmine Blossom Green	10.5 ± 0.3	9.7 ± 0.5
Premium Green	10.2 ± 0.4	11.4 ± 0.6
Premium Green decaffeinated	10.3 ± 0.3	11.5 ± 0.6
Lemon Spice Green	10.5 ± 0.5	9.9 ± 0.5
Sencha (Japanese green)	11.3 ± 0.1	9.9 ± 0.1
Dragonwell	12.5 ± 0.6	11.4 ± 0.4
specialty teas		
Silver Jasmine	6.9 ± 0.5	8.2 ± 0.2
Fusion Green and White	10.9 ± 0.5	11.8 ± 0.5
herbal tea		
Wild Raspberry caffeine-free	11.2 ± 0.4	10.5 ± 0.6

kinetic capillary chromatography (*21*), and nuclear magnetic resonance (NMR) spectroscopy (*22*).

For this study, we devised an HPLC method based on analysis of the derivative formed on reaction of theanine with 2,4-dinitrofluorobenzene to form the chromophoric dinitrophenyl (DNP) derivative. **Figure 2** illustrates the chromatographic separation of the DNP-theanine on the Inertsil column. The following determined analytical parameters for DNP-theanine ($n = 3$) demonstrate the sensitivity and the validity of the HPLC method: retention time on the column, 20.77 ± 0.5 min; recovery after spiking, 93.2 ± 4.7%; linearity range of concentration response, 31.2−156 ng; and limit of detection (LOD), 360 ± 5.2 pg.

Because, in addition to catechins and theaflavins, the amino acid theanine (**Figure 1**) present in teas is also reported to exhibit anticarcinogenic effects, we also determined the latter concentrations in the same 15 teas. **Table 1** shows the theanine levels of the 80% ethanol/water extracts ranged from 8.1 to 14.6 mg/g of tea leaves and those of the corresponding water extracts ranged from 6.1 to 13.7 mg/g of tea leaves. In contrast to flavonoids, theanine levels of the 80% ethanol extracts of tea leaves were generally similar to those of the water extracts. To our surprise, we observed that the Wild Raspberry caffeine-free herbal tea (not a member of the genus *Camellia sinensis*) also contained theanine (11.2 mg/g).

Anticarcinogenic Activities of Individual Tea Ingredients. *Catechins.* The MTT assay measures the decrease in mitochondrial activity of the cells, which in turn may reflect a decrease in cell proliferation. However, equally likely is that it reflects toxicity leading to loss of cell viability. Below, we discuss the inhibitory activities of tea compounds and tea extracts against cancer cells based on the results from the MTT assays. To place our findings in proper perspective, we also summarize recent studies that are relevant to the theme of this paper.

Table 2 shows the anticarcinogenic effects against three cancer cell lines (AGS stomach, MCF-7 breast, and PC-3 prostate) by four concentrations (50, 100, 200, and 400 μg/mL) of nine catechins. The data in this table show a wide range of activities by the different compounds. The susceptibilities of the three cell lines to the same catechin also varied widely. The following compounds exhibited high activity (percent cell death) against the AGS/MCF-7/PC-3 cells at the highest concentration: (−)-epicatechin (400 μg/mL = 1.38 μmol/mL), 89.7/79.9/85.0; (−)-gallocatechin gallate (400 μg/mL = 0.87 μmol/mL), 79.4/64.1/67.8; (−)-epigallocatechin (400 μg/mL = 1.31 μmol/mL), 71.6/56.5/65.6; (−)-catechin gallate (400 μg/mL = 0.90 μmol/mL), 64.5/67.4/71.1; and (−)-epigallocatechin gallate (400 μg/mL = 0.87 μmol/mL), 64.5/78.3/49.4. The data also show that the activities of these compounds are concentration-dependent. The other catechins exhibited low or no activities.

Table 2 also shows that the IC_{50} values for catechins against the AGS cells ranged from 170 μM for (−)-epigallocatechin-3-gallate (highest activity) to 500 μM for (−)-epigallocatechin. The corresponding range for the MCF-7 cells was from 150 μM for (−)-epigallocatechin-3-gallate to 850 μM for (−)-epigallocatechin, and that for the PC-3 cells was from 230 μM for (−)-catechin-3-gallate to 460 μM for (−)-epigallocatechin.

Anticarcinogenic effects of selected individual tea catechins, especially of epigallocatechin gallate (EGCG), have been extensively studied. A survey of the pertinent literature includes the following salient observations: (a) EGCG was the most potent of six green tea components against human tumor cell lines (*23*); (b) EGCG may exert part of its anticancer effect in human colon carcinoma HT29 cells by inhibiting angiogenesis (*24*); (c) EGCG down-regulated telomerase and induced apoptosis of human MCF-7 breast cancer cells but not in their normal counterparts (*25*, *26*); (d) orally administered EGCG inhibited intestinal tumorigenesis in mice, possibly through attenuation of the carcinogenic events (*27*); (e) EGCG exhibited antitumor properties against breast cancer cells in vitro and in vivo (*28*); and (f) combinations of EGCG and vanilloids (pepper capsaicinoids) acted synergistically against cancer cells (*29*).

The results obtained in the present study show that (a) although (−)-catechin exhibited low activity against the cancer cells, the isomeric (−)-epicatechin was highly active; (b) introduction of gallic acid moieties generally enhanced activity but not in any apparent systematic way; and (c) the three cell lines showed different susceptibilities to the structurally different catechins.

Theaflavins. **Table 3** shows inhibitory effects of three theaflavins against four cancer (HepG2 liver, HT29 colon, MCF-7 breast, and PC-3 prostate) and two normal (Chang liver and Hel299 lung) cell lines. The percent cell death at the highest concentration of theaflavin (400 μg/mL = 0.71 μmol/mL) ranged from 16.9 for HepG2 to 50.0 for HT29. The corresponding range for theaflavin-3-gallate (400 μg/mL = 0.56 μmol/mL) is from 33.9 for HT29 to 79.9 for PC-3. For theaflavin-3,3′-digallate (400 μg/mL = 0.46 μmol/mL), the range was from 49.2 for HT29 to 85.9 for PC-3.

The IC_{50} values ranged as follows: HepG2 cells, theaflavin-3,3′-digallate, 220 μM, and theaflavin-3-gallate, 580 μM; for HT29 cells, theaflavin-3,3′-digallate, 330 μM, and theaflavin, 720 μM; MCF-7 cells, theaflavin-3,3′-digallate, 280 μM; PC-3 cells, theaflavin-3,3′-digallate, 170 μM, and theaflavin-3-gallate, 280 μM; Chang cells, theaflavin-3,3′-digallate, 630 μM, and theaflavin, 1190 μM.

These results show that (a) adding gallate ester groups to the parent theaflavin molecule progressively increases activity, (b) the inhibitory potencies of theaflavin gallates are similar to those of the more active catechins, (c) theaflavins exhibited low activities against normal cells, and (d) as is the case with catechins, activities of theaflavins against cancer cells paralleled corresponding activities against bacteria (*3*).

Related studies have shown that black tea theaflavins inhibited

Table 2. Deaths of AGS, MCF-7, and PC-3 Cancer Cells Induced by Tea Catechins Listed Alphabetically [Listed Values Are Means ± SD ($n = 3$); Values in Parentheses Are Molecular Weights]

			cell death (%) of cancer cell line		
catechin	μg/mL	μmol/mL	AGS stomach	MCF-7 breast	PC-3 prostate
(–)-catechin (290)	400	1.38	7.1 ± 0.4*[a]	23.4 ± 1.7	31.7 ± 1.3
	200	0.69	–16.8 ± 0.8*	4.9 ± 0.1*	17.8 ± 0.5
	100	0.34	–17.4 ± 0.4*	–3.8 ± 0.3*	11.1 ± 0.3*
	50	0.17	–25.8 ± 0.8*	–4.3 ± 0.3*	–17.2 ± 0.2*
(+)-catechin (290)	400	1.38	6.4 ± 0.4*	19.6 ± 1.1	6.7 ± 0.4
	200	0.69	–13.5 ± 0.5*	2.2 ± 0.1*	–8.3 ± 0.6*
	100	0.34	–13.5 ± 1.7*	–7.1 ± 0.1*	–8.3 ± 1.1*
	50	0.17	–21.3 ± 1.7*	–13.0 ± 0.2*	–11.1 ± 0.9*
(±)-catechin (290)	400	1.38	–8.4 ± 0.5*	11.4 ± 0.6	20.0 ± 0.8
	200	0.69	–9 ± 0.4*	–2.7 ± 0.1*	14.4 ± 1.5
	100	0.34	–17.4 ± 1.1*	–15.8 ± 1.4*	–7.2 ± 0.7*
	50	0.17	–29 ± 0.4*	–25 ± 1.2*	–27.2 ± 2.5*
(–)-catechin-3-gallate (442)	400	0.90	64.5 ± 1.2	67.4 ± 2.2	71.1 ± 0.0
	200	0.45	46.5 ± 7.8	49.5 ± 12.2	60.6 ± 0.8
	100	0.23	41.9 ± 1.9	33.7 ± 10.2	51.1 ± 3.5
	50	0.11	3.2 ± 0.19*	7.1 ± 0.4*	20.6 ± 310.4*
IC_{50} (μM)			330	410	230
(–)-epicatechin (290)	400	1.38	89.7 ± 28.0	79.9 ± 19.4	85.0 ± 22.0
	200	0.69	85.8 ± 23.4	64.7 ± 4.0	82.8 ± 2.7
	100	0.34	52.3 ± 3.5	22.3 ± 2.6*	44.4 ± 32.2
	50	0.17	–0.6 ± 0.0*	7.1 ± 0.4*	3.9 ± 0.3*
IC_{50} (μM)			330	560	360
(–)-epicatechin-3-gallate (442)	400	0.90	52.9 ± 4.3	65.2 ± 17.3	57.8 ± 5.3
	200	0.45	51 ± 6.0	32.1 ± 4.9	45 ± 0.9
	100	0.22	23.9 ± 1.4	17.4 ± 2.7*	36.1 ± 0.3
	50	0.11	–18.7 ± 1.2*	2.7 ± 0.4*	–11.7 ± 0.6*
IC_{50} (μM)			470	670	400
(–)-epigallocatechin (306)	400	1.31	71.6 ± 6.5	56.5 ± 9.2	65.6 ± 3.2
	200	0.65	55.5 ± 18.5	49.5 ± 10.1	56.1 ± 2.1
	100	0.33	35.5 ± 2.5	20.1 ± 2.5*	40.6 ± 0.8
	50	0.16	1.3 ± 0.2*	19.0 ± 2.7*	14.4 ± 0.6
IC_{50} (μM)			500	850	460
(–)-epigallocatechin-3-gallate (306)	400	0.87	64.5 ± 1.2	78.3 ± 3.9	49.4 ± 3.8
	200	0.44	57.4 ± 1.7	75 ± 6.5	43.9 ± 2.6
	100	0.22	52.2 ± 0.7	70.7 ± 2.6	36.1 ± 3.1
	50	0.11	47.7 ± 1.2	21.2 ± 1.9*	10.6 ± 0.7*
IC_{50} (μM)			170	150	
(–)-gallocatechin-3-gallate (458)	400	0.87	79.4 ± 22.3	64.1 ± 3.9	67.8 ± 9.3
	200	0.44	54.2 ± 13.7	60.3 ± 7.4	63.9 ± 8.8
	100	0.22	44.5 ± 1.5	14.7 ± 0.5*	40.0 ± 3.7
	50	50	–14.2 ± 0.7*	16.8 ± 2.1*	1.1 ± 0.1*
IC_{50} (μM)			260	440	270

[a] The asterisk indicates the value is not significantly different at the 5% level from respective control using Dunnett's one-tailed test for a decrease in the OD value (number of cells) (inactive against cancer cells). Values without asterisks are different from control ($p < 0.05$) (active against cancer cells). IC_{50} = μmol/L (μM) that killed 50% of the cells under the test condition.

peroxide-induced cytotoxicity and DNA damage in rat normal liver epithelium RL-34 and human hepatoma HepG2 cell lines (*30*) as well as human gastric (MKN-28) cancer cells (*31*).

On balance, the results of the present and previous studies suggest that consumers may benefit more by drinking both green tea containing catechins and black tea containing theaflavins and that widely consumed so-called green tea dietary supplements sold as capsules and powders should contain catechins and theaflavins.

Theanine. **Table 3** shows that theanine at 400 μg/mL (2.28 μmol/mL) also induced cell death of four cancer cell lines. Inhibitory activity ranged from 58.8% for HepG2 liver cancer cells, to 65.1% for MCF-7 breast cells, to 77.1% for HT29 colon cells, to 89.3% for the PC-3 prostate cells. The data show also show that theanine inhibited the Chang normal liver cells (57.7%), but the Hel299 normal lung cells were not affected significantly. The IC_{50} values for theanine ranged from 170 μM for PC-3 prostate cancer cells to 1270 μM for the Chang normal liver cells.

Reported biological effects of theanine include enhancing antihepatoma effects of powdered green tea in rats (*32*) and of idarubacin-induced antitumor activity in mice (*33*). These observations and our data on the activities of theanine against the cancer cell lines suggest that theanine probably contributes to the beneficial effects teas.

Anticarcinogenic Activities of Tea Extracts. Table 4 shows the survival (percent cell death) of four cancer and two normal cell lines following exposure to four concentrations (50, 100, 200, and 400 μg/mL) of 15 commercial teas extracted either with 80% ethanol/water at 60 °C for 15 min and sonicated for 5 min or with boiled water for 5 min. The latter conditions are widely used to prepare tea infusion in the home. We evaluated the two different tea extracts because we had previously found

Table 3. Deaths of HepG2, HT29, MCF-7, and PC-3 Human Cancer Cells and Chang and Hel299 Human Normal Cells Induced by Theaflavins and Theanine [Values Are Means ± SD ($n = 3$); Numbers in Parentheses Are Molecular Weights]

			cell death (%) of cell line					
tea compound	μg/mL	μmol/mL	HepG2 liver cancer	HT29 colon cancer	MCF-7 breast cancer	PC-3 prostate cancer	Chang normal liver	Hel299 normal lung
theaflavin (564)	400	0.709	16.9 ± 0.3	50.0 ± 10.2	20.5 ± 0.9	22.2 ± 0.8	26.8 ± 2.6	32.7 ± 1.8
	200	0.354	8.1 ± 0.3	22.0 ± 3.3	14.5 ± 0.4	21.4 ± 0.5	9.3 ± 0.9*[a]	–1.8 ± 0.1*
	100	0.177	5.9 ± 0.3*	17.8 ± 0.73	7.2 ± 0.3*	20.1 ± 0.2	0 ± 0*	–7.3 ± 0.4*
	50	0.089	3.7 ± 0.2*	2.5 ± 0.3*	0 ± 0*	19.2 ± 0.7	–4.1 ± 0.1*	–10.9 ± 0.9*
IC_{50} (μM)				720			1190	
theaflavin-3-gallate (717)	400	0.558	47.8 ± 5.4	33.9 ± 1.3	47.0 ± 1.1	79.9 ± 8.5	41.2 ± 0.7	12.7 ± 1.1*
	200	0.279	27.2 ± 0.6	31.4 ± 2.3	38.6 ± 1.5	51.7 ± 5.5	36.1 ± 1.7	5.5 ± 0.2*
	100	0.14	6.6 ± 0.2*	11.0 ± 0.8*	22.9 ± 0.7	28.6 ± 1.2	17.5 ± 0.7	3.6 ± 0.3*
	50	0.07	5.9 ± 0.18*	6.8 ± 0.2*	6.0 ± 0.2*	27.4 ± 0.6	–9.3 ± 0.4*	0 ± 0*
IC_{50} (μM)			580			280		
theaflavin-3,3′-digallate (869)	400	0.46	77.9 ± 7.8	49.2 ± 1.6	51.8 ± 5.2	85.9 ± 10.4	46.4 ± 0.9	18.2 ± 2.4
	200	0.23	58.8 ± 2.1	57.6 ± 4.6	50.6 ± 2.5	70.1 ± 10.0	38.1 ± 5.1	9.1 ± 0.7*
	100	0.115	12.5 ± 0.21	19.5 ± 2.3	31.3 ± 1.6	25.2 ± 0.4	23.7 ± 1.3	–1.8 ± 0.2*
	50	0.058	4.4 ± 0.1*	33.9 ± 10.4	20.5 ± 1.5	12.4 ± 0.7	19.6 ± 1.8	–7.3 ± 0.9*
IC_{50} (μM)			220	330	280	170	630	
theanine (175)	400	2.283	58.8 ± 4.2	77.1 ± 2.9	65.1 ± 0	89.3 ± 7.1	57.7 ± 2.8	5.5 ± 0.3*
	200	1.142	19.1 ± 1.0	67.8 ± 1.8	60.2 ± 0	74.8 ± 26.6	52.6 ± 6.9	7.3 ± 0.4*
	100	0.571	2.2 ± 0.1*	31.4 ± 2.3	33.7 ± 1.8	29.9 ± 1.6	28.9 ± 2.1	7.3 ± 0.3*
	50	0.285	2.2 ± 0.1*	14.4 ± 3.0*	30.1 ± 3.6	9.8 ± 0.3	27.8 ± 2.0	21.8 ± 0
IC_{50} (μM)				800	900	760	1270	

[a] See footnotes to **Table 2**.

that the hydroalcoholic solvent extracted catechins and theaflavins more efficiently than did boiled water (*2*).

A statistical treatment using the Bonferroni adjustment for multiplicity defines tea levels that differed significantly (were more active) than the respective controls (see values in **Table 4** without a footnote). Inactive teas are designated by footnote *a* and 80% ethanol extracts, which were significantly more active than the corresponding water extracts, by footnote *b* in **Table 4**. Below, we discuss the trends for each cell line on the basis of the data listed in the table.

MCF-7 Breast Cancer Cells. The percent cell death of breast cancer cells by the highest concentration of the ethanolic tea extracts ranged from 26.5 for the Jasmine Blossom Green tea to 43.4 for Darjeeling Spring Black tea. The corresponding range for the water extracts ranged from 4.8 for the Wild Raspberry herbal tea to 61.4 for the Premium Green decaffeinated tea. Water extracts of English Breakfast Black, Jasmine Blossom Green, and Fusion Green and White teas with percent cell deaths of 68.7, 62.7, and 61.4, respectively, were also highly active against the breast cancer cells. The water extracts are more active than the ethanolic extracts. Both green and black tea extracts strongly inhibit the growth of breast cancer cells. It is also relevant that combined soybean and green tea diets inhibited estrogen-dependent human breast MCF-7 carcinoma in mice (*34*).

HT29 Colon Cancer Cells. The percent cell death of colon cancer cells by the highest concentration of the ethanolic tea extracts ranged from 19.5 for Wild Raspberry herbal tea to 71.2% for Sencha Japanese Green tea. The corresponding range for the water extracts is from 26.3 for Breakfast Blend Black organic tea to 73.7% for Exotica Dragonwell Green tea. Aqueous extracts of Exotica Silver Jasmine specialty tea with 72.9%, Fusion Green tea with 72.0%, and Premium Green tea with 67.8% were also highly effective against the colon cancer cells. Activities of the ethanol extracts were generally similar to those of the water extracts.

HepG2 Liver Cancer Cells. The percent cell death of the liver cancer cells by the highest concentration of the ethanol extract ranged from 14.0 for the Premium Green tea to 86.8 for Sencha Japanese Green tea, a 6-fold variation from the highest to lowest value. The corresponding range for the water extracts is from 8.8 for the Darjeeling Green organic tea to 58.8 for Wild Raspberry herbal tea, a 7-fold variation from highest to lowest value. The ethanol extracts are more effective in destroying the cancer cells than are the water extracts. A striking example is the 86.8 and 80.9% cell death caused by the ethanol extracts of Sencha Japanese Green and Sushi Bar Mild Green teas compared to 41.9 and 39.7% for the corresponding water extracts. The first column in **Table 4** shows that the total flavonoid content of the ethanol extracts of these two teas (94 and 116 mg/g of tea, respectively) was greater than the corresponding content of the water extracts (76.2 and 87.1 mg/g, respectively).

PC-3 Prostate Cancer Cells. The ethanol extracts exhibited exceptional activity against the prostate cancer cells, ranging from 56.0% cell death for Lemon Spice Green and Black tea to complete inhibition, within experimental error, by the following teas: Breakfast Blend Black organic, Darjeeling Summer Black, Exotica Dragonwell Green, Sencha Japanese Green, Sushi Bar Mild Green, and Wild Raspberry herbal teas. The water extracts ranged in activity from 17.1% for Darjeeling Spring Black to 83.8% for Darjeeling Summer Black tea. Water extracts with high activity include: Exotica Dragonwell Green, 82.5% cell death; Premium Green decaffeinated, 82.1% cell death; Wild Raspberry herbal, 81.6% cell death; Lemon Spice Green and Black, 74.4% cell death; Sushi Bar Mild Green, 73.9% cell death; Sencha Japanese Green, 71.8% cell death; and Breakfast Blend Black organic, 71.4% cell death.

A striking result is the high activity of both ethanol (87.2 ± 14.5%) and water extracts (81.6 ± 20.9%) of Wild Raspberry herbal tea. Because this so-called herbal tea contains only 3.5 mg of catechins/g and no theaflavins (**Table 4**), it is likely that its theanine content (11.2 mg/g) (**Table 1**) or other components may be responsible for the high potency against the prostate cells. The herbal tea extracts were less active against the other

Table 4. Relationship between Composition of Teas Determined by HPLC (*1*, *2*) and Anticarcinogenic Activities [C, Total Catechins; T, Total Theaflavins; F, Total Flavonoids, C + T, the First Number Represents the 80% Ethanol/Water Extract and the Second the Water Extract; Values Are Means ± SD ($n = 3$)]

		cell death (%) of cell line											
		HepG2 liver cancer		HT29 colon cancer		MCF-7 breast cancer		PC-3 prostate cancer		Chang normal liver		HEL299 normal lung	
tea name/composition (mg/g)	μg/mL	ethanol extract	water extract	ethanol extract	water extract	ethanol extract	water extract	ethanol extract	water extract	ethanol extract	water extract	ethanol extract	water extract
Breakfast Blend Black organic	400	69.1 ± 3.3^{b}	21.3 ± 1.2	54.2 ± 1.0^{b}	26.3 ± 3.6	31.3 ± 0.5	22.9 ± 1.4	87.2 ± 17.4^{b}	71.4 ± 7.5	56.7 ± 1.3^{b}	18.6 ± 1.4^{b}	21.8 ± 2.0^{b}	38.2 ± 1.1
C: 67.7 (ethanol), 42.3 (water)	200	26.5 ± 0.5^{b}	–3.7 ± 0.1^{a}	47.5 ± 3.1^{b}	–20.3 ± 1.4^{a}	9.6 ± 0.1^{a}	16.9 ± 1.5^{a}	61.1 ± 7.4^{b}	47.4 ± 1.5	41.2 ± 2.2^{b}	4.1 ± 0.7^{a}	12.7 ± 0.3^{b}	30.9 ± 1.6
T: 15.3, 5.1	100	–1.5 ± 0.0^{a}	–5.1 ± 0.2^{a}	22.0 ± 2.4^{b}	–28.8 ± 1.3^{a}	3.6 ± 0.3^{a}	4.8 ± 0.5^{a}	29.5 ± 0.9	23.5 ± 0.5	18.6 ± 0.2^{a}	–4.1 ± 0.7^{a}	7.3 ± 0.3^{a,b}	25.5 ± 0.6
F: 83, 47.4 (C + T)	50	–5.1 ± 0.0^{a}	–10.3 ± 0.8^{a}	19.5 ± 1.2^{a,b}	–39.8 ± 6.3	–3.6 ± 0.1^{a}	4.8 ± 0.4^{a}	14.5 ± 0.9	9.8 ± 0.1	16.5 ± 2.0^{a,b}	–20.6 ± 3.2^{a}	–5.5 ± 0.2^{a,b}	21.8 ± 3.5
Darjeeling Green organic	400	25.0 ± 1.5^{b}	8.8 ± 0.3^{a}	46.6 ± 2.2	54.2 ± 7.0	38.6 ± 0.0^{b}	51.8 ± 1.3	63.2 ± 3.7^{b}	36.3 ± 0.5	44.3 ± 1.6	44.3 ± 2.5	–10.9 ± 0.2^{a,b}	14.5 ± 0.3
C: 136, 100	200	0.7 ± 0.0^{a}	3.7 ± 0.1^{a}	17.8 ± 1.3^{a}	32.2 ± 4.0	33.7 ± 3.1	44.6 ± 1.9	30.8 ± 0.8	25.6 ± 0.2	35.1 ± 2.2	37.1 ± 3.6	–5.5 ± 0.3^{a}	1.8 ± 0.1^{a}
T: 0, 0	100	0 ± 0.0^{a}	2.2 ± 0.1^{a}	3.4 ± 0.1^{a}	5.9 ± 0.8^{a}	19.3 ± 2.9^{a}	21.7 ± 0.7	16.2 ± 0.7	16.2 ± 0.3	18.6 ± 1.2^{a}	29.9 ± 2.2	–10.9 ± 0.2^{a}	–14.5 ± 2.8^{a}
F: 136, 100	50	0 ± 0.0^{a}	0.7 ± 0.0^{a}	–32.2 ± 2.7^{a}	–8.5 ± 1.3^{a}	8.4 ± 0.4^{a}	15.7 ± 0.4^{a}	21.4 ± 1.2	12 ± 0.4	–9.3 ± 0.3^{a}	17.5 ± 1.7^{a}	–5.5 ± 0.3^{a}	–16.4 ± 3.1^{a}
Darjeeling Spring Black	400	43.4 ± 6.8^{b}	14 ± 0.2	50 ± 3.4	55.9 ± 5.4	43.4 ± 1.8	53.0 ± 1.4	84.2 ± 6.8^{b}	17.1 ± 0.2	45.4 ± 1.7	36.1 ± 1.2	34.5 ± 1.0^{b}	20 ± 0.9
C: 108, 63.4	200	10.3 ± 0.5	5.1 ± 0.1^{a}	43.2 ± 1.9	33.1 ± 0.8	38.6 ± 2.3	33.7 ± 3.1	61.1 ± 4.7^{b}	19.2 ± 0.5	27.8 ± 1.6	32.0 ± 3.9	–10.9 ± 2.1^{a}	0 ± 0.0^{a}
T: 5.6, 1.6	100	8.1 ± 0.2^{a}	0.7 ± 0.0^{a}	9.3 ± 1.4^{a}	–11 ± 0.2^{a}	7.2 ± 0.6^{a}	15.7 ± 0.9^{a}	23.5 ± 1.3	17.5 ± 1.2	15.5 ± 2.3^{a}	4.1 ± 0^{a}.	–27.3 ± 6.2^{a}	–7.3 ± 0.1^{a}
F: 113.6, 65	50	7.4 ± 0.2^{a}	0 ± 0.0^{a}	–6.8 ± 1.0^{a}	–49.2 ± 17.9^{a}	–1.2 ± 0.1^{a}	14.5 ± 0.8^{a}	17.9 ± 1.1	18.8 ± 1.4	–10.3 ± 1.8^{a}	0 ± 0.0^{a}	–25.5 ± 3.7^{a}	–10.9 ± 0.9^{a}
Darjeeling Summer Black	400	71.3 ± 11.0^{b}	32.4 ± 2.8	57.6 ± 2.3^{b}	69.5 ± 1.9	31.3 ± 1.6	31.3 ± 1.10	91.5 ± 4.6	83.8 ± 13.0	51.5 ± 2.2^{b}	19.6 ± 2.	5.5 ± 0.1^{a}	7.3 ± 0.6^{a}
C: 109, 63.0	200	25.7 ± 0.5^{b}	2.9 ± 0.1^{a}	54.2 ± 1.0	50.8 ± 2.6	14.5 ± 0.2	28.9 ± 2.4	70.1 ± 4.0^{b}	54.3 ± 1.0	42.3 ± 2.3^{b}	3.1 ± 0.6^{a}	–10.9 ± 0.4^{a}	1.8 ± 0.1^{a}
T: 6.8, 8.1	100	1.5 ± 0.1^{a}	0 ± 0.0^{a}	16.9 ± 0.7^{a}	16.1 ± 1.8	–12.0 ± 0.8^{a,b}	12.0 ± 1.1^{a}	29.9 ± 0.9	26.9 ± 0.9	12.4 ± 0.2^{a}	1.0 ± 0.1^{a}	–12.7 ± 0.6^{a}	0 ± 0.0^{a}
F: 115.8, 71.1	50	–6.6 ± 0.2^{a}	0 ± 0.0^{a}	0 ± 0.0^{a}	0.8 ± 0.2^{a}	–21.7 ± 0.9^{a,b}	9.6 ± 1.1^{a}	18.8 ± 0.4	16.7 ± 0.9	10.3 ± 0.4^{a}	–3.1 ± 0.1^{a}	–12.7 ± 0.6^{a}	0 ± 0.0^{a}
English Breakfast Black	400	43.4 ± 2.2^{b}	19.1 ± 2.3	58.5 ± 4.8	58.5 ± 7.2	28.9 ± 1.5^{b}	68.7 ± 2.6	81.6 ± 11.0^{b}	43.2 ± 1.9	50.5 ± 2.1	42.3 ± 3.0	–7.3 ± 0.4^{a}	25.5 ± 2.5
C: 54.0, 28.5	200	8.1 ± 0.1^{a}	7.4 ± 0.2^{a}	24.6 ± 1.4^{b}	56.8 ± 1.1	–3.6 ± 0.2^{a,b}	19.3 ± 0.6	47.0 ± 1.5^{b}	23.9 ± 1.9	33 ± 1.5	35.1 ± 1.1	–7.3 ± 0.4^{a,b}	10.9 ± 1.4^{a}
T: 15.4, 5.4	100	–10.3 ± 0.1^{a,b}	6.6 ± 0.3^{a}	–16.1 ± 0.7^{a,b}	16.1 ± 3.9^{a}	–18.1 ± 0.7^{a}	–2.4 ± 0.1^{a}	25.2 ± 0.9	15.8 ± 1.0	14.4 ± 2.3^{a}	26.8 ± 2.3	–7.3 ± 0.1^{a}	3.6 ± 0.4^{a}
F: 69.4, 33.9	50	–17.6 ± 0.4^{a,b}	3.7 ± 0.0^{a}	0 ± 0.0^{a}	12.7 ± 3.4^{a}	–16.9 ± 0.5^{a,b}	10.8 ± 1.9^{a}	11.5 ± 0.5	3.4 ± 0.1^{a}	–2.1 ± 0.5^{a}	9.3 ± 1.2^{a}	–10.9 ± 1.4^{a}	–7.3 ± 1.7^{a}
Exotica Dragonwell Green	400	69.9 ± 6.8^{b}	47.1 ± 6.5	59.3 ± 3.7	73.7 ± 2.4	30.1 ± 1.6^{b}	50.6 ± 2.5	92.7 ± 0.0^{b}	82.5 ± 26.2	52.6 ± 2.3^{b}	30.9 ± 0.9^{a}	5.5 ± 0.1^{a}	10.9 ± 0.4
C: 97.7, 74.4	200	17.6 ± 0.9	11.0 ± 1.4	55.9 ± 1.1	55.1 ± 8.3	21.7 ± 0.7	18.1 ± 3.5	62.8 ± 2.2	63.2 ± 8.8	36.1 ± 2.9	29.9 ± 2.6	–14.5 ± 0.2^{ab}	7..3 ± 0.3^{a}
T: 0, 0	100	–11.8 ± 0.2^{a,b}	2.9 ± 0.13^{a}	22.9 ± 1.5	24.6 ± 1.7^{a}	–1.2 ± 0.1^{a}	–3.6 ± 0.3^{a}	26.5 ± 0.5	25.6 ± 0.3	13.4 ± 1.7^{a}	11.3 ± 1.1^{a}	–14.5 ± 0.2^{a}	1.8 ± 0.1^{a}
F: 97.7, 74.4	50	–17.6 ± 0.5^{a,b}	0.7 ± 0.0^{a}	–7.6 ± 0.3^{a}	6.8 ± 0.8^{a}	–19.3 ± 2.5^{a}	–9.6 ± 1.3^{a}	14.1 ± 0.2	12.0 ± 0.2	6.2 ± 0.7^{a}	5.2 ± 1.0^{a}	–16.4 ± 1.0^{a}	0 ± 0.0^{a}
Exotica Silver Jasmine specialty	400	22.1 ± 1.0	28.7 ± 1.8	44.9 ± 4.1	72.9 ± 2.3	38.6 ± 0.0^{b}	50.6 ± 1.2	83.3 ± 12.8^{b}	73.5 ± 21.3	36.1 ± 1.2	29.9 ± 1.3	–12.7 ± 0.4^{a,b}	25.5 ± 0.6
C: 119, 83.1	200	3.7 ± 0.2^{a}	15.4 ± 1.2	28.0 ± 3.0	46.6 ± 5.9	34.9 ± 1.3	41.0 ± 1.7	37.2 ± 1.8^{b}	55.6 ± 2.7	36.1 ± 2.9	25.8 ± 2.5	–10.9 ± 0.5^{ab}	12.7 ± 0.5^{a}
T: 0, 0	100	1.5 ± 0.0^{a}	8.1 ± 0.6^{a}	–11.9 ± 0.6^{a}	–19.5 ± 0.4	30.1 ± 4.7^{b}	6.0 ± 1.1^{a}	14.5 ± 0.4	20.1 ± 0.4	33.0 ± 2.0^{b}	13.4 ± 0.8^{a}	–12.7 ± 1.0^{a}	3.6 ± 0.3^{a}
F: 119, 83.1	50	–0.7 ± 0.0^{a}	5.1 ± 0.1^{a}	–23.7 ± 2.3^{a}	–24.6 ± 1.8^{a}	26.5 ± 3.0^{b}	3.6 ± 0.1^{a}	0.4 ± 0.0^{a}	8.5 ± 0.5	12.4 ± 2.0^{a}	–8.2 ± 1.8^{a}	–12.7 ± 0.6^{a}	5.5 ± 0.4^{a}
Fusion Green and White	400	22.8 ± 1.1	17.6 ± 0.5	48.3 ± 3.2^{b}	72.0 ± 4.4	36.1 ± 1.4^{b}	61.4 ± 5.8	77.8 ± 4.5	68.8 ± 12.3	48.5 ± 1.94	45.4 ± 2.6	–5.5 ± 0.3^{a,b}	27.3 ± 0.0
C: 79.2, 67.0	200	3.7 ± 0.2^{a}	3.7 ± 0.1^{a}	42.4 ± 2.5	53.4 ± 3.9	27.7 ± 1.8	28.9 ± 0.5	39.7 ± 0.6	35.5 ± 1.2	33 ± 4.06	33.0 ± 2.0	–10.9 ± 1.1^{a,b}	12.7 ± 0.5^{a}
T: 0, 0	100	–3.7 ± 0.0^{a}	0.7 ± 0.0^{a}	–0.8 ± 0.1^{a}	4.2 ± 0.2^{a}	13.3 ± 0.7^{a}	16.9 ± 0.2^{a}	16.7 ± 0.8	12.8 ± 0.2	29.9 ± 2.2	27.8 ± 0.8	–12.7 ± 1.0^{a}	–1.8 ± 0.1
F: 79.2, 67.0	50	0 ± 0.0^{a}	0 ± 0.0^{a}	–12.7 ± 1.4^{a}	–8.5 ± 0.7^{a}	9.6 ± 0.6^{a}	12 ± 0.7^{a}	10.3 ± 0.4	4.3 ± 0.2^{a}	–6.2 ± 0.4^{a}	13.4 ± 0.6^{a}	–10.9 ± 0.9^{a}	–5.5 ± 0.1^{a}
Jasmine Blossom Green	400	52.9 ± 4.1^{b}	27.2 ± 2.5	59.3 ± 1.2^{b}	44.1 ± 4.0	26.5 ± 2.2^{b}	62.7 ± 2.0	89.7 ± 7.5^{b}	71.4 ± 9.6	51.5 ± 1.1^{b}	37.1 ± 3.6	0 ± 0^{a}	–5.5 ± 0.3^{a}
C: 108, 64.1	200	5.1 ± 0.2^{a}	2.2 ± 0.1^{a}	56.8 ± 1.1^{b}	38.1 ± 1.6	15.7 ± 0.7	31.3 ± 2.2	59.0 ± 3.7^{b}	45.7 ± 2.9	33.0 ± 3.0	22.7 ± 1.2	–5.5 ± 0.2^{a}	–18.2 ± 1.7^{a}
T: 0, 0	100	–8.1 ± 0.3^{a}	0.7 ± 0.0^{a}	22.0 ± 2.4	8.5 ± 0.3^{a}	–1.2 ± 0.1^{a}	8.4 ± 0.4^{a}	28.2 ± 1.2^{b}	16.2 ± 1.4	14.4 ± 1.7^{a}	10.3 ± 1.4^{a}	–5.5 ± 0.6^{a}	–25.5 ± 0.4^{a}
F: 108, 64.1	50	–14.0 ± 0.2^{a,b}	–0.7 ± 0.0^{a}	–4.2 ± 0.5^{a}	2.5 ± 0.3^{a}	–14.5 ± 2.9	7.2 ± 0.6^{a}	15.8 ± 0.5	13.2 ± 1.1	–2.1 ± 0.2^{a}	–6.2 ± 0.5^{a}	–10.9 ± 0.2^{a}	–27.3 ± 5.1^{a}
Lemon Spice Green and Black	400	11.8 ± 0.7^{b}	43.4 ± 3.9	41.5 ± 0.0	46.6 ± 5.2	38.6 ± 2.3^{b}	50.6 ± 7.4	56 ± 3.3^{b}	74.4 ± 2.5	41.2 ± 1.4	33.0 ± 1.0	–5.5 ± 0.2^{a,b}	23.6 ± 1.1
C: 42.1, 33.5	200	6.6 ± 0.2^{a}	15.4 ± 0.8	13.6 ± 0.8^{a}	5.9 ± 0.5^{a}	24.1 ± 1.9	21.7 ± 1.7	30.3 ± 1.5^{b}	49.1 ± 1.2	28.9 ± 2.9	29.9 ± 3.1	–9.1 ± 0.5^{a}	5.5 ± 0.2^{a}
T: 7.2, 2.6	100	6.6 ± 0.2^{a}	13.2 ± 1.3	–17.8 ± 1.8^{a}	–7.6 ± 0.5^{a}	19.3 ± 2.9^{a}	6.0 ± 0.5^{a}	17.9 ± 1.6	20.5 ± 0.8	5.2 ± 0.2^{a}	18.6 ± 1.4^{a}	–10.9 ± 1.6^{a}	–3.6 ± 0.4^{a}
F: 49.3, 36.1	50	5.1 ± 0.1^{a}	10.3 ± 0.3	–9.3 ± 1.2^{a}	–13.6 ± 0.3^{a}	18.1 ± 1.9	0 ± 0^{a}	15.4 ± 1.0	17.5 ± 0.3	–2.1 ± 0.1^{a}	6.2 ± 0.3^{a}	–16.4 ± 2.8	–9.1 ± 0.6^{a}
Premium Green	400	14.0 ± 1.7	15.4 ± 0.3	44.9 ± 3.4^{b}	67.8 ± 3.6	42.2 ± 0.9	36.1 ± 3.41	64.5 ± 5.4^{b}	24.4 ± 0.4	47.4 ± 6.5^{b}	30.9 ± 2.3	–10.9 ± 0.4^{ab}	29.1 ± 0.7
C: 96.8, 76.0	200	2.9 ± 0.2^{a}	6.6 ± 0.2^{a}	41.5 ± 0.6^{b}	58.5 ± 7.2	39.8 ± 2.4^{b}	15.7 ± 0.9^{a}	27.4 ± 1.9^{b}	16.2 ± 0.1	41.2 ± 2.2^{b}	19.6 ± 0.7^{a}	–10.9 ± 0.2^{ab}	23.6 ± 1.7
T: 0, 0	100	0 ± 0^{a}	4.4 ± 0.1^{a}	4.2 ± 0.3^{a}	20.3 ± 3.0^{a}	32.5 ± 1.7^{b}	0 ± 0.0^{a}	10.3 ± 0.5	15 ± 0.3	27.8 ± 1.2^{b}	6.2 ± 0.7^{a}	–10.9 ± 1.1^{ab}	18.2 ± 0.8
F: 96.8; 76.0	50	–4.4 ± 0.3^{a}	2..9 ± 0.0^{a}	–4.2 ± 1.3^{a}	–11.9 ± 1.4^{a}	27.7 ± 2.3^{b}	–9.6 ± 0.8^{a}	1..3 ± 0.0^{a,b}	12.8 ± 0.2	–18.6 ± 1.0^{a}	–18.6 ± 1.1^{a}	–23.6 ± 2.8^{a,b}	9.1 ± 1.1^{a}
Premium Green decaffeinated	400	19.9 ± 1.8^{b}	43.4 ± 2.2	47.5 ± 1.5^{b}	61.0 ± 6.6	41 ± 1.7^{b}	61.4 ± 0.0	70.9 ± 7.3^{b}	82.1 ± 3.9	45.4 ± 2.6^{b}	56.7 ± 1.3	–12.7 ± 1.4^{ab}	25.5 ± 1.9
C: 73.2, 32.5	200	–3.7 ± 0.2^{a}	6.6 ± 0.5^{a}	44.9 ± 8.3	47.5 ± 5.4	34.9 ± 1.3	39.8 ± 4.8	33.8 ± 1.5	43.6 ± 11.9	36.1 ± 2.9	34 ± 2.1	–3.6 ± 0.1^{a}	7.3 ± 0.1^{a}
T: 0, 0	100	–3.7 ± 0.2^{a}	5.9 ± 0.1^{a}	1.7 ± 0.2^{a}	5.1 ± 0.7^{a}	21.7 ± 1.3	22.9 ± 2.9	15 ± 0.1	20.9 ± 0.4	19.6 ± 0.2^{a}	15.5 ± 0.4^{a}	–1.8 ± 0.1^{a}	–5.5 ± 0.5^{a}
F: 73.2, 32.5	50	–4.4 ± 0.2^{a}	5.1 ± 0.3^{a}	3.4. ± 0.4^{a}	–7.6 ± 0.7^{a}	21.7 ± 1.0	16.9 ± 0.7	3.4 ± 0.1^{a,b}	20.1 ± 0.4	–6.2 ± 0.5^{a}	5.2 ± 0.2^{a}	1.8 ± 0.1^{a}	–9.1 ± 0.6^{a}
Sencha Japanese Green	400	86.8 ± 24.1^{b}	41.9 ± 10.6	71.2 ± 0.0^{b}	54.2 ± 1.0	32.5 ± 0.6	43.4 ± 0.9	96.2 ± 10.7^{b}	71.8 ± 18.5	53.6 ± 3.6	55.7 ± 3.9	1.8 ± 0.1^{a}	–5.5 ± 0.7^{a}
C: 94.0, 76.2	200	39.0 ± 0.9^{b}	15.4 ± 1.1	60.2 ± 2.6^{b}	24.6 ± 3.0	28.9 ± 0.49	28.9 ± 2.4	66.7 ± 5.1^{b}	45.3 ± 3.2	46.4 ± 0.9	49.5 ± 1.0	0 ± 0^{a}	–16.4 ± 2.0^{a}
T: 0, 0	100	–5.1 ± 0.0^{ab}	9.6 ± 0.4^{a}	24.6 ± 1.7^{b}	–14.4 ± 1.5^{a}	0 ± 0.00	12.0 ± 0.3^{a}	27.8 ± 0.5^{b}	14.5 ± 1.0	19.6 ± 1.5	20.6 ± 2.1	–7.3 ± 0.4^{a}	–23.6 ± 1.0^{a}
F: 94.0, 76.2	50	–17.6 ± 0.2^{a,b}	6.6 ± 0.3^{a}	14.4 ± 1.1^{a}	–5.9 ± 0.5^{a}	–8.4 ± 0.7^{a}	1.2 ± 0.1^{a}	14.5 ± 0.4	8.5 ± 0.1	13.4 ± 1.3^{a}	8.2 ± 0.5^{a}	–9.1 ± 0.3^{a}	–27.3 ± 1.1^{a}
Sushi Bar Mild Green	400	80.9 ± 9.3^{b}	39.7 ± 3.4	64.4 ± 3.1^{b}	71.2 ± 2.1	36.1 ± 1.4	37.3 ± 3.56	94.9 ± 7.9^{b}	73.9 ± 10.9	54.6 ± 1.2^{b}	39.2 ± 2.0	0 ± 0^{a}	16.4 ± 0.0
C: 116, 87.1	200	29.4 ± 1.5^{b}	8.8 ± 0.4^{a}	53.4 ± 1.9	50.0 ± 0.8	22.9 ± 0.7	12.0 ± 0.3^{a}	71.4 ± 7.5^{b}	45.3 ± 2.5	42.3 ± 1.5	30.9 ± 2.8	–3.6 ± 0.1^{a}	12.7 ± 0.8^{a}
T: 0, 0	100	–5.1 ± 0.0^{a}	7.4 ± 0.3^{a}	34.7 ± 1.8^{b}	0 ± 0^{a}	–7.2 ± 1.2^{a}	–1.2 ± 0.1^{a}	31.6 ± 1.6	23.1 ± 0.4	13.4 ± 1.4^{a}	–1 ± 0.1^{a}	–5.5 ± 0.5^{a}	3.6 ± 0.2^{a}
F: 116, 87.1	50	–8.1 ± 0.3^{a}	2.9 ± 0.1^{a}	10.2 ± 0.6^{a}	–6.8 ± 0.7^{a}	–20.5 ± 1.0	0 ± 0^{a}	16.7 ± 0.9	17.9 ± 0.3	–1.0 ± 0.1^{a}	–16.5 ± 0.9^{a}	–7.3 ± 0.5^{a}	1.8 ± 0.1
Wild Raspberry herbal	400	41.2 ± 1.0^{b}	58.8 ± 5.2	19.5 ± 2.0	30.5 ± 0.7	34.9 ± 0.6^{b}	4.8 ± 0.5^{a}	87.2 ± 14.5	81.6 ± 20.9	34.0 ± 1.6	25.8 ± 3.2	14.5 ± 1.2	21.8 ± 1.0
C: 0, 3.5	200	5.9 ± 0.3^{a}	18.4 ± 0.5	5.1 ± 0.4^{a}	–0.8 ± 0.0^{a}	24.1 ± 0.8^{b}	–2.4 ± 0.1^{a}	47.0 ± 4.2^{b}	65.0 ± 0.8	17.5 ± 2.0^{a}	5.2 ± 0.2^{a}	9.1 ± 0.4^{a}	18.2 ± 1.2
T: 0, 0	100	1.5 ± 0.0^{a}	–8.1 ± 0.2^{a}	–5.9 ± 0.2^{a}	–18.6 ± 0.8^{a}	13.3 ± 1.1^{ab}	–26.5 ± 0.8^{a}	19.7 ± 0.1^{b}	30.3 ± 0.9	9.3 ± 0.9^{a}	–5.2 ± 0.4^{a}	3.6 ± 0.1^{a}	1.8 ± 0.1^{a}
F: 0, 3.5	50	–2.2 ± 0.1^{a}	–8.8 ± 0.1^{a}	–12.7 ± 0.9^{a}	–22.0 ± 1.4^{a}	9.6 ± 0.6^{a,b}	–27.7 ± 0.8^{a}	18.8 ± 0.7	19.2 ± 0.5	8.2 ± 0.4^{a}	–16.5 ± 3.4^{a}	–1.8 ± 0.1^{a}	1.8 ± 0.0^{a}

[a] Not significantly different from control ($p > 0.05$) with Dunnett's one-tailed test (inactive). Values without superscript [a] are significantly different ($p < 0.05$) (active). [b] Anticarcinogenic activities of 80% ethanol/water extract significantly different from corresponding water extract ($p < 0.05$) using the Bonferroni adjustment for multiplicity.

cancer cells. Theanine may contribute to in vivo effects of teas against prostate cancer.

Other studies have reported that the consumption of tea is associated with decreased risk or progression of prostate cancer (*35*), possibly by inhibiting the overexpression of the cyclooxygenase (COX-2) enzyme (*36*).

Normal Chang Liver and HEL299 Lung Cells. **Table 4** shows that the tea extracts were also cytotoxic to normal liver but not to lung cells. The water extracts generally exhibited lower effects than did the ethanol extracts.

Relationship between the Composition of Teas and Anticarcinogenic Effects. Our attempts to relate levels of catechins and theaflavins of 15 teas determined by HPLC to anticarcinogenic effects were not successful. For example, for HT29 colon cancer cells, **Figure 3** shows the best correlations we observed with the sum of catechins and theaflavins ($R^2 = 0.3323$ for the water

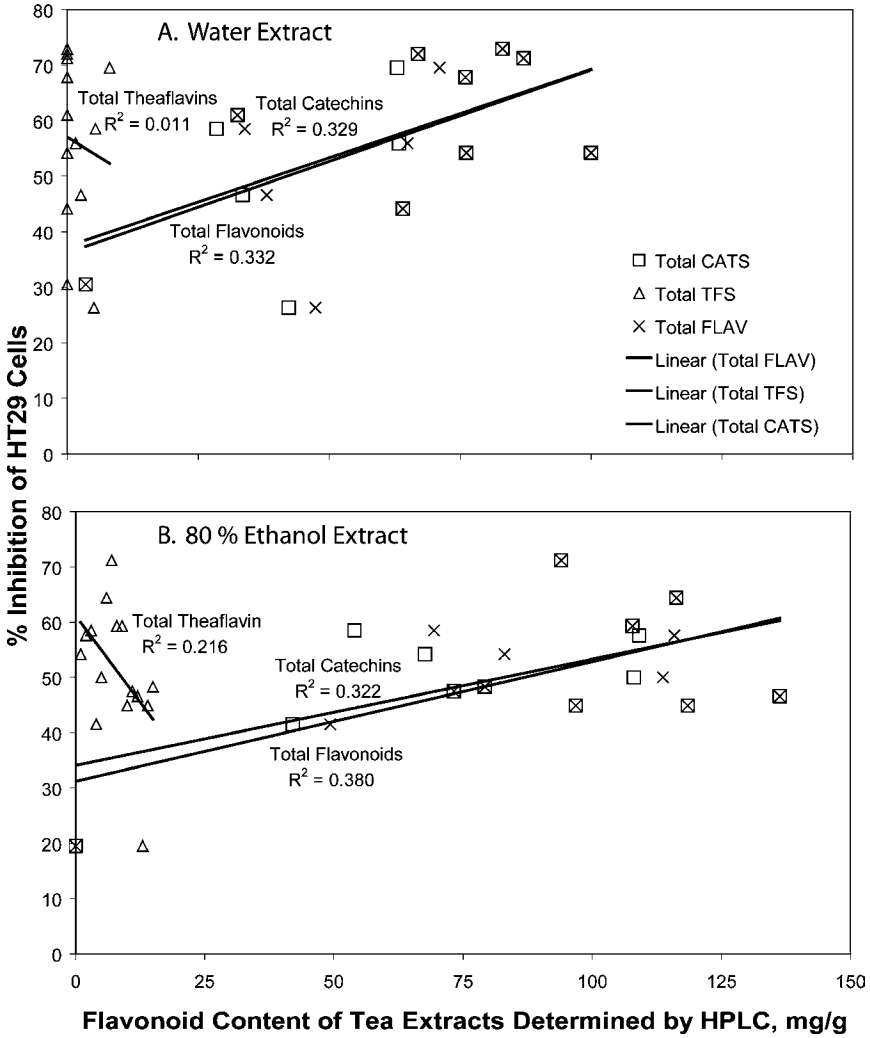

Figure 3. Relationship between the flavonoid content of teas determined by HPLC (*2*) and the antiproliferative activities against HT29 colon cancer cells shown in **Table 4**. The correlation coefficients were calculated by an Excel statistical program for least-squares fits.

extracts and 0.3797 for the 80% ethanol extracts). The correlation coefficients for the other cell lines (not shown) were lower than these low values. These observations suggest that other factors may contribute to the anticarcinogenic effects of teas. Such factors may include competitive, additive, and antagonistic interactions among the flavonoids at the molecular and cellular levels of the cancer cells.

Microscopy of Untreated and Treated Cancer Cells. The growth inhibition determined by the MTT assay was confirmed visually by microscopy of treated and untreated cancer cells, illustrated in **Figure 4**. This figure depicts the concentration-dependent decrease in the number of PC-3 human prostate cancer cells following exposure to the tea extracts. Both the MTT assay and microscopy show that the tea extracts were effective in killing the cancer cells. The disappearance of large numbers of cancer cells shown in the photomicrographs is consistent with an anticarcinogenic mechanism involving loss of cell viability as a result of cytotoxicity.

Mechanisms of Anticarcinogenic Effects of Flavonoids. Carcinogenesis is a sequential multistage cellular process consisting of tumor initiation, tumor promotion, and tumor progression. Because tumor promotion may be the only reversible event during cancer development, its suppression is regarded as an effective way to inhibit carcinogenesis (*37*). Because laboratory and epidemiological studies show that tea catechins and theaflavins and teas may protect animals and humans against breast (*38*), endometrial (*39*), esophageal (*40*), lung (*41*, *42*), prostate (*43*), stomach (*44*), and skin cancers (*45*), there is interest in defining the mechanisms by which these dietary ingredients exert their beneficial effects.

In addition to the mechanistic aspects mentioned earlier, published studies suggest that the antiproliferative effects of tea flavonoids result from multiple interactions at the molecular and genetic levels of cells. Thus, flavonoids are reported to (a) induce formation of reactive oxygen species (ROS) that trigger cell death (apoptosis) (*46*); (b) inhibit cytochrome P450 (CYP) enzymes involved in activation of procarcinogens and activate phase II enzymes such as glutathione-*S*-transferase and quinone reductase that catalyze the detoxification of carcinogens (*47*); (c) inhibit signal transduction proteins induced by tumor promoters including kinases causing arrest of the cell−cell cycle (*48*); (d) bind to plasma and other proteins that may then trigger an apoptosis cascade (*48*); (e) suppress secretion of metalloproteinases required for normal function of cells (*30*, *49*); (f) bind to and damage DNA and RNA of transcription factors involved in cancer promotion (*50*); (g) inhibit expression and release of the tumor necrosis factor (TNF)-α (*42*); and (h) inhibit angiogenesis (development of new blood vessels) in cancer cells (*49*). To what extent these events also occur in animal and human tissues merits further study.

Significance for the Human Diet. Although the bioavailability of flavonoids is low, multiple consumption of epigallo-

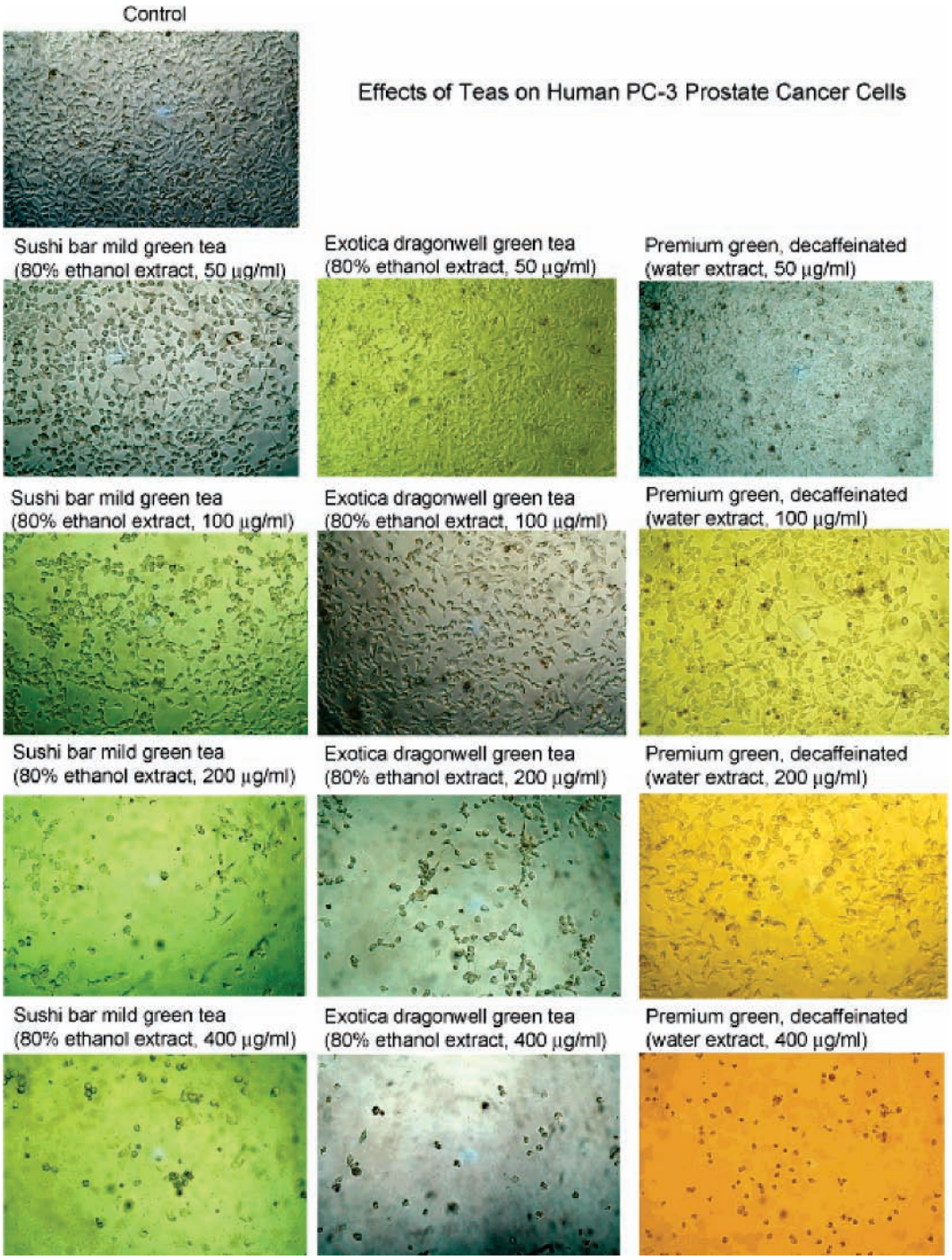

Figure 4. Photomicrographs showing the concentration-dependent destruction of human cancer cells by tea extracts.

catechin-3-gallate and of teas resulted in significant accumulation of catechins in most body organs with peak plasma levels of up to 7.5 μM (*51*−*54*). Moreover, human consumption of a tea preparation equivalent to 2−3 cups of tea resulted in saliva catechin levels ~100 times greater than peak plasma levels (*55*). These observations suggest that long-term consumption of tea can result in the absorption and retention of sufficient levels of flavonoids to exert beneficial effects directly in tissues or indirectly by modulating cell signaling pathways.

The results we obtained in this study extend our knowledge about the anticarcinogenic effects of catechins present in green teas, theaflavins present in black teas, and the amino acid theanine present in both tea categories. Because it may be risky to translate results from cell assays to in vivo effects, the observed destruction of a broad range of cancer cells suggests the need for animal and human studies designed to ascertain whether the observed wide variation in potencies of tea compounds and teas can predict corresponding effects in vivo.

The present study has not only demonstrated significant differences in anticarcinogenic potencies among the evaluated 13 tea compounds and 15 tea extracts but also shown that in most cases the ethanolic tea extracts generally induce cell death of cancer cells more effectively than do aqueous tea infusions. However, our attempts to relate the composition of tea extracts determined by HPLC to observed chemopreventive effects were not successful. To our knowledge, few previous studies tested biological effects of infusions of commercial teas consumed in the home or aqueous ethanol extracts containing higher flavonoid levels than the corresponding water extracts. We therefore suggest that aqueous ethanol should be used in the

preparation of concentrated black and green tea extracts for sale as dietary supplements in the form of powders and capsules.

Consumption of teas may also inhibit the growth of some normal liver cells. Therefore, a key consideration for the use of tea ingredients and teas in cancer prevention and treatment should be the ratio of effective preventive or therapeutic to toxic dose. Thus, although green tea extracts and their constituents were cytotoxic in rat hepatocytes (*56*), EGCG protected HepG2 normal liver cells against chronic alcohol-induced liver damage (*57*).

Whether tea flavonoids can additively or synergistically enhance anticarcinogenic effects of anticancer drugs, as they do antimicrobial activities of antibiotics (*58*), merits further study.

ACKNOWLEDGMENT

We thank Carol E. Levin for assistance with the figures and tables.

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Received for review August 7, 2006. Revised manuscript received October 11, 2006. Accepted November 2, 2006.

JF062276H